A phylogeny of the genus *Gasteracantha* Sundevall, 1833 (Araneae, Araneidae) with an examination of sexually dimorphic characters in the subfamily Gasteracanthinae

Steven Hoff Williams

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Abstract

The Arachnida subfamily Gasteracanthinae, commonly known as thorn or kite spiders due to their prominent abdominal spines, is mainly Pan-Tropical in distribution. Little has been published about the relationships and classification of the genera and there is still considerable taxonomic confusion about the status of some of the species in this subfamily; in particular, within the biggest genus: *Gasteracantha* Sundevall, 1833. This thesis represents the largest work on *Gasteracantha* since the studies by Dahl (1914) and Emerit (1974). By using both molecular and morphological cladistic analyses, the most comprehensive phylogeny to date of *Gasteracantha*, and the related genera, is inferred. By supplementing specimens from worldwide institutional collections this thesis highlights the need for both molecular and morphological data to classify these species' relationships and why a taxonomic knowledge of the genera is critical for understanding and interpreting these relationships. Additionally, a geometric morphometric analysis is conducted to quantify and examine the evolution of the abdominal shapes of taxa within the subfamily. With a phylogeny constructed, a biogeographical distribution analysis is conducted. This is followed by an analysis of the extreme sexual size dimorphism (eSSD) between Gasteracanthinae males and females, along with an examination of the evolution of several sexually dimorphic characters. Finally, taxonomic changes are proposed, suggestions around improving molecular and morphological work along with future areas of ecological study are made, and a novel approach to gathering images for future morphometric analysis is trialled. This thesis provides a resource that will help arachnologists worldwide on the future understanding of *Gasteracantha* and the rest of the subfamily taxa.

Acknowledgements

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This thesis is dedicated entirely to my late grandparents Dr Jack and Sheila Williams who would have loved reading it. It is also a special thank you to my grandad, Jack, who took me to OUMNH every week (for many years) so I could use the Entomology Department microscopes to identify British spiders and look at the dried *Gasteracantha* collection.

Diolch yn fawr.

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Chapter 1: Introduction and history

1.1 Introduction

In the current global biodiversity crisis, biological communities and their component taxa are increasingly vulnerable to negative anthropogenic impacts as highlighted by recent summaries produced by the International Union for Conservation of Nature (IUCN, 2022) and World Wide Fund For Nature (WWF, 2022). Consequently, extinction rates of species are increasing (Régnier *et al*., 2015). The ability to correctly define species and ascribe individuals to species (taxonomy) or even to distinct evolutionary taxonomic units and to understand the evolutionary relationships between taxa (phylogenetics) can contribute greatly to understanding biodiversity and its changes. Correct classification and a detailed understanding of a phylogeny allows for approaches to conservation based on evolutionary distinctiveness and the application of complementarity in where to focus conservation effort (Erwin, 1991; Vane-Wright, Humphries and Williams, 1991). As examples, the phylogenetic approach has been used to identify evolutionary distinct and endangered vertebrate species that require specific conservation actions (ZSL Edge Programme, 2022).

Phylogenetics has also been applied to diversity within species to identify distinct evolutionary lineages and specific conservation actions to maintain these lineages, as for example the butterfly fauna within the Tuscan Archipelago (Dapporto *et al*., 2017). There is, however, wide understanding that much of our knowledge of species relationships is limited and many species are undescribed or misclassified. Within the large groups of invertebrates, for example the insects, there are currently over 1 million described species and probably 80% more undescribed species (Stork, 2018). Amongst the arachnids there are over 100,000 described species and potentially an additional 500,000 that remain undescribed (Chapman,

2009). This impediment is being addressed, and new information is being continually assembled within global databases such as the Global Biodiversity Information Facility (GBIF, 2022) and National Center for Biotechnology Information (NCBI, 2022), with accompanying revisions to the classification and taxonomy of many taxa. In time this will allow meaningful phylogenies to be constructed, adding to understanding of biodiversity, its importance and how it has been shaped by past events over different time scales and how it is threatened by current anthropogenic activities.

Many invertebrate taxa have never been examined in any phylogenetic analysis. For these poorly researched groups their evolutionary relationships are currently unknown or conjectural. It is not uncommon to find large groups of species, genera or even families that have not had a recent taxonomic reclassification since their original descriptions. They have not had their respective species descriptions revised with modern techniques, instead they remain characterised by possibly unreliable methods and characters that are not good species dividers. This is particularly the case for some spider (Araneae) taxa, which are the subject of this thesis.

Historically, species were frequently ascribed to a genus or subfamily, the taxonomic grouping below family but above tribe or genus, and might have been considered closely related to other species due to single morphological characters that could have evolved in parallel. However, these older classifications were not attempting to imply evolutionary relationships. For many invertebrate taxa, taxonomic reclassifications have re-examined the relationships of groups of species or genera and now frequently include molecular data within them (for example: Benavides, Giribet and Hormiga, 2017; Castalanelli *et al.*, 2017; Huber, Eberle and Dimitrov 2018; Yan *et al.*, 2019). Research in a specific geographical region

might provide a reason to reclassify a group of species in that area, but with spider taxa that have extensive distributions it is uncommon to see full generic or family revisions.

With a well-supported phylogeny other research topics can be developed. These include the analysis of biodiversity in geographical locations, predictions of relationships with other taxa can be hypothesised, evolutionary development of characteristic or behavioural traits, and changes in species richness in the context of lineage changes (Sadava *et al*., 2011). Fundamentally, a well-supported phylogeny allows biodiversity and its spatial and temporal changes to be understood. When combined, taxonomy, classification and phylogenetics, form the study of systematics. Traditional taxonomy and classification have largely relied on morphological characteristics but molecular tools have revolutionised the study of systematics Molecular sequences available from cloud-based data storage sites (for example, BOLD (https://www.boldsystems.org/) and NCBI (https://www.ncbi.nlm.nih.gov/) can provide extensive datasets for inferring phylogenies facilitating revised understanding about relationships between species (Larabee *et al.*, 2016; Shi, Li and Li, 2021), assigning individuals to species, or distinct evolutionary units and revealing cryptic diversity (Carr *et al.*, 2011; Zhang and Li, 2014).

Whilst molecular tools are providing important advances in the understanding of species relationships (Cameron *et al.*, 2007; Schmidt, 2013; Larabee *et al.*, 2016; Shi, Li and Li, 2021), more traditional approaches to taxonomy still have important roles (Bybee *et al*., 2010; Giribet, 2015; Palandačić *et al*., 2017) as they can provide information to test hypotheses or add characters to predominantly molecular based phylogenies.

Morphology is still important to systematics because without it our understanding of species and what characteristics define them would be limited to molecular data and offer limited practicalities for field-based ecological work. Furthermore, it can be used to infer how and when specific characters evolve and arise or are lost within a clade. However, imprecise taxonomy or reliance on historical species descriptions for morphological characteristics can also lead to species misidentifications and impact interpretations. This can lead to inaccurate phylogenetic results or misunderstandings when examining character evolution and species relationships in both morphological and molecular based phylogenies. Examples of this can be found in insects and spiders (for example on the early divergence of pterygotes, Thomas *et al*, 2013; Gasteracanthinae, Tan *et al*., 2019; or the phylogeny of Phasmatodea, Bank *et al*., 2021). Therefore, having a taxonomic understanding of the study group can provide confidence in result interpretation and the testing of hypotheses.

This project examines the taxonomy, classification, and phylogeny of one taxonomic group of spiders: the subfamily Gasteracanthinae (Figure 1.1); with a focus on the largest genus in that group: *Gasteracantha*. Despite the group containing some exotic species and some members being used as models for behaviour, ecology and distribution (Bukowski, Linn and Christenson, 2001; Song, Zhu and Chen, 2001; Kim and Cho, 2002; Heinrichs and Barrion, 2004; Shin, 2007; Gawryszewski and Motta, 2012; Sato, 2012; Sen *et al*., 2015; White and Kemp, 2015, 2016; Roy, Saha and Raychaudhuri, 2017; White, 2017; Williams, 2017; Chamberland *et al*., 2020; Messas *et al*., 2021; Kemp, Edwards and White, 2022; Salgado-Roa *et al*., 2022) the evolutionary relationships of the group, their classification and their taxonomy are confused and require revision. This is what this thesis attempts to address.

Members of this group are morphologically diverse, and usually characterised by having long abdominal spines (Figure 1.1) and displaying extreme sexual dimorphism (Figure 1.1 and 1.2). They have a Pan-Tropical/Oriental/Australasian/New World distribution but, despite being studied for over 100 years, there are only very limited phylogenies available for small geographical regions (see section 1.2 for breakdown of previous work).

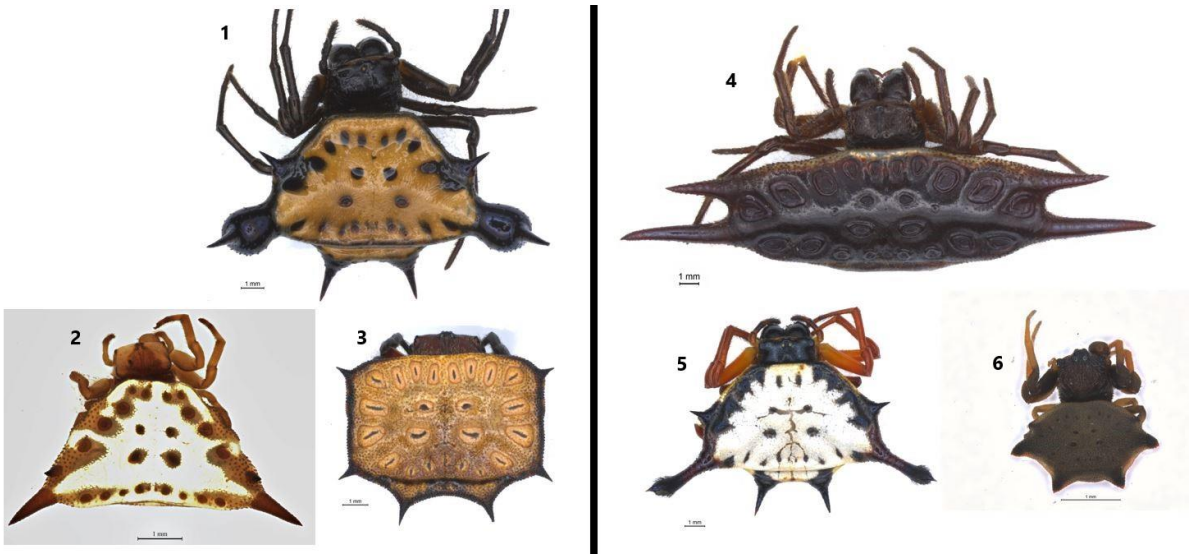

Figure 1.1 Examples of Gasteracanthinae, all in dorsal view, Left: Gasteracanthinae: 1. *Actinacantha globulata* (Walckenaer, 1841) (♀), 2. *Acrosomoides acrosomoides* (O. Pickard-Cambridge, 1879) (♀), 3. *Isoxya tabulata* (Thorell, 1859) (♀) and Right: *Gasteracantha*: 4. *G. mengei* Keyserling, 1864 (♀), 5. *G. clavatrix* (Walckenaer, 1841) (♀) and 6. *G. aciculata* (Pocock, 1898) (♂).

The Gasteracanthinae are members of the spider family Araneidae (Wheeler *et al.*, 2017). Araneidae is in the entelegyne subgroup of araneomorph spiders that is taxonomically defined by the female genital system, the eye arrangement, and the number of tarsal claws (Levi, 2002). Currently, the family is thought to comprise 180 genera (World Spider Catalog, 2022) that have a worldwide distribution (Jocqué and Dippenaar-Schoeman, 2006). Genera in

the family range from the common garden spiders: *Araneus*, to the large striped orb-weaving spiders: *Argiope*. Most genera construct webs to capture prey, although some have reduced webs or have entirely abandoned web building (Jocqué and Dippenaar-Schoeman, 2006). A history of Gasteracanthinae taxonomy, along with current knowledge and research featuring the group, is described in the next section.

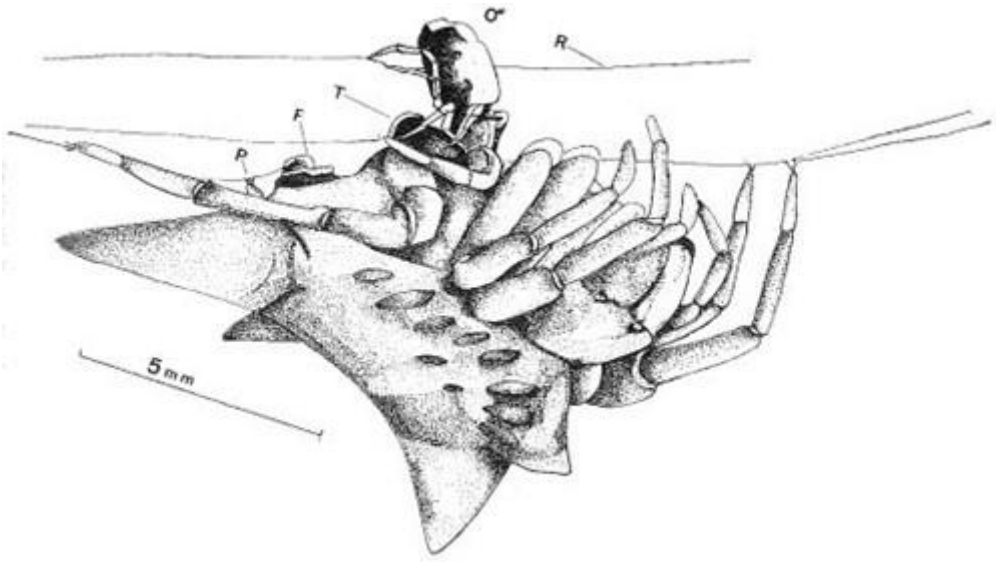

Figure 1.2 Line drawing of *Gasteracantha versicolor* (Walckenaer, 1841) male and female mating; from Emerit (1968)

1.2 Historical classification of the subfamily

The subfamily, Gasteracanthinae, is currently thought to comprise of seventeen genera: *Acrosomoides, Actinacantha, Aetrocantha, Afracantha, Aspidolasius, Austracantha, Augusta, Encyosaccus, Gasteracantha, Gastroxya, Hypsacantha, Isoxya, Macracantha, Madacantha, Parmatergus, Thelacantha* and *Togacantha* (some examples shown in Figure 1.1) (World Spider Catalog, 2022). Only four species and one sub-species are currently represented in the New World: *Afracantha camerunensis* (Thorell, 1899), *Aspidolasius branicki* (Taczanowski, 1879), *Gasteracantha cancriformi*s (Linnaeus, 1758), *G. cancriformis gertschi* Archer, 1941 and *G. flava* Nicolet, 1849 (World Spider Catalog, 2022). The genus *Gasteracantha*, the largest genus within Gasteracanthinae, is currently described as comprising 69 species and 18 sub-species (World Spider Catalog, 2022) but the subfamily classification has undergone some changes throughout its history.

Simon, in his 1895 *Histoire naturelle des Araignées*, listed the subfamily Gasteracanthinae as containing only *Gasteracantha* and *Encyosaccus*, but this has expanded over time. Historically there have been only three authors who published groupings for the Gasteracanthinae, all based on morphological characters and similarities in species distribution patterns: Simon (1864), Butler (1873) and Dahl (1914).

The 1864 publication by Simon was the first attempt at classifying the Gasteracanthinae into different species groups using simple features of gross morphology. The groupings were based on a limited range of morphological characters including spine shape and structure and abdominal shape. Simon split the 48 known species at the time into 8 species groups. In 1873 Butler provided a revision of *Gasteracantha* and included 113 species; many of these were later determined as synonyms. Butler (1873) divided these species into 13 species groups,

and, as with Simon, the division was based upon easily observed morphological characters such as spines and abdominal shape, along with some geographical distribution information.

By the time Dahl revised the genus in 1914 the species list had been reduced to 75 species due to the removal of synonyms, but Dahl split them into 16 species groups (Figure 1.3). Many of the names given in this publication are still valid and many of the species group names were subsequently used as generic names when Benoit (1962a, 1962b and 1964) and Emerit (1974) elevated members of the *Gasteracantha* to separate genera using more morphological character information.

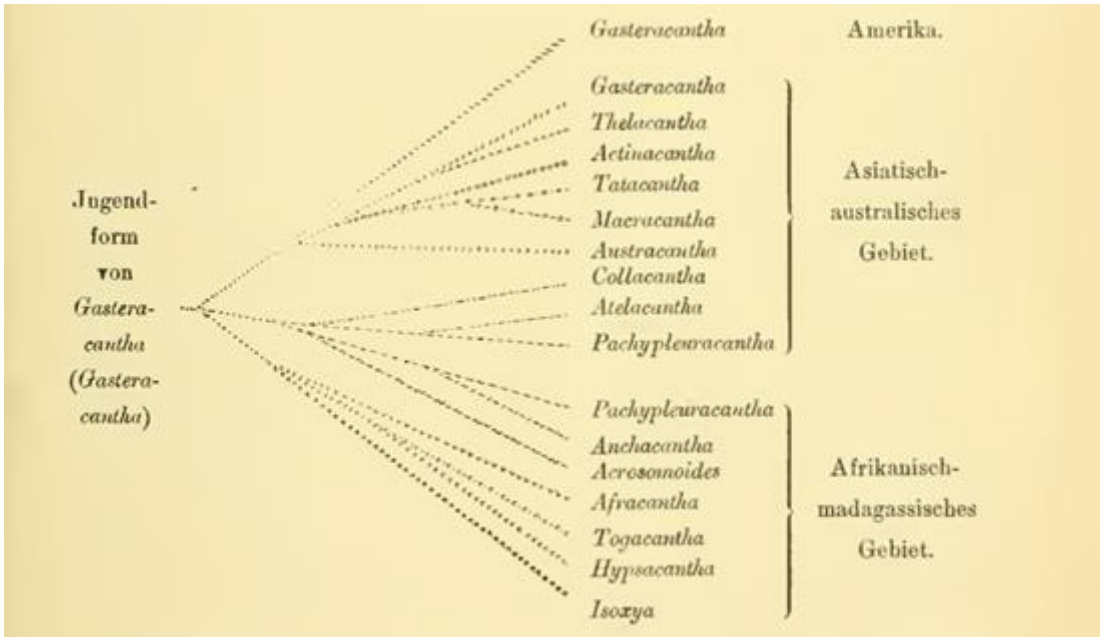

Figure 1.3 Dahl's sub-generic groupings of the Gasteracanthinae partly based on geographical distribution; from Dahl (1914)

After 1914 there have been no major world revision of the subfamily, but notable publications included Benoit (1962a, 1962b and 1964) who re-described species, described new species and assigned males of the African gasteracanthine fauna; Chrysanthus (1959,

1960, 1971) who only looked at the Solomon Islands and New Guinea species; Emerit (1968, 1973 and 1974) who revised the Madagascan species and confirmed or disagreed with Benoit's proposals for moving several species of *Gasteracantha* into their own new genera; and Tikader (1981; 1982) who revised the Indian species. These 20th Century publications started to explore the genus in detail by including fine line illustrations of the spiders and their genitalia and discussion about geographical location, possible relationships and, in the case of Emerit (1968, 1973 and 1974), included a very comprehensive description of all the above and their developmental biology. All these helped the taxonomic understanding of the genus but were limited by the geographic coverage of each of the studies.

Due to these limited revisions and differing opinions, the current taxonomy of the genus and closely related genera is questionable. Many species within *Gasteracantha* and the related genera are believed to be misplaced (Yong and Ono, 2009; Nentwig *et al*., 2019; Tan *et al*., 2019; Macharoenboon, Siriwut and Jeratthitikul 2021). The current recognised genera within Gasteracanthinae were formed when it was expanded by Scharff and Coddington (1997) to comprise the genera: *Gasteracantha*, *Augusta*, *Aetrocantha*, *Austracantha*, *Macracantha*, *Isoxya*, *Togacantha* and *Encyosaccus*. Within their research they included a large proportion of the Gasteracanthinae. It is apparent from their results that *Gasteracantha* is not monophyletic (a group of species all descended from the same common ancestor) but is probably paraphyletic (containing members of different genera and not all of the species of *Gasteracantha*). It is hypothesised here that there are species incorrectly classified as *Gasteracantha* and some splits into new genera may not be fully justified (see section 1.5). This is addressed within this thesis.

This thesis examines the relationship of the gasteracanthines, as referred to in Scharff *et al*. (2020), and aims to resolve a number of these currently questionable taxonomic splits. Since Scharff and Coddington (1997) other additions to the subfamily now include: *Acrosomoides*, *Actinacantha*, *Afracantha*, *Gastroxya*, *Hypsacantha*, *Madacantha*, *Aspidolasius* and *Thelacantha* (World Spider Catalog, 2022). There is currently no modern published list of the genera included in the Gasteracanthinae, the World Spider Catalog does not split into subfamily classifications, although "the *classic* composition is given in Platnick's book catalogue (Platnick 1989, 1993 and 1998), but not the on-line catalogue started in 2000" (Scharff *in litt*., 2016).

Large publications on Gasteracanthinae were scarce through the 2000s with only a new species (Mi and Peng, 2013), descriptions of distributions of species or environmental studies (Song, Zhu and Chen, 2001; Kim and Cho, 2002; Heinrichs and Barrion, 2004; Shin, 2007; Sato, 2012; Sen *et al*., 2015; Roy, Saha and Raychaudhuri, 2017; Williams, 2017), redescription of species and identification guides - predominantly from the Oriental region (Namkung, 2002, 2003; Kim and Kim, 2002; Kim and Park, 2007; Tanikawa, 2007, 2009; Zhu and Zhang, 2011; Yin *et al*., 2012; Kim and Lee, 2012; Kim *et al*., 2013; Mi and Peng, 2013; Sankaran, Jobi and Sebastian, 2015) colouration and prey capture (Gawryszewski and Motta, 2012), copulation and sperm release in *Gasteracantha cancriformis* (Bukowski, Linn and Christenson, 2001) (see Chapter 4 for further discussion on mating), and research publications featuring *Gasteracantha* as an outgroup taxon (a more distantly related group that serves as a reference when determining the evolutionary relationships of the taxa being studied) (Álvarez-Padilla and Hormiga, 2011; Magalhães and Santos, 2012).

In 2019 Tan *et al*. examined the phylogenetic relationships of four genera of Gasteracanthinae in Peninsular Malaysia using a small sample size of six Gasteracanthinae species from the 37 species that are found in the Oriental region. Their research put forward several hypotheses, based on molecular data alone, that had been discussed by arachnologists for many years. However, there was limited support for their suggested taxonomic changes, with a maximum of two species from each genus incorporated in their analysis. Unfortunately, in addition to limited taxon sampling, basic taxonomy was probably misinterpreted through inaccurate species identification. The species *Gasteracantha doriae* Simon, 1877 was mistakenly identified as a colour form of *G. diardi* (Lucas, 1835) and their finding were discussed, and hypotheses put forward based upon this incorrect identification. However, this was the first published attempt at examining the phylogenetic relationship between *Gasteracantha* species and other relatives. This thesis will aim to expand much further on the limited phylogeny that Tan *et al*. (2019) put forward by hypothesising a larger phylogeny based on molecular data and then supplementing this by creating a morphological-based phylogeny where molecular data cannot be obtained from species (see Chapter 2.2.1).

From 1899 to 1916, Strand described many species of *Gasteracantha* and some relatives and, in 2019, Nentwig *et al*. examined redundant names within these earlier works and raised several taxonomic questions and suggestions for future revisions that would be beneficial to understanding the status of some species. Their work focused on the taxonomy and literature surrounding the species and the conclusion resulted in the number of *Gasteracantha* being reduced to 70 species and 18 sub-species (World Spider Catalog, 2022). Their work also draws parallels with how the work of the American arachnologist Levi should be treated. Levi (1978a, 1978b, 1996, 2002) focused on the New World *Gasteracantha* species and their many variations, working on American orb-weaver keys and a basic family level phylogeny.

The standing of some of Levi's *Gasteracantha* synonyms, predominantly based upon only external female characters (Levi, 1978a), are dubious as he reduced the number of species and potentially "went too far" (Scharff *in litt.*, 2015) as some synonymies appear to warrant more taxonomic justification than Levi provided.

Research on gasteracanthines then started to branch out into non-taxonomic publications, for example: viral DNA in *Gasteracantha cancriformis* (Rosario *et al*., 2019), prey selection by *Macracantha* (then *Gasteracantha*) *hasselti* (C. L. Koch, 1837) (Michalko *et al*., 2020) and large studies of the phylogeography of *Gasteracantha cancriformis* (Chamberland *et al*., 2020; Salgado-Roa *et al*., 2022). *Gasteracantha cancriformis, G.quadrispinosa* and *G. fornicata* were also used to explore abdominal colouration and polymorphism in relation to prey capture and deception (Kemp *et al*., 2013, White and Kemp, 2015, 2016; White, 2017; Messas *et al*., 2021; Kemp, Edwards and White, 2022).

Scharff and Coddington continued their broader work and with others published, in 2020, a new phylogeny of the Araneidae. Although this only used molecular markers, and was almost entirely focused on the generic level, this publication produced interesting hypotheses about the standing of the different genera within the Gasteracanthinae. The nine species featured in the study were: *Acrosomoides acrosomoides*, Austracantha minax*, Augusta glyphica*, Gasteracantha cancriformis*, Isoxya mahafalensis, Macracantha arcuata*, Madacantha nossibeana*, Parmatergus sp.,* and *Thelacantha brevispina** (an * denotes that this is the genus type species). The results suggested that *Gasteracantha* and *Thelacantha* were closely related and that *Macracantha* was the sister, closest, generic relative to these two genera.

However, due to the limited number of included species from each genus, these relationships are not definitive. The inclusion of only one *Gasteracantha* from the New World, with none of the Oriental or African species included in the study means that the genera will warrant further examination to understand their relationships. Tan *et al.* (2019) hypothesised that *Macracantha* may be synonymous with *Gasteracantha* once more species are included in phylogenetic studies and Scharff *et al*. (2020) highlights how more taxa are needed for accurately resolving the generic and species relationships in this subfamily. One single *Gasteracantha* species will not necessarily provide results reflective of the relationships between the genera. The resulting relationships of the Gasteracanthinae genera from Scharff *et al.* (2020) raise questions that will be hypothesised and tested in this thesis.

Most recently, in 2021, Macharoenboon, Siriwut and Jeratthitikul published a review of the taxonomy of spiny-backed orb-weaving spiders of the subfamily Gasteracanthinae in Thailand. This had similarities with the work of Tan *et al*. (2019) but with a focus on Thailand, not Peninsular Malaysia. Their research used 3 molecular markers, mitochondrial CO1, ribosomal 16S and the nuclear gene H3 to create their phylogenies and used morphological characters to support these findings. As with Tan *et al*. (2019) they focused on a small number of Gasteracanthinae, 9 species in this case, and were unable to include more species from a wider geographical region which limited their results to localised taxa. However, Macharoenboon, Siriwut and Jeratthitikul suggested a transfer of one *Gasteracantha* species, *G. hasselti*, to *Macracantha* and suggested that there is a split within *Gasteracantha* that should form two separate generic clades. With only one *Gasteracantha* species available from this '*Macracantha* group' the suggestions and generic change seems premature and without more species from a wider geographical area the findings cannot be considered as conclusive. As with Tan *et al.* (2019), there are also minor taxonomic

misidentifications, including the suggestion that an immature specimen of *Gasteracantha doriae* might be a new species and with questionable identifications of *G. diadesmia* Thorell, 1887.

There is a need to expand much further on this limited phylogeny and determine if there are two separate clades that need to be reclassified as different generic groups as proposed by Macharoenboon, Siriwut and Jeratthitikul (2021). Figure 1.4 gives a summary of the current understanding of evolutionary relationships of the main Gasteracanthinae genera, produced by combining the publications of: Tan *et al*. (2019); Scharff *et al*. (2020); and Macharoenboon, Siriwut and Jeratthitikul (2021).

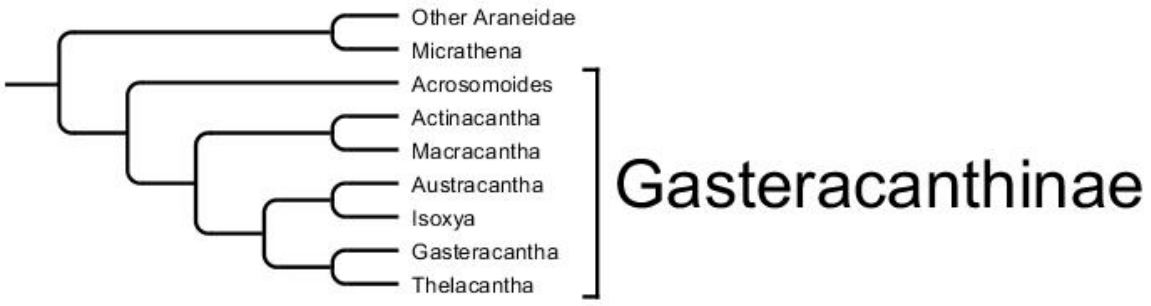

Figure 1.4 Summary tree of generic relationships (not representative of evolutionary distance) with the most common Gasteracanthinae genera included. Based upon combined: Tan *et al*. (2019), Scharff *et al*. (2020) and Macharoenboon, Siriwut and Jeratthitikul (2021).

1.3 Recent revisions of other taxonomic groups relevant to this work

Although the gasteracanthines are relatively understudied, there have been more recent revisions and reclassifications of other taxonomic groups which possess similar characteristics to the genera within the Gasteracanthinae. The following genera all provide a useful guide when embarking upon a new phylogenetic analysis.

1.3.1 *Micrathena*

In 1985 Levi published the currently definitive key to the genera *Micrathena* and *Chaetacis*. These genera are now synonymised and found within the Micrathinae, an Araneidae subfamily that also possesses abdominal spines. Levi's mammoth task of revising the two genera included over 100 comprehensive species descriptions, line drawing illustrations and a tentative phylogeny and paved the way for future research on the subfamily. In 2012 Magalhães and Santos published a phylogenetic analysis of *Micrathena* and *Chaetacis* using not only characters based upon Levi's work but also the theories that Levi put forward including: the relationship between the two genera, Levi's 8 species groups based upon 'somatic features of the females and genitalia features of both sexes', and the 'speculative tree representing the internal phylogeny of *Micrathena*' (Levi, 1985). This publication synonymised the genus *Chaetacis* into *Micrathena* and examined the evolution of the abdominal spines in *Micrathena*. This work is an informative example of what is possible when morphological data is used to create a concise phylogeny onto which the evolution of character traits can be mapped.

1.3.2 *Nephila*

Often used as a 'typical' example of tropical orb weaving spiders, the genera within Nephilidae - in particular the genus *Nephila*, are known for extreme sexual size dimorphism

as the females dwarf the males, being 6 times larger. Compared to *Micrathena,* more extensive work has been done on these giant orb weavers, comprising two comprehensive morphological phylogenetic analyses using hundreds of characters (Kuntner, 2006; Kuntner, Coddington, and Hormiga, 2008). This extensive work provides sources of characteristics that can be used in other analyses; a molecular phylogeny (Kuntner *et al*., 2013); and multiple publications on areas of evolution including gigantism in the largest nephilid species, extreme sexual size dimorphism (eSSD, see Chapter 4) across the subfamily and reproductive and sexual characteristics (Kuntner Coddington, and Hormiga, 2008; Kuntner *et al*., 2019; Kuntner and Coddington, 2009, 2020). The trait of eSSD in this extensive work is also apparent in the *Gasteracantha* and the material relating to the Nephilidae offers an approach that can be used for revisions of other taxa.

1.3.3 *Caerostris*

Another genus of spiders that shows eSSD is *Caerostris*. Grasshoff (1984) described many species using good illustrations of the characters in his work. In 2015 Gregorič *et al*. (2015b) described a new species of *Caerostris,* with high image quality and defined genitalia characters for all the species. Gregorič *et al.* (2015a) then presented a molecular phylogeny of *Caerostris*, using it to map evolution of the character traits of eSSD and specific web structures.

All these publications, especially those on *Nephila* and *Micrathena*, provide an excellent base and methodology for using multiple characteristics (molecular and morphological) within phylogenetic constructions.

1.4 Gasteracanthinae biogeography and ecology

Gasteracanthinae have a Pan-Tropical/Oriental/Australasian/Neotropical distribution band that stretches approximately between latitudes 30 degrees and -25 degrees (Figure 1.5). This distribution pattern raises the question of how the species dispersed and offers the potential for future research into the biogeography and ecology of the subfamily given their wide geographical range and varying habitats (Emerit, 1974; Tan *et al*., 2019; Chamberland *et al.*, 2020; Macharoenboon, Siriwut and Jeratthitikul, 2021; Salgado-Roa *et al*., 2022). The question of how the current distribution has arisen can only be partially answered prior to a phylogenetic analysis.

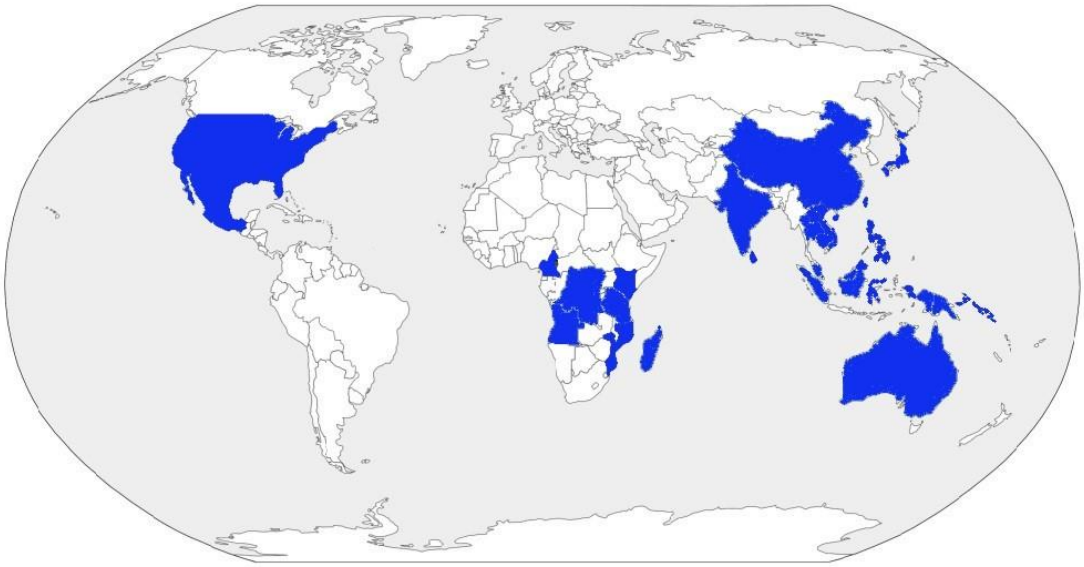

Figure 1.5 Map of approximate Gasteracanthinae distribution (blue highlights country distribution of taxa)

It has been suggested that the split of the Gondwana supercontinent (180m - Yr BP), may have been responsible for current species distributions being rooted millions of years ago (Cox and Moore, 2010). There are fossil and amber records of Araneae dating from the Carboniferous Period (359.2 to 299m Yr BP) (Guo, Selden and Ren, 2021) that support this theory. However, an amber fossil of a gasteracanthine specimen in Miocene sediments (23.03-5.33m Yr BP) from East Africa seen by Martin Pickford (*in litt*. with David Penney,

2016) and the publication of Dimitrov *et al*. (2016) suggest that *Gasteracantha* evolved after all the major land splits had occurred.

In addition to this, the common ballooning dispersal method of spiderlings (small male or immature females - before they reach over 1cm in body length), does facilitate long-distance dispersal. Ballooning involves sending out strands of silk which are then caught by air currents and if the silk and the spider's body provide enough drag force then the spiderling is lifted into the air (Foelix, 2011). Ballooning has enabled spiders to reach various islands and remote locations and enabled travel up to several hundred kilometres (Foelix, 2011), and further. Spiders have also been collected after they ballooned and landed on ships over a thousand miles from the nearest land. They have also been found floating 4,900m in the air (Platnick *et al*., 2020).

An example of the large distance spiders can balloon would be the *Tetragnatha* found in the volcanic Hawaiian archipelago. This is the most isolated archipelago in the world and is separated from the nearest continental land mass by nearly 4000 km of ocean (Gillespie, Croom and Palumbi, 1994). Despite this distance *Tetragnatha* has colonised Hawaii (Cotoras *et al*., 2018), indicating the ballooning dispersal ability of these spiders. This observation, and the fact that many Araneidae balloon (Walter, Bliss and Moritz, 2005; Blandenier, 2009; Wolz *et al*., 2020), suggest that ballooning by Gasteracanthinae is probably their primary dispersal mechanism. Gasteracanthine males are small, and the females can balloon in early development before they grow, as the large abdominal spines appear during female maturation (Emerit, 1974). There is also the possibility that anthropogenic introduction, again in the case of Hawaii where *Gasteracantha* was introduced in 1985 (Hofacker, Loomis and Fowler, 1990), could explain some *Gasteracantha* distributions.

Although the primary dispersal method appears to be ballooning, examinations into the biogeography, ecology and habitats of the group could provide interesting future research on this subfamily. The limited work on web structure, basic habitat and ecological studies and both predatory and defensive strategies does suggest there is a wealth of future research that could provide answers to wider biological questions.

There have been some studies looking at the structure, construction, and silk properties of webs by *Gasteracantha* (Peters, 1955; Emerit, 1968; Robinson and Robinson, 1975; Eberhard, 1982; Eberhard, 1986a; Edmunds and Edmunds, 1986; Kovoor, 1987; Lloyd and Elgar, 1997; Herberstein *et al*., 2000; Eberhard, 2003; Edwards *et al*., 2009; Briceño and Eberhard, 2012; and Eberhard, 2020), but the majority of these simply use *Gasteracantha* species as examples of orb-weaving spiders in their wider research. Behavioural traits such as attack behaviour (Uetz and Hartstock, 1987; Eberhard, 1989), leg movement (Eberhard, 2020), mating behaviour (Emerit, 1974) and developmental biology (Emerit, 1973; 1974) have also been briefly studied. Emerit (1974) also examined the ecology of the Madagascan species but in other cases ecological notes on *Gasteracantha* are limited to field data in publications; for example, the webs of *G. aciculata* being found in forest clearings at a relatively low level (Dahl, 1914).

Recent studies looked at polymorphism of abdominal colouration in relation to prey capture and deception using *Gasteracantha cancriformis* and *G. fornicata* (White and Kemp, 2015, 2016; White, 2017; Messas *et al*., 2021; Kemp, Edwards and White, 2022). These recent publications use *Gasteracantha* to examine the manner in which colouration, like the abdominal patterns on *Gasteracantha* (see Figure 2.9), can lure visually oriented prey, and

maximise the efficiency of the sit-and-wait hunting style of orb weavers. With polymorphism already known in *G. cancriformis* (Chamberland *et al*., 2020), and other species such as *Thelacantha brevispina*, there is the potential for further work to use a phylogeny to examine species lineages and investigate how abdominal colouration and lure traits have evolved (see Chapter 3).

However, to support further research, a phylogeny should be constructed to enable hypotheses to be suggested and tested and geographical areas of interest to be identified. Areas of high species richness for the Gasteracanthinae include Madagascar and the Solomon Islands (well-studied by Benoit in 1962a, 1962b and 1964, Chrysanthus in 1971 and Emerit in 1968, 1973, 1974, 1982a and 1982b). Both are regions of high biodiversity (Ganzhorn, *et al*., 2008; Furusawa, *et al.*, 2014).

The Gasteracanthinae of Madagascar are also still providing fascinating research topics, as Agnarsson *et al.* (in prep.) discovered a new species of *Isoxya* that appears to demonstrate lek-style behaviour for the first time in spiders; a prime example of why the group should be studied further. It is also clear that the biogeography of islands is still relevant to arachnology as Čandek *et al*. (2019) published on the biogeography of Caribbean *Cyrtognatha* spiders and discussed how species form an 'exclusively single island endemic pattern'. Work of this sort is yet to be undertaken on the Gasteracanthinae, but a reliable phylogeny would assist with interpreting results and provide a platform to build future hypotheses.

Additionally, museum or institutional collections can provide a wealth of distributional data. With recent publications (Čandek *et al*., 2019; Tan *et al*., 2019; Chamberland *et al*., 2020; Macharoenboon, Siriwut and Jeratthitikul, 2021; Salgado-Roa *et al*., 2022) being restricted to

limited geographical locations it highlights the need for accurate and up to date distribution data for the Gasteracanthinae. Through the process of data gathering for a phylogenetic analysis, more information can also be gathered to support future biogeographical and ecological work.

1.5 Aims of thesis and hypotheses

As the previous work on the Gasteracanthinae (see section 1.2) is mainly based upon historical taxonomy, this thesis aims to expand on the recent limited molecular Gasteracanthinae phylogenies that Tan *et al*. (2019) and Macharoenboon, Siriwut and Jeratthitikul (2021) put forward and to produce a more comprehensive revised molecular phylogeny. This molecular phylogeny will then be expanded upon by examining morphological data using a wide selection of species from all geographical areas to provide an understanding of the relationships between the species in the whole subfamily. Relationships between the genera can also be constructively examined and taxonomic changes suggested.

The specific aim of the study is to construct a molecular and morphological phylogeny of the Gasteracanthinae with focus on the genus *Gasteracantha.* Once completed the systematic position of the genera within Gasteracanthinae, their relationships and the implications of this for the classification of genera and *Gasteracantha* species can be re-examined, with the following hypotheses being tested:

- The subfamily Gasteracanthinae is monophyletic. For this hypothesis to be supported, all gasteracanthines following the phylogenetic analysis will be located in the same clade.
- The genus *Augusta* is incorrectly classified within the Gasteracanthinae due to gross external morphology (for example the lack of abdominal spines and abdominal shape). For this hypothesis to be supported the genus will be located outside the Gasteracanthinae clade and closer to the outgroup taxa in the phylogeny.
- *Thelacantha* is the sister genus to *Gasteracantha.* For this hypothesis to be supported *Thelacanatha* will be positioned as sister to *Gasteracantha* in the phylogeny.

- *Gasteracantha* is paraphyletic. For this hypothesis to be supported genera other than *Gasteracantha* will be found within the same clade.
- The genus *Macracantha* is synonomous with *Gasteracantha;* contrary to Macharoenboon, Siriwut and Jeratthitikul (2021). For this hypothesis to be supported *Macracantha* will be located in the same clade as *Gasteracantha* species.

These hypotheses of relationships outlined above will be tested by constructing phylogenetic trees from DNA sequence data and morphological data.

In addition to these, various aspects of character evolution, behaviour and biogeography will be investigated using the inferred phylogeny to test these specific hypotheses set out below:

- The species that are closely related (taxa from within the same clade inside the genus, or sister taxa) share the same abdominal shape as a result of selection pressures (currently unknown). For this hypothesis to be supported members of the same clade will share the same abdominal shape.
- Species of Gasteracanthinae from the same geographical region are related to each other likely due to short range dispersal (dispersal within the same biogeographical region). For this hypothesis to be supported members of the same clade will share the same geographical distribution.
- Extreme sexual size dimorphism is lost only within the genus *Isoxya*. For this hypothesis to be supported all gasteracanthines bar *Isoxya* will exhibit eSSD.
- As in *Micrathena*, the long abdominal spines in *Gasteracantha* and *Macracantha* evolved multiple times. For this hypothesis to be supported the long abdominal spines must evolve in different clades in the tree.

- The ventral tubercle is present within clades of Gasteracanthinae that exhibit the greatest sexual size dimorphism. For this hypothesis to be supported the tubercle will be present in the clades which show the greatest SSD.

Future work on the subfamily can draw upon the process and results from this thesis. By building upon the work that has gone before and learning from other groups, it is hoped that this project will be at the forefront of any future work on the subfamily. A cladistic approach (defined below) is taken in the construction of phylogenies within this thesis, therefore enabling the hypotheses to be tested.

1.6 Defining cladistics

Cladistics, also known as phylogenetic systematics, is a method of grouping organisms that share characters derived from a common ancestor (Lipscomb, 1998). A cladistic study requires the scoring of characters from the study organisms, then generating phylogenetic trees and testing their validity. A variety of different methods have been developed.

A list of definitions for frequent terms is provided here to assist with understanding (following Lipscomb, 1998; Williams and Ebach, 2020):

Autapomorphy – a derived character or trait that is unique to an organism

Branch – a line on a phylogenetic tree, that connects all the other parts of the tree, also representing the evolutionary history of the organisms

Character – any heritable attribute of an organism

Clade – a group of organisms believed to be made of all the evolutionary descendants of the same common ancestor

Homology – a similar characteristic inherited by two or more related organisms from a common ancestor

Homoplasy – a similar characteristic in two or more unrelated organisms due to convergent evolution

Ingroup - the organisms that are being classified

Internal nodes – the branching points on a tree

Monophyletic - a clade composed only of a common ancestor and all its lineal descendants

Nodes - point in a tree diagram at which lines intersect or branch

Outgroup - organisms that are not members of the group of organisms being classified

Polytomy – internal node of a cladogram with more than two immediate descendants

Root – the starting point or base of a tree

Synapomorphy – a derived (novel) character shared by 2 or more taxa and inherited from their most recent common ancestor

A cladistic analysis follows a basic process of distinct steps. The process starts by determining the primitive and derived character states from chosen outgroup taxa and chosen ingroup taxa. The scoring of characters follows specific principles and is covered in Chapter 2. Once the characters have been defined, they are analysed to reconstruct the species relationships and visualised using a phylogenetic tree diagram. Phylogenies can be constructed using molecular or morphological data (see Chapter 2), and even behavioural data can be used in analysis (Kuntner, Coddington and Hormiga, 2008). Tree construction follows various guidelines and rules that are covered here in Chapter 2. Once a tree is constructed it can then be used to test hypotheses about evolution in the group (Lipscomb, 1998); examples of this are presented within this thesis in Chapters 3 and 4.

Chapter 2: Molecular and morphological analysis

This chapter aims to test the following hypotheses as laid out in Chapter 1.5. The subfamily Gasteracanthinae is monophyletic due to the presence of the raised carapace, abdominal spines and the heavily sclerotised abdomen. The genus *Augusta* is incorrectly classified within the Gasteracanthinae due to the lack of prominent abdominal spines and unique abdominal shape. *Thelacantha* is the sister genus to *Gasteracantha,* a relationship obtained by Scharff *et. al.* (2020). *Gasteracantha* is paraphyletic by inclusion of various other genera within the same clades. The genus *Macracantha* is synonymous with *Gasteracantha,* contrary to the findings of Macharoenboon, Siriwut and Jeratthitikul (2021), due to morphological similarities in taxa that were not included in their work.

2.1 Introduction

Molecular markers have revolutionised phylogenetic analysis. This is particularly true for groups where morphological techniques cannot be applied. For example, molecular data has revolutionised our understanding of the evolution of microorganisms (Das and Dash, 2013). Rapid results and improving sets of programs to process molecular sequence data into phylogenies (Zhang *et al*., 2020) facilitate phylogenetic construction methods that would have been unthinkable when Dahl was constructing his 1914 taxonomic work on world *Gasteracantha*. The increased ease of extracting genomic DNA from many organisms (Tan and Yiap, 2009; Wang *et al.*, 2011; Ghatak, Muthukumaran and Nachimuthu, 2013; Bork, 2015; Aboul-Maaty and Oraby, 2019; Johnson, 2019; Tahir *et al*., 2019; Wang *et al*., 2019) and either amplifying specific molecular markers or sequencing the entire genome of species makes molecular analysis viable for many areas of study (Tan and Yiap, 2009; Tahir *et al*., 2019; Johnson, 2019; Hotaling *et al*., 2021). The quantity of data that can be gathered from

DNA sequencing will always outweigh that from morphological analysis (Wang, *et al*., 2019; Sharkey *et al.*, 2021).

Molecular data alone often produces studies that have resolved difficult taxonomic issues, contributed to the Tree of Life, revealed cryptic species (Bolzern, Burckhardt and Hänggi, 2013; Šašić *et al*., 2016) and divergence within taxa (Lawniczak *et al*., 2010; Hayashi and Sota, 2014). The quantity of characters available from molecular sequences in Araneidae surpasses any that can be generated from a solely morphological approach, for example, over 14,200bp were sequenced by Wang, *et al*. (2019). The Nephilidae morphological phylogeny of Kuntner, Coddington and Hormiga (2008) generated 197 characters, but by contrast the Nephilidae molecular phylogeny of Kuntner *et al*. (2013) used 4197 characters; over 20 times the amount of the previous morphological study.

A robust molecular phylogeny should not be constructed from a single molecular marker because different genomic regions evolve at different rates (Sadava *et al*., 2011). For example, ribosomal encoding genes (especially 16S) contain highly conserved regions and can be used to reveal deep rooted and ancient differentiation between clades (Woese and Fox, 1997). In contrast, CO1 mutates at approximately twice the rate of ribosomal genes, can reveal more recent differentiation between species (Dincă *et al*., 2021) and identify lineage differentiation within species (Hinojosa *et al.*, 2019). Additionally, the role of endosymbionts such as *Wolbacchia*, and lineage introgression can also cause problems of interpretation with limited molecular data, especially with recent divergence (Arif *et al*., 2021). There are also examples of recognised ‘good species’ where there is no divergence of even rapidly evolving molecular markers (Dapporto *et al.*, 2019).

Sequence data for species is of immense value for both phylogenetic and taxonomic work. The speed at molecular data can be generated enables phylogenies to be constructed quickly and efficiently, without the need to examine specimens for characteristics, if the taxa have been correctly identified. Although there is the risk with specimens of new species and curated material occasionally being destroyed in the attempt to acquire the tissue for molecular analysis, the inclusion of both morphological and molecular data in a combined analysis can provide more comprehensive results than either method alone (Bolzern, Burckhardt and Hänggi, 2013).

Without molecular data, a phylogeny will always have limitations and morphological based studies may include cryptic species that can only be split with molecular data (for example dung beetles Wilson and Angus, 2004; hoverflies Šašić *et al*., 2016; and spiders Bolzern, Burckhardt and Hänggi, 2013, Newton *et al*., 2020). A comprehensive phylogenetic analysis of the Gasteracanthinae would require the sequencing of several genes, or the entire genome (see Chapter 5). Molecular markers will provide a solid framework for the phylogeny before morphological characters are examined to promote further understanding and support.

2.1.1 The importance of morphology for phylogenetics

With the increased use of molecular sequence data, the relevance of traditional morphological methods to classification and phylogenetic analysis continues to be discussed (Hillis, 1987; Kluge 1989; Baker and Gatesy, 2002; Feng-Yi Su, Kutty and Meier, 2008; Giribet, 2010; Perkins, Martinsen and Falk, 2011; Lee and Palci 2015; Pyron, 2015; Wanninger, 2015; Goloboff *et al.*, 2019). Recently, Neumann *et al*. (2021) provided a comprehensive history of the advancement of morphological versus molecular data, and the two in combination, in phylogenetic studies. They concluded by reinforcing the importance of combining molecular and morphological data, a view which is still not completely supported in phylogenetic research (Oyston *et al*., 2022). Many studies over recent years have chosen to only use molecular data and ignore morphology as the ease of generating large volumes of molecular data is ideal for phylogenetic analyses.

A relevant example is Tan *et al*. (2019) who used 5 different molecular markers - Cytochrome C Oxidase I (CO1), Cytochrome C Oxidase subunit 2 (CO2), 16S ribosomal rDNA (16S), Histone H3.3 type A (H3A) and 18S ribosomal rDNA (18S) - to reconstruct the phylogeny of Malaysian *Gasteracantha.* Unfortunately, the authors made a misidentification (see Chapter 1.2) and based results and discussion on this error. Where there are doubts about a purely molecular based phylogeny, interpretations of character evolution may be erroneous if species are not correctly identified.

While molecular studies provide large amounts of data quickly, the lack of corresponding morphological data can hamper their use in the field of study, even with a concise and resolved molecular phylogeny. More recent work on the *Gasteracantha* of Thailand by Macharoenboon, Siriwut and Jeratthitikul (2021) used 3 genetic markers (CO1, 16S and H3) to reconstruct a phylogeny, but also used morphological characters to support these findings and to discuss the relationships between species. Although they did not incorporate any morphological characters into their phylogenetic analysis, they demonstrated why studying morphology was important for understanding species relationships and offers support when presenting taxonomic changes. When constructing a phylogeny from limited molecular data, morphological characters can also be used to provide additional characters.

In this study, morphological data was also used as specimens were not always available for molecular analysis (see 2.2.1). There are also recent examples of arachnological phylogenies that use both molecular and morphological characters (Wortley and Scotland 2006; Giribet, 2015; Lee and Palci 2015; Zhang and Maddison, 2015; Zanini *et al*., 2018). So, with this precedent, a combined molecular and morphological analysis was undertaken.

2.2 Materials and methodology – molecular analysis

This section details the taxa used for the molecular analysis followed by the selection of molecular markers, the methods of DNA extraction and the details of additional sequence data obtained from other sources.

2.2.1 Included taxa

The genus *Gasteracantha* is currently composed of 87 taxa (World Spider Catalog, 2022). One species is only known from the male, the rest known with either the mature female (the only exception being *G. picta* known from a juvenile female) or both sexes. 20 of the currently recognised species of *Gasteracantha* are known from both sexes. Only 22% of *Gasteracantha* have a male described. Although there are large collections of Gasteracanthinae specimens within museums and institutions, the primary issue faced in the molecular analysis was a lack of fresh material for DNA extraction. Museum collections have seen a general decline in *Gasteracantha* material being collected and deposited. The Nagoya Protocol (Convention on Biological Diversity, 2014) has certainly hindered the collection of *Gasteracantha*, and their geographic distribution covers a wide array of different countries with firm export legislation. These factors contribute to the lack of fresh material coming to museum collections in contrast to the past.

For this study, museum collections granted permission to attempt destructive DNA extractions from specimens ranging from approximately 55-85 years old from some of the more common species. Certain species: *Gasteracantha clarki* and *Gasteracantha regalis* Butler, 1873 for example, were not available for extraction attempts as these species are known only from the holotype. Even with non-destructive sampling methods used on these

valuable holotype specimens, such as those recommended by Bork (2015), it is highly unlikely that sequence data will be recovered from these specimens due to their age.

Specimens were loaned from the Natural History Museum London (NHM, UK), the EZ Lab (EZ, Slovenia) and the collections of I. Agnarsson (IA, USA), S. Danflous (SD, France) and S.H. Williams (SHW, UK) for use in the analyses. Most specimens were collected within 4-10 years of the extraction attempts and were stored in 96% ethanol at -20°c. Other specimens were stored in 70% ethanol at room temperature, with the oldest 4 specimens being between 60-80 years old. Although attempting extraction from these older specimens was ambitious (see Miller *et al*., 2013), museum collections are often required to "target species that have eluded current fieldwork" (Miller *et al*., 2013). When dealing with limited specimens of certain taxa there is often little choice regarding specimen age.

Museum arachnid spirit collections are stored at 70-75% ethanol (Beccaloni, 2016), however there can be situations where the ethanol percentage in these collections drops to 40% or even lower (Pickering, 1997; Moore, 1999). This does not provide suitable conditions for the preservation of genomic DNA because, as the ethanol concentration drops, water reacts with the DNA through hydrolysis (Obodovskiy, 2019). Once this process starts, the amount of useful genomic material that can be extracted decreases (Carew *et al*., 2017; Marquina *et al*. 2021) as the DNA become increasingly fragmented over time (Carew *et al*., 2017; Marquina *et al.* 2021). Therefore, specimens that were damaged, worn or stored in very low ethanol concentrations were avoided to give the maximum chance of successful extraction (Marquina *et al*., 2021).

Twenty-eight species were chosen from recently collected material (EZ, IA, SD and SHW) and from common or non-valuable museum specimens (NHM), see Table 2.3 and see Appendix 2.4 for full specimen details. The species used were: *Acrosomoides acrosomoides* (O. Pickard-Cambridge, 1879), *A. linnaei* (Walckenaer, 1841), *Aetrocantha falkensteini* Karsch, 1879, *Afracantha camerunensis* (Thorell, 1899), *Augusta glyphica* (Guérin, 1839), *Gasteracantha aciculata* (Pocock, 1898), *G. clavatrix* (Walckenaer, 1841), *G. curvispina* (Guérin, 1837), *G. diardi* (Lucas, 1835), *G. kuhli* C. L. Koch, 1837, *G. metallica* (Pocock, 1898), *G. milvoides* Butler, 1873, *G. quadrispinosa* O. Pickard-Cambridge, 1879, *G. rhomboidea madagascariensis* Vinson, 1863, *G. sanguinolenta* C. L. Koch, 1844, *G. scintillans* Butler, 1873, *G.* sp. nov. Williams, in prep., *G. sturi* (Doleschall, 1857), *G. unguifera* Simon, 1889, *G. versicolor formosa* Vinson, 1863, *Isoxya penizoides* Simon, 1887, *I. sp. nov.* Agnarsson *et al*., in prep, *I. tabulata* (Thorell, 1859), *Macracantha arcuata* (Fabricius, 1793), *M. hasselti* (C. L. Koch, 1837), *Micrathena schreibersi* (Perty, 1833), *Thelacantha brevispina* (Doleschall, 1857), *Togacantha nordvei* (Strand, 1913).

2.2.2 The selection of molecular markers

The three recent molecular analyses of Gasteracanthinae (Tan *et. al*., 2019, Scharff *et. al*., 2020; Macharoenboon, Siriwut and Jeratthitikul, 2021) used various combinations of mitochondrial protein coding genes (CO1 and CO2), ribosomal rDNA (16S) and nuclear genes (rDNA 18S and 28S, and histone H3) sequences, combining original sequence data and sequences from GenBank and BOLD. The most frequently used were CO1, 16S and H3 as they featured in all analyses. For the work presented here only CO1 and 16S were targeted for sequencing, representing both a variable and a more conserved molecular marker. H3 could not be used in this analysis due to limitations presented by the UK Covid-19 lockdowns, restricting laboratory time. In addition, paucity of H3, 18S and 28S sequence data

available from GenBank and BOLD affirmed this decision. Additional CO1 and 16S sequences were available from GenBank for some species that were unavailable for DNA extraction in this project (see 2.2.4).

2.2.3 Molecular methodology

All molecular work was undertaken at the Oxford Brookes University McGregor Laboratory with assistance from Dr A. Schonauer. Each specimen had 4 legs removed for crushing, except for *Afracantha camerunensis* and *G. quadrispinosa* which had 2 legs removed due to the species being less common in museum collections. Where specimens failed to provide enough or any DNA with this amount of tissue, the whole front of the spider was used, including the remaining legs. Again, the exception was made for *Afracantha camerunensis* and *G. quadrispinosa*, to prevent specimen destruction. Details of the DNA extraction protocol can be found in Appendix 1.1.

Following DNA extraction, 1ul of genomic DNA was run on a 1% agarose gel to determine the DNA quality (Figure 2.1). Additionally, the sample concentration was measured using a spectrophotometer (NanoDrop™ One/OneC Microvolume UV-Vis Spectrophotometer, Thermo Scientific™).

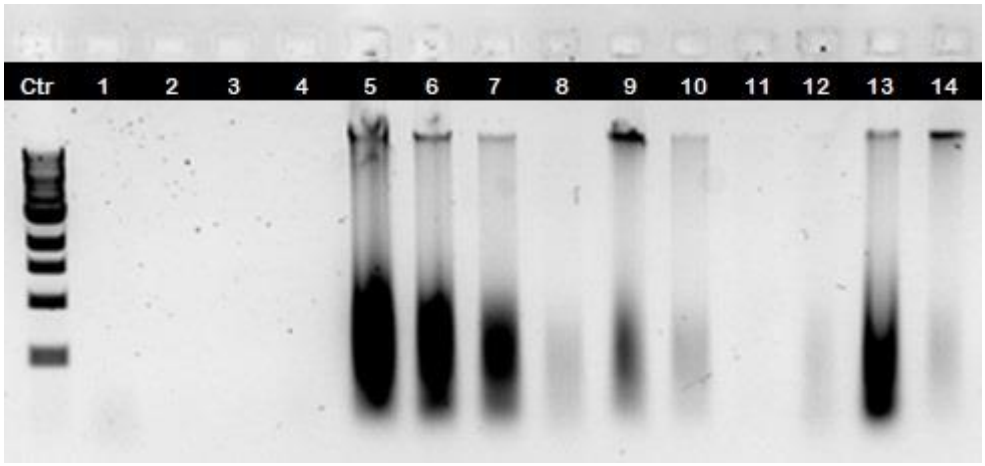

Figure 2.1 Image of gel showing the control (Ctr) (100bp DNA Ladder) (far left) and genomic DNA extractions 1-14 (left-right) from the first attempt at extraction. Note the absence of detectable genomic DNA in samples 1-4 from older museum specimens and in 11 (a small male *Gasteracantha* where specimen material was limited).

For the amplification of mitochondrial gene COI, the primers LCO 1490 (5' GGTCAA CAAATCATAAAGATATTGG 3') (Folmer *et al*., 1994) with C1-N-2776 (Maggie) (5' GGATAATCAGAATATCGTCGAGG 3') (Hedin and Maddison, 2001) were used (Figure 3.2). For the amplification of ribosomal gene 16S, the primers: LR-N-13398 (5' CGCCTGTTTATCAAAAACAT 3') (Simon et al. 1994) and LR-J-12864 (5' CTCCGGTTTGAACTCAGATCA 3') (Arnedo and Gillespie 2006) were used. These primers have been used in arachnid molecular analyses and are recognised as being suitable for the genes targeted (Simon *et al*., 1994; Arnedo *et al.*, 2004; Vidergar, Toplak and Kuntner, 2014; Tan *et al.*, 2019; Chamberland *et al*., 2020; Macharoenboon, Siriwut and Jeratthitikul 2021).

For PCR amplification of CO1 (Figure 2.2) and 16S sequences, respectively, One*Taq*® 2X Master Mix with Standard Buffer (New England Biolabs) was used as follows: 0.25ul of both 10uM forward and reverse primers, with between 50 and 100ng of genomic DNA, with 6.25ul of OneTaq 2x Master Mix, made up to 12.5ul of deionised water. Reactions were

assembled in PCR tubes on ice, then denatured at 94°C for 30 seconds, followed by 30 elongation cycles comprising 15 seconds at 94°C, 30 seconds at 58°C and 1 minute at 68°C, with a final extension for 5 minutes at 68°C.

The PCR products were purified using the GeneJET PCR Purification Kit (Thermo Scientific™) according to the manufacturer's guidelines. The PCR products were eluted in water, the concentration measured using a spectrophotometer (NanoDrop™ One/OneC Microvolume UV-Vis Spectrophotometer, Thermo Scientific™) and diluted to 10ng/ul (15ul total volume) for sequencing. Samples were sent premixed with primers (LCO 1490 or LR-N-13398, respectively) to Eurofins Genomics (UK) for Sanger Sequencing (https://www.eurofins.co.uk/).

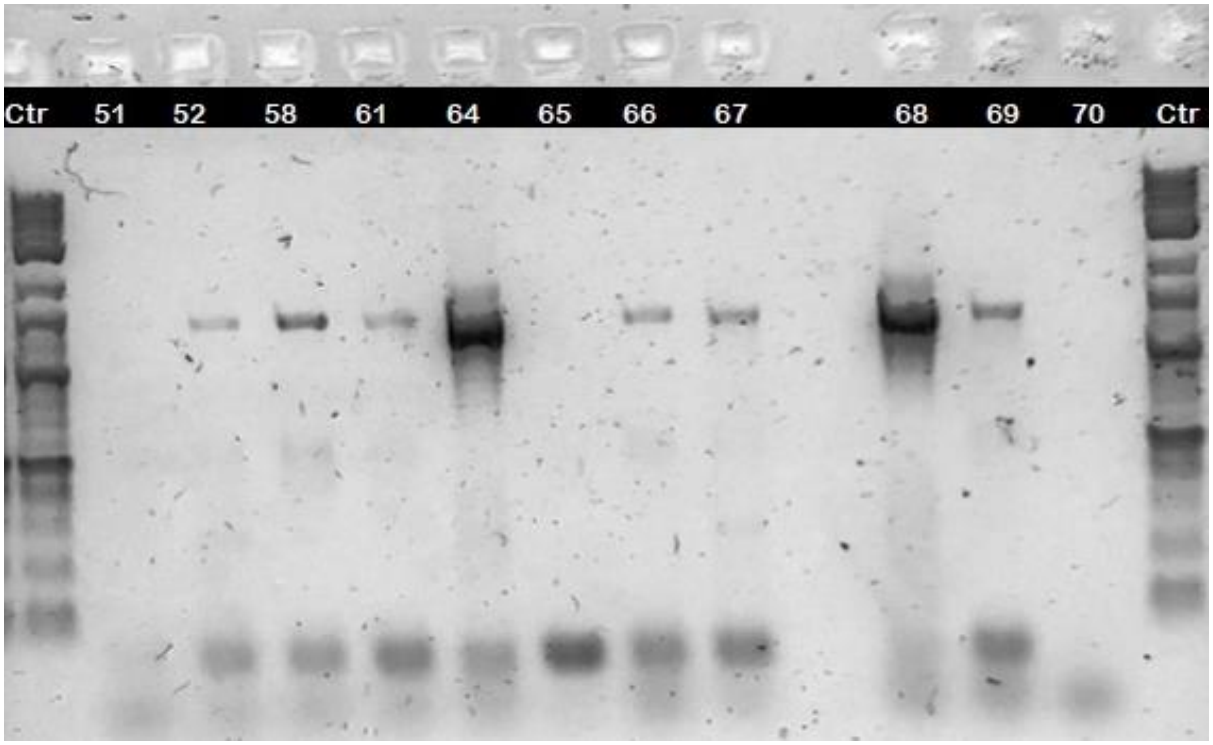

Figure 2.2 Image of PCR (CO1 amplification) for samples 51-52, 58, 61, 64-70. Control (Ctr) (1kb DNA Ladder) (far left and far right).

Sequence chromatograms were examined using the program ChromasPro 2.1.9 (Technelysium Pty. Ltd., 1996). These chromatograms were typically very clear and clean with little baseline noise for most of each sequence (Figure 2.3). Occasionally, there was noise or a loss of resolution at the start or end of a sequence, so the sequence was trimmed at a suitable point where the peaks could be interpreted (Figure 2.4). However, several sequences were discarded at this stage due to too much baseline disturbance or noisy signals where the peaks were not clear, and confirmation of the correct nucleotide is impossible (see Figure 2.5).

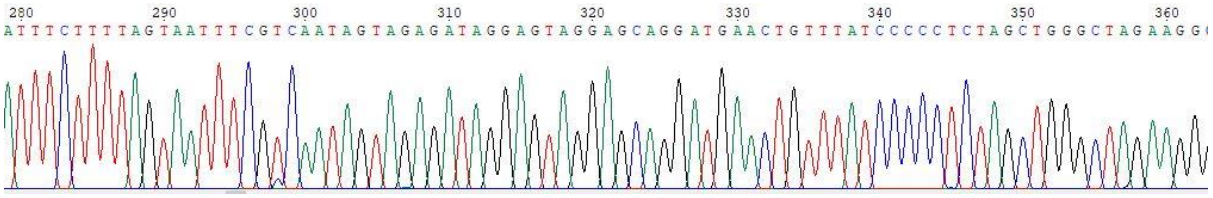

Figure 2.3 chromatogram of *Gasteracantha aciculata* (Pocock, 1898) CO1 - clear peaks with little or no baseline noise.

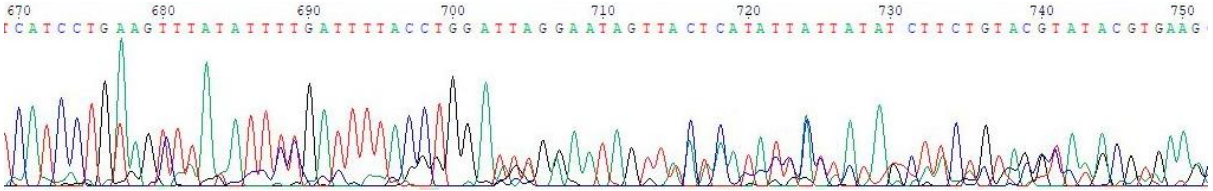

Figure 2.4 chromatogram of *Isoxya* sp. nov. CO1 - loss of resolution at end of the sequence.

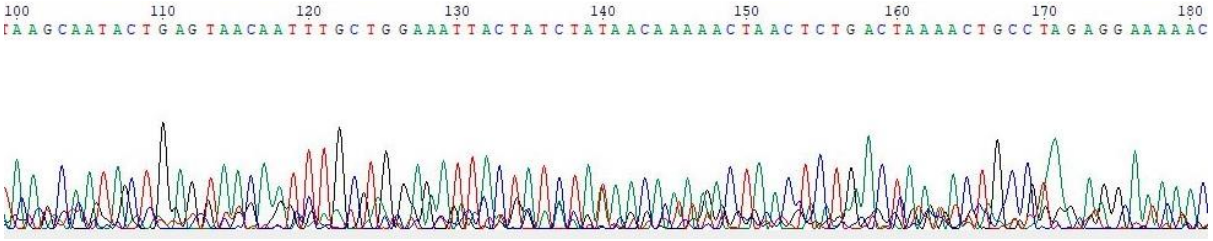

Figure 2.5 chromatogram of *Gasteracantha sanguinolenta* CO1 - discarded due to disturbance and noise.

After examination of each sequence and any trimming completed, sequences of between 376bp-782bp for CO1 and between 369-450bp for 16S were available for alignment. All sequence data was inputted into Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm that they were related to relevant taxa. Seventeen 16S sequences, but no CO1 sequences, had *Drosophila* or human contamination and were discarded. Time constraints on laboratory access due to COVID-19 did not permit repeat extractions for 16S. Specimens which yielded sequences for analysis and amplicon sizes are given in Table 2.3. It was not possible to extract DNA from *G. metallica, G. quadrispinosa, G. scintillans* and *G.* sp. nov. due to the age of the specimens (60-80 years old), their storage method within museum collections (70% ethanol, room temperature) and the amplicon length being too large.

2.2.4 Additional molecular data

Additional data was extracted from GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and from the Barcode of Life Data System (BOLD) (https://www.boldsystems.org/). Not every sequence could be confirmed as having a correct species identification and GenBank does not store sequence chromatograms, collection metadata or photographs (Meiklejohn, Damaso and Robertson, 2019). It was also discovered, when extracting sequence data, that BOLD was not always conclusive either. Many of the images in BOLD relating to gasteracanthines were not suitable for species identification between taxonomically close species. Although no effort was made to acquire the physical specimens for identification, this was only an issue when sequence data was from a very limited number of specimens for particular species. Where there were multiple options per sequence in Genbank or BOLD from known or trusted sources, these were prioritised along with those that had a higher number of base pairs for that gene.

2.2.5 Preparation of sequence data

Sequences were extracted from GenBank or BOLD in FASTA format and imported into a Mesquite v.3.7 (Maddison and Maddison, 2021) data matrix along with the sequences from this project's extractions. Separate matrices were made for CO1 and 16S.

A nexus file of the unaligned CO1 sequences was exported from Mesquite and uploaded to Clustal for alignment using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/ Madeira, Park, Lee, *et al.*, 2019). Clustal Omega is the current standard program in the Clustal series which are all used for multiple sequence alignment (MSA) and is a widely used alignment program (Sievers and Higgins, 2018). See Appendix 1.2 for parameter details. After the alignment was completed, it was exported, checked by eye in a new Mesquite v.3.7 matrix to ensure there were no gaps internally in the sequences or other alignment homology concerns.

For 16S, which showed very unequal lengths, the protocol of Gregoric *et al.* (2015a) was initially followed for alignment, using the multiple sequence alignment program MAFFT (version 7), for Multiple Alignment using Fast Fourier Transform, (MAFFT v.7: https://mafft.cbrc.jp/alignment/server/). MAFFT was chosen because it is designed for difficult to align sequences containing variable loop regions and offers alignment methods that are suited to smaller numbers of sequences; under 200 sequences (MAFFT v.7: https://mafft.cbrc.jp/alignment/software/). In addition to this, MAFFT has been used in recent arachnological phylogenetic analyses (Gregoric *et al*., 2015a; Eberle *et al*., 2018), the default parameters have recently been adjusted to increase the correct alignment of sequences (Katoh and Standley, 2016; Katoh, Rozewicki and Yamada, 2019) .MAFFT is suitable for sequences from closely related species (Katoh, Rozewicki and Yamada, 2019) and takes the secondary

structure of the rDNA into account by using a sequence, or consensus structure, from the alignment or from an external structural model as a reference sequence (Tourasse and Darfeuille, 2020) enabling the homology of the sequence to be predicted in the conserved regions.

The ‘strategy’ (algorithm) for alignment of 16S was chosen as L-INS-i. because it is known to be very accurate when aligning smaller numbers of sequences, with gaps in the data having minimal impact on alignment accuracy (Nuin, Wang and Tillier, 2006; Katoh and Standley, 2013; Long, Li and Fu, 2016). This alignment algorithm has also performed better in performance analysis tests on datasets with smaller evolutionary distances (Nuin, Wang and Tillier, 2006; Katoh and Standley, 2013) which suits this single gene dataset. Before an alignment of the 16S data was finalised, a sensitivity analysis of 42 different parameter value combinations was also conducted. The sensitivity analysis used various BLOSUM, gap open and gap extension penalty values, chosen to give a range of options across the various parameters, to discover the optimal settings. More details of this analysis can be found in Appendix 1.3.

Following the sensitivity analysis, the best parameters values were the default values. The alignments of 16S did not contain many unequal distributions of indels and it appeared that there were minimal divergent regions after the alignment had concluded. Then, following the sensitivity analysis, the L-INS-i alignment in MAFFT and confirmation by eye, the homology and alignment was deemed suitable for phylogenetic analyses following the criteria of Talavera and Castresana (2007). Relevant data was imported into a new Mesquite v3.7 matrix, and an additional combined CO1 and 16S matrix also constructed.

2.3 Materials and methodology – morphological analysis

This section covers the taxa used and scoring of the morphological characters, along with the comprehensive methods of scoring characters following cladistic guidelines, in preparation for the morphological analysis.

2.3.1 Included taxa

By comparison to the molecular data, the number of species available for analysis in the morphological analysis was greater (58). However, some taxa were included for the molecular analyses that were not available for the morphological analysis as physical specimens could not be located. To provide similar information on generic relationships in the molecular phylogeny suitable generic replacements were made where needed. For example, physical specimens of the taxa chosen for representation of the *Micrathena* outgroup were not always of the same species as the molecular sequence data, despite extensive searches to find appropriate specimens. The full list of taxa for the morphological analysis is available in Appendix A2.1 along with taxa excluded because of a lack of specimens, the only specimens available being immature or where a reliable identification could not be confirmed.

2.3.2 Examination of specimens

Specimens were examined using a Leica MZ75 microscope with a 7.9:1 zoom, x2 planapochromatic objective and x10 or x20 eyepieces. All images were taken with the Leica M165C (Leica, Germany) automontage photo stacking system at the Oxford University Museum of Natural History (OUMNH). Image stacks were acquired in Leica Application Suite (LAS V4.12) and then compiled using the program Helicon Focus (HF 7.5.8, Helicon Soft). Images were cropped and adjusted as needed in the photo editing software GIMP 2.10.3 (GIMP Dev. Team, 2019). Measurements were made using a x10 graticule eyepiece and confirmed using the measuring tools in LAS. Specimens were examined in either 70% or 96% ethanol depending on the respective storage method. As with the molecular data, the character matrix was generated in Mesquite v.3.7 (Maddison and Maddison, 2021).

2.3.3 Searching for reliable morphological characters

A common criticism of phylogenies constructed with morphological data is the limited number of characters available to create a dataset (Lee, 2004; Giribet, 2010; Lee and Palci 2015; Wanninger, 2015; Neumann *et al*., 2021; Oyston *et al*., 2022) and this is certainly true when compared to the 10,000s of characters that molecular sequence data can provide for phylogenetic analyses (Wang *et al*., 2019). To maximise the number of relevant characters for the analysis, a comprehensive study of characters used in previous publications was undertaken, along with thorough examination of specimens to locate novel characters. The primary method of character selection was to examine literature concerned with Gasteracanthinae or other orb-weaving species. Often the 19th and early 20th century publications used methods of describing characters that were non-transferable to a cladistic approach. Many of the more prominent publications required translation from French or German, but these publications often yielded more relevant data than those written in English due to their comprehensive taxonomic and morphological coverage.

More recent publications often include line drawings of key characteristics and genitalia and photographs of variations within species, making them useful for determining quantifiable characters. Both Benoit (1962a, 1962b, 1964, 1975) and Emerit (1968, 1973, 1974, 1975, 1982a, 1982b) provide clear morphological species descriptions and redescriptions and these works were key in understanding character variation between, and within, species.

A secondary method of locating characters was to critically examine specimens under a microscope to search for novel characteristics that varied between species. Once differences had been observed, series of specimens of the same species were used wherever possible to confirm that selected characters were consistent within the species. Attempts were made to acquire at least 5 examples of a male and female of each species for comparative purposes. This could not be achieved in the species that were rare within museum collections or, in the case of almost every species, where only one or two examples of males were available.

Most loaned specimens were either plentiful or of common species, for example the Natural History Museum in London (NHM) possesses a jar of over 700 examples of *Gasteracantha geminata* (Fabricius, 1798) from the same site, so risk of damaging or destroying the future value of such collections was minimal. However, for some species only a few specimens, or even only the type specimen is known. Because of this some morphological characters remained unscored in these species. For example, it was discovered that some type specimens from the NHM London had been loaned to other researchers in the past and had the epigyne (the external genital structure of female spiders) or the male palps, (the second pair of appendages which develop into a complex structure in mature males used to transfer sperm to the female during mating) removed but not re-associated with the specimens (Agnarsson *et al*., in prep).

This prevented these specimens yielding specific characters for the analysis, but it was sometimes possible to locate high quality line drawings of the genitalia within publications (by the same author who presumably did not return the dissection to the specimen), thus limiting the number of missing characters. This was also a productive method of gathering data from male Gasteracanthinae where physical specimens were not readily available.

Trials of using specimens stored in different ways were included in the search for morphological characters. Dried specimens of Gasteracanthinae are unique in that they can retain their abdominal shape, due to the abdominal sclerotization, almost like an insect, and they often show many of the morphological characters shown by specimens preserved in ethanol. However, dried material did present some difficulties. A lack of flexibility in the specimen means that any character that is obscured will either remain unseen or there is a risk of damaging the specimen to reveal it. The internal genitalia of dried female spiders are unusable for character scoring and the external genitalia features are often destroyed by the pin.

Within the Gasteracanthinae, any male-only phylogenetic analysis will be lacking in species data as less than 20% of species described from a female specimen have an associated male (see 2.2.2). Despite this, characters from even a small number of males may provide additional support to clades or species relationships from the female phylogeny in certain species groups. Locating male Gasteracanthinae in various collections was undertaken from 2015-2021. Researchers who were collecting in the areas of interest were encouraged to check webs for males whenever they encountered a female gasteracanthine and to actively look for males. The total numbers of mature male species and specimens found are given in Appendix A2.3 (Table A2.1). More details can be found in section 2.3.6.

Due to the lack of available males, the literature was consulted for additional information about the size ratio of males to females (total length, carapace length and width, and abdomen length and width). This data also contributed to the eSSD analysis (Chapter 4). Illustrations, or even basic photographs, of males also could be used to score head, carapace, and abdominal characters. The publications by Benoit (1962a, 1962b, 1964, 1975) and Emerit (1968, 1973, 1974, 1975, 1982a, 1982b) are both illustrated with high quality line drawings of abdomens, internal and external female genitalia and ventral views of male palps.

These publications were ideal for scoring characters where the specimen was damaged and provided male palp characters when specimens were unobtainable. For example, Emerit (1974) illustrated outlines of the median apophyses of several species (Figure 2.6) that could not be located as physical specimens and Benoit and Emerit (1975) also provided full specimen line drawings in addition those of the median apophysis (Figure 2.7).

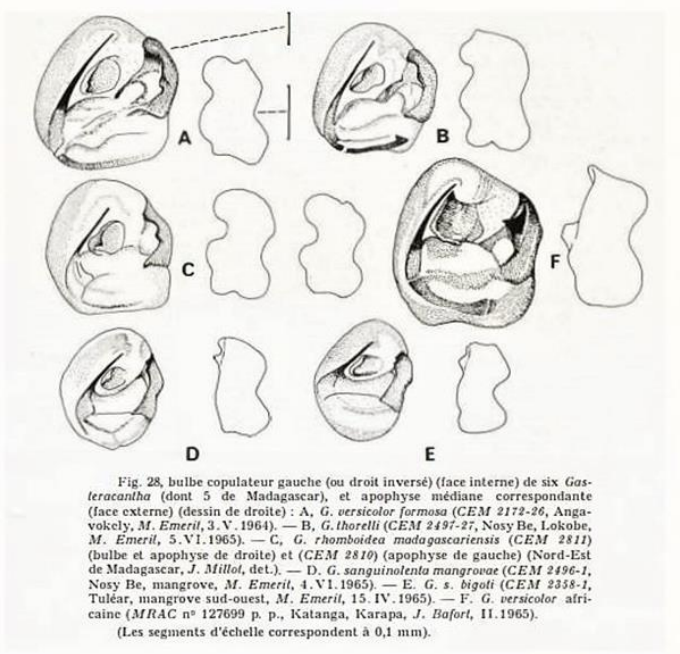

Fig. 28, bulbe copulateur gauche (ou droit inversé) (face interne) de six *Gasteracantha* (dont 5 de Madagascar), et apophyse médiane correspondante (face externe) (dessin de droite) : A, *G. versicolor formosa* (*CEM 2172-26*, Angavokely, *M. Emerit*, 3.V.1964). — B, *G. thorelli* (*CEM 2497-27*, Nosy Be, Lokobe, *M. Emerit*, 5.VI.1965). — C, *G. rhomboidea madagascariensis* (*CEM 2811*) (bulbe et apophyse de droite) et (*CEM 2810*) (apophyse de gauche) (Nord-Est de Madagascar, *J. Millot*, det.). — D. *G. sanguinolenta mangrovae* (*CEM 2496-1*, Nosy Be, mangrove, *M. Emerit*, 4.VI.1965). — E. *G. s. bigoti* (*CEM 2358-1*, Tuléar, mangrove sud-ouest, *M. Emerit*, 15.IV.1965). — F. *G. versicolor* africaine (*MRAC* nº 127699 p. p., Katanga, Karapa, *J. Bafort*, 11.1965).
(Les segments d'échelle correspondent à 0,1 mm).

Figure 2.6 Line drawing of ventral view of six male *Gasteracantha* palps and their median apophysis outlines; from Emerit (1974).

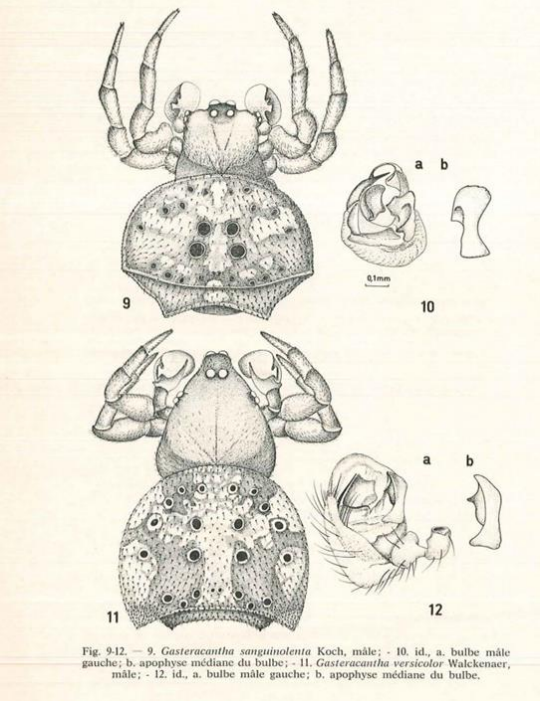

Fig. 9-12. — 9. *Gasteracantha sanguinolenta* Koch, mâle; - 10. id., a. bulbe mâle gauche; b. apophyse médiane du bulbe; - 11. *Gasteracantha versicolor* Walckenaer, mâle; - 12. id., a. bulbe mâle gauche; b. apophyse médiane du bulbe.

Figure 2.7 Line drawings of *Gasteracantha sanguinolenta* C. L. Koch, 1844 and *G. versicolor* males, ventral view of palps and median apophysis outlines; from Benoit and Emerit (1975).

After consulting the literature, only *Micrathena schreibersi* (Perty, 1833), *Isoxya penzoides* Simon, 1887, *Macracantha arcuata* (Fabricius, 1793*)*, *Gasteracantha kuhli* C. L. Koch, 1837 and *Gasteracantha theisi* Guérin, 1838 had significant missing male characters. Male specimens were not located for these taxa and the literature either did not provide suitable illustrative information or was simply without informative descriptions.

During the morphological study it became apparent that *Gasteracantha* females are extremely diverse in colour and spine structures but are limited in other informative characters, for example genitalia structures. In contrast, male *Gasteracantha*, who possess more diverse genitalia characters than the females, yielded more phylogenetically informative characters. Future work should aim to acquire male specimens as a priority to supplement any morphological studies (see Chapter 5.2).

2.3.4 Homology assessment and scoring reliable morphological characters

One of the most basic and important assumptions of cladistics and phylogenetic analyses, is that characters are homologous. Homology refers to a similar characteristic inherited by two or more related organisms from a common ancestor. The function of the character does not have to be the same, but the location or the structure of that character should be the same. This is referred to as 'topographical homology' (Jardine, 1969; Agnarsson and Coddington, 2008). Looking for the similarities in characters on the respective study taxa is therefore fundamental in assigning homology, but this is an arbitrary decision and must be guided by knowledge of the study taxa. Homology is a 'difficult and subtle problem' (Agnarsson and Coddington, 2008). The following section discusses the process of assigning homology and scoring the characters for the morphological analysis.

Once a potential character has been identified, primary homology can be proposed. If the character fulfils the topographical homology assessment of being in the same location on the taxa or being the same structure, then all the character states are transformationally homologous (Brower and Schawaroch, 1996). The character states can then be identified and hypothesised to be identical or not. 'All shared, identical character states represent conjunctures of potential homology' (Brower and Schawaroch, 1996) and provide evidence for relationships in phylogenetic analyses even if they are found to be plesiomorphic or symplesiomorphic; that is a character shared by all members of a clade that does not help to distinguish it from other clades.

Homology cannot be identified prior to a cladistic analysis, merely hypothesised. The adjudication of a character's homology, and if it is synapomorphic, is the result of cladogram construction (Brower and Schawaroch, 1996). Following de Pinna (1991) there are two typical homology hypotheses: primary and secondary. Primary, as listed above, is based upon educated theories prior to testing, but secondary homology is confirmed by a test of congruence, for example, a character that appears as a synapomorphy on a cladogram (Agnarsson and Coddington, 2008).

The study of Araneidae morphology, and the homology of characters in phylogenetic analyses has been well documented by various arachnologists. Most characters that were scored in this study fall under the criteria of primary homology. However, there were assessments of potential secondary homologous characters. These are listed in the character description and the results of the congruence testing of these characters is given either in the results and discussion of the morphological analyses, or in the character description as additional data following testing.

Publications concerning morphological phylogenetic analyses by Scharff and Coddington (1997), Kuntner, Coddington and Hormiga (2008) and Magalhães and Santos (2012) were the main points of reference as these are relevant, comprehensive studies that have withstood rigorous testing and scrutiny. They also featured taxa from the same, or closely related families, so these were the primary sources for character homology confirmation. These publications featured morphological phylogenies with large character datasets and limited disagreements of homology hypothesis assessments by the respective authors. Additional literature on general arachnid morphological phylogenies was also examined: Kuntner (2002), Schultz (2007), Lopardo and Hormiga (2015), Zhang and Maddison (2015) and Magalhães, Brescovit and Santos (2017).

The homology assignment of morphological characters in spiders is typically straightforward and, because the ingroup taxa in this study are all in the same subfamily, topographical homology assessment was clear in most cases. A substantial number of the characters scored in the dataset are present across various spider families as they are homologous structures that have the same or similar functions. In addition to providing support for the homology hypotheses of specific characters, the literature also provided suggestions for areas of the taxa that might yield additional characters.

Once a potential character has been identified it can be partitioned into discrete states (Pagel, 1999). These states can be simple (Freudenstein, 2005), for example character 2, Appendix A3.2.1 that scores the presence or absence of indentations on the cephalothorax. In some cases, these states might need quantifying, for example characters 64 and 65, Appendix A3.2.1 that score the width of part of the male genitalia. Not every character can be easily partitioned into discrete states however, as some express continuous variation, for example the curvature of spines in female *Gasteracantha* (see 2.3.5) where the ratios of curvature are continuous values (Pagel, 1999).

Most characters scored in this analysis were binary. State (0) was allocated to the character state as it was found in the primary outgroup taxon; *Nephila pilipes* (Fabricius, 1793) – where applicable. Where characters were found with more than two states, two methods were followed. Either the character was scored as a multistate character, a character with states (0), (1), (2) and so on (for example character 1, Appendix A3.2.1), or the character was split into two separate binary characters (0) and (1) but this left the requirement for the second character to have missing data for some taxa (for example characters 37 and 38, Appendix A3.2.1). In addition, multistate characters sometimes enabled potentially informative character states to remain and not be merged into a binary character where information could be lost.

How to score character states is one of the main issues when constructing a morphological character matrix. Maddison (1993) discussed various methods of scoring characters as either binary or multistate in detail. Maddison used an example of the colour of birds' tails (either a red tail, a blue tail, or no tail) to discuss methods of scoring characters. However, Maddison was not always critical of states that ultimately end in missing data. Using Maddison's (1993) example, the lack of a tail means there cannot be a colour assigned to the tail, so the state is marked with a '-' signifying missing data. The issue can occur when this is interpreted as 'missing' (normally marked with a '?') in the analysis. If all members of a clade, for example, had a character marked with the state '-', then this would not be problematic (Maddison, 1993). Examples of this might include the absence of spines in the outgroup taxa in this study. If the outgroup does not possess spines, then it would have no impact on the relationships within the outgroup.

Maddison was also writing when character weighting and ordering was a common method in phylogenetic analysis (Maddison, 1993), but this can lead to incorrectly weighting of character state changes. For example, in this study character 1 (see A3.2.1) scores the presence of a protrusion from the carapace that is split into two in most species, however it can be a single protrusion, or might not be present. Without knowing the direction which the character has transformed, (either from no protrusion, into one protrusion into two, or from no protrusion, to two, to one), the order cannot be unambiguously assigned. Additionally, Platnick (1979) noted that if we knew the entire character transformation series, we would also know the taxonomic phylogeny. Goloboff, Torres and Arias (2018), following a large study of various phylogenetic analyses methods, concluded in favour of "equal-weights parsimony" (see 2.4 for full discussion). Therefore, it was deemed appropriate to leave these multi-state characters as unordered when they were scored during this study.

When a potential multi-state character was instead split into two separate binary characters, the taxa that did not possess these new binary states were instead scored with a ‘-' to signify a gap. When taxa could not have a character scored, for example when a male of a species was unknown or the internal genitalia of a female type specimen could not be dissected, but the character state might be one that was already scored, a ‘?’ was scored to signify missing data. The treatment of these two states is covered in the analysis sections in 2.4.

Characters that required arbitrary partitioning, for example a larger than/smaller than character, were either informed by previous literature or careful judgements were made if this had not been considered. For example, care was taken to ensure that if measuring a distance in relation to another distance that the two were separate. For example, an abdominal spine length was not compared to another spine’s length. Specific details are provided on characters in Appendix A3.2.1.

Care was also taken to avoid redundant characters and not to duplicate scoring of the same character, therefore giving weight to species relationships unnecessarily. More information on specific character states or character location homology can be found in 2.3.6 and in the character list in Appendix A3.2.1. As new taxa were located and their characters added to the matrix during the project, this provided the opportunity for every character to have the scoring of its states confirmed, at least twice, in all taxa prior to the analyses.

2.3.5 Gasteracanthinae; unreliable morphological characters

With early, 19th century descriptions of species there are limitations that are quickly discovered when selecting characters for a cladistic analysis. Chrysanthus (1971) put it very clearly when he wrote: “many difficulties arise in the study of *Gasteracantha* species in consequence of the vagueness of many original descriptions and the different interpretations by subsequent authors”.

Many publications that describe species of Gasteracanthinae, up until around 1960, do not provide detailed descriptions of distinguishing characteristics beyond spine length and shape, abdominal shape, and often abdominal colour. All these characters can be variable depending on the age of the specimen, the development of the spider when collected or the manner and method of storage when preserved. Additional issues arise when publications are not written in a language one is familiar with and even the papers of Chrysanthus (1971) and Tikader (1985), written in English, still use basic descriptions, often not going further than: “abdomen...with large sigilla and armed with...[six] spines, spinnerets encircled by a horny ring” (Taikader, 1985). Characters like these that might assist in splitting common species from the same geographical area are not always suitable for inclusion in a cladistic analysis.

As an example, exact spine length and shape as characters in the morphological analysis were ruled out at an early stage. Exact spine curvature or spine shape in *Gasteracantha* females, following Chrysanthus (1971), Emerit (1974) and Tikader (1985) are useful for separating species in small geographical areas but are not easily coded as cladistic characters. Large variations were found in the same species, even in some cases in the pair of spines on the same specimen, and exact curvature of the spines was difficult to measure for the same reasons. Examples of individuals whose spines had not developed properly were found in museum collections (see Figure 2.8). These were probably due to an issue during the spider's moulting but could confusion to the scoring of these characters. The size of the female often, but not always, had a direct correlation to the size of the spines and there was an attempt to examine a long series of specimens for each taxon, to discover a method of quantifying the length of the spine with another abdominal structure as a ratio; see 2.3.6: *III. Female abdominal spines*.

Figure 2.8 Photograph of anterior/dorsal view of *Gasteracantha falcicornis* Butler, 1873 (♀) with deformed right median spine. From the Vienna Museum of Natural History (VMNH) collection.

Abdominal colour, although appearing in all publications, is often a highly variable character. The variation in abdominal colours can be extreme, for example in *Gasteracantha diardi* (Lucas, 1835), where individuals range from dark red (Figure 2.9) through to greens and yellows as live specimens, but these colours can fade in ethanol very easily (Kariko *et al*., 2018); see figure 2.4, *Gasteracantha diadesmia* Thorell, 1887. For most *Gasteracantha* species in particular, the colour red fades to yellow very quickly, but not always at the base of the spines (Williams, pers. obs.). Most specimens studied for the cladistic analysis were not representative of their true live colours. However, there is an exception. The unique metallic colouring of the endemic species found in the Solomon Islands is the same in live and in preserved specimens.

Chrysanthus (1971), Emerit (1974) and Tikader (1985) also use colour to discriminate between species; including Chrysanthus suggesting describing a sub-species based upon an apparent colour variation that has been found to just be related to the age of the specimens and the effect of ethanol on the specimens (Kariko *et al*., 2018; Marquina *et al*., 2021; Williams, in prep.). Certainly, when comparing museum specimens to fresh material the colour differences can be very marked and this led to the majority of considered colour-based characters being discarded early in the scoring process.

Figure 2.9 Left: Photograph of *Gasteracantha diardi* (♀) dark red colour variation live in situ (©Chien, 2021) Right: Photograph of *Gasteracantha diadesmia* (♀) dorsal view of abdomen showing faded colours from 70% ethanol storage, NHM London collection.

Abdominal shape is the final common feature of the publications pre-1960 where tentative taxonomic groupings were made based upon the shape of the abdomen (see Figures 1.1, 2.9, A3.6, A3.7, A3.8, A3.15 and A3.16 for examples of abdominal shapes). Although difficult to code as a cladistic character, the method of morphometric analysis employed later to investigate the evolution of abdominal shapes in the subfamily (see Chapters 3), has the potential of quantifying abdominal shape. Chrysanthus (1971), Emerit (1974) and Tikader (1985) each discuss abdominal shape but, once again, the geographical limitations of the respective studies mean that the over-emphasis on shape is not applicable to all the world fauna.

2.3.6 Scored morphological characters

A full list and description of the characters scored, and their relationships to previously described states by other authors where applicable, is given in Appendix 3.2.1. The character matrix used in the analysis is also available in Appendix 3.2.3. Several additional characters (number 67-79) are included in Appendix 3.2.2 but were not used in the morphological analysis as they were either uninformative, with only one taxon possessing a different state, or invariant amongst the examined species. These unused characters may be of value with examination of further material or in a more widespread taxonomic review. An overview of the characters is presented here with some details on homology. A glossary of morphological terminology can be found in Appendix 3.1 to aid with understanding.

Character list overview

I. Female carapace and cephalothorax: characters concerning the carapace, the cephalothorax and the area posterior to the eyes. Interpretation of homology of these characters is straightforward as they refer to features present in all spiders or, in the case of the protuberances, have obvious topographical correspondence. Characters such as fine white hairs present on freshly moulted material were rejected as specimen age can change the presence or absence of these. Chelicerae characters, both structural and relating to teeth, were also rejected. Despite considerable effort relating to counting and attempting to score teeth character states, these characters were too variable and provided no information about species or generic relationships.

II. Female abdomen surface: the surface of the spiny orb weavers is a hard, sclerotized structure, uncommon in the Araneidae, and more akin to the hard carapace found in most other spiders. Here characters score the abdominal surface structure and colouration in the chosen taxa. The abdomen is homologous in all Araneae and the sclerotized surface is found in several different generic groups.

III. Female abdominal spines: the external appearance of *Gasteracantha* females is often distinctively different even between species in a close geographical area, however these differences are often based on non-quantifiable characters. An example of this would be quantifying characters concerning the female abdominal spines. When comparing the spines of *G. curvispina* (Guérin, 1837) with *G. thorelli* Keyserling, 1864 a field guide, or key to species, might be able to provide a description that would split the two species based upon their second pair of spines. This method would not be applicable to a cladistic analysis as the difference would depend on the curvature of the spines and this ratio could not be quantified and split into discrete states due to variability.

The curvature, length and in some cases the angle to which the spines protrude all present problems when looking to quantify this variation. A new species of *Isoxya* will be described in Agnarsson *et al*. (in prep.) with notes on the abdominal variations that are found in recently moulted females versus older, gravid females. This changes the exact angle at which the abdominal spines protrude from the abdomen, so absolute angle measurements would be unsuitable.

The homology of the abdominal spines is clear as each pair of spines, regardless of shape or basal and terminal structure, has been confirmed to originate from the same location on the abdomen in all the species which exhibit these structures. Characters relating to the curvature of the abdominal spines were thoroughly scrutinised through the scoring process to confirm variability. Conclusions found that there were cases of ambiguity as specimens were found with spines that exhibited greater curvature than had been described or discussed in publications, or with one spine from a pair extremely curved and the opposite spine with minimal curvature, so the character was discarded. Large amounts of variation within a species were also found in relation to exact spine measurement. By using a fixed abdominal character (abdominal sigilla – see character 16, Appendix A3.2.1), the length of the spines could be quantified in a manner that did not change within a species.

IV. Female dorsal abdominal sigilla: sigilla are slightly depressed, sclerotized indentations on the external surface of the abdomen, marking points of internal muscle attachments. It is hypothesised that all spiders have these sigilla (Kuntner pers. comm., 2020) and they are just not normally noticeable due to the 'rather soft and sacklike' (Foelix, 2011) abdomen and lack of abdominal sclerotization in most species. The homology of these sigilla was documented in Scharff and Coddington (1997); Kuntner, Coddington and Hormiga (2008); and Gregorič *et al*. (2015b) in *Gasteracantha*, *Nephila* and *Caerostris* and the abdominal sigilla in the Gasteracanthinae are homologous with those.

V. Female legs: the homology of spider legs and their structure is not in doubt following decades of examination across all Araneae. More leg-specific characters were rejected than scored during the process due to high variability. For example, the exact leg colour within species appeared to be too variable, as different specimens of the same species were found with both black and brown legs or striped and un-striped legs. At family level the length of some legs is also not informative as the states remain constant.

VI. Female sternum: the homology of the sternum is clear as the structure is present in all spiders. See "*I. Female carapace and cephalothorax*" above for information on excluding characters like fine hairs. Additional sternum characters that were located from the literature were discarded during the scoring process as there was no variation in the taxa used in this study.

VII. Female venter: various characters on the ventral surface of the abdomen were scored. These included ventral condyles (round protuberances at the join of the cephalothorax to the abdomen) that are homologous and ventral sigilla; see dorsal sigilla above for more details about the homology of sigilla. Other characters included abdominal wrinkles, which are a structure found in the species with heavily sclerotized abdomens, and the ventral tubercle (character 32, Appendix A3.2.1) that, when present, is used by the males during mating.

VIII. Female Spinnerets: the homology of spider spinnerets has been discussed in previous studies (Scharff and Coddington, 1997; Kuntner, Coddington and Hormiga, 2008; Magalhães and Santos, 2012) and here many of these characters are similar to characters that have been used before. The specific characters for the Gasteracanthinae spinnerets are all in the same location on the spinneret structure so the homology of these characters is not in doubt.

IX. Female genitalia: the homology of the epigyne has been covered in previous studies. The sexual fertilization system and female genitalia of entelegyne spiders (the subgroup of the infraorder where Gasteracanthinae are located) can often assist in producing concise morphological phylogenies (Coddington, 1990; Kuntner, Coddington and Hormiga, 2008; Magalhães and Santos, 2012; Valdez-Mondragón, 2013; Valdez-Mondragón and Francke, 2015).

Two difficulties arose when scoring characters relating to the female genitalia. First were the apparent similarities between the genitalia, both external and internal, of the females for hypothesised closely related species. Within *Gasteracantha* 'female genitalia within the genus is rather uniform' showing little morphological difference (Scharff, *in litt*., 2015). The female genitalia internally, the spermatheca, is often very similar across different species. As Macharoenboon, Siriwut and Jeratthitikul (2021) showed with excellent line drawings (Figure A3.30), there can be apparent differences when examining a limited set of species. However, by contrast at a world level these differences quickly become harder, or impossible, to identify and quantify. That said, within the Gasteracanthinae there appears to be a clear divide between the external female genital structure of different genera. For example, *Isoxya* species all possess a heavily sclerotized double arch at the epigyne opening whereas *Gasteracantha* often have a scape-like lobe that protrudes away from the epigyne.

The second difficulty was concerning characters relating to sclerotization. During the examination process it was discovered that the sclerotization of the epigyne in recently moulted mature females is often incomplete. The epigyne, lobe, and area surrounding the epigyne can often be unsclerotized or have some partial sclerotization where on older females the areas are completely sclerotized. The maturity of the specimen, even during the adult moult, when it was captured has to be taken into account as this could provide confusion otherwise. This is raised in more detail in Agnarsson *et al*. (in prep.) where large numbers of the same species (100+), recently moulted and older, could be compared for variation.

X. Male somatic appearance: collecting *Gasteracantha* males is difficult as their maturity is very seasonal and requires an understanding of their behaviour to collect them effectively (Emerit, 1974). An effort was made by researchers from Slovenia, and their worldwide colleagues, to actively collect males when on fieldwork. However, several collection trips were disrupted by the Covid-19 pandemic, or no males were found. Future studies should look at raising young from egg-sacks and collaborating with as many worldwide arachnologists as possible to enable more male specimens to be examined. Therefore, due to a lack of specimens there are many taxa missing data from these characters (see Appendix 3.2.3). However, it is vital to score these characters because the male Gasteracanthinae can potentially yield a lot of taxonomic information.

The general appearance of the males was taken into consideration with head and carapace shape, some eyes on an extended protrusion, the abdominal structure and if spines were present, and the ratio of the full length of the male in relation to the female. The legs of the males, like the females, were targeted for characters relating to length, thickness, and specific structures. All the characters here are homologous and scored either in other publications or have the same homology as explained in the female character sections above.

XI. Male palp: it has been well documented that complex palp structures of mature male entelegyne spiders often yield numerous informative characters (Coddington,1990; Kuntner, Coddington and Hormiga, 2008; Magalhães and Santos, 2012; Valdez-Mondragón, 2013; Valdez-Mondragón and Francke, 2015).

The palps consist of hard, sclerotized parts and soft areas, there are also special protrusions (apophyses) that play an essential role during copulation (Foelix, 2011). The palps form a complex structure of homologous characters (Agnarsson and Coddington, 2008). The work by Scharff and Coddington (1997) explains the roles of the different structures and Kuntner, Coddington and Hormiga (2008) expanded further on this and discovered additional structures within the palp.

The high number of viable characters the male palps have yielded (often over 40 per publication in those examined during the study) in the larger recent works on other genera cannot be ignored, and any work on this complex structure could provide more support to any phylogenetic analysis. The male palp is therefore a valuable structure to gain morphological characteristics that relate directly to each species.

In basic terms, the male and female genitalia in Araneae have a mechanical function. For example, *Araneus* species copulate by the spine of the median apophysis hooking onto the epigyne scape, then the median apophysis twists, becoming enveloped by the scape. Other sclerites shift position to enable the embolus to situate in front of the epigynal opening before the hematotocha inflates causing the tegulum to rotate and press the conductor to one side and the embolus into the epigyne (Foelix, after Grasshoff (1973), 2011).

This reduces the chance of mating between species, although there are exceptions in other families (Kunz, Witthuhn and Uhl, 2015). The similarities between palp structures of *Araneus* and *Gasteracantha* suggest the copulation process should be similar. Detailed descriptions of Gasteracanthinae mating and courtship behaviour have been recorded in *Austracantha minax* (Thorell, 1859) by Mascord (1966) and *Gasteracantha versicolor* by Emerit (1974).

Some of the palp characters scored in this study were not obvious until the palp was expanded. Expansion was undertaken by placing the palp into a 5% Potassium hydroxide (KOH) and 95% distilled water solution for a few minutes. Then one or two drops of 100% distilled water were dropped onto the palp every few minutes with a pipette while gently pulling on the palp with forceps as it expanded.

Following the character scoring process, the final morphological dataset (Appendix A3.2.3) was split into three different analyses in three Mesquite v.3.7 matrices. The first forms a matrix of 66 informative characters with 58 species (Group 1), the second a matrix of 64 informative characters using 32 species (Group 2) and the third the same matrix of 64 informative characters but with 36 species (Group 3). These correspond respectively to: the full matrix of all species that could be analysed with all available female data for 58 species and that for males where it was available (contains quantities of missing data for both sexes) (Group 1); only males and females taxa included (Group 2) when both sexes have characters scored for them both (minimal missing data); and (Group 3) all the taxa from Group 2 with the addition of taxa that were included in molecular analyses (see 2.2.1) to enable easier tree comparison when the various methods of analysis are compared. The reduction in the number of characters for the second two groups reflects retention of only parsimony informative characters.

2.4 Phylogenetic analysis

This section covers the phylogenetic analyses of the molecular, morphological, and then combined data. A justification of the methods of phylogenetic analysis are first given, then the methods of analysis are described.

2.4.1 Methods of phylogenetic analysis

Two different methods of phylogenetic analysis were used as different methods might yield different sets of trees (see 2.5) (Rindal and Brower, 2010) and a combination of different methods can often increase confidence in clades and species relationships (Ribeiro *et al*., 2012). The two methods employed were: maximum parsimony (MP) analysis using PAUP* (Phylogenetic Analysis Using Parsimony *and other methods) and TNT (Tree analysis using New Technology), and a Bayesian inference (BI) analysis using MrBayes.

Parsimony is a philosophical principle that if everything is equal, the simplest explanation is preferable (Sadava *et al*., 2011; Franklin, 2015). In phylogenetic terms this means the model of parsimony finds the pattern, or patterns, that has the fewest evolutionary changes to infer the most parsimonious trees, or tree, out of those generated (Hall, 2011). This is the shortest, and therefore simplest, solution. The length of the tree is the total number of character state changes (or steps), required to support the relationships of the taxa shown. The better a tree fits the data, the lower the number of steps required. The most parsimonious tree will therefore be the one with the fewest steps (Lipscombe, 1998; Hall, 2011; Currie and Meade, 2014).

Many studies over the last 20 years both support and oppose parsimony. Goloboff, Torres and Arias (2018) concluded that trees generated using equal-weights parsimony are often preferable to ones generated by maximum likelihood. Additionally, Bayesian analysis produced trees 'about as good' as the ones inferred from equal-weights parsimony (Goloboff, Torres and Arias, 2018).

In addition to a MP analysis, a Bayesian Inference (BI) analysis of the same data was also conducted. BI is a statistical method of discovering the probability of a tree based upon Bayes' Theorem. Bayesian statistics uses prior beliefs to generate a hypothesis and then adds evidence, or data, to update those beliefs to produce a new, more probable, hypothesis that can be applied to any setting (Kurt, 2019).

The formula of Bayes' Theorem is:

$$Pr(A|B) = \frac{Pr(B|A)Pr(A)}{Pr(B)}$$

Pr = probability

A, B = events

$Pr(A|B)$ = the probability of A if B is true

$Pr(B|A)$ = the probability of B if A is true

$Pr(A), Pr(B)$ = the independent probabilities of A and B

In a phylogenetic setting, to calculate the posterior probability of the trees (that is the probability that the tree is correct based upon the information that has been provided) a combination of the prior likelihood information, the data likelihood information, and the specific chosen model (see Appendix 1.5) are used. This is ultimately displayed as the probability value for the relationships between species on the phylogenetic tree. This provides a realistic representation of what the inputted data can inform about the "evolutionary process that generated them" (Currie and Meade, 2014).

Other methods of inferring phylogenies are available, however these two were chosen because they are standard phylogenetic methods and allow straightforward comparison with results of other studies (Magalhães and Santos, 2012; Tarasov and Génier, 2015; Tarasov and Dimitrov, 2016; Shi, Li and Li, 2021).

2.4.2 Programs used

Three programs were used to analyse the molecular and morphological data. These were: PAUP* (Phylogenetic Analysis Using Parsimony *and other methods) (version 4.0a build 169) (Swofford, 2002), TNT (Tree analysis using New Technology) (version 1.5) (Goloboff, Farris and Nixon, 2008) and MrBayes (version 3.2.7a) (Ronquist *et al*., 2012).

All were run on a Windows 10 PC, with the addition of MrBayes occasionally being run through the online portal CIPRES Science Gateway v.3.3 (https://www.phylo.org/), as this provided the option to run each analysis at a much greater speed if required (for example during the sensitivity analysis of 16S). TNT was used for the molecular analyses instead of PAUP* as TNT was able to handle the molecular data within the memory limitations and was significantly faster than PAUP* (see Appendix 1.5). The specific details of the parameters used for each of the datasets, the limitations of each of the programs and the analyses run, are given in Appendix 1.4. In all trees *Nephila pilipes* was used as the outgroup to root the tree. This spider species is of a different family and morphologically very different to the Gasteracanthinae.

In the molecular analysis the MP analyses in TNT all ran for 100,000 replications. The BI analyses all stopped when the diagnostic stop value of split frequencies (set at 0.01) was met. This was 875,000 in CO1; 350,000 in 16S; and in combined (CO1+16S) 335,000. In the morphological analyses the MP analyses in PAUP* all ran for the requested 100,000 replications and the BI analyses all stopped when the diagnostic stop value of split frequencies (set at 0.01) was hit. This was 2,475,000 in Group 1; 1,300,000 in Group 2; and 1,565,000 in Group 3.

In the combined molecular and morphological analyses, the analysis on PAUP* completed all 100,000 replications and the BI analysis stopped when the diagnostic stop value of split frequencies (set at 0.01) was hit. This was at 60,000 generations. The result from the PAUP* analysis is not displayed as the output was comparable to the BI analysis.

In all trees the bootstrap replication values from the MP analysis are displayed at 60% cut-off and the Bayesian inference values are displayed at 0.6 (60%) cut-off. These values were chosen to highlight where support for clades, even if comparatively weak, was present in the trees.

All tree figures were constructed in the graphical editor TreeGraph2 (Stöver and Müller, 2010). The clade names used are the same in all analyses to aid with understanding and discussion. Occasionally, there may be clades that do not appear to warrant the name they have been allocated (for example: more representatives of other genera than *Gasteracantha* in the '*Gasteracantha* 1' clade within the molecular analysis) but these clade names have been selected to enable easier comparisons between the differing data analyses. In addition to this most trees are presented as cladograms, not respective of possible evolutionary age. The trees are displayed in this way to enable the clearest topological comparisons between species relationships. The combined molecular (CO1+16S) tree is also presented as a phylogram (Figure 2.16) with the branch lengths proportional to the amount of evolutionary change that has occurred.

2.5 Results

This section first presents the results of the DNA sequence alignments, followed by details of the variable and parsimony informative sites for each gene. A summary table is given of the sequence data obtained for each species. Then follows the results of the respective analyses, molecular, morphological, and then combined (molecular + morphology), in the form of inferred phylogenetic trees. The trees displayed are all the 50% majority rule consensus trees from the respective analyses, excluding the 16S maximum parsimony (MP), tree which only yielded 1 most parsimonious tree, and the combined molecular (CO1+16S) phylogram, displayed as a strict consensus tree. These were chosen over the strict consensus trees as in the morphological analyses the strict consensus trees had low species resolution and the 50% majority rule trees provided informative information.

2.5.1 DNA sequence alignments

The CO1 sequences aligned without issue and ranged in length from 376-1261bp. The CO1 sequence for *Afracantha camerunensis* was only 376bp, which was under half the length of the average length (780bp) across the other available sequences. Due to the high quantity of missing data this species was removed from the analysis. After this, *Acrosomoides acrosomoides* had the shortest sequence at 504bp and *Austracantha minax*, *Gasteracantha cancriformis* and *Isoxya mahafalensis* all were the longest at 1261bp. Following an examination of the CO1 data matrix, the CO1 sequences were trimmed at 29bp and at 740bp leaving a total of 711bp for analysis (Appendix 3.2.5). The trimming point was informed by looking to maximise the quantity of sequence data, while not affecting the regions of informative data. In addition to this, the starting cut point was made at the first codon position and a total of 711bp equated to 237 complete amino acid codons.

The same was performed for the 16S data. The 16S sequence data ranged from 369-450bp. The average number of base pairs was 431bp across 20 taxa with *Gasteracantha milvoides* Butler, 1873 having the lowest number at 369bp and various species from GenBank extraction having the highest number at 450bp. However, due to the gene possessing 9 variable regions (Johnson *et al*., 2019) and the alignment adjusting for this, the total number of base pairs following the alignment procedure was 556bp. Following an examination of the aligned 16S data the start of the 16S sequences were manually trimmed at 86bp and at 552bp, leaving a total of 467bp for the 16S analysis (Appendix 3.2.5). As with the CO1, the trimming points were informed by maximising the sequence data while minimising gaps and retaining informative data.

CO1 and 16S sequence information

Once an alignment had been performed, an analysis of the variable and informative sites in the CO1 and 16S data was conducted in PAUP* (Table 2.1).

Table 2.1
Number and proportion of variable and parsimony informative sites at each codon position in the *CO1* sequences

Codon position	Number of sites				
	Total	Variable		Informative	
		#	%	#	%
1	237	54	22.8	36	15.2
2	237	15	6.3	7	3.0
3	237	217	91.6	186	78.5
Total	711	286	40.2	229	32.2

The first two codons were less variable than the 3rd and, as a result, yielded less informative characters for phylogenetic analysis. The 3rd codon yielded a much greater number of informative characters as anticipated. With such a pronounced difference in character variability, the saturation of the 3rd codon was investigated as potentially this would impact any inferred phylogenies. The test for saturation of the 3rd codon was conducted in DAMBE7 (Xia, 2018). More information on the parameters chosen, and the results, can be found in Appendix 1.3. The results of the test showed that the 3rd codon was saturated (Figure A1.1). Further tests were conducted by inferring two phylogenies, one from the 1st and 2nd codon combined and one from the 3rd codon. These were performed to discover if the 3rd codon data had a phylogenetic signal consistent with that of the first two codon positions.

The trees produced with 1st and 2nd codon positions (Figure A1.2) and those using only the 3rd codon position (Figure A1.3) generated clades equivalent to those found in the full CO1 analysis (using all three codons) (Figure 2.10) and 16S analysis (Figure 2.13). However, the relative position of the clades was different. Because the full CO1 analysis (including all codon positions) recovered almost identical relationships to those recovered in the 16S analysis it was decided to retain the data for all three codons.

Table 2.2
Number and proportion of variable and parsimony informative sites in the *16S* sequences

Number of sites				
Total	Variable		Informative	
	#	%	#	%
467	226	48.4	161	34.5

The combined CO1 and 16S sequence data matrix was 1178bp long with 390 (33.1%) informative characters. The same 20 taxa that were featured in the 16S sequence analysis were in the combined matrix.

Table 2.3 Summary of DNA sequences generated in this study (with additional sequences derived from GenBank or BOLD)

Species name	CO1	CO1 amplicon	16S	16S amplicon	SHW Code	GenBank/BOLD reference
Acrosomoides acrosomoides	504bp	1309	-	-	SHW064	OQ150127
	658bp	-	-	-	-	MK420051
	-	-	447bp	-	-	MK420176
Actinacantha globulata	664bp	-	-	-	-	MG670112
	-	-	436bp	-	-	MG670140
Afracantha camerunensis	376bp	1309	-	-	SHW047	OQ150128
Araneus diadematus	918bp	-	-	-	-	JN018130
	-	-	449bp	-	-	KY467382
Aranoethra cambridgei	1261bp	-	-	-	-	MK420075
	-	-	450bp	-	-	MK420197
Augusta glyphica	659bp	-	-	-	-	MK420080
	-	-	450bp	-	-	MK420200
	-	-	431bp	485	SHW024	OQ148371
Austracantha minax	1261bp	-	-	-	-	MK420081
	-	-	448bp	-	-	MK420201
Gasteracantha aciculata	781bp	1309	-	-	SHW008	OQ150129
	729bp	1309	-	-	SHW010	OQ150130
	-	-	422bp	485	SHW010	OQ148372
G. cancriformis	1261bp	-	-	-	-	FJ525321
	-	-	397bp	-	-	MH258252
G. clavatrix	768bp	1309	-	485	SHW015	OQ150131
	651bp	1309	-	-	SHW016	OQ150132
G. diadesmia	675bp	-	-	-	-	MT584892
G. diardi	675bp	-	-	-	-	MT584896
	-	-	432bp	-	-	MG670141
	733bp	1309	-	-	SHW013	OQ150133
G. doriae	675bp	-	-	-	-	MT584890
*G. fornicata**	577bp	-	-	-	-	GCQT1837-17
	658bp	-	-	-	-	VAQTB027-11
G. kuhli	-	-	432bp	-	-	MG670147.1
	782bp	1309	-	-	SHW012	OQ150134
G. milvoides	722bp	1309	-	-	SHW035	OQ150135
	-	-	369bp	485	SHW060	OQ148373
G. rhomboidea madagascariensis	718bp	1309	-	-	SHW029	OQ150136
	-	-	412bp	485	SHW030	OQ148374
G. sp. (versicolor formosa)	658bp	-	-	-	-	RNOCF359-17
G. versicolor formosa	711bp	1309	-	-	SHW032	OQ674506
			438bp	485	SHW032	OQ148375
	710bp	1309	-	-	SHW033	OQ674516
*Isoxya mahafalensis**	1261bp	-	-	-	-	MK420117
	-	-	448bp	-	-	MK420234
I. penizoides	766bp	1309	-	-	SHW041	OQ150137
	762bp	1309	-	-	SHW042	OQ150138

Table 2.3 Continued.

Species name	CO1	CO1 amplicon	16S	16S amplicon	SHW Code	GenBank/BOLD reference
***I.* sp. nov.**	**634bp**	**-**	**-**	**-**	**-**	**MZ539942**
	679bp	**1309**	**-**	**-**	**SHW069**	**OQ150139**
I. sp.	**658bp**	**-**	**-**	**-**	**-**	**MW983524**
I. tabulata	**776bp**	**1309**	**-**	**-**	**SHW026**	**OQ150140**
Macracantha arcuata	**664bp**	**-**	**-**	**-**	**-**	**MG670122**
	-	**-**	**435bp**	**-**	**-**	**MG670149**
	788bp	**1309**	**-**	**-**	**SHW005**	**OQ150141**
	781bp	**1309**	**-**	**-**	**SHW006**	**OQ150142**
M. hasselti	**664bp**	**-**	**-**	**-**	**-**	**MG670120**
	-	**-**	**435bp**	**-**	**-**	**MG670148**
	574bp	**1309**	**-**	**-**	**SHW020**	**OQ150143**
	-	**-**	**432bp**	**485**	**SHW020**	**OQ148376**
Micrathena gracilis*	**636bp**	**-**	**-**	**-**	**-**	**MK420136**
	-	**-**	**450bp**	**-**	**-**	**MK420251**
M. schreibersi	**1114bp**	**-**	**-**	**-**	**-**	**KJ157317**
	-	**-**	**392bp**	**-**	**-**	**KJ157090**
	813bp	**1309**	**-**	**-**	**SHW063**	**OQ150144**
M. triangularispinosa*	**989bp**	**-**	**-**	**-**	**-**	**KX687314**
Nephila pilipes	**978bp**	**-**	**-**	**-**	**-**	**JN032337**
	-	**-**	**441bp**	**-**	**-**	**HQ441957**
Thelacantha brevispina	**664bp**	**-**	**-**	**-**	**-**	**MG670126**
	-	**-**	**445bp**	-	-	**MK420280**

* Denotes a species that was not available for morphological analysis.

2.5.2 Molecular analyses

Analyses of CO1 results

The CO1 analysis contained the most species in the molecular analyses (28) and used a matrix of 229 informative characters. The trees shown here (Figures 2.10 and 2.11) have strong resolution at the species level, as would be expected with this fast-evolving gene. The tree topologies generated by TNT (MP) and MrBayes (BI) are not identical, but broadly the groupings are.

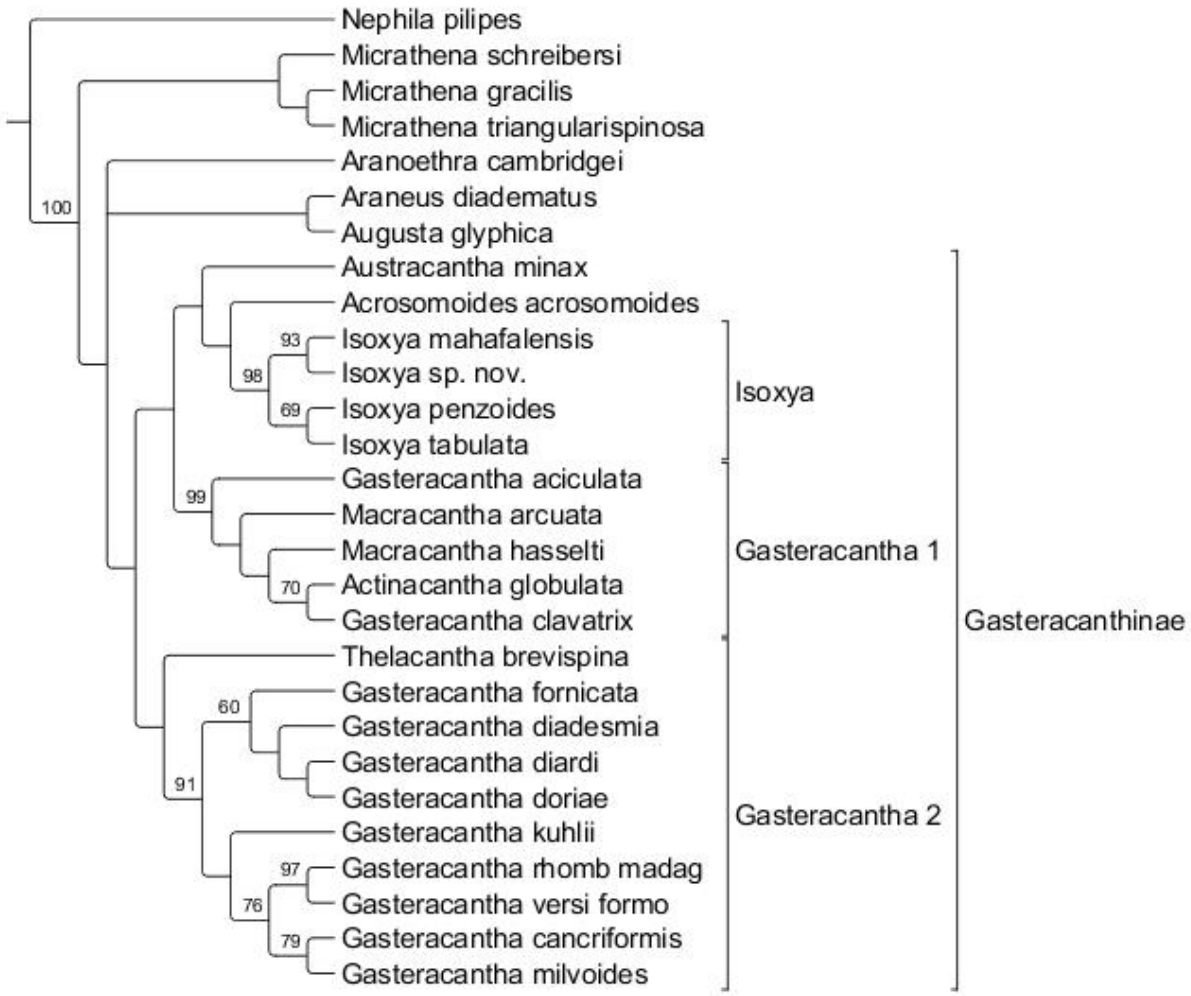

Figure 2.10 50% majority rule consensus tree of 2 equally parsimonious trees, inferred from maximum parsimony analysis of CO1 data, generated in TNT. Bootstrap value (BS) following 10,000 replications, displayed on relevant nodes at 60% cut-off.

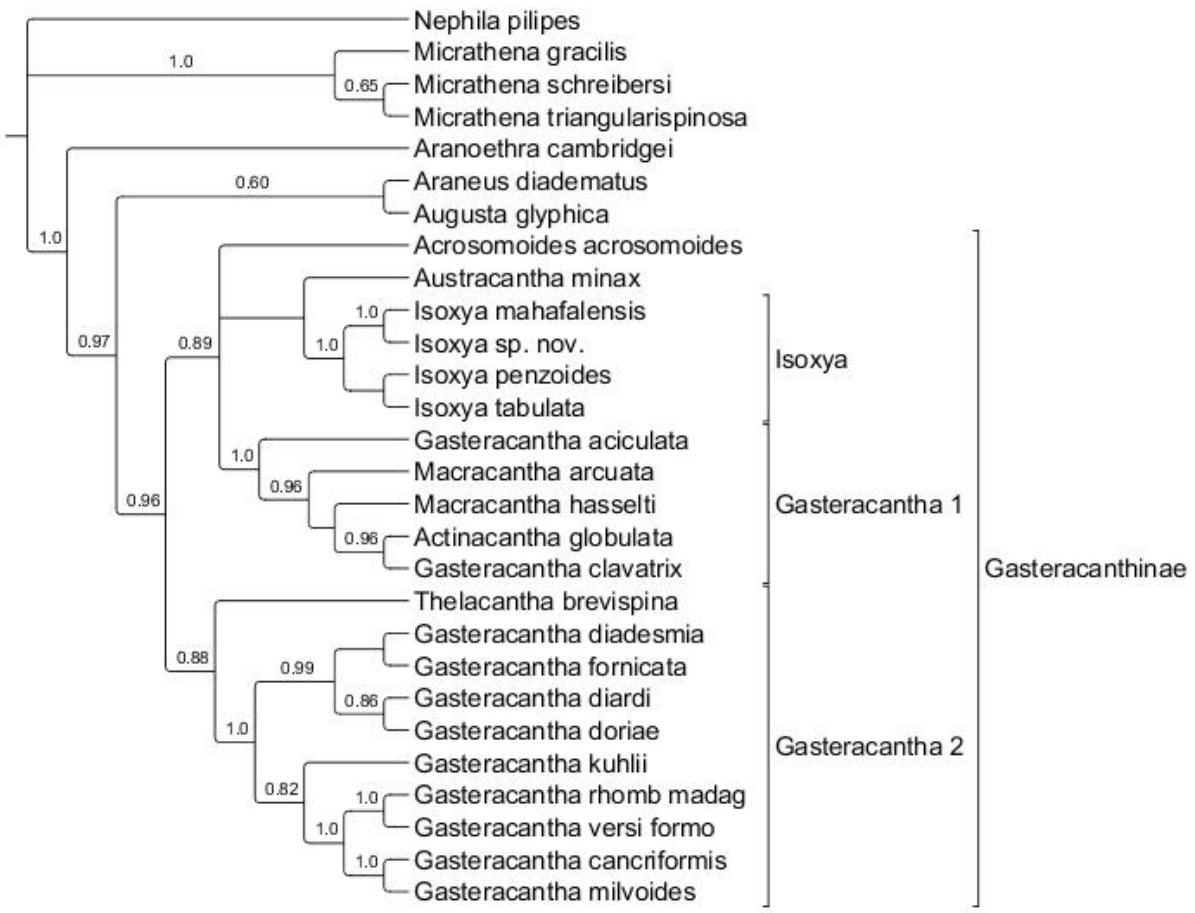

Figure 2.11 50% majority rule consensus of 1038 trees, inferred from Bayesian Inference analysis of CO1 data, generated in MrBayes. Posterior probability values displayed on relevant nodes at 0.6 (60%) cut-off.

In both phylogenies *Micrathena* form their own clade distinct from the Gasteracanthinae and *Araneus* and *Aranoethra* are also positioned outside the Gasteracanthinae. *Augusta* is located outside the subfamily Gasteracanthinae, providing evidence that the subfamily is not monophyletic. In both trees the Gasteracanthinae divide into two clades. The first clade contains a wide variety of genera and divides into two additional clades: '*Gasteracantha* 1' and a clade of *Isoxya* with *Acrosomoides* and *Austracantha* as unsupported sister taxa in the MP, and *Acrosomoides* forming a polytomy with the two clades in the BI. In both analyses *Isoxya* is monophyletic. The internal species relationships within '*Gasteracantha* 1' are not well supported in the MP results (Figure 2.10), however, there is strong signal at the basal node for this clade in both the BI and MP analyses. The second main clade: '*Gasteracantha* 2' is identical in both trees, comprising nine *Gasteracantha* species and the monotypic *Thelacantha* at a basal node of the clade.

Analyses of 16S results

The 16S trees (Figures 2.12 and 2.13) contain less taxa than the CO1 trees (20) and used a matrix of 161 informative characters. As with the CO1 analyses, the two methods of phylogenetic inference display broadly similar results. Although the alignment of the 16S required a sensitivity analysis (see Appendix 1.2), the trees displayed here follow most of the results of the CO1 analyses, regardless of the alignment parameter values (see Appendix 1.2). However, in the 16S analyses the Gasteracanthinae form a monophyletic group, because *Augusta* is located within the subfamily, unlike in the CO1 trees. The MP analysis, with only a single most parsimonious tree being generated, shows slightly more resolution than the BI analysis, but shows much less node support across the tree.

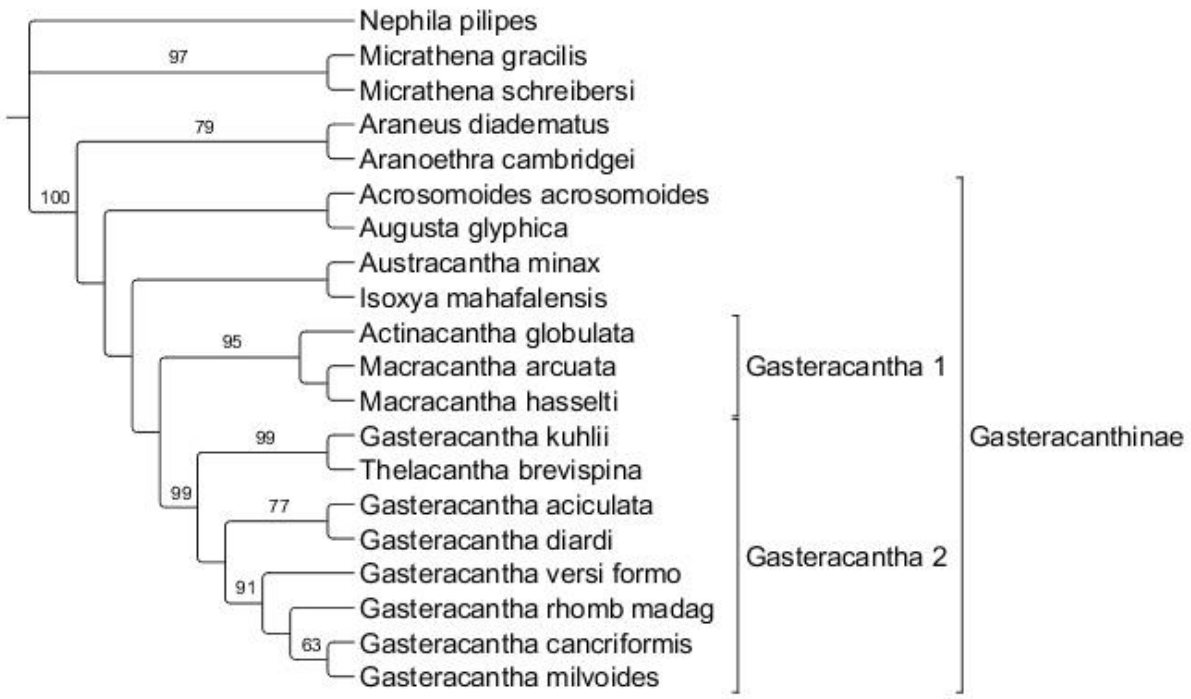

Figure 2.12 Single most parsimonious tree, inferred from maximum parsimony analysis of 16S data, generated in TNT. Bootstrap value (BS) following 10,000 replications, displayed on relevant nodes at 60% cut-off.

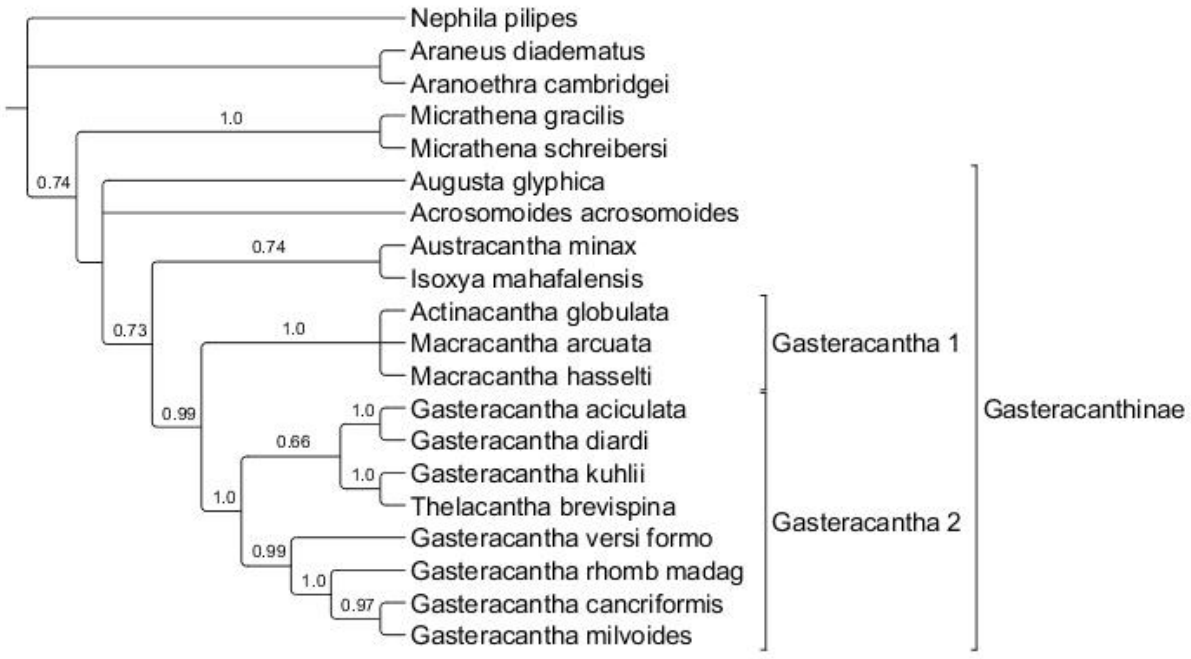

Figure 2.13 50% majority rule consensus of 223 trees, inferred from Bayesian Inference analysis of 16S data, generated in MrBayes. Posterior probability values displayed on relevant nodes at 0.6 (60%) cut-off.

In comparison to the CO1 analyses *Gasteracantha aciculata* is placed within the '*Gasteracantha* 2' group, not in '*Gasteracantha* 1'. However, all other members of '*Gasteracantha* 1' and '*Gasteracantha* 2' are the same, though the positions of individual species within these clades differs from those in the CO1 analysis. The two '*Gasteracantha*' clades remain sister to each other. Additionally, the relationships of *Micrathena*, *Acrosomoides* and *Augusta* are the same in the 16S and CO1 analyses. There are however, minor differences between the trees generated using the two sequences in the internal relationships of the species within the clades.

Analysis of combined DNA (CO1 and 16S) results

The combined molecular trees (Figures 2.14, 2.15 and 2.16) are generated using the CO1 and 16S sequence data (390 informative characters), restricting the analysis to the taxa that feature in the 16S trees (20). In Figure 2.16, the phylogram branch lengths are proportional to the amount of evolutionary change that has occurred. Specifically, MrBayes gives the branch lengths measured as the number of substitutions per site. In these combined trees there is a difference between the two analyses as the Gasteracanthinae are monophyletic in the BI analysis, with *Augusta* sister to the other gasteracanthines but not in the MP analysis; as *Augusta* is outside the subfamily, although this is unsupported. Additionally, *Augusta* has the longest branch in the phylogram.

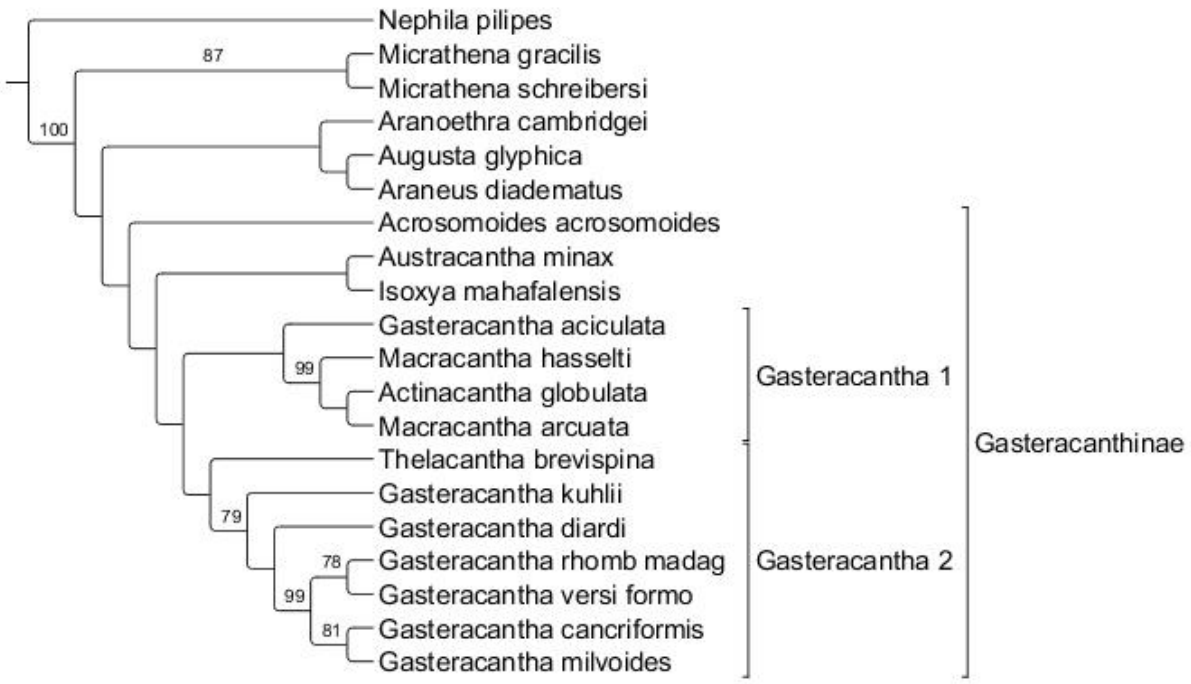

Figure 2.14 50% majority rule consensus of 2 equally parsimonious trees, inferred from maximum parsimony analysis of combined (CO1+16S) data, generated in TNT. Bootstrap value (BS) following 10,000 replications, displayed on relevant nodes at 60% cut-off.

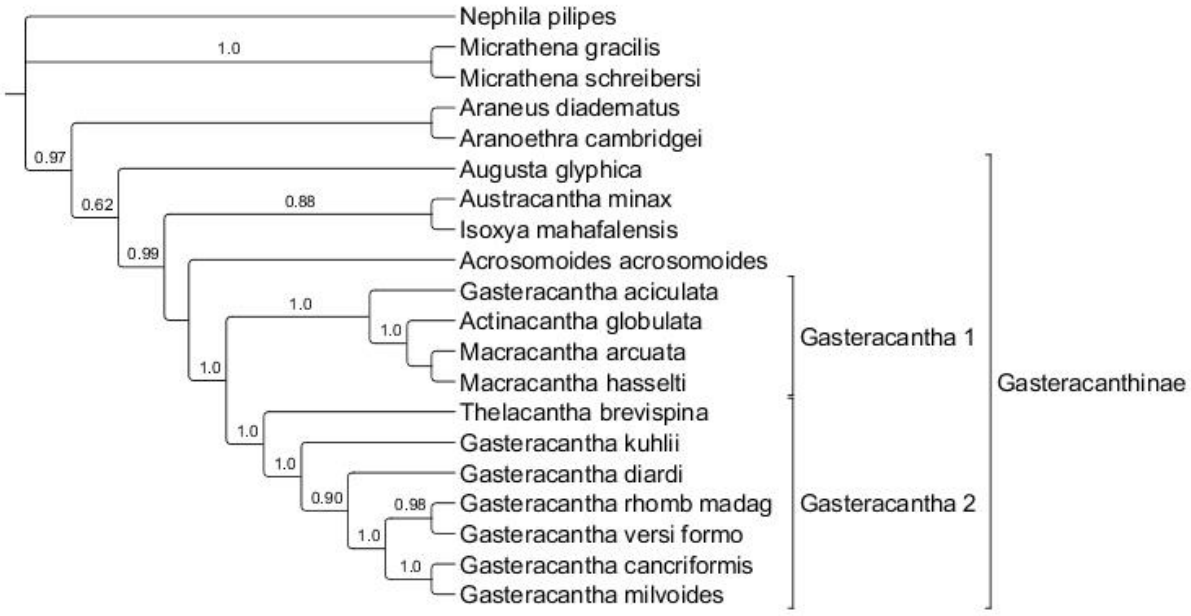

Figure 2.15 50% majority rule consensus of 153 trees, inferred from Bayesian Inference analysis of combined (CO1 + 16S) data, generated in MrBayes. Posterior probability values displayed on relevant nodes at 0.6 (60%) cut-off.

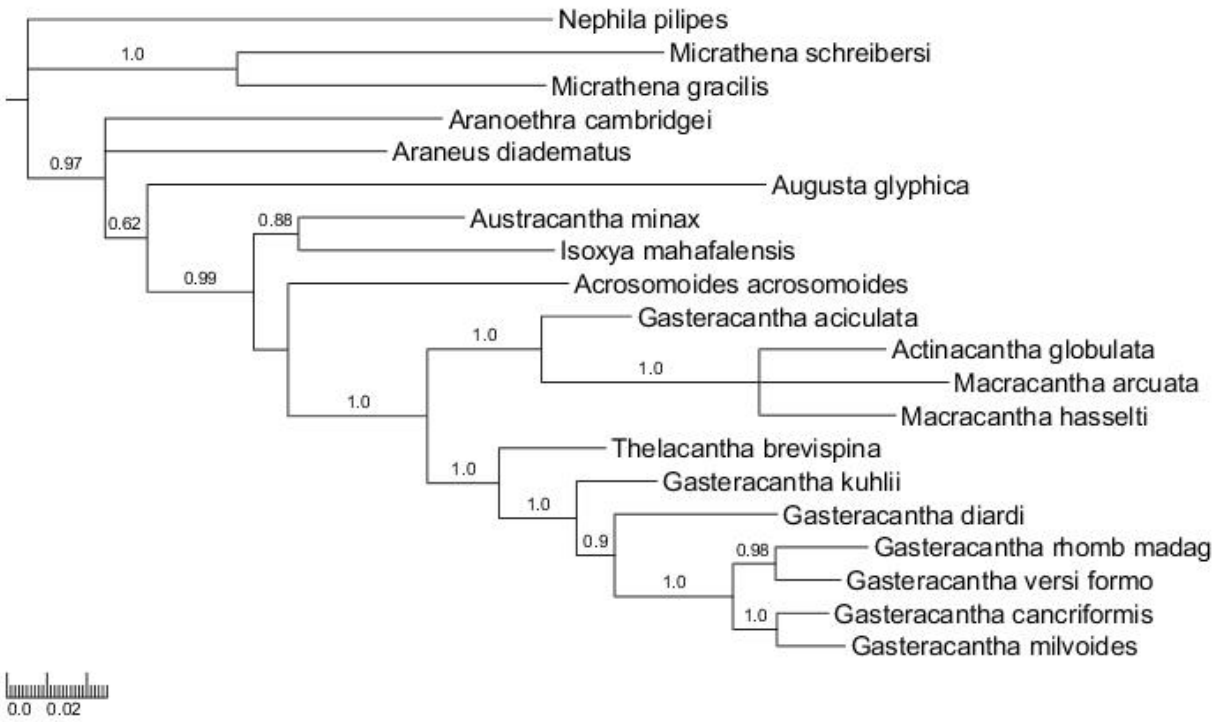

Figure 2.16 Strict consensus of 153 trees, inferred from Bayesian Inference analysis of combined (CO1 + 16S) data, generated in MrBayes. Posterior probability values displayed on relevant nodes at 0.6 (60%) cut-off. The scale bar represents the number of nucleotide substitutions per site.

Within the subfamily clade, that contains taxa that have been present in the Gasteracanthinae in both the CO1 analysis and 16S analysis, there is strong BI support at almost all nodes (Figure 2.15). The two '*Gasteracantha*' groups separate into their respective clades containing the same taxa as seen in the CO1 analysis, but at a deeper node within the subfamily, as in the 16S analysis.

The support for the clades '*Gasteracantha* 1' and '*Gasteracantha* 2' is strong in both combined molecular analysis methods. The final positioning of *Gasteracantha aciculata* is at the basal node of '*Gasteracantha* 1', with the other two genera *Actinacantha* and *Macracantha* deeper inside this clade. *Thelacantha brevispina* is located at the base of '*Gasteracantha* 2', with the remaining taxa forming well supported relationships inside it. The clade containing *G. rhomboidea madagascariensis G. versicolor formosa, G. cancriformis and G. milvoides* has the shortest branches in the phylogram suggesting they have diverged relatively recently. By combining the two genes, resolution of the positioning of these genera has become clearer and highlights that *Gasteracantha*, as currently defined, is not monophyletic.

2.5.3 Morphological analysis

The morphological data was split into three different analyses: Group 1, Group 2, and Group 3. As covered in 2.3.6, the groups correspond to the number of included taxa and characters used. Maximum Likelihood and Bayesian analyses are presented for each group. On each of the morphological trees the genus type species is denoted with a * after the species name. Additionally, various symbols mark selected nodes on the trees to aid discussion.

Group 1 morphological results

The Group 1 parsimony analysis resulted a large number (720) of equally parsimonious trees. Although almost every node is collapsed in the bootstrap resampling, the 50% majority rule consensus tree does show support for some clades within the equally parsimonious trees in Group 1 (MP, Figure 2.17). The outgroup genera: *Nephila*, *Araneus*, *Aranoethra* and *Micrathena* are consistently placed outside the Gasteracanthinae in both the ML and BI analyses (Figures 2.17 and 2.18).

The relationships between these taxa vary slightly depending on the analysis method but they always remain separate from the ingroup; the Gasteracanthinae. However, due to incomplete taxon sampling, the sister subfamily to Gasteracanthinae is not identified. Here the results confirm that *Micrathena* and *Gasteracantha*, though sharing some morphological characteristics, are separate genera as also identified in the molecular analyses. The MP analysis identifies three clades within the Gasteracanthinae: an '*Isoxya*' clade, a '*Gasteracantha* 1' clade, and a '*Gasteracantha* 2' clade, the last two including genera and species also identified as belonging to these clades in the molecular analyses, but with *Acrosomoides* and *Thelacantha* clades placed within '*Gasteracantha* 2'. The BI analysis identifies '*Gasteracantha* 1', *Isoxya, Thelacantha,* and *Acrosomoides* clades. Unlike the MP, the relationships of the majority of the *Gasteracantha* species are not resolved in the BI analysis.

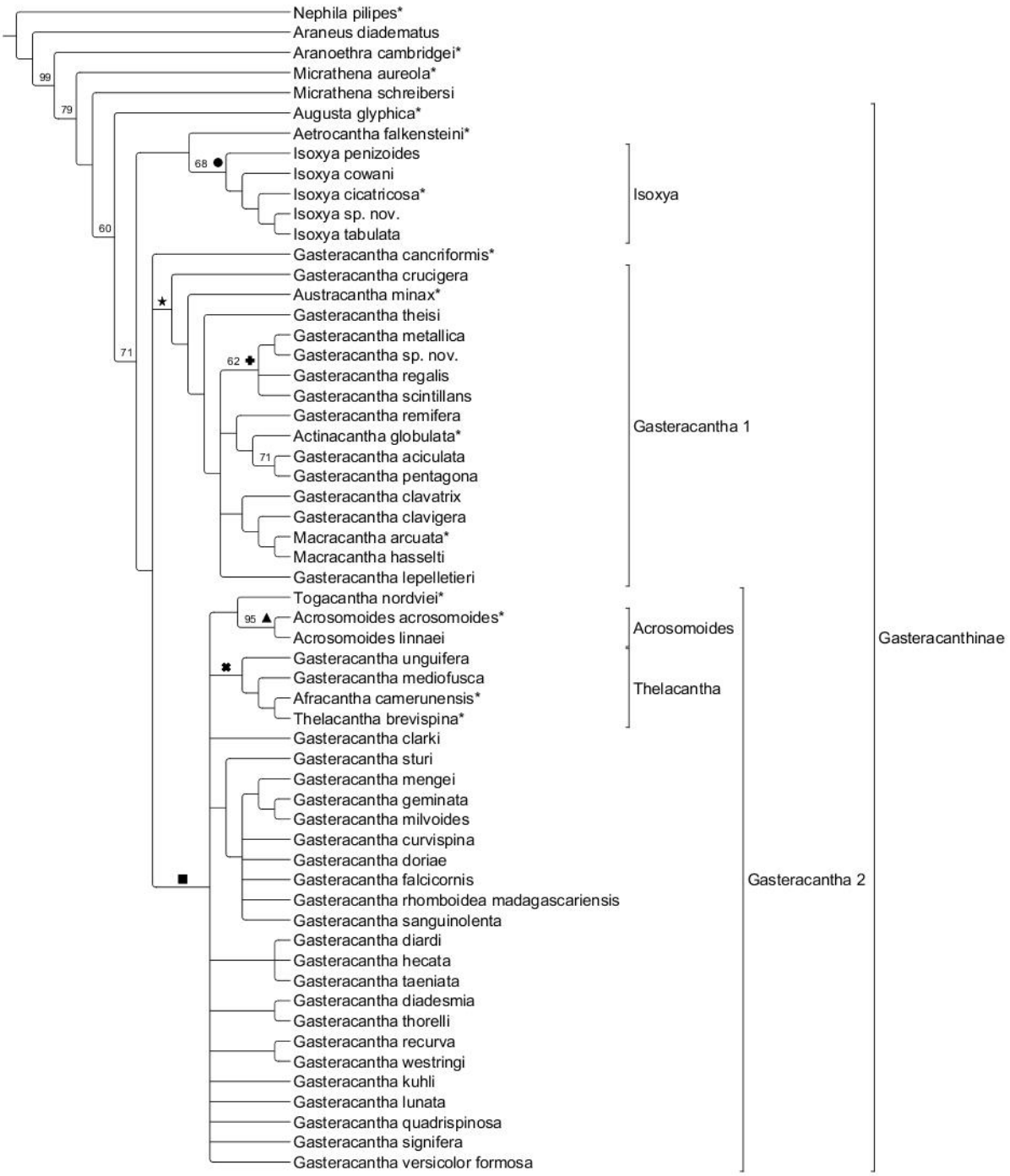
Nephila pilipes*
Araneus diadematus
Aranoethra cambridgei*
Micrathena aureola*
Micrathena schreibersi
Augusta glyphica*
Aetrocantha falkensteini*
Isoxya penizoides
Isoxya cowani
Isoxya cicatricosa*
Isoxya sp. nov.
Isoxya tabulata
Gasteracantha cancriformis*
Gasteracantha crucigera
Austracantha minax*
Gasteracantha theisi
Gasteracantha metallica
Gasteracantha sp. nov.
Gasteracantha regalis
Gasteracantha scintillans
Gasteracantha remifera
Actinacantha globulata*
Gasteracantha aciculata
Gasteracantha pentagona
Gasteracantha clavatrix
Gasteracantha clavigera
Macracantha arcuata*
Macracantha hasselti
Gasteracantha lepelletieri
Togacantha nordviei*
Acrosomoides acrosomoides*
Acrosomoides linnaei
Gasteracantha unguifera
Gasteracantha mediofusca
Afracantha camerunensis*
Thelacantha brevispina*
Gasteracantha clarki
Gasteracantha sturi
Gasteracantha mengei
Gasteracantha geminata
Gasteracantha milvoides
Gasteracantha curvispina
Gasteracantha doriae
Gasteracantha falcicornis
Gasteracantha rhomboidea madagascariensis
Gasteracantha sanguinolenta
Gasteracantha diardi
Gasteracantha hecata
Gasteracantha taeniata
Gasteracantha diadesmia
Gasteracantha thorelli
Gasteracantha recurva
Gasteracantha westringi
Gasteracantha kuhli
Gasteracantha lunata
Gasteracantha quadrispinosa
Gasteracantha signifera
Gasteracantha versicolor formosa
99
79
60
68
71
62
71
95
Isoxya
Gasteracantha 1
Acrosomoides
Thelacantha
Gasteracantha 2
Gasteracanthinae

Figure 2.17 50% majority rule consensus of 720 equally parsimonious trees (length = 273, *CI* = 0.282, *RI* = 0.662), inferred from maximum parsimony analysis of 66 morphological characters, generated in PAUP*. Bootstrap value (BS) following 1,000 replications, displayed on relevant nodes at 60% cut-off. Star symbol = node supported by character state changes in characters 18, 32, 34, 55 & 57; square symbol = node supported by character state changes in characters 9, 29 & 48; circle symbol = node supported by character state changes in characters 29, 32, 39, 42, 43, 47, 56, 63 & 64; plus symbol = node supported by character state changes in characters 7 & 26; triangle symbol = node supported by character state changes in characters 11, 26, 32, 37, 42, 43, 44, 54, 59 & 61; cross symbol = node supported by character state changes in characters 14, 15, 55 & 57.

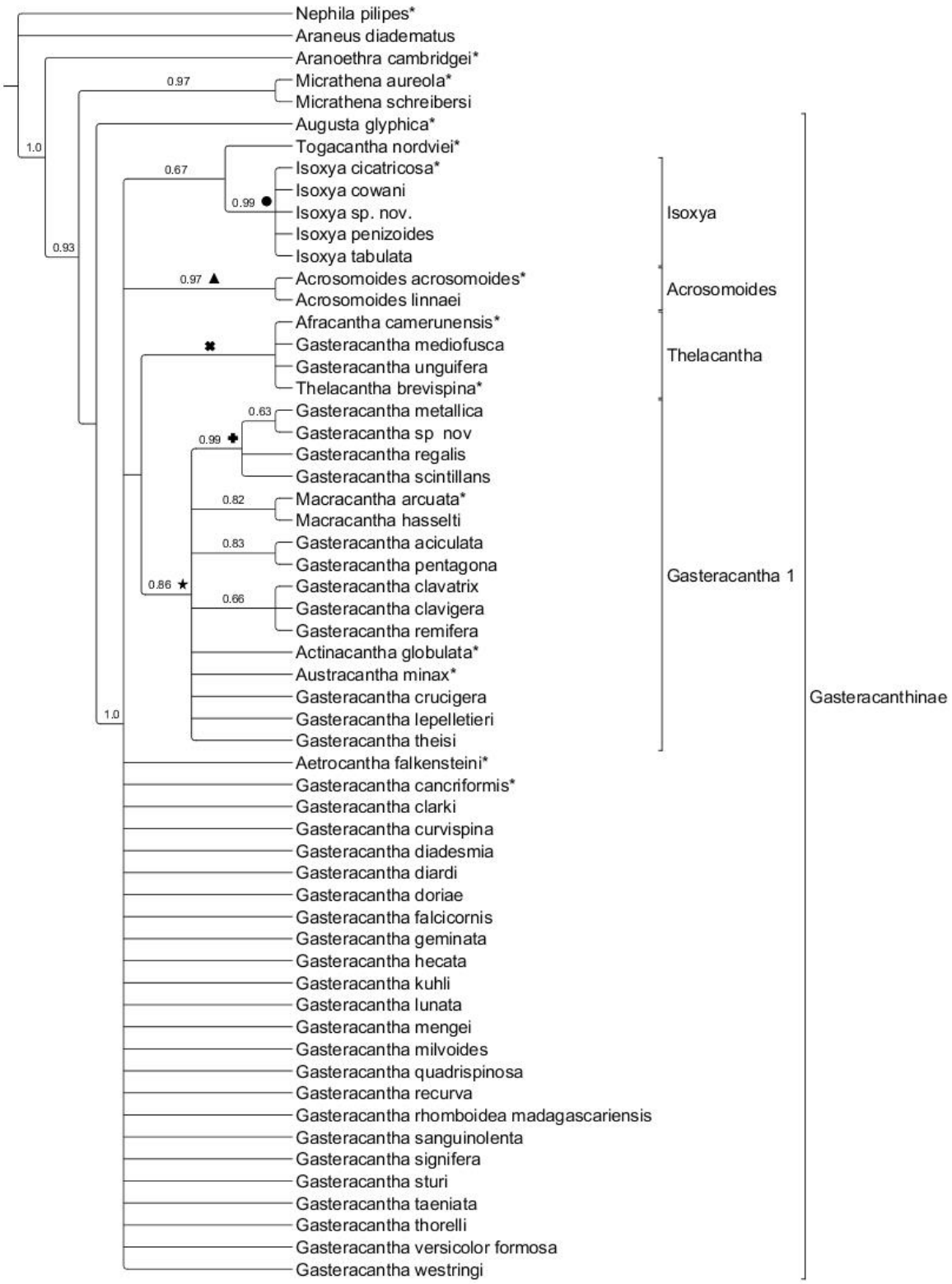
Nephila pilipes*
Araneus diadematus
Aranoethra cambridgei*
Micrathena aureola*
Micrathena schreibersi
Augusta glyphica*
Togacantha nordviei*
Isoxya cicatricosa*
Isoxya cowani
Isoxya sp. nov.
Isoxya penizoides
Isoxya tabulata
Acrosomoides acrosomoides*
Acrosomoides linnaei
Afracantha camerunensis*
Gasteracantha mediofusca
Gasteracantha unguifera
Thelacantha brevispina*
Gasteracantha metallica
Gasteracantha sp nov
Gasteracantha regalis
Gasteracantha scintillans
Macracantha arcuata*
Macracantha hasselti
Gasteracantha aciculata
Gasteracantha pentagona
Gasteracantha clavatrix
Gasteracantha clavigera
Gasteracantha remifera
Actinacantha globulata*
Austracantha minax*
Gasteracantha crucigera
Gasteracantha lepelletieri
Gasteracantha theisi
Aetrocantha falkensteini*
Gasteracantha cancriformis*
Gasteracantha clarki
Gasteracantha curvispina
Gasteracantha diadesmia
Gasteracantha diardi
Gasteracantha doriae
Gasteracantha falcicornis
Gasteracantha geminata
Gasteracantha hecata
Gasteracantha kuhli
Gasteracantha lunata
Gasteracantha mengei
Gasteracantha milvoides
Gasteracantha quadrispinosa
Gasteracantha recurva
Gasteracantha rhomboidea madagascariensis
Gasteracantha sanguinolenta
Gasteracantha signifera
Gasteracantha sturi
Gasteracantha taeniata
Gasteracantha thorelli
Gasteracantha versicolor formosa
Gasteracantha westringi
0.97
1.0
0.67
0.99
0.93
0.97
0.63
0.99
0.82
0.83
0.86
0.66
1.0
Isoxya
Acrosomoides
Thelacantha
Gasteracantha 1
Gasteracanthinae

Figure 2.18 50% majority rule consensus of 3714 trees, inferred from Bayesian inference (BI) analysis of 66 morphological characters, generated in MrBayes. Posterior probability values displayed on relevant nodes at 0.6 (60%) cut-off. Star symbol = node supported by character state changes in characters 18, 32, 34, 55 & 57; circle symbol = node supported by character state changes in characters 29, 32, 39, 42, 43, 47, 56, 63 & 64; plus symbol = node supported by character state changes in characters 7 & 26; triangle symbol = node supported by character state changes in characters 11, 26, 32, 37, 42, 43, 44, 54, 59 & 61; cross symbol = node supported by character state changes in characters 14, 15, 55 & 57.

In both analyses a Gasteracanthinae clade is present, with *Augusta glyphica* occupying the basal position within the clade (as sister to the other gasteracanthines), although with low BS or no BI statistical support. '*Isoxya*' forms a supported monophyletic clade in each of the analyses, with an unsupported sister relationship to *Aetrocantha* (in the MP analysis) and *Togacantha* (in the BI analysis). Deeper into the subfamily a large clade is formed that contains *Gasteracantha*, *Macracantha*, *Actinacantha*, *Thelacantha*, *Austracantha* and, depending on the analysis method, either *Togacantha* or *Aetrocantha*. This clade contains many resolved and unresolved species relationships.

In the Group 1 MP analysis (Figure 2.17) the large *Gasteracantha* clade is divided into two smaller clades '*Gasteracantha* 1' and '*Gasteracantha* 2' (as seen in the molecular analyses), along with *Gasteracantha cancriformis* together forming a polytomy. The '*Gasteracantha* 1' clade, is unsupported in the BS, but some unsupported resolution is seen in the majority rule tree and contains many species relationships identified in the molecular analyses. The exception is the inclusion of *Austracantha* inside the '*Gasteracantha* 1' clade, where state changes related to the third pair of spines (character 18), the absence of a ventral tubercle (character 32), and the spinneret sclerotised ring structure (character 34) and characters relating to the embolus (characters 55 and 57), denoted by ★, place this species with the '*Gasteracantha* 1' though this contradicts the molecular analysis and is unsupported in the BS replications.

The deeper nodes in '*Gasteracantha* 1' are generated by character synapomorphies such as those relating to median abdominal spines (character 17), the elongated spinneret cone (character 38), the presence of the terminal apophysis on the male palp (character 57) and shape of the median apophysis (character 66). This was confirmed by checking the character state change output in PAUP* (a list that displays the nodes where character states change). This large amount of data is not presented here. Although the clades are mostly unsupported, the character of the terminal apophysis (character 57) is a synapomorphy hypothesised to support '*Gasteracantha* 1'. This hypothesis can be tested by obtaining males of more species. Inside '*Gasteracantha* 1' a small clade is formed, with weak BS support, comprising the metallic coloured Solomon Island species, denoted by ✚. This clade is only supported by the species metallic abdominal colour (character 7) and state change in sternum colouration (character 26).

The other *Gasteracantha* clade, '*Gasteracantha* 2', is a mixture of unresolved species relationships and some small, mostly unsupported, clades. The '*Acrosomoides*' clade has one of the only supported nodes in the BS replications with support from character state changes for spines (character 11), sternum colour (character 26), the ventral tubercle (character 32), spinnerets (character 37) the epigyne (characters 42, 43 and 44), and male characters that differ from *Togacantha*, where known, (characters 54, 59 and 61), denoted by▲. Here *Togacantha*, a genus that has many character similarities with *Acrosomoides* and with *Isoxya*, is placed without support within '*Isoxya*' in the BI analysis and '*Acrosomoides*' in the MP analysis. '*Isoxya*' is supported in both analyses by state changes relating to ventral condyles (character 29), the lack of a ventral tubercle (character 32), the structure of the female genitalia (characters 39, 42, 43 and 47) and male palp (characters 56, 63 and 64), denoted by ●.

Group 2 morphological results

Group 2 comprises fewer taxa than Group 1 but with maximal character information. This was a deliberate choice to limit the amount of missing data in the hope that more resolution would be possible from the dataset. MP and BI trees are given in Figures 2.19 and 2.20. Greater resolution is also reflected in the lower number of equally parsimonious trees (172) in the MP analysis compared to the Group 1 analysis. Similar relationships and clades to those found in the Group 1 morphological analysis are also present.

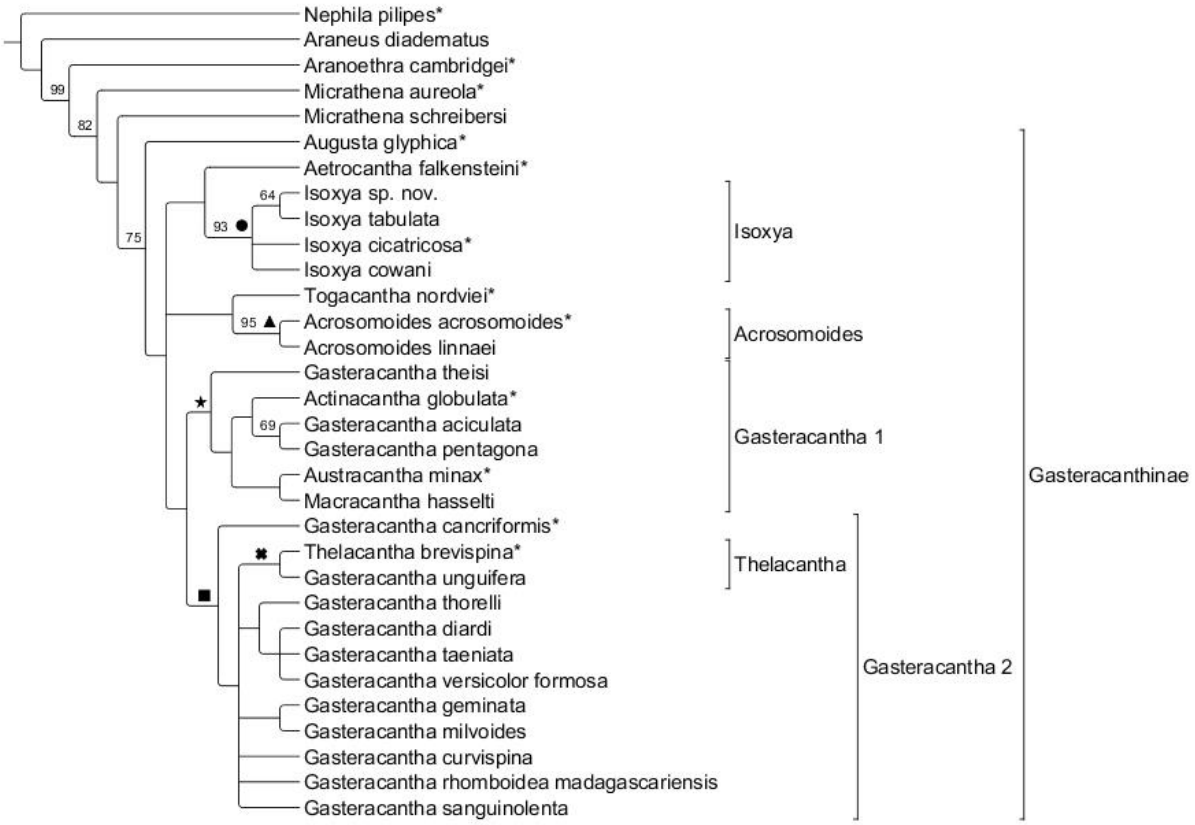

Figure 2.19 50% majority rule consensus of 172 equally parsimonious trees (length = 223, *CI* = 0.336, *RI* = 0.606), inferred from maximum parsimony analysis of 64 morphological characters, generated in PAUP*. Bootstrap value (BS) following 1,000 replications, displayed on relevant nodes at 60% cut-off. Star symbol = node supported by character state changes in characters 16, 19, 34, 38, 55 & 57; square symbol = node supported by character state changes in characters 32 & 49; circle symbol = node supported by character state changes in characters 9, 29, 32, 35, 39, 40, 41, 42, 43, 47, 51, 56 & 63; triangle symbol = node supported by character state changes in characters 11, 26, 32, 37, 42, 43, 44, 59, 61 & 63; cross symbol = node supported by character state changes in character 21.

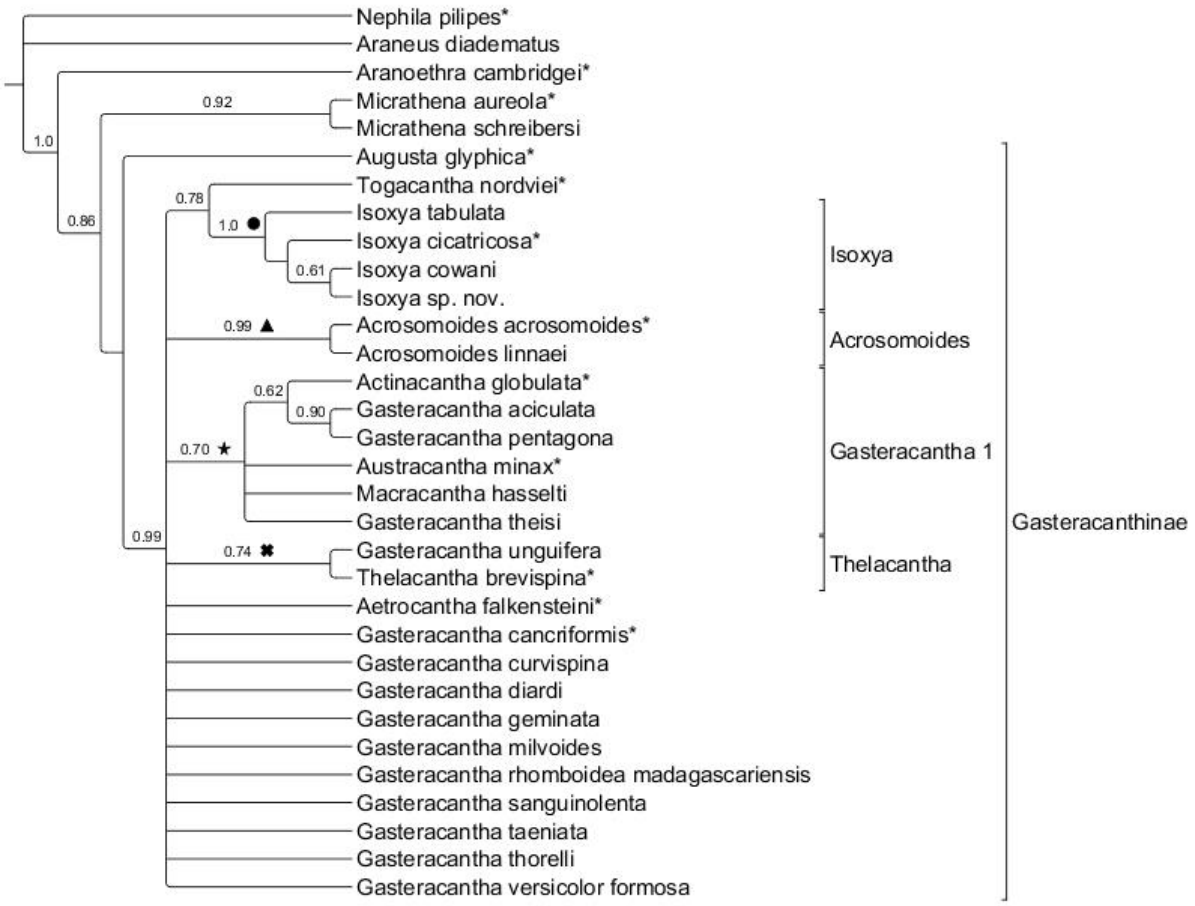

Figure 2.20 50% majority rule consensus of 1952 trees, inferred from Bayesian inference (BI) analysis of 64 morphological characters, generated in MrBayes. Posterior probability values displayed on relevant nodes at 0.6 (60%) cut-off. Star symbol = node supported by character state changes in characters 16, 19, 34, 38, 55 & 57; circle symbol = node supported by character state changes in characters 9, 29, 32, 35, 39, 40, 41, 42, 43, 47, 51, 56 & 63; triangle symbol = node supported by character state changes in characters 11, 26, 32, 37, 42, 43, 44, 59, 61 & 63; cross symbol = node supported by character state changes in character 21.

In the Group 2 analyses the Gasteracanthinae form a clade, with *Augusta* sister to the rest of the gasteracanthines. Within the Gasteracanthinae, the '*Isoxya*' and '*Acrosomoides*' clades are supported with *Togacantha* placed in either of these clades dependent on whether MP or BI analysis is undertaken. The remaining Gasteracanthinae form a '*Gasteracantha* 1' clade in both analyses. In the MP analysis there is a '*Gasteracantha* 2' clade, and a '*Thelacantha*' clade within this, although they are unsupported. *Gasteracantha cancriformis* is situated within '*Gasteracantha* 2' in the MP analysis, but not in the BI analysis. The remaining species in each clade are the same as in the Group 1 MP analysis, with less unresolved species relationships. In the BI analysis there are many unresolved relationships and '*Gasteracantha* 2' is not present.

As with the Group 1 BI analysis, there are still large numbers of unresolved relationships in the same '*Gasteracantha* 2' clade, but a supported '*Thelacantha*' clade does appear.

The large Gasteracanthinae clade has support in both analyses, supporting the subfamily's taxonomic validity. In the BI analysis the resolution across the whole tree is lacking in the same taxa as in the Group 1 analysis, despite full male data being included. These species appear morphologically very similar with only a few characters that differentiate them in the cladistic analyses.

Group 3 morphological results

Group 3 contains all the taxa from Group 2 with the addition of taxa that were included in molecular analyses and the few taxa that had some, but not all, male data scored. Once again in both MP (Figure 2.21) and BI analyses (Figure 2.22), the Gasteracanthinae form a clade and then, within the subfamily, divide into the same species clades found in the analysis of the Group 1 and 2. '*Isoxya*' stays as one well supported clade, '*Acrosomoides*' is strongly supported in the BS replications and BI values. The '*Gasteracantha* 1' clade is present again and '*Gasteracantha* 2' has some resolution in the MP analysis. It is unresolved in the BI, bar the '*Thelacantha*' clade, as in the analyses of Group 1 and Group 2.

Figure 2.21 50% majority rule consensus tree of 774 equally parsimonious trees (length = 238, *CI* = 0.319, *RI* = 0.625), inferred from maximum parsimony analysis of 64 morphological characters, generated in PAUP*. Bootstrap value (BS) following 1,000 replications, displayed on relevant nodes at 60% cut-off. Star symbol = node supported by character state changes in characters 18, 19, 32, 34, 38, 55 & 57; square symbol = node supported by character state changes in characters 32 & 49; circle symbol = node supported by character state changes in characters 29, 32, 35, 39, 41, 42, 43, 47, 56, 63 & 64; triangle symbol = node supported by character state changes in characters 11, 26, 32, 37, 42, 43, 44, 59, 61 and 63; cross symbol = node supported by character state changes in characters 14, 15, 26, 55, 57 & 65.

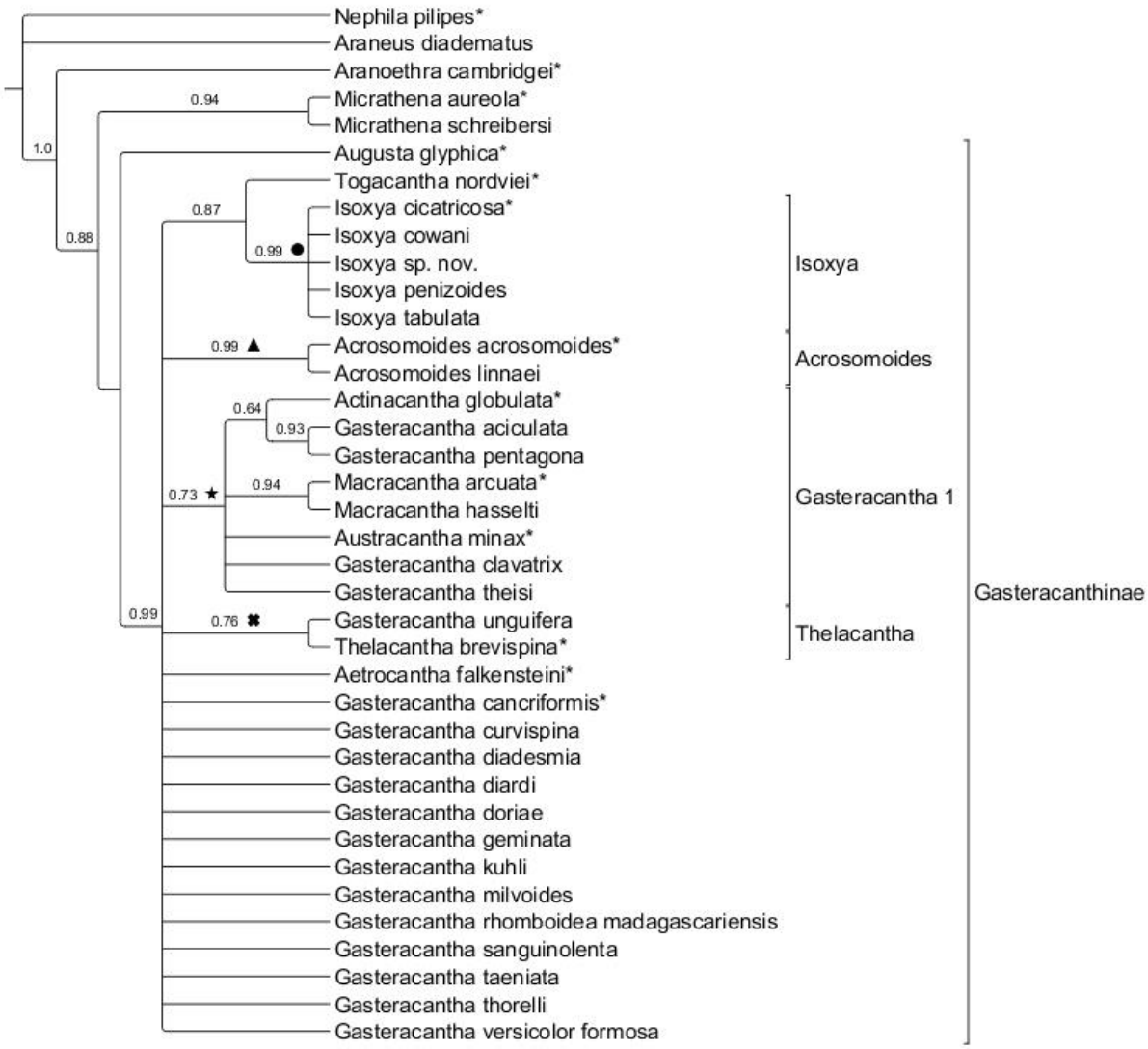

Figure 2.22 50% majority rule consensus tree of 2350 trees, inferred from Bayesian inference (BI) analysis of 64 morphological characters, generated in MrBayes. Posterior probability values displayed on relevant nodes at 0.6 (60%) cut-off. Star symbol = node supported by character state changes in characters 18, 19, 32, 34, 38, 55 & 57; circle symbol = node supported by character state changes in characters 29, 32, 35, 39, 41, 42, 43, 47, 56, 63 & 64; triangle symbol = node supported by character state changes in characters 11, 26, 32, 37, 42, 43, 44, 59, 61 and 63; cross symbol = node supported by character state changes in characters 14, 15, 26, 55, 57 & 65.

2.5.4 Combined molecular and morphological analysis

The molecular data (CO1+16S) and morphological data are combined in a BI analysis (Figure 2.23). Whilst the number of species included is limited, the combined molecular and morphological tree displays similar results to the combined molecular (CO1+16S) BI analysis tree (Figure 2.15). The only difference is the location of *Augusta*, which is located outside of the subfamily in the combined morphological and molecular analysis. The results from a MP analysis conducted in PAUP* (not shown) also displayed this. The combined morphological and molecular analysis did not provide any more information than the combined (CO1+16S) molecular analysis, as most characters were molecular data, and the signal of the morphological data did not significantly alter the results.

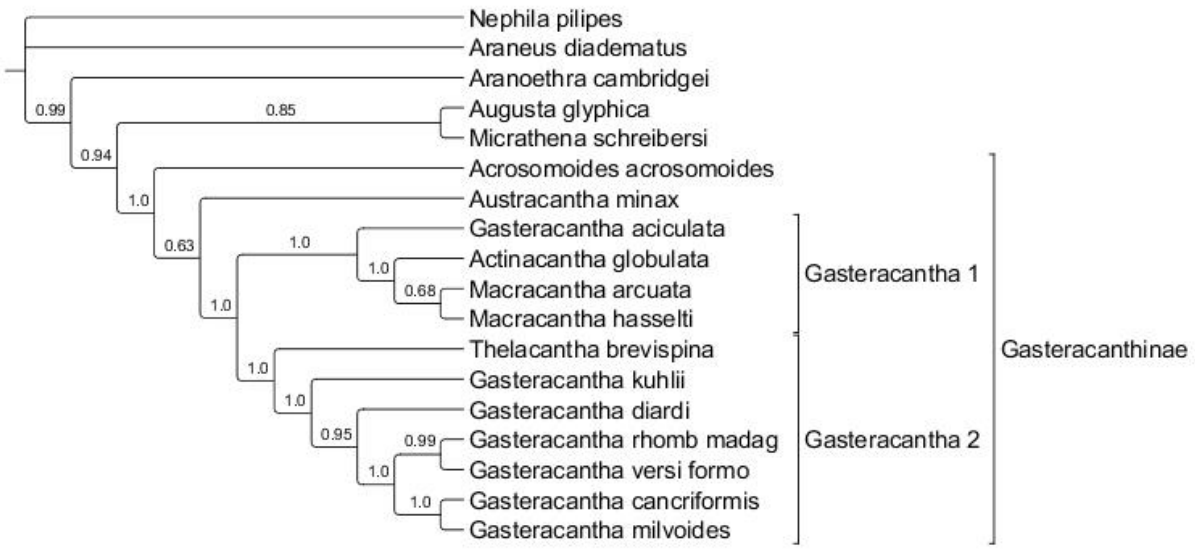

Figure 2.23 Combined analysis (morphological and molecular data) 50% majority rule consensus tree inferred from Bayesian inference (BI) analysis, generated in MrBayes. Posterior probability values displayed on relevant nodes at 0.6 (60%) cut-off.

Differences between the identification of clades in each of the molecular, morphological, and combined molecular and morphological analyses are summarised in Table 2.4.

Table 2.4 Clades present in each analysis

Clade Present	CO1 MP	CO1 BI	16S MP	16S BI	Com. MP	Com. BI	G1 MP	G1 BI	G2 MP	G2 BI	G3 MP	G3 BI	MM BI
Gasteracanthinae	X	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X
Isoxya	✓	✓	-	-	-	-	✓	✓	✓	✓	✓	✓	-
Acrosomoides	-	-	-	-	-	-	✓	✓	✓	✓	✓	✓	-
'*Gasteracantha* 1'	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Macracantha	✓	✓	✓	✓	X	✓	✓	✓	-	-	✓	✓	✓
Solomon Island *Gaster.*	-	-	-	-	-	-	✓	✓	-	-	-	-	-
'*Gasteracantha* 2'	✓	✓	✓	✓	✓	✓	✓	X	✓	X	✓	X	✓
***Thelacantha* + *Gasteracantha* spp.**	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

✓ = the clade is present; X = the clade is absent; - = that there is either none or only one species from that genus or group included in the analysis, so the clade is absent.

2.6 Discussion of all results

A comprehensive analysis of the Gasteracanthinae has been performed using both molecular and morphological data. The mitochondrial CO1 analyses contained the most species within any of the molecular analyses and showed strong resolution at the species level, as would be expected with this fast-evolving gene. Resolution at the species level was slightly less in analyses using the slower evolving 16S gene. However, all molecular analyses provided reliable and almost consistent discrimination of the subfamily.

Original molecular data was added to with sequences from GenBank and BOLD. GenBank is the largest repository of sequence data, with most sequences being accurately attributed to the correct species (Leray *et al*., 2019) and, if researchers are aware of potential issues, there is no reason why GenBank or BOLD data should not be used to supplement molecular datasets. The morphological analyses also provided additional evidence to support the monophyly of Gasteracanthinae. The *consistency* and *retention* indices of each character were produced for each Group. Tables in Appendix 3.2.4 show the average *ci* and *ri* scores of each character from the parsimony analyses. In these analyses the low values of average *ci* showed that there were many characters that have high levels of homoplasy. However, as the same characters often displayed a high average *ri,* they are evidently synapomorphies for clades.

In every analysis (molecular and morphological) members of the genera *Micrathena*, *Aranoethera* and *Araneus* are consistently placed outside of the Gasteracanthinae. This confirms the previous early separation of these genera by Simon (1864), Butler (1873) and Dahl (1914), which were primarily based on sometimes limited morphological evidence.

Following the work of Scharff and Coddington (1997), with later additions to the World Spider Catalog (2022), the Gasteracanthinae is described as including the following genera: *Acrosomoides*, *Actinacantha*, *Aetrocantha*, *Afracantha*, *Augusta*, *Austracantha*, *Gasteracantha*, *Isoxya*, *Macracantha*, *Thelacantha* and *Togacantha* (included in the analyses here) in addition: *Aspidolasius, Encyosaccus, Gastroxya*, *Hypsacantha*, and *Madacantha* (not included in the analyses here). Many of these genera had their taxonomic generic status based upon limited morphological characters (for example, *Actinacantha* possesses a globular base to the medial spines). There are currently no robust biological or ecological differences known between these genera, and their life-histories have not been recorded yet either. For most genera, even in *Gasteracantha* species (bar those studied by Emerit in the 1960-70s), very little is known past gross morphology and basic field data. The results of these analyses provide insight into the relationships between species and correct generic groupings that they belong to.

The predominant result within almost all the analyses is the support of the hypothesis (Chapter 1.5) that Gasteracanthinae is monophyletic. This is supported by the 16S and combined molecular (CO1+16S) analyses and all the morphological analyses. The only exceptions to this are found in the CO1 and combined molecular and morphological analyses, where the subfamily is not monophyletic, due to the placement of *Augusta glyphica*. The combined CO1 + 16S and all the morphological and combined morphology and molecular analyses place *Augusta* in the base of the Gasteracanthinae clade as sister to the remaining gasteracanthines. The long branch leading to *Augusta* in the phylogram (Figure 2.16) implies it diverged from the common gasteracanthine ancestor a long time ago, assuming a constant rate of mutation in the CO1 and 16S genes. If additional taxa are included with a closer relationship to *Augusta* the length of the branch may become shorter. For example, *Parmatergus* (included in Scharff *et al.*, 2020) and *Gastroxya* (see Chapter 5). The 16S analysis places the genus deeper within the subfamily clade.

Based upon the morphological and molecular analyses and the placement of *Augusta* in the Gasteracanthinae by Scharff *et al*. (2020), Gasteracanthinae should still be considered monophyletic. This is despite *Augusta* having a unique overall habitus compared to other gasteracanthines (Figure 2.23). There is insufficient evidence from this study to suggest *Augusta* should be excluded from Gasteracanthinae. However, additional data from more taxa and genetic markers should clarify the position. With additional data the taxonomic placement of species can be reassessed, for example *Aranoethra* was moved from Gasteracanthinae to Cyrtarachninae (Simon, 1885; Scharff *et al*., 2020). Based upon the results here, the hypothesis that *Augusta* was misplaced in the subfamily is rejected (Chapter 1.5). Therefore, there is no justification for a taxonomic reclassification of *Augusta*. In addition, this supports the hypothesis that Gasteracanthinae, as currently defined in the World Spider Catalog (2022), is monophyletic.

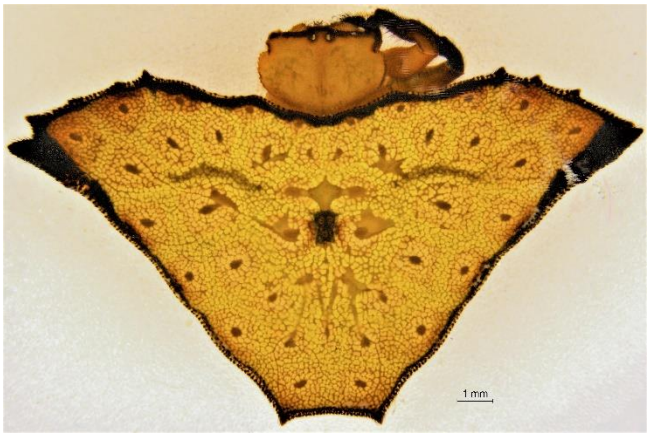

Figure 2.23 Photograph of *Augusta glyphica* (♀) dorsal view, showing unique inverted triangle abdominal shape.

The genera *Acrosomoides*, *Austracantha* and *Isoxya* are morphologically distinct from *Gasteracantha* based on various female abdominal characters (11, 26, 29, 34, 35 and 37) and male and female genitalia characters (39, 40, 42, 43, 44, 47, 48, 49, 54, 56, 59, 63 and 64). This distinction is supported by the molecular results. These three genera are situated within the Gasteracanthinae clade, and do not warrant any taxonomic changes based upon the findings presented here. The morphological analyses suggested various unsupported relationships, based upon some morphological characters that are shared with other genera (for example characters 14, 18, 19, 32, 34, 38). Future work could explore the taxonomic status of *Austracantha* in relation to *Isoxya*, a genus in which it has been placed previously. The monophyly of *Acrosomoides* was supported in all morphological analyses. The monophyly of *Isoxya* was supported in all morphological analyses and the CO1 analysis.

Gasteracantha, as currently understood, is paraphyletic by the inclusion of *Actinacantha*, *Macracantha* and *Thelacantha*. This supports the hypothesis from Chapter 1.5. The genera *Actinacantha*, *Gasteracantha*, *Macracantha* and *Thelacantha* clearly split into two separate clades, '*Gasteracantha* 1' and '*Gasteracantha* 2' in the molecular analyses. '*Gasteracantha* 1' comprises *Actinacantha*, *Macracantha* and *Gasteracantha* in part. '*Gasteracantha* 2' comprises *Thelacantha* and the remaining species of *Gasteracantha*.

'*Gasteracantha* 1' is also supported in the morphological analyses, but support is lacking for '*Gasteracantha* 2'. The clade '*Gasteracantha* 2' is equivalent to the taxonomic group *Gasteracantha* sensu stricto. Some support is given by morphological characters such as the female ventral tubercle (character 32) and the male eye location (character 49) for '*Gasteracantha* 2', denoted by ■ in the morphological trees. This clade has much stronger support in the posterior probability and bootstrap values in the molecular analyses.

The genus *Thelacantha* is placed either within '*Gasteracantha* 2' or with an unresolved relationship (for example Figure 2.21), but never in '*Gasteracantha* 1'. *Thelcacantha* has never been recovered as the sister genus to *Gasteracantha*. Therefore, the hypothesis that it *Thelacantha* is the sister genus to *Gasteracantha,* suggested by the results of Scharff *et al*. (2020), is not supported. Previous studies on the subfamily, by Tan *et al.* (2019), Scharff *et al*. (2020) and Macharoenboon, Siriwut and Jeratthitikul (2021), all focused on smaller numbers of non-African taxa. When additional African taxa are added to analyses, as in this study, it becomes evident that *Thelacantha* is synonomous with *Gasteracantha*.

Thelecantha is visually different to the majority of '*Gasteracantha* 2' species, with a protrusion on the top of the head, hexagonal abdomen and bulbous spine bases (characters 1, 14, 15). Morphologically *Thelacantha* is very similar to *Afracantha cameruensis*, *Gasteracantha mediofusca* (Doleschall, 1859) and *Gasteracantha unguifera* Simon, 1889 (for example characters 14, 15, 55 and 57) (section 2.4.2, Group 1 morphological analysis, denoted by ✖). It is also a common misidentification to find colour morphs of *G. mediofusca* identified as *T. brevispina* (Williams, pers. obs.) in museum collections. There is strong support for the '*Gasteracantha* 2' clade in the molecular analyses, and *Thelacantha's* relationship within it. These results justify a reclassification of *Thelacantha* back to *Gasteracantha*.

The molecular phylogenies offer strong support for the clade '*Gasteracantha* 1', and this is partially supported by the morphological data (MP analyses only). Morphological synapomorphies for the '*Gasteracantha* 1' clade are the inconspicuous embolus (character 55), terminal apophysis on the palp (character 57), vertical median abdominal spines (character 19), lack of a ventral tubercle and an extremely elongated spinneret tubercle (character 38), denoted by ★ in the morphological trees. As can be seen in Appendix 3.2.4, the abdominal spines characters (8-19) often exhibited low average *ci* values (between 0.091 – 0.500), indicating a high level of homoplasy across the tree. In particular, the characters relating to spine length (16-18) had extremely low average *ci* values (0.091-0.143). However, these spine characters exhibited higher average *ri* values (often 0.600 or higher). For example, character 19 (the horizontal or vertical median spines) in the Group 1 analysis had a *ci* = 0.200, as the state changed several times, but the *ri* = 0.789 as the character in part supported the clade '*Gasteracantha* 1' and *Isoxya* species relationships.

A hypothesis from Chapter 1.5 was that *Macracantha* is synonymous with *Gasteracantha,* supporting the suggestion made by Tan *et al*., (2019) that *Macracantha arcuata* could be synonymous with *Gasteracantha*. In all analyses *Macracantha* is located within '*Gastercantha* 1' along with *Actinacantha*, and various *Gasteracantha*. This supports the hypothesis that *Macracantha* is synonymous with *Gasteracantha*. This also contradicts the results of Macharoenboon, Siriwut and Jeratthitikul (2021) who assigned *Gasteracantha hasselti* to *Macracantha* based on limited taxon sampling. Although the two *Macracantha* species, *Actinacantha globulata* and *Gasteracantha aciculata* included in the molecular analyses are currently classified in different genera they have biological similarities. They possess triangular abdomens that taper to the spinnerets on the venter, lack the ventral tubercle and have similar abdominal markings and abdominal spine structure.

With all the information presented here across the molecular, morphological, and combined analyses the following suggestion is made. Additional taxa from the Group 1 morphological analysis that were inferred as being in the '*Gasteracantha* 1' clade (for example: *Gasteracantha clavatrix*, *G. clavigera* Giebel, 1863, *G. remifera* Butler, 1873 and the metallic species from the Solomon Islands: *G. metallica*, *G. regalis*, *G. scintillans* and *G. sp. nov.* – see Figure 2.17, denoted by ✚) are located and sequenced for molecular analysis. Based upon the morphological findings it is hypothesised that these taxa would remain in '*Gasteracantha* 1'. When more taxa and molecular data has been examined a full reclassification should be considered (see Chapter 5).

2.7 Conclusions

In conclusion, the work presented here has tested the hypotheses set out in Chapter 1.5. The results support a monophyletic Gasteracanthinae, although the placement of *Augusta* is still uncertain. *Gasteracantha*, as currently known, has been supported as paraphyletic based upon the relationships between current genera and two main clades have been identified: '*Gasteracantha* 1' and '*Gasteracantha* 2'. The hypothesis that *Thelacantha* is sister to *Gasteracantha* has been rejected, rather *Thelacantha* is synonymous with *Gasteracantha*. *Macracantha* is also supported as synonymous with *Gasteracantha*. Although there is not full resolution in the phylogenies, there is consistent signal that supports future taxonomic changes. Furthermore, these phylogenies can now be used to test other hypotheses (Chapters 3 and 4).

Chapter 3: Geometric morphometrics

Once a phylogenetic analysis has been completed the resulting phylogenetic trees can be used to investigate the evolution of character traits. This and the following chapter use the inferred molecular phylogenies (Figures 2.11 and 2.15) from Chapter 2 to examine various morphological characters. Here, a morphometric analysis of abdominal shape is conducted using the Gasteracanthinae taxa that featured in the molecular CO1 and combined (CO1+16S) analyses in Chapter 2. This analysis was performed to test the hypothesis from Chapter 1.5 that certain gasteracanthine clades have evolved particular abdominal shapes in response to selection pressures (currently unknown). The null hypothesis would be that these particular shapes would be randomly distributed within the tree. The results are presented and discussed in an evolutionary context.

3.1 Introduction to morphometrics

Geometric morphometric shape analysis is a method of quantitative analysis that examines shapes independent of size. Conventionally, analyses work by plotting fixed points, called landmarks, on the items being analysed then the distances and variation between these shapes are quantified. Analyses are often performed on fossils (Gray, *et al*. 2017; Dehon, *et al*. 2019) as well as skulls of animals like fish (Breno, Leirs and Van Dongen 2011; Stange, *et al*. 2018) or bird beaks (Bright, *et al*. 2016; Dalton, *et al*. 2017), and butterfly wing shape (Breuker *et al*., 2010). Morphometrics is an excellent way of quantifying variation and changes in shapes in a 2D plane and sometimes the 3D plane.

A traditional method of morphometric analysis is to use 2D photographic images of the organism of study that can then have the fixed landmark points plotted onto them (Sontigun

et al, 2017; Csősz *et al*., 2020; Wikantyoso *et al*., 2021). This approach is used in this analysis (see 3.2). This method is also easily repeated and additional data can be added to original datasets with minimal effort in the future.

3.1.1 Morphometrics in *Gasteracantha*

Emerit (1974) conducted a very brief 2D geometric morphometric analysis of several species of the Gasteracanthinae from Madagascar. He used the abdominal sigilla as the fixed points of measurement in a landmark-based morphometric analysis to map differences of shape between species (for example Figure 3.1) and illustrated them along with species descriptions and scientific drawings to aid species identification. Emerit noted difficulties on measuring absolute distances and how the biological development of the spiders measured had to be taken into consideration to avoid errors. Sub-adult gasteracanthines do not always possess clear sigilla, therefore adult specimens were chosen for the abdominal shape analysis here.

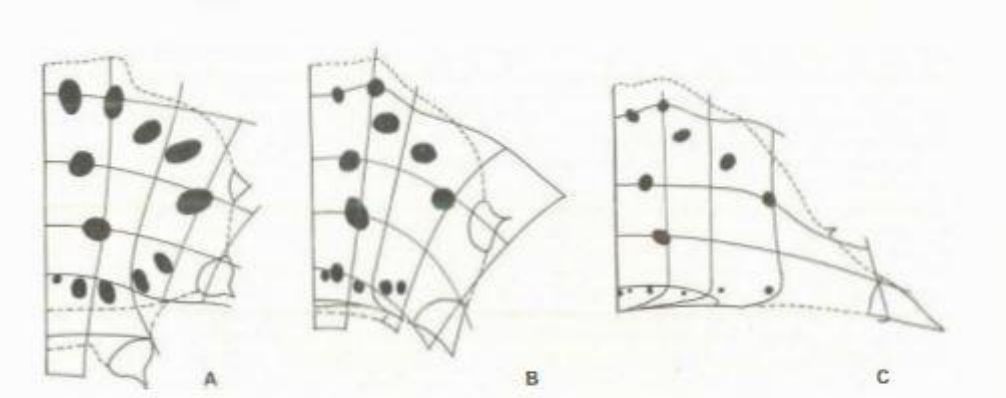

Fig. 26, réseaux structuraux de *Gasteracanthina* :
A, *Thelacantha brevispina*. — B, *Madacantha nossibeana*. — C, *Acrosomoides acrosomoides*
(Réseau figuré en traits pleins, contour de l'opisthosoma en tirets, sigilles en noir ; échelles différentes pour A, B, C).

Figure 3.1 From Emerit (1974) – basic morphometrics showing the differing abdominal shapes in three genera.

The Gasteracanthinae species studied in this thesis possess, even to a non-expert, four approximate abdominal shapes. These shapes are: rectangular, triangular, hexagonal, and inverted triangular. However, it is not possible to incorporate these shapes in a morphological cladistic analysis or map their evolution on a phylogenetic tree, as these shapes have not been quantified. Furthermore, there is variation within each shape. Not every taxon can be easily placed into one of these basic shapes, for example *Gasteracantha kuhli* could have been placed in hexagonal or rectangular prior to analysis. By using geometric morphometrics these shapes can be quantified into data that can be mapped onto a completed phylogeny. This can then highlight if there is a correlation between species relationships and the abdominal shape and test the theories of the early *Gasteracantha* publications (Simon, 1864; Butler, 1873; Dahl, 1914, see Chapter 1.2) that split and suggested closer species relationships based on abdomen shape. The hypothesis, from Chapter 1.5, was that when the abdominal shape of each species was mapped onto the final phylogeny there would be close similarities of abdominal shape between species that are closely related. This chapter examines how the abdominal shape, quantified with geometric morphometric methods maps onto the molecular Gasteracanthinae phylogenies.

3.2 Materials and methodology

This section covers the materials used in the morphometric analysis, along with the methods of preparing the taxa for analysis and methods of data analysis.

3.2.1 Included taxa

Twenty-one Gasteracanthinae species from the molecular analyses (Figures 2.11 and 2.15) that also had physical specimens available were imaged for the morphometric analysis (see Table 3.1). The single outgroup taxon *Aranoethra cambridgei* (see Table 3.1 for phylogenetic taxa grouping information) was also imaged as this species possesses the clear abdominal sigilla that were used as landmark points but is from a different subfamily. *A. cambridgei* displays more sigilla conspicuously than the gasteracanthines in this study. However, by focusing on the homologous sigilla and limiting the number of sigilla used to those found on the Gasteracanthinae species, *A. cambridgei* could be used as the outgroup without overwhelming the data with more sigilla than the ingroups. This trial used one specimen per species but exploration into species variation should certainly use this method with many more examples per species.

Table 3.1 Morphometric analysis data and results

Species name	Group (based upon phylogenetic results)	Abdominal Shape (post-analysis)	No. specimens measured
Acrosomoides acrosomoides	Gasteracanthinae	Triangle	1
Actinacantha globulata	*Gasteracantha* 1	Triangle	1
Aranoethra cambridgei	Outgroup	Rectangle	1
Augusta glyphica	Gasteracanthinae	Inverted Triangle	1
Austracantha minax	Gasteracanthinae	Hexagon	1
Gasteracantha aciculata	*Gasteracantha* 1	Hexagon	1
G. cancriformis	*Gasteracantha* 2	Rectangle	1
G. clavatrix	*Gasteracantha* 1	Triangle	1
G. diadesmia	*Gasteracantha* 2	Rectangle	1
G. diardi	*Gasteracantha* 2	Rectangle	1
G. doriae	*Gasteracantha* 2	Rectangle	1
G. kuhli	*Gasteracantha* 2	Hexagon	1
G. milvoides	*Gasteracantha* 2	Rectangle	1
G. rhomboidea madagascariensis	*Gasteracantha* 2	Rectangle	1
G. versicolor formosa	*Gasteracantha* 2	Rectangle	1
I. penizoides	*Isoxya*	Hexagon	1
Isoxya sp. nov.	*Isoxya*	Hexagon	1
I. tabulata	*Isoxya*	Hexagon	1
Macracantha arcuata	*Gasteracantha* 1	Triangle	1
M. hasselti	*Gasteracantha* 1	Triangle	1
Thelacantha brevispina	*Gasteracantha* 2	Hexagon	1

3.2.2 Photography

Images of the selected taxa were taken using a Leica M165C (Leica, Germany) automontage photo stacking system at OUMNH. Photo stacks were acquired using the software Leica Application Suite (LAS V4.12) and then compiled using the program Helicon Focus (HF 7.5.8). Minor edits and cropping of images were done in the photo editing software GIMP 2.10.3 (GIMP Dev. Team, 2019) when required.

Gasteracantha have specific abdominal characteristics, for example the elongated spinneret cone (character 40, Appendix A3.2.1) or vertical spines (character 19, Appendix A3.2.1) that often make it difficult to conventionally image in a dorsal or ventral view. In the case of larger specimens, or those with a steep ventral surface, a specially constructed dish was used which enabled the entire spider to sit in a flat position for photography. Alternatively, a suitable cut was made into a plastazote block to enable the abdomen to sit flat for imaging in either the lateral or dorsal planes (see Figure 3.2).

Figure 3.2 *Gasteracantha aciculata* Left: right lateral view of abdomen showing steep ventral surface; Right: flat dorsal view of abdomen ready for morphometric analysis

3.2.3 Software used

tpsUtil version 1.81 (TPS File Utility Program) (Rohlf, 2015) was used for the preparation of TPS (TiePie multi-channel Software) input files. These files were compressed and contained all the images for morphometric analyses. MorphoJ (Klingenberg, 2011), an integrated program package, was then used to conduct a geometric morphometric analysis.

3.2.4 Morphometric analysis

Images were imported into tpsUtil version 1.81 (Rohlf, 2015) to create a tps input file before being imported into tpsDig version 2.31 (Rohlf, 2015). Here the images were scaled using the measuring tool to correspond to the 1mm scale bar set on the Leica Application Suite (LAS V4.12) images. Then the landmark points (numbered 1-24) were affixed individually to each of the chosen 24 sigilla (see Figure 3.3) for each specimen. The landmark point of the deepest part of the sigilla was chosen, with the centre of the sigilla used if the deepest part was not obvious. As the sigilla are internal muscle attachment points that are conspicuous on the abdominal surface, the placement of the landmark was as consistent as possible throughout all the images.

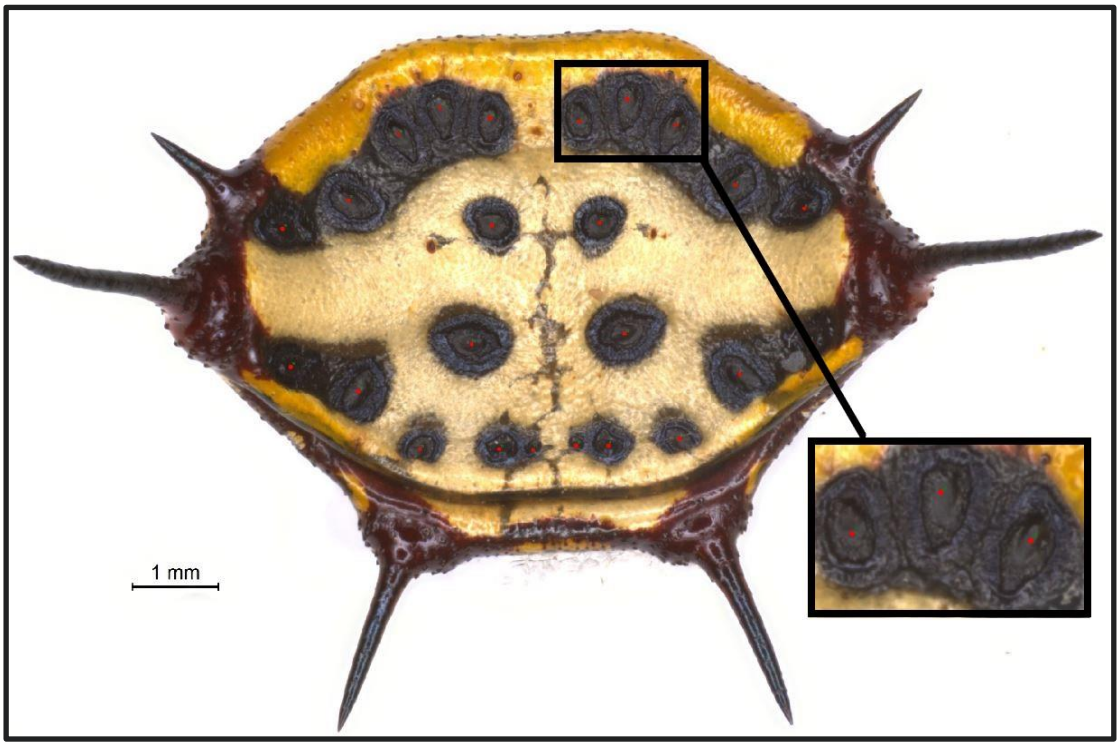

Figure 3.3 Fixing landmark points (red dots) in the centre of sigilla on image of *Gasteracantha aciculata* dorsal view of abdomen, in tpsDig.

The sigilla were numbered in a clockwise manner starting from the far right anterior sigilla group (Figure 3.4(A) as numbers 1-5 then down and round through the posterior right (Figure 3.4(B) 6-9, posterior central (Figure 3.4(D) 10-11, posterior left 12-15, anterior left 16-20, before moving to the central trapezoid (Figure 3.4(C), again clockwise, for 21-24.

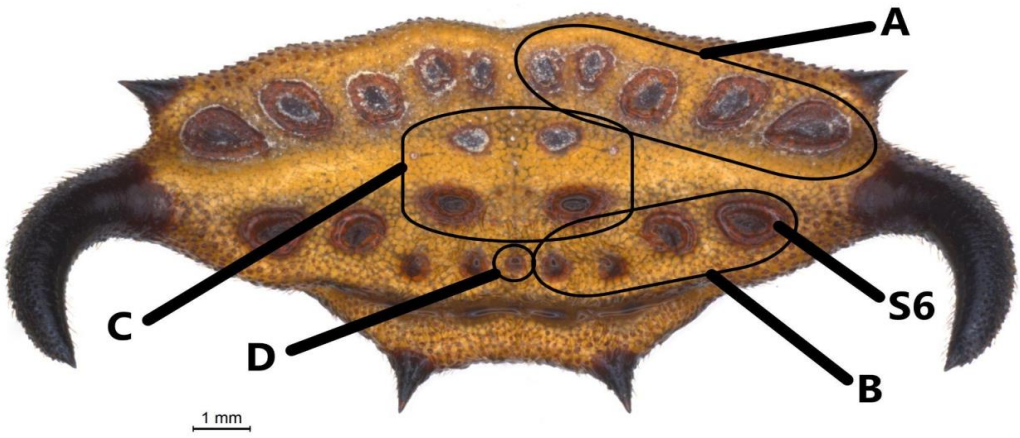

Figure 3.4 Copy of Figure A3.14 *Gasteracantha thorelli* Keyserling, 1864 abdomen dorsal view. Sigilla: anterior right (A), posterior right (B), central trapezoid (C), posterior central (D), sigilla "6" (S6).

If a consistent number of landmark points are not retained, then the analysis cannot function. For this reason, the central posterior sigilla (see character 21 in Appendix A3.2.1) that are sometimes fused into one sigilla were all fixed as two landmarks (numbers 10 and 11). Taxa where the sigilla are fused had the landmark points fixed side by side to enable the analysis to function, while scoring this morphological difference between species. Once all images had been annotated with landmarks the batch file was saved, and the number of landmarks confirmed as equal across all taxa, before being opened in the geometric morphometric program MorphoJ (Klingenberg, 2011).

Within MorphoJ a new dataset was created. This was followed by a Procrustes fit. A Procrustes fit is a statistical shape analysis that analyses the distribution of the shapes (Rohlf and Slice, 1990) and shows the mean of all the landmarks. In this case the mean of the abdominal shapes, defined by the landmarks set earlier, are shown as blue dots in Figure 3.5. The Procrustes residuals, the landmark points that were analysed to generate the mean, are shown as smaller black dots (Figure 3.5). The residuals offer information about the difference in shape between the taxa; for example, if one residual area was larger or if they were

confined to a particular region in the taxa (Polly *et al*., 2013; Polly, 2018). Here the abdominal shapes are similar enough that the residuals do not show very large differences from the mean Procrustes fit.

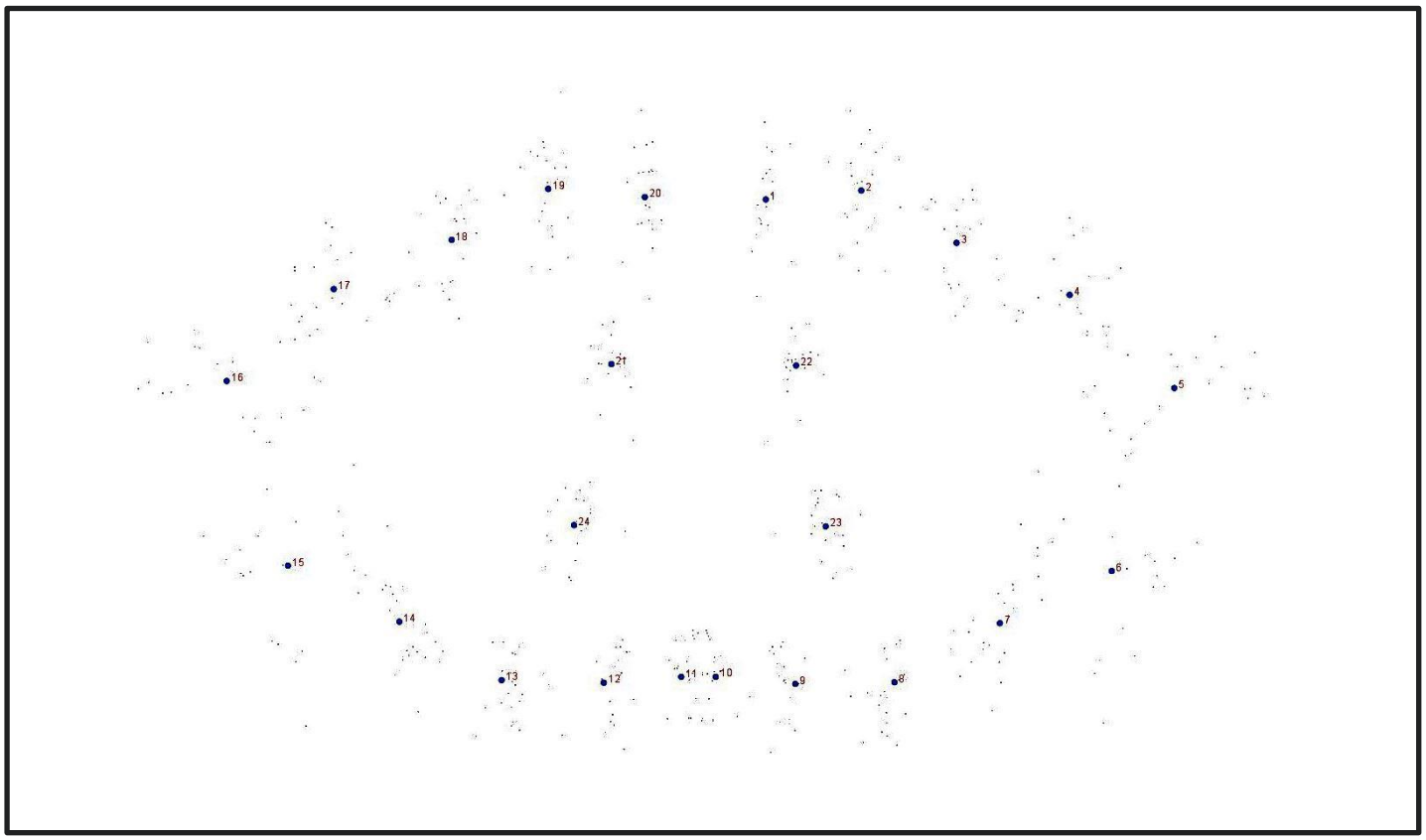

Figure 3.5 The Procrustes fit for the inputted images - the larger numbered blue dots referring to the mean landmark positioning, the small clusters of black dots show the Procrustes residuals.

Following this a classifier ‘Group’ is then set using states that refer to the taxa’s placement on the molecular trees (see Table 3.1). This can be used later in the analysis to demonstrate correlations between the two analyses. Additionally, the classifier ‘Shape’ was also retrospectively set after the morphometric analysis to enable the correlation between taxa to be highlighted clearly (see Figures. 3.8 and 3.9 below).

The sigilla landmarks 1-20 and 21-24 were continuously connected, along with an additional 2-5-6-15-16-19, in a ‘wireframe’ to enable ease of viewing in the ‘shape changes’ view (see Figure 3.6). Again, this was preparation for post analysis interpretation, providing a method of seeing the landmark points variation between the taxa with a clear outline rather than just the landmark points as dots.

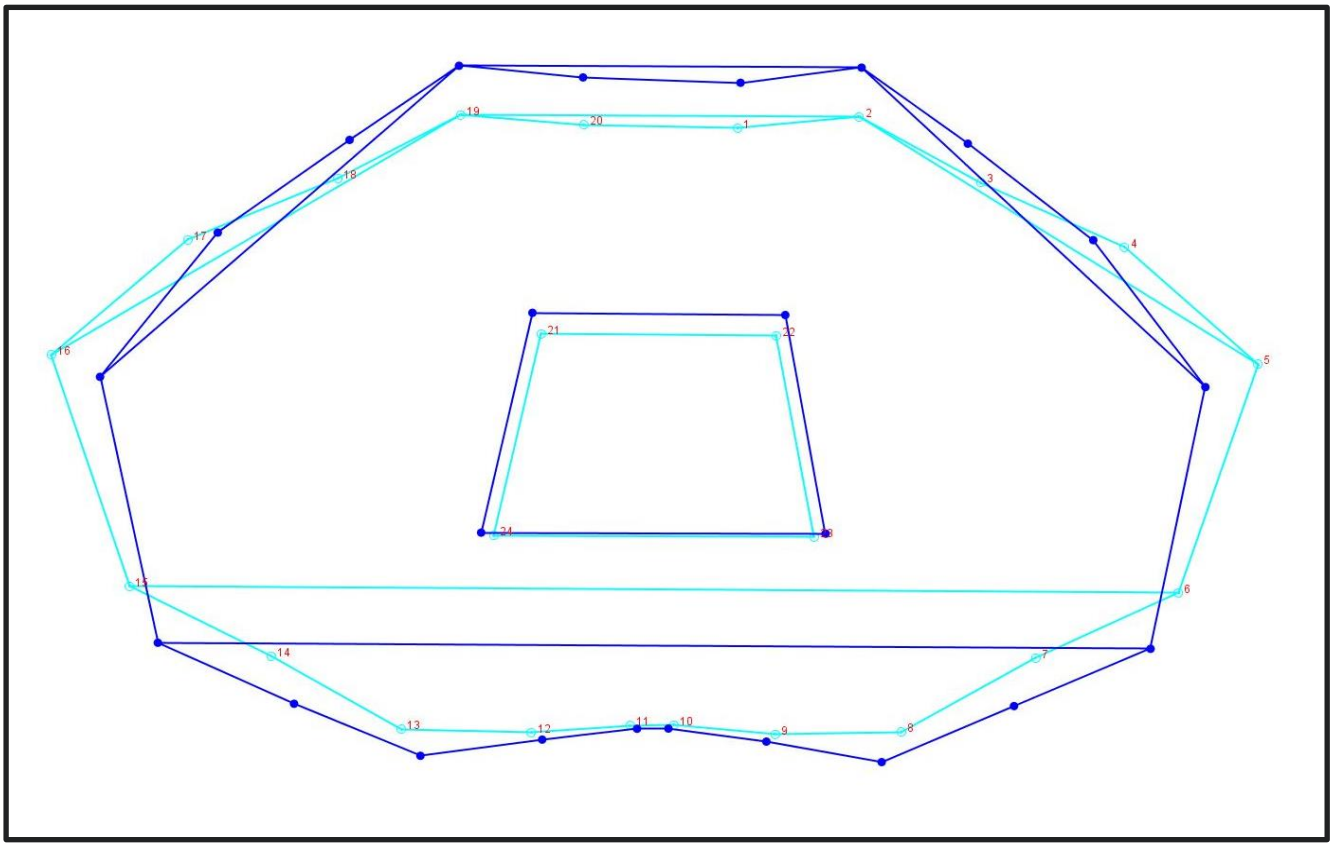

Figure 3.6 Example of wireframe - the PC 1 result view.

After this a covariance matrix was generated so that a Principal Component Analysis (PCA) could be performed. A covariance matrix measures the difference between the set variables, here measuring the difference between each fixed landmark’s location, and the details of the PCA are given below.

Once the Procrustes fit and covariance matrix was generated, MorphoJ was used to run a Principal Component Analysis (PCA). Eigenvectors and eigenvalues are the concepts that need to be computed from the covariance matrix to determine the principal components of the data. A PCA is a statistical procedure that converts a dataset of, here correlated, observations into a set of linearly uncorrelated variable values called Principal Components (PC). A PCA simplifies data and helps present the dataset in a compressed way (Polly *et al*., 2013; Polly, 2018; Savriama, 2018). Most of the information from within the initial variables is compressed into the first principal components. A PCA maximises information in the first component, then maximises remaining information in the second component and so on by retaining the components with higher information as the new variables. Thus PCA reduces multivariate data to a few axes that can be visualised and analysed, to identify groupings and differences within the original data.

This was a small dataset, and the results were straightforward to interpret. The output of the PCA used includes a lollypop graph of the shape changes with a PCA plot that can then have any classifiers, that were set earlier, superimposed over it and details of the eigenvalues which gives information about the amount of variation explained by each Principal Component (Figure 3.7).

3.3 Output and results

This section covers the data output from the PCA analysis, a summary of these results can be seen in Table 3.1, and the results from the analysis in the form of PCA plots and mapped onto the phylogeny.

3.3.1 Eigenvalues and PCA

In this analysis Principal Component 1 explained over 50% of variation in the data and Principal Component 2 30% of variation. All the other components explained less than 5% (Figure 3.7).

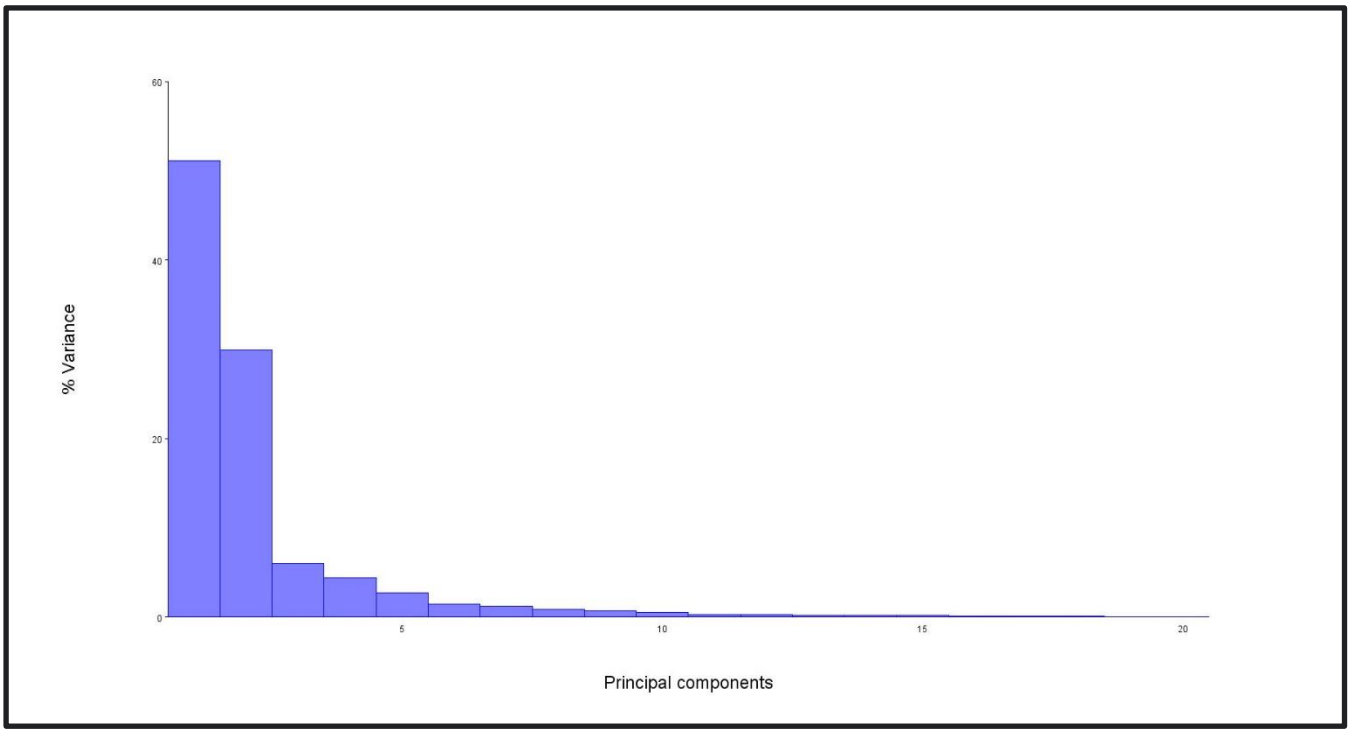

Figure 3.7 Eigenvalues bar chart showing percentage variance between the principal components that highlights PC1 and PC2 as the most variable.

The PCA plot (Figure 3.8) shows there are differences in the shapes of the different groups identified from the inferred BI CO1 and combined (CO1+16S) BI phylogenetic trees (see Chapter 2.4.1) and classified by 'Group' in the morphometric analysis. '*Gasteracantha* 1' species are circled and the points coloured in red, '*Gasteracantha* 2' in green, '*Isoxya*' in black and the Gasteracanthinae in blue. The solo outgroup species, *Aranoethra cambridgei*, is grey and is not circled as it is the only outgroup species shown here from the phylogenetic trees.

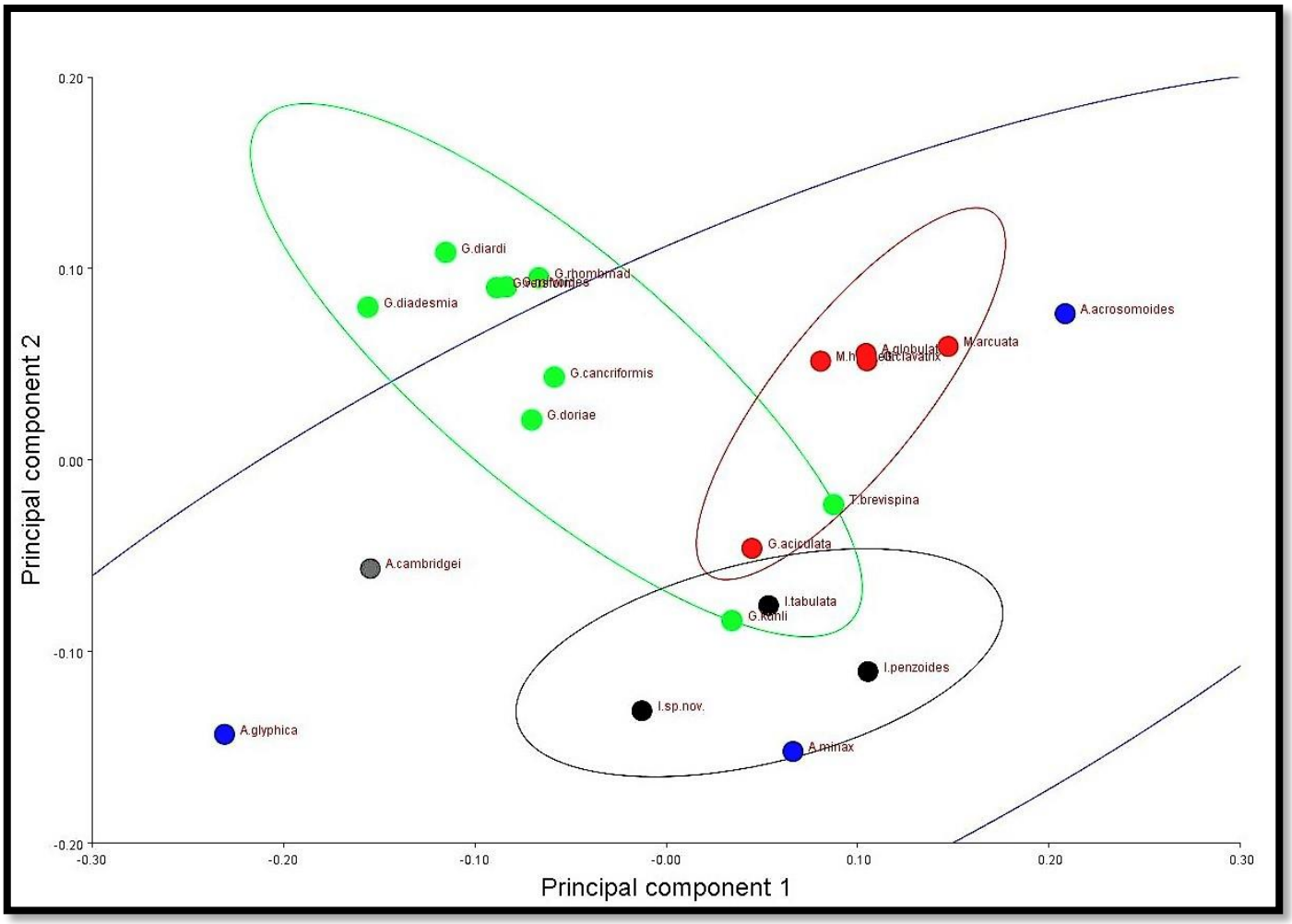

Figure 3.8 PCA plot of the species used in the morphological analysis. Taxa are circled by the classifier 'Group' that was set earlier in the analysis. Colours reflect 'Group': grey = Outgroup, black = *Isoxya*, blue = Gasteracanthinae, red = '*Gasteracantha* 1', green = '*Gasteracantha* 2'

There is overlap between the groups, four of the species are located within the overlapping central area of the plot: *Gasteracantha aciculata, G. kuhli, Isoxya tabulata* and *Thelacantha brevispina*.

The same PCA analysis with the species classified by 'Shape' (Figure 3.9), shows greater partitioning than when classified by 'Group'. 'Triangle' abdomen shaped species are circled, and points coloured red, 'Rectangle' is green, 'Hexagon' (which also includes the more

circular *Isoxya penzoides* abdomen as the sigilla pattern falls into this category) is black and *Augusta glyphica* 'Inverted Triangle' is grey and is the only one with this distinct shape so is not circled. These subjective shape descriptors are allocated based upon the findings of the morphometric analysis. As discussed earlier, some species would be harder to un-subjectively assign a descriptive shape but, by using the morphometric analysis, the shape can be quantified and allocated without accidental bias.

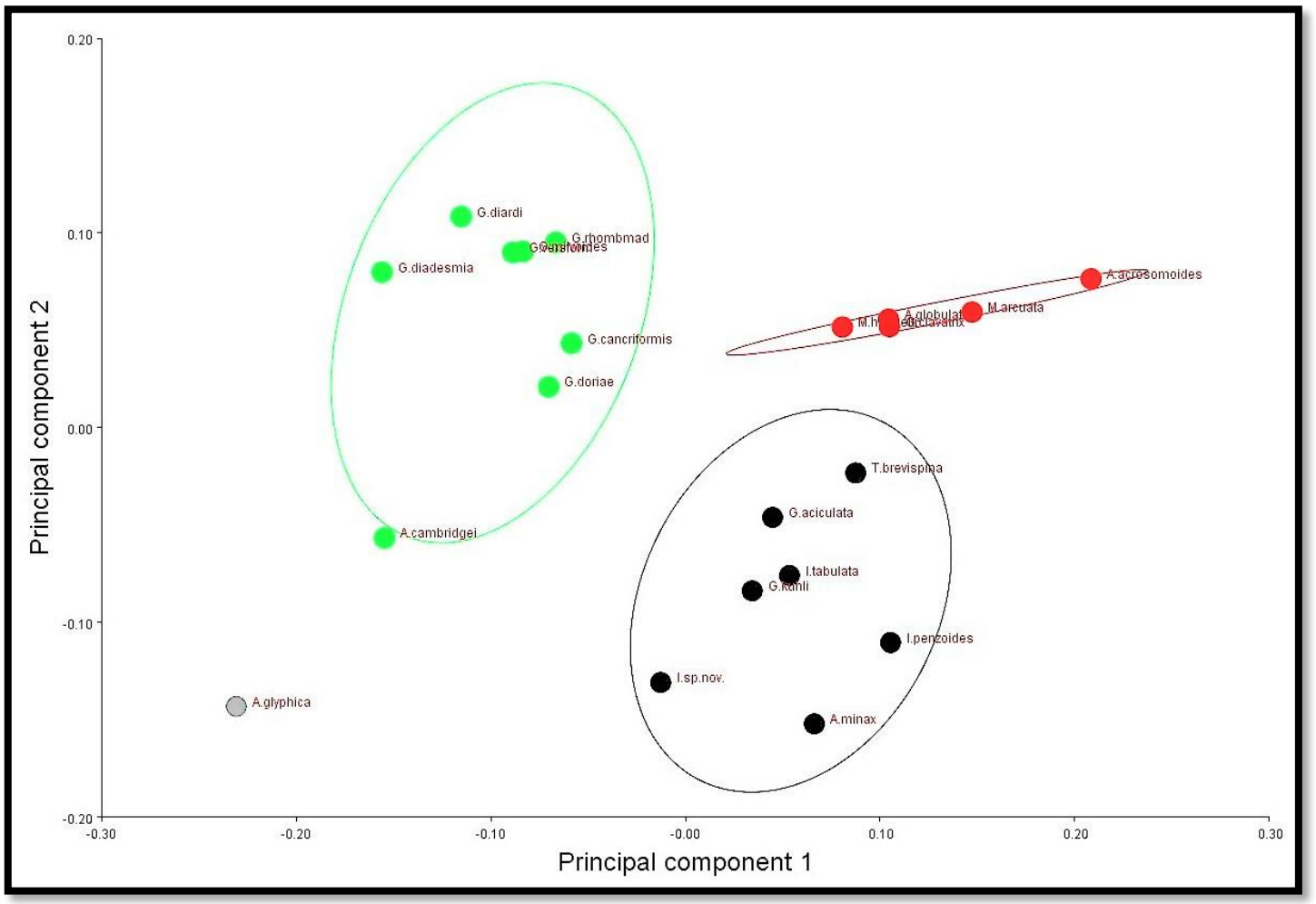

Figure 3.9 PCA plot of the species used in the morphological analysis. Taxa are circled by the classifier 'Shape' that was set earlier in the analysis. Colours reflect 'Shape': grey = inverted triangle, black = hexagon, red = triangle, green = rectangle.

There are three distinct groupings of abdominal shape, with the unique abdominal shape of *Augusta glyphica* as the fourth. Assignment of species to Groups and to Shapes is given in Table 3.1. The addition of other Gasteracanthinae genera that were not included in this study (see Chapter 4, eSSD analysis discussion and Chapter 5.2) might also reveal different abdominal shapes. However, for now just these four shapes are identified.

3.4 Discussion

Abdominal shapes have been mapped onto the molecular (CO1) phylogeny in Figure 3.10 and onto the combined molecular (CO1+16S) phylogeny in Figure 3.11. Because only one individual per species has been used in this trial, the results must be interpreted with caution as intra-specific variation has not been incorporated in the analysis. However, the results indicate that in this provisional morphometric analysis of abdominal shape there are distinct shape groups and that there is some correlation between abdominal shape and the relative positions of species within phylogenies produced using molecular data.

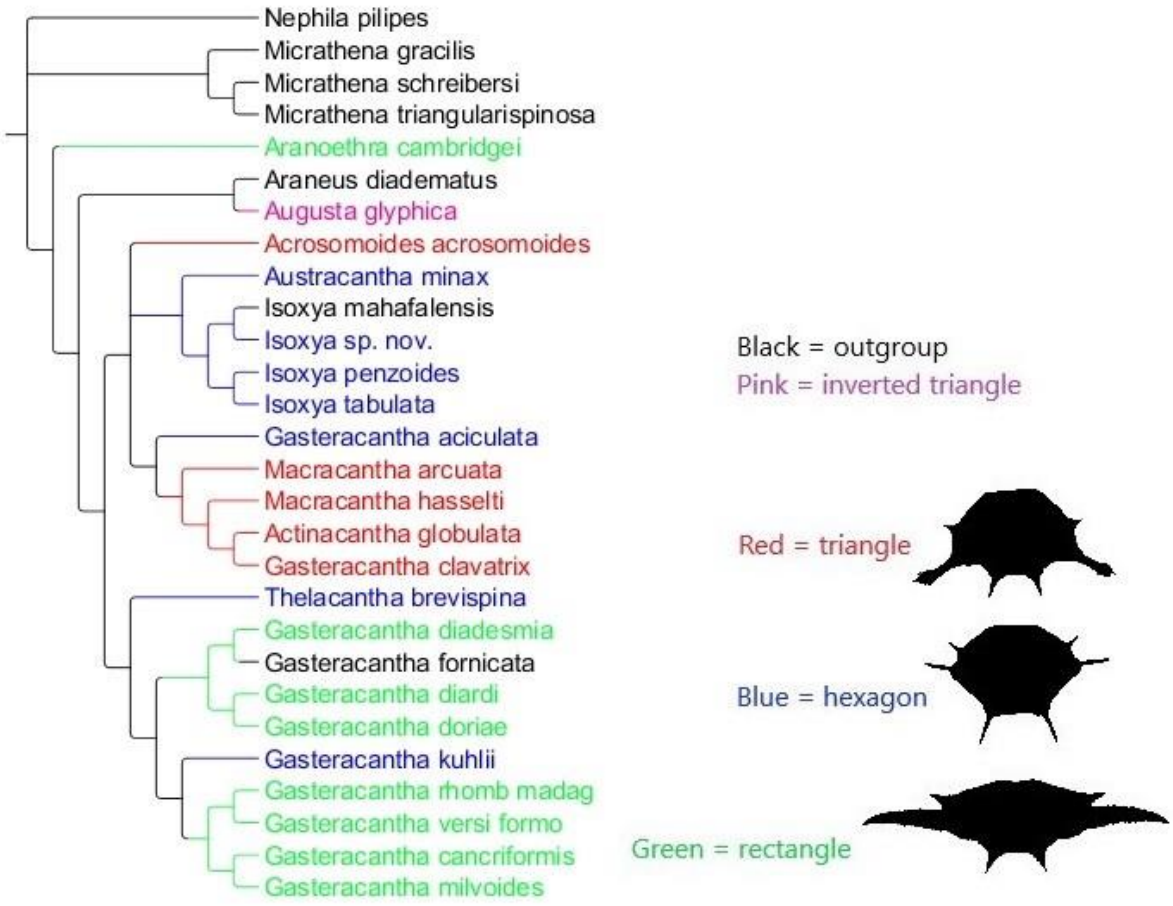

Figure 3.10 Copy of result tree from CO1 molecular BI analysis Figure 2.11 coloured to map abdominal shapes onto the tree with silhouettes of most common 3 shapes (black = outgroup and *G.fornicata* and *I.mahafalensis* [not scored], pink = inverted triangle, red = triangle, blue = hexagon, green = rectangle).

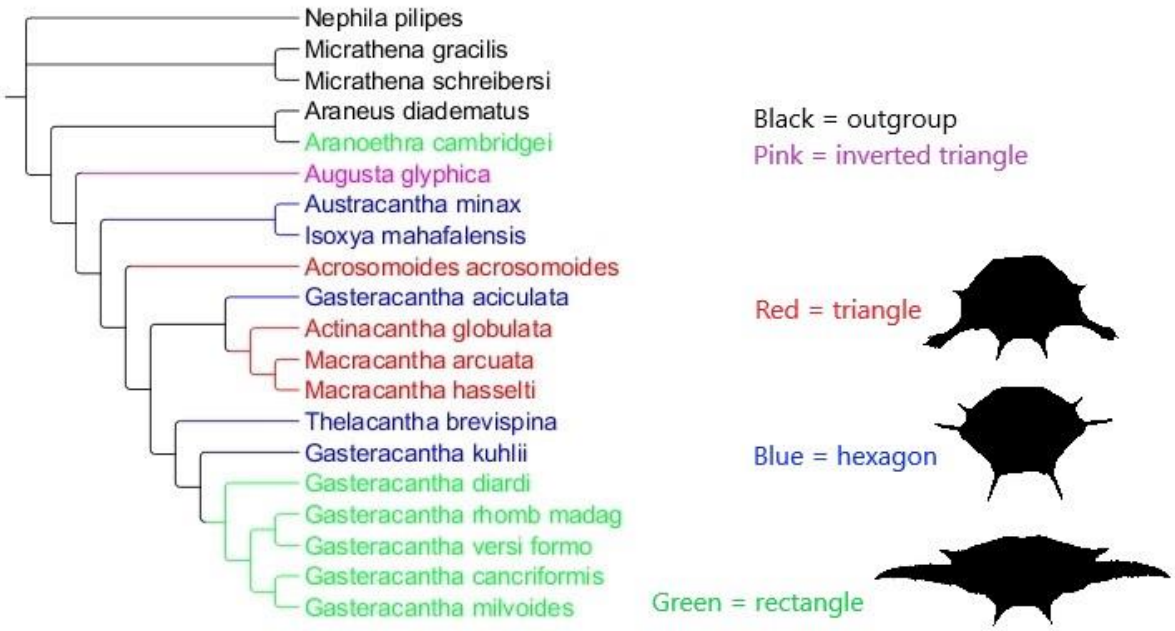

Figure 3.11 Copy of result tree from combined (CO1+16S) molecular BI analysis Figure 2.15. Coloured to reflect abdominal shape with silhouettes of most common 3 shapes (black = outgroup, pink = inverted triangle, red = triangle, blue = hexagon, green = rectangle). Note: *I. mahafalensis* was not scored in the morphometric analysis however it is coloured blue here to highlight the other *Isoxya* scored (see fig. 3.10) that were hexagonal.

In both trees there is some correlation between abdominal shape and species relatedness, although this could still be explained by chance. The most prominent results are seen in the '*Gasteracantha* 1' group where the more derived species within this clade share a similar abdominal shape, and with '*Gasteracantha* 2' where there are many taxa with similar abdominal shapes. However, each clade is not comprised of only a single abdominal shape. The species *Gasteracantha aciculata, G. kuhli, Thelacantha brevispina* which are located at the base of their respective clades all possess a hexagonal abdomen shape. This finding is interesting and poses the question why do the taxa at the basal nodes of each of the respective clades within *Gasteracantha* possess the same abdominal shape? The results suggest the ancestral abdominal state is hexagonal in Gasteracanthinae. However, the results do not

support the hypothesis that the same abdominal shape would be found in closely related species (Chapter 1.5).

Questions are therefore posed as to why the derived taxa within the respective clades would possess such differing abdominal shapes from one another. In the absence of detailed ecological information on the groups, there is no obvious answer. However, current work on abdominal patterns and prey lure (White and Kemp, 2015, 2016; White, 2017; Messas *et al*., 2021; Kemp, Edwards and White, 2022) have focused only on gasteracanthine taxa with rectangular abdomen shapes. Future studies could explore if the specific prey lure abdominal patterning is present only in taxa with rectangular abdomens and what, if any, techniques do other species with different abdominal shapes use. *Thelacantha brevispina* is also a species that exhibits a range of colour polymorphism, with a hexagonal abdomen, that is yet to be studied in detail. The common thought that abdominal colouration is associated to flower mimicry in orb weavers (White and Kemp, 2015) could also be examined in conjunction to gasteracanthines abdominal shape. More discussion on various selection pressures can be found in Chapter 4.

There is an anomaly in the abdominal shape of *Acrosomoides*. They possess a triangular abdomen, like '*Gasteracantha* 1' species, but otherwise few shared characters with that clade and are located in a more basal position in the subfamily (see Figures 3.10 and 3.11). This suggests that abdominal shape might not be homologous and future studies might reveal parallel evolution between the two different groups. The homology of abdominal shapes should be examined carefully in future studies and before taxonomic reclassifications.

The abdominal shape was not used as a character in the morphological analyses. The fact that taxa roughly group by abdominal shape in the molecular phylogenies presented is also testimony to Simon's (1864), Butler's (1873) and Dahl's (1914) interpretation. These authors, working on classifications not based on evolutionary relationships, used abdominal shape, geographical distribution, and spine lengths to allocate sub-generic groupings. The results demonstrate that their taxonomic groups based on abdominal shapes are in fact natural groups. The mapping of geographical distribution and the evolution of spine length is considered in Chapter 4.

Future consideration should be made regarding the use of sigilla as the fixed landmark. There is a limitation to using this character as it prevented the use of certain outgroup taxa lacking conspicuous sigilla. However, by selecting the homologous sigilla that were chosen for the landmarks, any taxon that possesses sigilla should be suitable for comparison and analysis. Other genera were not included, for example *Caerostris* (see Chapter 1.3), that are known to exhibit these homologous sigilla. Despite these limitations, this trial has highlighted similarities between closely related taxa and demonstrates that morphometrics is a useful tool for quantitative analysis of abdominal shape. If a method of exposing the inconspicuous muscle attachment points could be developed for taxa that do not have conspicuous sigilla (see Chapter 5 and Appendix 4) then this approach could be viable for morphometric analysis on all spiders.

3.4 Conclusions

In conclusion, this chapter demonstrates that morphometric analysis can quantify a complex morphological characteristic (the abdominal shape). The results show the ancestral abdominal state of Gasteracanthinae is hexagonal. However, the hypothesis that closely related species (taxa from within the same clade within the genus or sister taxa) share the same abdominal shape has not been supported.

Chapter 4: Biogeography and evolution of sexually dimorphic characters

This chapter aims to test the following hypotheses as laid out in Chapter 1.5. Species of Gasteracanthinae from the same biogeographical region are closely related likely due to short range dispersal. The trait of extreme sexual size dimorphism is only lost within the genus *Isoxya,* and ecological or other biological reasons are hypothesised for this loss. As in *Micrathena*, the long abdominal spines in *Gasteracantha* and *Macracantha* evolved multiple times. The ventral tubercle is present within clades of Gasteracanthinae that exhibit the greatest sexual size dimorphism. All these hypotheses are discussed in relation to various molecular phylogenies from Chapter 2.

4.1 Biogeography of the Gasteracanthinae

Geographical distributions, combined with phylogenetic information can be used to make inferences about the origins and historical biogeography of taxonomic groups. As discussed in Chapter 1.4, the amber fossil of *Gasteracantha* and the publication of Dimitrov *et al*. (2016) suggest that *Gasteracantha* evolved after the breakup of Gondwana. Ballooning is likely to be the Gasteracanthinae's dispersal method based on research in other Araneidae dispersal (Walter, Bliss and Moritz, 2005; Blandenier, 2009; Wolz *et al*., 2020) and Emerit's work on *Gasteracantha* developmental biology (1974). The hypothesis suggested in Chapter 1.5 was that species of Gasteracanthinae from the same geographical region would be closely related due to short range dispersal.

4.1.1 Methodology

Distribution data for the species included in the molecular analyses (Chapter 2) was extracted from the World Spider Catalog (2022) and the individual specimen data (Appendix 2). Species distributions were classified into the following broad regions (see Figure 4.1): Oriental region (from India through to the Philippines, including Indonesia and China); African region (Africa and Madagascar); Australasian region (Australia and New Guinea); Nearctic and Neotropical regions (America and Mexico); Various – referring specifically to *Thelacantha* distribution (Madagascar, Mauritius, Pakistan to Japan, New Guinea, Australia, Fiji, French Polynesia, and Hawaii). Species distributions were then mapped onto the CO1 phylogeny, the combined molecular (CO1+16S) phylogeny and the Group 1 morphological phylogeny (Chapter 2).

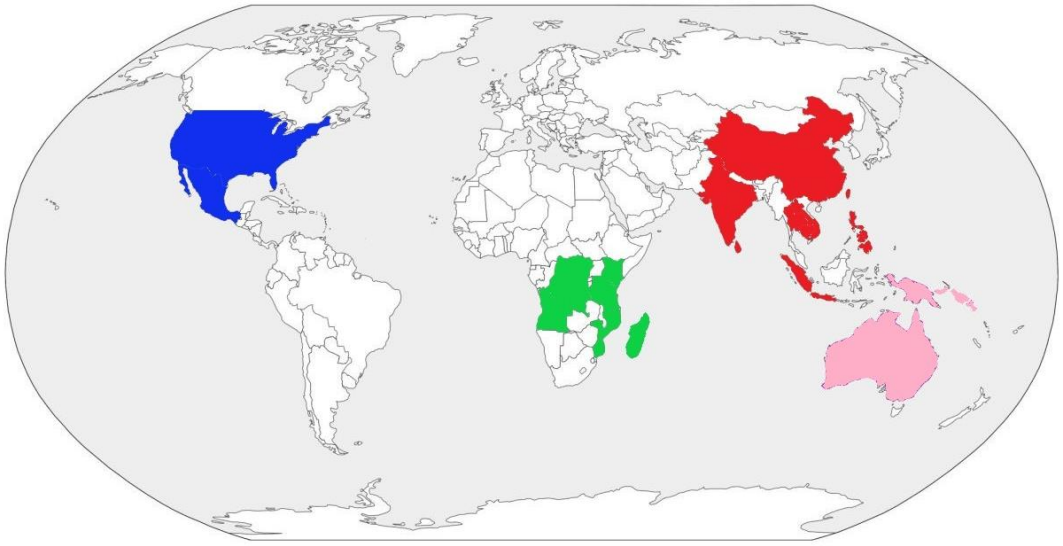

Figure 4.1 Map of approximate Gasteracanthinae distribution coloured by geographical region (red = Oriental, green = Africa, pink = Australasia, dark blue = Nearctic and Neotropical). Various (see above in 4.1.1) is not shown as coloured due to the overlap with other regions. Borneo.

4.1.2 Results

The results show there is an Oriental distribution for all but one species within the '*Gasteracantha* 1' clade in the molecular analyses (Figures 4.2 and 4.3) with *Gasteracantha aciculata* occurring in the neighbouring region of Australasia. The additional taxa from the morphological analysis also show distribution in these two regions (Figure 4.4). The distributions of the '*Gasteracantha* 2' clade members are more diverse (Figure 4.2 and 4.3). Basal species in this clade have an Oriental distribution in the combined analysis (Figure 4.2) with derived taxa having African and New World distributions. *Thelacantha brevispina* is the only taxon with a very extensive geographical range and it is located at the basal node of '*Gasteracantha* 2' in both the CO1 and combined trees. However, in the morphological tree (Figure 4.4) various geographical distributions are interspersed in '*Gasteracantha* 2'. Many of the remaining mapped species within the Gasteracanthinae have a predominantly African distribution, for example *Isoxya* in the CO1 and morphological trees (Figures 4.3 and 4.4).

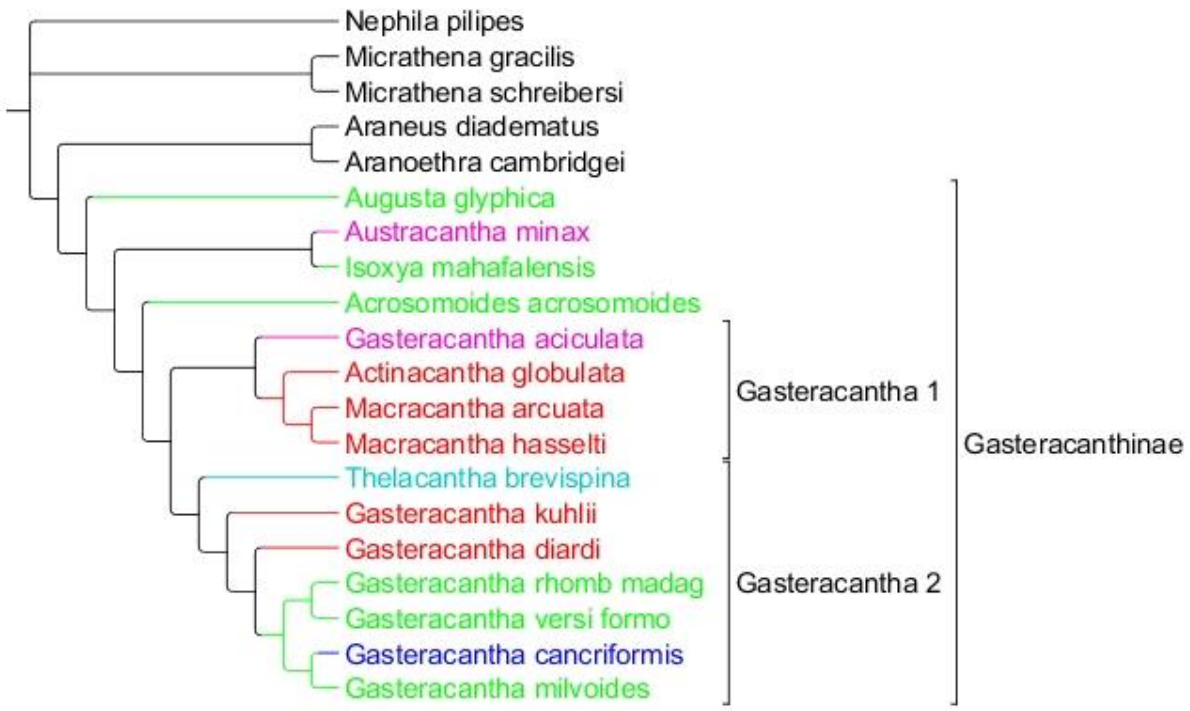

Figure 4.2 Copy of combined (CO1+16S) molecular BI analysis 50% majority rule consensus tree, coloured to highlight geographical species distribution (red = Oriental, green = Africa, pink = Australasia, dark blue = Neotropic, light blue = various, black = outgroup taxa).

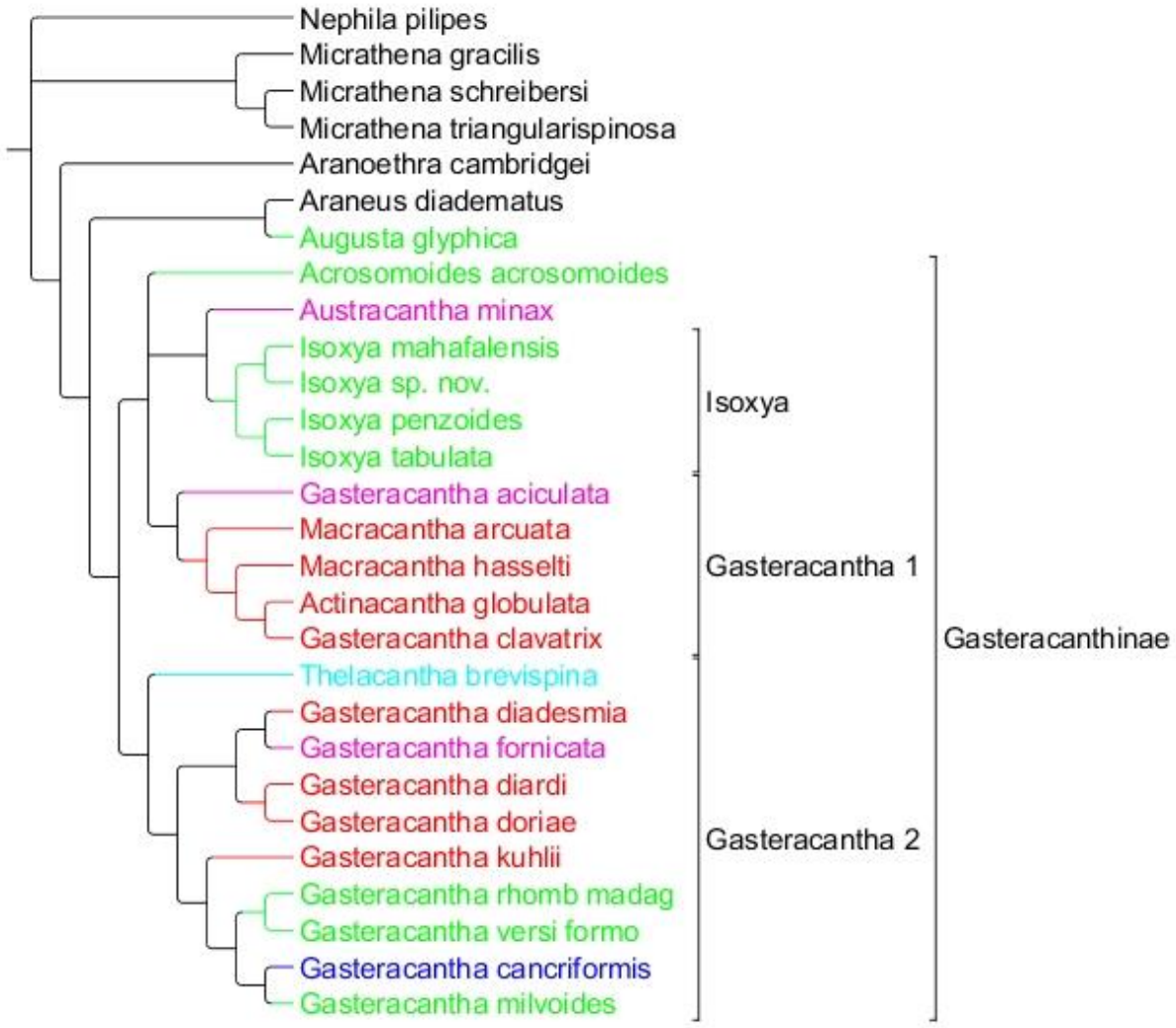

Figure 4.3 Copy of CO1 molecular BI analysis 50% majority rule consensus tree, coloured to highlight geographical species distribution (red = Oriental, green = Africa, pink = Australasia, dark blue = Neotropic, light blue = various, black = outgroup taxa).

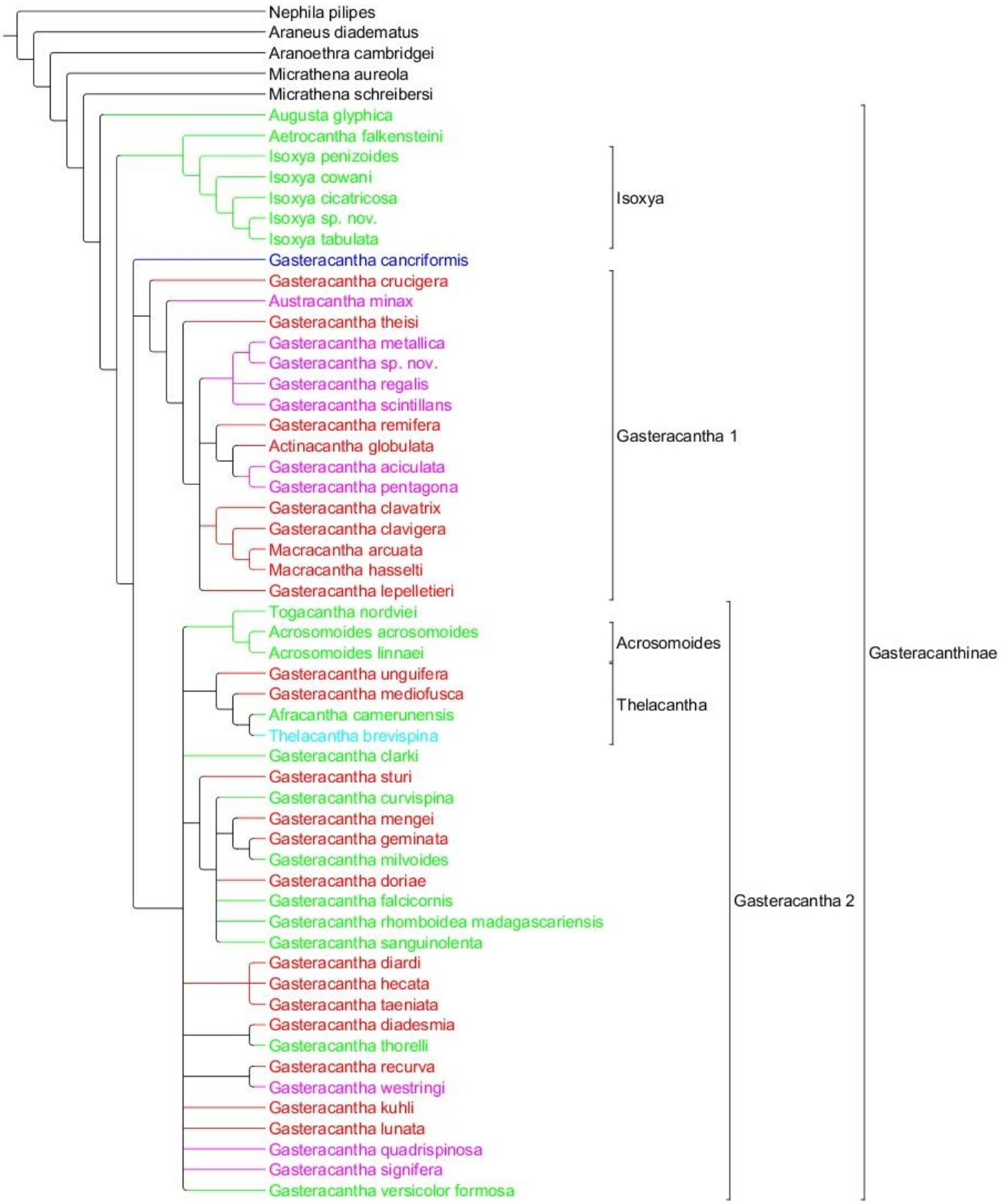

Figure 4.4 Copy of morphological Group 1 MP analysis 50% majority rule consensus tree, coloured to highlight geographical species distribution (red = Oriental, green = Africa, pink = Australasia, dark blue = Neotropic, light blue = various, black = outgroup taxa).

4.1.3 Discussion

Taxa in '*Gasteracantha* 2' show a varied distribution in all analyses with taxa that have Oriental, African and New World distributions. The molecular trees (Figure 4.2 and 4.3) show taxa from the Oriental region located at the basal nodes of the clade, before derived taxa show an African and New World distribution. Here the phylogenetic location of *Gasteracantha cancriformis*, with a Nearctic and Neotropical distribution, is interesting as it is derived within a clade of African species. The molecular phylogenetic results placed this species within '*Gasteracantha* 2', and the morphological analyses was inconclusive on its location (Chapter 2). The placement the New World *G. cancriformis* within a clade of African species suggests a long-range dispersal event has occurred between Africa and the New World.

This then raises the question, has a single dispersal event to the New World from Africa occurred or have multiple dispersal events occurred? This can be answered in future analyses by including the remaining New World *Gasteracantha* species and discovering their phylogenetic location. If all the New World taxa are located in the same clade, then this would indicate a single dispersal. If the taxa are spread across various clades, then this would indicate multiple dispersal events. There are examples of other orb-weaver genera that have species in both the Neotropical and African regions, for example *Araneus* and *Neoscona* (World Spider Catalog, 2022) and long-range dispersal has been seen in other Araneidae for example, *Argiope* and *Nuctenea* (Agnarsson *et al.*, 2016; Scharff *et al*., 2020).

The remaining clades in the molecular phylogenies show single or close geographical distributions, for example '*Gasteracantha* 1', Oriental and Australasian distribution, or *Isoxya*, African distribution. These findings support the hypothesis from Chapter 1.5 that

closely related species would be from the same geographical region likely due to short range dispersal. However, this is not the case within '*Gasteracantha* 2' due to the long-range dispersal of *G. cancriformis*.

The biogeographical analysis has highlighted a long-range dispersal event from Africa to the New World and suggested future work to discover if this was a single occurrence or happened multiple times. Although not all taxa from a geographical location have been inferred as closely related, most clades have supported the hypothesis from Chapter 1.5, except for the clade containing *G. cancriformis*. Remarkably, this also partially supports Dahl's 1914 subgeneric classification method based upon geographical distribution.

4.2 Evolution of sexual size dimorphism and other sexually dimorphic characters

This section takes the inferred molecular phylogenies (CO1 and combined CO1+16S) and explores the evolution of sexually dimorphic characters that are found in the subfamily. Firstly, the hypothesis of extreme sexual size dimorphism loss in the genus *Isoxya* is explored using the CO1 tree.

4.2.1 Introduction to sexual size dimorphism

Sexual dimorphism occurs when the female and male of the same species exhibit different characteristics for example mandibles (Okada *et al.*, 2021). This can also relate to traits like colouration or size. Sexual size dimorphism (SSD), therefore refers to the difference between the size of the male and the female of the same species. Extreme sexual size dimorphism, as discussed here, is where the size of the female is much greater than that of the male. Spider groups that demonstrate eSSD are rare, but when this occurs, they exhibit the largest eSSD among terrestrial animals. Apart from spiders, eSSD is rare in the animal kingdom (Kuntner and Coddington, 2020) although it can also be found in other arthropods such as beetles, moths and orchid mantids (Blanckenhorn, Meier and Teder, 2009), and marine animals, with some female fish reaching eight times the size of their male counterpart. It has been previously noted and confirmed that gasteracanthines exhibit SSD (Pickard-Cambridge, 1879; Simon, 1895; Hormiga, Scharff and Coddington, 2000; Kuntner and Coddington 2020) but, due once again to the lack of male specimens, published data is limited.

In spider eSSD, the female to male body length is ≥2.0. This threshold is an arbitrary number (Kuntner and Coddington, 2020), but female spiders can often be 3–10 times larger than males (Kuntner and Coddington, 2020). There is an example of male-biased SSD, achieved through higher growth rates in males (Lång, 2001), in the linyphiid spider *Linyphia*

triangularis (Clerck, 1758). SSD is frequently achieved by the different maturation times between males and females in both insects (Esperk *et al.,* 2007) and spiders (Levy, 1970; Elgar*,* Ghaffar and Read, 1990; Vollrath and Parker, 1992; Head, 1995; Legrand and Morse, 2000; Foelix, 2011; Magalhães & Santos, 2011; Lemaître *et al*., 2020). A range of hypotheses have been proposed and tested to explain SSD and eSSD in spiders, insects, and other animals (for example Fairbairn, Blanckenhorn and Szekély, 2007).

Reproductive success is one of the most common theories proposed to account for SSD and eSSD in insects and spiders (Prenter, Elwood and Montgomery, 1999). Larger females produce a greater number of offspring (Capinera, 2008) but this does not explain the relative size of the male in eSSD. Ecological pressures can also produce eSSD. For example, eSSD has evolved several times in deep-sea ceratioid anglerfishes where the much smaller males exhibit sexual parasitism to various degrees. These range from obligate parasitism to temporary non-parasitic attachment (Pietsch, 2005). This phenomenon has evolved because anglerfish males and females live at very low population densities so do not meet very often.

In spiders, eSSD has been correlated with morphological or behavioral traits like sexual cannibalism, male self-sacrifice, emasculation and remote copulation, genital mutilation and plugging, traumatic insemination, mate binding, male accumulation, mate guarding and opportunistic mating (Kuntner and Coddington, 2020). However, due to lack of study, little is known about how these relate to gasteracanthines. One explanation could be related to avoiding sexual cannibalism because a smaller male can more easily escape the female. Sexual cannibalism is common in spiders that exhibit eSSD (*Nephila, Trichonephila, Argiope*, and *Latrodectus*) and can generally explain small males (Kuntner and Coddington, 2020).

Spider mating can be complex (Foelix, 2011). A male spider will construct a special web, the sperm web, before depositing a drop of fluid containing sperm onto it. Then the male draws the sperm into his elaborately structured pedipalps (see Chapter 2). This process can occur either prior to, or after locating a female. In most spiders elaborate courtship patterns have evolved, with the probable aim of protecting the male from being mistaken for prey. In orb-weavers the male will rhythmically pluck at the threads of the female's web to encourage the female to approach. After this the male will mount the female before inserting his palps one after another. Small Gasteracanthine males will stand on the ventral tubercle (see Figure 4.9) if it is present.

Male spiders usually die soon after, or even during, the mating process. Females of some species can bite into the abdomen of the male during mating or, in some instances, even consume males after mating (for example the male of *Latrodectus* sp. can be captured and eaten by the female), but many males escape sexual cannibalism. Like nephilid males, gasteracanthines appear to place their bodies far from female jaws by using the ventral tubercle for mating (Figure 4.9).

Additionally, male spiders must search, walk, climb, and traverse to find females, as female web spiders are sedentary. Studies have found smaller male spiders can climb faster than larger males; the gravity hypothesis (Foellmer and Moya Laraño, 2009). As female Gasteracanthinae will build webs off the ground in the typical orb-weaver manner, this hypothesis could explain Gasteracanthinae males being smaller. Survival when searching for females could also be improved by smaller male size. Emerit (1974) noted that males often drop to the ground when disturbed and blend into the leaf-litter on the ground. The success of camouflage might then favour smaller, inconspicuous males with this behaviour trait. A final

point to note is in the instance of many orb-weavers, the theories of sexual size dimorphism that relate to direct male contest can safely be ruled out as males have not evolved a large body size to defeat competitors.

4.2.2 Material and methods

The taxa used for this study were those Gasteracanthinae genera featured in the CO1 analysis. Measurements were made directly from individuals of both sexes, but when male specimens were not available for measurement the information was extracted from species descriptions in the literature. The measurement used was the total length of the specimen excluding abdominal spines (character 52, Appendix A3.2.1). Directly measured specimens are listed in Appendix 2.6. Specimens were measured using Leica Application Suite (LAS V4.12) at OUMNH.

Supplementation was made from publications (Benoit, 1962b; Benoit and Emerit, 1975; Emerit, 1973, 1974, 1982a, 1982b) where it was possible to extract the male measurements. Either the authors had given the measurements in text, or it was possible to gain the measurements using the scale bar next to illustrations. The total length measurements (with some exceptions which are listed) were absolutes that were calculated into a mean value if more than one specimen was available. The maximum number of specimens per species measured was 5 (as in the morphological analysis) and the minimum was 1, either from publication data or physical specimen data (see Table 4.1).

As the datasets and phylogenies were small, the characters were manually mapped onto the phylogenies in the graphical editor TreeGraph2 (Stöver and Müller, 2010). The CO1 and combined molecular analysis phylogenetic trees were used because they did not use any

morphological information in their production and the dataset would therefore not influence results in mapping morphological traits.

4.2.3 Results

Male/female length ratios were analysed at the generic level (Table 4.1) within the Gasteracanthinae with most of this data original, and unpublished (see Appendix 2.6 for full table of species measurement data).

Table 4.1 Generic level data for SSD analysis

Genus	Size Ratio	Specimens measured
Acrosomoides	**≥ 3**	**6 (2M, 4F)**
Actinacantha	**≥ 4**	**4 (1M, 3F)**
Augusta	**≥ 2.5**	**7 (2M, 5F)**
Austracantha	**≥ 2**	**9 (4M, 5F)**
Gasteracantha	**≥ 3**	**50 (20M 30F)**
Isoxya	**≥ 1.5**	**32 (14M, 18F)**
Macracantha	**≥ 3.5**	**7 (2M, 5F)**
Thelacantha	**≥ 3.5**	**8 (4M, 4F)**
Togacantha	**≥ 3**	**5 (1M, 4F)**

Acrosomoides with a size ratio of ≥ 3 clearly exhibits eSSD as the females are substantially larger than the males in the case of both species measured. However, there are limited male specimens from *Acrosomoides* with only the male of one species currently described; with another to be described (Williams, in prep.). *Actinacantha* exhibited the largest average size ratio in the subfamily at ≥ 4. More data will be required to confirm if this really is the average but eSSD is clearly present. Currently the male of *Actinacantha globulata* is undescribed, but the presence of eSSD is apparent in relation to the only known associated male (Williams, in prep.). The genus *Augusta* has a size ratio of ≥ 2.5 with clear presence of eSSD.

The *Austracantha* size ratio is ≥ 2. The eSSD ratio is not as large in this genus compared to other Gasteracanthinae but is still apparent and recorded in various publications (Mascord,

1966; Hickman, 1967; Davies, 1988) and many specimens were available for measurement (Appendix 2.6). The *Gasteracantha* size ratio averages ≥ 3 however, *Gasteracantha* demonstrates the greatest eSSD range in the subfamily. Some smaller females were recorded as around 2.5 times larger than the males and the largest ratios reached ≥ 4 in some of the larger species (for example *Gasteracantha diardi*).

The *Isoxya* size ratio average is the lowest in the subfamily at ≥ 1.5 indicating *Isoxya* do not exhibit eSSD. *Isoxya tabulata* and *Isoxya penzoides* had the largest ratio within the genus at 1.9; near the arbitary threshold of eSSD.

Macracantha exhibits a size ratio of ≥ 3.5 with a clear presence of eSSD. No data was obtained for *Macracantha arcuata* (the genus type of *Macracantha*) and this ratio is based only upon the newly classified (Macharoenboon, Siriwut, and Jeratthitikul, 2021) *Macracantha hasselti* (formerly *Gasteracantha*). The *Thelacantha* size ratio is ≥ 3.5 and eSSD is apparent with the same substantial female size ratio as seen in other members of the subfamily. Finally, *Togacantha* exhibits a size ratio of ≥ 3. Again, this is a clear indication of eSSD.

The inferred CO1 tree (Figure 4.5) is presented here annotated with the sexual size dimorphism ratios plotted onto the phylogeny where possible. This tree is used to examine the SSD and eSSD because the number of *Isoxya* species available in the CO1 analysis is greater than in the combined molecular phylogeny. The reason for this is that the significant change in Gasteracanthinae SSD occurs within the *Isoxya* species and therefore requires more taxa to be examined. In the combined molecular tree (not shown annotated here) only one

species of *Isoxya* was used and this does not highlight the SSD change as clearly when only one *Isoxya* species is present.

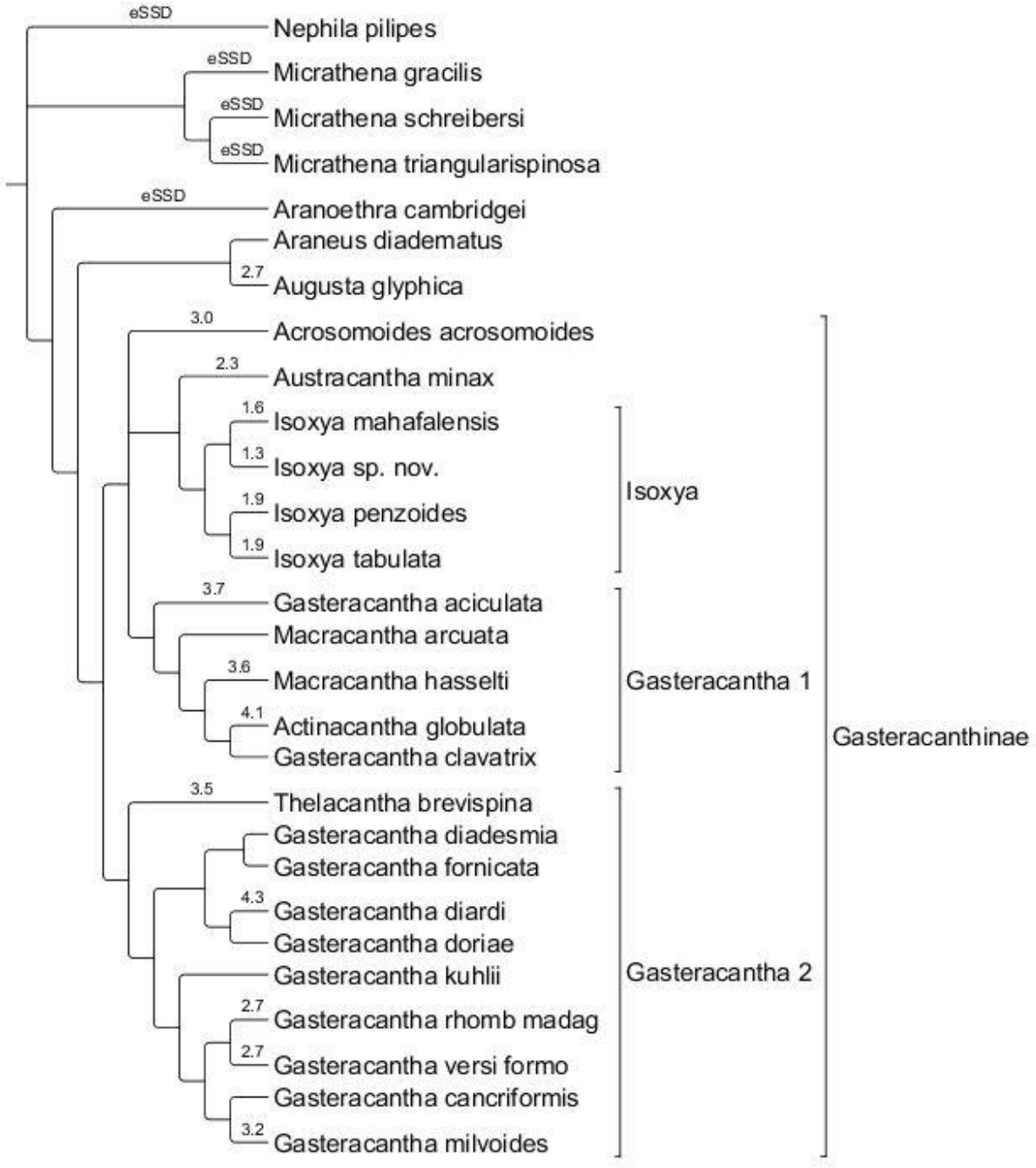

Figure 4.5 Copy of result tree from CO1 analysis. Nodes labelled with species SSD ratio where it is known and "eSSD" if species proved in other studies to exhibit eSSD, but exact ratio not known from data here.

4.2.4 Discussion

Sexual size dimorphism is common and appears to be the rule for orb-weaver spiders in general and particularly araneid spiders (Elgar, Ghaffar and Read 1990; Hormiga, Scharff and Coddington, 2000). However, due to the high quantity of outgroup taxa in this study that exhibit eSSD (Figure 4.5), the trait of extreme sexual size dimorphism, that has evolved independently at least four times (Hormiga, Scharff and Coddington, 2000) and is present in at least 16 spider clades (Kuntner and Coddington, 2020), appears more common than it would be if a full Araneae phylogeny was used (for example Wheeler *et al*., 2017). If a more comprehensive phylogeny of various subfamilies was used to examine the trait, then there would be more examples of sexual monomorphism in the results. Most members of the Gasteracanthinae, exhibit eSSD. This confirms and expands upon the previous works on the subfamily (Hormiga, Scharff and Coddington, 2000; Kuntner and Coddington 2020). On the annotated phylogeny (Figure 4.5) taxa without a number on the node means the male is not currently known or there was no suitable data available. The implications of lacking comparative male and female data in the morphological analysis (Chapter 2) were felt greatest here where the hypothesis surrounding SSD could not be fully tested, and the final conclusions and data presented are still primary.

The annotated phylogeny highlights Gasteracanthinae exhibit extreme sexual size dimorphism; though this is unsurprising if the presumed ancestral state for the gasteracanthines is to exhibit eSSD. All taxa included here bar the *Isoxya* and the species with no male data, support the presence of eSSD in the subfamily. Currently there is also no indication that any of the gasteracanthines missing male data would not also have the presence of eSSD recorded in that species. For example, it is hypothesised *Macracantha arcuata* males would also be at least 3 times smaller than the respective female. This is based

upon the similarities between female Gasteracanthinae biology, including in some cases the ventral tubercle (see Figure 4.11), and the typical size of females being similar in many of the genera.

Regardless of the confirmation of eSSD in the subfamily, the standout discovery from the analysis can be found in the *Isoxya* group. The only members of a clade within the Gasteracanthinae that have lost the eSSD trait is the genus *Isoxya*. This supports the hypothesis from Chapter 1.5 of eSSD loss. The question of why this trait has been lost is currently unknown at present due to lack of data and evidence. However, a reason could be the unique behaviour of *I. sp. nov*. males described in Agnarsson *et al.* (in prep.). A unique mating system in gasteracanthines appears to have evolved with *Isoxya* males living in conjunction with each other and possible lekking behaviour exhibited. This data is still preliminary and will warrant future examination.

Absent from the studies in this thesis was the genus *Hypsacantha* (Figure 4.6). Like *Isoxya* above, *Hypsacantha* seem to display a lower SSD ratio. In *Hypsacantha* the size ratio is ≥ 1.6 (only based upon abdominal length data in publications: Emerit, (1973) and Benoit and Emerit (1975). Future phylogenetic analyses of the subfamily should include this genus to determine its phylogenetic position in relation to *Isoxya* and then examine the SSD. *Hypsacantha* is hypothesised here to be closely related to *Isoxya*, based upon morphological similarities.

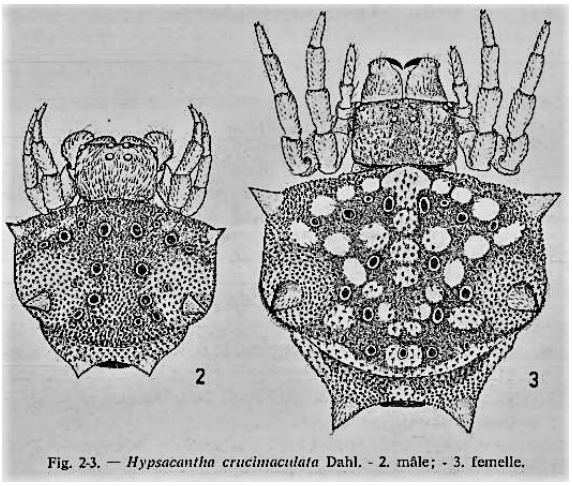

Figure 4.6 Line drawing of *Hypsacantha* (♂ left, ♀ right) from Benoit and Emerit (1975)

In conclusion all genera within Gasteracanthinae, bar *Isoxya*, exhibit eSSD. This supports the hypothesis from Chapter 1.5 and expands upon previous studies, and here ratios are mapped onto the Gasteracanthinae phylogeny. Extreme SSD has been lost from *Isoxya*, but the precise reason(s) for this cannot be determined without detailed ecological and behavioural studies. Suggestions have also been made that the smaller size of Gasteracanthinae males might be to avoid sexual cannibalism.

4.3 Evolution of prominent sexually dimorphic characters

This section discusses two specific sexually dimorphic character trait hypotheses relating to female Gasteracanthinae. The long abdominal spines in *Gasteracantha* and *Macracantha* evolved multiple times and that the ventral tubercle is present within the Gasteracanthinae clades that exhibit the greatest sexual size dimorphism. Again, these traits are mapped onto a molecular phylogeny and the results are discussed individually

4.3.1 Large median abdominal spines

Female gasteracanthines possess highly sexually dimorphic abdominal spines and it is currently unknown why, in particular the medial spines, have evolved. The current thinking is that they offer protection against predators by acting as sharp and harmful structures that can hinder the storage of these spiders in the nests of mud dauber wasps (Edmunds and Edmunds 1986; Cloudsley-Thompson, 1995; Elgar and Jebb, 1999). This was also proposed for *Micrathena* (Peckham, 1889; Edmunds and Edmunds, 1986; Cloudsley-Thompson, 1995). Egg-carrying females can be slower moving and can suffer higher predation rates (Okada *et al.*, 2021). In the Gasteracanthinae the sclerotised abdomen surface also offers a defence for females against wasp predation, but the abdominal patterns appear to be related to prey capture and not aposematic colouration (White and Kemp, 2015, 2016; White, 2017; Messas *et al*., 2021; Kemp, Edwards and White, 2022). The females are likely to have evolved anti-preadtor measures because they are larger and live longer than the males and carry the eggs. The spines are also probably not part of an elaborate camouflage system to hide alongside debris in orb webs like *Cyclosa* spp. do (Gan *et al*., 2010) as abdominal spines do not blend into the web structure and gasteracanthines are not known to place debris in their webs.

The spines are visually prominent and an instantly identifiable characteristic of the Gasteracanthinae. The size of the abdominal spines, as discussed in Chapter 2.3.5, can range from small protrusions through to spines that may be 3-4 times longer than the width of the abdomen. Some species have spines as long, thin structures and others shorter, stout protrusions with varying degrees of curvature (see Figure 4.7).

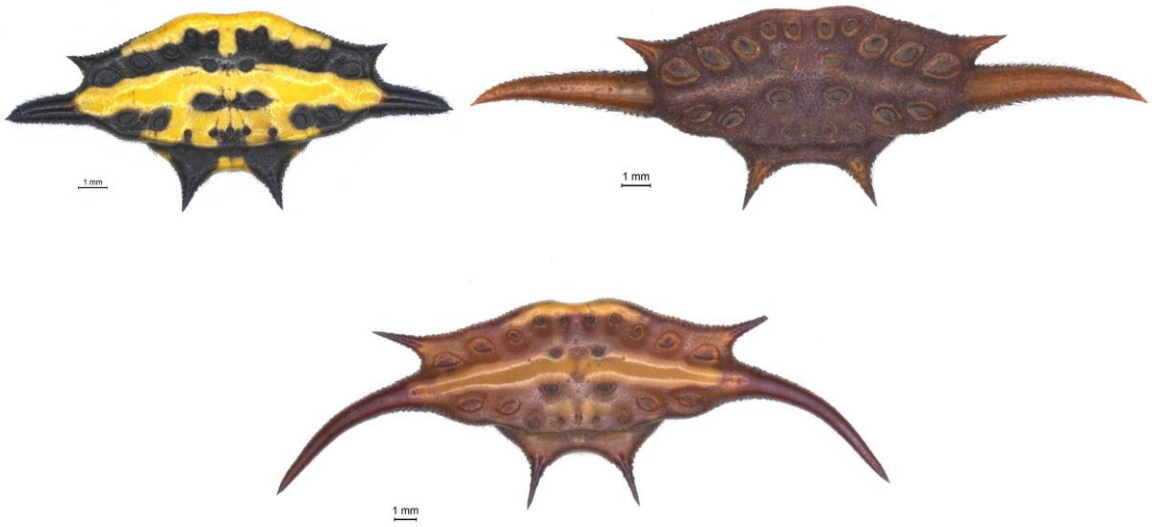

Figure 4.7 Examples of *Gasteracantha* median abdominal spines. Left to right, top to bottom: Dorsal view of *G. rhomboidea madagascariensis* Vinson, 1863 abdomen (short spines), dorsal view of *G. versicolor formosa* abdomen (long spines), dorsal view of *G. milvoides* abdomen (long spines).

4.3.2 Methodology

Magalhães and Santos (2012) used exact measurements for scoring the spines in their morphological *Micrathena* phylogeny; characters 10 and 11. In the morphological analysis in this study (Chapter 2) a less than or more than method was used, defined by fixed points on the abdomen. The measurement ratio chosen for the median spines was related to abdomen width with the state (0) Less than half of abdomen width; and the state (1) Equal to/more than half of abdomen width. The variation of exact spine length within species can be high in some Gasteracanthinae, but this ratio remains consistent between mature individuals of the same species. Here in this examination of median abdominal spine length 'large' is quantified following state (1) (character 17, Appendix 3.2.1) where the spine is equal to/more than half of abdomen width as was scored during the morphological analysis. Spine length was mapped onto the combined molecular (CO1+16S) phylogeny (Figure 4.8). This tree is used to map the evolution of the median spines which are the largest of the three pairs of abdominal spines in most Gasteracanthinae.

4.3.3 Results

Long median abdominal spines are present in two clades within the Gasteracanthinae. The more derived species of the '*Gasteracantha* 1' clade possess the character with no loss. This state was also present in most of the Oriental and Australasian species that were in the '*Gasteracantha* 1' clade in the Group 1 morphological analysis (Figure 2.17 and 2.18).

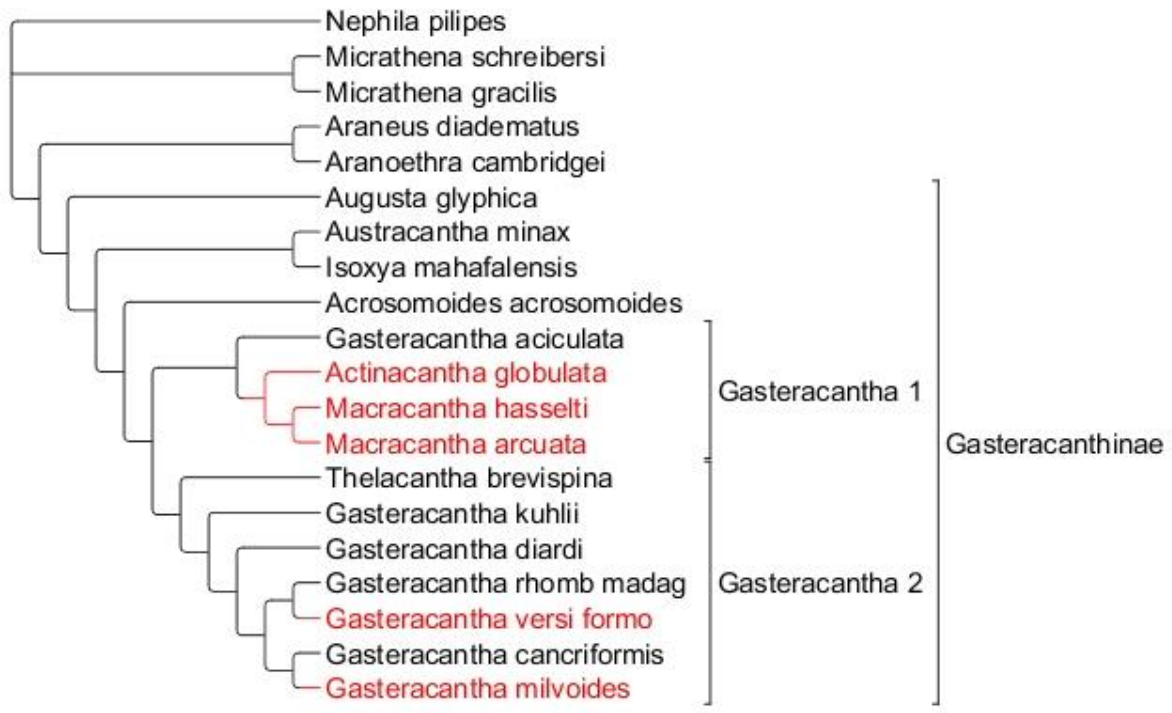

Figure 4.8 Copy of combined (CO1+16S) molecular BI analysis 50% majority rule consensus tree. Gasteracanthinae species with long medial spines (red), no spines or small spines (black). Constructed in TreeGraph2.

The other *Gasteracantha* that show long median spines are in the more derived species of '*Gasteracantha* 2'. Along with *Gasteracantha curvispina* (Guérin, 1837) and *Gasteracantha falcicornis*, from the Group 1 morphological analysis (see Figure 2.17 and 2.18), these are the only Gasteracanthinae with long median spines not located in '*Gasteracantha* 1'.

4.3.4 Discussion

Long median abdominal spines occur in the derived species in the '*Gasteracantha* 1' clade. They also occur in two species in '*Gasteracantha* 2'. Therefore, this character state appears to have evolved independently on two occasions. This supports the hypothesis from Chapter 1.5 that the long abdominal spines have evolved more than once. This has similarities to the

work on *Micrathena* abdominal spines by Magalhães and Santos (2012) who suggested that various abdominal spines have evolved independently in the genus.

Without ecological information, the cause or mechanism for the evolution of this trait are unknown. Future work should focus on examining the environment occupied by species with these long spines to discover common factors between the Oriental and African species that exhibit the character. For example, are all these species highly targeted by wasps and the spines mean they are unable to be placed inside mud burrows? The fact that long abdominal spines have evolved multiple times suggests there could be similar pressures acting in parallel within the two geographical regions.

In conclusion, this analysis has identified that the long abdominal spines in *Gasteracantha* and *Macracantha* evolved multiple times and supported the hypothesis from Chapter 1.5. This character was seen to exhibit homoplasy in the morphological analyses (see Chapter 2.5.2 and 2.6) but is a synapomorphy for the derived species in '*Gasteracantha* 1' (see Chapter 2.5.2 and 2.6). More species should be included in future analyses to discover where the long abdominal spines first evolve in the '*Gasteracantha* 1' clade and the African species that possess the trait.

4.3.5 Ventral Tubercle

This section tests the hypothesis from Chapter 1.5 that the ventral tubercle is present within clades of Gasteracanthinae that exhibit the greatest sexual size dimorphism ratio. This hypothesis was generated based upon the observation by various authors that the structure is utilised by small males when mating with much larger females. The ventral tubercle, present on many of the Gasteracanthinae, is a projection on the underside of the female, which the

male stands on or grasps during mating (Emerit, 1968; Levi, 2002; Bradley, 2013) (see Figures 4.9 and 4.10). This homologous structure is often used to discriminate between species in taxonomic keys.

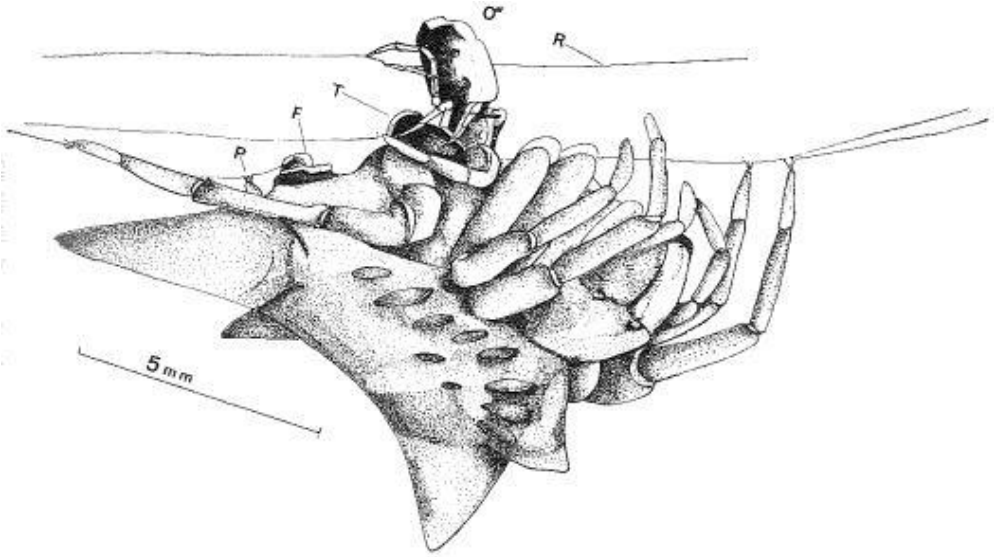

Figure 4.9 Line drawing of male and female *Gasteracantha versicolor* mating, from Emerit (1968)

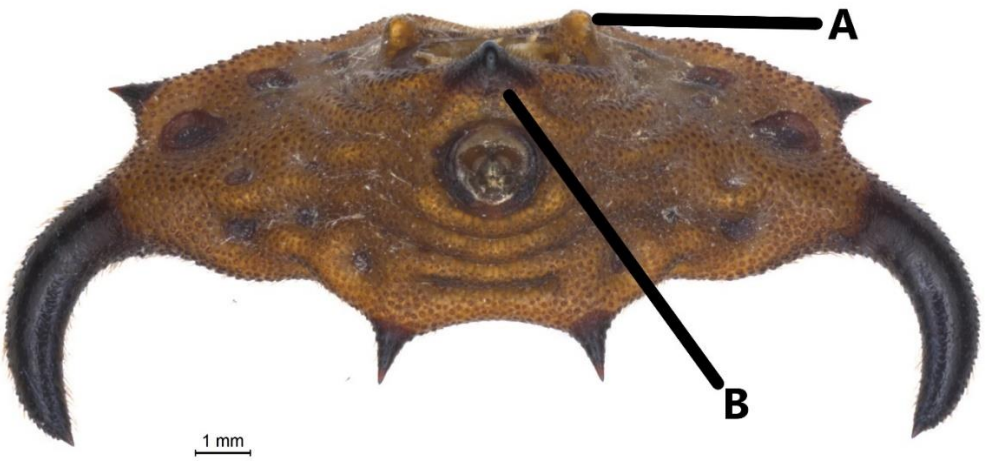

Figure 4.10 Copy of Figure A3.19 showing ventral condyles (A) and ventral tubercle (B) on the ventral side of *Gasteracantha thorelli* (♀).

4.3.6 Methodology

As with the medial spines, the character is mapped onto the combined molecular (CO1+16S) phylogeny.

4.3.7 Results

The character of the ventral tubercle is present within '*Gasteracantha* 2'. All the other taxa in this molecular phylogeny, except *Acrosomoides acrosomoides* lack this character.

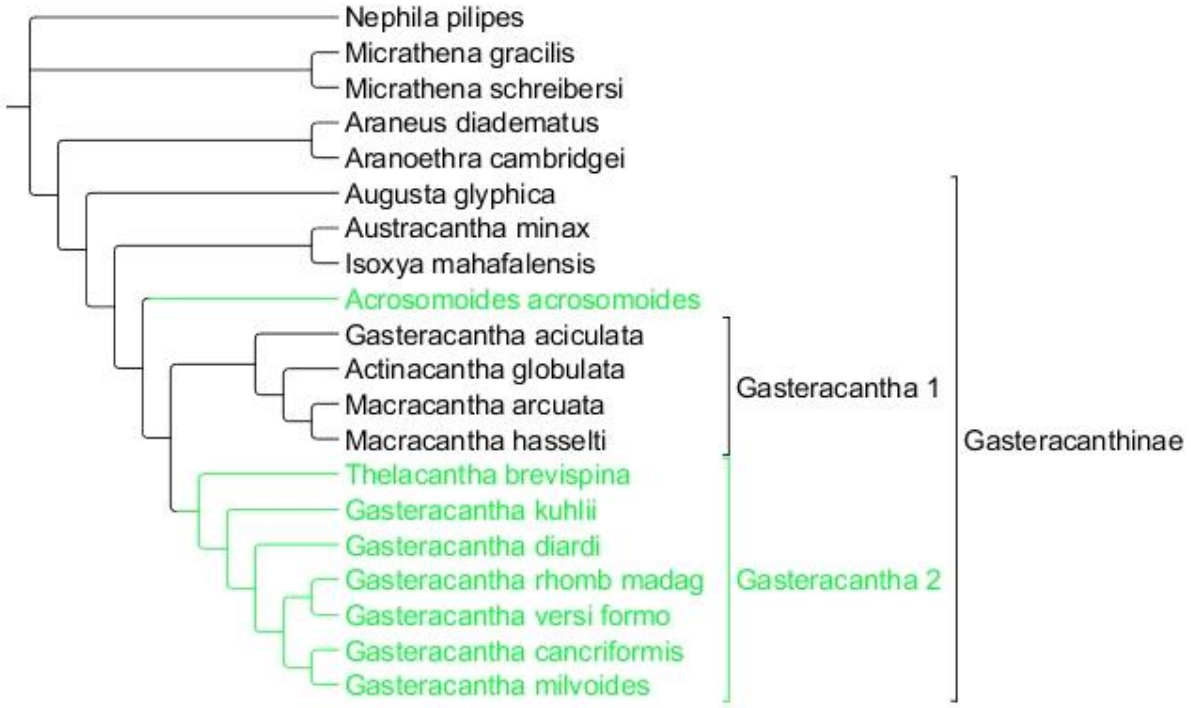

Figure 4.11 Copy of combined (CO1+16S) molecular BI analysis 50% majority rule consensus tree. Coloured to show species with ventral tubercle (green) and no tubercle (black).

4.3.8 Discussion

The presence of the ventral tubercle is almost entirely restricted to '*Gasteracantha* 2'. Its presence in *Acrosomoides acrosomoides* and absence in '*Gasteracantha* 1' may indicate that it is a character that evolved in a common ancestor and later was lost from '*Gasteracantha* 1'.

An alternative explanation would be that the ventral tubercle is not homologous within the two groups; although the tubercle is in the same location on all taxa that possess it. It does not occur in currently known *Isoxya* species (based on examination when generating the character matrix and known species descriptions).

When the results are examined alongside the eSSD data from section 4.1, the hypothesis that the Gasteracanthinae with the greatest eSSD ratio would possess the tubercle can be rejected as it is unsupported. The females in '*Gasteracantha* 1' do not possess the tubercle although they exhibit significant (>3.0-4.0) eSSD and *Austracantha* and some '*Gasteracantha* 2' species, with a smaller size ratio (c. >2.5), possess the tubercle. As seen in section 4.2, eSSD is exhibited throughout the subfamily, but there is no apparent correlation between the presence of the tubercle and the eSSD size ratio.

When the results of the morphometric analysis (Figures 3.10 and 3.11) are taken into consideration it appears that there is a correlation between abdominal shape and the presence of a tubercle. The rectangular species from '*Gasteracantha* 2', even when including the taxa that have unresolved relationships in the morphological analysis, all possess the tubercle. As discussed in Chapter 2, the presence of the tubercle is a synapomorphy of the '*Gasteracantha* 2' clade.

By contrast the taxa within '*Gasteracantha* 1', with either a triangular or hexagonal abdominal shape, do not possess this character (character 32, Appendix 3.2.1). The venter of taxa with triangular and hexagonal abdomens is elongated with prominent wrinkles down to the spinnerets. It is possible that these wrinkles in members of '*Gasteracantha* 1' (for example *Gasteracantha scintillans* Butler, 1873 – see Figure A3.5) can act in place of the

ventral tubercle for the smaller males to stand on. Therefore, the need for a tubercle would be redundant. This theory could be tested by observing species that lack the tubercle, either in the field or under laboratory conditions, to discover where the male is positioned during mating.

Although there are apparent correlations between abdominal shape, geographical distribution and the presence of the tubercle, there are some exceptions. *Thelacantha* and *Acrosomoides* both possess the tubercle but do not have a rectangular abdominal shape. The Oriental species in '*Gasteracantha* 2' along with the African and New World species all possess the tubercle, so its presence is not related to geographical distribution either. Likewise large ratios of eSSD are not always accompanied by the presence of a tubercule in the female.

In conclusion, the tubercle is a character that appears to have been inherited from a common ancestor, before being lost in '*Gasteracantha* 1'. There are no correlations between the presence of the tubercle and eSSD ratios and the hypothesis that eSSD ratio would correlate to the character has been rejected.

4.4 Conclusions

The results presented in this chapter demonstrate that with a molecular phylogeny the biogeography of a group and the evolution of selected morphological characters can be hypothesised. In this work, geographic signals have been seen within the molecular phylogeny, for example the '*Gasteracantha* 1' clade has an Oriental and Australasian distribution, and an occurrence of long-distance dispersal has been identified from Africa to the New World. eSSD has been supported as lost within *Isoxya* while remaining present in the rest of the subfamily. The presence of long medial spines has been supported as evolving multiple times and links between eSSD and the presence of an abdominal tubercle have been tested and rejected. All these conclusions should remain tentative until more taxa with sequence information are added to the analyses, but this Chapter has demonstrated how hypotheses can be tested with a phylogeny.

Chapter 5: Conclusions and future work

5.1 Summary of thesis results

The aim of this work was to construct a phylogeny of the Gasteracanthinae using DNA sequence and morphological data. This could then be used to examine the species relationships and the validity of previously proposed relationships. As previous studies had proposed relationships by using molecular data limited by narrow geographic coverage (Tan *et al.*, 2019; Macharoenboon, Siriwut and Jeratthitikul, 2021), or were based on sometimes limited morphological criteria (see Chapter 1.2), a re-examination was timely. This also provided an opportunity for developing the wider understanding of a subfamily of under-studied arachnids once the phylogeny was constructed.

This study used sequence data for CO1and 16S, to construct phylogenies, based on each gene alone and in combination. These were then compared to morphological trees, inferred using some previously reliable characters and a larger group of novel characters. A combined molecular (CO1+16S) and morphology tree was also constructed. Phylogenetic trees were constructed using Bayesian Inference and Maximum Parsimony methods in all datasets.

Results showed the resolution of the molecular trees was strongest when the most taxa and the most rapidly evolving gene (CO1) were used (Figures 2.10 and 2.11). The trees inferred from 16S (Figures 2.12 and 2.13), a gene that probably evolves at a slower rate, did reveal deeper divisions and basal generic relationships within the phylogeny. A few species were placed in different clades in the 16S analysis compared to the CO1 analysis. A minority of species switched between clades, but these differences were minimal. The combined molecular trees (Figures 2.14, 2.15 and 2.16) provided similar resolution to the CO1 trees. The morphological trees (Figures 2.17-2.22) showed similar results to the molecular analyses, though relationships were less supported in most cases. This was anticipated from the number of scored morphological characters (66 informative characters) in relation to molecular sequence characters (390 informative characters).

The different phylogenies, despite using unique datasets, were broadly consistent. Once they had been constructed the hypotheses surrounding relationships, presented in Chapter 1.5, were tested. The hypothesis that Gasteracanthinae would be recovered as a monophyletic group was tested and supported. The analyses also supported previous studies (for example Scharff *et al*., 2020) by confirming that *Micrathena*, here used as an outgroup, is not closely related to the gasteracanthines. The hypothesis that *Augusta* should not be regarded as a gasteracanthine was rejected following the molecular analyses. The genus should still be considered a gasteracanthine based upon these findings but warrants further examination to confirm the exact placement and its relationship to the other Gasteracanthinae.

In molecular analyses the clade '*Isoxya*' was recovered from the CO1 dataset, and two main clades were identified within the Gasteracanthinae ('*Gasteracantha* 1' and '*Gasteracantha* 2') in all three datasets. The composition of these two clades was also broadly consistent between analyses. The clades '*Isoxya*' and '*Gasteracantha* 1' were also recovered in the morphological analyses, but '*Gasteracantha* 2' was not due to lack of support. However, the morphological studies did identify additional distinct clades where molecular data could not be obtained. These were a '*Thelacantha*' clade, residing within '*Gasteracantha* 2', and an '*Acrosomoides*' clade. There were some Gasteracanthinae genera for which it was not possible to confirm their phylogenetic placement using morphology. These were *Aetrocantha*, *Afracantha* and *Togacantha*. These genera still require more examination to discover their relationships within the subfamily and should be included in future molecular analyses when sequences can be obtained either through fresh material or other DNA extraction methods (see section 5.2).

Whilst these results demonstrate that phylogenies can differ when different datasets are used, some universal conclusions can be drawn from these analyses. These include the support of the hypothesis that *Gasteracantha* is paraphyletic due to the inferred relationships of other genera. In addition to this, analyses results rejected the hypothesis that *Thelacantha* is sister to *Gasteracantha* by the placement of this species within '*Gasteracantha* 2'. The results indicated that a taxonomic change of *Thelacantha* back to *Gasteracantha* is required. This would ultimately create a monophyletic '*Gasteracantha* 2' clade.

The results of all analyses have also indicated that major taxonomic reclassifications will be required for the taxa within '*Gasteracantha* 1'. This includes support for the hypothesis that *Macracantha* is synonymous with *Gasteracantha*, due to the relationship of the genus to

Gasteracantha species. This study discovered that *Actinacanatha* should be synonymised too. Discussion of these reclassifications can be found in section 5.2 along with diagnoses based upon the phylogenetic placement.

The phylogenies were not restricted to relationship-based hypotheses. A 2D morphometric study of female abdominal shape was used in conjunction with the molecular phylogenies to test the hypothesis that closely related species would share the same abdominal shape. A Principal Component Analysis revealed four basic abdominal shapes, but the hypothesis was rejected as not all species within clades exhibited the same abdominal shapes.

The biogeography of the subfamily was examined to test the hypothesis that species found in the same geographical region would be closely related to each other due to short-range dispersal. '*Gasteracantha* 1' has an Oriental and Australasian distribution. The clades of '*Gasteracantha* 2' also demonstrated many examples of close-range distribution. However, the study also revealed an occurrence of long-range dispersal as the New World species *Gasteracantha cancriformis* was located within a clade of African taxa. It is apparent from the findings that more work is required to discover how many long-range dispersal events have occurred. The geographical origins of the Gasteracanthinae are worthy of future study too.

Extreme sexual size dimorphism was also quantified and plotted onto the CO1 molecular phylogeny. The hypothesis that *Isoxya* would exhibit a loss of eSSD, while the remaining gasteracanthines would still show this trait, was accepted. This supported other studies (for example Hormiga *et al*., 2000; Kuntner and Coddington, 2020) and expanded upon their findings with additional data, ratios and genera included here. The reason for the loss of eSSD must remain speculative until more behavioural and ecological information on Isoxya becomes available. The addition of other gasteracanthine genera to the phylogeny might reveal other gasteracanthines that have lost the eSSD trait too.

Finally, hypotheses around specific morphological characters (the long abdominal spines and the ventral tubercle) were tested. The hypothesis that the long abdominal spines, present in some *Gasteracantha* and *Macracantha*, evolved multiple times was supported. When compared to the biogeographical analysis, there is a suggestion that an ecological pressure is the reason for the long spines. Taxa in the Oriental, Australasian, and African regions exhibit this character. Then the hypothesis that the ventral tubercle, associated with the mechanics of mating small gasteracanthine males (Emerit, 1968; Levi, 2002; Bradley, 2013), would be present in the clades which showed the greatest eSSD ratios, was rejected. There were no correlations between the eSSD ratios and the clade in which taxa that possess the tubercle are found. However, there was one instance of the character being lost, or evolving independently as a non-homologous structure, that could be explored in the future.

This thesis has constructed the most comprehensive gasteracanthine phylogenies to date and used them to test relationship-based, biogeographical, and character-based hypotheses. Following the results of these analyses, areas of future work have been highlighted and new hypotheses to test have been discovered. These, along with future approaches to data gathering for phylogenetic studies, are discussed in section 5.2.

This thesis provides a framework and a base for future work on the Gasteracanthinae. The molecular and morphological datasets (see Appendix 3) will be invaluable to any future study of the Gasteracanthinae. Although more data is required before a phylogeny of the complete subfamily can be constructed, it is hoped that this thesis provides a robust starting point for this goal. The results here have also provided a phylogenetic background to any forthcoming ecological and behavioural studies in the group. However, the methods used, results, and hypotheses tested should not be regarded as restricted to this subfamily.

This thesis highlights the importance of morphological data to identify clades when molecular sequences are unavailable. Any phylogeny which requires the inclusion of taxa that might lack molecular data could explore the morphological methods presented here. However, the phylogenies based upon molecular datasets were superior in the number of informative characters and support for the inferred relationships. Additionally, excluding molecular data will not provide full resolution in many cases and an attempt at molecular phylogenetics should always be attempted as priority. However, the similarities between the two datasets should be viewed as a positive result for the morphological data.

This thesis has conducted a morphometric analysis using fixed landmarks that could be applied to all spiders, if the sigilla are conspicuous or can be located on spiders with soft

abdomens (see 5.2). In addition to this biogeographical analysis has revealed a case of long-range dispersal between Africa and the New World. Sexually dimorphic characters have been examined and gasteracanthines could be used as a group to explore the eSSD phenomenon or other extreme character dimorphisms.

This thesis has demonstrated what is possible once a phylogeny has been constructed. Hypotheses can be tested in conjunction with an understanding of the phylogenetic relationships between the subject taxa. Discussions can elaborate upon more than the results from these tests as species relationships or biological traits are incorporated. By combining all these aspects, a greater understanding of the taxa can be achieved. This can be applied to any group, and it is hoped that this thesis will encourage research into many areas of biology.

5.2 Future work

This section outlines future work that has been identified during this thesis and future hypotheses that could be tested. Some of these suggestions will require a comprehensive phylogeny of the subfamily prior to testing and this is discussed first.

5.2.1 DNA extractions and future morphological characters

Whilst the major findings from the phylogenetic analyses are statistically robust, they should still be regarded as tentative. There is a need to include more taxa, and more molecular markers. Here only two markers were used, with CO1 extractions more successful than the 16S extractions and more CO1 data available externally. This meant phylogenies using 16S data were limited to the number of species that had sequences and taxa with only CO1 sequences were removed.

The success of the CO1 extractions had a significant positive weighting towards samples that had been stored in 96% ethanol, stored at -20°c (Table 2.1) and collected within the last 5-10 years prior to sampling. However, specimens of various ages yielded some genomic data. Specimens stored in 70% ethanol at room temperature sometimes yielded genomic data, but less than the specimens stored in 96% ethanol. The samples that were from museum collections yielded no sequence data during this process. This is likely due to the lower ethanol percentage, storage conditions and specimen degradation over time. The differences between the storage conditions or ethanol percentage in the samples that yielded 16S were the same as in the CO1.

As DNA extraction from museum specimens is largely dependent on preservation methods (Burrell, Disotell and Bergey, 2015), a concerted effort to use fresh specimens should be

made. Alternatively, a method of extracting genomic data could be explored for use on specimens where no fresh material can be collected. Genomic data from museum specimens could fill crucial sample gaps and provide a more comprehensive geographic sampling of taxa. The results of this thesis have already highlighted the importance of a wide geographical range of taxa for understanding gasteracanthine relationships. Historical museum specimens are also the only source of genetic data for type specimens and for most rare taxa. These specimens can aid in resolving long-standing taxonomic uncertainty (Roycroft *et al.,* 2022).

DNA from museum specimens is often highly degraded and fragmented, so extraction and analyses are becoming focused on shorter amplicons to extract sequences that can then be combined to create a longer sequence. Success has been seen over recent years (Rohland, Siedel and Hofreiter, 2010; Burrell, Disotell and Bergey, 2015; Raxworthy and Smith, 2021; Roycroft *et al.,* 2022) and now genomic sequence data is being generated from specimens up to 180 years old (Roycroft *et al.*, 2022)

As degraded DNA extracts have an average molecule size of 100-200bp a PCR approach will need to target regions <300bp or even <200bp to mitigate allelic dropout (Burrell, Disotell and Bergey, 2015). These short sequences can then be combined to create a larger sequence suitable for phylogenetic analysis. This might be most successful with CO1 extraction because mitochondrial DNA is present in relatively large quantities within living cells by contrast to nuclear DNA (Rohland, Siedel and Hofreiter, 2010). However, this approach can be expensive and time consuming. There is also a risk of contamination when working with degraded DNA (Burrell, Disotell and Bergey, 2015), as was experienced in this study.

There are also more options, for example whole genome shot sequencing can work on museum specimens (yielding 40%, or higher, of expected genomic coverage in relation to fresh material) and result in data suitable for systematics (Burrell, Disotell and Bergey, 2015). Alternatively, RAD-Seq techniques, using restriction enzymes to fragment DNA before sequencing the fragmented products (Burrell, Disotell and Bergey, 2015; Raxworthy and Smith, 2021) or next-generation sequencing, using fragmented DNA which is then sequenced simultaneously using a single PCR (Fletcher, 2019), might be an option for historical museum specimens.

It is clear a DNA extraction method that optimizes DNA yields from low-quality samples will be required for producing a comprehensive phylogeny of Gasteracanthinae. Getting the right approach and method of extraction could be time consuming but if sequences can be recovered from irreplaceable museum specimens, then it will be worthwhile. This is a rapidly evolving field and should be explored for use in future studies.

For a comprehensive phylogeny a minimum of 5 genes should be used, as in Scharff *et al*. (2020), but ideally the whole genome. The whole genome is yet to be sequenced in gasteracanthines, but has been used in other phylogenies, for example in Diptera: Cameron *et al.* (2007) and Shi, Li and Li (2021). Additionally, more original molecular data should be collected to avoid identification errors from GenBank and BOLD data (Meiklejohn, Damaso and Robertson, 2019; Pentinsaari *et al*. 2020). In this study, sequences from reliable researchers, or reliable published works, were prioritised. The other option is to obtain the specimen for identification.

Fortunately, minimal numbers of sequences could not be confirmed as reliable or could not be cross-checked to other identified taxa's sequences, but some species were excluded from those that were available. For example, the only available sequence of CO1 for *Gasteracantha geminata* (Fabricius, 1798) (MH194248), listed as "UNVERIFIED", was deemed unsuitable as the specimen could not be confirmed as being correctly identified. This meant that the species did not feature in any molecular analyses.

An additional issue with sequences from these sites is that an examination of sequence quality cannot be performed for GenBank sequences because the site does not store chromatograms. BOLD does occasionally have the chromatogram available, so adjustments can be made. However, without this option reliance must be made on the data being interpreted accurately by the uploader. Data concerning the primers used is available, but this does not provide information on how successful or unsuccessful the extractions were or how much baseline noise, for example, was present in each chromatogram. Material correctly identified and extracted by trusted sources must be a priority.

The collection of fresh material for molecular extractions might also highlight new species or unknown males. Male *Gasteracantha* provided viable characters for the morphological analyses. Without these a full morphological phylogenetic analysis of the gasteracanthines will always be lacking (see Chapter 2). Despite extensive exploration of museum and private collections, there was a scarcity of males for analysis. Due to the limited number of males currently known, and publications featuring palp illustrations focused on the open ventral view and median apophysis, there is potential for new characters to be discovered. Males need to be located for around 80% of the gasteracanthine taxa.

There were some currently recognised Gasteracanthinae taxa where no mature specimens could be obtained. For example, the 4 *Gastroxya* species (World Spider Catalog, 2022) and *Hypsacantha*, were not found in any of the institutions or collections that were accessed. Although unavailable to this study, these genera should be included in future studies. The phylogenetic placement of the *Gastroxya* species is currently unknown and not easily hypothesised based on gross morphology. As with *Augusta*, they may ultimately be located at the base of the gasteracanthine clade. *Hypsacantha* and *Gastroxya* could also provide more data around the loss of eSSD in the gasteracanthines. Without including them, this remains unknown. The missing species from the New World (*Afracantha camerunensis, Aspidolasius branicki, Gasteracantha cancriformis gertschi* and *G. flava*) should also be included to discover if there are multiple long-range dispersal events from Africa (see Chapter 4).

If data from more sequences, taxa and males can be generated as outlined above, then a comprehensive phylogeny of the gasteracanthines can be inferred. This will then enable the future work discussed in the following sections to be undertaken.

5.2.2 Reclassification proposal

The analyses in Chapter 2 have revealed the possibility that a large taxonomic change is required for the species within the clade of *Gasteracantha* which is split into '*Gasteracantha* 1' and '*Gasteracantha* 2'. *Gasteracantha* was supported as paraphyletic due to the inclusion of *Actinacantha*, *Macracantha* and *Thelacantha* in clades containing *Gasteracantha* species in all analyses. The key question from the results is whether *Gasteracantha* should be split into two subgeneric groups within the same genus, or if '*Gasteracantha* 1' and '*Gasteracantha* 2' need to be taxonomically reclassified as separate sister genera. As the relationships currently stand, in a classification system the two clades should remain as one

genus. This contradicts the work of Macharoenboon, Siriwut and Jeratthitikul (2021), who were quick to assign a generic change of *Gasteracantha hasselti* to *Macracantha* without the inclusion of many taxa that shared morphological characters and a close geographical distribution.

Due to the phylogenetic location of the taxa, the following reclassification is therefore proposed. *Gasteracantha* is divided into two subgeneric groups: *Gasteracantha (Gasteracantha),* which would be referred to as *Gasteracantha* sensu stricto., and *Gasteracantha (Macracantha)*. The following species would then be synonymised with *Gasteracantha*: *Thelacantha brevispina = Gasteracantha (Gasteracantha) brevispina*; *Actinacantha globulata = Gasteracantha (Macracantha) globulata*; *Macracantha arcuata = Gasteracantha (Macracantha) arcuata*; and *Macracantha hasselti = Gasteracantha (Macracantha) hasselti*.

Taxonomic diagnoses of the two *Gasteracantha* subgeneric groupings are presented here. *Gasteracantha (Gasteracantha)* can be diagnosed with the following characters: females possess a ventral tubercle; males possess a conspicuous embolus and a smooth anterior edge to the median apophysis. *Gasteracantha (Macracantha)* can be diagnosed with the following characters: females possess an extremely elongated spinneret tubercle; males possess a terminal apophysis and a rough or toothed anterior edge to the median apophysis.

Prior to these reclassifications being published, more taxa should be included in a comprehensive molecular phylogeny to avoid making any incorrect taxonomic changes. The hypothesis would be that the proposed reclassification would be accepted based upon a comprehensive molecular phylogeny and the morphological taxonomic diagnoses presented above. There is morphological support for the changes, but further molecular support is required to confirm these relationships. It has been proved in this thesis that morphology alone, and a small number of sample taxa, cannot resolve the relationships within this subfamily.

5.2.3 Biological and ecological work

By generating a phylogeny, this thesis has been able to test various hypotheses and discover various areas of future work. The Gasteracanthinae are an interesting group of orb weavers with many biological and ecological study applications that are yet to be explored.

Gasteracanthine mating behaviour, as discussed in Chapter 4, could reveal more about the eSSD phenomenon. An examination of mating in gasteracanthine species that lack the ventral tubercle, that is then compared to mating between those who do possess a tubercle, could provide the answer to why the tubercle has been lost within *Gasteracantha* and why gasteracanthines exhibit eSSD. In addition to this, *Isoxya*, with a loss of eSSD, requires detailed ecological and biological studies to discover if there are behavioural traits that have caused the loss. This could correlate to a wider study on invertebrates who either exhibit or have lost eSSD or SSD traits. Comprehensive work has already been conducted into nephilid sexual dimorphism. The construction of a gasteracanthine phylogeny offers an opportunity to replicate these studies within the Araneidae, targeting the bigger subfamilies, like Gasteracanthinae, that exhibit eSSD.

An opportunity could arise to simultaneously examine the defence mechanisms of species while during current research work. For example, during studies monitoring and examining species abdominal colour lures, colour morphs success against chromatic and achromatic backgrounds, and how the time of the day, abdominal patterns, or the angle of the spider when in the web can affect prey capture (White and Kemp, 2015, 2016; White, 2017; Messas *et al*., 2021; Kemp, Edwards and White, 2022). While species are under field observation or being collected for studies in laboratory conditions, tests could be undertaken to discover the exact purpose of the abdominal spines and sclerotization of the abdomen. Many previous studies have only presented hypotheses and an exact answer is yet to be confirmed, beyond the defence against mud dauber wasps.

Additionally, the work on colour lures should examine the unique species endemic to the Solomon Islands that possess the metallic abdominal colouration (see Chapter 2.5 and Figure A3.5). The reason behind the metallic colouration would be fascinating to discover and could further enhance the wider topic of colour lures. These species would also fit studies exploring the reason for the long abdominal spines, overlapping with defence mechanisms.

There is clearly an opportunity for large ecological and environmental studies to explore the relationships between gasteracanthines and their environment. Questions arise and hypotheses could be tested within areas of high speciation, like Madagascar, to discover if, the key to Gasteracanthinae species co-existence may be due to fine-scale microhabitat partitioning and/or prey specialisation as hypothesised in Kemp, Edwards and White (2022).

Finally, the long-range dispersal of *Gasteracantha cancriformis* should be explored and, by including the remaining New World taxa, the possibility of multiple dispersal events from Africa to the New World can be supported or rejected. These are just a selection of future studies that would enhance the understanding of the subfamily or wider scientific topics.

5.2.4 Morphometrics

Within this study work a novel method of generating 3D images for morphometric analysis using micro-computed tomography (micro-CT) was trialled. This method offers opportunities to develop upon the morphometric study in Chapter 3. Micro-CT scanning is an established method for generating images to use in morphometric analysis and other analyses across many taxa (Shi, Westeen and Rabosky, 2018, Ege *et al*. 2020, Günther *et al*. 2021). Spiders have been micro-CT scanned for various studies, including creating 3D images of fossilised arachnids (Garwood *et al.*, 2016), 2D images of eye structure (Fenk *et al.*, 2010), 3D images of eye structure (Sumner-Rooney, in prep.) and silk (Weissbach, Neugebauer and Joel, 2021). This was the first time this method of image generation was attempted for a morphometric analysis of spider abdominal shape.

Micro-CT uses X-rays to see outside and, more typically, inside objects by image slices. These 2D slices can then be used to either view internal structures as individual slices or, as in the case here, processed and then rendered into 3D models for analysis of either external or internal structures. 3D rendering is a computer graphical process that can be used to create a 3D model in a digital file format. Micro-CT provides a usually non-destructive method of viewing the structure of objects or organisms and it also provides a way of creating 3D images that then can be further processed (Simonsen and Kitching, 2014). Occasionally samples may require staining to enable clearer projections (Gignac *et al*., 2016).

Micro-CT is comparable with medical CT scan imaging but on a much smaller scale with a much higher resolution (Postnov *et al.*, 2006). The micro-CT scanner works by generating X-rays which are then transmitted through the sample and recorded as a 2D projection image. This is then repeated as the sample is rotated until the whole sample has been imaged in these slices. The slices can then be reconstructed and rendered into a 3D graphical model which can offer information that normally would require specimen destruction (Ngan-Tillard and Huisman, 2017). Quantitative data for comparison between samples can easily be obtained from micro-CT scanned images, for example simple measurements in a morphometric analysis through to examining bone density in humans, and this makes it useful for many fields of science (Simonsen and Kitching, 2014; Smith *et al*. 2016).

With the abdomen shapes that can be seen in various planes in *Gasteracantha,* 3D rendered models from a micro-CT scan of the spiders or abdomens can make fixing the landmark points easier. Multiple views that are hard to photograph, for example the lateral view (as seen in Chapter 3, Figure 3.2) which might have inconspicuous sigilla hidden inside the abdominal wrinkles, can be captured easily and the image can be enhanced to define the structures clearly. The examples seen in Figures 5.1-5.4) demonstrate how clearly the sigilla can be seen by simply adjusting the rendered specimen colour and enhancing the definition on the abdomen structures. It also provides the opportunity to produce high quality images of the rendered models that are eye-catching and useful for promoting the taxa to a wider audience including citizen science and outreach events.

Specimen handling and preparation is critical for this to be successful and, due to Covid-19 restrictions, accessing the specimens during this preparatory stage was impossible (see Appendix 4). This resulted in a data set with an insufficient quantity of specimens for analysis.

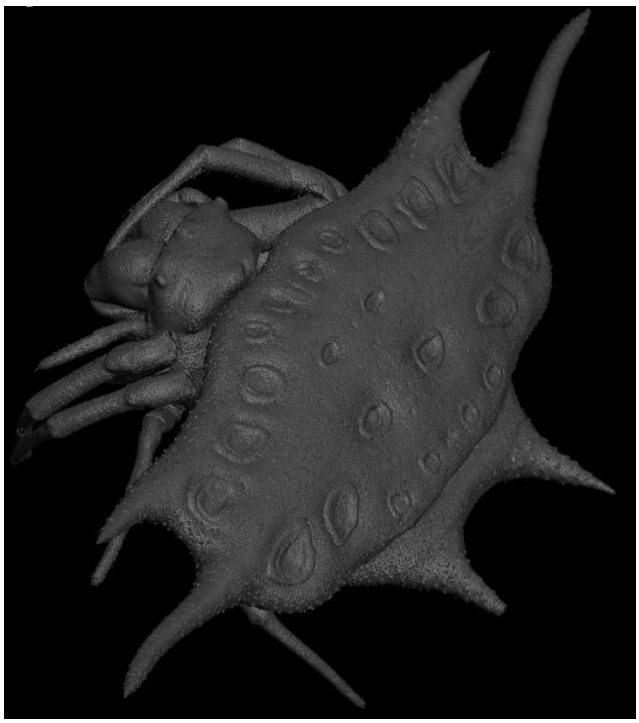

Figure 5.1 *Gasteracantha doriae* Simon, 1877 angled dorsal view. Micro-CT scan, rendered into 3D image using Amira 5 coloured in grey, dorsal abdominal sigilla clearly present.

All the figures (Figures 5.1-5.4) are examples of how clear the abdominal sigilla are on a rendered 3D *Gasteracantha* and how different angles can be simply achieved by rotating the 'floating' 3D spider in Amira 5 and exporting the desired image.

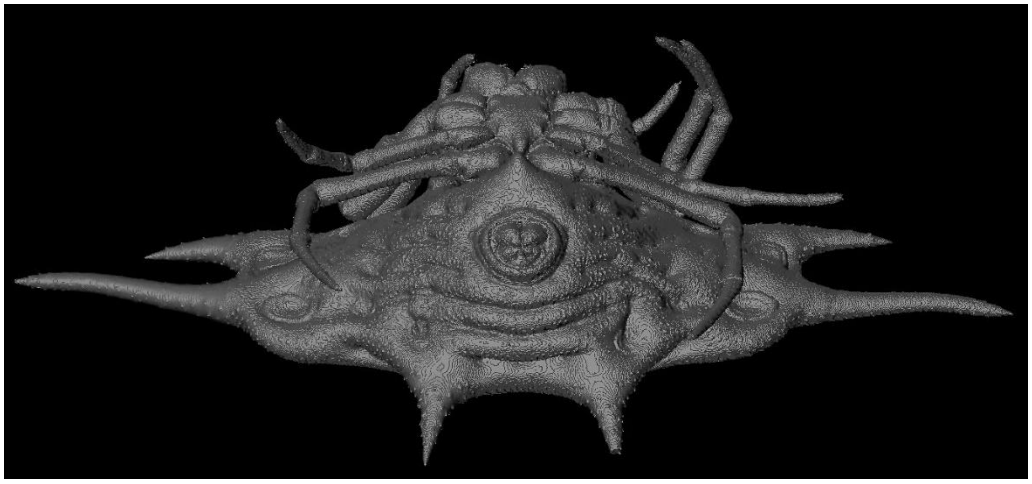

Figure 5.2 *Gasteracantha doriae* angled ventral view. Micro-CT scan, rendered into 3D image using Amira 5 coloured in grey, ventral abdominal sigilla clear - including within abdominal wrinkles - and suitable for morphometric analysis.

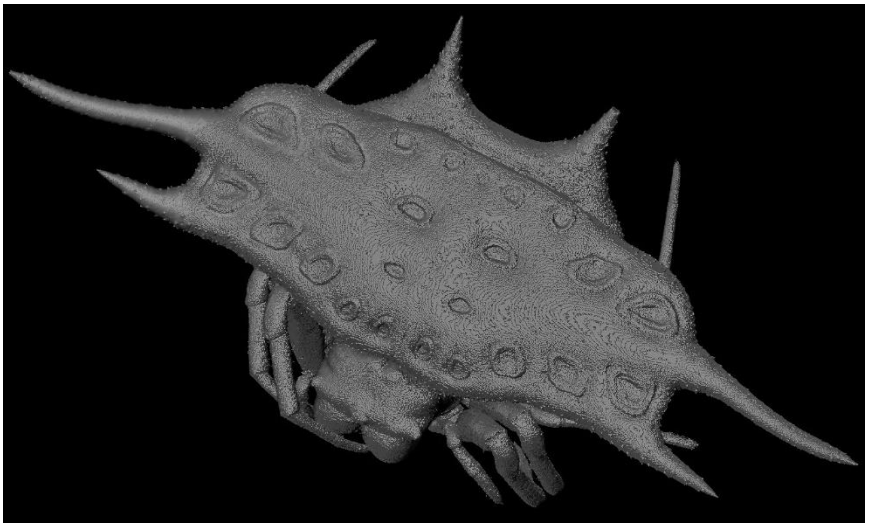

Figure 5.3 *Gasteracantha doriae* angled dorsal view. Micro-CT scan, rendered into 3D image using Amira 5 coloured in grey, dorsal abdominal sigilla clear, slight disturbance on dorsal surface but suitable for morphometric analysis.

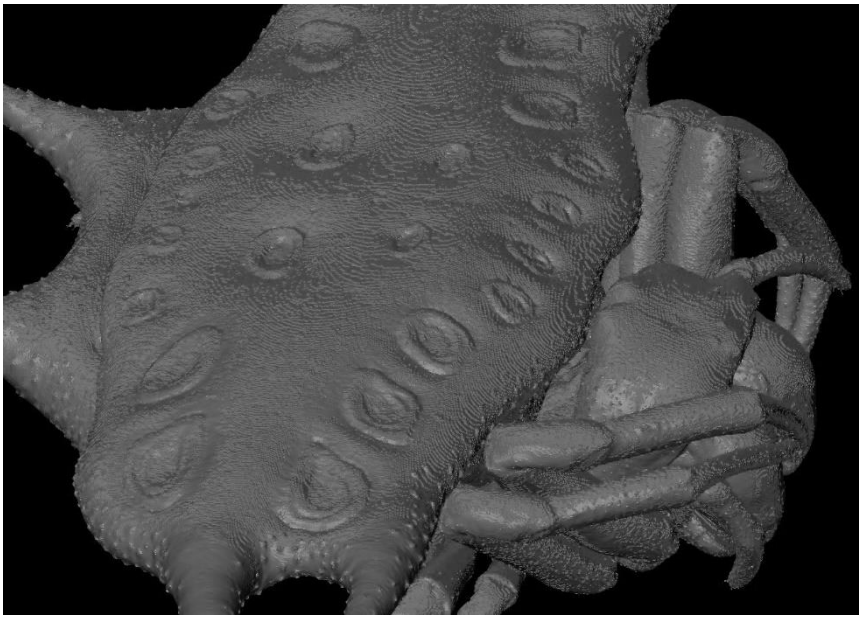

Figure 5.4 *Gasteracantha doriae* angled dorsal view. Micro-CT scan, rendered into 3D image using Amira 5 coloured in grey, zoomed in to highlight clear dorsal abdominal sigilla, slight disturbance on dorsal surface but suitable for morphometric analysis.

Although the 3 successful 3D scanned models were not used for analysis here, they do offer an alternative to the standard 2D photographic approach (see Chapter 3). The process of scanning will need to be honed, but it has highlighted a potential tool that should be explored in the future to assist in further investigations into the abdominal morphometrics of the group. Additionally, as highlighted in Chapter 3, most species have inconspicuous muscle attachment points, but these might become visible if the internal structure of the abdomens from species with inconspicuous sigilla could be visualised using micro-CT scanning. This would enable the techniques trialled in Chapter 3 to become viable for all spiders and open the option of morphometric analysis to many more taxa.

3D morphometric analysis could also be undertaken to examine the abdominal structures of *Gasteracantha* and other genera to discover if there are more viable shape-based characters. Micro-CT scanning can even be used to examine the male genitalia, as seen in Lepidoptera

taxonomy (Simonsen and Kitching, 2014), and could offer an alternative method of morphometric analysis for the median apophysis shape in *Gasteracantha* palps.

3D scanning also offers a more refined method for analysis using landmarks, compared to 2D studies. The use of three planes in a coordinate based morphometrics study allows the subtly of shape differences to be determined with more precision than analysis in two planes permits.

Although the trial highlighted some issues with the procedure, some exacerbated by the Covid-19 protocols, it also demonstrated how, when successful, the viability of 3D scanning for examining a complex character such as abdominal shape. The images above illustrate how the 3D scan of the taxa can be used to score a morphometric analysis in various views. The possibility for scoring male palp characters also becomes viable. As discussed in 5.2.2, specific shapes on the complex median apophysis could yield more morphological data. Quantitative characters could be scored from the palp if a 3D image was used. This could provide more morphological data in preparation for the complete phylogeny reconstruction.

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Appendix 1: Additional information from Chapter 2

A1.1 DNA extraction protocol (Chapter 2.2.3)

The DNA extraction protocol was modified from Miller, Dykes and Polesky (1988). First the removed legs were placed in a 1.5 ml tube with 200 µl homogenizing buffer (160mM Sucrose, 80mM EDTA (pH 8.0), 100mM Tris-HCl (pH 8.0), 0.5% SDS, 0.10mg/ml Proteinase K (Roche) with 1/100 14mg/ml Proteinase K added just before addition of the specimen material. The legs were then crushed with a pipette tip and incubated at 58°C on a heating block overnight. For DNA precipitation 100 µl of 4.5 M NaC1 per sample was added and mixed briefly. Then 225 µl of chloroform was added, mixed for 10 minutes and the sample centrifuged at 15000 rpm for 10 minutes. Approximately 100 µl of the supernatant was then transferred to a new 1.5ml tube, avoiding the interphase. The DNA was precipitated by adding an equal volume of 100% isopropanol, shaken thoroughly and immediately centrifuged at 15000 rpm for 10 minutes. The supernatant was then discarded, and the DNA pellet washed with 0.5ml 70% EtOH, incubated at room temperature for 15 minutes and centrifuged at 15000 rpm for 10 minutes. The supernatant was then decanted, and the DNA pellet dried at room temperature (for approximately 5 minutes) and then dissolved in 20-30 µl deionized water.

A1.2 Clustal Omega (Chapter 2.2.5)

Clustal Omega generates alignments by starting with two of the sequences and identifying all possible similar regions between them. These are usually the closest in the dataset. The alignment dataset is then built up by aligning either grouped aligned sequences or individual sequences with each other. This alignment follows the topology of a stepwise constructed guide tree, which is based upon the clustering of sequences (Sievers and Higgins, 2018). All sequences are compared to each other during this process. Then finally the MSA is produced using the progressive alignment HHalign package, aligning two profile hidden Markov models (HMM) which score the homology of the alignments (Daugelaite, O'Driscoll and Sleator, 2013; Sievers and Higgins, 2018) and make the alignments suitable for a phylogenetic analysis.

Alignment parameters for CO1 dataset

Input - DNA; Output - FASTA; Dealign input sequences - no [the sequences were not aligned prior to submission]; Mbed-like clustering guide-tree – yes; Iteration – yes; Number of combined iterations – default (0); Max guide tree iterations – default; Max HMM iterations – default; Order - aligned.

A1.3 MAFFT (Chapter 2.2.5)

In MAFFT, an initial alignment is constructed by the progressive method (Feng and Doolittle, 1987), where a rough distance between every pair of input sequences is quickly calculated and a guide tree is constructed before the input sequences are progressively aligned following the branching order of the guide tree (Katoh *et al*., 2005). Then the L-INS-i algorithm uses an iterative refinement, in which local pairwise alignment information (Smith-Waterman, 1981) is incorporated into an objective function (Katoh and Standley, 2013), to obtain a more accurate output alignment.

A variety of BLOSUM (BLOcks SUBstitution Matrix) matrices are available that can be used to assist in aligning evolutionary divergent sequences or sequences that contain gaps due to variable regions. BLOSUM assists in the evaluation of the alignments and clusters the closer related sequences based upon their percentage similarity following the scoring of the sequence positions. The BLOSUM62 matrix is set as the default scoring matrix, meaning the sequences in the BLOSUM will each cluster if they are 62%, or more, identical when two sequences are aligned (O'Connor, 2021).

BLOSUM80, where the sequences will cluster if they are 80% or more identical, is typically used for closer related sequences. However, because of the inclusion of non Gasteracanthinae taxa, the fact BLOSUM62 is the default because it can detect weak similarities (Henikoff and Henikoff, 1992) and that there was no clear difference between the two when tested in the sensitivity analysis, the default BLOSUM62 was retained.

During sequence alignment gaps may need to be inserted into the sequence to enable alignment (Mount, 2008). The gap open penalty is the cost for opening and inserting a gap in the alignment and the higher this value is the less frequent the gaps will be. The gap extension penalty is the cost for extending a gap by one residue. Again, increasing this value will make the gaps shorter but the terminal gaps, on either end, do not get penalised. The gap open and gap extension penalties of the default parameters both correspond with suggestions on where to set the gap penalties for closely related sequences (Katoh and Standley, 2016). Once again, various mixed values were tested in the sensitivity analysis to provide a range of options and there were very limited differences.

The separate parameter combinations alignments were generated individually and exported to their respective output files. The values tested were based upon sampling a wide a range of parameter options while not testing every single possible combination as this would have been overly time-consuming. Once a file was generated for each of the tested 42 parameter value combinations the data was run as an analysis in MrBayes to generate a phylogenetic tree using the protocol as listed in A1.5.8. These trees were then individually examined, and the clades shown in each recorded. A summary of these results of this analysis can be seen at the end of this section below.

The results of the sensitivity analysis showed 100% of trees displayed Gasteracanthinae separate from the outgroups and the relationships of the species within '*Gasteracantha* 2' (see 2.5.2) remained the same too in all tests. 93% of trees in the sensitivity analysis displayed the species relationships of '*Gasteracantha* 1' as in Figure 2.13. The only major difference in the sensitivity analysis was the positioning of the 4 single Gasteracanthinae generic representatives outside the *Gasteracantha* groups. In 67% of trees, the relationships were displayed as in Figure 2.13. The remaining 33% of trees kept the genera within the subfamily but added an additional node as one genus split from the others. Therefore, with the majority of trees following the same results, the default parameters were selected; this also aids in replication of results and informs future analyses parameters recommendations.

Table A1.1 Sensitivity analysis of 16S alignment

Sensitivity Analysis	BLOSUM Value	Gap Open Penalty	Gap Extension Penalty	Sensitivity Analysis	BLOSUM Value	Gap Open Penalty	Gap Extension Penalty
1.	62	1.0	0.10	**22.**	80	1.0	0.10
2.	62	1.1	0.14	**23.**	80	1.1	0.14
3.	62	1.2	0.20	**24.**	80	1.2	0.20
4.	62	1.3	0.25	**25.**	80	1.3	0.25
5.	62	1.4	0.30	**26.**	80	1.4	0.30
6.	62	1.5	0.14	**27.**	80	1.5	0.14
7.	62	1.6	0.20	**28.**	80	1.6	0.20
8.	62	1.7	0.25	**29.**	80	1.7	0.25
9.	62	1.8	0.30	**30.**	80	1.8	0.30
10.	62	1.9	0.10	**31.**	80	1.9	0.10
11.	62	2.0	0.14	**32.**	80	2.0	0.14
12.	62	2.1	0.20	**33.**	80	2.1	0.20
13.	62	2.2	0.25	**34.**	80	2.2	0.25
14.	62	2.3	0.30	**35.**	80	2.3	0.30
15.	62	2.4	0.10	**36.**	80	2.4	0.10
16.	62	2.5	0.14	**37.**	80	2.5	0.14
17.	62	2.6	0.20	**38.**	80	2.6	0.20
18.	62	2.7	0.25	**39.**	80	2.7	0.25
19.	62	2.8	0.30	**40.**	80	2.8	0.30
20.	62	2.9	0.10	**41.**	80	2.9	0.10
21.	62	3.0	0.14	**42.**	80	3.0	0.14

Alignment parameters for 16S dataset (Chapter 2.2.5)

Output Order – aligned; BLOSUM62; Gap open penalty - 1.5; Offset value (gap extension penalty) - 0.14.

A1.4 DAMBE (Chapter 2.2.5)

DAMBE7 (Xia, 2018) is a program that can perform various functions surrounding molecular sequences and phylogenies. Here it was used to test the saturation of the 3rd codon in the CO1 sequences used in the molecular analyses.

A1.4.1 Parameters

The CO1 matrix was imported into DAMBE and the 'Protein-coding Nuc. Seq.' option chosen with 'InvMtDNA (Trans_Table=5)' selected as this was the most suitable option for arachnid sequences. Unresolved bases were left as they were. The 3rd codon was then selected to work on, excluding the 1st and 2nd codons. The 'Measure Substitution Saturation' analysis was then selected following 'Xia, *et al.*' and all sites included. The 'transition and transversion' graph was generated using TN93 substitution model and is presented here (Figure A1.1). It is worth noting that the results did not differ when the 'Standard (Trans_Table=1)' option was selected or any of the other substitution models available.

A1.4.2 Results

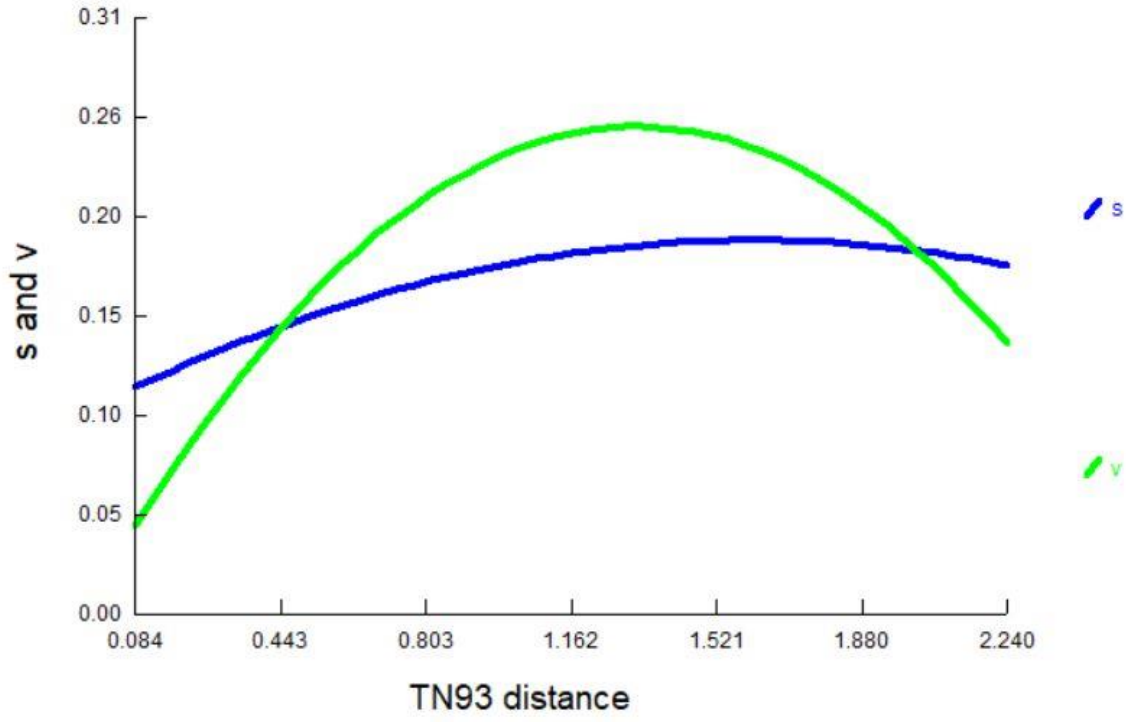

Figure A1.1 The graph displaying the results of the saturation test in the 3rd codon CO1 data. S = transitions (blue) and V = transversions (green).

The graph in Figure A1.1 shows the results of the saturation test. For sequences that are more genetically similar (lower TN93 distance) the number of transitions were greater than transversions. With greater genetic distance the number of transitions plateaus quickly, soon outnumbered by transitions. This indicates that there is saturation at the 3rd codon position (Chapter 2.5.1).

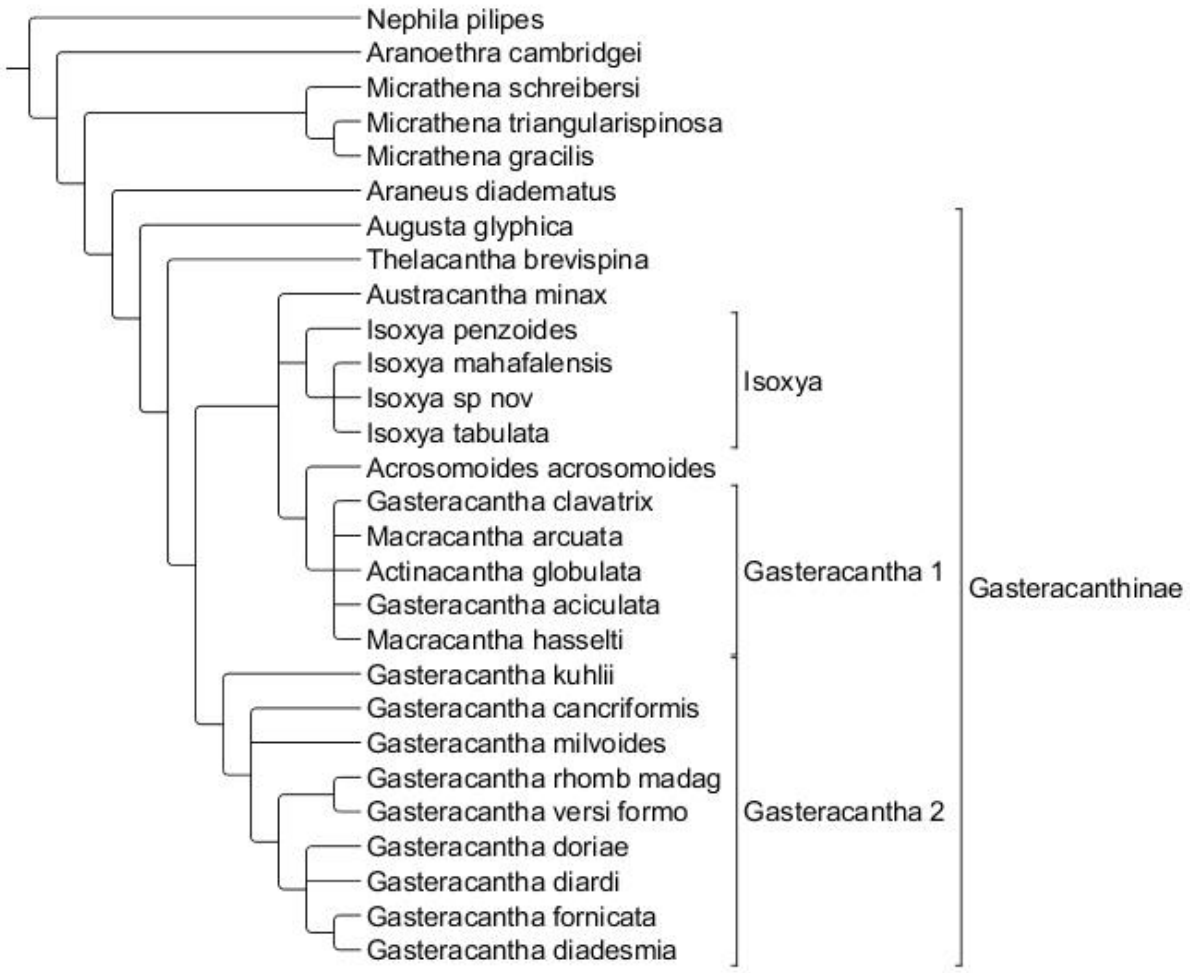

Figure A1.2 50% majority rule consensus tree of 131 equally parsimonious trees, inferred from maximum parsimony analysis of codon positions 1 & 2 in the CO1 data, generated in PAUP*.

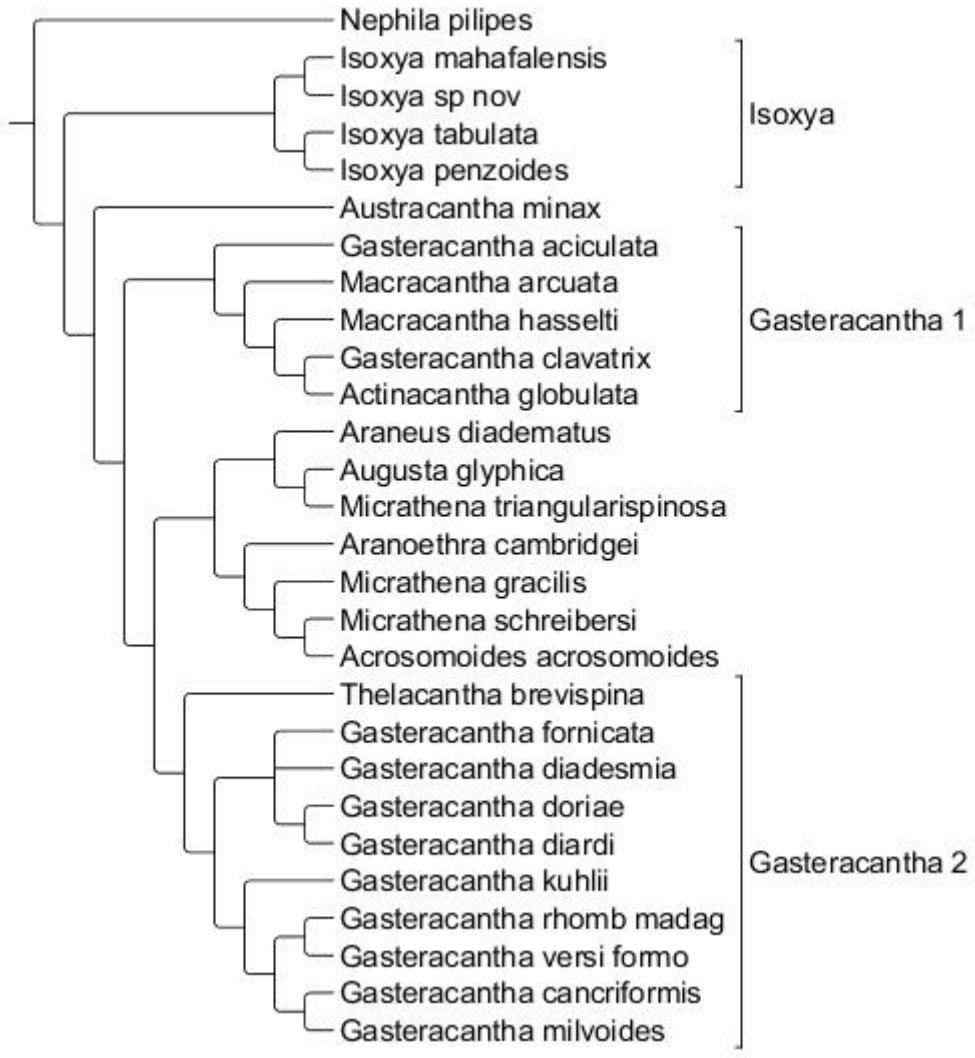

Figure A1.3 50% majority rule consensus tree of 2 equally parsimonious trees, inferred from maximum parsimony analysis of codon position 3 in the CO1 data, generated in PAUP*.

The trees inferred using 1st and 2nd codon positions (Figure A1.2) generated many more equally parsimonious trees but this was to be expected with less characters than the tree inferred from the 3rd codon position (Figure A1.3). Both the trees recovered the clades '*Gasteracantha* 1', '*Gasteracantha* 2' and '*Isoxya*' with the same Gasteracanthinae species in each. The relationship between these clades is equivalent and the difference is that the outgroup taxa have been recovered inside the '*Gasteracantha* 2' clade in the tree inferred from the 3rd codon position.

A1.5 Programs for phylogenetic analysis (Chapter 2.4.2)

The analyses of the morphological data were performed using PAUP*, TNT and MrBayes. The molecular analyses were performed using TNT and MrBayes. The combined analysis was performed on MrBayes. Information on each of the programs is provided in the next sections. Information on the morphological analyses is given first as the batch files were larger and this avoids repetition in the description.

The purpose of analysing the datasets with different methods was to rigorously test the data and provide the most comprehensive analysis to reveal the most informative phylogeny. TNT was used for the molecular analyses instead of PAUP* as TNT was able to handle the molecular data within the memory limitations and was significantly faster than PAUP* (see A1.4.3).

A1.5.1 PAUP*

PAUP* is a program used for inferring phylogenies using the optimality criterion of maximum parsimony. For the morphological analyses the data matrices were exported from Mesquite v.3.7 as nexus files, with the character states 0-3 and missing characters set as either '?' or '-' depending on whether the character exists in the species or not. However, under the parsimony criterion, PAUP* deals with missing characters by assigning, to the taxon, the character state that would offer the most parsimonious outcome in relation to its placement on the tree.

Next a batch file was created, an example of which is shown in A1.5.2, to provide details of the commands used and explain the process of the phylogeny construction. This was preferred to inputting commands line by line into the command line as it enabled multiple files to be stored and the information retained for each one, so analyses and methods could be compared.

A1.5.2 PAUP* batch file example

```
#nexus
Begin paup;
set autoclose=yes warntree=no warnreset=no increase=auto;
log start file=filename.log;
execute filename.nex;
cstatus full=yes;
outgroup Nephila_pilipes;
tstatus full=yes;
Set criterion=parsimony;
hsearch start=stepwise addseq=random nreps=100000 nchuck=2 chuckscore=2 swap=tbr;
hsearch start=current chuckscore=no;
set root=outgroup;
roottrees;
savetrees file=filename.tre brlens=yes root=yes;
savetrees file=filename.tre brlens=no root=yes;
pscores / single=all ci=yes ri=yes
scorefile=M1treescores.txt;
contree all / strict=yes treefile=filename.tre;
contree all / majrule=yes percent=50 treefile=filename.tre;
cleartrees;
bootstrap nreps=1000 search=heuristic/ addseq=random nchuck=10 chuckscore=1 nreps=10;
savetrees file=filename.tre from=1 to=1 savebootp=nodelabels maxdecimals=0;
log stop;
end;
```

In all trees *Nephila pilipes* was set as the outgroup taxon using the command 'outgroup=Nephila_pilipes;'. 'hsearch' is a command that begins a tree search using the heuristic algorithm. An initial tree is generated which then undergoes branch swapping where rearrangements, during the branch swapping, are generated by cutting clades off the main tree and reinserting them back into the tree. These clades can potentially be rerooted at available points during this stage. 'start=stepwise addseq=random' are the commands used to generate the starting trees. The taxa are randomly ordered at the start of the analysis and a tree is constructed using maximum parsimony with the first three taxa. Then additional taxa are added in a stepwise manner one by one following a random order with each taxa added to the tree in the most parsimonious position (Kitching *et al.*, 1998). 'nreps=100000' specifies 100,000 replications of the stepwise addition method.

'nchuck=2 chuckscore=2 swap=tbr' are the commands that control the branch swapping. 'nchuck=2' means that no more than two trees are retained with a score that is greater than the 'chuckscore' value. In this case it retains the two shortest trees from each of the 100,000 stepwise replicates. 'swap=tbr" refers to the tree bisection and reconnection (TBR) method of branch swapping that is used in PAUP*. The PAUP* tbr algorithm works by cutting a clade from the tree and reconnecting it to each of the branches of the tree that remains, ultimately evaluating all possible bisections and reconnections (Swofford and Olsen, 1990). 'hsearch start=current chuckscore=no' performs more branch swapping on the trees that remain from the initial swapping procedure. The analysis of Group 1, see Chapter 2.5, did not include this additional heuristic search as, when run in trials including it, the number of trees generated entered an endless cycle of tree generation and there was no resolution.

All the shortest trees in the morphological analysis, and later the molecular analysis, were rooted with the outgroup taxa *Nephila pilipes*. These shortest trees were then summarised by a calculation of the strict consensus tree, using the 'contree all / strict=yes', and a 50% majority rule 'contree all / majrule=yes percent=50'. Large numbers of equally parsimonious trees are hard to examine separately and a strict consensus method, that only displays the nodes present in all the equally parsimonious trees, is a constructive method of condensing many resulting equally parsimonious trees into one tree. However, the strict consensus tree can have low species resolution as any nodes and clades not common to all the trees are collapsed. A majority rule consensus, typically set at 50%, where the nodes retained are those that feature in the set percentage number of trees, can provide additional information in the inferred phylogeny.

The data was assessed for homoplasy by using the commands 'pscores / single=all ci=yes ri=yes' that generates a separate file with the consistency index and retention index for all the characters (see tables in A3.2.4) and the length of the retained equally parsimonious trees.

The consistency index (*ci*) can be used as a measure of how the individual character fits a phylogenetic tree. Additionally, the same process can be applied for the tree consistency index (CI) to measure the relative amount of homoplasy, character state changes, across the whole tree (Lipscombe, 1998). The relevant tree index is noted on the trees in the results section (Chapter 2.5).

The formula is:

$$ci = \frac{exp.steps}{obs.steps}$$

This gives the *ci*. A character with a lower number can indicate that the character has high amounts of homoplasy, (parallel or convergent evolution of the same character for various reasons) as it may have a larger number of state changes (observed steps) (Lipscombe, 1998).

However, because a character has high homoplasy in a tree does not always mean that it is not a synapomorphy, a character possessed by two or more taxa that defines a clade. To measure this the retention index (*ri*) is used. Again, this formula can also calculate the overall retention index (RI) for the desired tree. The tree RI measures the amount of synapomorphy expected from the dataset, that is retained as synapomorphy on the cladogram. (Lipscombe, 1998).

The formula is:

$$ri = \frac{max.steps - obs.steps}{max.steps - exp.steps}$$

These two sums provide a measure of the character homoplasy as well as explaining the present synapomorphies (see tables in A3.2.4 for a full breakdown of character *ci* and *ri*).

To provide a measure of how well supported the nodes on the resulting trees were by the dataset, a bootstrap replication analysis was performed using the 'bootstrap' command. Bootstrap analysis starts by sampling, at random, the characters in the original data matrix and creating a desired number of pseudoreplicate matrices. Trees are then constructed, again using the maximum parsimony criterion, and each clade is given a value. The bootstrap value for the clade reflects how frequently the clade appears in all the replications. The clades supported by a higher number of characters will be allocated a higher value, with 100 being the highest, as they have a greater support than clades that may only be supported by a small number of characters.

A1.5.3 TNT

TNT (Tree analysis using New Technology) (Goloboff, Farris and Nixon, 2008), like PAUP*, is a program used for inferring phylogenies using maximum parsimony. For the morphological TNT analysis, the data matrices were exported from Mesquite v.3.7 as TNT files using the option 'gaps not converted to 'missing'' with the character states 0-3 and missing characters set as '?' and gaps as '-'. In DNA datasets in TNT the gaps can be scored either as missing or as a fifth state depending on user defined parameters.

A1.5.4 TNT (morphological analysis)

In TNT, the menu-based interface was used, which offers checkbox options as opposed to a command line like PAUP*. The 'traditional analysis' was selected as the 'new technology' option differs from the 'traditional analysis' firstly, by offering different heuristic search options other than the tree bisection and reconnection (TBR) method of branch swapping, for example: Ratchet (Nixon, 1999), Tree Drifting (Goloboff, 1999), Sectorial Searches (Goloboff, 1999), and Tree Fusing (Goloboff, 1999) and, secondly, is suited towards datasets with a greater number of taxa, 80 or more, (Giribet, 2005) and these datasets did not contain that many taxa.

The 'new technology' function offers fast analyses. However, due to the fact TNT can estimate the consensus in this function by user defined parameters (Giribet, 2005) the number of equal length trees might not always be accurate. Therefore, as the maximum parsimony analysis was desired (in part to compare the results with PAUP*), the 'traditional analysis' was selected in TNT.

The starting trees were set as 'Wagner', which makes the lowest number of assumptions about the number of evolutionary steps resulting in the most parsimonious hypothesis, with a random seed (chosen at random), and replications set to 100,000, to provide a comprehensive analysis of the data without overwhelming the memory and to mirror the PAUP* analysis as much as possible. The swapping algorithm was set to 'tree bisection reconnection'. The trees saved per replication set as 2, as in PAUP*, and 'replace existing trees' selected.

TNT offers a faster alternative to PAUP* (Goloboff, Farris and Nixon, 2008) but memory limitations in the software can occur. Here the limitations of TNT were then reached as TNT was unable to complete the searches in the Group 1 and Group 3 datasets due to the maximum memory capacity being reached, even when the maximum settings were applied. This was due to the high number of equally parsimonious trees that were found. PAUP*, although taking longer to complete the analyses, did not suffer from a lack of memory and, due to wanting an accurate number of equally parsimonious trees, PAUP* was prioritised over TNT for this stage of the analysis.

Another factor in favour of PAUP* is that the output is easily accessed (for example the *ci* and *ri* of characters can easily be displayed) even if the analysis is comparatively slower. Despite not offering the full output results it was possible to compare the tree output from both PAUP* and TNT to confirm that no anomaly had occurred. This was confirmed as all the consensus trees from both analyses were the same, or very similar, with no major differences in species relationships; this was expected as the settings applied in TNT were consciously set as comparable to PAUP*, all analyses used the same datasets and parsimony analysis.

Once consensus trees were created a bootstrap analysis was run in TNT, as had been in PAUP*. Although the number of equally parsimonious trees was not accurate, TNT is able to run large numbers of bootstrap replications much faster than PAUP* (Goloboff and Pol, 2007), and this difference is most clearly seen in the larger Group 1 dataset. Therefore, bootstrap resampling was undertaken in TNT to confirm that there were no anomalies in the PAUP* bootstrap replication results. Once again, there were no major differences between the two outputs, but the 50% majority rule trees and bootstrap values presented in the results (Chapter 2.5) are those from the PAUP* analysis.

A1.5.5 TNT (molecular analysis)

For the molecular analyses the three data matrices were individually exported from Mesquite v.3.7 as TNT files with gaps converted to "missing" as any missing molecular sequence data can be scored in this way; unlike the morphological data where a character may not be present at all in a species. In this analysis gaps were treated as missing data as opposed to the other option which is called the '5th state'. The '5th state' turns the missing data into an additional character. Therefore, if taxa share multiple gap positions in the dataset that are longer than one base, then these taxa will appear to share multiple characters when gaps correlate. This produces questionable results (Ogden and Rosenberg, 2007) as the parsimony assumption assumes that each character is independent and can be misled when the 5th state is included in molecular analyses.

For all three datasets the ‘traditional analysis’ was selected with the starting trees set as ‘Wagner’ with a ‘random seed’ chosen at random, and ‘replications’ set to 100,000. The swapping algorithm was set to ‘tree bisection reconnection’, the ‘trees saved per replication’ set as 2 and ‘replace existing trees’ selected; this was the same as in the morphological analysis.

Unlike with some of the morphological data, the number of equally parsimonious trees in the molecular analysis was very low (see results Chapter 2.5). This meant that TNT was suitable as it handled the data swiftly without exceeding the tree memory capacity and PAUP* was not used. A bootstrap replication analysis was also performed in TNT, with 10,000 replications as in the morphological analysis.

A1.5.6 MrBayes

MrBayes is a program that uses Bayesian inference, an application of Bayes’ theorem (see 2.4.1), to infer the probability of the tree nodes. The version used here is 3.7.2a for Windows, with a command line interface. The commands were added to the nexus file that was generated by Mesquite v.3.7 when the data was exported. As with the PAUP* batch file above, the details of the commands are listed below. More details can also be accessed within MrBayes itself by using the command ‘help;’ which displays a list of commands with a short descriptive line of text.

For the morphological analysis on MrBayes, the three data matrices were individually exported from Mesquite v.3.7 as Nexus for MrBayes files, with names simplified for MrBayes as in the morphological analysis. Gaps were also set as missing and not an additional character state. The parameters of the molecular analysis mirrored those of the morphological analysis bar the differences listed in A1.5.2.

A1.5.7 MrBayes batch file example (morphological analysis)

```
begin mrbayes;
set autoclose=yes; nowarn=yes;
outgroup=Nephila_pilipes;
lset nst=1 coding=variable rates=gamma;
showmodel;
mcmc ngen=10000000 samplefreq=1000 printfreq=1000 nruns=2 nchains=4
temp=0.1 stoprule=yes stopval=0.01 savebrlens=yes;
sumt relburnin=yes burninfrac=0.25 contype=halfcompat;
end;
```

The command ‘lset’ controls the model, and the parameters, chosen for the data analysis. MrBayes offers two models to handle morphological data, however the default, and recommended (Ronquist *et al*., 2020) method is the Standard Discrete (Morphology) Model (Lewis, 2001). The Parsimony Model offered by MrBayes was not selected as it is not recommended by the developers (Ronquist *et al*., 2020), as MrBayes was designed for BI. Additionally, it would be a duplication of methodology that has already been tested in TNT and PAUP*; programs designed for the parsimony model.

The Standard Discrete Model specifies that there is a single rate of change between character states which is implemented in the command by ‘nst=1’. The command ‘coding=variable’ is a standard for morphological data that states only variable characters were sampled, and MrBayes automatically recognises character states numbered 0-9. The states of - and ? for gaps and missing data respectively, were defined in the nexus matrix when it was exported from Mesquite. ‘rates=gamma’ then sets the parameter of the rate of change within the characters. This was arbitrarily chosen for this analysis, and it is the default recommended setting.

‘showmodel;’ is a command that was included to confirm that the model selected earlier had been implemented prior to analysis. The command ‘mcmc’ then instructs MrBayes on how to run the analysis. The Markov chain Monte Carlo (MCMC) is a procedure that estimates the posterior distribution of parameter values. The parameter values are sampled in relation to their posterior probability. This means that once convergence has been reached the more probable values will be sampled more frequently (Currie and Meade, 2014). In this case, the more frequently a clade occurs, the more times it will be found and the more support it will receive.

The Markov chain starts by drawing a tree at random from the tree sample, with approximate probable values. This is often a point far away from the posterior distribution. The likelihood of this data is then calculated, then a new tree is proposed at the next step and again the likelihood of this new tree, with new parameter values, is calculated. Here, either the new values are accepted as more probable, or the old values are retained. This stage is then repeated over a set number of replications 'ngen'. The initial stages of this search are referred to as hill-climbing as the Markov chain moves from a low likelihood area of the parameter space and eventually samples from the areas that provide the greatest likelihoods more frequently (Currie and Meade, 2014). This is described as converging on the posterior distribution.

To avoid large amounts of output data the command 'samplefreq=1000' was used to sample the chain every 1000 generations, also known as thinning. To increase the speed of convergence a process called metropolis coupling is implemented (Ronquist and Huelsenbeck, 2003). This process runs additional chains that have a raised posterior probability, enabling them to explore areas of the probability distribution more easily. This is called 'heating'. Additionally, a swap is made between a heated and the original 'cold' chain at intervals resulting in the cold chain locating more trees with similar likelihoods but differing topographical results. The default values of 'nchains=4' and 'temp=0.1' were used to define the number of heated chains and the amount of heating to use.

The default ‘nruns=2’ was also used to run two independent analyses at the same time. These runs can then be compared to confirm convergence, by calculating the average standard deviation of the frequency of selected clades from the trees acquired in each run. This is known as splitting. This analysis can be stopped by the command ‘stoprule=yes’ and the ‘stopval=0.01’ sets the stop point for when this average standard deviation of split frequencies reaches the value of less than 0.01.

The ‘sumt’ command defines how the results of the analysis are reported. MrBayes is instructed on how many initial tree samples to discard. As discussed above, because the Markov chain is started by a random tree, the initial pre-convergence samples have a low posterior probability before the hill climbing moves into the areas of high likelihood and the chain stabilises (Currie and Meade, 2014). These initial trees with low probability are discarded from this starting burn-in phase using the commands ‘relburnin=yes’ and ‘burninfrac=0.25’ to set the value at 25% of initial trees to be discarded.

Then, as with PAUP*, a 50% majority rule consensus tree was implemented to summarise the trees generated from the analysis with the command ‘contype=halfcompat;’. The posterior probability of each clade is calculated by MrBayes and automatically displayed on the consensus tree.

A1.5.8 MrBayes batch file example (molecular analysis)

```
begin mrbayes;
        set autoclose=yes nowarn=yes;
        outgroup=Nephila_pilipes;
        lset nst=6 rates=invgamma;
        showmodel;
        mcmc ngen=1000000 printfreq=1000 samplefreq=1000 nruns=2 nchains=4
        temp=0.1 stoprule=yes stopval=0.01; savebrlens=yes;
        sumt relburnin=yes burninfrac=0.25 contype=halfcompat;
end;
```

Here the model of sequence evolution 'lset nst=6' was chosen. This is the Generalised Time Reversible (GTR) model that incorporates different rates of change, in this case 6, between the nucleotides and nucleotide frequencies. It is regarded as a suitable parameter-rich model for inferring tree topology and the ancestry of sequences (Abadi *et al*., 2019) and fits real data better than other alternative models (Arenas, 2015). It has been seen to perform well in comparative analysis testing and revealing the cladistic relationships between taxa (Abadi *et al*., 2019) and has been commonly used in phylogenetic studies (Sumner *et al*., 2012; Arenas, 2015).

The rate of change variation was set to a standard distribution of 'rates=invgamma' which sets the parameter of the rate of change within the characters to a gamma distribution, but with a proportion of invariable sites. Again, this was common practice in relation to the GTR model (Tarasov and Génier, 2015; Tarasov and Dimitrov, 2016; Ronquist *et al.*, 2020).

A1.5.9 Combined analysis

Following the morphological and molecular analysis in Chapter 2, a combined analysis was run in MrBayes featuring both morphological and molecular data in one dataset. The same dataset was also run in PAUP* but is not displayed (see Chapter 2.5). These programs were selected as it is possible to run partitioned data in both. Taxa that featured in the combined DNA analysis were the only ones used, to maximise the amount of data available. As no morphological data was scored for *Micrathena gracilis* and *Isoxya mahafalensis* these taxa were excluded.

A1.5.10 MrBayes (combined analysis)

First the data matrix is preceded by 'format datatype=mixed (Standard:1-66,DNA:67-1247)' to partition the data. Then, in the batch file, the commands 'charset morphology = 1-66;' and 'charset DNA = 67-1247' are listed followed by '(standard: 1-66, DNA:67-1247)' and 'partition favored = 2: morphology, DNA;' to inform MrBayes that there are two partitions, two different types of data. These are then automatically set by the command 'set partition=favored;' so MrBayes recognises the first 66 characters are morphological data and the remaining are molecular characters which were then set to follow the respective models from the analyses in Chapter 2. This adjustment to the batch file was straightforward and performs the same standard parameters on the morphological data and the same GTR model on the molecular data before combining the results.

Appendix 2: Taxa list, material used and data tables

A2.1 List of included and excluded taxa

The 58 taxa used in the morphological analysis were:

Nephila pilipes (Fabricius, 1793), *Araneus diadematus* (Linnaeus, 1758), *Aetrocantha falkensteini* Karsch, 1879, *Aranoethra cambridgei* (Butler, 1873), *Micrathena aureola* (C. L. Koch, 1836), *M. schreibersi* (Perty, 1833), *Augusta glyphica* (Guérin, 1839), *Isoxya cicatricosa* (C. L. Koch, 1844), *I. cowani* (Butler, 1883), *I. penizoides* Simon, 1887, *I.* sp. nov. Agnarsson *et al*., in prep, *I. tabulata* (Thorell, 1859), *Acrosomoides acrosomoides* (O. Pickard-Cambridge, 1879), *A. linnaei* (Walckenaer, 1841), *Actinacantha globulata* (Walckenaer, 1841), *Afracantha camerunensis* (Thorell, 1899), *Austracantha minax* (Thorell, 1859), *Macracantha arcuata* (Fabricius, 1793), *M. hasselti* (C. L. Koch, 1837), *Thelacantha brevispina* (Doleschall, 1857), *Togacantha nordviei* (Strand, 1913), *Gasteracantha aciculata* (Pocock, 1898), *G. cancriformis* (Linnaeus, 1758), *G. clarki* Emerit, 1974, *G. clavatrix* (Walckenaer, 1841), *G. clavigera* Giebel, 1863, *G. crucigera* Bradley, 1877, *G. curvispina* (Guérin, 1837), *G. diadesmia* Thorell, 1887, *G. diardi* (Lucas, 1835), *G. doriae* Simon, 1877, *G. falcicornis* Butler, 1873, *G. geminata* (Fabricius, 1798), *G. hecata* (Walckenaer, 1841), *G. kuhli* C. L. Koch, 1837, *G. lepelletieri* (Guérin, 1825), *G. lunata* Guérin, 1838, *G. mediofusca* (Doleschall, 1859), *G. mengei* Keyserling, 1864, *G. metallica* (Pocock, 1898), *G. milvoides* Butler, 1873, *G. pentagona* (Walckenaer, 1841), *G. quadrispinosa* O. Pickard-Cambridge, 1879, *G. recurva* Simon, 1877, *G. regalis* Butler, 1873, *G. remifera* Butler, 1873, *G. rhomboidea madagascariensis* Vinson, 1863, *G. sanguinolenta* C. L. Koch, 1844, *G. scintillans* Butler, 1873, *G. signifera* Pocock, 1898, *G.* sp. nov. Williams, in prep., *G. sturi* (Doleschall, 1857), *G. taeniata* (Walckenaer, 1841), *G. theisi* Guérin, 1838, *G. thorelli* Keyserling, 1864, *G. unguifera* Simon, 1889, *G. versicolor formosa* Vinson, 1863, *G. westringi* Keyserling, 1864.

Many Gasteracanthinae were excluded from the phylogenetic analysis. This was due to: lack of specimens, the only specimens available being immature, or where a reliable identification could not be confirmed. The validity of many of the species are in doubt, for example many of Strand's names as seen in Nentwig *et al*. (2019).

Excluded taxa were:

Gasteracantha acutispina Dahl, 1914, *G. audouini* Guérin, 1838, *G. aureola* Mi & Peng, 2013, *G. beccarii* Thorell, 1877, *G. biloba* (Thorell, 1878), *G. cancriformis gertschi* Archer, 1941, *G. curvistyla* Dahl, 1914, *G. cuspidata* C. L. Koch, 1837, *G. dalyi* Pocock, 1900, *G. fasciata* Guérin, 1838, *G. flava* Nicolet, 1849, *G. fornicata* (Fabricius, 1775), *G. frontata* Blackwall, 1864, *G. gambeyi* Simon, 1877, *G. interrupta* Dahl, 1914, *G. irradiata* (Walckenaer, 1841*), G. janopol* Barrion & Litsinger, 1995, *G. martensi* Dahl, 1914, *G. notata* Kulczyński, 1910, *G. panisicca* Butler, 1873, *G. parangdiadesmia* Barrion & Litsinger, 1995, *G. picta* (Thorell, 1892), *G. rhomboidea* Guérin, 1838, *G. rhomboidea comorensis* Strand, 1916, *G. rubrospinis* Guérin, 1838, *G. rufithorax* Simon, 1881, *G. sacerdotalis* L. Koch, 1872, *G. sanguinea* Dahl, 1914, *G. sanguinolenta andrefanae* Emerit, 1974, *G. sanguinolenta bigoti* Emerit, 1974, *G. sanguinolenta emeriti* Roberts, 1983, *G. sanguinolenta insulicola* Emerit, 1974, *G. sanguinolenta legendrei* Emerit, 1974, *G. sanguinolenta mangrovae* Emerit, 1974, *G. sapperi* Dahl, 1914, *G. sauteri* Dahl, 1914, *G. signifera bistrigella* Strand, 1911, *G. signifera heterospina* Strand, 1915, *G. signifera pustulinota* Strand, 1911, *G. simoni Emerit, 1974*O. Pickard-Cambridge, 1879, *G. sororna* Butler, 1873, *G. subaequispina* Dahl, 1914, *G. taeniata analispina* Strand, 1911, *G. taeniata anirensis* Strand, 1911, *G. taeniata lugubris* Simon, 1898, *G. taeniata novahannoveriana* Dahl, 1914, *G. thomasinsulae* Archer, 1951, *G. tondanae* Pocock, 1897, *G. transversa* C. L. Koch, 1837, *G. versicolor* (Walckenaer, 1841), *G. versicolor avaratrae* Emerit, 1974, *Acrosomoides tetraedrus* (Walckenaer, 1841), *Austracantha minax astrigera* (L. Koch, 1871), *A. minax hermitis* (Hogg, 1914), *A. minax leonhardii* (Strand, 1913), *A. minax lugubris* (L. Koch, 1871), *Isoxya basilewskyi* Benoit & Emerit, 1975, *I. mahafalensis* Emerit, 1974, *I. milloti* Emerit, 1974, *I. mossamedensis* Benoit, 1962, *I. mucronata* (Walckenaer, 1841), *I. nigromutica* (Caporiacco, 1939), *I. reuteri* (Lenz, 1886), *I. semiflava* Simon, 1887, *I. somalica* (Caporiacco, 1940), *I. stuhlmanni* (Bösenberg & Lenz, 1895), *I. testudinaria* (Simon, 1901) and *I. yatesi* Emerit, 1973.

A2.2 Specimens used in morphological analysis

Specimens used for scoring characters in the morphological analysis comprised the entire Gasteracanthinae collections, including unidentified material, of EZ, MM, NMH, OUMNH and VMNH so specimens are not listed if not used for morphological analysis to avoid confusion. A maximum of 5, in most cases, were used for scoring in the morphological analysis. Data is transcribed from labels and databases where possible. Due to the historical nature of some museum collections, site and collection data is limited in some cases. Sources and specimens were:

EZ Lab, Slovenia (EZ):

1♀ *Acrosomoides acrosomoides* ARA2000, SE Africa: Madagascar, Toamasina, Andasibe Mantadia NP, Anivokely, -18.93717 48.42005, 900-1000alt, Agnarsson I., Kuntner M., 19.iv.2008; 1♀ 1♂ *Acrosomoides acrosomoides* ARA2014, SE Africa: Madagascar, Toamasina, Andasibe Mantadia NP, 16.iv.2008; 1♀ 1♂ *Augusta glyphica* ARA2019, SE Africa: Madagascar, Toamasina, Andasibe Mantadia NP, Analamazoatra, -18.93717 48.42005, 900-1000alt, Agnarsson I., Kuntner M., 31.iii.2008; 1♀ *Augusta glyphica* ARA2025, SE Africa: Madagascar, Toamasina, Andasibe Mantadia NP, Analamazoatra, -18.93717 48.42005, 900-1000alt, Agnarsson I., Kuntner M., 31.iii.2008; 1♀ *Gasteracantha aciculata* ARA2040, Australasia: Papua New Guinea, New Britain, camp1, I. Agnarsson, 5.iv.2009; 1♀ *Gasteracantha aciculata* ARA2041, Australasia: Papua New Guinea, New Britain, riverbank, I. Agnarsson, 9.iv.2009; 2♀ 2♂ *Gasteracantha aciculata* ARA2043, Australasia: Papua New Guinea, New Britain, camp1, I. Agnarsson, 4-9.iv.2009; 2♀ 1♂ *Gasteracantha aciculata* ARA2044, Australasia: Papua New Guinea, New Britain, camp1, I. Agnarsson, 4.iv.2009; 2♀ 2♂ *Gasteracantha aciculata* ARA2046, Australasia: Papua New Guinea, New Britain, camp2, I. Agnarsson, 11.iv.2009; 1♂ *Gasteracantha aciculata*

ARA2047, Australasia: Papua New Guinea, New Britain, I. Agnarsson, 8.iv.2009; 5♀ *Gasteracantha clavatrix* ARA2011, SE Asia: Indonesia, Sulawesi, Bitung, 1.48347 125.12720, 251alt, Kuntner M., Gregorič M., 13.vii.2007; 1♀ 1♂ *Gasteracantha diardi* ARA2008, SE Asia: Malaysia, Pahang, Bukit Fraser, 3.72069 101.74038, 1253alt, M in F web, M. Kuntner, 4.vi.2007; 1♀ *Gasteracantha diardi* ARA2010, SE Asia: Malaysia, Pahang, Bukit Fraser, 3.72069 101.74038, 1253alt, Kuntner, 4.vi.2007; 1♀ *Gasteracantha hasselti* ARA1982, E Asia: China, Yunnan, Xishuangbanna, Mengla, Green Stone Forest, 21.91697 101.29075, 598alt, Green stone, Kuntner M., Li D.Q., Gregorič M., 27.xii.2010; 1♀ *Gasteracantha hasselti* ARA1987, E Asia: China, Hainan, Lingshui Li Autonomous County, 18.73200 109.88578, 915alt, Kuntner M., 21.vi.2011; 1♀ 1♂ juv. *Gasteracantha kuhli* ARA2005, SE Asia: Male and Female in the same web, SP10, 16-18.v.2005; 1♀ *Gasteracantha milvoides* ARA2033, S Africa: South Africa, Sod.B out RD., 18.iv.2006; 1♀ *Gasteracantha milvoides* ARA2034, S Africa: South Africa, KwaZulu-Natal, Hluhluwe-iMfolozi NP, forest, 20.iv.2008; 1♀ *Gasteracantha milvoides* ARA2035, S Africa: South Africa, KwaZulu-Natal iSimangaliso, Wetland Park, Cape Vidal, 28.iv.2008; 1♀ *Gasteracantha milvoides* ARA2036, S Africa: South Africa, Sod. B. Mgob., 17.iv.2006; 1♀ *Gasteracantha milvoides* ARA2042, South Africa, Sod. B. Mgob., 17.iv.2006; 1♀ *Gasteracantha sturi* ARA1983, China: Yunnan, Xishuangbanna, Baka forest, 21.71368 100.78302, 695alt, Baka, Kuntner M., Li D.Q., Gregorič M., 27.xii.2010; 1♀ *Gasteracantha sturi* ARA1984; China: Yunnan, Xishuangbanna, Mengla, Mengyuan, 119 farm, 21.64703 101.41668, 816alt, Kuntner M., Li D.Q., Gregorič M., 31.xii.2010; 1♀ *Gasteracantha sturi* ARA1986, China: Yunnan, Xishuangbanna, Baka forest, 21.71368 100.78302, 695alt, Baka, Kuntner M., Li D.Q., Gregorič M., 29.xii.2010; 2♀ *Gasteracantha rhomboidea madagascariensis* ARA2026, SE Africa: Madagascar, Toamasina, Andasibe Mantadia NP, Analamazoatra, Agnarsson I., Kuntner M., 4.iv.2008; 1♀ *Gasteracantha rhomboidea*

madagascariensis ARA2027, SE Africa: Madagascar, Antsiranana, Montagne d'Ambre NP, I. Agnarsson & M. Kuntner, 4.iv.2008; 1♀ *Gasteracantha versicolor formosa* ARA2012, S Africa: South Africa, Sod. B. Mgob., 17.iv.2006; 1♀ 1♂ *Gasteracantha versicolor formosa* ARA2018, SE Africa: Madagascar, Toamasina, Andasibe Mantadia NP, Analamazoatra, -18.93717 48.42005, 900-1000alt, Agnarsson I., Kuntner M., 31.iii.2008; 1♀ *Gasteracantha versicolor formosa* ARA2020, SE Africa: Madagascar, Toamasina, Andasibe Mantadia NP, Analamazoatra, -18.93717 48.42005, 900-1000alt, Agnarsson I., Kuntner M., 29.iii.2008; 1♀ 1♂ *Gasteracantha versicolor formosa* ARA2021, SE Africa: Madagascar, Toamasina, Andasibe Mantadia NP, Analamazoatra, -18.93717 48.42005, 900-1000alt, MF same web, Agnarsson I., Kuntner M., 31.iii.2008; 1♀ 1♂ *Gasteracantha versicolor formosa* ARA2022, SE Africa: Madagascar, Toamasina, Andasibe Mantadia NP, Analamazoatra, -18.93717 48.42005, 900-1000alt, M & F, Agnarsson I., Kuntner M., 31.iii.2008; 1♀ 1♂ *Gasteracantha versicolor formosa* ARA2023, SE Africa: Madagascar, Toamasina, Andasibe Mantadia NP, Analamazoatra, -18.93717 48.42005, 900-1000alt, Agnarsson I., Kuntner M., 31.iii.2008; 1♀ 1♂ *Isoxya tabulata* ARA1993, S Africa: South Africa, 17.iv.2006; 2♀ 1♂ *Isoxya tabulata* ARA1995, S Africa: South Africa, 14.iv.2006; 1♀ 1♂ *Isoxya tabulata* ARA1997, S Africa: South Africa, 17.iv.2006; 1♀ *Isoxya tabulata* ARA2037, S Africa: South Africa, KwaZulu-Natal, Hluhluwe-iMfolozi NP, Hilltop at station, 20.iv.2006; 2♀ *Macracantha arcuata* ARA1985, E Asia: China, Yunnan, Xishuangbanna, Botanical garden, 21.92392 101.19792, 620alt, XI Shuangbanna Bot. Garden, Kuntner M., Li D.Q., Gregorič M., 26-30.xii.2010; 1♀ *Macracantha arcuata* ARA2001, SE Asia: Malaysia, Selangor, Gombak, forest around field station, 3.32485 101.75292, 272alt, Macrac., Kuntner M., Gregorič M., 6.i.2010; 1♀ *Macracantha arcuata* ARA2002, SE Asia: Malaysia, Selangor, Gombak, forest around field station, 3.32485 101.75292, 272alt, Macrac., Kuntner M., Gregorič M., 7.i.2010;

1♀ *Macracantha arcuata* ARA2003, SE Asia: Malaysia, Selangor, Gombak, forest around field station, 3.32485 101.75292, 272alt, Macrac., Kuntner M., Gregorič M., 7.i.2010; 1♀ *Macracantha arcuata* ARA2004, SE Asia: Malaysia, Selangor, Gombak, forest around field station, 3.32485 101.75292, 272alt, Macrac., Kuntner M., Gregorič M., 6.i.2010; 1♂ *Nephila pilipes* ARA2112, Pulau Ubin, 1.ii.2019; 1♂ *Nephila pilipes* ARA2113, Pulau Ubin, 1.ii.2019; 1♀ *Thelacantha brevispina* ARA1983, E Asia: China, Yunnan, Xishuangbanna, Baka forest, 21.71368 100.78302, 695alt, Baka, Kuntner M., Li D.Q., Gregorič M., 27.xii.2010; 1♀ *Thelacantha brevispina* ARA1998, Indian Ocean: Mauritius, Black River Gorge NP, Brise Fer Station, secondary rain forest, -20.37628 57.44339, 602alt, Agnarsson I., Kuntner M., 20.iv.2008; 1♀ *Thelacantha brevispina* ARA2032, Indian Ocean, Mauritius, Black River Gorge NP, Brise Fer Station, secondary rain forest, -20.37628 57.44339, 602alt, I. Agnarsson & M. Kuntner, 20.iv.2008.

Ingi Agnarsson (private collection), USA (IA):

5♀ 5♂ *Isoxya* sp. nov. coll. Agnarsson I., Kuntner, M., Gregoric M. Eastern Madagascar, Toamasina province, along the road leading from Andasibe to Mantadia (Andasibe-Mantadia National Park at -18.82663, 48.43333), 5-8. December 2017

Manchester Museum, UK (MM):

1♀ *Gasteracantha cancriformis* Mexico, Baja Calif, S.Ignacio Lag., saltbush assocn., Coll. P.Brown, 16.Feb.83, G7572.1021 or 10821, Murphy Colln.; 1♂ *Gasteracantha hasselti* Malaysia, Johor, Layang-Layang, 100m, garden (Corley) 21.Jul.79, G7572.1045 or 7640 Murphy Colln.; 1♂ *Gasteracantha hasselti* G7579.9; 1♀ *Gasteracantha mediofusca* P.N.G., Morobe, Wau, 1300m, Coll. E.W.Classey, 26.Jul.74, G7572.1030 or 2420, MurphyColln.; 1♂ *Gasteracantha milvoides* Kenya, Coast, Gedi, 20m, litter, shrubs, 10.Sep.80, G7572.1024

or 9276 Murphy Colln.; 1♂ *Gasteracantha milvoides* Kenya, Coast, Gedi, 10m, forest, leaf litter, 14.Sep.84, G7572.1013 or 12423, Murphy Colln.; 1♂ *Gasteracantha milvoides* Kenya, Coast, Kilifi, 30m, litter, (B.Fulton), Aug.80, G7572.1017 or 8957, Murphy Colln.; 1♂ *Gasteracantha pentagona* Malaysia, W.Pahang, Genting, 700m, shrubs, Feb.01, G7572.1058 or 23791, Murphy Colln.; 1♂ *Gasteracantha pentagona* Malaysia, W.Pahang, Genting, 700m, shrubs, Feb.01, G7572.1051 or 23808, Murphy Colln.; 1♂ *Gasteracantha taeniata* Sabah, Kinabalu N.P., 1800m, rainforest paths, 30.Jul.79, G7572.1014 or 7893, Murphy Colln.; 1♂ *Gasteracantha taeniata* & 1♂ *G. sp*. (different species) Australia, Queensland, C.Tribulation, 5m, coastal rainforest, 24.Jul.92, G7572.1056 or 21095, Murphy Colln.; 1♂ 1 juv♂ *Gasteracantha taeniata* Australia, Queensland, Kuranda, 500m, bush, (Caravan Pk), 25.Oct.97, G7572.1057 or 22682, Murphy Colln.; 2♂ 1 juv♂ *Gasteracantha taeniata* Malaysia, Johor, Layang-Layang, 100m, garden, (Corley), 21.Jul.79, G7572.1028 or 7641, Murphy Colln.; 1♀ *Togacantha nordviei* Kenya, Rift Val., nr.M.Longonot, 2500m, jungle, Coll. Julia Barnley, Sep.81, G7572.1018 or 9639, Murphy Colln.

Natural History Museum London, UK (NHM):

3♀ 1♂ *Actinacantha globulata* Sarawak: Gunong Mulu N.P., Environs of base camp 65m, from trees, Batu Hill, 29.iv.1978, coll. F. Wanless, R.G.S. Mulu Exped. 1977-78; 1♀ *Actinacantha globulata* Malaysia: Sarawak, Mulu Exped. March 1978, Hidden Valley, P. Chapman coll.; 7♀ *Actinacantha metallica* 87.8, Solomon Islands, purchased C.M.Woodford, Type; 16♀ *Aetrocantha falkensteini* 1907.5.9.1-600 (part), Uganda, Sunion (c); 1♀ *Afracantha cameruensis* Uganda, (Sunion), 1907.5.9.1.600; 1♀ *Aranoethra cambridgei* Accra, G.A. Hoglett, 8.7.148; 1♀ *Aranoethra cambridgei* Cameroon, Bates; 1♀ *Aranoethra cambridgei* Bibiani, Gold Coast, Dr. H.G.F. Spuitell; 2♂ *Austracantha minax* 1924.III.1.1.520-23, Gawler Ranges, S. Austr., Hogg Coll.; 2♀ *Austracantha minax*

B.M.N.H. Reg No. 1931.7.30.22-23, Bridport, Tasmania, In orb webs decorated with tufts of silk, V.V .Hickman, 26.1.29; 4♀ 1♂ *Austracantha minax* 1915.3.5.1966-69, Koch Coll., Sydney; 1♂ *Austracantha minax* 1924.III.1.1477, Loc?, Hogg Coll.; 3♀ *Gasteracantha cancriformis* 1937.3.31.87-89., Pulch Sanghi, Lord Maynes Exped., Coll. Hon. A. Chaplin; 4♀ *Gasteracantha cancriformis* 1899.6.20.13-16, Nanau, Bahamas, J.L.Bonhote; 1♀ *Gasteracantha clarki* EM 2670 HOLOTYPE, Cascade Entate, Mahe, 28.xi.05, 900ft, 1983.3.22.4; 1♀ *Gasteracantha clavatrix* Sulawesi: Solato river, oven, 15.ii.80; 3♀ *Gasteracantha clavatrix* Indonesia: Sulawesi, Utara, Dumoga-Bone N.P., Nov. 1985, In elephant grass, around sulphur springs, P. Hillyaro Leg.; 1♀ *Gasteracantha clavigera* 1896.12.16.36, Leite, Philippines, Whitehead Expedition; 2♀ *Gasteracantha clavigera* Philippines: Manila, 10.ix.1914, A.E. Wikman leg; 2♀ *Gasteracantha crucigera* 1924.6.24.4-5, New Guinea I.B.E., P.J. de Vertenie; 1♀ *Gasteracantha crucigera* New Guinea: Madang District, Finisterre Mts., Naho River Valley, Damanti, 3550', M.E. Bacchus Coll. No. 30, 2-7.x.1964, British Museum (Nat. Hist.) and Univ. of Newcastle-upon-Tyne Exped., 1964-65; 1♀ *Gasteracantha crucigera* New Guinea: Lae Lower Bewapi Creek, Womersley's Garden; 4♀ *Gasteracantha crucigera* 1937.12.13.113-116., Dutch New Guinea, 1936, L.E. Cheesman, No.8; 1♀ *Gasteracantha diadesmia* 95.9.21.690, Tharrawaddy, E.W.Oates; 1♀ *Gasteracantha diardi* 15.3.6.1952, c.Herrich Schaffer; 2♀ *Gasteracantha doriae* 1898.10.18.12-13., Singapore, H.N. Ridley; 1♀ *Gasteracantha geminata* 1924.VII.22.8-91, Malabar, India, E.E.E. Fisher (a.a.p.); 23♀ *Gasteracantha hasselti* 1894.12.15.18-23, Deli, Sumatra, Dr. Wloesch; 2♀ *Gasteracantha hasselti* West Malaysia, Seremban, on oil palm, via C.I.E. 14.11.83; 2♀ *Gasteracantha hecata* 1890.7.1.6099-6100, Lugon, Keyserling Coll.; 7♀ *Gasteracantha kuhli* 1901.3.20.34-36, Singapore, H.N.Ridley; 2♀ *Gasteracantha kuhli* 1924.3.1.604.605 KY1, Dran, Langbian, S.Annam, L.Boden Floss, Hogg Coll.; 1♀ *Gasteracantha kuhli* N. India: Kalimpong Sikkim, 27.iii.1924, 6,000ft, Maj R.W.G.

Hingston; 1♀ *Gasteracantha kuhli* In orb web in forest, Maewa Khola: Sanghu (27°21'N87°33'E), 29.10.1961, 6,000ft., Coll. No. 114, Brit. Mus. Nepal Exped. 1961-1962, K.H.Hyatt coll.; 1♀ *Gasteracantha lepelletieri* Amboena, Beccari;

1♀ *Gasteracantha lepelletieri* Aru Isl., Beccari coll.; 8♀ *Gasteracantha lepelletieri* New Guinea: Morobe District, Herzog Mts., Vagau, 4,000ft, M.E. Bacchus Coll. No. 30, 2-7.x.1964, British Museum (Nat. Hist.) and Univ. of Newcastle-upon-Tyne Exped., 1964-65; 2♀ *Gasteracantha lepelletieri* New Guinea: Madang District, Finisterre Mts., Naho River Valley, Damanti, 3550', M.E. Bacchus Coll. No. 30, 2-7.x.1964, British Museum (Nat. Hist.) and Univ. of Newcastle-upon-Tyne Exped., 1964-65; 1♀ *Gasteracantha lepelletieri* 77.29, Torres Straits, Mc Farlane; 5♀ *Gasteracantha lepelletieri* 1896.5.15.10-14., Morok, Papua; 2♀ *Gasteracantha lunata* 1897.11.1.82-83, Ternate (Temate), W. Kukenthal; 5♀ *Gasteracantha lunata* 1897.11.1.87-91, Patani, Halmaheira, W. Kukenthal; 1♀ *Gasteracantha mediofusca* 1912.3.6.1; Chandigarh, Nimah, India, Bombay, Nat. Hist. Soc.; 1♀ *Gasteracantha mediofusca* 1924.III.1.1.520-23, Gawler Ranges, S. Austr., Hogg Coll.; 1♀ *Gasteracantha pentagona* 1907.2.21 1-10 pt., New Guinea, Fakfak, May, 1905, A.E. Pratt; 1♀ *Gasteracantha pentagona* 1937.12.13.445, Dutch New Guinea, Humboldts Bay, 7.iv.1936, N0.142, L.E. Cheesman; 1♀ *Gasteracantha quadrispinosa* 1937.12.13.437, Dutch New Guinea, Humboldts Bay, 200ft, iv.1936 no.129, L.E.Cheesman; 2♀ *Gasteracantha recurva* N.Luzon., L. Whitehead, 95.5.11.2.3; 1♀ *Gasteracantha regalis* HOLOTYPE BM.1864.45, New Hebrides, Ex. Dry Collection; 2♀ *Gasteracantha remifera* 1924.VII.22.8-91, Malabar, India, E.E.E. Fisher (a.a.p.); 1♀ *Gasteracantha remifera* Peradensia, 01.7.10.4; 2♀ *Gasteracantha remifera* Central Celebes: north of Gulf of Bone; 2♀ *Gasteracantha remifera* 1906.11.14.31-36., Ceylon, Dr. A. Willey (P.); 1♀ *Gasteracantha scintillans* HOLOTYPE BM.1855.69.264, Det. N.Scharff, Ex. Dry collection; 2♀ *Gasteracantha scintillans* 1936.11.13.25-28, Tenaru, Guadalcanal, British Solomon Is., xi.1935, R.J.A.W.

Levet; 1♀ *Gasteracantha scintillans* Royal Society: B.S.I.P. 1965, Coll. No: G2, Lower Camp Area, Mt. Gallego, Guadalcanal, B.M.(N.H.) Acc.No: 2182 (Moll); 10♀ *Gasteracantha scintillans* 1955.11.25.6-15, Solomon Islands, Guadalcanal, Tapenangie, 10-20th December, 1953, J.D.Bradley coll.; 1♀ *Gasteracantha scintillans* Royal Soc. B.S.I.P. 1965, Coll No. VV6. Foliage near, River. Leg E.F.P, Kolombangara, New Georgia Group, B.M.(N.H.) Acc.No.2183; 1♀ *Gasteracantha signifera* Rubiana, New Georgia, Solomon Is. 8.6.1897, A.Willey; 1♀ *Gasteracantha signifera* 1936.11.13.48, Tulagi, British Solomon Is., 14.1.1935, R.J.A.W. Levet; 10♀ *Gasteracantha signifera* Iles Salomon: New Georgia, R.A.Levet, 1931; 4♀ *Gasteracantha sp. nov.* Royal Society: B.S.I.P. 1965, Colln. No. Arachnida, Leg. P.Hunt, Alasa'a, Malaita, B.M.(N.H.) Acc. No. 2181 (Moll.); 2♀ *Gasteracantha sturi* 1937.12.13.124-125, Dutch New Guinea, 1936, No.10, L.E. Cheesman; 45♀ *Gasteracantha taeniata* Goroka, E.Highl., N.Guinea, June 4. 1956, coll. Dr. J. Szent-Ivanx; 1♀ *Gasteracantha theisi* New Guinea: Madang District, Finisterre Mts., Naho River Valley, Budemu, 4150', M.E. Bacchus Coll. No. 51, 15-25.x.1964, British Museum (Nat. Hist.) and Univ. of Newcastle-upon-Tyne Exped., 1964-65;2♀ *Gasteracantha unguifera* Swept in insect net by R.L.Coe - Small patch forest, Maewa Khola: Sanghu (27°21'N87°33'E), 30.10.1961 6,000ft., Coll No. 127, Brit. Mus. Nepal Exped. 1961-62, K.H. Hyatt coll.; 1♀ *Gasteracantha unguifera* In orb-webs whilst marching between Leware 28°19'N 83°52'E and Dharam Bazar, 28°18.5'N 83°46.5'E, K.H. Hyatt. Coll. No. 106A, Brit. Mus. Nepal Exped. 1954., 14 May; 1♀ *Gasteracantha unguifera* Collected whilst marching between Tilhar (28°17'N 83°51'E) and Naudhara, 28°17'N 83°51'E), K.H. Hyatt. Coll. No. 247, Brit. Mus. Nepal Exped. 1954., 29 July; 1♀ *Gasteracantha unguifera* 1934.2.28.124., Tsuntang, Sikkim, N.India, 6,000ft. 24.4.1924., Maj R.W.G.Hingston., Mt. Everest Explor. Exped. 1924; 11♀ *Gasteracantha westringi* 1924.ii.1.46-53, Port N'gea, Noumea, New Caledonia, Jan.4.1913. no. 5., P.D. Montague; 8♀ 1♂ *Isoxya cicatricosa* 1901.3.6.1-8,

Kowie, nr. Grahamstown Natal, F. Pym., Jan. 1895; 1♀ *Isoxya cowani* 82.25, Central Madagascar, Rev. D.Cowan, Type; 2♂ *Isoxya cowani* 1919.12.7.3-10 (pt), Ankerimadinika Forest, Central Madagascar, 12.9.1913, R.Beck; 6♀ *Isoxya cowani* 1919.12.7.3-10, Ankerimadinika Forest, Central Madagascar – Web about 5ft from ground, particles of leaves as mimicry in lower part of nest, 12.9.1913, R.Beck; 1♀ *Macracantha arcuata* Sarawak: Gunong Mulu N.P. Environs of base camp 65m. In web, open, near kitchen doorway. Coll. F. Wanless June, 1978, R.G.S. Mulu Expedition 1977-8; 1♀ *Nephila pilipes* Sandahan, D. Cator, BMNH 1895.7.20.26.29; 1♀ *Nephila pilipes* Sandahan, D. Cator, BMNH 1895.7.20.26.29; 6♀ 6♂ *Thelacantha brevispina* Table Island (Andamans), Oates.

Oxford University Museum of Natural History, UK (OUMNH):

20♀ *Chaetacis aureola* Jar 1157 O.P.C, Rogers, Minas Gereas; 1♂ *Chaetacis aureola* Jar 1157 O.P.C, Rogers, Minas Gereas; 1♂ *Chaetacis aureola* Jar 1158 O.P.C, Traill, Amazons; 5♀ *Gasteracantha diardi* Jar 1124 O.P.C, Cutter, Borneo; 1♀ *Gasteracantha falcicornis* World Aranea, OUMNH-2010-098, Mehrabi, Coals, Harrison & Offord, vii-viii.2010, Mozambique; 2♀ *Gasteracantha falcicornis* World Aranea, OUMNH-2010-098, Mehrabi, Coals, Harrison & Offord, 10.viii.2010, Mozambique; 1♀ *Gasteracantha falcicornis* World Aranea, OUMNH-2010-098, Mehrabi, Coals, Harrison & Offord, 31.vii-02.viii.2010, Mozambique; 58♀ *Gasteracantha geminata* Jar 1125 O.P.C, Ceylon; 1♀ *Gasteracantha mengei* Jar 1124 O.P.C, Borneo; 37♀ *Gasteracantha scintillans* Jar 1126 O.P.C, C.M.Woodford 1892, Solomon Islands.

Sam Danflous (private collection), France (SD):

1♀ *Acrosomoides linnaei* République centrafricaine (RCA), Sangha Mbaéré, Ripisylve, sous-bois, Lac 1, Bord de lac, 2,480322 N 16,215608 E to 2,481672 N 16,222751 E, 20/11/10,

Nuit, 403alt, sans code; 1♀ *Acrosomoides linnaei* République centrafricaine (RCA), Sangha Mbaéré, Lac 7, Tour lac 7, Végétation aquatique, 2°27'53.6" 16°13'30.5", 2.464888889 16.22513889, 28/11/10, Jour, 414alt, OM1; 1♀ *Aetrocantha falkensteini* République centrafricaine (RCA), Sangha Mbaéré, Sous-bois forêt, Lac 1, Camp, 2° 28' 49,2" 16° 12' 56,2", 2.480322 16.215608, 06/02/12, Nuit, 401alt, TU; 1♀ *Aetrocantha falkensteini* République centrafricaine (RCA), Sangha Mbaéré, Lac 1, Camp de base, 2°28'49.5" 16°12'55.9", 2.480416667 16.21552778, 24/11/10, Jour, 392alt, NL; 1♀ *Aetrocantha falkensteini* République centrafricaine (RCA), Sangha Mbaéré, Dans un, Azobé (Lophira alata, Ochnaceae) à 40 m du sol, Lac 1, Camp, 2° 28' 49,5" 16° 12' 55,9", 2.480417 16.215528, 13/02/12, Jour, 392alt, XI; 1♀ *Aetrocantha falkensteini* République centrafricaine (RCA), Sangha Mbaéré, Camp 3, Camp, 02°50'01.8" 16°08'13.7", 2.833833333 16.13713889, 05/02/05, Jour, 375alt, EQ; 1♀ *Aetrocantha falkensteini* République centrafricaine (RCA), Sangha Mbaéré, Abords camp de base lac 1, Sous-bois forêt, 2° 28' 49,3" 16° 12' 57", 2.480373 16.215838, 30/01/12, Nuit, 392alt, SH; 1♀ *Aetrocantha falkensteini* République centrafricaine (RCA), Sangha Mbaéré, Après dortoirs camp de base lac 1, Chablis n°2 en bas du Mboko Battage, 2° 28' 39,2" 16° 12' 58,6", 2.477559 16.216269, 22/02/12, Jour, 425alt, AAU; 1♀ *Afracantha cameruensis* République centrafricaine (RCA), Sangha Mbaéré, Sous-bois forêt, Lac 7, Prospection lac, 2° 27' 47,8" 16° 13' 29,4", 2.463277 16.224823, 18/02/12, Jour, 404alt, ZQ; 1♀ *Gasteracantha curvispina* République centrafricaine (RCA), Sangha Mbaéré, Camp transit 2 au bord du Kénié, 2°27'37.8" 16°08'33.2", 17/11/10, Jour, 376alt, MB2a; 2♀ *Gasteracantha curvispina* République centrafricaine (RCA), Sangha Mbaéré, Ripisylve, sous-bois, Lac 1, Bord de lac, 2,480322 N 16,215608 E to 2,481672 N 16,222751 E, 20/11/10, Nuit, 403alt, sans code; 1♀ *Gasteracantha curvispina* République centrafricaine (RCA), Sangha Mbaéré, Lac 1, Camp, Sous-bois forêt, 2° 28' 49,2" 16° 12' 56,2", 2.480322 16.215608, 14/02/12, Jour,

401alt, XL; 1♀ *Gasteracantha curvispina* République centrafricaine (RCA), Bénin, Forêt de Houéyogbé, dépt. Mono, commune Houéyogbé, 82alt, 06°33'43"N 01°51'02"E, J.-P. Maurel, 20.X.2014; 1♀ *Gasteracantha sanguinolenta* République centrafricaine (RCA), Sangha Mbaéré, Molongo, Alentours du village, Strate arbustive, 2°27'11.6" 16°04'58.7", 2.453222222 16.08297222 14-15/11/10, Nuit, 343alt, LQ; 1♀ 1♂ *Gasteracantha sanguinolenta* République centrafricaine (RCA), Sangha Mbaéré, Berges de la Sangha, Molongo-Nyangouté, Bord Sangha, Samuel Danflous, prospection en pirogue, 2°25'04.9" 16°06'22.3", 2.418027778 16.10619444, 16/11/10, Jour, 360alt, LYa; 1♀ *Gasteracantha sanguinolenta* République centrafricaine (RCA), Sangha Mbaéré, M'boki, Nord du village, Bras mort de la Sangha, 2° 28' 19,1" 16° 48' 44,6", 2.471964 16.081238, 25/01/12, Jour, 382alt; 1♀ *Isoxya penzoides* République centrafricaine (RCA), Sangha Mbaéré, Sous-bois forêt, Lac 4, Prospection lac (Bilos), 2° 28' 23,7" 16° 13' 25,4", 11/02/12, Jour, 395alt, WE; 1♀ *Isoxya penzoides* République centrafricaine (RCA), Sangha Mbaéré, Forêt, sous-bois, Camp transit 1 vers camp transit 2 (au bord du Kénié), 2°26'47.4"N 16°05'25.1"E to 2°27'37.8"N 16°08'33.2"E / UTM 33 N : 624492-271937 to 627031-272015, 17/11/10, Jour, 390/376alt, MB1; 1♀ *Isoxya penzoides* République centrafricaine (RCA), Sangha Mbaéré, Végétation aquatique, Tour lac 3, 2°29'19.1" 16°13'58.4", 2.488638889 16.23288889, 23/11/10, Jour, 401alt, MY1; 1♀ *Isoxya penzoides* République centrafricaine (RCA), Sangha Mbaéré, Sous-bois forêt, Lac 7, Prospection lac, 2° 27' 47,8" 16° 13' 29,4", 2.463277 16.224823, 18/02/12, Jour, 404alt, ZQ; 1♀ *Isoxya penzoides* République centrafricaine (RCA), Sangha Mbaéré, Lac 1, Camp de base, Dans un Azobé (Lophira alata, Ochnaceae) à 38 m du sol, UV canopée, 2°28'49.5" 16°12'55.9", 2.480416667 16.21552778, 22-23/11/10, Nuit, 392alt, NB; 1♀ *Togacantha nordvei* République centrafricaine (RCA), Sangha Mbaéré, Ripisylve, sous-bois, Lac 1, Bord de lac, 2,480322 N 16,215608 E to 2,481672 N 16,222751 E, 20/11/10, Nuit, 403alt, sans code; 1♀ *Togacantha nordvei* République centrafricaine

(RCA), Sangha Mbaéré, Forêt, sous-bois, 2°28'08.6"N 16°13°32.1"E to 2°28'06.7"N 16°13'29.6"E / UTM 33 N: 636260-272970 to 636186-272911, 27/11/10, Jour, 385/405alt, OE1; 2♀ *Togacantha nordvei* République centrafricaine (RCA), Sangha Mbaéré, Forêt, sous-bois, Camp transit 1 vers camp transit 2 (au bord du Kénié), 2°26'47.4"N 16°05'25.1"E to 2°27'37.8"N 16°08'33.2"E / UTM 33 N : 624492-271937 to 627031-272015, 17/11/10, Jour, 390/376alt, MB1; 1♀ *Togacantha nordvei* République centrafricaine (RCA), Sangha Mbaéré, Lac 7, Prospection lac, Végétation aquatique, 2° 27' 47,8" 16° 13' 29,4", 2.463277 16.224823 02/02/12, Jour, 404alt, SW7.

Steven Hoff Williams (private collection), UK (SHW):

4♀ 4♂ *Araneus diadematus* England, Eynsham, Oxfordshire, 51.780469, -1.373194, coll. Steven Hoff Williams, September 2018; 3♀ *Gasteracantha milvoides* Mount Lico, Mozambique, -15.79358, 37.36116, 921m, coll. Gabriella Bittencourt, May 2018; 2♀ *Gasteracantha milvoides* Mount Socone, Mozambique, -15.73623, 37.28815, 1159m, coll. Gabriella Bittencourt, May 2018; 6♀ *Gasteracantha milvoides* Mount Lico, Mozambique, -15.79358, 37.36116, 921m, coll. Gabriella Bittencourt, May 2018; 3♀ 1 juv♂ *Isoxya tabulata* Mount Lico, Mozambique, -15.79358, 37.36116, 921m, coll. Gabriella Bittencourt, May 2018; 3♀ *Micrathena schreibersi* French Guiana, Gite D'Angouleme, N05.40843°, W53.65457°, Feb.2018; 3♀ *Micrathena schreibersi* French Guiana, Camp Patowa, Mar.-Apr. 2018, coll. Gabriella Bittencourt.

Vienna Museum of Natural History, Austria (VMNH):

1♂ *Austracantha minax* NHMW 28877, Sidney, 1884; 1♀ *Gasteracantha falcicornis* NHMW 28812, Risangile (O-Ustafrica), 1900; 1♀ *Gasteracantha lunata* NHMW 28898, Amboina; 1♀ *Gasteracantha scintillans* NHMW 28866, H. Geong-Ins. (Solom.), Albatros

15./2.1897; 2♀ *Gasteracantha simoni* NHMW28810, Tanganyika; 2♀ *Gasteracantha thorelli* NHMW 28811, Nossi Be (Madagascar); 2♀ *Isoxya cicatricosa* NHMW 28808, Ustafr: Ugano, 1936.

A2.3 Male specimens located

Table A2.1

Male specimens located for study - additional male data extracted from literature

Species name	Number of specimens	Institution/ Collection
Nephila pilipes	2	EZ
Araneus diadematus	4	SHW
Micrathena aureola	2	OUMNH
Augusta glyphica	1	EZ
Isoxya cicatricosa	1	NHM
Isoxya cowani	2	NHM
Isoxya sp. nov.	5	IA
Isoxya tabulata	3	EZ
*Acrosomoides acrosomoides**	1	EZ
*Actinacantha globulata**	1	NHM
Austracantha minax	3	NHM, VMNH
Macracantha hasselti	2	MM
Thelacantha brevispina	6	NHM
*Gasteracantha aciculata**	6	EZ
*Gasteracantha diardi**	1	EZ
Gasteracantha milvoides	3	MM
Gasteracantha pentagona	2	MM
Gasteracantha rhomboidea madagascariensis	1	EZ
Gasteracantha sanguinolenta	1	SD
Gasteracantha taeniata	5	MM
Gasteracantha versicolor formosa	4	EZ

(* denotes a currently undescribed male but all are: Williams, in prep.)

A2.4 Material used in molecular analysis

The specimens used for extractions in molecular analysis by S.H.W. and A.S. at Oxford Brookes University - McGregor Lab.

Sources and specimens were:

EZ Lab, Slovenia (EZ):

1♀ *Acrosomoides acrosomoides* ARA2000, SE Africa: Madagascar, Toamasina, Andasibe Mantadia NP, Anivokely, -18.93717 48.42005, 900-1000alt, Agnarsson I., Kuntner M., 19.iv.2008; 1♀ *Acrosomoides acrosomoides* ARA2014, SE Africa: Madagascar, Toamasina, Andasibe Mantadia NP, 16.iv.2008; 1♀ 1♂ *Augusta glyphica* ARA2019, SE Africa: Madagascar, Toamasina, Andasibe Mantadia NP, Analamazoatra, -18.93717 48.42005, 900-1000alt, Agnarsson I., Kuntner M., 31.iii.2008; 1♀ *Gasteracantha aciculata* ARA2041, Australasia: Papua New Guinea, New Britain, riverbank, I. Agnarsson, 9.iv.2009; 1♀ 1♂ *Gasteracantha aciculata* ARA2046, Australasia: Papua New Guinea, New Britain, camp2, I. Agnarsson, 11.iv.2009; 2♀ *Gasteracantha clavatrix* ARA2011, SE Asia: Indonesia, Sulawesi, Bitung, 1.48347 125.12720, 251alt, Kuntner M., Gregorič M., 13.vii.2007; 2♀ *Gasteracantha diardi* ARA2010, SE Asia: Malaysia, Pahang, Bukit Fraser, 3.72069 101.74038, 1253alt, Kuntner, 4.vi.2007; 1♀ *Gasteracantha hasselti* ARA1982, E Asia: China, Yunnan, Xishuangbanna, Mengla, Green Stone Forest, 21.91697 101.29075, 598alt, Green stone, Kuntner M., Li D.Q., Gregorič M., 27.xii.2010; 1♀ *Gasteracantha hasselti* ARA1987, E Asia: China, Hainan, Lingshui Li Autonomous County, 18.73200 109.88578, 915alt, Kuntner M., 21.vi.2011; 1♀ 1♂ juv. *Gasteracantha kuhli* ARA2005, SE Asia: Male and Female in the same web, SP10, 16-18.v.2005; 1♀ *Gasteracantha milvoides* ARA2033, S Africa: South Africa, Sod.B out RD., 18.iv.2006; 1♀ *Gasteracantha milvoides* ARA2034, S Africa: South Africa, KwaZulu-Natal, Hluhluwe-iMfolozi NP, forest, 20.iv.2008; 1♀

Gasteracantha sturi ARA1984; China: Yunnan, Xishuangbanna, Mengla, Mengyuan, 119 farm, 21.64703 101.41668, 816alt, Kuntner M., Li D.Q., Gregorič M., 31.xii.2010; 1♀ *Gasteracantha sturi* ARA1986, China: Yunnan, Xishuangbanna, Baka forest, 21.71368 100.78302, 695alt, Baka, Kuntner M., Li D.Q., Gregorič M., 29.xii.2010; 1♀ *Gasteracantha rhomboidea madagascariensis* ARA2026, SE Africa: Madagascar, Toamasina, Andasibe Mantadia NP, Analamazoatra, Agnarsson I., Kuntner M., 4.iv.2008; 1♀ *Gasteracantha rhomboidea madagascariensis* ARA2027, SE Africa: Madagascar, Antsiranana, Montagne d'Ambre NP, I. Agnarsson & M. Kuntner, 4.iv.2008; 1♀ 1♂ *Gasteracantha versicolor formosa* ARA2018, SE Africa: Madagascar, Toamasina, Andasibe Mantadia NP, Analamazoatra, -18.93717 48.42005, 900-1000alt, Agnarsson I., Kuntner M., 31.iii.2008; 1♀ 1♂ *Gasteracantha versicolor formosa* ARA2021, SE Africa: Madagascar, Toamasina, Andasibe Mantadia NP, Analamazoatra, -18.93717 48.42005, 900-1000alt, MF same web, Agnarsson I., Kuntner M., 31.iii.2008; 1♀ 1♂ *Isoxya tabulata* ARA1993, S Africa: South Africa, 17.iv.2006; 1♀ *Isoxya tabulata* ARA2037, S Africa: South Africa, KwaZulu-Natal, Hluhluwe-iMfolozi NP, Hilltop at station, 20.iv.2006; 2♀ *Macracantha arcuata* ARA1985, E Asia: China, Yunnan, Xishuangbanna, Botanical garden, 21.92392 101.19792, 620alt, XI Shuangbanna Bot. Garden, Kuntner M., Li D.Q., Gregorič M., 26-30.xii.2010; 1♀ *Macracantha arcuata* ARA2004, SE Asia: Malaysia, Selangor, Gombak, forest around field station, 3.32485 101.75292, 272alt, Macrac., Kuntner M., Gregorič M., 6.i.2010; 1♀ *Thelacantha brevispina* ARA2032, Indian Ocean, Mauritius, Black River Gorge NP, Brise Fer Station, secondary rain forest, -20.37628 57.44339, 602alt, I. Agnarsson & M. Kuntner, 20.iv.2008.

Ingi Agnarsson (private collection), USA (IA):

1♀ 1♂ *Isoxya* sp. nov. coll. Agnarsson I., Kuntner, M., Gregoric M. Eastern Madagascar, Toamasina province, along the road leading from Andasibe to Mantadia (Andasibe-Mantadia National Park at -18.82663, 48.43333), 5-8. December 2017.

Natural History Museum London, UK (NHM):

1♀ *Actinacantha metallica* 87.8, Solomon Islands, purchased C.M.Woodford, [not type specimen]; 1♀ *Gasteracantha quadrispinosa* 1937.12.13.437, Dutch New Guinea, Humboldts Bay, 200ft, iv.1936 no.129, L.E.Cheesman; 1♀ *Gasteracantha scintillans* Royal Society: B.S.I.P. 1965, Coll. No: G2, Lower Camp Area, Mt. Gallego, Guadalcanal, B.M.(N.H.) Acc.No: 2182 (Moll); 1♀ *Gasteracantha sp. nov.* Royal Society: B.S.I.P. 1965, Colln. No. Arachnida, Leg. P.Hunt, Alasa'a, Malaita, B.M.(N.H.) Acc. No. 2181 (Moll.); 1♀ *Gasteracantha unguifera* Collected whilst marching between Tilhar (28°17'N 83°51'E) and Naudhara, 28°17'N 83°51'E), K.H. Hyatt. Coll. No. 247, Brit. Mus. Nepal Exped. 1954., 29 July; 1♀ *Gasteracantha unguifera* 1934.2.28.124., Tsuntang, Sikkim, N.India, 6,000ft. 24.4.1924., Maj R.W.G.Hingston., Mt. Everest Explor. Exped. 1924.

Sam Danflous (private collection), France (SD):

1♀ *Acrosomoides linnaei* République centrafricaine (RCA), Sangha Mbaéré, Ripisylve, sous-bois, Lac 1, Bord de lac, 2,480322 N 16,215608 E to 2,481672 N 16,222751 E, 20/11/10, Nuit, 403alt, sans code; 1♀ *Acrosomoides linnaei* République centrafricaine (RCA), Sangha Mbaéré, Lac 7, Tour lac 7, Végétation aquatique, 2°27'53.6" 16°13'30.5", 2.464888889 16.22513889, 28/11/10, Jour, 414alt, OM1; 1♀ *Aetrocantha falkensteini* République centrafricaine (RCA), Sangha Mbaéré, Sous-bois forêt, Lac 1, Camp, 2° 28' 49,2" 16° 12' 56,2", 2.480322 16.215608, 06/02/12, Nuit, 401alt, TU; 1♀ *Aetrocantha falkensteini*

République centrafricaine (RCA), Sangha Mbaéré, Lac 1, Camp de base, 2°28'49.5" 16°12'55.9", 2.480416667 16.21552778, 24/11/10, Jour, 392alt, NL; 1♀ *Afracantha cameruensis* République centrafricaine (RCA), Sangha Mbaéré, Sous-bois forêt, Lac 7, Prospection lac, 2° 27' 47,8" 16° 13' 29,4", 2.463277 16.224823, 18/02/12, Jour, 404alt, ZQ; 1♀ *Gasteracantha curvispina* République centrafricaine (RCA), Sangha Mbaéré, Camp transit 2 au bord du Kénié, 2°27'37.8" 16°08'33.2", 17/11/10, Jour, 376alt, MB2a; 1♀ Gasteracantha curvispina République centrafricaine (RCA), Bénin, Forêt de Houéyogbé, dépt. Mono, commune Houéyogbé, 82alt, 06°33'43"N 01°51'02"E, 6.561944444444444 N 1.850555555555556 E, J.-P. Maurel, 20.X.2014; 1♀ *Gasteracantha sanguinolenta* République centrafricaine (RCA), Sangha Mbaéré, Molongo, Alentours du village, Strate arbustive, 2°27'11.6" 16°04'58.7", 2.453222222 16.08297222 14-15/11/10, Nuit, 343alt, LQ; 1♀ 1♂ *Gasteracantha sanguinolenta* République centrafricaine (RCA), Sangha Mbaéré, Berges de la Sangha, Molongo-Nyangouté, Bord Sangha, Samuel Danflous, prospection en pirogue, 2°25'04.9" 16°06'22.3", 2.418027778 16.10619444, 16/11/10, Jour, 360alt, LYa; 1♀ *Isoxya penzoides* République centrafricaine (RCA), Sangha Mbaéré, Sous-bois forêt, Lac 4, Prospection lac (Bilos), 2° 28' 23,7" 16° 13' 25,4", 11/02/12, Jour, 395alt, WE; 1♀ *Isoxya penzoides* République centrafricaine (RCA), Sangha Mbaéré, Végétation aquatique, Tour lac 3, 2°29'19.1" 16°13'58.4", 2.488638889 16.23288889, 23/11/10, Jour, 401alt, MY1; 1♀ *Togacantha nordvei* République centrafricaine (RCA), Sangha Mbaéré, Forêt, sous-bois, 2°28'08.6"N 16°13°32.1"E to 2°28'06.7"N 16°13'29.6"E / UTM 33 N: 636260-272970 to 636186-272911, 27/11/10, Jour, 385/405alt, OE1; 1♀ *Togacantha nordvei* République centrafricaine (RCA), Sangha Mbaéré, Lac 7, Prospection lac, Végétation aquatique, 2° 27' 47,8" 16° 13' 29,4", 2.463277 16.224823 02/02/12, Jour, 404alt, SW7.

Steven Hoff Williams (private collection), UK (SHW):

1♀ *Gasteracantha milvoides* Mount Lico, Mozambique, -15.79358, 37.36116, 921m, coll. Gabriella Bittencourt, May 2018; 1♀ *Isoxya tabulata* Mount Lico, Mozambique, -15.79358, 37.36116, 921m, coll. Gabriella Bittencourt, May 2018; 1♀ *Micrathena schreibersi* French Guiana, Camp Patowa, Mar.-Apr. 2018, coll. Gabriella Bittencourt.

A2.5 Measurement data for SSD (Chapter 4)

Measurement data (all values = mm) M1-M5 = male 1-5, F1-F5 = female 1-5, MM = male mean, FM = female mean (data original by S.H.W or converted from Emerit (1973, 1974, 1982b), Sankaran *et al*. (2015) and Williams (2017) * = abdomen data only

Species						
	M1	M2	M3	M4	M5	MM
Acrosomoides acrosomoides	2.4	-	-	-	-	2.4
	F1	F2	F3	F4	F5	FM
Acrosomoides acrosomoides	7.4	7.2	-	-	-	7.3
	M1	M2	M3	M4	M5	MM
Acrosomoides linnaei	2.4	-	-	-	-	2.4
	F1	F2	F3	F4	F5	FM
Acrosomoides linnaei	7.1	7.3	-	-	-	7.2
	M1	M2	M3	M4	M5	MM
Actinacantha globulata	2.1	-	-	-	-	2.1
	F1	F2	F3	F4	F5	FM
Actinacantha globulata	9.1	9.0	8.8	-	-	8.9
	M1	M2	M3	M4	M5	MM
Augusta glyphica	3.4	3.5	-	-	-	3.4
	F1	F2	F3	F4	F5	FM
Augusta glyphica	8.7	8.7	10.6	9.6	9.0	9.3
	M1	M2	M3	M4	M5	MM
Austracantha minax	3.3	3.5	3.5	3.4	-	3.4
	F1	F2	F3	F4	F5	FM
Austracantha minax	5.6	8.5	8.5	9.3	7.2	7.8
	M1	M2	M3	M4	M5	MM
Gasteracantha aciculata	2.0	2.1	2.1	2.0	-	2.0
	F1	F2	F3	F4	F5	FM
Gasteracantha aciculata	7.7	7.5	7.6	6.5	7.9	7.4
	M1	M2	M3	M4	M5	MM
Gasteracantha diardi	2.2	-	-	-	-	2.2
	F1	F2	F3	F4	F5	FM
Gasteracantha diardi	10.3	8.7	-	-	-	9.5
	M1	M2	M3	M4	M5	MM
Gasteracantha geminata	3.3	-	-	-	-	3.3
	F1	F2	F3	F4	F5	FM
Gasteracantha geminata	6.4	6.6	-	-	-	6.5
	M1	M2	M3	M4	M5	MM
Gasteracantha milvoides	2.6	2.4	3.0	-	-	2.6
	F1	F2	F3	F4	F5	FM
Gasteracantha milvoides	7.4	8.8	8.9	8.8	8.9	8.5

Measurement data (all values = mm) M1-M5 = male 1-5, F1-F5 = female 1-5, MM = male mean, FM = female mean (data original by S.H.W or converted from Emerit (1973, 1974, 1982b), Sankaran *et al.* (2015) and Williams (2017) * = abdomen data only

Species						
	M1	**M2**	**M3**	**M4**	**M5**	**MM**
Gasteracantha pentagona	**2.4**	**2.4**	**-**	**-**	**-**	**2.4**
	F1	**F2**	**F3**	**F4**	**F5**	**FM**
Gasteracantha pentagona	**8.0**	**8.9**	**-**	**-**	**-**	**8.4**
	M1	**M2**	**M3**	**M4**	**M5**	**MM**
Gasteracantha rhom. madag.	**2.4**	**-**	**-**	**-**	**-**	**2.4**
	F1	**F2**	**F3**	**F4**	**F5**	**FM**
Gasteracantha rhom. madag.	**5.3**	**7.2**	**7.6**	**6.3**	**6.8**	**6.6**
	M1	**M2**	**M3**	**M4**	**M5**	**MM**
Gasteracantha taeniata	**2.1**	**2.3**	**2.1**	**2.2**	**1.9**	**2.1**
	F1	**F2**	**F3**	**F4**	**F5**	**FM**
Gasteracantha taeniata	**6.8**	**6.8**	**8.4**	**7.0**	**6.7**	**7.1**
	M1	**M2**	**M3**	**M4**	**M5**	**MM**
Gasteracantha versi. form.	**2.3**	**2.6**	**2.4**	**-**	**-**	**2.4**
	F1	**F2**	**F3**	**F4**	**F5**	**FM**
Gasteracantha versi. form.	**5.5**	**6.0**	**6.9**	**7.6**	**-**	**6.5**
	M1	**M2**	**M3**	**M4**	**M5**	**MM**
Hypsacantha crucimaculata*	**2.2**	**-**	**-**	**-**	**-**	**2.2**
	F1	**F2**	**F3**	**F4**	**F5**	**FM**
Hypsacantha crucimaculata*	**3.6**	**-**	**-**	**-**	**-**	**3.6**
	M1	**M2**	**M3**	**M4**	**M5**	**MM**
Isoxya cicatricosa	**4.0**	**-**	**-**	**-**	**-**	**4.0**
	F1	**F2**	**F3**	**F4**	**F5**	**FM**
Isoxya cicatricosa	**6.0**	**-**	**-**	**-**	**-**	**6.0**
	M1	**M2**	**M3**	**M4**	**M5**	**MM**
Isoxya cowani	**4.0**	**3.5**	**3.4**	**-**	**-**	**3.6**
	F1	**F2**	**F3**	**F4**	**F5**	**FM**
Isoxya cowani	**5.5**	**5.3**	**5.4**	**5.4**	**5.4**	**5.4**
	M1	**M2**	**M3**	**M4**	**M5**	**MM**
Isoxya mahafalensis	**3.4**	**-**	**-**	**-**	**-**	**3.4**
	F1	**F2**	**F3**	**F4**	**F5**	**FM**
Isoxya mahafalensis	**5.4**	**-**	**-**	**-**	**-**	**5.4**
	M1	**M2**	**M3**	**M4**	**M5**	**MM**
Isoxya penzoides*	**2.3**	**-**	**-**	**-**	**-**	**2.3**
	F1	**F2**	**F3**	**F4**	**F5**	**FM**
Isoxya penzoides*	**4.6**	**-**	**-**	**-**	**-**	**4.6**

Measurement data (all values = mm) M1-M5 = male 1-5, F1-F5 = female 1-5, MM = male mean, FM = female mean (data original by S.H.W or converted from Emerit (1973, 1974, 1982b), Sankaran *et al.* (2015) and Williams (2017) * = abdomen data only

Species						
	M1	**M2**	**M3**	**M4**	**M5**	**MM**
Isoxya sp. nov.	**3.9**	**5.1**	**4.9**	**4.9**	**4.5**	**4.6**
	F1	**F2**	**F3**	**F4**	**F5**	**FM**
Isoxya sp. nov.	**5.3**	**6.0**	**6.9**	**6.2**	**6.1**	**6.1**
	M1	**M2**	**M3**	**M4**	**M5**	**MM**
Isoxya tabulata	**3.4**	**3.4**	**3.1**	**-**	**-**	**3.3**
	F1	**F2**	**F3**	**F4**	**F5**	**FM**
Isoxya tabulata	**5.7**	**6.6**	**6.0**	**6.8**	**6.5**	**6.3**
	M1	**M2**	**M3**	**M4**	**M5**	**MM**
Macracantha hasselti	**2.0**	**2.5**	**-**	**-**	**-**	**2.2**
	F1	**F2**	**F3**	**F4**	**F5**	**FM**
Macracantha hasselti	**8.0**	**7.9**	**8.1**	**8.7**	**7.4**	**8.0**
	M1	**M2**	**M3**	**M4**	**M5**	**MM**
Thelacantha brevispina	**2.1**	**2.1**	**2.1**	**2.0**	**-**	**2.1**
	F1	**F2**	**F3**	**F4**	**F5**	**FM**
Thelacantha brevispina	**7.2**	**8.6**	**7.5**	**6.7**	**-**	**7.5**
	M1	**M2**	**M3**	**M4**	**M5**	**MM**
Togacantha nordvei	**2.2**	**-**	**-**	**-**	**-**	**2.2**
	F1	**F2**	**F3**	**F4**	**F5**	**FM**
Togacantha nordvei	**6.6**	**6.8**	**6.8**	**6.8**	**-**	**6.7**

Appendix 3: Morphological Matrix, Full Character List and Molecular Matrices

A3.1 Glossary of morphological terminology

A glossary of morphological terminology is presented here (based upon Coddington, 1990 (figures 56-67); Scharff and Coddington, 1997; Kuntner, Coddington and Hormiga, 2008 (figure 16D); and Nentwig *et al*., 2022).

Abdomen – Posterior major body part.

Anterior – Front, nearer to the head.

Book lung - Anterior respiration organ of most spiders.

Carapace - Dorsal shield covering the surface of prosoma.

Cephalothorax - Anterior major body part; a fusion of the head and thorax.

Chelicerae - The most anterior pair of extremities of prosoma in arachnids.

Conductor – sclerotised part of the embolic division that serves as protection and guidance for the embolus.

Condyles – an articular prominence (in the case of Gasteracanthinae these are present in a similar manner to tubercles)

Coxa – First segment of leg or palp.

Cymbium – the tarsus of the male palp that covers the palp bulb dorsally.

Dorsal - Faced opposite to the mouth (the back or upper surface).

Embolic division – distal part of the palp bulb that consists of the embolus and additional sclerites.

Embolus – sclerotised organ that transfers sperm.

Epigyne – sclerotised external plate covering the female reproductive opening.

Epigyne opening – the copulatory opening for the male embolus.

Eyes – Anterior Lateral Eyes (ALE), Anterior Median Eyes (AME), Posterior Lateral Eyes (PLE), Posterior Median Eyes (PME)

Femur – Third segment of legs and palps.

Hematotocha – soft area of the palp that expands hydraulically.

Lateral – (situated) on the side.

Lobe – like, but not homologous with, the scape (see below).

Median – In the middle or midline.

Median apophysis – a median sclerite of the palp bulb that is separated from the tegulum.

Paracymbium – a structure that is either completely or partly detached from the cymbium.

Paramedian apophysis – a sclerite basal extension of the conductor.

Pars pendula - a membranous connector between the ejaculatory duct and the sclerotized embolus.

Posterior – further back in position or nearer the rear or hind end.

Prosoma - Anterior body part, consisting of six (merged) segments.

Scape – appendage of anterior epigynal margin.

Sclerite – hardened area of cuticle, like the apophyses.

Septum – sclerotised division between the two epigyne openings.

Sigilla – Externally visible muscle attachments

Spermatheca - sperm storage organ of the vulva of female.

Spermophor – the sperm duct, in which sperm can be taken up, stored, and expelled during copulation.

Spinneret - Modified extremities of the abdomen that produce silk.

Spiracle - The opening of the trachea on the ventral side of the body.

Sternum - Ventral plate of the prosoma.

Subterminal apophysis – sclerite located under the terminal of the embolic division of the palp bulb.

Tegulum – the largest median sclerite of the bulb that contains the spermophor.

Terminal apophysis – sclerite located on the terminal of the embolic division of the palp bulb.

Trachea - Finely branching internal tubes, part of the respiratory system. Normally, the posterior respiratory organ in spiders.

Venter/Ventral - The side of the body where the mouth is located (belly or lower side).

A3.2 Morphological characters scored list details

A full list of characters scored and used in the analysis is presented here, followed by a list of additional characters that were scored but not used in the analysis (due to being uninformative or invariant). A full matrix of characters (both included and excluded) is also presented in A3.2.3, and the average consistency and retention indices for all characters in the Group 1-3 maximum parsimony analyses in PAUP* are presented in A3.2.4.

Sometimes character states were scored as missing where specimens could not have characters scored but the character might be present; for example, in species where males have been described but no specimens were available to study. Gaps are left when a character is confirmed to not be present in certain species; for example, character 12 cannot be scored in species without spines.

A description of the character states follows, concluding with a description of the character if further information is required. Notes on the function of the character, where known, and the character's homology hypothesis are also included where required. Where characters have been used before in previous works they are listed as "Author/s (date) character #" to distinguish from the character they are referring to here.

A3.2.1 Scored characters used in morphological analysis

Character List

I. Female carapace and cephalothorax:

1. *Central posterior carapace tubercle (Female)*

(0) Flat; (1) Conspicuously raised tubercle, split; (2) Conspicuously raised tubercle, single.

Unordered. This character was scored as unordered because the single protuberance (2) is an autapomorphy for *Thelacantha brevispina* (Doleschall, 1857) Figure A3.1 and is currently only seen in this species, therefore it is not clear if the state transformed from flat into a tubercle that then divides or if the states independently evolved. Magalhães and Santos (2012) character 26, Scharff and Coddington (1997) character 47 and Levi (1985, 2002) all refer to the raised tubercle on the carapace present in *Gasteracantha* and members of the Gasteracanthinae. There is a generic split between *Thelacantha* and *Gasteracantha* with the carapace raised tubercle split into two or remaining as one. The single protuberance is an apomorphy for the species *Thelacantha brevispina* (2) Figure A3.1(A), but the raised carapace is a synapomorphy for Gasteracanthinae and is hypothesized to be a homologous character due to its location; for example: *Gasteracantha aciculata* (1) Figure A3.2(A).

Figure A3.1 Photograph of the anterior view of *Thelacantha brevispina* (♀) carapace showing state (2) of character 1 single raised tubercle (A).

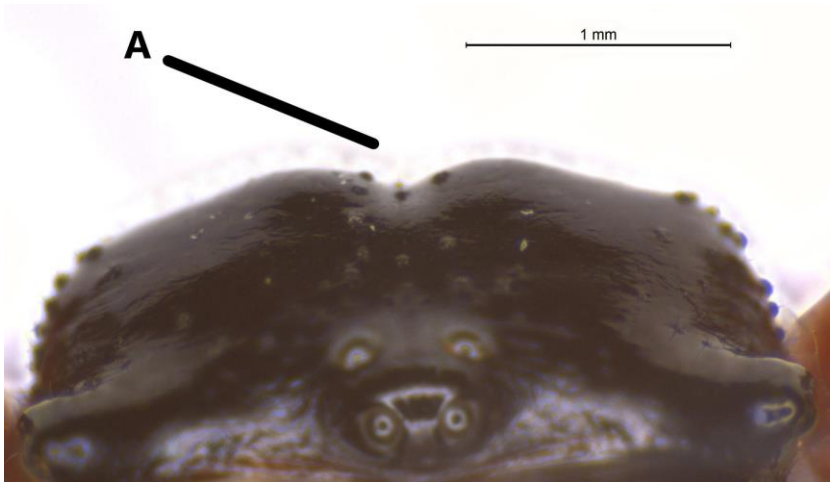

Figure A3.2 Photograph of the anterior view of *Gasteracantha aciculata* (♀) carapace showing state (1) of character 1 double raised tubercle (A).

2. *Multiple indentations on cephalothorax surface (Female)*

(0) Absent; (1) Present.

Taken directly from Magalhães and Santos (2012) character 32. Exact numbers of dimples and shapes not used (characters 33 and 34 in Magalhães and Santos) as the characters would be uninformative as the dimples are not present in the Gasteracanthinae, only in the *Micrathena* in this study; for example: *Micrathena schreibersi* (1) Figure A3.3(A).

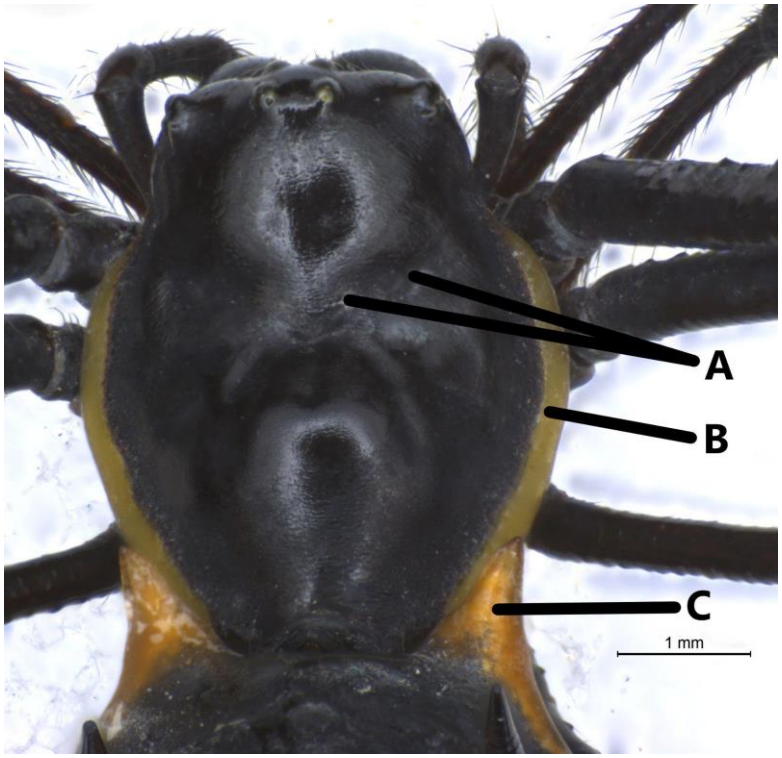

Figure A3.3 Photograph of the dorsal view of *Micrathena schreibersi* (♀) cephalothorax highlighting state (1) of character 2 indentations on cephalothorax (A), state (1) of character 5 thoracic rim (B) and state (1) of character 8 front facing spines (C).

3. *Carapace shape (Female)*

(0) Longer than wide; (1) Wider than long or equal length.

Scharff and Coddington (1997) character 42. This character is taken from Scharff and Coddington to score the structure of the "square" carapace seen in the Gasteracanthinae, for example: *Gasteracantha thorelli* (1) Figure A3.4(A), which separates them from other Araneidae genera.

4. *Indentation posterior to posterior median eyes (PME) on head (Female)*

(0) Flat; (1) Conspicuous indentation.

A homologous indentation is present behind the PME but before the raised cephalothorax in many taxa; for example: *Gasteracantha thorelli* (1) Figure A3.4(B). No other form of indentation in the eye area was recorded in the studied taxa.

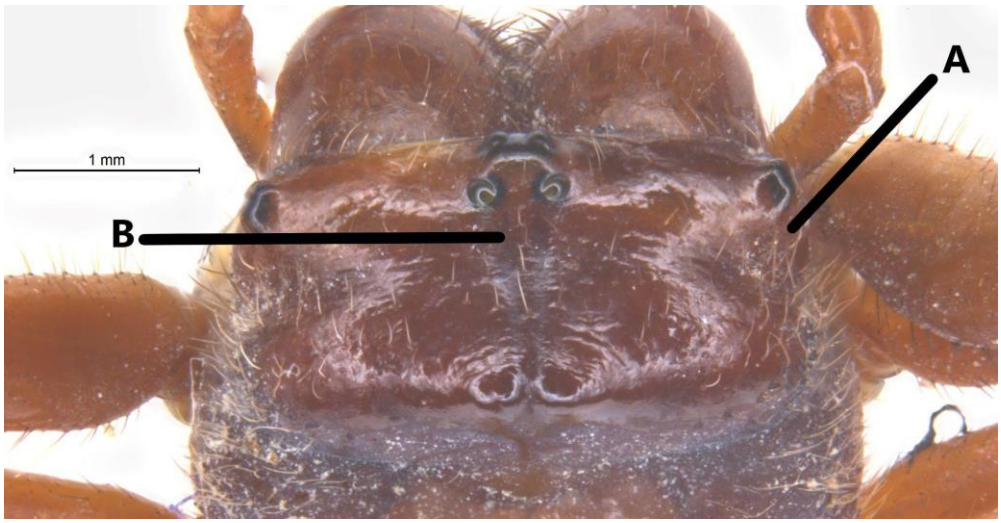

Figure A3.4 Photograph of the dorsal view of *Gasteracantha thorelli* (♀) carapace showing state (1) of character 3 square carapace (A) and state (1) of character 4 indentation posterior to PME (B).

5. *Carapace thoracic rim (Female)*

(0) Absent; (1) Present.

Taken from Magalhães and Santos (2012) character 3 as a character that relates to *Micrathena*, for example: *Micrathena schreibersi* (1) Figure A3.3(B), and not the other spiny orb weavers in this study. The rim is an addition to the carapace in some taxa.

II. Female abdomen surface:

6. *Abdomen surface structure (Female)*

(0) Soft; (1) Sclerotized.

This character is similar to character 44 from Magalhães and Santos (2012), following the protocol of using forceps to test abdomen sclerotization. Here this is simplified, as the first two outgroup species have typically soft abdomens and the Gasteracanthinae and *Micrathena* have the sclerotized abdominal structure.

7. *Abdomen colouring (Female)*

(0) Non metallic; (1) Metallic.

Gasteracantha species from the Solomon Islands possess a clearly metallic-coloured abdomen that appears homologous. There is no intermediate state, they are either metallic or not and do not lose colouration in ethanol unless the specimen is extremely damaged (Williams, pers. obs.); for example: *Gasteracantha scintillans* (1) Figure A3.5. For a discussion about abdominal colouring see Chapter 2 and Chapter 4.

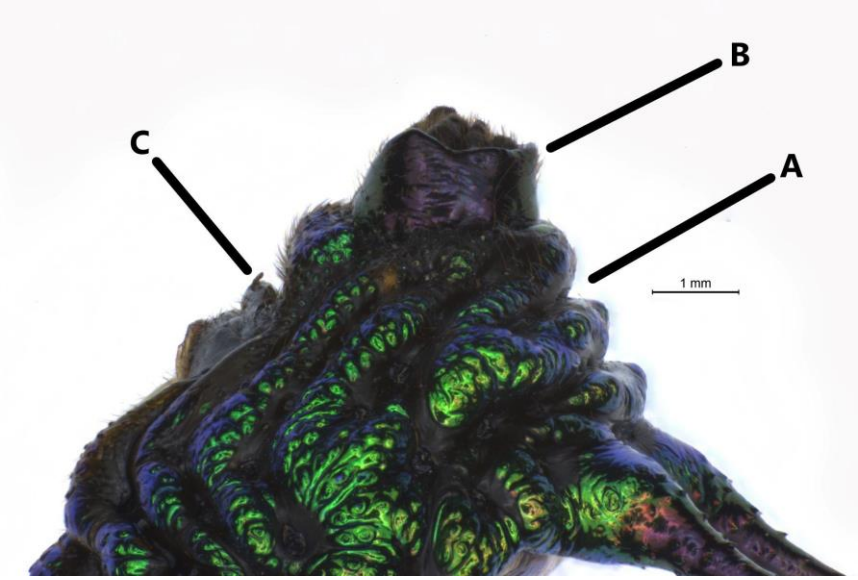

Figure A3.5 Photograph of the right lateral view of *Gasteracantha scintillans* (♀) showing state (1) of character 7 metallic colouration (whole abdomen), state (1) abdominal wrinkles (A) of character 31, state (1) extremely elongated spinneret tubercle (B) of character 38, state (1) projected epigye lobe (C) of character 44.

III. Female abdominal spines:

8. *Abdominal spines facing head (Female)*

(0) Absent; (1) Present.

Rather than focusing on the specific numbers of spines per species, the front facing pair is scored as a single character regardless of number; for example: *Micrathena schreibersi* (1) Figure A3.3(C). This character is similar to Magalhães and Santos (2012) character 50 but simplified here as only a few taxa in this study possess any front facing spines.

9. *Abdominal spines anterior (Female)*

(0) Absent; (1) Present.

This, and the following two characters, are very similar to characters 50-54 from Magalhães and Santos (2012). Spines are very prominent features on many of the species; for example: *Gasteracantha aciculata* (1) Figure A3.6 and *Gasteracantha thorelli* (1) Figure A3.7. This character is also scored in Scharff and Coddington (1997) character 62, but as a combination of all 3 pairs of spines. Due to the absence of different abdominal spines in various studied taxa the split into 3 separate characters is used here, opposed to the single character in Scharff and Coddington (1997).

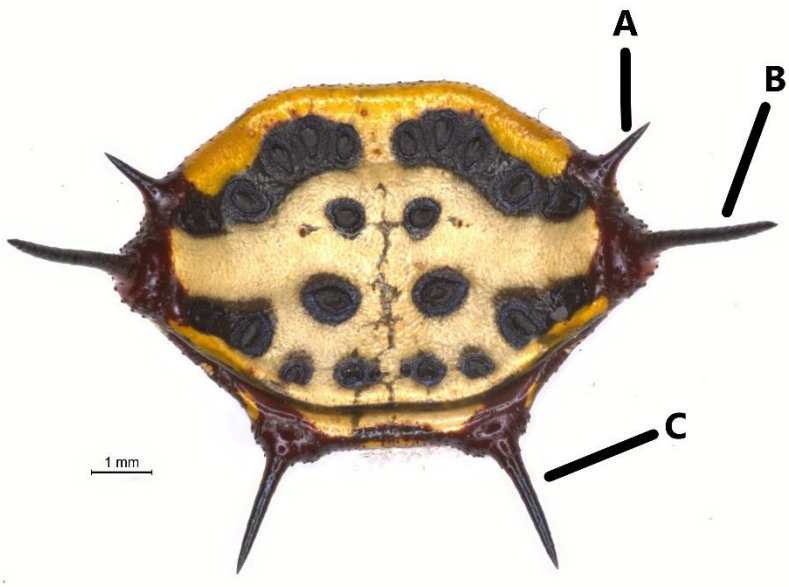

Figure A3.6 Photograph of the dorsal view of *Gasteracantha aciculata* (♀) abdomen with anterior (A), median (B) and posterior (C) spines present.

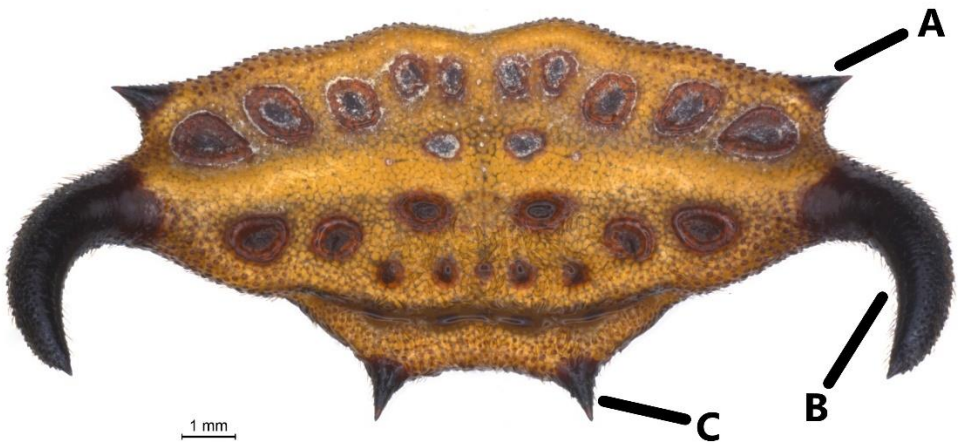

Figure A3.7 Photograph of the dorsal view of *Gasteracantha thorelli* (♀) abdomen with anterior (A), median (B) and posterior (C) spines present.

10. *Abdominal spines median (Female)*

(0) Absent; (1) Present.

See character 9 above.

11. *Abdominal spines posterior (Female)*

(0) Absent; (1) Present.

See character 9 above. The third pair of spines can be difficult to observe in some species as often they are the smallest pair, but clear absence can be confirmed for species which lack this character state; for example: *Gasteracantha mengei* Keyserling, 1864 (0) Figure A3.8.

Figure A3.8 Photograph of the dorsal view of *Gasteracantha mengei* (♀) whole spider, with anterior (A), median (B) spines present and no posterior spines.

12. *Abdominal spines median terminal structure (Female)*

(0) Tapering; (1) Bulbous.

The most common spine structure is tapering to a point with some species having a bulbous apex of the spine; for example: *Gasteracantha clavatrix* (1) Figure A3.9.

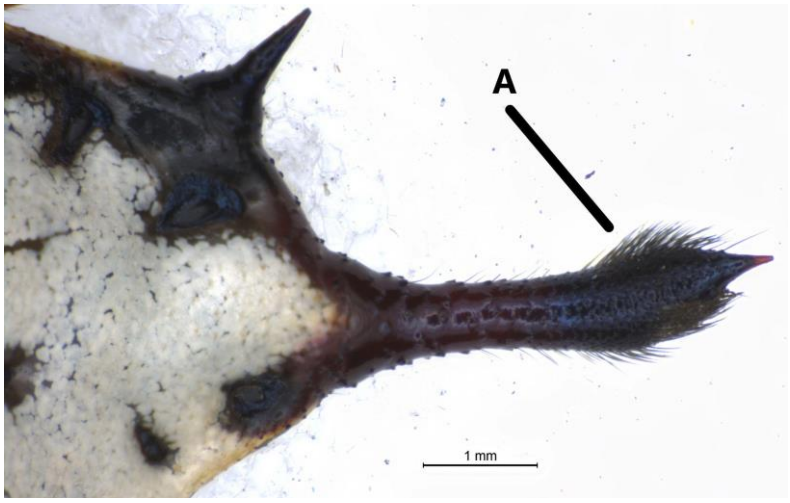

Figure A3.9 Photograph of the dorsal view of *Gasteracantha clavatrix* (♀) showing state (1) of character 12 bulbous terminal structure of spine (A).

13. *Basal structure of anterior abdominal spines (Female)*

(0) No basal structure; (1) Bulbous; (2) Fused.

Unordered. Species of *Gasteracantha* and members of the Gasteracanthinae can often have a homologous bulbous base structure before the spine tapers; for example: *Thelacantha brevispina* (1) Figure A3.10(A). *Gasteracantha geminata* has fused spines pair 1 and 2 at the base structure; *Gasteracantha geminata* (2) Figure A3.11. The fusing of spines 1 and 2 is currently only known in *G. geminata*.

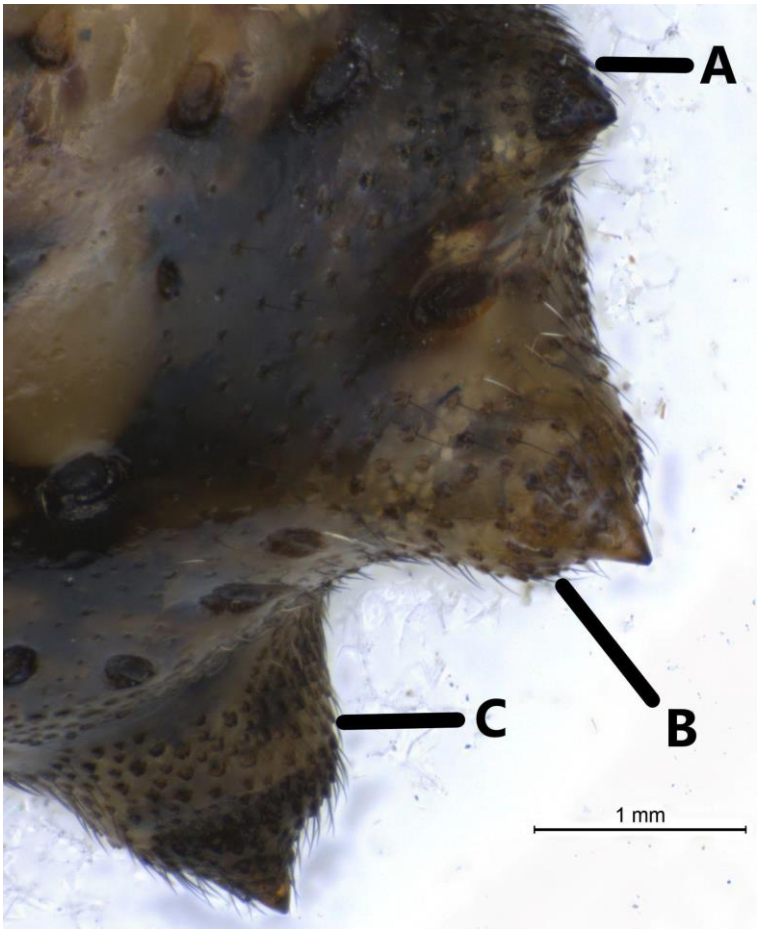

Figure A3.10 Photograph of the dorsal view of *Thelacantha brevispina* (♀) spines showing spines anterior (A), median (B), posterior (C).

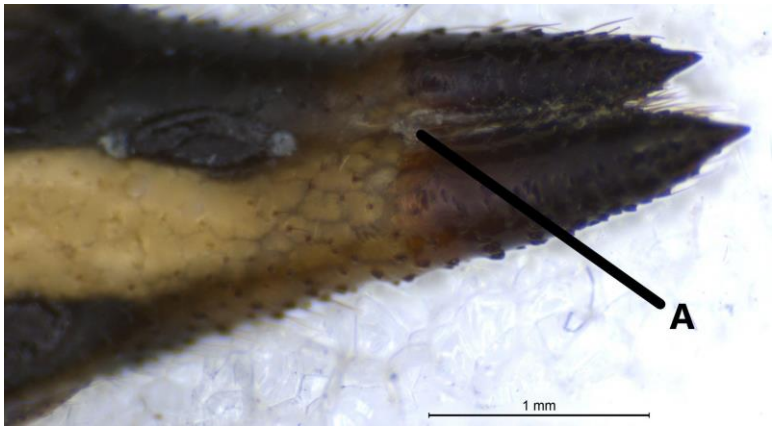

Figure A3.11 Photograph of the dorsal view of *Gasteracantha geminata* (♀) spines showing state (2) of characters 13 and 14 fused spines (A).

14. *Basal structure of median abdominal spines (Female)*

(0) No basal structure; (1) Bulbous; (2) Fused.

Unordered. See character 20 above. For example: *Actinacantha globulata* (Walckenaer, 1841) (1) Figure A3.12(A).

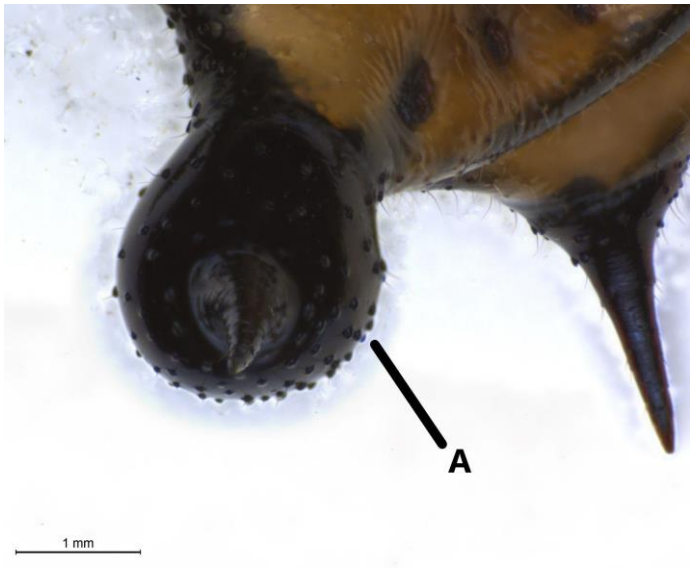

Figure A3.12 Photograph of the dorsal view of *Actinacantha globulata* (♀) spines showing state (1) of character 14 bulbous median spine base (A).

15. *Basal structure of posterior abdominal spines (Female)*

(0) No basal structure; (1) Bulbous.

See character 20 above but without state (2) referring to fusing spines, as no currently known species have a fused 3rd spine as these are in a different area of the abdomen to spines pair 1 and 2; for example: *Thelacantha brevispina* (1) Figure A3.10(C).

16. *Length of anterior abdominal spines (Female)*

(0) Less than half of central sigilla trapezoid width; (1) Equal to/more than half of central sigilla trapezoid width.

Spine length is seen in Magalhães and Santos (2012) characters 10 and 11, but the exact measurements used there are not used here. Instead, a less than or more than fixed points on the abdomen method is used. The point chosen is the width between the central trapezoid sigilla, the sclerotised muscle attachment points that are present on the external surface of the abdomen, at the widest point (see Figure A3.14(C)). This distance was chosen arbitrarily as the central trapezoid is topographically homologous and sigilla are present and developed in the adult *Gasteracantha* (Emerit, 1974) and no variation in these sigillae positioning was seen in over 10,000 specimens (Williams, pers. obs.). The variation of exact spine length within species can be high in some Gasteracanthinae, but this ratio remains consistent between individuals of the same species. These characters were scored, but with the hypothesis that, following the work by Magalhães and Santos (2012), the large spines may have evolved multiple times so might exhibit a high homoplasy and consistency index in the resulting analyses (see Chapter 2.5 and 2.6).

17. *Length of median abdominal spines (Female)*

(0) Less than half of abdomen width; (1) Equal to/more than half of abdomen width.

See Character 16 above but with different points of comparison.

18. *Length of posterior abdominal spines (Female)*

(0) Less than central sigilla trapezoid width; (1) Equal to/more than central sigilla trapezoid width.

See Character 16 above but with different comparative ratio.

19. *Abdominal spines median spines plane (Female)*

(0) Horizontal; (1) Vertical.

Magalhães and Santos (2012) use characters for spine positions, character 51, and the arrangement character 60. The variability of the median spines' position in a horizontal or vertical plane is very low within species but varies between species. Care was taken when scoring gravid females as the abdomen becomes slightly distorted. For example: *Gasteracantha thorelli* (0) Figure A3.7 and *Gasteracantha aciculata* (1) Figure A3.13. The other two pairs of spines are not scored in this way as they are uninformative characters for this study as they do not change planes in this dramatic manner in the currently known species.

Figure A3.13 Photograph of the right lateral view of *Gasteracantha aciculata* (♀) abdomen.

IV. Female dorsal abdominal sigilla:

20. *Dorsal abdominal sigilla - central trapezoid, anterior and posterior left and right (Female)*

(0) Inconspicuous; (1) Extremely conspicuous.

Scharff and Coddington (1997) characters 56 and 57 refer to basic sigilla presence/absence and some positioning as does Kuntner, Coddington and Hormiga (2008), character 72. Here the sigilla are referred to as areas more akin to the suggestion of Macharoenboon, Siriwut and Jeratthitikul (2021) but still scored as a single character. For example: *Gasteracantha thorelli* (1) Figure A3.14(A, B, C, D). Future studies might yield methods of scoring these specific locations (like character 21) with potentially more variation recorded in missing taxa.

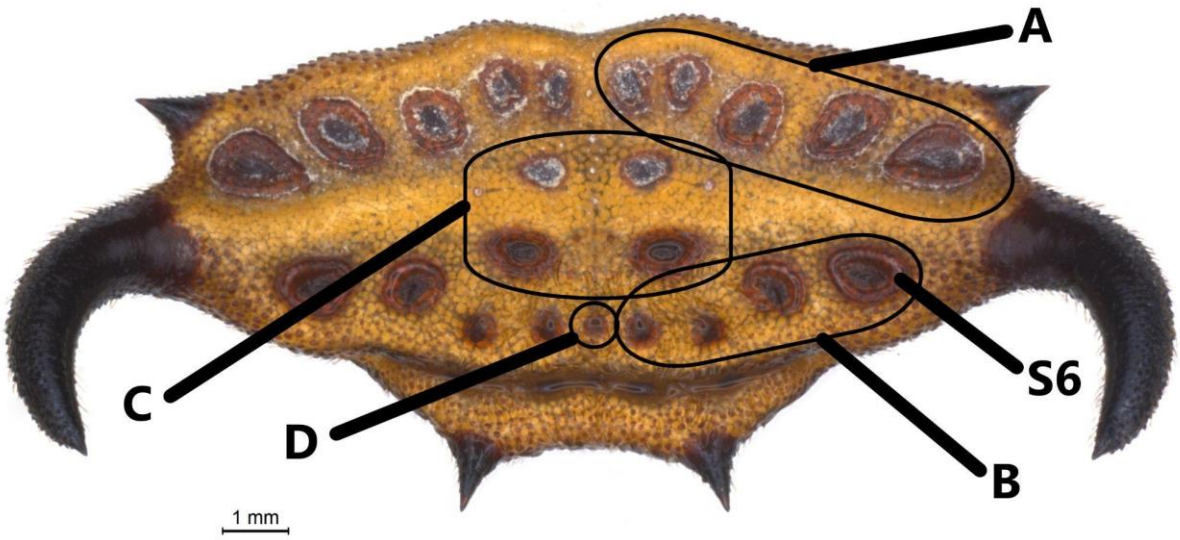

Figure A3.14 Photograph of *Gasteracantha thorelli* (♀) dorsal abdomen showing Sigilla: anterior right (A), posterior right (B), central trapezoid (C), posterior central (D), sigilla "6" (S6).

21. *Dorsal abdominal sigilla - posterior central sigilla structure (Female)*

(0) Separate into two; (1) Fused together as one.

The posterior central sigilla are either fused together into one or split as two separate sigilla; for example: *Gasteracantha thorelli* (0) Figure A3.14(D), *Gasteracantha mengei* (1) Figure A3.8. The sigilla can be almost touching in some species but they are either split or fused. This variation between taxa is scored only when sigilla are conspicuous.

22. *Dorsal abdominal sigilla - position of sigilla 'number 6' (Female)*

(0) Sigilla number 6 under spine 2; (1) Sigilla number 6 between spine 1 and spine 2.

Emerit (1974) discussed the position of sigilla 'number 6' (following Emerit, 1974) as a synapomorphy for some Gasteracanthinae genera from Africa and Madagascar; for example: *Gasteracantha thorelli* (0) Figure A3.14(S6), *Isoxya* sp. nov. (Agnarsson *et al*., in prep.) (1) Figure A3.15.

Figure A3.15 Photograph of *Isoxya* sp. nov. (♀) dorsal, highlighting state (1) Sigilla "6" (S6) of character 22.

23. *Dorsal abdominal sigilla - shape of sigilla 'number 6' (Female)*

(0) Trapezoid; (1) Circular/oval.

Macharoenboon, Siriwut and Jeratthitikul (2021) examined the shape of the dorsal sigilla and here is expanded upon with a focus on sigilla 6 due to Emerit's findings on generic splits relating to this character (1974). Sigilla shape is hypothesised here to be a character that would be worth further investigation but would require viewing vast numbers of specimens to check variability in the specific sigillae that have not been studied. Here the scoring is split into two distinct states as highlighted in: *Gasteracantha thorelli* (0) Figure A3.16(S6), *Acrosomoides acrosomoides* (O. Pickard-Cambridge, 1879) (1) Figure A3.16(S6).

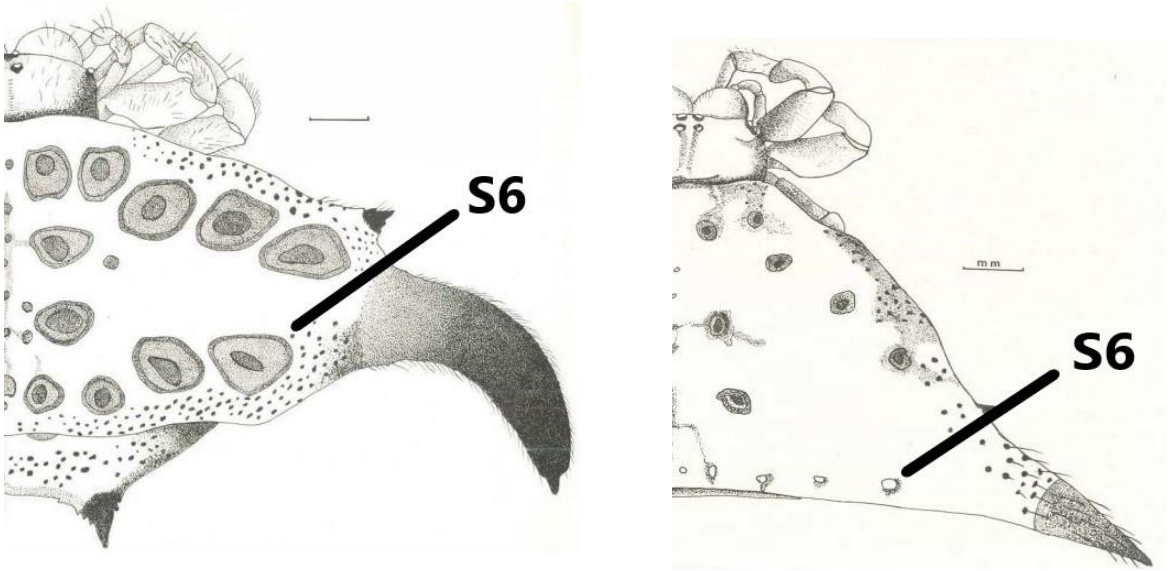

Figure A3.16 Copy of figures from Emerit (1974) – Gasteracantha thorelli (♀) (left) highlighting state (0) Sigilla "6" (S6) of character 23, and *Acrosomoides acrosomoides* (♀) (right) highlighting state (1).

V. Female legs:

24. *Leg I, following Jocqué and Dippenaar-Schoeman (2006), femur spines (Female)*

(0) Many leg spines present femur I; (1) No leg spines present femur I.

Magalhães and Santos (2012) use the character 43 of "Leg IV, coxae, texture" taken from Levi (1985); for example: *Araneus diadematus* (Linnaeus, 1758) (0) Figure A3.17(A).

Figure A3.17 *Araneus diadematus* (♀) showing state (0) leg IV, femur spines (A) of character 24.

25. *Femur 1 length (Female)*

(0) Longer than femur IV; (1) Shorter than femur IV.

Magalhães and Santos (2012) character 7 is similar, but the exact length of the femur is not recorded here, simply in relation to a different leg following Levi (2002).

VI. Female sternum:

26. *Sternum colouration (Female)*

(0) Solid dark; (1) Dark with light spots on edge of sternum; (2) Dark with central spot of light colouration; (3) Full sternum bright colouration.

Unordered. Adaptation of Magalhães and Santos (2012) character 36 with different states reflective of the various Gasteracanthinae colouration as opposed to *Micrathena*; for example: *Gasteracantha aciculata* (3) Figure A3.18(A). The state (1) refers to species that have small spots of colour on the edge of the sternum, typically 5 spots, with a dark central area. This is different from (2) because this state refers to a single spot of colour in the central area of the sternum. The homology of this character is questionable, depending on the biochemical basis of the pigments, and this character is an example of secondary homology.

It was contemplated scoring this as an ordered transformation, but this was decided against as the transformation order is not known. The character is so prominent, and probably relates to protection against predators (Chamberland *et al*., 2020) or prey lure (White and Kemp, 2015, 2016; White, 2017; Messas *et al*., 2021; Kemp, Edwards and White, 2022), that it has been scored even though the homoplasy of this character is expected to be high following the test of congruence in the inferred phylogenies.

Following the analyses, results were generated: In the Group 1 analysis this character provided a *ci* of 0.188 which was very low, but the *ri* was 0.567. Group 2 and 3 were comparable, with: *ci* = 0.250 and 0.231 and *ri* = 0.400 and 0.474 respectively. This would imply that the character homoplasy assumption was correct, however further investigation into the scoring of this character will be required before a conclusive result can be determined.

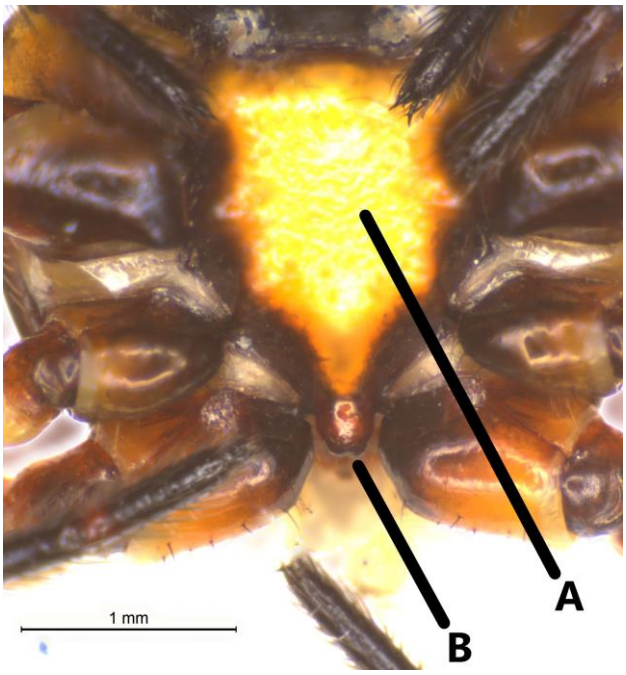

Figure A3.18 Photograph of *Gasteracantha aciculata* (♀) Showing state (3) full sternum bright sternum colour (A) of character 26, and state (1) posterior end shape pointed (B) of character 28.

27. *Sternum ventral surface (Female)*

(0) Not flat; (1) Flat

Directly taken from Magalhães and Santos (2012) character 38, with the alteration to 'not flat' rather than multi-state for species that do not show a flat sternum due to low variation; for example: *Gasteracantha aciculata* (1) Figure A3.18 (A).

28. *Sternum posterior end shape (Female)*

(0) Not pointed; (1) Pointed/elongated.

Directly taken from Magalhães and Santos (2012) character 39; for example: *Gasteracantha aciculata* (1) Figure A3.18(B).

VII. Female venter:

29. *Ventral condyles (Female)*

(0) Absent; (1) Present.

Directly taken from Scharff and Coddington (1997) character 58; for example: *Gasteracantha thorelli* (1) Figure A3.19(A) and A3.23(A). These homologous structures appear to have a correlation, in part, to the width of the abdomen. Typically, if the abdomen is wider than its length these condyles are present, hypothesised as a structure to support the spider and prevent it tipping too far in either direction when walking (Williams, pers. obs.).

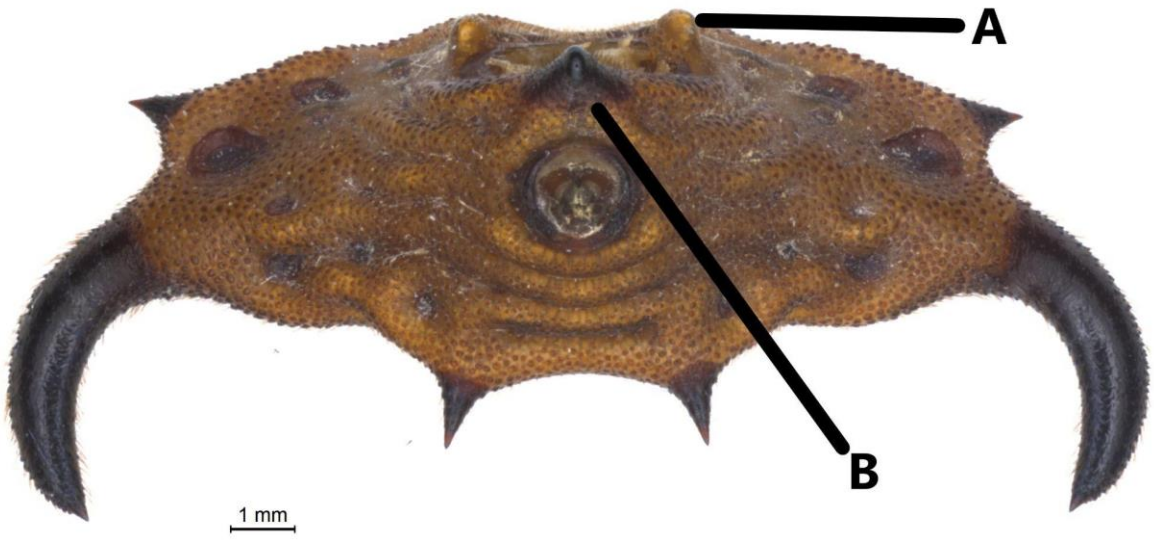

Figure A3.19 Photograph of *Gasteracantha thorelli* (♀) ventral, showing state (1) ventral condyles (A) present in character 29, and state (1) ventral tubercle (B) of character 32.

30. *Venter sigilla (Female)*

(0) Inconspicuous; (1) Conspicuous.

See character 20 above for similarities and homology discussion but this is the first time ventral sigilla have been used in a study of *Gasteracantha*. Future studies could examine the exact positioning of these sigilla or their shape; see Chapter 4 for more details; see Figures A3.14-16.

31. *Venter wrinkles (Female)*

(0) Absent; (1) Conspicuous small grooves.

Ventral surface of Gasteracanthinae is sclerotized into wrinkles (same structure as the rest of the abdomen) and scored here; for example: *Gasteracantha scintillans* (1) Figure A3.5(A).

32. *Ventral tubercle in front of spinnerets (Female)*

(0) Absent; (1) Present.

Used by Scharff and Coddington (1997) character 27, and by Magalhães and Santos (2012) character 63. Also noted by Levi (2002) and stated by Bradley (2013) that "there is also a small projection from the underside of the abdomen that the male grasps while mating"; for example: *Gasteracantha thorelli* (1) Figure A3.19(B), A3.23(B). The function relates to the mating process (see Chapter 4 for more details).

VIII. Female Spinnerets:

33. *Sclerotized ring around spinnerets (Female)*

(0) Absent; (1) Present.

Magalhães and Santos (2012) character 65, Scharff and Coddington (1997) character 65 and Levi (1985) all reference the sclerotized ring around the spinnerets; for example: *Gasteracantha scintillans* (1) Figure A3.20(A).

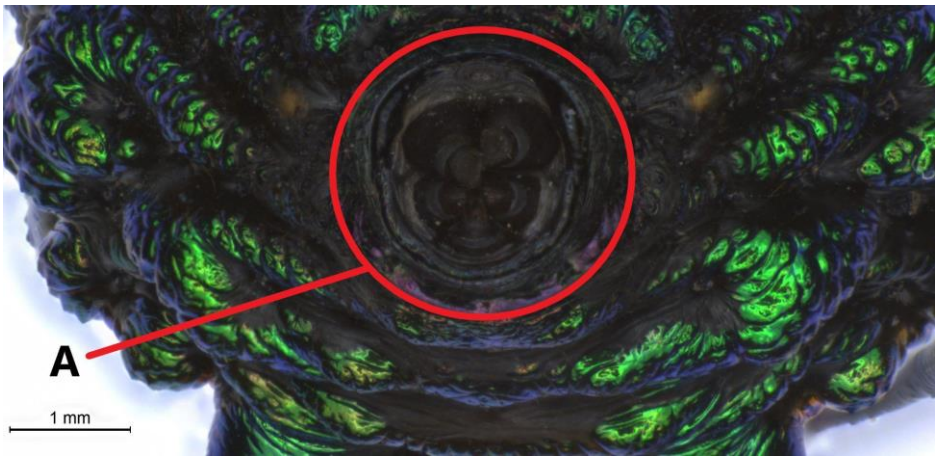

Figure A3.20 Photograph of *Gasteracantha scintillans* (♀) ventral, focused on state (1) ring around spinnerets (A) of character 33 and state (0) of character 34 and 35.

34. *Sclerotized ring structure (Female)*

(0) Full; (1) Broken; (2) One third missing.

Unordered. The sclerotized ring has a different structure depending on the different genera but remains homologous in all. The differences are clear and unmistakable; for example: *Gasteracantha scintillans* (0) Figure A3.20(A) *Gasteracantha sturi* (1) Figure A3.21(A).

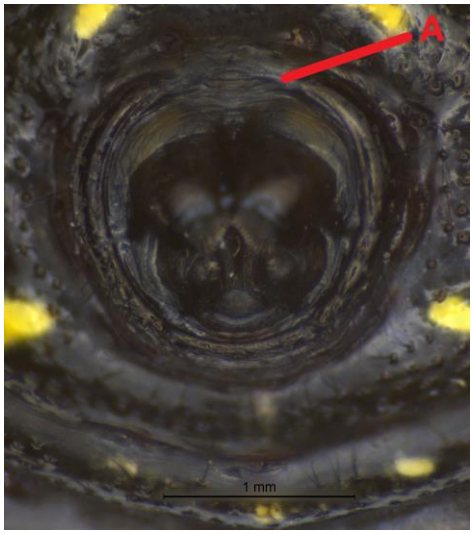

Figure A3.21 Photograph of *Gasteracantha sturi* (♀) ventral, focused on state (1) broken ring (A) of character 34.

35. *Sclerotized ring plates (Female)*

(0) 1 plate; (1) 3 plates; (2) 5 plates.

Unordered. Emerit (1974) noted in his key to the Gasteracanthinae of Madagascar and the surrounding area: “Anneau chitineux des filières dissocié en petites plaques indépendantes”. There are also plates within the *Gasteracantha* sclerotized ring structure; for example: *Gasteracantha scintillans* (0) Figure A3.20 and *Gasteracantha sturi* (1) Figure A3.21. By partitioning the number of plates into discrete states this enables potential information to not get lost within a binary character. As there was no variation within species for the number of plates present, this was deemed suitable. This also provides the option for unstudied or currently unknown species to possess different numbers of plates and additional states added in future studies.

36. *Sclerotized spinneret tracheal spiracle plate (Female)*

(0) Unsclerotised; (1) Sclerotized.

This respiratory organ character is from Scharff and Coddington (1997) character 66; for example: *Gasteracantha thorelli* (1) Figure A3.22(A).

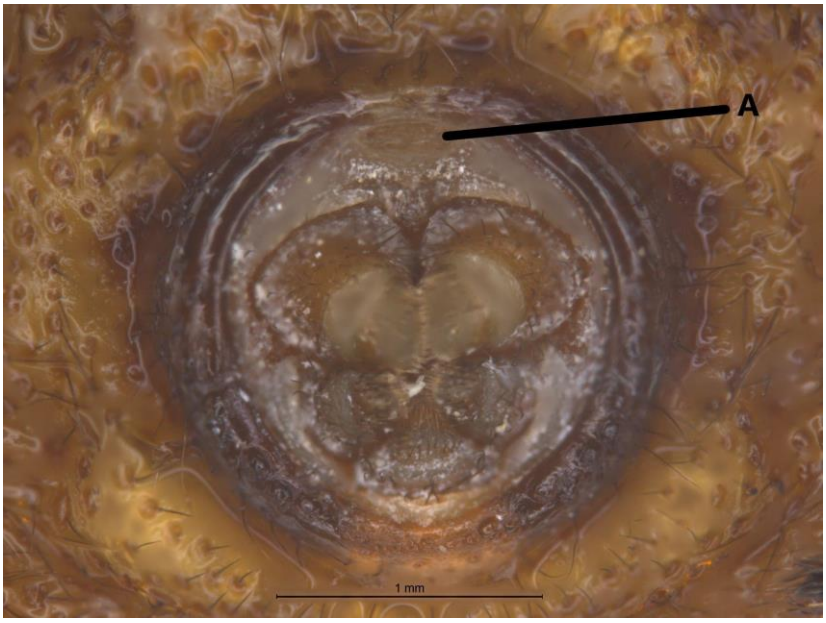

Figure A3.22 Photograph of *Gasteracantha thorelli* (♀) dorsal, focused on state (1) spiracle plate (A) of character 36.

37. *Spinnerets position (Female)*

(0) Against abdomen; (1) On raised tubercle.

Magalhães and Santos (2012) character 12 describes the *Micrathena* elongated spinneret cone length and this is homologous with the *Gasteracantha* spinneret cone. However, due to some outgroups having differing spinneret positioning this character is then expanded upon in the following character.

38. *Raised spineret tubercle (Female)*

(0) Raised; (1) Extremely elongated.

Magalhães and Santos (2012) character 12 describes elongated spinneret cone length. Within this dataset the exact measurement is not required as the taxa used have either an elongated or not elongated spinneret cone; for example: *Gasteracantha thorelli* (0) Figure A3.23(C), *Gasteracantha scintillans* (1) Figure A3.5(B). The elongation consists of the same sclerotization as the spinneret ring.

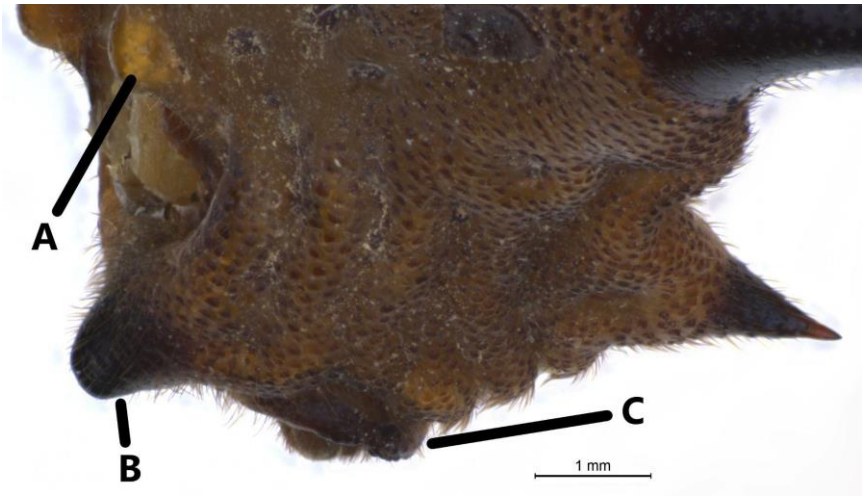

Figure A3.23 Photograph of *Gasteracantha thorelli* (♀) left lateral view, showing state (1) ventral condyles (A) of character 29, state (1) ventral tubercle (B) of character 32, and state (0) spinneret tubercle raised (C) of character 38.

IX. Female genitalia:

39. *Sclerotized epigyne anterior edge shape (Female)*

(0) No prominent shape; (1) Heavily sclerotized in two humps.

Benoit (1962a, 1962b, 1975) and Emerit (1974, 1975) both discuss the two humps that give the genus *Isoxya*, in particular, a distinctive external epigyne shape; for example: *Isoxya* sp. nov. (1) Figure A3.24(A).

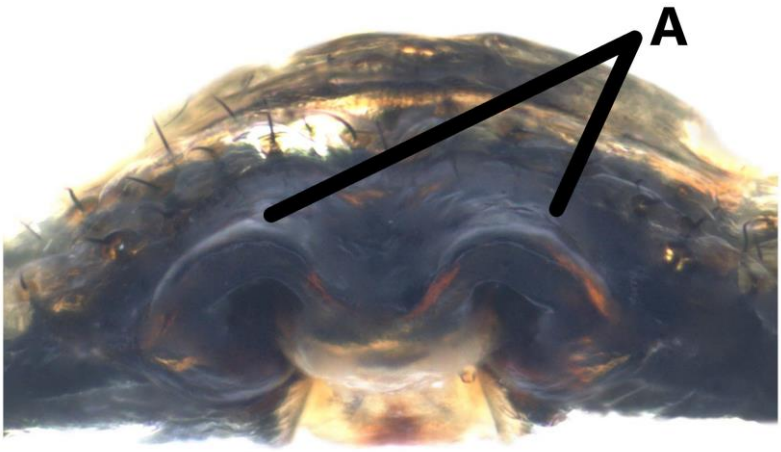

Figure A3.24 Photograph of *Isoxya* sp. nov. (♀) ventral epigyne, showing state (1) sclerotized humps on epigyne (A) of character 39

40. *Epigyne opening (Female)*

(0) Chambers; (1) Slit.

This character is similar to Kuntner, Coddington and Hormiga (2008) character 91 which discusses homologous epigyne openings. Magalhães and Santos (2012) character 84 also discusses the copulatory openings but there is less variety within *Micrathena* than *Gasteracantha*; for example: *Gasteracantha aciculata* (0) Figure A3.25(A).

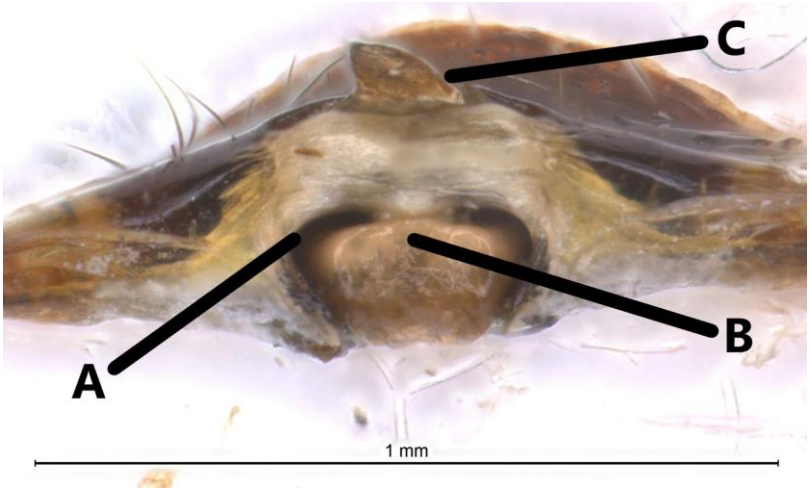

Figure A3.25 Photograph of *Gasteracantha aciculata* (♀) ventral epigyne, showing state (0) chambers (A) of character 40, (1) medial (A) of character 41, (0) wide septum (B) of character 42, (1) projected lobe (C) of character 44.

41. *Orientation of epigyne opening (Female)*

(0) Lateral; (1) Medial.

This character is similar to Kuntner, Coddington and Hormiga (2008) character 92 referring to the copulatory openings within chambers position; for example: *Gasteracantha thorelli* (0) Figure A3.26(A) and *Gasteracantha aciculata* (1) Figure A3.25(A).

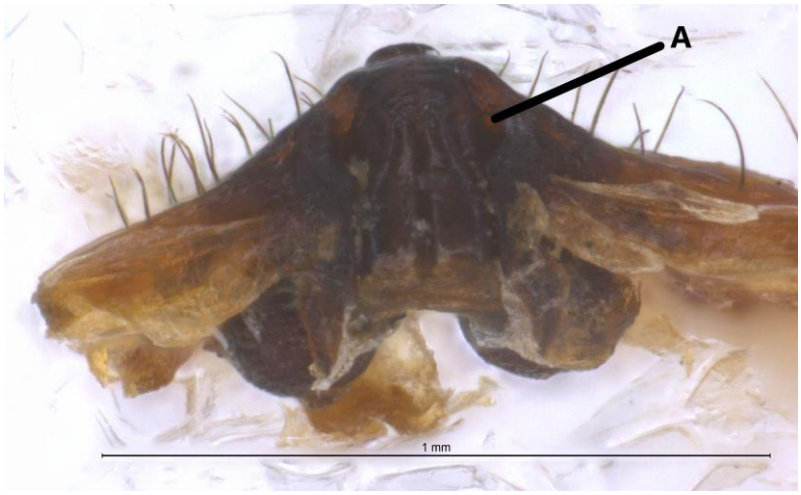

Figure A3.26 Photograph of *Gasteracantha thorelli* (♀) ventral epigyne, showing state (0) lateral opening (A) of character 41.

42. *Top of septum width (Female)*

(0) Wide; (1) Thin.

Kuntner, Coddington and Hormiga (2008) discuss the septum in characters 93 and 94. Here the widest point of the septum is used as reference for the top of the septum width, keeping the ratio within the same species; for example: *Gasteracantha aciculata* (0) Figure A3.25(B) and *Acrosomoides acrosomoides* (1) Figure A3.27(A).

43. *Septum shape (Female)*

(0) Rectangular; (1) Triangular; (2) Inverted triangle; (3) Hourglass.

This character has similarities to Kuntner, Coddington and Hormiga (2008) character 94 describing the shape of the septum; for example: *Acrosomoides acrosomoides* (1) Figure A3.27(A) and *Togacantha nordvei* (Strand, 1913) (3) Figure A3.28(A).

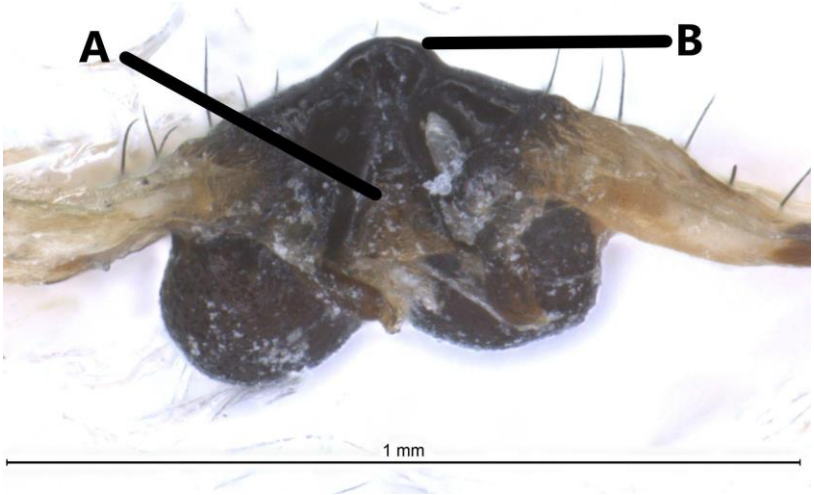

Figure A3.27 Photograph of *Acrosomoides acrosomoides* (♀) ventral epigyne, showing state (1) septum (A) of characters 42 and 43, and state (0) rounded lobe (B) of character 44.

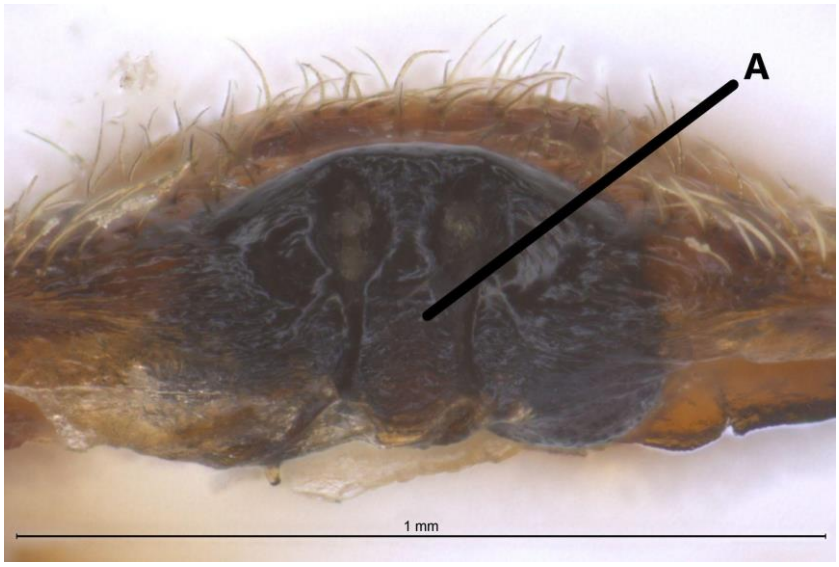

Figure A3.28 Photograph of *Togacantha nordvei* (♀) ventral epigyne, showing state (3) septum hourglass shape (A) of character 43.

44. *Epigyne lobe (Female)*

(0) Rounded/Absent; (1) Conspicuously projected.

Kuntner, Coddington and Hormiga (2008) character 96 discusses the scape in various orb-weavers as does Scharff and Coddington (1997) character 28. However, Magalhães and Santos (2012) character 76 states: "we do not consider the *Araneus* scape as homologous to the lobe present in several araneid genera sampled in this study because is it a long, membranous structure attached to the anterior face of the epigynal bulge". In this study the lobe is also considered to not be homologous with the *Araneus* scape due to the differences listed in Magalhães and Santos (2012). However, the Gasteracanthinae lobes are considered homologous with *Micrathena* lobes; for example: *Gasteracantha scintillans* (1) Figure A3.5(C), *Gasteracantha aciculata* (1) Figure A3.25(C).

45. *Lobe shape (Female)*

(0) Gradual; (1) Steep.

Magalhães and Santos (2012) characters 78 and 79 discuss epigynum lobe shape. The shape of the projected lobe, when present, can be either wide and shallower as in *Acrosomoides acrosomoides* (0) Figure A3.27(B) or steeply rising as in *Gasteracantha scintillans* (1) Figures A3.5(C) and A3.29(A).

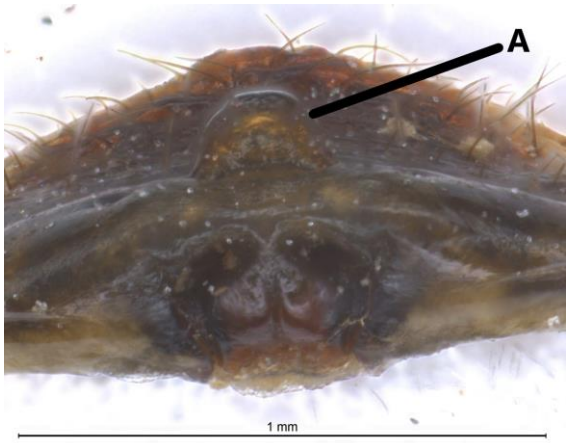

Figure A3.29 Photograph of *Gasteracantha scintillans* (♀) ventral epigyne, showing state (1) steep lobe (A) of character 45.

46. *Spermathecae separation (Female)*

(0) Small or none; (1) Wide.

Kuntner, Coddington and Hormiga (2008) character 108 refers to the size of separation between spermathecae; for example: *Macracantha arcuata* (1). See Macharoenboon, Siriwut and Jeratthitikul (2021), page 50 figure 12 C, for line drawing. Here (1) is separated by the width of a spermathecae. Although the spermatheca location is homologous, care was taken to not distort its position when dissecting the internal genitalia.

47. *Spermatheca shape (Female)*

(0) Oval; (1) Reniform (kidney bean shaped).

The spermatheca shape is used in Kuntner, Coddington and Hormiga (2008) character 107 and Magalhães and Santos (2012) character 85. The recent publication by Macharoenboon, Siriwut and Jeratthitikul (2021) featured illustrations of spermathecae from various species in Thailand and made suggestions on a new classification based upon these shapes. For example: *Macracantha hasselti* (1) Figure A2.30.

Figure A3.30 Photograph of *Macracantha hasselti* (♀) dorsal reniform spermatheca, state (1) of character 47.

X. Male somatic appearance:

48. *Carapace shape (Male)*

(0) Oval; (1) Square; (2) Rounded.

Unordered. This character is similar to Magalhães and Santos (2012) character 89 and Scharff and Coddington (1997) character 43, scoring the carapace shape; for example: *Isoxya* sp. nov. (1) Figure A3.31(A) and *Gasteracantha aciculata* (2) Figure A3.32(A).

Figure A3.31 Photograph of *Isoxya* sp nov (♂) dorsal, showing state (1) square carapace of character 48.

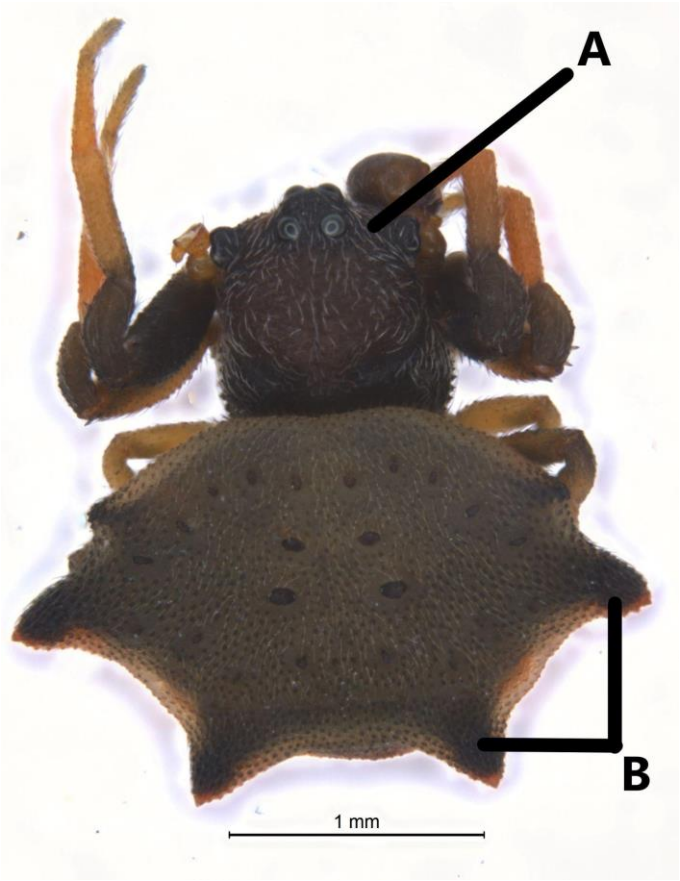

Figure A3.32 Photograph of *Gasteracantha aciculata* (♂) dorsal, showing state (2) rounded carapace (A) of character 48, and state (1) spines (B) of character 51.

49. *Posterior median eyes (PME) and anterior median eyes (AME) on extended protrusion (Male)*

(0) Absent; (1) Present.

An extension from carapace shape character 52 above to score the PME and AME eye location that varies within males in the Gasteracanthinae but does not duplicate the character above.

50. *Abdomen surface structure (Male)*

(0) Soft; (1) Sclerotized.

Similar to character 11 above but for the males.

51. *Abdominal spines (any) (Male)*

(0) Absent; (1) Present.

This character is similar to Scharff and Coddington (1997) character 62 (all 3 pairs of spines). Due to a lack of somatic male data, unlike with female Gasteracanthinae, a decision was made to score the presence of abdominal spines as a single character. Spines that were present in the subfamily appear homologous, but the quantity of missing data and duplicating characters was deemed too high. With more male somatic data this could be a character division in the future, as was seen here in characters 9-11 changed from 1 character in Scharff and Coddington (1997). For example: *Augusta glyphica* (Guérin, 1839) (0) Figure A3.33 and *Gasteracantha aciculata* (1) Figure A3.32(B).

Figure A3.33 Photograph of *Augusta glyphica* (♂) dorsal.

52. *Male to female ratio of size - total length (Male)*

(0) Male less than half of female; (1) Male equal to or more than half of female.

Scharff and Coddington (1997) character 61 and Kuntner, Coddington and Hormiga (2008) character 122 are very similar to this. The Gasteracanthinae show a large sexual size dimorphism between males and females with some *Isoxya* exceptions. Different measurements were made (total length, carapace length and width and abdominal length and width) to compare the size of males to females and in all measurements the same ratios were obtained so the presence or absence of eSSD was scored as one character here using total length as the measure.

53. *Femur pair 1 (Male)*

(0) Thin; (1) Thick.

Magalhães and Santos (2012) and Scharff and Coddington (1997) both use specific leg characters, here this character ties in with descriptions of species and differences within the Aranaidae. The thickness of femur I is prominent in species where it is present; for example: *Gasteracantha aciculata* (1) Figure A3.34.

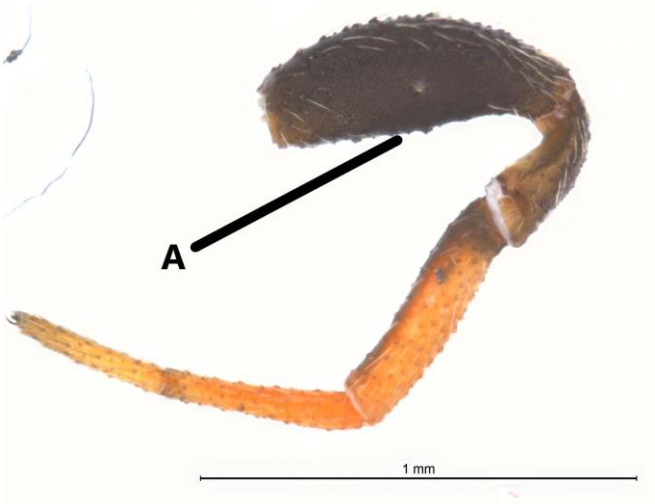

Figure A3.34 Photograph of *Gasteracantha aciculata* (♂) leg I, showing state (1) femur thick (A) of character 53.

54. *Coxa 1 hook, femur 2 groove (Male)*

(0) Absent; (1) Present.

Following Kuntner, Coddington and Hormiga (2008) and Magalhães and Santos (2012), as stated by them both, Scharff and Coddington (1997) use two characters (33 and 34) and here they are merged into one as they are biologically dependent integrated functional complexes (Wilkinson, 1995); see Figure A3.35.

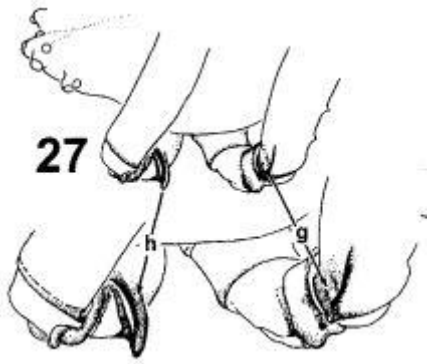

Figure A3.35 Line drawing taken from Scharff and Coddington (1997) to highlight hook (h) and groove (g) here seen in the species *Erivptiom edax* (♂) state (1) of character 54.

XI. Male palp:

55. *Palp embolus (Male)*

(0) Conspicuous; (1) Inconspicuous.

Example: *Gasteracantha taeniata* (Walckenaer, 1841) (0) Figure A3.36(A). The embolus of the palp, within the studied species here, is either conspicuous or not.

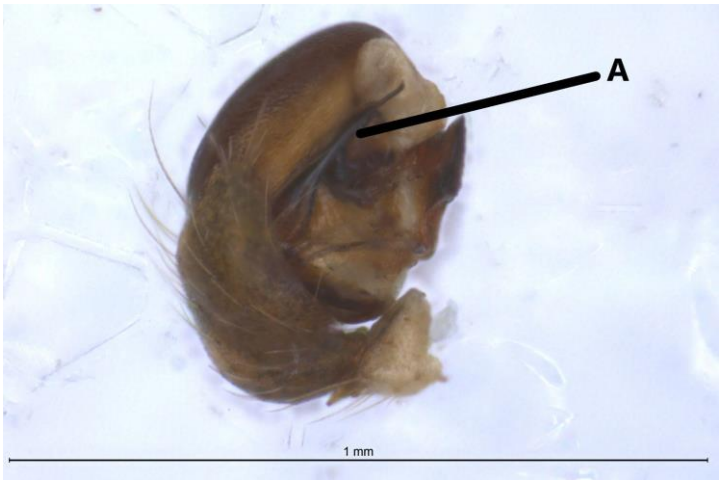

Figure A3.36 Photograph of *Gasteracantha taeniata* (♂) dorsal view, showing state (0) embolus conspicuous (A) of character 55.

56. *Pars pendula (Male)*

(0) Absent/inconspicuous; (1) Present/conspicuous.

Kuntner, Coddington and Hormiga (2008) character 157 describes the pars pendula and its homology. It is conspicuously present here in species of *Isoxya*; for example: *Isoxya* sp. nov. (1) Figure A3.37(A).

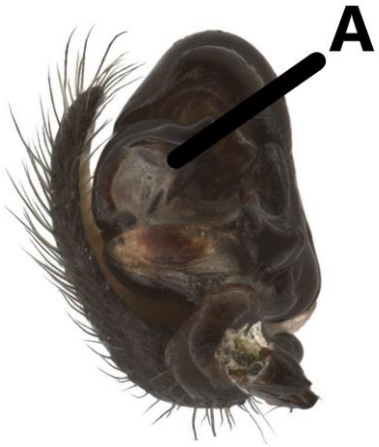

Figure A3.37 Photograph of *Isoxya* sp. nov. (♂) dorsal view, showing state (1) pars pendula (A) of character 56.

57. *Terminal apophysis (Male)*

(0) Absent; (1) Present.

Directly taken from Scharff and Coddington (1997) character 22 and Kuntner, Coddington and Hormiga (2008) character 197. The terminal apophysis is either present or not, for example: *Gasteracantha aciculata* (1) Figure A3.38(A). This is an apophysis that is a synapomorphy for a clade of Oriental *Gasteracantha* (see Chapter 5).

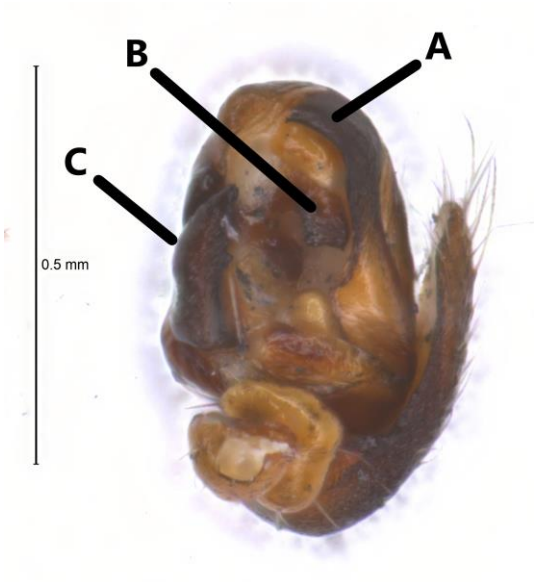

Figure A3.38 Photograph of *Gasteracantha aciculata* (♂) dorsal view showing Palp apophyses: terminal apophysis (A), paramedian apophysis (B), median apophysis (C). State (1) of character 57 (A), state (1) of character 60 (B), and state (1) of character 62 and 66 (C).

58. *Palp shape (Male)*

(0) Spherical; (1) Oval.

This character scores the basic shape of the whole palp bulb when in its natural, unexpanded state; for example: *Gasteracantha aciculata* (1) Figure A3.38.

59. *Paracymbium (Male)*

(0) Absent; (1) Present.

Directly taken from Scharff and Coddington (1997) character 6 and Kuntner, Coddington and Hormiga (2008) character 144. The paracymbium is either present in a homologous location or absent with no mid-point, for example: *Isoxya* sp. nov. (1) Figure A3.39(A).

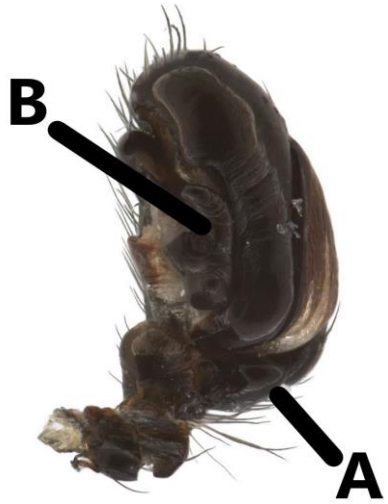

Figure A3.39 Photograph of *Isoxya* sp. nov. (♂) retrolateral view showing state (1) paracymbium (A) of character 59, and state (1) median apophysis forked (B) of character 63.

60. *Paramedian apophysis (Male)*

(0) Absent; (1) Present.

Directly taken from Scharff and Coddington (1997) character 18. The paramedian apophysis is either present or not, for example: *Gasteracantha aciculata* (1) Figure A3.38(B).

61. *Cymbium (Male)*

(0) Dorsal; (1) Mesal.

Directly taken from Scharff and Coddington (1997) character 5, but with an additional state: lateral. Also used in Kuntner, Coddington and Hormiga (2008) character 140 minus the lateral state like here; for example: *Gasteracantha aciculata* (1) Figure A3.32.

62. *Median apophysis (MA) (Male)*

(0) Absent; (1) Present.

Directly taken from Scharff and Coddington (1997) character 10 and Kuntner, Coddington and Hormiga (2008) character 158. As with the other apophyses the MA is either present or not, for example: *Gasteracantha aciculata* (1) Figure A3.38(C).

The following 4 characters (63-66) all describe the shape of the same functional sclerite, the median apophysis. It was observed that the characters are independent of each other (i.e., not linked in a cladistic sense), such that the presence or absence of particular states in one character does not affect the presence or absence of states in another. Therefore, this was deemed a valid approach to scoring this complex structure. Future work could examine the possibility of using morphometrics to quantify the shape of the median apophysis (see Chapter 5 and Appendix 4).

63. *MA distal edge (Male)*

(0) Rounded; (1) Forked.

This character is like Scharff and Coddington (1997) character 14, but here the focus is only on the distal edge and if there is a fork in the MA; for example: *Gasteracantha aciculata* (0) Figure A3.38(C), *Isoxya sp. nov.* (1) Figure A3.39(B). Taxa that did not possess a MA were scored with a '–' to represent a gap in characters 63-66.

64. *MA base width (Male)*

(0) Wide; (1) Thin.

Here the width is scored in relation to the width of the base to the width of the MA at its widest point. See annotated images *Acrosomoides acrosomoides* and *Isoxya sp. nov.* (1) Figure A3.40 for details. This, and character 65, could be refined in future studies with a morphometric analysis of the MA in *Gasteracantha* (see Chapter 5).

65. *Median apophysis tip width (Male)*

(0) Wide; (1) Thin.

As Character 64 above.

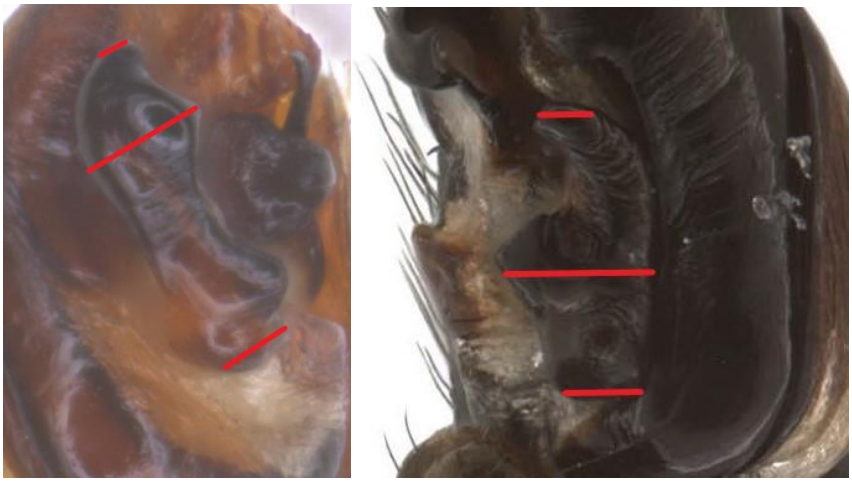

Figure A3.40 Photograph of retrolateral view (close up on MA) Left: *Acrosomoides Acrosomoides* (♂), Right: *Isoxya sp. nov.* (♂) Palp: median apophysis measurement points at tip, widest point, base - highlighted by red lines (characters 64 and 65).

66. *MA anterior edge (Male)*

(0) Smooth sclerotized; (1) Rough sclerotized/or with teeth.

This character is similar to Scharff and Coddington (1997) character 15 but adds to the (1) state by adding the rough sclerotization which is not clearly formed teeth but is conspicuously not smooth. Here the surface of the anterior edge is the focus with either a smooth or rough/toothed edge; for example: *Acrosomoides acrosomoides* (O. Pickard-Cambridge, 1879) (0) Figure A3.41(A), *Gasteracantha aciculata* (1) Figure A3.38(C).

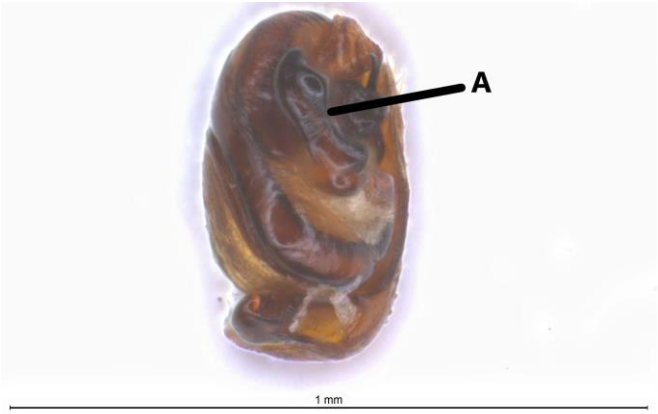

Figure A3.41 Photograph of *Acrosomoides acrosomoides* (♂) retrolateral view palp, showing state (0) median apophysis (A) of character 66.

A3.2.2 Scored characters not used in morphological analysis

XII. Excluded uninformative characters (not parsimony informative as only one species possessed character state 1 following scoring)

67. *Femur 4 length (Female)*

(0) Longer than femur 3; (1) Shorter than femur 3.

Magalhães and Santos (2012) character 8 is similar, but the exact length of the femur is not recorded here, simply in relation to a different leg following Levi (2002). Only *Augusta glyphica* was scored with state (1), the rest of the taxa scored as (0), so the character was parsimony uninformative; by comparison to Character 25 where *Nephila pilipes* and *Araneus diadematus* were both scored with state (0), and the remaining taxa (1), yielding an informative character.

68. *Booklung cover surface (Female)*

(0) Sclerotized; (1) Not sclerotized.

This character here is very simple by comparison to others in the examined literature due to lack of variation in the species included here.

69. *Sclerotized plate anterior to epigyne (Female)*

(0) Present; (1) Absent.

Directly taken from Magalhães and Santos (2012) character 73 and similar to Kuntner, Coddington and Hormiga (2008) character 88. The plate is conspicuous when present. The exact function of the plate is unknown but might be related to the mating process of small Gasteracanthinae males.

70. *Epigyne posterior edge (Female)*

(0) Sclerotized; (1) Not sclerotized.

This character refers to the sclerotization surrounding the epigyne. The spiny orb-weavers with sclerotized abdomens and sclerotized spinneret tubercles often possess heavily sclerotized epigynes. Care was taken to confirm if the specimens were older mature females as discussed earlier.

71. *Epigyne anterior edge (Female)*

(0) Sclerotized; (1) Not sclerotized.

See character 70 above.

72. *Palp embolus location (Male)*

(0) Extended externally away from palp; (1) Extended within palp.

The embolus can be prominently extended away from the rest of the palp or within the confines of the palp. If within the confines of the palp, the embolus can be both conspicuous or inconspicuous. Here it was uninformative as only one taxon had state (0).

73. *Elongated tegulum from palp bulb (Male)*

(0) Not elongated; (1) Clearly elongated.

The elongated tegulum is present in species previously belonging to the genus *Chaetacis*, before their taxonomic generic change to *Micrathena*, and some species of *Micrathena*. Levi (1985) provided detailed illustrations of the palps that possess this structure.

74. *Subterminal apophysis (Male)*

(0) Absent; (1) Present.

Directly taken from Scharff and Coddington (1997) character 20 and Kuntner, Coddington and Hormiga (2008) character 196. Subterminal apophysis either present or not but here it was uninformative as only one taxon had state (1).

XIII. Excluded characters (the same character states scored for all taxa)

75. *Eyes - PLE & ALE (Female)*

(0) Joined; (1) Separate.

76. *Eyes - Distance between PME (Female)*

(0) Less than between AME; (1) More than between AME.

77. *Chelicerae - Protrusion on anterior edge (Female)*

(0) Present; (1) Absent.

78. *Sternum - Shape (Female)*

(0) Shield; (1) Other.

79. *Femur 1 length (Male)*

(0) Longer than femur 2; (1) Shorter than femur 2.

A3.2.3 Matrix of morphological characters (1-79)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Nephila pilipes	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-	0
Araneus diadematus	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-	0
Aetrocantha falkensteini	1	0	1	1	0	1	0	0	0	1	1	0	0	0	0	-	0	0	0	1
Aranoethra cambridgei	0	1	0	0	0	1	0	1	1	1	1	0	0	0	0	1	0	0	0	1
Micrathena aureola	0	1	0	1	1	1	0	1	1	1	1	0	0	0	0	0	0	0	0	1
Micrathena schreibersi	0	1	0	1	1	1	0	1	1	1	1	0	0	0	0	0	1	1	0	1
Augusta glyphica	0	0	0	0	0	1	0	1	1	1	1	0	0	0	0	0	0	0	1	1
Isoxya cicatricosa	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	1
Isoxya cowani	1	0	1	1	0	1	0	0	1	1	1	0	1	1	1	0	0	0	1	1
Isoxya penizoides	1	0	1	1	0	1	0	0	0	0	0	-	-	-	-	-	-	-	-	1
Isoxya sp. nov.	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	1	0	1	1	1
Isoxya tabulata	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	1	0	1	0	1
Acrosomoides acrosomoides	1	0	1	1	0	1	0	0	1	1	0	0	0	0	-	0	0	-	0	1
Acrosomoides linnaei	1	0	1	1	0	1	0	0	1	1	0	0	0	0	-	0	0	-	0	1
Actinacantha globulata	1	0	1	1	0	1	0	0	1	1	1	0	0	1	0	1	1	1	1	1
Afracantha camerunensis	1	0	1	1	0	1	0	0	1	1	1	0	0	1	1	0	0	0	1	1
Austracantha minax	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	0	0	1	1	1
Macracantha arcuata	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	0	1	0	1	1
Macracantha hasselti	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	1	1	1	1	1
Thelacantha brevispina	2	0	1	1	0	1	0	0	1	1	1	0	0	1	1	0	0	0	1	1
Togacantha nordviei	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	1
Gasteracantha aciculata	1	0	1	1	0	1	0	0	1	1	1	0	1	1	1	1	0	1	1	1
Gasteracantha cancriformis	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	1
Gasteracantha clarki	1	0	1	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	1
Gasteracantha clavatrix	1	0	1	1	0	1	0	0	1	1	1	1	0	0	0	0	1	1	1	1
Gasteracantha clavigera	1	0	1	1	0	1	0	0	1	1	1	1	0	0	0	0	1	0	1	1
Gasteracantha cruciger a	1	0	1	1	0	1	0	0	1	1	1	0	0	1	0	0	0	1	0	1
Gasteracantha curvispina	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	1	1	1	0	1
Gasteracantha diadesmia	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	1
Gasteracantha diardi	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	0	0	1	0	1
Gasteracantha doriae	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	1	0	1	0	1
Gasteracantha falcicornis	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	1	1	0	0	1
Gasteracantha geminata	1	0	1	1	0	1	0	0	1	1	1	0	2	2	0	1	0	0	0	1
Gasteracantha hecata	1	0	1	1	0	1	0	0	1	1	0	0	0	0	-	0	0	0	0	1
Gasteracantha kuhli	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	1
Gasteracantha lepelletieri	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	1	1	1	1	1
Gasteracantha lunata	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	1
Gasteracantha mediofusca	1	0	1	1	0	1	0	0	1	1	1	0	1	1	1	0	0	0	0	1
Gasteracantha mengei	1	0	1	1	0	1	0	0	1	1	0	0	0	0	-	1	0	-	0	1
Gasteracantha metallica	1	0	1	1	0	1	1	0	1	1	1	0	0	0	0	0	1	1	1	1
Gasteracantha milvoides	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	1	1	1	0	1
Gasteracantha pentagona	1	0	1	1	0	1	0	0	1	1	1	0	1	1	1	1	0	1	1	1
Gasteracantha quadrispinosa	1	0	1	1	0	1	0	0	1	1	0	0	0	0	-	0	0	-	0	1
Gasteracantha recurva	1	0	1	1	0	1	0	0	1	1	0	0	0	0	-	0	0	0	0	1
Gasteracantha regalis	1	0	1	1	0	1	1	0	1	1	1	0	0	0	0	1	1	1	1	1
Gasteracantha remifera	1	0	1	1	0	1	0	0	1	1	1	1	0	0	0	1	1	1	1	1
Gasteracantha rhomboidea madagascariensis	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	1
Gasteracantha sanguinolenta	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	1	0	1	0	1
Gasteracantha scintillans	1	0	1	1	0	1	1	0	1	1	1	0	0	0	0	1	1	1	1	1
Gasteracantha signifera	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	1
Gasteracantha sp. nov.	1	0	1	1	0	1	1	0	1	1	1	0	0	0	0	0	1	1	1	1
Gasteracantha sturi	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	1
Gasteracantha taeniata	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	0	0	1	0	1
Gasteracantha theisi	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	1	0	0	1	1
Gasteracantha thorelli	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	1
Gasteracantha unguifera	1	0	1	1	0	1	0	0	1	1	1	0	0	1	1	0	0	0	0	1
Gasteracantha versicolor formosa	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	0	1	1	0	1
Gasteracantha westringi	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	1
	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Nephila pilipes	-	-	-	0	0	0	0	0	0	0	0	0	0	-	-	0	0	-	0	0
Araneus diadematus	-	-	-	0	0	0	1	0	0	0	0	0	0	-	-	0	0	-	-	0
Aetrocantha falkensteini	1	1	0	1	1	0	1	1	0	1	1	1	1	1	2	1	0	-	0	0
Aranoethra cambridgei	1	0	1	1	1	0	1	0	0	1	1	0	1	1	0	0	0	-	0	0
Micrathena aureola	1	1	1	0	1	0	1	1	0	1	1	0	1	0	0	0	1	1	0	0
Micrathena schreibersi	0	1	1	0	1	0	0	1	0	1	1	0	1	1	2	0	1	1	0	0
Augusta glyphica	0	0	0	1	1	0	1	1	0	1	0	0	0	-	-	0	1	0	0	0
Isoxya cicatricosa	0	1	0	1	1	3	1	1	1	1	1	0	1	1	0	1	0	-	1	1
Isoxya cowani	1	1	0	1	1	0	1	1	1	1	1	0	1	1	0	1	0	-	1	1
Isoxya penizoides	1	-	0	1	1	0	1	1	1	1	1	0	1	0	1	1	0	-	1	0
Isoxya sp. nov.	1	1	0	1	1	1	1	1	1	1	1	0	1	1	0	1	0	-	1	0
Isoxya tabulata	1	1	0	1	1	1	1	1	1	1	1	0	1	0	0	1	0	-	1	0
Acrosomoides acrosomoides	0	0	1	1	1	2	1	1	1	1	1	1	1	2	1	1	0	-	0	0
Acrosomoides linnaei	0	0	1	1	1	0	1	1	1	1	1	1	1	2	1	1	0	-	0	0
Actinacantha globulata	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	0	0
Afracantha camerunensis	1	0	1	1	1	0	1	1	1	1	1	0	1	1	0	1	1	0	0	0
Austracantha minax	0	0	1	1	1	3	1	1	0	1	1	0	0	-	-	1	0	-	0	1
Macracantha arcuata	1	0	1	1	1	1	1	1	0	1	1	0	1	1	0	1	1	1	0	0
Macracantha hasselti	0	0	1	1	1	1	1	1	0	1	1	0	1	0	0	1	1	1	0	0
Thelacantha brevispina	1	0	0	1	1	0	1	1	1	1	1	1	1	1	0	1	1	0	0	0
Togacantha nordviei	0	0	1	1	1	1	1	1	1	1	1	0	1	2	1	1	1	0	0	0
Gasteracantha aciculata	0	0	1	1	1	3	1	1	1	1	1	0	1	0	0	1	1	1	0	0
Gasteracantha cancriformis	0	0	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	0	0	0
Gasteracantha clarki	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	?
Gasteracantha clavatrix	0	0	1	1	1	1	1	1	0	1	1	0	1	0	0	1	1	1	0	0
Gasteracantha clavigera	0	0	0	1	1	1	1	1	0	1	1	0	1	0	0	1	1	1	0	0
Gasteracantha cruciger a	0	0	1	1	1	1	1	1	0	1	1	0	1	0	0	1	1	1	0	0
Gasteracantha curvispina	1	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	1
Gasteracantha diadesmia	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	0
Gasteracantha diardi	1	0	0	1	1	0	1	1	1	1	1	1	1	0	0	1	1	0	0	1
Gasteracantha doriae	1	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	1
Gasteracantha falcicornis	1	0	0	1	1	2	1	1	1	1	1	1	1	1	0	1	1	0	0	1
Gasteracantha geminata	1	0	0	1	1	3	1	1	1	1	1	1	1	1	0	1	1	0	0	0
Gasteracantha hecata	1	0	0	1	1	2	1	1	1	1	1	1	1	0	0	1	1	0	0	1
Gasteracantha kuhli	1	0	0	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0	1
Gasteracantha lepelletieri	0	0	1	1	1	1	1	1	0	1	1	0	1	0	0	1	1	1	0	0
Gasteracantha lunata	1	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	1
Gasteracantha mediofusca	0	0	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	0	0	0
Gasteracantha mengei	1	0	0	1	1	0	1	1	1	1	1	1	1	1	0	1	1	0	0	0
Gasteracantha metallica	0	0	0	1	1	0	1	1	0	1	1	0	1	0	0	1	1	1	0	0
Gasteracantha milvoides	0	0	0	1	1	0	1	1	1	1	1	1	1	0	0	1	1	0	0	0
Gasteracantha pentagona	0	0	1	1	1	1	1	1	0	1	1	0	1	0	0	1	1	1	0	0
Gasteracantha quadrispinosa	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	1
Gasteracantha recurva	1	0	0	1	1	0	1	1	1	1	1	1	1	0	0	1	1	0	0	0
Gasteracantha regalis	0	0	1	1	1	0	1	1	0	1	1	0	1	0	0	1	1	1	0	0
Gasteracantha remifera	0	0	1	1	1	1	1	1	1	1	1	0	1	0	0	1	1	1	0	0
Gasteracantha rhomboidea madagascariensis	1	0	1	1	1	3	1	1	1	1	1	1	1	1	0	1	1	0	0	1
Gasteracantha sanguinolenta	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	1
Gasteracantha scintillans	0	0	1	1	1	0	1	1	0	1	1	0	1	0	0	1	1	1	0	0
Gasteracantha signifera	1	0	1	1	1	0	1	1	1	1	1	1	1	0	0	1	1	0	0	1
Gasteracantha sp. nov.	0	0	1	1	1	0	1	1	0	1	1	0	1	0	0	1	1	1	0	0
Gasteracantha sturi	1	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	1
Gasteracantha taeniata	0	0	0	1	1	2	1	1	1	1	1	1	1	0	0	1	1	0	0	1
Gasteracantha theisi	0	0	1	1	1	1	1	1	0	1	1	0	1	0	0	1	1	1	0	0
Gasteracantha thorelli	0	0	0	1	1	1	1	1	1	1	1	1	1	0	2	1	1	0	0	1
Gasteracantha unguifera	1	0	1	1	1	0	1	1	1	1	1	1	1	1	2	1	1	0	0	1
Gasteracantha versicolor formosa	0	0	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1
Gasteracantha westringi	0	0	0	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0	1

	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Nephila pilipes	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Araneus diadematus	0	0	0	1	1	1	0	0	0	0	0	1	0	1	1	0	1	0	1	0
Aetrocantha falkensteini	0	1	1	0	-	0	0	1	0	1	0	1	1	?	0	0	0	1	?	1
Aranoethra cambridgei	0	0	3	1	0	1	0	0	0	1	0	0	0	?	0	0	0	0	?	0
Micrathena aureola	1	1	2	1	1	1	0	2	0	1	1	1	1	1	0	0	0	1	1	0
Micrathena schreibersi	0	0	0	0	-	0	1	0	0	1	0	0	1	0	0	0	0	1	1	0
Augusta glyphica	1	1	1	0	-	0	0	0	0	1	0	0	1	1	0	0	0	1	1	1
Isoxya cicatricosa	1	0	3	0	-	0	1	1	0	1	1	1	1	1	0	1	0	1	1	1
Isoxya cowani	1	0	3	0	-	0	1	1	0	1	1	1	1	1	0	1	0	1	1	0
Isoxya penizoides	1	0	3	0	-	0	1	1	0	1	0	1	1	?	?	?	?	?	?	?
Isoxya sp. nov.	1	0	3	0	-	0	1	1	0	1	1	1	1	1	0	1	0	1	1	0
Isoxya tabulata	1	0	3	0	-	0	1	1	0	1	1	0	1	1	0	1	0	1	?	1
Acrosomoides acrosomoides	1	1	1	1	0	0	0	?	0	1	1	0	1	0	0	0	0	1	1	1
Acrosomoides linnaei	1	1	1	1	1	0	0	2	0	1	1	0	1	?	0	0	0	1	1	1
Actinacantha globulata	1	1	1	1	1	1	0	2	0	1	1	0	1	1	1	0	1	1	0	1
Afracantha camerunensis	1	0	3	0	-	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?
Austracantha minax	1	1	1	0	-	1	1	1	0	1	1	0	1	1	1	0	1	1	0	1
Macracantha arcuata	1	0	2	0	-	1	1	?	?	?	?	?	?	?	?	?	?	?	?	?
Macracantha hasselti	1	0	2	0	-	0	1	1	0	1	1	0	1	1	1	0	1	1	0	1
Thelacantha brevispina	1	1	1	1	0	1	0	2	1	1	1	0	1	1	1	0	1	1	1	1
Togacantha nordviei	1	0	3	0	-	0	0	2	0	1	1	0	1	?	?	?	0	1	0	1
Gasteracantha aciculata	1	0	0	1	1	1	0	2	1	1	1	0	1	1	1	0	1	1	0	1
Gasteracantha cancriformis	1	1	1	1	1	1	0	1	1	1	1	0	1	?	0	0	0	1	0	1
Gasteracantha clarki	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Gasteracantha clavatrix	1	1	1	1	1	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?
Gasteracantha clavigera	1	0	2	1	1	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?
Gasteracantha cruciger a	1	1	1	1	1	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?
Gasteracantha curvispina	1	1	1	1	1	1	0	2	1	1	1	0	1	?	0	0	0	1	0	1
Gasteracantha diadesmia	0	1	1	1	0	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?
Gasteracantha diardi	1	1	1	1	0	1	0	2	1	1	1	0	1	1	0	0	0	1	0	1
Gasteracantha doriae	0	1	1	1	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?
Gasteracantha falcicornis	1	1	1	1	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?
Gasteracantha geminata	1	0	1	0	-	0	0	2	1	1	1	0	1	?	0	0	0	1	0	1
Gasteracantha hecata	1	1	1	1	0	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?
Gasteracantha kuhli	0	1	1	1	0	0	0	2	1	1	1	0	1	?	?	?	?	?	?	?
Gasteracantha lepelletieri	1	1	1	1	1	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?
Gasteracantha lunata	0	1	1	1	1	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?
Gasteracantha mediofusca	1	1	1	1	0	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?
Gasteracantha mengei	0	1	1	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Gasteracantha metallica	1	1	1	1	1	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?
Gasteracantha milvoides	1	1	1	0	-	0	0	2	1	1	1	0	1	1	0	0	0	1	0	1
Gasteracantha pentagona	1	1	1	1	1	1	0	2	0	1	1	0	1	1	1	0	1	1	0	1
Gasteracantha quadrispinosa	1	1	1	1	1	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?
Gasteracantha recurva	0	0	0	1	1	-	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Gasteracantha regalis	1	1	1	1	1	-	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Gasteracantha remifera	1	1	1	1	1	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?
Gasteracantha rhomboidea madagascariensis	1	?	1	1	0	0	0	1	1	1	1	0	1	?	0	0	0	1	0	1
Gasteracantha sanguinolenta	1	1	1	1	0	1	0	1	0	1	1	0	1	?	0	0	0	1	0	1
Gasteracantha scintillans	1	1	1	1	1	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?
Gasteracantha signifera	0	1	1	1	0	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?
Gasteracantha sp. nov.	1	1	1	1	1	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?
Gasteracantha sturi	1	1	1	1	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?
Gasteracantha taeniata	1	1	1	1	0	0	0	2	1	1	1	0	1	1	0	0	0	1	0	1
Gasteracantha theisi	1	1	1	1	1	1	0	1	0	1	1	0	1	?	1	0	1	1	0	1
Gasteracantha thorelli	0	1	1	1	0	1	0	2	1	1	1	0	1	?	0	0	0	1	0	1
Gasteracantha unguifera	0	1	1	1	0	0	1	1	0	1	1	0	1	?	1	0	1	1	0	1
Gasteracantha versicolor formosa	0	1	1	1	0	1	0	2	1	1	1	0	1	0	0	0	0	1	0	1
Gasteracantha westringi	0	0	1	1	1	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?

	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	
Nephila pilipes	0	0	-	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0	0	
Araneus diadematus	2	1	0	0	1	0	0	1	1	1	1	1	0	1	0	0	0	0	0	
Aetrocantha falkensteini	?	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	
Aranoethra cambridgei	2	?	?	?	?	?	0	0	0	0	0	1	0	0	0	0	0	0	0	
Micrathena aureola	?	0	-	-	-	-	0	0	0	0	0	1	1	0	0	0	0	0	0	
Micrathena schreibersi	?	1	?	?	?	0	0	0	0	0	0	1	0	0	0	0	0	0	0	
Augusta glyphica	2	1	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	
Isoxya cicatricosa	2	1	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	
Isoxya cowani	2	1	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	
Isoxya penizoides	?	?	?	?	?	?	0	0	0	0	0	?	?	?	0	0	0	0	?	
Isoxya sp. nov.	2	1	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	
Isoxya tabulata	?	1	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	
Acrosomoides acrosomoides	1	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	
Acrosomoides linnaei	1	1	0	?	?	0	0	0	0	0	0	1	0	0	0	0	0	0	0	
Actinacantha globulata	2	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	
Afracantha camerunensis	?	?	?	?	?	?	0	0	0	0	0	?	-	-	0	0	0	0	0	
Austracantha minax	2	1	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	
Macracantha arcuata	?	?	?	?	?	?	0	0	0	0	0	?	?	?	0	0	0	0	?	
Macracantha hasselti	2	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	
Thelacantha brevispina	2	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	
Togacantha nordviei	2	1	1	0	1	0	0	0	0	0	0	?	0	0	0	0	0	0	0	
Gasteracantha aciculata	2	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	
Gasteracantha cancriformis	?	1	0	0	1	?	0	0	0	0	0	1	0	0	0	0	0	0	0	
Gasteracantha clarki	?	?	?	?	?	?	0	0	0	0	0	?	?	?	0	0	0	0	?	
Gasteracantha clavatrix	?	?	?	?	?	?	0	0	0	0	0	?	?	?	0	0	0	0	?	
Gasteracantha clavigera	?	?	?	?	?	?	0	0	0	0	0	?	?	?	0	0	0	0	?	
Gasteracantha cruciger a	?	?	?	?	?	?	0	0	0	0	0	?	?	?	0	0	0	0	?	
Gasteracantha curvispina	2	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	
Gasteracantha diadesmia	?	?	?	?	?	?	0	0	0	0	0	?	?	?	0	0	0	0	?	
Gasteracantha diardi	2	1	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	
Gasteracantha doriae	?	?	?	?	?	?	0	0	0	0	0	?	?	?	0	0	0	0	?	
Gasteracantha falcicornis	?	?	?	?	?	?	0	0	0	0	0	?	?	?	0	0	0	0	?	
Gasteracantha geminata	?	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	
Gasteracantha hecata	?	?	?	?	?	?	0	0	0	0	0	?	?	?	0	0	0	0	?	
Gasteracantha kuhli	?	?	?	?	?	?	0	0	0	0	0	?	?	?	0	0	0	0	?	
Gasteracantha lepelletieri	?	?	?	?	?	?	0	0	0	0	0	?	?	?	0	0	0	0	?	
Gasteracantha lunata	?	?	?	?	?	?	0	0	0	0	0	?	?	?	0	0	0	0	?	
Gasteracantha mediofusca	?	?	?	?	?	?	0	0	0	0	0	?	?	?	0	0	0	0	?	
Gasteracantha mengei	?	?	?	?	?	?	0	0	0	0	0	?	?	?	0	0	0	0	?	
Gasteracantha metallica	?	?	?	?	?	?	0	0	0	0	0	?	?	?	0	0	0	0	?	
Gasteracantha milvoides	2	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	
Gasteracantha pentagona	2	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	
Gasteracantha quadrispinosa	?	?	?	?	?	?	0	0	0	0	0	?	?	?	0	0	0	0	?	
Gasteracantha recurva	?	?	?	?	?	?	0	0	0	0	0	?	?	?	0	0	0	0	?	
Gasteracantha regalis	?	?	?	?	?	?	0	0	0	0	0	?	?	?	0	0	0	0	?	
Gasteracantha remifera	?	?	?	?	?	?	0	0	0	0	0	?	?	?	0	0	0	0	?	
Gasteracantha rhomboidea madagascariensis	?	1	0	0	0	0	0	0	0	0	0	1	0	?	0	0	0	0	?	
Gasteracantha sanguinolenta	2	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	
Gasteracantha scintillans	?	?	?	?	?	?	0	0	0	0	0	?	?	?	0	0	0	0	?	
Gasteracantha signifera	?	?	?	?	?	?	0	0	0	0	0	?	?	?	0	0	0	0	?	
Gasteracantha sp. nov.	?	?	?	?	?	?	0	0	0	0	0	?	?	?	0	0	0	0	?	
Gasteracantha sturi	?	?	?	?	?	?	0	0	0	0	0	?	?	?	0	0	0	0	?	
Gasteracantha taeniata	2	1	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	
Gasteracantha theisi	?	1	0	0	1	0	0	0	0	0	0	1	0	?	0	0	0	0	?	
Gasteracantha thorelli	?	1	0	0	1	0	0	0	0	0	0	1	0	?	0	0	0	0	?	
Gasteracantha unguifera	2	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	
Gasteracantha versicolor formosa	2	1	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	
Gasteracantha westringi	?	?	?	?	?	?	0	0	0	0	0	?	?	?	0	0	0	0	?	

A3.2.4 Average consistency and retention indices for characters from maximum parsimony morphological analysis conducted in PAUP* (Chapter 2.5.2)

Average character consistency index and retention index (720 trees Group 1 dataset)

Character	*ci*	*ri*	Character	*ci*	*ri*	Character	*ci*	*ri*
1	1.000	1.000	23	0.111	0.704	45	0.167	0.722
2	0.500	0.500	24	0.500	0.667	46	0.111	0.667
3	1.000	1.000	25	1.000	1.000	47	0.200	0.556
4	0.500	0.667	26	0.188	0.567	48	0.333	0.750
5	0.500	0.000	27	0.500	0.000	49	0.200	0.636
6	1.000	1.000	28	1.000	1.000	50	1.000	1.000
7	1.000	1.000	29	0.167	0.750	51	0.333	0.667
8	0.500	0.667	30	1.000	1.000	52	0.250	0.500
9	0.250	0.250	31	0.500	0.500	53	1.000	1.000
10	0.500	0.500	32	0.200	0.857	54	0.250	0.000
11	0.143	0.250	33	0.333	0.333	55	0.500	0.875
12	0.500	0.500	34	0.182	0.654	56	1.000	1.000
13	0.500	0.333	35	0.333	0.333	57	0.500	0.875
14	0.400	0.625	36	1.000	1.000	58	1.000	1.000
15	0.333	0.667	37	0.250	0.727	59	0.250	0.667
16	0.125	0.611	38	0.500	0.938	60	0.333	0.667
17	0.143	0.600	39	1.000	1.000	61	1.000	1.000
18	0.091	0.545	40	0.125	0.632	62	0.500	0.000
19	0.200	0.789	41	0.111	0.467	63	0.333	0.600
20	1.000	1.000	42	0.111	0.529	64	0.333	0.667
21	0.091	0.583	43	0.333	0.571	65	0.500	0.750
22	0.333	0.667	44	0.125	0.533	66	0.500	0.750

Average character consistency index and retention index (172 trees Group 2 dataset)

Character	*ci*	*ri*	Character	*ci*	*ri*	Character	*ci*	*ri*
1	1.000	1.000	23	0.167	0.615	45	0.250	0.625
2	0.500	0.500	24	0.500	0.667	46	0.143	0.571
3	1.000	1.000	25	1.000	1.000	47	0.250	0.571
4	0.500	0.667	26	0.250	0.400	48	0.333	0.733
5	0.500	0.000	27	0.500	0.000	49	0.250	0.700
6	1.000	1.000	28	1.000	1.000	50	1.000	1.000
7	n/a	n/a	29	0.200	0.636	51	0.333	0.600
8	0.500	0.667	30	1.000	1.000	52	0.250	0.400
9	0.500	0.500	31	0.500	0.500	53	1.000	1.000
10	1.000	1.000	32	0.333	0.857	54	0.250	0.000
11	0.500	0.667	33	0.333	0.333	55	0.333	0.750
12	n/a	n/a	34	0.250	0.455	56	1.000	1.000
13	0.667	0.500	35	0.400	0.400	57	0.333	0.750
14	0.500	0.600	36	1.000	1.000	58	1.000	1.000
15	0.333	0.500	37	0.250	0.700	59	0.250	0.667
16	0.143	0.455	38	0.500	0.833	60	0.333	0.667
17	0.167	0.000	39	1.000	1.000	61	1.000	1.000
18	0.167	0.615	40	0.200	0.600	62	0.500	0.000
19	0.200	0.556	41	0.167	0.286	63	0.333	0.600
20	1.000	1.000	42	0.143	0.455	64	0.200	0.333
21	0.143	0.500	43	0.429	0.556	65	0.333	0.500
22	0.333	0.667	44	0.167	0.583	66	0.333	0.500

Average character consistency index and retention index (774 trees Group 3 dataset)

Character	*ci*	*ri*	Character	*ci*	*ri*	Character	*ci*	*ri*
1	1.000	1.000	23	0.143	0.647	45	0.250	0.667
2	0.500	0.500	24	0.500	0.667	46	0.111	0.529
3	1.000	1.000	25	1.000	1.000	47	0.250	0.667
4	0.500	0.667	26	0.231	0.474	48	0.333	0.750
5	0.500	0.000	27	0.500	0.000	49	0.250	0.727
6	1.000	1.000	28	1.000	1.000	50	1.000	1.000
7	n/a	n/a	29	0.200	0.692	51	0.333	0.667
8	0.500	0.667	30	1.000	1.000	52	0.250	0.500
9	0.333	0.333	31	0.500	0.500	53	1.000	1.000
10	0.500	0.500	32	0.333	0.882	54	0.250	0.000
11	0.333	0.500	33	0.333	0.333	55	0.333	0.750
12	n/a	n/a	34	0.200	0.429	56	1.000	1.000
13	0.667	0.500	35	0.333	0.333	57	0.333	0.750
14	0.500	0.600	36	1.000	1.000	58	1.000	1.000
15	0.333	0.500	37	0.250	0.727	59	0.250	0.667
16	0.143	0.500	38	0.500	0.875	60	0.333	0.667
17	0.143	0.143	39	1.000	1.000	61	1.000	1.000
18	0.143	0.600	40	0.200	0.667	62	0.500	0.000
19	0.200	0.636	41	0.125	0.300	63	0.333	0.600
20	1.000	1.000	42	0.143	0.538	64	0.250	0.500
21	0.125	0.563	43	0.429	0.636	65	0.333	0.500
22	0.333	0.667	44	0.167	0.643	66	0.333	0.500

A3.3 Matrices of molecular characters (CO1 and 16S)

Complete CO1 sequence data

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Nephila_pilipes               ------------GGAACTGCTATAAGAGTTTTGATTCGGATTGAATTGGGTCAAGTTGGAAGATTATTAGGAGATGATCAGTTGTATAATGTAATTGTTA
Araneus_diadematus            ---------------------------GTATTGATTCGAATTGAATTAGGTCAGCCTGGGAGATTTATTGGAGATGATCAACTTTATAATGTTATTGTAA
Aranoethra_cambridgei         GCAGCTATAATAGGAACTGCAATAAGAGTATTAATTCGAATTGAATTAGGACAAGGAGGAAGATTTTTAGGAGATGATCAATTATATAATGTAATTGTTA
Micrathena_gracilis           TCTGCTATAGTAGGTACTGCTATAAGTGTTTTAATCCGTATTGAATTAGGACAGATAGGTAGATTTATAGGAGATGACCAGTTATATAATGTTATTGTAA
Micrathena_schreibersi        ----------------------------------------------------------TGGTAGGTTTATAGGAGACGATCAGCTGTATAATGTAGTAGTGA
Micrathena_triangularispinosa ------------------------------------------------------------------------------------------------
Acrosomoides_acrosomoides     TCAGCTATAGTAGGAACCGCAATAAGGGTTTTAATTCGGATTGAGCTTGGTCAACCTGGAAGATTTATTGGGGATGATCAATTATATAATGTGGTAGTAA
Actinacantha_globulata        TCTGCAATAATTGGGACAGCAATAAGAGTTTTAATCCGAATTGAACTTGGTCAACCTGGAAGATTCATTGGGGATGACCAGTTGTATAATGTTGTAGTAA
Augusta_glyphica              TCTGCTATAATTGGTACAGCTATAAGAGTATTAATTCGAATTGAATTAGGACAATCTGGAAGATTTTTTGGTGATGATCAGCTTTATAATGTAATTGTTA
Austracantha_minax            TCTGCTATAGTAGGGACTGCAATAAGAGTATTAATTCGAATTGAGCTTGGTCAACCAGGAAGATTTATTGGGGATGATCAATTGTATAATGTAGTGGTAA
Isoxya_mahafalensis           GCTGCTATAGTAGGAACGGCTATAAGAGTTTTAATTCGTATTGAACTTGGACAACCTGGAAGATTCATTGGGGATGATCAGTTATATAATGTGGTAGTAA
Isoxya_penzoides              GCGGCTATAGTAGGAACAGCTATAAGAGTTTTAATTCGAATTGAACTTGGGCAGCCTGGGAGATTTATTGGGGATGATCAGTTATATAATGTGGTGGTGA
Isoxya_sp_nov                 GCTGCTATAGTAGGAACGGCTATAAGAGTTTTAATTCGTATTGAACTTGGGCAACCTGGTAGGTTCATTGGGGATGATCAGTTATATAATGTGGTGGTAA
Isoxya_tabulata               GCTGCTATGGTGGGGACGGCTATAAGAGTTTTAATTCGAATTGAACTTGGGCAGCCTGGTAGATTTATTGGGGATGATCAGTTATATAATGTGGTGGTAA
Macracantha_arcuata           GTCTGCATAATTGGAACAGCAATAAGAGTTTTAATTCGAATTGAACTTGGTCAGCCCGGAAGATTTATCGGAGATGATCAATTGTATAATGTTGTAGTAA
Macracantha_hasselti          TCTGCGATAATTGGAACAGCAATAAGAGTTTTAATTCGAATTGAACTTGGTCAACCTGGAAGATTTATTGGGGATGACCAGTTGTATAATGTTGTAGTAA
Thelacantha_brevispina        TCAGCAATAGTTGGAACTGCAATAAGAGTGTTAATTCGAATTGAATTAGGTCAGCCAGGGAGATTTATTGGGGATGATCAGTTATATAATGTAATTGTAA
Gasteracantha_aciculata       TCTGCAATAATTGGAACAGCGATAAGAGTATTAATTCGAATTGAACTTGGTCAACCTGGAAGGTTTATTGGGGATGATCAATTATATAATGTTGTAGTAA
Gasteracantha_cancriformis    TCAGCAATGGTCGGTACTGCAATAAGAGTATTAATTCGAATTGAATTAGGACAGCCAGGTAGATTTATTGGTGATGATCAATTATATAATGTGGTAGTAA
Gasteracantha_clavatrix       ---------TGTGTGACAGCAATAAGGGTATTAATTCGAATTGAACTTGGCCAACCTGGAAGATTTATCGGGGATGACCAATTATATAATGTCGTCGTAA
Gasteracantha_diadesmia       TCAGCAATAGTTGGTACTGCAATAAGAGTATTGATTCGAATTGAATTAGGACAACCAGGGAGGTTTATTGGTGATGACCAATTATATAATGTGGTAGTGA
Gasteracantha_diardi          TCAGCAATAGTTGGTACGGCAATAAGAGTATTGATTCGAATTGAATTAGGGCAACCAGGGAGGTTTATTGGTGATGACCAATTATATAATGTGGTAGTGA
Gasteracantha_doriae          TCAGCAATAGTTGGTACTGCAATAAGAGTATTGATTCGAATTGAATTAGGGCAACCAGGAAGGTTTATTGGTGATGACCAATTATACAATGTGGTAGTGA
Gasteracantha_fornicata       TCAGCAATAGTTGGTACTGCAATAAGAGTATTGATTCGAATTGAATTGGGACAACCGGGGAGCTTTATTGGTGATGACCAATTATATAATGTGGTAGTTA
Gasteracantha_kuhlii          TCAGCAATAGTAGGAACTGCTATAAGAGTATTAATTCGAATTGAATTAGGGCAACCAGGCAGATTTATTGGTGATGACCAATTATATAATGTGGTGGTTA
Gasteracantha_milvoides       TCAGCAATGGTCGGTACTGCTATAAGAGTATTGATTCGAATTGAATTAGGACAACCAGGTAGATTTATTGGTGATGATCAATTATATAATGTGGTAGTAA
Gasteracantha_rhomb_madag     TCCGCAATGGTTGGTACTGCTATAAGAGTTTTAATTCGAATTGAATTGGGGCAACCTGGTAGGTTTATTGGTGATGACCAGTTATATAATGTAGTAGTTA
Gasteracantha_versi_formo     TCAGCAATGGTTGGTACTGCTATAAGAGTTTTGATCCGAATTGAGTTAGGACAACCGGGTAGATTTATTGGTGATGACCAGTTGTATAATGTAGTAGTTA
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Nephila_pilipes               CAGCTCATGCTTTTGTAATAATTTTTTTTATAGTTATACCTATTTTAATTGGGGGTTTTGGTAATTGATTGGTTCCTTTAATATTGGGGGCTCCTGATAT
Araneus_diadematus            CTGCGCATGCGTTTGTAATAATTTTTTTTATAGTTATACCTATTTTAATTGGGGGATTTGGAAATTGATTAGTGCCTTTAATGTTAGGGGCTCCTGATAT
Aranoethra_cambridgei         CTGCTCATGCATTTGTAATAATTTTTTTTATAGTAATACCAATTTTAATTGGGGGGTTTGGTAATTGATTAGTTCCGTTAATATTAGGTGCTCCTGATAT
Micrathena_gracilis           CTGCTCATGCTTTTATTATAATTTTTTTTATGGTTATACCAATTATAATTGGTGGTTTTGGAAATTGGTTAGTTCCTTTAATATTAGGGGCTCCAGATAT
Micrathena_schreibersi        CTGCTCATGCTTTTGTAATAATCTTTTTCATAGTGATACCAATTTTAATTGGAGGTTTTGGGAATTGATTGGTGCCGCTAATATTAGGAGCACCGGATAT
Micrathena_triangularispinosa ------------------------------------------------TTGGGGGATTCGGAAATTGGTTAGTTCCTTTAATGTTAGGCGCTCCTGATAT
Acrosomoides_acrosomoides     CTGCTCATGCTTTTGTAATAATTTTTTTTATAGTGATACCAATTTTAATTGGGGGGTTTGGGAATTGGTTGATTCCAATAATGTTAGGGGCTCCTGATAT
Actinacantha_globulata        CAGCTCATGCTTTTGTAATAATTTTTTTTATAGTAATACCTATTTTAATTGGGGGATTTGGTAATTGATTAGTACCATTAATGTTAGGCGCTCCGGATAT
Augusta_glyphica              CAGCTCATGCTTTTGTAATGATTTTTTTTATAGTAATACCTATAATAATTGGTGGGTTTGGAAATTGGTTAGTTCCTTTGATGTTAGGTGCGCCTGATAT
Austracantha_minax            CTGCTCATGCTTTTGTAATAATTTTTTTTATAGTTATACCTATTTTAATTGGGGGGTTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCTCCTGATAT
Isoxya_mahafalensis           CTGCTCATGCATTTGTAATAATTTTTTTTATAGTAATACCGATTTTAATTGGGGGGTTTGGAAATTGATTAGTTCCGTTAATATTGGGTGCTCCTGATAT
Isoxya_penzoides              CTGCTCATGCATTTGTAATAATTTTTTTTATAGTTATACCTATTTTAATTGGGGGGTTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCACCTGATAT
Isoxya_sp_nov                 CTGCTCATGCATTTGTAATAATTTTTTTTATAGTAATACCGATTTTGATTGGAGGGTTTGGGAATTGATTAGTTCCATTAATACTAGGTGCTCCTGATAT
Isoxya_tabulata               CTGCTCATGCATTTGTAATAATTTTTTTTATAGTTATACCAATTTTGATTGGAGGGTTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCTCCTGATAT
Macracantha_arcuata           CTGCACATGCTTTTGTAATAATTTTTTTTATAGTTATACCTATTTTGATTGGTGGATTTGGAAATTGGTTGGTTCCTTTAATATTAGGTGCTCCGGATAT
Macracantha_hasselti          CTGCTCATGCTTTTGTAATAATTTTTTTTATAGTAATGCCTATTTTAATTGGAGGGTTTGGTAATTGATTAGTACCCTTAATATTAGGGGCTCCAGATAT
Thelacantha_brevispina        CTGCACATGCATTTGTTATAATTTTTTTTATAGTAATACCTATTTTAATTGGGGGGTTTGGGAATTGGTTAGTTCCTTTAATATTAGGGGCCCCTGATAT
Gasteracantha_aciculata       CTGCTCATGCTTTTGTAATAATTTTTTTTATAGTAATACCTATCCTGATTGGGGGATTTGGGAATTGATTAGTTCCTTTAATATTAGGCGCTCCGGATAT
Gasteracantha_cancriformis    CTGCTCATGCTTTTGTTATAATTTTTTTTATAGTAATACCAATCCTAATTGGTGGTTTTGGGAATTGATTGGTACCTTTAATGTTAGGAGCACCAGATAT
Gasteracantha_clavatrix       CGGCACATGCTTTTGTAATAATTTTTTTTATAGTAATACCTATCTTAATCGGGGGATTTGGCAATTGATTAGTACCATTAATATTAGGCGCTCCGGATAT
Gasteracantha_diadesmia       CTGCTCATGCTTTTGTTATAATTTTTTTTATAGTTATACCTATTTTAATTGGGGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGGGCTCCTGATAT
Gasteracantha_diardi          CTGCTCATGCTTTTGTTATAATTTTTTTTATAGTTATGCCTATTTTAATTGGGGGATTTGGAAATTGACTAGTTCCTTTAATATTAGGGGCCCCTGATAT
Gasteracantha_doriae          CTGCTCATGCTTTTGTTATAATTTTTTTTATAGTAATACCTATTTTAATTGGGGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGGGCTCCTGATAT
Gasteracantha_fornicata       CTGCTCATGCTTTTGTTATAATTTTTTTTATAGTAATACCTATTTTAATTGGAGGATTTGGTAACTGGTTAGTCCCTTTAATGTTGGGAGCTCCTGATAT
Gasteracantha_kuhlii          CTGCCCATGCTTTTGTTATAATTTTTTTTATAGTTATACCCATTTTAATTGGAGGTTTTGGTAATTGATTAGTACCTCTAATATTAGGGGCACCTGATAT
Gasteracantha_milvoides       CTGCTCATGCTTTTGTTATAATTTTTTTTATAGTAATACCAATTTTAATTGGTGGTTTTGGAAATTGATTGGTACCTTTAATGCTAGGAGCTCCAGATAT
Gasteracantha_rhomb_madag     CTGCTCATGCTTTTATTATAATTTTTTTTATAGTAATACCTATTTTAATTGGAGGTTTTGGGAATTGATTGGTACCTCTAATGTTAGGAGCACCAGATAT
Gasteracantha_versi_formo     CTGCTCATGCTTTTATTATAATTTTTTTTATAGTGATACCTATCTTAATTGGAGGTTTTGGAAATTGATTGGTACCTTTAATGTTAGGGGCACCAGATAT
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Nephila_pilipes               AGCTTTTCCTCGCATAAATAATTTAAGATTTTGATTATTACCCCCTTCATTATTTTTATTGTTTATTTCATCAATAGTAGAAATAGGTGTAGGTGCAGGA
Araneus_diadematus            AGCGTTTCCTCGAATAAATAATTTAAGATTTTGATTACTTCCTCCATCTTTATTTCTTTTGATTGTTTCTTCAATAGTTGAGATAGGAGTTGGTGCAGGG
Aranoethra_cambridgei         AGCTTTTCCTCGAATAAATAATTTAAGATTTTGATTATTACCTCCATCTTTATTTCTTTTAATTAATTCTTCAATAGTAGAAATAGGAGTAGGGGCTGGT
Micrathena_gracilis           AGCTTTTCCGCGAATAAATAACTTGAGATTTTGATTATTACCTCCCTCGTTATTAATATTAATTATTTCTTCAATAGTTGAAATAGGGGTTGGGGCTGGA
Micrathena_schreibersi        GGCTTTTCCACGAATAAATAATTTGAGTTTTTGGTTGTTACCTCCATCTTTATTAATATTAATTATTTCTTCAATGGTTGAAATGGGGGTAGGTTCCGGG
Micrathena_triangularispinosa AGCTTTTCCTCGAATAAATAATTTAAGGTTTTGATTATTGCCTCCATCTTTATTAATATTAATCATTTCTTCTATAGTAGAAATGGGAGTGGGTGCTGGG
Acrosomoides_acrosomoides     AGCTTTTCCTCGAATAAATAATTTAAGATTTTGATTACTTCCTCCTTCTTTATTTTTATTAGTAGTATCCTCAATGGTTGAAATAGGGGTAGGTGCTGGA
Actinacantha_globulata        AGCATTTCCTCGAATAAATAATTTGAGATTTTGATTATTACCTCCTTCATTAATACTTTTAGTAATCTCATCTATAGTAGAAATGGGAGTCGGAGCGGGG
Augusta_glyphica              AGCTTTTCCGCGAATAAATAATTTAAGATTCTGACTGTTGCCGCCTTCTTTATTTTTTTTAATTTCATCTTCTATAGTAGAAATAGGAGTGGGAACAGGG
Austracantha_minax            AGCTTTTCCTCGAATAAATAATTTAAGATTTTGATTATTACCTCCTTCATTATTTCTTTTAGTAATTTCATCAATAGTTGAGATAGGGGTGGGAGCAGGA
Isoxya_mahafalensis           AGCTTTTCCTCGAATAAATAATTTAAGGTTTTGACTTCTTCCCCCTTCATTATTTCTTTTAGTAATTTCATCAATAGTAGAGATAGGGGTAGGAGCAGGA
Isoxya_penzoides              AGCTTTTCCTCGTATGAATAATTTAAGATTTTGACTTCTTCCCCCTTCTTTATTTCTTTTAGTAATTTCATCAATAGTTGAAATAGGGGTAGGGGCAGGA
Isoxya_sp_nov                 AGCTTTTCCTCGAATAAATAATTTAAGGTTTTGACTTCTCCCCCCTTCGTTATTTCTTTTAGTAATTTCGTCAATAGTAGAGATAGGAGTAGGAGCAGGA
Isoxya_tabulata               AGCTTTTCCTCGAATGAATAATTTAAGATTTTGACTTCTTCCTCCTTCTTTATTTCTTTTAGTCATTTCATCAATAGTTGAAATGGGAGTGGGGGCAGGA
Macracantha_arcuata           AGCATTTCCTCGGATAAATAATTTAAGATTTTGACTATTACCACCTTCATTAATACTTTTAATAATCTCATCTATAGCGGAAATAGGGGTGGGGGCGGGA
Macracantha_hasselti          AGCATTTCCTCGAATAAACAATTTGAGATTTTGATTATTGCCTCCTTCATTAATACTTTTAGTAATTTCATCTATAGTAGAAATAGGAGTTGGGGCAGGA
Thelacantha_brevispina        AGCTTTTCCACGAATAAATAATTTAAGATTTTGATTATTACCACCATCTTTATTTCTTTTAGTAATTTCTTCTATGGTAGAAATAGGAGTAGGGGCAGGA
Gasteracantha_aciculata       AGCATTCCCCCGTATAAATAATTTAAGATTTTGATTATTACCTCCCTCATTAATACTTTTAGTAATTTCATCTATAGTAGAAATAGGAGTCGGGGCAGGA
Gasteracantha_cancriformis    GGCTTTTCCTCGGATAAATAATTTAAGATTTTGGTTATTACCTCCTTCTTTACTACTTTTAGTAATTTCTAGAATAGTGGAAATGGGAGTGGGGGCTGGT
Gasteracantha_clavatrix       AGCGTTTCCTCGAATAAATAATTTGAGATTTTGGTTATTACCCCCTTCATTAATACTCTTAGTAATTTCATCAATAGTAGAGATAGGAGTTGGAGCGGGA
Gasteracantha_diadesmia       AGCTTTTCCTCGAATGAATAATTTAAGATTTTGGTTATTACCCCCATCTTTATTACTTTTAGTAATTTCTAGAATAGTAGAAATAGGGGTTGGGGCTGGT
Gasteracantha_diardi          AGCTTTTCCTCGAATGAATAATTTAAGATTTTGGTTATTACCTCCATCTTTATTACTTTTAGTAATTTCTAGAATAGTAGAAATAGGGGTTGGGGCTGGT
Gasteracantha_doriae          AGCTTTTCCTCGAATAAATAATTTAAGGTTTTGATTATTACCCCCATCTTTATTACTTTTAGTAATTTCTAGAATAGTAGAAATAGGGGTTGGGGCTGGT
Gasteracantha_fornicata       AGCTTTTCCTCGGATAAATAATTTAAGATTTTGGTTATTACCCCCTTCTTTATTACTATTAGTAATTTCTAGAATAGTAGAGATAGGAGTTGGAGCTGGT
Gasteracantha_kuhlii          AGCTTTTCCTCGTATAAATAATTTGAGATTTTGGTTATTACCTCCATCTTTATTACTTTTAGTAATTTCTAGAATAGTAGAAATAGGTGTAGGGGCTGGT
Gasteracantha_milvoides       AGCTTTTCCTCGGATAAATAATTTAAGATTTTGGTTATTACCCCCTTCTCTATTACTTTTAGTAATTTCTAGAATAGTAGAAATAGGTGTGGGGGCTGGT
Gasteracantha_rhomb_madag     GGCTTTTCCTCGTATAAATAATTTAAGATTTTGGTTATTACCCCCGTCTTTGTTACTTTTAGTAATTTCTAGAATAGTAGAAATAGGTGTGGGGGCTGGT
Gasteracantha_versi_formo     AGCTTTTCCTCGAATAAATAATTTAAGATTTTGGTTATTACCTCCGTCTTTGTTACTTTTAGTAATTTCTAGGATAGTAGAGATAGGTGTGGGGGCTGGT
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Nephila_pilipes               TGAACTGTATATCCTCCATTGGCTTCTTTAGAAGGTCATGCTGGAAGATCTGTAGATTTTGCTATTTTTTCTTTACATTTAGCGGGTGCTTCTTCAATTA
Araneus_diadematus            TGGACTGTATATCCTCCTTTAGCCGGATTAGAGGGTCATGCTGGAAGATCAGTGGATTTTGCAATTTTTTCTTTGCATTTAGCGGGGGCTTCTTCTATTA
Aranoethra_cambridgei         TGAACAATCTATCCTCCTTTAGCAAGATTAGAAGGACATGCTGGTAGATCAGTAGATTTTGCAATTTTTTCTTTACATTTAGCAGGAGCTTCATCAATTA
Micrathena_gracilis           TGAACTGTTTATCCCCCTTTAGCTTCACTAGAAGGACATGCTGGAAGATCAGTAGATTTTGCTATTTTTTCTTTACATTTAGCAGGGGCTTCTTCTATTA
Micrathena_schreibersi        TGAACTATTTATCCCCCCCTAGCGTCTTTAGACGGACATGCTGGGAGATCAGTAGATTTTGCTATTTTTTCGCTTCACTTAGCTGGTGCCTCTTCTATTA
Micrathena_triangularispinosa TGAACTGTCTATCCTCCTTTAGCTTCTTTAGAAGGACATGCTGGGAGATCTGTAGATTTTGCAATTTTTTCTTTGCATTTAGCTGGAGCTTCATCTATTA
Acrosomoides_acrosomoides     TGAACTGTTTATCCCCCTTTGGCCGGATTAGAAGGTCATTCTGGAAGATCGGTGGATTTTGCAATTTTTTCGTTACATTTAGCAGGGGCTTCTTCAATTA
Actinacantha_globulata        TGAACTATTTACCCTCCTTTAGCCAGATTAGAAGGTCATTCTGGGAGTTCAGTTGACTTTGCAATTTTTTCTCTCCATTTAGCGGGGGCTTCTTCAATTA
Augusta_glyphica              TGAACTATATATCCGCCTTTAGCAGGGATAGACGGACATTCTAGAATGTCCGTCGATCTTGCTATTTTTTCTCTTCATTTAGCTGGGGGATCTTCTATTA
Austracantha_minax            TGAACTGTTTATCCTCCTTTAGCTGGTTTGGAAGGGCATGCCGGAAGATCTGTTGATTTTGCTATTTTTTCTCTTCATTTAGCGGGAGCTTCTTCAATTA
Isoxya_mahafalensis           TGAACTGTTTATCCTCCTCTAGCTGGGTTAGAAGGGCATGCAGGGAGATCTGTAGATTTTGCTATTTTTTCTCTTCATTTGGCTGGGGCTTCTTCTATTA
Isoxya_penzoides              TGAACTGTTTATCCCCCTTTAGCTGGGTTAGAAGGGCATGCCGGAAGATCTGTAGATTTTGCTATTTTTTCTCTTCATTTGGCTGGAGCTTCTTCTATTA
Isoxya_sp_nov                 TGAACTGTTTATCCCCCTCTAGCTGGGCTAGAAGGGCATGCTGGGAGATCTGTGGATTTTGCTATTTTTTCTCTTCATTTGGCTGGGGCTTCTTCTATTA
Isoxya_tabulata               TGAACTGTTTATCCCCCTCTAGCTGGGTTAGAGGGGCATGCCGGAAGATCTGTGGATTTTGCTATTTTTTCTCTTCATTTGGCTGGGGCTTCTTCTATTA
Macracantha_arcuata           TGAACTATTTATCCTCCTTTAGCTAGATTAGAAGGACATTCTGGTAGTTCCGTTGATTTTGCAATTTTTTCTCTTCATTTAGCGGGAGCTTCTTCGATTA
Macracantha_hasselti          TGGACTATTTATCCACCTTTAGCAAGATTAGAGGGACATTCTGGAAGTTCAGTTGATTTCGCAATTTTTTCTTTACACTTAGCGGGAGCTTCTTCAATTA
Thelacantha_brevispina        TGAACTGTGTATCCTCCTCTTGCTAGTTTAGAAGGTCATGCGGGGAGCTCTGTAGATTTTGCAATTTTTTCTCTTCATTTAGCTGGGGCTTCTTCGATTA
Gasteracantha_aciculata       TGAACTATTTATCCTCCTTTAGCTAGATTAGAAGGACATTCTGGAAGTTCAGTTGATTTTGCAATTTTTTCTTTACATTTAGCAGGAGCTTCTTCAATTA
Gasteracantha_cancriformis    TGAACTGTGTACCCTCCATTAGCTAGATTAGAGGGACATGCCGGAAGATCAGTTGATTTTGCAATTTTCTCTTTACATTTAGCAGGGGCTTCTTCAATTA
Gasteracantha_clavatrix       TGGACTATTTATCCTCCTTTAGCCAGATTAGAAGGGCATTCTGGGAGTTCGGTTGATTTTGCAATTTTTTCTCTTCATTTAGCAGGGGCATCTTCAATTA
Gasteracantha_diadesmia       TGGACTGTTTATCCCCCATTAGCAAGATTAGAGGGTCATGCAGGAAGTTCGGTAGATTTTGCAATTTTTTCTCTTCATTTAGCTGGGGCTTCTTCAATTA
Gasteracantha_diardi          TGGACTGTTTACCCTCCATTAGCAAGATTAGAGGGTCATGCAGGAAGATCAGTAGATTTTGCAATTTTTTCTTTGCATCTAGCTGGGGCTTCTTCAATTA
Gasteracantha_doriae          TGGACTGTTTACCCACCATTAGCAAGATTAGAGGGTCATGCAGGAAGTTCAGTAGATTTTGCAATTTTTTCTTTACATTTAGCTGGGGCTTCTTCAATTA
Gasteracantha_fornicata       TGGACTGTTTATCCCCCGCTAGCTAGATTAGAGGGTCATGCTGGAAGCTCAGTAGATTTTGCAATTTTTTCTCTTCATTTAGCTGGGGCCTCCTCAATTA
Gasteracantha_kuhlii          TGGACTGTTTATCCTCCATTAGCTAGATTAGAGGGTCATGCAGGAAGTTCAGTGGATTTTGCAATTTTTTCTCTACATTTGGCTGGGGCTTCTTCAATTA
Gasteracantha_milvoides       TGAACTGTATACCCTCCTCTAGCTAGATTAGAGGGACATGCCGGAAGATCAGTCGATTTCGCAATTTTTTCTTTGCATTTAGCGGGAGCTTCTTCAATTA
Gasteracantha_rhomb_madag     TGAACTGTTTATCCCCCATTAGCTAGATTAGAAGGGCATGCCGGGAGTTCAGTAGATTTTGCAATTTTTTCTTTACATTTAGCAGGGGCTTCTTCAATTA
Gasteracantha_versi_formo     TGAACTGTGTATCCTCCATTAGCTAGATTGGAGGGGCATGCCGGAAGTTCAGTAGATTTTGCAATCTTTTCTTTACATTTAGCAGGGGCTTCTTCAATTA
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Nephila_pilipes               TAGGGGCTATTAATTTTATTTCAACAATTTTAAATATGCGATCATATGGAATATCTATAGAGAAAATTCCTTTATTTGTATGATCTGTATTGATTACTGC
Araneus_diadematus            TAGGGGCTATTAATTTTATTTCTACAATTATTAATATGCGTTTTTATGGAATAACAATAGAAAAAGTTCCTTTATTTGTGTGGTCTGTATTAATTACGGC
Aranoethra_cambridgei         TAGGTGCAATTAATTTCATTTCTACTATTTTAAATATACGTTTTTATGGAATAACAATAGAAAAAATTCCTTTATTTGTTTGATCTGTTTTGATTACTGC
Micrathena_gracilis           TAGGGGCTATTAATTTTATTTCTACTATTTTAAATATACGAATGTTAGGAATAACAATGGAAAAGGTTCCTTTGTTTGTATGATCTGTTTTTATTACTGC
Micrathena_schreibersi        TAGGAGCAATTAATTTTATTTCGACAATTATAAATATGCGTTTAGTAGGGGTAACTATAGATAAGGTTCCTTTATTTGTCTGATCTGTGTTAATTACAGC
Micrathena_triangularispinosa TAGGGGCTATTAACTTTATTTCTACAATTTTAAATATGCGATTAATAGGAATAAGAATAGAAAAGGTTCCATTATTTGTCTGGTCTGTATTGATTACAGC
Acrosomoides_acrosomoides     TAGGGGCAATTAATTTTATTTCTACAATTTTAAATATACGGTTTTATGGGATAACTATAGAAAAGATTCCTTTATTTGTATGGTCTGTCTTAATCACTGC
Actinacantha_globulata        TAGGGGCTATTAATTTTATTTCAACTATTATTAATATACGTTTTTATGGGATAACTATAGAGAAAATCCCTTTATTTGTTTGATCTGTCTTAATTACTGC
Augusta_glyphica              TAGGGGCTATTAATTTTATTTCTACTATTATAAATATGCGTTATTTTGGGATGACAATAGATAAAGTTCCATTATTTGTGTGATCAGTATTGGTTACAGC
Austracantha_minax            TAGGGGCTATTAATTTTATTTCTACTATTATTAATATACGATCTTACGGAATAAGAATAGAAAAGGTTCCTTTATTTGTTTGGTCTGTTTTAATTACTGC
Isoxya_mahafalensis           TAGGAGCTATTAATTTTATTTCTACAATTATTAATATACGGTTTTATGGAATGACTATAGAAAAAGTTCCTTTATTTGTATGATCAGTTTTAATTACTGC
Isoxya_penzoides              TAGGGGCTATTAATTTTATTTCTACAATTATTAATATACGATTTTATGGGATAACTATAGAAAAAGTTCCTTTATTTGTTTGATCCGTATTAATTACTGC
Isoxya_sp_nov                 TGGGAGCCATTAATTTTATTTCTACAATCATTAATATACGGTTTTATGGAATGACTATAGAAAAAGTTCCTTTATTTGTATGATCAGTCTTAATTACTGC
Isoxya_tabulata               TAGGGGCTATTAATTTTATTTCTACAATTATTAATATACGGTTTTACGGAATGACTATAGAAAAAGTTCCTTTATTTGTGTGATCTGTATTAATTACTGC
Macracantha_arcuata           TGGGTGCTATTAATTTTATTTCAACTATTATTAATATACGTTTTTATGGAATAACTATAGAAAAAATTCCTTTATTTGTTTGGTCTGTTTTAATTACTGC
Macracantha_hasselti          TAGGAGCTATTAATTTTATTTCAACTATTATTAATATACGTTTTTATGGGATAACTATAGAAAAAATTCCTTTATTTGTTTGATCTGTTTTAATTACTGC
Thelacantha_brevispina        TAGGGGCTATTAATTTTATTTCCACCATTATTAATATACGATTTTATGGAATATCTATAGAAAAGGTTCCATTATTTGTTTGGTCAGTTTTGATTACTGC
Gasteracantha_aciculata       TAGGAGCTATTAATTTTATTTCTACTATTATTAATATACGATTTTATGGAATAACTATAGAAAAAATTCCTTTATTTGTTTGATCTGTTTTAATTACTGC
Gasteracantha_cancriformis    TAGGGGCTATTAATTTTATTTCAACTATTATTAATATACGATTTTATGGAATAACTATAGAAAAGGTTCCTTTATTTGTTTGATCTGTATTAATTACTGC
Gasteracantha_clavatrix       TAGGGGCTATTAATTTTATTTCAACTATTATTAATATACGTTTTTACGGAATAACTATAGAGAAAATTCCTTTATTTGTTTGGTCTGTTCTAATTACTGC
Gasteracantha_diadesmia       TAGGAGCTATTAATTTTATTTCAACGATTATTAATATACGATTTTATGGAATGACTATAGAAAAAATTCCTTTGTTTGTTTGATCTGTGTTAATCACTGC
Gasteracantha_diardi          TAGGGGCTATTAATTTTATTTCAACGATTATTAATATACGATTTTACGGAATGACTATAGAAAAAATCCCTTTGTTTGTTTGATCTGTGTTGATTACTGC
Gasteracantha_doriae          TAGGGGCAATTAATTTTATTTCAACGATTATTAATATACGGTTTTATGGGATGACTATAGAAAAAATTCCTTTGTTTGTTTGATCGGTGTTAATTACTGC
Gasteracantha_fornicata       TAGGAGCTATCAATTTTATTTCTACAATTATTAATATACGATTTTACGGGATGACTATAGAAAAAATTCCTTTGTTTGTTTGATCTGTTTTAATTACTGC
Gasteracantha_kuhlii          TAGGGGCTATTAATTTTATTTCAACTATTATTAATATACGATTTTACGGTATAACTATAGAAAAAGTTCCTTTATTTGTTTGATCTGTATTAATTACTGC
Gasteracantha_milvoides       TAGGGGCTATCAATTTTATTTCAACTATTATTAATATACGATTTTATGGAATAACTATAGAAAAAGTTCCTCTGTTTGTTTGATCTGTATTAATTACTGC
Gasteracantha_rhomb_madag     TAGGGGCTATTAATTTTATTTCAACTATTATTAATATGCGGTTTTATGGAATAACTATAGAAAAGGTTCCTTTATTTGTATGATCTGTGTTGATTACTGC
Gasteracantha_versi_formo     TAGGGGCTATTAATTTTATTTCAACTATTATTAATATGCGGTTTTATGGGATAACTATAGAAAAGGTCCCTTTATTTGTTTGATCTGTGTTAATTACTGC
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Nephila_pilipes               TGTATTACTTTTACTTTCATTACCAGTATTAGCTGGTGCAATTACAATATTATTAACTGATCGAAATTTTAATACTTCTTTTTTTGACCCTTCTGGGGGT
Araneus_diadematus            TGTTTTACTATTACTTTCTTTACCCGTTTTGGCAGGTGCTATTACTATATTATTAACTGACCGAAATTTTAATACATCATTTTTTGATCCTTCGGGAGGG
Aranoethra_cambridgei         GGTATTATTATTATTATCTTTACCTGTATTAGCTGGAGCAATTACAATATTATTAACTGATCGAAATTTTAATACTTCTTTTTTTGATCCTTCAGGAGGA
Micrathena_gracilis           TATTCTTTTGCTTTTATCTTTGCCAGTATTGGCTGGGGCTATTACTATATTATTAACTGATCGAAATTTTAATACCTCTTTTTTTGACCCTTCAGGGGGA
Micrathena_schreibersi        TGTATTATTATTACTGTCTTTACCTGTATTGGCAGGAGCAATTACTATATTGTTGACAGATCGGAATTTCAATACTTCGTTTTTTGATCCTTCAGGAGGA
Micrathena_triangularispinosa AGTTCTTTTGCTATTATCTTTGCCAGTATTAGCTGGGGCTATTACAATATTATTAACAGATCGAAATTTTAATACTTCTTTTTTTGATCCGTCAGGTGGA
Acrosomoides_acrosomoides     TGTTTTATTACTTTTGTCTCTTCCTGTTTTAGCAGGGGCTATTACAATATTATTAACTGACCGAAATTTTAATACTTCTTTTTTTGACCCTTCTGGAGGA
Actinacantha_globulata        TGTTTTATTACTTTTGTCTCTCCCAGTTTTAGCCGGAGCTATCACGATATTGTTAACAGATCGAAATTTTAATACATCATTTTTTGACCCGAGAGGTGGT
Augusta_glyphica              GGTTCTTTTATTGTTGTCTCTTCCTGTTTTAGCTGGAGCTATTACTATATTATTAACTGATCGGAATTTTAATACTTCTTTTTTTGATCCTTCAGGAGGT
Austracantha_minax            TGTTTTACTTTTATTATCATTACCTGTATTAGCAGGGGCTATTACTATATTATTAACTGATCGAAATTTTAATACCTCTTTCTTTGACCCTTCGGGCGGA
Isoxya_mahafalensis           GGTTTTGTTATTATTATCTCTTCCAGTGTTGGCTGGTGCTATTACAATATTATTAACAGACCGAAATTTTAATACTTCATTTTTTGACCCTTCTGGGGGT
Isoxya_penzoides              AGTTTTGTTATTGTTATCCCTACCAGTGTTGGCTGGTGCTATTACGATATTGTTAACAGATCGAAATTTTAATACTTCATTCTTTGATCCTTCGGGTGGG
Isoxya_sp_nov                 GGTTTTATTATTATTATCTCTTCCAGTATTGGCTGGTGCTATTACAATATTATTAACAGATCGAAATTTTAATACTTCATTTTTTGATCCTTCTGGGGGT
Isoxya_tabulata               AGTCTTGTTATTATTATCTCTACCAGTATTGGCGGGTGCTATTACAATACTATTAACAGATCGAAATTTTAATACTTCATTTTTTGACCCTTCGGGTGGG
Macracantha_arcuata           CGTTTTATTATTATTATCTCTGCCAGTACTAGCTGGAGCTATCACAATATTATTAACAGATCGTAATTTTAATACGTCATTTTTTGACCCAAGAGGTGGT
Macracantha_hasselti          TGTTTTATTGCTTTTATCTCTTCCAGTACTAGCTGGGGCTATCACAATATTATTAACAGATCGAAATTTTAATACATCGTTTTTTGACCCAAGAGGTGGT
Thelacantha_brevispina        TGTATTATTATTATTATCTCTTCCTGTATTAGCAGGAGCAATTACTATATTATTAACTGATCGAAATTTTAATACTTCTTTTTTTGACCCTTCTGGGGGA
Gasteracantha_aciculata       TGTTTTATTACTATTATCTCTTCCAGTATTAGCTGGGGCTATTACTATATTATTAACAGATCGAAATTTTAATACATCTTTTTTTGACCCAAGTGGGGGG
Gasteracantha_cancriformis    TGTATTACTACTTTTATCTCTTCCTGTTTTAGCGGGGGCTATTACTATATTACTAACTGACCGAAATTTTAATACTTCTTTTTTTGATCCTTCTGGGGGG
Gasteracantha_clavatrix       TGTTTTACTACTTTTATCACTTCCAGTGTTAGCTGGGGCTATTACTATATTGTTAACAGATCGAAATTTTAATACTTCATTTTTTGACCCAAGAGGTGGT
Gasteracantha_diadesmia       TGTTTTATTACTTTTATCTTTACCTGTTTTAGCAGGAGCTATTACTATATTGTTAACTGACCGAAATTTTAATACTTCATTT------------------
Gasteracantha_diardi          TGTTCTATTACTTTTATCTTTACCTGTTCTAGCAGGGGCTATTACTATATTATTAACGGATCGAAATTTTAATACTTCTTTTTTTGACCCTTCGGGTGGG
Gasteracantha_doriae          TGTTTTATTACTTTTATCTTTACCTGTTTTAGCAGGGGCTATTACTATGTTGTTAACGGATCGAAATTTTAATACTTCTTTTTTTGACCCTTCAGGGGGA
Gasteracantha_fornicata       TGTATTATTACTTTTATCATTACCTGTTTTAGCAGGAGCTATTACTATATTATTAACTGACCGAAATTTTAACACTTCTTTTTTTGACCCTTCTGGGGGA
Gasteracantha_kuhlii          TGTTTTATTACTTTTATCTCTTCCTGTTTTAGCCGGGGCCATTACTATATTATTAACTGATCGAAATTTTAATACTTCTTTTTTTGATCCTTCTGGGGGA
Gasteracantha_milvoides       TGTATTATTACTTTTATCTCTTCCTGTTTTAGCGGGGGCTATTACTATGTTATTAACTGATCGAAATTTTAATACTTCTTTTTTTGACCCTTCTGGGGGA
Gasteracantha_rhomb_madag     TGTATTATTACTTTTATCTTTACCGGTTTTAGCGGGGGCTATTACTATATTATTGACTGATCGAAATTTCAATACTTCTTTTTTTGACCCTTCAGGGGGG
Gasteracantha_versi_formo     TGTATTATTACTTTTATCTTTACCTGTTTTAGCGGGGGCTATTACTATATTATTGACTGATCGAAATTTTAATACTTCTTTTTTTGACCCTTCAGGGGGT
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Nephila_pilipes               GGGGATCCTATTTTATTTCAACATTTATTTTGATTTTTTGGTCATCCTGAAGTTTATATTTTAATTTTACCAGGATTTGGTATTGTTTCTCATATTATTA
Araneus_diadematus            GGAGATCCTATTTTATTTCAACATTTATTTTGATTTTTTGGTCATCCGGAGGTTTATATTTTAATTTTGCCGGGATTTGGAATTGTCTCTCATATTATTA
Aranoethra_cambridgei         GGGGATCCTATTTTATTTCAACATTTATTT???????????TCATCCTGAGGTATATATTTTAATTTTACCTGGATTTGGAATTGTTTCTCATATTATTA
Micrathena_gracilis           GGGGATCC--------------------------------------------------------------------------------------------
Micrathena_schreibersi        GGAGACCCTATTTTATTTCAACATTTATTTTGATTTTTTGGCCATCCGGAGGTATATATTTTAATTCTCCCAGGTTTTGGGATTGTATCACATATTATTA
Micrathena_triangularispinosa GGGGATCCAATTTTATTTCAGCACTTGTTTTGATTTTTTGGGCATCCTGAAGTATATATTTTAATTCTCCCGGGATTTGGGATTGTTTCGCATATTATTA
Acrosomoides_acrosomoides     GGGGATCCTATTTTGTTTCAGCATTTATTT-----------------------------------------------------------------
Actinacantha_globulata        GGGGACCCTATTTTATTCCAACATTTATTTTGATTTT-----------------------------------------------------------
Augusta_glyphica              GGGGATCCAATTTYATTTCAACATTTGTTTT-----------------------------------------------------------------
Austracantha_minax            GGGGATCCTATTTTATTTCAACATTTATTT???????????TCATCCAGAAGTTTATATTTTGATTTTGCCTGGATTTGGTATAGTATCTCATATTATTA
Isoxya_mahafalensis           GGGGATCCTATTTTGTTTCAACATTTGTTT???????????TCATCCTGAAGTTTATATTTTGATTTTACCTGGATTTGGAATAGTTTCTCATATTATTA
Isoxya_penzoides              GGGGATCCTATTTTGTTTCAACATTTGTTTTGATTTTTTGGTCACCCTGAAGTTTATATTTTGATTTTACCTGGATTTGGGATAGTTTCTCATATTATTA
Isoxya_sp_nov                 GGGGACCCTATTTTGTTTCAACATTTATTTTGATTTTTTGGTCATCCTGAAGTTTATATTTTGATTTTACCTGGATT-----------------------
Isoxya_tabulata               GGGGATCCTATTTTGTTTCAACATTTGTTTTGATTTTTTGGTCACCCTGAGGTTTATATTTTGATTTTACCTGGATTTGGGATAGTTTCACATATTATTA
Macracantha_arcuata           GGTGATCCTATTTTATTTCAACATTTATTTTGATTTTTTGGTCATCCCGAGGTTTATATTTTGATTTTACCTGGATTTGGAATAGTTTCTCATATTATTT
Macracantha_hasselti          GGTGATCCTATTTTATTTCAACATTTATTTTGATTTT-----------------------------------------------------------
Thelacantha_brevispina        GGGGACCCTATTTTATTTCAACATTTGTTTTGATTTT-----------------------------------------------------------
Gasteracantha_aciculata       GGGGATCCAATTTTATTTCAACATTTGTTTTGATTTTTTGGTCATCCTGAAGTTTATATTTTAATTTTACCTGGGTTTGGAATGGTATCCCATATTATTA
Gasteracantha_cancriformis    GGGGACCCTATTTTATTTCAACATTTATTTTGATTTT????TCATCCTGAAGTTTATATTTTAATTTTACCTGGATTCGGGATAGTATCCCATATTATTA
Gasteracantha_clavatrix       GGGGACCCTATTTTATTTCAACATTTGTTTTGGTTTTTTGGTCATCCTGAAGTTTACATTTTAATTTTACCTGGGTTTGGTATAGTTTCTCATATTATTA
Gasteracantha_diadesmia       -----------------------------------------------------------------------------------------------
Gasteracantha_diardi          GGGGATCCTATTTTATTCCAACATTTGTTTTGATTTTTTGGTCATCCTGAGGTTTATATTTTAATTTTACCAGGGTTTGGTATAGTCTCTCATATTATTA
Gasteracantha_doriae          GGGGATCCTATTTTATTTCAACATTTATTTTGATTTTTTGGGCATCCT------------------------------------------------
Gasteracantha_fornicata       GGAGACCCTATTTTATTTCAACATTTATTC-------------------------------------------------------------------
Gasteracantha_kuhlii          GGAGACCCTATTTTATTTCAACATTTATTTTGATTTTTTGGGCATCCTGAAGTTTATATTTTAATTTTGCCAGGATTTGGTATAGTTTCTCATATTATTA
Gasteracantha_milvoides       GGAGACCCTATTTTATTTCAACATTTATTTTGATTCTTTGGTCATCCTGAAGTTTACATTTTAATTTTACCTGGATTTGGGATGGTATCTCATATTATCA
Gasteracantha_rhomb_madag     GGGGACCCAATTTTATTTCAACATTTATTTTGATTTTTTGGGCATCCTGAAGTTTATATTTTAATTTTACCAGGGTTTGGGATAGTATCCCATATTATTA
Gasteracantha_versi_formo     GGGGACCCAATTTTATTTCAACATTTATTTTGATTTTTTGGGCACCCTGAAGTCTATATTTTAATTTTACCAGGGTTTGGGATAGTATCTCATATTATTA
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Nephila_pilipes               GAGCTTCTGTA
Araneus_diadematus            GTTCTTCTGTA
Aranoethra_cambridgei         GGTCTTCTGTA
Micrathena_gracilis           -----------
Micrathena_schreibersi        GAGCTTCAGTA
Micrathena_triangularispinosa GGTCTTCTGTT
Acrosomoides_acrosomoides     -----------
Actinacantha_globulata        -----------
Augusta_glyphica              -----------
Austracantha_minax            GGGCTTCTGTT
Isoxya_mahafalensis           GAGCTTCTGTA
Isoxya_penzoides              GGGCTTCTGTA
Isoxya_sp_nov                 -----------
Isoxya_tabulata               GGGCCTCTGTT
Macracantha_arcuata           GTGCTTCCGTA
Macracantha_hasselti          -----------
Thelacantha_brevispina        -----------
Gasteracantha_aciculata       GTGCTTCTGTA
Gasteracantha_cancriformis    GATCTTCTGTT
Gasteracantha_clavatrix       GAGCTTCTGTA
Gasteracantha_diadesmia       -----------
Gasteracantha_diardi          GGTCTTCTGTA
Gasteracantha_doriae          -----------
Gasteracantha_fornicata       -----------
Gasteracantha_kuhlii          GATCTTCTGTA
Gasteracantha_milvoides       GATCTTCTGTT
Gasteracantha_rhomb_madag     GTTCTTC----
Gasteracantha_versi_formo     GATCATCTGTT
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Complete 16S sequence data

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Nephila_pilipes             --------------GTAATTAGTAACACCTGCTCCATGTA-AAATAAATAGCCGCAGAATTTTTACTGTGCTAAGGTAGCATAATCATTAGCCTTTTAAT
Araneus_diadematus          TTCTTACTGCTTTTATTGTAAGTAACACCTGCTCAATGAA-TTTTAAATAGCCGCGGAAATTTAACCGTGCTAAGGTAGCATAATCATTAGCCTCTTAAT
Aranoethra_cambridgei       TTCTTAAAGATTTTATTTTAAGTAACACCTGCTCAATGAG-CTTTAAATAGCCGCAGAAATTTAACTGTGCTAAGGTAGCATAATCATTAGTCCCCTAAT
Micrathena_gracilis         TTCTTATTAAAATTATAATAAGTAACATCTGCTCAATGAA-TATTTAATAGCCGCGGAATTTTAACCGTGCTAAGGTAGCATAATCATTAGCCCTATAAT
Micrathena_schreibersi      -------------------------CATCTGCTCAATGAACTTTTAAATAGCCGCGGAAATTTAACCGTGCTAAGGTAGCATAATCATTAGCCCCTTAAT
Acrosomoides_acrosomoides   TTCTTAATGAAATAATTTTAAGTAATACCTGCTCAATGA--TTTTAAATAGCCGCAGAATTTAACTGTGCTAAGGTAGCATAATCATTAGCCCTTTAAT
Actinacantha_globulata      ------------TAATATTAAGTATCTCCTGCTCAATGAA-CCTTAAATAGCCGCAGAAAATTAACTGTGCTAAGGTAGCATAATCATTAGCCCTTTAAT
Augusta_glyphica            TTCTTACTGAATTAAAAGTAAGTAATATCTGCTCAATGAA-ATTTAAATAGCCGCGGAAACTTAACTGTGCTAAGGTAGCATAATCATTAGCCTTTTAAT
Austracantha_minax          TTCTTAATGAATTTATATTAAGTATTACCTGCTCAATGAA-ATTTAAATAGCCGCAGAAATTTAACTGTGCTAAGGTAGCATAATCATTAGTCCTTTAAT
Isoxya_mahafalensis         TTCTTAATGAAATAATTTTAAGTAATGCCTGCTCAATGAA-TATTAAATAGCCGCAGAAATTTAACTGTGCTAAGGTAGCATAATCATTAACCCTTTAAT
Macracantha_arcuata         -------------TATATTAAGTATCTCCTGCCCAATGAA-ATTTGAAGAGCCGCAGAAAATTAACTGTGCTAAGGTAGCATAATCATTAGCCCTTTAAT
Macracantha_hasselti        ------------TAATATTAAGTATATTCTGCTCAATGAA-CTTTAAATAGCCGCAGAAAATTTACTGTGCTAAGGTAGCATAATCATTAGCCCTTTAAT
Thelacantha_brevispina      CTCTTAACGAAATATTGTTAAGTATCACCTGCTCAATGAATTTTTTAATAGCCGCAGAAATTTAACTGTGCTAAGGTAGCATAATCATTAGCTCTTTAAT
Gasteracantha_aciculata     TTCTTAACGTAATATTGTTAAGTATTACCTGCTCAATGAC-CATTTAATAGCCGCAGAAATTTAACTGTGCTAAGGTAGCATAATCATTAGCTCTTTAAT
Gasteracantha_cancriformis ------------------------------------------------TAGCCGCAGAAATTTAACTGTGCTAAGGTAGCATAATCATTAGCTCTTTAAT
Gasteracantha_diardi        ------------TATTATTAAGTATTACCTGCTCAATGAA-TCTTTAATAGCCGCAGAAATTTAACTGTGCTAAGGTAGCATAATCATTAGCTCTTTAAT
Gasteracantha_kuhlii        ------------TATTGTTAAGTATCACCTGCTCAATGAATTTTTTAATAGCCGCAGAAATTTAACTGTGCTAAGGTAGCATAATCATTAGCTCTTTAAT
Gasteracantha_milvoides     CTCTTAATATT-TCTTATTAAGTATAACCTGCTCAATGACCAATTTAATAGCCGCAGAAATTTAACTGTGCTAAGGTAGCATAATCATTAGCTCTTTAAT
Gasteracantha_rhomb_madag   CTCTTAACGTTATATTGTTAAGTATAGCCTGCTCAATGATCAATTTAATAGCCGCAGAAATTTAACTGTGCTAAGGTAGCATAATCATTAGCTCTTTAAT
Gasteracantha_versi_formo   CTCTTAATGTAATATTGTTAAGTATCACCTGCTCAATGATCAATTTAATAGCCGCAGAAATTTAACTGTGCTAAGGTAGCATAATCATTAGCTCTTTAAT

Nephila_pilipes             TAAAGGCTTGTATGAAAGGTGAAACGTTT---AAATTTCTTTATTTTATTATT--ACCTCCAAAATTCTCTTAA-GAATTAAAAGATTCTTATTAAATAA
Araneus_diadematus          TAGAGGCTTGTATGAACGGTGAAACATTT---CAACTTCTTTATTAATTAATT--TTTTTTAAATTTAAATTAA-GAATAAAAATGTTCTTATAAAAAAA
Aranoethra_cambridgei       TAGGGACTTGTATGAAAGGTGAAACATTT---TAATTTCTTTATTAATATATTTATATTTTTAATTTAAATTAA-AAATAAAAATGTTTTTATAAATAAA
Micrathena_gracilis         TGAGGGCTTGTATGAATGATGAAACATTTCAATAACTTTTTTAATATTTCTAA------TTTAACTTTAATTAA-AAGTTAAAATACTTTTATATTAATA
Micrathena_schreibersi      TAGTGGCTTGTATGAAGGATGAAACATTTGGATATCTTTTTTAATTTTTTTCT------TTTAACTTAAATTAA-GAATAAAAATATTCTTATTTATTTA
Acrosomoides_acrosomoides   TAAGGGCTTGTATGAAAGGTAAAACATTT---CATTTTCTATAACTAATAATT--TTAACTAAAATTAAATTAA-GAATAAAAATGTTCTTATCCATAAA
Actinacantha_globulata      TAAGGGCTCGTATGAATGGATAAACATTT---TGTATACTATAAATTATTTGA-TTATATTAAATTTTGATTAAGGTATAAAAATATAATTATAAAAAAA
Augusta_glyphica            TAAAGGCTCGCATGAAAGATAAAACATTT---TATTTACTATAATTATTTAAT--TATTTTAAACTTTAATTAA-GAATAAAAATATTCTTATATAAAAA
Austracantha_minax          TAAGGGCTCGTATGAATGGTAATACACTT---CATATCCTATAAATTTTAATC---CTTTTAAAATTAAATCAA-GAATAAAAATGTTCTTATTCTAAAA
Isoxya_mahafalensis         TAAGGGCTAGTATGAATGGTAATACAATT---TATTTTCTATAATTTTTAATT---ATTTTTAACTTTAATTAA-GAATAAAAATGTTCTTATTATTAAA
Macracantha_arcuata         TAAGGGCTTGCATGAATGGATAAACATTT---TATATCCTGTAACTAATTTAA-TTATATTGAATTTTAATTAA-ATGTAAAAATACATTTATGCTAAAA
Macracantha_hasselti        TAAGGGCTCGTATGAAAGAATAAACATTT---TATATTCTGTAATTTATTAAT--CTTATCAAATTTTGATTAAAAAATAAAAATATAATTATATTAAAA
Thelacantha_brevispina      TAAGGGCTCGTATGAATGGTTTAACATTT---TATATACTATTATATTTTAAA--TAAATTAAAATTTAATTAA-GAATAAAAATGTTCTTATAATAAAA
Gasteracantha_aciculata     TAAGGGCTCGTATGAATGGTTAAACATTT---TATACACTATAATCCTTTTTA-ATAAATCAAAATTTAATTAA-GAATAAAAATGTTCTTATAATTAAA
Gasteracantha_cancriformis TAAGAGCTCGTATGAAAGGTTTAACATTA---TATATACTATAACTTTTTTAA--TAAATCAAAATTTAACTAA-GAATCAAAATGTTCTTATTATATAA
Gasteracantha_diardi        TAAGGGCTCGTATGAATGGTTAAACATTT---TATACACTATAATTCTTTTAA--TTAATAAAAATTTAACTAA-GAATAAAAATGTTCTTATAACTTAA
Gasteracantha_kuhlii        TAAGGGCTCGTATGAATGGTTTAACATTT---TATATACTATTATATTTTAAA--TAAATTAAAATTTAATTAA-GAATAAAAATGTTCTTATAATAAAA
Gasteracantha_milvoides     TAAGAGCTCGTATGAAAGGTTAAACATTA---TATATACTATAACTTTTTTAA--TAAATCAAAATTTAACTAA-GAATCAAAATGTTCTTATTATGTAA
Gasteracantha_rhomb_madag   TAAGAGCTCGTATGAAAGGTTAAACATTA---TATATTCTGTAATATTTTTAA--TAAATCAAAATTTAACTAA-GAATCAAAATGTTCTTATTTTATAA
Gasteracantha_versi_formo   TAAGAGCTCGTATGAAAGGTTAAACATTT---TATATACTGTAAC-CTTTTAA--TAAATCAAAATTTAACTAA-GAATAAAAATGTTCTTATTATAAAA
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Nephila_pilipes             CCAGACGACAAGACCCTATTGAACTTTACTGAAGTTTAACTGGGGCAGTTAATTAATAATCATTTTA-ATTTATA---TTATTATATAAAT-GACCCAAT
Araneus_diadematus          TTAGACAACAAGACCCTTTTGAACTTCACTTTTGTTTAACTGGGGCAGTTAAAAAATAAAAATCTTT-TTTAAAT---TCTTTTAATAAAG-GAACCAA-
Aranoethra_cambridgei       TTAGACGACAAGACCCTATTGAACTTTACTTTTGTTTAGCTGGGGCAGCTAAAAAATGATTATTTTT-TTAAAAA---AAATAAAATAAAT-GATCCAA-
Micrathena_gracilis         ATAGACGACAAGACCCTAATGAACTTCACTTTAGTTTAGCTGGGGCAGCAAAACAAAAATAATTTTTTTTTAAAT---TTTTTAAATAAAT-GACCCAT-
Micrathena_schreibersi      ATAGACGACAAGACCCTACTGAACTTTACTTTAGTTTCGCTGGGGCAGCGAAATAATAATATTTTTTTTTCTACA---ATATTTACTAAAT-GACCCAT-
Acrosomoides_acrosomoides   ATAGACGATAAGACCCTATTGAACTTTACTTAAGTTTAGCTGGGGCAGCTAAAAAATAATAATCTTA-TTAAATA---TTTTTCTTTAAAC-GACCCAA-
Actinacantha_globulata      AAAGACGACAAGACCCTATTGAACTTTACTTTAGTTCAGCTGGGGCAGCTAGACAAAAATAATTTTA-AACAAAT---TAGTTATATAAAA-GACCCGA-
Augusta_glyphica            AAAGACGACAAGACCCTATTGAACTTTACTAAAGTTTAGCTGGGGTAGCTATAAAAATATTATTTTC-TATAATATGCATATATTTTAGAA-GAACCAA-
Austracantha_minax          ACAGACGATAAGACCCTATTGAACTTAACTTTAGTTTAGCTGGGGCAGCTATAAAAAAATAATCTTA-TTTCATC---TTTTTAAATAAAC-GACCCAA-
Isoxya_mahafalensis         ATAGACGATAAGACCCTATTGAATTTTACTTTTGTTTAGCTGGGGCAGCTAAAAAAAAATAATCTTT-TTTTATT---TTATTAAATAAATAGACCCAA-
Macracantha_arcuata         AAAGACGACAAGACCCTATTGAACTTTACTTTAGTTTAGCTGGGGCAGCTAAATAAAAAAAAATTTTTAAACATTA---TTATTATATAAAA-GAACCAA-
Macracantha_hasselti        AAAGACGACAAGACCCTATTGAACTTTACTTCAGTTTAGCTGGGGCAGCTAAAAAAAAATAATTTTT-AATAATA---TATTTATATAAAA-GACCCAA-
Thelacantha_brevispina      AAAGACGATAAGACCCTATTGAACTTTACTTTAGTTTAACTGGGGCAGTTAACTAATAATAATCTTA-CTTACTA---TTTTTTTATAAAT--ACCCAA-
Gasteracantha_aciculata     ATAGACGATAAGACCCTATTGAACTTTACTTTAGTTTAACTGGGGCAGTTAACTAATAATAATCTTAATTTTTCA---TCATTTCATAAAT--ATCCAA-
Gasteracantha_cancriformis  ATAGACGATAAGACCCTATTGAACTTTACTTTAGTTTAACTGGGGCAGTTAATTAATAATTATCTTA-ATCAACC---TCTTTTTATAAAA--ATCCAA-
Gasteracantha_diardi        ATAGACGATAAGACCCTATTGAACTTTACTTTAGTTTAACTGGGGCAGTTAACTAATAATAATCTTA-ATTTTTA---TAATTTTATAAAT--ACCCAA-
Gasteracantha_kuhlii        AAAGACGATAAGACCCTATTGAACTTTACTTTAGTTTAACTGGGGCAGTTAACTAATAATAATCTTA-CTTACTA---TTTTTTTATAAAT--ACCCAA-
Gasteracantha_milvoides     ATAGACGATAAGACCCTATTGAACTTTACTTTAGTTTAACTGGGGCAGTTAACTAATAATAATCTTA-ATTAACC---TCTTTTTATAAAA--ATCCAA-
Gasteracantha_rhomb_madag   ATAGACGATAAGACCCTATTGAACTTTACTTTAGTTTAACTGGGGCAGTTAACTAATAATAATCTTA-ATAAAAT---TCTTTTTATAAAA--ACCCAA-
Gasteracantha_versi_formo   ATAGACGATAAGACCCTATTGAACTTTACTTTAGTTTAACTGGGGCAGTTAACTAATAATAATCTTA-ATTAACC---TTTTTTTATAAAA--GTCCAA-

Nephila_pilipes             TACCATTGATTACATGAATTAAGTTACCATAGGGATAACAGCGTAATTTTTTTTTTAAAGTTCATATAACAAAAAA--AGATTGCGACCTCGATGTTGAA-
Araneus_diadematus          TATAATTGATTATATGAA-CAAGTTACCATAGGGATAACAGCGTAATATTTTTTTAAAGCTCTTATTAAAAAAAA--TGATTACGACCTCGATGTTGAA-
Aranoethra_cambridgei       TAAAATTGATTAATTGAT-CAAGTTACCATAGGGATAACAGCGTAATATTTTATTAAAGATCTTATTTTCTAAAA--TGATTGCGACCTCGATGTTGAA-
Micrathena_gracilis         TACAATTGATTATTTGTT-CAAGTTACCATAGGGATAACAGCGTAATTAATTTTTGAAGCTCTTATTTAAAAATATTAGATTGCGACCTCGATGTTGAA-
Micrathena_schreibersi      TATAAATGAATATTTGCT-TAAGTTACCATAGGGATAACAGCGTAATTAATTTTTGAAGCTCTTATTTAAAAAAA--TGATTGCGACCTCGATGTTGAA-
Acrosomoides_acrosomoides   ATTGTTTGAGTTTTTGAA-TAAGTTACCATAGGGATAACAGCGTAATATTTTTTTGAAGCTCTTATTTAAAAAAA--AGAKTGCGACCTCGATGTTGAA-
Actinacantha_globulata      CATAGTTGAGTTTATGAA--AAGTTACCATAGGGATAACAGCGTAATTTATTTTTTAAGCTCTTATTATAAGATA--AGATTGCGACCTCGATGTTGAA-
Augusta_glyphica            TATAATTGACTTTTCGAA-AAAGTTACCATAGGGATAACAGCGTAATATTTTTTTGAAGCTCTTATTTTTAAAAA--AGATTGCGACCTCGATGTTGAA-
Austracantha_minax          TAAAATTGAATATATGAA-AAAGTTACCATAGGGATAACAGCGTAATATTTTTTTAAAGTTCTTATTTAAAAAAA--AGATTGCGACCTCGATGTTGAA-
Isoxya_mahafalensis         TATAATTGAGTAAATGAA-AAAATTACCATAGGGATAACAGTGTAATATCTTTCTAAAGCTCTTATTTAAAAAGA--AGATTGCAACCTCGATGTTGAA-
Macracantha_arcuata         TAAAATTGAGTTTATGAA--AAGTTACCATAGGGATAACAGCGTAATTTATTTTTTAAGCTCTTATTACAAAATA--AGATTGCGACCTCGATGTTGAA-
Macracantha_hasselti        TATAATTGAGTATGTGAT--AAGTTACCATAGGGATAACAGCGTAATTTATTTTTTAAGCTCTTATTATAAAATA--AGATTGCGACCTCGATGTTGAA-
Thelacantha_brevispina      TTAATTTGATT---TGAA-TAAGTTACCATAGGGATAACAGCGTAATTTGTTTCTAAAGCTCTTATTTAAAAACA--AGATTGCGACCTCGATGTTGAA-
Gasteracantha_aciculata     TCAATTTGATT--ACGAA-TAAGTTACCATAGGGATAACAGCGTAATTTGTT-------------------------------------------------
Gasteracantha_cancriformis  TCTATTTGAAT--TTGAA-TAAGTTACCATAGGGATAACAGCGTGATTTGTTTCTAAAGTTCTTATTTAAAAACA--AGATTGCGACCTCGATGTTGAA-
Gasteracantha_diardi        TCAATTTGATT--TCGAA-TAAGTTACCATAGGGATAACAGCGTAATTTGTTTCTAAAGCTCTTATTTAAAAACA--AGATTGCGACCTCGATGTTGAA-
Gasteracantha_kuhlii        TTAATTTGATT---TGAA-TAAGTTACCATAGGGATAACAGCGTAATTTGTTTCTAAAGCTCTTATTTAAAAACA--AGATTGCGACCTCGATGTTGAA-
Gasteracantha_milvoides     TCTATTTGAAT--TTGAA-TAAGTTACCATAGGGATAACAGCGTAATTTGTTTCTAAAGTTCTTAT-----------------------------------
Gasteracantha_rhomb_madag   TTTATTTGAAT--TTGAA-TAAGTTACCATAGGGATAACAGCGTAATTTATTTCTAAAGCTCTTATTTAAAAACA--AGATTGCGACCTCGATGTTGAGA
Gasteracantha_versi_formo   TCTATTTGAAT--TTGAA-TAAGTTACCATAGGGATAACAGCGTAATTTGTTTCTAAAGCTCTTATTTAAAAACA--AGATTGCGACCTCGATGTTGAAT
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Nephila_pilipes             TTATTGTTACATATTAAGGCGCAAAAGCTT-ATAATGTGGGTCTGTTCGACCTTTAAAACTTTACGT
Araneus_diadematus          TTAGTGTAACTTTATAG-ATGCAGTAATTT-TAAAAGTAGGTCTGTTCGACCATTAAAACACTACGT
Aranoethra_cambridgei       TTATTGTAACTTTATAA-ATGCAGCAGTTT-TAAAAGTGGGTCTGTTCGACCCTTAATACATTACAT
Micrathena_gracilis         TTCTTGTTACTATTTTA-ACGCAACCGTTA-AATAAGTGGGTCTGTTCGACCCTTAAACCAATACAT
Micrathena_schreibersi      TTATTGTTACTATCTTA-ACGCAACAGTTA-ATAA--------------------------------
Acrosomoides_acrosomoides   TTAGTATTACTTTATTA-ATGCACCTATTA-TTAAAGCGGGTCTGTTCGACCTTTAAAATACTACAT
Actinacantha_globulata      TTAGTGTTTCTTTTTAA-ATGCAACAATTT-TTAAAGTAGGTCTGTTCGACCTTTAAAACATTACA-
Augusta_glyphica            TTAGTGTAACTT-ATTA-ATGCAATAATTA-TTAAAGTAGGTCTGTTCGACCTATAAAACACTACAT
Austracantha_minax          TTAATGTACCTTTATAA-ATGCAGTAATTTATTAAAGTGGGTCTGTTCGACCCTTAAAACATTACAT
Isoxya_mahafalensis         TTAATGTTCCTTTGTAA-ACGCAGTAGTTT-ACAAAGTAGGTCTGTTCGACCTTTAAAACATTACAT
Macracantha_arcuata         TTAGTGTTCCTTTATAG-ATGCAATAATTT-TTAAAGTAGGTCTGTTCGACCTTTAAAACATTACA-
Macracantha_hasselti        TTAGTGTTCCTTTATAG-ATGCAATAATTT-TTAAAGTAGGTCTGTTCGACCTTTAATACATTACA-
Thelacantha_brevispina      TTAATGATCCTTCTTAA-ATGCAACAATTT-ATAAAGTAGGTCTGTTCGACCTTTAAATCATTACAT
Gasteracantha_aciculata     ------------------------------------------------------------------
Gasteracantha_cancriformis  TTAGTGTTCCTTTTTAA-ATGCAATAATTT-TAAAAGTAGGTCTGTTCGACCTTTAAAACATTACA-
Gasteracantha_diardi        TTAATAGTTCTTTTTAA-ATGCAACCATTT-TAAAAGTAGGTCTGTTCGACCATTAAAACATTACA-
Gasteracantha_kuhlii        TTAATGATCCTTCTTAA-ATGCAACAATTT-ATAAAGTAGGTCTGTTCGACCTTTAAATCATTACA-
Gasteracantha_milvoides     ------------------------------------------------------------------
Gasteracantha_rhomb_madag   TTAGTGTAACTTTTTAA-ATGCAACA----------------------------------------
Gasteracantha_versi_formo   TAATGATTCCTTTTTAA-ATGCAACAATTT--AAAAGTA----------------------------
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Matrix of variable CO1 characters

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Nephila_pilipes               ----------GATTATGTGATGTAGTTAAATAATTGTGTAATTATTGAATTATATTTATGTTTTATGGTTTAATGGTTATTTCATTAATATATACTATAT
Araneus_diadematus            ---------------AGTAATATGCCTGATATATTACTTTATATGGGAATTATATTTATGATATATAGGTTAGTAGTTAGTTAATTAATATACTTATTAT
Aranoethra_cambridgei         GCAGCTAATAGATAAAATAATAAAGGAAATTAATTATATAATTTTAGAATTAAAATTATGGTTTATAGTGTAATATTTATTTAATTAATATATATATTAT
Micrathena_gracilis           TCTGCTAGTAGTTTTTACTATAAGATATATAAATCGTATTATATTTATATTGTAATAATTTTATGTAGTTTAATAGTAATTGAATCGATATATATCGTAT
Micrathena_schreibersi        --------------------------TTGTAAACTGCGTAGAGTTTGAACCAGAATTATATTGTATGGGGCAATAAAGGTTAAATTGTTGTGTATATTAT
Micrathena_triangularispinosa ------------------------------------------------------------TGACATGTAGTTTAGTACTTATTTAATTAGTATATGTATTAT
Acrosomoides_acrosomoides     TCAGCTAGTAGACAGTATGGCTTACCTAATATGTTATATGGAATTTGAATTAGAATTATGGTGTGTGATAAAGTAGTTATTTAATTAATATACTTTTTAT
Actinacantha_globulata        TCTGCAAATTGGAAATACAACTTACCTAACATGTCGTGTTGAAATTGAATTAAATTTATGATTTATAGAATAGTACTGAATTAATTGATATATATTATAA
Augusta_glyphica              TCTGCTAATTGTATAAATAATAAATCTAATTTTTTGCTTAATTATTGAGTTAAATAAATTGTATGTAGTTTGGTATGTATTGAATTAACACGTGGTTTAT
Austracantha_minax            TCTGCTAGTAGGTAAAATAGCTTACCAAATATGTTATGTAGGATTTGAATTATATTTATGGTATATAGTTTAATAATTATTTAATTAATATATATTATAT
Isoxya_mahafalensis           GCTGCTAGTAGAGTATATTACTAACCTAACATGTTGTATGGAATTAGAATTAAAGTTATGGTATATAGTGTAATGTTTATTTAATTAGTACTCTCTATAT
Isoxya_penzoides              GCGGCTAGTAGAATATATAACTGGCCTGATATGTTGTATGGGGTTAGAATTATATTTATGGTATATAGTTTAATAAATATTTTGTTAATACTCTCTTTAT
Isoxya_sp_nov                 GCTGCTAGTAGAGTATATTACTGACCTTGCATGTTGTATGGGATTAGAATTAAAGTTGTAGTGTATAGTATAACATTTATTTAATTAGTACTCCCTGTAT
Isoxya_tabulata               GCTGCTGGTGGGGTATATAACTGGCCTTATATGTTGTATGGGATTAGAATTATAATTGTAGTATATAGTTTAATAATTATTTAGTTAATACTCTTTTTAT
Macracantha_arcuata           GTCTGCAATTGAAAATATAACTTGCCCAATACATTATGTTGAATATGAATTATATTTGTTATATGTGGTTTAATATTGAATTGATTAATACATAATATAA
Macracantha_hasselti          TCTGCGAATTGAAAATATAACTTACCTAATATGTCGTGTTGAATTTGAATTAAGTTTATAGTTTATAGACTAATAGTAAATTAACTGATATATGTTATAA
Thelacantha_brevispina        TCAGCAAGTTGATAAGATAATATGCCAGATATGTTGTATAATATAAGTATTAAATTTATGGTGTGTAGTTTAATAGCTATTAAATTAATATATAAATTAT
Gasteracantha_aciculata       TCTGCAAATTGAAGAAATAACTTACCTAGTATGTTATATTGAATTTGAATTAAATCCGTGATGTATAGTTTAATACTGAACCTATTAATATATATCATAA
Gasteracantha_cancriformis    TCAGCAGGTCGTTAAAATAATAAGCCATATATTTTATATGGAATTTGTATTAAAACCATTTTGTATGGATTAGTAAAAGTTTGATTAATGTATATTTTAC
Gasteracantha_clavatrix       -------TGTTGAAGAATAACTCACCTAATACGTCATATCGCAGATGAATTAAATCTACGATCTATAGAATAATACTGAGTTAATTGATGTATACTATAA
Gasteracantha_diadesmia       TCAGCAAGTTGTTAAAGTAATAAACCAGGTATTTCATATGGAGTTTGTATTATATTTATGATATATAGTTTAATAGTTATTTAGTTAATGTATACATTAT
Gasteracantha_diardi          TCAGCAAGTTGTGAAAGTAATAGACCAGGTATTTCATATGGAGTTTGTATTATGTTTATGATATACAGTTTAATAGCTATTTAGTTAATGTATATATTAT
Gasteracantha_doriae          TCAGCAAGTTGTTAAAGTAATAGACCAAGTATTTCATACGGAGTTTGTATTAAATTTATGATATATAGTTTAATAGTTATTTAATTAGTATATACATTAT
Gasteracantha_fornicata       TCAGCAAGTTGTTAAAGTAATGAACCGGCTATTTCATATGGATTTTGTATTAAATTTATAATTCGTAGCTTAGTGATTATTTGATTAATGTATACTTTAT
Gasteracantha_kuhlii          TCAGCAAGTAGATTAAATAATAGACCACATATTTCATATGGGTTCTGTATTATACTTATATTTTATAGATCAATAGATATTTTATTGATGTATATATTAT
Gasteracantha_milvoides       TCAGCAGGTCGTTTAAGTAATAAACCATATATTTTATATGGAATTTGTATTAAAATTATTTTATATGGATTAGCAATAATTTGATTAATGTATACTTCAT
Gasteracantha_rhomb_madag     TCCGCAGGTTGTTTATATAATGGACCTTGTATTTCGTATAGATTTTATATTAAATTTATATTGTATGGATCAGTAAAAGTTTTATTAATGTATACGTTGT
Gasteracantha_versi_formo     TCAGCAGGTTGTTTATGCAGTAAACCGTATATTTCGTGTAGATTTTATATTAGATCTATATTATATGGATTAGTAGAAATTTAATTAATGTATATGTTGT
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Nephila_pilipes               TTAGTTATTATCAATAAATATGAAATGATTATGTTCTTAATGTGAGATATTTTTTTTATTAGTCTTAAGTTTTAATTAGATCATATAAATCTAGAATTTA
Araneus_diadematus            TCTGATGTTTTCAATTGAATTGAGGTGATTTTACGGATAGTGTGAGAAGTTTATTTTGTTAGGCTTTAGTTTTTATATGTTTTTATAAAACAAAAGTTTA
Aranoethra_cambridgei         TCTAATAATTTCAATAAAAAGGTTAAACTTTTAAAGATAAAGTGTGAAATTTATTTTATTAAACTAAATATTCTTTTAATTTTTATAAAACAAAAATTTA
Micrathena_gracilis           AAAAATATTTTCAATTAAGTGGTAATGTTCTTATTCACAAAGTGAGAAATTTTTTTTATTAAGCTTTAGTTTTTTTTAAAATGTTAAAAACAGAGGTTTG
Micrathena_schreibersi        AAAAATATTTTCAGTTAGGATTCGATATTCCCAGTCTTACAGTGGGAAATTTTTTGCTCTATTCCTTAAATTTGATAAGTTTAGTAGGAACTATGGTTTA
Micrathena_triangularispinosa AAAAACATTTTCTATAAGAGTGTGATGCTTTTATTCTTAAAGTGGGATATTTATTTTGTTATACTATAGTTCTTATTAGATTAATAAAAAGAAAGGTATA
Acrosomoides_acrosomoides     TTAAGAGTACTCAGTTAAGATGTAATGTTCTTGCGGATAATTTGAGAGGTTTATTGTATTAAGCTTAAGATTTTATTAAGTTTTATGAAACTAAGATTTA
Actinacantha_globulata        ACTAGAATCATCTATAAGACAGGGATATCTTTACAGATAATTTGGGTATCTTATTTCCTTAGGCTTAAGTTTTATTATATTTTTATGAAACTAGAACTTA
Augusta_glyphica              TTTAATTCATTCTATAAAAGAAAGATAATGTTAAGGGAACATTAATGCCTCTTTTTCTTTATGGATTAGTTTTTTTAAGTTATTTTGAGACAATAGTATA
Austracantha_minax            TCTAGAATTATCAATTGAGGAGAAATGTTTTTATGGTTGAGGCGAGATTTTTTTTTCTTTAGACTTAAGTTTTTTTATAATCTTACAAAAGAAAGGTTTA
Isoxya_mahafalensis           TCTAGAATTATCAATAGAGAAGAAATGTTTTCATGGGTAAGGAGGGATATTTTTTTCTTTGTGCTTTAATTTTTATATAGTTTTATAAGACTAAAGTTTA
Isoxya_penzoides              TCTAGAATTATCAATTAAGAGGAAATGTTCTTATGGGTAAGGCGAGATATTTTTTTCTTTGTACTTTAGTTTTTATATAATTTTATGAAACTAAAGTTTA
Isoxya_sp_nov                 TCTAGAATTGTCAATAGAAAAGAAATGTTCTCATGGGCAAGGTGGGATGTTTTTTTCTTTGTGCTTTGACTTTTACATAGTTTTATAAGACTAAAGTTTA
Isoxya_tabulata               TCTAGCATTATCAATTAGAGGGAAATGTTCTCATGGGTAGGGCGAGATGTTTTTTTCTTTGTGCTTTAGTTTTTATATAGTTTTACAAGACTAAAGTTTA
Macracantha_arcuata           ACTAAAATCATCTACGAAGGGGGAATATTTTTATAGATAAATTGTGTCTTTTATTTCTTTAGACTTGGTTTTTATTATATTTTTATAAAACTAAAATTTA
Macracantha_hasselti          ACTAGAATTATCTATAAAATGGAAGTATTATTAAAGATAGATTGAGTATTTCATTTTACTAGACTTAAATTTTATTATATTTTTATGAAACTAAAATTTA
Thelacantha_brevispina        TCTAGAATTTTCTGTAAAAAGGAAATGGTTTCTTAGTTAATGGGGGCTATTTATTTCTTTATGCTTGAGTTTTCCTATAATTTTATAAATCTAAGGTATA
Gasteracantha_aciculata       ACTAGAATTATCTATAAAACGGAAATATTTTTATAGATAAATTGAGTATTTTATTTTATTAAACTTAAATTTTTTTATAATTTTATAAAACTAAAATTTA
Gasteracantha_cancriformis    ACTAGAATTTAGAATGAGAGGGTTATGGCTATATAGATAGAGCGAGAATTTTATCTTATTAAGCTTAAGTTTTATTATAATTTTATAAAACTAAGGTTTA
Gasteracantha_clavatrix       ACCAGAATTATCAATAGAATAGGAGTATTTTTACAGATAAGTTGGGTGTTTTATTTCTTTAAGCATAAGTTTTATTATATTTTTACAAAACTAGAATTTA
Gasteracantha_diadesmia       ACTAGAATTTAGAATAAAGTGGTTGTGTTCATAAAGATAGTGAGAGTGATTTATTTCTTTATGCTTAAATTTTAGTATAATTTTATAAGACTAAAATTTG
Gasteracantha_diardi          ACTAGAATTTAGAATAAAGTGGTTGTGTCTATAAAGATAGTGAGAGAAATTTATTTTGTCATGCTTAAGTTTTAGTATAATTTTACAAGACTAAAACTTG
Gasteracantha_doriae          ACTAGAATTTAGAATAAAGTGGTTGTGTCAATAAAGATAGTGAGAGTAATTTATTTTATTATGCTTAAGATTTAGTATAGTTTTATGAGACTAAAATTTG
Gasteracantha_fornicata       ACAAGAATTTAGAATAGAATAGTTGTGTTCGCATAGATAGTGTGAGCAATTTATTTCTTTATGCCCAAATCTTTATATAATTTTACGAGACTAAAATTTG
Gasteracantha_kuhlii          ACTAGAATTTAGAATAAATAGGTTGTGTTTATATAGATAGTGAGAGTAGTTTATTTCATTGTGCTTAAGTTTTATTATAATTTTACTAAACTAAAGTTTA
Gasteracantha_milvoides       ACTAGAATTTAGAATAAATGGGTTATGACTTCATAGATAGAGCGAGAACTTCATTTTGTTAGACTTAAGTCTTATTATAATTTTATAAAACTAAAGTTCG
Gasteracantha_rhomb_madag     ACTAGAATTTAGAATAAATGGGTTATGTTCATATAGATAAGGCGGGTAATTTATTTTATTAAGCTTAAGTTTTATTATGGTTTTATAAAACTAAGGTTTA
Gasteracantha_versi_formo     ACTAGAATTTAGGATAGATGGGTTATGGTTATATAGATGGGGCGAGTAATTTACTTTATTAAGCTTAAGTTTTATTATGGTTTTATGAAACTAAGGCTTA
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Nephila_pilipes               AATATGATTTGATACTTACTATAAATATTATAATATATTATTTTTCTTCTGTGTTATATATATTTTATTATAAATTTTTTAAGTTA
Araneus_diadematus            GGTATAATGTGTTACATACTTTACTTGATTTTATATATCATTAATTTTCGAGATTATATATATTTGGTTATGGATATCTTATTTTA
Aranoethra_cambridgei         TATTTGATTGGATATATATATTATATATAATAATATATTATTTTTTTTCAAAGTTATATAT??TTTGATATATATATTTTAGTTTA
Micrathena_gracilis           AATTTTATTTATCTTGCTTATTGAATGTGTTTATATATTATTCTTCTTCAGAGT--------------------------------
Micrathena_schreibersi        CATGTAATATGATATATACGTTATATGAAATTATGTGATGCTTGTTTTCAAAACTATATATATCTGGATACCATTGTAATAAGTAA
Micrathena_triangularispinosa CGTATGATAAGTCTTGCATATTGAATATGTTAATATAATATTTTTTGTCATAGTAATGCGTATGTTAATACCGATGTTGTAGTTTT
Acrosomoides_acrosomoides     AGTCTAACTTGTTATACTTGTCTTTTAAGTTAATATATCATTTTTCTTCTAAGTTGTGTAT--------------------------
Actinacantha_globulata        TATCTAATTTGTTATACTTGTCCATTACATCGATGTAATATTAATCGAGATTGCTACATATAT-------------------------
Augusta_glyphica              GAAATGGTAGGTCTTATGTGTCTTTTATATTTATATATTGTTTTTTTTTCAATGTAATATGT--------------------------
Austracantha_minax            TGTTTAATTTGTTACTTATAATATATAAGTTTATATATTATTCTCCTTCGCAGTTATATAT??TTAATTGTGTATTAATTAGGTTT
Isoxya_mahafalensis           AAATTAATTGGTTGTATATATCTAGTGTTTTAATATAACATTTATCTTCTGTGTTGTATGT??TTTATTGTATATAATTTAAGTTA
Isoxya_penzoides              TACATAATTAGTTGTATGTACCAAGTGTTTTGATGTAATATTTACTTTCGTGGTTGTATGTATTCTATTGTATATGATTTAGGTTA
Isoxya_sp_nov                 AAACTAATTGGTTATATATATCTAATGTTTTAATATAATATTTATTTTCTGTGCTGTATATATTTTATTGTATA------------
Isoxya_tabulata               GATATAATTAGCTGTATATATCAAATGGTTTAACATAATATTTATCTTCGTGGTTGTATGTATTCTGTTGTATATGATATAGGCTT
Macracantha_arcuata           TGTTTAATTCGTTATATATATCGAACATATCAATATAATTTTGATCAAGATTTTTATATATATTTCGTTGTATATAATTTTTGTCA
Macracantha_hasselti          TATTTAATTTGTTATGCTTATCTAACATGTCAATATAATATTAGTCAAGATTTTTATATATAT-------------------------
Thelacantha_brevispina        TGATTGATTTGATATATATATCTTATAAAATTATATATTATTTTTCTTCTGAGCTATATGTAT-------------------------
Gasteracantha_aciculata       TATTTAATTTGTTATACATATCTAATATGTTTATATAATATTATTCAAGTGGGTAATATGTATTTTATTATATGTAGACTATGTTA
Gasteracantha_cancriformis    TATATAATTTGATACACTTATCTTTTAGGTTTATACATCATTTTTTTTCTGGGCTATATATATTTTATTATATACGAACTAATTTT
Gasteracantha_clavatrix       TGTTCAATTTGTTACACTTAACTAGTATGTTTATGTAATATTTATCAAGATTGCTATATGTGTTTTATCATATGTTATTTAAGTTA
Gasteracantha_diadesmia       TATGTAACTTGTTATACTTATTATTTAAATTTATGTATCATTTAT---------------------------------------
Gasteracantha_diardi          TATGTGATTTGTCATACTTATTATTCAAGTTTATATAGTATTTTTCTTCGTGGTTACATGTATTTTGTTATAAGTTACTTAGTTTA
Gasteracantha_doriae          TAGGTAATTTGTTATACTTATTATTTAAGTTTGTGTAGTATTTTTCTTCAGAGTTATATATATGTT-----------------------
Gasteracantha_fornicata       TATTTAATTTGATATACTTAATATTTAAATTTATATATCATCTTTCTTCTGAACTATATAC--------------------------
Gasteracantha_kuhlii          TATATAATTTGTTATACTTATCTTTTACGCTTATATATTATTTTTTTTTCTGAACTATATATATGTTATTATGAATTATTTAATTTA
Gasteracantha_milvoides       TATATAATTTGATATACTTATCTTTTAGGTTTGTATATTATTTTTCTTCTGAACTATATATACTTTATCATATATGGATCAATTTT
Gasteracantha_rhomb_madag     AATGTGATTTGATATACTTATTAGTTAGGTTTATATGTTACTTTTCTTCAGGGCAATATATATGTTATTATAAGTGAACTATTT--
Gasteracantha_versi_formo     TATGTAATTTGATATACTTATTATTTAGGTTTATATGTTATTTTTCTTCAGTGCAATATATATGCTACTATAAGTGAATTAATATT
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Matrix of variable 16S characters

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Nephila_pilipes             --------GTAATACACTCTA-AAAATATTTTGCCTTTAAAGGTTAGTGAAGTT--AAATTTCTATTTTATTATT-ACCTCCAACTCTT-GAATGATTCT
Araneus_diadematus          TCTGCTTTATTGAACACTAAA-TTTATGATACGCCTCTAGAGGTTCGTGAAATT--CAACTTCTATTAATTAATT-TTTTTTATAAATT-GAAATGTTCA
Aranoethra_cambridgei       TAAGATTTATTTAACACTAAG-CTTATAATATGTCCCCAGGGATTAGTGAAATT--TAATTTCTATTAATATATTATATTTTTTAAATT-AAAATGTTTA
Micrathena_gracilis         TTTAAAATATAAAACATTAAA-TATTTGTTACGCCCTAGAGGGTTTATGAAATTCATAACTTTTAATATTTCTAA-----TTTCTAATT-AAGTTACTTA
Micrathena_schreibersi      --------------CATTAAACTTTATGATACGCCCCTAGTGGTTGATGAAATTGGTATCTTTTAATTTTTTTCT-----TTTCAAATT-GAAATATTCT
Acrosomoides_acrosomoides   TATGAAAAATTTAATACTAA--TTTATATTATGCCCTTAAGGGTTAGTAAAATT--CATTTTCAAACTAATAATT-TTAACTAAAAATT-GAAATGTTCC
Actinacantha_globulata      -------AATATATCTCTAAA-CCTATAAAATGCCCTTAAGGGCTTGATAAATT--TGTATACAAAATTATTTGATTATATTATTGATTGGTAATATAAA
Augusta_glyphica            TCTGAATAAAAGAATATTAAA-ATTATGACATGCCTTTAAAGGCCAATAAAATT--TATTTACAAATTATTTAAT-TATTTTACTAATT-GAAATATTCA
Austracantha_minax          TATGAATTATATATTACTAAA-ATTATAATATGTCCTTAAGGGCTTGTAATACT--CATATCCAAAATTTTAATC--CTTTTAAAAATC-GAAATGTTCT
Isoxya_mahafalensis         TATGAAAAATTTAATGCTAAA-TATATAATATACCCTTAAGGGATTGTAATAAT--TATTTTCAAATTTTTAATT--ATTTTTCTAATT-GAAATGTTCT
Macracantha_arcuata         -------TATATATCTCCAAA-ATTGGAAAATGCCCTTAAGGGTCTGATAAATT--TATATCCGAACTAATTTAATTATATTGTTAATT-ATGATACATG
Macracantha_hasselti        -------AATATATATTTAAA-CTTATAAATTGCCCTTAAGGGCTAAATAAATT--TATATTCGAATTTATTAAT-CTTATCATTGATTAAAAATATAAA
Thelacantha_brevispina      CACGAAAATTGTATCACTAAATTTTTTAATATGCTCTTAAGGGCTTGTTTAATT--TATATACATATATTTTAAA-TAAATTAATAATT-GAAATGTTCA
Gasteracantha_aciculata     TACGTAAATTGTATTACTAAC-CATTTAATATGCTCTTAAGGGCTTGTTAAATT--TATACACAAATCCTTTTTAATAAATCAATAATT-GAAATGTTCA
Gasteracantha_cancriformis  --------------------------TAATATGCTCTTAAGAGCTAGTTTAATA--TATATACAAACTTTTTTAA-TAAATCAATAACT-GAACTGTTCT
Gasteracantha_diardi        -------ATTATATTACTAAA-TCTTTAATATGCTCTTAAGGGCTTGTTAAATT--TATACACAAATTCTTTTAA-TTAATAAATAACT-GAAATGTTCA
Gasteracantha_kuhlii        -------ATTGTATCACTAAATTTTTTAATATGCTCTTAAGGGCTTGTTTAATT--TATATACATATATTTTTAAA-TAAATTAATAATT-GAAATGTTCA
Gasteracantha_milvoides     CATATT-CTTATATAACTAACCAATTTAATATGCTCTTAAGAGCTAGTTAAATA--TATATACAAACTTTTTTAA-TAAATCAATAACT-GAACTGTTCT
Gasteracantha_rhomb_madag   CACGTTAATTGTATAGCTAATCAATTTAATATGCTCTTAAGAGCTAGTTAAATA--TATATTCGAATATTTTTAA-TAAATCAATAACT-GAACTGTTCT
Gasteracantha_versi_formo   CATGTAAATTGTATCACTAATCAATTTAATATGCTCTTAAGAGCTAGTTAAATT--TATATACGAAC-CTTTTAA-TAAATCAATAACT-GAAATGTTCT

Nephila_pilipes             AAATACCGCATCTGAATAACTTATTTATCATA-ATTTATATTATATAATACAATACCATTACATAATGCATTTTTATAAACAAAAAGGA-TATTGTTAAT
Araneus_diadematus          AAAAATTACTTCCTTTTAACTTAAATAAAACT-TTTAAATTCTTTAAAGAAAATATAATTATATAACGCAATTTTACTTAAAAAATAGA-TAGTGTAATT
Aranoethra_cambridgei       AATAATTGCATCTTTTTAGCCTAAATGTTATT-TTAAAAAAAAAAAAAATATAATAAAATTAATTATCGCAATTATAATTTTCTAATGGA-TATTGTAATT
Micrathena_gracilis         TTAATATGCAACCTTATAGCCAAACAATAATTTTTTAAATTTTTAAAATACATTACAATTATTTTTCGCATAATTGCTTTAAAATAGGA-TCTTGTTATA
Micrathena_schreibersi      TATTTATGCACCTTTATCGCCGAATTATATTTTTTCTACAATATTACATACATTATAAAAATTTCTTGCATAATTGCTTTAAAAATGGA-TATTGTTATA
Acrosomoides_acrosomoides   CATAAATGTATCTTAATAGCCTAAATATAACA-TTAAATATTTTCTTACACAAATTGTTGTTTTAATGCAATTTTGCTTTAAAAAAGGA-TAGTATTATT
Actinacantha_globulata      AAAAAAAGCATCTTTACAGCCTGACAATAATA-AACAAATTAGTATAAAACGACATAGTGTTATAA-GCATTATTTCTTATAAGTAGGA-TAGTGTTTTT
Augusta_glyphica            TAAAAAAGCATCTAAATAGTCTTAAATTTATC-TATAATAATAATTTGAAAAATATAATCTTTCAAAGCAATTTTGCTTTTTAAAAGGA-TAGTGTAATT
Austracantha_minax          CTAAAACGTATCATTATAGCCTTAAAATAACA-TTTCATCTTTTAAAACACAATAAAATAATATAAAGCAATTTTATTTTAAAAAAGGA-TAATGTACTT
Isoxya_mahafalensis         ATTAAATGTATTTTTTTAGCCTAAAAATAACT-TTTTATTTTATAAAATACAATATAATGAAATAAAATAATCTCACTTTAAAAGAGAA-TAATGTTCTT
Macracantha_arcuata         CTAAAAAGCATCTTTATAGCCTAATAAAAATTAAACATTATTATATAAAAAAATAAAATGTTATAA-GCATTATTTCTTACAAATAGGA-TAGTGTTCTT
Macracantha_hasselti        TTAAAAAGCATCTTCATAGCCTAAAAATAATT-AATAATATATTATAAAACAATATAATGATGTAT-GCATTATTTCTTATAAATAGGA-TAGTGTTCTT
Thelacantha_brevispina      ATAAAAAGTATCTTTATAACTTACTTATAACA-CTTACTATTTTTTAATACAATTAATTT---TAATGCATTGTCACTTTAAAACAGGA-TAATGATCTT
Gasteracantha_aciculata     ATTAAATGTATCTTTATAACTTACTTATAACAATTTTTCATCATTCAATATAATCAATTT--ACAATGCATTG----------------------------
Gasteracantha_cancriformis  ATATAATGTATCTTTATAACTTATTTATTACA-ATCAACCTCTTTTAAAATAATCTATTA--TTAATGCGTTGTCATTTTAAAACAGGA-TAGTGTTCTT
Gasteracantha_diardi        ACTTAATGTATCTTTATAACTTACTTATAACA-ATTTTTATAATTTAATACAATCAATTT--TCAATGCATTGTCACTTTAAAACAGGA-TAATAGTTTT
Gasteracantha_kuhlii        ATAAAAAGTATCTTTATAACTTACTTATAACA-CTTACTATTTTTTAATACAATTAATTT---TAATGCATTGTCACTTTAAAACAGGA-TAATGATCTT
Gasteracantha_milvoides     ATGTAATGTATCTTTATAACTTACTTATAACA-ATTAACCTCTTTTAAAATAATCTATTA--TTAATGCATTGTCATT----------------------
Gasteracantha_rhomb_madag   TTATAATGTATCTTTATAACTTACTTATAACA-ATAAAATTCTTTTAAAACAATTTATTA--TTAATGCATTATCACTTTAAAACAGGGATAGTGTAATT
Gasteracantha_versi_formo   ATAAAATGTATCTTTATAACTTACTTATAACA-ATTAACCTTTTTTAAAGTAATCTATTA--TTAATGCATTGTCACTTTAAAACAGGATAATGATTCTT
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Nephila_pilipes             ATAAGCAAAGCTATATTGTTAACTTG
Araneus_diadematus          TAAGATGTAATTTAAATAATAACACG
Aranoethra_cambridgei       TAAAATGCAGTTTAAATGCTTACATA
Micrathena_gracilis         TTTAACACCGTAAATATGCTACCAAA
Micrathena_schreibersi      TCTAACACAGTAATA-----------
Acrosomoides_acrosomoides   TATAATCCTATATTAACGTTAATACA
Actinacantha_globulata      TTAAATACAATTTTAATATTAACATA
Augusta_glyphica            -ATAATATAATATTAATATAAACACA
Austracantha_minax          TAAAATGTAATTTTAATGCTAACATA
Isoxya_mahafalensis         TGAAACGTAGTTACAATATTAACATA
Macracantha_arcuata         TAAGATATAATTTTAATATTAACATA
Macracantha_hasselti        TAAGATATAATTTTAATATTTACATA
Thelacantha_brevispina      CTAAATACAATTATAATATTATCATA
Gasteracantha_aciculata     --------------------------
Gasteracantha_cancriformis  TTAAATATAATTTAAATATTAACATA
Gasteracantha_diardi        TTAAATACCATTTAAATAATAACATA
Gasteracantha_kuhlii        CTAAATACAATTATAATATTATCATA
Gasteracantha_milvoides     --------------------------
Gasteracantha_rhomb_madag   TTAAATACA-----------------
Gasteracantha_versi_formo   TTAAATACAATT-AAATA--------
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Appendix 4: Micro-CT scanned images trial methods

A4.1 Trial of micro-CT scan

Female specimens (no type material) were prepared for micro-CT scanning by transferring them from their housing into uniform size glass or plastic tubes filled with 70% or 96% ethanol, depending on which collection they were being loaned from. The tubes were all 16mm diameter, barring some glass tubes for species which required larger tubes due to the size of the specimen. There was no staining of the specimens with an iodine-based contrast agent (Gignac *et al*, 2016) prior to the scans. The decision to not stain the specimens was for several reasons. It was hypothesized that, given the structures under examination are external to the spider, the staining would not be necessary as iodine is typically used to make internal soft-tissue structures more visible (Gignac *et al*., 2016). The iodine solutions are a purple colour, and it was unclear if the staining process would damage the colour of the preserved specimens permanently.

Additionally, exposing specimens to an incorrect concentration of iodine solution could potentially lead to destruction of the specimens themselves (Gignac *et al*., 2016); most of which were loaned from museum collections. So, for these reasons and the fact that due to the procedure needing to be performed by a professional outside the project due to Covid-19 restrictions, it was decided not to include this extra step of staining. Prior to scanning, the ethanol was drained out of each tube and specimens were left to air dry for approximately 20 minutes. This ultimately proved to be not enough time for some of the specimens, which might have required drying for longer or by hand (see next section).

The specimens, within numbered tubes, were transported to the University of Bristol where they were handed to the team there to perform the scans. Due to Covid-19 protocols at the time it was impossible for any external visitors to enter the university buildings, so the scans were performed by Dr Liz Martin-Silverstone in the Department of Earth Sciences.

Approximately 1200-1500 slice images were taken for each scan (a random example of a slice is given in Figure A4.2). The exact number of slices varied due to the size and positioning of the tubes. To save time, the smaller tubes containing specimens were grouped together in threes for the scans, stabilised on a dense foam board. This process was repeated for each of the samples and the images stored on an external hard drive.

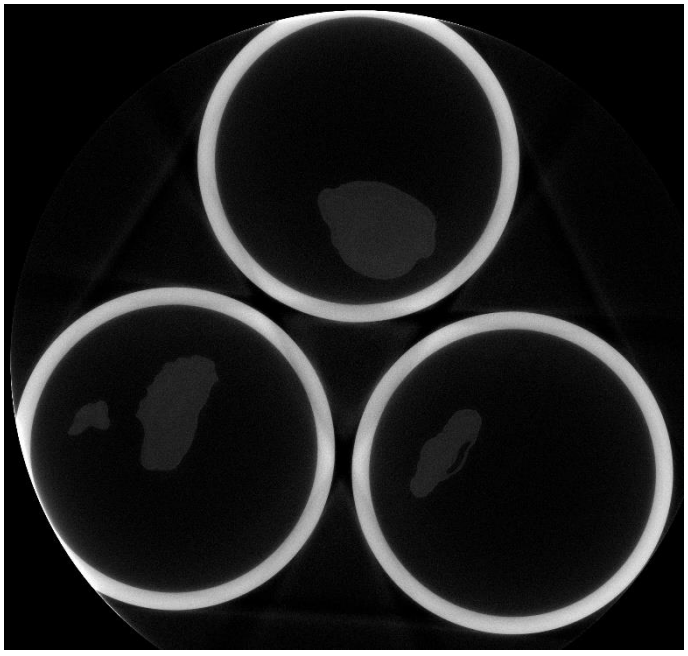

Figure A4.1 Example slice from Micro-CT scan process showing 3 tubes (white colour) containing various specimens (light grey colour).

A4.2 Rendering the 3D models

The Micro-CT scan images were stacked in ImageJ (Rasband, 2018) before cropping, for example from 3 tubes (Figure A4.1) to 1 tube (Figures A4.2 and A4.3). Images with too much background noise, for example at the base of the tube (Figure A4.3), were also cut at this stage to make the rendering process simpler and not obscure the spider as much. These new files were then stored and compressed into one file, ready for rendering into 3D models.

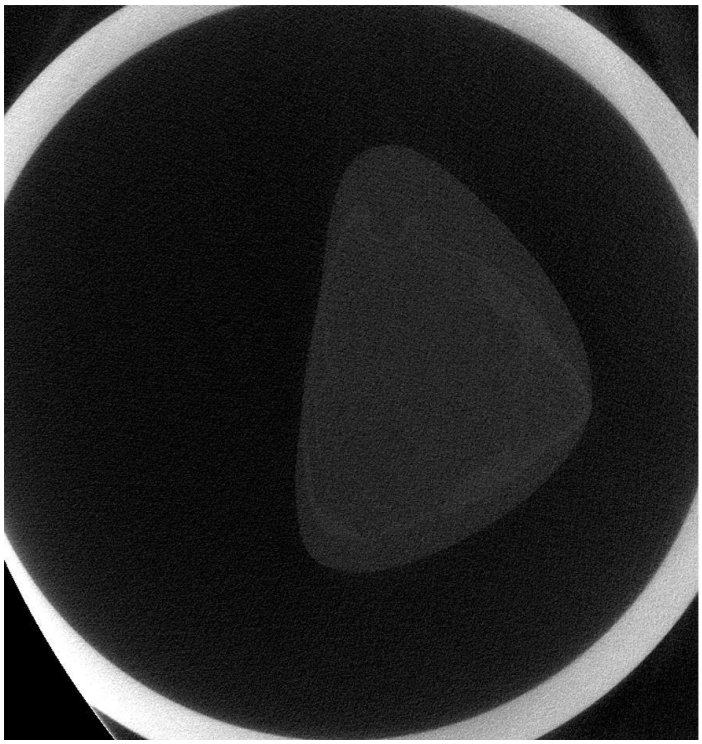

Figure A4.2 Example of cropped slice from Micro-CT scan process showing 1 tube (white colour) containing a specimen (light grey colour) and light spots of disturbance (light grey near edges of tube).

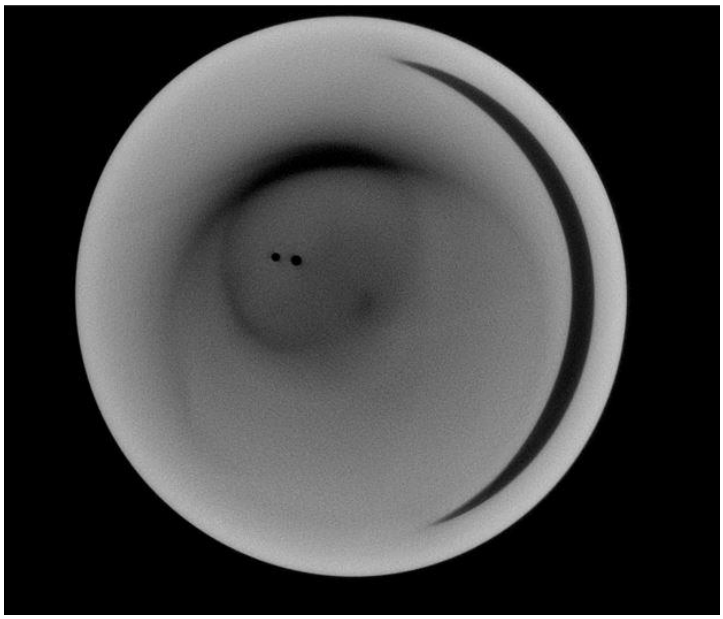

Figure A4.3 Example of cropped slice from Micro-CT scan process showing 1 tube (white colour) with noise from the base (white colour with darker rings).

The process of rendering the images into a 3D model was undertaken in Amira 5 (Stalling, Westerhoff and Hege, 2005). A brief overview of the rendering process is described here. The combined slice images from ImageJ are imported and the spider is then manually selected and cut out from each of the slices. The threshold limits, to separate the glass or plastic tube from the spider were manipulated here and a shortcut method of selecting the same shape across multiple slices also assisted in making this process faster. Once only the spider is selected the 3D model was then generated, the results of the successful trial scan of *Gasteracantha doriae* can be seen in the following section. The exact specifications, for example the threshold of separating materials and the cropping, for each scan is not provided here as they are specific for each specimen.

Unfortunately, the process of scanning did not always respond well to the glass surface of the tubes. The threshold of the glass and the specimens was too similar in the scans, and was often connected by unevaporated ethanol, so a lot of noise and confusion was created as the

glass appears to connect to the specimen via the ethanol. This meant the thresholds would not automatically remove the tube from the spider's body. Most specimens were in glass tubes but when plastic tubes were used the scans were clearer even when ethanol was not fully evaporated.

For future scanning, plastic tubes and thorough drying is recommended prior to scanning. The possibility of staining with iodine should also be taken into consideration. During the scanning process the X-ray filament broke and this caused additional disturbance in the scans making the rendering process more difficult. The threshold limit to distinguish the spider from the edges of the tube and the background becomes harder when the scans are not clear, this is also the case when a specimen is not completely dry of ethanol.

Once it was discovered that most of the trial scans were unusable the rendering process was abandoned as the time required to generate the rendered images was deemed too high if it was unclear if they would ultimately be usable. 3 species, 2 of which were scanned in plastic tubes, did yield rendered images that could be used in a morphometric analysis: *Gasteracantha cancriformis*, *G. doriae* and *G. sturi* (Doleschall, 1857). However, to avoid using two separate types of data, photographs of real specimens and rendered 3D models, the latter were not used in the analysis in Chapter 3.

Appendix 5: Publications during thesis

A5.1 New faunistic records of *Gasteracantha* Sundevall, 1833 and *Macracantha* Simon, 1864 species (Araneae: Araneidae) from Vietnam