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Phylogenetic relationships of *Actinacantha* Simon, *Gasteracantha* Sundevall, *Macracantha* Hasselt and *Thelacantha* Simon spiny orb-weavers (Araneae: Araneidae) in Peninsular Malaysia

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Abstract. Spiny orb-weavers of the genera *Actinacantha*, *Gasteracantha*, *Macracantha*, and *Thelacantha* are spiders with rigid abdomens and prominent spines that are mostly endemic to Asia. The taxonomy and phylogeny of these spiders are poorly studied due to their intraspecific morphological variations, the rarity of male specimens in collections and the lack of distinctive morphological characters in older descriptions. Therefore, this study has employed for the first time, a multigene approach using three mitochondrial (CO1, CO2 and 16S) and two nuclear-encoded (H3A and 18S) molecular markers to aid in the identification and phylogenetic reconstruction of these spiny orb-weavers collected from Peninsular Malaysia. The genus *Gasteracantha* as presently circumscribed is not monophyletic, with *G. hasselti* C. L. Koch, 1837 forming a lineage with *A. globulata* (Walckenaer, 1841) and *M. arcuata* (Fabricius, 1793). Our results also revealed two different morphotypes of *G. diardi* which are morphologically different from *G. mengei* (Keyserling, 1864). The epigynal structures of *G. diardi* (Lucas, 1835), *G. hasselti*, *G. kuhli* C. L. Koch, 1837, *G. mengei*, *M. arcuata*, and *T. brevispina* (Doleschall, 1857) are illustrated. The morphology of *G. diardi* and *G. mengei* is detailed for the first time.

Key words. Gasteracanthinae, orb-weaving, spider, molecular, phylogeny, Malaysia

INTRODUCTION

Spiny orb-weavers of the genera *Actinacantha* Simon, *Gasteracantha* Sundevall, *Macracantha* Hasselt and *Thelacantha* Simon are spiders of the subfamily Gasteracanthinae mostly found in Asia which have evolved rigid and generally broad abdomens armed with up to three pairs of prominent spines (Yin et al., 1997; Koh & Leong, 2013). Female spiny orb-weavers often display striking abdominal colors and distinct sigilla patterns associated with prey attraction (Craig & Ebert, 1994; Hauber, 2002). On the basis of morphological data, Scharff & Coddington (1997) showed that the Old-World spiny orb-weavers constituted a monophyletic subfamily Gasteracanthinae comprising of *Gastroxva* Benoit, *Augusta* O. Pickard-Cambridge, *Macracantha*, *Isoxya* Simon, *Austracantha* Dahl, *Togacantha* Dahl, *Gasteracantha*, and *Aetrocantha* Karsch. This subfamily is a separate lineage from Micratheninae which consisted of New-World spiny orb-weavers (Scharff & Coddington, 1997). The taxonomic characters of the genus *Gasteracantha* were first outlined by Sundevall (1833) and later refined by Dahl (1914), Pickard-Cambridge (1879), Chrysanthus (1971), and others who had access to collections rich in specimens from the Malay Archipelago. These taxonomic studies emphasised more on female individuals due to their relative abundance in collections. Dahl (1914) categorised all his *Gasteracantha* specimens into 16 subgenera according to their abdominal structure, sigilla patterns, spine position and arrangement. These subgenera include *Macracantha*, *Tatacantha*, *Actinacantha*, *Hypsacantha*, *Austracantha*, *Togacantha*, *Afracantha*, *Isoxya*, *Acrosomoides*, *Atelacantha*, *Collacantha*, *Thelacantha*, *Anchacantha*, *Pachypleuracantha*, *Gasteracantha*, and *Tetracantha*, some of which have since then been erected to genus level. In terms of morphological characters, it was established by Pickard-Cambridge (1879), Dahl (1914) and subsequent authors that the dimensions of the abdomen, spines, and sigilla were not reliable in the identification of the females of a species as they were highly variable within the same species. On the other hand, spine position and arrangement, sigilla number and pattern, abdominal coloration and epigyne structure were shown to be useful in species discrimination (Pickard-Cambridge, 1879; Dahl, 1914; Chrysanthus, 1959, 1971; present study).

Currently, the genera *Actinacantha*, *Macracantha*, and *Thelacantha* are each monotypic, whereas *Gasteracantha* is comprised of 101 taxonomically accepted species (World Spider Catalog, 2018). Nine species of spiny orb-weavers have been recorded from Peninsular Malaysia, viz., *Actinacantha*

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globulata (Walckenaer), *Gasteracantha cuspidata* C. L. Koch, *G. diardi* (Lucas), *G. doriae* Simon, *G. hasselti* C. L. Koch, *G. kuhli* C. L. Koch, *G. mengei* Keyserling, *Macracantha arcuata* (Fabricius), and *Thelacantha brevispina* (Doleschall) (Walckenaer, 1841; Koh, 1989; Murphy & Murphy, 2000; Norma-Rashid & Li, 2009; Koh & Leong, 2013). Four other species were recorded only from East Malaysia, namely *G. crucigera* Bradley, *G. diadesmia* Thorell, *G. frontata* Blackwall, and *G. sturi* (Doleschall) (Doleschall, 1857; Pickard-Cambridge, 1879; Murphy & Murphy, 2000; Yong & Ono, 2009). However, the taxonomy of these spiny orb-weavers remain poorly understood due to the unavailability of male specimens in collections, poorly preserved type specimens, intraspecific morphological plasticity and the lack of distinctive morphological characters for a number of species in older descriptions (Pickard-Cambridge, 1879; Dahl, 1914; Chrysanthus, 1959; Tikader, 1982). While the taxonomy of these Old-World spiny orb-weavers will be better elucidated with increased sampling and documentation, the accurate identification of especially male and juvenile individuals would likely benefit from the employment of molecular approaches. Recent efforts in molecular phylogeny, phylogenomics and comparative transcriptomics have provided valuable insights into the evolution of spiders, particularly orb-weavers (Garrison et al., 2016; Fernández et al., 2018; Kallal et al., 2018). In the case of Gasteracanthinae and Micratheninae, results from these studies have revealed each subfamily to be monophyletic but distantly related. Although genetic data is available for *G. hasselti* and *M. arcuata*, the DNA of other species are yet to be sequenced at present. The inclusion of other species especially those of *Actinacantha* and *Thelacantha* would provide better insights into the phylogenetic relationships of these taxonomically challenging groups of spiders. Therefore, the present study aims to (i) elucidate the phylogeny of *Actinacantha*, *Gasteracantha*, *Macracantha*, and *Thelacantha* spiny orb-weavers collected from Peninsular Malaysia using a multigene approach; and (ii) illustrate the morphology of these spiny orb-weavers with emphasis on their reproductive structures.

MATERIAL AND METHODS

Ethics statement. The research on *Actinacantha*, *Gasteracantha*, *Macracantha*, and *Thelacantha* spiny orb-weavers is approved by the Universiti Tunku Abdul Rahman (UTAR) Scientific and Ethical Review Committee (SERC) and was carried out in accordance to the guidelines stated by SERC.

Specimen collection. Specimens were collected using a sweeping net. Two to four legs of each specimen were preserved in 95% ethanol for DNA extraction, while the specimen itself was preserved in 75% ethanol. Voucher specimens were deposited in the arachnid collection of UTAR Insect Repository (UIR), Kampar, Perak, Malaysia.

Sample identification. The specimens were examined using a Motic SMZ-161 stereomicroscope and a Labo AXL microscope. Specimens were identified based on published literature (Walckenaer, 1841; Doleschall, 1857; Simon, 1864; Bradley, 1877; Dahl, 1914; Chrysanthus, 1971; Jäger & Praxaysombath, 2009; Yin et al., 2012; Koh & Leong, 2013; Sen et al., 2015; Roy et al., 2017; Williams, 2017). Only the holotype material of *G. mengei* (British Museum of Natural History, BMNH) was examined. All measurements were taken using Motic Images Plus 2.0 following calibration. Photos were then taken separately using a mounted Samsung Galaxy S7 Edge to be digitally traced using a Wacom Bamboo CTL471 tablet. Leg and palp measurements are shown as: total length (femur, patella, tibia, metatarsus, tarsus). Abbreviations of morphological structures: AL= abdominal length; ALE= anterior lateral eye; AME= anterior median eye; AS= average length of anterior spines; AW= abdominal width; CL= cephalothoracic length; CW= cephalothoracic width; MS= average length of median spines; PLE= posterior lateral eye; PME= posterior median eye; PS= average length of posterior spines; TL= total length.

DNA extraction and amplification. The ethanol-preserved legs were ground using sterile micropestles followed by DNA extraction using an i-genomic G-Spin Total DNA Extraction Kit (iNtRON Biotechnology, Korea).

Five molecular markers were used in the present study, namely the cytochrome *c* oxidase subunit I (CO1), cytochrome *c* oxidase subunit II (CO2), 16S ribosomal RNA (16S), histone H3 family A (H3A), and 18S ribosomal RNA (18S). The three mitochondrial (CO1, CO2, and 16S) and two nuclear-encoded (H3A and 18S) genetic markers were amplified using Polymerase Chain Reaction (PCR) based on primers and PCR parameters described by Colgan et al. (1998), Folmer et al. (1994), Simon et al. (1994), Giribet et al. (1996), Whiting et al. (1997), Su et al. (2011), Cheng & Kuntner (2014, 2015) and Tan et al. (2016). Primer details are summarized in Supplementary Table S1. PCR was performed using i-Taq™ Plus DNA Polymerase Kits (iNtRON Biotechnology, Korea) and a FlexCycler2 PCR thermal cycler (Analytik Jena, Germany). The amplified PCR products were electrophoresed and visualised using a 1.0% agarose gel stained with ethidium bromide before specimens were purified and sent for sequencing (Genomics Bioscience & Technology, Taiwan).

Phylogenetic analyses. DNA sequences were assembled into contigs using ChromasPro V1.5 (Technelysium Pty Ltd). Relevant DNA sequences of Gasteracanthinae spiny orb-weavers were downloaded from GenBank for comparison (Table 1). *Argiope* and *Cyrtophora* of the family Araneidae were included as the outgroup taxa for the rooting of all phylogenetic trees based on (i) their relatedness to Gasteracanthinae within the 'argiopoid clade' as reported by Scharff & Coddington (1997); and (ii) the complete set of DNA sequences for each genetic marker used in the present study. However, additional DNA sequences of *Micrathena* and *Neoscona* were used as the outgroup for the CO1 dataset in line with recent reports that they are closely related to Gasteracanthinae (Garrison et al., 2016; Kallal et al., 2018). Each individual marker and their concatenated datasets (i.e.,

Table 1. Samples used in this study.

No.	Species	Voucher	Sex (M/F)	Details	Genetic marker				
					16S	CO1	CO2	H3A	18S
1	*Actinacantha globulata*	AGLO1	Sub-F	Semenyih, Selangor, Malaysia	MG670140	MG670112	MG670127	MG670170	MG670155
2	*Gasteracantha diardi*	GDIA1	F	Gunung Jerai, Kedah, Malaysia	KU055746	KU055841	KU056030	MG670171	MG670156
3	*Gasteracantha diardi*	GDIA2	Sub-F	Gunung Jerai, Kedah, Malaysia	MG670141	MG670113	MG670128	MG670172	MG670157
4	*Gasteracantha diardi*	GDIA3	F	Penang Hill, Penang, Malaysia	MG670142	MG670114	MG670129	MG670173	MG670158
5	*Gasteracantha diardi*	GDIA4	F	Penang Hill, Penang, Malaysia	MG670143	MG670115	MG670130	MG670174	MG670159
6	*Gasteracantha diardi* white morph	GDIA5	Sub-F	Kuala Sepetang, Perak, Malaysia	MG670144	MG670116	MG670131	MG670175	MG670160
7	*Gasteracantha diardi* yellow morph	GDIA6	F	Kuala Sepetang, Perak, Malaysia	MG670145	MG670117	MG670132	MG670176	MG670161
8	*Gasteracantha kuhli*	GKUH1	F	Kepong, Selangor, Malaysia	KU055747	KU055842	KU056031	KU055935	MG015860
9	*Gasteracantha kuhli*	GKUH2	F	Semenyih, Selangor, Malaysia	MG670146	MG670118	MG670133	MG670177	MG670162
10	*Gasteracantha kuhli*	GKUH3	F	Sungai Relau, Pahang, Malaysia	MG670147	MG670119	–	MG670178	MG670163
11	*Gasteracantha hasselti*	GHAS1	F	Sungai Sedim, Kedah, Malaysia	MG670148	MG670120	MG670134	MG670179	–
12	*Gasteracantha mengei*	GMEN1	F	Sungai Sedim, Kedah, Malaysia	–	–	–	–	–
13	*Macracantha arcuata*	MARC1	F	Semenyih, Selangor, Malaysia	MG670149	MG670121	MG670135	MG670180	MG670164
14	*Macracantha arcuata*	MARC2	F	Semenyih, Selangor, Malaysia	MG670150	MG670122	MG670136	MG670181	MG670165
15	*Macracantha arcuata*	MARC3	F	Sungai Sedim, Kedah, Malaysia	MG670151	MG670123	MG670137	MG670182	MG670166
16	*Thelacantha brevispina*	TBRE1	F	Penang Hill, Penang, Malaysia	MG670152	MG670124	MG670138	MG670183	MG670167
17	*Thelacantha brevispina*	TBRE2	F	Penang Hill, Penang, Malaysia	MG670153	MG670125	MG670139	MG670184	MG670168
18	*Thelacantha brevispina*	TBRE3	F	Gunung Jerai, Kedah, Malaysia	MG670154	MG670126	–	–	MG670169
19	*Argiope dang*	ADAN1	F	Perak, Malaysia	KU055667	KU055762	KU055951	KU055857	MG015850
20	*Cyrtophora moluccensis*	CMOC1	F	Penang, Malaysia	KU055745	KU055840	KU056029	KU055934	MG015859

Table 1. (Continued)

GenBank Sequences								
No.	**Species**	**Locality**	**Reference**	**Genetic Marker**				
				16S	**CO1**	**CO2**	**H3A**	**18S**
1	*Gasteracantha kuhli*	Taiwan	Cheng & Kuntner 2014	–	KJ957963	KJ958063	KJ958011	–
2	*Gasteracantha kuhli*	Japan	Tanikawa et al., 2006	–	DQ518416	–	–	–
3	*Gasteracantha kuhli*	Japan	Tanikawa et al., 2014	–	AB910447	–	–	–
4	*Gasteracantha kuhli*	–	–	JN816531	JN817164	–	–	–
5	*Gasteracantha cancriformis*	Hispanolia	McHugh et al., 2014	–	KJ157212	–	–	–
6	*Gasteracantha cancriformis*	Hispanolia	McHugh et al., 2014	–	KJ157213	–	–	–
7	*Gasteracantha cancriformis*	Bahamas	Kartzinel & Pringle, 2015	KP253913	KP253807	–	–	–
8	*Gasteracantha cancriformis*	Puerto Rico	McHugh et al., 2014	KJ156990	KJ157214	–	–	–
9	*Gasteracantha cancriformis*	–	Alvarez-Padilla et al., 2009	EU003256	EU003287	–	EU003319	–
10	*Gasteracantha cancriformis*	–	Agnarsson & Blackledge, 2009	FJ525354	FJ525321	–	FJ525340	–
11	*Thelacantha brevispina*	French Polynesia	Ramage et al., 2017	–	KX055036	–	–	–
12	*Thelacantha brevispina*	French Polynesia	Ramage et al., 2017	–	KX055037	–	–	–
13	*Thelacantha brevispina*	Japan	Yamada et al., 2015	–	AB969824	–	–	–
14	*Micrathena miles*	South America	Magalhaes et al., 2017	–	KX687317	–	–	–
15	*Micrathena embira*	South America	Magalhaes et al., 2017	–	KX687311	–	–	–
16	*Neoscona nautica*	–	Wang et al., 2017	–	JN817147	–	–	–

"–" dashes indicate non-applicable/available data

all combinations between the 16S, CO1, CO2, H3A and 18S markers) were analysed to identify the datasets that best represent the phylogeny of *Actinacantha*, *Gasteracantha*, *Macracantha*, and *Thelacantha* spiny orb-weavers in (i) Peninsular Malaysia and (ii) across the globe. The alignment of DNA sequences was performed using default MUSCLE parameters at https://www.ebi.ac.uk/Tools/msa/muscle/ (Edgar, 2004), while the editing and truncation of multiple sequence alignment blocks were carried out using Bioedit v7.2.5 (Hall, 1999). File conversions were carried out using either ALTER (Glez-Pena et al., 2010) or Geneious v11.0.5 (https://www.geneious.com).

Phylogenetic trees were computed based on Maximum Likelihood (ML), Maximum Parsimony (MP), and Bayesian (BI) algorithms. Partition Finder v2.1.1 (Lanfear et al, 2018) was used to determine the optimal partitioning schemes and substitution models of each dataset using the Akaike information criterion (AIC). ML trees were generated using the IQ TREE web server (Nguyen et al., 2015; Trifinopoulos et al., 2016) at http://iqtree.cibiv.univie.ac.at/. Clade support was computed based on 1,000 Ultrafast bootstrap replicates.

Due to the small number of taxa, parsimony analyses were carried out in TNT (Goloboff & Catalano, 2016) using the implicit enumeration option in tree search over 2,000 standard bootstrap replicates. Larger datasets with more than 30 taxa were analysed using the traditional search option with multiple addition sequences followed by branch swapping, also resampled over 2,000 standard bootstrap replicates (Goloboff et al., 2008).

BI analysis was performed using Mr. Bayes v3.2.6 (Ronquist et al., 2012) based on two runs of 20,000,000 generations from four Markov independent chains with trees sampled every 100th generation. Markov chain Monte Carlo (MCMC) runs were assessed for convergence of log likelihood values using Tracer v1.5 (http://196 tree.-bio.ed.ac.uk/software/tracer/), and the first 25 % of samples were discarded as burn-in. The resulting phylogenetic trees were visualised and edited using Figtree v1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

Uncorrected pairwise genetic distances were estimated using PAUP 4.0b10 (Swofford, 2003) to determine the intra- and interspecific genetic difference between specimens. This was performed for the (i) 16S+CO1+CO2+H3A dataset with the most complete set of DNA data; (ii) CO1 dataset which included DNA sequences of Gasteracanthinae spiny orb-weavers from elsewhere in the world; and (iii) other genetic markers, i.e., CO2, 16S, H3A and 18S.

RESULTS

Field sampling and sample identification. A total of 18 female specimens of the genera *Actinacantha*, *Gasteracantha*, *Macracantha* and *Thelacantha* were collected from Peninsular Malaysia (Fig. 1, Table 1). A total of seven species of spiny orb-weavers were identified, namely *A. globulata, G. diardi*, *G. hasselti*, *G. kuhli*, *G. mengei*, *M. arcuata*, and *T. brevispina*. The taxonomy and natural history of these spiders are summarized at the end of the manuscript. The morphology and epigynal structures of *G. diardi* and *G. mengei* are detailed for the first time.

Molecular analyses. The details of each DNA sequenced specimen are summarised in Table 1 together with their associated GenBank accession numbers. DNA amplification was not successful for the poorly preserved *Gasteracantha mengei* (GMEN1) specimen. Several DNA regions also failed to amplify for *G. hasselti* GHAS1, *G. kuhli* GKUH3, and *Thelacantha brevispina* TBRE3. The final MSA lengths of the 16S, CO1, CO2, H3A, and 18S genetic markers were 449 bp, 664 bp, 792 bp, 354 bp, and 917 bp, respectively; whereas the number of parsimoniously informative sites for these markers were 138, 184, 234, 50, and 42, respectively. The data partitions and substitution models used for the phylogenetic inference of these genetic markers are summarised in Table 2. Generally, a relatively lower resolution was observed for phylogenetic trees computed using individual genetic markers compared to concatenated ones. As similar tree topologies were observed for the 16S+CO1+CO2, CO1+CO2+H3A, 16S+CO1+CO2+H3A, and 16S+CO1+CO2+H3A+18S trees, only the 16S+CO1+CO2+H3A tree (Fig. 3) is discussed here as it has the most complete set of DNA sequences of specimens collected in the present study (see Table 1). Additionally, the CO1 tree (Fig. 4) which incorporated a larger number of DNA sequences from GenBank is illustrated to infer the phylogeny of these spiny orb-weavers on a larger and wider scale. The 16S+CO1+CO2+H3A+18S tree is shown as supplementary Fig. S1 while all other phylogenetic trees are available upon request. The monophyly of the Old-World spiny orb-weavers, i.e., *Actinacantha*, *Gasteracantha*, *Macracantha*, and *Thelacantha*, was supported in all concatenated datasets using *Argiope* and *Cyrtophora* as outgroup taxa. This was also observed in the CO1 tree which included the DNA sequences of New-World spiny orb-weaver *Micrathena* and other closely-related genera.

Based on the 16S+CO1+CO2+H3A tree (Fig. 3), the genus *Gasteracantha* was inferred to be paraphyletic with respect to *Actinacantha*, *Macracantha* and *Thelacantha* (ML = 100%; MP = 100%; BI = 1.00). *G. diardi* (taxa A1 and A2) and *G. kuhli* (species A3) were sister to one another and collectively constituted a monophyletic clade A (ML = 100%; MP = 95%; BI = 1.00). On the other hand, *T. brevispina* (ML = 100%; MP = 100%; BI = 1.00) was sister to clade A. *A. globulata* and *M. arcuata* were sister to each other with low support (ML = 67%; MP = – %; BI = 0.55), together forming a clade C with *G. hasselti* (ML = 100%; MP = 100%; BI = 1.00).

The topology of the CO1 tree (Fig. 4) was similar to that of 16S+CO1+CO2+H3A but showed generally lower nodal supports. Additional DNA sequences of *Gasteracantha kuhli, G. cancriformis* (Linnaeus) and *Thelacantha brevispina* were also included. *G. cancriformis* sequences formed a monophyletic group A4 (ML = 100%; MP = 99%; BI = 1.00). Two *G. kuhli* specimens from Japan (DQ518416 and

Table 2. Summary of data partitions and substitution models used for phylogenetic inference based on the 16S, CO1, CO2, H3A and 18S markers.

DNA Marker	Data Subset	Substitution Model (IQ-TREE)	Substitution Model (MrBayes)
16S	–	TIM2+F+G4	GTR + gamma
CO1	Position 1	TN+F+I	GTR + gamma
	Position 2	F81+F	F81 + I
	Position 3	TN+F+G4	GTR + gamma
CO2	Position 1	F81+F+I	GTR + I
	Position 2	TN+F+G4	GTR + gamma
	Position 3	HKY+F+I	HKY + I
H3A	Position 1	K2P	JC + I
	Position 2	TIM2e+G4	GTR + gamma
	Position 3	K2P	GTR
18S	–	K2P+I	GTR + I

Table 3. Uncorrected "p" distance (%) between selected DNA sequences of *Actinacantha*, *Gasteracantha*, *Macracantha*, and *Thelacantha* based on the 16S+CO1+CO2+H3A dataset.

	Sample	1	2	3	4	5	6	7	8
1	*Actinacantha globulata* AGLO1								
2	*Gasteracantha hasselti* GHAS1	8.10							
3	*Macracantha arcuata* MARC1	9.54	8.66						
4	*Gasteracantha diardi* GDIA1	15.13	14.60	15.72					
5	*Gasteracantha diardi* GDIA3	15.19	14.71	15.82	0.63				
6	*Gasteracantha diardi* GDIA5	14.47	14.04	15.24	3.58	3.75			
7	*Gasteracantha kuhli* GKUH1	14.61	13.77	14.53	8.02	8.27	7.95		
8	*Thelacantha brevispina* TBRE1	16.01	14.86	16.24	12.22	12.37	12.42	11.89	

AB910447) and a conspecific sequence of unknown origin (JN817164) appeared to be genetically distinct from the *G. kuhli* from Malaysia and Taiwan (KJ957963). Genetic variation was also observed between conspecific individuals collected from different states in Peninsular Malaysia.

The uncorrected "p" distance (%) between selected samples of *Actinacantha*, *Gasteracantha*, *Macracantha* and *Thelacantha* based on the 16S+CO1+CO2+H3A dataset (Table 3) will be used as the main reference unless stated otherwise. The genetic distance matrix of the 16S+CO1+CO2+H3A+18S dataset is provided as Supplementary Data (Table S2) due to incomplete DNA data (of especially *G. hasselti*). According to Table 3, a genetic distance of 13.77−14.86% was recorded between *G. hasselti* and other *Gasteracantha* spp. collected in Peninsular Malaysia. *G. hasselti* and *A. globulata* showed a genetic difference of 8.10% while *G. hasselti* and *M. arcuata* differed by 8.66%. *Gasteracantha diardi* GDIA1, GDIA3, and GDIA5 differed genetically by 3.58−3.75%. On the other hand, individuals of *G. diardi* demonstrated a genetic difference of 7.95−8.27% in comparison with *G. kuhli*. The genetic distance matrix of the CO1 dataset is summarised in Supplementary Data (Table S3) to enable genetic comparisons with specimens from other locations. The "p" distance matrices of the 16S and CO2 markers are tabulated as Tables S4 and S5, respectively, while those of the H3A and 18S markers are available upon request.

DISCUSSION

Molecular results. Genetic information often serves as an additional and independent set of data to support the identification or evolutionary relationships of many organisms including spiders (Scharff & Coddington, 1997; Bond et al., 2014; Cheng & Kuntner, 2014, 2015; Fernández et al., 2014; Magalhaes et al., 2017). As DNA can be extracted and amplified from juvenile spiders, spiderlings or even damaged or incomplete appendages, the employment of such molecular approach is especially valuable in the study and identification of spiders with confusing morphological characteristics such as the spiny orb-weavers.

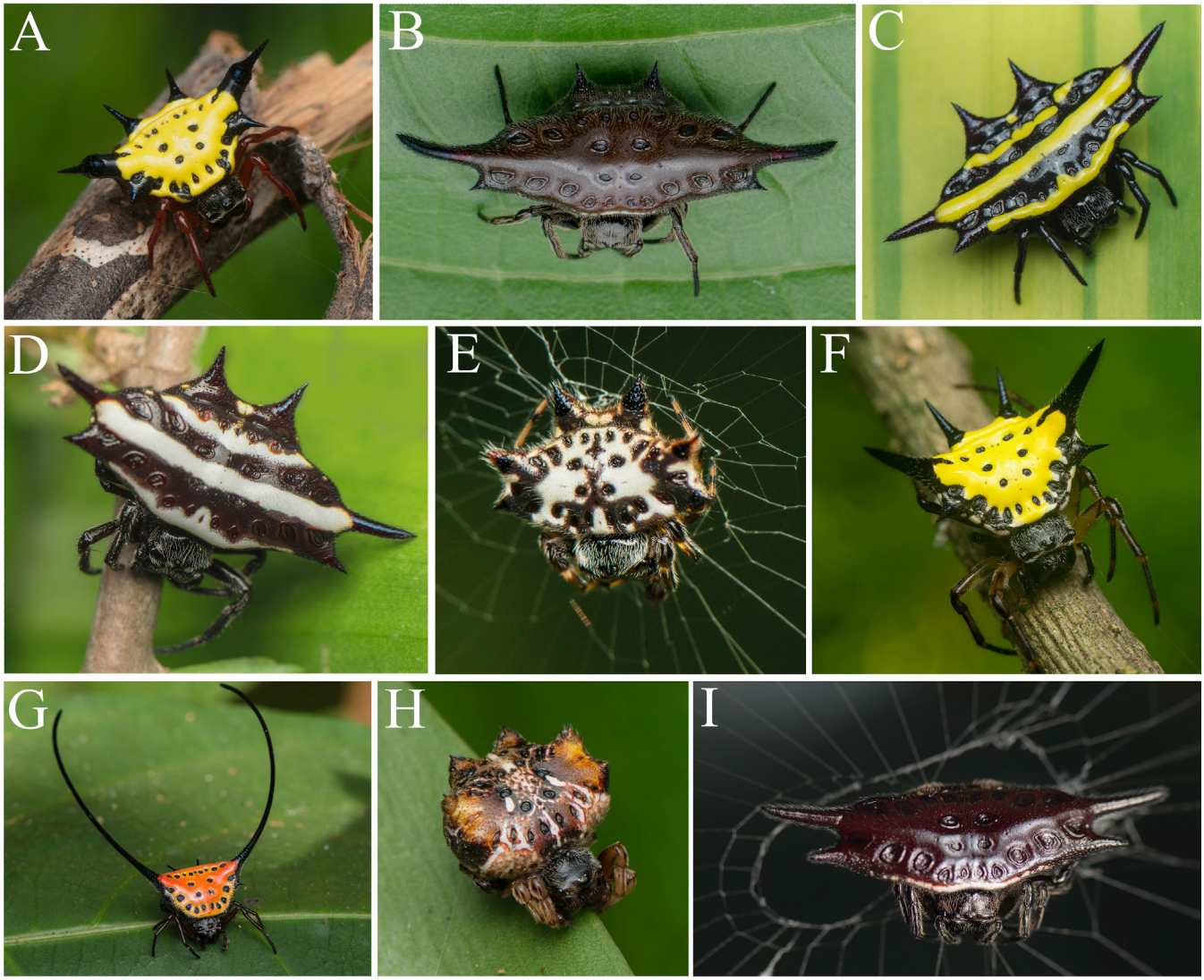

Fig. 1. General habitus. A, *Actinacantha globulata* (Walckenaer, 1841), sub-female; B, *Gasteracantha diardi* (Lucas, 1835), female; C, *G. diardi* yellow morph, female; D, *G. diardi* white morph, sub-female; E, *G. kuhli*, female; F, *Gasteracantha hasselti* C. L. Koch, 1837, female; G, *Macracantha arcuata* (Fabricius, 1793), female; H, *Thelacantha brevispina* (Doleschall, 1857), female; I, *G. mengei* Keyserling, 1864, female. Photos by Tan Ji.

The present study infers for the first time the phylogenetic relationships of *Actinacantha*, *Gasteracantha*, *Macracantha*, and *Thelacantha* spiny orb-weavers. Preliminary phylogenetic results were generated based on 18 spiny orb-weaver specimens collected from Peninsular Malaysia, as well as the limited number of genetic data available in GenBank. The monophyly of these members of the subfamily Gasteracanthinae is well supported based on the analyses of three mitochondrial (16S, CO1, and CO2) and two nuclear-encoded markers (H3A and 18S). Our genetic support is in line with their unique morphology otherwise absent in other members of Araneidae. Notably, these spiny orb-weavers differ from Neotropical *Micrathena* which displays generally longer than wide prosoma and abdomen, as well as longer abdominal appendages (Levi, 1978). The genus *Micrathena* was also shown to be phylogenetically distant from *Gasteracantha* and *Macracantha* by Garrison et al. (2016) based on a phylogenomic approach. The phylogenetic relationships of two other genera of the Australian spiny orb-weavers *Austracantha* and *Poecilopachys* Simon were not studied although similarities in gross morphology (Emerit, 1974; Davies, 1988) may indicate their relatedness to *Gasteracantha* and *Thelacantha*.

The paraphyletic nature of *Gasteracantha hasselti* with respect to other members of the genus *Gasteracantha* was apparent in all concatenated trees (ML ≥ 98.5%; MP ≥ 98%; BI = 1.00). Genetic data also indicated that *A. globulata* and *G. hasselti* are more closely related to *M. arcuata* ("p" = 8.66−9.54%) than the other *Gasteracantha* spp. ("p" = 13.77−14.86%) used in this study. This is supported by the morphological similarities observed between the three species (Figs. 1A, F, G), i.e., dorsally trapezoidal abdomen with unique sigilla patterns, striking abdominal coloration of yellow to orange to red, prominent or well-developed median spines and reddish-brown leg coloration. All three species also lack a central bulge on the venter of the abdomen between the genital groove and spinnerets- a character generally observed in *Gasteracantha* and *Thelacantha*. These observations supported the assignment of *G. crucigera* (now *A. globulata*) and *G. hasselti* under the subgenus of *Actinacantha* by Simon (1864) and Dahl (1914), although

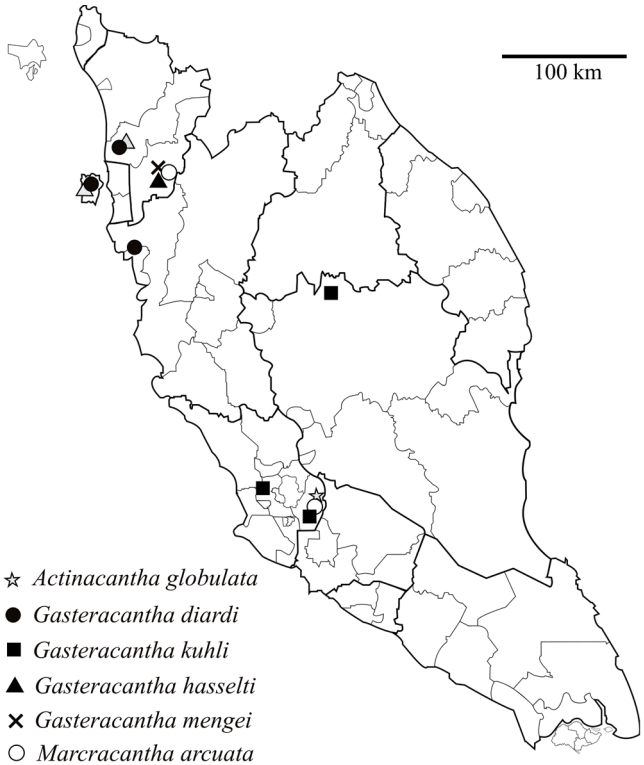

Fig. 2. Locations of specimens collected in this study.

these two taxa do not form a monophyletic clade either based on the phylogenetic tree (Fig. 3). This may be due to the simple taxonomic key back then which may not be sufficient to clearly define a genus or species. For example, *M. arcuata* was considered as a monotypic species by Dahl (1914) based mainly on the unique, long and hardened spines in adult females. The lack of well-defined morphological characters again emphasized on the need for a taxonomic revision when more specimens becomes available. On the other hand, *A. globulata* was inferred to be sister to *M. arcuata* based on the 16S+CO1+CO2+H3A dataset albeit with low support (ML = 67%; MP = – %; BI = 0.55). This may suggest that the bulbous bases of the middle spines of *A. globulata* and the extended middle abdominal spines of *M. arcuata* evolved from a common ancestor with "less-derived" spinal features that was closely related to *G. hasselti*. Similar observations of spine elongation were reported to have occurred multiple times based on the phylogeny of *Micrathena* (Magalhães & Santos, 2012).

The erection of *Thelacantha* by Benoit (1964) using *Gasteracantha brevispina* as type species was supported based on the 16S+CO1+CO2+H3A data (Fig. 3) which inferred this species as sister to all *Gasteracantha* spp. excluding *G. hasselti*. Two different genotypes were observed in *G. diardi* specimens: phenotype A1 specimens without

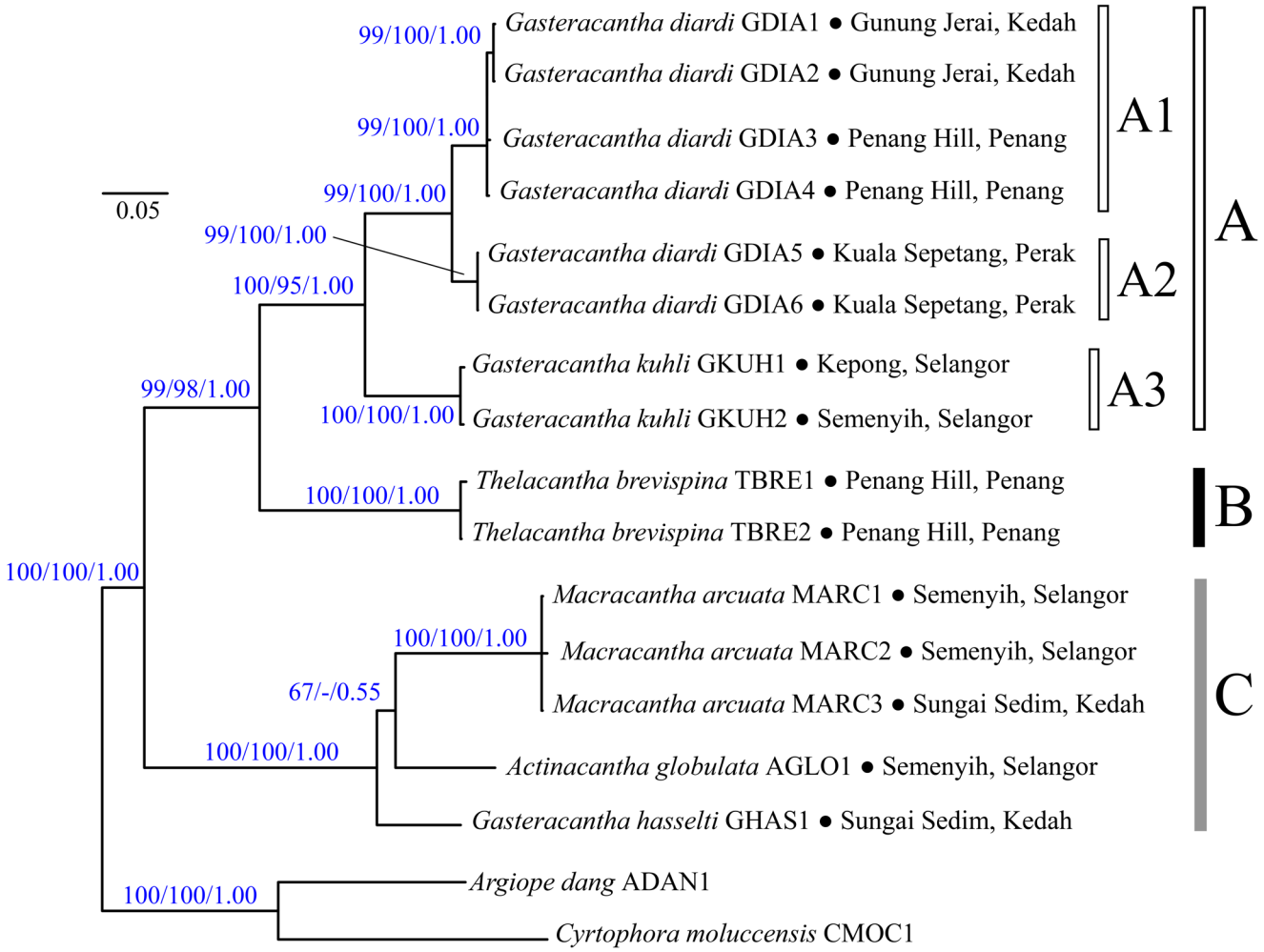

Fig. 3. Bayesian tree based on the concatenated 16S+CO1+CO2+H3A dataset of samples used in the present study. Numeric values at nodes arranged in an order of Ultrafast ML bootstrap support/MP bootstrap support/Bayesian posterior probability.

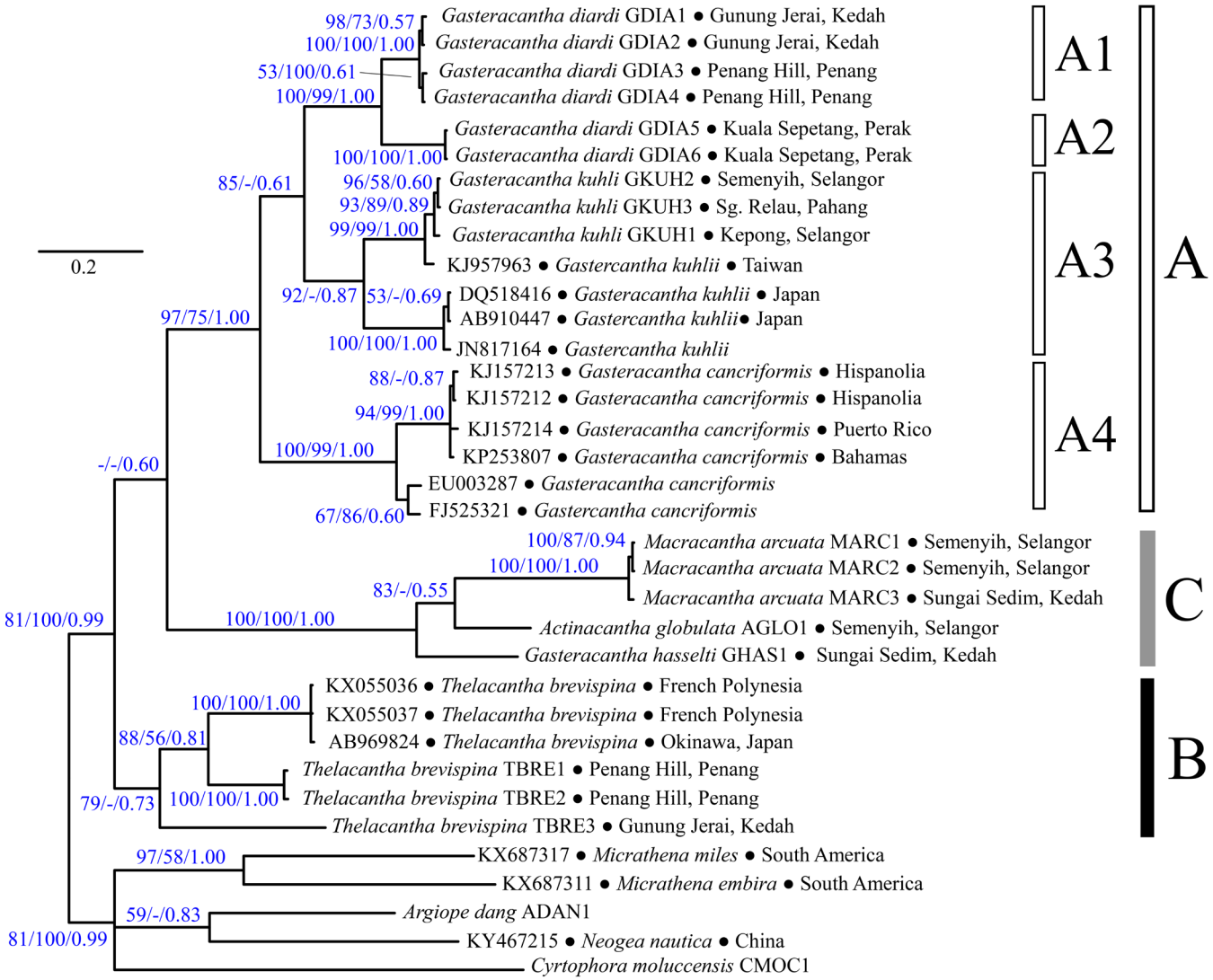

Fig. 4. Bayesian tree based on the CO1 dataset of *Actinacantha*, *Gasteracantha*, *Macracantha* and *Thelacantha*. Numeric values at nodes arranged in an order of Ultrafast ML bootstrap support/MP bootstrap support/Bayesian posterior probability.

abdominal colorations and phenotype A2 specimens with horizontal abdominal stripes of yellow or white. However, the 16S+CO1+CO2+H3A genetic distance of 3.58–3.75% between these two genotypes was lower than the interspecific distance of 7.95% between *G. diardi* and *G. kuhli* (Table 3). A similar trend was observed in the CO1 distance matrix (Supplementary Table S3), with *G. diardi* sequences displaying an intraspecific distance of less than 5.6%, and an interspecific difference of 8–12%. These values fell within the intra- and interspecific CO1 ranges of 0–6.7% and 4–22% reported for Araneidae (Čandek & Kuntner, 2015). In addition to similar abdominal spine arrangement and epigyne structure, it is suggested that *G. diardi* phenotypes A1 and A2 are likely still conspecific. The taxonomic status of *G. diardi* will become evident when additional adult specimens are examined.

Genetic analyses were also carried out with the inclusion of GenBank DNA sequences of *G. cancriformis* and *G. kuhli*, currently the only two species that were previously sequenced. *G. cancriformis* endemic to America and Hawaii was shown to be genetically distinct from *G. diardi* (CO1 "p" = 11.0–11.8%) and *G. kuhli* (CO1 "p" = 8.4–8.9%) from Asia. On the other hand, the CO1 tree (Fig. 4) inferred a *G. kuhli* specimen from Taiwan (KJ957963) to be closely related to those from Malaysia (CO1 "p" = 0.82%). However, they were genetically distinct from the *G.* "*kuhli*" specimens (DQ518416, AB910447 and JN817164) from a sister clade. The relatively high genetic difference between these two groups suggest that the latter specimens may be another closely related species which requires careful morphological examination. Interestingly, individuals of the same species within Peninsular Malaysia appear to be genetically different according to locality for *G. diardi*, *M. arcuata*, and *Thelacantha brevispina* (Fig. 4). However, the relatively scarce number of specimens sequenced herein hindered the identification of any definitive distributive patterns for spiny orb-weavers in Peninsular Malaysia. Nevertheless, it was observed that the populations of these spiders are often localised (pers. observ.). Future studies on genetic diversity and phylogeography will shed light on the gene flow and distribution patterns of spiny orb-weavers in this region.

In conclusion, the present study has established the genetic records for *A. globulata, G. diardi*, *G. hasselti*, *G. kuhli*, *M. arcuata*, and *T. brevispina* which proved useful in their

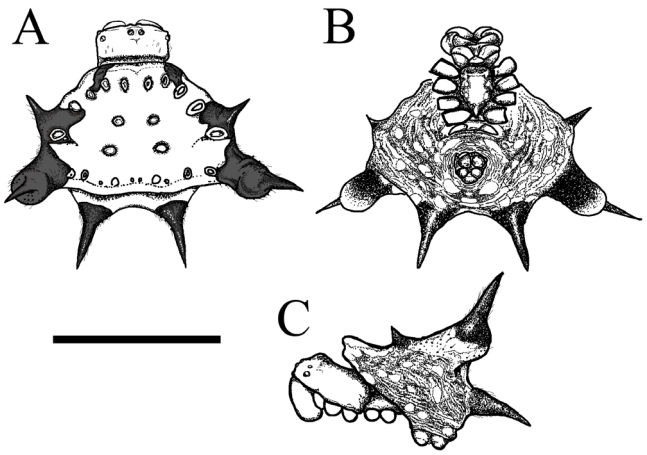

Fig. 5. Sub-female *Actinacantha globulata* (Walckenaer, 1841). A, Dorsal habitus; B, Ventral habitus; C, Lateral habitus. Scale = 5 mm.

identification. Although molecular data have provided valuable insights into the phylogeny of these spiders, the present results are limited by taxon sampling and geographical coverage, especially so for the diverse genus of *Gasteracantha*. Genetic data supported the earlier revisions of *Thelacantha* and *Macracantha*, and also revealed the paraphyletic nature of *Gasteracantha*. The epigynal structures of several species of spiny orb-weavers including *G. diardi* and *G. mengei* were also illustrated for future reference. The inclusion of other *Gasteracantha* spp. (i.e., *G. clavigera*, *G. cuspidata*, *G. doriae*, *G. diadesmia*, *G. frontata*, and *G. sturi*) as well as a wider sampling approach would greatly contribute to a better documentation and taxonomic resolution of Gasteracanthinae spiny orb-weavers in Southeast Asia.

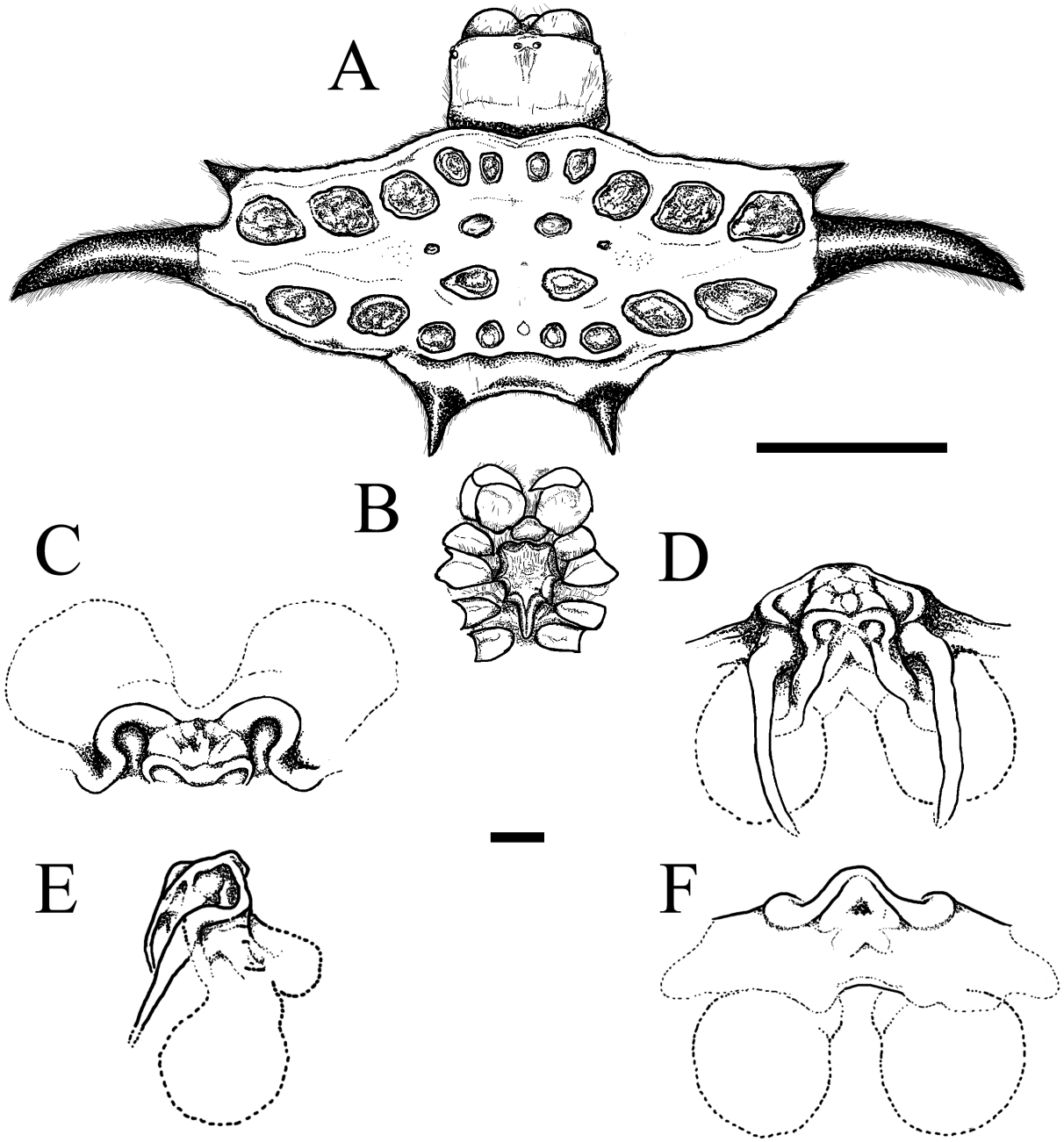

Fig. 6. *Gasteracantha diardi* (Lucas, 1835). A, Dorsal habitus; B−E, Epigynal structure. A, Ventral view; B, Posterior view; C, Lateral view; D, Dorsal view; E, Anterior view. Scale A = 5 mm; B−E = 100 µm. S = Spermatheca.

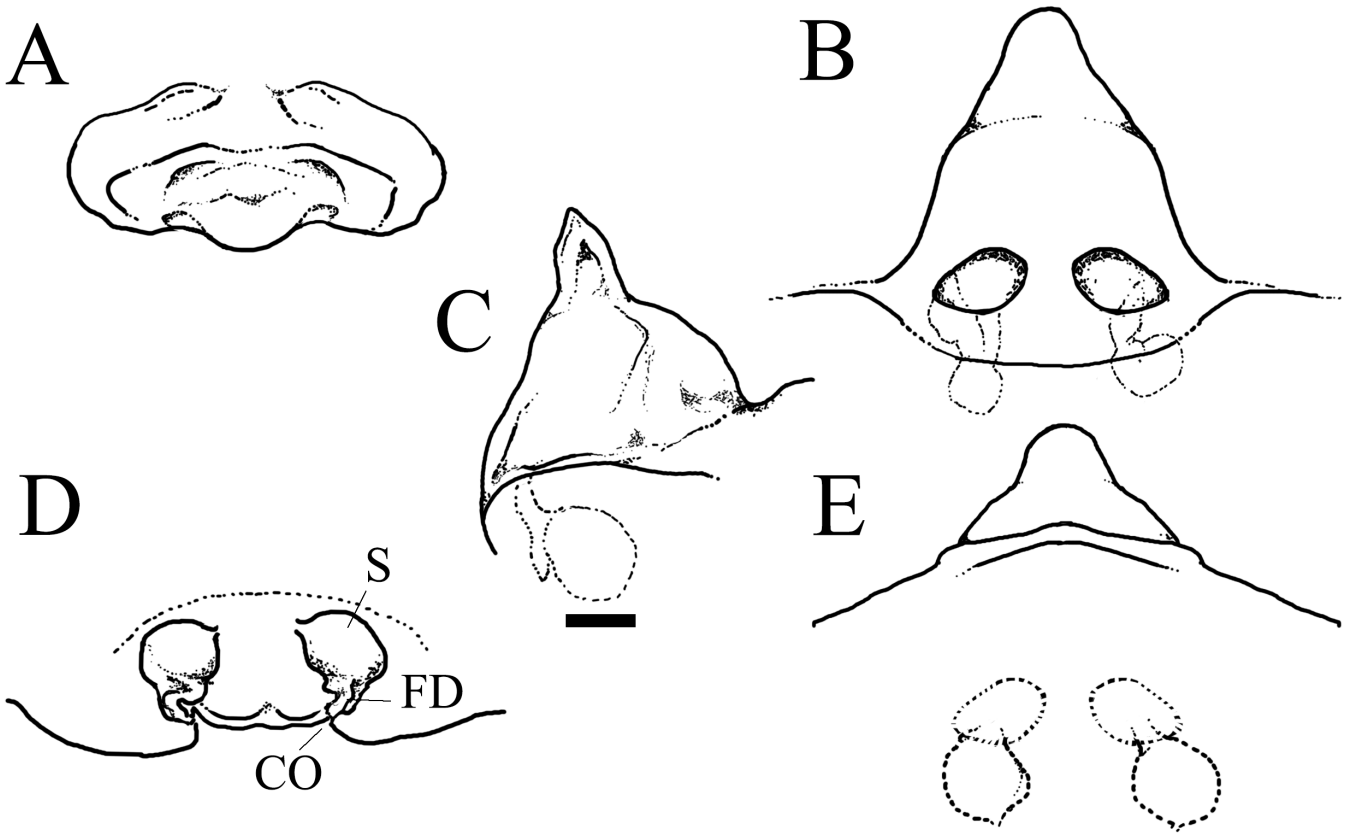

Fig. 7. Epigynal structure of *Gasteracantha hasselti* C. L. Koch, 1837. A, Ventral view; B, Posterior view; C, Lateral view; D, Dorsal view; E, Anterior view. Scale = 100 μm. CO = Copulatory opening; FD = Fertilisation duct; S = Spermatheca.

TAXONOMY

In 1914, Dahl categorised all his *Gasteracantha* specimens into 16 subgenera based on their abdominal structure, sigilla patterns, spine position and arrangement. These subgenera include *Macracantha*, *Tatacantha*, *Actinacantha*, *Hypsacantha*, *Austracantha*, *Togacantha*, *Afracantha*, *Isoxya*, *Acrosomoides*, *Atelacantha*, *Collacantha*, *Thelacantha*, *Anchacantha*, *Pachypleuracantha*, *Gasteracantha*, and *Tetracantha*. Although the delineation of a few of these subgenera of *Gasteracantha* appeared to be reasonable, e.g., *Actinacantha*, *Macracantha* (Emerit, 1974) and *Thelacantha* (Benoit, 1964; Emerit, 1974), the authors wondered if all these subgenera would be better grouped under the somewhat general diagnosis of the genus *Gasteracantha* (*vide infra*) or erected individually to various monophyletic or monotypic genera – a decision to be made when more species over a greater geographical range are examined. The present study has shown that spine position and arrangement, sigilla number and pattern, abdominal coloration and epigyne structure were useful in species discrimination, which agreed with reports from several studies (Pickard-Cambridge, 1879; Dahl, 1914; Chrysanthus, 1959, 1971), although the latter character is more often used to differentiate between very closely related species. Currently, the two main issues in the species identification of female Gasteracanthinae spiny orb-weavers lie in the (i) significant morphological difference between the adults and juvenile; and (ii) lack of reliable identification characters for sub-female individuals, e.g., the lack of well-developed spines prevent an accurate differentiation of *G. diardi*, *G. mengei*, *G. doriae*, etc. While a more robust identification key of both female and male species is expected with increased sampling and documentation, the accurate identification of juvenile individuals would likely still rely on molecular identification.

FAMILY Araneidae Clerck, 1757

GENUS *Actinacantha* (Walckenaer, 1841)

Actinacantha globulata (Walckenaer, 1841)

(Figs. 1A, 5A−C)

Plectana globulata Walckenaer, 1841: 151
Actinacantha globulata Simon, 1864: 286.
Gasteracantha globulata van Hasselt, 1882: 12

Material examined. MALAYSIA. 1 sub-female (reg. UIR150116-AGLO1), Semenyih., Selangor, coll. Chan ZY, 15 Jan.2016.

Diagnosis. Adult female *A. globulata* is differentiated from those of *Gasteracantha hasselti* and *Macracantha arcuata* based on the unique bulbous bases from which the middle spines originate.

Natural history. A sub-female was collected from a web in the understorey of a primary tropical forest. The species appears to be uncommon and very localised in the Malay Peninsula.

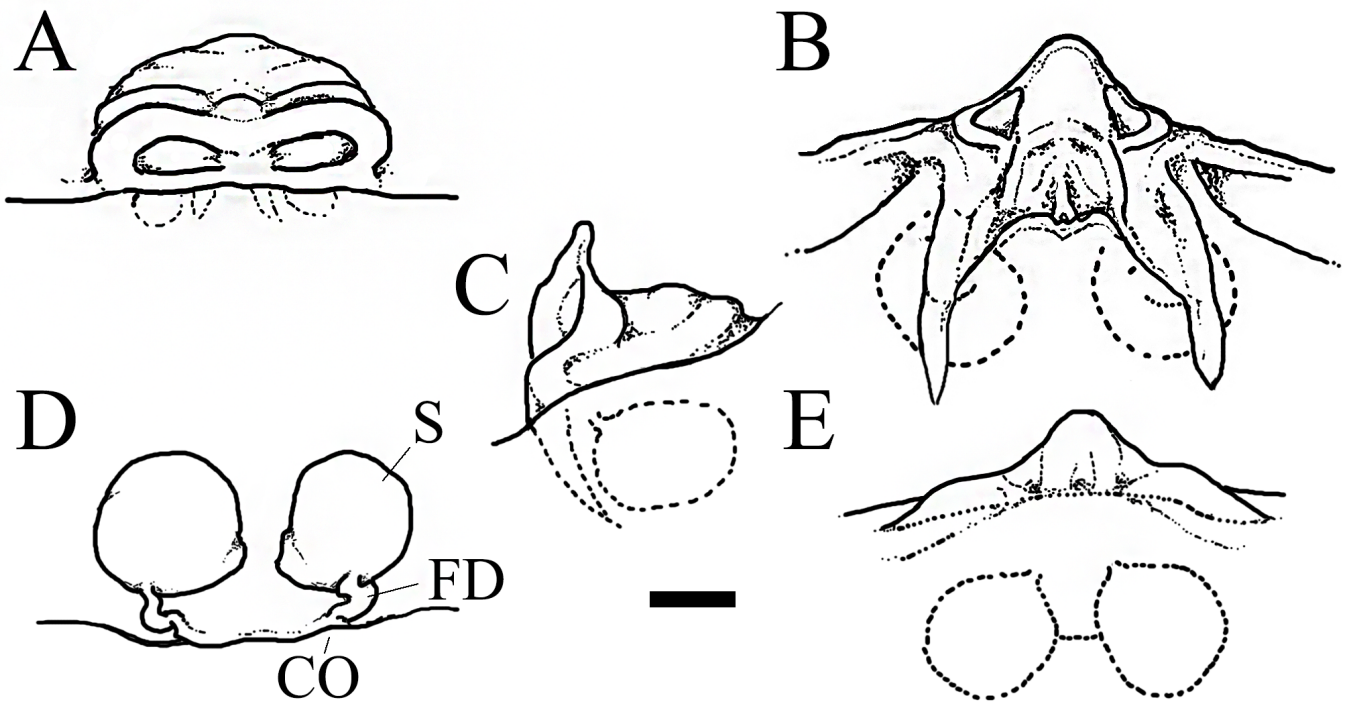

Fig. 8. Epigynal structure of *Gasteracantha kuhli* C. L. Koch, 1837. A, Ventral view; B, Posterior view; C, Lateral view; D, Dorsal view; E, Anterior view. Scale = 100 μm. CO = Copulatory opening; FD = Fertilisation duct; S = Spermatheca.

Distribution. Peninsular Malaysia, Borneo, and Indonesia (Sumatra and Java).

Remarks. The similarities in the morphological descriptions and geographical distribution of *A. globulata* and *Gasteracantha crucigera* suggest that they may likely be synonymous (Walckenaer, 1841; Simon, 1864; Bradley, 1877; Chrysanthus, 1971; Dahl, 1914; Yong & Ono, 2009). However, a solid conclusion cannot be made based on the morphology and DNA sequence of only one *A. globulata* sub-adult female in its penultimate molt. This limited information may overlook cryptic or sibling species existing between Peninsular Malaysia, Borneo, and Papua New Guinea. The recent employment of molecular data has already revealed the existence of species complexes in spider and insect taxa which may well be possible among geographically isolated *A. globulata* and *G. crucigera* (Franzini et al., 2013; Gregorič et al., 2015; Yong et al., 2015). Nevertheless, the morphological and molecular characterisation of more specimens of both species are expected to resolve this taxonomic uncertainty.

Genus *Gasteracantha* Sundevall, 1833

According to Dahl (1914) and Pickard-Cambridge (1879), members of the genus *Gasteracantha* are characterised by a cephalothorax that is no longer than broad and strongly arched in the front but flattened in the posterior half and covered by the anterior part of the abdomen. The abdomen is composed of a chitin skin armed with 2–6 spines arising from different points of the abdominal margin. The abdomen is marked with sigilla (previously called sigil or ocelli) on the upperside (and occasionally underside) which are likely points of attachment of muscular fibers (Pickard-Cambridge, 1879). Four of the sigilla form a trapezoid pattern at the middle of the abdomen while the others are around the edge. There are generally two transverse rows of sigilla at the rear edge of the abdomen although the most posterior row often occurs within the posterior abdominal fold in some species and is thus invisible. Dahl (1914) reported that the epigynum was hardly usable as a morphological character as (i) it often displays fewer tangible features; and (ii) it is variable depending on duration since the ultimate molt. However, Chrysanthus (1959, 1971) advocated the examination of the epigynal structures in species identification and have illustrated numerous reproductive structures of *Gasteracantha* spp. from New Guinea. In this study, the epigynal structure was shown to be valuable information in species identification, especially between closely related species.

Gasteracantha diardi (Lucas, 1835)

(Figs. 1B–D, 5A–F)

Epeira diardi Lucas, 1835: 70, pl. 149, fig. 4.
Gasteracantha pavesi O. Pickard-Cambridge, 1879: 282, pl. 26, fig. 4.
Gasteracantha diardi Dahl, 1914: 285.
Gasteracantha diardi Kolosváry, 1931: 1057, pl. 30, fig. 1.

Material examined. MALAYSIA. 2 females (reg. UIR270115-GDIA1&2), Penang Hill, Penang, coll. Tan J, 31 Oct.2013; 2 females (reg. UIR270115-GDIA3&4), Gunung Jerai, Kedah, coll. Tan J and Chan ZY, 9 June.2014; 2 females (reg. UIR270115-GDIA5&6), Kuala Sepetang, Taiping, Perak, coll. Tan J and Chan ZY, 12 May.2014.

Measurements (female). CL = 2.45; CW = 3.72; AL = 6.39; AW = 25.56 (including median spines); TL = 8.80. AS = 1.15; MS = 6.23; PS = 1.58.

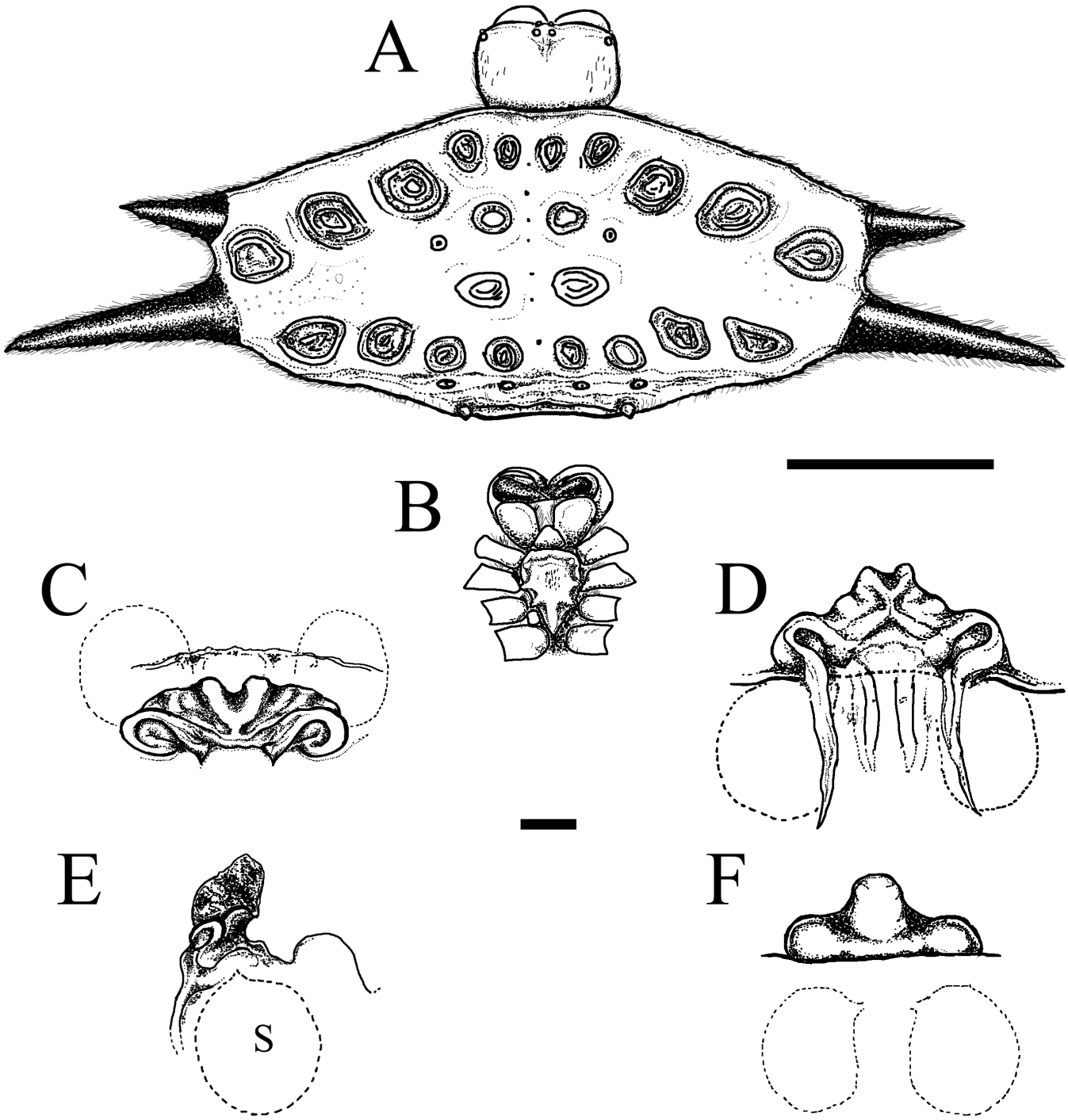

Fig. 9. *Gasteracantha mengei* Keyserling, 1864. A, Dorsal habitus; B−E, Epigynal structure. A, Ventral view; B, Posterior view; C, Lateral view; D, Dorsal view; E, Anterior view. Scale A = 5 mm; B−E = 100 µm. S = Spermatheca.

Interocular distance. AME – AME = 0.21; ALE – AME = 1.44; ALE – ALE = 3.47; PME – PME = 0.26; PLE – PME = 1.36; PLE – PLE = 3.49; ALE – PLE = 0.04; AME – PME = 0.12.

Legs. I 10.47 (3.02, 1.40, 2.71, 1.76, 1.58); II 10.16 (3.00, 1.23, 2.39, 1.92, 1.62); III 7.50 (2.68, 0.83, 1.44, 1.51, 1.04); IV 12.80 (5.00, 1.28, 2.72, 2.48, 1.32). Leg formula 4123.

Diagnosis. Female individuals of *G. diardi* display tiny angular anterior abdominal spines and well-developed lateral median spines which may be straight or slightly bent backwards (Lucas, 1835; Dahl, 1941). The epigyne of *G. diardi* is illustrated for the first time in Fig. 6, which is shorter than wide and appears M-shaped in ventral view (Fig. 6C), with two tiny openings that extend internally and a forked septum with two apparent depressions.

Intraspecific variation. Two different abdominal patterns of female *G. diardi* were observed in this study: (i) dark-colored abdomen with three horizontally transverse stripes (white and yellow; Fig. 1C, D) or (ii) without stripes (Fig. 1B). Both morphotypes display similar epigyne structure. Spine curvature and bristles more conspicuous in full adults.

Fig. 10. Holotype sample of *Gasteracantha mengei* Keyserling, 1864 deposited in the Natural History Museum, London. A, Dorsal view of abdomen; B, Posteroventral view of epigyne. Scale not available. (Photo reproduced with permission from Joseph Koh).

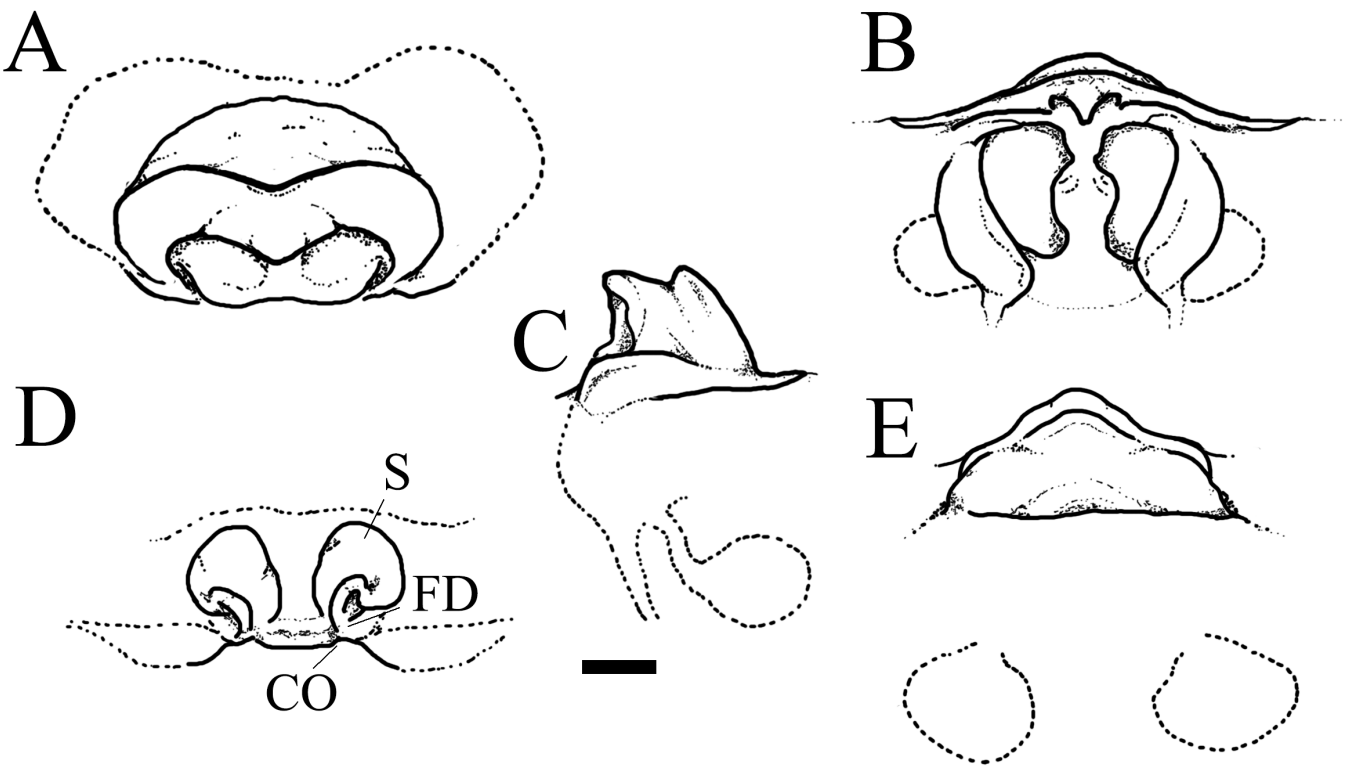

Fig. 11. Epigynal structure of *Macracantha arcuata* (Fabricius, 1793). A, Ventral view; B, Posterior view; C, Lateral view; D, Dorsal view; E, Anterior view. Scale = 100 µm. CO = Copulatory opening; FD = Fertilisation duct; S = Spermatheca.

Natural history. In Peninsular Malaysia, the dark form of female *G. diardi* (Fig. 1B) appeared to be prevalent in the highlands, while two striped female individuals (Fig. 1C, D) were collected from the fringes of a mangrove forest. The webs of female individuals were conspicuous and often constructed at 1−2 m above ground. Males not observed.

Distribution. China, Thailand, Malaysia, Borneo and Indonesia.

Gasteracantha hasselti C. L. Koch, 1837
(Figs. 1F, 7A−E)

Gasteracantha hasseltii C. L. Koch, 1837: 29, fig. 267
Gasteracantha hasseltii Dahl, 1914: 247. 250
Gasteracantha hasselti Song, Zhu & Chen, 1999: 281, figs. 168E, F, K, L, Q, R
Gasteracantha hasselti **Yin et al., 2012**: 581, figs. 279a−f
Gasteracantha hasselti Sen et al., 2015: 108, figs. 617−621, pl. 21
Gasteracantha hasselti Williams, 2017: 249, figs. 1−7

Material examined. MALAYSIA. 1 female (reg. UIR270115-GHAS1), Sungai Sedim, Kedah, coll. Tan J and Chan ZY, 27 January 2015.

Diagnosis. Adult female *G. hasselti* is differentiated from those of *Actinacantha globulata* and *Macracantha arcuata* based on the typically attenuated median spines which hardly exceeds half the width of the abdomen.

Natural history. A female was collected from a web amid the shrubs of a disturbed forest. Female individuals tend to construct webs in dryer areas. Males not observed.

Distribution. India, China, Indochina, Peninsular Malaysia, Singapore, Indonesia.

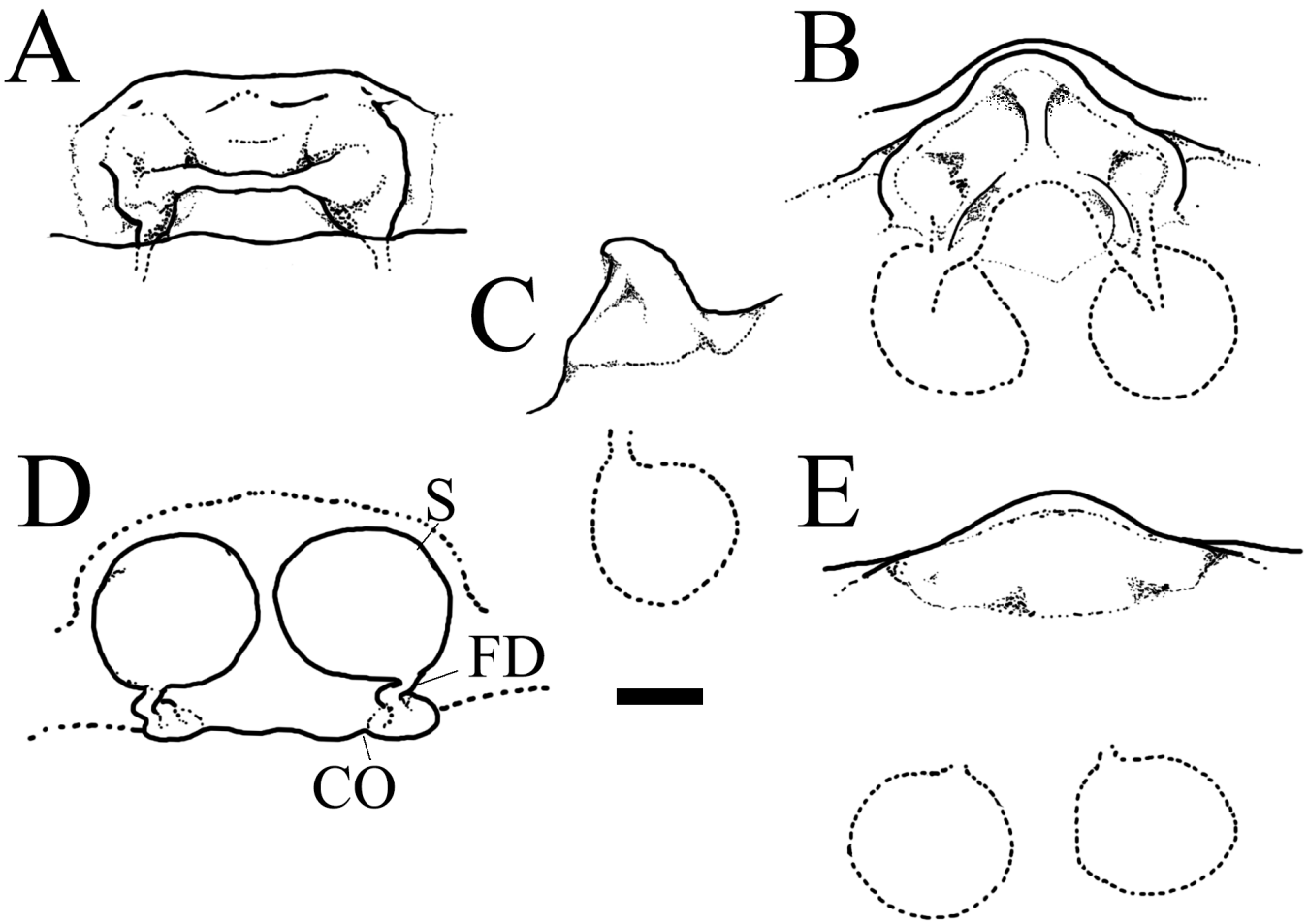

Fig. 12. Epigynal structure of *Thelacantha brevispina* (Doleschall, 1857). A, Ventral view; B, Posterior view; C, Lateral view; D, Dorsal view; E, Anterior view. Scale = 100 μm. CO = Copulatory opening; FD = Fertilisation duct; S = Spermatheca.

***Gasteracantha kuhli* C. L. Koch, 1837**
(Figs. 1E, 8A−E)

Gasteracantha kuhlii C. L. Koch, 1837: 20, fig. 262
Gasteracantha kuhli Dahl, 1914: 262
Gasteracantha kuhli Yin et al., 2012: 582, figs. 280a−f
Gasteracantha kuhli Kim & Lee, 2012: 74, figs. 53A−D, pl. 13
Gasteracantha kuhlii Sen et al., 2015: 108, figs. 622−626, pl. 21
Gasteracantha kuhlii Roy, Saha & Raychaudhuri, 2017: 6, figs. 7−12, 167

Material examined. MALAYSIA. 1 female (reg. UIR0200414-GKUH1), Kepong, Selangor, coll. Tan J, 20 Apr.2014; 1 female (reg. UIR031014-GKUH2), Sungai Tekala, Hulu Langat, Selangor, coll. Tan J, 03 Oct.2014; 1 female (reg. UIR091116-GKUH3), Sungai Relau, Pahang, coll. Tan J, 09 Nov.2016.

Diagnosis. Female individuals of *G. kuhli* are tiny (body length 6−8 mm) and display a dorsally white abdomen with several pairs of black patches at the borders which may or may not be interconnected. All three pairs of abdominal spines are short, with the posterior pair similar or slightly longer than that of the median spines.

Natural history. Female individuals were collected from conspicuous webs in parks, and disturbed forests. In Peninsular Malaysia, this common species is observed in the lowlands and at high elevations (< 1,200 m). Males not observed.

Distribution. India, Japan, and Southeast Asia.

***Gasteracantha mengei* Keyserling, 1864**
(Figs. 1I, 9A−F, 10A, B)

Gasteracantha mengei Keyserling, 1864: 67, pl. 1, fig. 5
Gasteracantha malayensis van Hasselt, 1882: 13
Gasteracantha mengei Dahl, 1914: 270, fig. 10

Material examined. Holotype – female, Malacca, Peninsular Malaysia, "Det. N. Scharff Ex. Dry Collection", no date, BMNH.

Others: 1 female (damaged abdomen) (reg. UIR270615-GMEN1), Sungai Sedim, Kedah, coll. Tan J and Chan ZY, 27 June.2015.

Diagnosis. Female individuals of *G. mengei* can be differentiated from female *G. diardi* and *G. diadesmia* Thorell (see Fig. 4, p.33 in Roy et al., 2017 and Fig. 615 in Sen et al., 2015) by the somewhat parallel arrangement of its anterior and median spines in dorsal view as well as the inconspicuous posterior spines (Figs. 1I, 9A, 10A). In terms of epigynal structure, the protruding Y-shaped structure

of the epigyne in posterior view (Fig. 9D) can be used to distinguish *G. mengei* from *G. diardi*.

Natural history. One female individual was collected from a large web (~ 1 m) built across two trees near a river in a disturbed forest.

Distribution. Thailand, Malaysia, Singapore and Borneo.

Remarks. *G. mengei* can be confused with *G. diardi* as the females are similar in terms of size, appearance and geographical distribution. Despite the failure to obtain any DNA data for *G. mengei* GMEN1, a cross-check with the holotype specimen (Fig. 10) clearly showed that these two species are different. It is worth noting that the four tiny posterior sigilla (arrow in Fig. 10) observed in the *G. mengei* holotype (then '*G. mengii*', labelled 'Det. N. Scharff Ex. Dry Collection' from Malacca, Peninsular Malaysia) were not illustrated or described in Keyserling (1864). This may have led to the incorrect synonymy of two morphologically similar species, namely *G. mengei* and *G. malayensis* (Simon, 1864) as the subsequent authors, i.e., Dahl (1914) and van Hasselt (1882) often relied on illustrations for identification. Both *Gasteracantha* species were also coincidentally from the same type locality of Malacca. These two species are unique within the genus as they both lack conspicuous posterior abdominal spines. However, they can be distinguished based on the presence of the row of four posterior sigilla as well as the angle and position of the median and posterior spines. According to Simon (1864), the two pairs of spines of *G. malayensis* are 'straight and parallel' (p. 285 and Fig. 130 in Simon, 1864), unlike the abdominal spines of *G. mengei* which are slightly angled towards the posterior (Figs. 9A, 10A). These morphological characters also distinguish the females of these two species from those of *G. quadrispinosa* O. Pickard-Cambridge, 1879 from Australia and New Guinea, a species thought to be conspecific with the former two due to the lack of information on abdominal colors at that time (Pickard-Cambridge, 1879; Dahl, 1914; Chrysanthus, 1971; World Spider Catalog, 2018). Photographic records suggest that both *G. malayensis* and *G. mengei* are extant in Peninsular Malaysia and more sampling is required for validation, considering the possibility of ethanol preservation affecting not only the color but also the natural direction of the spines as pointed out by Pickard-Cambridge (1879).

Genus *Macracantha* Simon, 1864

Macracantha arcuata (Fabricius, 1793)

(Figs. 1G; 11A–E)

Aranea arcuata Fabricius, 1793: 425
Gasteracantha arcuata Dahl, 1914: 242, fig. 1
Gasteracantha arcuata C. L. Koch, 1837: 34, fig. 270
Gasteracantha arcuata Yin et al., 1997: 94, figs. 23a–c
Gasteracantha arcuata Song, Zhu & Chen, 1999: 281, figs. 167N–P, 168A, B, O

Material examined. MALAYSIA. 2 females (reg. UIR031014-MARC1&2), Sungai Tekala, Hulu Langat, Selangor, coll. Tan J and Chan ZY, 3 Oct.2014; 1 female (reg. UIR270615-MARC3), Sungai Sedim, Kedah, coll. Tan J and Chan ZY, 27 June.2015.

Diagnosis. Adult female *Macracantha arcuata* is differentiated from those of *Actinacantha globulata* and *Gasteracantha hasselti* based on the unique long median spines that are multiple times the width of the abdomen.

Natural history. Female individuals were collected in the understorey of disturbed forests, where they resided in an inverted position at the center of their horizontal webs built directly above or near (< 2 m) water bodies. The reddish-orange abdominal color was observed to fade with age, turning white. The long abdominal spines of females retain some flexibility post-molt and are angled farther apart in gravid and satiated individuals. Males not observed and female populations appeared to be seasonal.

Distribution. India, China, and Southeast Asia.

Genus *Thelacantha* Hasselt, 1882

Thelacantha brevispina (Doleschall, 1857)

(Figs. 1H, 12A–E)

Plectana brevispina Doleschall, 1857: 423
Gasteracantha brevispina Workman & Workman, 1892: 8, pl. 8
Thelacantha brevispina Benoit, 1964
Gasteracantha mammosa Barrion & Litsinger, 1995: 554, figs. 341a–e
Thelacantha brevispina Yin et al., 2012: 584, figs. 281a–i

Material examined. MALAYSIA. 2 females (reg. UIR010214-TBRE1&2), Penang Hill, Penang Island, coll. Tan J, 1 Feb.2014; 1 female (reg. UIR090614-TBRE3), Gunung Jerai, Kedah, coll. Tan J and Chan ZY, 9 June.2014.

Diagnosis. Female individuals of *T. brevispina* are identified based on the two large longitudinally ovoid depressions on the abdomen as well as posterior spines that occur on swollen tubercles.

Natural history. In Peninsular Malaysia, female individuals appear to be abundant in the highlands close to human settlements. Sub-females may display lighter and vibrant abdominal coloration. Males not observed.

Distribution. Madagascar, India, Southeast Asia, Australia and French Polynesia.

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SUPPLEMENTARY DATA

Fig. S1. Bayesian tree based on the 16S+CO1+CO2+H3A+18S dataset of *Actinacantha*, *Gasteracantha*, *Macracantha*, and *Thelacantha*. Numeric values at nodes arranged in an order of Ultrafast ML bootstrap support/MP bootstrap support/Bayesian posterior probability.

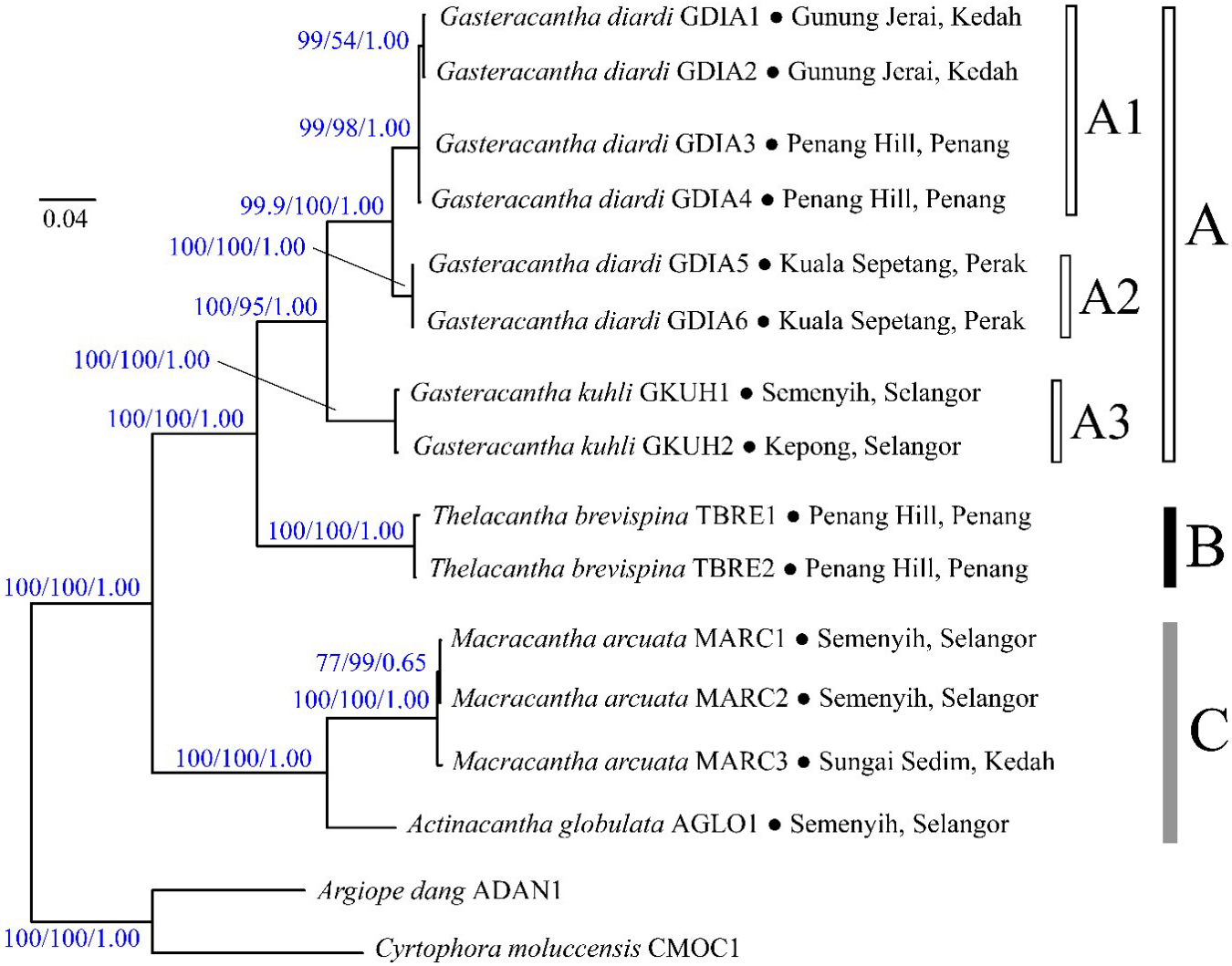

Table S1. Details of primers used in this study.

Genetic Marker	Primer Sequence	Annealing Temperature	Reference
16S	LR-J-12887: 5′-CCG GTC TGA ACT CAG ATC ACG T-3′ LR-N-13398: 5′-CGC CTG TTT AAC AAA AAC AT-3′	48°C	Simon et al. (1994); Su et al. (2011); Tan et al. (2016)
CO1	LCO-J-1490: 5′-GGT CAA CAA ATC ATA AAG ATA TAT TGG-3′ HCO-N-2198: 5′-TAA ACT TCA GGG TGA CCA AAA AAA TCA-3′	47°C	Su et al. (2011); Folmer et al. (1994)
CO2	COII-FWD: 5′_-CCT CGA CGA TAT TCT GAT TAT C-3′ C2-N-3661r: 5′-CCA CCA ATT TCA GAA CAT TGA CC-3′	47°C	Simon et al. (1994); Cheng & Kuntner (2014, 2015)
H3A	H3aF2: 5′-ATG GCT CGG TAC CAA GCA GAC-3′ H3aR1: 5′-ATA TCC TTR GGC ATR ATR GTG AC-3′	47°C	Colgan et al. (1998); Cheng & Kuntner (2014, 2015)
18S	3F: 5′- GTT CGA TTC CGG AGA GGG A-3′ 18Sbi: 5′- GAG TCT CGT TCG TTA TCG GA-3′	48°C	Giribet et al. (1996); Whiting et al. (1997)

Table S2. Uncorrected "p" distance (%) between selected DNA sequences of *Actinacantha*, *Gasteracantha*, *Macracantha*, and *Thelacantha* based on the 16S+CO1+CO2+H3A+18S dataset.

	Sample	1	2	3	4	5	6	7	8	9	10	11	12	13
1	*Actinacantha globulata* AGLO1													
2	*Macracantha arcuata* MARC1	6.87												
3	*Macracantha arcuata* MARC3	6.81	0.38											
4	*Macracantha arcuata* MARC2	6.90	0.09	0.35										
5	*Thelacantha brevispina* TBRE1	12.00	12.10	12.04	12.07									
6	*Thelacantha brevispina* TBRE2	11.87	11.88	11.88	11.91	0.35								
7	*Gasteracantha diardi* GDIA5	10.87	11.35	11.35	11.32	9.03	9.00							
8	*Gasteracantha diardi* GDIA6	10.87	11.35	11.35	11.32	9.03	9.00							
9	*Gasteracantha diardi* GDIA2	11.28	11.67	11.67	11.70	8.84	8.65	2.63	2.63					
10	*Gasteracantha diardi* GDIA3	11.38	11.76	11.77	11.79	9.00	8.81	2.66	2.66	0.44				
11	*Gasteracantha diardi* GDIA1	11.33	11.69	11.66	11.69	8.89	8.73	2.54	2.54	0.19	0.44			
12	*Gasteracantha diardi* GDIA4	11.35	11.76	11.77	11.79	8.97	8.78	2.63	2.63	0.41	0.10	0.41		
13	*Gasteracantha kuhli* GKUH1	10.81	10.69	10.63	10.66	8.59	8.56	5.71	5.71	5.80	5.93	5.75	5.96	
14	*Gasteracantha kuhli* GKUH2	10.75	10.72	10.66	10.69	8.43	8.43	5.67	5.67	5.77	5.83	5.72	5.86	0.32

Table S3. Uncorrected "p" distance (%) between CO1 DNA sequences of selected *Actinacantha*, *Gasteracantha*, *Macracantha*, and *Thelacantha*.

	Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	*G. cancriformis* EU003287																											
2	*G. cancriformis* KP253807	4.12																										
3	*G. cancriformis* FJ525321	1.52	3.96																									
4	*G. kuhlii* DQ518416	9.44	10.40	9.66																								
5	*G. kuhlii* AB910447	9.49	10.45	9.80	0																							
6	*G. kuhlii* JN817164	10.58	11.21	10.73	1.29	1.28																						
7	*A. globulata* AGLO1	14.58	15.66	14.76	14.83	15.03	16.03																					
8	*G. hasselti* GHAS1	13.84	15.06	14.16	14.51	14.74	15.60	8.13																				
9	*M. arcuata* MARC1	15.36	16.29	15.21	14.50	14.75	15.75	9.64	9.04																			
10	*M. arcuata* MARC2	15.36	16.29	15.21	14.50	14.75	15.75	9.64	9.04	0																		
11	*M. arcuata* MARC3	15.37	16.29	15.21	14.67	14.91	15.58	9.64	9.04	0.60	0.60																	
12	*T. brevispina* TBRE1	11.10	11.87	10.69	10.11	10.26	11.82	14.76	13.40	15.81	15.81	15.81																
13	*T. brevispina* TBRE2	11.25	12.02	10.84	10.26	10.41	11.97	15.06	13.40	15.81	15.81	15.81	0.30															
14	*T. brevispina* TBRE3	12.62	14.00	12.35	11.21	11.22	12.76	15.81	15.06	13.86	13.86	13.86	9.34	9.49														
15	*G. diardi* GDIA3	10.49	11.87	10.09	9.34	9.47	10.11	14.61	13.70	15.51	15.51	15.51	12.20	12.20	12.65													
16	*G. diardi* GDIA4	10.33	11.71	10.24	9.19	9.31	9.96	14.46	13.55	15.66	15.66	15.66	12.05	12.05	12.50	0.15												
17	*G. diardi* GDIA2	10.03	11.71	10.24	9.03	9.15	9.80	14.61	13.40	15.66	15.66	15.66	12.05	12.05	12.50	0.45	0.30											
18	*G. diardi* GDIA1	10.18	11.87	10.39	9.03	9.15	9.80	14.61	13.40	15.51	15.51	15.51	12.20	12.20	12.65	0.30	0.15	0.15										
19	*G. diardi* GDIA6	10.18	11.56	10.39	8.42	8.54	9.33	13.10	12.35	14.00	14.00	14.31	11.90	12.20	13.40	5.57	5.42	5.42	5.27									
20	*G. diardi* GDIA5	10.18	11.56	10.39	8.42	8.54	9.34	13.10	12.35	14.01	14.01	14.31	11.90	12.20	13.40	5.57	5.42	5.42	5.27	0								
21	*G. kuhli* GKUH3	8.67	8.68	8.13	5.93	6.03	7.17	14.00	12.80	13.86	13.86	13.86	10.54	10.84	11.60	8.13	8.28	8.43	8.43	8.28	8.28							
22	*G. kuhli* GKUH2	8.67	8.68	8.13	5.93	6.03	7.17	14.00	12.80	13.86	13.86	13.86	10.54	10.84	11.60	8.13	8.28	8.43	8.43	8.28	8.28	0						
23	*G. kuhli* GKUH1	8.52	8.83	8.28	5.93	6.03	7.17	14.31	12.65	13.70	13.70	13.70	10.69	10.99	11.90	8.43	8.58	8.43	8.43	8.28	8.28	0.30	0.30					

	Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
24	*G. cancriformis* KJ157213	4.16	0. 84	4.29	9.92	10.05	11.10	15.40	14.07	15.72	15.72	15.72	11.22	11.39	13.09	11.42	11.26	11.25	11.42	11.09	11.09	8.42	8.42	8.58				
25	*G. cancriformis* KJ157212	4.09	0.68	4.23	10.21	10.35	11.44	15.36	14.16	15.50	15.50	15.51	11.38	11.55	13.68	11.77	11.60	11.59	11.77	11.25	11.25	8.65	8.65	8.82	0.17			
26	*G. cancriformis* KJ157214	4.42	0.98	4.38	10.23	10.35	11.39	15.59	14.59	16.38	16.38	16.54	11.50	11.66	13.93	11.69	11.53	11.53	11.69	11.68	11.68	8.74	8.74	8.90	1.15	1.00		
27	*G. kuhlii* KJ957963	8.28	8.78	8.35	5.69	5.78	7.27	14.05	12.87	13.86	13.86	14.04	10.80	11.13	12.04	8.69	8.52	8.36	8.36	8.20	8.20	0.82	0.82	0.82	8.33	8.49	8.82	

Table S4. Uncorrected "p" distance (%) between selected DNA sequences of *Actinacantha*, *Gasteracantha*, *Macracantha*, and *Thelacantha* based on the 16S marker.

	Sample	1	2	3	4	5	6	7	8
1	*Gasteracantha diardi* GDIA3								
2	*Gasteracantha diardi* GDIA1	0.00							
3	*Gasteracantha diardi* GDIA5	1.39	1.39						
4	*Gasteracantha kuhli* GKUH1	6.98	6.98	7.68					
5	*Thelacantha brevispina* TBRE1	14.35	14.35	14.12	13.23				
6	*Gasteracantha hasselti* GHAS1	16.52	16.52	16.75	14.67	16.74			
7	*Macracantha arcuata* MARC1	17.39	17.39	18.09	16.04	16.24	9.23		
8	*Actinacantha globulata* AGLO1	15.78	15.78	16.48	15.36	16.94	9.19	10.36	

Table S5. Uncorrected "p" distance (%) between selected DNA sequences of *Actinacantha*, *Gasteracantha*, *Macracantha*, and *Thelacantha* based on the CO2 marker.

	Sample	1	2	3	4	5	6	7	8
1	*Gasteracantha hasselti* GHAS1								
2	*Actinacantha globulata* AGLO1	10.48							
3	*Macracantha arcuata* MARC1	11.11	12.75						
4	*Gasteracantha diardi* GDIA1	17.23	18.24	17.99					
5	*Gasteracantha diardi* GDIA3	17.11	18.24	18.11	0.38				
6	*Gasteracantha diardi* GDIA5	16.60	17.48	17.73	4.44	4.44			
7	*Gasteracantha kuhli* GKUH1	16.09	16.97	16.59	10.90	10.90	10.52		
8	*Thelacantha brevispina* TBRE1	17.42	19.57	19.44	14.85	14.98	16.13	15.76	