ZooKeys 1032: 1–15 (2021)
doi: 10.3897/zookeys.1032.64917
https://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Mountainous millipedes in Vietnam. I. Two new species of the family Paradoxosomatidae from Mount Fansipan (Diplopoda, Polydesmida)

Anh D. Nguyen[1,2], Dai Dac Nguyen[1,3], Katsuyuki Eguchi[3]

[1] Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18, Hoangquocviet Rd., Caugiay District, Hanoi, Vietnam [2] *Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18, Hoangquocviet Rd., Caugiay District, Hanoi, Vietnam* [3] *Department of Biological Sciences, Faculty of Sciences, Tokyo Metropolitan University, Hachioji-shi, Tokyo Prefecture, Japan*

Corresponding author: Anh D. Nguyen (ducanh.iebr@gmail.com)

Academic editor: D. V. Spiegel | Received 24 February 2021 | Accepted 23 March 2021 | Published 16 April 2021

http://zoobank.org/E45C61A8-6565-4AB5-B5FC-AD907A9B4738

Citation: Nguyen AD, Nguyen D-D, Eguchi K (2021) Mountainous millipedes in Vietnam. I. Two new species of the family Paradoxosomatidae from Mount Fansipan (Diplopoda, Polydesmida). ZooKeys 1032: 1–15. https://doi.org/10.3897/zookeys.1032.64917

Abstract

Two new paradoxosomatid millipede species were discovered at the high elevations of Mount Fansipan in northwestern Vietnam. They are named as *Orthomorphoides sapa* **sp. nov.** and *Hylomus solenophorus* **sp. nov.** In addition to their morphological descriptions, sequences from fragments of two mitochondrial genes, *COI* and *16S rRNA*, are also provided for both new species.

Keywords

Biodiversity, *COI*, *16S rRNA*, new species, northwest Vietnam, taxonomy

Introduction

At least three-quarters of Vietnam's landscape is covered by mountains and hills (Le Ba Thao 2017). Not surprisingly then, this mountainous region is characterised by high biodiversity with many new species and genera being discovered annually (Sterling et al. 2006). Access to the high mountains is, however, very difficult resulting in limited biodiversity investigations, with millipedes from mountainous regions being particularly poorly known (Nguyen et al. 2019).

Table 1. The known millipede species on Mt. Fanxipan, northwestern Vietnam.

Order	Family	Species
Glomerida	Glomeridae	*Hyleoglomeris fanxipan* Nguyen, Hwang & Eguchi, 2019
		Hyleoglomeris sapa Nguyen, Hwang & Eguchi, 2019
Sphaerotheriida	Zephroniidae	*Sphaerobelum separatum* (Attems, 1953)
Platydesmida	Andrognathidae	*Pseudodesmus camptotrichus* Attems, 1938
Polyzoniida	Siphonotidae	*Dawydoffia kalonota* Attems, 1953
Julida	Julidae	*Nepalmatoiulus fan* (Enghoff, 1987)
		Nepalmatoiulus pan (Enghoff, 1987)
Spirobolida	Spirobolellidae	*Physobolus annulatus* Attems, 1953
Chordeumatida	Kashmireumatidae	*Vieteuma topali* Golovatch, 1984
Polydesmida	Cryptodesmidae	*Trichopeltis kometis* (Attems, 1938)
Polydesmida	Paradoxosomatidae	*Chapanella rubida* Attems, 1953
		Hylomus cervarius (Attems, 1953)
		Hylomus proximus (Nguyen, Golovatch & Anichkin, 2005)
		Kronopolites montanus Golovatch, 2009
		Oxidus gigas (Attems, 1953)
		Sapamorpha complexa Golovatch, 2009
		Sellanucheza variata (Attems, 1953)
		Tylopus crassipes Golovatch, 1984
		Tylopus magicus Golovatch, 1984
		Tylopus nodulipes (Attems, 1953)
		Tylopus provurcus Golovatch, 1984
		Tylopus sapaensis Nguyen, 2012
		Tylopus sigma (Attems, 1953)

Among the high mountains in Vietnam, Mount Fansipan is very well-known as Vietnam's highest peak, 3143 m a.s.l. It is located in the Hoang Lien Son Mountain Range, the southeasternmost extension of the Himalaya Range. The biodiversity of this mountain is very rich, containing both temperate and subtropical elements and both low- and highland species (Sterling et al. 2006). Little is known about the millipede fauna of Mount Fansipan. To date, only 23 species have been reported from this mountain and almost all of them are considered to be endemic to the region (Attems 1938, 1953; Golovatch 1984, 2009; Enghoff 1987; Golovatch and Enghoff 1993, 1994; Nguyen et al. 2005, 2019; Nguyen 2012) (Table 1).

This work will contribute to a better understanding of the millipede fauna of Mount Fansipan by describing two new paradoxosomatid species found there. Both morphological and molecular data are provided for the new species.

Material and methods

Material was collected from high elevations of Mount Fansipan, northwestern Vietnam, and preserved in 90% ethanol. Specimens were observed under an Olympus SZX10 microscope.

Images at various focal planes were taken under both normal and ultraviolet (UV) light using a micro-optics imaging system coupled with a Nikon D5100 camera (see Sierwald et al. 2019 for a detailed description of the UV imaging technique).

Multiple images were processed in Adobe Lightroom, then stacked using Helicon Focus v. 4.0 and assembled using Adobe Photoshop CS6.

For the purposes of scanning electron micrography, gonopods were dissected, mounted on aluminium stubs, coated with gold and then studied using the LEO EVO 60 SEM system (Carl Zeiss) in the Field Museum of Natural History. After SEM imaging, the gonopods were returned and preserved with their specimen.

Total DNA was extracted from several midbody legs using the QIAGEN DNeasy Blood & Tissue Kit. Fragments of the mitochondrial *cytochrome c oxidase subunit I* (*COI*) and *16S rRNA* genes were amplified using two pairs of primers: COI-1F (5'-ACTCTACTAATCATAAGGAT-3') and COI-1R (5'-TAAACCTCCGGGTGACCAA-3'), 16S-1F (5'-CCGGTTTGAACTCAGATCA-3') and 16S-1R (5'-TGACTGTTTAGCAAAGACAT-3'). The amplification protocol followed a previously published method by Nguyen et al. (2017). Each successfully amplified and sequenced fragment was assembled using ChromasPro v. 2.1.8 and confirmed by BLAST searches (Zhang et al. 2000). All nucleotide sequences were deposited in GenBank.

All holotypes and paratypes were deposited in the Department of Soil Ecology, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Hanoi, Vietnam.

Results

Taxonomy

Order Polydesmida Pocock, 1881

Family Paradoxosomatidae Daday, 1889
Genus *Orthomorphoides* Likhitrakarn, Golovatch & Panha, 2011

***Orthomorphoides sapa* sp. nov.**
http://zoobank.org/3BBAA752-06DC-47AD-894A-FAD9AD6E8283
Figs 1–5

Material examined. ***Holotype***: male (IEBR-Myr 710H), Vietnam, Lao Cai Province, Hoang Lien National Park, natural forest, 22.32250°N, 103.77081°E, 2478 m a.s.l., 7 July 2018, coll. Nguyen Dac-Dai. ***Paratype***: 1 female (IEBR-Myr 710P), together with holotype.

Diagnosis. The species is easily recognized by having a black sub-moniliform body, small and crest-shaped paraterga, a long and slender gonofemorite, a simple solenophore with neither modifications nor additional processes, a poorly developed lamina medialis, a well-developed lamina lateralis with a tongue-shaped process, and the gonopod tip with a broad apical lobule.

Description. Holotype body length about 22.1 mm, width of pro- and metazona 1.6 mm and 2.1 mm, respectively.

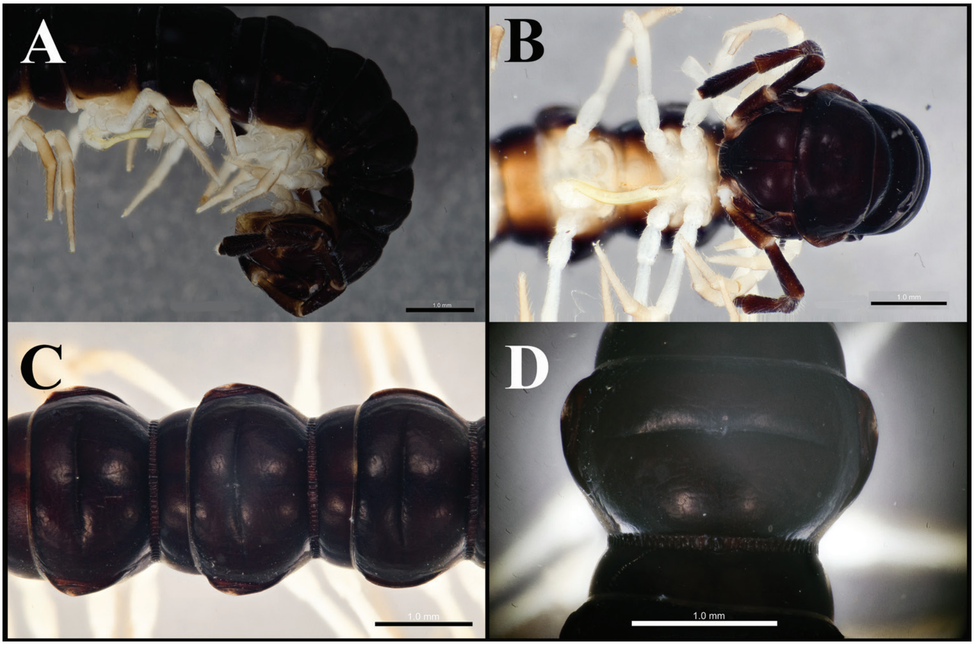


Figure 1. *Orthomorphoides sapa* sp. nov., holotype (IEBR-Myr 710H) anteriormost body segments, lateral view (**A**), ventral view (**B**) segments 8–10, dorsal view (**C**) segment 10, dorsal view (**D**).

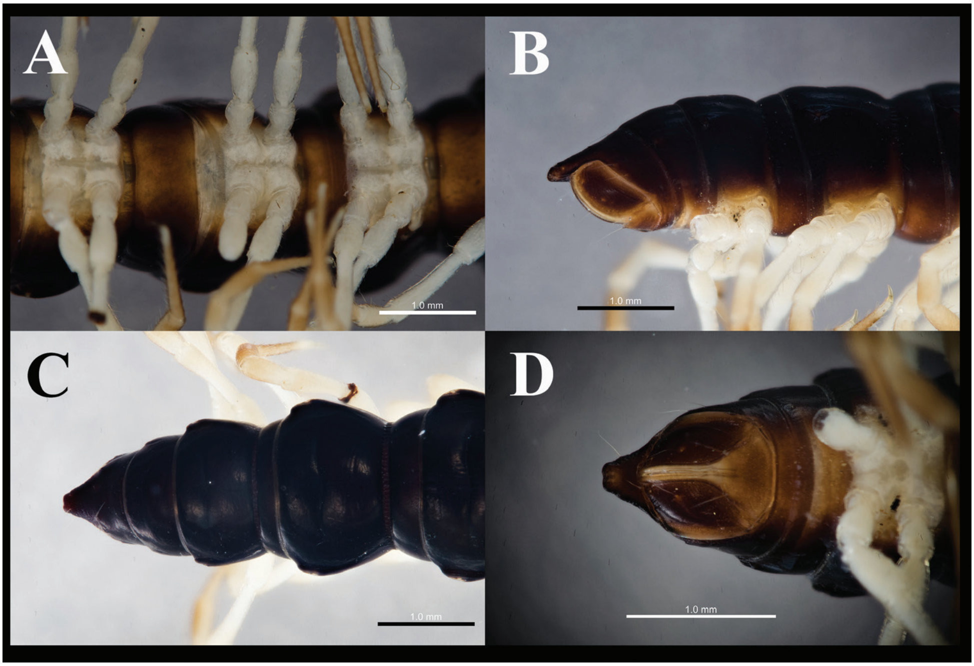


Figure 2. *Orthomorphoides sapa* sp. nov., holotype (IEBR-Myr 710H) body segments 8–10, ventral view (**A**) posteriormost segment, lateral view (**B**), dorsal view (**C**) telson, ventral view (**D**).

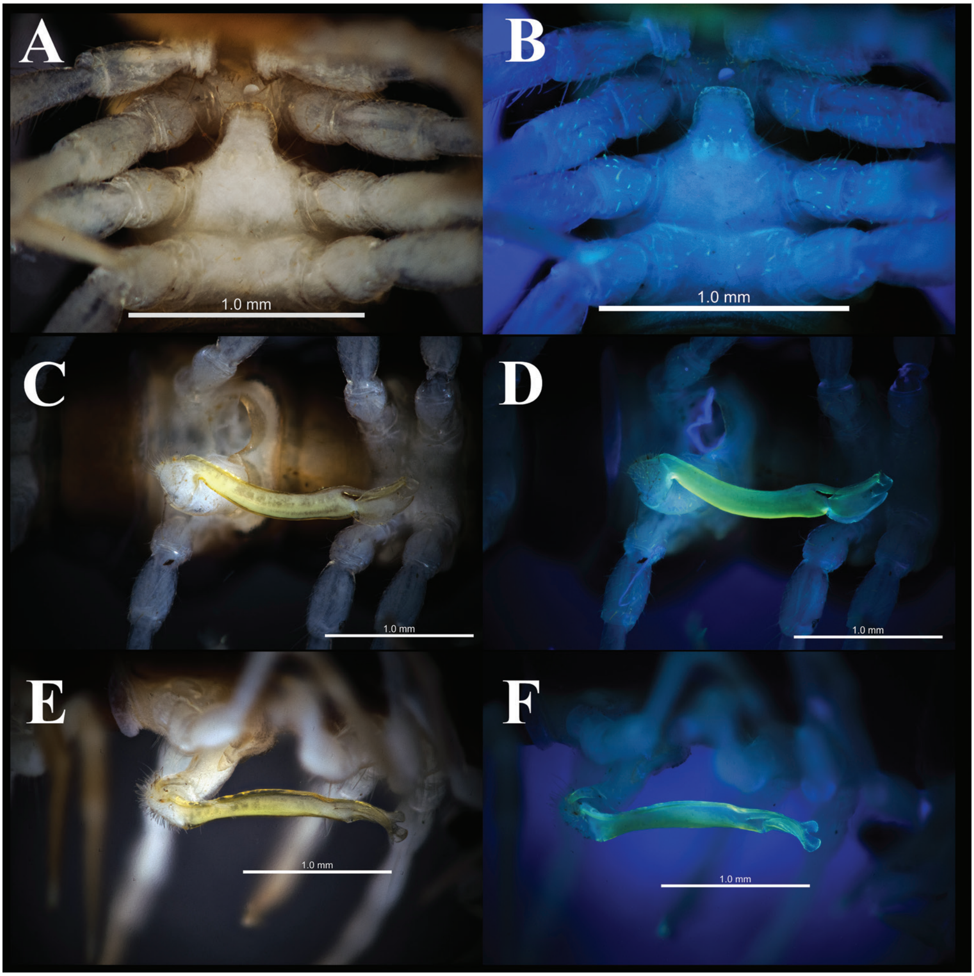


Figure 3. *Orthomorphoides sapa* sp. nov., holotype (IEBR-Myr 710H) sternal process between coxae 4, subventral view, normal light (**A**), UV light (**B**) right gonopod, ventral view, normal light (**C**), UV light (**D**), submesal view, normal light (**E**), UV light (**F**).

Colouration (Figs 1–2): body black except whitish yellow legs and sterna; several podomeres light brown.

Head (Fig. 1A, B) somewhat larger than collum, clypeolabral region densely setose, vertex sparsely setose. Epicranial suture distinct; frons with 2+2 setae along epicranial suture. Antenna long, slender, reaching segment 3 if stretched laterally; antennomere 2>3=4=5=6>1>7; tip with four sensory cones.

Collum (Fig. 1B) semicircular; surface smooth, shining with two rows of setae: 4+4 anterior and 1+1 intermediate; transverse sulcus present, but short, indistinct. Paratergum present, crest-shaped.

Body sub-moniliform (Figs 1C, D, 2C). Surface smooth and shining, without metatergal setae. Transverse metatergal sulci deep, line-shaped and present on all

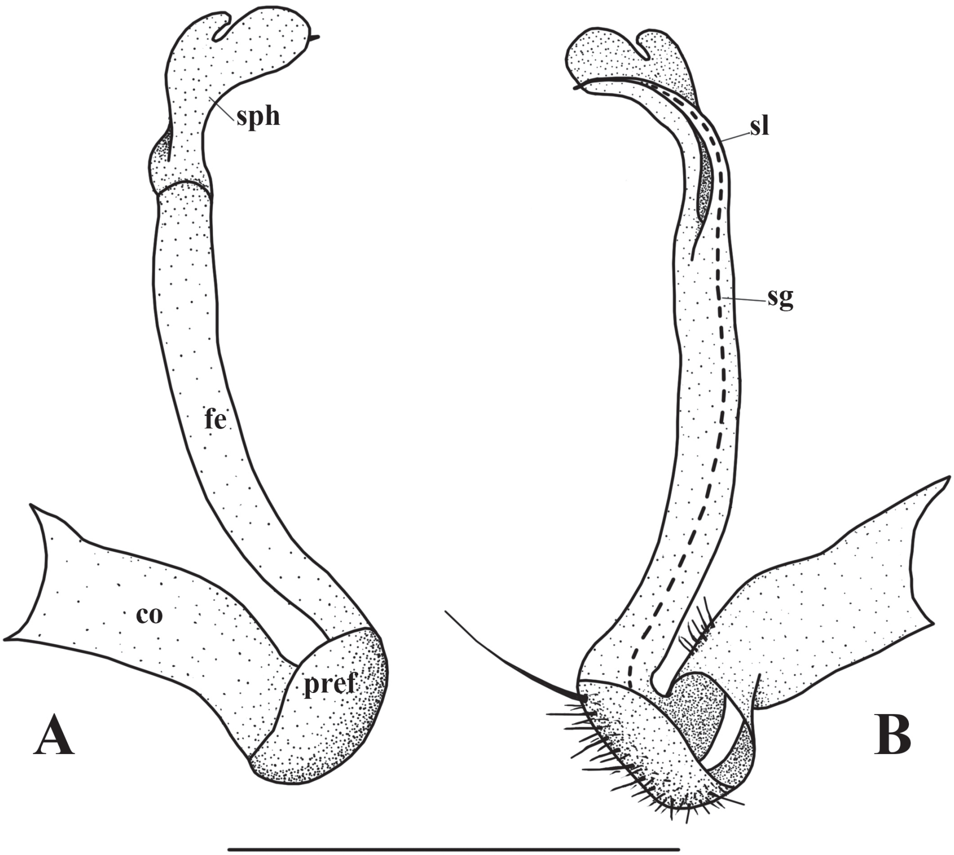


Figure 4. *Orthomorphoides sapa* sp. nov., holotype (IEBR-Myr 710H) left gonopod, lateral view (**A**), mesal view (**B**) co = coxite, pref = prefemorite, fe = femorite, sl = solenomere, sph = solenophore, sg = seminal groove. Scale bar: 0.5 mm.

segments. Waist between pro- and metazonae relatively deep, striolate. Paraterga present, but small, crest-shaped, lying lower than metatergal surface. Pleurosternal carinae present as complete crests on segments 2–3, reduced to caudal teeth on segments 4–7, and missing on subsequent segments. Axial line missing.

Epiproct (Fig. 2B–D) broadly truncated, flattened dorsoventrally; tip with four spinnerets. Hypoproct subtriangular, with two distolateral, separated setiferous knobs (Fig. 2D).

Sterna sparsely setose, with distinct cross-impressions, without modifications except for a setiferous, broadly tongue-shaped lobule between coxae 4 (Fig. 3A, B).

Legs slender, long about 1.6–1.8 times as long as midbody height. Femora without modifications. Prefemora not swollen. Tarsal brushes absent.

Gonopods simple (Figs 3C–F, 4, 5). Coxite long, cylindrical, distoventral part sparsely setose. Prefemorite densely setose, set off from femorite by an oblique sulcus laterally. Femorite long, slender, without processes or modifications. The demarcation

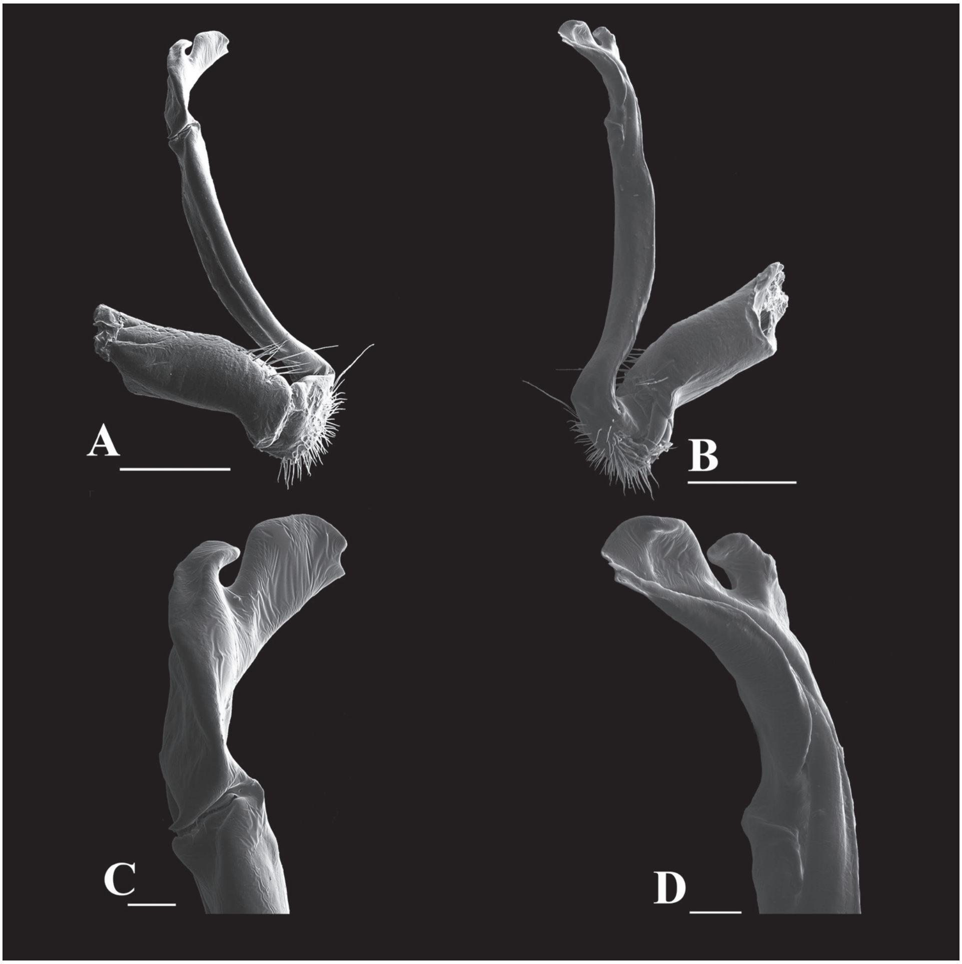


Figure 5. *Orthomorphoides sapa* sp. nov., holotype (IEBR-Myr 710H) left gonopod, lateral view (**A**), mesal view (**B**) postfemoral region of gonopod, lateral view (**C**), mesal view (**D**). Scale bars: 400 µm (**A, B**); 60 µm (**C, D**).

between postfemoral region and femorite present laterally. Lamina medialis of solenophore poorly developed while lamina lateralis well developed, with a tuberculiform process. Tip of gonopod broadly rounded lobule. Seminal groove running entirely on mesal side of femorite, then entering a flagelliform solenomere sheathed by solenophore.

DNA barcoding. Fragments of *COI* and *16S rRNA* genes were uploaded to GenBank with the accession numbers: MW647898 and MW648327, respectively. The new species has a close *COI* gene similarity with *Orthomorphoides setosus* (KU234720) of 87.17%. It also shares 74.04% and 72.68% of its *16S rRNA* gene sequence with *Asiomorpha coarctata* (KU721885) and *Pogonosternum nigrovirgatum* (KU745218), respectively.

Etymology. Named after Sapa, the type locality. It is a noun in apposition.

Remarks. The genus *Orthomorphoides* was extracted from the genus *Orthomorpha* by Likhitrakarn et al. (2011) for two species: *Orthomorpha setosa* (Attems, 1937) and *Orthomorpha exarata* (Attems, 1953). The genus, therefore, currently contains only two species, *Orthomorphoides setosus* from Lam Dong Province (Vietnam) and *Orthomorphoides exaratus* from Xieng Khouang (Laos).

This new species obviously belongs to the genus *Orthomorphoides* because of its generic characters, such as: long and slender femorite without visible modifications and processes, and the solenomere being sheathed by a solenophore except for the exposed tip. The new species does, however, clearly differ from the two known *Orthomorphoides* species in body shape and the degree of development of the paraterga. In addition, the solenophore of the new species carries a tuberculiform process laterally while neither *O. setosus* nor *O. exaratus* possess additional processes on the solenophore.

The two previously described *Orthomorphoides* species were found only in high mountains in southcentral Vietnam and Xieng Khouang (>1500 m a.s.l.) (Attems 1953). The discovery of this new species, found in Sapa, may further support the mountainous distribution of this genus, although this must be confirmed with additional field studies and possible discoveries.

Genus *Hylomus* Cool & Loomis, 1924

Hylomus solenophorus sp. nov.

http://zoobank.org/2BAC98BA-8221-4C8B-B1B5-63ACD3534CE9
Figs 6–10

Material examined. ***Holotype.*** male (IEBR-Myr 712), Vietnam, Lao Cai Province, Hoang Lien National Park, natural forest, 22.32250°N, -103.77081°E, 2478 m a.s.l., 7 July 2018, coll. Nguyen Dac-Dai. ***Paratype.*** 1 male, 1 female (IEBR-Myr 714), Lao Cai Province, Hoang Lien National Park, natural forest, 22.32129°N, -103.77094°E, 2547 m a.s.l., 7 July 2018, coll. Nguyen Dac-Dai.

Diagnosis. The species differs from its congeners by having wing paraterga, two rows of 2+2 and 2+2 knobs on the metaterga, and a well-developed, broad gonopod solenophore with an additional distal process.

Description. Holotype body length about 16.0 mm, width of pro- and metazona 1.2 mm and 2.0 mm, respectively.

Coloration (Figs 6, 7): whole body blackish brown or black, except labrum, sterna and legs yellowish brown. Projected caudal corners of paraterga yellowish brown.

Head (Fig. 6C, D) slightly smaller than body segments, but somewhat larger than collum. Epicranial suture clearly distinct; frons with 2+2 setae along epicranial suture. Clypeolabral region moderately setose. Antenna extremely long, reaching segment 5 if stretched laterally; antennomere 3=4=5>2>6>1>7; tip with four sensory cones.

Collum (Fig. 6A) slightly smaller than segment 2, semicircular; surface not smooth, with 3 rows of setiferous knobs: 3+3 anterior, 2+2 intermediate and 1+1 posterior; transverse sulcus present, located near a row of 1+1 posterior knobs. Paratergum broadly triangular, well developed, with a lateral incision (Fig. 6A).

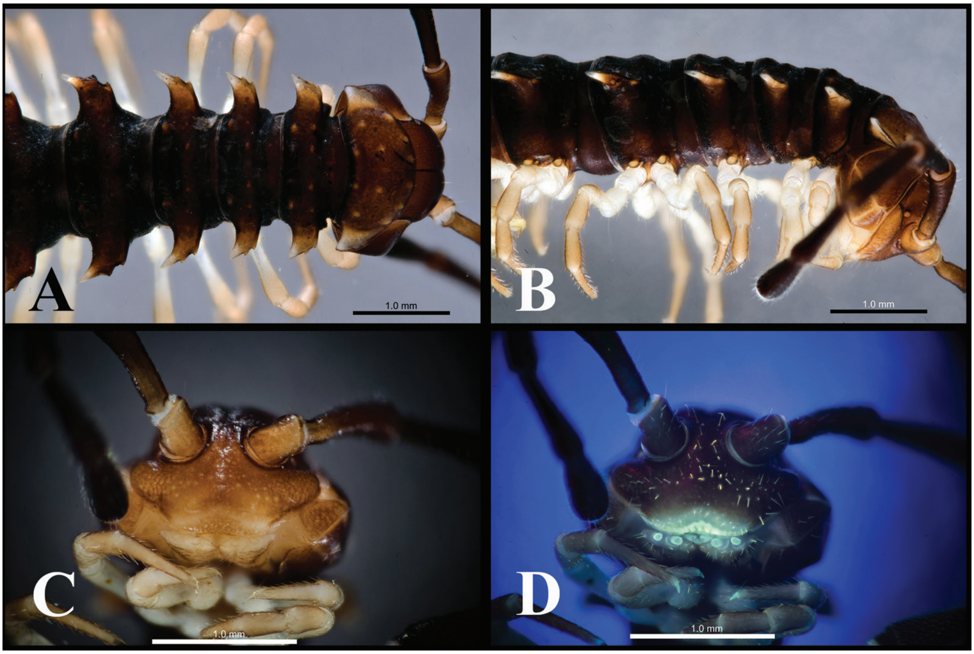


Figure 6. *Hylomus solenophorus* sp. nov., holotype (IEBR-Myr 712H) anteriormost body segments, dorsal view (**A**), lateral view (**B**) head, anterior view (**C, D**).

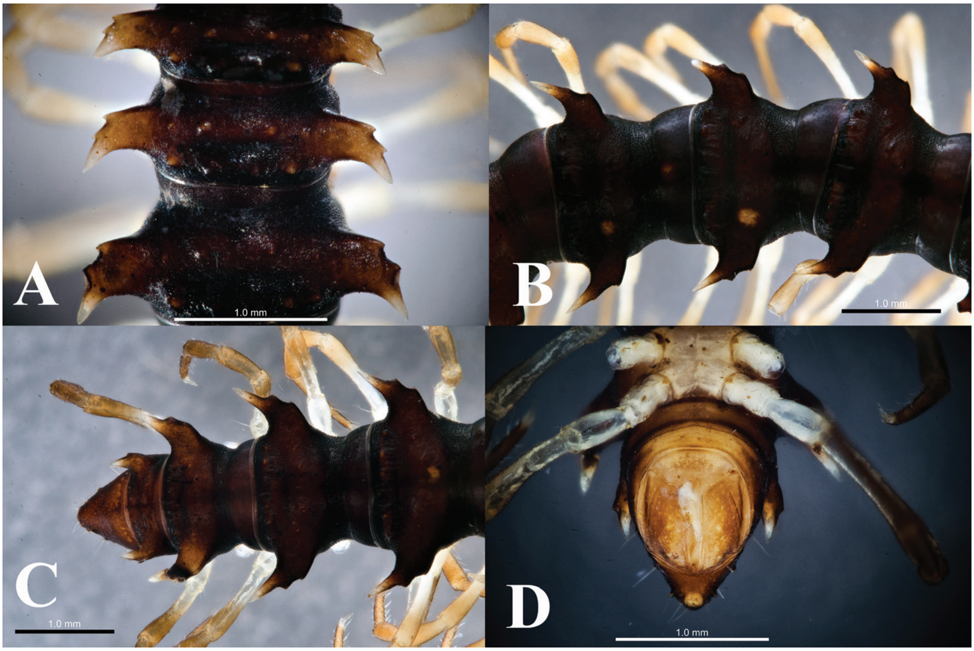


Figure 7. *Hylomus solenophorus* sp. nov., holotype (IEBR-Myr 712H) segments 8–10, dorsal view (**A, B**); posteriormost segments, dorsal view (**C**); telson, ventral view (**D**).

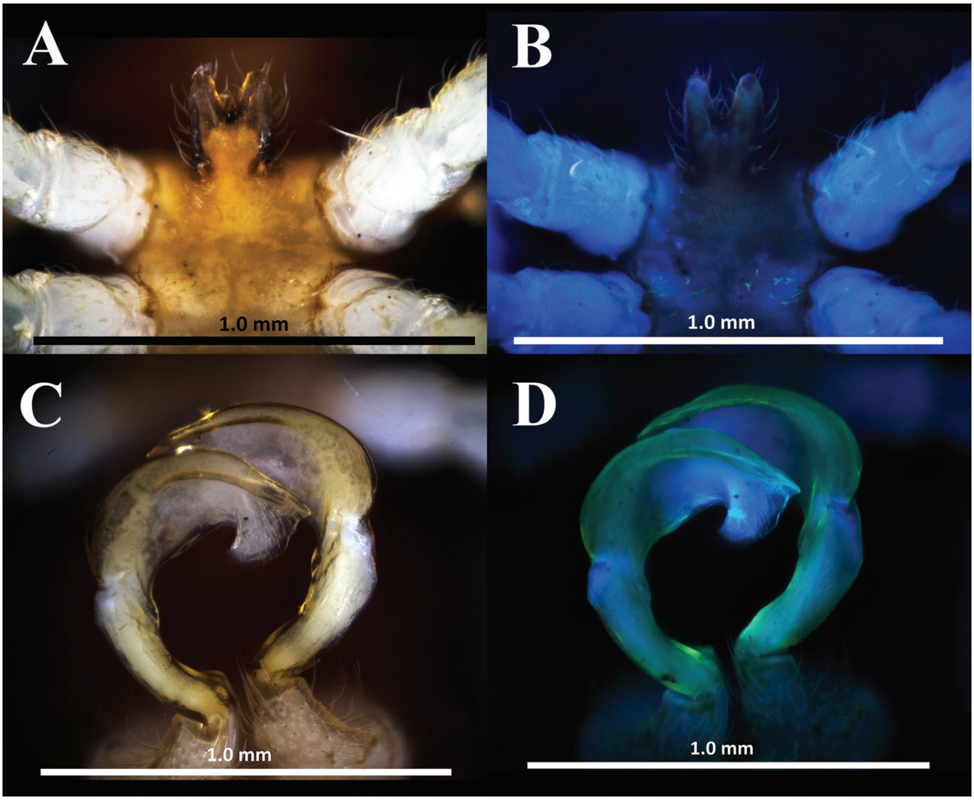


Figure 8. *Hylomus solenophorus* sp. nov., holotype (IEBR-Myr 712H) sternal process between coxae 4, subventral view, normal light (**A**), UV light (**B**) gonopods, ventral view, normal light (**C**), UV light (**D**).

Body segment 3<4<2=5–17, thereafter gradually tapering toward telson (Figs 6A, B, 7A–C). Prozonae smooth, shagreened while metazonae densely covered with microgranulation. Transverse metatergal sulci shallow, broad, present on all segment. Metaterga 2–4 with two rows of setiferous knobs: 2+2 and 2+2 in front of and behind transverse sulcus, respectively; other metaterga with two rows of 2+2 and 3+3 setiferous knobs. Axial line distinct, thin. Waist between pro- and metazonae indistinct, shallow, and broad. Pleurosternal carinae absent.

Paraterga (Figs 6A, 7A–C)) wing-shaped, with pointed, projected caudal corners, lying horizonally, but reduced as broad-base spine with two lateral incisions on segments 7–18.

Epiproct long, broadly truncated, flattened dorsoventrally; tip with four spinnerets (Fig. 7D). Hypoproct trapeziform, with two distolateral, separated setiferous knobs (Fig. 7D).

Sterna sparsely setose, with distinct cross-impressions, without modifications except for an anterioventrally directed, large, strongly bi-lobuled process between coxae 4 (Fig. 8A, B).

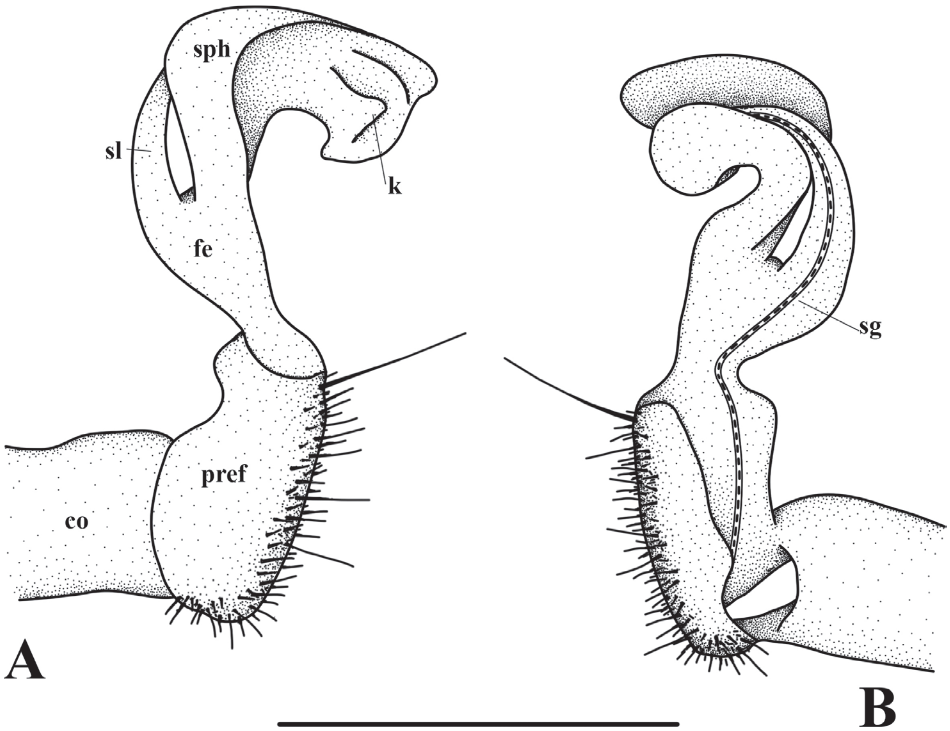


Figure 9. *Hylomus solenophorus* sp. nov., holotype (IEBR-Myr 712H) left gonopod, lateral view (**A**), mesal view (**B**) co = coxite, pref = prefemorite, fe = femorite, sl = solenomere, sph = solenophore, sg = seminal groove, k = distoapical process k. Scale bar: 1 mm.

Legs slender, long about 1.5–1.7 times as long as midbody height. Prefemora not swollen. Femora without modifications. Tarsal brushes absent.

Gonopods simple (Figs 8C, D, 9, 10). Coxite cylindrical, distoventral part sparsely setose. Prefemorite densely setose, set off from femorite by an oblique sulcus laterally. Femorite slightly curved mesad, somewhat enlarged distally, without modifications and processes. Demarcation between femorite and postfemoral region absent. Postfemoral region consisting of solenophore and solenomere. Femorite and solenophore subequal in length. Solenophore simple, broad, slightly spiral and somewhat curved down. Tip of gonopod broadly round, with an additional distoapical process. Seminal groove running entirely on mesal side of femorite, then entering the flagelliform solenomere sheathed by solenophore.

DNA characters. Fragments of COI and 16S rRNA were uploaded to GenBank with accession numbers: MW647899 and MW648328, respectively. The new species has a close COI identity with *Hylomus cervarius* (MG669370) and *Hylomus enghoffi* (MG669369) of 85.1% and 83.45%, respectively. It also shares 79.57% and 78.72% of its *16S rRNA* gene sequence with *Hylomus cervarius* (MG564329) and *Hylomus enghoffi* (MG564330), respectively.

Figure 10. *Hylomus solenophorus* sp. nov., holotype (IEBR-Myr 712H) left gonopod, ventral view (**A**), lateral view (**B**), mesal view (**C**) postfemoral region, mesal view (**D**) tip of gonopod, ventral view (**E**) sl = solenomere, sph = solenophore, k = distoapical process k. Scale bars: 100 μm (**A–C, E**); 40 μm (**D**).

Etymology. An epithet "*solenophorus*" is used to emphasize the well-developed, broad solenophore carrying a triangular distoapical process.

Remarks. The genus *Hylomus* Cook & Loomis, 1924 used to be a synonym of the genus *Desmoxytes* Chamberlin, 1923 (Golovatch and Enghoff 1994), but it was recently re-evaluated by Srisonchai et al. (2018) and currently comprises 39 species (Sierwald and Spelda 2021). The new species differs distinctly from the majority of known *Hylomus* species (except for *H. spectabilis*) in having wing-shaped paraterga (vs. antler-like or spiniform paraterga). As already stated, *Hylomus solenophorus* sp. nov. is closely similar to *H. spectabilis* (Attems, 1937) from central Vietnam by having wing-shaped paraterga and the same gonopod conformation. On the other hand, the newly described species differs clearly from *H. spectabilis* by being smaller in size (length: 16 mm vs. 30 mm; width of metazona: 2.0 mm vs. 6.1 mm) and having darker body coloration (darkish brown or black vs. brown). Furthermore, leg femora of the new species possess no visible modifications, metaterga contain two rows of 2 + 2 and 3 + 3 setiferous knobs and the gonopod has no spine z, but possesses a triangular distoapical process, instead. By comparison, the 7th femur of the *H. spectabilis* male has a ventral hump, metaterga are characterised by two transversal rows of 2 + 2 well-developed spines and 1 + 1 smaller spines and the gonopod is characterised by well-developed spine z, but no process k.

Discussion

The Hoang Lien Son Mountain Range with Mt. Fansipan plays a very important role in general geodistribution of animals in Vietnam, particularly of millipedes (Sterling et al. 2006). This mountain range is considered the southeasternmost extension of the Himalaya Range containing both Indian and Chinese zoological features. The Hoang Lien Son granites date from 80 to 29 million years ago, while the uplift of the range by tectonic activity began around 65 million years ago and continues to this day. This is also an interesting place for both ecological and biodiversity studies (Sterling et al. 2006). Despite its important role, the fauna of the mountain, in particular, the soil invertebrates, is still poorly known.

As described by Attems (1937, 1953), Enghoff (1987), Golovatch (1984, 2009), Nguyen et al. (2005) and Nguyen (2012), few millipede species have been discovered in this mountain. The recent record of two new species, *Hyleoglomeris hoanglien* (Nguyen, Eguchi & Hwang, 2019) and *Hyleoglomeris fanxipan* (Nguyen, Eguchi & Hwang, 2019) at very high elevation of 2800 m, proves that this region needs to be more intensively surveyed in order to obtain a comprehensive knowledge of the local fauna (Nguyen et al. 2019).

The Paradoxosomatidae is a huge family containing more than 1000 species distributed in about 220 genera (Nguyen and Sierwald 2013). Phylogenetic relationships between the paradoxosomatid genera and species within the family are still questionable. There are, however, some available DNA data for members of this family (183 *COI* gene records and 53 *16S rRNA* gene records in GenBank). It is not considered very practical to analyse the relationships between the two new species (belonging to different genera) and other known paradoxosomatid species using two short fragments of *COI* and *16S rRNA* genes. As a result, although the DNA barcoding data are provided here for the two new species, neither the genetic divergence nor phylogenetic relationships are taken into account. The provided DNA barcoding data should be considered an additional characterisation tool for the new species descriptions and identification.

Conclusion

Most of World's high-mountain regions are very difficult to access, but their natural habitats are well conserved. It is, therefore, highly expected that more intensive surveys in those regions will reveal many new taxa, not only of millipedes but also of other invertebrates.

Acknowledgements

This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 106.05-2019.320 to Anh D. Nguyen. The work is also partly supported by Tokyo Metropolitan University Fund for TMU Strategic Research (Leader: Prof. Noriaki Murakami; FY2020-FY2022) and Asahi Glass Foundation 933 (Leader: Katsuyuki Eguchi; FY2017-FY2020). The first author sincerely thanks Dr Petra Sierwald and Ms Stephanie Ware for kindly helping to take photographs during his visit to the Field Museum of Natural History, Chicago (USA). Drs Sergei Golovatch (Russia) and Catherine Car (Australia) are acknowledged for their invaluable comments and suggestions to improve the English.

References

Attems C (1937) Myriapoda 3. Polydesmoidea I. Fam. Strongylosomidae. Das Tierreich 68: 1–300. https://doi.org/10.1515/9783111567099

Attems C (1938) Die von Dr. C. Dawydoff in französisch Indochina gesammelten Myriopoden. Mémoires du Muséum national d'histoire naturelle N.S. 6(2): 187–353.

Attems C (1953) Myriopoden von Indochina. Expedition von Dr. C. Dawydoff (1938–1939). Mémoires du Muséum National d'Histoire Naturelle {N.S., Sér. A, Zool.} 5(3): 133–230.

Enghoff H (1987) Revision of *Nepalmatoiulus* Mauries 1983 – a Southeast Asiatic genus of millipedes (Diplopoda: Julida: Julidae). Courier Forschungsinstitut Senckenberg 93: 241–331.

Golovatch SI (1984) Contribution to the millipede fauna of Vietnam (Diplopoda), II. Acta Zoologica Hungarica 30(1–2): 53–77.

Golovatch SI (2009) On several new or poorly-known Oriental Paradoxosomatidae (Diplopoda: Polydesmida), IX. Arthropoda Selecta 18(3/4): 119–124. https://kmkjournals.com/upload/PDF/ArthropodaSelecta/18/18_3_119_124_Golovatch.pdf

Golovatch SI, Enghoff H (1993) Review of the genus *Tylopus*, with descriptions of new species from Thailand (Diplopoda, Polydesmida, Paradoxosomatidae). Steenstrupia 19(3): 85–125.

Golovatch SI, Enghoff H (1994) Review of the dragon millipede, genus *Desmoxytes* Chamberlin, 1923 (Diplopoda, Polydesmida, Paradoxosomatidae). Steenstrupia 20(2): 1–71.

Le Ba Thao (2017) Vietnam, the country and its geographical regions. The Gioi Publishers, 620 pp.

Likhitrakarn N, Golovatch SI, Panha S (2011) Revision of the Southeast Asian millipede genus *Orthomorpha* Bollman, 1893, with the proposal of a new genus (Diplopoda, Polydesmida, Paradoxosomatidae). ZooKeys 131: 1–161. https://doi.org/10.3897/zookeys.131.1921

Nguyen AD (2012) *Tylopus* millipedes in Vietnam (Diplopoda: Polydesmida: Paradoxosomatidae: Sulciferini), with descriptions of five new species. The Raffles Bulletin of Zoology 60(2): 289–31. https://lkcnhm.nus.edu.sg/wp-content/uploads/sites/10/app/uploads/2017/06/60rbz289-311.pdf

Nguyen AD, Eguchi K, Hwang UW (2019) Two new pill millipedes (Diplopoda: Glomerida: Glomeridae) from high mountains of Vietnam. Journal of Natural History 53(21–22): 1369–1384. https://doi.org/10.1080/00222933.2019.1646338

Nguyen AD, Golovatch SI, Anichkin AE (2005) Dragon millipedes (Polydesmida, Paradoxosomatidae, genus *Desmoxytes*) in Vietnam. Arthropoda Selecta 13(3): 251–257. https://kmkjournals.com/upload/PDF/ArthropodaSelecta/14/14_3%20251_257%20Nguyen.pdf

Nguyen AD, Korsós Z, Jang KH, Hwang UW (2017) A revision and phylogenetic analysis of the millipede genus *Oxidus* Cook, 1911 (Polydesmida: Paradoxosomatidae). European Journal of Taxonomy 293: 1–22. https://doi.org/10.5852/ejt.2017.293

Nguyen AD, Sierwald P (2013) A worldwide catalog of the family Paradoxosomatidae Daday, 1889 (Diplopoda: Polydesmida). CheckList 9(6): 1132–1353. https://doi.org/10.15560/9.6.1132

Sierwald P, Hennen DA, Zahnle XJ, Ware S, Marek PE (2019) Taxonomic synthesis of the eastern North American millipede genus *Pseudopolydesmus* (Diplopoda: Polydesmida: Polydesmidae), utilizing high-detail ultraviolet fluorescence imaging, Zoological Journal of the Linnean Society 187(1): 117–142. https://doi.org/10.1093/zoolinnean/zlz020

Sierwald P, Spelda J (2021) MilliBase. *Hylomus* Cook & Loomis, 1924. http://www.millibase.org/aphia.php?p=taxdetails&id=893579 [Accessed on 2021-02-09]

Srisonchai R, Enghoff H, Likhitrakarn N, Panha S (2018) A revision of dragon millipedes I: genus *Desmoxytes* Chamberlin, 1923, with the description of eight new species (Diplopoda, Polydesmida, Paradoxosomatidae). Zookeys 761: 1–177. https://doi.org/10.3897/zookeys.761.24214

Sterling EJ, Hurley MM, Le DM (2006) Vietnam: a natural history. Yale University Press, 448 pp.

Zhang Z, Schwartz S, Wagner L, Miller W (2000) A greedy algorithm for aligning DNA sequences. Journal of Computational Biology 7(1–2): 203–214. https://doi.org/10.1089/10665270050081478

ZooKeys 1032: 17–62 (2021)
doi: 10.3897/zookeys.1032.62001
https://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

A review of the taxonomy of spiny-backed orb-weaving spiders of the subfamily Gasteracanthinae (Araneae, Araneidae) in Thailand

Kongkit Macharoenboon[1], Warut Siriwut[1], Ekgachai Jeratthitikul[1]

[1] *Animal Systematics and Molecular Ecology Laboratory, Department of Biology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*

Corresponding author: Ekgachai Jeratthitikul (Ekgachai.jer@mahidol.edu)

Academic editor: I. Agnarsson | Received 13 December 2020 | Accepted 17 March 2021 | Published 16 April 2021

http://zoobank.org/EBF7586A-9EF7-48FE-BFB5-3D8F1E372120

Citation: Macharoenboon K, Siriwut W, Jeratthitikul E (2021) A review of the taxonomy of spiny-backed orb-weaving spiders of the subfamily Gasteracanthinae (Araneae, Araneidae) in Thailand. ZooKeys 1032: 17–62. https://doi.org/10.3897/zookeys.1032.62001

Abstract

Spiny-backed orb-weaving spiders of the subfamily Gasteracanthinae are broadly distributed in the Old World. Despite their use as a model species in biology, evolution, and behavior because of their extraordinary characteristics, the systematics of this group of spiders are still poorly understood. This study elucidates the systematics of Gasteracanthinae in Thailand based on morphological and molecular-based analyses. In total, seven species from three genera, namely *Gasteracantha*, *Macracantha*, and *Thelacantha*, were recorded in Thailand. Shape of abdominal spines, pattern of sigilla, and female genitalia are significant characters for species identification. In contrast, coloration shows highly intraspecific variation in most species within Gasteracanthinae. A phylogenetic tree based on partial sequences of COI, 16S, and H3 genes recovered Gasteracanthinae as a monophyletic group and supports the existence of three clades. *Gasteracantha hasselti* is placed as a sister taxon to *Macracantha arcuata*. Hence, we propose to transfer *G. hasselti* to *Macracantha.* Moreover, molecular species delimitation analyses (ABGD, bPTP, and GMYC) using 675 bp of COI gene support all nominal species, with evidence of possible additional cryptic species.

Keywords

Gasteracanthinae, molecular phylogeny, species delimitation, taxonomy, Thailand

Introduction

Spiny-backed orb-weaving spiders are a group of spiders typically featuring an abdomen decorated with conspicuous spines and of notable coloration (Pickard-Cambridge 1879; Simon 1892; Dahl 1914; Emerit 1974; Barrion and Litsinger 1995). These spiders are currently classified into two subfamilies, Micratheninae and Gasteracanthinae (Scharff and Coddington 1997). Micratheninae is widespread in the New World, while most species within Gasteracanthinae are broadly distributed in the Old World (Scharff and Coddington 1997). It has been suggested that the abdominal spines of members of both Gasteracanthinae and Micratheninae serve as defensive structures (Peckham 1889; Cloudsley-Thompson 1995), whereas the distinct coloration possibly plays a role in prey attraction (Kemp et al. 2013) and aposematism (Cloudsley-Thompson 1995). Some species also exhibit intraspecific color polymorphism, for example, *Gasteracantha cancriformis* (Linnaeus, 1758), *Gasteracantha fornicata* (Fabricius, 1775), and *Thelacantha brevispina* (Doleschall, 1857) (Truong 2012; Kemp et al. 2013; Salgado-Roa et al. 2018; Chamberland et al. 2020). This polymorphism suggests different adaptive advantages for each morph and/or the effect of frequency-dependent selection (Punzalan et al. 2005; Ishii and Shimada 2010; Cotoras et al. 2016; White and Kemp 2016). Moreover, these spiders are well known for their sexual dimorphism: the males are extremely reduced in size, and their spines are poorly developed (Pickard-Cambridge 1879; Hormiga et al. 2000). Due to such extraordinary characteristics of Gasteracanthinae, they are frequently used as species models for evolutionary, biological, ecological, and behavioral studies (i.e., Yoshida 1989; Jaffé et al. 2006; Gawryszewski and Motta 2012; Kemp et al. 2013).

The taxonomy of Gasteracanthinae was first proposed by Simon in 1892. The author placed almost all old-world spiny-backed orb-weavers in the tribe Gasteracantheae, which feature distinct morphological characters, i.e., a hard-sclerotized abdomen that overlaps the cephalothorax, the presence of conspicuous sigilla on dorsal abdomen, and prominent abdominal spines (Simon 1892). Subsequently, Dahl (1914) classified *Gasteracantha*, the predominant genus in Gasteracanthinae, into 16 subgenera based on the shape and position of abdominal spines, structure of abdomen, and sigilla pattern. Since then, several subgeneric names or junior synonyms of *Gasteracantha* have been revalidated (Benoit 1962, 1964; Emerit 1974). Scharff and Coddington (1997) reconstructed the phylogeny of Araneidae and revealed that spiny-backed orb-weaving spiders did not represent a monophyletic group, but were instead separated into two monophyletic clades, consisting of species from the Old World and New World, respectively. Based on these results, the authors classified all new-world genera into subfamily Micratheninae, and placed *Gasteracantha* and the rest of the old-world genera in subfamily Gasteracanthinae. The distant relationship between Micratheninae and Gasteracanthinae was later supported by several molecular and transcriptomic studies (Álvarez-Padilla et al. 2009; Dimitrov et al. 2017; Wheeler et al. 2017; Fernández et al. 2018; Kallal et al. 2018; Scharff et al. 2020).

Thailand is located within two significant biodiversity hotspots, Indo-Burma and Sundaland, and is home to a high biodiversity of flora and fauna (Myers et al. 2000). At the time of writing, 43 species of spiders from four genera of Gasteracanthinae (*Actinacantha* Simon, 1864; *Gasteracantha* Sundevall, 1833; *Macracantha* Fabricius, 1793; and *Thelacantha* Hasselt, 1882) have been recorded in Southeast Asia, of which ten species from three genera were recorded in Thailand (World Spider Catalog 2020), including *Gasteracantha clavigera* Giebel, 1863; *Gasteracantha diadesmia* Thorell, 1887; *Gasteracantha diardi* (Lucas 1835); *Gasteracantha frontata* Blackwall, 1864; *Gasteracantha irradiata* (Walckenaer, 1841); *Gasteracantha kuhli* C. L. Koch, 1837; *Gasteracantha hasselti* C. L. Koch, 1837; *Gasteracantha rubrospinis* Guérin, 1838; *Macracantha arcuata* (Fabricius, 1793); and *Thelacantha brevispina* (Doleschall, 1857). However, the taxonomy of Gasteracanthinae at the species level remains unclear because of the general scarcity of male specimens for morphological and molecular study, the lack of morphological characters for the identification of sub-adults and male spiders, and intraspecific morphological variation and morphological resemblance among closely related species (Pickard-Cambridge 1879; Dahl 1914; Tan et al. 2019). Molecular approaches in terms of DNA barcoding and species delimitation can resolve these taxonomic issues. These techniques were successfully applied in several studies of different spider groups, and can be especially helpful in differentiating among morphologically similar taxa (Zhang and Li 2014; Hedin 2015; Ortiz and Francke 2016). However, molecular data of Gasteracanthinae in South East Asia are still lacking. Only the study by Tan et. al. (2019) has focused on phylogeny of Gasteracanthinae at species/population levels.

The objective of this study is to elucidate the taxonomy of spiny-backed orb-weavers in subfamily Gasteracanthinae, specifically in *Gasteracantha*, *Macracantha*, and *Thelacantha*, based on the morphological and molecular analyses of specimens from Thailand.

Materials and methods

Specimen sampling

Spiders were collected throughout Thailand by visual searching in several types of habitats, including rainforest, dipterocarp forest, paddy field, mangrove forest, and areas with human development. Specimens were euthanized following methods of Cooper (2011). Animal use in this study was approved by the Faculty of Science, Mahidol University Animal Care and Use Committee SCMU-ACUC (MUSC62-002-466). All specimens were preserved in 95% (v/v) ethanol and kept at -20 °C for molecular work and long-term storage. The dorsal and ventral views of each morphotype were photographed using Nikon D7200 + Nikon AF-S Nikkor 105 mm f/2.8G IF-ED VR Micro. All voucher specimens were deposited at Mahidol University of Natural History Museum, Salaya, Thailand (**MUMNH**).

Species identification

Species identification was primarily based on external and internal morphology, with emphasis on the characteristics of shape and position of abdominal spines, color pattern on abdomen, sigilla pattern, and epigynal structure. The morphology of each species was

Table 1. Primers used for the PCR reaction and sequencing in this study.

Genes	Primer	Reference
COI	LCO-1490: 5'-GGT CAA CAA ATC ATA AAG ATA TAT TGG-3'	Folmer et al. (1994), Simon et al. (1994)
	NANCY: 5'-CCC-GGT-AAA-ATT-AAA-ATA-TAA-ACT-TC-3'	
16S	16Sa: 5'-CGC-CTG-TTT-ATC-AAA-AAC-AT-3'	Palumbi et al. (1991)
	16Sb: 5'-CTC-CGG-TTT-GAA-CTC-AGA-TCA-3'	
H3	H3aF: 5'-ATG-GCT-CGT-ACC-AAG-CAG-ACV-GC-3'	Colgan et al. (1998)
	H3aR: 5'-ATA-TCC-TTR-GGC-ATR-ATR-GTG-AC-3'	

examined by using complete adult female specimens. Previous taxonomic publications including original descriptions were used as reference for species identification (Simon 1877; Pickard-Cambridge 1879; Thorell 1887; Pocock 1900; Dahl 1914; Emerit 1974; Tikader 1982; Barrion and Litsinger 1995; Sen et al. 2015; Roy et al. 2017). In order to observe female reproductive organs, the ventral plate was removed using an insect pin. It was immersed in saturated 5% (v/v) KOH for 5–10 minutes to clean off remaining soft tissue, then washed in distilled water. Internal and external morphology of specimens was observed under Nikon stereoscopic zoom microscope SMZ745. All measurements were taken from the left side of the body and recorded in millimeters. Leg measurements are provided as total length (femur, patella, tibia, metatarsus, and tarsus).

Abbreviations for female genitalia are: **S** = spermatheca, **CD** = copulatory duct, **FD** = fertilization duct, **EF** = epigastric furrow, **UP** = upper patch (the sclerotized plate on the top of epigynum), and **SC** = scape.

Molecular analyses

A total of 32 individuals were selected. Fragments of two mitochondrial genes, Cytochrome c oxidase subunit I (COI) and 16S rRNA (16S); and one nuclear marker, Histone subunit 3 (H3) were amplified as molecular markers. Genomic DNA was extracted from four right legs of each spider by using NucleoSpin tissue kit (MACHEREY-NAGEL, Germany). Primer sets used for PCR amplification are summarized in Table 1. PCR reactions were performed using T100 thermal cycle (Bio-Rad Laboratories, USA) with the following conditions: 5 min at 94 °C; 30 cycles of denaturation for 60 s at 94 °C, annealing for 45 s at 48–51 °C, and elongation for 90 s at 72 °C; pre-denaturation for 3 min at 94 °C, and post-elongation for 4 min at 72 °C. The final total PCR volume was 30 µl, consisting of 15 µl of EmeraldAmp PCR Maser Mix (TAKARA BIO INC.), 1.5 µl of both forward and reverse primers, 9 µl of distilled water, and 3 µl of template DNA (at least 25 ng). PCR products were checked by running a 1.5% agarose gel electrophoresis, and were purified by PEG precipitation. Purified samples were sequenced by Sanger method using automated sequencer (ABI prism 3730XL).

Phylogenetic analyses

Sequences were automatically aligned in MEGA X (Kumar et al. 2018) using MUSCLE alignment (Edgar 2004), then manually checked and edited. Edited sequences

Table 2. Samples used in this study, with specimen vouchers and GenBank accession numbers.

species	Voucher	Locality	Accession number			Reference
			COI	16S	H3	
Gasteracantha diadesmia	MUMNH-ARA-GAS011	Nakhon Ratchasima, Thailand	MT584892	MT584924	-	This study
	MUMNH-ARA-GAS047	Mae Hong Sorn, Thailand	MT584893	MT584925	MT584953	This study
	MUMNH-ARA-GAS067	Surat Thani, Thailand	MT584894	MT584926	MT584954	This study
	MUMNH-ARA-GAS117	Loei, Thailand	MT584895	MT584927	MT584955	This study
Gasteracantha diardi	MUMNH-ARA-GAS021	Chumpon, Thailand	MT584896	MT584928	MT371076	This study
	MUMNH-ARA-GAS104	Nakhon Si Thammarat, Thailand	MT584897	MT584929	MT584956	This study
	MUMNH-ARA-GAS127	Phumi Pôpôk Vil, Cambodia	MT584898	MT584930	MT584957	This study
	MUMNH-ARA-GAS129	Chiangmai, Thailand	MT584899	MT584931	-	This study
	MUMNH-ARA-GAS132	Nakhon Ratchasima, Thailand	MT584900	MT584932	-	This study
	GDIA1	Kedah, Malaysia -	KU055841	KU055746	MG670171	Tan et al. 2019
	GDIA3	Penang, Malaysia	MG670114	MG670142	MG670173	Tan et al. 2019
Gasteracantha doriae	MUMNH-ARA-GAS053	Trat, Thailand	MT584901	MT584933	MT584958	This study
	MUMNH-ARA-GAS068	Suratthani, Thailand	MT584902	MT584934	MT584959	This study
	MUMNH-ARA-GAS130	Rayong, Thailand	MT584890	MT584922	MT584951	This study
	MUMNH-ARA-GAS131	Rayong, Thailand	MT584891	MT584923	MT584952	This study
	GDIA5	Perak, Malaysia	MG670116	MG670144	MG670175	Tan et al. 2019
	GDIA6	Perak, Malaysia	MG670117	MG670145	MG670176	Tan et al. 2019
Gasteracantha kuhli	MUMNH-ARA-GAS007	Surat Thani, Thailand	MT584909	MT584941	-	This study
	MUMNH-ARA-GAS029	Ratchaburi, Thailand	MT584910	MT584942	MT371077	This study
	MUMNH-ARA-GAS033	Samut Prakan, Thailand	MT584911	MT584943	MT584962	This study
	MUMNH-ARA-GAS042	Krabi, Thailand	MT584912	MT584944	MT584963	This study
	MUMNH-ARA-GAS101	Chiangmai, Thailand	MT584913	MT584945	-	This study
	GKUH2	Selangor, Malaysia	MG670118	MG670146	MG670177	Tan et al. 2019
	GKUH3	Pahang, Malaysia	MG670119	MG670147	MG670178	Tan et al. 2019
Gasteracantha cancriformis	787198	Hispaniola	KJ157212	KJ156989	-	McHugh et al. 2014
	782149	Puerto Rico	KJ157214	KJ156990	-	McHugh et al. 2014
	N/A	N/A	FJ525321	FJ525354	FJ525340	Agnarsson and Blackledge 2009
Macracantha arcuata	MUMNH-ARA-MAC005	Krabi, Thailand	MT584914	MT584946	MT584964	This study
	MUMNH-ARA-MAC008	Prachuab Khiri Khan, Thailand	MT584915	MT584947	MT584965	This study
	Mar-02	Selangor, Malaysia	MG670122	MG670150	MG670181	Tan et al. 2019
	Mar-03	Kedah, Malaysia	MG670123	MG670151	MG670182	Tan et al. 2019
	ZMUC00008513	Naknon Sri Thammarat, Thailand	MK420123	MK420239	MK420339	Scharff et al. 2020
	MUMNH-ARA-MAC011	Chiangmai, Thailand	MT584916	MT584948	MT584966	This study
	MUMNH-ARA-MAC021	Phumi Pôpôk Vil, Cambodia	MT584917	MT584949	MT584967	This study
Macracantha hasselti	MUMNH-ARA-GAS016	Ubon Ratchathani, Thailand	MT584903	MT584935	-	This study
	MUMNH-ARA-GAS018	Saraburi, Thailand	MT584904	MT584936	MT371075	This study
	MUMNH-ARA-GAS025	Phetchaburi, Thailand	MT584905	MT584937	-	This study
	MUMNH-ARA-GAS037	Phetchaburi, Thailand	MT584906	MT584938	MT584960	This study
	MUMNH-ARA-GAS050	Mae Hong Sorn, Thailand	MT584907	MT584939	MT584961	This study
	MUMNH-ARA-GAS065	Chumpon, Thailand	MT584908	MT584940	-	This study
	GHAS1	Kedah, Malaysia	MG670120	MG670148	MG670179	Tan et al. 2019
Thelacantha brevispina	MUMNH-ARA-THE004	Phetchaburi, Thailand	MT584918	-	MT584968	This study
	MUMNH-ARA-THE005	Surat Thani, Thailand	MT584919	-	MT584969	This study
	MUMNH-ARA-THE007	Loei, Thailand	MT584920	-	MT584970	This study
	MUMNH-ARA-THE008	Samut Prakan, Thailand	MT584921	MT584950	MT584971	This study
	TBRE1	Penang, Malaysia	MG670124	MG670152	MG670183	Tan et al. 2019
	TBRE2	Penang, Malaysia	MG670125	MG670153	MG670184	Tan et al. 2019
	TBRE3	Kedah, Malaysia	MG670126	MG670154	-	Tan et al. 2019
	sc06156	French Polynesia	KX055041	-	-	Ramage et al. 2017
	sc05514	French Polynesia	KX055044	-	-	Ramage et al. 2017
	Gam_Ok01	Okinawa, Japan	AB969824	-	-	Yamada et al. 2015
Actinacantha globulata	AGLO1	Semenyih, Selangor, Malaysia	MG670112	MG670140	MG670170	Tan et al. 2019
Outgroup						
Cyclosa caroli	n92	USA: Florida, Gainesville	MK420091	MK420211	MK420316	Scharff et al. 2020

Cyclosa turbinata	CA	USA: California, Encinitas	MK420092	MK420212	MK420317	Scharff et al. 2020
Cyclosa walckenaeri	n94	USA: California, Big Sur	MK420093	MK420213	MK420318	Scharff et al. 2020
Micrathena gracilis	102	USA: Ohio	MK420136	MK420251	MK420349	Scharff et al. 2020
Micrathena gracilis	N/A	N/A	FJ525326	FJ525359	FJ525343	Agnarsson and Blackledge 2009
Micrathena horrida	784351	Cuba	KJ157243	KJ157016	-	McHugh et al. 2014
Micrathena sagittata	7	USA: Florida, Gainesville, 7.vii.1997	MK420137	MK420253	-	Scharff et al. 2020
Herennia etruscilla	N/A	N/A	KC849074	KC849118	KC849033	Kuntner et al. 2013
Herennia multipuncta	N/A	N/A	KC849075	KC849119	KC849034	Kuntner et al. 2013
Nephila pilipes	N/A	N/A	KC849088	KC849130	KC849045	Kuntner et al. 2013
Nephila clavate	N/A	N/A	KC849082	KC849125	KC849041	Kuntner et al. 2013
Nephila clavipes	N/A	N/A	FJ525328	FJ525361	FJ525344	Agnarsson and Blackledge 2009
Nephila senegalensis	N/A	N/A	KC849090	KC849132	KC849047	Kuntner et al. 2013
Nephilengys dodo	N/A	N/A	KC849097	KC849138	KC849053	Kuntner et al. 2013
Nephilengys malabarensis	N/A	N/A	KC849099	KC849140	KC849055	Kuntner et al. 2013

were deposited in GenBank; the accession numbers and related information are summarized in Table 2.

In this study, we included sequences from previous publications as outgroups and some sequences of Gasteranthinae from Thailand and adjacent countries as ingroups (Table 2). The outgroups were the subfamily Nephilinae, which is considered as a sister clade of the rest of Araneidae (Hormiga and Griswold 2014); genus *Micrathena*, another spiny orb-weaver from Neotropical regions; and genus *Cyclosa*, which is considered to be closely related to Gasteracanthinae (Wheeler et al. 2017). Phylogenetic analyses were conducted based on maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI). Each gene was independently partitioned to find the best-fit models for nucleotide substitution using KAKUSAN4 (Tanabe 2007) with Bayesian Information Criterion (BIC) (Schwarz 1978). Only COI was further partitioned by codon position into three partitions. The best-fit models for each partition were GTR+G for the first and the third codon positions of COI and for 16S; F81+G for the second codon position of COI; and HKY85+G for H3.

For MP analyses, multiple sequences were used to generate molecular matrices using GB2TNT (Goloboff and Catalano 2012). Maximum parsimonious tree was constructed with TNT v. 1.5 (Goloboff and Catalano 2016). TNT searches were run with 5,000 replications of traditional heuristic search. Trees were saved twice per replicate. Branch-swapping was conducted with tree bisection-reconnection (TBR). Support for nodes was accessed using Jackknifing (Farris 1997) with 1,000 pseudoreplications, and set character removal probability equal to 36% under the traditional search. ML analyses were executed in RAxML v.8.2.12 (Stamatakis 2014). Due to limitations in best-fit model selection in RAxML, all analyses were performed under GTRGAMMA model. Support clades were assessed with 1,000 bootstrap replications. BI analyses were performed in MRBAYES v3.2.6 (Ronquist et al. 2012) on the online CIPRES Science Gateway server (Miller et al. 2010), using Markov chain Monte Carlo (MCMC), and sampling for 20,000,000 generations. Each run contained four chains with the temperature setting of 0.05. Trees were sampled every 200 generations. The first 25% of trees were discarded as burn-in. The results of MCMC sampling were monitored using Tracer v. 1.7 (Rambaut et al. 2018) to ensure that Markov chains had run to become stationary, the standard deviation of split frequencies was below 0.01, and effective sampling size (ESS) exceeded 200 for all parameters after burn-in.

Genetic distances between species within Gasteracanthinae were examined using COI sequence (675 bp) via uncorrected pairwise genetic distance as implemented in MEGA X (Kumar et al. 2018). The examined taxa were grouped following the clusters from species delimitation results.

Species delimitations

Species delimitations were analyzed via computational methods to examine whether each lineage (or putative species) in the phylogenetic tree was statistically significant as a distinct species. The sequence matrices of the COI gene (675 bp), 16S gene (454 bp), and H3 gene (328 bp) were used as DNA barcoding. Each dataset consisted of 52, 46, and 38 individuals, respectively. Delimitation of each taxa was executed using Automatic barcode gap discovery (ABGD), Bayesian Poisson tree processes (bPTP), and Generalized mixed Yule coalescent (GMYC). Firstly, Automatic barcode gap discovery (ABGD) analysis (Puillandre et al. 2011) was run on the online sever: https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html. Default parameters were used, except the relative gap width, which was set at 1.0. Kimura-2-parameter was used as substitution model (Kimura 1980). Secondly, the Bayesian Poisson tree processes (bPTP) was carried out using the bPTP server: https://species.h-its.org/. The ML tree reconstructed from RAxML was used as input data (Zhang et al. 2013). The analysis was run as rooted with outgroups removed, sampling MCMC 500,000 generations, 500 of thinning, and burn-in as 0.1. Thirdly, the Generalized mixed Yule coalescent (GMYC; Pons et al. 2006) was performed using the BI tree from BEAST v.2.6.2 (Bouckaert et al. 2014) under Yule speciation model. The analysis was run for 5,000,000 generations. Trees were sampled every 1,000 generations. Sampled trees from BEAST were summarized onto a single tree using TreeAnnotator v.2.6 (BEAST package), with 25% of samples discarded as burn-in. The GMYC analysis was conducted with the 'splits' package using R v.3.6 (available at http://r-forge.rproject.org/projects/splits). The species delimitations by these three methods were compared for consistency with (1) morphological characters between OTUs based on original descriptions and previous taxonomic reviews, (2) uncorrected genetic distance between OTUs by using COI sequence (675 bp), and (3) molecular phylogenetic analyses based on partial sequences of COI, 16S, and H3 genes.

Results

Morphological study

A total of 342 spiders from 93 localities was morphologically identified to seven species from three genera: *Gasteracantha diardi* (Lucas, 1835), *Gasteracantha diadesmia* Thorell, 1887, *Gasteracantha doriae* Simon, 1877, *Gasteracantha kuhli* Koch, 1837, *Gasteracantha hasselti* Koch, 1837, *Macracantha arcuata* (Fabricius, 1793), and *Thelacantha brevispina* (Doleschall, 1857). Distribution maps of all species are presented in Fig. 1. We were unable to obtain specimens of four species previously recorded and/or described from Thailand for this study: *Gasteracantha frontata* Blackwall, 1864, *Gasteracantha irradiata* Walckenaer, 1842, *Gasteracantha rubrospinis* Guérin, 1838, and *Gasteracantha clavigera* Giebel, 1863 (Giebel 1863; Pocock 1897; Simon 1886; Dahl 1914).

The number of dorsal sigilla in most species is equal, but the arrangement, shape and size are variable among species. To describe the number and position of sigilla on the abdomen, we divide the abdominal sigilla into four groups according to their position (Fig. 2): (i) the anterior edge sigilla form a row near the anterior border of the dorsal abdomen, (ii) the posterior edge sigilla form a row near the posterior border of dorsal abdomen, (iii) the median sigilla are situated in the middle of the dorsal abdomen, arranged in a trapezoid shape, and (iv) the outer posterior edge sigilla form a row behind the posterior border of the dorsal abdomen.

Phylogenetic analyses and genetic divergence

The total length of the concatenated alignment was 1457 bp, consisting of 675 bp of COI, 454 bp of 16S rRNA and 328 bp of H3. The concatenated dataset had 288, 252, and 105 variable sites and 252, 202, and 83 parsimonious informative sites, for COI, 16S, and H3, respectively. The three phylogenetic methods recovered some differences in branching patterns. Here, only the topology from the ML tree is selected to guide the discussion (Fig. 3). Phylogenetic trees from MP and BI analyses are available as a Suppl. material 1 (Suppl. material 1: Figs S1, S2).

The phylogenetic tree recovered Gasteracanthinae as a monophyletic group with high nodal support for all analyses (Fig. 3, node 1: MP=99/ML=100/BI=1.00). All nominal species within Gasteracanthinae form a well-supported clade. The Gasteracanthinae clade can be divided into three major clades, consisting of (I) a clade of *A. globulata*, *G. hasselti*, and *M. arcuata* (Fig. 3, node 2: MP=100/ ML=100/ BI=1.00); (II) a clade of five *Gasteracantha* species, including *G. cancriformis*, *G. kuhli*, *G. diardi*, *G. diadesmia*, and two lineages that were morphologically identified as *G. doriae* (Fig. 3, node 5: MP=99/ ML=99/ BI=1.00); and (III) a clade of *Thelacantha* (Fig. 3, node 7: MP=97/ ML=100/ BI=1.00). Clade II forms a sister relationship with clade III (Fig. 3, node 4: MP=97/ ML=100/ BI=1.00), while clade I is a sister to clade II + clade III. The only known new-world *Gasteracantha* species, *G. cancriformis*, is placed in a basal position to the rest of *Gasteracantha*. The broad-abdomen *Gasteracantha*, consisting of *G. diadesmia*, *G. diardi*, and two clades of *G. doriae*, form a monophyletic group (Fig. 3, node 6:

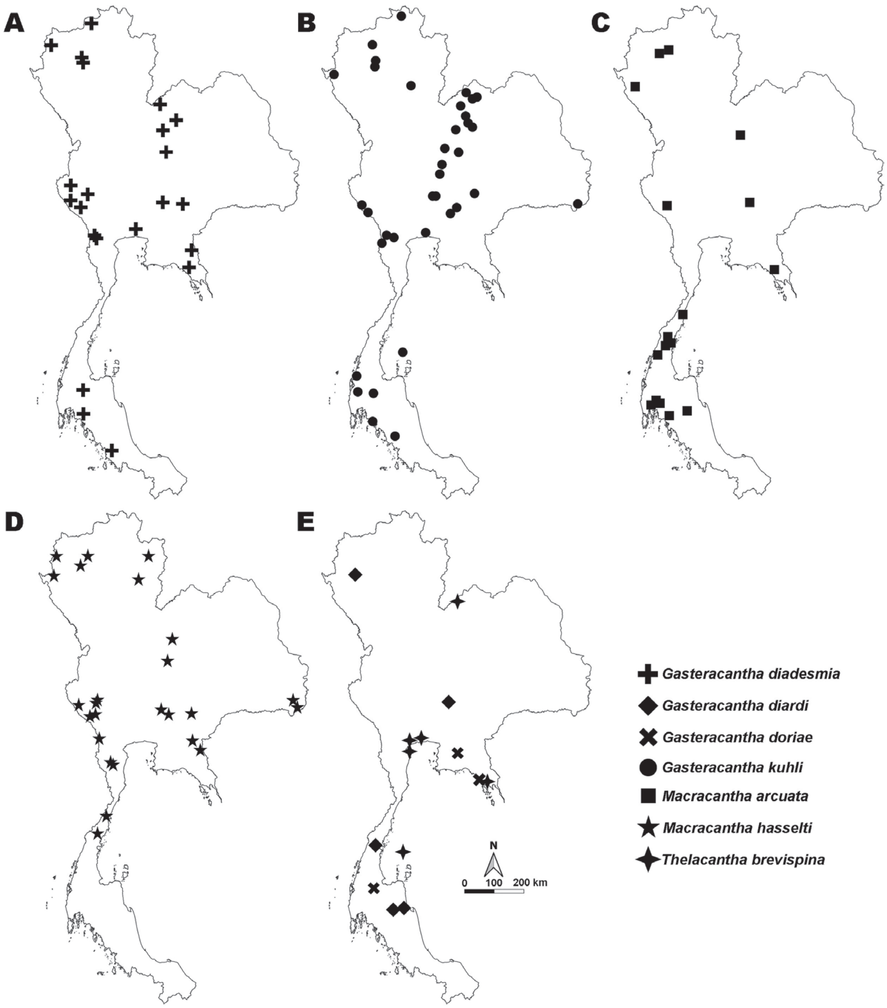


Figure 1. Distribution map of Gasteracanthinae in Thailand **A** *Gasteracantha diadesmia* **B** *G. kuhli* **C** *Macracantha arcuata* **D** *M. hasselti* **E** *G. diardi*, *G. doriae*, and *Thelacantha brevispina*.

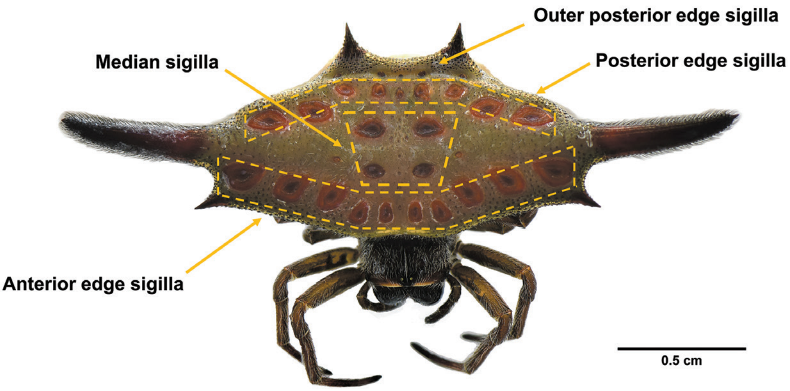


Figure 2. Female *Gasteracantha diardi* with proposed names of the abdominal sigilla groups used in this study.

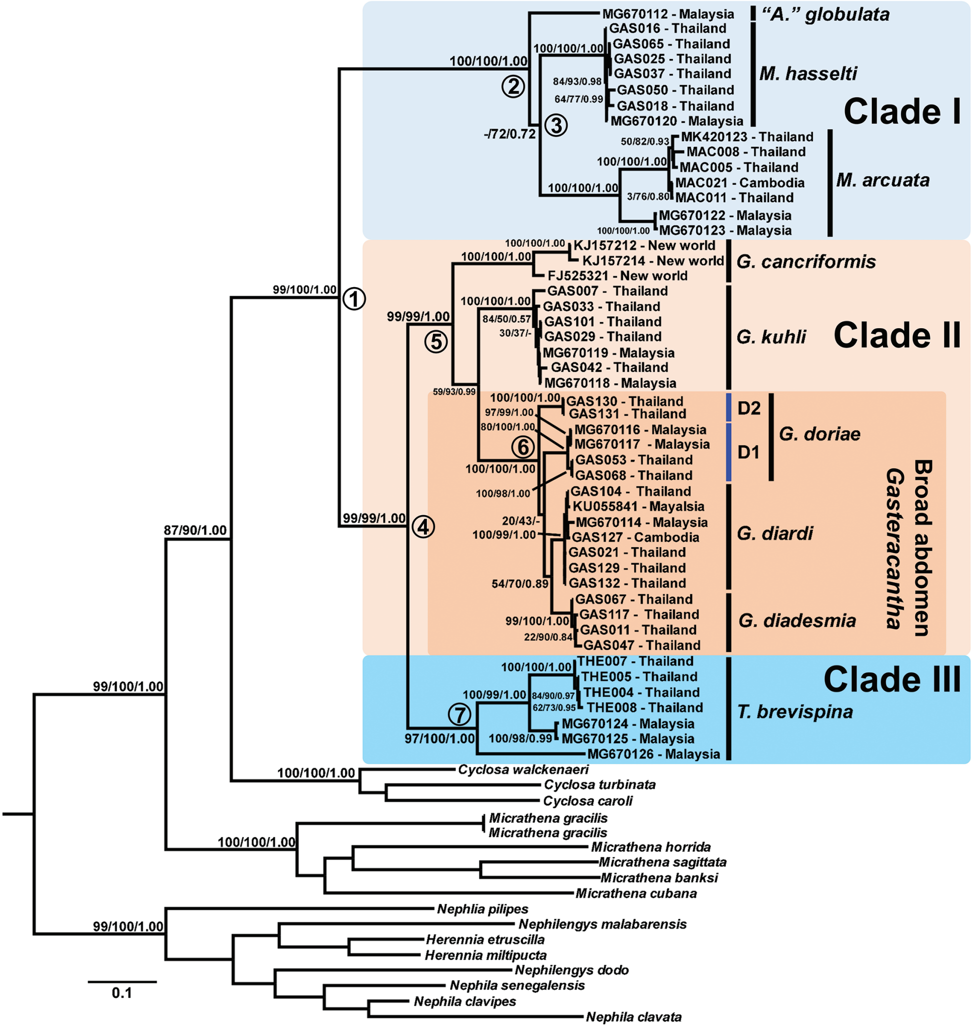


Figure 3. Maximum Likelihood phylogenetic tree reconstructed from COI+16S+H3 genes of Gasteracanthinae and outgroups. Nodal support values are labeled as Jackknife support/ML bootstrap values/Bayesian posterior probability.

MP=100/ ML=100/ BI=1.00). Subclades with deep genetic divergences within nominal taxa are detected in *G. cancriformis*, *M. arcuata*, and *T. brevispina*, whereas two clades of *G. doriae* are recovered with a distant relationship. The *G. doriae* clade D1 consists of specimens from Malaysia and juvenile females from Trat and Surat Thani provinces, Thailand; while the Clade D2 contains individuals from Rayong Province, Thailand.

In addition, polyphyly of *Gasteracantha* is revealed. Phylogenetic analyses nest *G. hasselti* together with *M. arcuata* (Fig. 3, node 3), although their phylogenetic relationship is supported only by the ML analysis. The genetic distance also shows that *G. hasselti* is more closely related to *M. arcuata* than other *Gasteracantha* (Table 3). Therefore, we propose to move *G. hasselti* to the genus *Macracantha* as *Macracantha hasselti* (C. L. Koch, 1837) comb. nov. The supporting evidence is further discussed below.

Genetic distances of COI gene ranged from 3.77 to 16.49% (average = 10.89%) between taxa, and from 0.15 to 1.30% (average = 0.53%) within taxa (Table 3).

Species delimitation

All three statistical approaches based on the COI gene dataset generated congruent results for 15 OTUs, corresponding to nine nominal species and six possible cryptic species (Fig. 4). These cryptic species were detected within nominal species: one lineage each in *G. cancriformis*, *G. doriae*, and *M. arcuata*, and three lineages in *T. brevispina*. The delimitation based on the 16S gene generated 14 OTUs for ABGD, 15 OTUs for bPTP, and 10 OTUs for GMYC (Suppl. material 1: Fig. S5). The delimitation based on the H3 gene generated ten OTUs for ABGD, nine OTUs for bPTP, and six OTUs for GMYC (Suppl. material 1: Fig. S6). The delimitation results from the COI dataset were the most consistent with the morphological identification; also, 16S and H3 sequences of some individuals were unavailable for this study. Therefore, only the results from the COI dataset are used for the discussion.

Diversity of Gasteracanthinae in Thailand

In summary, seven species from three genera, which are *Gasteracantha*, *Macracantha*, and *Thelacantha*, were collected in this study. They are *G. diadesmia*, *G. diardi*, *G. doriae*, *G. kuhli*, *M. arcuata*, *M. hasselti*, and *T. brevispina*. Four other species previously recorded from Thailand, *G. clavigera*, *G. frontata*, *G. irradiata*, and *G. rubrospinis*, were not found during surveys. Therefore, there are eleven named species of Gasteracanthinae present in Thailand including those from previous historical records.

Order Araneae Clerck, 1757
Family Araneidae Clerck, 1757
Subfamily Gasteracanthinae Scharff & Coddington, 1997

Key to species of spiny-backed orb-weaving spiders subfamily Gasteracanthinae in Thailand

Only species for which specimens were available in this study are included.

1 Ventral tubercle present. Anterior margin of abdomen forming slight arch between anterior spines. Spinnerets encircled by black sclerotized ring. Spermathecae round or oval.......... **2**

– Ventral tubercle absent. Anterior margin of abdomen forming strong arch between anterior spines. Spinnerets placed on elevated black sclerotized structure. Shape of spermathecae not as above.......... **6**

2 Abdomen much wider than long. Median spines different from other spines. Large trapezoid-shaped sigilla present **3**

– Abdomen slightly wider than long. Each pair of spines quite similar in shape. Large trapezoid-shaped sigilla absent........................ **5**

3 Median spine very large, long, covered with hairs, and arched posteriorly with few marginal spikes. Median sigilla with two small sigilla beside the large trapezoid-shaped sigilla .... ***G. diardi***

– Median spine large, with scattered hairs, not arched or slightly arched posteriorly with conspicuous marginal spikes. Median sigilla without two small sigilla beside the large trapezoid-shaped sigilla........................ **4**

4 Median spine large, thick, plate-like, and directed horizontally. The angle between anterior and posterior spines narrow. Two dark horizontal bands on abdomen straight .... ***G. diadesmia***

– Median spines long, thin, less conical, and slightly arched backward. The angle between anterior and posterior spines relatively obtuse. Two dark horizontal bands on abdomen sinuous .... ***G. doriae***

5 Abdominal spines conical, the bases of anterior and median spines fused. Dorsal abdomen with black and white patches, usually arranged in inverse Y-band. Sternal band hoof-shaped .... ***G. kuhli***

– Abdominal spines tubercle-shaped with small projection at the tip. Abdomen various in color. Two large white spots usually present on dorsal abdomen. Sternal band not as above........................ ***T. brevispina***

6 Anterior and posterior spines poorly developed. Median spines very long, at least three times the width of abdomen, slender, and strongly arched........................ ***M. arcuata***

– Anterior and posterior spines well developed, sharp. Median spine straight, longest, but less than two times the width of abdomen, thick at the base and tapering toward the tip .... ***M. hasselti***

Taxonomic account

Genus *Gasteracantha* Sundevall, 1833

Type species. *Aranea cancriformis* Linnaeus, 1758.

Diagnosis. Cephalic region highly elevated near the middle, abruptly sloped downward posteriorly. Median ocular quadrangle wider behind than in front. Cephalothorax overlapping anterior abdomen. Sternum heart-shaped, pointed posteriorly, concave anteriorly below labium. Abdomen wider than long, with prominent coloration, three pairs of spines, and sigilla on dorsal and ventral sides. Four median sigilla arranged in trapezoid. Dorsal sigilla in three rows, situated near the anterior edge, posterior edge, and behind the posterior edge. Spinnerets encircled by a black sclerotized ring. IV femora elongated.

Table 3. Average interspecific uncorrected p-distance (%±S.E.) based on the 675 bp COI gene fragment sequences between species within Gasteracanthinae. Average intraspecific distances within each taxon are marked in bold.

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. *Actinacantha globulata*	**N/A**														
2. *Gasteracantha cancriformis* (C1)	15.43±1.46	**1.00±0.40**													
3. *Gasteracantha cancriformis* (C2)	14.76±1.40	4.26±0.79	**N/A**												
4. *Gasteracantha diadesmia*	14.08±1.35	10.85±1.24	9.84±1.14	**0.84±0.25**											
5. *Gasteracantha diardi*	14.48±1.36	11.41±1.30	10.12±1.20	3.77±0.70	**0.31±0.14**										
6. *Gasteracantha doriae* (D1)	13.03±1.29	11.28±1.25	10.19±1.16	5.13±0.79	5.40±0.82	**0.45±0.21**									
7. *Gasteracantha doriae* (D2)	13.55±1.34	11.65±1.27	10.43±1.17	4.48±0.76	5.15±0.85	5.53±0.82	**0**								
8. *Gasteracantha kuhli*	14.05±1.38	8.68±1.11	8.19±1.02	7.75±0.96	8.36±1.03	7.89±0.99	7.73±0.99	**0.58±0.19**							
9. *Macracantha arcuata* (M1)	10.72±1.14	16.49±1.49	15.59±1.40	15.28±1.36	16.45±1.40	15.09±1.34	16.00±1.41	16.12±1.43	**1.30±0.29**						
10. *Macracantha arcuata* (M2)	9.64±1.11	15.96±1.50	15.21±1.34	14.19±1.32	15.58±1.38	14.01±1.31	15.06±1.39	13.68±1.32	7.02±0.95	**0.60±0.30**					
11. *Macracantha hasselti*	8.33±1.04	14.13±1.39	14.05±1.30	13.06±1.26	13.69±1.30	12.56±1.26	12.81±1.26	12.83±1.26	9.46±1.06	9.21±1.07	**0.72±0.21**				
12. *Thelacantha brevispina* (T1)	14.95±1.40	12.91±1.31	12.03±1.22	12.30±1.23	13.29±1.30	12.43±1.26	12.26±1.25	11.86±1.23	15.73±1.37	15.47±1.40	14.08±1.30	**0.17±0.12**			
13. *Thelacantha brevispina* (T2)	14.91±1.40	11.53±1.28	10.77±1.20	11.48±1.18	12.05±1.22	11.90±1.24	11.30±1.20	10.56±1.15	16.20±1.38	15.81±1.36	13.58±1.29	5.69±0.91	**0.30±0.15**		
14. *Thelacantha brevispina* (T3)	15.12±1.43	13.71±1.38	13.29±1.33	10.78±1.17	11.57±1.22	11.16±1.22	10.86±1.20	11.12±1.21	15.12±1.37	14.21±1.32	13.25±1.29	8.92±1.10	8.19±1.09	**0.15±0.15**	
15. *Thelacantha brevispina* (T4)	15.81±1.47	13.77±1.37	12.35±1.25	12.27±1.27	12.63±1.28	13.33±1.31	12.35±1.28	11.64±1.24	14.70±1.37	13.86±1.36	15.06±1.36	9.60±1.15	9.41±1.14	10.25±1.21	**N/A**

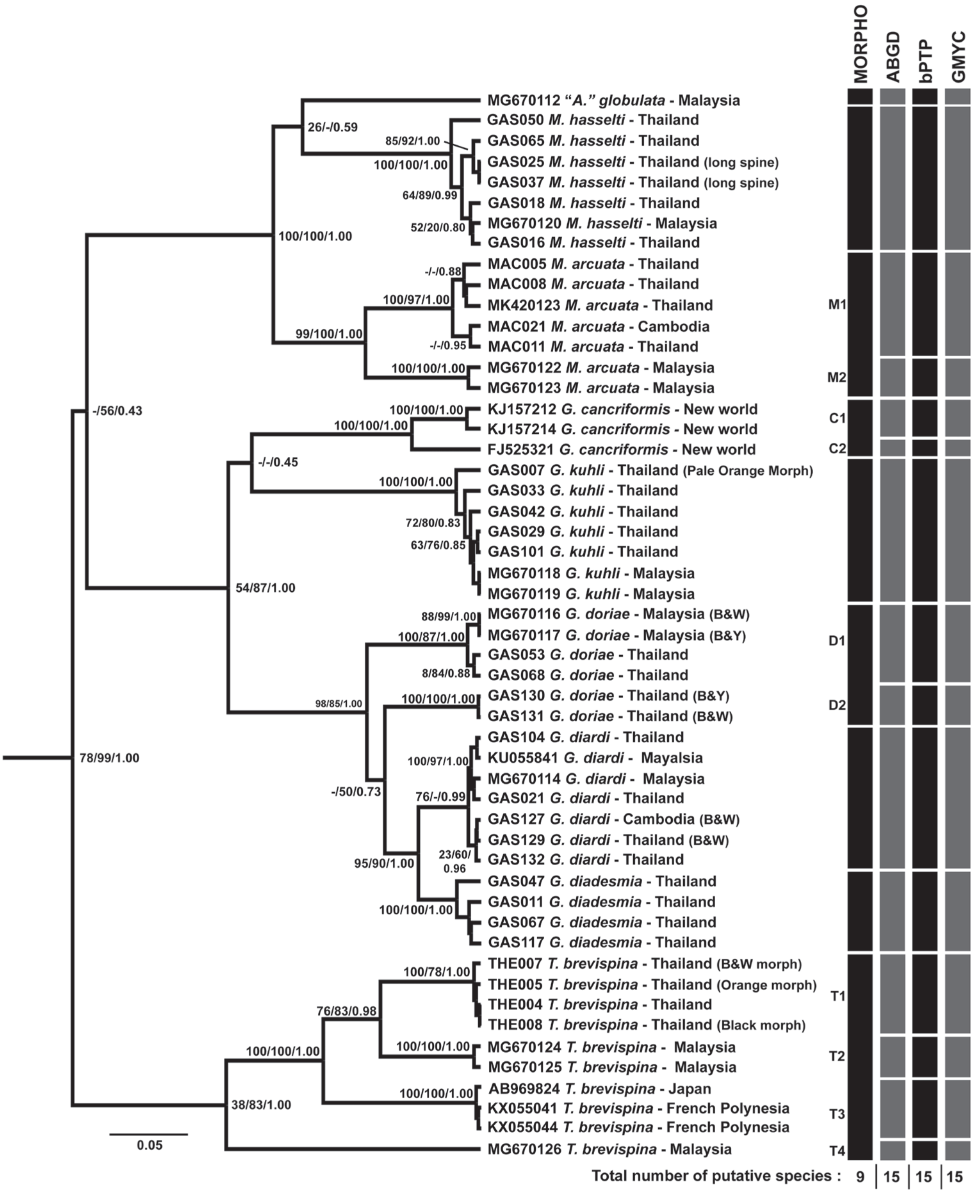


Figure 4. Ultrametric tree generated by BEAST from 675 bp of COI gene showing clusters of OTUs as suggested by morphological identification, and three molecular species delimitation algorithms, ABGD, bPTP, and GMYC. Nodal support values are labeled as MP Jackknife support/ML bootstrap values/Bayesian posterior probability. Gene tree from MP and ML analyses are available as Suppl. material 1 (Suppl. material 1: Figs S3, S4).

Remarks. The genus *Gasteracantha* was first described by Sundevall (1833), and subsequently was revised by many authors (Pickard-Cambridge 1879; Dahl 1914; Benoit 1962, 1964; Emerit 1974; Barrion and Litsinger 1995; Levi 1996). Currently, *Gasteracantha* comprises 88 valid species worldwide (World Spider Catalog 2020).

Gasteracantha diadesmia Thorell, 1887

Figures 5, 11A–C

Gasteracantha diadesmia Thorell, 1887: 225. Type locality: Myanmar, Bhamo.
Full list of synonyms and usage of the name available in World Spider Catalog (2020).

Material. THAILAND • 3 ♀; Nakhon Ratchasima Province, Wang Nam Khiao District; 14°32.57'N, 101°58.22'E; MUMNH-ARA-GAS009 • 3 ♀; Nakhon Ratchasima Province, Pak Chong District, Phaya Yen; 14°36.97'N, 101°15.90'E; MUMNH-ARA-GAS011 • 2 ♀; Satun Province, Thung Wa District, Khantiphon Cave; 07°05.08'N, 99°47.92'E; MUMNH-ARA-GAS012 • 3 ♀; Ratchaburi Province, Suan Phueng District; 13°33.03'N, 99°17.14'E; MUMNH-ARA-GAS028; MUMNH-ARA-GAS030 • 1 ♀; Kanchanaburi Province, Sai Yok District; 14°24.93'N, 98°52.54'E • 5 ♀; Ratchaburi Province, Suan Phueng District, Pachi Stream; 13°31.18'N, 99°18.88'E; MUMNH-ARA-GAS031 • 1 ♀; Chiang Mai Province, Mueang District; 18°47.00'N, 98°57.13'E; MUMNH-ARA-GAS034 • 3 ♀; Krabi Province, Mueang District, Krabi Noi; 08°07.54'N, 98°55.40'E; MUMNH-ARA-GAS043 • 1 ♀; Bangkok Province, Ratchathewi District, Santiphap Park; 13°45.68'N, 100°32.42'E; MUMNH-ARA-GAS045 • 4 ♀; Mae Hong Son Province, Mueang District, Pang Mu; 19°18.12'N, 97°57.58'E; MUMNH-ARA-GAS047 • 1 ♀ juvenile; Surat Thani Province, Khiri Rat Nikhom District, Wang Badarn Cave; 08°54.52'N, 98°57.08'E; MUMNH-ARA-GAS067 • 2 ♀; Chanthaburi Province, Soi Dao District; 13°06.67'N, 102°12.30'E; MUMNH-ARA-GAS076 • 2 ♀; Chanthaburi Province, Mueang District, Khlong Narai; 12°35.45'N, 102°09.48'E; MUMNH-ARA-GAS077 • 4 ♀; Kanchanaburi Province, Si Sawat District, Na Suan, Ong-ju Canal; 14°48.45'N, 99°05.53'E; MUMNH-ARA-GAS082 • 1 ♀; Phetchabun Province, Lom Sak District; 16°43.74'N, 101°20.22'E; MUMNH-ARA-GAS086 • 2 ♀; Chaiyaphum Province, Phakdi Chumphon District, Ban Chiang, Wua Daeng Cave; 16°04.55'N, 101°26.46'E; MUMNH-ARA-GAS096 • 1 ♀; Loei Province, Nong Hin District; 17°02.41'N, 101°44.18'E; MUMNH-ARA-GAS099 • 2 ♀; Chiang Mai Province, Mae Rim District; 18°55.10'N, 98°54.51'E; MUMNH-ARA-GAS102 • 2 ♀; Kanchanaburi Province, Mueang District, Li Chia Cave; 15°04.50'N, 98°33.96'E; MUMNH-ARA-GAS107 • 3 ♀; Kanchanaburi Province, Thong Pha Phum District, Huai Kayeng; 14°37.85'N, 98°34.32'E; MUMNH-ARA-GAS108 • 5 ♀; Loei Province, Phu Ruea District; 17°31.55'N, 101°15.33'E; MUMNH-ARA-GAS117 • 3 ♀; Chiang Mai Province, Fang District; 19°57.46'N, 99°12.17'E; MUMNH-ARA-GAS122.

Diagnosis. Sternum dark brown with median yellow spot. Abdomen much wider than long. Dorsal side of abdomen with three yellow abdominal horizontal bands: first band on anterior edge near base of anterior spines, second band running between median spines, and third band behind middle sigilla reaching posterior edge. Edge of abdomen with serrated spikes, obvious on spines. Spines dark brown to orange. Anterior spines smallest, obliquely directed. Median spines longest, thick, plate-like, horizontally pointed. Posterior spines conical, pointed backward. Two median yellow spots between the bases of posterior spines. Ventral side of abdomen blackish with scattered yellow spots and small black granules. Ten anterior edge sigilla in total: four

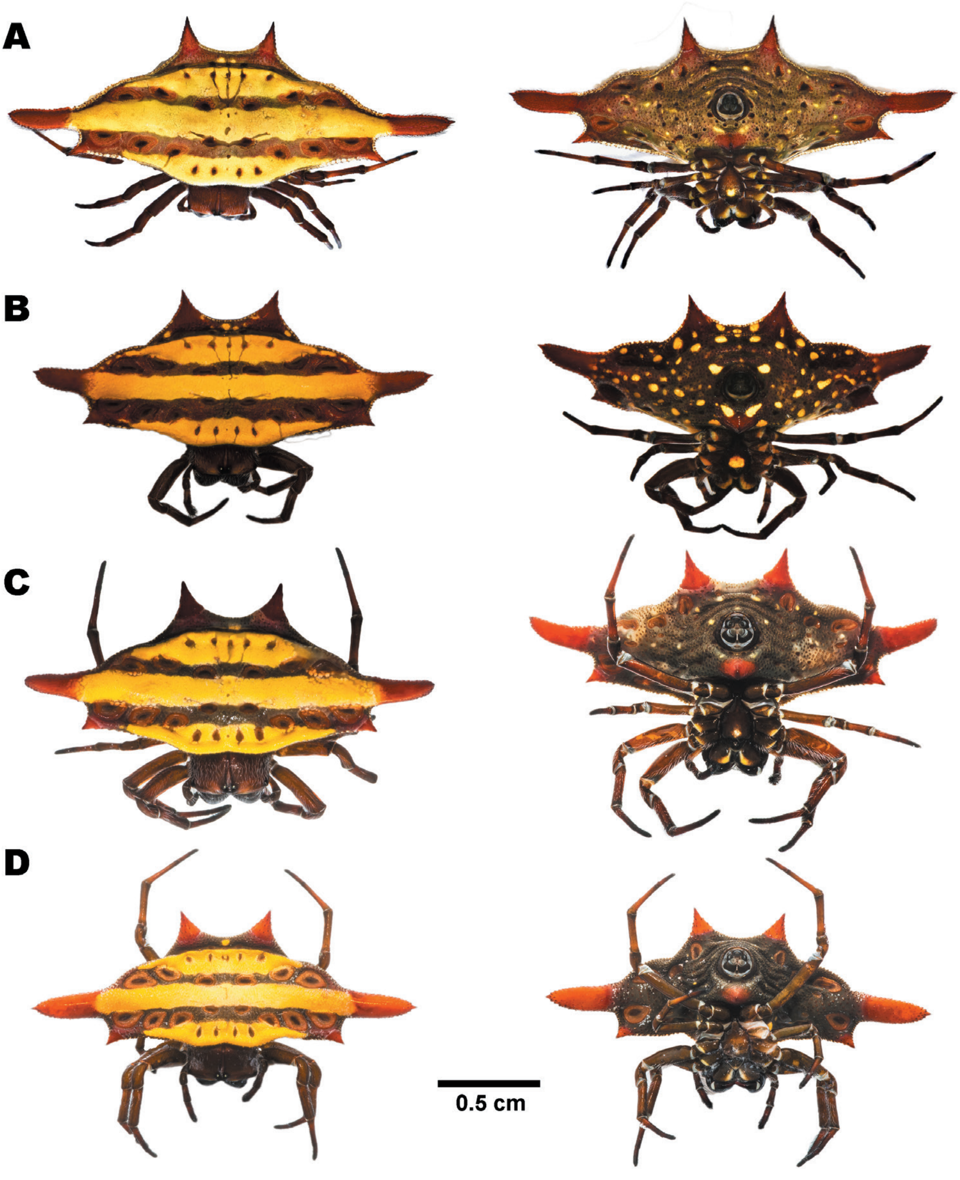


Figure 5. Females of *Gasteracantha diadesmia* showing dorsal view (left) and ventral view (right) **A** specimen from Mae Hong Son (MUMNH-ARA-GAS047) **B** specimen from Nakhon Ratchasima (MUMNH-ARA-GAS011) **C** specimen from Loei (MUMNH-ARA-GAS117) **D** specimen from Chanthaburi (MUMNH-ARA-GAS076).

sigilla in the middle small, forming a straight line, three sigilla on each side larger, trapezoid. Four median sigilla arranged in trapezoid. Ten posterior edge sigilla in total: six sigilla in the middle, forming a straight line, the pair in the middle close together; two sigilla on each side larger, trapezoid. Five outer posterior edge sigilla, placed near posterior spines. Epigynum subtriangular with two lateral dark patches (Fig. 11A). Scape large, pointed posteriorly, divided into three curves (Fig. 11A, C). Spermathecae round (Fig. 11A), ventrally partially overlapped by wing-shaped sclerotized structure (Fig. 11B). Copulatory ducts encapsulated by sclerotized structure (Fig. 11A). Fertilization ducts emerging posteriorly from spermathecae (Fig. 11A).

Variation. Dorsal dark horizontal bands, spines, and ventral abdomen either reddish (Fig. 5A, D) or blackish (Fig. 5B, C) in some specimens. Median spines in some specimens slightly pointed backwards (Fig. 5C).

Remarks. *Gasteracantha diadesmia* resembles *Gasteracantha sturi* (Doleschall, 1857), but black horizontal bands of *G. diadesmia* are wider than in *G. sturi* (Doleschall 1857; Kolosváry 1931). Moreover, median spines of *G. diadesmia* are large, thick, and plate-like, while median spines of *G. sturi* are very blunt. *Gasteracantha diadesmia* is distinguished from other Thai species by having broader anterior yellow horizontal band and thick, plate-like, and horizontally pointed median spines. Barrion and Litsinger (1995) reported another form with discontinuous dark horizontal bands from the Philippines. However, this morphotype might belong to another species because the shape of spines and color pattern are different.

Distribution and habitat. India, Myanmar, China, Thailand, Vietnam, Philippines, and Andaman and Nicobar Islands (Yin et al. 1997; World Spider Catalog 2020). *Gasteracantha diadesmia* are commonly found in mixed deciduous forest and dipterocarp forest. The spiders usually construct a vertical web between shrubs in open areas, and sit at the center of the web.

***Gasteracantha diardi* (Lucas, 1835)**

Figures 6, 11D–F

Epeira diardi Lucas, 1835: 70, pl.149, fig. 4. Type locality: Indonesia, Java.
Full list of synonyms and usage of the name available in World Spider Catalog (2020).

Material. Thailand • 3 ♀; Chumphon Province, Sawi District, Wisai Tai; 10°22.38'N, 99°03.61'E; MUMNH-ARA-GAS021 • 3 ♀; Nakhon Ratchasima Province, Pak Chong District, Phaya Yen; 14°36.97'N, 101°15.90'E; MUMNH-ARA-GAS022 • 1 ♀; Nakhon Si Thammarat Province, Chang Klang District; 08°19.27'N, 99°35.39'E; MUMNH-ARA-GAS104 • 4 ♀; Nakhon Si Thammarat Province, Phra Phrom District; 08°22.59'N, 099°52.72'E; MUMNH-ARA-GAS105 • 1 ♀; Chiang Mai Province, Mae Chaem District; 18°28.81'N, 98°22.96'E; MUMNH-ARA-GAS129 • 4 ♀; Nakhon Ratchasima Province, Pak Chong District, Phaya Yen; 14°36.97'N, 101°15.90'E; MUMNH-ARA-GAS132. Cambodia • 3 ♀; Kampot Province; 10°34.92'N, 104°07.21'E; MUMNH-ARA-GAS127.

Diagnosis. Sternum dark brown with small median yellow spot. Abdomen much wider than long. Dorsal side of abdomen dark brown. Edge of abdomen with few serrated spikes. Spines dark brown to orange. Anterior spines smallest, slightly directed

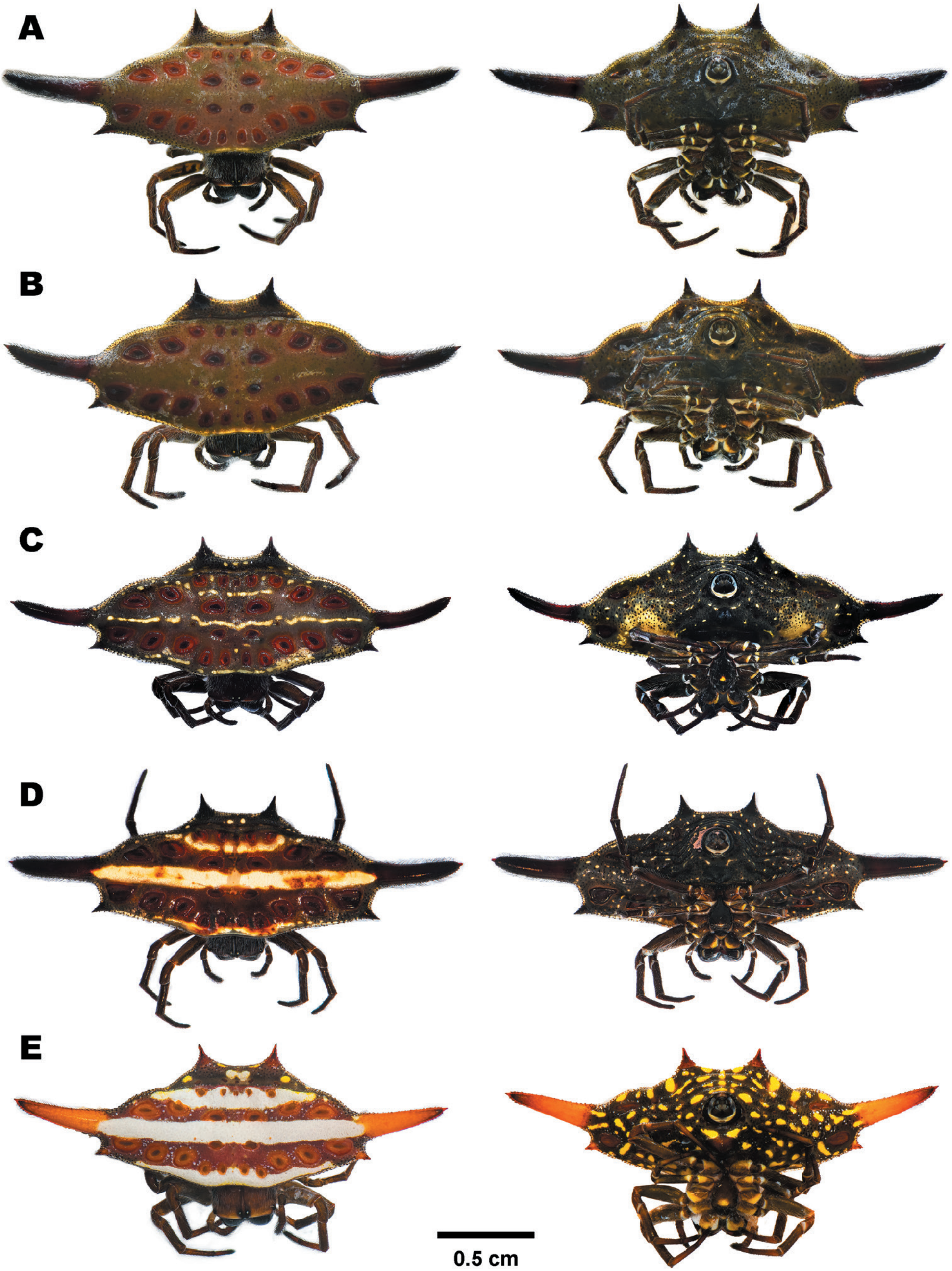


Figure 6. Females of *Gasteracantha diardi* showing dorsal view (left) and ventral view (right) **A, B** dark brown morph **A** specimen from Nakhon Si Thammarat (MUMNH-ARA-GAS104) **B** specimen from Nakhon Si Thammarat (MUMNH-ARA-GAS105) **C** dark red with stripes morph, specimen from Nakhon Ratchasima (MUMNH-ARA-GAS132) **D** narrow horizontal band morph, specimen from Chiang Mai (MUMNH-ARA-GAS129) **E** broad horizontal band morph, specimen from Cambodia, Kampot (MUMNH-ARA-GAS127).

obliquely. Median spines very large, covered with hairs, and arched backward. Posterior spines conical, pointed backward. Ventral side of abdomen dark brown with scattered yellow spots and small black granules. Ten anterior edge sigilla in total: four sigilla in the middle smaller, forming a straight line, three sigilla on each side larger, trapezoid. Four median sigilla arranged in a trapezoid, with two small sigilla situated on both lateral sides. Posterior edge with ten sigilla in total: six sigilla in the middle forming a straight line, the pair in the middle closely placed; two sigilla on each side larger, trapezoid. Outer posterior edge with five sigilla near posterior spines. Epigynum subtriangular with two lateral dark patches (Fig. 11D). Scape trapezoid, pointed posteriorly (Fig. 11F). Spermathecae round (Fig. 11D), ventrally partially overlapped by wing-shaped sclerotized structure (Fig. 11E). Copulatory ducts encapsulated by sclerotized structure (Fig. 11D). Fertilization ducts emerging posteriorly from spermathecae (Fig. 11D).

Variation. Four morphotypes were found in this study: (1) the dark brown morph is the most common in Thailand. The dorsal abdomen is plain dark brown (Fig. 6A, B). This form is concordant with the description by Lucas (1835). (2) A dark red with stripes morph bears three thin yellow stripes near the anterior margin, between median spines, and in front of posterior sigilla (Fig. 6C). (3) A narrow horizontally banded morph bears three white and three black horizontal lines on dorsal abdomen (Fig. 6D). The first and the third white bands are very narrow. (4) A broad horizontally banded morph possesses three white and two black horizontal lines on dorsal abdomen (Fig. 6E). The median spines are bright orange. Ventral side of abdomen is decorated by bright yellow spots.

Remarks. *Gasteracantha diardi* can be distinguished from other broad-abdomen *Gasteracantha* by its large and posteriorly arched median spines, and two additional small sigilla beside the median trapezoid-shaped sigilla. The original description of *G. diardi* describes the plain dark brown morph specimens (Lucas 1835). In this study, we report three additional color morphs other than the original description. These color morphs are confirmed by molecular phylogenetic analysis in this study (Fig 4).

Distribution and habitat. India, China, Laos, Thailand, Malaysia, and Indonesia (Java, Borneo, and Sumatra) (Butler 1873; Pickard-Cambridge 1879; Dahl 1914; World Spider Catalog 2020). *Gasteracantha diardi* usually constructs a vertical web between trees, at a height of approximately 2 meters above ground in open areas, and sits at the center of the web.

Gasteracantha doriae Simon, 1877

Figures 7, 11G–I

Gasteracantha doriae Simon, 1877: 232, pl.3, fig. 3. Type locality: Sarawak, Borneo Island.

Full list of synonyms and usage of the name available in World Spider Catalog (2020).

Material. Thailand • 3 ♀ juvenile; Trat Province, Laem Ngop District; 12°10.39'N, 102°24.33'E; MUMNH-ARA-GAS053 • 1 ♀ juvenile; Surat Thani Province, Khiri

Rat Nikhom District, Wang Badarn Cave; 08°54.52'N, 98°57.08'E; MUMNH-ARA-GAS068 • 5 ♀; Rayong Province, Wang Chan District, Pa Yup Nai; 13°01.27'N, 101°26.83'E; MUMNH-ARA-GAS130, MUMNH-ARA-GAS131.

Diagnosis. Sternum brownish black with large yellow spot at the center. Abdomen much wider than long. Dorsal side of abdomen with two black and three white horizontal bands. Two black abdominal horizontal bands arched with sinuous margins. First black horizontal band slightly hollow at the anterior middle. Edge of abdomen with serrated spikes, obvious on spines. Anterior spines smallest, directed obliquely. Posterior spines conical, pointed backward. Median spines longest, less conical, and slightly arched backward. One large median spot between the bases of posterior spines, and one lateral spot on each side. Ventral side of abdomen blackish with chalk-white spots and small black granules. Sigilla reddish brown. Anterior edge with ten sigilla: four sigilla in the middle smaller, forming a straight line, three sigilla on each side larger, trapezoid-shaped. Four median sigilla arranged in trapezoid shape. Posterior edge with ten sigilla: six sigilla in the middle smaller, forming a straight line, with the pair in the middle close together; two sigilla on each side larger, trapezoid. Outer posterior edge with five sigilla near posterior spines. Epigynum with a pair of hook-shaped sclerotized structures between spermathecae, visible in posterior view (Fig. 11I). Spermathecae round (Fig. 11G), ventrally partially overlapped by wing-shaped sclerotized structure (Fig. 11H). Scape long, pointed posteriorly, flanked by lateral sclerotized plates (Fig. 11I). Copulatory ducts encapsulated by sclerotized structure (Fig. 11G). Fertilization duct emerging posteriorly from spermathecae (Fig. 11G).

Variation. Two color morphs are observed consisting of the black-white banded morph (Fig. 7A) and the black-yellow banded morph (Fig. 7B). The black bands in the B&Y morph are less sinuous than in the B&W morph.

Remarks. This species resembles *G. frontata*, *G. diadesmia*, and *G. sturi*. These species can be distinguished from each other by abdominal spines and abdominal color pattern. The median spines of *G. doriae* are longer and less conical than *G. frontata*. The median spines of *G. diadesmia* are thicker and wider than *G. doriae*. *Gasteracantha doriae* differs from *G. sturi* in having longer and pointed median spines and wider black horizontal bands. Additionally, the angle between anterior and median spines of *G. doriae* is more obtuse than other species. Although the type specimen of *G. frontata* is without horizontal bands (Blackwall 1864; Pickard-Cambridge 1879), there are some reports stating that *G. frontata* contains abdominal horizontal bands (Pickard-Cambridge 1879; Pocock 1900). Pocock (1900) reported that the first horizontal band of *G. frontata* reaches the base of the anterior spine, whereas the first horizontal band of *G. doriae* terminates before the base of the anterior spine.

Two *Gasteracantha* species with abdominal horizontal bands that were previously recognized as *G. diardi* by Tan et al. (2019) are grouped separately from other Thai *G. diardi* with high nodal support. In addition, these two individuals are morphologically different from other *G. diardi* specimens from Thailand by having smaller size of median spines, as well as different color pattern (horizontal bands morph). By comparing photographs in Tan et al. (2019) and previous taxonomic publications (Simon 1877;

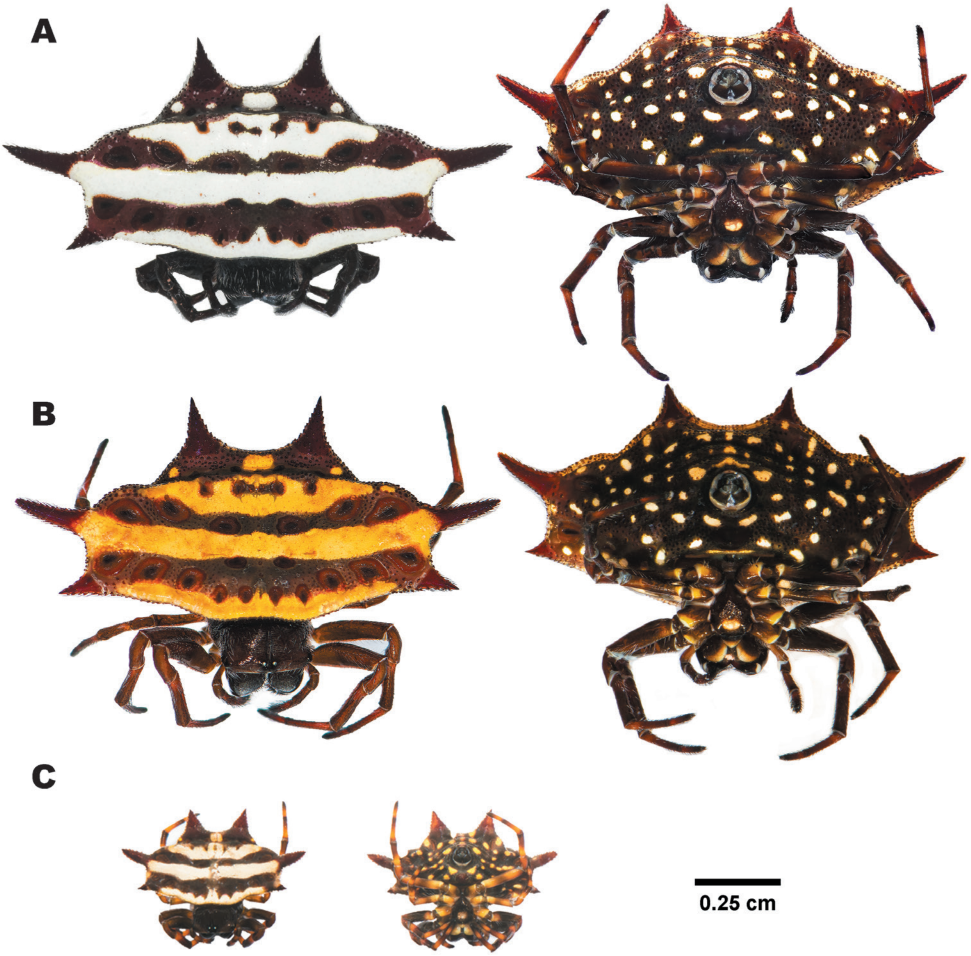


Figure 7. Females of *Gasteracantha doriae* **A** black-white bands morph, specimen from Rayong (MUMNH-ARA-GAS131) **B** black-yellow bands morph, specimen from Rayong (MUMNH-ARA-GAS130-1) **C** juvenile, specimen from Trat (MUMNH-ARA-GAS053) **A, B** belong to clade D2 and **C** from clade D1 in Fig. 3.

Workman and Workman 1892), we propose that these two individuals were *Gasteracantha doriae*. Unfortunately, our specimens (Fig. 7C) in D1 clade that were placed in the same clade with *G. doriae* s.s. Tan et al. (2019) were still juvenile, and therefore we were unable to examine the genitalia.

Interestingly, the phylogenetic tree and species delimitation results suggest another distinct clade in *G. doriae* (clade D2 in Figs 3 and 4). These two clades of *G. doriae* show a distant relationship and potentially are cryptic species. Only a couple of morphological differences can be detected. Morphological characters of *G. doriae* D1, which we observed via photographs in Tan et al. (2019), is similar to the original description (Simon 1877), while *G. doriae* D2 shows morphological variation. The horizontal black bands of *G. doriae* D1 are rather straight with smooth margin, whereas the horizontal black bands of *G. doriae* D2 are curved and with apparently sinuous margin (Figs 7A, B). In addition, *G. doriae* D1 possesses three horizontal black bands, while *G. doriae* D2 presents only two horizontal black bands. The angle between anterior and median spines of *G. doriae* D2 is more obtuse than in *G. doriae* D1. All molecular analyses (i.e., phylogenetic analyses, species delimitation, and genetic distance) in this study strongly suggest that the two lineages are distinct species. However, due to unavailability of adult specimens of *G. doriae* D1, we were unable to compare the female genitalia structure between *G. doriae* D1 and D2, which is usually used as a reliable and distinguishable character in *Gasteracantha* species. Further investigation of adult female specimens from the type locality is necessary to resolve this taxonomic problem.

Distribution and habitat. Indonesia (Borneo), Malaysia, and Thailand (World Spider Catalog 2020). Adult spiders were collected from shrubs and trees. The female spider builds a vertical web between shrubs or trees in open areas. They sit at the center of the web with head directed downward.

Gasteracantha kuhli C. L. Koch, 1837

Figures 8, 11J–L

Gasteracantha kuhli C. L. Koch, 1837: 20, fig. 262. Type locality: Indonesia, Java.
Full list of synonyms and usage of the name available in World Spider Catalog (2020).

Material. Thailand • 5 ♀; Nakhon Ratchasima Province, Pak Chong District; 14°31.10'N, 101°24.00'E; MUMNH-ARA-GAS002 • 5 ♀; Ubon Ratchathani Province, Na Chaluai District, Dom Yai Canal; 14°41.25'N, 105°09.27'E; MUMNH-ARA-GAS003 • 2 ♀; Kanchanaburi Province, Si Sawat District; 14°26.49'N, 99°08.06'E; MUMNH-ARA-GAS004 • 2 ♀; Surat Thani Province, Ko Pha-ngan District, Koh Tao Is.; 10°04.04'N, 99°49.10'E; MUMNH-ARA-GAS005 • 5 ♀; Samut Prakan Province, Phra Pradaeng District, Bang Kachao; 13°41.50'N, 100°33.46'E; MUMNH-ARA-GAS005 • 1 ♀; Surat Thani Province, Phanom District, Khlong Sok; 08°54.20'N, 98°31.81'E; MUMNH-ARA-GAS007 • 4 ♀; Kanchanaburi Province, Sai Yok District; 14°24.93'N, 98°52.54'E; MUMNH-ARA-GAS027 • 5 ♀; Ratchaburi Province, Suan Phueng District; 13°33.03'N, 99°17.14'E; MUMNH-ARA-GAS029 • 5 ♀; Ratchaburi Province, Suan Phueng District, Pachi Stream; 13°31.18'N, 99°18.88'E; MUMNH-ARA-GAS030 • 5 ♀; Samut Prakan Province, Phra Pradaeng District, Bang Kachao; 13°41.85'N, 100°33.93'E; MUMNH-ARA-GAS033 • 4 ♀; Trang Province, Mueang District, Ban Pho; 07°34.18'N, 99°39.16'E; MUMNH-ARA-GAS039 • 2 ♀; Ranong Province, Suk Samran District, Na Kha; 09°23.75'N, 98°25.75'E; MUMNH-ARA-GAS040 • 5 ♀; Krabi Province, Mueang District, Krabi Noi; 08°07.54'N, 98°55.50'E; MUMNH-ARA-GAS042 • 5 ♀; Phrae Province, Mueang District; 18°08.58'N, 100°07.81'E; MUMNH-ARA-GAS046 • 4 ♀; Mae Hong Son Province, Mueang District, Pang Mu; 19°18.12'N, 97°57.58'E; MUMNH-

ARA-GAS048 • 2 ♀; Mae Hong Son Province, Mae La Noi District, Mae La Luang; 18°32.31'N, 97°53.83'E; MUMNH-ARA-GAS049 • 1 ♀; Loei Province, Mueang District, Kok Thong; 17°30.53'N, 101°35.83'E; MUMNH-ARA-GAS054 • 1 ♀; Loei Province, Pak Chom District, Huai Bo Suen; 17°44.78'N, 101°58.33'E; MUMNH-ARA-GAS055 • 5 ♀; Udon Thani Province, Nam Som District; 17°46.93'N, 102°06.02'E; MUMNH-ARA-GAS056 • 1 ♀; Nakhon Ratchasima Province, Mueang District, Suranari University; 14°52.97'N, 102°01.27'E; MUMNH-ARA-GAS057 • 5 ♀; Prachuap Khiri Khan Province, Bang Saphan District, Khao Ma Rong Cave; 11°12.17'N, 99°29.65'E; MUMNH-ARA-GAS060 • 2 ♀; Chumphon Province, Tha Sae District, Pisadarn Cave; 10°45.60'N, 99°13.77'E; MUMNH-ARA-GAS063 • 2 ♀; Surat Thani Province, Khiri Rat Nikhom District, Wang Badarn Cave; 08°54.52'N, 98°57.83'E; MUMNH-ARA-GAS066 • 1 ♀; Sa Kaeo Province, Khlong Hat District, Phet Pho Thong Cave; 13°24.80'N, 102°19.55'E; MUMNH-ARA-GAS072 • 1 ♀; Lopburi Province, Mueang District, Kok Toom; 14°48.80'N, 100°47.63'E; MUMNH-ARA-GAS074 • 1 ♀; Lopburi Province, Mueang District, Phra Tad Cave; 14°48.40'N, 100°49.48'E; MUMNH-ARA-GAS075 • 1 ♀; Chanthaburi Province, Mueang District, Khlong Narai; 12°35.48'N, 102°09.45'E; MUMNH-ARA-GAS078 • 2 ♀; Nakhon Nayok Province, Pak Phli District, Khun Dan Prakarn Chon Dam; 14°18.88'N, 101°19.27'E; MUMNH-ARA-GAS080 • 4 ♀; Kanchanaburi Province, Si Sawat District, Ong-ju Canal; 14°48.45'N, 99°05.53'E; MUMNH-ARA-GAS084 • 1 ♀; Phetchabun Province, Si Thep District, 15°28.52'N, 100°58.53'E; MUMNH-ARA-GAS085 • 1 ♀; Phetchabun Province, Lom Sak District; 16°43.73'N, 101°20.22'E; MUMNH-ARA-GAS087 • 2 ♀; Loei Province, Wang Saphung District, Pha Bing, 17°14.05'N, 101°45.63'E; MUMNH-ARA-GAS089 • 4 ♀; Phetchabun Province, Wichian Buri District, Wat Tham Thep Bandan, 15°45.42'N, 101°02.27'E; MUMNH-ARA-GAS090 • 2 ♀; Loei Province, Wang Saphung District, Pha Bing; 17°14.47'N, 101°44.25'E; MUMNH-ARA-GAS091 • 2 ♀; Loei Province, Chiang Khan District, Bu Hom; 17°55.05'N, 101°45.13'E; MUMNH-ARA-GAS092 • 1 ♀; Loei Province, Phu Kradueng District, Pha Nok Khao; 16°53.65'N, 101°57.28'E; MUMNH-ARA-GAS093 • 2 ♀; Phetchabun Province, Mueang District, Wat Nam Pang Cave; 16°14.77'N, 101°08.17'E; MUMNH-ARA-GAS094 • 3 ♀; Chaiyaphum Province, Phakdi Chumphon District, Wua Daeng Cave; 16°04.55'N, 101°26.45'E; MUMNH-ARA-GAS095 • 3 ♀; Loei Province, Nong Hin District; 17°02.42'N, 101°44.18'E; MUMNH-ARA-GAS098 • 3 ♀; Chiang Mai Province, Mae Rim District, Mae Raem; 18°55.10'N, 98°54.52'E; MUMNH-ARA-GAS101 • 1 ♀; Chiang Mai Province, Mueang District; 18°46.93'N, 98°57.53'E; MUMNH-ARA-GAS103 • 2 ♀; Kanchanaburi Province, Thong Pha Phum District, Huai Kayeng; 14°37.85'N, 98°34.32'E; MUMNH-ARA-GAS110 • 1 ♀; Ratchaburi Province, Mueang District, Nam Phu; 13°33.47'N, 99°36.97'E; MUMNH-ARA-GAS114 • 2 ♀; Chiang Rai Province, Mae Fa Luang District; 20°14.23'N, 99°49.42'E; MUMNH-ARA-GAS123 • 3 ♀; Chiang Mai Province, Chiang Dao District; MUMNH-ARA-GAS124.

Diagnosis. Sternum black with dull yellow hoof-shaped patch. Abdomen octagonal, slightly wider than long. Dorsal side of abdomen with black and white patches.

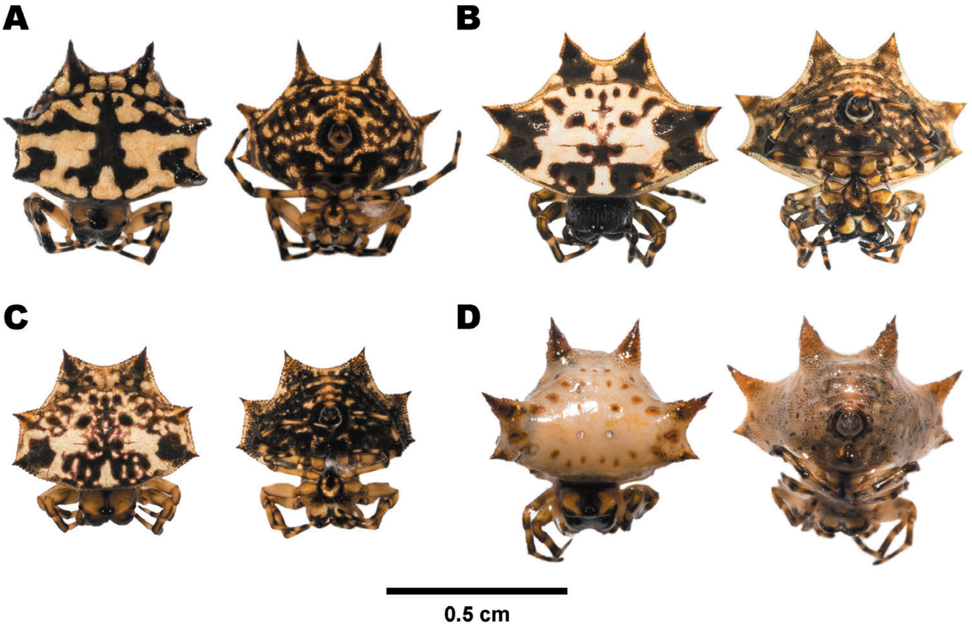


Figure 8. Females of *Gasteracantha kuhli* showing dorsal view (left) and ventral view (right) **A–C** black-white morph **A** specimen from Samut Prakan (MUMNH-ARA-GAS033) **B** specimen from Lopburi (MUMNH-ARA-GAS074) **C** specimen from Ratchaburi (MUMNH-ARA-GAS029) **D** pale orange morph, specimen from Surat Thani (MUMNH-ARA-GAS007).

Edge of abdomen smooth. Three pairs of spines similar in shape. Bases of anterior spines and median spines fused. Ventral side of abdomen blackish brown with scattered chalky yellow stripes. Anterior edge with ten sigilla in total: four sigilla in the middle, three sigilla on each side, placed near the base of anterior spines. Four median sigilla arranged in trapezoid shape. Posterior edge with ten sigilla in total: six sigilla in the middle near posterior margin, forming a straight line, the pair in the middle closely placed. Outer posterior edge with five sigilla, placed near posterior spines. Epigynum subtriangular with small subtriangular scape (Fig. 11F). Spermathecae round (Fig. 11G), ventrally partially overlapped by unconnected sclerotized structures on each side (Fig. 11H). Copulatory ducts encapsulated by sclerotized structure (Fig. 11G). Fertilization ducts emerging posteriorly from spermathecae (Fig. 11G).

Variation. Color patterns on the abdomen of *G. kuhli* are variable, but commonly with inverse Y-band markings on the dorsal abdomen (Fig. 8A-C). Another morph is pale orange (Fig. 8D). This morph is newly discovered in this study. Its description is as follows: cephalothorax blackish brown with large dull yellow patches on each side, slightly longer than wide, clothed with short white hairs. Cephalic region highly elevated and abruptly sloped downward posteriorly, thoracic region overlapped by anterior side of abdomen. Eight eyes arranged into two rows subequal in size, located above the frontal margin: four median eyes form a trapezoid and are placed on a small protuberance at the middle of frontal margin, lateral eyes on each side placed on a tubercle near corner of frontal margin. Sternum dark brown with large hoof-shaped patch. Abdomen slightly wider than long, pale beige with small brown spots on margin. Six abdominal spines orangish brown, conical, tapering toward the tip. Anterior spines smallest, directed obliquely. Median spines pointed obliquely. Posterior spines largest, pointed backward with small brown spots near the bases. Ventral side of abdomen pale orange with scattered brown granules. Sigilla orangish brown. Ten anterior edge sigilla in total: six sigilla in the middle, two sigilla on each side near the base of anterior spines. Four median sigilla arranged in trapezoid. Ten posterior edge sigilla in total, the pair in the middle placed close together. Outer posterior edge with five sigilla near posterior spines.

Distribution and habitat. Bhutan, China, Japan, Korea, Hong Kong, Taiwan, Cambodia, Thailand, Myanmar, Andaman and Nicobar Islands, Indonesia (Java, and Sumatra), Phi1ippines, and Singapore (Barrion and Litsinger 1995; Sen et al. 2015; World Spider Catalog 2020). *Gasteracantha kuhli* can be found in several habitats such as paddy fields, dipterocarp forest, dry evergreen forest and agriculture areas. The female spider builds a vertical web between shrubs or trees in open areas. The spiders sit at the center of web with head pointed downwards.

Gasteracantha clavigera Giebel, 1863

Gasteracantha clavigera Giebel, 1863: 307. Type locality: Siam.
Full list of synonyms and usage of the name available in World Spider Catalog (2020).

Remarks. The abdomen of *G. clavigera* is octagonal, slightly wider than long. Color of the abdomen is yellow, with black stripes near the anterior edge. The appearance of this species is similar to *M. hasselti* and *M. arcuata*. However, tips of median spines of *G. clavigera* are club-shaped, and decorated with a tuft of hairs (Giebel 1863; Butler 1873; Simon 1877).

Gasteracantha clavigera was described by Giebel (1863). However, only the name "Siam" [= Thailand] was mentioned, without any location details. *Gasteracantha clavigera* has been reported in the Malay Archipelago. Based on its distribution records from previous study, this species might be found in the southern part of Thailand (World Spider Catalog 2020).

Distribution. Thailand, Philippines (Luzon, Manilla, and Samar), and Indonesia (Sulawesi) (Dahl 1914; World Spider Catalog 2020).

Gasteracantha frontata Blackwall, 1864

Gasteracantha frontata Blackwall, 1864: 40. Type locality: East Indies.
Full list of synonyms and usage of the name available in World Spider Catalog (2020).

Remarks. The abdomen of *G. frontata* is wider than long. Color of the abdomen is brownish yellow. Median spines of *G. frontata* are conical, and not elongated compared to other *Gasteracantha* species with a broad abdomen (Blackwall 1864; Pickard-Cambridge 1879). *Gasteracantha frontata* were reported from Chanthaburi and Rayong provinces (Simon 1886). However, we failed to obtain specimens from either area in this study.

Distribution. East Indies, India, Thailand, Myanmar, Vietnam, and Indonesia (Simon 1886; World Spider Catalog 2020).

Gasteracantha irradiata (Walckenaer, 1841)

Plectana irradiata Walckenaer, 1841: 170. Type locality: Cochinchina.
Full list of synonyms and usage of the name available in World Spider Catalog (2020).

Remarks. The abdomen of *G. irradiata* is oval and wider than long. Color of the abdomen is yellowish. The anterior edge of the abdomen is strongly curved backwards. Abdominal sigilla are very small. Abdominal spines are reddish. Anterior spines are shortest. Median spines are longest (Walckenaer 1841; Merian 1911; Dahl 1914).

The specimens of *G. irradiata* collected from Thailand belong to Dahl's collection (Dahl 1914). However, the sampling locality was only noted as "Siam." Based on its distribution records from previous study, it is possible that *G. irradiata* might be found in the southern and/or eastern parts of Thailand (World Spider Catalog 2020). We failed to collect *G. irradiata* in this study.

Distribution and habitat. Vietnam, Thailand, and Indonesia (Sulawesi, Sumatra, Lombok, and Java) (Dahl 1914; World Spider Catalog 2020).

Gasteracantha rubrospinis Guérin, 1838

Gasteracantha rubrospinis Guérin, 1838: 53. Type locality: Waigiou [Waigeo Island].
Full list of synonyms and usage of the name available in World Spider Catalog (2020).

Remarks. The abdomen of *Gasteracantha rubrospinis* is wider than long. This species can be distinguished from other Thai *Gasteracantha* by characteristics of their spines and the color pattern on the dorsal abdomen. The abdomen is bright yellow, with a large and incomplete horizontal black transverse band near the anterior edge. The abdominal spines are wider at the base, tapered toward the tip, and ending with a sharp point (Guérin 1838; Simon 1877; Pocock 1897). The reported specimens of *G. rubrospinis* from Thailand belong to Pocock's collection (Pocock 1897). The locality was listed as "Patani" [= Pattani Province], the southernmost province of Thailand. No specimens were obtained in this study.

Distribution and habitat. Indonesia (Moluccas, Sulawesi, Lombok), New Caledonia, Guam, Thailand (Pattani Province) (Pocock 1897; World Spider Catalog 2020).

Genus *Macracantha* Simon, 1864

Type species. *Aranea arcuata* Fabricius, 1793

Diagnosis. Cephalic region highly elevated near the middle, abruptly sloped downward posteriorly. Median ocular quadrangle wider behind than in front. Cephalothorax overlapping anterior abdomen. Sternum heart-shaped, pointed posteriorly, concave anteriorly below labium. Abdomen octagonal with three pairs of spines, and sigilla on dorsal and ventral sides. Anterior edge of abdomen curved between median spines. Dorsal sigilla teardrop-shaped, subequal in size, arranged in three rows, and situated near the anterior edge, posterior edge, and behind the posterior edge. Four median sigilla arranged in a trapezoid. Median spines well developed, elongated. Ventral tubercle is absent. Spinnerets placed on elevated black sclerotized structure, forming a shape like a shield volcano. Legs elongated.

Remarks. *Macracantha* was formerly classified as a subgenus of *Gasteracantha*, but later elevated to an independent genus by Emerit (1974). This genus now consists of two species, *M. arcuata* (World Spider Catalog 2020) and *M. hasselti* (this study). The latter species is currently transferred to *Macracantha* according to phylogenetic analyses and anatomical evidence in this study.

Macracantha arcuata (Fabricius, 1793)

Figures 9A, B, 12A–C

Aranea arcuata Fabricius, 1793: 425. Type locality: East Indies.
Full list of synonyms and usage of the name available in World Spider Catalog (2020).

Material. Thailand • 4 ♀; Krabi Province, Mueang District, Krabi Noi; 08°07.45'N, 98°55.45'E; MUMNH-ARA-MAC002 • 1 ♀; Phang-nga Province, Thap Put District, 08°35.58'N, 98°40.08'E; MUMNH-ARA-MAC003 • 2 ♀; Nakhon Ratchasima Province, Pak Chong District; 14°31.10'N, 101°24.00'E; MUMNH-ARA-MAC004 • 5 ♀; Krabi Province, Plai Phraya District, Khao Khao Hua Ling; 08°30.88'N, 98°45.57'E; MUMNH-ARA-MAC005 • 1 ♀; Ranong Province, Mueang District, Hat Som paen; 09°57.55'N, 98°39.57'E; MUMNH-ARA-MAC007 • 4 ♀; Prachuap Khiri Khan Province, Bang Saphan District, Khao Ma Rong Cave; 11°12.17'N, 99°29.65'E; MUMNH-ARA-MAC008 • 2 ♀; Phang-nga Province, Mueang District; 08°26.57'N, 98°30.95'E; MUMNH-ARA-MAC009 • 2 ♀; Phetchabun Province, Lom Sak District; 16°43.74'N, 101°20.22'E; MUMNH-ARA-MAC010 • 2 ♀; Chiang Mai Province, Mae Rim District, Pong Yaeng; 18°53.93'N, 98°51.58'E; MUMNH-ARA-MAC011 • 1 ♀; Nakhon Si Thammarat Province, Chang Klang District; 08°19.27'N, 99°35.38'E; MUMNH-ARA-MAC012 • 4 ♀; Kanchanaburi Province, Sai Yok District; 14°24.93'N, 98°52.53'E; MUMNH-ARA-MAC013 • 1 ♀; Chumphon Province, Mueang District, Ban Na; 10°27.43'N, 99°02.58'E; MUMNH-ARA-MAC015 • 2 ♀; Mae Hong Son Province, Mae Sariang District, Mae Ho, 18°03.78'N, 98°02.20'E; MUMNH-ARA-

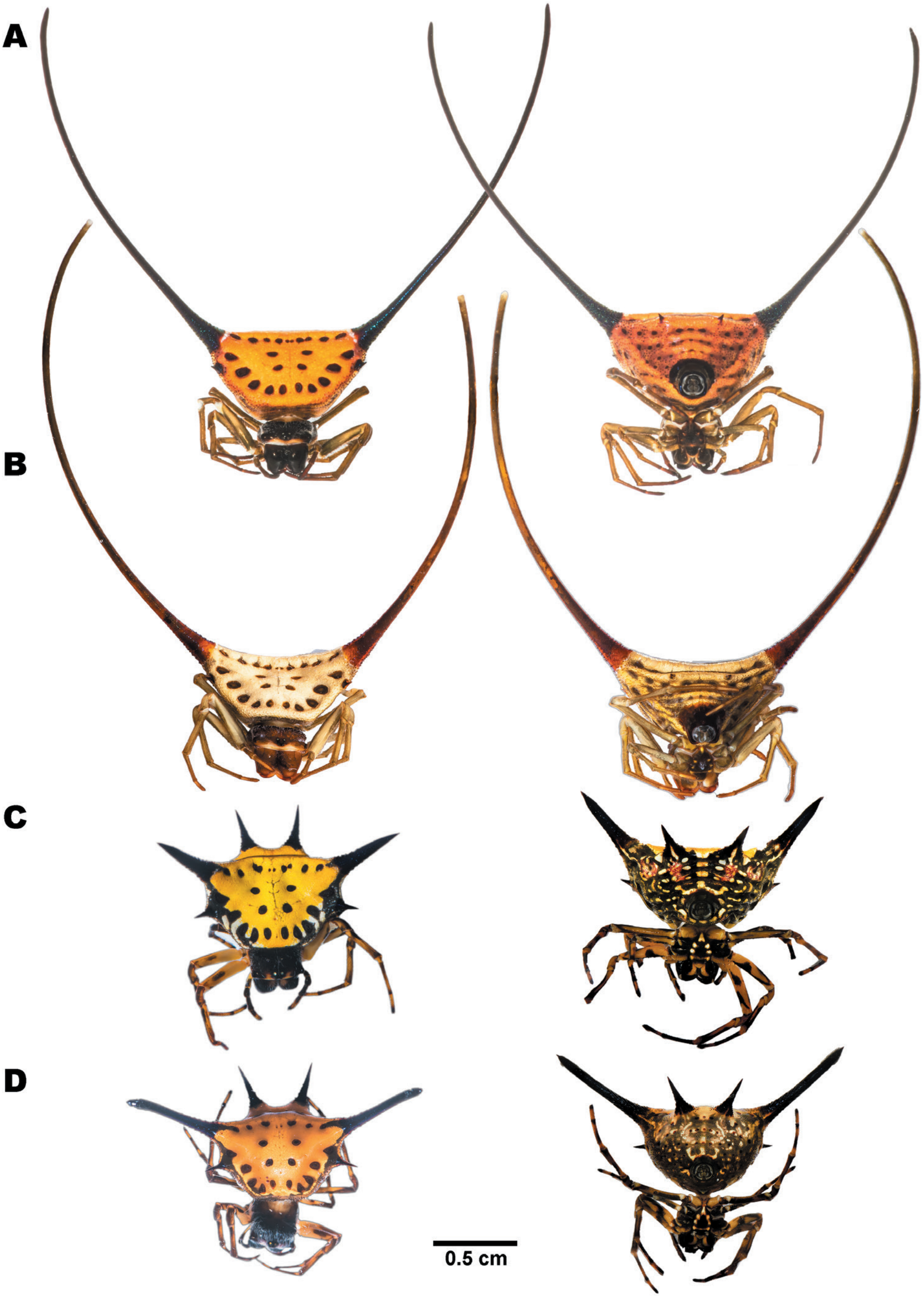


Figure 9. Females of **A, B** *Macracantha arcuata* and **C, D** *M. hasselti* showing dorsal view (left) and ventral view (right) **A** orange morph, specimen from Prachuap Khiri Khan (MUMNH-ARA-MAC008) **B** white morph, specimen from Kanchanaburi (MUMNH-ARA-MAC013-W1) **C** sharp spines morph, specimen from Saraburi (MUMNH-ARA-GAS018) **D** long spines morph, specimen from Phetchaburi (MUMNH-ARA-GAS025).

MAC016 • 2 ♀; Chiang Mai Province, Mae Taeng District; 19°10.71'N, 98°54.95'E; MUMNH-ARA-MAC017 • 3 ♀; Chumphon Province, Sawi District, Nam Lot Cave; 10°14.03'N, 98°56.68'E; MUMNH-ARA-MAC019 • Chumphon Province, Mueang District, Wat Tham Sing; 10°25.58'N, 99°03.63'E; MUMNH-ARA-MAC020 • 1 ♀; Chanthaburi Province, Laem Sing District; 12°31.12'N, 102°10.23'E; MUMNH-ARA-MAC022. Cambodia • 2 ♀; Cambodia Province, Kampot District; 10°34.92'N, 104°07.22'E; MUMNH-ARA-MAC021.

Diagnosis. Sternum black with yellow patches near anterior edge, coxae II and III, and the apex. Abdomen octagonal, orange, and slightly wider than long. Anterior edge of abdomen curved between anterior spines. Median spines very long, slender, and strongly arched, three times the abdomen width. Anterior and posterior spines poorly developed. Ventral side of abdomen orange. Spinnerets placed on strongly elevated black sclerotized structure. Ten anterior edge sigilla subequal in size. Four median sigilla arranged in a trapezoid. Ten posterior edge sigilla arranged in a straight line, closely spaced together. Outer posterior edge with nine sigilla: five sigilla placed near posterior spines, two sigilla on each side. Epigynum wider than long, with transparent median groove, visible in ventral view (Fig. 12B). Scape tongue-shaped, with strongly recurved tip, visible in ventral view (Fig. 12B). Spermathecae reniform (Fig. 12A). Copulatory ducts bulging distally, encapsulated by sclerotized structure (Fig. 12A, C). Fertilization ducts emerging posteriorly from spermathecae (Fig. 12A).

Variation. Two plain color morphs were found in this study, consisting of an orange morph (Fig. 9A), and a white morph (Fig. 9B). The orange morph was the most common, whereas the white morph was found rarely within some populations.

Distribution and habitat. India, Sri Lanka, China, Myanmar, Malaysia, Thailand, Cambodia, and Indonesia (Java and Sumatra) (Tikader 1982; Yin et al. 1997; World Spider Catalog 2020). *Macracantha arcuata* builds a vertical web under the shade of large trees or thick bushes. The female spider hangs at the underside of the web.

Macracantha hasselti **(C. L. Koch, 1837) comb. nov.**

Figures 9C–D, 12D–I

Gasteracantha hasseltii C. L. Koch, 1837: 29, fig. 267. Type locality: Indonesia, Java. Full list of synonyms and usage of the name available in World Spider Catalog (2020).

Material. Thailand • 3 ♀; Nakhon Ratchasima Province, Wang Nam Khiao District; 14°32.57'N, 101°58.22'E; MUMNH-ARA-GAS013 • 2 ♀; Ratchaburi Province, Suan Phueng District, 13°34.88'N, 99°10.79'E; MUMNH-ARA-GAS014 • 2 ♀; Nan Province, Tha Wang Pha District; 19°08.45'N, 100°45.38'E; MUMNH-ARA-GAS15 • 5 ♀; Ubon Ratchathani Province, Na Chaluai District, Wat Phupansoong; 14°30.30'N, 105°16.33'E; MUMNH-ARA-GAS016 • 1 ♀; Nakhon Ratchasima Province, Pak Chong District; 14°31.58'N, 101°22.13'E; MUMNH-ARA-GAS017 • 2 ♀; Saraburi Province, Kaeng Khoi District, Tha Maprang; 14°29.85'N, 101°08.25'E;

MUMNH-ARA-GAS018 • 4 ♀; Phrae Province, Rong Kwang District, Huai Rong Waterfall; 18°26.51'N, 100°27.01'E; MUMNH-ARA-GAS019 • 5 ♀; Ubon Ratchathani Province, Det Udom District, Non Sombun; 14°47.44'N, 105°06.16'E; MUMNH-ARA-GAS020 • 2 ♀; Nakhon Ratchasima Province, Pak Chong District, Phaya Yen; 14°36.97'N, 101°15.90'E; MUMNH-ARA-GAS024 • 2 ♀; Phetchaburi Province, Kaeng Krachan District; 12°53.41'N, 99°39.32'E; MUMNH-ARA-GAS025 • 1 ♀; Phetchaburi Province, Kaeng Krachan District; 12°54.68'N, 99°38.45'E; MUMNH-ARA-GAS037 • 2 ♀; Chiang Mai Province, Mae Taeng District; 19°08.51'N, 98°54.94'E; MUMNH-ARA-GAS038 • 4 ♀; Mae Hong Son Province, Mueang District, Pang Mu; 19°18.12'N, 097°57.73'E; MUMNH-ARA-GAS049 • 5 ♀; Mae Hong Son Province, Mae La Noi District, Mae La Luang; 18°32.31'N, 97°53.83'E; MUMNH-ARA-GAS050 • 3 ♀; Prachuap Khiri Khan Province, Bang Saphan District, Wat Tham Khao Wong; 11°17.47'N, 99°29.72'E; MUMNH-ARA-GAS062 • 5 ♀; Chumphon Province, Tha Sae District, Pisadarn Cave; 10°45.60'N, 99°13.77'E; MUMNH-ARA-GAS065 • 5 ♀; Sa Kaeo Province, Khlong Hat District, Saeng Tian Cave; 13°18.93'N, 102°19.91'E; MUMNH-ARA-GAS070 • 5 ♀; Sa Kaeo Province, Khao Chakan District, Wat Tham Khao Chan; 13°34.73'N, 102°05.56'E; MUMNH-ARA-GAS073 • 5 ♀; Kanchanaburi Province, Si Sawat District, Ong-ju Canal; 14°48.45'N, 99°05.53'E; MUMNH-ARA-GAS083 • 4 ♀; Phetchabun Province, Lom Sak District; 16°43.74'N, 101°20.22'E; MUMNH-ARA-GAS088 • 3 ♀; Chaiyaphum Province, Phakdi Chumphon District, Wua Daeng Cave; 16°04.55'N, 101°26.46'E; MUMNH-ARA-GAS097 • 3 ♀; Chiang Mai Province, Mae Rim District, Mae Raem; 18°55.10'N, 98°54.51'E; MUMNH-ARA-GAS100 • 3 ♀; Kanchanaburi Province, Thong Pha Phum District, Huai Kayeng; 14°37.85'N, 98°34.32'E; MUMNH-ARA-GAS109 • 1 ♀; Kanchanaburi Province, Sai Yok District, Tha Sao; 14°21.14'N, 98°57.28'E; MUMNH-ARA-GAS113 • 1 ♀; Kanchanaburi Province, Si Sawat District, Khao Chot; 14°48.26'N, 99°10.93'E; MUMNH-ARA-GAS121 • 3 ♀; Kanchanaburi Province, Si Sawat District, Tha Kradan; 14°22.41'N, 99°09.02'E; MUMNH-ARA-GAS125.

Diagnosis. Sternum black with yellow patches near anterior edge, coxae II and III, and the apex. Abdomen octagonal. Anterior edge of abdomen curved between anterior spines. Dorsal side of abdomen yellow with black and white patches near anterior margin. Anterior and posterior spines small, and sharp at the tips. Median spines longest, tapering toward the tip. Ventral side of abdomen black with scattered yellow stripes. Spinnerets placed on strongly elevated black sclerotized structure. Ten anterior edge sigilla subequal in size. Four median sigilla arranged in a trapezoid. Ten posterior edge sigilla arranged in a straight line, with the first pair and the second and third sigilla from the middle close together. Outer posterior edge with nine sigilla in total: five sigilla placed near posterior spines, two sigilla on each side. Epigynum subtriangular with sock-shaped structures, opposite to each other (Fig. 12D, G). Scape very long, tongue-shaped, pointed posteriorly (Fig. 12E, H). Spermathecae balloon-shaped (Fig. 12D, G). Copulatory ducts bulging distally, encapsulated by sclerotized structure (Fig. 12D, G). Fertilization ducts emerging posteriorly from spermathecae (Fig. 12D, G).

Variation. The patch near abdominal anterior margin is narrow or absent in some specimens. Two morphs are found in this study: a sharp spines morph (Figs 9C; 12D–F) with its morphology as in the diagnosis, and a long spines morph (figs 9D; 12G–I), which is characterized by the six abdominal spines being longer than in the sharp spines morph. The median spines are longest, straight without tapering, and with spikes at the bases. The epigynum of the two morphs are similar in shape.

Remarks. *Macracantha hasselti* was once classified in genus *Gasteracantha* (World Spider Catalog 2020). However, the phylogenetic tree in this study recovered a sister relationship between *M. arcuata* and *M. hasselti*, which is supported by their synapomorphic characters (see discussion) in both external and internal morphologies. Based on this evidence, we propose to reclassify these two species in the same genus.

The long spines morph resembles *Gasteracantha dalyi* Pocock, 1900, especially as their female genital structures are identical (Tikader 1982). They are differentiated from each other by the morphology of abdominal spines. Anterior and posterior spines of *M. hasselti* are longer and the median spines are shorter than in *G. dalyi* (Tikader 1982).

Distribution and habitat. India, China, Cambodia, Vietnam, Myanmar, Thailand, Malaysia, Singapore, and Indonesia (Java, and Sumatra) (Yin et al. 2012; Sen et al. 2015; World Spider Catalog 2020). *Macracantha hasselti* builds a vertical web under the shade of large trees or thick shrubs.

Genus *Thelacantha* Hasselt, 1882

Type species. *Plectana brevispina* Doleschall, 1857.

Diagnosis. Cephalic region highly elevated in middle, abruptly sloping downward posteriorly. Median ocular quadrangle wider behind than in front. Cephalothorax overlapping abdomen. Sternum heart-shaped, pointed posteriorly, and concave anteriorly below labium. Abdomen octagonal, with sigilla on dorsal and ventral sides. Three pairs of abdominal spines, tubercle, with a small protuberance at the tip. Dorsal sigilla in three rows, situated near the anterior edge, posterior edge, and behind the posterior edge. Four median sigilla arranged in a trapezoid. Ventral tubercle is present. Spinnerets encircled by black sclerotized rings.

Remarks. *Thelacantha* was a subgenus of *Gasteracantha*, but later proposed to be a genus (Benoit 1964; Emerit 1974), which is now monotypic (World Spider Catalog 2020).

Thelacantha brevispina (Doleschall, 1857)

Figures 10, 12J–L

Plectana brevispina Doleschall, 1857: 423. Type locality: Indonesia, Ambon Island.
Full list of synonyms and usage of the name available in World Spider Catalog (2020).

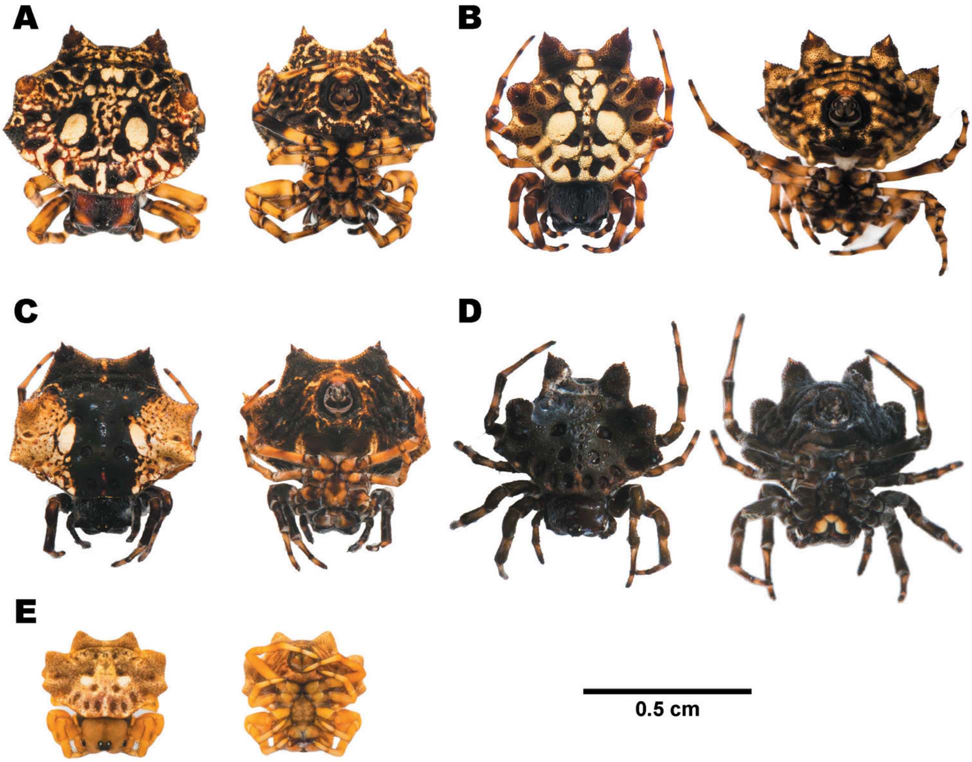


Figure 10. Females of *Thelacantha brevispina* showing dorsal view (left) and ventral view (right) **A, B** multi-color morph **A** specimen from Phetchaburi (MUMNH-ARA-THE004) **B** specimen from Samut Songkhram (MUMNH-ARA-THE008) **C** black-white morph, specimen from Loei (MUMNH-ARA-THE007) **D** black morph, specimen from Samut Songkhram (MUMNH-ARA-THE008) **E** orange morph, specimen from Surat Thani (MUMNH-ARA-THE005).

Material. Thailand • 4 ♀; Samut Sakhon Province, Khok Kham District; 13°29.27'N, 100°20.13'E; MUMNH-ARA-THE003 • 3 ♀; Phetchaburi Province, Ban Laem District; 13°02.55'N, 100°05.55'E; MUMNH-ARA-THE004 • 5 ♀, 2 ♀ juvenile; Surat Thani Province, Ko Pha-ngan District, Koh Tao Is.; 10°04.07'N, 99°49.16'E; MUMNH-ARA-THE005 • 5 ♀; Loei Province, Phu Ruea District, Lat Khang; 17°31.55'N, 101°15.33'E; MUMNH-ARA-THE007 • 5 ♀; Samut Songkhram Province, Mueang District, Bang Kaeo; 13°23.18'N, 100°02.18'E; MUMNH-ARA-THE008 • 2 ♀; Trat Province, Laem Ngop District, 12°10.38'N, 102°24.33'E; MUMNH-ARA-THE009.

Diagnosis. Sternum black. Sternal band various in shape. Abdomen octagonal, slightly wider than long. Color pattern on dorsal abdomen various but frequently with two large white spots. Three pairs of abdominal spines similar in shape, tubercle with small protuberance at the tip. Ventral side of abdomen black, with scattered yellowish stripes. Ten anterior edge sigilla subequal in size. Four median sigilla arranged in a trapezoid. Ten posterior edge sigilla, the middle pair very small, and close together. Outer

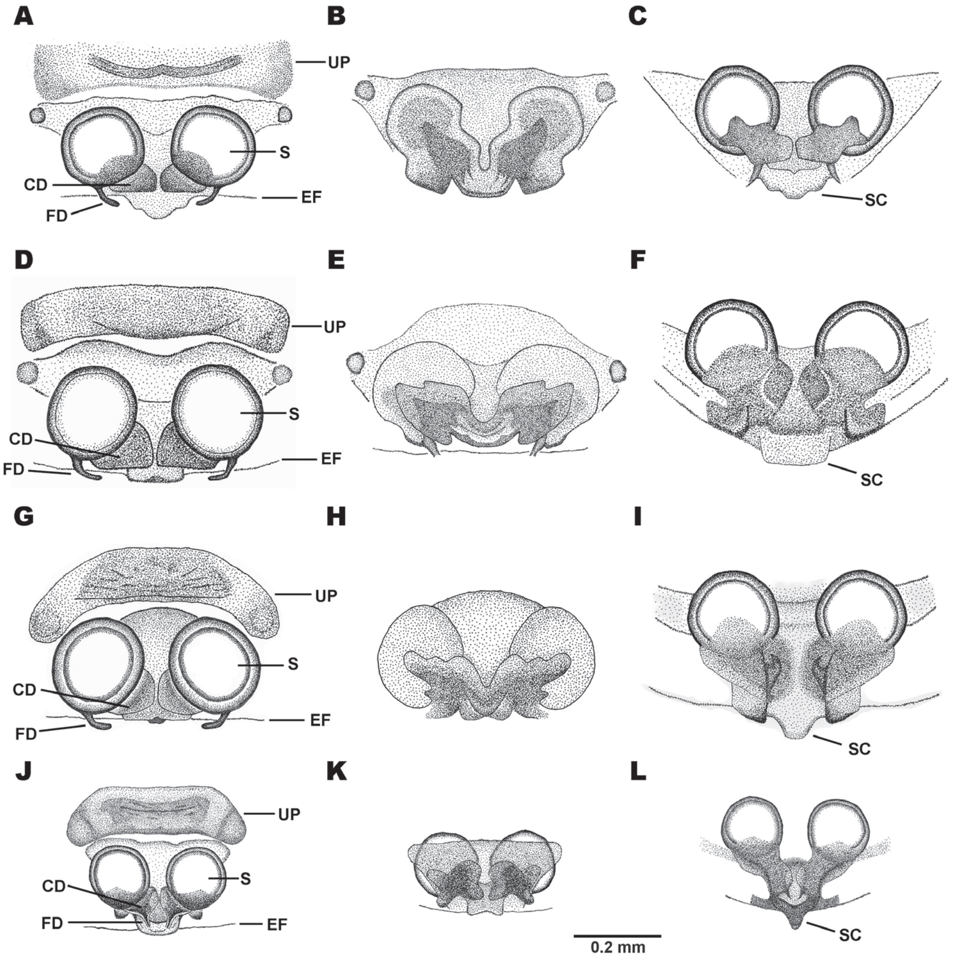


Figure 11. Female genitalia of **A–C** *Gasteracantha diadesmia* **D–F** *G. diardi* **G–I** *G. doriae* **J–L** *G. kuhli*. Genitalia are shown in dorsal view (**A, D, G, J**), ventral view from external (**B, E, H, K**), and posterior view (**C, F, I, L**).

posterior edge with five sigilla, located near posterior spines. Epigynum relatively simple in shape with bracket-shaped scape (Fig. 12K). Spermathecae oval, placed close together (Fig. 12J, K). Fertilization duct short, emerging posteriorly from spermathecae (Fig. 12J).

Variation. *Thelacantha brevispina* shows high color variation on abdomen. Four color morphs were found in this study: (1) the multi-colored morph (Fig. 10A, B) is decorated with white, black, and red patches on the dorsal abdomen; (2) the black-white morph (Fig. 10C) possesses a vertical central black line from the anterior to the base of the posterior spines with white areas on each side; (3) the black morph (Fig. 10D) shows a completely black abdomen without the two large white spots; (4) the orange morph (Fig. 10E) is characterized by a bright orange abdomen with two white spots. Such morphotypes are found in adult spiders, except in the orange morph,

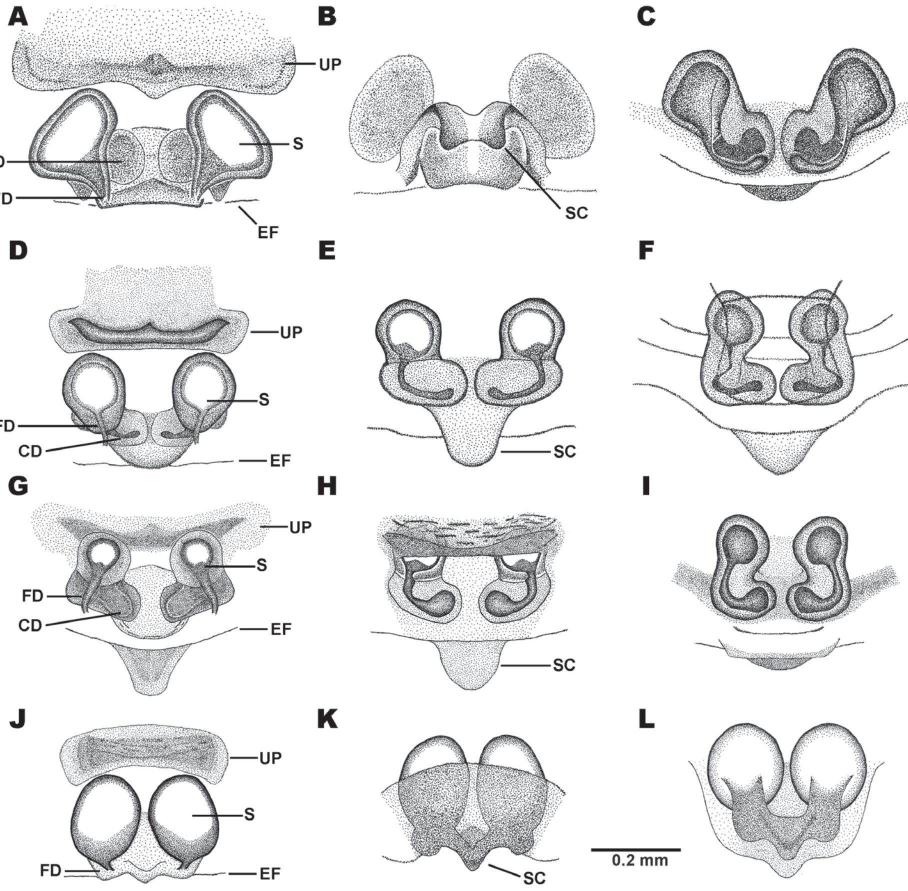


Figure 12. Female genitalia of **A–C** *Macracantha arcuata* **D–I** *M. hasselti* **J–L** *Thelacantha brevispina*. Genitalia are shown in dorsal view (**A, D, G, J**), ventral view from external (**B, E, H, K**), and posterior view (**C, F, I, L**).

which was a juvenile specimen. The multi-colored morph was found in every population, whereas the other morphs were relatively rare.

Remarks. *Thelacantha brevispina* has been noted for the two large, distinct white spots on its abdomen (Pickard-Cambridge 1879; Chrysanthus 1959; Emerit 1974; Tikader 1982; Barrion and Litsinger 1995; Yin et al. 1997; Dierkens and Charlat 2011). Some color morphs in this study have been reported in previous works such as the Multi-color morph (Dierkens and Charlet 2011) and the Black-White morph (Tikader 1982). *Thelacantha brevispina* is widely distributed on a global scale. It has been recorded from Madagascar to Australia and also oceanic islands such as French Polynesia, and Fiji (Emerit 1974; Barrion and Litsinger 1995; Dierkens and Charlat 2011). Currently, it is classified as a monotypic species (World Spider Catalog 2020). However, the results of species delimitation have demonstrated four distinct species in the *T. brevispina* lineage (Fig. 4, T1–T4). Worldwide taxon sampling may reveal a large number of cryptic species, and elucidate their taxonomic status.

Distribution and habitat. India, Pakistan, Bangladesh, Sri Lanka China, Taiwan, Japan, Korea, Myanmar, Thailand, Malaysia, Indonesia (Ambon, Java, Sumatra, and Sulawesi), Philippines, New Guinea, Australia, Fiji, Mauritius, French Polynesia, Hawaii, and Madagascar (Emerit 1974; Tikader 1982; Yin et al. 1997, 2012; World Spider Catalog 2020). In this study, *Thelacantha brevispina* was found widely dispersed in coastal areas. They were commonly found in mangrove forests along the Inner Gulf of Thailand, but one population was found in the mountainous area in Phu Ruea District, Loei Province, which is far from the sea. These spiders build a vertical web between trees in open areas and sit at the center of the web.

Discussion

Spiny-backed orb-weaving spiders exhibit high intraspecific variation and also morphological similarities among closely related species (Pickard-Cambridge 1879; Dahl 1914; Chrysanthus 1959; Benoit 1964). Thus, species delimitation is always challenging. This study used molecular approaches to guide the delimitation of species boundaries, and to confirm the morphological classification. The genetic distances based on the COI gene among 15 OTUs show that intraspecific divergence between members of Gasteracanthinae is less than the interspecific divergence, with no overlap between intra- and interspecific distances (Table 3). The gap between intra- and interspecific distance was 1.31–3.76%. The interspecific genetic difference between Gasteracanthinae was 20.55 times than that of the intraspecific genetic difference. This value is higher than the ten times difference originally proposed by Hebert et al. (2004). Moreover, all of the estimates of interspecific genetic distance between species of Gasteracanthinae in this study were greater than 3%, which is the suggested barcoding threshold value for species delineation in arachnids (Barrett and Hebert 2005).

The delimitation results based on the COI gene in all analyses (ABGD, bPTP, and GMYC) confirm 15 distant lineages for the dataset of *Actinacantha*, *Gasteracantha*, *Thelacantha*, and *Macracantha* in the present study. These species delimitation methods are congruent with morphological identification of at least seven examined Thai lineages, consisting of *G. diadesmia*, *G. diardi*, *G. doriae* (D2), *G. kuhli*, *M. arcuata* (M1), *M. hasselti*, and *T. brevispina* (T1). This suggests that the characters of shape and position of abdominal spines, as well as the epigynal structure are useful in delimiting species boundaries in Gasteracanthinae.

In addition, among the 15 discovered lineages, six lineages nested within *T. brevispina*, *M. arcuata*, *G. cancriformis*, and *G. doriae* are likely to be cryptic species (Fig. 4). Apart from the case of *G. doriae*, which has been discussed in the previous taxonomic section, cryptic speciation in other taxa is discussed here. *Thelacantha brevispina* is separated into four different lineages. These lineages are from Thailand (Fig. 4, T1), French Polynesia and Japan (Fig. 4, T3), and two lineages are from Malaysia (Fig. 4, T2 and T4). The clade from Thailand exhibits various color patterns on the abdomen, although their genetic distance is relatively low (0.17%). Furthermore, each color morph is restricted to a single locality, suggesting that each population might have independently evolved their color pattern recently. Also, two specimens of *T. brevispina* from French Polynesia and Japan are grouped into the same lineage; these two islands are geographically distant. This suggests that human activity introduced non-native species from one island to the other (Dawson et al. 2017).

Similarly, *Macracantha arcuata* is separated into two lineages, one from Thailand and Cambodia (Fig. 4, M1), and another from Malaysia (Fig. 4, M2). Deep divergence in both *T. brevispina* and *M. arcuata* corresponds to their geographic distribution. They can be divided into Indochinese (M1, T1) and Sundaic lineages (M2, T2, T4). The biogeographic partition between Indochinese and Sundaic lineages has been observed in other animals such as freshwater shrimp (De Bruyn et al. 2005), amphibians (Emerson et al. 2000), reptiles (Brown, et al. 2012), and birds (Dejtaradol et al. 2016; Manawatthana et al. 2017), as well as in plants (Van Steenis 1950). This phenomenon might suggest a strong paleogeographic barrier between the northern and southern regions of the Southeast Asia mainland (Woodruff 2003, 2010) and/or many colonization events in the area. Two zoogeographical lines, the Isthmus of Kra and the Kangar-Pattani line, are considered as the transition zone between Indochinese and Sundaic biogeographic regions (Woodruff 2003). The results from this study tend to support the Kangar-Pattani line as the boundary line for Gasteracanthinae. However, further model testing and biogeographic study with more samples of Gasteracanthinae from the region should be conducted in order to support our hypothesis.

Deep divergence detected in this study also indicates the possibility of cryptic speciation disguising several species within a nominal name. Unfortunately, we were unable to investigate the type series of *G. cancriformis*, *M. arcuata*, and *T. brevispina*, and topotypes of these species were unavailable, particularly their molecular data. Hence, there was not enough evidence to indicate the taxonomic placement of such distinct lineages. Consequently, we are only able to report such high diversification as a deep divergence within each species.

Based on the phylogenetic tree constructed in this study (Fig. 3), the monophyletic origin of Gasteracanthinae (Fig. 3, node 1) and the great phylogenetic distance between Gasteracanthinae and Micratheninae are congruent with previous studies (Scharff and Coddington 1997; Wheeler et al. 2017; Kallal et al. 2018; Tan et al. 2019; Scharff et al. 2020). Three major clades indicated in the tree are also supported by morphological evidence. Conspecific members in Clade I (Fig. 4, node 2) exhibit metallic bluish black spines, with highly-modified median spines that differ from anterior and posterior spines. They also lack large trapezoid-shaped sigilla on the dorsal abdomen (Tan el al. 2019).

The synapomorphic character common to clade II and III (Fig. 3, node 4) is the presence of a ventral tubercle. Clade III (Fig. 3, node 7) possesses oval spermathecae (Fig. 12J) and six tubercle spines (Fig. 10) as unique characters. In clade II (Fig. 3, node 5), the round spermathecae constitutes a synapomorphic character. The shape of median spines of the broad-abdomen *Gasteracantha* (Fig. 3, node 6) is variable among species, whereas the anterior and posterior spines are similar in shape and direction.

They possess large trapezoid-shaped sigilla at the anterior edge, middle, and posterior edges, and small sigilla forming a straight line at the middle of anterior and posterior edges. Taxonomically, their species boundary is difficult to delimit because of morphological similarity (Butler 1873; Pickard-Cambridge 1879; Thorell 1887). Moreover, most broad-abdomen *Gasteracantha* are color polymorphic species, and the horizontal bands morph tends to be conserved within this group. These factors might create confusion for identification. However, the spine character and female genitalia seem to be sufficient to separate the three species of this genus examined in this study. Because this study consists of few members of Gasteracanthinae, further investigation that includes more taxon sampling is needed to indicate phylogenetic relationships among the whole subfamily Gasteracanthinae.

'*Gasteracantha hasseltii* C. L. Koch, 1837' has long been placed in genus *Gasteracantha* (World Spider Catalog 2020). However, molecular phylogenetic analysis in this study suggests reclassifying it to genus *Macracantha*. The close phylogenetic relationship between *M. hasselti* and *M. arcuata* is supported by their synapomorphic characters. They share the characteristics of well-developed and elongated median spines, similar pattern of sternal bands, and a concave anterior edge of abdomen. Their posterior edge sigilla are similar in shape and arrangement. Their spinnerets are situated on an elevated black sclerotized structure, forming a shape like a shield volcano (Fig. 9). In the female reproductive organ, the spermathecae of *M. hasselti* and *M. arcuata* exhibit a complex shape (Fig. 12A, D, G), whereas the spermathecae of other Thai *Gasteracantha* in this study are simply round (Fig 11A, D, G, J). Both species also lack a ventral tubercle, a protuberance between epigynum and spinnerets, while this character is present in other *Gasteracantha* species from Thailand compared in this study. Based on both morphological and molecular-based evidence, it is appropriate to classify these two species in the same genus.

The monophyletic relationship between "*A.*" *globulata* and *Macracantha* is highlighted by the phylogenetic tree in this study with high nodal support. Therefore, it may be appropriate to transfer "*A.*" *globulata* to the genus *Macracantha*. While "*A.*" *globulata* has a distinct characteristic of the tuberculous base of median spines, it also shares morphological characteristics with other *Macracantha* species, i.e., elongated median spines, curved anterior abdomen, sternal band, posterior sigilla that are arranged in a straight line, and the absence of a ventral tubercle (Walckenaer 1841; Hasselt 1882; Tan et al. 2019). Unfortunately, some morphological features of "*A.*" *globulata*, especially the female genitalia structure are still unavailable; only external features of one sub-adult female are illustrated in Tan et al. (2019). Fresh materials of adult females are essential to confirm this hypothesis.

In addition, there are other *Gasteracantha* species that share some morphological characteristics with members of *Macracantha* and potentially should be transferred to the genus, including *Gasteracantha clavatrix* (Walckenaer, 1841), *Gasteracantha clavigera* Giebel, 1863, *Gasteracantha dalyi* Pocock, 1900, *Gasteracantha janopol* Barrion & Litsinger, 1995, *Gasteracantha remifera* Butler, 1873, *Gasteracantha sororna* Butler,

1873. These species exhibit elongated median spines, elevated spinnerets, concave anterior abdomen, and absence of ventral tubercle (Walckenaer 1841; Giebel 1863; Butler 1873; Simon 1877; Pocock 1900; Tikader 1982; Barrion and Litsinger 1995). Their taxonomic placement should be investigated in further study when fresh material of complete adult specimens and their molecular data are available.

Moreover, in this study, the comparative study of abdominal spines in Gasteracanthinae indicated shape variability, especially for a pair of median spines that differ from anterior and posterior spines in many species. The high modification of median spines may have convergently occurred at least twice in clade I and in the clade of broad-abdomen *Gasteracantha*, as well as the for the tubercle spines in *A. globulata* and *T. brevispina*. These examples might be similar to the convergent evolution of long spines in spiny orb-weaving spiders of subfamily Micratheninae, in which the long spine has evolved independently several times within Micratheninae (Magalhaes and Santos 2012). Despite the distant relationship between Micratheninae and Gasteracanthinae, *M. arcuata* (Fabricius, 1793) shows morphological similarity with *Micrathena cyanospina* (Lucas, 1835). Both species possess remarkably long spines, which are very similar in shape (Levi 1985).

Conclusions

Although intraspecific morphological variation in Gasteracanthinae has been highlighted by some authors (Pickard-Cambridge 1879; Dahl 1914; Kolosváry 1931; Chrysanthus 1959; Benoit 1964; Emerit 1974), our morphological study has demonstrated that the shape and position of abdominal spines, sigilla pattern, and the female genitalia structure are significant characters for species identification and classification. In this study, seven species from three genera, *Gasteracantha*, *Macracantha*, and *Thelacantha*, were identified by both morphological examination and confirmed by molecular approaches. By including previous historical records, we find that there are eleven species of Gasteracanthinae present in Thailand. We transfer '*Gasteracantha hasselti*' to the genus *Macracantha* according to molecular phylogeny and morphological evidence. Most species within Gasteracanthinae exhibit highly intraspecific color polymorphism. Hence, molecular-based analyses provide an applicable tool for indicating species boundaries, and insight into evolutionary history through phylogenetic relationships among taxa. The molecular species delimitation suggests the existence of nine putative species, along with six hidden lineages that seem to be represented as distinct species. Consequently, the number of species in Gasteracanthinae might be underestimated. A comprehensive revision by including more species sampling of both female and male spiders in the future would lead to the discovery more cryptic diversity and lead to a better understanding of the evolutionary history of abdominal spines, intraspecific color polymorphism, sexual dimorphism, as well as phylogeography. These insights will extend the perspectives of colonization patterns of arachnids in Southeast Asia.

Acknowledgements

First of all, specimens in this project were collected and donated by many contributors. We cannot express enough thanks to all participants, especially the members of Animal Systematics and Molecular Ecology Laboratory, Mahidol University, and Animal Systematics Research Unit, Chulalongkorn University. We sincerely thank Dr. Natapot Warrit and Dr. Chalita Kongrit for all useful suggestions. This study was supported by The Thailand Research Fund under grant number TRF-DBG 6080011. The authors would like to express our grateful thanks to reviewers and editors for their constructive comments that improved the manuscript. We also thank Mr. David John Anderson for his linguistic work.

References

Agnarsson I, Blackledge TA (2009) Can a spider web be too sticky? Tensile mechanics constrains the evolution of capture spiral stickiness in orb-weaving spiders. Journal of Zoology 278(2): 134–140. https://doi.org/10.1111/j.1469-7998.2009.00558.x

Álvarez-Padilla F, Dimitrov D, Giribet G, Hormiga G (2009) Phylogenetic relationships of the spider family Tetragnathidae (Araneae, Araneoidea) based on morphological and DNA sequence data. Cladistics 25(2): 109–146. https://doi.org/10.1111/j.1096-0031.2008.00242.x

Barrett RDH, Hebert PDN (2005) Identifying spiders through DNA barcodes. Canadian Journal of Zoology 83(3): 481–491. https://doi.org/10.1139/z05-024

Barrion AT, Litsinger JA (1995) Riceland Spiders of South and Southeast Asia. CAB International Wallingford, UK, 700 pp.

Benoit PLG (1962) Monographie des Araneidae-Gasteracanthinae africains (Araneae). Annales, Musée Royal de l'Afrique Centrale, Sciences Zoologiques 112: 1–70.

Benoit PLG (1964) Nouvelle contribution à la connaissance des Araneidae-Gasteracanthinae d'Afrique et de Madagascar (Araneae). Publicações Culturais da Companhia de Diamantes de Angola 69: 41–52.

Blackwall J (1864) Descriptions of seven new species of East Indian spiders received from the Rev. O. P. Cambridge. Annals and Magazine of Natural History 14(3): 36–45. https://doi.org/10.1080/00222936408681653

Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu CH, Xie D, Suchard MA, Rambaut A, Drummond AJ (2014) BEAST 2: a software platform for Bayesian evolutionary snalysis. PLOS Computational Biology 10: e1003537. https://doi.org/10.1371/journal.pcbi.1003537

Brown RM, Siler CD, Lee GL, Das I, McGuire JA (2012) Phylogeny and cryptic diversification in Southeast Asian flying geckos. Molecular Phylogenetics and Evolution 65(2): 351–361. https://doi.org/10.1016/j.ympev.2012.06.009

Butler AG (1873) A monographic list of the species of *Gasteracantha* or crab-spiders, with descriptions of new species. Transactions of the Entomological Society of London 1873: 153–180. https://doi.org/10.1111/j.1365-2311.1873.tb00640.x

Chamberland L, Salgado-Roa FC, Basco A, Crastz-Flores A, Binford GJ, Agnarsson I (2020) Phylogeography of the widespread Caribbean spiny orb weaver *Gasteracantha cancriformis*. PeerJ 8: e8976. https://doi.org/10.7717/peerj.8976

Chrysanthus P (1959) Spiders from south New Guinea II. Nova Guinea N.S. 10: 197–206.

Cloudsley-Thompson J (1995) A review of the anti-predator devices of spiders. Bulletin of the British Arachnological Society 10(3): 81–96.

Colgan DJ, McLauchlan A, Wilson GDF, Livingston SP, Edgecombe GD, Macaranas J, Cassis G, Gray MR (1998) Histone H3 and U2 snRNA DNA sequences and arthropod evolution. Australian Journal of Zoology 46(5): 419–437. https://doi.org/10.1071/ZO98048

Cooper JE (2011) Anesthesia, Analgesia, and Euthanasia of Invertebrates. ILAR Journal 52(2): 196–204. https://doi.org/10.1093/ilar.52.2.196

Cotoras DD, Brewer MS, Croucher PJP, Oxford GS, Lindberg DR, Gillespie RG (2016) Convergent evolution in the colour polymorphism of *Selkirkiella* spiders (Theridiidae) from the South American temperate rainforest. Biological Journal of the Linnean Society 120(3): 649–663. https://doi.org/10.1111/bij.12908

Dahl F (1914) Die Gasteracanthen des Berliner Zoologischen Museums und deren geographische Verbreitung. Mitteilungen aus dem Zoologischen Museum in Berlin 7: 235–301.

Dawson W, Moser D, van Kleunen M, Kreft H, Pergl J, Pyšek P, Weigelt P, Winter M, Lenzner B, Blackburn TM, Dyer EE, Cassey P, Scrivens SL, Economo EP, Guénard B, Capinha C, Seebens H, García-Díaz P, Nentwig W, García-Berthou E, Casal C, Mandrak NE, Fuller P, Meyer C, Essl F (2017) Global hotspots and correlates of alien species richness across taxonomic groups. Nature Ecology & Evolution 1: e0186. https://doi.org/10.1038/s41559-017-0186

De Bruyn M, Nugroho E, Hossain MM, Wilson JC, Mather PB (2005) Phylogeographic evidence for the existence of an ancient biogeographic barrier: The Isthmus of Kra Seaway. Heredity 94: 370–378. https://doi.org/10.1038/sj.hdy.6800613

Dejtaradol A, Renner SC, Karapan S, Bates PJJ, Moyle RG, Päckert M (2016) Indochinese-Sundaic faunal transition and phylogeographical divides north of the Isthmus of Kra in Southeast Asian Bulbuls (Aves: Pycnonotidae). Journal of Biogeography 43(3): 471–483. https://doi.org/10.1111/jbi.12662

Dierkens M, Charlat S (2011) Contribution à la connaissance des araignées des îles de la Société (Polynésie française). Revue Arachnologique 17(5): 63–81.

Dimitrov D, Benavides LR, Arnedo MA, Giribet G, Griswold CE, Scharff N, Hormiga G (2017) Rounding up the usual suspects: a standard target-gene approach for resolving the interfamilial phylogenetic relationships of ecribellate orb-weaving spiders with a new family-rank classification (Araneae, Araneoidea). Cladistics 33(3): 221–250. https://doi.org/10.1111/cla.12165

Doleschall L (1857) Bijdrage tot de kennis der Arachniden van den Indischen Archipel. Natuurkundig Tijdschrift voor Nederlandsch-Indie 13: 339–434. https://doi.org/10.5962/bhl.title.66068

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32(5): 1792–1797. https://doi.org/10.1093/nar/gkh340

Emerit M (1974) Arachnides araignées Araneidae Gasteracanthinae. Faune Madagascar 38: 1–215.

Emerson SB, Inger RF, Iskandar D (2000) Molecular systematics and biogeography of the fanged frogs of Southeast Asia. Molecular Phylogenetics and Evolution 16(1): 131–142. https://doi.org/10.1006/mpev.2000.0778

Fabricius JC (1793) Entomologiae systematica emendata et aucta, secundum classes, ordines, genera, species adjectis synonimis, locis, observationibus, descriptionibus. Hafniae 2: 407–428. https://doi.org/10.5962/bhl.title.122153

Farris JS (1997) The future of phylogeny reconstruction. Zoologica Scripta 26(4): 303–311. https://doi.org/10.1111/j.1463-6409.1997.tb00420.x

Fernández R, Kallal RJ, Dimitrov D, Ballesteros JA, Arnedo MA, Giribet G, Hormiga G (2018) Phylogenomics, diversification dynamics, and comparative transcriptomics across the spider tree of life. Current Biology 28(9): 1489–1497. https://doi.org/10.1016/j.cub.2018.03.064

Folmer O, Black M, Hoeh WR, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial Cytochrome C oxidase subunit I from diverse metazoan invertebrates. Molecular marine biology and biotechnology 3(5): 294–299.

Gawryszewski FM, Motta PC (2012) Colouration of the orb-web spider *Gasteracantha cancriformis* does not increase its foraging success. Ethology Ecology & Evolution 24(1): 23–38. https://doi.org/10.1080/03949370.2011.582044

Giebel CG (1863) Drei und zwanzig neue und einige bekannte Spinnen der Hallischen Sammlung. Zeitschrift für die gesammten Naturwissenschaft 21: 306–328.

Goloboff PA, Catalano SA (2012) GB-to-TNT: facilitating creation of matrices from GenBank and diagnosis of results in TNT. Cladistics 28(5): 503–513. https://doi.org/10.1111/j.1096-0031.2012.00400.x

Goloboff PA, Catalano SA (2016) TNT version 1.5, including a full implementation of phylogenetic morphometrics. Cladistics 32(3): 221–238. https://doi.org/10.1111/cla.12160

Guérin-Méneville FE (1838) Histoire naturelle des Crustacés, Arachnides et Insectes recueillis dans le Voyage autour du Monde de la Corvette de Sa Majesté, La Coquille, exécuté pendant les anées 1822–1825 sous le commandement du Capitaine Duperry. Paris 2(1: Zoologie): 51–56.

Hasselt AWM (1882) Araneae. In: Veth PJ (Ed.) Midden-Sumatra 4A(11). Reizen en onderzoekingen der Sumatra-expeditie, uitgerust door het aardrijkskundig genootschap, 1877–1879. Brill, Leiden, 1–56 pp.

Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM (2004) Identification of birds through DNA barcodes. PLOS biology 2(10): e312. https://doi.org/10.1371/journal.pbio.0020312

Hedin M (2015) High-stakes species delimitation in eyeless cave spiders (Cicurina, Dictynidae, Araneae) from central Texas. Molecular Ecology 24: 346–361. https://doi.org/10.1111/mec.13036

Hormiga G, Griswold CE (2014) Systematics, phylogeny, and evolution of orb-weaving Spiders. Annual Review of Entomology 59(1): 487–512. https://doi.org/10.1146/annurev-ento-011613-162046

Hormiga G, Scharff N, Coddington JA (2000) The phylogenetic basis of sexual size dimorphism in orb-weaving Spiders (Araneae, Orbiculariae). Systematic Biology 49(3): 435–462. https://doi.org/10.1080/10635159950127330

Ishii Y, Shimada M (2010) The effect of learning and search images on predator–prey interactions. Population ecology 52: 27–35. https://doi.org/10.1007/s10144-009-0185-x

Jaffé R, Eberhard W, De Angelo C, Eusse D, Gutierrez A, Quijas S, Rodríguez A, Rodríguez M (2006) Caution, webs in the way! Possible functions of silk stabilimenta in *Gasteracantha cancriformis* (Araneae, Araneidae). The Journal of arachnology 34(2): 448–455. https://doi.org/10.1636/S04-28.1

Kallal RJ, Fernández R, Giribet G, Hormiga G (2018) A phylotranscriptomic backbone of the orb-weaving spider family Araneidae (Arachnida, Araneae) supported by multiple methodological approaches. Molecular Phylogenetics and Evolution 126: 129–140. https://doi.org/10.1016/j.ympev.2018.04.007

Kemp DJ, Holmes C, Congdon BC, Edwards W (2013) Color polymorphism in spiny spiders (*Gasteracantha fornicata*): testing the adaptive significance of a geographically clinal lure. Ethology 119(12): 1126–1137. https://doi.org/10.1111/eth.12172

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111–120. https://doi.org/10.1007/BF01731581

Koch CL (1837) Die Arachniden. C. H. Zeh'sche Buchhandlung, Nürnberg, Dritter Band, 105–119 [pls 106–118].

Kolosváry G (1931) Variations-Studien über "*Gasteracantha*" und "*Argyope*" Arten. Archivio Zoologico Italiano 16: 1055–1085.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35(6): 1547–1549. https://doi.org/10.1093/molbev/msy096

Kuntner M, Arnedo MA, Trontelj P, Lokovšek T, Agnarsson I (2013) A molecular phylogeny of nephilid spiders: evolutionary history of a model lineage. Molecular Phylogenetics and Evolution 69(3): 961–979. https://doi.org/10.1016/j.ympev.2013.06.008

Levi HW (1985) The spiny orb-weaver genera *Micrathena* and *Chaetacis* (Araneae: Araneidae). Bulletin of the Museum of Comparative Zoology at Harvard College 150: 429–618.

Levi HW (1996) The American orb weavers *Hypognatha*, *Encyosaccus*, *Xylethrus*, *Gasteracantha*, and *Enacrosoma* (Araneae, Araneidae). Bulletin of the Museum of Comparative Zoology at Harvard College 155: 89–157.

Linnaeus C (1758) Systema naturae per regna tria naturae, secundum classes, ordines, genera, species cum characteribus differentiis, synonymis, locis. Editio decima, reformata. Holmiae, 821 pp. https://doi.org/10.5962/bhl.title.559

Lucas H (1835) Article: "*Epeira*." Dictionnaire pittoresque d'histoire naturelle. Guérin. Paris 3: 69–70.

Magalhaes I, Santos A (2012) Phylogenetic analysis of *Micrathena* and *Chaetacis* spiders (Araneae: Araneidae) reveals multiple origins of extreme sexual size dimorphism and long abdominal spines. Zoological Journal of the Linnean Society 166: 14–53. https://doi.org/10.1111/j.1096-3642.2012.00831.x

Manawatthana S, Laosinchai P, Onparn N, Brockelman WY, Round PD (2017) Phylogeography of bulbuls in the genus *Iole* (Aves: Pycnonotidae). Biological Journal of the Linnean Society 120(4): 931–944. https://doi.org/10.1093/biolinnean/blw013

McHugh A, Yablonsky C, Binford G, Agnarsson I (2014) Molecular phylogenetics of Caribbean *Micrathena* (Araneae: Araneidae) suggests multiple colonisation events and single island endemism. Invertebrate Systematics 28(4): 337–349. https://doi.org/10.1071/IS13051

Merian P (1911) Die Spinnenfauna von Celebes. Beiträge zur Tiergeographie im Indoaustralischen Archipel. Zoologische Jahrbücher, Abteilung für Systematik, Geographie und Biologie der Tiere 31: 165–354.

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Paper presented at the 2010 Gateway Computing Environments Workshop (GCE). https://doi.org/10.1109/GCE.2010.5676129

Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca G, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403: 853–858. https://doi.org/10.1038/35002501

Ortiz D, Francke OF (2016) Two DNA barcodes and morphology for multi-method species delimitation in Bonnetina tarantulas (Araneae: Theraphosidae). Molecular Phylogenetics and Evolution 101: 176–193. https://doi.org/10.1016/j.ympev.2016.05.003

Palumbi SR, Martin A, Romano S, McMillan WO, Stice L, Grabowski G (1991) The simple fool's guide to PCR : version 2.0. University of Hawaii, Honolulu: privacy published complied by S. Palumbi, 28 pp.

Peckham EG (1889) Protective resemblances in spiders. Occasional Papers of the Natural History Society of Wisconsin 1(2): 61–113.

Pickard-Cambridge O (1879) On some new and little known species of Araneidea, with remarks on the genus *Gasteracantha*. Proceedings of the Zoological Society of London 47(2): 279–293. https://doi.org/10.1111/j.1096-3642.1879.tb02656.x

Pocock RI (1897) Spinnen (Araneae). In: Kükenthal W (Ed.) Ergebnisse einer zoologische Forschungsreise in dem Molukken und Borneo. Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft 23: 591–629.

Pocock RI (1900) The fauna of British India, including Ceylon and Burma. Arachnida. (Vol. I). Taylor and Francis, London, 279 pp. https://doi.org/10.5962/bhl.title.17227

Pons J, Barraclough TG, Gomez-Zurita J, Cardoso A, Duran DP, Hazell S, Kamoun S, Sumlin WD, Vogler AP (2006) Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Systematic Biology 55: 595–609. https://doi.org/10.1080/10635150600852011

Puillandre N, Lambert A, Brouillet S, Achaz G (2011) ABGD, Automatic Barcode Gap Discovery for primary species delimitation. Molecular Ecology 21(8): 1864–1877. https://doi.org/10.1111/j.1365-294X.2011.05239.x

Punzalan D, Rodd FH, Hughes KA (2005) Perceptual processes and the maintenance of polymorphism through frequency-dependent predation. Evolutionary Ecology 19(3): 303–320. https://doi.org/10.1007/s10682-005-2777-z

Ramage T, Martins-Simoes P, Mialdea G, Allemand R, Duplouy A, Rousse P, Davies N, Roderick GK, Charlat S (2017) A DNA barcode-based survey of terrestrial arthropods in the society islands of French Polynesia: host diversity within the SymbioCode Project. European Journal of Taxonomy 272: 1–13. https://doi.org/10.5852/ejt.2017.272

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67(5): 901–904. https://doi.org/10.1093/sysbio/syy032

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Roy TK, Saha S, Raychaudhuri D (2017) On the araneid fauna (Araneae: Araneidae) of the tea estates of Dooars, West Bengal, India. World Scientific News 67(1): 1–67.

Salgado-Roa FC, Pardo-Diaz C, Lasso E, Arias CF, Solferini VN, Salazar C (2018) Gene flow and Andean uplift shape the diversification of *Gasteracantha cancriformis* (Araneae: Araneidae) in Northern South America. Ecology and Evolution 8(14): 7131–7142. https://doi.org/10.1002/ece3.4237

Scharff N, Coddington JA (1997) A phylogenetic analysis of the orb-weaving spider family Araneidae (Arachnida, Araneae). Zoological Journal of the Linnean Society 120(4): 355–434. https://doi.org/10.1111/j.1096-3642.1997.tb01281.x

Scharff N, Coddington JA, Blackledge TA, Agnarsson I, Framenau VW, Szűts T, Hayashi CY, Dimitrov D (2020) Phylogeny of the orb-weaving spider family Araneidae (Araneae: Araneoidea). Cladistics 36(1): 1–21. https://doi.org/10.1111/cla.12382

Schwarz G (1978) Estimating the dimension of a model. The Annals of Statistics 6(2): 461–464. https://doi.org/10.1214/aos/1176344136

Sen S, Dhali DC, Saha S, Raychaudhuri D (2015) Spiders (Araneae: Arachnida) of reserve forests of Dooars: Gorumara National Park, Chapramari Wildlife Sanctuary and Mahananda Wildlife Sanctuary. World Scientific News 20: 1–339.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87(6): 651–701. https://doi.org/10.1093/aesa/87.6.651

Simon E (1864) Histoire naturelle des araignées (aranéides). Paris, 540 pp. https://doi.org/10.5962/bhl.title.47654

Simon E (1877) Etudes arachnologiques. 6e mémoire. X. Arachnides nouveaux ou peu connus. Annales de la Société entomologique de France 7(5): 225–242.

Simon E (1886) Arachnides recueillis par M. A. Pavie (sous-chef du service des postes au Cambodge) dans le royaume de Siam, au Cambodge et en Cochinchine. Actes de la Société Linnéenne de Bordeaux 40: 137–166.

Simon E (1892) Histoire Naturelle des Araignées I. Librairie Encyclopédique de Roret, Paris, 1084 pp. https://doi.org/10.5962/bhl.title.51973

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Sundevall CJ (1833) Conspectus Arachnidum. C.F. Berling, Londini Gothorum, 39 pp.

Tan J, Chan ZJ, Ong CA, Yong HS (2019) Phylogenetic relationships of *Actinacantha* Simon, *Gasteracantha* Sundevall, *Macracantha* Hasselt and *Thelacantha* Simon spiny orbweavers (Araneae: Araneidae) in Peninsular Malaysia. Raffles Bulletin of Zoology 67: 32–55. https://doi.org/10.26107/RBZ-2019-0003

Tanabe AS (2007) KAKUSAN: a computer program to automate the selection of a nucleotide substitution model and the configuration of a mixed model on multilocus data. Molecular Ecology Notes 7(6): 962–964. https://doi.org/10.1111/j.1471-8286.2007.01807.x

Thorell T (1887) Viaggio di L. Fea in Birmania e regioni vicine. II. Primo saggio sui ragni birmani. Annali del Museo Civico di Storia Naturale di Genova 25: 5–417.

Tikader BK (1982) The fauna of India. Spiders: Araneae. Vol. II. Part 1 Family Araneidae (= Argiopidae) typical orb-weavers. Part 2 Family Gnaphosidae. Zoological Survey of India, Calcutta, 293 pp.

Truong H (2012) Coloration in relation to ecology in the Asian spiny-backed spider, *Thelacantha brevispina* (Araneae, Araneidae) on Moorea, French Polynesia. https://escholarship.org/content/qt5524p5x6/qt5524p5x6.pdf?t=meyblz

Van Steenis CGGJ (1950) The delimitation of Malesia and its main plant geographical divisions. Flora Malesiana 1(1): 70–75.

Walckenaer CA (1841) Histoire naturelle des Insects. Aptères. Tome deuxième. Roret, Paris, 549 pp.

Wheeler WC, Coddington JA, Crowley LM, Dimitrov D, Goloboff PA, Griswold CE, Hormiga G, Prendini L, Ramírez MJ, Sierwald P, Almeida-Silva L, Alvarez-Padilla F, Arnedo MA, Benavides SLR, Benjamin SP, Bond JE, Grismado CJ, Hasan E, Hedin M, Izquierdo MA, Labarque FM, Ledford J, Lopardo L, Maddison WP, Miller JA, Piacentini LN, Platnick NI, Polotow D, Silva-Dávila D, Scharff N, Szűts T, Ubick D, Vink CJ, Wood HM, Junxia Z (2017) The spider tree of life: phylogeny of Araneae based on target-gene analyses from an extensive taxon sampling. Cladistics 33(6): 574–616. https://doi.org/10.1111/cla.12182

White TE, Kemp DJ (2016) Color polymorphic lures target different visual channels in prey. Evolution 70(6): 1398–1408. https://doi.org/10.1111/evo.12948

Woodruff DS (2003) Neogene marine transgressions, palaeogeography and biogeographic transitions on the Thai–Malay Peninsula. Journal of Biogeography 30(4): 551–567. https://doi.org/10.1046/j.1365-2699.2003.00846.x

Woodruff DS (2010) Biogeography and conservation in Southeast Asia: how 2.7 million years of repeated environmental fluctuations affect today's patterns and the future of the remaining refugial-phase biodiversity. Biodiversity and Conservation 19: 919–941. https://doi.org/10.1007/s10531-010-9783-3

Workman T, Workman ME (1892) Malaysian spiders. Belfast, 8 pp.

World Spider Catalog (2020) World Spider Catalog. Version 21.0. Natural History Museum Bern. http://wsc.nmbe.ch [accessed on 10 Nov 2020]

Yamada K, Yamada A, Kawanishi Y, Gurung R, Sasaki T, Tokuda G, Maekawa H (2015) Widespread distribution and evolutionary patterns of mariner-like elements among various spiders and insects. Journal of Insect Biotechnology and Sericology 84(2): 29–41. https://doi.org/10.11416/jibs.84.2_029

Yin CM, Wang JF, Zhu MS, Xie LP, Peng XJ, Bao YH (1997) Fauna Sinica: Arachnida: Araneae: Araneidae. Science Press, Beijing, 460 pp.

Yin CM, Peng XJ, Yan HM, Bao YH, Xu X, Tang G, Zhou QS, Liu P (2012) Fauna Hunan: Araneae in Hunan, China. Hunan Science and Technology Press, Changsha, 1590 pp.

Yoshida M (1989) Predatory Behavior of *Gasteracantha mammosa* C. Koch (Araneae; Araneidae). Acta Arachnologica 37(2): 57–67. https://doi.org/10.2476/asjaa.37.57

Zhang J, Kapli P, Pavlidis P, Stamatakis A (2013) A general species delimitation method with applications to phylogenetic placements. Bioinformatics 29(22): 2869–2876. https://doi.org/10.1093/bioinformatics/btt499

Zhang Y, Li S (2014) A spider species complex revealed high cryptic diversity in South China caves. Molecular Phylogenetics and Evolution 79: 353–358. https://doi.org/10.1016/j.ympev.2014.05.017

Supplementary material 1

Figures S1–S6

Authors: Kongkit Macharoenboon, Warut Siriwut, Ekgachai Jeratthitikul

Data type: phylogenetic trees

Explanation note: **Fig. S1.** Maximum parsimonious phylogenetic tree reconstructed from COI+16S+H3 dataset. **Fig. S2.** Bayesian inference phylogenetic tree reconstructed from COI+16S+H3 dataset. **Fig. S3.** Maximum parsimonious phylogenetic tree reconstructed from COI gene. **Fig. S4.** Maximum likelihood phylogenetic tree reconstructed from COI gene. **Fig. S5.** Ultrametric tree reconstructed from 454 bp of 16S gene showing clusters of OTUs as suggested by morphological identification, and three molecular species delimitation algorithms. **Fig. S6.** Ultrametric tree reconstructed from 328 bp of H3 gene showing clusters of OTUs as suggested by morphological identification, and three molecular species delimitation algorithms.

Link: https://doi.org/10.3897/zookeys.1032.62001.suppl1

ZooKeys 1032: 63–77 (2021)
doi: 10.3897/zookeys.1032.63431
https://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Rhodolitica on rhodoliths: a new stoloniferan genus (Anthozoa, Octocorallia, Alcyonacea)

Odalisca Breedy[1,3], Leen van Ofwegen[2],
Catherine S. McFadden[4], Catalina Murillo-Cruz[3,5]

1 *Centro de Investigación en Ciencias del Mar y Limnología; Museo de Zoología, UCR, Universidad de Costa Rica, P. O. Box 11501-2060, San José, Costa Rica; Smithsonian Tropical Research Institute, Republic of Panama* **2** *Netherlands Center for Biodiversity Naturalis, P.O. Box 9517, 2300, RA Leiden, The Netherlands* **3** *Centro de Investigación en Estructuras Microscópicas, Universidad de Costa Rica, P. O. Box 11501-2060, San José, Costa Rica* **4** *Department of Biology, Harvey Mudd College, Claremont, CA 91711-5990, USA* **5** *Escuela de Medicina, Departamento de Bioquímica, Universidad de Costa Rica, P. O. Box 11501-2060, San José, Costa Rica*

Corresponding author: Odalisca Breedy (odalisca.breedy@ucr.ac.cr)

Academic editor: B.W. Hoeksema | Received 20 January 2021 | Accepted 9 February 2021 | Published 16 April 2021

http://zoobank.org/D07ED053-E397-49B8-9F10-70A4AB2C2214

Citation: Breedy O, van Ofwegen L, McFadden CS, Murillo-Cruz C (2021) *Rhodolitica* on rhodoliths: a new stoloniferan genus (Anthozoa, Octocorallia, Alcyonacea). ZooKeys 1032: 63–77. https://doi.org/10.3897/zookeys.1032.63431

Abstract

Rhodolitica occulta **gen. nov. et sp. nov.** (Clavulariidae) is described from Cocos Island National Park, Pacific Ocean, Costa Rica. The species was found at various islets and rocky outcrops around the island, 20–55 m in depth. The genus is characterised by tubular, single, erect anthosteles interconnected by thin basal ribbon-like stolons on the surfaces of living rhodoliths. The anthosteles are devoid of fused sclerites, which are only present in the stolons. Coenenchymal sclerites are mostly spindles of various shapes, with a characteristic cylindrical warty type in the outer layer, crosses and radiates. Anthocodiae are armed with points, lacking collarets. Colonies and sclerites are red. Using an integrative taxonomic approach, we separate the new genus from similar genera through both morphological comparison and a molecular phylogenetic analysis. This research is a contribution to the knowledge of the octocoral biodiversity in Cocos Island and marine biodiversity in the eastern tropical Pacific.

Keywords

Biodiversity, Cocos Island, new species, oceanic island, soft corals, taxonomy

Introduction

Cocos Island, Costa Rica, an oceanic eastern tropical Pacific (ETP) island, has been considered a biodiversity and endemism hot-spot for marine organisms (Cortés 2016). The shallow-water octocoral fauna is poorly represented here, with only three gorgonian species reported, one of them endemic (Breedy and Cortés 2008, 2011). The mesophotic and deep regions of the island have shown a richer unknown octocoral diversity. In 2012, a new family, a new genus, and a new species of Alcyoniina, order Alcyonacea, were described (Breedy et al. 2012) representing the first record of this octocoral group. Every new site or habitat explored around Cocos Island is a potential treasure trove of new species.

In 2007, during a CIMAR expedition aboard the R/V *Proteus*, while dredging at depths of 40–45 m in Chatham Bay, scientists collected rhodoliths with a red stoloniferous octocoral. Rhodoliths are found at several points around Cocos Island (Fernández 2008; Sibaja-Cordero 2012) except in the southwest area. The rhodolith beds extend to 90 m depth, forming dense beds between 20 and 30 m (Cortés 2016). Rhodoliths are composed of several species of calcareous algae and support rich associated biota that ranges from foraminiferans to small fishes (Solano-Barquero 2011).

The Stolonifera include a group of octocorals that consist of individual tubular polyps that arise separately from ribbon-like stolons and present a series of transitional forms, from solitary separated polyps to united polyps joined at their bases in a common extended coenenchyme (Williams 1989; Fabricius and Alderslade 2001). The octocoral group Stolonifera is represented in Cocos Island by the common shallow-water species *Carijoa riisei* (Duchassaing & Michelotti, 1860) in the family Clavulariidae. The stoloniferous octocoral found on rhodoliths was first reported by Breedy and Cortés (2008), tentatively identified as a species of the genus *Rhodelinda* Bayer, 1981, or as a species related to the genus *Paratelesto* Utinomi, 1958. Herein, we describe it as a new genus within the family Clavulariidae. We use an integrative taxonomic approach, combining morphological and molecular analyses in order to phylogenetically position this monospecific species within Octocorallia.

Materials and methods

Study site and collection methods

The specimens were collected by scuba diving down to 30 m depth, by dredging, and by the submersible HOV 'DeepSee' of the M/V 'Argo' to 55 m depth. Cocos Island National Park is an oceanic island located between 5°30'–5°34'N and 87°01'–87°06'W in the eastern Tropical Pacific (ETP) approximately 500 km southwest of Costa Rica and more than 600 km northeast of the Galápagos Islands, Ecuador (Cortés 2016). The collected specimens were found on rhodoliths at various points along Chatham Bay (NW of the Island) from Manuelita Islet to Punta Ulloa, and off Manuelita at the Everest pinnacle (Fig. 1). They were preserved in 95% ethanol.

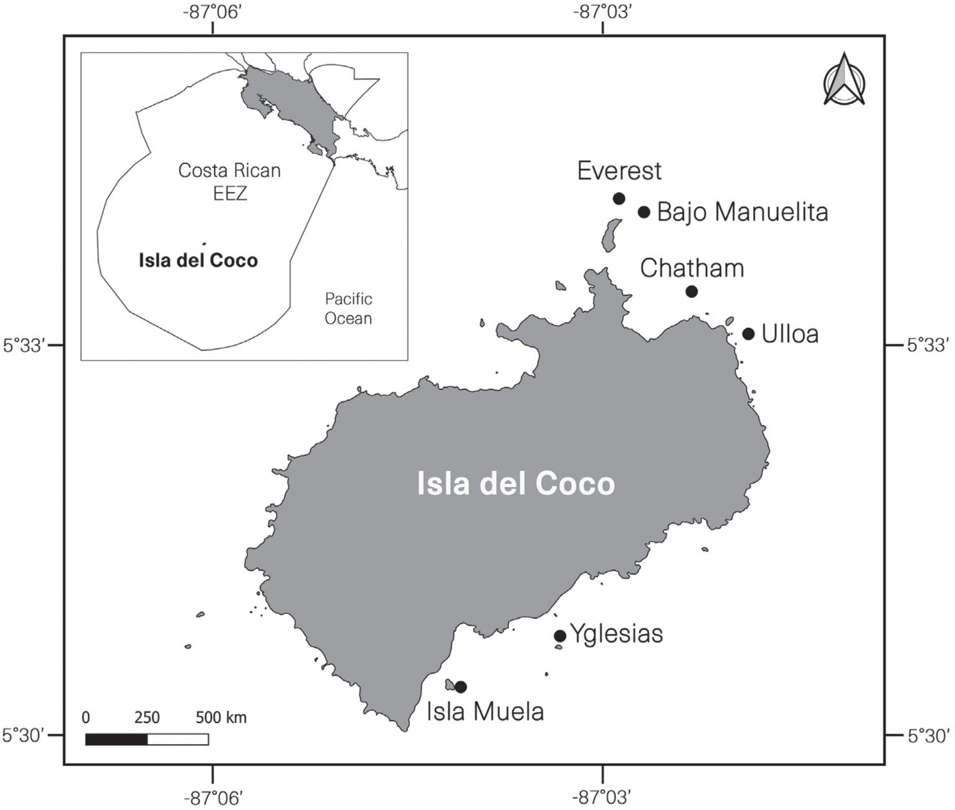


Figure 1. Map showing the collecting sites around Cocos Island, Costa Rica. Map by Beatriz Naranjo, UCR.

Morphological analysis

Preserved specimens were morphologically analysed and photographed. For taxonomic identification, sclerites from different parts of the colonies (polyp, coenenchyme, base and stolons) were obtained by dissolving the tissue in 5% sodium hypochlorite; dissociated sclerites were washed several times in distilled water until organic matter was completely removed, dehydrated with 100% ethanol, and subsequently dried in an oven. Sclerites were prepared for light microscopy, mounted in glycerine, and photographed with an Olympus LX 51 inverted microscope. For scanning electron microscopy (SEM), sclerites were mounted on SEM stubs by double stick carbon tape and silver paint, then sputter-coated with gold, 30–60 nm layer, in an Eiko IB-5 Ion Coater; the images were obtained using a Hitachi SEM S-3700N (at 15kV). All specimens were preserved with the anthocodiae partially or totally retracted therefore the description is based on polyp dissection. Measurements of the sclerites were obtained from the SEM images. The holotype and paratypes are deposited at the Zoology Museum, University of Costa Rica, Costa Rica (**MZUCR**).

Molecular phylogenetic analysis

DNA was extracted from ethanol-preserved tissues with the NucleoSpin® Tissue kit (Macherey-Nagel, Germany) according to the manufacturer's instructions, and kept at –20 °C until further processing. A partial region of the mitochondrial mismatch repair gene (mtMutS) was amplified with ND42599F (5'-GCCATTATGGTTAACTATTAC-3'; France and Hoover 2002) and MUT3458R (5'-TSGAGCAAAAGCCACTCC-3'; Sánchez et al. 2003), the mitochondrial cytochrome oxidase I gene (COI) with the primers COII8068F (5'-CCATAACAGGACTAGCAGCATC-3'; McFadden et al. 2004) and COIOCTR (5'-ATCATAGCATAGACCATACC-3'; France and Hoover 2002); and the 28S nuclear ribosomal gene with 28S-Far (5'-CACGAGACCGATAGCGAACAAGTA-3') and 28S-Rar (5'-TCATTTCGACCCTAAGACCTC-3') (McFadden and Ofwegen 2012). All the reactions were carried out in 50 µl volume with 10-50 ng DNA, 2.5 units Taq DNA polymerase (DreamTaq, Thermo Scientific, Waltham, MA), 1X DreamTaq Buffer, 0.2 mM of each dNTP, 0.3 µM of each primer and 50 µg of BSA. The amplification protocol for mtMutS consisted of 2 min of initial denaturation at 94 °C followed by 35 cycles of 30 sec at 94 °C, annealing at 50 °C for 30 sec, extension at 72 °C for 30 sec and a final extension at 72 °C for 5 min, and for COI and 28S was 5 min of initial denaturation at 94 °C followed by 35 cycles of 60 sec at 94 °C, annealing at 46 °C for 90 sec, extension at 72 °C for 60 sec and a final extension at 72 °C for 10 min. The resulting PCR products were purified and sequenced by Macrogen Inc. (Seoul, Korea), using the same forward and reverse PCR primers. Sequences from MZUCR2513 have been deposited in GenBank under accession numbers MW491885 (28S rDNA), MW491886 (mtMutS) and MW491887 (COI).

Sequences obtained from MZUCR 2513 were aligned with reference sequences from a wide range of octocoral taxa (Suppl. material 1: Table S1) using the FFT-NS-i method in MAFFT (Katoh et al. 2005). JModeltest (Darriba et al. 2012) was used to identify the best model of evolution for each gene region based on the AIC. GTR+I+G was identified as the best substitution model for mtMutS and 28S, with the similar TrN+I+G suggested for COI. Consequently, GTR+I+G was specified for all three gene regions in both maximum likelihood and Bayesian analyses. Maximum likelihood trees were constructed for each gene region separately using PhyML (Guindon and Gascuel 2003) with 100 bootstrap replicates (Suppl. material 2–4). No conflict was found between tree topologies so all three gene regions were concatenated for subsequent analyses; specimens for which data for one or more genes were missing were not included in the combined analysis. RAxML v8 (Stamatakis 2014) was run with 200 rapid bootstrap replicates, which allows a search for the best-scoring tree and bootstrapping in a single run. Bayesian analyses were conducted using MrBayes v3.2.1 (Ronquist et al. 2012), run for 4×10^6 generations (until standard deviation of split partitions < 0.01) with a burn-in of 25% and default Metropolis coupling parameters.

Results

Systematics

Class Anthozoa Ehrenberg, 1831
Subclass Octocorallia Haeckel, 1866
Order Alcyonacea Lamouroux, 1812
Family Clavulariidae Hickson, 1894

Genus *Rhodolitica* gen. nov.

http://zoobank.org/12B6B038-9830-4209-94B6-BA453693E194

Diagnosis. Colonies composed of tubular, single, erect anthosteles up to 8 mm tall and closely spaced, 1–3 mm apart forming interconnected groups of 2–15 anthosteles. Anthosteles arise directly from thin basal ribbon-like stolons that encrust irregular surfaces of living rhodoliths. Stolons composed of conspicuous red brittle fused sclerites not extending into anthostelar walls. Bright red coenenchymal sclerites compose the anthostelar walls, including long warty spindles, up to 0.27 mm in length; complexly warted cylindrical spindles up to 0.32 mm long; bent spindles and smaller crosses and radiates. Polyps whitish to transparent. Anthocodiae retractile, armed with eight interseptal points of slender warty spindles, up to 0.24 mm long, and small orange biscuit-like rods along the tentacles. Collaret absent. Collected specimens were preserved with the anthocodiae partially or totally retracted therefore a full description of polyps was not possible.

Colour of colonies bright red. Azooxanthellate.

Type species. *Rhodolitica occulta* spec. nov. by original designation.

Etymology. The generic name is in reference to the substratum to which the colonies were attached: rhodolith rocks.

Rhodolitica occulta sp. nov.

http://zoobank.org/B7B0E8AA-C5DE-433D-A2BC-0E73B386D45E
Figures 2, 3

Material examined. ***Holotype.*** MZUCR 2514, lot 1, ethanol preserved, Cocos Island, Chatham Bay, 05°33.347'N, 87°02.336'W, dredging, 45 m depth, J. Cortés, J. Sibaja-Cordero, Proteus-CIMAR Expedition, R/V Proteus, 13 January 2007. ***Paratypes.*** MZUCR 2514, lots 2, 3, ethanol preserved, same geographical data as the holotype. MZUCR 2734, lot ethanol preserved, Roca Chatham Bay, Langosta, Punta Ulloa, 05°33.222'N, 87°02.053'W, dredging 39–44 m depth, J. Cortés, J. Sibaja-Cordero, Proteus-CIMAR Expedition, R/V Proteus, 17 January 2007. MZUCR 2513 (GenBank accession numbers MW491885 (28S rDNA), MW491886 (mtMutS) and

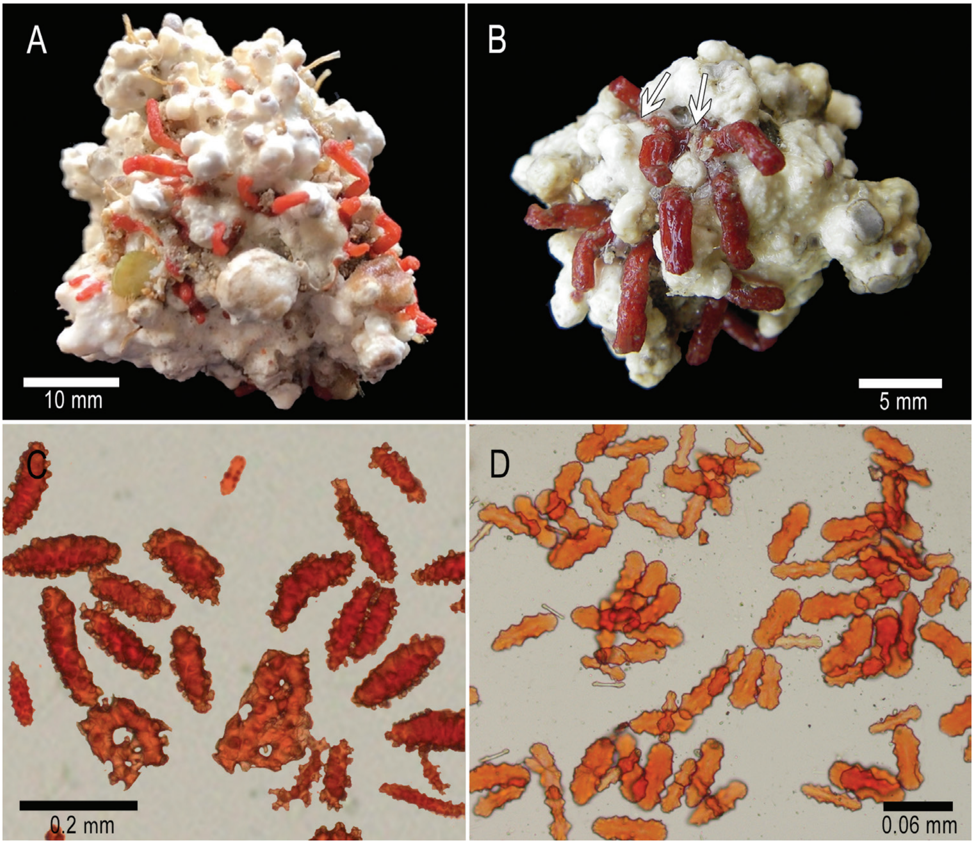


Figure 2. *Rhodolitica occulta* sp. nov. **A** holotype MZUCR 2514 **B** paratype MZUCR 2513, ribbon-like stolons can be observed at the upper cluster of polyps (white arrows) **C** unsorted sclerites of the holotype **D** tentacular sclerites.

MW491887 (COI)), ethanol preserved, Chatham Bay, Punta Ulloa, 05°33.222'N, 87°02.063'W dredging 45 m depth, J. Sibaja-Cordero, 14 April 2008. MZUCR 3301, ethanol preserved, Bahía Iglesias, Muela Rock, 23 m depth, J. Cortés, 14 January 2007. MZUCR 3302, lot preserved in ethanol, Chatham Bay, 05°32'43.6"N, 87°01'41.7"W, 20 m depth, J. Cortés, 3 April 2009. MZUCR 3303, lot ethanol preserved, off Cocos Island, Everest pinnacle, 55 m depth, HOV DeepSee Dive 2375, A. Klapfer, 25 October 2015.

Type locality. Chatham Bay, Cocos Island, Costa Rica, 45 m depth.

Description. The holotype is composed of several bright red tubular, single anthosteles extending up to 8 mm above the surface of a 4 cm-diameter rhodolith (from one lot of five rhodoliths, 4–7 cm diameter) (Fig. 2A). The tubular anthosteles are slightly expanded distally, forming groups of 10–12, and closely spaced, with the bases 1–2 mm apart. The anthosteles arise directly from thin basal stolons (Fig. 2B). The stolons are ribbon-like, approximately 1 mm in width, and extending in irregular pat-

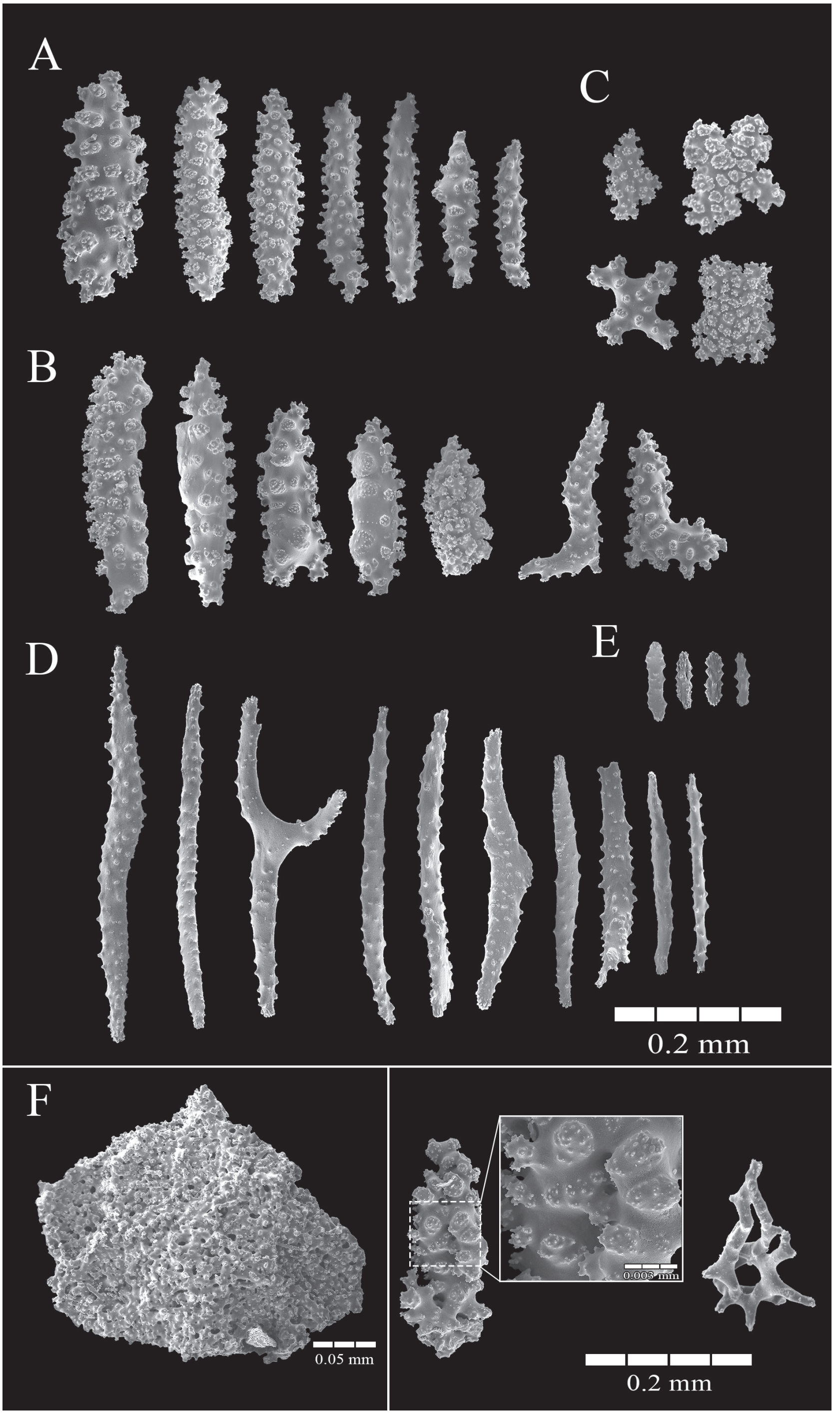


Figure 3. *Rhodolitica occulta* sp. nov., holotype MZUCR 2514 **A, B** outer coenenchymal sclerites **C** inner coenenchymal sclerites **D** anthocodial sclerites **E** tentacular sclerites **F** fused and partially fused sclerites from stolons, details at the right.

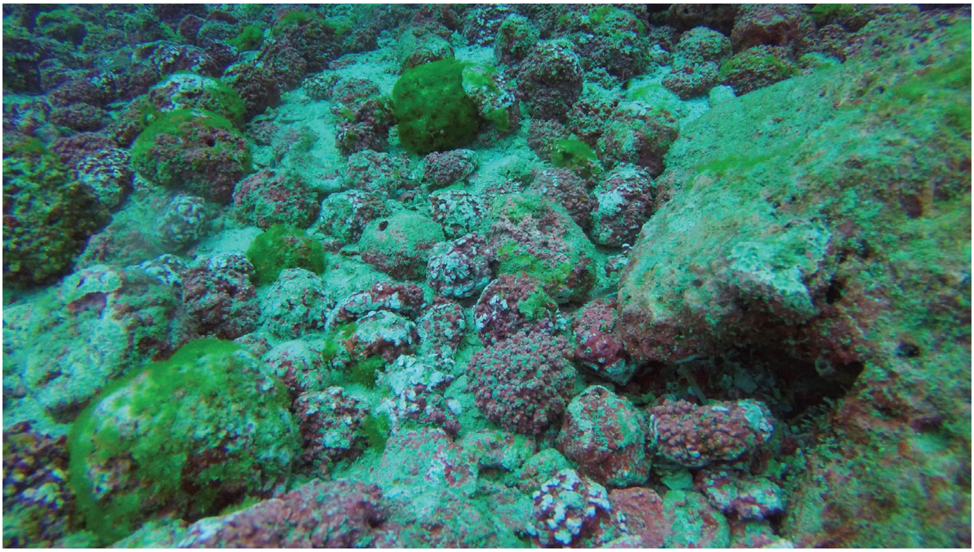


Figure 4. Rhodoliths bed, Pájara Islet, Cocos Island, at 22 m in depth. Photograph by Beatriz Naranjo, UCR.

terns on the surface of the rhodolith. Anthostelar walls are composed of bright red, variable coenenchymal sclerites: long warty spindles, 0.17–0.27 mm in length, 0.047–0.07 mm wide (Figs 2C, 3A); complexly warted cylindrical spindles with fused tubercles on one surface, and warty on the other, 0.11–0.32 mm long and 0.045–0.08 mm wide (Fig. 3B); bent spindles, 0.19–0.23 mm long, 0.01–0.013 mm wide (Fig. 3B, end of the row); smaller crosses, radiates up to 0.15 mm long, and transitional forms (Fig. 3C) from the inner coenenchyme. Conspicuous fused or partly fused sclerites are present in the stolons (Figs 2C, 3F); Figure 3F (right side) shows details of sclerites fused together. These brittle sclerites can partially dissociate during the dissolution process, forming small or large aggregations 0.2–1.6 mm long (Figs 2C, 3F). They are present only in the stolons, and do not extend into the anthostelar walls. Anthocodiae are completely retractile into the anthosteles. The anthocodiae have eight interseptal points, composed of slender thorny spindles, 0.23–0.5 mm long and 0.018–0.05 mm wide (Figs 2C, 3D) arranged 'en chevron', with very few transverse spindles, not forming a collaret. The points are composed of 10–15 spindles. Orange biscuit-like rods, 0.047–0.095 mm long and 0.014–0.026 mm wide (Figs 2D, 3E) are along the tentacles. Unfortunately, all specimens have been preserved with the anthocodiae partially or totally retracted therefore a full description is not possible.

Variability. The paratypes were found on rhodoliths of 3–9 cm in diameter. Some variation in colour was found in a few paratypes. They present red and orange anthosteles in the same colony. All other characteristics were consistent with those of the holotype.

Habitat and distribution. The colonies were found only associated with living rhodoliths, from 20 to 55 m depth. The rhodoliths were in aggregations, forming extensive

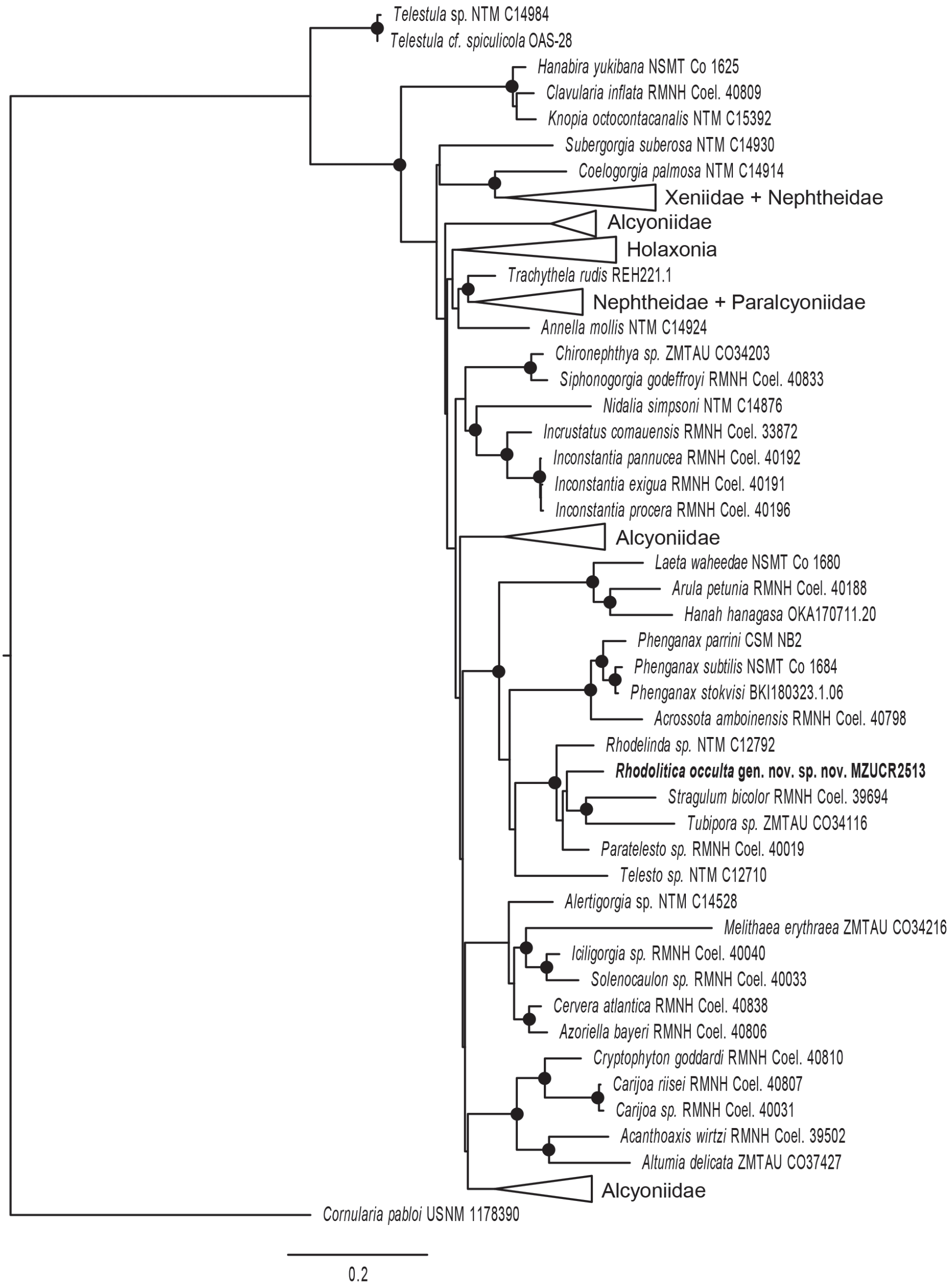


Figure 5. Maximum likelihood reconstruction (2417 nt of concatenated mtMutS, COI, 28S rDNA) of the Holaxonia-Alcyoniina clade of Octocorallia. Two stoloniferous taxa belonging to the Calcaxonia-Pennatulacea clade (*Cornularia*, *Telestula*) were included to root the tree. Clades that did not include any stoloniferans have been collapsed to triangles to facilitate readability. Solid circles on nodes indicate strong support from both maximum likelihood and Bayesian analyses (bootstrap > 70%, pp > 0.95)

beds or dispersed on sandy bottoms (Fig. 4). Specimens were also collected from a dead shell and from a dead piece of coral partially covered by rhodoliths, making it evident that this species is associated with living rhodoliths. In some colonies anthosteles are occupied by several epizoic organisms, especially bryozoans and small hydroids.

The species is presently only known from Cocos Island.

Etymology. An adjective (L), *occultus*, meaning hidden, secret, mysterious. Historically, numerous expeditions to Cocos Island seeking pirate treasures buried somewhere in the mysterious island were completely unsuccessful. The new species, out of sight, hidden amongst rhodoliths, shows that the real treasure of the island is its biodiversity.

Phylogenetic analysis

All phylogenetic analyses placed MZUCR 2513 in a well-supported clade (ML bootstrap (bs) = 95%; Bayesian posterior probability (pp) = 1.0) that included families Tubiporidae, Acrossotidae, Arulidae, and several genera of Clavulariidae (Fig. 5). Within that larger clade of stoloniferans, both ML and Bayesian methods recovered MZUCR 2513 in a clade with *Paratelesto* sp., *Rhodelinda* sp. and [*Tubipora*+*Stragulum*] (bs = 100%; pp = 1.0) with no support (bs < 50%, pp < 0.90) for the sister relationships among those taxa.

Discussion

From the morphological point of view, *Rhodolitica* gen. nov. is related to *Rhodelinda*, *Paratelesto*, and *Stragulum* Ofwegen & Haddad, 2011, all within the family Clavulariidae, a conclusion that is supported by the phylogenetic analysis. Morphologically, *Paratelesto* grows in bushy clusters, the colony branches many times, up to four times with secondary polyps (Fabricius and Alderslade 2001); this characteristic separates it from *Rhodolitica* and the other two genera. Although the external layer of large complexly warted cylindrical sclerites in *Paratelesto* is somewhat similar to that in *Rhodolitica* (Fig. 3B), none of the other features are shared. The main difference between the new genus and *Rhodelinda* is that the anthostelar armature in *Rhodelinda* consists of fused sclerites forming a brittle tube (Bayer 1981; Williams 1989; Pastorino and Ituarte 1996). In *Rhodolitica* the fused sclerites are only present in the stolons. In addition, the anthocodial and coenenchymal sclerites are different: neither the conspicuous cylindrical sclerites and spindles forming the external layer of the coenenchyme (Fig.3A, B) nor the internal coenenchyme radiates (Fig. 3C) of *Rhodolitica* are present in *Rhodelinda*, and the anthocodial points are shorter and sharper in *Rhodolitica* (Fig. 3D) (Pastorino and Ituarte 1996). As in *Rhodolitica*, *Stragulum* does not have inseparably fused sclerites forming the anthosteles. The types of sclerites found in the basal layer of the coenenchyme in *Stragulum* are similar to those that form the stolons in *Rhodolitica*; however, *Stragulum* colonies form encrusting sheets instead of stolons. Also, the characteristic anthostelar cylindrical sclerites of *Rhodolitica* are not present. Phylogenetically, *Tubipora* Linnaeus, 1758, in the family Tubiporidae, is also close to the above mentioned genera, but morphologically it is different from the other three genera. The anthostele walls of *Tubipora* form rigid tubes of solidly fused sclerites and the polyp tubes are joined laterally by an elevated series of transverse platforms (Fabricius and Alderslade 2001), producing complex structures. The genus is also zooxanthellate (Fabricius and Alderslade 2001) in contrast to the other three.

The morphological distinction of *Rhodolitica* from *Rhodelinda*, *Stragulum*, and *Tubipora* is also supported by phylogenetic analyses. Although all analyses strongly supported the placement of *Rhodolitica* in a clade with *Rhodelinda*, *Paratelesto*, *Stragulum*, and *Tubipora*, the relationships among those genera remained poorly resolved. Lack of clear evidence for a sister relationship between *Rhodolitica* and any one of these other related but morphologically distinct genera supports the decision to establish a new genus for *R. occulta* sp. nov., and highlights the importance of the integrative approach to octocoral taxonomic work.

Acknowledgements

We are grateful to Bert Hoeksema (Naturalis Biodiversity Center, Leiden, the Netherlands) and the anonymous reviewers for their suggestions and comments that definitely improved our publication. We thank the Servicio Nacional de Áreas Protegidas de Costa Rica and the personnel of the Parque Nacional Isla del Coco for allowing our research at the Island. We express our appreciation to the crew of the R/V Proteus, 2007 trip, and the submersible *DeepSee* and M/V *Argo* (UnderSea Hunter Group). We thank Jeffrey Sibaja and Jorge Cortés for collecting the samples. We appreciate Luis Solís and Andrea Moya (UCR) for their contribution in this manuscript. The research was funded by Vicerrectoría de Investigación, Universidad de Costa Rica (projects B5172, B6773 and B5159), Marviva Foundation and Conservation International.

References

Bayer FM (1981) On some genera of stoloniferous Octocorals (Coelenterata: Anthozoa), with descriptions of new taxa. Proceedings of the Biological Society of Washington 94(3): 878–901.

Breedy O, Cortés J (2008) Octocorals (Coelenterata: Anthozoa: Octocorallia) of Isla del Coco, Costa Rica. Revista de Biología Tropical 56 (Supplement 2): 71–77.

Breedy O, Cortés J (2011) Morphology and taxonomy of a new species of *Leptogorgia* (Cnidaria: Octocorallia: Gorgoniidae) in Cocos Island National Park, Pacific Costa Rica. Proceedings of the Biological Society of Washington 124: 62–69. https://doi.org/10.2988/10-18.1

Breedy O, van Ofwegen LP, Vargas S (2012) A new family of soft corals (Anthozoa, Octocorallia, Alcyonacea) from the aphotic tropical eastern Pacific waters revealed by integrative taxonomy, Systematics and Biodiversity 10(3): 351–359. https://doi.org/10.1080/14772000.2012.707694

Cortés J (2016) Isla del Coco: Coastal and marine ecosystems. In: Kappelle M (Ed.) Costa Rican ecosystems. University of Chicago Press, Chicago and London, 162–191. https://doi.org/10.7208/chicago/9780226121642.003.0007

Cortés J (2019) Isla del Coco, Costa Rica, Eastern Tropical Pacific. In: Loya, Y, Puglise KA, Bridge TCL (Eds) Mesophotic Coral Ecosystems. Coral Reefs of the World 12. Springer Nature, Switzerland, 465–474. https://doi.org/10.1007/978-3-319-92735-0_26

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9(8): e772. https://doi.org/10.1038/nmeth.2109

Duchassaing P, Michelotti G (1860) Mémoire sur les Coralliaires des Antilles. Memorie della Reale Accademia delle Scienze di Torino (ser. 2) 19: 279–365.

Ehrenberg CG (1831) Symbolae physicae, seu icones et descriptiones corporum naturalium novorum. Pars Zoologica, 4. Berlin.

Fabricius K, Alderslade P (2001) Soft Corals and Sea Fans: A Comprehensive Guide to the Tropical Shallow Water Genera of the Central West Pacific, the Indian Ocean and the Red Sea. Australian Institute of Marine Science, Townsville, 264 pp.

Fernández C (2008) Flora marina del Parque Nacional Isla del Coco, Costa Rica, Pacífico Tropical oriental. Revista de Biología Tropical 56(2): 57–69.

France SC, Hoover LL (2002) DNA sequences of the mitochondrial COI gene have low levels of divergence among deep-sea octocorals (Cnidaria: Anthozoa). Hydrobiologia 471: 149–155. https://doi.org/10.1023/A:1016517724749

Haeckel E (1866) Generelle Morphologie der Organismen. Berlin, 1036 pp.

Hickson SJ (1894) A revision of the genera of the Alcyonaria Stolonifera, with a description of one new genus and several new species. Transactions of the Zoological Society of London 13(9): 325–347. https://doi.org/10.1111/j.1096-3642.1894.tb00052.x

Lamouroux JVF (1812) Extrait d'un mémoire sur la classification des polypiers coralligènes non entièrement pierreux. Nouveau Bulletin des Sciences par la Société Philomatique, Paris 3(63): 181–188.

Linnaeus C (1758) Systema naturae. Editio decima, reformata. 1: 824 pp. [Holmiae.]

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52: 696–704. https://doi.org/10.1080/10635150390235520

Katoh K, Kuma K, Toh H, Miyata T (2005) MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Research 33: 511–513. https://doi.org/10.1093/nar/gki198

Konar B, Riosmena-Rodriguez R, Iken K (2006) Rhodolith bed: a newly discovered habitat in the North Pacific Ocean. Botanica Marina 49: 355–359. https://doi.org/10.1515/BOT.2006.044

McFadden CS, Tullis ID, Hutchinson MB, Winner K, Sohm JA (2004) Variation in coding (NADH dehydrogenase subunits 2, 3, and 6) and noncoding intergenic spacer regions of the mitochondrial genome in Octocorallia (Cnidaria: Anthozoa). Marine Biotechnology 6: 516–526. https://doi.org/10.1071/IS12035

McFadden CS, van Ofwegen LP (2012) Stoloniferous octocorals (Anthozoa, Octocorallia) from South Africa, with descriptions of a new family of Alcyonacea, a new genus of Clavulariidae, and a new species of *Cornularia* (Cornulariidae). Invertebrate. Systematics 26: 331–356. https://doi:10.10071/is12035

van Ofwegen LP, Breedy O, Cairns SD (2009) Octocorallia-Octocorals. In: Häussermann V, Försterra G (Eds) Marine Benthic Fauna of Chilean Patagonia. Nature in Focus, Santiago, Chile, 177–214.

van Ofwegen LP, Haddad MA (2011) A probably invasive new genus and new species of soft coral (Octocorallia: Alcyonacea: Clavulariidae) from Brazil. Zootaxa 3107: 38–46. https://doi.org/10.11646/zootaxa.3107.1.2

Pastorino G, Ituarte C (1996) *Rhodelinda gardineri* (Gohar, 1940) (Coelenterata: Anthozoa) a stoloniferous octocoral from Patagonian shallow waters. Bulletin of Marine Science 58(1): 196–202.

Ronquist F, Teslenko M, van der Mark P, Ayres D, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Sánchez JA, McFadden CS, France SC, Lasker HR (2003) Molecular phylogenetic analyses of shallow-water Caribbean octocorals. Marine Biology 142: 975–987. https://doi.org/10.1007/s00227-003-1018-7

Sibaja-Cordero JA, Troncosa JS, Benavides-Vrela C, Cortés J (2012) Distribution of shallow water soft and hard bottom seabeds in the Isla del Coco National Park, Pacific Costa Rica. Revista de Biología Tropical 60(3): 53–66.

Solano-Barquero A (2011) Macrofauna asociada a rodolitos en el Parque Nacional Isla del Coco, Costa Rica. Thesis, Universidad de Costa Rica, San Pedro, Costa Rica.

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post–analysis of large phylogenies. Bioinformatics 30(9): 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Utinomi H (1958) On some octocorals from deep waters of Prov. Tosa. Sikoku. Publications of the Seto Marine Biological Laboratory 7(1): 89–110. https://doi.org/10.5134/174598

Williams G (1989) A comparison of the stoloniferous octocorallian genera *Scyphopodium*, *Stereotelesto*, *Bathytelesto* and *Rhodelinda*, with description of a new species from south eastern Africa (Anthozoa, Clavulariidae). Journal of the Zoological Society of London 219: 621–635. https://doi.org/10.1111/j.1469-7998.1989.tb02604.x

Supplementary material 1

Table S1. Sequence data used in phylogenetic analysis

Authors: Odalisca Breedy, Leen van Ofwegen, Catherine S. McFadden, Catalina Murillo-Cruz

Data type: Phylogenetic

Explanation note: **Table S1** contains the sequence data of Octocorallia used in the phylogenetic analysis for a new genus and species: *Rhodolitica occulta* gen. nov. sp. nov.

Link: https://doi.org/10.3897/zookeys.1032.63431.suppl1

Supplementary material 2

Fig. S1

Authors: Odalisca Breedy, Leen van Ofwegen, Catherine S. McFadden, Catalina Murillo-Cruz

Data type: Phylogenetic

Explanation note: Maximum likelihood tree for mtMutS (738 nt). Only the stoloniferan clade that includes *Rhodolitica occulta* gen. nov. sp. nov. is shown, rooted to *Clavularia inflata*. Numbers on branches are bootstrap values (% of 100 replicates)

Link: https://doi.org/10.3897/zookeys.1032.63431.suppl2

Supplementary material 3

Fig. S2

Authors: Odalisca Breedy, Leen van Ofwegen, Catherine S. McFadden, Catalina Murillo-Cruz

Data type: Phylogenetic

Explanation note: Maximum likelihood tree for COI (693 nt). Only the stoloniferan clade that includes *Rhodolitica occulta* gen. nov. sp. nov. is shown, rooted to *Clavularia inflata*. Numbers on branches are bootstrap values (% of 100 replicates).

Link: https://doi.org/10.3897/zookeys.1032.63431.supp3

Supplementary material 4

Fig. S3

Authors: Odalisca Breedy, Leen van Ofwegen, Catherine S. McFadden, Catalina Murillo-Cruz

Data type: Phylogenetic

Explanation note: Maximum likelihood tree for 28S rDNA (813 nt). Only the stoloniferan clade that includes *Rhodolitica occulta* gen. nov. sp. nov. is shown, rooted to *Clavularia inflata*. Numbers on branches are bootstrap values (% of 100 replicates).

Link: https://doi.org/10.3897/zookeys.1032.63431.suppl4

ZooKeys 1032: 79–90 (2021)
doi: 10.3897/zookeys.1032.63905
https://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Definition of the *Gonioctena subgeminata* species group (Coleoptera, Chrysomelidae, Chrysomelinae), with descriptions of two new species from China and Vietnam

Hee-Wook Cho[1]

[1] *Department of Zoology, Nakdonggang National Institute of Biological Resources, Sangju, 37242, South Korea*

Corresponding author: Hee-Wook Cho (lampides@gmail.com)

Academic editor: Astrid Eben | Received 3 February 2021 | Accepted 25 March 2021 | Published 16 April 2021

http://zoobank.org/830085C4-73AB-403B-BD87-DDD1E0C2C892

Citation: Cho H-W (2021) Definition of the *Gonioctena subgeminata* species group (Coleoptera, Chrysomelidae, Chrysomelinae), with descriptions of two new species from China and Vietnam. ZooKeys 1032: 79–90. https://doi.org/10.3897/zookeys.1032.63905

Abstract

This paper defines and reviews the *Gonioctena subgeminata* species group of the subgenus *Asiphytodecta* Chen, 1935. The group contains the following five species, including two new to science: *G. subgeminata* (Chen, 1934), *G. tonkinensis* (Chen, 1934), *G. oudai* Cho & Borowiec, 2016, *G. allardi* **sp. nov.**, and *G. mantillerii* **sp. nov.** The species group restricted to China and Vietnam is characterized by a labrum without a tooth-like projection, elytral punctures arranged in discernible rows, and a setose aedeagus with a broad apical process. *Gonioctena tonkinensis* is newly reported as an ovoviviparous species. Habitus photographs, illustrations of diagnostic characters of each species, and a key to the species are provided.

Keywords

Asiphytodecta, leaf beetle, ovoviviparity, taxonomy

Introduction

The subgenus *Asiphytodecta* Chen, 1935, is the second most speciose group of the nine subgenera in the genus *Gonioctena*, with approximately 25 species occurring in the Oriental Region and Palearctic China (Cho and Borowiec 2016a). More than half of the *Asiphytodecta* species present a tooth-like projection at the anterior margin of the labrum and completely irregular punctures on the elytra, which are unique and remarkable characters in *Gonioctena*. There are two species groups within the subgenus, *tredecimmaculata* and *flavoplagiata*, based on the morphological characters of adults. Bezděk (2002) proposed the *G. tredecimmaculata* species group for five species occurring in the Indochinese Peninsula and China, characterized by a black median spot on the pronotum and 12 black spots on the elytra varying from small, separated spots to large, transversely merged spots. Recent taxonomic work on *Asiphytodecta* by Cho et al. (2016) and Sprecher-Uebersax and Daccordi (2016) indicated that four more species should be added to the group: *G. fraudulenta* Sprecher-Uebersax & Daccordi, *G. taiwanensis* (Achard), *G. ohmomoi* Cho, Takizawa & Borowiec, and *G. riyuetanensis* Cho, Takizawa & Borowiec. The *G. flavoplagiata* species group proposed by Cho and Borowiec (2016a) includes five species from China, Laos, and Vietnam and is easily recognized by the strongly widened and flattened last four antennomeres and two pairs of yellowish brown spots on the elytra.

In contrast, members of other *Asiphytodecta* species lacking the above unique characters have been relatively poorly studied. Moreover, an identification key for known species has not been provided, and the subgeneric placement of several species remains uncertain. This study is the first attempt to solve these taxonomic problems, and the *Gonioctena* (*Asiphytodecta*) *subgeminata* species group classification is proposed for five species distributed in China and Vietnam, characterized by the labrum lacking a tooth-like projection, elytral punctures arranged in discernible rows, and a setose aedeagus with a broad apical process. Two new species of the *G. subgeminata* species group were discovered by recent examination of material from the Museum National d'Histoire Naturelle, Paris and are described here.

Materials and methods

Descriptions were prepared using Nikon SMZ800 and Nikon Eclipse E600 microscopes. Male and female genitalia were dissected from adult specimens, softened in a closed Petri dish with wet tissue paper for 12–24 hours, cleared in 10% sodium hydroxide solution, and rinsed in distilled water. Photographs were taken using the Nikon D5200 digital camera attached to the Nikon SMZ1500 microscope and were edited in Helicon Focus 5.3.12 and Adobe Photoshop CS5. Line drawings were made from photographs in Adobe Photoshop CS5 using the Wacom Intuos4 graphics tablet.

The specimens examined in this study are deposited in the following collections:

BPBM	Bernice Pauahi Bishop Museum, Honolulu, Hawaii, USA;
HCC	Hee-Wook Cho private collection, Yecheon, South Korea;
HTC	Hauro Takizawa private collection, Hasuda, Japan;
LMC	Lev N. Medvedev private collection, Moscow, Russia;
MNHN	Museum National d'Histoire Naturelle, Paris, France;
NHMB	Naturhistorisches Museum Basel, Basel, Switzerland;
NHMUK	The National History Museum, London, UK;

NMPC	Národní Muzeum, Prague, Czech Republic;
TLMF	Horst Kippenberg private collection, Tiroler Landesmuseum Ferdinandeum, Innsbruck, Austria;
ZMHB	Museum für Naturkunde der Humboldt-Universität, Berlin, Germany.

Taxonomic account

Gonioctena (*Asiphytodecta*) *subgeminata* species group

Differential diagnosis. Body length 5.7–8.6 mm. Body short-oval to oval, strongly convex. Ground color reddish brown to orange with black spots or sinuate transverse bands on dorsum. Last four or five antennomeres blackish brown and legs entirely reddish brown. Anterior margin of the labrum almost straight without a tooth-like projection. Elytra covered with regular rows of punctures including partially irregular ones or rather irregular punctures arranged in confused single or double rows. Aedeagus concave and setose apicolaterally, with apical process broad, produced laterally. Spermatheca C-shaped and thick, with apex widely rounded. The *Gonioctena tredecimmaculata* species group is similar to members of the *Gonioctena subgeminata* species group in body shape and coloration but differs in its completely irregular punctures on the elytra and a tooth-like projection at the anterior margin of the labrum. *Gonioctena medogana* Wang differs in the aedeagus with a very thin and glabrous apical process.

Key to the species of the *Gonioctena* (*Asiphytodecta*) *subgeminata* species group

1 Elytra covered with regular punctures arranged in single rows, partially irregular (Figs 2, 3) **2**
– Elytra covered with irregular punctures arranged in confused single or double rows (Figs 1, 4, 5) **3**
2 Pronotum with a pair of obscure spots (Fig. 8); apical process of aedeagus approximately 1.5 × wider than median lobe (Fig. 16). China (Sichuan) .... ***G. oudai*** **Cho & Borowiec**
– Pronotum without obscure spots (Fig. 7); apical process of aedeagus approximately 1.2 × wider than median lobe (Fig. 15). China (Sichuan) .... ***G. mantillerii*** **sp. nov.**
3 Pronotum with a pair of basal triangular black markings (Fig. 10). China (Guangxi), Vietnam .... ***G. tonkinensis*** **(Chen)**
– Pronotum without black markings (Figs 6, 9) **4**
4 Elytra with 10 black spots, sometimes narrowly connected, but not merged into a band (Fig. 4). China (Anhui, Fujian, Hunan, Jiangxi, Sichuan), Taiwan .... ***G. subgeminata*** **(Chen)**
– Elytra with two basal spots and two sinuate transverse bands (Fig. 1). Vietnam .... ***G. llardi*** **sp. nov.**

Gonioctena (*Asiphytodecta*) *allardi* sp. nov.

http://zoobank.org/7A746AF3-58A8-4B6C-9E5A-B87E64BB98A9
Figures 1, 6, 11, 12

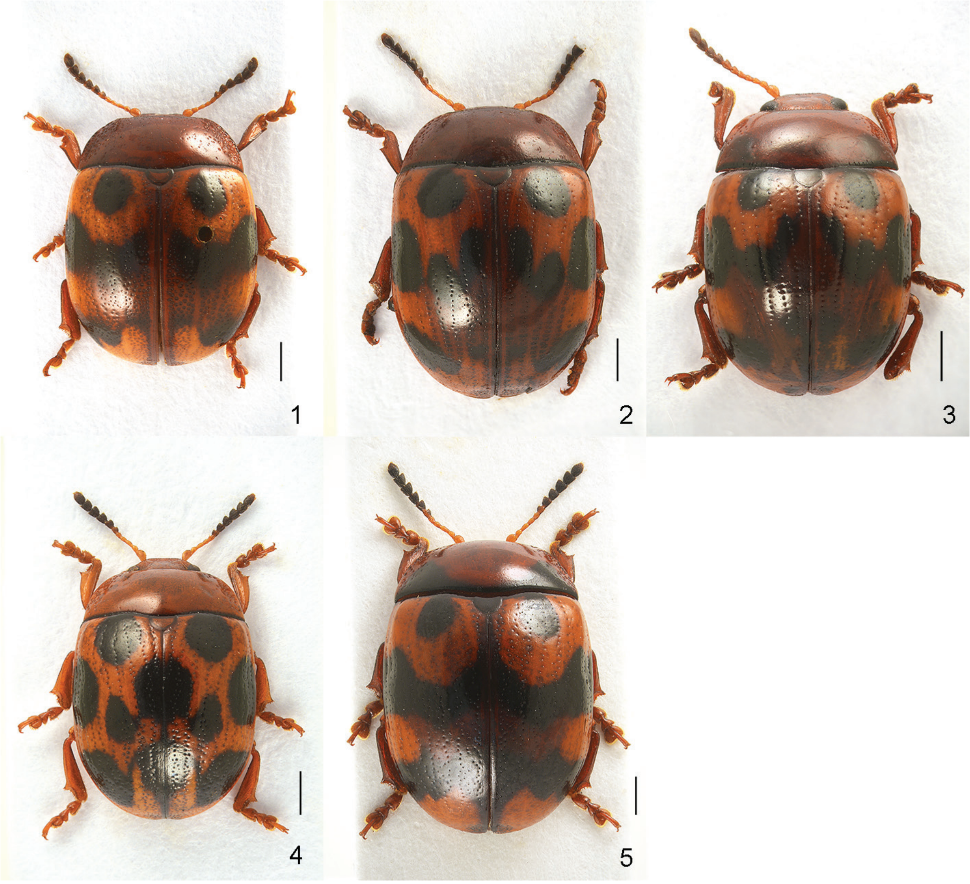
Type locality. Vietnam: Hanoi, Son Tay.

Type material. ***Holotype*:** ♂ (MNHN), "Son Tay // Ex. Musaeo E. Allard 1899 // MUSEUM PARIS 1952 Coll. R. Oberthur // HOLOTYPUS *Gonioctena* (*A.*) *allardi* sp. nov. Cho & Borowiec 2014". ***Paratype*:** 1♀ (MNHN), same data as for holotype.

Diagnosis. This new species is similar to *Gonioctena mantillerii* sp. nov. and *G. oudai* in body shape and coloration. However, *G. allardi* sp. nov. can be distinguished by the following characters: elytra covered with rather irregular punctures arranged in confused single or double rows (regular punctures arranged in single rows, partially irregular in other species); pronotum without spots (same in *G. mantillerii* sp. nov., a pair of lateral obscure spots present in *G. oudai*); aedeagus with an apical process widened to lateral tooth-like projections, 1.2 × wider than the median lobe (widened to lateral blunt projections, 1.2 × wider than the median lobe in *G. mantillerii* sp. nov., very large, 1.5 × wider than the median lobe in *G. oudai*).

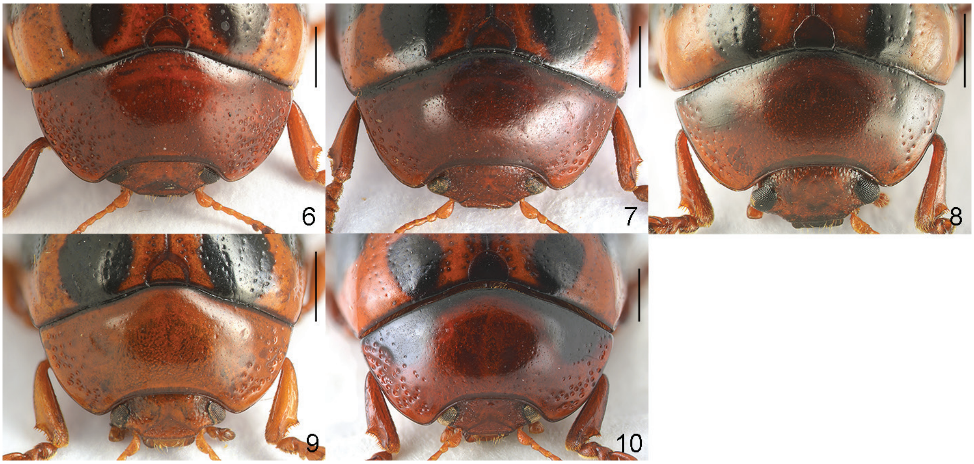
Description. Measurements in mm (n = 2): length of body: 6.50–7.50 (mean 7.00); width of body: 5.00–5.80 (mean 5.40); height of body: 3.30–4.00 (mean 3.65); width of head: 1.95–2.10 (mean 2.03); interocular distance: 1.25–1.35 (mean 1.30); width of apex of pronotum: 2.30–2.40 (mean 2.35); width of base of pronotum: 4.42–5.00 (mean 4.71); length of pronotum along midline: 2.00–2.25 (mean 2.13); length of elytra along suture: 4.90–5.90 (mean 5.40).

Body short oval and strongly convex (Fig. 1). Head reddish brown, with labrum partially dark brown, apex of mandibles black. Antennomeres I–VI yellowish brown, VI partially darkened, VII–XI blackish brown. Pronotum reddish brown, basal margin black. Scutellum reddish brown. Elytra orange, with a pair of black spots and sinuate transverse bands, dark area between bands, tip partially darkened. Venter and legs entirely reddish brown. ***Head.*** Vertex weakly convex, covered with sparse punctures, becoming coarser and denser toward sides. Frontal suture V-shaped, reaching anterior margin, coronal suture rather short. Frons flat, suddenly depressed at anterior margin, covered with dense punctures. Clypeus very narrow and trapezoidal. Anterior margin of labrum almost straight. Mandibles with two sharp apical teeth and deep excavation for apical maxillary palpomere on outer side. Maxillary palps four-segmented, with apical palpomere slightly widened, truncated apically. Antennae reaching pronotal base; antennomere I robust; antennomere II shorter than III; antennomere III longer than IV; antennomeres VII–X widened, VIII–X each almost as long as wide; antennomere XI longest, ~ 1.51 × as long as wide (Fig. 11). ***Pronotum.*** Lateral sides widest at base, strongly and roundly narrowed anteriorly, anterior angles strongly produced (Fig. 6). Anterior and lateral margins bordered; lateral margins hardly visible in dorsal view. Trichobothria absent on both anterior and posterior angles. Disc covered with sparse punctures; lateral sides covered with much larger and denser punctures; interspaces covered with fine and sparse punctures. Scutellum distinctly wider than



Figures 1–5. Dorsal habitus **1** *Gonioctena allardi* sp. nov., holotype **2** *G. mantillerii* sp. nov., holotype **3** *G. oudai* **4** *G. subgeminata* **5** *G. tonkinensis*. Scale bars: 1.0 mm.

long, narrowed posteriorly. ***Elytra.*** Lateral sides slightly widened posteriorly, widest before middle, thence roundly narrowed posteriorly. Humeral calli well developed. Disc covered with rather irregular punctures arranged in confused single or double rows in median region, regular punctures arranged in rows in lateral region, dense punctures between second and third striae in apical half; interspaces covered with fine and sparse punctures. Epipleura visible except near base in lateral view. Hind wings well developed. ***Venter.*** Hypomera weakly rugose, with few punctures near antero-lateral corners of prosternum. Prosternum covered with coarse and dense punctures bearing long setae; prosternal process enlarged apically, bordered laterally, with sparse punctures. Metasternum covered with small and sparse punctures in median region, large and dense punctures in lateral region. Abdominal ventrites covered with sparse or moderately dense punctures bearing short setae. ***Legs.*** Moderately robust. Tibiae widened apically, with a tooth-like projection. Fore legs with tarsomere I slightly narrower than III in male and distinctly narrower than III in female. Tarsal claws appendiculate.



Figures 6–10. Head and pronotum **6** *Gonioctena allardi* sp. nov., holotype **7** *G. mantillerii* sp. nov., holotype **8** *G. oudai* **9** *G. subgeminata* **10** *G. tonkinensis*. Scale bars: 1.0 mm.

Genitalia. Aedeagus rather thin, subparallel-sided, weakly narrowed before apical process, setose apicolaterally, with apical process widened to lateral tooth-like projections in dorsal view; moderately curved, apex pointed in lateral view (Fig. 12). Spermatheca C-shaped, swollen basally, with apex rounded (Fig. 13).

Etymology. Named after its collector, E. Allard.

Distribution. Vietnam (Hanoi).

Gonioctena (*Asiphytodecta*) *mantillerii* sp. nov.

http://zoobank.org/7BE92F7A-9B6A-4299-A95D-43A49969FDF1

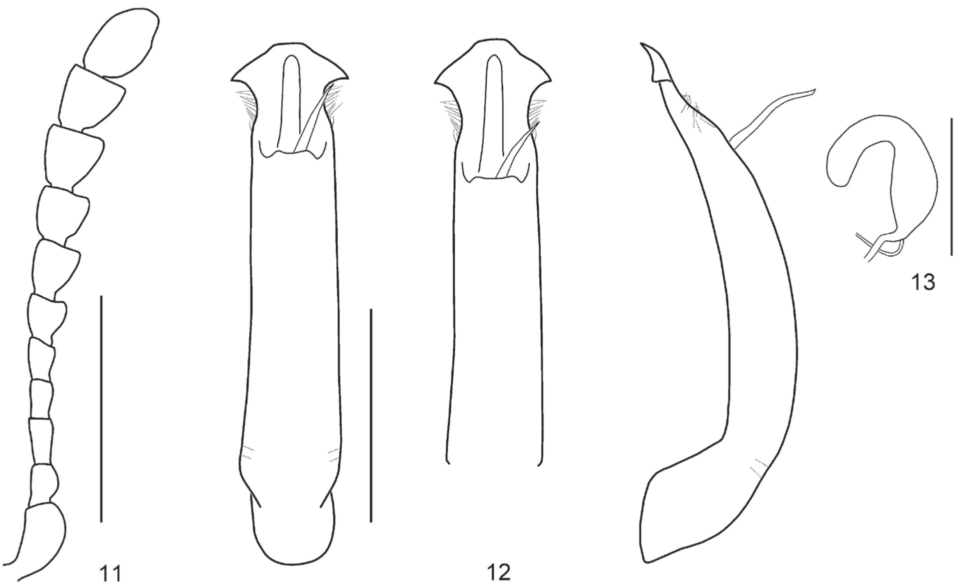
Figures 2, 7, 14, 15

Type locality. China: Sichuan.

Type material. *Holotype*: ♂ (MNHN), "Su-Tchuen, Chasseurs Indigènes 1903 // HOLOTYPUS *Gonioctena* (*A.*) *mantillerii* sp. n. Cho & Borowiec 2014".

Diagnosis. This new species is similar to *Gonioctena allardi* sp. nov. and *G. oudai* in body shape and coloration. However, *G. mantillerii* sp. nov. can be distinguished by the following characters: elytra covered with regular punctures arranged in single rows, partially irregular (rather irregular punctures arranged in confused single or double rows in *G. allardi* sp. nov., similar in *G. oudai*); pronotum without spots (same in *G. allardi* sp. nov., a pair of lateral obscure spots present in *G. oudai*); aedeagus with an apical process widened to lateral blunt projections, 1.2 × wider than the median lobe (widened to lateral tooth-like projections, 1.2 × wider than the median lobe in *G. allardi* sp. nov., very large, 1.5 × wider than the median lobe in *G. oudai*).

Description. Measurements in mm (n = 1): length of body: 6.75; width of body: 4.85; height of body: 3.30; width of head: 2.00; interocular distance: 1.27; width of

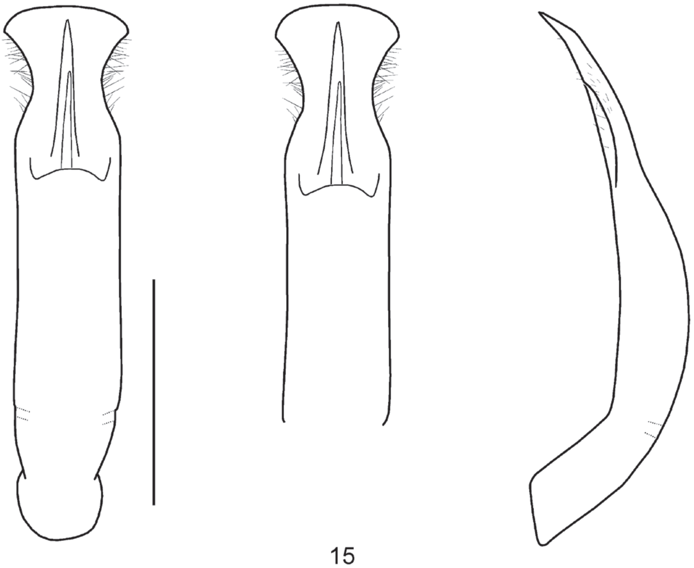


Figures 11–13. *Gonioctena allardi* sp. nov., holotype **11** antenna **12** aedeagus (dorsal view, apex in dorsal view, lateral view) **13** spermatheca. Scale bars: 1.0 mm (**11**, **12**); 0.5 mm (**13**).

apex of pronotum: 2.35; width of base of pronotum: 4.42; length of pronotum along midline: 1.95; length of elytra along suture: 5.10.

Body short oval and strongly convex (Fig. 2). Head reddish brown, with apex of mandibles black. Antennomeres I–VI yellowish brown, VI partially darkened, VII–XI blackish brown. Pronotum reddish brown, basal margin black. Scutellum reddish brown. Elytra orange, with six pairs of black spots, dark area in median region. Venter and legs entirely reddish brown. ***Head.*** Vertex weakly convex, covered with sparse punctures, becoming coarser and denser toward sides. Frontal suture V-shaped, reaching anterior margin, coronal suture rather short. Frons flat, suddenly depressed at anterior margin, covered with dense punctures. Clypeus very narrow and trapezoidal. Anterior margin of labrum almost straight. Mandibles with two sharp apical teeth and deep excavation for apical maxillary palpomere on outer side. Maxillary palps four-segmented, with apical palpomere slightly widened, truncate apically. Antennae reaching pronotal base; antennomere I robust; antennomere II shorter than III; antennomere III longer than IV; antennomeres VII–X widened, VII–VIII slightly longer than wide, IX–X almost as long as wide; XI longest, ~ 1.65 × as long as wide (Fig. 14). ***Pronotum.*** Lateral sides widest at base, strongly and roundly narrowed anteriorly, anterior angles strongly produced (Fig. 7). Anterior and lateral margins bordered; lateral margins well visible in dorsal view. Trichobothria absent on both anterior and posterior angles. Disc covered with very sparse punctures; lateral sides covered with much larger and denser punctures; interspaces covered with fine and sparse punctures. Scutellum distinctly wider than long, narrowed posteriorly. ***Elytra.*** Lateral sides subparallel, widest near middle, thence roundly narrowed posteriorly. Humeral calli well developed. Disc covered with eleven regular rows of large punctures, including a short

14



15

Figures 14, 15. *Gonioctena mantillerii* sp. nov., holotype **14** antenna **15** aedeagus (dorsal view, apex in dorsal view, lateral view). Scale bars: 1.0 mm.

scutellar row, punctures partially irregular; interspaces covered with fine and sparse punctures. Epipleura visible except near base in lateral view. Hind wings well developed. ***Venter.*** Hypomera weakly rugose, with few punctures near anterolateral corners of prosternum. Prosternum covered with coarse and moderately dense punctures bearing long setae; prosternal process enlarged apically, bordered laterally, with sparse punctures. Metasternum covered with small and sparse punctures in median region, large and dense punctures in lateral region. Abdominal ventrites covered with sparse or moderately dense punctures bearing short setae. ***Legs.*** Moderately robust. Tibiae widened apically, with a tooth-like projection. Fore legs with tarsomere I slightly narrower than III. Tarsal claws appendiculate. ***Genitalia.*** Aedeagus rather thin, subparallel-sided, strongly concave in apical 1/4, setose apicolaterally, with the apical process widened to lateral blunt projections in dorsal view; moderately curved, apex pointed in lateral view (Fig. 15).

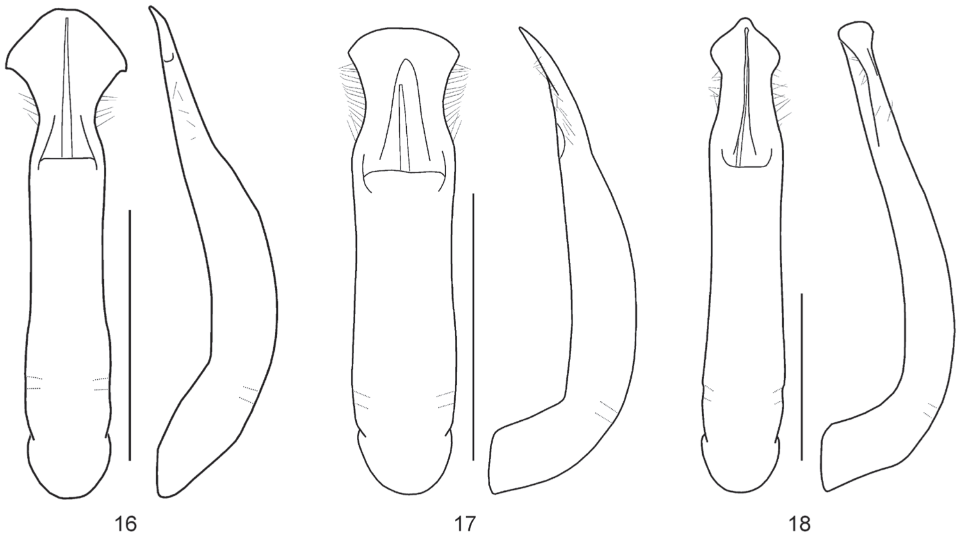
Etymology. Dedicated to Antoine Mantilleri, the curator of beetles at the Museum National d'Histoire Naturelle in Paris.

Distribution. China (Sichuan).

***Gonioctena* (*Asiphytodecta*) *oudai* Cho & Borowiec, 2016**

Figures 3, 8, 16

Gonioctena (*Asiphytodecta*) *oudai* Cho & Borowiec, 2016b: 170 (type locality: China, Sichuan, Leshan, Mt. Emei).



Figures 16–18. Aedeagus, dorsal, and lateral views (after Cho and Borowiec 2016b) **16** *Gonioctena oudai* **17** *G. subgeminata* **18** *G. tonkinensis*. Scale bars: 1.0 mm.

Type material. ***Holotype***: ♂ (NMPC), "China, SW Sichuan, Mt. Emei, 1000–2000 m, 6.VI.1997, Ouda M. lgt. // HOLOTYPUS *Gonioctena* (*A.*) *oudai* sp. n. Cho & Borowiec 2015". ***Paratype***: 1♂ (LMC), "China: Sichuan, Mt. Emei, 600–1050 m, 5–19.V.1989, Lad. Bocak lgt. // PARATYPUS *Gonioctena* (*A.*) *oudai* sp. n. Cho & Borowiec 2015".

Distribution. China (Sichuan).

***Gonioctena* (*Asiphytodecta*) *subgeminata* (Chen, 1934)**

Figures 4, 9, 17

Phytodecta subgeminatus Chen, 1934: 71, 75 (type locality: China, Guangdong, Guangzhou), 1938: 290, 294.

Phytodecta (*Asiphytodecta*) *subgeminatus*: Chen 1935: 131, 1936: 88; Chûjô 1958: 67.

Asiphytodecta subgeminatus: Chen and Young 1941: 208.

Gonioctena (*Asiphytodecta*) *subgeminata*: Gressitt and Kimoto 1963: 365; Kimoto and Gressitt 1981: 385, 386; Kippenberg 2010: 432; Yang et al. 2014: 388; Yang et al. 2015: 54; Cho and Borowiec 2016b: 170, 174.

Gonioctena (*Asiphytodecta*) *subgeminata subgeminata*: Gressitt and Kimoto 1963: 360.

Gonioctena (*Asiphytodecta*) *subgeminatus*: Kimoto and Chu 1996: 52; Kimoto and Takizawa 1997: 158, 295, 369; Kimoto 2003: 79.

Gonioctena subgeminata: Yu et al. 1996: 68.

Type material. Type probably lost.

Other material. China – **Anhui**: 1♂ (TLMF), Tianzhushan env., 30.75N, 116.45SE, 11–14.V.2004, Jaroslav Turna leg.; **Fujian**: 1♂ (NHMB), Kuatun (2300 m), 27,40 n. Br. 117,40ö., 2.VI.1938, L.J. Klapperich leg.; 1♂ (BPBM), Shaowu, Taohulan, 21.II.1943, K.S. Liu leg.; **Guangdong:** 1♀ (BPBM), Yaoshan (Mt. range), Linhsien (District), 3.V.1934, F.K. To leg.; **Hunan:** 1♂ (NHMUK), mts. Dalongshan, XinHua, 1600 m, VII.2004, Jing leg.; 1♂ (NHMUK), mts. Wugongshan, ad An-Fu 1650 m, VIII.2004; **Jiangxi:** 2♀♀ (NHMUK), mts. Tianiangshan, ad Xin-Huan, 1600 m, VII.2004; **Sichuan:** 1♀ (NHMUK), Nanping, 6.VI.2001, E. Kučera leg.; 1♂ (HCC), Nanping-Jiuzhaigou, 7–12.VI.2009, E. Kučera leg.; 1♂, 1♀ (HCC), Nanping-Jiuzhaigou, 11–14.VI.2011, E. Kučera leg.; **Zhejiang:** 2♂♂ (NHMB), Tienmuschan; **Taiwan:** 1♀ (HTC), Guandaoxi, 5.V.1973, S. Nakamura leg.

Distribution. China (Anhui, Fujian, Guangdong, Hunan, Jiangxi, Sichuan, Zhejiang), Taiwan.

Remarks. Chen (1934) proposed this species based on two specimens from ZMHB with a reddish brown pronotum and elytra with eleven black spots including the obscure spot near the apex. Searching the ZMHB collection, I could not find any specimen matching the original description. However, the original description of *G. subgeminata*, with an illustration of the habitus, allowed its identification without any doubt.

***Gonioctena* (*Asiphytodecta*) *tonkinensis* (Chen, 1934)**

Figures 5, 10, 18

Phytodecta subgeminatus var. *tonkinensis* Chen, 1934: 76 (type locality: Vietnam, Lang Son, Loc Binh, Mt. Mau Son), 1938: 295; Kimoto and Gressitt 1981: 386 (synonymized with *G. subgeminata*); Kippenberg 2010: 432 (as synonym of *G. subgeminata*).

Phytodecta (*Asiphytodecta*) *subgeminatus* var. *tonkinensis*: Chen 1935: 131, 1936: 88.

Asiphytodecta subgeminatus tonkinensis: Chen and Young 1941: 208.

Gonioctena (*Asiphytodecta*) *subgeminata tonkinensis*: Gressitt and Kimoto 1963: 360.

Gonioctena (*Asiphytodecta*) *tonkinensis*: Cho and Borowiec 2016b: 181 (resurrected as a valid species).

Type material. ***Lectotype*** (designated by Cho and Borowiec 2016b): ♀ (ZMHB), "Tonkin, Montes Mauson, April, Mai 2–3000', H. Fruhstorfer // Type // TYPE // *Phytodecta subgeminata* Chen // LECTOTYPUS *Phytodecta subgeminata* var. *tonkinensis* Chen, 1934 des. H.W. Cho 2015". ***Paralectotype*:** 1♂ (ZMHB), same data as for lectotype.

Distribution. China (Guangxi), Vietnam (Vinh Phuc).

Remarks. *Gonioctena subgeminata tonkinensis* (Chen, 1934) was synonymized with *G. subgeminata* (Chen, 1934) by Kimoto and Gressitt (1981). However, this name was removed from synonymy with *G. subgeminata* and raised to the species level by Cho and Borowiec (2016b). Several larvae were dissected from a female specimen; therefore, this species is ovoviviparous.

Acknowledgements

I thank Michael Geiser (NHMUK), Shepherd Myers (BPBM), Hauro Takizawa (Saitama, Japan), Lev N. Medvedev (LMC), Isabelle Zürcher-Pfander (NHMB), Lukáš Sekerka (NMPC), Horst Kippenberg (Herzogenaurach, Germany), and Bernd Jaeger (ZMHB) for allowing me to examine specimens under their care. I also thank Antoine Mantilleri (MNHN) for the loan of specimens and his generous assistance during my stay in his institution. The English in this document has been checked by at least two professional editors, both native speakers of English. For a certificate, please see: http://www.textcheck.com/certificate/index/HWLHrT. The University of Wrocław supported this work under scientific grant No. 2125/M/KBTE/15. The Nakdonggang National Institute of Biological Resources (NNIBR) supported article processing charges under project No. NNIBR-2020-01101.

References

Bezděk J (2002) A review of the *Gonioctena tredecimmaculata* (Jacoby, 1888) group (Coleoptera, Chrysomelidae, Chrysomelinae). Entomologica Basiliensia 24: 7–22.

Chûjô M (1958) A taxonomic study on the Chrysomelidae (Insecta, Coleoptera) from Formosa. Part X. Subfamily Chrysomelinae. Quarterly Journal of the Taiwan Museum 11: 1–85.

Chen SH (1934) Recherches sur les Chrysomelinae de la Chine et du Tonkin. Thèses présentées Faculté des Sciences de l'Université de Paris, 1re thèse, Paris, 105 pp.

Chen SH (1935) Classification of Asiatic *Phytodecta* (Col. Chrysomelinae). Chinese Journal of Zoology 1: 125–133.

Chen SH (1936) Catalogue des Chrysomelinae de la Chine, de l'Indochine et du Japon. Notes d'Entomologie Chinoise 3: 63–102.

Chen SH (1938) Recherches sur les Chrysomelinae de la Chine et du Tonkin. Annales de la Société Entomologique de France 106: 283–323.

Chen SH, Young B (1941) The coleopterous genus *Asiphytodecta* Chen. Sinensia 12: 199–210.

Cho H-W, Borowiec L (2016a) Revision of the *Gonioctena flavoplagiata* species-group (Coleoptera: Chrysomelidae: Chrysomelinae), with descriptions of two new species from China and Laos. Acta Entomologica Musei Nationalis Pragae 56: 755–768.

Cho H-W, Borowiec L (2016b) On the genus *Gonioctena* Chevrolat (Coleoptera: Chrysomelidae: Chrysomelinae), with descriptions of seven new species from the Oriental region and Palaearctic China. Zootaxa 4067: 168–184. https://doi.org/10.11646/zootaxa.4067.2.3

Cho H-W, Takizawa H, Borowiec L (2016) Notes on *Gonioctena tredecimmaculata* (Jacoby, 1888), with descriptions of two new species from Taiwan (Coleoptera: Chrysomelidae: Chrysomelinae). Annales Zoologici (Warszawa) 66: 357–369. https://doi.org/10.3161/00034541ANZ2016.66.3.002

Gressitt JL, Kimoto S (1963) The Chrysomelidae (Coleopt.) of China and Korea, Part 2. Pacific Insects Monograph 1B: 300–1026. http://hbs.bishopmuseum.org/pim/pdf/pim1B.pdf

Kimoto S (2003) The leaf beetles (Chrysomelidae) of Thailand and Indochina. Tokai University Press, Tokyo, 150 pp.

Kimoto S, Chu Y-I (1996) Systematic catalog of Chrysomelidae of Taiwan (Insecta: Coleoptera). Bulletin of the Institute of Comparative Studies of International Cultures and Societies 16: 1–152.

Kimoto S, Gressitt JL (1981) Chrysomelidae (Coleoptera) of Thailand, Cambodia, Laos and Vietnam, II. Clytrinae, Cryptocephalinae, Chlamisinae, Lamprosomatinae and Chrysomelinae. Pacific Insects 23: 286–391. http://hbs.bishopmuseum.org/pi/pdf/23(3)-286.pdf

Kimoto S, Takizawa H (1997) Leaf beetles (Chrysomelidae) of Taiwan. Tokai University Press, Tokyo, 581 pp.

Kippenberg H (2010) New acts and comments, Chrysomelidae: Chrysomelinae. In: Löbl I, Smetana A (Eds) Catalogue of Palaearctic Coleoptera, Volume 6 Chrysomeloidea. Apollo Books, Stenstrup, 67–73. [390–443.]

Sprecher-Uebersax E, Daccordi M (2016) Leaf-beetles of the subfamily Chrysomelinae of Laos (Coleoptera: Chrysomelidae). Entomologica Basiliensia et Collectionis Frey 35: 455–485.

Yang X, Ge S, Nie R, Ruan Y, Li W (2015) Chinese leaf beetles. Science Press, Beijing, 507 pp.

Yang X, Ge S, Wang S, Li W, Cui J (2014) Fauna Sinica, Insecta (Vol. 61). Coleoptera Chrysomelidae Chrysomelinae. Science Press, Beijing, 641 pp.

Yu P, Wang S, Yang X (1996) Economic Insect Fauna of China, Fasc. 54, Coleoptera: Chrysomeloidea (II). Science Press, Beijing, 324 pp.