Acta Arachnologica, 67(2): 87–90, December 31, 2018

A new species of *Cyclosa* (Araneae: Araneidae) from Southeast Asia

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Abstract — A new species of *Cyclosa* is described under the name of *Cyclosa bulla* n. sp. using specimens collected from Thailand, Singapore and Brunei. Females of the species can be easily distinguished from other congeners by the shape of the abdomen, which has a globose posterior end. In contrast, males cannot be distinguished from those of *Cyclosa bifida*, which seems to be the most closely related species, even by the shape of the palpal organ. In this study, male specimens are identified by DNA barcoding.

Key words — *Cyclosa bulla*, taxonomy, COI, barcoding, Thailand, Singapore, Brunei

Introduction

The genus *Cyclosa* Menge 1866 comprises 174 species that have been described worldwide, and 27 species of these have been known to Southeast Asian fauna (WSC ver. 19.5, 2018). Recently, several interesting specimens of the genus were obtained during a survey of spider fauna in the southern region of Thailand, Singapore and Brunei, which will be described as a new species in this paper. It is the 28th species of the genus in this area.

Materials and methods

The specimens were preserved in a 75% ethanol at room temperature. The morphological characteristics were examined under a stereoscopic microscope (M3Z, Wild Heerbrugg AG, Heerbrugg, Switzerland), and photographs were taken using an EOS Kiss X7 attached to the microscope. All measurements are given in mm. The type specimens designated in this paper are deposited in the collection of the Natural History Museum, National Science Museum, Thailand (THNHM), Thammasat University and National Museum of Nature and Science, Tokyo (NSMT).

We conducted DNA barcoding using mt-COI sequencing data to confirm the pair of male and female specimens. The specimens used in molecular analysis are shown in the appendix. Genomic DNA was extracted from the right third leg using a FavorPrep Tissue Genomic DNA Extraction Mini Kit (Favorgen Biotech Corp, Ping-Tung, Taiwan). Mt-COI was amplified using the primer set LCO1498: 5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′ and HCO2198: 5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′ (Folmer et al. 1994). The reactants were initially denatured for 2 min at 94°C, followed by 40 cycles of 15 s at 94°C, 20 s at 47°C, and 30 s at 72°C. PCR products were purified using ExoSAP-IT (GE Healthcare Bio-Sciences, Little Chalfont, England). Purified PCR products were sequenced using a BigDye terminator cycle sequencing kit (ver. 3.1, Applied Biosystems, Foster City, CA, USA) and analyzed on an ABI 3130xl automated DNA sequencer (Applied Biosystems). Chromatograms were checked using MEGA ver. 7 (Kumar et al. 2016). Obtained sequences were aligned using MUSCLE (Edgar 2004) in MEGA. The phylogenetic tree was constructed by the neighbor-joining (NJ) method using MEGA.

Results

Female specimens of the species could be distinguished from any other known species by their posteriorly globose abdomen. The shape of epigyne of these specimens are quite similar to that of *C. bifida*, but the shape of abdomen is more useful for the identification than the shape of epigyne in case of *Cyclosa* species (e.g. *C. octotuberculata, C. monticola* and *C. laticauda*; Tanikawa 1992). Therefore, we concluded that the species in question is new to science. Male specimens collected together with females had an abdomen of a different shape that closely resembles that of *Cyclosa bifida* (Doleschall 1859). Therefore, we confirmed the pair of male and female specimens by DNA barcoding as following.

We obtained 577 bp of mt-COI partial sequences from the specimens for molecular analysis. Haplotype names and

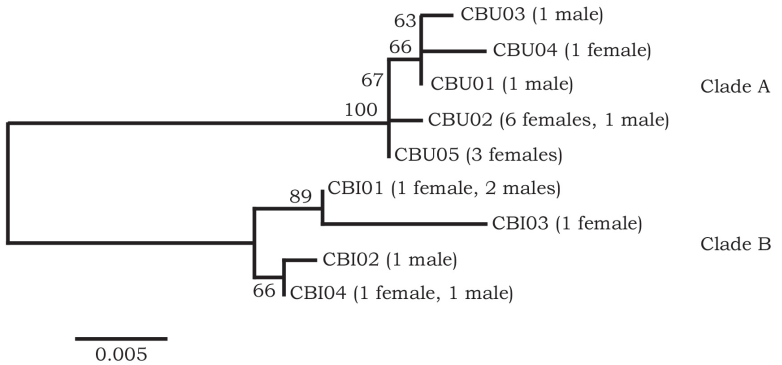

Fig. 1. An unrooted bootstrap consensus tree obtained by the neighbor-joining method for the detected haplotypes. Scores at each node are bootstrap values (1000 replicates). Specimens of clade A are identified as *Cyclosa bulla* n. sp. and those of clade B as *Cyclosa bifida*. The scale bar shows substitutions per site.

Fig. 2. *Cyclosa bulla* n. sp. A, female (holotype), dorsal view; B, same, lateral view; C, paratype (male), dorsal view; D, epigyne, ventral view (holotype); E, same, lateral view; F, male left palp, prolateral view (paratype); G, same, retrolateral view (paratype). Scales: A–C, 1 mm; D–G, 0.25 mm.

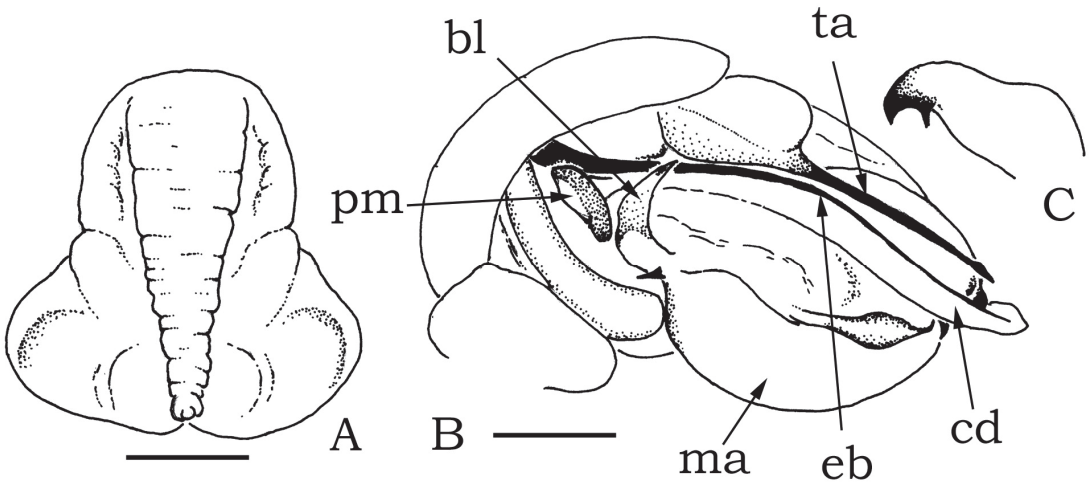

Fig. 3. Copulatory organs of *Cyclosa bulla* n. sp. A, epigyne, ventral view (holotype); B, male left palp, prolateral view (paratype), C, median apophysis of male palp (paratype). bl, basal lamella of median apophysis; cd, conducter; eb, embolus; ma, median apophysis; pm, paramedian apophysis; ta, terminal apophysis; Scales: 0.25 mm.

accession numbers are shown in the appendix. The inferred phylogenetic tree consists of two major clades (Fig. 1, clades A and B). Clade B includes all the females of *Cyclosa bifida* and clade A includes all of females of the new species. The four male specimens included in clade B (haplotypes: CBI01, 02, 04) were identified as *Cyclosa bifida*, and the three males included in clade A (haplotypes CBU01–03) were from the new species. Even after identification by DNA barcoding, we were unable to recognize the morphological difference between males of *Cyclosa bifida* and the new species.

Description of the new species
Cyclosa bulla new species
[Thai name: Mangmoum-Taai-Klom-Cyclosa]
(Figs. 2–3)

Type series. Holotype: ♀, Hala-Bala Wildlife Sanctuary, Narathiwat Province, Thailand (5.80179N 101.82836E), 5-III-2018 (THNHM-I-01245, COI haplotype: CBU05). Paratypes: 6♀2♂, same data as the holotype (3♀1♂: THNHM-I-01246 – 01249, COI haplotypes: CBU02, 03, 04, 05. 3♀1♂: NSMT, COI haplotypes: CBU02, 05). 1♀, Hala-Bala Wildlife Sanctuary (5.807004N, 101.845026E), 6-III-2018 (Thammasat University, COI haplotype: CBU02). All the type specimens were collected by A. Tanikawa, B. Petcharad, V. Saksongmuang and Y. Suzuki.

Other specimens examined. THAILAND: 2♀, the same locality as the holotype, 19-II-2016, A. Tanikawa, B. Petcharad and S. Koshi-ishi leg. SINGAPORE: 1♂, Central Catchment Nature Reserve, Rifle Range Link (1.354605N 103.796414E), 30-XI-2017, J. K. H. Koh leg. BRUNEI: Belait Melilas, Sungai Ingei Mammal Trap Trail (4.150000N 114.717500E), 14-IV-2012, J. K. H. Koh leg.

Etymology. The specific name is derived from the knob-like shaped posterior part of the abdomen; "bulla" is a Latin word that means "globe".

Diagnosis. The new species seems to be closely related to *Cyclosa bifida*, but females can be easily distinguished from *Cyclosa bifida* by the posteriorly globose abdomen (Figs. 2A–B). Males of these species cannot be separated morphologically, even by the shape of the palpal organ. Male specimens of the new species can be identified only by DNA sequencing data, e.g. mt-COI.

Description. Based on the holotype female and 1 male paratype.

Coloration and markings. Female (Figs. 2A–B) and male (paratype, Fig. 2C): carapace black. Dorsum of abdomen black with silver marking.

Measurements. Female / male. Body 5.06 / 3.32 long. Carapace 1.65 / 1.45 long; 1.05 / 0.93 wide. Length of legs [tarsus + metatarsus + tibia + patella + femur = total]: I, 0.48 + 0.88 + 0.95 + 0.55 + 1.38 = 4.24 / 0.44 + 0.73 + 0.80 + 0.43 + 1.08 = 3.48; II, 0.45 + 0.81 + 0.88 + 0.53 + 1.28 = 3.95 / 0.40 + 0.63 + 0.68 + 0.40 + 0.99 = 3.10; III, 0.38 + 0.55 + 0.55 + 0.35 + 0.90 = 2.73 / 0.33 + 0.44 + 0.46 + 0.26 + 0.71 = 2.20; IV, 0.45 + 0.98 + 1.10 + 0.50 + 1.49 = 4.52 / 0.40 + 0.73 + 0.73 + 0.35 + 1.08 = 3.29. Abdomen 3.08 / 1.73) long; 1.36 / 1.03 wide.

Body and legs. Female / male. Carapace longer than wide [length divided by width 1.57 / 1.56]. Median ocular area wider than long [length divided by width 1.57 / 1.57]; wider in front than behind [anterior width divided by posterior width 1.32 / 1.64]. Labium wider than long [length divided by width 0.65 / 0.86]. Sternum longer than wide [length divided by width 1.30 / 1.32]. Length of leg I divided by

length of carapace 2.57 /2.40. Abdomen longer than wide [length divided by width 2.26 / 1.68]; in female, posteriorly globose; in male, oval.

Genital organs. Epigyne as in Figs. 2D–E, 3A, with wrinkled slim scape. Male palp: as in Figs. 2F–G, 3B–C: basal lamella of median apophysis projecting, paramedian apophysis visible in prolateral view, embolus filiform, median apophysis apically bent and bifurcated.

Variations. Female body length 3.78–5.44; male body length 2.40–3.32.

Distribution. Thailand, Singapore and Brunei.

Acknowledgments

We wish to express our heartfelt thanks to the following people for their kind help with our study. Joseph K. H. Koh, Singapore; Venus Saksongmuang, Prince of Songkla University; Yuya Suzuki, University of Tsukuba; Sunate Karapan and Kumaree Nachalame, Hala-Bala Wildlife Sanctuary; Tadashi Miyashita, the University of Tokyo.

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Received September 8, 2018 / Accepted September 26, 2018

Appendix. Locality, haplotype and accession numbers of specimens used for the molecular analysis. The unknown males with haplotype names beginning with CBU were identified as *Cyclosa bulla* n. sp. and those begin with CBI were identified as *Cyclosa bifida*.

species	sex	locality	haplotype	accession no.
unknown	male	Central Catchment Nature Reserve, Rifle Range Link, Singapore	CBU01	LC415140
unknown	male	Hala-Bala Wildlife Sanctuary, Narathiwat Province, Thailand	CBU02	LC415141
unknown	male	Hala-Bala Wildlife Sanctuary, Narathiwat Province, Thailand	CBU03	LC415142
unknown	2 males	Nam Tok Yong National Park, Nakhon Si Thammarat Province, Thailand	CBI01	LC415136
unknown	male	Khao Pu-Khao Ya National Park, Phatthalung Province, Thailand	CBI02	LC415137
unknown	male	Hala-Bala Wildlife Sanctuary, Narathiwat Province, Thailand	CBI04	LC415139
C. bulla	6 females	Hala-Bala Wildlife Sanctuary, Narathiwat Province, Thailand	CBU02	LC415141
C. bulla	female	Hala-Bala Wildlife Sanctuary, Narathiwat Province, Thailand	CBU04	LC415143
C. bulla	3 females	Hala-Bala Wildlife Sanctuary, Narathiwat Province, Thailand	CBU05	LC415144
C. bifida	female	Hala-Bala Wildlife Sanctuary, Narathiwat Province, Thailand	CBI01	LC415136
C. bifida	female	Nam Tok Yong National Park, Nakhon Si Thammarat Province, Thailand	CBI03	LC415138
C. bifida	female	Nam Tok Yong National Park, Nakhon Si Thammarat Province, Thailand	CBI04	LC415139
C. bifida	female	Hala-Bala Wildlife Sanctuary, Narathiwat Province, Thailand	CBI04	LC415139