Integrative taxonomy of araneomorph spiders: Breathing new life into an old science
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First records and three
new species of the family
Symphytognathidae (Arachnida:
Araneae) from Thailand, and
the circumscription of the genus
Crassignatha Wunderlich, 1995

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Abstract

The family Symphytognathidae is reported from Thailand for the first time. Three new species: *Anapistula choojaiae* sp. nov., *Crassignatha seeliam* sp. nov., and *Crassignatha seedam* sp. nov. are described and illustrated. Distribution is expanded and additional morphological data are reported for *Patu shiluensis* Lin & Li, 2009. Specimens were collected in Thailand between July and August 2018. The newly described species were found in the north mountainous region of Chiang Mai, and *Patu shiluensis* was collected in the coastal region of Phuket. DNA sequences are provided for all the species here studied. The relations of these symphytognathid species were tested using previously published phylogenetic analyses on micro orb-weavers. Also, we used micro CT analysis to build 3D models of the male genitalia and somatic characters of two species of *Crassignatha* Wunderlich, 1995. The molecular phylogeny and 3D models were used to discuss the taxonomy and circumscription of the currently valid symphytognathid genera, with focus on *Crassignatha* and *Patu* Marples 1951. Based on this, three new combinations are suggested: *Crassignatha bicorniventris* (Lin & Li, 2009) comb. nov., *Crassignatha quadriventris* (Lin & Li, 2009) comb. nov., and *Crassignatha spinathoraxi* (Lin & Li, 2009) comb. nov. A new record of *Crassignatha danaugirangensis* Miller et al, 2014 is reported from Brunei.

Keywords: 3D reconstruction, *Anapistula*, Borneo, Computed tomography, micro-CT, *Patu*, Sabah, Symphytognathoids

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Introduction

The family Symphytognathidae includes some of the tiniest spiders known. According to a recent "Spider World Record" study [1], this family holds the records for the smallest female, smallest male and smallest web. The Symphytognathidae has traditionally been put together with other small size araneoids (Anapidae, Mysmenidae and Theridiosomatidae, sometimes along with synaphrids and micropholcommatids) in a group informally called the symphytognathoids [2,3]. Although phylogenetic relationships among the Symphytognathidae have not been directly studied, some representatives have been used as part of other phylogenetic studies targeting the family Mysmenidae [4,5], as well as a broad scope analysis of the whole order Araneae [6,7]. Symphytognathids can be separated from other relatives by the following combination of characters: the loss of the posterior median eyes, reducing eye number to six (with the further loss of the anterior median eyes in the case of the four-eyed genus *Anapistula*), fusion of the chelicerae (but see below), extreme reduction or loss of female pedipalp, the labium being much wider than long, loss of the colulus, sternum broadly truncated posteriorly, the absence of book lungs, and the presence of one or two promarginal cheliceral teeth originating from a common base [3,4,8–10].

The family is widespread in the tropics and subtropical regions, with most species described from the southern hemisphere. At present 8 genera and 74 species are recorded worldwide. In Asia, six genera and 29 species have been recorded [11]. From these, 19 species have been recorded from China [10,12–15] and six from South East Asia (Indonesia, Malaysia and Vietnam) [16–19]. Here, the family Symphytognathidae is formally reported from Thailand for the first time, although Lopardo et al. [4] did include a Thai symphytognathid in their study, designated SYMP-004-THAI , which was later identified as *Crassignatha* (Lopardo, *pers. comm.*). We describe three new species of the genera *Anapistula* and *Crassignatha* and expand the known distribution of *Patu shiluensis.* We used a combination of newly generated sequences and sequences available in GeneBank to build a molecular phylogeny of the Symphytognathidae —and related micro orb-weaver families— in order to test the familial placement of our new species. Additionally, we discuss the taxonomy of the Symphytognathidae with emphasis on the genera *Crassignatha* and *Patu*.

Material and Methods

Fieldwork — The symphytognathid specimens reported here were collected in Chiang Mai and Phuket, Thailand, between July 16th and August 6th 2018. All the specimens were captured using methods optimized for ground dwelling spiders: leaf litter sifting, Winkler extractors, pitfall traps and direct collecting on ground, and among sifted leaf litter.

Molecular data — To test the relationships and position of the novel species within the Symphytognathidae, we selected one specimen from each species we collected and used all four right legs to extracted genomic DNA and sequence six gene fragments: COI, H3, 12S, 16S, 18S and 28S (primers in SM1) following [6,20] protocols. Sequences were edited in Geneious Prime 2020.0.5 and deposited in GenBank; accession numbers are reported in Table 1. We used these sequences and a selection of taxa previously used to test the phylogeny of mysmenid spiders [4,5]. A total of 47 species of "symphytognathoids" from the families Anapidae, Mysmenidae, Symphytognathidae and Theridiosomatidae were used. Two more species of Tetragnathidae were used as an outgroup to the symphytognathoids. We used MAFFT v.7.450 online (https://mafft.cbrc.jp/alignment/server/) with default parameters to align the sequences. Matrix was built using in Sequence Matrix v.1.8 (http://www.ggvaidya.com/taxondna/); matrix available in SM1. Each locus was treated as a partition and examined with jModelTest2 [21] in CIPRES [22] to get the best model fit for each; GTR+I+G was selected in all cases. Our datasets were analyzed using MEGA X [23] for Maximum Parsimony (SPR, default values, bootstrap= 1000); RaXML [24] in CIPRES for Maximum Likelihood (GTR, bootstrap= 1000) and MrBayes v. 3.2.6 [25] in CIPRES for the Bayesian Inference (GTR+I+G, two independent runs with one cold and three heated chains, mcmc=50,000,000 gen, samplefreq=1000, burnin=2500; partitions are indicated in the NEXUS file). The program Tracer v. 1.7.1 [26] was used to analyze the performance of our BI analyses.

Table 1. GenBank accession numbers of DNA sequences generated for the present work.

Species	COI	H3	16s	12s	18s	28s
Anapistula choojaiae	MT712393	MT782018		MT711286	MT711238	MT711242
Crassignatha seedam	MT712396	MT782021			MT711241	
Crassignatha seeliam	MT712394	MT782019			MT711239	
Patu shiluensis	MT712395	MT782020	MT711285		MT711240	

Morphological data — Specimens were photographed with a Nikon DS-Ri2 camera attached to a Leica DM 2500 microscope. Specimens were observed in ethanol using semi-permanent slide preparations [27]. Female genitalia were dissected, digested using pancreatine solution [28], and cleared with methyl salicylate. For the 3D scans, whole male spiders were stained in 1% Iodine -70% et- OH for 24 hours. Specimens were fixed in a modified 10ul pipette tip and scanned using a Zeiss X-radia 520 versa. 3D model and subsequent segmentation of the internal ducts of male pedipalps were done in Avizo 9.5.0. All the specimens have been deposited in the collection of the Naturalis Biodiversity Center, Leiden, the Netherlands. Additionally, two males of *Crassignatha danaugirangensis* Miller et al., 2014, recently collected in Brunei, were analyzed using micro-CT scanning. 3D reconstructions were used to clarify some anatomical details of this species and the genus *Crassignatha*, including the internal and external structure of the male pedipalp, cheliceral armature, and carapace texture.

Nomenclature of the genital structures was based on Harvey [17] and Lin, Tao, and Li [14] for *Anapistula*, and Lin and Li [13] and Miller, Griswold, and Yin [10] for *Crassignatha* and *Patu.* Abbreviations in text and figures: A – Epigynal atrium; AME – Anterior median eyes; BI – Bayesian Inference; C – Conductor; C1 – Conductor, anterior projection; C2 – conductor, posterior projection; Cd – Copulatory duct; Ch – Chelicera; ChT– cheliceral tooth; Co – Copulatory opening; Ct – cymbial tooth; Cy – Cymbium; E – Embolus; Em– Embolic membrane; EMD – Epigynal median duct; F – Femur; Fd – Fertilization duct; Lb – lateral branch of the EMD; LE – lateral eyes; Mcl– male leg II mating clasper; ML – Maximum Likelihood; MP – Maximum Parsimony; Pa – Patella; Pc – Paracymbium; PME – Posterior median eyes; S – Spermatheca; Sa – Secretory ampulla; Sc – Epigynal scape; Sd – Spermatic duct; T – Tibia.

Results

Phylogenetic Analysis

Tree topologies inferred by the different phylogenetic analyses performed (Figs. 5.1–5.3) show some consistencies in several groupings; however, low support values are common, especially in the MP and ML trees. There is an inconsistent and problematic placement of the Symphytognathidae in relation to the Anapidae. All tree analyses recovered Mysmenidae as monophyletic and a sister group of Anapidae + Symphytognathidae. Theridiosomatidae is recovered as monophyletic in the MP and ML analyses with medium to high support (Figs. 5.1–5.2); nevertheless, in the BI the position of this family is not resolved (Fig. 5.3). Similarly, the position of Micropholcommatinae, currently considered part of the Anapidae, is not clear, being found as paraphyletic in the MP, unresolved in the BI, and a poorly supported monophyletic clade in the ML analysis (Fig. 5.1–5.3). The Anapidae is closely related to the Symphytognathidae in all our trees (with the notable exception of the two micropholcommatines in the ML and BI); however, it appears as a poorly supported monophyletic group in the ML (Fig. 5.2), and paraphyletic in the MP and BI (Fig. 5.1, 5.3). The Symphytognathidae appear monophyletic with moderate to high support in all the analyses (Figs. 5.1–5.2). In the BI analysis, this family is monophyletic and highly supported but found in an unresolved branch that includes the paraphyletic Anapidae (Fig. 5.3). The internal relations of the Symphytognathidae are similar in all our trees forming one clade that includes *Symphytognatha picta*, one species (SYMP_008_DR) identified as S*ymphytognatha*, one as *Patu* (*Patu*_SYMP_001_DR), and one more (SYMP_005_AUST) that remained unidentified. The other clade recovers the rest of the *Patu* species + *Crassignatha.* Here, two terminals (SYMP_002_MAD and SYMP_003_MAD) are closer to *Patu shiluensis* —and related to the three *Crassignatha* representatives—; and two other (SYMP_006_ AUS and SYMP_007_AUS) are consistently found outside of the *Crassignatha + Patu* clade*.* SYMP-004-THAI consistently clusters with *Crassignatha seeliam* sp. nov., and unpublished morphological observations (Lopardo, pers. comm.) are consistent with the possibility that these are conspecific.
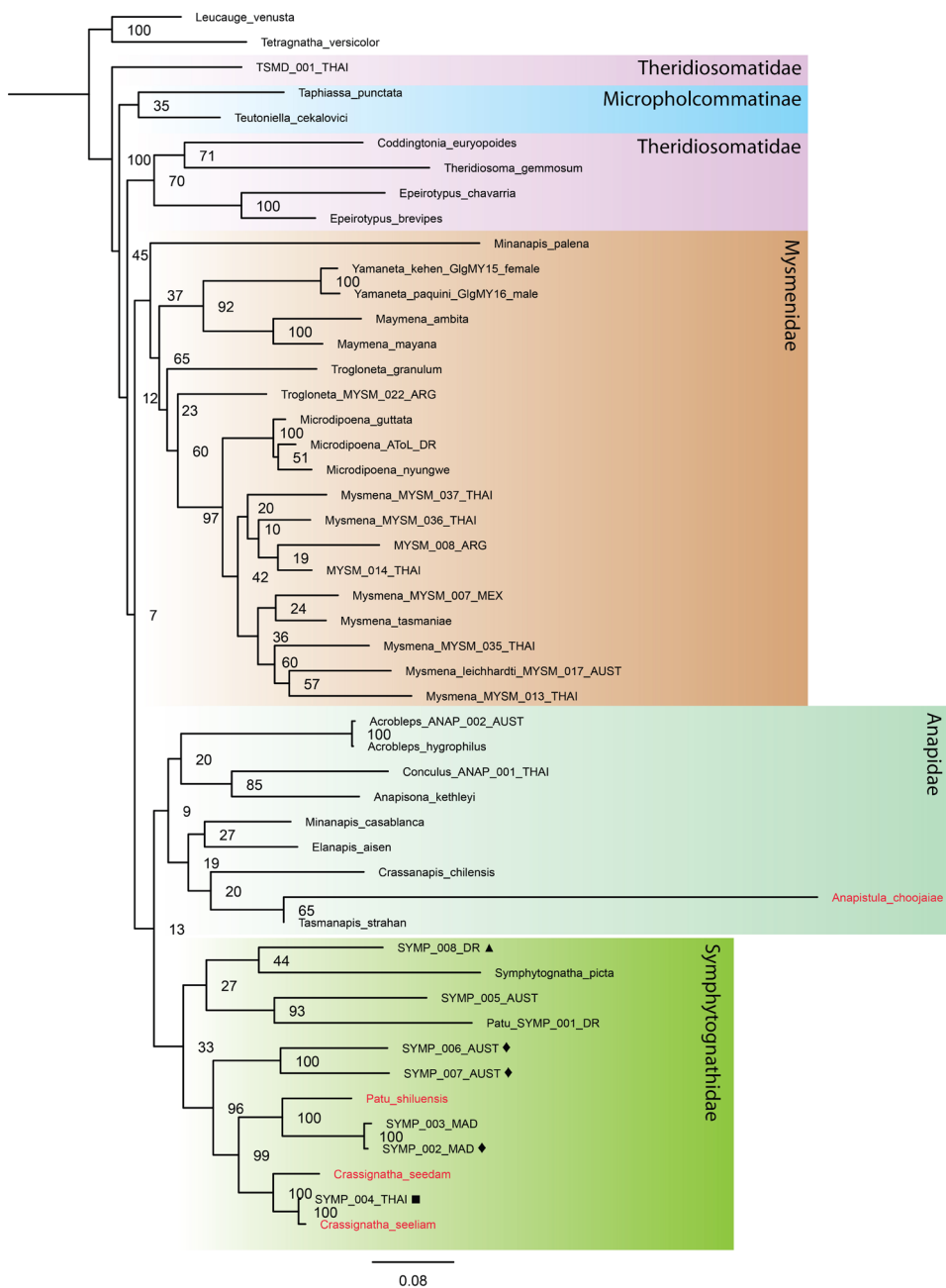
Figure 5.1.– Maximum Parsimony Tree. Obtained in MEGA-X using a modified version of Lopardo et al. [4] and Feng et al. [5] plus the four symphytognathid species from our study (in red). Numbers at nodes indicate bootstrap support. Note the paraphyly of Anapidae and the high support of *Crassignatha* and *Patu* in the Symphytognathidae. Molecular vouchers used for previous "symphytognathoid" studies [4,31] identified to genus level by L. Lopardo (pers. comm.) as follows: ■ *Crassignatha* (apparently conspecific with *C. seeliam*); ◆*Patu*; and ▲*Symphytognatha*.

Figure 5.2.– Maximum Likelihood Tree. Obtained in RAxML using a modified version of Lopardo et al. [4] and Feng et al. [5] plus the four symphytognathid species from our study (in red). Numbers at nodes indicate bootstrap support. Note the long branch of *Anapistula* and its position within Anapidae; and the high support of *Crassignatha* and *Patu* in the Symphytognathidae. Molecular vouchers used for previous "symphytognathoid" studies [4,31] identified to genus level by L. Lopardo (pers. comm.) as follows: ■ *Crassignatha* (apparently conspecific with *C. seeliam*); ◆*Patu*; and ▲*Symphytognatha*.
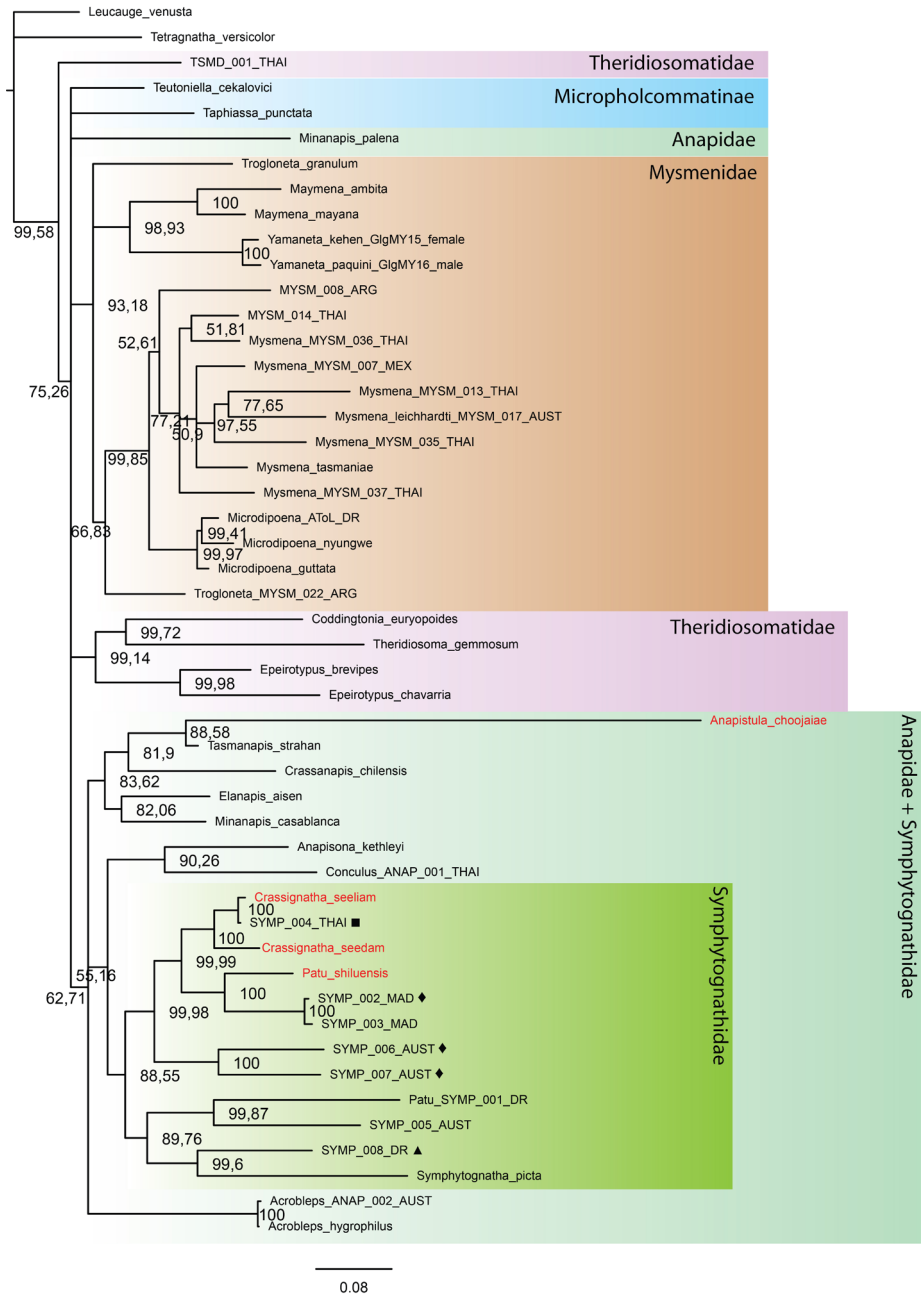
Figure 5.3.– Bayesian Inference Tree. Obtained in Mr. Bayes using a modified version of Lopardo et al. [4] and Feng et al. [5] plus the four symphytognathid species from our study (in red). Numbers at nodes indicate percent posterior probabilities. Note the unresolved relations of the Anapidae and the highly supported monophyly of Symphytognathidae. Molecular vouchers used for previous "symphytognathoid" studies [4,31] identified to genus level by L. Lopardo (pers. comm.) as follows: ■ *Crassignatha* (apparently conspecific with *C. seeliam*); ◆*Patu*; and ▲*Symphytognatha*.
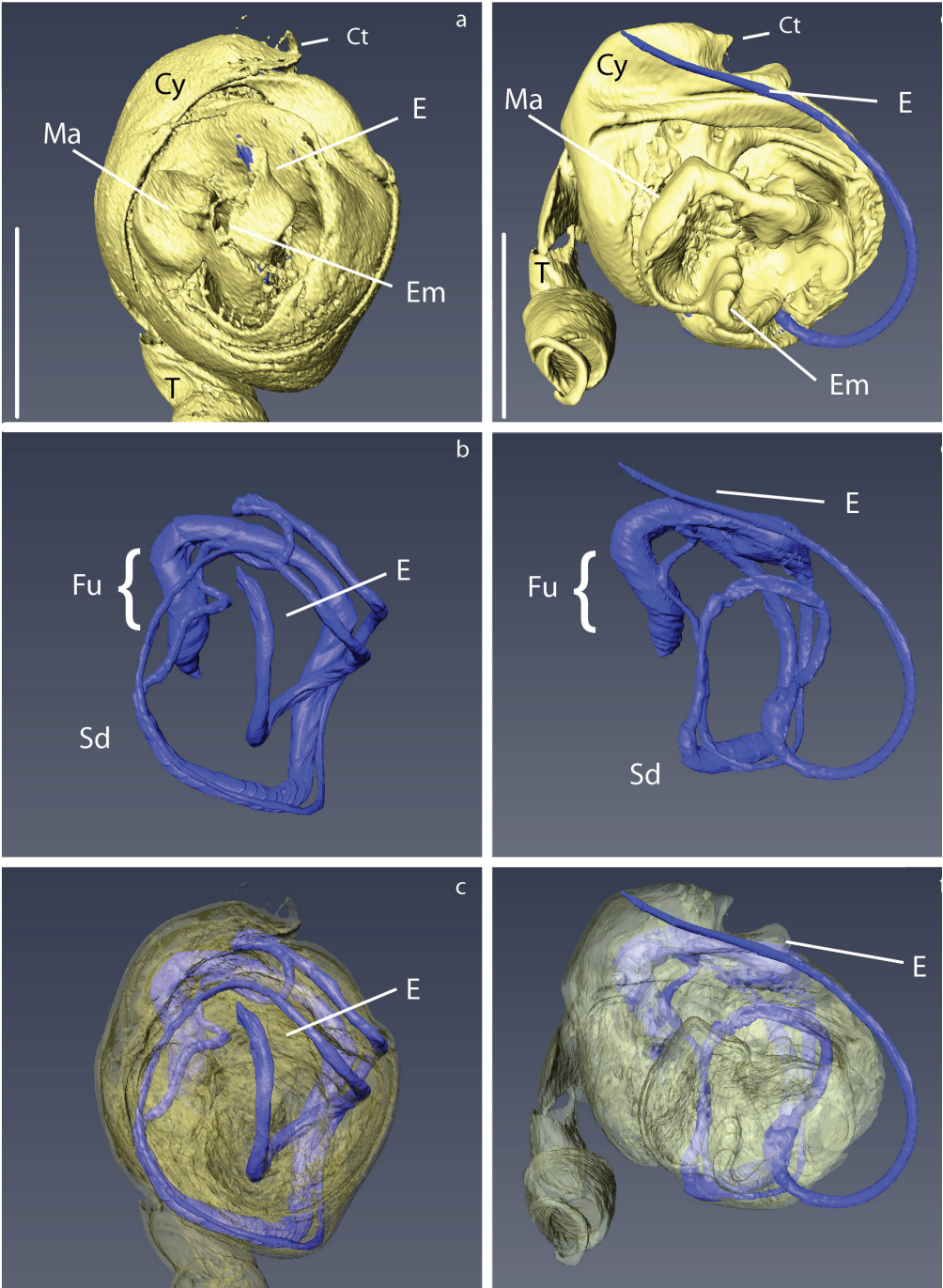
Figure 5.4.- 3D reconstruction of the male palp of *Crassignatha*. With detail in the spermatic ducts: a–c *C. seeliam* sp. n; d–f *C. danaugirangensis*. Scale bars: 0.1 mm. Ct – Cymbial tooth; Cy – Cymbium; E – Embolus; Em– Embolic membrane; Fu – Fundus; MA – Median apophysis; Pa – Patella; Sd – Spermatic duct; T – Tibia.
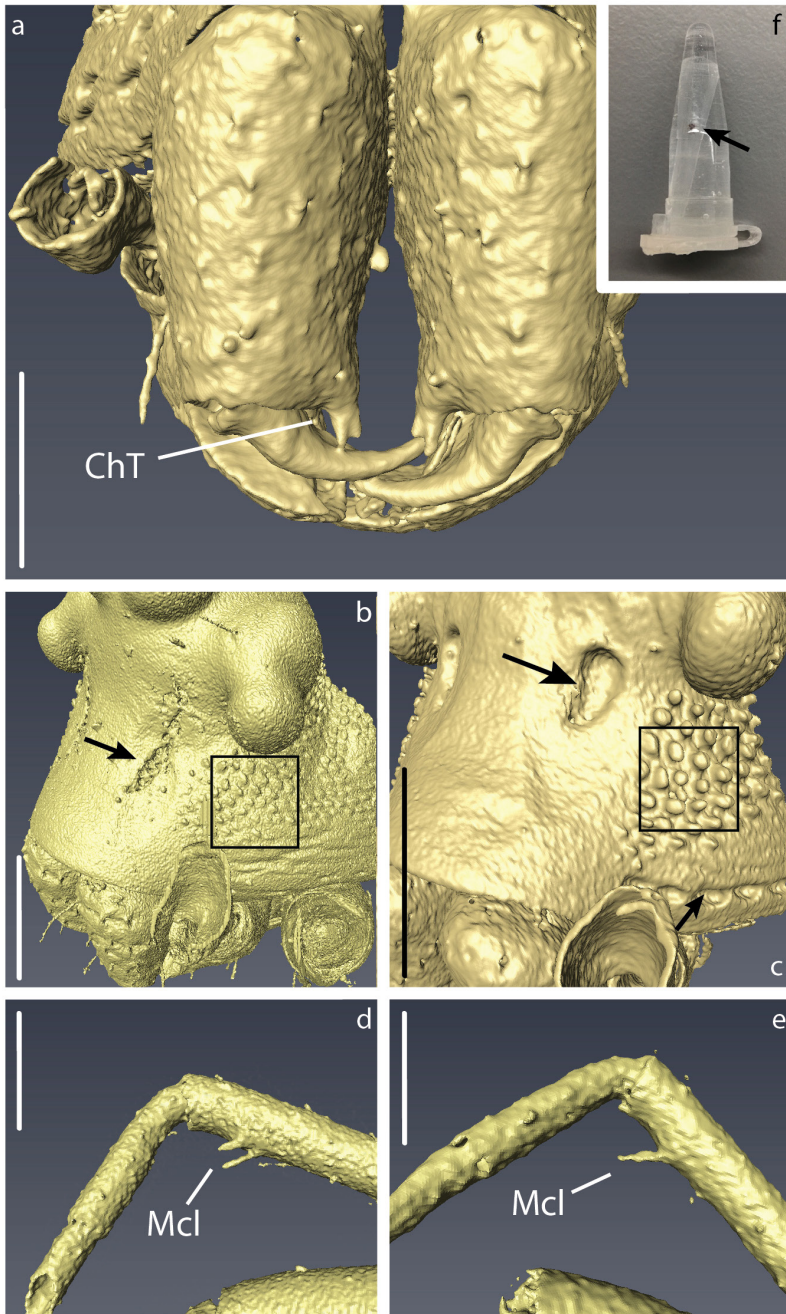
Figure 5.5.- 3D reconstruction of some diagnostic characters of *Crassignatha*. a, c, e *C. danaugirangensis*. b, d *C. seeliam* sp. n.; a chelicerae, arrow pointing at the bifurcated tooth; b, c detail of the carapace; cephalothorax tubercles (in the squares), and pore bearing sulcus (arrows); d, e Male leg II clasper; f whole male specimen of *C. danaugirangensis* prepared for micro-CT inside a modified 10ul pipette tip and a 0.5ml Eppendorf tube filled with 70% Et-OH. Scale bars: 0.06 mm (a); 0.1 mm (b–e). ChT– cheliceral tooth; Mcl– male leg II mating clasper.
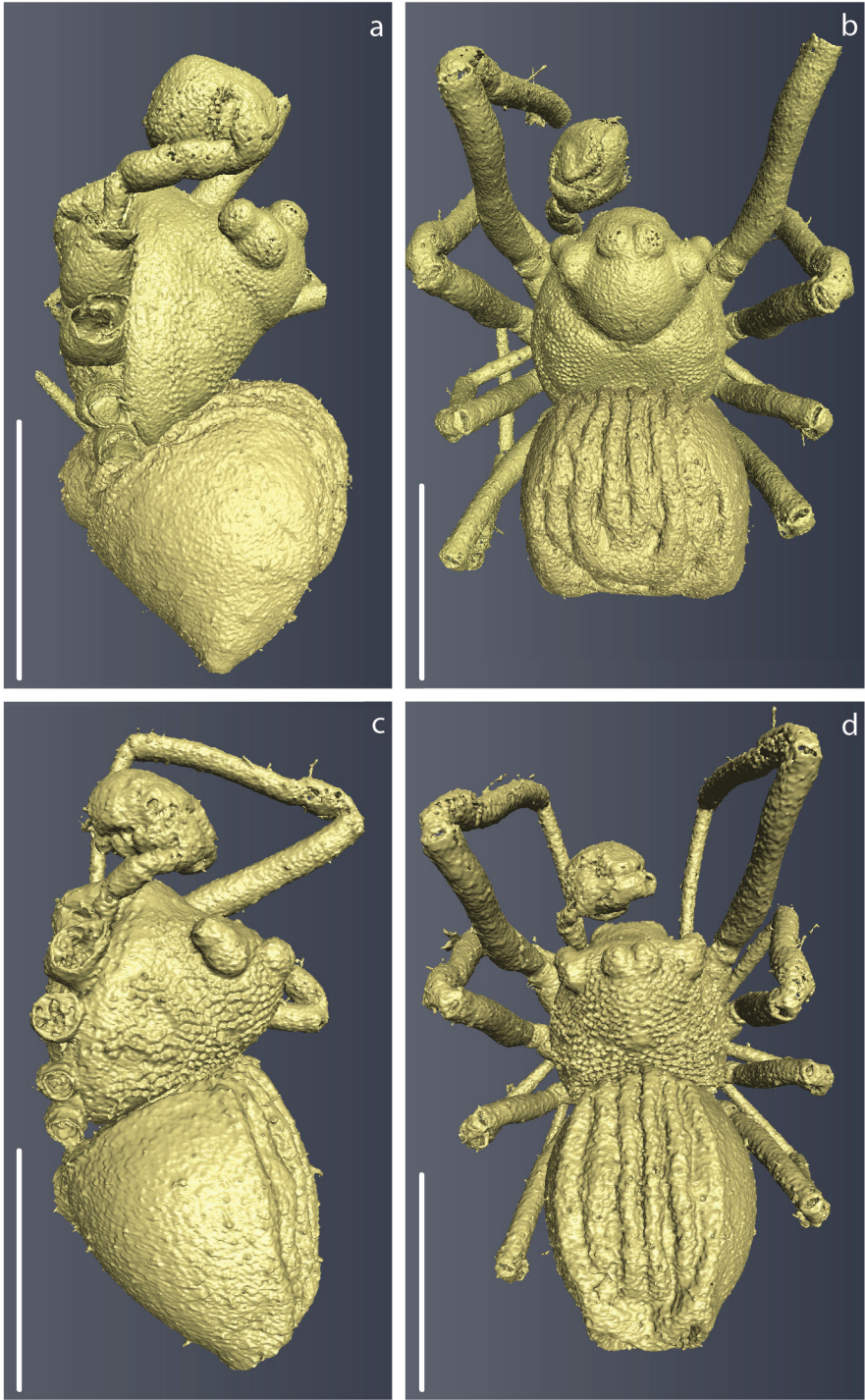
Figure 5.**6**.- **3D reconstruction of the habitus of *Crassignatha.*** males: **a, b** *C. seeliam* sp. n; **c, d** *C. danaugirangensis.* Right pedipalp was dissected previous to the scanning. Scale bars: 0.3 mm.

Micro-CT and 3D modelling

The micro computed tomography scans allowed us to observe in detail small structures of the surface and internal ducts of the male genitalia (Figs. 5.4a–f). Structures like the cheliceral teeth (Fig. 5.5a), cephalothorax tubercles (Figs. 5.5b, c), and mating clasper on male tibia II (Figs. 5.5d, e) were also observed. We reconstructed 3D models of the whole body surface of *Crassignatha seeliam* (Figs. 5.6a–b) and *Crassignata danaugirangensis* (Figs. 5.6c–d). All of these images were important to examine, interpret and clarify the diagnostic characters of the genus *Crassignatha*. Additional views of the pedipalps, spermatic ducts and habitus can be found in the Supplementary Material (SM2, SM3)

Taxonomy

Family Symphytognathidae Hickman, 1931
Genus *Anapistula* Gertsch, 1941
   Anapistula Gertsch, 1941: 2. Type species *Anapistula secreta* Gertsch, 1941.
***Anapistula choojaiae* sp. nov.**
   Figs. 5.7–5.9

Holotype: THAILAND • ♂; Chiang Mai*,* Pha Daeng National Park. Riparian tropical forest; 19º37.768'N, 98º57.257'E. 560m; July 16-19, 2018; Booppa Petcharad, Jeremy Miller, F. Andres Rivera-Quiroz leg.; Winkler extractor; RMNH.ARA.18442. **Paratypes:** THAILAND • ♀ allotype; same data as holotype • 1♂ 1♀; same data as holotype; RMNH.5106639 • 2♀; Pha Daeng National Park. Bamboo forest; 19º37.668'N, 98º57.131'E. 573m, same dates and collectors as holotype; RMNH.ARA.18443.

Etymology: The species epithet is a Latinized matronym of the second authors' daughter.

Diagnosis: Female genitalia in *Anapistula* show little morphological variation between congeneric species making it generally difficult to tell species apart. However, *A. choojaiae* sp. nov. can be distinguished from most *Anapistula* species by the presence of an epigynal atrium; *A. aquytabuera* Rheims & Brescovit, 2003, *A. pocaruguara* and *A. ybyquyra* Rheims & Brescovit, 2003 —from Brazil—, *A. panensis* Lin, Tao, and Li 2013 and *A. zhengi* Lin, Tao, and Li 2013 —from China—, and *A. seychellensis* Saaristo, 1996 —from the Seychelles— also share this character. *A. choojaiae* differs from all of these by the relative size and shape of the atrium, the width of the EMD and the bifurcation of the Lb (compare Fig. 5.8d and 5.9c to figs. 16, 18, 21: [29]; figs. 3, 4, 8, 9:[14]; fig. 3: [30]).
   Male pedipalp of *A. choojaiae* similar to *A. panensis* in the overall shape of the palp and in having C1 and C2 roughly the same length, but differs on the width of C1 in respect to C2 and the length of the E in relation to C1 (compare Figs. 5.7c, 5.9a to fig. 1-2:[14]).
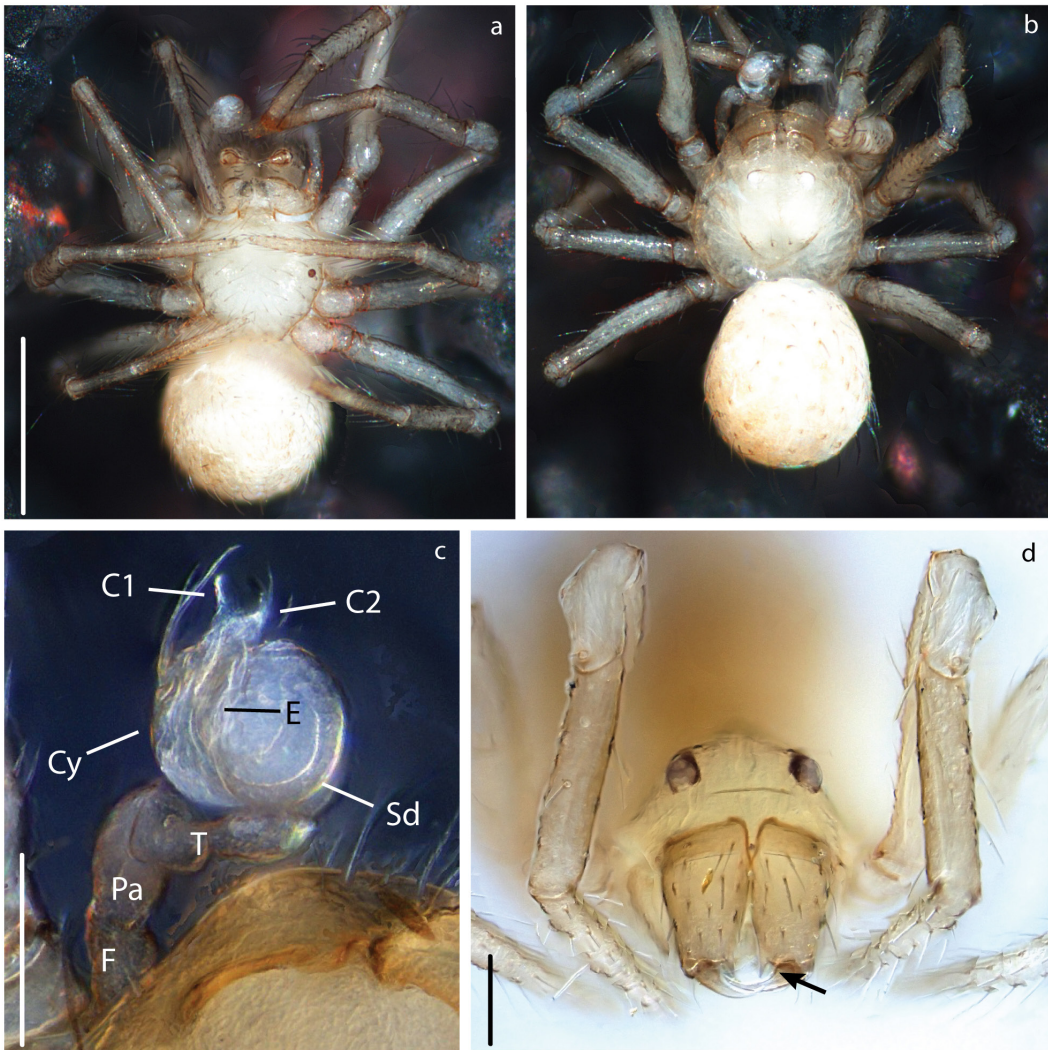
Figure 5.**7.-** *Anapistula choojaiae* sp. n. male: Habitus: **a** ventral view; b dorsal view. Palp: **c** ventral view. Female: Prosoma: **d** anterior view. Scale bars: 0.2 mm (**a, b**); 0.07 mm (**c**); 0.06 mm (**d**). Arrow pointing to the cheliceral teeth. C1 – Conductor, anterior projection; C2 – Conductor, posterior projection; Cy – Cymbium; E – Embolus; F – Femur; Pa – Patella; Sd – Spermatic duct; T – Tibia.

Description: Carapace ovoid, yellowish-white with smooth texture (Figs. 5.7a, b; **5.**8a, b). AME absent (Fig. 5.7d). Male LE without pigmentation (Figs. 5.7b; **5.**8b). Chelicerae with two promarginal teeth (Fig. 5.7d). Legs same color as carapace with slightly darker color on distal segments. Abdomen sub-spherical with small sparse sclerotized patches, some bearing long setae (Figs. 5.7b; 5.8b). Scuta absent in both sexes.

Male palp: Weakly sclerotized (Fig. 5.7c). Semicircular from ventral view (Figs. 5.7c; **5.**9a). With one wide sheet shaped conductor that presents two projections, here called C1 and C2 (Fig. 5.9a; b). Embolus short and transparent located posteriorly to C; very difficult to see (Figs. 5.7c; 5.9a).

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Figure 5.8.- *Anapistula choojaiae* sp. n. female: Habitus: **a** ventral view; **b** dorsal view. Epigynum: **c** ventral view; **d** dorsal view, cleared. Scale bars: 0.2 mm (**a, b**); 0.06 mm (**c**); 0.03 mm (**d**). A – Atrium; Cd – Copulatory duct; Co – Copulatory opening; MD – Epigynal median duct; Fd – Fertilization duct; Lb – lateral branch of the EMD; S – Spermatheca.

Vulva: Epigynal plate flat, without scape. Atrium semi-circular as wide as inner distance between S (Fig. 5.8c). Spermathecae spherical, heavily sclerotized in relation to the rest of the body (Fig. 5.8d). Cd easy to distinguish inside the EMD. LB diverging from the EMD forming a "Y" (Figs. 5.8d; 5.9c). Fertilization ducts very short and difficult to see, they appear as small bumps on the distal portion of Lb (Fig. 5.9c).

Male: Total length 0.4; carapace 0.2 long, 0.21 wide; clypeus 0.03; Chelicera 0.1 long, 0.06 wide; Leg I: femur 0.26, patella 0.1, tibia 0.17, metatarsus 0.09 tarsus 0.17; leg formula IV-I-II-III; abdomen 0.21 long, 0.21 wide.

Female: Total length 0.43, carapace 0.2 long, 0.21 wide; clypeus 0.3; Chelicera 0.1 long, 0.05 wide; Leg I: femur 0.20, patella 0.09, tibia 0.14, metatarsus 0.16, tarsus 0.1; leg formula IV-I-II-III; abdomen 0.24 long, 0.23 wide.
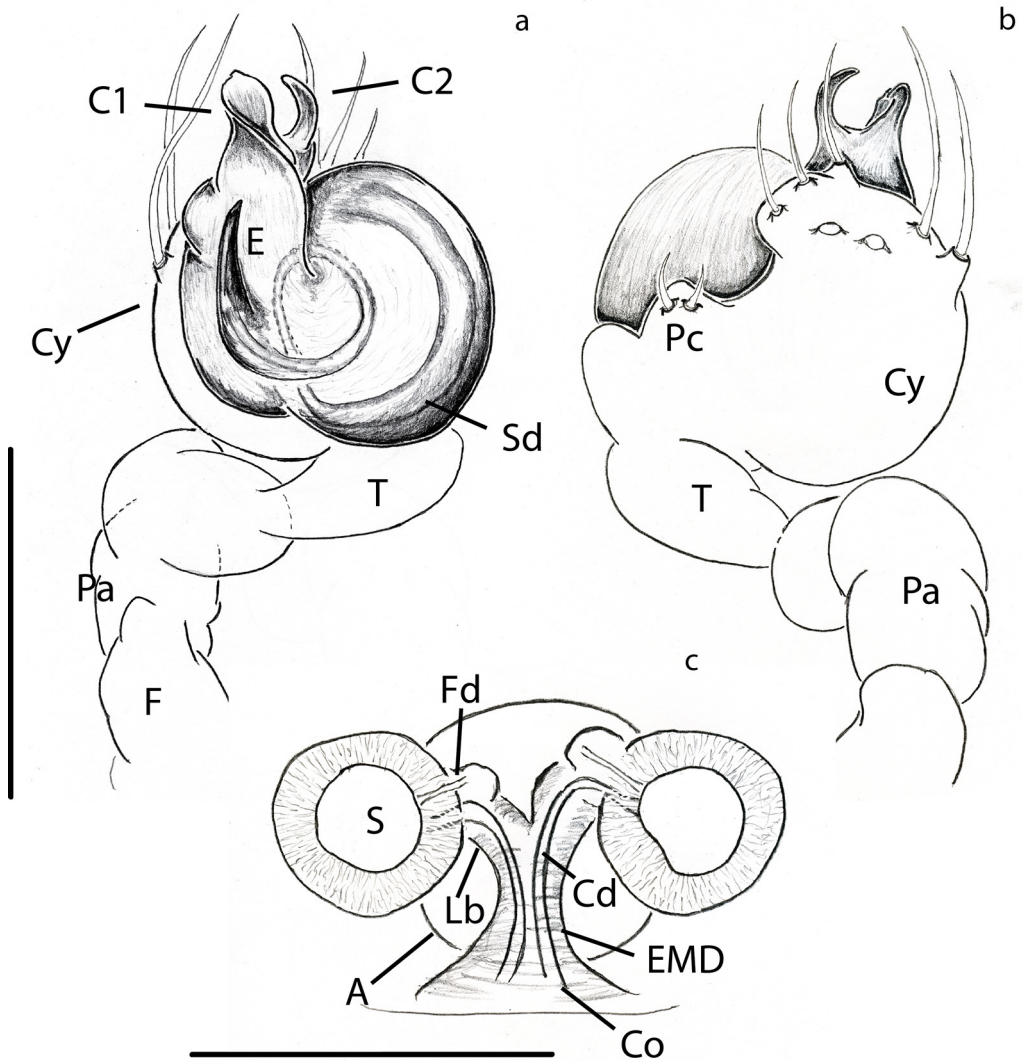
Figure 5.9.- *Anapistula choojaiae* **sp. n., genitalia**. Palp: a ventral view; b dorsal view. Epigynum, cleared: c dorsal view. Scale bars: 0.07 mm (a, b); 0.06 mm (c). A – Atrium; C1 – Conductor, anterior projection; C2 – Conductor, posterior projection; Cd – Copulatory duct; Co – Copulatory opening; Cy – Cymbium; E – Embolus; F – Femur; Fd – Fertilization duct; Lb – lateral branch of the EMD; MA – Median apophysis; MD – Epigynal median duct; Pa – Patella; Pc – Paracymbium; S – Spermatheca; Sa – Sececretory ampullae; Sc – Scape; Sd – Spermatic duct; T – Tibia.

Genus *Crassignatha* Wunderlich, 1995

Crassignatha Wunderlich, 1995: 547. Type species *Crassignatha haeneli* Wunderlich, 1995.

Crassignatha seeliam sp. nov.

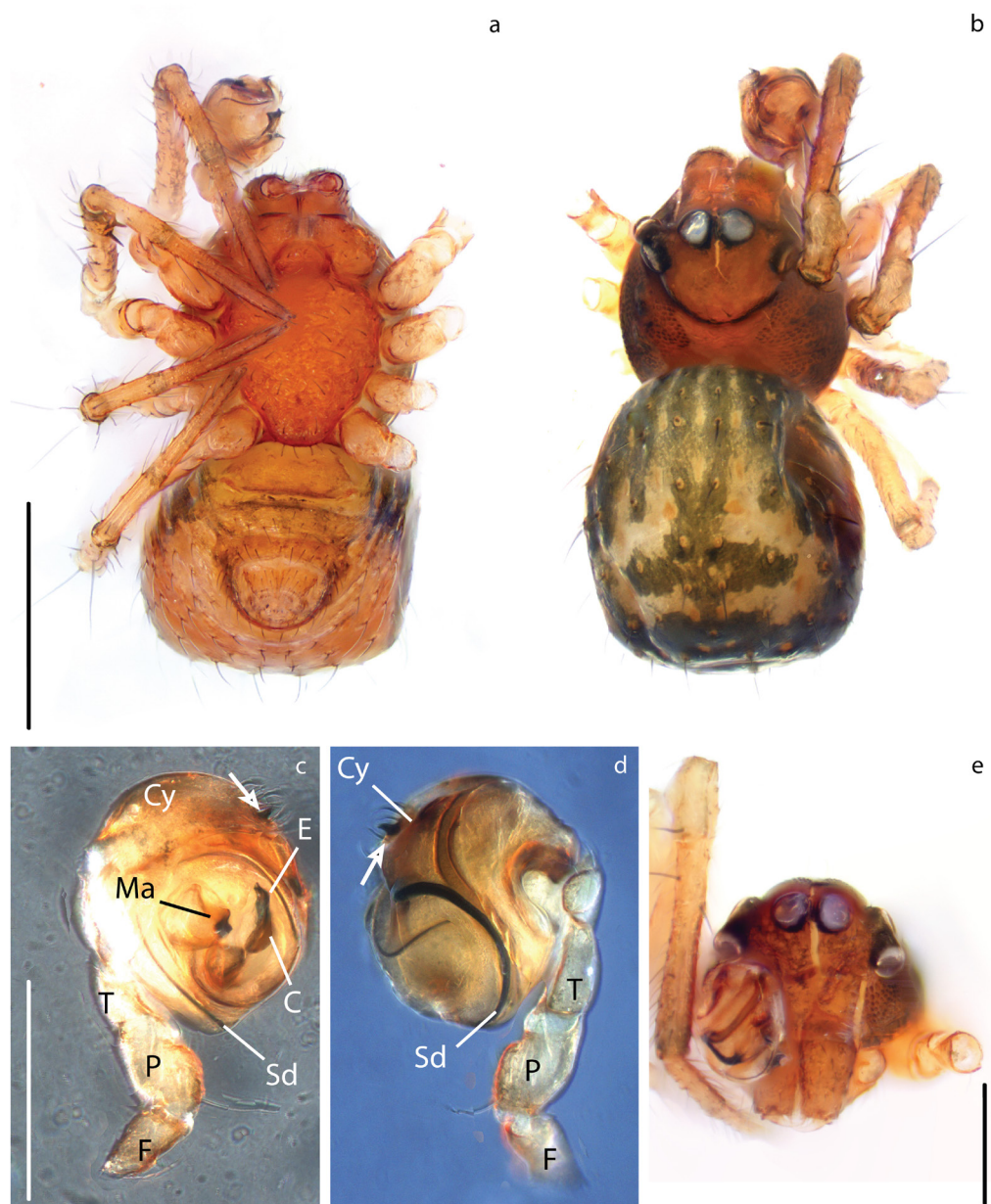Figs. 5.4a-c; 5.5b, d; 5.6a, b; 5.10–5.12.

Figure 5.**10**.- *Crassignatha seeliam* sp. n., male: Habitus: **a** ventral view; **b** dorsal view. Palp: **c** ventral view; **d** retrolateral view. Prosoma: **e** anterior view. Scale bars: 0.3 mm (**a, b**); 0.15 mm (**c–e**). Arrow pointing at the Cymbial tooth. Ct – Cymbial tooth; Cy – Cymbium; C – Conductor; E – Embolus; Em– Embolic membrane; F – Femur; MA – Median apophysis; Pa – Patella; Sd – Spermatic duct; T – Tibia.

Holotype: THAILAND • ♂: Chiang Mai, Doi Inthanon National Park. Montane evergreen forest; 18º30.454'N, 98º30.584'E. 1605m; July 21-24, 2018;
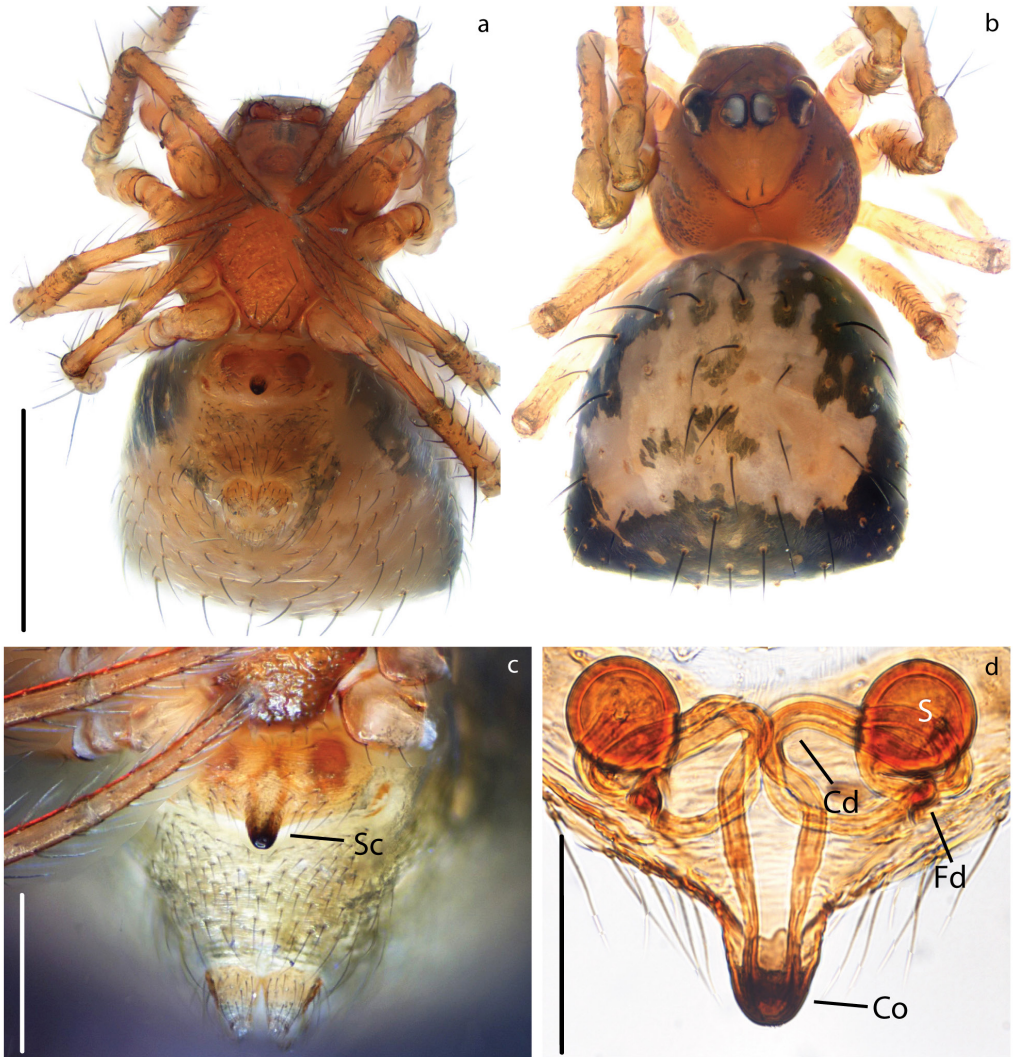
Figure 5.**11.**- *Crassignatha seeliam* sp. n. female: Habitus: **a** ventral view; **b** dorsal view. Epigynum: **c** ventral view; **d** dorsal view, cleared. Scale bars: 0.4 mm (**a, b**); 0.15 mm (**c**); 0.07 mm (**d**). Cd – Copulatory duct; Co – Copulatory opening; Fd – Fertilization duct; S – Spermatheca; Sa – Sececretory ampullae; Sc – Scape.

Booppa Petcharad, Jeremy Miller, F. Andres Rivera-Quiroz leg.; direct hand coll.; RMNH.ARA.18444. **Paratypes**: THAILAND • ♀ allotype; same data as holotype • 8 ♀; same data as holotype; RMNH.5106641• ♂ and ♀ Chiang Mai, Doi Suthep National Park. Montane evergreen forest with pine; 18º48.502'N, 98º53.528'E. 1409m; July 24-28, 2018; same collectors as holotype; pitfall traps. RMNH.ARA.18445.

Etymology: The species epithet is a derivation of the Thai *seeliam* (square), in reference to the shape of the abdomen in dorsal view.
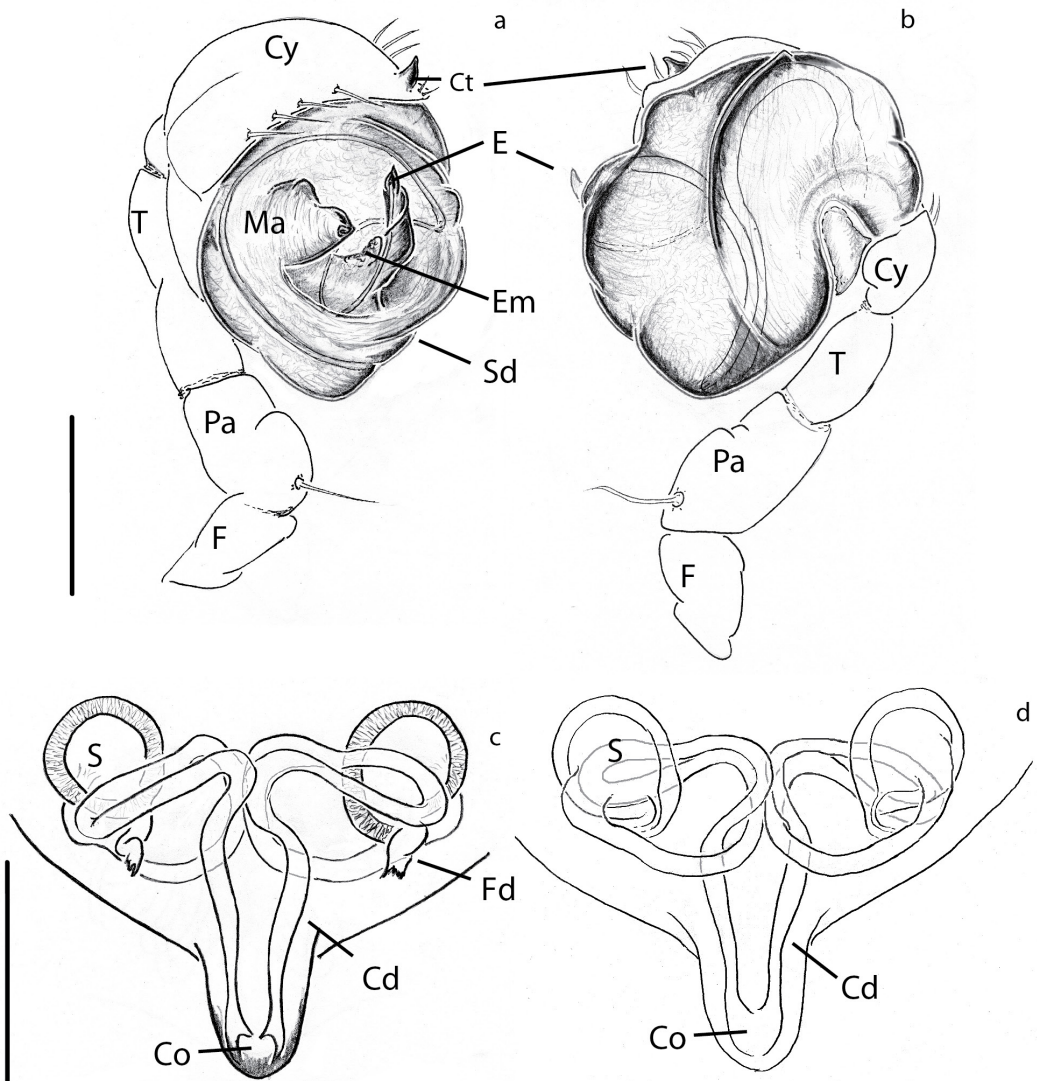
Figure 5.12.- *Crassignatha seeliam* **sp. n., genitalia.** Palp: a ventral view; b dorsal view. Epigynum, cleared: c dorsal view, d ventral view. Scale bars: 0.1 mm (a, b); 0.07 mm (c, d). Cd – Copulatory duct; Co – Copulatory opening; Ct – Cymbial tooth; Cy – Cymbium; E – Embolus; Em– Embolic membrane; F – Femur; Fd – Fertilization duct; MA – Median apophysis; Pa – Patella; S – Spermatheca; Sa – Sececretory ampullae; Sc – Scape; Sd – Spermatic duct; T – Tibia.

Diagnosis: Distinguished from other *Crassignatha* species except *Crassignatha quadriventris* [13] by the semi-squared posterior of the abdomen in dorsal view (Figs. 5.10b; 5.11b). Female can be separated from *C. quadriventris* by the coiling of the copulatory ducts in the epigynum (compare Figs. 5.11d and 5.12c, d to fig. 10: [13]). Male differs on the size of tegular sclerites and the cymbial tooth being short and stout instead of hook- shaped (compare Figs. 5.10c,d and 5.12a, b to fig. 8: [13]).

Figure 5.**13**.- *Crassignatha seedam* sp. n. female: Habitus: **a** ventral view; **b** dorsal view. Epigynum: **c** ventral view; **d** dorsal view, cleared. Scale bars: 0.3 mm (**a, b**); 0.1 mm (**c, d**); 0.05 mm (**d**). Cd – Copulatory duct; Co – Copulatory opening; Fd – Fertilization duct; S – Spermatheca; Sa – Sececretory ampullae.

Description: Carapace coloration orange-brown covered by small tubercles (Figs. 5.6a,b; 5.10a, b; 5.11a, b). Legs same color, slightly darker on distal portion its segments. Male Tibia II with two spines (mating claspers) (Fig. 5.5d). Abdomen black with light red patches; squared posteriorly, with sparse sclerotized patches, some bearing long setae (Figs. 5.10b; 5.11b). Male with posterior scutum wrapping the abdomen. Male palp: slightly less sclerotized than carapace. Semicircular from ventral view (Figs. 5.10c; 5.12a). Cymbium with distal

tooth. Median apophysis as big as Ct (Fig. 5.12a). Embolus filiform, exposed when palp is expanded (Fig. 5.12c). Spermatic duct very long and coiling two times inside the bulb (Fig. 5.4b, c).

Vulva: Epigynum with wide scape directed ventrally, heavily sclerotized at the tip (Fig. 5.11c). Copulatory opening at the tip of scape (Figs. 5.11d; 5.12c, d). Spermathecae spherical, slightly more sclerotized than epigynum, separated by approximately two times their diameter (Fig. 5.11d). Copulatory ducts very long, coiling over themselves before connecting toS. Fertilization ducts as long as S width, projecting dorsally (Figs. 5.11d, 5.12c).

Male: Total length 0.68; carapace 0.36 long, 0.30 wide; clypeus 0.13; Chelicera 0.1 long, 0.07 wide; Leg I: femur 0.28, patella 0.12, tibia 0.37, metatarsus 0.17, tarsus 0.22; leg formula I-II-IV-III; abdomen 0.42 long, 0.38 wide.

Female: Total length 0.69, carapace 0.44 long, 0.39 wide; clypeus 0.12; Chelicera 0.15 long, 0.1wide; Leg I: femur 0.42, patella 0.15, tibia 0.53, metatarsus 0.22, tarsus 0.27; leg formula I-II-IV-III abdomen 0.44 long, 0.43 wide.

Crassignatha seedam sp. nov.

Figs. 5.13; 5.15b, d

Holotype: THAILAND • ♀ Chiang Mai, Doi Suthep National Park. Montane evergreen forest with pine; 18º48.502'N, 98º53.528'E. 1409m; July 24-28, 2018. Booppa Petcharad, Jeremy Miller, F. Andres Rivera-Quiroz leg.; direct hand coll.; RMNH.5106640. **Male Unknown.**

Etymology: The species epithet is a derivation of the Thai *seedam* (black), in reference to the dark coloration of this species.

Diagnosis: *Crassignatha seedam sp. nov.* differs from other *Crassignatha* species by having a nearly round abdomen instead of triangular or squared, and having the epigynum bulging ventro-posteriorly but not forming an scape (compare Figs. 5.13d and 15b, d to Fig. 5.12c, and fig. 10: [13] and fig. 76d, h: [10]).

Description: Carapace brown with smooth texture (Fig. 5.13b). Legs light brown, slightly darker on the distal portion its segments. Abdomen sub-spherical, darker than carapace with sparse light patches (Figs. 5.13a, b).

Vulva: Epigynum weakly sclerotized but covered by small dark patches (Fig. 5.13d), bulging ventrally. Copulatory openings broad but not forming an atrium (Fig. 5.15b). Spermathecae spherical, much more sclerotized than epigynum, separated by 0.5 times their diameter (Fig. 5.13d). Copulatory ducts long, coiling over themselves before connecting to S. Fertilization ducts as long as S width, connecting very close to Cd and projecting dorsally (Figs. 5.15b, d).

Female: Total length 0.56, carapace 0.28 long, 0.26 wide; clypeus 0.06; Chelicera 0.1 long, 0.07 wide; Leg I: femur 0.3, patella 0.1, tibia 0.22, metatarsus 0.13, tarsus 0.19; leg formula I-II-IV-III; abdomen 0.47 long, 0.41 wide.
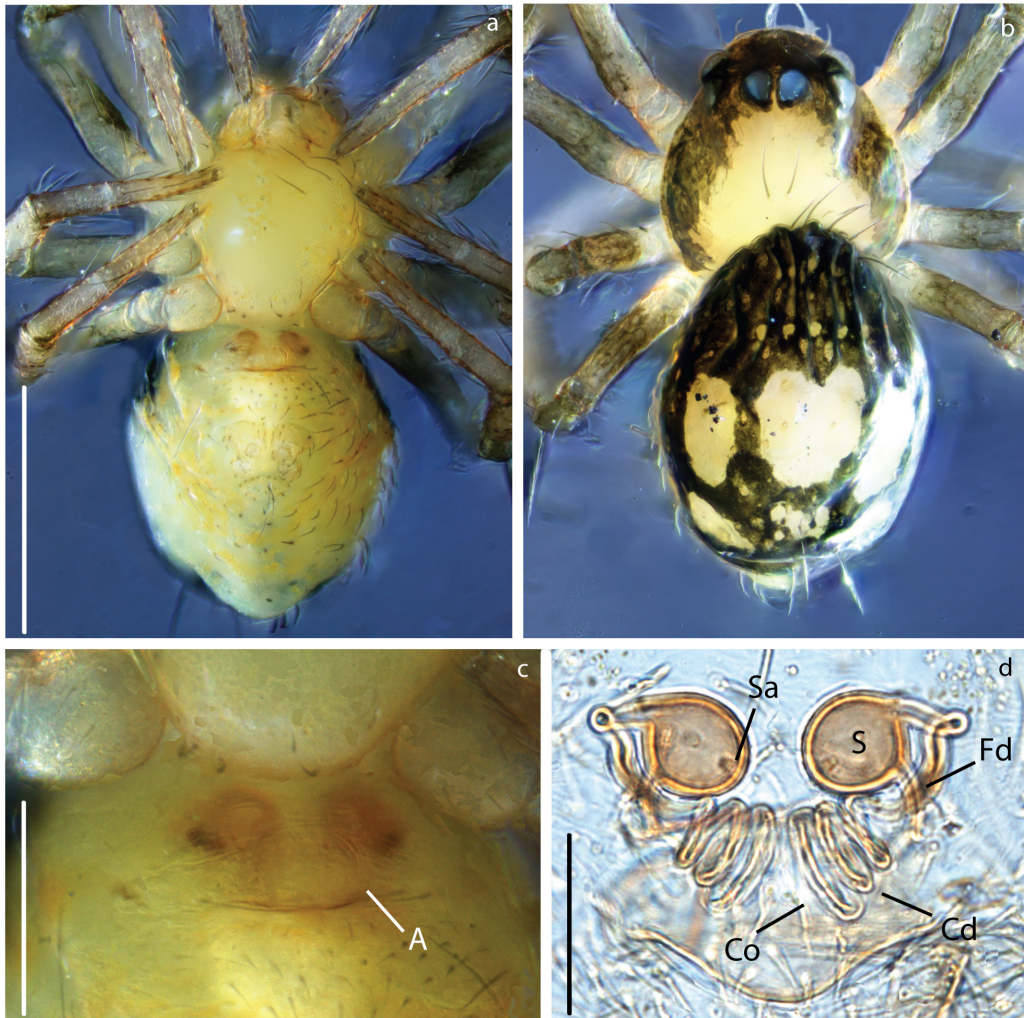
Figure 5.14.- *Patu shiluensis* **Lin & Li, 2009 female**. Habitus: **a** ventral view; **b** dorsal view. Epigynum: **c** ventral view; d dorsal view, cleared. Scale bars: 0.2 mm (**a, b**); 0.06 mm (**c**); 0.03 mm (**d**). A – Atrium; Cd – Copulatory duct; Co – Copulatory opening; MD – Epigynal median duct; Fd – Fertilization duct; Lb – lateral branch of the EMD; S – Spermatheca Sa – Secretory ampullae.

Crassignatha danaugirangensis Miller et al., 2014

Crassignatha danaugirangensis Miller et al., 2014: 4. f. 1a–f, 3, 4.Figs. 5.4d–f; 5.5a, c, e; 5.6c, d.

New records. BRUNEI • 2♂; Temburong, Huala Belalong Field Studies Centre; 4.545ºN 115.157ºE, 150m; September 26 – October 6, 2018; *Taxon Expeditions 2018* leg.; Winkler extractor; RMNH.5106643.

Genus *Patu* Marples, 1951

Patu Marples, 1951: 47. Type species *Patu vitiensis* Marples, 1951.

Patu shiluensis Lin & Li, 2009.

Patu shiluensis Lin & Li, 2009: 59, f. 11A-B, 12A-B, 13A-D.
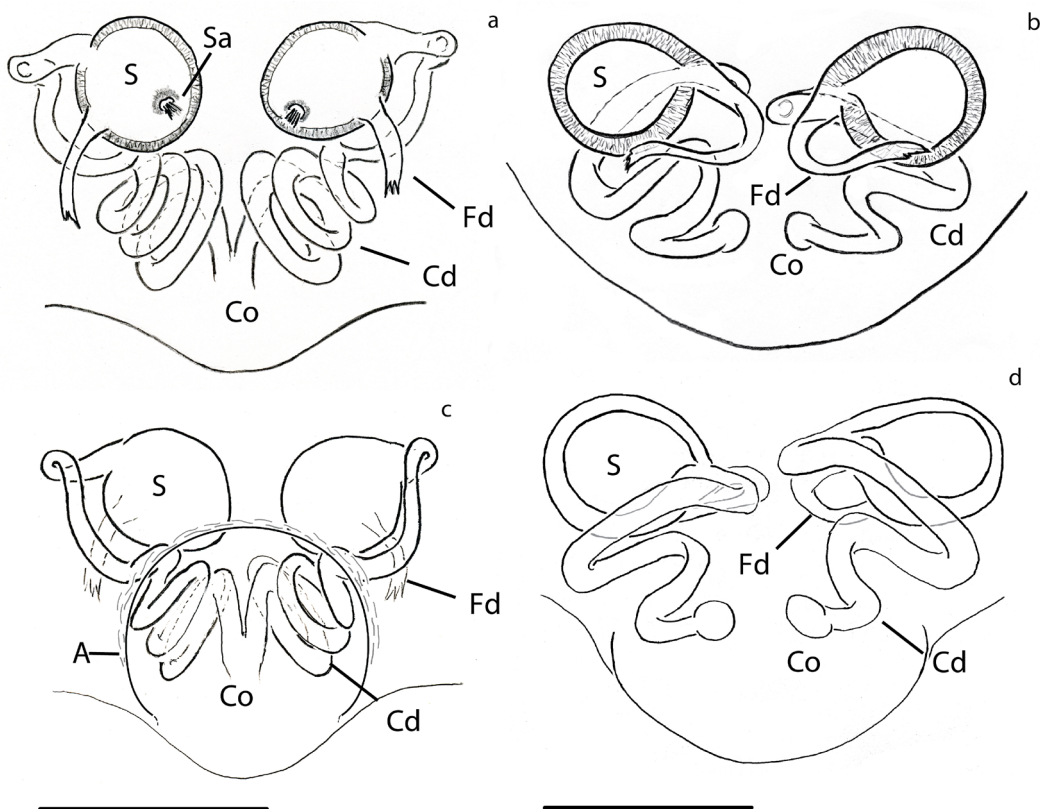
Figs. 5.14, 5.15a, c.

Figure 5.**15**.- **Epigynum, cleared. a, c** *Patu shiluensis* Lin & Li, 2009: **b, d** *Crassignatha seedam* sp. n. : **a, b** dorsal view; **c, d** ventral view. Scale bars: 0.03 mm (**a, c**); 0.05 mm (**b, d**). A – Atrium; Cd – Copulatory duct; Co – Copulatory opening; Fd – Fertilization duct; S – Spermatheca; Sa – Sececretory ampullae.

Collected material: THAILAND • 4♀; Phuket Province, Siray Island. Mixed tropical forest; 7º53.355'N, 98º26.083'E. 132m; August 02-06, 2018; Booppa Petcharad, Jeremy Miller, F. Andres Rivera-Quiroz leg.; Winkler extractor; RMNH.5106642.

Distribution: Known only from its type locality, Shilu Town, Hainan Province, China and the specimens collected for the present work.

Morphological remarks: Carapace pale yellow with black margin, smooth texture (Fig. 5.14b). Legs black and semi-transparent. Abdomen oval, longer than wide (Figs. 5.14a, b). Ventrally same color as carapace, dorsally, darker with pale yellow patches.
Vulva: Epigynum weakly sclerotized, semi-transparent (Fig. 5.14c). Atrium semi-circular slightly wider than inner distance between S (Figs. 5.14c; 5.15c). Spermathecae spherical slightly more sclerotized than epigynum, separated by 0.5 times their diameter (Fig. 5.14d). Copulatory ducts spring-like, spiraling three times over themselves.

Fertilization ducts as long as S width, projecting posteriorly (Figs. 5.14d; **5.**15a, c).

Female: Total length 0.52, carapace 0.21 long, 0.2 wide; clypeus 0.04; Chelicera 0.07 long, 0.05 wide; Leg I: femur 0.15, patella 0.07, tibia 0.1, metatarsus 0.07, tarsus 0.1; leg formula I-II-IV-III; abdomen 0.34 long, 0.28 wide.

Notes: Small somatic variations can be seen between the specimen we collected in Thailand and the ones previously described from China (compare Fig. 5.14b to fig. 11: [13]). However, we did not find any objective differences in the female genitalia.

Secretory ampullae (Figs. 5.14d, 5.15a) were very evident in our specimens; these glandular structures might be homologous to the accessory glands in Lopardo and Hormiga [31]. These structures were found in one anapid (*Tasmanaspis*) and several mysmenids, but scored as absent or unknown for all the symphytognathids.

The authors of this species mentioned it to be close to *Patu silho* Saaristo, 1996 from Seychelles. The possibility of *P. silho* not being a true *Patu* was discussed by its author [30,32] mentioning evident differences on somatic and sexual characters between *P. silho* and other *Patu* species. Nevertheless, the author deemed appropriate to place it in this genus. We also consider this species might be misplaced in *Patu* but would need further and more detailed analysis out of the scope of this work to clarify it (see discussion on *Patu* relationships below).

Discussion

The monophyly of the Symphytognathidae and its relations to other symphytognathoid spiders have resulted in complications and inconsistencies across different studies. The symphytognathoids were first recognized in a morphological study being formed by four putatively monophyletic families Anapidae, Symphytognathidae, Mysmenidae and Theridiosomatidae [2]. The monophyly of this clade has been tested several times using different molecular approaches targeting specific families [4,5,33], the Orbiculariae [34], and the whole order Araneae [6,7]. However, only a few representatives of the family Symphytognathidae have been used rendering their position and relations largely unexplored. Here, we built on two previous studies that used 9 species of Symphytognathidae to test the relations of the Mysmenidae [4,5]. Similarly to Feng et al. [5] low node supports were common in our trees, especially for MP and ML; still, the topologies we observed when including our 4 species are consistent with the results from these studies. All of our analyses showed a close relationship between the Symphytognathidae and the Anapidae (Figs. 5.1–**5.**3). This relationship has also been recovered in previous works [2,4–6]. Although tenuous due to the few terminals included, our study fails to recover the monophyly of the Anapidae and the position of micropholcommatids within this family. Our BI tree could not fully resolve the relations between the Anapidae and Symphytognathidae; similar issues have been observed before for the symphytognathoids [4,5,33–35]. This has been explained by either the limited set of loci and the relatively low taxon sampling [5] or an indication of the polyphyly of the "symphytognathoids" as suggested by three broad scoped phylogenies [6,34,35]. Nevertheless, Symphytognathoids were found to be a highly supported monophyletic group in a recent study that used ultraconserved elements (UCE) from 16 species across the four principal symphytognathoid families [7]

The internal relations of the Symphytognathidae in our analyses are still unresolved. Most of Lopardo's identifications (*pers. comm.*) are found in the *Crassignatha + Patu* clade. From these, SYMP_004_THAI (identified to *Crassignatha;* presumably conspecific to *C. seeliam*), and SYMP_002_MAD and SYMP_003_MAD (*Patu*) group together with the other representatives of the genera they were identified to. But the placing of two more, SYMP_006_AUS and SYMP_007_AUS (*Patu*), is more ambiguous being found outside of the *Crassignatha + Patu* clade rendering *Patu* paraphyletic. Thhis cladeand its internal relations are highly supported in all our trees (Figs. 5.1–5.3). Other two sequences, SYMP_008_DR (*Symphytognatha*) and Patu_SYMP_001_DR, are consistently grouped in another branch of the Symphytognathidae together with *Symphytognatha picta* and other unidentified symphytognathid (Figs. 5.1–5.3) suggesting that Patu_SYMP_001_DR might be misidentified. The position of *Anapistula* within the Symphytognathidae is also problematic. *Anapistula choojaiae* has a very long branch that is recovered as a sister to *Tasmanapis strahan* Platnick & Forster, 1989 with moderate to high support in the ML and BI (Figs. 5.2, 5.3). In these two analyses, this branch is related to other Anapidae having much higher support values in the BI than the ML (Figs. 5.2, 5.3). Nevertheless, the recent UCE study by Kulkarni, et al. [7] places this genus next to *Patu* in a highly supported but taxonomically limited Symphytognathidae. Solving the internal relations of the families Anapidae and Symphytognathidae, and clarifying their delimitations would need a much more detailed examination with a broader taxonomic sample.

The minute size of the symphytognathid spiders complicates the observation of diagnostic traits. Examination and interpretation of many characters require higher magnifications than those a dissection microscope can give. Therefore, SEM images have been previously used in the taxonomy of this family [8,10,29]. Unfortunately, the process for getting SEM images is destructive; therefore, rare specimens or short series are not usually prepared in this way and some characters cannot be properly observed. Here we used micro-CT scanning to overcome this issue and get clear views of important characters without damaging the specimens. 3D reconstruction has been used before to elucidate surfaces and internal structures of spider genitalia [36–38]. Nevertheless, ours are, to the best of our knowledge, the smallest palps that have been processed using this method. This was challenging in itself since we wanted to preserve the samples without critical point drying, a method commonly used in micro-CT scanning [37,39–41]. The tiny size of the palps, less than 0.2mm wide, did not allow to properly fix the dissected organ and keep it from moving during the scanning process. We attempted to fix the palp in agarose but the contrast of the resulting scans was too low to allow any observations. This problem was solved by scanning the entire spider (without dissecting the palp) in Et-OH 70% inside a modified 10ul pipette tip that was in turn inside a 0.5ml Eppendorf tube (Fig. 5.5f) in a similar fashion to Lipke, Hammel, and Michalik (2015), and Sombke et al. (2015). With this approach we were able to reconstruct the long and complicated internal ducts of the male genitalia (Figs. 5. 4b, c, e, f), as well as the surface of the external somatic and genital morphology (Figs. 5.4a, b; 5.5a-e; 5.6a-d; SM2, 3). Other internal structures of the male palp —probably glands— could be observed

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but would require more detailed examination out of the scope of the present work to accurately determine their nature; therefore, they are not shown in our 3D models. Images obtained through 3D reconstruction were used to interpret and discuss the diagnostic characters of the genus *Crassignatha* and compare them to other Symphytognathid genera in Table2.

Forster and Platnick [8] reviewed the Symphytognathidae and its component genera. Five of the eight currently recognized symphytognathid genera were included: *Anapistula* Gertsch, 1941, *Curimagua* Forster & Platnick, 1977, *Globignatha* Balogh & Loksa, 1968, *Patu* Marples, 1951, and *Symphytognatha* Hickman, 1931. *Crassignatha* Wunderlich, 1995 was described based on a single male specimen from peninsular Malaysia. This genus has been associated with several families (Synaphridae, Anapidae, Mysmenidae, Symphytognathidae; [9, 10, 31, 55]) and is currently considered a symphytognathid. Two other genera currently cataloged as Symphytognathidae, *Iardinis* Simon, 1899 *Anapogonia* Simon, 1905, are unrecognizable [8, 31, 46, 52]. Although spider taxonomy generally relies heavily on genitalia, little in the way of descriptive text or helpful depictions of genitalic characters was offered in Forster & Platnick's [8] revision. Table 2 summarizes some important diagnostic characters of the currently accepted symphytognathid genera in an attempt to clarify the taxonomic inconsistencies in this family.

Other than their small size, the characteristic that is perhaps most strongly associated with the Symphytognathidae was the fusion of the chelicerae [8]. But the degree of fusion is variable across the family and is particularly problematic in the genus *Patu*. The two species originally placed in *Patu* were reported as having the chelicerae fused for about half their length, but the degree of fusion was apparently less extensive in the genotype *Patu vitiensis* than in *Patu samoensis*, the other species described [48]. Subsequent authors have generally characterized *Patu* as having the chelicerae fused only at the base (Forster & Platnick, 1977). Curiously, Forster [54] made no mention of cheliceral fusion in *Patu*, but he did report basal fusion of the chelicerae in two genera (*Pseudanapis* and *Textricella*) that were subsequently transferred to Anapidae. So, assessing the presence or absence of basal cheliceral fusion is not always straight forward in practice. Some (but not all) *Patu* species known from males have a number of ventral distal macrosetae on tibia II, a characteristic scored as present in Lopardo's *Patu* specimens SYMP_002_MAD and SYMP_006_AUS and absent in Patu_SYMP_001_DR and *Symphytognatha picta* [31]. The this leg II clasper is otherwise found only in *Crassignatha.*

Genotype Crassignatha haeneli Wunderlich, 1995 features a textured carapace and a distinctive ventral spur on tibial II (Figs. 5.5d, e; figs. 14, 15, 17:[16]). The chelicerae are not conspicuously fused and are armed with a single bifid tooth (Fig. 5.5a); a character also scored for three species (SYMP_002_MAD, SYMP_006_AUS and SYMP_007_AUS, later on identified as Patu) used in Lopardo and Hormiga [31]. Miller et al. [10, 19] placed several additional species in Crassignatha, including the first

Table 2- Overview of diagnostic characters of the currently accepted genera of the Symphytog-nathidae.

	Anapistula Gertsch, 1941	*Anapogonia* Simon, 1905	*Crassignatha* Wunderlich, 1995	*Curimagua* Forster & Platnick, 1977
Sexes known	♀♂	♀	♀♂	♀♂
Species	25	1	9	2
Nomenclatural status	Valid	Valid	Valid	Valid
Female genitalia, internal	Pair of round spermathecae connected by t-shaped duct	--	Large spermathecae, convoluted duct path (Figs. 5.12c-d)	Ducts follow nearly straight path posteriorly from round spermathecae
Female genitalia, external	Transverse rounded lip overhanging furrow	--	Short robust scape (Fig. 5.11c-d)	Transverse rounded lip overhanging furrow
Tarsal claws	Homogeneous	--	Homogeneous	--
Cheliceral fusion	Near the base	Absent	Near the base	Near the base
Cheliceral teeth	Two (Fig. 5.7d)	--	Single asymmetrically bifid tooth, or two teeth (Fig. 5.5a)	Absent
Male tibia II clasper	Absent	N.A.	1-4 (Fig. 5.5d, e)	Absent
Male abdominal scutum	Absent except in *A. boneti*	N.A.	Surrounding the posterior part of the abdomen. Usually present, except in *C. haeneli*	Absent
Pars cephalica	Usually only slightly raised, strongly raised in *A. Boneti*	--	Strongly raised	Strongly raised
Eye arrangement	Usually four eyes (Fig. 5.8b), median eyes present in *A. boneti*	Six eyes in triads	Six eyes in diads (Figs. 5.10b, e; 5.11b)	Six eyes in triads
Female palp	Absent	--	Absent	Vestigial
Carapace texture	Mostly smooth	--	Generally covered with tubercles (Fig. 5.5b, c)	Mostly smooth
Abdomen shape	Subspherical	--	Subspherical, sometimes with postero-lateral lobes (Fig. 5.6)	Subspherical
Cymbium	With stong setae but without teeth or denticles	N.A.	With cymbial tooth (Fig. 5.4b, d)	With small bumps or denticles (figs. 66: [8])
Spermatic duct	Coiling 1.5 times over itself (Fig. 5.9a)	N.A.	Long, coiling several times around itself (Fig. 5.4b, e)	--
Embolus	Short less than 0.5 times the diameter of the bulb (Figs. 5.7c, 5.9a)	N.A.	Variable, short (Fig. 5.4 c) or long, about the diameter of the palp (Fig. 5.4 f)	Short about 0.5 times the diameter of the bulb (figs. 67, 68: [8])
Relevant literature	[8,17,29,43,44]	[45,46]	[9, 10, 31]	[8]

Number of species is based on the WSC [11].

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Table 2- Overview of diagnostic characters of the currently accepted genera of the Symphytog-nathidae (Continuation).

	Globignatha Balogh & Loksa, 1968	*Iardinis* Simon, 1899	*Patu* Marples, 1951	*Symphytognatha* Hickman, 1931
Sexes known	♀	♂	♀♂	♀♂
Species	2	(2)	18	15
Nomenclatural status	Valid	Nomen dubium*	Valid	Valid
Female genitalia, internal	Spermathecae twisted anteriorly	N.A.	Spermathecae variable, sometimes elongate or reniform	Copulatory ducts loop around elongate spermathecae (figs. 1-6, plate 1, fig. 2: [47])
Female genitalia, external	Transverse rounded lip overhanging furrow	N.A.	Transverse rounded lip overhanging furrow, or a flexible scape (figs. 1d, 2e: [48])	Transverse rounded lip overhanging furrow
Tarsal claws	Homogeneous	--	Homogeneous	Multidentate only in anterior legs (figs. 6, 7: [8]; fig. 2: [47]; fig. 3: [15])
Cheliceral fusion	Almost entirely fused with no visible suture line (figs. 41, 42: [8])	--	Fused basally to about half their length	Fused for most of their length, with visible suture line
Cheliceral teeth	One large, two short (fig. 43: [8])	One (fig. 6: [49])	Usually a single large tooth with 1-3 peaks	Two sinuous teeth (figs. 3, 32, 36: [8]; Figs. 5.2B, 5.2C : [15]; fig. 122A: [31])
Male tibia II, clasper	N.A.	--	Sometimes 1-2	Absent
Male abdominal scutum	N.A.	--	Absent	Absent
Pars cephalica	Strongly raised	Strongly raised	Strongly raised	Strongly raised
Eye arrangement	Six eyes in diads	Six eyes in triads	Six eyes in diads (Fig. 5.14b)	Six eyes in diads
Female palp	Absent	N.A.	Absent	Absent
Carapace texture	Mostly smooth	--	Mostly smooth	Mostly smooth
Abdomen shape	Subspherical	--	Subspherical, sometimes with lobes	Subspherical
Cymbium	N.A.	--	--	--
Spermatic duct	N.A.	Coiling 1.5 times over itself (fig. 7: [49]; fig 135a: [31])	--	--
Embolus	N.A.	long, 0,5–1,5 the diameter of the bulb (fig. 7: [49]; figs. 1,2: [50])	long about 1 time the diameter of the bulb(figs. 1e, 1f: [48]; fig. 19: [51])	Short about 0.5 times the diameter of the bulb (figs. 8, 9: [8])
Relevant literature	[8]	[8, 31, 50–52]	[8, 30, 48, 53, 54]	[8,15,31,47]

◄Number of species is based on the WSC [11]. *Type species *Iardinis weyersi* Simon, 1899 considered *nomen dubium*; two species placed in this genus by Brignoli [48,51] remain cataloged here [11].

descriptions of females. In all of Miller's species where males are known, they possess a unique abdominal scutum surrounding the abdomen laterally and posteriorly. In most *Crassignatha* species, the female genitalia consists of a pair of robust round spermathecae separated by about their diameter, copulatory ducts that loop and switchback along their path, and a short, robust scape (figs. 76, 79, 89A-89D:[10]); only *C. longtou* and *C. seedam* sp. nov have a transverse bulge and not a scape (figs. 89E, 89F, 91F:[10]).

Wunderlich [16] stated that *Crassignatha haeneli* lacked an abdominal scutum, and among the Symphytognathidae, only *Anapistula boneti* and Miller's *Crassignatha* species have a scutum (but see *Patu spinathoraxi*, below). A dissection of *Crassignatha* chelicerae indicated that they were indeed fused at the base (fig. 78A:[10]). It is however worth noting that the 3D scan of *Crassignatha* presented here do not appear to indicate cheliceral fusion (Fig. 5.5a). It was also determined that most of these *Crassignatha* species have an asymmetrical split in the cheliceral tooth with a small peak on the mesal side of the tooth; only *C. longtou* has two subequal teeth. *Crassignatha* species known from the male all have a group of 1-3 strong ventral setae on male tibia II (figs. 74E, 77D, 80E, 83E:[10]). One species had the abdomen modified with a pair of posteriolateral lobes (figs. 86D-F:[10]), not as conspicuous in other species (Figs. 5.6b, d), or generally round or oblong.

Modern symphytognathid taxonomy in Asia – 2009 was a big year for little spiders in Asia. Four papers described a total of 18 symphytognathid species from China, Japan, and Vietnam [10, 13, 18, 56]. These were distributed across the genera *Anapistula*, *Crassignatha*, and *Patu*. Lin and Li [13] described five new *Patu* species from China. Again, fusion of the chelicerae only near the base was declared as a characteristic of *Patu*. Chelicerae of all species were illustrated as fused, but no details were provided in the text. Of these five species, three show characters that match the diagnostic characters of *Crassignatha* instead of *Patu*:

Patu bicorniventris Lin & Li, 2009, known from the female only, has an asymmetrically bifid cheliceral tooth (figs. 2C, 2D: [13]) resembling those typical of *Crassignatha* (fig. 78A: [10]). It also has modifications to the abdomen consisting of two posteriolateral lobes and a straight posterior margin, resembling *Crassignatha ertou* (figs. 86D-86F: [10]). The female genitalia of *Patu bicorniventris* resembles most *Crassignatha* females described in Miller et al. [10], featuring conspicuous spermathecae with convoluted copulatory ducts leading to a knob-like median scape.

Patu quadriventris Lin & Li, 2009 shares with *P. bicorniventris* an abdomen that is truncated posteriorly, but lacks the posteriolateral lobes. The female genitalia is consistent with *Crassignatha*. The cymbium of the male pedipalp has a distal apophysis (CS in fig. 9C: [13]) that strongly resembles the Ct in *Crassignatha* (figs. 9a; 13a, d; figs. 75, 77B, 81, 82B, 84, 87, 88: [10]).

Patu spinathoraxi Lin & Li, 2009 has distinctive spikey tubercles covering the carapace. It closely resembles (but is not conspecific with) *Crassignatha longtou* Miller, Griswold & Yin, 2009, which was described from the female only. The female genitalia of both species are similar, featuring round spermathecae with ducts that run ectally before turning back toward the middle and terminate in a pair of conspicuous posterior openings; they contrast with *Crassignatha* in that they lack a robust scape. The male has a medially split abdominal scutum, a single ventral macroseta on tibia II, and a distal apophysis of the cymbium similar to those found in *Crassignatha* (CS in fig. 16C: [13]). These two species are clearly congeneric; whether they are best placed together in *Crassignatha*, or in their own new genus, is debatable.

Current status and proposed changes – Of the eight valid symphytognathid genera, *Anapistula*, *Curimagua*, *Globignatha*, *Symphytognatha*, and *Crassignatha* seem morphologically coherent and recognizable; *Anapogonia* and *Iardinis* are currently unrecognizable; *Patu* remains problematic. However, some species currently placed in *Patu* show clear affinities with *Crassignatha*. We propose the following taxonomic changes: *Crassignatha bicorniventris* (Lin & Li, 2009) **comb. nov.**, *Crassignatha quadriventris* (Lin & Li, 2009) **comb. nov.**, and *Crassignatha spinathoraxi* (Lin & Li, 2009) **comb. nov.**

Acknowledgments

References

1.   Mammola S, Michalik P, Hebets EA, Isaia M. (2017). Record breaking achievements by spiders and the scientists who study them. *PeerJ* **5**, e3972 DOI: 10.7717/peerj.3972.

2.   Griswold CE, Coddington JA, Hormiga G, Scharff N. (1998). Phylogeny of the orb-web building spiders (Araneae, Orbiculariae: Deinopoidea, Araneoidea). *Zool. J. Linn. Soc.* **123**, 1–99 DOI: 10.1111/j.1096-3642.1998.tb01290.x.

3.   Hormiga G, Griswold CE. (2014). Systematics, Phylogeny, and Evolution of Orb-Weaving Spiders. *Annu. Rev. Entomol.* **59**, 487–512 DOI: 10.1146/annurev-ento-011613-162046.

4.   Lopardo L, Giribet G, Hormiga G. (2011).

Morphology to the rescue: Molecular data and the signal of morphological characters in combined phylogenetic analyses-a case study from mysmenid spiders (Araneae, Mysmenidae), with comments on the evolution of web architecture. *Cladistics* **27**, 278–330 DOI: 10.1111/j.1096-0031.2010.00332.x.

5.   Feng C, Miller JA, Lin Y, Shu Y. (2019). Further study of two chinese cave spiders (Araneae, mysmenidae), with description of a new genus. *Zookeys* **870**, 77–100 DOI: 10.3897/zookeys.870.35971.

6.   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 Silva LR, 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, Szüts T, Ubick D, Vink CJ, Wood HM, Zhang J. (2017). The spider tree of life: phylogeny of Araneae based on target-gene analyses from an extensive taxon sampling. *Cladistics* **33**, 574–616 DOI: 10.1111/cla.12182.

7.   Kulkarni S, Wood H, Lloyd M, Hormiga G. (2020). Spider-specific probe set for ultraconserved elements offers new perspectives on the evolutionary history of spiders (Arachnida, Araneae). *Mol. Ecol. Resour.* **20**, 185–203 DOI: 10.1111/1755-0998.13099.

8.   Forster RR, Platnick NI. (1977). A review of the spider family Symphytognathidae (Arachnida, Araneae). *Am. museum Novit.* **2619**, 1–29.

9.   Wunderlich J. (2004). The fossil spiders of the family Anapidae s. l. (Aeaneae) [sic] in Baltic, Dominican and Mexican amber and their extant relatives, with the description of the new subfamily Comarominae. *Beiträge zur Araneologie* **3**, 1020–1111.

10.   Miller JA, Griswold CE, Yin C. (2009). The symphytognathoid spiders of the Gaoligongshan, Yunnan, China (Araneae: Araneoidea): Systematics and diversity of micro-orbweavers. *Zookeys* **11**, 9–195 DOI: 10.3897/zookeys.11.160.

11.   WSC. http://www.wsc.nmbe.ch/ (2020) World Spider Catalog Version 21.0. *Natural History Museum Bern, online at http://wsc.nmbe.ch* DOI: 10.24436/2.

12.   Tong Y, Li S. (2006). Symphytognathidae (Araneae), a spider family newly recorded from China. *Zootaxa* **1259**, 33–38.

13.   Lin Y, Li S. (2009). First described Patu spiders (Araneae, Symphytognathidae) from Asia. *Zootaxa* **2154**, 47–68.

14.   Lin Y, Tao Y, Li S. (2013). Two new species of the genus *Anapistula* (Araneae, Symphitognathidae) from Southern China. *Acta Zootaxonomica Sin.* **38**, 53–58.

15.   Lin Y. (2019). First report of the spider genus Symphytognatha from Asia (Araneae, Symphytognathidae). *Zootaxa* **4638**, 291–295.

16.   Wunderlich J. (1995). Drei bisher unbekannte Arten und Gattungen der Familie Anapidae (s.l.) aus Süd-Afrika, Brasilien und Malaysia (Arachnida: Araneae). *Beiträge zur Araneologie* **3**, 543–551.

17.   Harvey MS. (1998). A review of the Australasian species of Anapistula Gertsch (Araneae: Symphytognathidae). *Rec. West. Aust. Museum* **19**, 111–120.

18.   Lin Y, Pham DS, Li S. (2009). Six new spiders from caves of Northern Vietnam (Araneae: Tetrablemmidae: Ochyroceratidae: Telemidae: Symphytognathidae). *Raffles Bull. Zool.* **57**, 323–342.

19.   Miller JA, Schilthuizen M, Burmester J, van der Graaf L, Merckx V, Jocqué M, Kessler P, Fayle T, Breeschoten T, Broeren R, Bouman R, Chua W-J, Feijen F, Fermont T, Groen K, Groen M, Kil N, de Laat H, Moerland M, Moncoquet C, Panjang E, Philip A, Roca-Eriksen R, Rooduijn B, van Santen M, Swakman V, Evans M, Evans L, Love K, Joscelyne S, Tober A, Wilson H, Ambu L, Goossens B. (2014). Dispatch from the field: ecology of ground-web-building spiders with description of a new species (Araneae, Symphytognathidae). *Biodivers. Data J.* **2**, e1076 DOI: 10.3897/bdj.2.e1076.

20.   Miller JA, Griswold CE, Haddad CR. (2010). Taxonomic revision of the spider family Penestomidae (Araneae, Entelegynae). *Zootaxa* 1–36 DOI: 10.11646/zootaxa.2534.1.1.

21.   Darriba D, Taboada GL, Doallo R, Posada D. (2012). JModelTest 2: More models, new heuristics and parallel computing. *Nat. Methods* **9**, 772 DOI: 10.1038/nmeth.2109.

22.   Miller MA, Pfeiffer W, Schwartz T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. in *2010 Gateway*

5

Computing Environments Workshop, GCE 2010 1–8 DOI: 10.1109/GCE.2010.5676129.

23.   Kumar S, Stecher G, Li M, Knyaz C, Tamura K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* **35**, 1547–1549 DOI: 10.1093/molbev/msy096.

24.   Stamatakis A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**, 1312–1313 DOI: 10.1093/bioinformatics/btu033.

25.   Ronquist F, Huelsenbeck JP. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574 DOI: 10.1093/bioinformatics/btg180.

26.   Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Syst. Biol.* **67**, 901–904 DOI: 10.1093/sysbio/syy032.

27.   Coddington JA. (1983). A temporary slide-mount allowing precise manipulation of small structures. *Verhandlungen des Naturwissenschaftlichen Vereins Hambg.* **26**, 291–292.

28.   Alvarez-Padilla F, Hormiga G. (2007). A protocol for digesting internal soft tissues and mounting spiders for scanning electron microscopy. *J. Arachnol.* **35**, 538–542 DOI: 10.1636/Sh06-55.1.

29.   Rheims C, Brescovit AD. (2003). Description of six new species of *Anapistula* Gertsch (Araneae, Symphytognathidae) from Brazil. *Bull. Br. Arachnol. Soc.* **12**, 324–330.

30.   Saaristo MI. (1996). Symphytognathidae (Arachnida, Araneae), a new spider family for the granitic islands of Seychelles. *Phelsuma* **4**, 53–56.

31.   Lopardo L, Hormiga G. (2015). Out of the twilight zone: Phylogeny and evolutionary morphology of the orb-weaving spider family Mysmenidae, with a focus on spinneret spigot morphology in symphytognathoids (Araneae, Araneoidea). *Zool. J. Linn. Soc.* **173**, 527–786 DOI: 10.1111/zoj.12199.

32.   Saaristo MI. (Press Manchester, UK, 2010). Araneae. in *Arachnida and Myriapoda of the Seychelles islands* (eds. Gerlach, J. & Marusik, Y.) 306.

33.   Rix MG, Harvey MS, Roberts JD. (2008). Molecular phylogenetics of the spider family Micropholcommatidae (Arachnida: Araneae) using nuclear rRNA genes (18S and 28S). *Mol. Phylogenet. Evol.* **46**, 1031–1048 DOI: 10.1016/j.ympev.2007.11.001.

34.   Fernández R, Hormiga G, Giribet G. (2014). Phylogenomic analysis of spiders reveals nonmonophyly of orb weavers. *Curr. Biol.* **24**, 1772–1777 DOI: 10.1016/j.cub.2014.06.035.

35.   Dimitrov D, Lopardo L, Giribet G, Arnedo MA, Álvarez-Padilla F, Hormiga G. (2012). Tangled in a sparse spider web: Single origin of orb weavers and their spinning work unravelled by denser taxonomic sampling. *Proc. R. Soc. B Biol. Sci.* **279**, 1341–1350 DOI: 10.1098/rspb.2011.2011.

36.   Lipke E, Hammel JU, Michalik P. (2015). First evidence of neurons in the male copulatory organ of a spider (Arachnida, Araneae). *Biol. Lett.* **11**, 20150465 DOI: 10.1098/rsbl.2015.0465.

37.   Sentenská L, Müller CHG, Pekár S, Uhl G. (2017). Neurons and a sensory organ in the pedipalps of male spiders reveal that it is not a numb structure. *Sci. Rep.* **7**, 12209 DOI: 10.1038/s41598-017-12555-5.

38.   Dederichs TM, Müller CHG, Sentenská L, Lipke E, Uhl G, Michalik P. (2019). The innervation of the male copulatory organ of spiders (Araneae) - A comparative analysis. *Front. Zool.* **16**, 1–14 DOI: 10.1186/s12983-019-0337-6.

39.   Keklikoglou K, Faulwetter S, Chatzinikolaou E, Wils P, Brecko J, Kvaček J, Metscher B, Arvanitidis C. (2019). Micro-computed tomography for natural history specimens: a handbook of best practice protocols. *Eur. J. Taxon.* **522**, 1–55 DOI: 10.5852/ejt.2019.522.

40.   Steinhoff POM, Sombke A, Liedtke J, Schneider JM, Harzsch S, Uhl G. (2017). The synganglion of the jumping spider Marpissa muscosa (Arachnida: Salticidae): Insights from histology, immunohistochemistry and microCT analysis. *Arthropod Struct. Dev.* **46**, 156–170 DOI: 10.1016/j.asd.2016.11.003.

41.   Steinhoff POM, Uhl G, Harzsch S, Sombke A. (2020). Visual pathways in the brain of the jumping spider Marpissa muscosa. *J. Comp. Neurol.* **528**, 1883–1902 DOI: 10.1002/cne.24861.

42.   Sombke A, Lipke E, Michalik P, Uhl G, Harzsch S. (2015). Potential and limitations of X-Ray micro-computed tomography in arthropod neuroanatomy: A methodological and comparative survey. *J. Comp. Neurol.* **523**, 1281–1295 DOI: 10.1002/cne.23741.

43.   Dupérré N, Tapia E. (2017). On some minuscule spiders (Araneae: Theridiosomatidae,

Symphytognathidae) from the Chocó region of Ecuador with the description of ten new species. *Zootaxa* **4341**, 375–399 DOI: 10.11646/zootaxa.4341.3.3.

44.	Rubio GD, González A. (2010). The first Symphytognathidae (Arachnida: Araneae) from Argentina, with the description of a new species of Anapistula from the Yungas Mountain rainforest. *Rev. Chil. Hist. Nat.* **83**, 243–247 DOI: 10.4067/S0716-078X2010000200005.

45.	Simon E. (1905). Arachnides de Java, recueillis par le Prof. K. Kraepelin en 1904. *Mitteilungen aus dem Naturhistorischen Museum Hambg.* **22**, 49–73.

46.	Platnick NI, Forster RR. (1989). A revision of the temperate South American and Australasian spiders of the family Anapidae (Araneae, Araneoidea). Bulletin of the AMNH ; no. 190. *Bull. Am. museum Nat. Hist.*

47.	Hickman V V. (1931). A new family of spiders. *Proc. Zool. Soc. London* **101**, 1321–1328.

48.	Marples BJ. (1951). Pacific Symphytognathid Spiders. *Pacific Sci.* **5**, 47–51.

49.	Brignoli PM. (1978). Spinnen aus Nepal, IV. Drei neue Symphytognathidae (Arachnida: Araneae). *Senckenb. Biol.* **59**, 247–252.

50.	Brignoli PM. (1980). On few Mysmenidae from the Oriental and Australian regions (Araneae). *Rev. Suisse Zool.* **87**, 727–738.

51.	Gertsch WJ. (1960). Descriptions of American spiders of the family Symphytognathidae. *Am. Museum Novit.* **1981**, 1–40.

52.	Levi HW, Levi LR. (1962). The genera of the spider family Theridiidae. *Bull. Museum Comp. Zool.* **127**, 1–71.

53.	Marples BJ. (1955). Spiders from Wesern Samoa. *J. Linn. Soc. London, Zool.* **42**, 453–504.

54.	Forster RR. (1959). The spiders of the family Symphytognathidae. *Trans. Proc. R. Soc. New Zeal.* **86**, 263–329.

55.	Marusik YM, Lehtinen PT. (2003). Synaphridae Wunderlich, 1986 (Aranei: Araneoidea), a new family status, with a description of a new species from Turkmenistan. *Arthropoda Sel.* **11**, 143–152.

56.	Shinkai E. (Tokai University Press, 2009). Two new species of the Genera *Wendilgarda* and *Patu* from Japan (Araneae: Theridiosomatidae and Symphytognathidae). in *The spiders of Japan* (ed. Ono, H.) 75–77.

Supplementary Material

Supplementary Material 1. List of primers used in our study, alignment of DNA sequence data used in phylogenetic analyses in nexus format, and Trace plot and histograms for both runs of the BI analysis observed in Tracer 1.7.1.

Supplementary Material 2. 3D reconstructions *Crassignatha seeliam* male pedipalp and habitus.

Supplementary Material 3. 3D reconstructions *Crassignatha danaugirangensis* male pedipalp and habitus.

(Included in the original publication in ZooKeys)