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A new genus of Pseudospirobolellidae (Diplopoda, Spirobolida) from limestone karst areas in Thailand, with descriptions of three new species

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Abstract

A new genus of the millipede family Pseudospirobolellidae, *Siliquobolellus* **gen. nov.**, is described from limestone mountains in Thailand, based on three new species, viz. *Siliquobolellus amicusdraconis* **gen. et sp. nov.** from Uthaithani Province, *Siliquobolellus constrictus* **gen. et sp. nov.** from Prachuap Khiri Khan Province and *Siliquobolellus prasankokae* **gen. et sp. nov.** (type species) from Lampang Province. The descriptions are based on gonopod morphology and mitochondrial DNA data (COI barcodes). The COI barcodes grouped the three new species in a well-supported *Siliquobolellus* **gen. nov.** clade. The mean interspecific COI sequence divergence among the three new species was 12% (range: 8–15%). The mean intergeneric COI sequence divergence between *Siliquobolellus* **gen. nov.**, *Coxobolellus* Pimvichai, Enghoff, Panha & Backeljau, 2020, and *Pseudospirobolellus* Carl, 1912 was 19% (range: 14–23%). Three conspicuous gonopodal synapomorphies differentiate *Siliquobolellus* **gen. nov.** from other pseudospirobollellid genera: (1) the telopodital part of the posterior gonopod forms a deep concavity, (2) the telopodite of the anterior gonopod is directed distad and does not reach the tip of the coxal part of the anterior gonopod, and (3) the tip of the anterior gonopod coxa is narrowed, curving mesad. As such, the monophyly of the new genus is well supported by both morphological and mitochondrial DNA data. A distribution map and an identification key to the species are provided.

Key Words

COI, gonopod, monophyly, synapomorphy

Introduction

Hitherto, the small SE Asian spirobolidan millipede family Pseudospirobolellidae comprised < 20 species in 2 or 3 genera, viz. *Pseudospirobolellus* Carl, 1912, *Coxobolellus* Pimvichai, Enghoff, Panha & Backeljau, 2020, and *Benoitolus* Mauriès, 1980 (Enghoff et al. 2015; Pimvichai et al. 2020). Yet, recent mitochondrial DNA sequence analyses suggested that the genera *Pseudospirobolellus* and *Coxobolellus* form a well-supported clade, while the genus *Benoitolus* is nested within the family Pachybolidae (whose monophyly is not well-corroborated), though with limited support and obliterating the otherwise strong support for the genus *Litostrophus* Chamberlin, 1921 (Pimvichai et al. 2020). Thus, the monophyly and taxonomic composition of the family Pseudospirobolellidae are still unclear and need more attention.

In this context, the present study uses gonopod morphology and mitochondrial DNA sequence data to describe three new species in a new genus of Pseudospirobolellidae from limestone karst areas in Thailand.

Materials and methods

Live specimens were hand-collected and were partly preserved in 70% ethanol for morphological study and partly placed in a freezer at –20 °C for DNA analysis.

This research was conducted under the approval of the Animal Care and Use regulations (numbers U1-07304-2560 and IACUC-MSU-037/2019) of the Thai government.

Morphology

Gonopods were photographed with a digital camera. Samples for scanning electron microscopy (SEM: Hitachi TM4000Plus) were air-dried directly from alcohol and sputter-coated for 60 s with gold (Hitachi: MC1000). Scanning electron micrographs were taken at the Central Lab of Mahasarakham University. Drawings were made using a stereomicroscope and photographs. Voucher specimens were deposited in the collections of CUMZ.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from legs of single specimens of *Siliquobolellus amicusdraconis* gen. et sp. nov. (Hub Pa Tard, Uthaithani Province; CUMZ-D00149), *Siliquobolellus constrictus* gen. et sp. nov. (Ban Yang Chum, Prachuap Khiri Khan Province; CUMZ-D00150) and *Siliquobolellus prasankokae* gen. et sp. nov. (Pha Thai, Lampang Province; CUMZ-D00148) using the NucleoSpin Tissue kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. PCR amplifications and sequencing of the standard mitochondrial COI DNA barcoding fragment (Hebert et al. 2003) were done as described by Pimvichai et al. (2020). The COI fragment was amplified with the primers LCO-1490 and HCO-2198 (Folmer et al. 1994). The new COI nucleotide sequences have been deposited in GenBank under accession numbers OP174621− OP174623. Sample data and voucher codes are provided in Table 1.

Alignment and phylogenetic analysis

The COI data included 56 specimens, representing 18 genera and 46 nominal species of ingroup taxa (Table 1). Three species of the order Spirostreptida, viz. *Anurostreptus barthelemyae* Demange, 1961 (Harpagophoridae), *Chonecambala crassicauda* Mauriès & Enghoff, 1990 (Pericambalidae) and *Thyropygus allevatus* (Karsch, 1881) (Harpagophoridae) were used as outgroup.

CodonCode Aligner (ver. 4.0.4, CodonCode Corporation) was used to assemble the forward and reverse sequences and to check for errors and ambiguities. All sequences were checked with the Basic Local Alignment Search Tool (BLAST) provided by NCBI and compared with reference sequences in GenBank. All sequences were aligned using MUSCLE (ver. 3.6, see http://www.drive5.com/muscle; Edgar 2004). The alignments consisted of 660 bp. The sequences were checked for ambiguous nucleotide sites, saturation and phylogenetic signal using DAMBE (ver. 5.2.65. see http://dambe.bio.uottawa.ca/DAMBE/dambe_install_win.aspx; Xia 2018). MEGA (ver. 11.0.10, see http://www.megasoftware.net; Tamura et al. 2021) was used to (1) check for stop codons, (2) translate sequences into amino acids, and (3) calculate uncorrected pairwise p-distances among sequences by computing the proportion of nucleotide sites by which two sequences differ.

Phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian inference (BI). The shape parameter of the gamma distribution, based on 16 rate categories, was estimated using maximum-likelihood analysis. ML trees were inferred with RAxML (ver. 8.2.12, see http://www.phylo.org/index.php/tools/raxmlhpc2_tgb.html; Stamatakis 2014) through the CIPRES Science Gateway (Miller et al. 2010) using a GTR+G substitution model and 1000 bootstrap replicates to assess branch support. BI trees were constructed with MrBayes (ver. 3.2.7a, see http://www.phylo.org/index.php/tools/mrbayes_xsede.html; Huelsenbeck and Ronquist 2001). Substitution models were inferred using jModeltest (ver. 2.1.10, see https://www.github.com/ddarriba/jmodeltest2/releases; Darriba et al. 2012) applying Akaike Information Criterion (AIC) weights as selection criterion. This yielded TIM2+ I+G (–lnL = 11998.1218, gamma shape = 0.4620) as best model.

BI trees were run for 2 million generations (heating parameter: 0.02), sampling every 1000 generations. Convergences were confirmed by verifying that the standard deviations of split frequencies were below 0.01. Then the first 1000 trees were discarded as burn-in, so that the final consensus tree was built from the last 3002 trees. Support for nodes was assessed by posterior probabilities.

For ML we consider branches with bootstrap values (BV) of ≥ 70% to be well supported (Hillis and Bull 1993) and < 70% as poorly supported. For BI trees, we consider branches with posterior probabilities (PP) of ≥ 0.95 to be well supported (San Mauro and Agorreta 2010) and below as poorly supported.

Results

The uncorrected p-distances between the sequences ranged from 0.00 to 0.26 (Suppl. material 1: Table S1). The mean interspecific sequence divergence within *Siliquobolellus* gen. nov. was 0.12 (range: 0.08–0.15). The mean intergeneric sequence divergences between *Siliquobolellus* gen. nov. and the other putative pseudospirobolellid genera were: 0.17 (range: 0.14–0.20) for *Coxobolellus*, 0.21 (range: 0.20–0.23) for *Pseudospirobolellus*, and 0.23 (range: 0.22–0.24) for *Benoitolus birgitae* (Hoffman, 1981). The mean intergeneric sequence divergence between *Siliquobolellus* gen. nov., *Coxobolellus*, and *Pseudospirobolellus* was 0.19 (range: 0.14–0.23).

Table 1. Specimens from which the COI gene fragment was sequenced. CUMZ, Museum of Zoology, Chulalongkorn University, Bangkok, Thailand; NHMD, Natural History Museum of Denmark; NHMW, Naturhistorisches Museum, Vienna, Austria; NHM, The Natural History Museum, London, United Kingdom. Names of countries are in CAPITALS. Abbreviations after species names refer to the isolate of each sequence. GenBank accession numbers are indicated for each species.

	Voucher code	Locality	COI
Genus *Siliquobolellus* gen. nov.			
S. amicusdraconis gen. et sp. nov.	CUMZ- D00149	Hub Pa Tard, Lan-Sak, Uthaithani, THAILAND	OP174621
S. constrictus gen. et sp. nov.	CUMZ- D00150	Ban Yang Chum, Kui Buri, Prachuap Khiri Khan, THAILAND	OP174622
S. prasankokae gen. et sp. nov.	CUMZ- D00148	Pha Thai, Ngao, Lampang, THAILAND	OP174623
Genus *Apeuthes*			
A. maculatus Amc	NHMW-Inv. No.2395	South Annam, VIETNAM	MF187404
A. maculatus Am26	NHMD-621697	Nha Trang, Bao Dai Villas Hotel, in garden, VIETNAM	MZ567159
A. fimbriatus BMP	CUMZ-D00144	Bach Ma Peak, Da Nang, VIETNAM	MZ567160
A. longeligulatus TPP	CUMZ-D00140	Tham Phet Po Thong, Klong Hard, Sa Kaeo, THAILAND	MZ567161
A. pollex SMR	CUMZ-D00141	Sra Morakot, Klongthom, Krabi, THAILAND	MZ567162
A. pollex SML	CUMZ-D00142	Koh 8, Similan islands, Phang-Nga, THAILAND	MZ567163
A. pollex WTS	CUMZ-D00143	Tham Sue Temple, Muang, Krabi, THAILAND	MZ567164
?*A. spininavis* ABB	CUMZ-D00145	Air Banun, Perak, MALAYSIA	MZ567165
Genus *Atopochetus*			
A. anaticeps SVL	CUMZ- D00091	Srivilai temple, Chalermprakiet, Saraburi, THAILAND	MF187405
A. dollfusii DOL	NHM	Cochinchina, VIETNAM	MF187412
A. helix SPT	CUMZ- D00094	Suan Pa Thong Pha Phum, Kanchanaburi, THAILAND	MF187416
A. moulmeinensis TAK	CUMZ- D00095	Km 87, Tha Song Yang, Tak, THAILAND	MF187417
A. setiferus HPT	CUMZ- D00097	Hub Pa Tard, Lan-Sak, Uthaithani, THAILAND	MF187419
A. spinimargo Ton27	NHMD- 00047013	Koh Yo, Songkhla, THAILAND	MF187423
A. truncatus SML	CUMZ- D00101	Koh 8, Similan islands, Phang-Nga, THAILAND	MF187424
A. uncinatus KMR	CUMZ- D00102	Khao Mar Rong, Bangsapan, Prachuapkhirikhan, THAILAND	MF187425
A. weseneri Tos29	NHMD-00047003	Supar Royal Beach Hotel, Khanom, Nakhonsrithammarat, THAILAND	MF187431
Genus *Aulacobolus*			
A. uncopygus Auc	NHMW-Inv. No.2375	Nilgiris, South India, INDIA	MF187433
Genus *Benoitolus*			
B. birgitae BBG	NHMD 621687	Chiang Dao, Chiang Mai, THAILAND	MT328992
Genus *Coxobolellus*			
C. albiceps Stpw	CUMZ-D00121	Tham Pha Tub, Muang District, Nan Province, THAILAND (green individual)	MT328994
C. albiceps Stpl	CUMZ-D00122	Tham Pha Tub, Muang District, Nan Province, THAILAND (small, brown individual)	MT328993
C. albiceps TPB	CUMZ-D00123	Wat Tham Bampen Bun, Pan District, Chiang-Rai Province, THAILAND	MT328996
C. albiceps Stvd	CUMZ-D00124	Tham Wang Daeng, Noen Maprang District, Phitsanulok Province, THAILAND	MT328995
C. compactogonus SKR	CUMZ-D00134	Sakaerat Environmental Research Station, Wang Nam Khiao District, Nakhon Ratchasima Province, THAILAND	MT328998
C. compactogonus KLC	CUMZ-D00135	Khao Look Chang, Pak Chong District, Nakhon Ratchasima Province, THAILAND	MT328997
C. fuscus HKK	CUMZ-D00133	Kroeng Krawia waterfall, Sangkhla Buri District, Kanchanaburi Province, THAILAND	MT328999
C. nodosus SPW	CUMZ-D00126	Chao Por Phawo Shrine, Mae Sot District, Tak Province, THAILAND	MT329000
C. serratus KKL	CUMZ-D00132	Khao Kalok, Pran Buri District, Prachuap Khiri Khan Province, THAILAND	MT329001
C. simplex TNP	CUMZ-D00136	Tham Pha Pha Ngam, Mae Prik District, Lampang Province, THAILAND	MT329002
C. tenebris KWP	CUMZ-D00119	Wat Khao Wong Phrohm-majan, Ban Rai District, Uthai Thani Province, THAILAND	MT329003
C. tenebris TPL	CUMZ-D00120	Wat Tham Phrom Lok Khao Yai, Sai Yok District, Kanchanaburi Province, THAILAND	MT329004
C. tigris TKP	CUMZ-D00130	Wat Tham Khao Plu, Pathio District, Chumphon Province, THAILAND	MT329005
C. tigris TYE	CUMZ-D00131	Tham Yai I, Pathio District, Chumphon Province, THAILAND	MT329006
C. transversalis Stpg	CUMZ-D00125	Tham Pha Tub, Muang District, Nan Province, THAILAND	MT329007
C. valvatus TCD	CUMZ-D00127	Wat Tham Chiang Dao, Chiang Dao District, Chiang-Mai Province, THAILAND	MT329009
C. valvatus BRC	CUMZ-D00128	Tham Borichinda, Chom Thong District, Chiang-Mai Province, THAILAND	MT329008
C. valvatus TST	CUMZ-D00129	Tham Sam Ta, Muang District, Mae Hong Son Province, THAILAND	MT329010
Genus *Leptogoniulus*			
L. sorornus BTN	CUMZ- D00109	Botanical Garden, Penang, MALAYSIA	MF187434
Genus *Litostrophus*			
L. chamaeleon PPT	CUMZ- D00111	Phu Pha terb, Mukdahan, THAILAND	MF187436
L. saraburensis PKS	CUMZ- D00113	Phukhae Botanical Garden, Saraburi, THAILAND	MF187438
L. segregatus Ls19	NHMD 621686	Koh Kut, Trad, THAILAND	MF187440
Genus *Macrurobolus*			
M. macrurus INT	CUMZ- D00147	Wat Tham Inthanin, Mae Sot District, Tak Province, THAILAND	MZ905519
Genus *Madabolus*			
M. maximus Mm4	NHMD-00047007	de Toliara Province, Parc National de Bermaraha, South Bank of Manambolo River, Near Tombeau Vazimba, MADAGASCAR	MF187441
Genus *Narceus*			
N. annularis			NC_003343.1

	Voucher code	Locality	COI
Genus *Parabolus*			
P. dimorphus Pd34	NHMD-00047004	Dar es Salaam, TANZANIA	MF187442
Genus *Paraspirobolus*			
P. lucifugus			AB608779.1
Genus *Pelmatojulus*			
P. tigrinus Pt2	NHMD-00047008	Southern part of the Comoé N.P., 30 km north of Kakpin, CÔTE d'IVOIRE	MF187443
P. togoensis Pto6	NHMD-00047006	Biakpa, GHANA	MF187444
Genus *Pseudospirobolellus*			
Pseudospirobolellus avernus GPG	CUMZ-D00117	Gua Pulai, Gua Musang, Kelantan, MALAYSIA	MT329011
Pseudospirobolellus sp. KCS	CUMZ-D00118	Koh Chuang, Sattahip, Chonburi, THAILAND	MT329012
Genus *Rhinocricus*			
R. parcus Rp49	NHMD-00047009	Puerto Rico, USA	MF187449
Genus Trachelomegalus			
T. sp. Tr54	NHMD-00047012	Borneo Sabah, MALAYSIA	MF187445
Genus *Trigoniulus*			
T. corallinus Tco15	NHMD-00047010	Vientiane, LAOS	MF187446
Outgroup			
Genus *Anurostreptus*			
A. barthelemyae Tlb	CUMZ-D00003	Thale-Ban N.P., Khuan-Don, Satun, THAILAND	KC519469
Genus *Chonecambala*			
C. crassicauda Ttp	CUMZ-D00001	Ton-Tong waterfall, Pua, Nan, THAILAND	KC519467
Genus *Thyropygus*			
T. allevatus Bb	CUMZ-D00013	BangBan, Ayutthaya, THAILAND	KC519479

The mean intergeneric sequence divergences between *Siliquobolellus* gen. nov. and various pachybolid genera were: 0.20 (range: 0.18–0.23) for *Apeuthes* Attems, 1938, 0.23 (range: 0.20–0.26) for *Atopochetus* Attems, 1953, 0.21 (range: 0.18–0.22) for *Litostrophus* Chamberlin, 1921, and 0.20 (range: 0.19–0.22) for *Pelmatojulus* De Saussure, 1860.

The mean intergeneric sequence divergences between *Siliquobolellus* gen. nov. and the other Spirobolida families were: 0.22 (range: 0.21–0.24) for *Narceus annularis* (Rafinesque, 1820) (Spirobolidae) and 0.24 (range: 0.23–0.26) for *Paraspirobolus lucifugus* (Gervais, 1836) (Spirobolellidae).

Both the BI and ML trees (Fig. 1) showed *Siliquobolellus* gen. nov., *Coxobolellus* and *Pseudospirobolellus* as three well-supported clades, which jointly formed a well-supported Pseudospirobolellidae clade (though without *Benoitolus birgitae*). However, the sister group relationship between *Siliquobolellus* gen. nov. and *Coxobolellus* was poorly supported by both BI and ML.

Taxonomy

Class Diplopoda de Blainville in Gervais, 1844
Order Spirobolida Bollman, 1893
Suborder Spirobolidea Bollman, 1893
Family Pseudospirobolellidae Brölemann, 1913

Genus *Siliquobolellus* gen. nov.

https://zoobank.org/A2CA57E0-53B4-4060-B621-E8A3322EAFB4

Fig. 2

Etymology. From Latin *siliqua* = pod, and referring to the podlike shape of the gonopod telopodite.

Type species. *Siliquobolellus prasankokae* gen. et sp. nov.

Other included species. *Siliquobolellus amicusdraconis* gen. et sp. nov. and *Siliquobolellus constrictus* gen. et sp. nov.

Diagnosis. *Siliquobolellus* species are characterised by (1) the unique shape of the telopodital part of their posterior gonopod, which forms a deep concavity. In contrast, the telopodital part of the posterior gonopod is extremely slender and sickle-shaped in *Pseudospirobolellus*, does not form a deep concavity in *Coxobolellus*, or is slender with a broad apical canopy in *Benoitolus*; (2) the telopodite of anterior gonopod (at) simple, directed distad, not reaching tip of coxal part of the anterior gonopod (cx). In contrast, the telopodite of anterior gonopod (at) is overreaching coxa in the other three genera; and (3) tip of anterior gonopod coxa narrowed, curving mesad. In contrast the tip of the anterior gonopod coxa directed distad in *Coxobolellus*, or is rounded in *Pseudospirobolellus* and *Benoitolus*.

General description. Head capsule smooth. Occipital furrow extending down between, but not beyond eyes; clypeal furrow reaching level of antennal sockets. Area below antennal sockets and eyes impressed, forming part of antennal furrow. Incisura lateralis (IL) open (Fig. 2A). 2+2 labral teeth, a row of labral setae, 3+3 supralabral setae. Diameter of eyes ca half of interocular space; 7 vertical rows of ommatidia, 4 horizontal rows, 24–26 ommatidia per eye. Antennae short, not reaching beyond collum when stretched back, accommodated in a shallow furrow composed of a horizontal segment in the head capsule and a vertical segment in the mandibular cardo and stipes. Antennomere lengths 2 > 6 > 1 > 5 > 3 = 4 > 7 in *Siliquobolellus amicusdraconis* gen. et sp. nov. and *S. constrictus* gen. et sp. nov. and 2 > 1 > 6 > 5 > 3 = 4 > 7 in *S. prasankokae* gen. et sp. nov.; antennomere 1 glabrous, 2 and 3 with some ventral setae, 4, 5 and 6 densely setose; 4 apical sensilla. Mandibles: stipes (Mst)

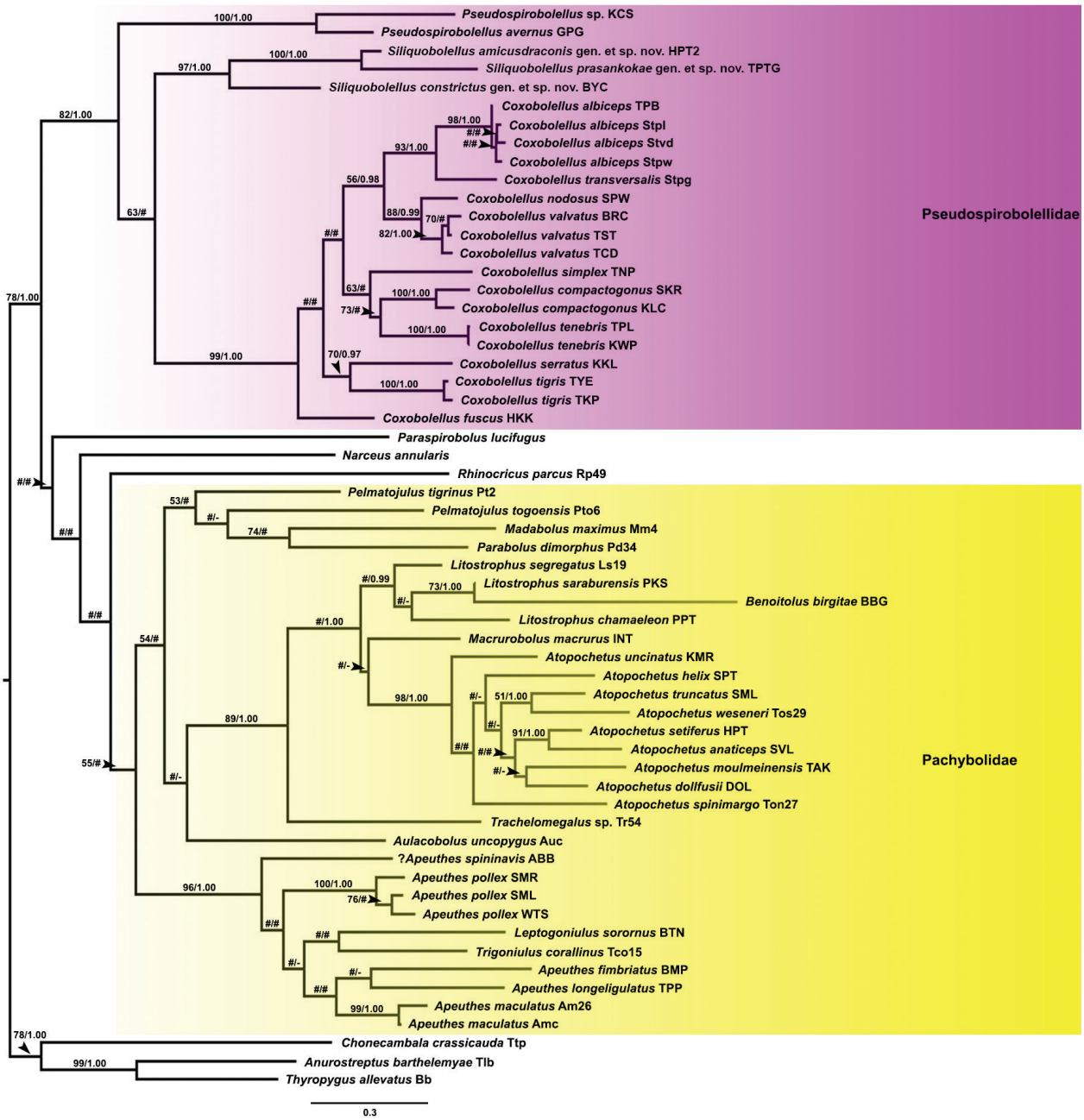

Figure 1. Phylogenetic relationships of *Siliquobolellus* gen. nov. and several other spirobolidan millipede taxa based on maximum likelihood analysis (ML) and Bayesian inference (BI) of a 660 bp COI gene fragment. Numbers at nodes indicate branch support based on bootstrapping (ML) / posterior probabilities (BI). Scale bar = 0.3 substitutions/site. # indicates branches with < 50% ML bootstrap support or < 0.95 posterior probability, - indicates non-supported branches. The colored areas mark the families Pseudospirobolellidae (minus *Benoitolus*) (purple) and Pachybolidae (plus *Benoitolus*) (yellow).

broad at base, apically gradually narrowed, triangular. Gnathochilarium (Fig. 2B): each stipe (Gst) with 3 apical setae; each lamella lingualis with 2 setae, one behind the other. Basal part of mentum (Me) transversely wrinkled; basal part of stipites longitudinally wrinkled.

Collum smooth, with a marginal furrow along lateral part of anterior margin; lateral lobes narrowly rounded, extending as far ventrad as the ventral margin of body ring 2.

Body rings 2–3 ventrally concave, hence with distinct ventrolateral "corners". Body rings very smooth, parallel-sided in dorsal view. Prozona smooth. 'Tergo-pleural' suture visible on pro- and mesozona; mesozona ventrally with fine oblique striae, dorsally punctate; metazona ventrally with fine longitudinal striae, otherwise smooth. "Pleural" parts of rings with fine oblique striae. Sterna transversely striate. Ozopores from ring 6, situated in mesozona, ~1/2 pore diameter in front of metazona.

Telson smooth; preanal ring (Pre) with slightly recurved dorsal profile, with short process protruding to vertical tangent to anal valves or slightly beyond (Fig. 2C–E). Anal valves (Av) smooth, rounded (Fig. 2C–E). Subanal scale (Sub) broadly triangular (Fig. 2F).

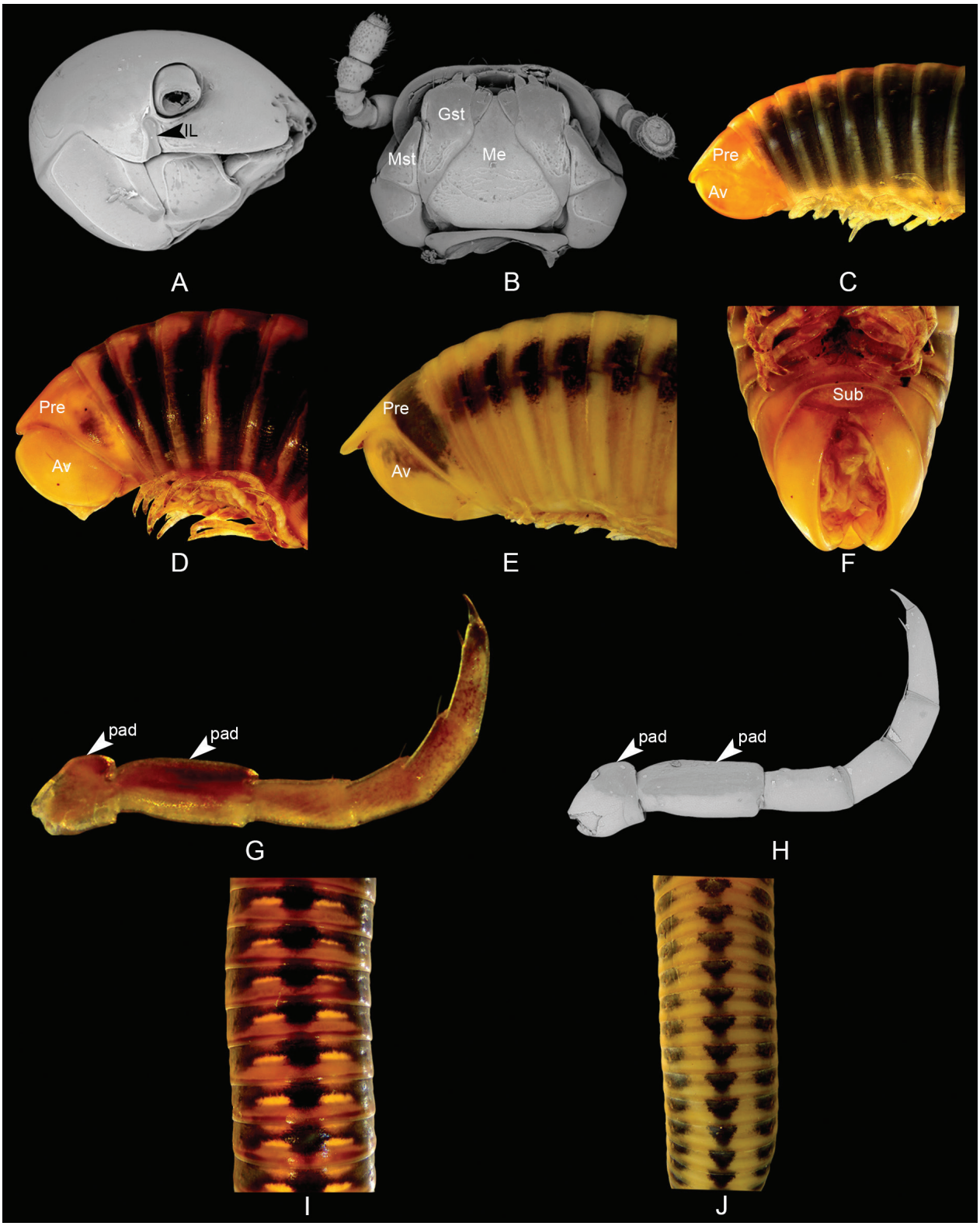

Figure 2. External morphology of *Siliquobolellus* gen. nov. **A–C.** *S. amicusdraconis* gen. et sp. nov. A. Head, lateral view; **B.** Head, ventral view; **C.** Posterior end, lateral view; **D.** Posterior end, lateral view (*S. prasankokae* gen. et sp. nov.); **E.** Posterior end, lateral view (*S. constrictus* gen. et sp. nov.); **F–I.** *S. prasankokae* gen. et sp. nov.; **F.** Posterior end, ventral view; **G, H.** Male leg, latero-ventral view; **I.** Body rings, dorsal colour pattern; **J.** *S. constrictus* gen. et sp. nov., body rings, dorsal colour pattern. Av = anal valves; Gst = gnathochilarial stipes; IL = incisura lateralis Me = mentum; Mst = Mandibular stipes; Pre = preanal ring; Sub = subanal scale.

Legs (Fig. 2G, H): length of midbody legs 55–60% of body diameter in males, 40–48% of body diameter in females. Prefemur basally constricted and longer than other podomeres. First and second legs with 2 or 3 prefemoral, 2 or 3 femoral, 2 or 3 postfemoral, and 2–4 tibial setae, and 4 or 5 ventral and 1 dorsal apical setae on tarsi, numbers of setae reaching constancy from pair 3: in males each podomere from postfemur to tibia with 1 seta; tarsi with 1ventral apical and 1 dorsal apical seta; in females each leg podomere from coxae to tibia with 1 seta, tarsi with 1–3 ventral and 1 dorsal apical setae. Claw slender and sharp.

Male sexual characters. Apical part of coxa and entire ventral surface of prefemur from third to the last body rings with large ventral soft pad. Body ring 7 entirely fused ventrally, no trace of a suture. Tip of anterior gonopods visible when the animal is stretched out (not when it is rolled up).

Anterior gonopods (Figs 3–5A, B, D, E) with a fairly small triangular mesal sternal process (st). Coxa broad at base, apically gradually narrowed, curving mesad, posterior surface folding over telopodite, for accommodation of telopodite. Telopodite gradually narrowed towards tip, directed distad, not reaching tip of anterior gonopod coxa (cx).

Posterior gonopods (Figs 3–5C, F) simple, rounded, with short, smooth coxal part (pcx); with prominent opening of efferent groove (oeg) distomesally of pcx; telopodital part (pt) twice the length of the pcx, lateral margin folding mesad, forming a deep concavity.

Female vulvae (Figs 3L, 4K, 5K) simple, valves prominent.

Species descriptions

Siliquobolellus amicusdraconis gen. et sp. nov.

https://zoobank.org/5D598D47-C70C-438C-8AAB-E083A7E7053B

Figs 2, 3, 6, 7

Material studied. ***Holotype.*** 1 male (CUMZ-D00149-1), Thailand, Uthaithani Province, Lan-Sak District, Hub Pa Tard; 15°22'37.13"N, 99°37'49.98"E; 119 m a.s.l.; 26 July 2020; P. Pimvichai, T. Backeljau and P. Prasankok leg.

Paratypes. 3 males (CUMZ-D00149-3), 3 females (CUMZ-D00149-2); same data as holotype.

Etymology. The species epithet means "friend of the dragon" and refers to the type locality, which is shared with the "shocking pink dragon millipede", *Desmoxytes purpurosea* Enghoff, Sutcharit & Panha, 2007.

Diagnosis. Differing from other species in the genus by having the tip of anterior gonopod crossing over with tip of opposite side, the two together delimiting a drop-shaped "window", whereas in the other two species the tips of anterior gonopod are separated from each other; externally differing by its bright orange color on the anterior and posterior ends. In contrast, *S. constrictus* gen. et sp. nov. has a row of triangular dark brown spots middorsally on its body rings, while *S. prasankokae* gen. et sp. nov. has a row of rectangular dark brown spots middorsally on the body rings.

Description. Adult males with 41 or 42 podous rings, 1 apodous ring. Length ~3 cm, diameter 2.7–2.9 mm. Adult females with 41–43 podous rings, 1–3 apodous rings. Length ~3 cm, diameter 2.8–3.1 mm.

Colour. Living animal mainly dark brown. Head, dorsal part of the first four body rings and telson orange, antenna light brown, middorsal metazona orange (Fig. 6A, B).

Anterior gonopods (Fig. 3A, B, D, E) with extremely small triangular process between coxae (st), with high coxae, apically narrow, curving mesad, tip crossing over with tip of opposite side, the two together delimiting a drop-shaped "window". Telopodite flattened apically rounded, curving backward, directed distad, as a thumb-like process (Fig. 3B, arrow).

Posterior gonopods (Fig. 3C, F–K) simple, rounded, with short, smooth coxal part (pcx); telopodital part (pt) twice the length of the pcx, lateral margin folding mesad, forming a deep concavity, the inner lateral margin expanded into rounded lamella, apically forming a canopy (Fig. 3J, arrow).

Female vulvae (Fig. 3L): simple, valves prominent, the right valve slightly larger than the left valve.

DNA barcode. The GenBank accession number of the COI barcode of the paratype is OP174621 (voucher code CUMZ-D00149).

Habitat. Found under leaf litter and on rocks.

Distribution. Known only from the type locality in Uthaithani Province, Thailand (Fig. 7).

Siliquobolellus constrictus gen. et sp. nov.

https://zoobank.org/FF7A3AFE-2FBB-469D-9A29-55F70DDE12F8

Figs 2, 4, 6, 7

Material studied. ***Holotype.*** 1 male (CUMZ-D00150-1), Thailand, Prachuap Khiri Khan Province, Kui Buri District, Ban Yang Chum; 12°04'19.64"N, 99°42'57.63"E; 149 m a.s.l.; 7 August 2014; C. Sutcharit leg.

Paratypes. 1 male (CUMZ-D00150-2), 2 females (CUMZ-D00150-3); same data as holotype.

Etymology. The species epithet is a Latin adjective derived from the verb constringere = "to tighten" and refers to the constricted anterior gonopod coxa.

Diagnosis. Differing from other species in the genus by having the anterior gonopod coxae constricted at middle of lateral margin, while the other two species have no constriction at middle of lateral margin of anterior gonopod coxae; externally differing by having a middorsal row of triangular dark brown spots, with dark brown bands on both lateral sides running parallel to the dorsal one.

Description. Adult males with 41 or 42 podous rings, 1 apodous ring. Length ~3 cm, diameter 3.6–3.7 mm. Adult females with 42 or 43 podous rings, 1 apodous ring. Length ~3 cm, diameter 4.1–4.5 mm.

Colour. After 8 years in alcohol overall beige with 3 stripes from head to telson: a mid-dorsal row of triangular dark brown spots, with dark brown bands on both lateral side running parallel to the dorsal one (Fig. 2J).

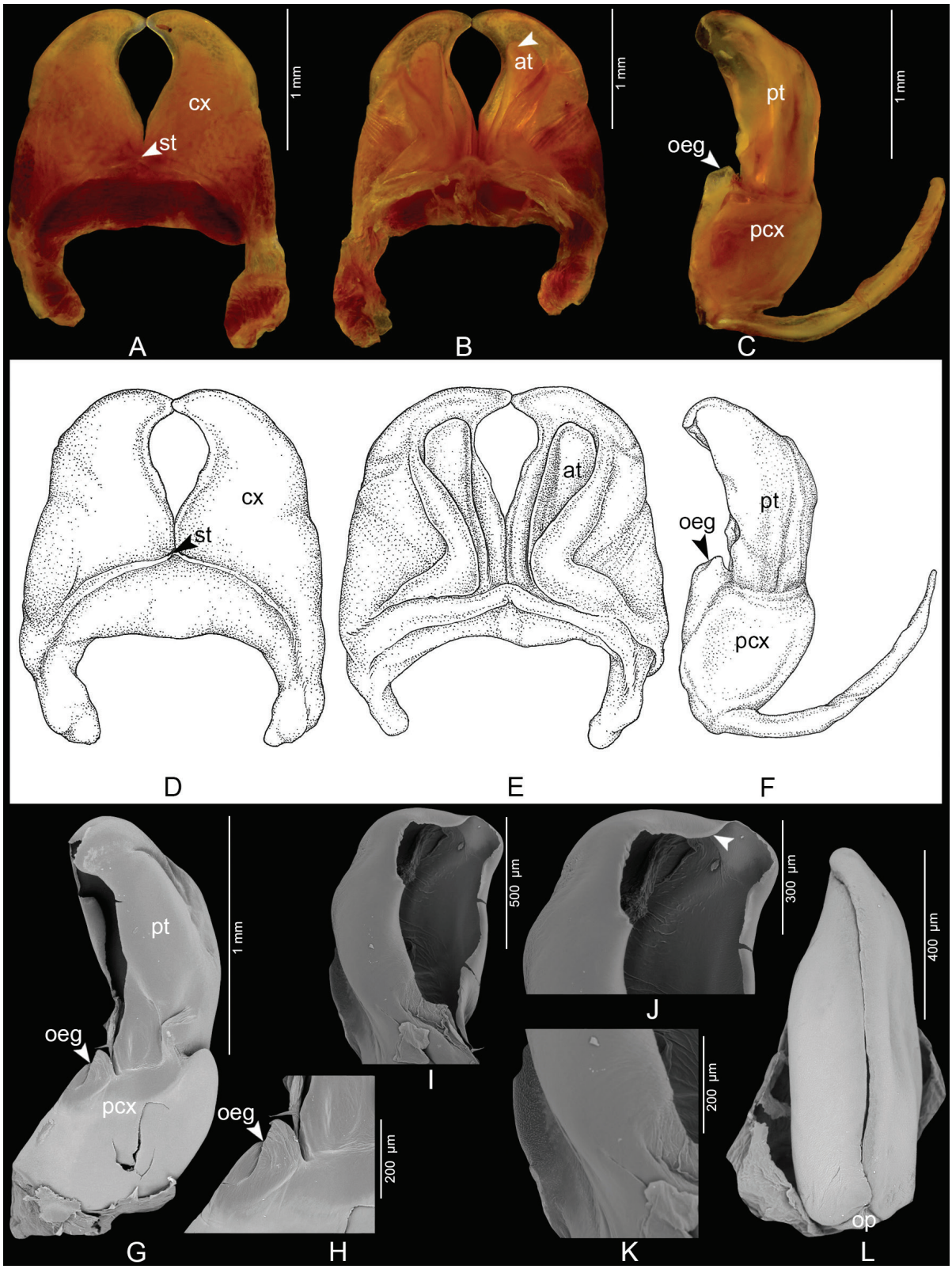

Figure 3. *Siliquobolellus amicusdraconis* gen. et sp. nov., holotype, gonopods (specimen from Hub Pa Tard, CUMZ-D00149-1). **A.** Anterior gonopod, anterior view; **B.** Anterior gonopod, posterior view, arrow indicates a thumb-like process; **C.** Right posterior gonopod, lateral view; **D.** Anterior gonopod, anterior view; **E.** Anterior gonopod, posterior view; **F.** Right posterior gonopod, lateral view; **G.** SEM, Right posterior gonopod, latero-mesal view; **H.** SEM, Mesal part of right posterior gonopod, lateral view; **I.** SEM, Right posterior gonopod, latero-mesal view; **J.** SEM, Tip of right posterior gonopod, latero-mesal view, arrow indicates a canopy; **K.** SEM, lateral part of pt, latero-mesal view; **L.** SEM, left female vulva, posterior mesal view. at = anterior gonopod telopodite; cx = coxa; oeg = opening of efferent groove; op = operculum of vulva; pcx = coxal part of the posterior gonopod telopodite; pt = telopodital part of the posterior gonopod telopodite; st = sternal process.

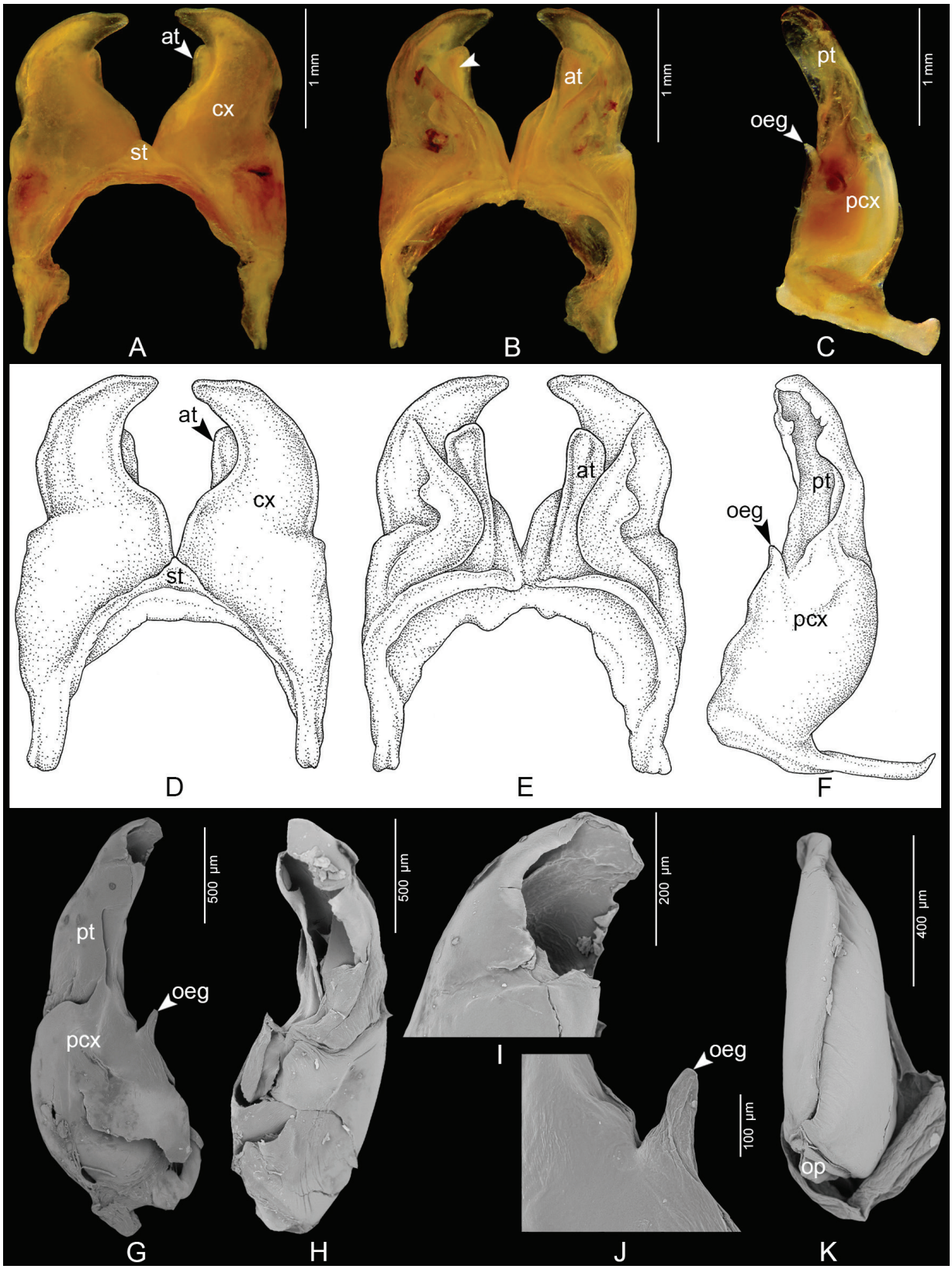

Figure 4. *Siliquobolellus constrictus* gen. et sp. nov., holotype, gonopods (specimen from Ban Yang Chum, CUMZ-D00150-1). **A.** Anterior gonopod, anterior view; **B.** Anterior gonopod, posterior view, arrow indicates a thumb-like process, with longitudinal ridge; **C.** Right posterior gonopod, lateral view; **D.** Anterior gonopod, anterior view; **E.** Anterior gonopod, posterior view; **F.** Right posterior gonopod, lateral view; **G.** SEM, Left posterior gonopod, lateral view; **H.** SEM, Left posterior gonopod, latero-mesal view; **I.** SEM, tip of left posterior gonopod, latero-mesal view; **J.** SEM, Mesal part of left posterior gonopod, lateral view; **K.** SEM, left female vulva, posterior mesal view. at = anterior gonopod telopodite; cx = coxa; oeg = opening of efferent groove; op = operculum of vulva; pcx = coxal part of the posterior gonopod telopodite; pt = telopodital part of the posterior gonopod telopodite; st = sternal process.

Anterior gonopods (Fig. 4A, B, D, E) with a small triangular process between coxae (st), with high coxae, broad at base, gradually narrowing towards tip, distinctly constricted at middle of lateral margin, apical part flattened, curving mesad, mesal margin basally straight, curving towards tip, posterior surface folding cover more than half of telopodite. Telopodite small, directed distad, thumb-like process, with longitudinal ridge from base to tip (Fig. 4B, arrow).

Posterior gonopods (Fig. 4C, F–J) simple, rounded, with short, smooth coxal part (pcx); telopodital part (pt) twice the length of the pcx, lateral margin expanded, folding mesad, forming a concavity (not so deep as in the other two species).

Female vulvae (Fig. 4K): simple, valves prominent, the left valve slightly larger than the right valve.

DNA barcode. The GenBank accession number of the COI barcode of the paratype is OP174622 (voucher code CUMZ-D00150).

Habitat. Found under leaf litter.

Distribution. Known only from the type locality in Prachuap Khiri Khan Province, Thailand (Fig. 7).

Siliquobolellus prasankokae gen. et sp. nov.

https://zoobank.org/E6927A6E-1532-4746-9679-4391A1F4C4C9

Figs. 2, 5, 6, 7

Material studied. ***Holotype.*** 1 male (CUMZ-D00148-1), Thailand, Lampang Province, Ngao District, Pha Thai; 18°36'01.05"N, 99°54'01.00"E; 350 m a.s.l.; 28 July 2020; P. Pimvichai, P. Prasankok and S. Saratan leg.

Paratypes. 6 females (CUMZ-D00148-2); same data as holotype.

Etymology. The species honors Associate Professor Dr. Pongpun Prasankok, biologist and devoted millipede collector.

Diagnosis. Differing from other species in the genus by having the posterior gonopod apically with a short, sharp protrusion; externally differing by having a row of rectangular dark brown spots middorsally on the body rings.

Description. Adult male with 41 podous rings, 1 apodous ring. Length ~3 cm, diameter ~3 mm. Adult females with 40 or 41 podous rings, 1 apodous ring. Length ~3 cm, diameter 3.2–3.6 mm.

Color of living animal: collum and telson beige; antenna and leg light brown; dorsal part of body rings yellowish brown, with a row of rectangular dark brown spots middorsally on the body rings; head and lateral part of body rings dark brown (Fig. 2I, 6C, D).

Anterior gonopods (Fig. 5A, B, D, E) with high coxae, apically narrow, curving mesad, mesal margin sigmoid, posterior surface folding to cover more than half of telopodite, with strong ridge laterally. Telopodite with thick process, with strong ridge distomesally (Fig. 5B, arrow).

Posterior gonopods (Fig. 5C, F–J) simple, rounded, with short, smooth coxal part (pcx); telopodital part (pt) twice the length of the pcx, lateral margin folding mesad, forming a deep concavity, the inner lateral margin expanded into a rounded lamella, apically forming a canopy (Fig. 5I, arrow), apically with a short, sharp protrusion (Fig. 5C, G, arrows).

Female vulvae (Fig. 5K): simple, valves prominent, the right valve slightly larger than the left valve.

DNA barcode. The GenBank accession number of the COI barcode of the paratype is OP174623 (voucher code CUMZ-D00148).

Habitat. Found under leaf litter and inside a rotten log.

Distribution. Known only from the type locality in Lampang Province, Thailand (Fig. 7).

Key to Pseudospirobolellidae genera (based on adult males)

1 Telopodital part of the posterior gonopod forming a deep concavity; telopodite of anterior gonopod not reaching tip of coxal part of anterior gonopod; tip of anterior gonopod coxa narrowed, curving mesad ..... *Siliquobolellus* gen. nov.
– Telopodital part of the posterior gonopod simple, not forming a deep concavity; telopodite of anterior gonopod protruding above the tip of coxal part of anterior gonopod 2
2 The coxae of the 3rd male leg-pair with extremely large, protruding processes ..... *Coxobolellus* Pimvichai, Enghoff, Panha & Backeljau, 2020
– The coxae of the 3rd male leg-pair without large processes 3
3 The posterior gonopod extremely slender, sickle-shaped ..... *Pseudospirobolellus* Carl, 1912
– The posterior gonopod slender, apically with broad canopy ..... *Benoitolus* Mauriès, 1980

Key to species of the genus *Siliquobolellus* gen. nov. (based on adult males)

1 Tip of anterior gonopod coxa crossing over with tip of opposite side (Fig. 3A), telopodite of anterior gonopod flattened, apically rounded, curving backward (Fig. 3B, E) ..... *S. amicusdraconis* gen. et sp. nov.
– Tip of anterior gonopod coxa separate from that of the opposite side 2
2 Posterior gonopod telopodite apically with a short, sharp protrusion (Fig. 5C, H), mesal margin of anterior gonopod telopodite with a strong ridge (Fig. 5B, E) ..... *S. prasankokae* gen. et sp. nov.
– Posterior gonopod telopodite apically without a protrusion, anterior gonopod coxae constricted at middle of lateral margin (Fig. 4A, D) ..... *S. constrictus* gen et sp. nov.

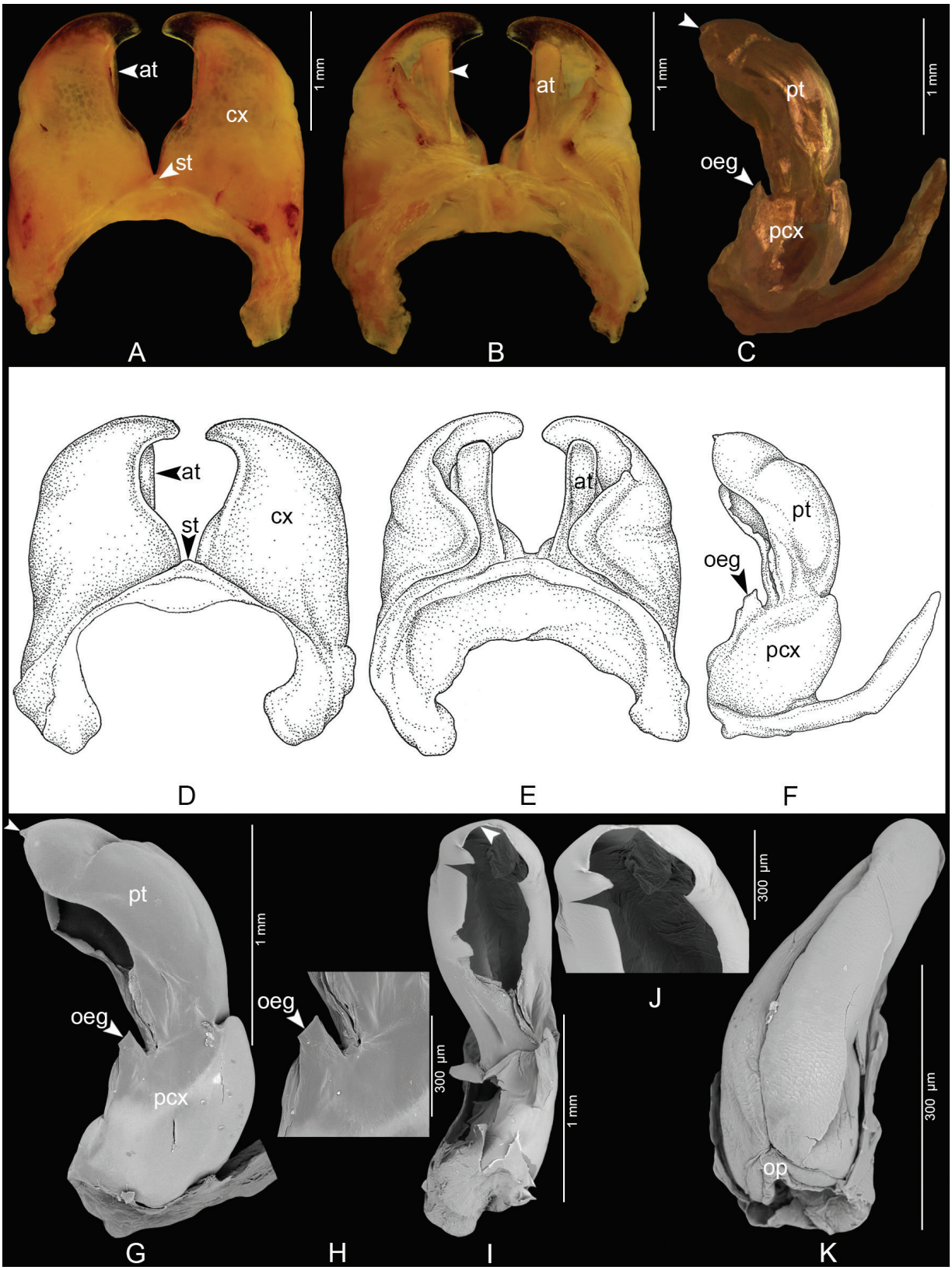

Figure 5. *Siliquobolellus prasankokae* gen. et sp. nov., holotype, gonopods (specimen from Pha Thai, CUMZ-D00148-1). **A.** Anterior gonopod, anterior view; **B.** Anterior gonopod, posterior view, arrow indicates a a thumb-like process with strong ridge distomesally; **C.** Right posterior gonopod, lateral view, arrow indicates a short, sharp protrusion; **D.** Anterior gonopod, anterior view; **E.** Anterior gonopod, posterior view; **F.** Right posterior gonopod, lateral view; **G.** SEM, Right posterior gonopod, lateral view, arrow indicates a short, sharp protrusion; **H.** SEM, Mesal part of right posterior gonopod, lateral view; **I.** SEM, Right posterior gonopod, mesal view, arrow indicates a canopy; **J.** SEM, Tip of right posterior gonopod, mesal view; **K.** SEM, left female vulva, posterior mesal view. at = anterior gonopod telopodite; cx = coxa; oeg = opening of efferent groove; op = operculum of vulva; pcx = coxal part of the posterior gonopod telopodite; pt = telopodital part of the posterior gonopod telopodite; st = sternal process.

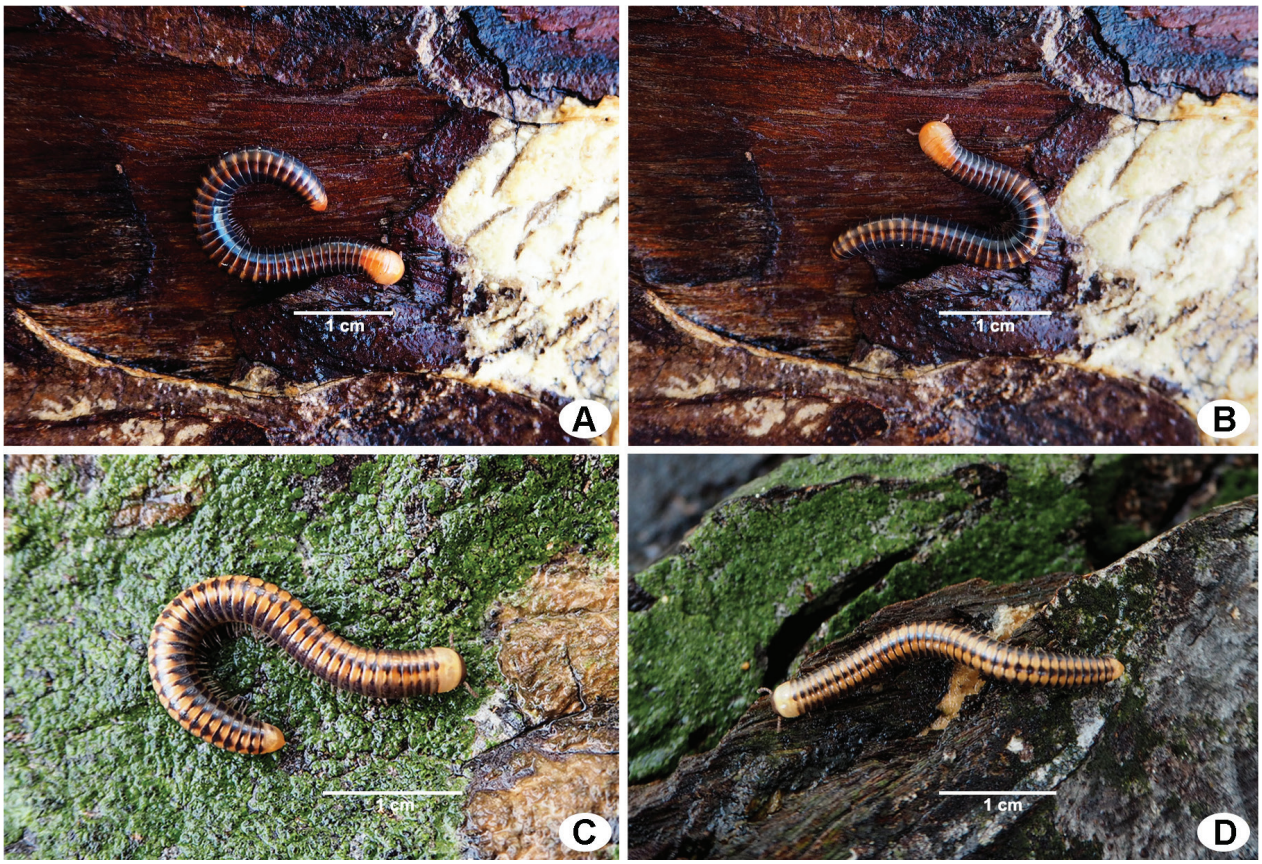

Figure 6. Live *Siliquobolellus* gen. nov. species from Thailand. **A, B.** *S. amicusdraconis* gen. et sp. nov. (from Hub Pa Tard), female (paratype, CUMZ-D00149-2); **C, D.** *S. prasankokae* gen. et sp. nov. (from Pha Thai), male (holotype, CUMZ-D00148-1).

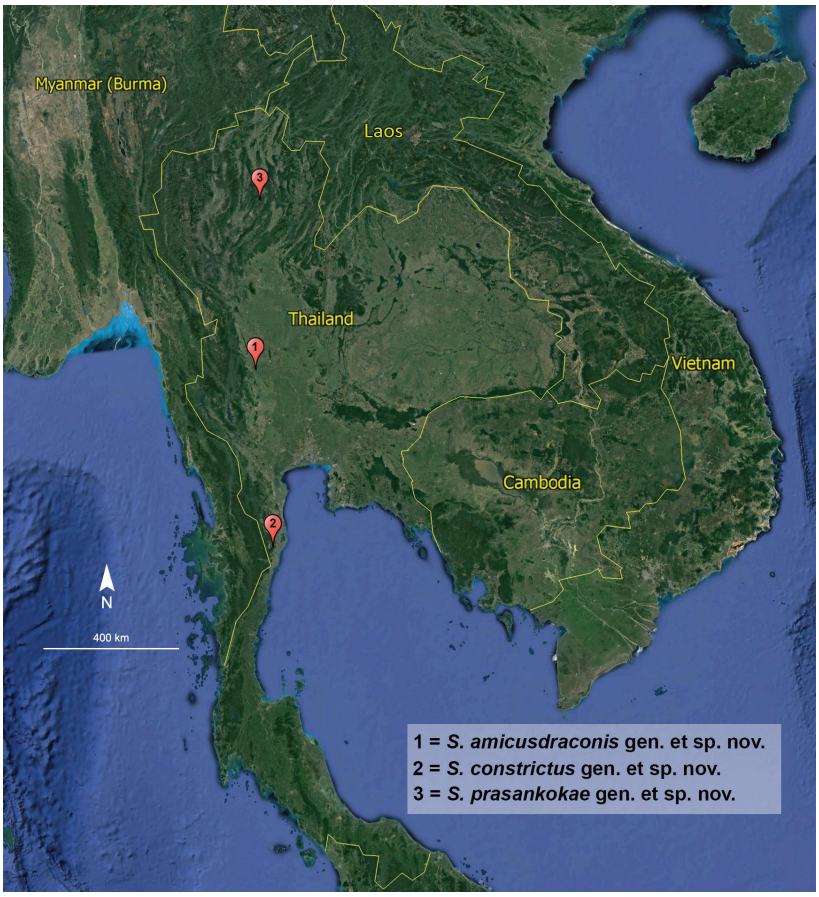

Figure 7. Geographic location of the type localities of *Siliquobolellus* gen. nov. species.

Discussion

Morphologically, the three new species clearly belong to the Pseudospirobolellidae because they share the unique characters of the family viz., (1) prefemora of male legs with large ventral soft pads, (2) posterior gonopod simple and independent of each other, (3) small and slender species, (4) anterior gonopods sternum slightly fused with coxae or present only by a small inward pleat, and (5) tracheal apodeme is articulated at the base of the coxae (Brölemann 1913; Hoffman 1981). The placement in Pseudospirobolellidae is also suggested by the phylogenetic ML and BI trees, which show a well-supported COI grouping of the three new species with the genera *Coxobolellus* and *Pseudospirobolellus*. The creation of the new genus is based on three lines of evidence: (1) the three new species share three conspicuous gonopodal synapomorphies by which they consistently differ from the other pseudospirobolellid genera, viz. (i) the telopodital part of their posterior gonopod forms a deep concavity, (ii) the telopodite of anterior gonopod is directed distad and does not reach the tip of coxal part of anterior gonopod, and (iii) the tip of the anterior gonopod coxa is narrowed, curving mesad, (2) the three new species form a well-supported, rather deeply diverging clade with respect to the genera *Coxobolellus* and *Pseudospirobolellus*, and (3) yet, this clade reveals no well-supported sister group relationship to either of these two genera, suggesting that the three new species are phylogenetically not convincingly linked to *Coxobolellus* or *Pseudospirobolellus*. Therefore, it is appropriate to assign the three new species to their own, well-defined genus, viz. *Siliquobolellus* gen. nov.

While the new pseudospirobolellid genus seems to be well-founded, it does show a remarkably high mean interspecific COI sequence divergence of 12% (range: 8–15%). Yet, this appears to be a common phenomenon in millipedes, with reported mean interspecific COI divergences between congeneric species of, for example, 11% (range: 6–15%) in *Coxobolellus* (Pimvichai et al. 2020), 14% (range: 9–17%) in *Atopochetus* Attems, 1953 (Pimvichai et al. 2018), 11% (range: 9–11%) in *Litostrophus* Chamberlin, 1921 (Pimvichai et al. 2018), 15% (range: 15–16%) in *Anurostreptus* Attems, 1914 (Pimvichai et al. 2014), 14% (range: 5–18%) in *Thyropygus* Pocock, 1894 (Pimvichai et al. 2014), and 12.9–15.9% in *Glomeris* Latreille, 1802 (Reip and Wesener 2018). Nevertheless, these high interspecific COI divergences between congeneric species are still substantially lower than many mean intergeneric COI divergences. This is well-illustrated by the four pseudospirobollelid genera whose mean intergeneric COI divergence is 20% (range: 14–24%) with *Benoitolus*, and 19% (range: 14–23%) without *Benoitolus*.

The new genus and the three new species constitute an important expansion of the family Pseudospirobolellidae, but at the same time further expose the problem of the highly divergent position of *Benoitolus birgitae* within Pachybolidae, instead of Pseudospirobolellidae (Pimvichai et al. 2020, 2022). However, its long branch and disruptive effect on the monophyly of *Litostrophus* means that the *B. birgitae* sequence may be suspected to reflect an artefact, caused by insufficient DNA and/or taxon sampling. Hence future work on pseudospirobolellid phylogeny and taxonomy will have to take this point into account, the more so as new tentative pseudospirobolellid species are lining up for description, including two undescribed species from China (Pitz and Sierwald 2010), and several *Coxobolellus*-like species from northern India (material in Natural History Museum of Denmark).

Acknowledgements

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Supplementary material 1

Table S1

Authors: Piyatida Pimvichai, Henrik Enghoff, Somsak Panha, Thierry Backeljau

Data type: excel file

Explanation note: Estimates of COI sequence divergences within and among *Siliquobolellus* gen. nov. species and related taxa expressed as uncorrected p-distances (rounded off to two decimals).

Link: https://doi.org/10.3897/zse.98.90032.suppl1