Integrative description of new giant pill-millipedes from southern Thailand (Diplopoda, Sphaerotheriida, Zephroniidae)

Trine ROSENMEJER 1, Henrik ENGHOFF 2,*, Leif MORITZ 3 & Thomas WESENER 4

1,2 Natural History Museum of Denmark, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen OE, Denmark.

3,4 Zoological Research Museum Alexander Koenig, Leibniz Institute for Animal Biodiversity, Adenauerallee 160, D-53113 Bonn, Germany.

3 Institute of Evolutionary Biology and Ecology, University of Bonn, An der Immenburg 1, 53121 Bonn, Germany.

* Corresponding author: henghoff@snm.ku.dk

1 Email: trinehansen@hotmail.com

3 Email: moritz.leif@gmail.com

4 Email: twesener@leibniz-zfmk.de

Abstract. Two new species of giant pill-millipedes, Zephronia viridisoma Rosenmejer & Wesener sp. nov. and Sphaerobelum aesculus Rosenmejer & Wesener sp. nov., are described based on museum samples from southern Thailand. Zephronia viridisoma sp. nov. comes from Khao Lak, while the type locality of S. aesculus sp. nov. is on Phuket Island. Both species are described integratively, combining light microscopy, scanning electron microscopy, multi-layer photography, micro-CT scans and genetic barcoding. Genetic barcoding was successfully conducted for holotypes of both new species, which could be added to a dataset of all published sequences of the family Zephroniidae, including all described species from Thailand, Laos and Cambodia up to 2020. Genetic barcoding of the COI gene revealed another female of S. aesculus sp. nov., 160 km east of the type locality. Both new species are genetically distant from all other Zephroniidae from Thailand and surrounding countries, showing uncorrected p-distances of 16.8–23.1%.

A virtual cybertype of a paratype of Z. viridisoma sp. nov. was created and made publically accessible.

Keywords. Cybertype, CT Scan, DNA Barcoding, biodiversity, soil fauna.

Introduction

Thailand harbours an extremely rich and highly endemic biota. This unique biodiversity, which to a high extent is associated with limestone karsts, is under threat, mainly from anthropogenic habitat destruction. There is thus a race against time to describe and protect the microendemic Thai endangered fauna (Clements et al. 2006). Millipedes (Diplopoda) constitute a conspicuous element in Thailand’s biodiversity, including some of the largest terrestrial invertebrates, as well as many strikingly coloured species; see, e.g., Enghoff et al. (2007), Srisonchai et al. (2016) and Pimvichai et al. (2018). The millipede fauna of Thailand has received considerable attention in recent years. From the time when the first species were recorded from “Siam” by Karsch (1881) and until the first comprehensive list of Thai millipedes was published by Enghoff (2005), 105 species had been recorded, but due to a massive effort by Professor Somsak Panha and his (now former) students from Chulalongkorn University in Bangkok, in collaboration with foreign specialists, the following 14 years saw a dramatic increase to 228 species (Likhitrakarn et al. 2019), and the number is still growing: with the contributions by Pimvichai et al. (2020), Wesener et al. (2021) and Likhitrakarn et al. (2021), and including the two species described here, 243 millipede species are now known from Thailand. The increased knowledge of Thai millipedes has mainly concerned the orders Polydesmida Leach, 1815, Spirobolida Bollman, 1893 and Spirostreptida Brandt, 1833. In contrast, the giant pill-millipedes, order Sphaerotheriida Brandt, 1833, have largely been neglected although they are common and apparently very diverse in Thailand. Until now, only five species of Sphaerotheriida have been recorded from Thailand, viz., Zephronia siamensis Hirst, 1907 (Hirst 1907), Z. cf. viridescens Attems, 1936 (recorded by Wongthamwanich et al. 2013), Sphaerobelum truncatum Wongthamwanich, 2012 (Wongthamwanich et al. 2012), and – very recently – Zephronia lannaensis Likhitrakarn & Golovatch, 2021, and Z. phrain Likhitrakarn & Golovatch, 2021 (Likhitrakarn et al. 2021).

The present paper is a first step of an effort to bring the knowledge of the Thai giant pill-millipede fauna up to a similar level as those of neighbouring Vietnam (e.g., Semenyuk et al. 2020) and Laos (Wesener 2019). We describe two new giant pill-millipede species from Thailand integratively, combining morphology with genetic barcodes, and creating a freely accessible cybertype for one of the new species.

Material and methods

Specimens

Details of the studied specimens, which were all hand-collected, are summarized in Table 1.

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ATOL</td>
<td>Assembling the Tree Of Life project</td>
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<tr>
<td>CT</td>
<td>computer tomography</td>
</tr>
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<td>MHNG</td>
<td>Muséum d’Histoire naturelle de la Ville de Genève, Geneva, Switzerland</td>
</tr>
<tr>
<td>NHMD</td>
<td>Natural History Museum of Denmark, University of Copenhagen, Denmark</td>
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<tr>
<td>SEM</td>
<td>scanning electron microscopy</td>
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<td>ZFMK</td>
<td>Zoological Research Museum A. Koenig, Leibniz Institute for Animal Biodiversity, Bonn, Germany</td>
</tr>
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</table>

Map

The localities of the described species were mapped in QGis ver. 3.8.0 Zanzibar (QGIS Development Team 2018).
Dissecting, light microscopy, illustrations

Dissecting and pencil drawings were done under a Zeiss Discovery V8 with a camera lucida. Illustrations were produced by hand on paper, with a camera lucida mounted on a Zeiss Discovery V8. Drawings were afterwards scanned, and digitally touched up. All illustrations and pictures were edited in Adobe Photoshop CC. The backgrounds have been smoothed out, to reduce any disruptive visuals and to make them more similar in overall appearance. All terminology follows the recent literature (Wesener 2016a).

Scanning electron microscopy

For scanning electron microscopy (SEM), the endotergum and head were dissected. The samples were cleaned and dehydrated via an ethanol series (2× 96%, 3× 100%) before being mounted on aluminum stubs and dried overnight. The stub was sputter-coated with gold in a Cressington 108 auto sputter coater. Images were obtained using a Zeiss Sigma 300 VP SEM at the ZFMK. After the study, dry coated SEM material was removed from stubs and returned to alcohol.

Micro-computed tomography

Volumetric data from a female paratype of *Zephronia viridisoma* Rosenmejer & Wesener sp. nov. (ZFMK MYR8787) in 75% ethanol was obtained using micro-computed tomography (micro-CT). Micro-CT data was obtained with a SkyScan 1272 (Bruker microCT, Kontich, Belgium) at the ZFMK using the following scanning parameters: source voltage = 60 kV, source current = 160 μA, filter = AL 0.5 mm, pixel size = 6.515728 μm, exposure = 2508 ms, rotation = 180°, rotation step = 0.1°, averaging = 7, random movement = 15, flat field correction = ON, geometrical correction = on. Digital sections were reconstructed with NRecon ver. 1.7 (Bruker microCT, Kontich, Belgium) and modified in Fiji Image J 1.52g (Schindelin et al. 2012). Volume rendering was performed in Drishti ver. 2.6.3 (Limaye 2012). The image sequence of the reconstructed micro-CT-scan, i.e., the cybertype, is deposited in Zenodo and can be accessed by https://doi.org/10.5281/zenodo.4548243. The Voucher is stored at ZFMK (ZFMK MYR8787).
DNA barcoding

In order to help with identifying related species, identifying females, and investigate the intraspecific and interspecific genetic (COI) distances, a molecular genetic barcoding study (Hebert et al. 2003) was conducted. DNA extraction, amplification, and sequencing of the cytochrome c oxidase subunit 1 (COI) gene was done as in previous studies (Sagorny & Wesener 2017; Wesener 2019), using the degenerate primer pair HCO-JJ/LCO-JJ (HCOJJ AWACTTGVGGRTGVCAAAARAATCA / LCOJJ CHACWAAYCATAAAAGATATYGG) (Astrin & Stüben 2008). BLAST searches (Altschul et al. 1997) were performed as nucleotide blast on 19 July 2019 to confirm sequence identities and to check for contaminations. Sequences were concatenated by hand or utilizing the software Seqman (DNASTAR Inc.). The whole dataset was translated into amino acids to rule-out the accidental amplification of pseudogenes. The six new sequences have been uploaded to GenBank under the accession codes MW898737–MW898742 (Table 2).

Phylogenetic and distance analyses

The six new COI sequences were added to a dataset from a previous study, containing all published sequences of the Zephroniidae Gray, 1843 (as per June 2020) in addition to far and near outgroups (Wesener 2019). All sequences were aligned in Bioedit (Hall 1999). The final dataset contained 34 terminals (Table 1) and 671 base pairs as four base pairs were excluded because of too much missing data.

The number of base differences per site between sequences (p-distances) was calculated (see Supp. file 1). Codon positions included were 1st+2nd+3rd. All ambiguous positions were removed for each sequence pair. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013). The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model (Nei & Kumar 2000). The tree with the highest log likelihood (-9133.8776) is shown (Fig. 1). The percentage of trees (based on 1000 replicates) in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. The topology with a superior log likelihood value was selected. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.566)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 36.56% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis included 34 nucleotide sequences. Codon positions included were 1st+2nd+3rd. All positions with less than 85% site coverage were eliminated. That is, fewer than 15% alignment gaps, missing data, and ambiguous bases were allowed at any position.

Results

Tree description and genetic distances between species

The gene tree might not be suitable for reconstructing deeper phylogenetic splits, but the order Sphaertheriida and the family Zephroniidae were recovered as monophyletic taxa. Genetic distances were high, interspecific distances in our dataset of Zephroniidae varied between 13.6–24.4% (Supp. file 1). The maximum likelihood tree based on the COI barcoding fragment showed low support (51%) for the family Zephroniidae, with Cryxus in a basal position to a trichotomy (Fig. 1). Bootstrap support was generally low, with only a few species-groups, like the Zephyria ‘sensu stricto’ group encompassing Z. ovalis, Z. laotica, Z. dawydoffi and Z. siamensis, receiving high support values (Fig. 1). Most Sphaerobelum species grouped together, albeit with low support. Sphaerobelum aesculus Rosenmejer & Wesener sp. nov. was retrieved in an isolated position and differed from the closest related species (Fig. 1), a Zephroniidae species (Zephroniidae_spII) of an unknown genus obtained from pet trade of Malaysia,
Table 2. Genetically analysed specimens, voucher and GenBank number. Specimens marked by an * have been newly sequenced. Abbreviations: MHNG = Muséum d’Histoire naturelle de la Ville de Genève, Geneva, Switzerland; NHMD = Natural History Museum of Denmark, University of Copenhagen; QVMAG = Queen Victoria Museum and Art Gallery, Tasmania, Australia; SMF = Senckenberg Museum Frankfurt, Germany; ZFMK = Zoological Research Museum Koenig, Bonn, Germany.

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by 17.9% (Supp. file 1). The two specimens of *S. aesculus* sp. nov., found 160 km apart (Fig. 2), differed by 6.1%. Holotype and paratype of *Zephronia viridisoma* sp. nov. showed no intraspecific distance and differed by 16.7% from the genetically closest taxon, an undescribed *Zephronia* from Aow Noi Temple (Mueang district, Prachuap Khiri Khan Province).

**Fig. 1.** The evolutionary history of the analyzed Sphaerotheriidae Brandt, 1833 inferred by using the Maximum Likelihood method analyzed with the General Time Reversible model based on the COI gene. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Codon positions included were 1st+2nd+3rd. All positions with less than 5% site coverage were eliminated. Yellow and green boxes mark the newly described species. Numbers below branches mark bootstrap values (1000 replicates). For specimen information see Table 2.
Taxonomy

Class Diplopoda de Blainville in Gervais, 1844
Order Sphaerotheriida Brandt, 1833
Family Zephroniidae Gray, 1843

Remarks
See Wesener (2016a) for a catalogue of the family.

Genus Sphaerobelum Verhoeff, 1924

Type species
Sphaerobelum clavigerum Verhoeff, 1924, from Vietnam.

Other taxa included
18 species including the one described below (Semenyuk et al. 2018, 2020; Wesener 2019; Zhao et al. 2020).

Distribution
Vietnam, Thailand, Laos, China.

Sphaerobelum aesculus Rosenmejer & Wesener sp. nov.
urn:lsid:zoobank.org:act:84D3714E-5CF8-4063-8A4D-40BF8CBEBFB0
Figs 2A, 3–5, 6A

Diagnosis
Differs from all other species of the genus Sphaerobelum in the shape of the posterior telopod, where there is a swelling at the tip of the immovable finger, but the swelling does not extend above the margin (Fig. 5B arrow). Such a swelling is currently unknown from any other giant pill-millipede species.

Derivatio nominis
Named after the horse chestnut tree Aesculus hippocastanum L., for the resemblance of the rolled-up female to a horse chestnut. Noun in apposition.

Material examined
Holotype

Other material
THAILAND • 1 ♀; Nakhon Si Thammarat Prov., Khao Luang NP; 8°43′25.2″ N, 99°40′7.7″ E, 355 m a.s.l.; 10–12 Oct. 2003; ATOL Expedition 2003 leg.; NHMD 621694.

Description (based on holotype)
Size. Length 23.8 mm. Width of thoracic shield 10.5 mm, of widest segment (9) 11.1 mm. Height of thoracic shield 6.2 mm, Height of highest segment (7) 7 mm.

Colour. Head, antenna, legs and tergites golden light brown. Posterior margin of tergites and paratergite tips medium brown. Anal shield medially brown, with lighter colour at edges (Fig. 2A).
Fig. 2. Map of Thailand, collection localities of *Sphaerobelum aesculus* Rosenmejer & Wesener sp. nov. and *Zephronia viridisoma* Rosenmejer & Wesener sp. nov., and habitus photographs: *S. aesculus* Rosenmejer & Wesener sp. nov., ♀ (NHMD 621694); *Z. viridisoma* Rosenmejer & Wesener sp. nov., paratype, ♀ (ZFMK MYR8787). Scale bars = 1 cm.
HEAD. Number of ommatidia 55. Antennae (Fig. 3A–C): Antennomere lengths: 6>1>2=3=4=5. Antennae short, barely reaching first leg pair. Sixth antennomere apically slightly swollen (Fig. 3A), number of apical cones 52/56 (Fig. 3B). Mandible: not dissected. Gnathochilarium: lingual lamella with numerous long setae, medially glabrous.

COLLUM. With few setae spread thinly along borders.

THORACIC SHIELD. Thoracic shield grooves wide and deep, with 3 sclerotized ledges along inner ridge.

TERGITES. Paratergite tips on posterior half projecting backwards. Tergites glabrous with dull leather-like surface (Fig. 3D–E). At high magnification tiny setae become visible.

ENDOTERGUM (Fig. 6A). With a regular flat margin. Outer zone with two rows of irregular marginal setae. Not extending beyond posterior margin, but reaching ⅔ of the outer area. Anterior part of marginal ridge flat. Intersegmental membrane smooth, without cones and with a row of setae with large glabrous gaps.

FIRST STIGMATIC PLATE (Fig. 4A). With a well-rounded apex.

PLEURITES. Pleurite 1 projecting posteriorly with sharp apex. Pleurite 2 with rounded apex projecting slightly.

LEGS. Ventral spines on leg 1 4/4, on leg 2 3/5, on leg 3 3/4. Apical spine absent on leg 3. Three apical and 4 or 5 ventral spines on midbody legs (Fig. 4C). Inner margin of femur with 8–12 small teeth, but not excavated. Femur 2, tarsus 4 times as long as wide.

ANAL SHIELD. Well-rounded and glabrous. Ventral side with a single small locking carina, placed ⅓ from pleurite.

MALE GONOPORE. Located at mesal margin of coxa (Fig. 4B), large, covered by a membranous plate.

ANTERIOR TELPODS (Fig. 4D–F). Podomere 1 rectangular, as long as wide, covered with setae anteriorly, with extra long setae medially, and posteriorly glabrous. Podomere 2 with long immovable finger visible laterally in anterior view. Finger curving inwards against podomere 3. Podomere 3 cylindrical twice as long as wide. Suture between podomere 3 and 4 barely visible in posterior and lateral views. Podomere 4 very short, just ¼ of length of podomere 3, with one short and dark spine on apex.

POSTERIOR TELPODS (Fig. 5A–B). Podomere 1 as long as wide, covered in setae anteriorly, and nearly glabrous posteriorly. Podomere 2 with wide immovable finger. Immovable finger straight, only slightly tapering towards apex, bearing an oval membranous spot on posterior side. The membrane between podomere 2 and 3 with single membranous lobe, angled. Podomere 3 with sparse setation. Podomere 3 ca 3 times longer than wide, towards immovable finger with membranous ledge, a single spine, posterior side with row of 9 or 10 crenulated teeth. Podomere 4 short, straight, ca 2.5 times as long as wide, with a short membranous ledge and 2 spines towards immovable finger.

**Female from Khao Luang NP**

Length 45 mm. Width of thoracic shield 23 mm, of widest segment (9) 24.6 mm. Height of thoracic shield 12.6 mm, of highest segment (8) 15.2 mm. Antennae reaching leg pair 2. Apical cones on antennae 43/34. Subanal plate well rounded. Vulvae (Fig. 5C): small, with a narrow operculum (Op), only reaching bottom part of prefemur. External lateral plate (EP) and inner mesal plate (IP) almost completely fused.
Fig. 3. *Sphaerobelum aesculus* Rosenmejer & Wesener sp. nov., holotype, ♂ (NHMD 621693), scanning electron micrographs. **A.** Right antenna, lateral view. **B.** Right antenna, disc. **C.** Detail of apical cone. **D.** Tergite surface of midbody ring. **E.** Tergite surface at very high magnification. Abbreviations: ac = apical cone; sb = sensilla basiconica.
Fig. 4. *Sphaerobelum aesculus* Rosenmejer & Wesener sp. nov., holotype, ♂ (NHMD 621693), drawings.  
A. First left coxa with stigmatic plate, posterior view.  
B. Second left coxa with gonopore, posterior view.  
C. Left leg 9, posterior view.  
D. Left anterior telopod, posterior view.  
E. Left anterior telopod, anterior view.  
F. Left anterior telopod, lateral view on podomeres 2–4.  
Abbreviations: as = apical spine; cl = claw; Cx = coxa; Fe = femur; Go = gonopore; Po = postfemur; Pre = prefemur; St = stigmatic plate; Ta = tarsus; Ti = tibia; vs = ventral spines. Scale bars = 1 mm.
Fig. 5. *Sphaerobelum aesculus* Rosenmejer & Wesener sp. nov. drawings. A–B. Holotype, ♂ (NHMD 621693), C. ♀ (NHMD 621694). A. Left posterior telopod, anterior view. B. Left posterior telopod, posterior view, arrow points to swelling of immovable finger. C. Left second coxa and prefemur with female vulva. Abbreviations: Cx = coxa; EP = external, lateral plate; IP = inner, mesal plate; Op = operculum; Pre = prefemur. Scale bars = 1 mm.
Distribution
If the female from Khao Luang (Fig. 2A) is indeed conspecific with the male holotype, the species appears to have a wide area of distribution stretching from Phuket Island at least 160 km to the east (Fig. 2). Unpublished data from a larger inventory project (see, e.g., Wesener et al. 2021) of giant pill-millipedes in the surroundings of Krabi, half way between Phuket Island and Khao Luang, did not recover this species among the numerous specimens, hinting at a patchy distribution and specific microhabitat requirements of *S. aesculus* sp. nov.

Genus *Zephronia* Gray, 1832

Type species
*Zephronia ovalis* Gray, 1832.

Other taxa included
44 species, including the one described below (Wesener 2016a, 2019; Semenyuk et al. 2018, 2020).

Distribution
NE India, Nepal, Myanmar, with a few species also in SE Asia.

Remark
Likhitrakarn et al. (2021) provided an updated diagnosis of this genus including the presence of several apical tarsal spines. The new species described here necessitates a slight modification, as it has only one apical tarsal spine.

Fig. 6. Endoterga of midbody tergites, scanning electron micrographs. A. *Sphaerobelum aesculus* Rosenmejer & Wesener sp. nov., holotype (NHMD 621693). B. *Zephronia viridisoma* Rosenmejer & Wesener sp. nov., holotype, ♂ (NHMD 621695). Abbreviations: cp = cuticular patterns; iA = inner area.
Zephronia viridisoma Rosenmejer & Wesener sp. nov.
urn:lsid:zoobank.org:act:272E06C8-2DE5-4722-A7D5-D85002D68424
Figs 2B, 6B–12

Diagnosis
Posterior telopod typical for the genus, not differing from those of other Zephronia species. Small (25–28 mm long) green species (Fig. 2B), surface appearing glabrous, dull, with a single medium sized locking carina at the anal shield and a strongly projecting pleurite 1 (Fig. 9A). One of the few Zephronia species with just a single apical spine on the legs (Fig. 9B), differing in this character from all other described Thai Zephronia species which have 2–5 apical tarsal spines. Male antennomere 6 swollen (Fig. 7A) but not axe-shaped, with <50 apical cones. Endotergum with three dense rows of long marginal setae (Fig. 6B). Palpi of gnathochilarium with sensory cones arranged in clusters (Fig. 8C–D). Anterior telopod podomere 3 with an elevated process at posterior side carrying sclerotized teeth. Podomere 4 short and narrow.

Derivatio nominis
Named after the overall green colour of living individuals of the species, noun in apposition.

Material examined

Holotype
THAILAND • ♂; Nakhon Si Thammarat Province, Sichon District, Khao Lark Waterfall; 9°03′6″ N, 99°47′24″ E; 25 Aug. 2007; Chulalongkorn University expedition of millipede workshop leg.; dense jungle on limestone; NHMD 621695.

Paratypes
THAILAND • 2 ♂♂, 4 ♀♀; same collection data as for holotype; NHMD 621696 • 1 ♂; same collection data as for holotype; ZFMK MYR8786 • 1 ♀; same collection data as for holotype; CT scan voucher; ZFMK MYR8787.

Description (based on holotype)

SIZE. Length 25.6 mm. Width of thoracic shield 12.1 mm, of widest segment (8) 13 mm. Height of thoracic shield 7.1 mm, of highest segment (8) 7.7 mm.

COLOUR. Head medium brown, faded from green. Antennae medium brown. Legs medium brown, tarsal claws apically dark brown. Tergites light brown, faded green medially, with darker brown posterior margin. Paratergite tips medium brown with darker edges. Anal shield dark green, edges faded to brown (Fig. 2B).

HEAD (Fig. 7A–C). Number of ommatidia 50 (Fig. 7B). Organ of Tömösváry placed midway between ocelli and antennal groove (Fig. 7A), with its typical coral-like inner structure (Fig. 7C).

ANTENNAE (Fig. 7A, D). Reaching leg pair 3, 6 visible antennomeres. Antennomere lengths: 6>1>2=3>4=5. Antennomere 6 apically swollen, number of apical cones 32/44.

EPIPHARYNX (Fig. 8A). With an extraordinarily large inner tooth.

MANDIBLE (Fig. 8E–F). Inner tooth 3-cusped. Pectinate lamellae with 6 or 7 rows of teeth. Condylus at anterior margin with two ridges.
Fig. 7. *Zephyronia viridisoma* Rosenmejer & Wesener sp. nov., paratype, ♂ (ZFMK MYR8786), head, scanning electron micrographs. **A**. Lateral view. **B**. Right eye. **C**. Organ of Tömösváry. **D**. Left antenna, lateral view. Abbreviations: Ant = antenna; IL = incisura lateralis; O = ommatidia; TO = organ of Tömösváry.
**Fig. 8. Zephyronia viridisoma** Rosenmejer & Wesener sp. nov., paratype, ♂ (ZFMK MYR8786), mouthparts, scanning electron micrographs. **A.** Epipharynx, ventral view. **B.** Gnathochilarium, underside. **C.** Gnathochilarium frontal view at lateral palps and central pads. **D.** Detail of sensory cones at lateral palps. **E.** Right mandible, dorsal view. **F.** Right mandible, mesal view. Abbreviations: 3iT = 3-combed inner tooth; Co = condylus; cP = central pads; Endo = endochilarium; eT = external tooth; iA = inner area; LL = lamellae linguales; LP = lateral palpi; Me = mentum; Mp = molar plate; pL = pectinate lamellae; St = stipes; T = tooth of epipharynx.
Fig. 9. *Zephyronia viridisoma* Rosenmejer & Wesener sp. nov., paratype, ♂ (ZFMK MYR8786), legs and pleurite (laterotergite), scanning electron micrographs. A. First pleurite. B. Left midbody leg, posterior view. C. Leg, femur, detail of sclerotized teeth. D. Surface of midbody tergite. Abbreviations: Cx = coxa; Fe = femur; Led = ledge; Po = postfemur; Pr = protuberance; Pre = prefemur; Sp = small triangular spines; Ta = tarsus; Tib = tibia.
Gnathochilarium (Fig. 8B–D). Lamellae linguales with numerous long setae, medially glabrous. Stipes and mentum with numerous long setae in a regular pattern. Sensory cones on gnathochilarium palps in clusters (Fig. 8D).

Collum. With short setation, evenly distributed across surface.

Thoracic shield. Thoracic shield grooves wide and deep, with 9 sclerotized ledges along inner ridge of grooves.

Tergites (Fig. 9D). Tergites glabrous, with dull orange skin like surface. At high magnification tiny setae and knobs become visible. Paratergite tips on posterior half projecting backwards (Fig. 12A–B).

Endotergum (Fig. 6B). With a regular flat margin. Outer zone with three dense rows of irregular marginal setae, some of them extending beyond posterior margin. A single row of rounded cuticular impressions present next to marginal ridge. Intersegmental membrane smooth, without cones and with very few setae.

Stigmatic plate (Fig. 10A). First plate with a rounded sub-triangular apex. More posterior plates similar to those of other representatives of the family, half-covered by the pleurites (Fig. 12C).

Pleurites (Figs 9A, 12D). Pleurite 1 projecting strongly, with sharp apex. Pleurite 2 projecting slightly less than 1 and with more rounded apex (Fig. 12D). Projection absent from pleurite 3 (Fig. 12D).

Legs. Ventral spines on leg 1 1/1, on leg 2 3/2, on leg 3 4/4. Apical spine on leg 3 absent. A single apical spine and 5 or 6 ventral spines on midbody legs (Fig. 9B). Femur regularly shaped, 2.2 times longer than wide, sclerotized ledge of medium length, inner margin apically with 5 or 6 small rounded triangular spines on ventral side (Fig. 9B–C). Tarsus 4.2 times longer than wide.

Anal shield. Well-rounded and glabrous. Ventral side with dark coloured. A medium-sized locking carina placed slightly closer to pleurite than margin (Fig. 12D).

Male gonopore (Fig. 10B). Inconspicuous, consisting of large membranous opening located directly at mesal margin.

Anterior telopods (Fig. 10C–E). With long setae medially on the first 2 podomeres, covering posterior part on podomere 2. Podomere 1 rectangular, as long as wide. Podomere 2 in anterior view as wide as but slightly narrower than podomere 1. Immovable finger barely visible in anterior view (Fig. 10D), short, not protruding up to podomere 4 (Fig. 10C). Podomere 3 long and wide, with rounded projection on posterior side, bearing 3 crenulated teeth. Podomere 4 very narrow and short, with 3 large spines near apex, and a smaller spine near edge to podomere 3.

Posterior telopods (Fig. 10F–G). Podomere 2 setose, immovable finger straight, apically tapering, with rows of small circular sclerotized spots. Membranous lobe between podomere 3 and 4 bearing 2 elongated processes, fused at base. Podomere 3 with 8 or 9 small crenulated teeth on posterior side. Membranous lobe on podomere 3 with single spine. Podomere 4 slightly curved towards immovable finger, membranous lobe with 2 spines. Setation on podomere 2 primarily on anterior side.

Description of female paratype
Length 25.6 mm. Width of thoracic shield 12.3 mm, of widest tergite (8) 13.7 mm. Height of thoracic shield: 8.4 mm, of highest tergite (8) 9.3 mm. Antennae reaching leg pair 3. Apical cones left/right:
Fig. 10. *Zephronia viridisoma* Rosenmejer & Wesener sp. nov., holotype, ♂ (NHMD 621695), drawings. A. Coxa of first left leg with stigmatic plate, posterior view. B. Coxa of second left leg with gonopore, posterior view. C. Left anterior telopod, lateral view on podomeres 2–4. D. Left anterior telopod, anterior view. E. Left anterior telopod, posterior view. F. Right posterior telopod, posterior view. G. Right posterior telopod, anterior view. Abbreviations: cr-t = crenulated teeth; Cx = coxa; Go = gonopore; St = stigmatic plate. Scale bars = 1 mm.
25/25. Subanal plate well rounded. Vulvae (Fig. 11): with a large pointed operculum (Op). External lateral plate (EP) and inner mesal plate (IP) fused at bottom.

Another female paratype was used for microcomputed tomography. The resulting volume rendering is shown in Fig. 12. While this approach revealed no significant additional details it serves to illustrate morphology of the specimen in an excellent way, much better than any photograph.

Discussion

Identity and biogeography of the genus *Sphaerobelum*

*Sphaerobelum aesculus* sp. nov. is only tentatively placed in the genus *Sphaerobelum* as it differs in some characters from the other species of the genus. The immovable finger of the posterior telopod in *S. aesculus* sp. nov. is only weakly swollen, and the swelling is not projecting above the telopod margins (Fig. 5B) in contrast to all other species of *Sphaerobelum* (see Wongthamwanich et al. 2012). *Sphaerobelum aesculus* sp. nov. represents the southern-most known occurrence for the genus, with other species of *Sphaerobelum* having been collected at least 1000 km further north. *Sphaerobelum aesculus*

**Fig. 11.** *Zephyronia viridisoma* Rosenmejer & Wesener sp. nov., paratype, ♀ (NHM D621696), drawings. A. Second right coxa and prefemur with vulva, posterior view. Abbreviations: Cx = coxa; Op = operculum; Pre = prefemur. Scale bar = 1 mm.
Sphaerobelum aesculus \textit{sp. nov.} differs clearly from the giant pill-millipede genera occurring further south, \textit{Tigridosphaera} Jeekel, 2000, \textit{Sphaeropoeus} Brandt, 1833 and \textit{Castanotherium} Pocock, 1895. \textit{Sphaerobelum aesculus} \textit{sp. nov.} differs clearly from all known \textit{Castanotherium} species in which the posterior telopod only consists of three joints, not four as in the new species described here. \textit{Sphaerobelum aesculus} \textit{sp. nov.} differs from \textit{Sphaeropoeus} (see Wesener 2016b) as well as \textit{Tigridosphaera} (see Jeekel 2000; Enghoff \textit{et al.} 2015; Wesener 2016a) in details of the anterior telopod. In \textit{Tigridosphaera} the last podomere of the anterior telopod is extending backwards producing a sharp process (very similar to the species of \textit{Prionobelum}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure12.png}
\caption{\textit{Zephyronia viridisoma} Rosenmejer & Wesener \textit{sp. nov.}, paratype, \(\varphi\) (ZFMK MYR8787), volume rendering based on micro-computed tomography. Stored as cybertype under https://doi.org/10.5281/zenodo.4548243. \textbf{A.} Habitus, lateral view. \textbf{B.} Habitus, ventro-lateral view. \textbf{C.} Habitus, ventral view. \textbf{D.} Body-ring architecture, cross section through midbody-ring. Abbreviations: \textit{Ant} = Antennae; \textit{As} = anal shield; \textit{ts} = thoracic shield; \textit{Col} = collum; \textit{Cx} = Coxa; \textit{Fe} = femur; \textit{Gn} = gnathochilarium; \textit{Go} = gonopore; \textit{IL} = incisura lateralis; \textit{Lc} = locking carina; \textit{O} = ommatidia; \textit{Pl} = pleurite; \textit{Po} = postfemur; \textit{Pp} = prothorax; \textit{Pre} = prefemur; \textit{Pt} = paratergite; \textit{st} = stigma, \textit{Ta} = tarsus; \textit{Te} = tergite; \textit{Tib} = tibia; \textit{Tr} = tracheal apodeme; \textit{ts} = thoracic shield. Not to scale.}
\end{figure}
Verhoeff, 1924 (see Mauriès 2001 for a revision), while in Sphaeroptoeus the third podomere of the anterior telopod has a characteristic process (see Wesener 2016b for a revision). Genetically, S. aesculus sp. nov. does group, albeit weakly supported (Fig. 1), with a specimen of an unknown genus from Malaysia. Therefore, S. aesculus sp. nov. is either a distant branch of Sphaerobelum, with potentially additional species occurring in southern Thailand or Malaysia, or belongs to an as yet undescribed genus.

**Intra- and interspecific distances of the COI gene**

The barcoding studies of Zephroniidae giant pill-millipedes are currently biased towards interspecific distances, as only a few intraspecific sequences, often from the same locality, are available. Therefore any interpretation of intraspecific genetic variation in species of the Zephroniidae is currently difficult. The 6.1% p-distance in the COI barcoding gene observed between the two different populations of S. aesculus sp. nov. are certainly high for intraspecific distances, higher than the up to 5% observed in the geographically widespread European pill millipede species Glomeris marginata (Villers, 1789) and G. klugii Brandt, 1833 (Reip & Wesener 2018; Wesener & Conrad 2016). Only future studies, once females of the species become available from the type locality, or male specimens from Khao Luang, will show if the 6.1% correspond to intra- or interspecific distances. The observed interspecific distances of the two new Thai Zephroniidae species towards related species are slightly higher (minimum 16.8%) than those observed in giant pill-millipedes from Madagascar, where the species of Sphaeromimus de Saussure & Zehntner, 1902 show interspecific distances of 8.3–20.8% (Wesener et al. 2014; Moritz & Wesener 2017), while the interspecific distances in the less well-sampled genus Zoolamba are 9.1–20% (Sagorny & Wesener 2017; Wesener & Anilkumar 2020; Wesener & Sagorny 2021). One reason behind the large interspecific distances in our two new species from southern Thailand might be that their closest relatives have not been discovered or sampled yet.

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**Supplementary material**

**Supp. file 1.** Estimates of Evolutionary Divergence between Sequences. The number of base differences per site from between sequences are shown. The analysis involved 34 nucleotide sequences. Codon positions included were 1\textsuperscript{st} + 2\textsuperscript{nd} + 3\textsuperscript{rd}. All ambiguous positions were removed for each sequence pair. There were a total of 674 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura *et al.* 2013). https://doi.org/10.5852/ejt.2021.762.1457.4821