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Discovery of another fern-feeding group of moths: the larvae of Hoploscopini (Insecta: Lepidoptera: Pyraloidea) from Borneo

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Abstract. We report the discovery of Hoploscopini larvae (Lepidoptera: Crambidae: Heliothelinae) on ferns at the southern slopes of Mount Kinabalu (Sabah, Borneo). The COI barcode of the larvae assigns them to the genus *Hoploscopa*. We provide the first detailed description of the larval stage for this tribe. Among Crambidae, these larvae are most similar to Crambinae larvae but differ in the presence of two L setae on A9, a character state that is present in Acentropinae and Schoenobiinae. We discuss the presence and distribution of L setae on A9 in Crambidae. Our observations of these larvae on this host plant and published host plant data support our hypothesis that larvae of the entire tribe Hoploscopini may be fern-feeders.

Key words. larva, chaetotaxy, *Hoploscopa*, fern, DNA barcoding

INTRODUCTION

Hoploscopini is comprised of two genera, *Hoploscopa* Meyrick, 1886 and *Perimeceta* Turner, 1915, with a total of 20 described and more than 60 undescribed species in the Oriental Region, Wallacea, New Guinea and northern Queensland (Robinson et al., 1994; Nuss, 1998, 1999; Nuss et al., 2003–2016). Hoploscopini are still poorly studied, and preimaginal stages as well as larval food plants were unknown until very recently (Miller et al., 2015), but had not been assigned to this tribe. The nocturnal adults are recorded from mountainous habitats and are attracted by artificial light. The forewing length of the moths ranges from 7 to 10 mm in *Hoploscopa* and from 11 to 13 mm in *Perimeceta*. Forewing colouration is reddish-brown with various markings of diagonal stripes, ellipses, or silvery spots (Robinson et al., 1994; Nuss, 1998).

The classification of the group is somewhat controversial. Robinson et al. (1994) established the Hoploscopini within Scopariinae without an explanation for doing so, but Nuss (1998) pointed to the lack of synapomorphies supporting this grouping. Later, Hoploscopini were included in Heliothelinae based on the conspicuous, inwardly directed spine in the corpus bursae of the female genitalia (Nuss, 1998, 1999). The Heliothelinae, originally established as a tribe within Scopariinae (Amsel, 1961) and later elevated to subfamily rank by Minet (1982), were subsequently synonymised with Scopariinae by Munroe & Solis (1998) and retained in synonymy by Solis & Maes (2003). Future phylogenetic analyses may show whether one of these or even another classification might be supported.

The objective of this paper is to record the discovery of five hoploscopine larvae on fern fronds at Mount Kinabalu (Sabah, Borneo) and to compare these findings with the available information of food plants of Hoploscopini.

MATERIAL & METHODS

Five larvae were found on Mount Kinabalu at an altitude of 1,680 m during the night of 13 June 2015 sitting on the undersides of fern fronds, which were unfolded and unwebbed. They were taken with the plants on which they were found for rearing purposes down to Kota Kinabalu at sea level, where rearing efforts were continued using fern species from the lowlands. None of the larvae accepted this alteration in food, climate, and elevation, and all the larvae died. Two of the larvae were kept in 96% natural ethanol for subsequent morphological and genetic analyses and are deposited at the University Museum of Bergen, Norway.

Genetic analysis was performed by extraction of DNA from the whole body of one of the larvae using Qiagen's DNEasy blood & tissue kit. PCR amplification of the DNA barcoding region of the mitochondrial cytochrome C oxidase subunit I (COI) gene was done using the primers LCO (Folmer et al., 1994) and Nancy (Wahlberg & Wheat, 2008) in combination with a universal T7/T3 tail (Wahlberg & Wheat, 2008). We used 25 µl of PCR volume containing 0.75u TaKaRa Ex Taq Hot-Start DNA polymerase, 2.5 µl 10 × buffer, 400 nM of each primer, 800 nM dNTP mix and 2 µl DNA extract.

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Cycling conditions were as follows: initial denaturation for 5 min at 95°C, 40 cycles with (1) 30 s at 95°C, (2) 30 s at 48°C, (3) 90 s at 70°C, final extension of 10 min at 70°C. PCR success was evaluated via gel electrophoresis on a 1% agarose gel using GelRed (Biotium). For clean-up of the successfully amplified PCR products, 0.5u of each the Exonuclease (Exo) and Shrimp Alkaline Phosphatase (SAP) enzymes were added to 8 µl of PCR product and the mixture was incubated in a thermocycler for 15 min at 37°C before inactivating the enzymes for 30 min at 80°C. The sequencing PCR was performed with BigDye, using 160 nM of T7/T3 sequencing primers and 0.5–2 µl PCR product. Sequencing was done at the Sequencing Facility, University of Bergen.

The alignment of the DNA sequence data was done with PhyDE version 0.9971 (Müller et al., 2010). MEGA 7 (Kumar et al., 2016) was used to find the best-fitting DNA model, which resulted in the GTR+G+I model. A Maximum Likelihood (ML) analysis of the sequence data was done with RAxML 7.4.2 (Stamatakis, 2006), using the raxmlGUI 1.3 interface (Silvestro & Michalak, 2012). The ML analysis included a bootstrap test with 1,000 replications. The resulting ML tree was edited in TreeGraph version 2.11.1-654 beta (Stöver & Müller, 2010). We used the BIN numbers of the Barcode of Life Database (BOLD, http://v4.boldsystems.org; Ratnasingham & Hebert, 2007) as DNA-barcoded taxa may not have been identified to species level.

In addition to the "BC MTD" Barcode samples provided by MN, BOLD was mined for further relevant records. Due to the different opinions regarding the classification of Hoploscopini we included Scopariinae in our search.

After DNA extraction, the exoskeleton of the larva was cut laterally, flattened, and preserved together with the head capsule in Euparal on a microscopic slide for further examination. The second larva was left intact in order to study the length and direction of the setae. Terminology of larval morphology, especially chaetotaxy, follows Hasenfuss (1963). Thoracic segments are abbreviated as T1–T3, abdominal segments as A1–A10. Drawings were done using Adobe Illustrator CS6, version 16.0.3.

The larval food plant was identified using Raciborski (1898) and Beaman & Edwards (2007).

RESULTS

A total of five individuals of larvae were found on fern fronds at 1,680 m altitude on the southern slope of Mount Kinabalu in Mesilau (see Fig. 1 for two of the larvae). Weather conditions were cloudy, but not rainy, with high humidity and temperature at about 20°C.

Material examined. Two larvae: Malaysia, Sabah, Mount Kinabalu National Park, Mesilau, western edge of Mount Kinabalu Golf Club, 6°01′38″N 116°35′32″E, 1,680 m, 13.vi.2015, leg. Théo Léger & Richard Mally (University Museum of Bergen, Norway).

Fig. 1. Two *Hoploscopa* larvae (centre and right) on the underside of a fragment of their food plant, *Dicranopteris linearis* (Burman, 1768) Underwood, 1907. Scale: one square measures 5 mm.

Molecular identification of the larva. Sequencing of one of the larvae yielded a 655 bp fragment of the 5′ part of the COI gene. A search for similar sequences with the NCBI nucleotide blast tool resulted in a closest match with three specimens of "Scopariinae sp." originating from Papua New Guinea (Miller et al., 2015). The corresponding images of these specimens on BOLD allowed us to identify these three and another seven specimens, altogether forming four barcode-species, as belonging to the genus *Hoploscopa*, and the information was corrected accordingly in the BOLD database.

Subsequently, we analysed all sequences available for *Hoploscopa* and *Perimeceta* available to us on BOLD in a ML analysis. The species whose larva we describe here is sister to the species pair *Hoploscopa* AAU5240 + *Hoploscopa* AAU5241, both from North Sumatra (Sumatera Utara), in the ML tree (Fig. 3). The adult *Hoploscopa* specimens that we collected in Mount Kinabalu National Park (see Fig. 2 for representatives) appear as five separate species (BOLD BINs ADE1420, ADE3896, ADE3897, ADE4123 and ADE4125 in Table 1 and Fig. 3). The larvae could not be matched to any of the adults of the 15 DNA-barcoded species of *Hoploscopa* (Fig. 3).

Perimeceta, the other genus of Hoploscopini with four species included in this analysis, is sister to *Hoploscopa* in the ML tree (Fig. 3).

All included COI barcode sequences (Table 1) are publicly available on NCBI's GenBank and the European Nucleotide Archive (ENA) via the accession numbers as well as on BOLD.

Morphological description of the larvae. The larvae are identified as Pyraloidea by the presence of two setae in the prespiracular group of the prothorax and three subventral setae on abdominal segments 3 to 6 as well as by the crochets forming a complete circle (Solis, 2006).

In the larval key on European Pyraloidea by Hasenfuss (1960), our larvae are identified as Crambidae ("Crambinae" sensu Hasenfuss, 1960), but they match none of the treated subgroups in this family. The closest similarity is found with Crambinae ("Crambini" sensu Hasenfuss, 1960), agreeing

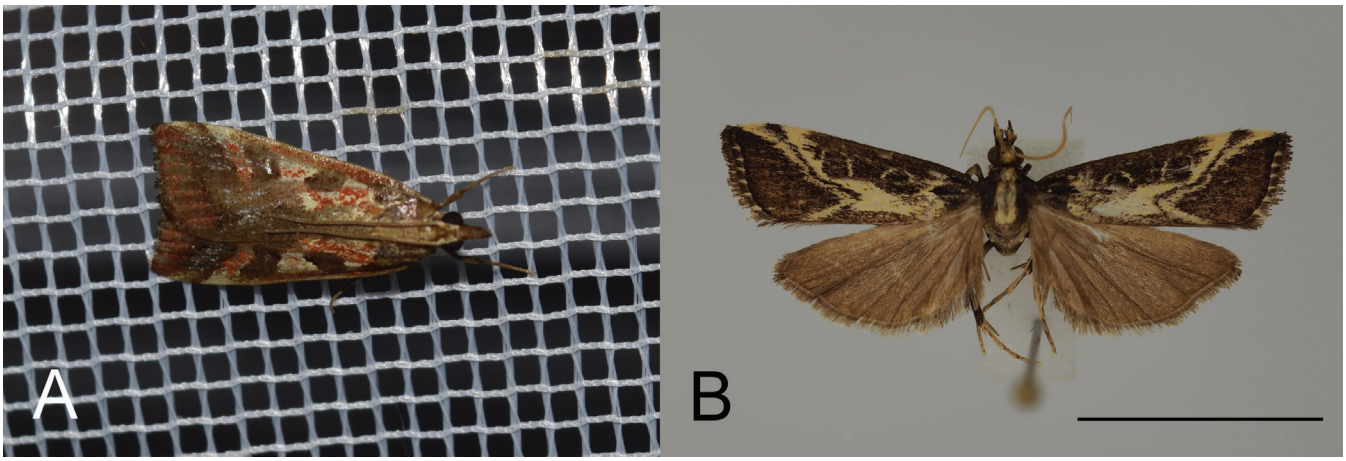

Fig. 2. Adult specimens of *Hoploscopa* from Mount Kinabalu. A, an unidentified specimen taken on 05 June 2015 at night by T. Léger near Mount Kinabalu park headquarters; B, specimen BC MTD Lep 03004 of *Hoploscopa* ADE1420 from the genetic analysis, abdomen removed for DNA extraction and genitalia dissection. Note that neither of the two adult specimens is conspecific with the described larvae. Scale: A, one mesh square measures 1.2 × 1.2 mm; B, scale bar measures 10 mm.

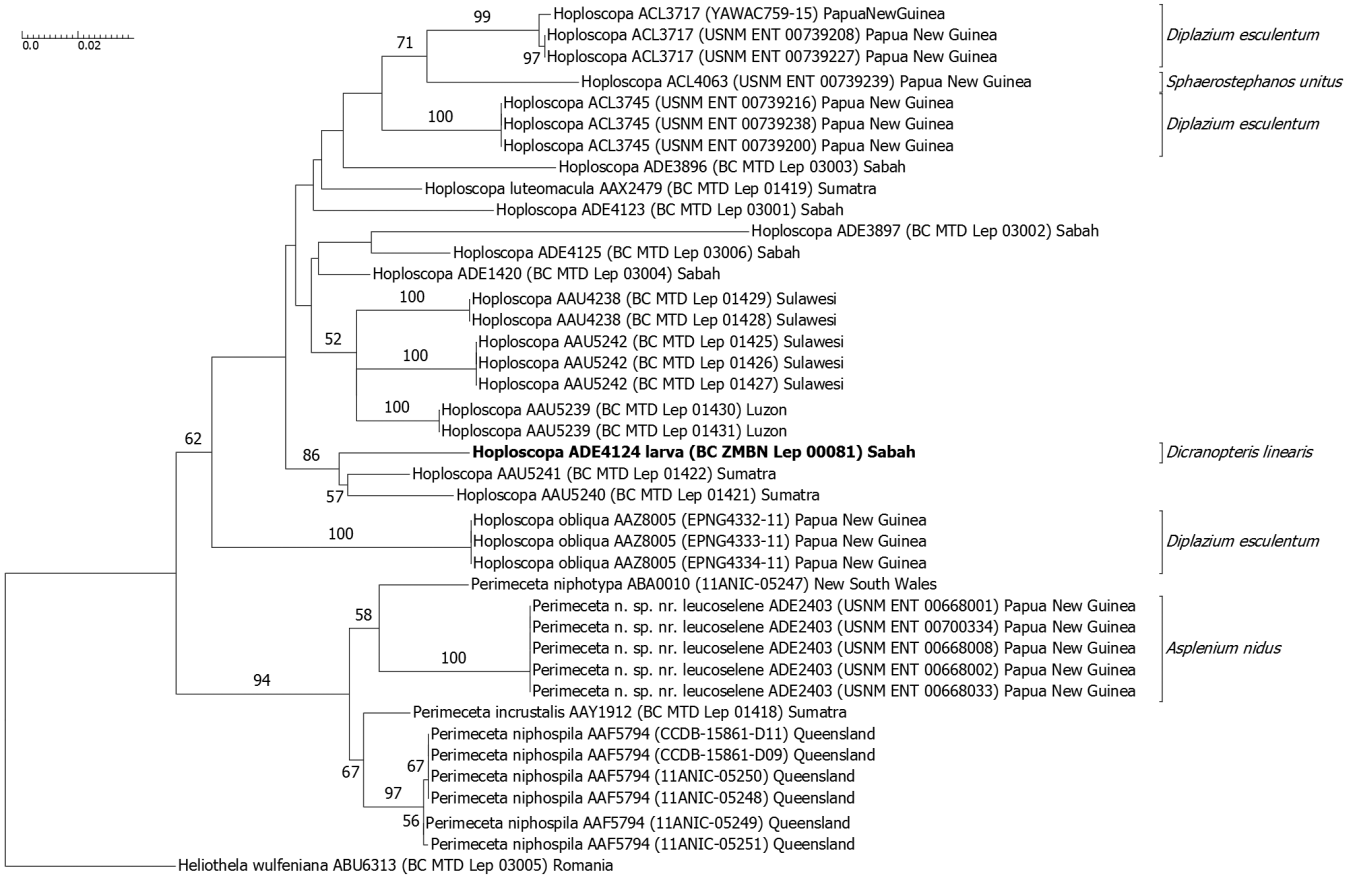

Fig. 3. Maximum likelihood tree of Hoploscopini DNA barcodes (549–654 bp), based on the GTR+G+I model, with *Heliothela wulfeniana* (Heliothelini; 509 bp) as outgroup and the sequenced larva from Mount Kinabalu marked in bold. BOLD BIN numbers are indicated behind the taxon names, followed by the BOLD Sample ID in brackets, and the geographical origin of the sample. Known host plants are indicated on the right; the numbers above the branches represent bootstrap values ≥ 50% based on 1,000 bootstrap replicates. Scale bar represents number of substitutions per site.

in these character states with Hasenfuss' (1960) diagnosis: epicranial index 1–1.7; adfrontals span max half of the length of the coronal suture; AF2 more ventral than P2; stemma 5 posterodorsal of stemma 6; stemmata 4–6–5 in a sharp to right angle; O3 ventral of line connecting stemmata 4 and 5; O3 considerably closer to SO3 than to O2; O1–O2–O3 in a sharp to right angle; A1 with XD2–SD1–SD2 in a right to obtuse angle; L2 macroscopic on meso- and metathorax; abdominal legs normal developed, crochets with two to three hook rows; A1–8 with distance D2–D2 considerably longer than D1–D1, and L1 posterodorsal of L2; on A8 the stigma is located caudal of the lines connecting D1 and SD1 as well as SD1 and L1; A9 with D1 near to and anterodorsally to DS1, and D2 of both sides on a single pinaculum; well developed

Table 1. Samples of DNA barcoded specimens included in this study. For taxa without species identification the respective BOLD BIN number was used as species epithet.

Taxon	BOLD BIN (No. of Sampled Specimens)	Geographical Origin	GenBank Accession Number	BOLD Sample ID
Heliothela wulfeniana	ABU6313 (n = 1)	Romania	KY080439	BC MTD Lep 03005
Hoploscopa luteomacula	AAX2479 (n = 1)	Indonesia, Sumatra	KX843698	BC MTD Lep 01419
Hoploscopa obliqua	AAZ8005 (n = 3)	Papua New Guinea	KX783025 KX783026 KX783027	USNM ENT 00665932 USNM ENT 00514750 USNM ENT 00514731
Hoploscopa ADE4124	ADE4124 (n = 1)	Malaysia, Sabah	KY080442	BC ZMBN Lep 00081
Hoploscopa AAU5242	AAU5242 (n = 3)	Indonesia, Sulawesi	JN272552 JN272553 JN272554	BC MTD Lep 01425 BC MTD Lep 01426 BC MTD Lep 01427
Hoploscopa AAU5239	AAU5239 (n = 2)	Philippines, Luzon	JN272557 JN272558	BC MTD Lep 01430 BC MTD Lep 01431
Hoploscopa AAU4238	AAU4238 (n = 2)	Indonesia, Sulawesi	JN272555 JN272556	BC MTD Lep 01428 BC MTD Lep 01429
Hoploscopa ADE4125	ADE4125 (n = 1)	Malaysia, Sabah	KY080444	BC MTD Lep 03006
Hoploscopa ADE1420	ADE1420 (n = 1)	Malaysia, Sabah	KY080440	BC MTD Lep 03004
Hoploscopa AAU5240	AAU5240 (n = 1)	Indonesia, Sumatra	JN272550	BC MTD Lep 01421
Hoploscopa AAU5241	AAU5241 (n = 1)	Indonesia, Sumatra	JN272551	BC MTD Lep 01422
Hoploscopa ADE4123	ADE4123 (n = 1)	Malaysia, Sabah	KY080441	BC MTD Lep 03001
Hoploscopa ADE3896	ADE3896 (n = 1)	Malaysia, Sabah	KY080445	BC MTD Lep 03003
Hoploscopa ACL3745	ACL3745 (n = 3)	Papua New Guinea	KP850086 KP850401 KP850535	USNM ENT 00739216 USNM ENT 00739238 USNM ENT 00739200
Hoploscopa ACL4063	ACL4063 (n = 1)	Papua New Guinea	KP850867	USNM ENT 00739239
Hoploscopa ACL3717	ACL3717 (n = 3)	Papua New Guinea	KP850187 KP850609 KX842727	USNM ENT 00739208 USNM ENT 00739227 YAWCATCR0759
Hoploscopa ADE3897	ADE3897 (n = 1)	Malaysia, Sabah	KY080443	BC MTD Lep 03002
Perimeceta incrustalis	AAY1912 (n = 1)	Indonesia, Sumatra	KX843699	BC MTD Lep 01418
Perimeceta niphospila	AAF5794 (n = 6)	Australia, Queensland	KF388782 KF391745 JN272547 JN272548 KF390107 KF392415	11ANIC-05248 11ANIC-05249 11ANIC-05250 11ANIC-05251 CCDB-15861-D09 CCDB-15861-D11

Taxon	BOLD BIN (No. of Sampled Specimens)	Geographical Origin	GenBank Accession Number	BOLD Sample ID
Perimeceta niphotypa	ABA0010 (n = 1)	Australia, New South Wales	KF391291	11ANIC-05247
Perimeceta sp. near *leucoselene*	ADE2403 (n = 5)	Papua New Guinea	KY034067	USNM ENT 00668001
			KY034068	USNM ENT 00668002
			KY034066	USNM ENT 00668008
			KY034070	USNM ENT 00668033
			KY034069	USNM ENT 00700334

anal plate; A10 with distance II–II smaller than or equal to II–III, IIIa macroscopic. Our larvae differ from Hasenfuss' (1960) diagnosis of Crambinae in these character states: AF2 lateral of bifurcation of epicranial suture (instead of more dorsal than bifurcation); A1 with only two SV setae (instead of three); A9 with L2 present (instead of absent); distance V1–V1 on A10 larger than on A9 (instead of smaller); and on A10 distance V1–VIId smaller than VIIc–VIIb (instead of larger).

Head. (Fig. 4) Orthognathous, brown; epicranial suture present; vertex with microsetae V1–3 in a line; pore Va variable in position, slightly lateral between V2 and V3 or lateral of V3; front with P1 close to AF1, P2 between P1 and V1, pore Pb ventral of P2, pore Pa in the centre of P1, L1 and A3; AF1 slightly dorsal of the centre of adfrontal area, AF2 at level of lower end of central suture, pore AFa between AF1 and AF2, closer to AF2; F1 ventral of AFa halfway of dorsoventral expanse of the frontal area, pore Fa medioventral of F1; ventral clypeus margin slightly undulated, C1 on lateral end, C2 halfway between C1 and sagittal plane dorsal of slight ventrad protrusion; A1, A2 and A3 in an arched line, distance A2–A3 approximately twice the distance A1–A2; L1 central on lateral head, pore La posterodorsal of L1; microseta G1 at level of P1 and L1, pore Ga anteroventral of G1; six stemmata in an oval semicircle, O1 in its centre, O2 posterior of stemma 1, O3 well posterior of stemma 6, pore Oa posteroventral of stemma 6; SO1 ventral of stemma 5 posterior of antennal socket, SO2 ventral of stemma 6, SO3 ventral of pore Oa, pore SOa anterior of SO3.

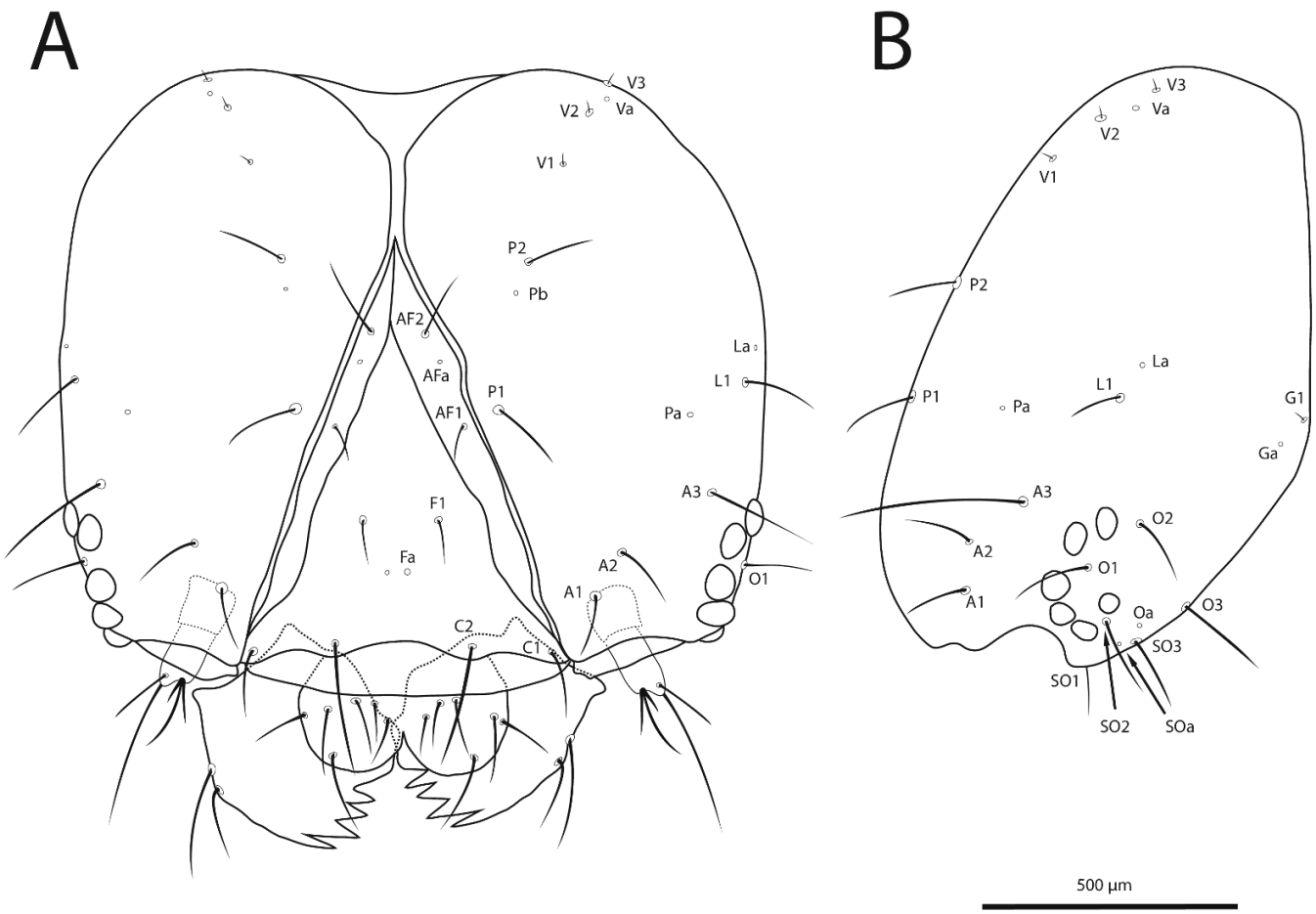

Fig. 4. Chaetotaxy of the postcranial body, sinistral view. Chaetal terminology after Hasenfuss (1963). Abbreviations: s.pp., seta paraproctalis.