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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

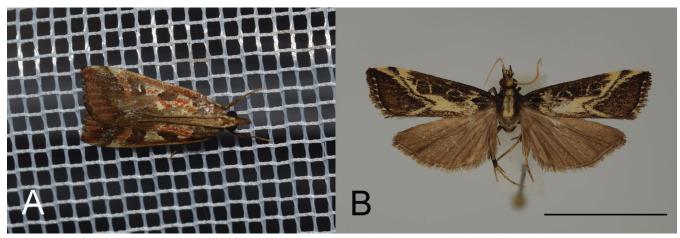


Fig. 2. Adult specimens of Hoploscopa from Mount Kinabalu. A, an unidentified specimen taken on 05 June 2015 at night by T. Léger near Mount Kinabalu park headquarters; B, specimen BC MTD Lep 03004 of Hoploscopa ADE1420 from the genetic analysis, abdomen removed for DNA extraction and genitalia dissection. 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.

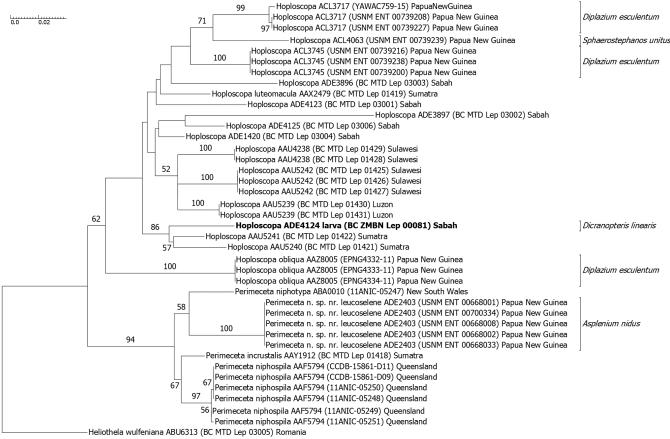


Fig. 3. Maximum likelihood tree of Hoploscopini DNA barcodes (549–654 bp), based on the GTR+G+I model, with *Heliothela wulfeniana* (Heliothelini; 509 bp) as outgroup and the sequenced larva from Mount Kinabalu marked in bold. BOLD BIN numbers are indicated behind the taxon names, followed by the BOLD 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 \geq 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

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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 KY034068 KY034066 KY034070 KY034069	USNM ENT 00668001 USNM ENT 00668002 USNM ENT 00668008 USNM ENT 00668033 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.

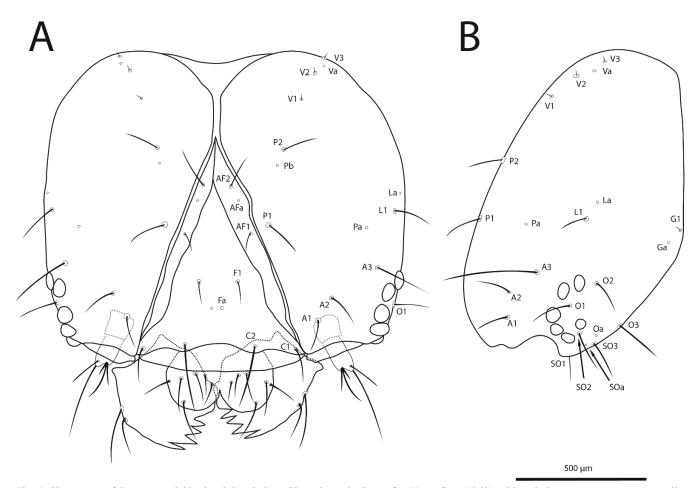


Fig. 4. Chaetotaxy of the postcranial body, sinistral view. Chaetal terminology after Hasenfuss (1963). Abbreviations: s.pp., seta paraproctalis.

Thorax. (Fig. 5) Prothoracic and prespiracular shield as well as dorsal, subdorsal and lateral pinacula strongly sclerotised, black, subventral and ventral pinacula less so. Prothoracic shield with roughly equally long D1 and D2, XD1, XD2, SD1 and SD2, three pores (a-c) present, pore a posterodorsal and pore b posterior of XD1, pore c dorsal of XD2, microseta MXD1 at posterior edge of prothoracic shield between D1 and D2; prespiracular shield anterior to spiracle bisetose (L1–2), extending posteroventrally around spiracle; subventral pinaculum bisetose (SV1-2); coxa of prothoracic leg with five setae, MV2 microseta on anterodorsal end of coxal band, MV3 microseta between the two anterior coxal macrosetae, a third microseta posterodorsally close to posterior coxal macroseta; V1 posteroventral of leg. T2 and T3 identical: bisetose dorsal and subdorsal pinacula (D1+D2, SD1+SD2), D2 about twice the length of D1; MD1 microseta anterior of D2, MSD1-2 microsetae anteroventral of subdorsal pinaculum; two lateral pinacula with anterior bisetose (L1+L2) and the posterior unisetose (L3), posterior lateral pinaculum including L3 is missing on one body side in one of the two investigated specimens; subventral pinacula unisetose (SV1); coxal band with MV2 and MV3 as in prothorax, plus MV1 microseta anterodorsally of MV2; V1 posteroventral of leg.

Abdomen. (Fig. 5) A1-8: Two unisetose (D1, D2) dorsal pinacula and the unisetose (SD1) subdorsal pinaculum strongly sclerotised, subventral pinacula less so; D1, D2 and SD1 about the same length; MD1 microseta anterior to D2 on anterior segment edge; SD2 microseta anterior of spiracle; two lateral pinacula below spiracle, anterodorsal one bisetose (L1-2), the posteroventral unisetose (L3), setae of approximately equal length; subventral group bisetose (SV1, SV3) in A1 and A7, trisetose (SV1-3) in A2 and A3-6 (at anteriodorsal base of prolegs), and unisetose (SV1) in A8 and A9, SV1-2 are absent on left side of A4 in one larva; V1 at posteromedial end of abdominal segments; MV3 microseta on anterior segment edge between subventral group and V1. A9 with a large unpaired dorsal pinaculum covering the dorsum, bearing one long seta (D2) on each side; subdorsal pinaculum trisetose (D1, SD1, L2), with SD1 in a less strongly sclerotised semicircular part of pinaculum;

MD1 on anterior segment edge between dorsal and subdorsal pinaculum; lateral pinaculum unisetose (L1); one subventral (SV1) and one ventral (V1) seta, with MV3 on anterior segment edge between SV1 and V1. Unpaired anal shield (segment A10) with four setae (I, II, III, IIIa) on each side, seta I being the shortest; short Seta paraproctalis (S.ppr.) posteroventral of anal shield on dorsal tip of a lanceolate, weakly sclerotised area; V1 close to ventral base of terminal legs. Prolegs on A3–6 with crochets in a complete circle of three concentric rows of outward-directed hooks, the outer circle bearing the shortest hooks and the inner circle the longest; prolegs on A10 forming a semicircle.

Food plant records. The larvae were found on *Dicranopteris linearis* (Burman, 1768) Underwood, 1907 (Gleicheniaceae; Fig. 6). The larvae were sitting on the underside of intact fronds, which did not show any traces of frass. In captivity, they fed on this plant species from the outer edge of the pinnate leaves towards the mid-ribs (Fig. 1). After we left the collecting area at 1,680 m we continued to feed the caterpillars with non-Gleicheniaceae ferns growing in the lowland of Kota Kinabalu, but these were rejected and the larvae died.

The search for related species on BOLD resulted in the recognition of five barcoded Hoploscopinae species collected in Yawan, Papua New Guinea which are found to feed on ferns as well: *Hoploscopa* ACL3745, *Hoploscopa* ACL3717 and *Hoploscopa* obliqua (Rothschild, 1915) feed on *Diplazium esculentum* (Retzius in Retzius & König, 1791) Swartz, 1803 (Athyriaceae), *Hoploscopa* ACL4063 feeds on *Sphaerostephanos unitus* (Linnaeus, 1759) Holttum, 1794 (Thelypteridaceae), and *Perimeceta* sp. near *leucoselene* (Hampson, 1919) feeds on *Asplenium nidus* Linnaeus, 1753 (Aspleniaceae) (S. Miller, C. Redmond & T. Whitfield, pers. comm.; Botanical Research Institute of Texas, 2003–2009).

DISCUSSION

All known larval host plant records for Hoploscopinae belong to six species, and all are included in our analysis (Fig. 3). The larvae are feeding on fern species of

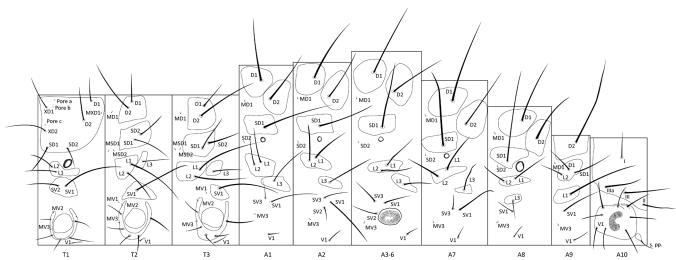


Fig. 5. Chaetotaxy of the postcranial body, sinistral view. Chaetal terminology after Hasenfuss (1963). Abbreviations: s.pp., seta paraproctalis.



Fig. 6. *Dicranopteris linearis* (Burman, 1768) Underwood, 1907, the food plant of the investigated *Hoploscopa* larvae; photo taken at the collecting site of the larvae.

Gleicheniaceae (Gleicheniales), Aspleniaceae, Athyriaceae or Thelypteridaceae (all three Polypodiales). These repeated findings from ferns as food plants suggest that Hoploscopini are fern feeders. This fern-feeding habit contrasts with the feeding habit of the supposed sister-group Heliothelini. For one of its species, Heliothela wulfeniana (Scopoli, 1763), the angiosperm genera Viola Linnaeus, 1753 (Violaceae) and Mentha Linnaeus, 1753 (Lamiaceae) are recorded as larval food plants (Nuss, 2005). Other fern-feeders among Crambidae are known in Musotiminae (e.g. Munroe, 1972; Kirk, 1978; Phillips & Solis, 1996; Solis et al., 2004, 2005a, 2005b; Yen et al., 2004), in Phenacodes Turner, 1937, treated in Scopariinae by Munroe (1958) and Munroe & Solis (1998), and provisionally placed in Cybalomiinae by Nuss (1999), and in the Spilomelinae Herpetogramma sphingealis Handfield & Handfield, 2011, H. aeglealis (Walker, 1859) and *H. platycapna* (Meyrick, 1897) (Kirk, 1978; Solis, 2008; Handfield & Handfield, 2011), Diasemiopsis ramburialis (Duponchel, 1833) (Farahpour-Haghani et al., 2016) and Udea decrepitalis (Herrich-Schäffer, 1848) (Lhomme, 1935).

Based on the diagnostic characters of the chaetotaxy provided by Hasenfuss (1960), the Crambinae are the group that shares the most larval similarities with *Hoploscopa*. One character that differs in *Hoploscopa* is the presence of an L2 seta on A9, grouped on a pinaculum together with D1 and SD1. Among Crambidae, a second L seta on A9 is yet only known to occur in Acentropinae (Hasenfuss, 1960, 1963) and Schoenobiinae (Passoa & Habeck, 1987; Passoa, 1988). This character state may turn out to be a synapomorphy for a group comprising Acentropinae, Schoenobiinae and Hoploscopini. A comprehensive comparison of the chaetotaxy of all crambid subfamilies is currently not possible, as descriptions of larvae of Cathariinae, Lathrotelinae, Linostinae, Midilinae, and Heliothelini are still lacking.

The molecular data analysis reveals 16 DNA barcode-species for *Hoploscopa*. This is precisely the number of species so far described in the genus, but some known species are recorded

from other geographical places (Nuss et al., 2003–2016) and therefore at least some of them are probably not conspecific with those included in our analysis. This, as well as the long terminal branches of some taxa in our analysis, point to a large proportion of still undescribed species in *Hoploscopa*, and in fact Robinson et al. (1994) already report more than 50 undescribed species of *Hoploscopa* based on material in entomological collections. The inability to link the larvae from Mount Kinabalu with any of the collected adults during the same field trip suggests that different *Hoploscopa* species on Mount Kinabalu may have different flight times, or species may not be attracted by artificial light. Future study of altitudinal and chronological occurrence as well as of food plant usage in Hoploscopini will contribute to our understanding of speciation in this still poorly studied group.

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