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Nepticulidae from the Amazon rainforest: first discovery of females of the enigmatic genus *Dvidulopsis* and description of the *Acalypha*-feeding *Stigmella mariusi* sp. nov.

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Upon the first discovery, this publication provides the results of the examination and documentation of the female genitalia of *Dvidulopsis* Stonis & Diškus, an endemic Neotropical genus previously known only from male specimens. It also describes *Stigmella mariusi* Stonis & Diškus, sp. nov., a previously overlooked *Acalypha*-feeding species of Nepticulidae from the western pre-montane Amazon.

Keywords: *Acalypha* L., Ecuador, leaf mines, Neotropical fauna, new species, pygmy moths

INTRODUCTION

Pygmy moths (Nepticulidae) are among the smallest lepidopterans in the world, currently comprising about 1,035 species globally. Their larvae are predominantly leaf miners, although some mine in stems, bark, or buds, and occasionally induce galls.

Based on the South African and Northern European faunas, excellent characterisations of the family were provided by Scoble (1983) and Johansson et al. (1990). Initial attempts to review the Neotropical Nepticulidae were undertaken by Puplesis & Robinson (2000) and van Nieukerken

et al. (2016), and an updated illustrative review was later presented in the monograph *Neotropical Nepticulidae* by Stonis et al. (2022). The latter publication also provides numerous references that have steadily updated the list of Neotropical Nepticulidae over the last decade.

Despite these recent reviews, several additions to the Neotropical fauna have since been documented. These include thirteen new species of *Stigmella* Schrank and *Acalyptris* Meyrick from Honduras (Stonis et al., 2024, 2025b, 2025c), one new species from the Yungas province of Peru (Stonis et al., 2025c), and the introduction of a new genus, *Dvidulopsis* Stonis & Diškus, along with one new Central American species described in this genus (Stonis et al., 2025a).

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However, despite the description of these fifteen new species from Central America and the Peruvian tropical Andes, the species composition in the Amazon has remained unchanged when compared with the most recent list by Stonis et al. (2025a), in which all Nepticulidae species known so far from the Amazon were already listed and mapped.

The recently described genus *Dvidulopsis* was erected primarily on the basis of earlier-described species previously assigned to an exclusive group within *Acalyptaris*, the *A. latipennata* species group (Stonis, Diškus, 2018). *Dvidulopsis* is a distinctive taxon, characterised by remarkable features of the male genitalia: the uncus and gnathos are fully divided (paired) or at least partially divided; the valva bears an inner (median) process or lobe; the phallus lacks cornuti but possesses three distinctive, elongated carinae; and the phallus tube is basally constricted. Molecular data from new mtDNA COI-5' sequences further support the genus as a distinct phylogenetic lineage: in published phylogenetic trees (Stonis et al., 2025a), sequenced species of *Dvidulopsis* clearly differ from *Acalyptaris* and *Fomoria* Beirne, forming a sister clade to these two genera (*Acalyptaris* + *Fomoria*).

The genus, currently comprising eight species, is endemic to lowland tropical humid forests, typically occurring at elevations of 10–400 m. Half of the known species are equatorial, inhabiting the humid forests of the Amazon Basin, while the remainder are found in the humid forests of Central America. In the paper erecting *Dvidulopsis* (Stonis et al., 2025a), it was noted that the genus appears to be rare: all known species occur at low densities and are therefore difficult to encounter. Consequently, it is perhaps unsurprising that many species of the genus were described from singletons and remain known only from such material. This may also explain why no female specimens of the genus have been described until now.

Given the unique morphology of the male genitalia and the distinctive molecular evidence, it might be expected that the female genitalia also exhibit features differing from those of re-

lated genera and are potentially unique within the group.

Owing to forthcoming structural changes, we carried out a comprehensive and detailed inventory of all collection materials deposited at the Biosystematics Research Group and the Nature Research Centre (transferred from the former Lithuanian University of Educational Sciences, LEU), including material temporarily held on loan from other institutions. During this inventory, two previously neglected and unidentified female specimens were discovered. Although these specimens had been collected in 2000 from the lowland Amazon Basin (Yasuní National Park, Ecuador), they remained unpublished and now appear to belong to the recently described genus *Dvidulopsis*. In addition, one unidentified *Stigmella* species from the Amazonian province of Ecuador was detected.

The aim of the present study was to examine and provide the first photographic documentation of the female genitalia of *Dvidulopsis*, and to describe one new *Stigmella* species, a previously overlooked taxon of *Acalypha*-feeding Nepticulidae from the western premontane Amazon.

MATERIALS AND METHODS

The material examined in this study, including the specimens forming the basis for the description of the new species and the first documentation of *Dvidulopsis* females, derives from fieldwork carried out in Ecuador by J. R. Stonis (formerly Rimantas Puplesis) and S. R. Hill. These collections were made during collaborative research projects with Ecuadorian partners, beginning with the 2000 fieldwork conducted in collaboration with Professor Giovanni Onore, the Ecuadorian counterpart at the Pontifical Catholic University of Ecuador (PUCE), Quito.

Protocols for field collecting, species identification, and descriptive procedures generally follow Puplesis (1994); Puplesis, Robinson (2000); and Diškus, Stonis (2022). Abdomens were macerated in 10% KOH, cleaned, and dissected. Male genital capsules were removed

from the abdominal pelt and mounted with the ventral side uppermost; the phallus was not separated from the genital capsule. Mounts of female genitalia were prepared in the same manner as those of males, following procedures outlined in Stonis et al. (2022), although female genitalia and abdominal pelts were stained with Chlorazol Black (Direct Black 38 / Azo Black).

Permanent slides were examined and photographed using a Leica DM2500 compound microscope equipped with a Leica DFC420 digital camera. Terminology for morphological structures follows Puplesis, Robinson (2000), except that the terms 'aedeagus' and 'cilia' are here replaced by 'phallus' and 'fringe', respectively.

Abbreviations for institutions and depositories. BRG – Biosystematics Research Group, currently based at the NRC, Vilnius, Lithuania; LEU – Lithuanian University of Educational Sciences, Vilnius, Lithuania (formerly abbreviated as VPU), now closed, with scientific collections transferred to ZMUC (Zoological Museum, Natural History Museum of Denmark, Copenhagen) or MfN, or temporarily held by the BRG and NRC; MfN – Museum für Naturkunde, formerly known as the Museum für Naturkunde der Humboldt-Universität zu Berlin, Germany; NHMUK (formerly BMNH) – the Natural History Museum, London, United Kingdom; NRC – State Scientific Research Institute Nature Research Centre, Vilnius, Lithuania; PUCE – Pontificia Universidad Católica del Ecuador, Quito, Ecuador.

RESULTS

Discovery and first documentation of *Dvidulopsis* females

(Figs 1, 2)

Material examined. 1 ♀, Ecuador, Napo Region, SE of Coca, Yasuní National Park, near Río Tiputini, Amazon humid forest (rainforest), 260 m, at light, 15–25.i.2000, leg. R. Puplesis & S. R. Hill, genitalia slide no. AD0331 (formerly VPU, now transferred to NRC); 1 ♀, same label data, genitalia slide no. AD0332 (formerly VPU, now transferred to NRC).

Thorax and basal two-fifths of the forewing dark grey-brown, glossy, with green and purple iridescence; from other angles, thorax and forewing base appear glossy metallic grey. Along the costa, forewing with a pale brownish-yellow stripe. Apically, forewing with two partially merging (coalescent) shiny metallic-grey patches showing strong purple iridescence; a few dark brown scales occur between these patches. Dark black-brown scales form a distinct oblique shadow anterior to the apical markings. Fringe pale grey, glossy, with scattered dark brown scales; fringe line absent. Forewing underside pale ochreous brown with light purple iridescence, lacking spots or androconia. Hindwing pale ochre to greyish ochre (depending on angle), upper side and underside without androconia. Legs glossy golden ochre.

Female genitalia (Fig. 2). Total length 920 µm (genitalia slide no. AD0332) to 935 µm (genitalia slide no. AD0331). Abdominal apex 240–250 µm wide, only slightly rounded, almost truncated. Both anterior and posterior apophyses slender and relatively short (approx. 105–110 µm). Vestibulum slightly folded, without a vaginal sclerite. Corpus bursae elongated, oval, bearing two long signa that occupy nearly the entire length of the corpus bursae; signa composed of distinctive, cell-like structures. Ductus spermathecae with 2.5–3 coils and an irregular, lobate vesicle; proximal part of ductus spermathecae sclerotised and strongly folded in genitalia slide no. AD0332.

Remarks. The external description and photographs are based entirely on the specimen associated with genitalia slide no. AD0331. The second specimen, bearing genitalia slide no. AD0332 but collected at the same locality and on the same date, differs slightly in external appearance, probably due to some scale rubbing and forewings looking somehow worn-out. The female genitalia of both specimens agree in most structural features, although the proximal part of the ductus spermathecae shows notable differences (see Fig. 2a–c vs. Fig. 2d–f). For this reason, it cannot be stated with full confidence whether the two examined females represent a single species or quite possibly two closely related species.



Fig. 1. Habitats and the female adult of *Dvidulopsis* sp. discovered in the Amazon rainforest: a, b – collecting locality in Yasuní National Park, near Río Tiputini; c–g – details of the adult photographed under different illumination and from different angles of view

The wide forewing and the characteristic scaling pattern observed in the studied female specimens fully correspond with the general appearance of *Dvidulopsis*. When compared with all known representatives of the genus, the two females may represent an undescribed species – or potentially two species – within *Dvidulopsis*. A comparison of these females with the males of the known *Dvidulopsis* spe-

cies revealed no exact match, not even with those whose males were collected in the same or neighbouring localities in the Amazon.

Both females were collected in Amazonian rainforest in eastern Ecuador at an elevation of approximately 260 m (Fig. 1a, b). Adults appear to be active in January and can be attracted to light. No further biological information is available; the host plant and larval mine remain unknown.

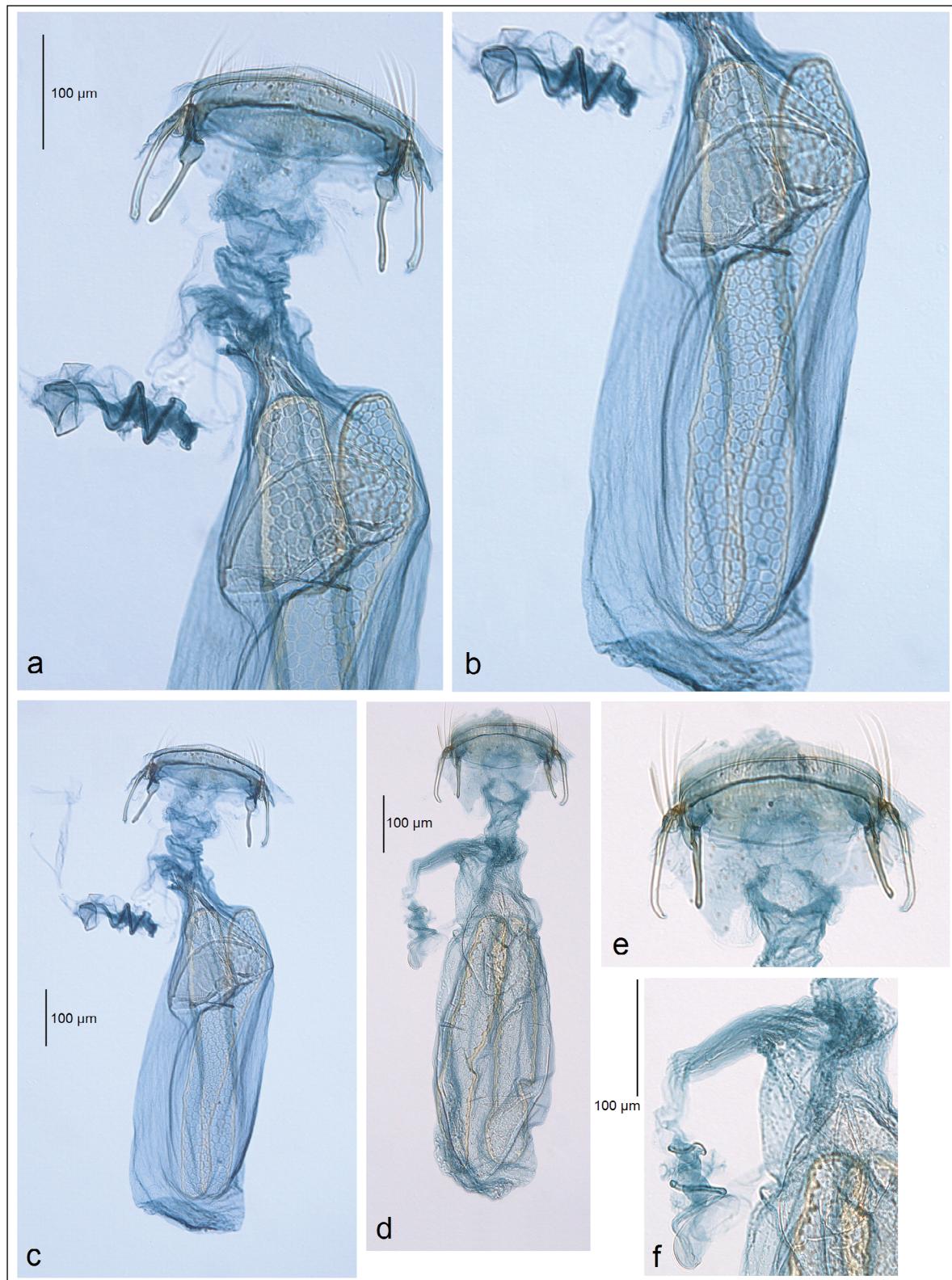


Fig. 2. First documentation of the female genitalia of *Dvidulopsis* Stonis & Diškus: a – genitalia slide no. AD0331, abdominal tip, apophyses, ductus spermathecae, and caudal half of corpus bursae; b – same, corpus bursae; c – same, general view; d – genitalia slide no. AD0332, general view; e – same, abdominal tip and apophyses; f – ductus spermathecae (sclerotised proximally)

Description of *Stigmella mariusi* Stonis & Diškus, sp. nov.

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(Figs 3–5)

Type material. Holotype: ♂, Ecuador, Napo Province, 17 km SE of Tena, Misahualli, right bank of Río Napo, 400 m, 22–31.i.2001; larvae on *Acalypha* sp., sample no. 4740 (BRG), leg. R. Puplesis; genitalia slide no. AD0345 (currently BRG, with subsequent transfer to MfN).

Diagnosis. *Stigmella mariusi* sp. nov. belongs to the *S. marmorea* group, one of the largest (and possibly paraphyletic) species groups in the Neotropics. Externally and in the male genitalia, the new species appears most closely related to another equatorial, *Acalypha*-feeding species, *S. montanotropica* Puplesis & Diškus, 2002 (described and illustrated by Puplesis et al. 2002: 23, figs 5, 29–30) (see Remarks).

Although in thoracic scaling and forewing colouration adults of the new species resemble several other *Stigmella* species with uniformly coloured, shiny brown thoraces and forewings lacking a transverse fascia, *S. mariusi* sp. nov. is readily distinguished from all previously described Neotropical *Stigmella*, including *S. montanotropica*, by the presence of a distinctive glossy cream apical spot on the forewing (Fig. 3d, e).

In the male genitalia, *S. mariusi* sp. nov. is easily separated from all other *Stigmella* species (including *S. montanotropica*) by the presence of a unique, large, distally rounded, horn-like cornutus. Additional diagnostic features include a clearly bilobed uncus with relatively wide lateral lobes, a weakly developed second apical process of the valva, and characteristic clusters of large, spine-like cornuti surrounding the unique horn-like cornutus (Fig. 4).

The leaf mines of *S. mariusi* sp. nov. are rather distinctive (Fig. 5a–d), though they may be easily confused with the very similar mines of *S. montanotropica*, another *Acalypha*-feeding species.

Male. Forewing length 1.7 mm; wingspan 3.9 mm ($n = 1$). Frons, palpi, and scape golden cream. Frontal tuft beige-orange. Collar relatively short, composed of golden-cream lamellar scales. Antenna approximately half the length of

the forewing; flagellum with about 29–30 segments, glossy, pale grey dorsally and golden cream ventrally. Thorax, tegula, and forewing unicolorous greyish brown, golden-glossy, with some purple iridescence, especially prominent apically; no forewing fascia present. Fringe grey-brown, except for a contrasting, triangular glossy-cream patch at the apex. Forewing underside dark brown, without spots or androconia. Hindwing and fringe grey-brown above and below, without androconia. Legs brownish cream, golden-glossy, with light purple iridescence.

Female. Unknown.

Male genitalia (Fig. 4). Genital capsule distinctly longer (215 μ m) than wide (140 μ m). Vinculum with two small, triangular lateral lobes. Uncus bilobed, the lobes deeply separated and relatively wide (Fig. 4a). Gnathos forming a large angular plate with two slender caudal processes. Valva 150 μ m long, with a large, pointed apical process and a prominent inner lobe; the caudal margin of the inner lobe bears a small irregular extension resembling a second apical process. Transtilla with slender, medium-long sublateral processes. Phallus 165 μ m long, 80 μ m wide; vesica armed with numerous large spine-like cornuti and one exceptionally large, horn-like cornutus, unusually rounded rather than pointed apically. Manica absent.

Bionomics. The host plant is *Acalypha* L. (Euphorbiaceae: Acalyphoideae, Acalyphinae), identified by Alvaro Pérez (PUCE). Eggs are laid on the underside of the leaf, usually close to a lateral vein and only occasionally away from the venation. The egg case is glossy, very small, and barely visible even with a hand lens. Larvae mine the leaves in January, and judging from old, empty mines, larval activity also occurs in late December. The larva is pale yellow, with a pale brown intestine and a pale brown head. The leaf mine is a slender, often contorted or sometimes sinuous gallery, widening gradually and only slightly. Occasionally, the mine follows the leaf margin. In the early part of the mine, the frass is green-black to brown-black and almost fills the width of the gallery; later, the frass becomes black, but the clear margins of the gallery remain unfilled and slender. Some variability in

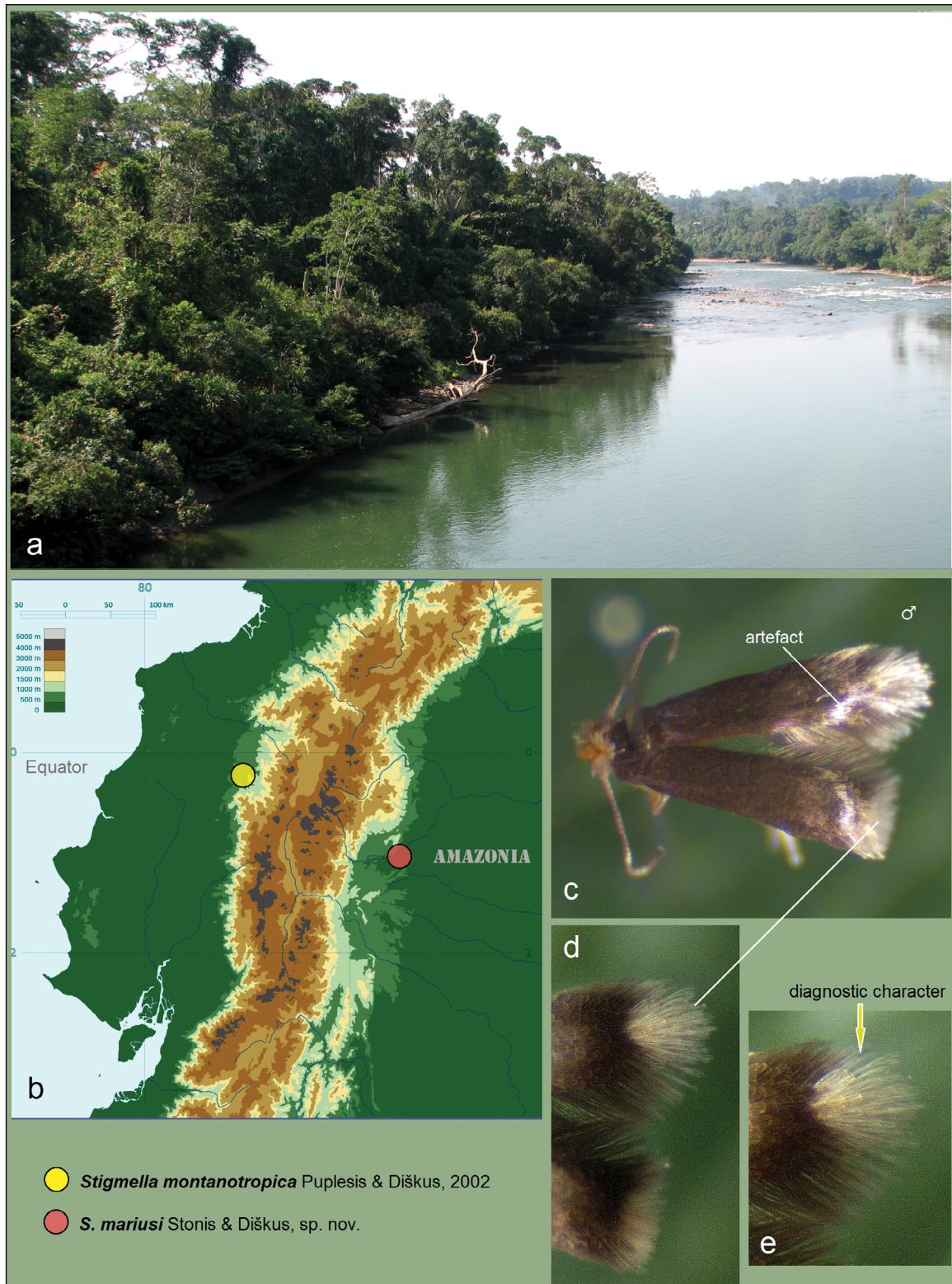


Fig. 3. Habitats, the distribution map and a male adult of *Stigmella mariusi* Stonis & Diškus, sp. nov., discovered in premontane Amazon rainforest: a – collecting locality in Ecuador, Napo Province, 17 km SE of Tena, Misahualli, a bank of Río Napo, at an elevation of about 400 m; b – the distribution map of *Stigmella mariusi* Stonis & Diškus, sp. nov., and the related *Acalypha*-feeding *S. montanotropica* Puplesis & Diškus; c–e – details of the male adult of *S. mariusi*, sp. nov.

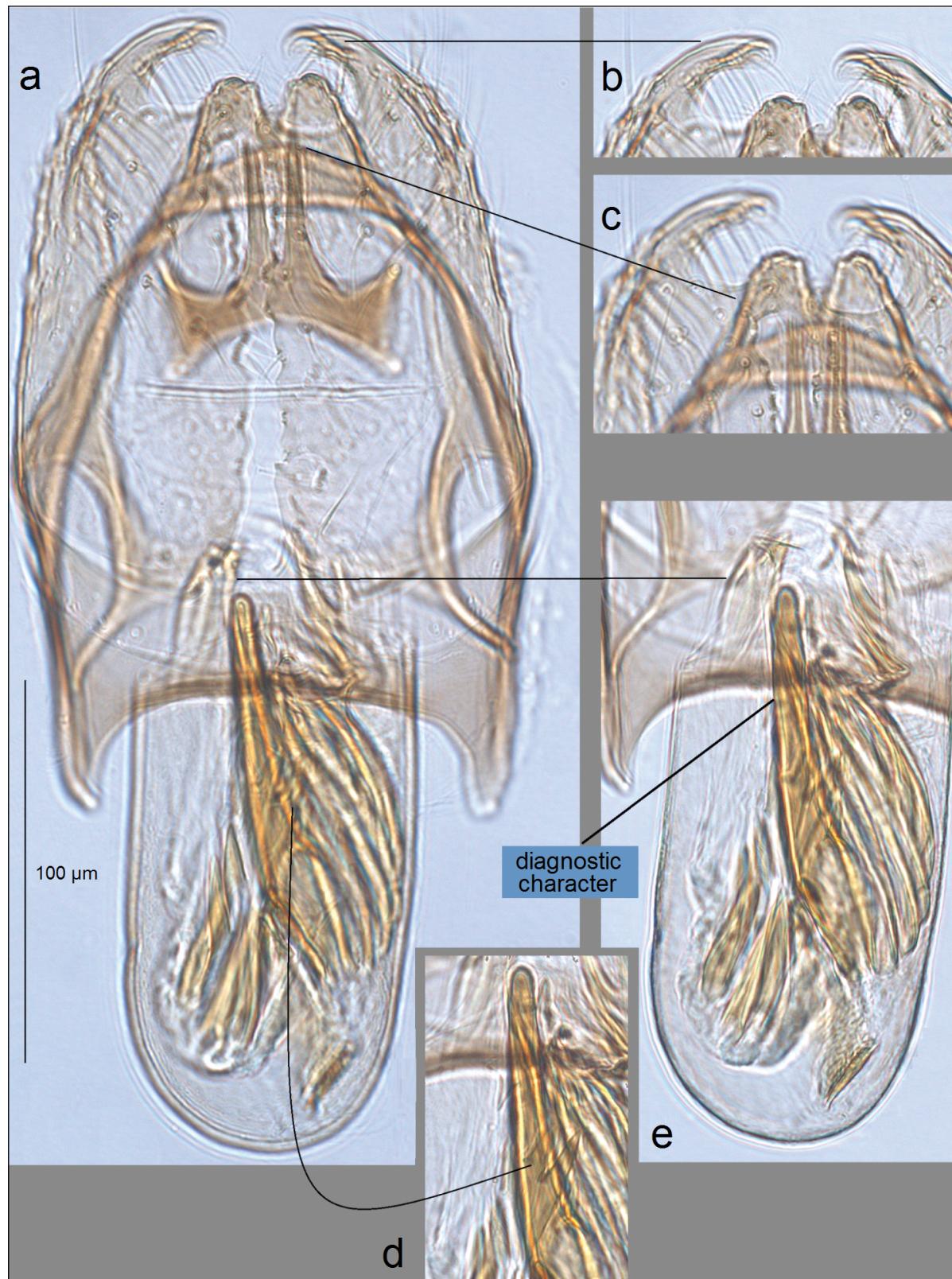


Fig. 4. Male genitalia of *Stigmella mariusi* Stonis & Diškus, sp. nov.: a – general view of capsule with phallus inside; b, c – details of valva and uncus; d, e – details of phallus with different focus on cornuti

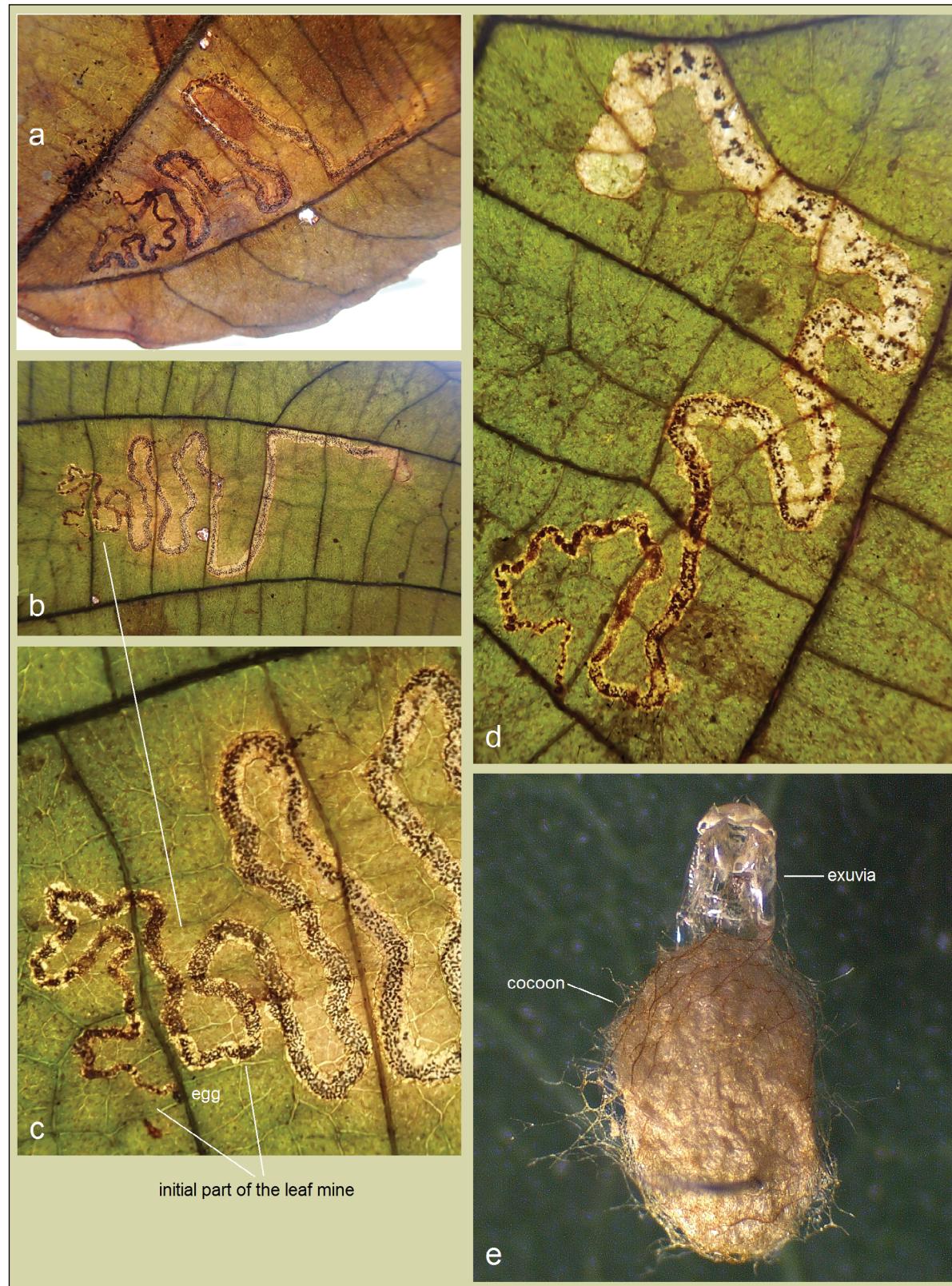


Fig. 5. Bionomics of *Stigmella mariusi* Stonis & Diškus, sp. nov.: a-d – vacated leaf mines of *Acalypha* sp. (Euphorbiaceae), Ecuador, Napo Province, 17 km SE of Tena, Misahualli, the right bank of Río Napo, 400 m, sample no. 4740 (BRG); e – cocoon and exuvia of the holotype specimen

mine shape was observed in the field: while most mines remain slender and contorted (Fig. 5a, b), a few were shorter and noticeably wider in their terminal part (Fig. 5d). Unfortunately, it is not known with certainty from which specific leaf mine the holotype was reared, and it is only presumed that the mine illustrated in Fig. 5d is the probable source. The cocoon (Fig. 5e) is oval, 2.2 mm long and 1.3 mm wide, yellowish beige, with an irregular network of dark brown threads. Adults are active in January.

Distribution. The new species occurs in tropical humid forest – the Amazon premontane rainforest of Ecuador – at an elevation of about 400 m (Fig. 3a, b).

Etymology. The species is named in honour of Marius Grigonis (Vilnius, Lithuania), acknowledging his valuable assistance in sorting historical collection material (BRG) prior to its transfer to the MfN, Berlin.

Remarks. In addition to the holotype of *S. montanotropica* Puplesis & Diškus – another equatorial, *Acalypha*-feeding species – deposited in the collection of NHMUK, we also examined the following paratype specimen during the present study: 1 ♀ (paratype), Ecuador, E of Santo Domingo de los Colorados, Tandapi, 1200 m, montane tropical forest, 3–6.ii.2000, larva on *Acalypha*, leg. R. Puplesis, genitalia slide no. AD0336 (formerly VPU, now scheduled for transfer to MfN).

CONCLUSIONS

1. The first discovery and examination of females of *Dvidulopsis* Stonis & Diškus revealed that the female genitalia possess two large and distinctive signa reticulata but lack vaginal sclerites, i.e., structures so characteristic of *Acalyptis* Meyrick and *Fomoria* Beirne; the abdominal tip of the studied *Dvidulopsis* is wide and almost truncated.

2. The Ecuadorian *Acalypha*-feeding *Stigmella mariusi* sp. nov. is a distinctive species readily separated from all other *Stigmella*, including the related Ecuadorian *Acalypha*-feeder *S. montanotropica* Puplesis & Diškus, by its conspicuous glossy cream apical spot on the forewing and

the large, distally rounded, horn-like cornutus in the male genitalia.

ACKNOWLEDGEMENTS

We are grateful to Professor Dr Giovanni Onore, a former professor at the Pontifical Catholic University of Ecuador (Quito, Ecuador), for facilitating the preparations required to obtain permits for collecting material in Ecuador and for his generous collaboration over the years. We also express our deep appreciation to the late Dr Gaden S. Robinson (Natural History Museum, London) for his early inspiration for the Neotropical project and for his sustained support throughout its development.

We further thank Marius Grigonis (Vilnius, Lithuania) for his valuable assistance in inventorying historical and recent collection material (BRG) prior to its transfer to the NRC (Vilnius) and MfN (Berlin).

Received 12 December 2025

Accepted 22 December 2025

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**MAŽIEJI GAUBTAGALVIAI (NEPTICULIDAE)
IŠ AMAZONIJOS: PIRMĄ KARTĄ APTIKTOS
DVIDULOPSIS GENTIES PATELĖS IR MOKSLUI NAUJA ACALYPHA AUGALUS MINUOJANTI RŪSIS STIGMELLA MARIUSI SP. NOV.**

Santrauka

Iki šiol drėgnuose neotropinio regiono miškuose aptinkama endeminė gentis *Dvidulopsis* Stonis & Diškus buvo žinoma išskirtinai tik pagal patinų požymius. Šioje publikacijoje pirmą kartą dokumentuojamas *Dvidulopsis* patelių genitalinės struktūros. Atlikti tyrimai atskleidė, kad *Dvidulopsis* patelių genitalijoms būdinga trumpa, bet plati kiaušdėtė ir du itin dideli, korėti kopuliacinių maišelių dariniai (*signa*), tačiau nėra vaginalinių skleritų, t. y. morfologinių struktūrų, itin būdingų *Acalyptris* Meyrick ir *Fomoria* Beirne gentims. Taip pat straipsnyje aprašoma nauja mažųjų gaubtagalvių rūsis – *Stigmella mariusi* Stonis & Diškus, sp. nov., kurios viškrai minuoja *Acalyptha* L. (Euphorbiaceae) augalų lapus. Ši Amazonijos prieškalnių miškuose aptikta rūsis yra savita. *S. mariusi* sp. nov. pagal šviesią apikalinę priekinio sparno dėmę ir didelį užapvalintą patinų genitalijų rago formos spagli (cornutus) gali būti lengvai išskiriama iš visų kitų *Stigmella* Schrank rūsių (taip pat labiau giminiškos *Acalyptha* minuojančios *S. montanotropica* Puplesis & Diškus rūšies).

Reikšminiai žodžiai: *Acalyptha* L., Ekvadoras, lapų minos, mažieji gaubtagalviai, mitybiniai augalai, neotropinė fauna