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Refining the Neotropical genus *Coptotrichoides* (Tischeriidae): discovery of three *Serjania*-feeding species, including *C. criminalis* sp. nov., collected under hazardous circumstances

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This study describes three distinctive new *Serjania*-feeding species of the enigmatic Neotropical genus *Coptotrichoides* Diškus & Stonis (Lepidoptera: Tischeriidae), which is trophically associated with host plants of the family Sapindaceae. In addition, new mitochondrial DNA barcode sequences are provided, together with molecular considerations relevant to the generic distinctiveness and phylogenetic placement of *Coptotrichoides*. Newly generated molecular data from additional material also resulted in the discovery of previously unreported distribution records of several species of the genera *Neotischeria* Diškus & Stonis and *Astrotischeria* Puplesis & Diškus in South America. Molecular analyses incorporating the newly obtained *Coptotrichoides* sequences further confirm the distinctiveness of the genus and again suggest that *Coptotrichoides* is more closely related to the genus *Dishkeya* Stonis than to *Coptotriche* Walsingham and especially to *Tischeria* Zeller, although this relationship remains insufficiently resolved. The article is illustrated with photographs of male and female genitalia, leaf mines and exuviae as well as a molecular phylogenetic tree and a mitotype network based on mtDNA CO1-5' sequences.

Keywords: leaf mines, leaf miners, Neotropical fauna, Sapindaceae, trumpet moths

INTRODUCTION

Tischeriidae, commonly known as trumpet leaf-miner moths or trumpet moths, constitute a distinct family within the earliest-diverging (monotrysian) lineages of extant Lepidoptera and occupy a comparatively basal phylogenetic position within the order (for a detailed discussion, see Regier et al., 2015).

The larvae of trumpet moths are obligate leaf miners throughout all instars (Fig. 1a), and pupation also takes place within the leaf mine.

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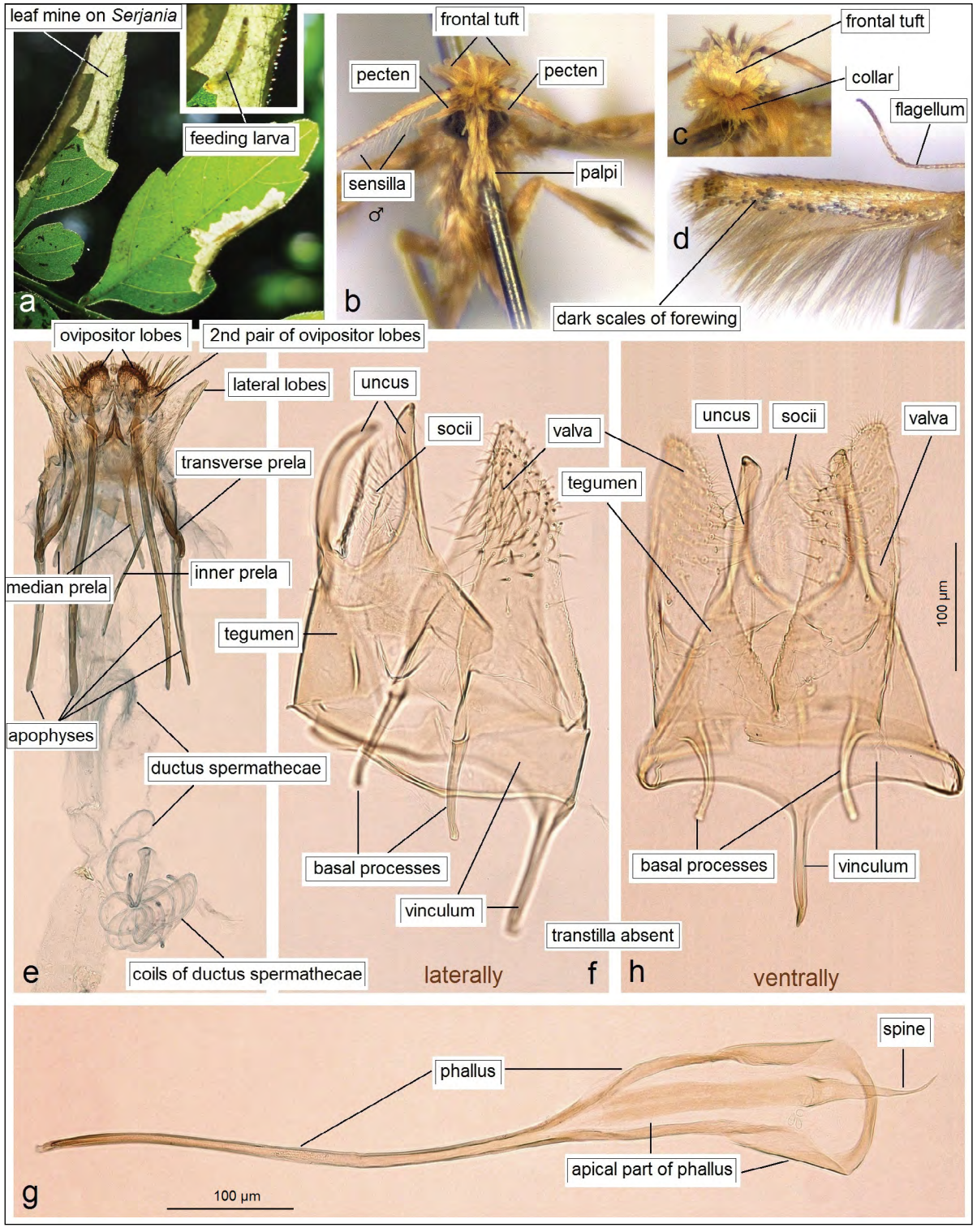

Fig. 1. *Coptotrichoides sapindoidum* Diškus & Stonis, a type species of the genus *Coptotrichoides* Diškus & Stonis, known from western Honduras: a – leaf mines with larva or pupa in the folded part of the leaf mine; b – head, frontal view; c – same, dorsal view; d – left forewing and hindwing; e – female genitalia, slide no. D1091, paratype (MfN); f, h – male genitalia, capsule, slide no. AD537, holotype (MfN); g – same, phallus (after Stonis et al., 2023, modified)

The pioneering American entomologist Annette Frances Braun (1884–1978) was the first to publish an exhaustive monograph on the family, based on the North American fauna (Braun, 1972). Subsequent characterisations of Tischeriidae were provided by Davis (1999) and by Puplesis, Diškus (2003). For a comprehensive overview of the family's morphology, species diversity, and, in particular, the most recent taxonomic circumscription of its genera, we refer to the monograph by Stonis et al. (2023).

Despite forming a relatively small and clearly monophyletic family, Tischeriidae are taxonomically more diverse than might be expected, especially at the generic level. Adult moths exhibit remarkably uniform external morphology, often sharing very similar or even indistinguishable wing venation and other diagnostic characters. Nevertheless, recent comparative studies of male and female genitalia, supplemented by biological observations and molecular data, have revealed a total of eleven genera within the family. These investigations also resulted in the description of the genus *Coptotrichoides* Diškus & Stonis, with *C. sapindoidum* Diškus & Stonis designated as the type species (Stonis et al., 2023) (Fig. 1).

Externally, species of *Coptotrichoides* (Fig. 1b–d) may be confused with other speckled Tischeriidae, particularly representatives of the genus *Coptotriche* Walsingham. The forewing in *Coptotrichoides* is usually irregularly speckled with dark scales (Fig. 1d) and may occasionally bear bright yellow-ochre patches. In male genital morphology, species of *Coptotrichoides* also show overall similarity to *Coptotriche*; however, the genus is readily distinguished by the absence of the transtilla and anellus, the apically tapered valva, a slender uncus, and a relatively elongate tegumen (Fig. 1f, h).

Species of *Coptotrichoides* are trophically associated with host plants of the family Sapindaceae. At present, *Coptotrichoides* is known exclusively from the Neotropics, ranging from Central America to South America as far south as tropical northern Argentina (Stonis et al., 2023). Prior to the present study, the genus comprised six species.

Recent fieldwork conducted in Honduras between 2023 and 2025, together with the examination of previously neglected material collected in Colombia, has revealed the presence of three additional species of *Coptotrichoides*. These findings considerably expand the known diversity of the genus and provide new insights into its morphological and biological variation.

The aim of the present study is to describe three new species, *Coptotrichoides elaboratum* sp. nov., *C. criminalis* sp. nov., and *C. marinum* sp. nov., and to compare them with all previously recognised congeners. This includes (1) detailed formal descriptions of adult morphology, genitalia, and diagnostic characters, and (2) an assessment of how their morphological, biological, and molecular traits refine the current concept of this recently established yet taxonomically and biologically enigmatic Neotropical genus. In addition, we report new distributional data for several other species of Tischeriidae.

We hope that this study contributes to a better understanding of *Coptotrichoides* and, more broadly, draws attention to the still insufficiently studied Neotropical Tischeriidae.

MATERIALS AND METHODS

Material. The material examined in this study was collected by the first author. Since 2023, fieldwork has been conducted in Honduras during visits associated with voluntary biodiversity research in forested areas, with a particular focus on leaf-mining Lepidoptera. Sampling formed part of broader long-term cooperation frameworks between the European Union and Honduras addressing sustainable forest management and biodiversity conservation, with the involvement of the Honduran Institute of Forest Conservation, Protected Areas, and Wildlife (ICF).

Following publication, all new material collected in Honduras and used in the present study will be deposited in the collection of the Museum für Naturkunde (MfN), Berlin, Germany.

Previously neglected material from Colombia, collected by the first author, was also examined. During a recent comprehensive inventory of Lepidoptera holdings at the Biosystematics Research Group (BRG) and the State Scientific Research Institute Nature Research Centre (NRC) – including material transferred from the former Lithuanian University of Educational Sciences and specimens on loan from other institutions – several unidentified Tischeriidae specimens collected in 2019 from Santa Marta, Colombia, were discovered. Here, one of these specimens, which had remained unpublished, is identified as belonging to *Coptotrichoides*. The material is currently housed at the NRC and will likely be permanently deposited in the collection of the Laboratorio de Entomología, UNESIS, Departamento de Biología, Pontificia Universidad Javeriana, Bogotá, Colombia (MPUJ).

In addition, the study examined and identified several other Tischeriidae specimens belonging to the genera *Neotischeria* Diškus & Stonis and *Astrotischeria* Puplesis & Diškus, collected in Honduras, Ecuador, Peru, and Bolivia. It provides new data on the geographical distribution of these species.

Collecting methods. For reasons that remain unclear, light trapping proved ineffective for collecting *Coptotrichoides* in both Honduras and Colombia. Consequently, all adult specimens examined in this study were obtained through rearing from leaf-mining larvae. The applied rearing technique, specifically adapted for Tischeriidae, has been described in detail by Stonis et al. (2018) and more recently summarised by Stonis et al. (2024). Severely disturbed, open habitats – such as village dirt roads and paths along plantations, or in other rural areas – were identified as the most typical environments for locating *Coptotrichoides* larvae (Fig. 2a).

Leaf mines (Fig. 2b–d) were located by careful inspection of known or potential host plants. In all cases, Tischeriidae mines were found within the leaf lamina and typically contained a single larva (Fig. 2c); only occasionally two or three mines were observed on a single leaf. Especially during early larval instars, larvae were sometimes difficult to detect even under a stereoscopic microscope, as they remained concealed within the initial, narrow portion of the mine. When disturbed, actively feeding mature larvae rapidly retreated towards this initial section of the mine, presumably as a defensive response.

Rearing was carried out indoors under ambient conditions, with temperatures of approximately 26–30°C and relative humidity comparable to outdoor levels. Unlike standard rearing practices commonly used for Nepticulidae (Stonis et al., 2022), mined leaf portions were not excised. Instead, entire twigs or shoots bearing active mines were collected. Larvae were allowed to continue feeding for several days up to one or two weeks, until pupation occurred. Medium-sized or larger plastic containers were used exclusively, as test tubes and Petri dishes proved less suitable for successful larval development.

As pupation takes place within the leaf mine, mines were regularly examined under a stereoscopic microscope to detect pupae. Dark-coloured pupae were usually visible within the slender gallery of the mine when viewed under transmitted light. Each pupa was carefully excised together with a 3–4 cm section of the surrounding leaf tissue and transferred to a smaller transparent container (Fig. 2e). Leaf fragments were arranged with sufficient spacing to minimise mould formation. In cases where desiccation posed a risk, a moistened cotton wad (Fig. 2e) or fresh leaves (Fig. 2f) were placed in a corner of the container. Excess moisture or condensation was reduced by periodic ventilation of the container (Stonis et al., 2018, 2024).

Containers were kept in a shaded or dark environment at room temperature and protected from direct sunlight until adult emergence. Newly emerged adults were individually captured in small glass tubes and euthanised by briefly immersing the tube containing the specimen in hot water for 1–2 s.

Fig. 2. Collecting and rearing of *Coptotrichoides* adults from feeding larvae: a – a typical open habitat along the dirt road in a rural area where *Coptotrichoides* larvae were found; b – a leaf mine with a hidden larva; c–d – sculptured and partially stained upper epidermis of the leaf mine; e – maintenance of moisture using a moistened cotton wad (and fresh leaves); f – same, with added fresh leaves (ideally of the host plant, which is not the case in the photographs)

Specimen dissection and documentation. Methods and protocols for specimen dissection, species identification, and description followed those outlined in earlier monographs (Puplesis, Diškus, 2003; Stonis et al., 2022). For genitalia preparations, abdomens were macerated in a 10% potassium hydroxide (KOH) solution, after which the male genital capsule and female genitalia were carefully extracted, cleaned, and mounted with the ventral side uppermost. In many cases, the phallus was dissected from the genital capsule and mounted separately under an individual cover slip on the same microscope slide. Abdominal pelts were consistently retained and preserved.

Permanent genitalia preparations were examined and photographed using a Leica DM2500 compound microscope equipped with a Leica DFC420 digital camera. Exuviae were studied under a Lomo MBS-10 stereoscopic microscope, and images were captured using a Leica S6D stereomicroscope fitted with a Leica DFC290 digital camera. Specimens

were illuminated using a Ring Light LED 60 mounted directly on the stereomicroscope objective.

Most leaf mines were photographed *in situ* using a smartphone equipped with a high-resolution camera (Samsung Galaxy). Additional images of leaf mines were obtained using an Olympus DP-26 digital camera.

Molecular analysis. Air-dried or 96% ethanol-preserved larvae, pupae, and imagoes were used for DNA extraction with the GeneJet Genomic DNA Purification Kit (Thermo Fisher Scientific Baltics), following the manufacturer's protocol. The extracted DNA samples were deposited in the collection of the NRC. Universal primers LCO1490 (5'-ggtcaacaaatcataaagatattgg-3') and HCO2198 (5'-taaacttcagggtgaccaaaaaatca-3') (European and Mediterranean Plant Protection Organization, 2016), synthesised by Metabion (Planegg), were used to amplify a partial fragment of the mitochondrial cytochrome c oxidase subunit 1 gene (mtDNA CO1-5'). The polymerase chain reaction (PCR) mixture consisted of 12.5 μL of 2× Green DreamTaq PCR Master Mix (Thermo Fisher Scientific Baltics), 2.5 μL of each primer (10 pmol/μL), 2 μL of extracted DNA, and deionised water to a final volume of 25 μL. PCR conditions were as follows: an initial denaturation for 5 min at 95°C; 45 cycles of 40 s at 94°C, 40 s at 45°C, and 1 min at 72°C; followed by a final extension for 5 min at 72°C. Horizontal electrophoresis of PCR products was carried out on 1.5% agarose gel (Thermo Fisher Scientific Baltics) stained with Roti Gelstain Red (Karl Roth) and submerged in 0.5× TBE buffer (Thermo Fisher Scientific Baltics). Visualisation was performed under 254 nm UV light. Fragment sizes were estimated using a GeneRuler Express DNA Ladder (Thermo Fisher Scientific Baltics). Successfully amplified PCR products were purified with 1.25 μL of exonuclease I (20 U/μL) and 4.5 μL of FastAP thermosensitive alkaline phosphatase (1 U/μL) (Thermo Fisher Scientific Baltics). Bidirectional Sanger sequencing was performed by Macrogen Europe (Amsterdam, The Netherlands) using an ABI 3730xl 96-capillary DNA Analyzer (Applied Biosystems). Sequence alignment was carried out by BioEdit v.7.2.5 (Hall, 1999). Newly obtained DNA sequences were deposited in the NCBI GenBank database (Benson et al., 2013) under the accession numbers PX660105–PX660125. Related species, genera, and outgroup taxa previously acquired by the authors in earlier studies (Stonis et al., 2021, 2023, 2025a, b; Orlovskytė et al., 2023) were included in the analysis. In addition, sequences of *Dishkeya bifurcata* (Braun) and *Coptotriche purinosella* (Chambers) were obtained from the BOLD platform (Ratnasingham, Hebert, 2007). Phylogenetic analyses were conducted using MEGA v.7 (Kumar et al., 2016) to construct Neighbor-Joining (NJ; TN93 + G model, 10,000 bootstrap replicates) and Maximum Likelihood (ML; GTR + G + I model, 1000 and 10,000 bootstrap replicates) trees. Bayesian analysis was performed by MrBayes v.3.2.3 (Ronquist, Huelsenbeck, 2003) under the GTR + G + I model, with 10 million generations. Fasta-to-Nexus format conversion was carried out using ClustalX2 (Larkin et al., 2007). Species delimitation was assessed using the bPTP method (Zhang et al., 2013). A mitotype network was constructed using the Median Joining algorithm (Bandelt et al., 1999) implemented in PopArt v.1.7 (Leigh, Bryant, 2015).

Abbreviations for institutions. 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 mainly to ZMUC or MfN, or temporarily held by the BRG and NRC; MfN – Museum für Naturkunde, formerly known as the Museum der Naturkunde für Humboldt Universität zu Berlin or Museum für Naturkunde / Leibniz-Institut für Evolutions und Biodiversitätsforschung, Berlin, Germany; MPUJ – Collection of Laboratorio de Entomología, UNESIS, Departamento de Biología, Pontificia Universidad Javeriana, Bogotá, Colombia; NRC – State Scientific Research Institute Nature Research Centre (NRC), Vilnius, Lithuania.

RESULTS

Descriptions of discovered new species of *Coptotrichoides* Diškus & Stonis

***Coptotrichoides elaboratum* Stonis & Diškus sp. nov.**

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(Figs 3–5)

Type material. Holotype: ♂, HONDURAS, Lempira Department, Gracias, via Gualtepeque, 14°35'35"N, 88°34'36"W, elevation 750 m, mining larva on *Serjania* sp. (Sapindaceae), 28–31.iii.2024, leg. J. R. Stonis, genitalia slide no. AD1188 (MfN). Paratypes: 2 ♀, same label data as holotype, genitalia slide no. AD1187♀ (MfN). (Note: three specimens with a label 'Francisco Morazán Department, Cantarranas (Sierita Piedra), right bank of Río Grande o Choluteca, 14°15'25"N, 87°0'54"W, elevation 665 m, 22.iii.2025, larvae and pupae on *Serjania* sp., ex p. iv.2025, leg. J. R. Stonis' were taken for DNA extraction and no pinned specimens or genitalia slides are preserved).

Diagnosis. Externally, this new species may be confused with other sparsely speckled species of *Coptotrichoides* or with superficially similar Tischeriidae. In the male genitalia, *C. elaboratum* sp. nov. is immediately recognisable by the shape of the phallus, which bears a unique combination of two large, curved lateral horns and clusters of lateral and apical spines. The female genitalia are likewise distinctive, characterised by the combination of an unusually long median prela and a specifically modified inner prela (see Fig. 5b).

DNA barcodes. We sequenced five specimens: three from Cantarranas (Honduras, right bank of Río Grande o Choluteca, 14°15'25"N, 87°0'54"W, elevation 665 m, 22.iii.2025, larvae and pupae on *Serjania* sp., ex p. iv.2025, leg. J. R. Stonis) and two from Gracias (Honduras, Lempira Department, via Gualtepeque, 14°35'35"N, 88°34'36"W, elevation 750 m, mining larva on *Serjania* sp., 28–31.iii.2024, leg. J. R. Stonis). All DNA sequences were deposited in GenBank (accession IDs: PX660111–660115).

Description. *Male*. Forewing length 3.0 mm; wingspan 6.5 mm ($n = 1$). Head: frons, palpi, and pecten glossy yellowish cream; frontal tuft glossy beige-cream; collar composed of slender lamellar scales, mostly pale ochreous beige, intermixed with some glossy brown-grey scales; antenna approximately half the length of the forewing or slightly longer; flagellum glossy yellowish grey on upper side. Tegula glossy brown-grey. Thorax brown-grey medially, pale ochreous beige to yellow-beige laterally. Forewing pale ochreous beige, sparsely speckled with glossy brown-grey scales along margins and apically; fringe pale grey; fringe line indistinct or absent; underside of forewing brown-grey, without androconia. Hindwing and fringe grey. Legs pale yellow-beige, densely speckled with brown-grey scales dorsally. Abdomen brown-grey dorsally; ventrally yellow-beige, speckled with brown-grey scales.

Female (Fig. 3g–i). Similar to male.

Male genitalia (Fig. 4). Genital capsule about 590 μm long, 240–260 μm wide. Uncus composed of two very slender, elongate lobes. Socii (Fig. 4f, g) large, paired, densely covered with fine spines. Tegumen moderately long, without spines. Valva (Fig. 4e) 305–310 μm long, gradually tapering towards apex, except for basal two-fifths, which are slightly but abruptly widened; basal processes slender and relatively short. Anellus indistinct or absent. Vinculum very short, bearing a very slender, elongate, spine-like anterior process (Fig. 4h). Phallus about 700 μm long, very slender, but apically widened and highly elaborated, with two large, curved, horn-like lateral processes (Fig. 4c, i) and three distinct sets of spines: two lateral and one apical (Fig. 4a–d).

Female genitalia (Fig. 5). Genitalia about 1230 μm long. Ovipositor lobes moderately large, rounded, densely covered with peg-like setae; second pair of ovipositor lobes three to four times smaller than the primary lobes but bearing numerous long setae; lateral lobes short. Anterior apophyses slightly longer than posterior apophyses. Prela consisting of three pairs of distinctive, rod-like and lobe-like projections: transverse prela (Fig. 5a) relatively wide,

Fig. 3. *Coptotrichoides elaboratum* Stonis & Diškus, sp. nov.: a–d – leaf mines, Gracias, Lempira Department, Honduras; e – exuvia, lateral view; f – abdominal part of exuvia; g–i – adult of female paratype, Gracias, Lempira Department, Honduras (MfN)

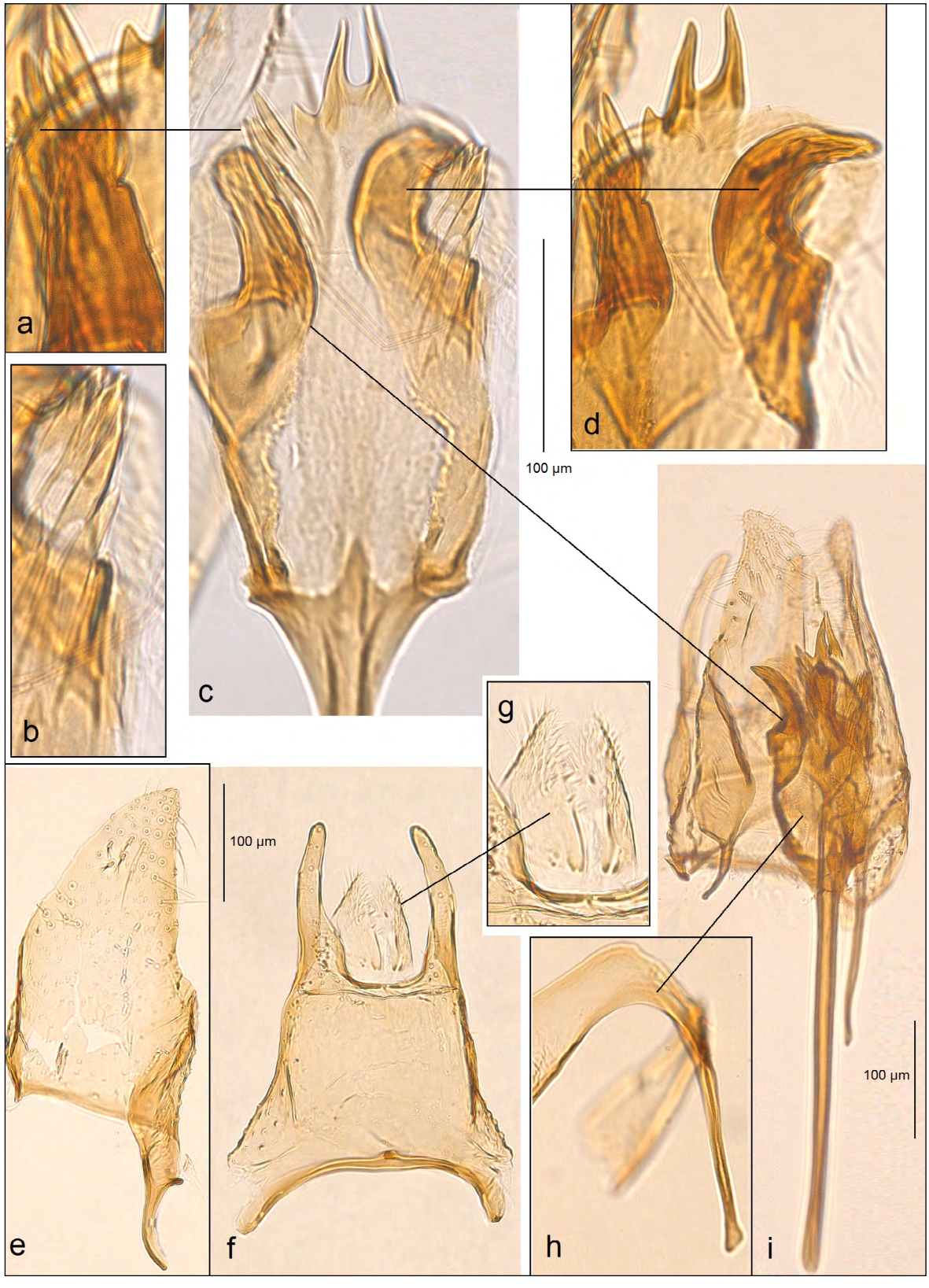

Fig. 4. *Coptotrichoides elaboratum* Stonis & Diškus, sp. nov., holotype, male genitalia, slide no. AD1188 (MfN): a, b – lateral spines of the phallus; c, d – details of apical part of the phallus; e – valva, lateral view; f – tegumen, uncus, and socii; g – socii; h – anterior spine-like process of the vinculum, lateral view; i – general view of the capsule with phallus inside (photographed before dissection and permanet fixation in Euparal)

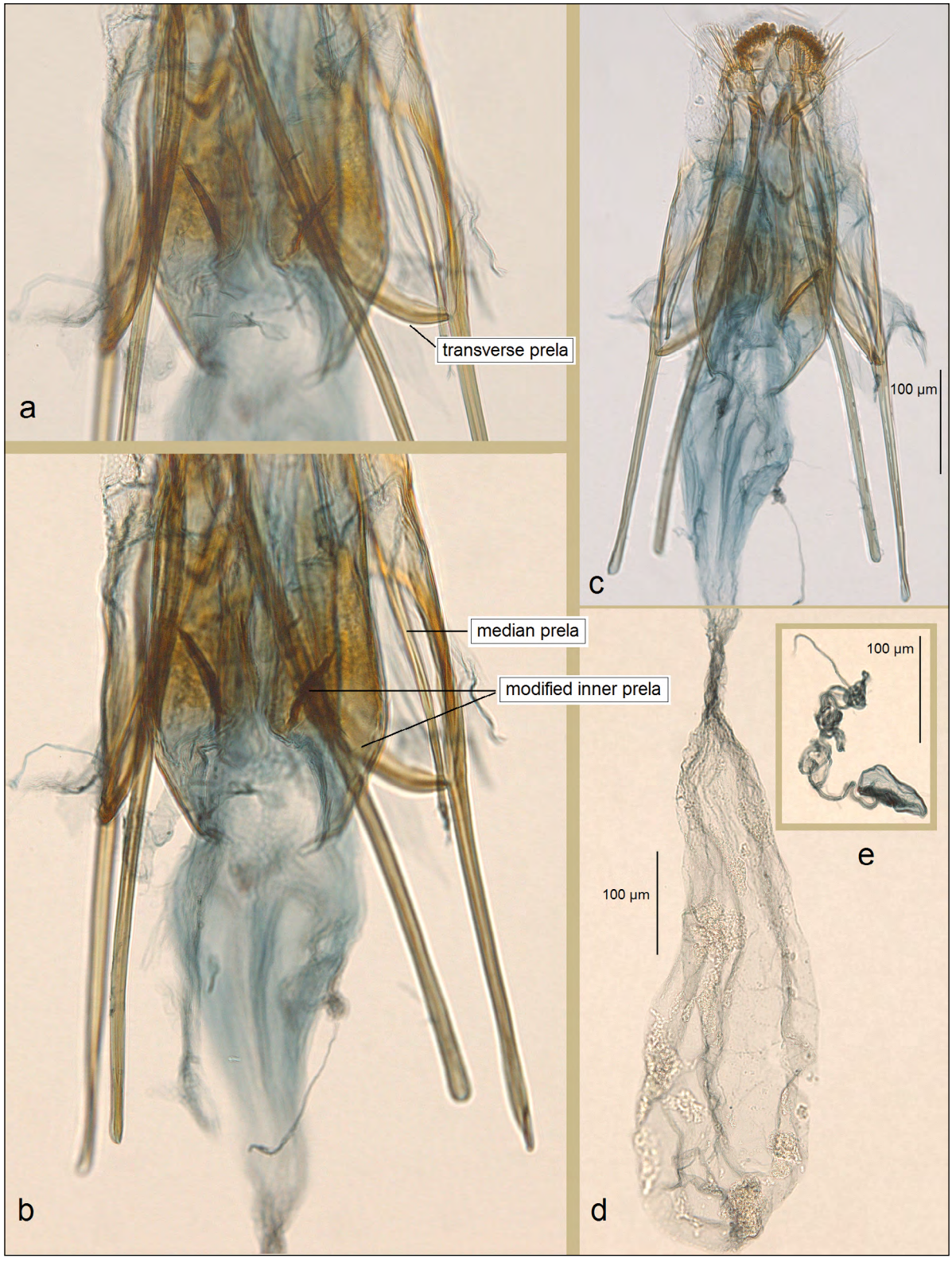

Fig. 5. *Coptotrichoides elaboratum* Stonis & Diškus, sp. nov., paratype, female genitalia, slide no. AD1187 (MfN): a, b – apophyses and prela; c, d – general view of the genitalia; e – ductus spermathecae

slightly curved, and distally pointed; median prela (Fig. 5b) very long and slender; inner prela (Fig. 5b) strongly modified, unusually large, lobe-like, and medially elaborated. Corpus bursae relatively short (540–610 μm); pectinations very sparse and mostly indistinct. Ductus spermathecae (broken but not lost in genitalia slide AD1187) short and very slender, with numerous small, irregular coils and a relatively large, irregularly triangular vesicle (Fig. 5c).

Bionomics (Fig. 3a–f). Host plant: *Serjania* Mill. (Sapindaceae). Larvae mine leaves in March. The trumpet-like, slightly elongate leaf mines are markedly shorter than those of *C. criminalis* sp. nov. (described below), whitish and transparent, and lack frass. The nidus is narrowly oval, not visible through the epidermis; therefore, dissection of the mine is necessary to observe it. Adults occur in April.

Distribution. This species is known from two localities in valleys of the mountainous mainland of Honduras: near Gracias, Lempira Department, at an elevation of 750 m, and near Cantarranas, Francisco Morazán Department, at approximately 670 m. Vacant leaf mines resembling those of *C. elaboratum* sp. nov. were also observed along the Pacific coast of Honduras; however, in the absence of reared specimens or molecularly examined larvae, there is no reliable evidence confirming the occurrence of *C. elaboratum* sp. nov. in the tropical dry forests of the Pacific coastal region.

Etymology. The species name is derived from the Latin *elaboratus* ('elaborated'), referring to the highly elaborated structure of the male genitalia, particularly the phallus with its lateral horn-like processes and lateral and apical spines, as well as to the distinctly elaborated, lobe-like inner prela of the female genitalia.

Coptotrichoides criminalis Stonis & Diškus sp. nov.

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(Figs 6–8)

Type material. Holotype: 1 ♂, HONDURAS, Atlántida Department, E of Tela, Colonia Tres de Mayo, [close to Las Palmas], 15°46'64"N, 87°23'15"W, elevation 30 m, mining larva on *Serjania* sp. (Sapindaceae), 13–19.iv.2024, leg. J. R. Stonis, genitalia slide no. AD1190 (MfN). Paratypes (5 ♂, 2 ♀): 2 ♂, 2 ♀, same label data as holotype, genitalia slide nos. AD1178♂, AD1189♀ (MfN); 1 ♂, Atlántida Department, E of Tela, Colonia Tres de Mayo, 15°46'9"N, 87°23'14"W, elevation 30 m, 25.iv.2025, larvae and pupae on *Serjania* sp., leg. J. R. Stonis, genitalia slide no. AD1247♂ (from adult in pupal skin, without pinned adults preserved) (MfN); 1 ♂, Francisco Morazán Department, Cantarranas, 14°16'7"N, 87°1'17"W, elevation 700 m, pupa on *Serjania* sp., 20.iii.2025, leg. J. R. Stonis, genitalia slide no. AD1238♂ (from adult in pupal skin, without pinned adults preserved) (MfN); 1 ♂, same label data, mining larva on *Serjania* sp., 17.iv.2025, genitalia slide no. AD1237♂ (from adult in pupal skin, without pinned adults preserved) (MfN).

Diagnosis. Externally, females – and especially males – of *Coptotrichoides criminalis* sp. nov. may be confused with other sparsely speckled species of *Coptotrichoides* or other superficially similar genera of Tischeriidae. However, compared with related species, *C. criminalis* sp. nov., particularly the females, tends to be darker and more densely speckled with blackish scales. In the male genitalia, the species is immediately recognisable by the shape of the phallus, which bears a unique, long, curved, and pointed apical spine (Fig. 7e); in addition, the valva possesses a short basal lobe (Fig. 7c). The female genitalia are likewise distinctive, characterised by the unusually modified, lobate inner prela (see Fig. 8e, f).

DNA barcodes. We sequenced six specimens: four from Cantarranas (Honduras, right bank of Río Grande o Choluteca, 14°15'25"N, 87°0'54"W, elevation 665 m, 11.ii.2025, 20.iii.2025, and 22.iv.2025, larvae and adult from *Serjania* sp., leg. J. R. Stonis) and two from Tela (Honduras, Atlántida Department, E of Tela, Colonia Tres de Mayo, 15°46'9"N, 87°23'14"W, elevation 30 m, 25.iv.2025, pupae from *Serjania* sp., leg. J. R. Stonis). All DNA sequences were deposited in GenBank (accession IDs: PX660105–660110).

Fig. 6. *Coptotrichoides criminalis* Stonis & Diškus, sp. nov.: a–d – leaf mines, Tela, Atlántida Department, Honduras; e, f – adult of the female paratype associated with genitalia slide no. AD1189, E of Tela (Colonia Tres de Mayo), Honduras, 25.iv.2025 (MfN)

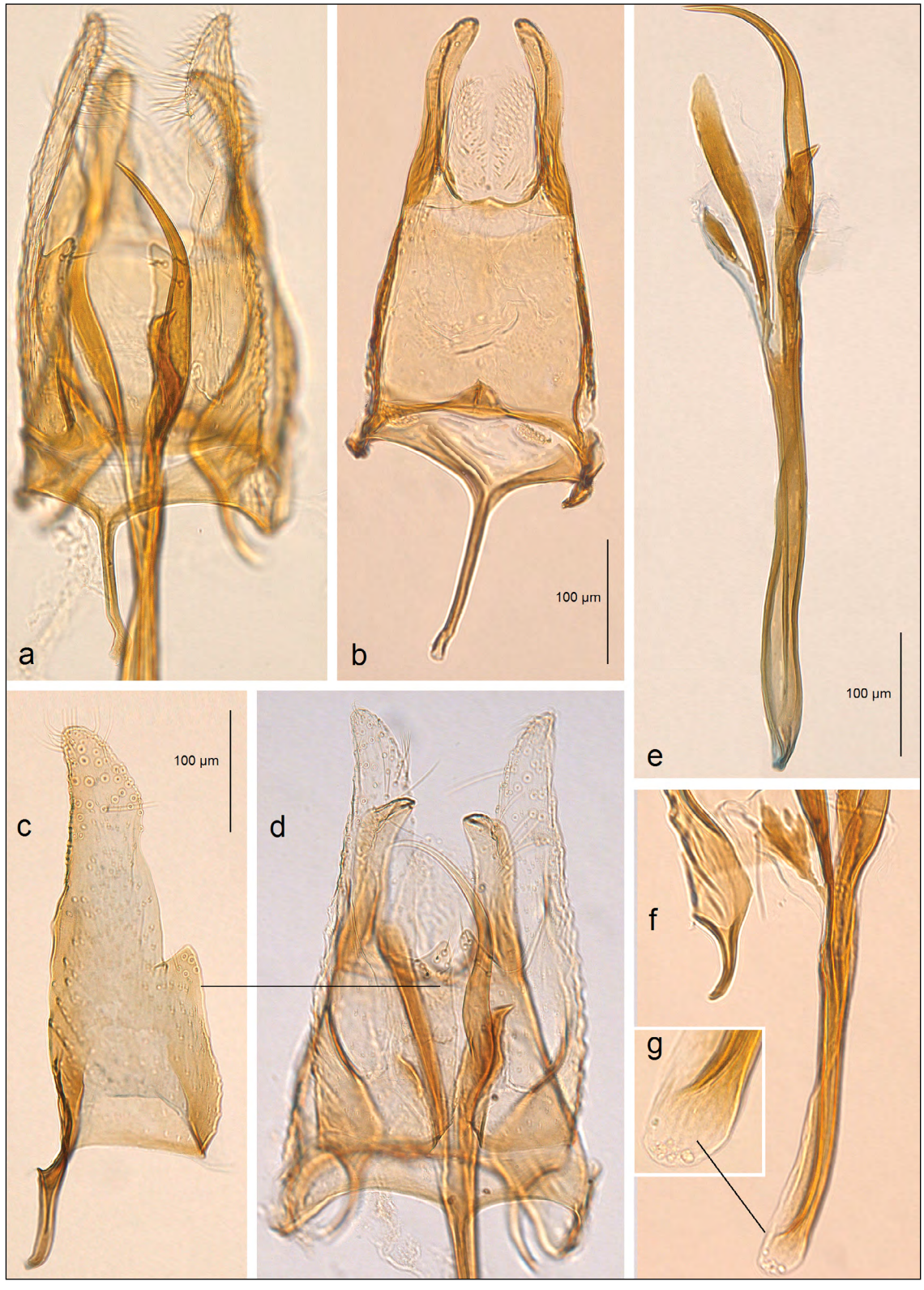

Fig. 7. *Coptotrichoides criminalis* Stonis & Diškus, sp. nov., male genitalia: a – general view with the phallus inside, holotype, Tela, Honduras, slide no. AD1190 (MfN); b – capsule with valva and phallus removed, paratype, Cantarranas, Honduras, slide no. AD1237 (MfN); c – valva, lateral view, paratype, Tela, slide no. AD1178 (MfN); d – general view, paratype, Tela, slide no. AD1247 (MfN); e – phallus, paratype, Tela, slide no. AD1178 (MfN); f, g – basal part of the phallus, paratype, Cantarranas, slide no. AD1237 (MfN)

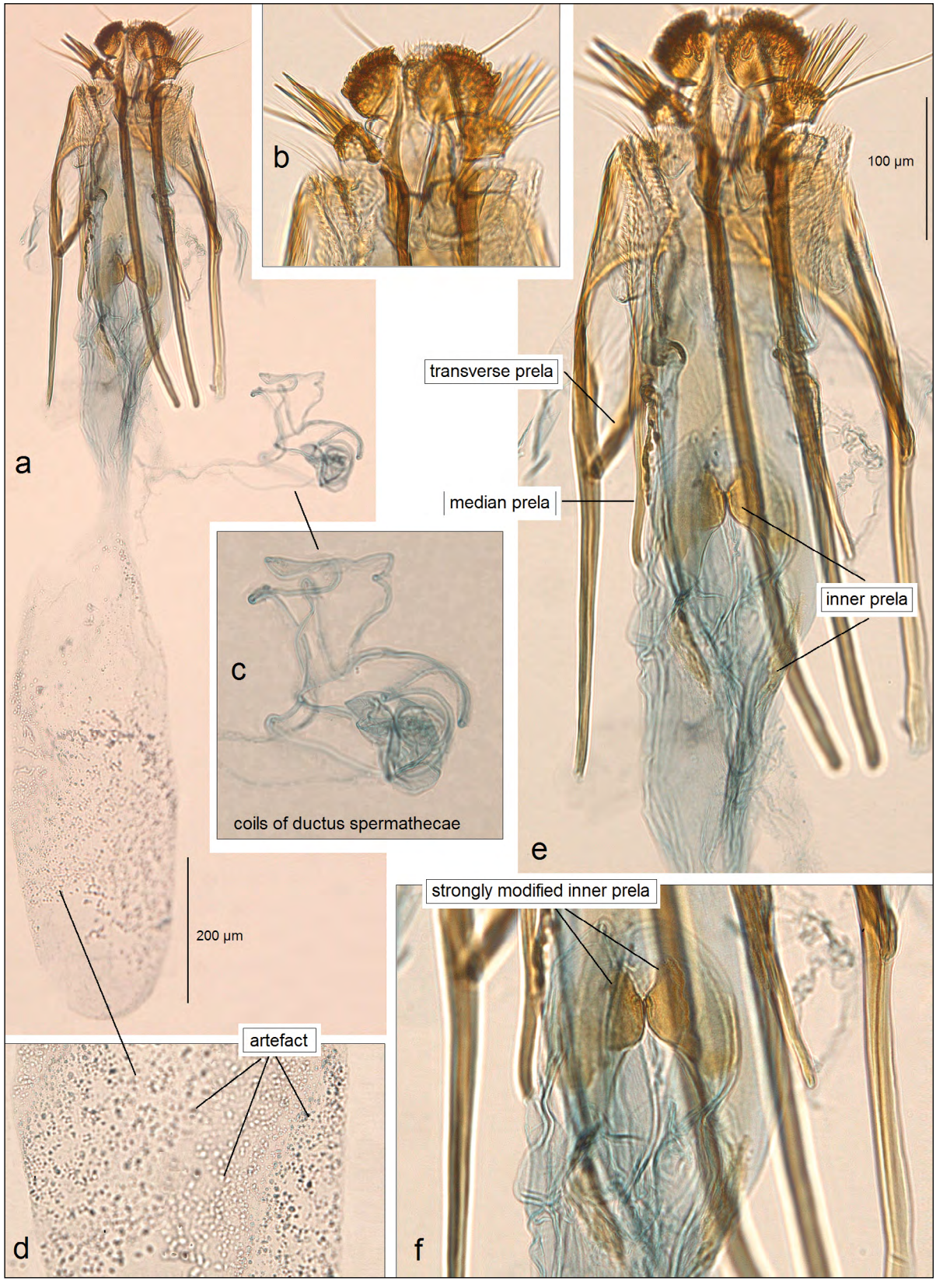

Fig. 8. *Coptotrichoides criminalis* Stonis & Diškus, sp. nov., female genitalia, paratype, genitalia slide no. AD1189 (MfN): a – general view; b – ovipositor lobes; c – ductus spermathecae; d – fragment of corpus bursae; e, f – apophyses and prela

Description. *Male.* Forewing length 2.9–3.1 mm; wingspan 6.4–6.6 mm (n = 3). Head: palpi golden cream; frons glossy ochre-cream; pecten composed of numerous long ochre-cream scales; frontal tuft golden cream; collar composed of slender lamellar scales, predominantly yellow-cream, with some grey-brown scales medially; antenna approximately half the length of the forewing or slightly longer; flagellum glossy dark grey, ochre-cream basally (presumably bearing androconial ochre-cream scales); sensilla approximately three to four times longer than the width of the flagellum. Tegula covered with glossy brown-grey scales, proximally yellow-beige. Thorax brown-grey, yellow-beige laterally. Forewing pale ochreous beige, speckled with glossy brown-grey scales along margins and apically; fringe pale grey; fringe line indistinct or absent; underside of forewing brown-grey, without androconia. Hindwing and fringe grey. Legs pale yellow-beige, densely speckled with brown-grey scales dorsally. Abdomen brown-grey dorsally; ventrally yellow-beige, speckled with brown-grey scales.

Female (Fig. 6e, f). Similar to male but darker and more densely speckled with blackish scales. Forewing length 2.7–2.8 mm; wingspan 6.1–6.2 mm (n = 2).

Male genitalia (Fig. 7). Genital capsule 530–540 μm long, 200–240 μm wide. Uncus composed of two very slender, elongate lobes. Socii large, distinctly paired, densely covered with fine spines. Tegumen moderately long, without spines. Valva (Fig. 7e) ca. 350 μm long, gradually tapering towards the apex, with a triangular basal lobe (Fig. 7c); basal process slender and relatively short. Anellus indistinct or absent. Vinculum very short, bearing a very slender, elongate, spine-like anterior process (Fig. 7b). Phallus (Fig. 7a, e–g) 530–640 μm long, slender, apically pointed and slightly curved, with one long and two shorter, process-like apical sclerites (Fig. 7a, e).

Female genitalia (Fig. 8). Genitalia about 1410 μm long. Ovipositor lobes moderately large, rounded, densely covered with peg-like setae; second pair of ovipositor lobes two to three times smaller than primary lobes but bearing numerous long setae; lateral lobes short and indistinct. Anterior and posterior apophyses equal in length. Prela consisting of three pairs of distinctive, rod-like and lobe-like projections (Fig. 8e, f): transverse prela slender; median prela relatively long and slender; inner prela strongly modified, lobe-like, medially rounded and sclerotised. Corpus bursae relatively short (about 720 μm); pectinations very sparse and mostly indistinct (Fig. 8d). Ductus spermathecae very slender, with numerous large but irregular, sinuous coils and an irregular, weakly sclerotised vesicle (Fig. 8c).

Bionomics (Fig. 6a–d). Host plant: *Serjania* Mill. (Sapindaceae). Larvae mine leaves in April. The elongate trumpet-like leaf mines are markedly longer than those of *C. elaboratum* sp. nov. (described above) and *C. marinum* sp. nov. (described below), whitish and transparent, and lack frass. Rarely, leaf mines occur close to the leaf margin, and the mining larva folds the margin of the mined leaf prior to pupation. The nidus is narrowly oval, not visible through the epidermis; therefore, dissection of the mine is necessary to observe it. Adults occur from late April to early May.

Distribution. This species is known from two localities in Honduras: near Tela, Atlántida Department, at an elevation of approximately 30 m, and near Cantarranas, Francisco Morazán Department, at about 670 m.

Etymology. The species name is derived from the Latin *criminalis* ('criminal'), referring to the hazardous and life-threatening circumstances under which the type material was collected, during an armed robbery encountered in the field. Further details of this incident are provided in the Appendix.

Coptotrichoides marinum Diškus & Stonis, sp. nov.

urn:lsid:zoobank.org:act:D0027E5D-1DF6-492B-8253-9E16CBE1ADAB

(Figs 9–11)

Type material. Holotype: ♀, COLOMBIA: Magdalena Department, 33 km E Santa Marta, Zona Hotelera (near vda. Los Naranjos),

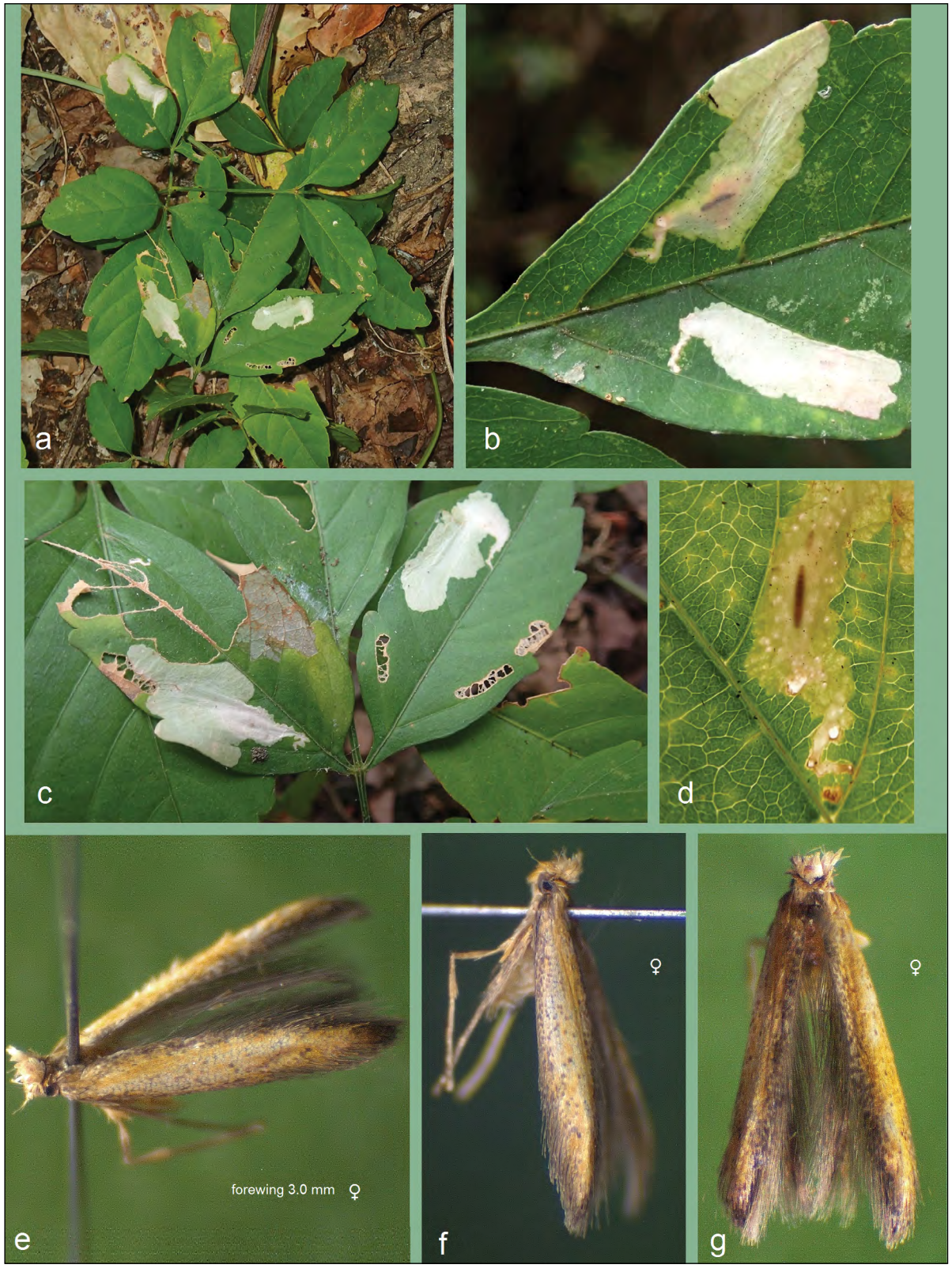

Fig. 9. *Coptotrichoides marinum* Diškus & Stonis, sp. nov.: a–d – leaf mines on *Serjania* sp., Sapindaceae, E of Santa Marta, Magdalena Department, Colombia; e–g – adult of the female holotype associated with genitalia slide AD1250

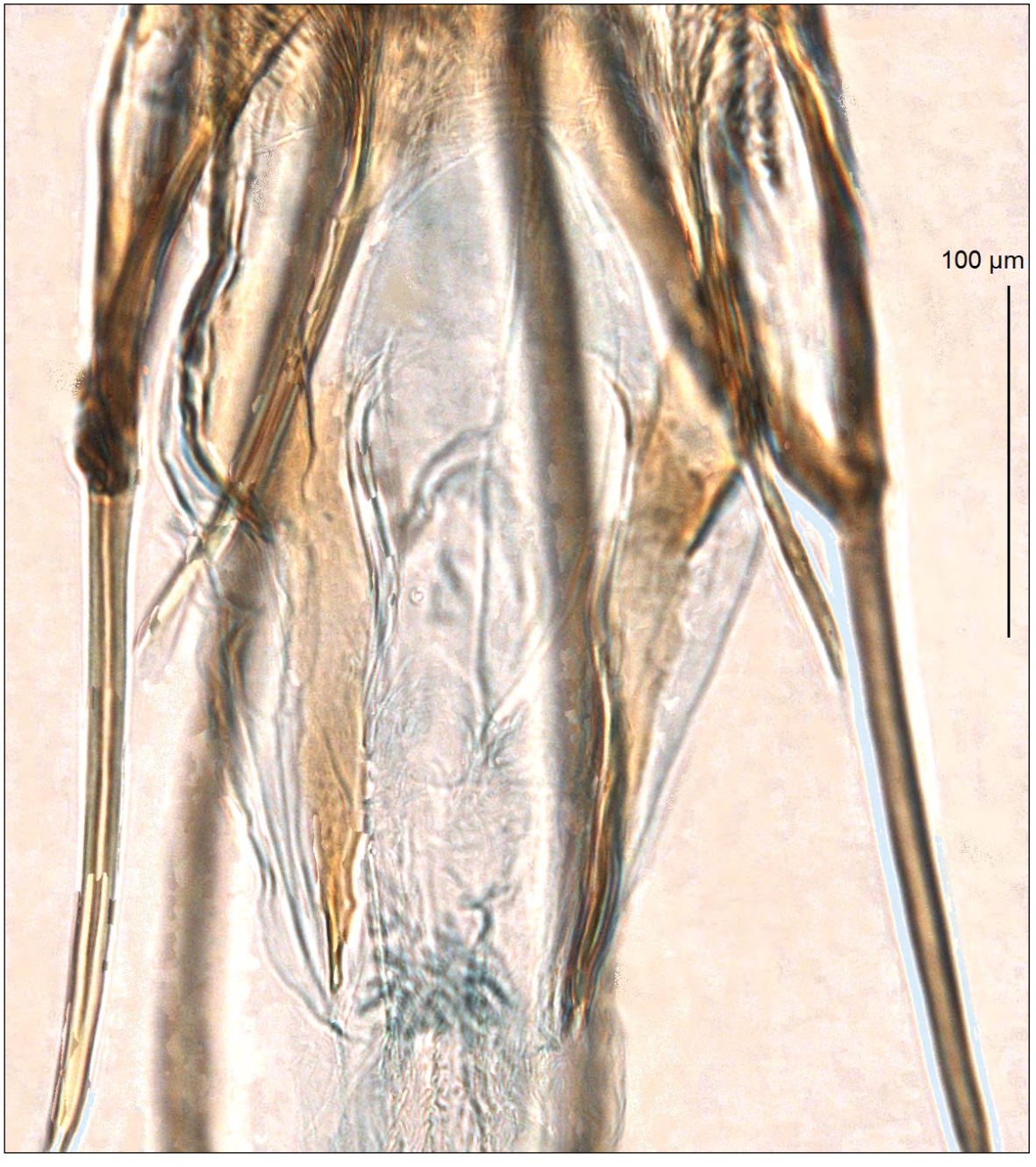

Fig. 10. *Coptotrichoides marinum* Diškus & Stonis, sp. nov., female genitalia, inner prela, genitalia slide no. AD1250

11°17'34"N, 73°53'30"W, elevation 40 m, pupa on *Serjania* sp. (Sapindaceae), 7.ii.2019, ex pupa 11.ii.2019, leg. J. R. Stonis, genitalia slide no. AD1250 (NRC; intended for subsequent deposition in MPUJ).

Diagnosis. Externally, this new species may be confused with other sparsely speckled species of *Coptotrichoides* or other superficially similar genera of Tischeriidae; however, in the female genitalia, *C. marinum* sp. nov. is readily distinguished by the uniquely large and pointed inner prela (see Fig. 10).

DNA barcode. Female holotype specimen was sequenced; the resulting DNA barcode was deposited in GenBank (accession ID: PX660116).

Description (external characters based on a female specimen shown in Fig. 9e–g). Forewing length 3.0 mm; wingspan 6.6 mm (n = 1). Head: frons and palpi yellowish cream; pecten long and slender, beige-cream; frontal tuft glossy beige-cream; collar small, composed of slender, pale beige lamellar scales; antenna unknown (broken). Tegula densely speckled with brown-grey scales laterally. Thorax grey-black medially, yellowish beige laterally. Forewing glossy yellowish beige, speckled with grey-black scales laterally and apically; fringe dark grey; fringe line indistinct or absent; underside of forewing blackish brown, without androconia. Hindwing and fringe dark grey. Legs glossy beige-cream, densely speckled with grey-black scales dorsally.

Female genitalia (Figs 10, 11). Genitalia about 1030 µm long. Ovipositor lobes large,

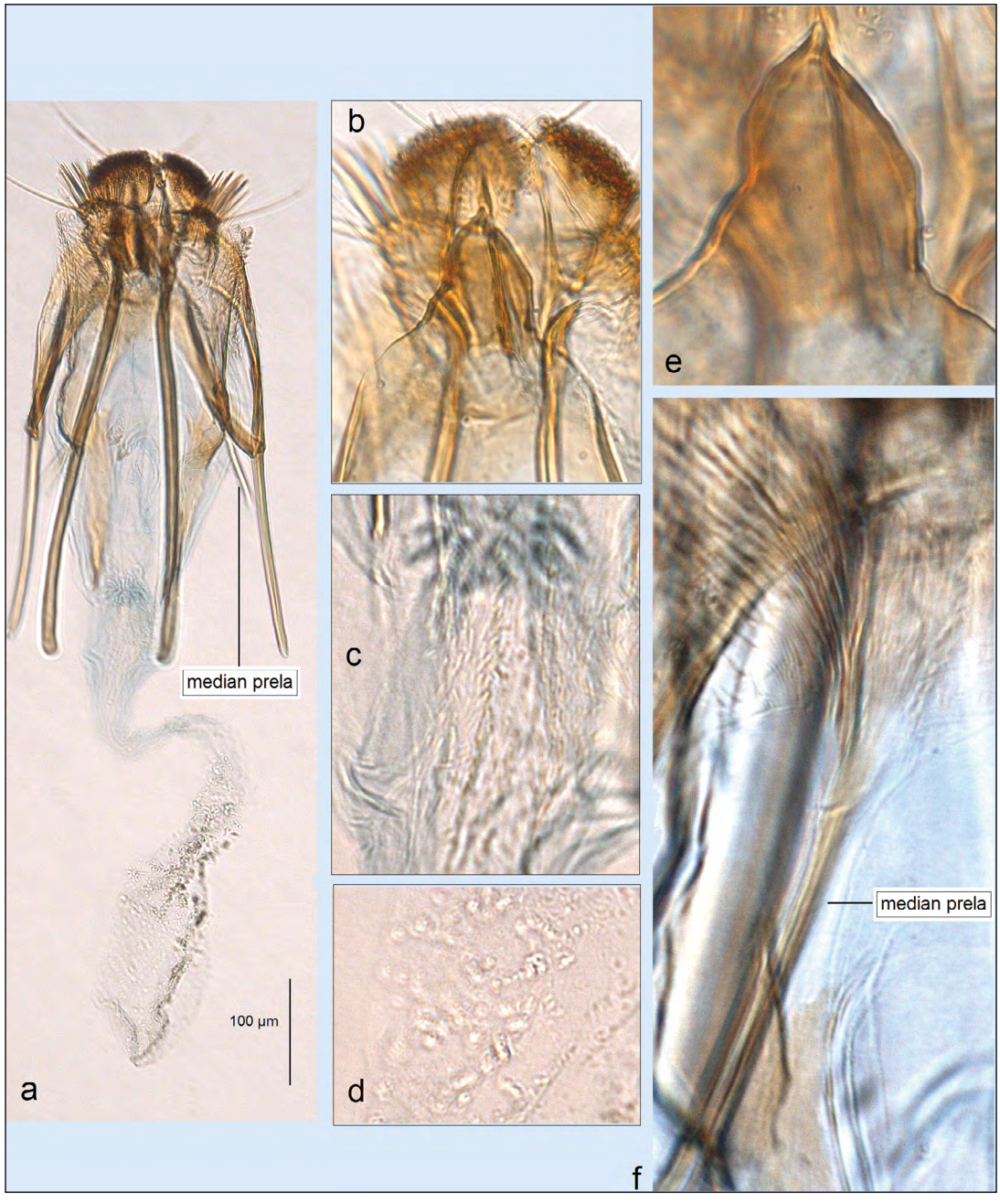

Fig. 11. *Coptotrichoides marinum* Diškus & Stonis, sp. nov., female genitalia, slide no. AD1250: a – general view; b, e – caudal sclerite; c, d – details of corpus bursae; f – median prela

rounded, densely covered with peg-like setae; second pair of ovipositor lobes two to three times smaller than primary lobes but bearing numerous long setae. Anterior and posterior apophyses equal in length. Prela consisting of three pairs of distinctive projections: transverse prela relatively wide and distally truncate; median prela relatively long and very slender; inner prela strongly modified, forming a pair of elongate, pointed lobes. Corpus bursae unusually short; pectinations indistinct or absent. Ductus spermathecae unknown (broken and lost).

Bionomics (Fig. 9a–d). Host plant: *Serjania* Mill. (Sapindaceae). Larvae mine leaves in early February and, based on the presence of vacant leaf mines, apparently also in January. The trumpet-like mines are wide but distinctly shorter (and wider) than that of *C. criminalis* sp. nov. (described above); the leaf mines have a characteristic whitish appearance, are transparent, and lack frass. The nidus is narrowly oval, not visible through the epidermis; therefore, dissection of the mine is necessary to observe it. Adults occur in February.

Distribution. This species is known from a single locality in Colombia near the coast of the Caribbean Sea: Magdalena Department, at an elevation of approximately 40 m.

Etymology. The species name is derived from the Latin adjective *marinus*, -a, -um ('marine; of the sea'), used here in the neuter form *marinum*, and refers to the occurrence of the species in coastal habitats along the Caribbean Sea in Colombia.

Molecular considerations of *Coptotrichoides*

In this study, twelve partial 657 bp mitochondrial DNA sequences of the CO1-5' gene region of *Coptotrichoides* were successfully obtained for the first time. In addition, our previously published barcode data for *C. serjaniphaga* (Remeikis & Stonis) were included in the analysis (Stonis et al., 2021, 2023). To assess the systematic position of the recently established *Coptotrichoides* genus (Stonis et al., 2023), selected representatives of the related genera *Dishkeya* Stonis, *Rytietia* Diškus, Xu & Dai, *Coptotriche* Walsingham, and *Tischeria* Zeller were incorporated into the dataset.

Molecular analysis indicated that the three new species described herein consistently and reliably formed separated branches supported by all applied analytical approaches (Fig. 12). The genetic distinctiveness of *C. criminalis* sp. nov. and *C. elaboratum* sp. nov. was approved by the bPTP species delimitation method (bPTP support value of *C. criminalis* sp. nov. = 91%; *C. elaboratum* sp. nov. = 78%); for the delimitation of *C. marinum* sp. nov., more specimens of this species should be included in the analysis.

Despite the geographical differences, the Colombian *C. marinum* sp. nov. showed a closer genetic affinity to the Honduran *C. elaboratum* sp. nov. (the pairwise distance varied between 5.07 ± 1.10% and 5.47 ± 1.16%) than that observed between the two Honduran species *C. elaboratum* sp. nov. and *C. criminalis* sp. nov. (they differed by 9.69 ± 1.71% – 10.42 ± 1.80%). Together, these three newly described species formed a well-supported monophyletic clade (NJ = 99%, ML = 99%, Bayesian posterior probability = 100%). However, the addition of *C. serjaniphaga* made the branch, though monophyletic, but statistically unsupported. This species occupying the basal position within the genus may retain more ancestral molecular characters. This pattern may be explained, at least in part, by the markedly different geographical and ecological setting of *C. serjaniphaga*, which occurs in the Andean region of Peru at an elevation of approximately 2,700 m, whereas other *Coptotrichoides* species are known from tropical lowland or subtropical premontane habitats of Central America.

The overall topology of the resulting molecular tree (Fig. 12) is largely congruent with that presented in our previous study on *Dishkeya* (Stonis et al., 2025). With the inclusion of newly generated sequences of *C. criminalis* sp. nov., *C. elaboratum* sp. nov., and *C. marinum* sp. nov., the present analysis further reinforces the molecular distinctiveness, though yet unsupported, of *Coptotrichoides* and improves our understanding of its phylogenetic relationships. In both the previous and

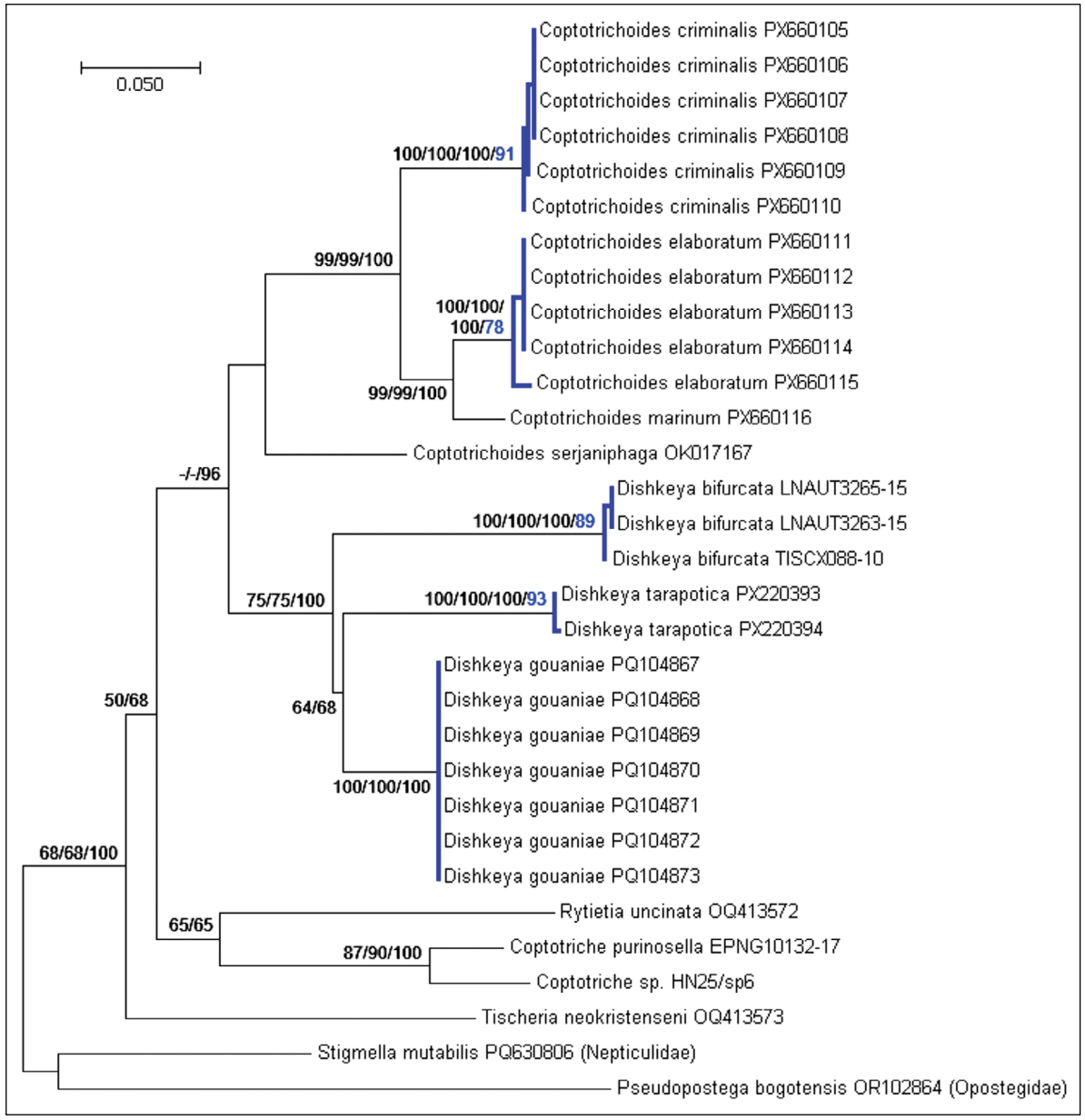

Fig. 12. The topology of the new *Coptotrichoides* Diškus & Stonis species and related genera based on the 657 bp-long CO1-5' sequences. Numbers represent the bootstrap values obtained for Neighbor-Joining (10,000 bootstrap replicates) / Maximum Likelihood (1000 replicates) / Bayesian inference (10,000,000 generations) / bPTP (blue) in %. Values below 50 are not shown. *Stigmella mutabilis* Stonis & Remeikis (Nepticulidae) and *Pseudopostega bogotensis* Vargas (Opostegidae) were included as an outgroup

current analyses, the genus *Dishkeya* appears to be the closest relative of *Coptotrichoides*, rather than *Coptotriche*; however, robust statistical support for this topology was provided only by the Bayesian inference (96%), whereas other approaches yielded incongruent support. These results suggest interpreting the placement of *Coptotrichoides* with caution. Consequently, resolving phylogenetic relationships among *Coptotrichoides* and other related genera (*Coptotriche*, *Rytietia*, *Tischeria*) will require additional analyses incorporating broader taxonomic sampling and additional molecular markers.

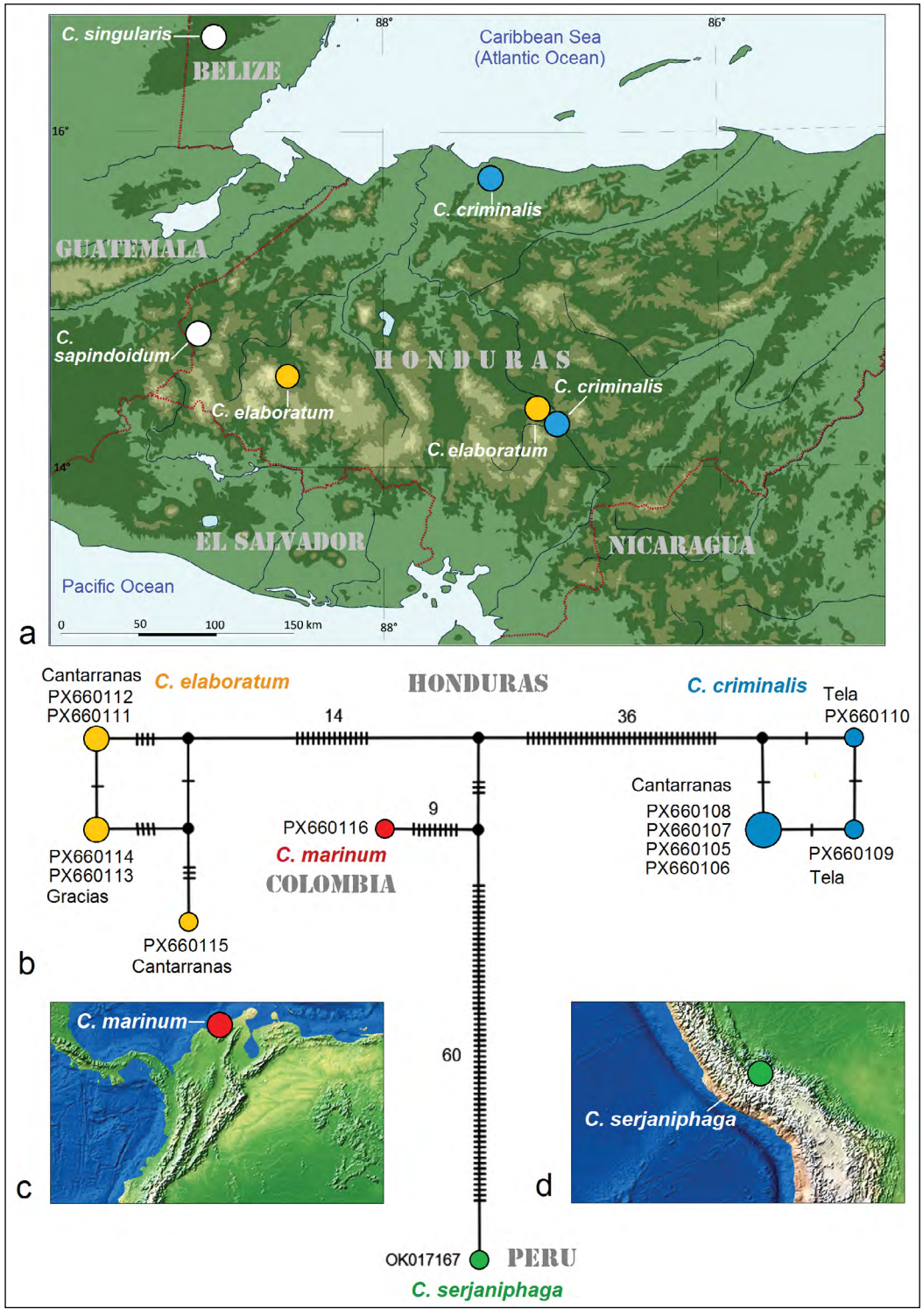

Fig. 13. Distribution data and molecular considerations of *Coptotrichoides* Diškus & Stonis: a, c, d – distribution of the species. The white dots indicate morphologically distinct but molecularly unstudied species; b – the mitotype network of *Coptotrichoides* based on 657 bp-long mtDNA CO1-5' sequences, constructed by the Median Joining Network. The circles represent mitotypes; the circle size is proportional to the frequency of each mitotype. The mitotypes not sampled in the study but predicted to be are depicted by smaller unnamed circles. The dashes on the lines connecting mitotypes and numbers next to them indicate hypothesized mutational steps

To further explore evolutionary relationships among *Serjania*-feeding *Coptotrichoides* species and to visualise intraspecific and interspecific genetic variation, a mitotype network was constructed (Fig. 13). It highlighted remarkably large genetic distances between all included species: it ranged from 30–31 hypothesised mutational steps between *C. elaboratum* sp. nov. and Colombian *C. marinum* sp. nov. to 100–101 steps between *C. criminalis* sp. nov. and Peruvian *C. serjaniphaga*; 55–57 mutational steps separated Honduran *C. elaboratum* sp. nov. and *C. criminalis* sp. nov. specimens. These patterns indicate clear genetic divergence among the analysed species and are fully congruent with the results of the phylogenetic reconstruction and the bPTP species delimitation approach (Fig. 12). The differences were detected within species, too: both *C. criminalis* sp. nov. and *C. elaboratum* sp. nov., represented by multiple specimens, were characterised by intraspecific divergence, which reached 0.31 ± 0.21% and 1.28 ± 0.45%, respectively.

New distribution data for selected Tischeriidae based on newly obtained molecular sequences

During the present study, we additionally sequenced nine specimens belonging to four species of the genera *Neotischeria* Diškus & Stonis and *Astrotischeria* Puplesis & Diškus, including the Ecuadorian *A. plagifera* (Meyrick) (Fig. 14). The molecular analyses resulted in new geographical distribution records for *A. selvica* Diškus, Carvalho-Filho & Stonis, *A. trilobata* Diškus & Stonis, 2018, and *Neotischeria neotropicana* (Diškus & Stonis).

Neotischeria neotropicana: 1 ♀, 1 dead larva, HONDURAS **(first record for Honduras)**, Atlántida Department, Tela [Colonia Tres de Mayo, near Las Palmas], 15°46'64"N, 87°23'15"W, elevation 30 m, larva on *Sida* sp. (Malvaceae), 6–18.iv.2024, leg. J. R. Stonis, DNA sequences have been deposited in GenBank (accession IDs: PX660120 and PX660121); 2 ♂, 1 ♀, PERU (**new distribution data within Peru**), San Martin Province, Tarapoto, 6°27'52"S, 76°19'29"W, elevation 700 m, mining larva on *Sida* sp., 7.ii.2025, leg. A. Diškus, DNA sequences have been deposited in GenBank (accession IDs: PX660117 (♂), PX660118 (♂), PX660119 (♀)).

Astrotischeria trilobata: 1 ♀, PERU (**first record for Peru**), Province de Bongará, Cocachimba, 6°3'19"S, 77°53'34"W, elevation 1820 m, from feeding larva, 29.i.2025, leg. A. Diškus, DNA sequence has been deposited in Genbank (accession ID: PX660122); 1 ♀, BOLIVIA, Nor Yungas Province, Coroico, 16°12'24"S, 67°43'54"W, elevation 1680 m, mining larva on *Austroeupatorium* sp. (Asteraceae), 07–16.vi.2018, card no. 5237, leg. A. Diškus and J. R. Stonis, DNA sequence has been deposited in Genbank (accession ID: PX660123).

Astrotischeria selvica: 1 ♀, PERU (**first record for Peru**), San Martin Province, Tarapoto, 6°27'29"S, 76°21'3"W, 480 m, from feeding larva, 11.ii.2025, leg. A. Diškus, DNA sequence has been deposited in Genbank (accession ID: PX660124).

Astrotischeria plagifera (Meyrick): 1 ♀, ECUADOR, Loja Province, 45 km S Loja, western environments of Vilcabamba, 4°17'42"S, 79°13'15"W, elevation 1950 m, mining larvae on *Rhysolepis incana* (Pers.) H. Rob. & A. J. Moore (Asteraceae), 23.i.2017, leg. A. Diškus, DNA sequence has been deposited in Genbank (accession ID: PX660125).

Analysis of *Neotischeria* and *Astrotischeria* topologies based on mtDNA CO1-5' sequences highlights several important points (see numbered notes 1–4 indicated by pink squares in Fig. 14).

1) Although the geographical distribution of Tischeriidae species remains poorly studied, *Neotischeria neotropicana* has been regarded as the Tischeriidae species with the widest known distribution in the Neotropical region (Diškus, Stonis, 2015). Subsequent records from Mexico and Guatemala further expanded its known range (Stonis et al., 2020), and the species is now reported from Mexico southwards to Peru and Bolivia. As these distributional data have so far been based exclusively on morphological examinations of genitalia and leaf mines, the question arises as to whether *N. neotropicana* represents

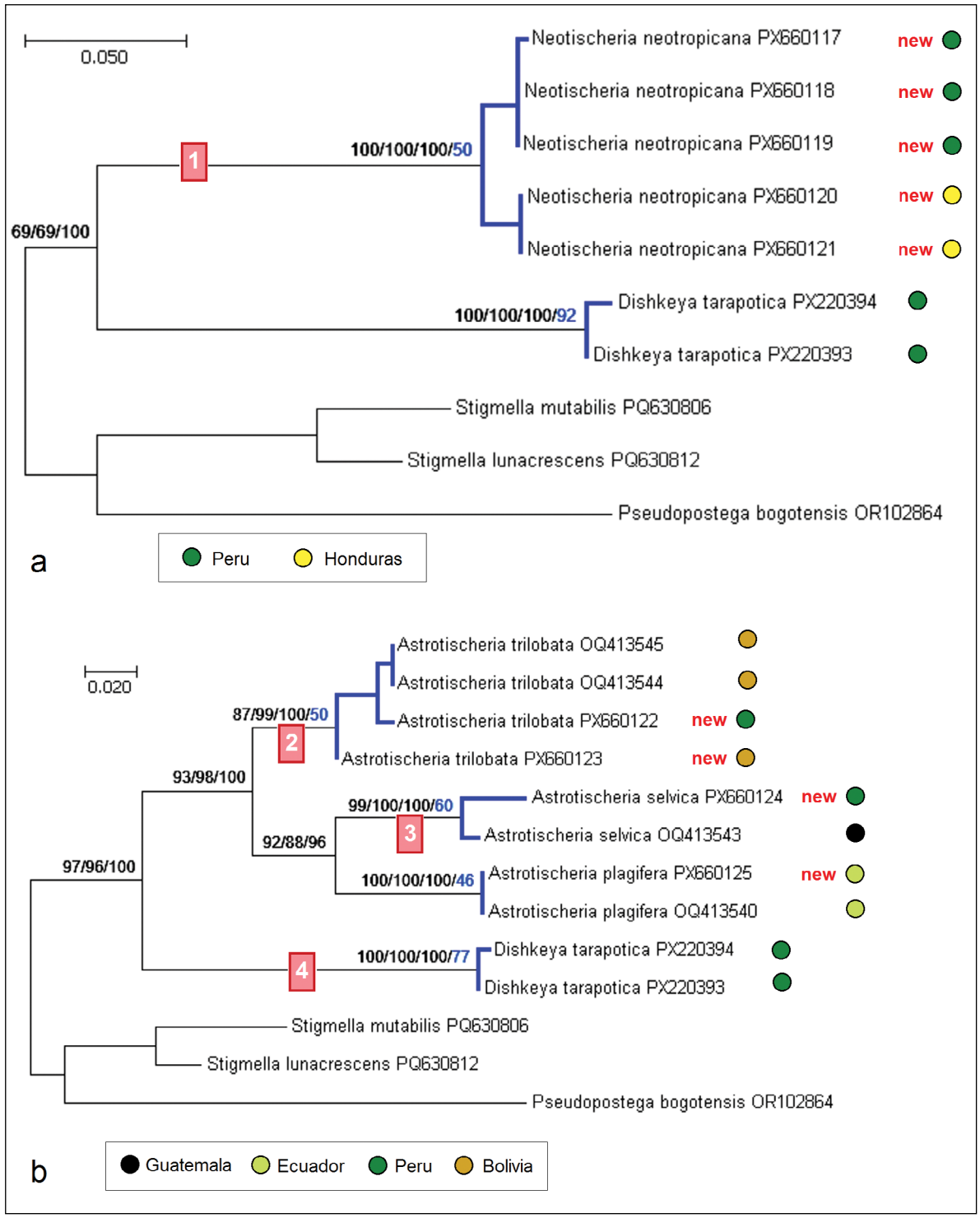

Fig. 14. Topologies based on the mtDNA CO1-5' sequences: a – topology of *Neotischeria* Diškus & Stonis species in comparison with selected other taxa; b – topology of *Astrotischeria* Puplesis & Diškus species. Numbers at nodes represent support values for Neighbor-Joining (10,000 bootstrap replicates) / Maximum Likelihood (10,000 bootstrap replicates) / Bayesian inference (10,000,000 generations), expressed as percentages. Blue colour indicates species delimitation inferred using the bPTP method. *Stigmella lunacrescens* Stonis & Remeikis, *S. mutabilis* Stonis & Remeikis (Nepticulidae), and *Pseudopostega bogotensis* Vargas (Opostegidae) were included as outgroups. Numbers 1–4 in pink squares refer to the numbered notes in the text

a genetically homogeneous species across this vast range. The comparison of DNA sequences from Honduras (Central America) and Peru (South America) reveal detectable genetic differences between populations ranging from 2.29 ± 0.61% to 2.47 ± 0.63%, though the applied bPTP species delimitation algorithm considers them to belong to the same species (Fig. 14a).

2) Until recently, *Astrotischeria trilobata* had been known only from Bolivia (Coroico) (Stonis et al., 2018). This distributional data was supplemented in 2025 by the discovery of a feeding larva of this species in Peru (Cocachimba). DNA sequencing of these specimens, along with two previously obtained Bolivian sequences, resulted in the recovery of a distinct *A. trilobata* clade, which was supported by all applied molecular approaches (NJ = 87%, ML = 99%, Bayesian posterior probability = 100%) and their conspecificity was approved by bPTP (Fig. 14b).

3) *Astrotischeria selvica* was originally described from Belize, Guatemala, and eastern Brazil and had not previously been recorded elsewhere (Stonis et al., 2018). Our data now confirm its presence in Peru (Tarapoto). Comparison of the newly obtained Peruvian DNA sequence with the previously published sequence from Guatemala revealed a rather high intraspecific divergence (2.76% ± 0.99%), though the applied bPTP method confirmed that both sequences belong to a single species (Fig. 14b). From a morphological perspective, *A. selvica* is closely related to *A. melantheranella* Heppner, a species occurring in Florida (USA), and the differentiation between these two allied species was discussed in detail by Stonis, Diškus (2024). However, our attempts to recover a partial mtDNA CO1-5' sequence from older type material of *A. melantheranella* were unsuccessful, and its barcode therefore remains unavailable.

4) *Dishkeya* Stonis (Stonis, Solis, 2020) is an enigmatic and morphologically distinctive genus of Tischeriidae characterised by unusually slender or branched leaf mines on Rhamnaceae. Based on currently available data, *Dishkeya* is endemic to the Americas, with a distribution ranging from California and Arizona (USA) to Bolivia but remains species-poor (Stonis et al., 2025). Until recently, only two species of this genus had been sequenced. During our latest study, an additional species from the Amazon Basin, *Dishkeya tarapotica* Diškus & Stonis, was barcoded; however, these molecular data have already been published separately (Stonis et al., 2025).

DISCUSSION

The present study resulted in the discovery and description of three species new to science: *Coptotrichoides elaboratum* Stonis & Diškus, sp. nov., *C. criminalis* Stonis & Diškus, sp. nov., and *C. marinum* Diškus & Stonis, sp. nov. While the addition of three species to a recently established genus is itself noteworthy, the most striking outcome of this study is not merely the increase in species number, but the unexpectedly high diversity of morphological structures observed in the male and female genitalia.

In principle, each newly discovered species in an insufficiently studied and species-poor genus has the potential to refine our understanding of that genus as a whole. In the case of *Coptotrichoides*, the newly described species are particularly informative, as they exhibit highly distinctive genital morphologies in both sexes. An additional strength of the present study lies in the generation of molecular data, which remain extremely scarce for this genus. Until recently, DNA barcode data were available for only a single species of *Coptotrichoides*. The inclusion of molecular sequences for additional species therefore represents a substantial advance and allows both the diagnosis and the broader concept of the genus to be reassessed.

The newly examined species confirm several diagnostic characters proposed in the original description of *Coptotrichoides* (Stonis et al., 2023). In particular, the adult head morphology remains consistent across the genus: the frontal tuft overlaps the frons and is composed of long, relatively wide lamellar scales; the pecten is distinctly elongate and conspicuous; and the collar is weakly paired or unpaired, consisting of very slender, almost piliform lamellar scales. With

regard to the forewing pattern, earlier studies noted that dark scales are usually irregularly distributed, being most abundant in the apical half of the wing. The newly discovered species demonstrate that dark scaling may also be well developed along the costal and dorsal margins of the forewing. Exceptionally, in *C. suprafasciata* (Diškus & Stonis), the forewing bears two bright yellow-ochre antemedian and postmedian patches. The hindwing remains slender in all species, and androconia are still unknown in the genus.

New morphological evidence necessitates a refinement of the generic diagnosis of the male genitalia. The uncus consistently consists of two elongate, slender lateral lobes (except basally). The socii range from moderately large to distinctly large, are membranous, weakly paired to distinctly paired, and are densely covered with fine spines. The tegumen is moderately long and lacks spines on the diaphragm; only *C. singularis* (Stonis & Diškus) is known to possess a slightly spinose diaphragm (Stonis et al., 2023). The pseudognathos is absent. The valva, in lateral and ventral view, generally tapers towards the apex and is basally widened; in some species it bears a small, inwardly directed apical process. Newly described species demonstrate that a basal lobe on the inner margin of the valva may be present (e.g. *C. criminalis* sp. nov.), and that the basal part of the valva may be abruptly bulged (e.g. *C. elaboratum* sp. nov.). The basal process of the valva is consistently short. A constant and diagnostic feature of *Coptotrichoides* male genitalia is the absence of the transtilla, in contrast to the genus *Coptotriche*. The anellus is absent or indistinct, and the juxta is always absent.

The present study also confirms that the vinculum is always short to very short but bears a median anterior process that varies from moderately long to extremely long. The greatest expansion of knowledge concerns the morphology of the phallus. In *Coptotrichoides*, the phallus is long to very long and rod-like, often with paired lateral lobes and a distinctive apical modification. In several species, the phallus is highly elaborated, bearing curved horn-like lateral processes and an extended apical portion armed with lateral or apical spines. Earlier studies suggested that lateral spines were absent in the genus; the newly discovered species clearly demonstrate that this assumption was incomplete.

In the female genitalia, ovipositor lobes are large to moderately large, with a relatively narrow gap between them. Whereas lateral lobes were previously described as distinctly elongate and proximally wide, all three newly described species possess very short, weakly developed lateral lobes. The anterior and posterior apophyses are usually similar in length, although slight differences occur among species. Earlier descriptions characterised the prela as three pairs of rod-like projections; recent discoveries reveal that the inner prela may be variously modified and lobe-like. The caudal sclerite, typically strongly thickened and inverted V-shaped distally, is weakly developed in *C. criminalis* sp. nov. The antrum is always absent. The accessory sac ranges from slender and strongly folded to weakly developed, and the ductus spermathecae is slender and coiled, lacking the wide, folded proximal portion typical of *Coptotriche*. The vesicle, previously regarded as small and indistinct, is relatively large in *C. elaboratum* sp. nov. The corpus bursae is short; although earlier studies described a long, slender 'neck', the newly discovered species indicate that this structure is shorter than previously assumed.

Recent discoveries have also contributed significantly to our understanding of the bionomics and taxonomic composition of *Coptotrichoides*. The genus currently comprises nine species, all trophically associated with Sapindaceae. Host-plant relationships remain unknown for *C. deliquescens* (Meyrick) from Guyana and *C. braziliensis* (Diškus & Stonis) from Brazil. Other species show more specific associations: *C. singularis* feeds on *Cardiospermum grandiflorum* Sw., *C. suprafasciata* on *Allophylus edulis* (A St. Hil.) Radlk., while the remaining species are associated with various species of *Serjania* Mill. Larvae mine leaves, producing irregular blotch-like or elongated trumpet-shaped mines; in some cases, larvae

fold the leaf margin prior to pupation. The genus is endemic to the Neotropical region and is currently known from Belize southwards to tropical northern Argentina.

The present study further demonstrates that species diversity of *Coptotrichoides* is particularly high in the region surrounding the Caribbean Sea. Of the nine currently recognised species, five occur in this region, three of them in Honduras (*C. sapindoidum*, *C. elaboratum* sp. nov., *C. criminalis* sp. nov.). Notably, these Honduran species exhibit striking differences in male genital morphology, highlighting a previously underestimated level of morphological diversification within the genus.

Molecular analysis consistently supports the distinctiveness of the three newly described *Coptotrichoides* species – *C. elaboratum* sp. nov., *C. criminalis* sp. nov., and *C. marinum* sp. nov., confirming the suitability of the CO1-5' partial sequence as a DNA barcode for animal species identification (Hebert et al., 2003). Together with *C. serjaniphaga*, these species form the *Coptotrichoides* clade that is separated from other Tischeriidae genera; however, due to insufficiently supported topology, the systematic position of the recently established *Coptotrichoides* genus (Stonis et al., 2023) still requires clarification including additional specimens and molecular characters.

Finally, beyond the focal genus, this study generated new molecular data for four species of *Neotischeria* and *Astrotischeria*, which provided a valuable reference for future identification and genetic variation of these species. Molecular data also yielded new distribution records for *A. selvica* and *A. trilobata* in Peru, and for *Neotischeria neotropicana* in Peru and Honduras, further emphasising the importance of integrative approaches combining morphology, molecular data, and field observations.

CONCLUSIONS

1. Recent discoveries demonstrate that species diversity of the Neotropical endemic genus *Coptotrichoides* Diškus & Stonis is particularly high in the region surrounding the Caribbean Sea. Of the nine species currently recognised in the genus, five are known exclusively from this region: *C. singularis* Stonis & Diškus, *C. sapindoidum* Diškus & Stonis, *C. elaboratum* Stonis & Diškus, sp. nov., *C. criminalis* Stonis & Diškus, sp. nov., and *C. marinum* Diškus & Stonis, sp. nov.

2. Leaf mines of *Serjania*-feeding *Coptotrichoides* species have limited diagnostic value for reliable species identification. Although adults of this genus are also externally very similar and difficult to distinguish, the morphology of male and female genitalia provides clear, consistent, and reliable characters by which all species of the genus – including *Serjania*-feeding taxa – can be readily separated.

3. The discovery of new species has enabled refinement of the generic diagnosis of *Coptotrichoides*. In male genitalia, species of this genus resemble those of *Coptotriche* Walsingham; however, *Coptotrichoides* is characterised by the consistent absence of the transtilla and anellus, the presence of an apically tapering valva, a slender uncus, a relatively longer tegumen, and an unspined or weakly spined teguminal diaphragm. In the female genitalia, in contrast to *Coptotriche*, *Coptotrichoides* is characterised by a slender ductus spermathecae and usually variously modified prela.

4. Molecular analyses revealed that all *Coptotrichoides* species for which mtDNA CO1-5' sequences are available are genetically distinct. Mitotype network analyses visualising intraspecific variability and evolutionary distances indicate relatively large genetic divergences among species. The genus *Dishkeya* Stonis appears to be the closest known relative of *Coptotrichoides*, rather than *Coptotriche*; however, support for this relationship remains moderate and not yet conclusive.

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APPENDIX: Why one species was named *criminalis*

By the collector and the first author: 'April, 2025. In the middle of the day, in a forested area near a village by the Choluteca River, I was collecting Tischeriidae leaf mines – enjoying a peaceful afternoon and completely absorbed in my work. Then, in an instant – like something straight out of an action movie – everything changed. Four fully masked bandits, armed with guns and machetes, appeared out of nowhere. One pointed a gun at my head and demanded that I hand over everything I had.

So, I experienced an armed robbery – deeply shocking, to say the least. I lost not only some money and valuables, like the watch torn from my wrist, but more importantly, my expensive mobile phone, along with all valuable data, contacts, and irreplaceable photos it contained (many of which, unfortunately, hadn't been backed up to the cloud).

The ordeal lasted about 15–20 minutes. But surprisingly, it ended on a somewhat fortunate note, especially considering how much worse it could have been. Sadly, word has reached me that killings and kidnappings remain all too common in this region. They could have injured me and left me bleeding, pushed me into the river where no one would have ever found me, or taken me hostage to extort ransom money from my family...

There are detailed guidelines for personnel caught in such high-risk situations, and I did my best to follow them. I did not resist, but instead tried to negotiate, calmly repeating 'somos amigos' [we are friends]. Eventually, the attackers became somewhat 'benevolent': I asked them to return my water (they did), my collected larvae (they dropped the jar at my feet), and – at my request – even my work documents and the key to the room I was staying in. Finally, in a strange gesture of parting courtesy, they even left me a few of my cigarettes.

It was a frightening and disheartening experience – one that clouded my appreciation for this beloved country. But as I walked back to the village in the sweltering heat, drinking water and smoking those last cigarettes, I felt a strange sense of relief, especially knowing that the precious larvae had been returned.

A couple weeks later, those larvae developed into adult moths, and to my astonishment, they turned out to belong to a previously unknown species of Tischeriidae. We named it *Coptotrichoides criminalis* – a small reminder that even amid violence, science sometimes finds a way forward. Well... some come with wings. Others come with guns and machetes.'

Jonas R. Stonis, Arūnas Diškus,
Svetlana Orlovskytė

NEOTROPIKŲ GENTIES *COPTOTRICHOIDES* (TISCHERIIDAE) PATIKSLINIMAS, REMIANTIS TRIJŲ NAUJŲ, *SERJANIA* AUGALUS MINUOJANČIŲ RŪŠIŲ ATRADIMU, ĮSKAITANT *C. CRIMINALIS* SP. NOV., SURINKTĄ PAVOJINGOMIS SĄLYGOMIS

Santrauka

Naujausi tyrimai rodo, kad neotropikų endeminės genties *Coptotrichoides* Diškus & Stonis rūšių įvairovė Karibų jūros regione yra itin didelė: iš devynių šiuo metu aprašytų šios genties rūšių net penkios aptiktos išimtinai šiame regione: *C. singularis* Stonis & Diškus, *C. sapindoidum* Diškus & Stonis, *C. elaboratum* Stonis & Diškus, sp. nov., *C. criminalis* Stonis & Diškus, sp. nov. ir *C. marinum* Diškus & Stonis, sp. nov. Naujų rūšių atradimas leido patikslinti bendrą *Coptotrichoides* genties diagnostiką ir nustatyti naujus morfologinius požymius. Dalinių mitochondrinės DNR sekų tyrimai patvirtino atskiras naujai aprašytas rūšis, nors ir nepakankamai patikimai išskyrė *Coptotrichoides* gentį bei atskleidė gana didelius genetinius skirtumus tarp *Coptotrichoides* rūšių. Be to, šiame tyrime gautos naujos *Neotischeria* Diškus & Stonis ir *Astrotischeria* Puplesis & Diškus genčių DNR sekos suteikė naujų žinių apie *A. selvica* Diškus, Carvalho-Filho & Stonis ir *A. trilobata* Diškus & Stonis geografinį paplitimą Peru bei *Neotischeria neotropicana* (Diškus & Stonis) paplitimą Peru ir Hondūre.

Reikšminiai žodžiai: lapų minos, mažieji šeriuotaūsiai, neotropinė fauna, Sapindaceae, vabzdžiai minuotojai