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Refining the concept of the enigmatic genus *Dishkeya* Stonis (Lepidoptera: Tischeriidae) upon discovery of a new species with remarkably branched leaf mines on *Gouania* Jacq. (Rhamnaceae) from the Amazon Basin

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Abstract

This study aims to describe a new species of *Dishkeya* Stonis, *D. tarapotica* Diškus & Stonis, **sp. nov.**, and to assess how its characteristics contribute to the morphological, biological, and molecular understanding of this small, enigmatic, and understudied genus. Despite the similarity of leaf mines between *D. tarapotica* **sp. nov.** and *D. gouaniae* (Stonis & Diškus), the new species is a distinct taxon that differs clearly from all other *Dishkeya* species—including those feeding on the same host-plant genus, *Gouania* Jacq. (Rhamnaceae)—due to notable differences in genital morphology. Key diagnostic characters include the small female corpus bursae, a wide male phallus with strongly developed lateral processes and exceptionally long carinae, an elaborately shaped valva, and most notably, the sclerotized socii (*pseudognathos*). These characters support the current concept of the genus *Dishkeya*. In contrast, the presence of long antennal sensilla trichodea in females of *D. tarapotica* **sp. nov.** and the related *D. gouaniae* represents a unique and striking trait—an atypical character within the family Tischeriidae, but one that contributes to the diagnostic characteristics of the genus *Dishkeya*. Molecular analysis incorporating newly generated sequences of *D. tarapotica* **sp. nov.** further confirmed its distinctiveness and once again raised the possibility that *Dishkeya* is more closely related to the genera *Coptotrichoides* Diškus & Stonis and *Coptotriche* Walsingham, rather than to *Tischeria* Zeller.

Key words: barcode, *Coptotrichoides, Coptotriche, Dishkeya tarapotica,* leaf miners, Neotropics, *Tischeria,* trumpet leafminer moths

Introduction

Tischeriidae, commonly known as trumpet leafminer moths or trumpet moths, represent a distinct family within the earliest (monotrysian) lineages of extant Lepidoptera. Phylogenetically, they occupy a basal position among Lepidoptera (for detailed discussion, see Regier *et al*. 2015) and are characterized by a number of highly distinctive morphological traits (Davis 1999, Puplesis & Diškus 2003, Stonis *et al*. 2023). The larvae of trumpet moths are internal leaf miners throughout all instars, and pupation also takes place within the mine.

Since the legendary American entomologist Annette Frances Braun (1884–1978) published the first exhaustive monograph on Tischeriidae (Braun 1972), our understanding of the family's morphology, species diversity, and especially the taxonomic composition of its genera has changed dramatically. For a recent review of the eleven currently recognized genera of Tischeriidae worldwide, along with a complete list of relevant references, see Stonis *et al*. (2023).

The genus *Dishkeya* Stonis, in Stonis & Solis 2020, is a recent addition to scientific classification. Figuratively speaking, this small genus was extracted from the iconic—and, for a long time, the only recognized—genus in the family Tischeriidae, *Tischeria* Zeller, 1839. It was observed that two previously described *Tischeria* species, *T. bifurcata* Braun, 1915 and *T. gouaniae* Stonis & Diškus, 2007, did not conform to the morphological diagnosis of *Tischeria*. As a result, these species were excluded from *Tischeria*, and a new genus, *Dishkeya*, was erected to accommodate them along with one newly discovered species.

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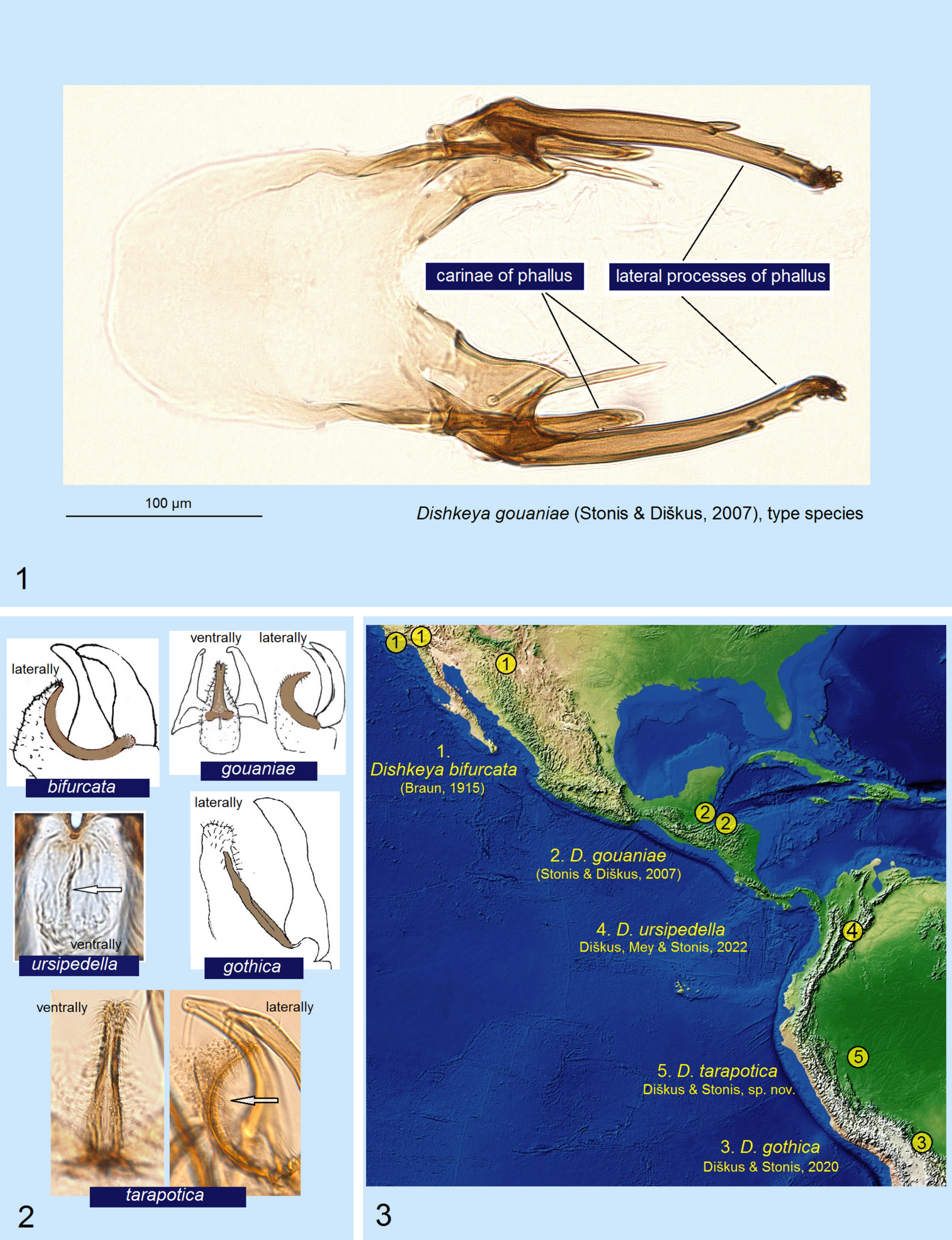

FIGURES 1–3. Genus *Dishkeya* Stonis: 1, major diagnostic characters of the phallus, type species of the genus, *D. gouaniae* (Stonis & Diškus, 2007), Honduras, genitalia slide no. AD1183 (MfN); 2, sclerotized socii (brown painted or arrowed) of *Dishkeya* species forming a pseudognathos; 3, distribution of the currently known species.

This genus of trumpet moths is highly distinctive and fascinating. Its larvae mine the leaves of Rhamnaceae, developing leaf mines that are unusual among Tischeriidae—slender and branching (Stonis *et al*. 2024)—whereas the vast majority of tischeriid species produce trumpet-shaped or irregular blotch-like mines. The male genitalia also exhibit unique characters: the phallus is notably wide and equipped with long lateral processes. Unlike *Tischeria*, where the presence of a juxta is a major diagnostic character (Stonis *et al*. 2023), *Dishkeya* instead possesses well-developed, often long and elaborate carinae of the phallus (Stonis & Solis 2020) (Fig. 1). However, perhaps the most striking and presumably derived character involves the socii, which are sclerotized to varying degrees and transformed into a unique structure termed *pseudognathos* by Stonis & Solis (2020) (Fig. 2).

In the female genitalia, the genus is not marked by highly distinctive features, but small ovipositor lobes and rather large lateral lobes have previously been considered potentially diagnostic for *Dishkeya* (Stonis *et al*. 2023).

Based on the first available molecular sequences, the primarily morphological concept of *Dishkeya* proposed by Stonis & Solis (2020) has recently been expanded by incorporating the genus into the phylogeny of Tischeriidae (Stonis *et al*. 2023, 2024). This molecular analysis suggested that *Dishkeya* is closely related to the Sapindaceae-feeding genus *Coptotrichoides* Diškus & Stonis, in Stonis *et al.* 2023, as well as to *Coptotriche* Walsingham, 1890, but not to *Tischeria*, from which *Dishkeya* was originally “extracted”.

In light of current species distribution data, the genus *Dishkeya* is endemic to the Americas, ranging from California and Arizona (USA) to Bolivia, but remains species-poor (Fig. 3). Until now, only four species were known, and the discovery of additional species was anticipated. The recent identification of a new species from the Amazon Basin is a remarkable and exciting development—not only because of the novel geographic region but also due to the highly distinctive nature of the new species.

The goal of this study is to introduce a new species and to compare it with previously known species within the genus. This includes (1) a detailed formal description of the new species, and (2) an assessment of how its characteristics contribute to the morphological, biological, and molecular understanding of this recently established, yet taxonomically and biologically enigmatic genus.

In theory, each newly discovered species in an understudied, low-diversity genus can enhance our understanding of the genus as a whole. In this case, the new species is particularly remarkable, exhibiting highly distinctive external morphology in females alongside prominent and well-defined genital characters in both sexes. Furthermore, this species was discovered in a region where *Dishkeya* had not previously been recorded, offering multiple new insights.

An additional advantage of this study is the availability of a small but exceptionally valuable set of material: both sexes are represented, with a male and a female reared from the same host plant and reliably associated. Furthermore, molecular sequences have been obtained—an especially significant contribution, given the critical shortage of such data in *Dishkeya*. Until now, molecular data were available for only two known species within the genus. Now, having one more species along with their molecular sequences, we can update the genus characteristics and broaden our knowledge on *Dishkeya* in general.

Material and Methods

Material. The description of the new species is based on material collected in Tarapoto, San Martín Department, San Martín Region, PERÚ, 6°26'57"S, 76°20'42"W, elevation 520 m. This material is currently deposited at BRG but will be transferred to the collection of the Museum für Naturkunde (MfN), Berlin, Germany, following publication. Comparative material examined: 4 ♂, holotype and paratypes of *Tischeria gouaniae*, now *Dishkeya gouaniae* (Stonis & Diškus), BELIZE, Cayo District, Chiquibul Forest Reserve, Las Cuevas, 16°43'53"N, 88°59'11"W, elevation 550 m, mining larvae on *Gouania polygama* (Jacq.) Urb. (Rhamnaceae), 25.ix.1997 and 19.iii–3.vii.1998, leg. O. T. Lewis; genitalia slide nos 31442♂ (holotype), 31443♂ (NHMUK); 2 ♂, 2 ♀, non-type specimens of *Dishkeya gouaniae*, HONDURAS, Atlántida Department, Tela, near Las Palmas, elevation 30 m, 15°46'56"N, 87°23'15"W; larvae feeding on *Gouania polygama* (Rhamnaceae); 13–19.iv.2024, leg. J. R. Stonis, genitalia slide nos AD1183♂, AD1192♂, AD1198♀ (MfN); 2 ♂, holotype and paratype of *Dishkeya ursipedella* Diškus, Mey & Stonis, COLOMBIA, Cundinamarca, Choachí (La Chorrera Waterfall), 4°35'33"N, 73°57'36"W, elevation 2,500 m, 1–2.ii.2017, leg. W. Mey, genitalia slide nos AD1119 (holotype), AD1120 (paratype) (currently deposited at MfN, with the holotype to be subsequently transferred to UNC); 1 ♂, holotype of *Dishkeya gothica* Diškus & Stonis, BOLIVIA, Nor Yungas Province, Coroico, 16°12'25"S, 67°43'53"W, elevation 1,680 m, mining larva on *Gouania lupuloides* (L.) Urb. (Rhamnaceae), 15.vi.2018, field card no. 5260, leg. A. Diškus, genitalia slide no. AD1010♂ (genitalia from adult in pupal skin, no pinned adult preserved) (ZMUC).

Methods. Terminology used for genitalia followed by Stonis *et al.* (2023). Collecting methods and techniques for rearing adults from mining larvae are described in detail in our previous publications (Stonis *et al.* 2024). Genitalia were prepared following the protocol outlined by Puplesis & Diškus (2003). Abdomens were macerated in 10% KOH, and the genital capsules, as well as the phallus, were mounted in Euparal, with the ventral side uppermost. Genitalia slides were examined and photographed using a Leica DM2500 microscope equipped with a Leica DFC420 digital camera. Adults were studied and measured under a Lomo-10 stereoscopic microscope and photographed using a Leica S6D stereomicroscope with an attached Leica DFC290 digital camera. Forewing length was measured along the costa, from the wing base to the apex of the fringe. To calculate wingspan, the measured forewing length was doubled, and the thorax width was added. For specimen illumination, a ring light LED 60 was mounted directly to the stereomicroscope lens, offering adjustable intensity and a colour temperature range of 7,000 to 11,000 K, delivering 8,000 Lux at a 100 mm working distance. Leaf-mine photographs were taken in the field using an Olympus TG-2 camera with the SuperMacro function.

Molecular Analysis. Total genomic DNA was extracted from an air-dried adult male (holotype) preserved in pupal skin with the abdomen dissected, and from two legs of the female paratype using the GeneJet Genomic DNA Purification Kit (Thermo Fisher Scientific Baltics,Vilnius, Lithuania), following the manufacturer's protocol. The extracted samples of DNA are deposited in the collection of the State Scientific Research Institute Nature Research Centre (NRC) (Vilnius, Lithuania). The universal barcoding primers LCO1490 (5'-ggtcaacaaatcataaagatattgg-3') and HCO2198 (5'-taaacttcagggtgaccaaaaaatca-3') (European and Mediterranean Plant Protection Organization 2016, 2021), synthesized at Metabion (Planegg, Germany), were applied for the amplification of the partial cytochrome c oxidase subunit 1 of the mitochondrial DNA (mtDNA CO1-5'). The mixture of polymerase chain reaction (PCR) contained 12.5 µL of 2× DreamTaq PCR Master Mix (Thermo Fisher Scientific Baltics, Vilnius, Lithuania), 2.5 µL of each primer (10 pmol/µL), 2 µL of extracted DNA, and 5.5 µL deionised H_2O. The PCR conditions were as follows: an initial denaturation of DNA sequences at 95 °C for 5 min; 45 cycles of denaturation at 94 °C for 40 s, annealing of primers at 45 °C for 40 s, extension of sequences at 72 °C for 1 min; final extension at 72 °C for 5 min. Horizontal electrophoresis of PCR products was carried out on 1.5% agarose (Thermo Fisher Scientific Baltics, Vilnius, Lithuania) gel stained with the Roti Gelstain Red dye (Karl Roth, Karlsruhe, Germany) submerged into 0.5× TBE buffer (Thermo Fisher Scientific Baltics, Vilnius, Lithuania). The visualisation was performed under 254 nm UV light. The size of the amplified DNA was measured with the GeneRuler Express DNA ladder (Thermo Fisher Scientific Baltics, Vilnius, Lithuania). Both successfully amplified PCR products were purified each 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, Vilnius, Lithuania). Afterwards, they were sent for the automatic Sanger sequencing in both directions with the 3730xl DNA analyzer (Applied Biosystems, Waltham, Massachusetts, USA) to Macrogen Europe (Amsterdam, The Netherlands). The alignment of obtained sequences was performed by the BioEdit v.7.2.5 program (Hall 1999). Both successfully aligned new DNA sequences were deposited in the NCBI GenBank database (Benson *et al*. 2013) under the accession numbers PX220393 and PX220394. For molecular analysis, we included seven of our own sequences of *Dishkeya gouaniae*, as well as one sequence from each of the following genera: *Coptotrichoides* Diškus & Stonis, *Rytietia* Diškus, Xu & Dai, *Tischeria* Zeller, and *Pseudopostega* Kozlov. These sequences were obtained in earlier studies and have already been used in our previous publications (Stonis *et al.* 2021, 2023, 2024). In addition, sequences of *Dishkeya bifurcata* (Braun) and *Coptotriche purinosella* (Chambers), used in our analysis, were obtained from the BOLD platform (Ratnasingham & Hebert 2007). The most likely nucleotide substitution model was selected (GTR+G+I) from the list of models available in MEGA v.7 (Kumar *et al*. 2016). The reconstruction of phylogenetic relationships was carried out by the Maximum Likelihood (ML) (10,000 bootstrap replicates) method included in MEGA, and the Bayesian inference (10 million generations) implemented into MrBayes v.3.2.3 (Ronquist & Huelsenbeck 2003). The ClustalX2 software (Larkin *et al*. 2007) was applied for the Fasta format conversion to Nexus. Species delimitation was performed by the bPTP method (Zhang *et al*. 2013). The mitotype network was constructed using the TCS Network (Clement *et al*. 2002) with PopArt v.1.7 (Leigh & Bryant 2015). Codes along each entity in Fig. 36 refer to GenBank and BOLD.

Abbreviation for Institutions and Specimen Depositories. BRG—Biosystematics Research Group, currently based at the State Scientific Research Institute Nature Research Centre (NRC), Vilnius, Lithuania; MfN—Museum für Naturkunde, formerly known as the Museum für Naturkunde der Humboldt-Universität zu Berlin or Museum für Naturkunde / Leibniz-Institut für Evolutions- und Biodiversitätsforschung, Berlin, Germany; NHMUK (formerly BMNH)—the Natural History Museum, London, United Kingdom; NRC—State Scientific Research Institute Nature Research Centre, Vilnius, Lithuania; UNC—Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, Colombia; ZMUC—Zoological Museum, Natural History Museum of Denmark, Copenhagen, Denmark.

Description of a new species

Dishkeya tarapotica Diškus & Stonis, sp. nov.

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Figs 4–28

Type material. Holotype: ♂, PERÚ: San Martín Region, San Martín Department, Tarapoto, 6°26'57"S, 76°20'42"W, elevation 520 m, developed pupa in a leaf mine on *Gouania polygama* (Jacq.) Urb. (Rhamnaceae), 3.ii.2025, ex pupa 7.ii.2025, field card no. 5386, A. Diškus, genitalia slide no. AD1225♂ (genitalia from adult in pupal skin, no pinned adult preserved) (MfN).

Paratype: 1 ♀, same label data as holotype, genitalia slide no. AD1233♀ (MfN). The original label data of the specimens are given with the term 'Province' instead of 'Department'.

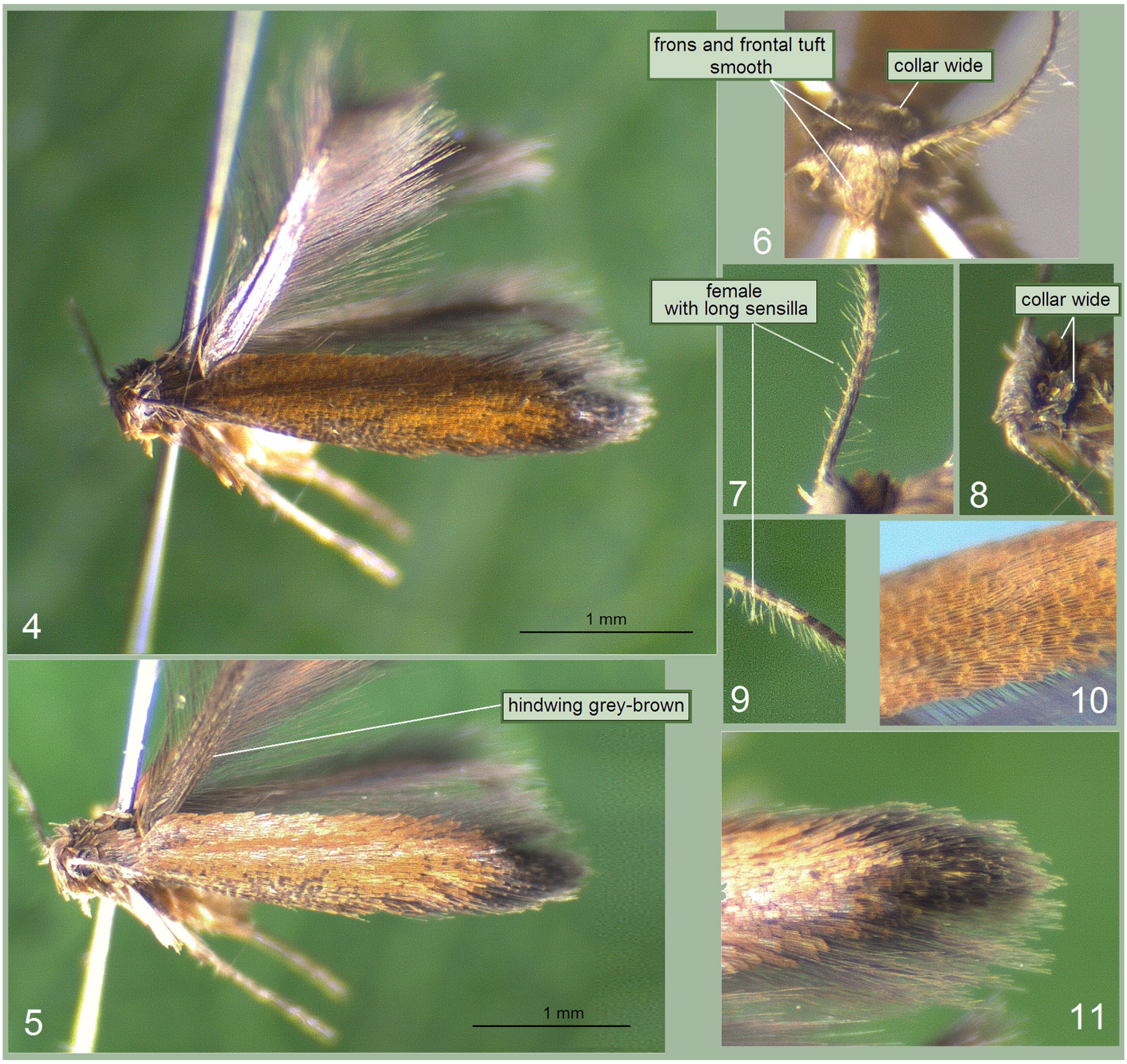
Diagnosis. Dishkeya tarapotica **sp. nov.** is externally distinguished by its intensely dark beige-orange forewings—a coloration notably darker and more intensely orangish than that of other known *Dishkeya* species. In females, the presence of unusually long antennal sensilla—approximately three times the width of the flagellum—is a particularly striking and diagnostic character (Fig. 7). In male genitalia, the species can be readily identified by the lateral processes of the phallus with large spines and by the exceptionally long carinae, which clearly separate it from all congeneric species (Figs 15–17, 20).

Barcodes. We barcoded the male holotype and the female paratype; the sequence have been deposited in GenBank (accession IDs: PX220393 and PX220394).

External characters (Figs 4–11) (see *Remarks*). Forewing length approx. 2.8 mm; wingspan approx. 6.1 mm (n = 1). Head: palpi pale yellowish beige; frons smoothly scaled, pale greyish beige speckled with brown and dark brown scales; pecten small, slender, pale greyish beige; frontal tuft glossy dark grey, smoothly overlapping head; collar large and wide, comprised of glossy blackish grey lamellar scales; antennal flagellum glossy blackish grey; in females, antennal sensilla about three times longer than width of flagellum. Thorax: tegula greyish beige, proximally covered with grey-black scales with some purple iridescence; thorax glossy, predominantly dark grey beige sparsely irrorated with grey-black scales; forewing glossy, predominantly intense dark yellow-beige with blue-purple iridescence; costal margin irrorated with grey-black scales; apex marked with black scales showing purple iridescence; fringe grey to blackish grey, without fringe line; forewing underside black-brown, lacking spots or androconia. Hindwing without androconia, dark brown-grey on upper side and underside though under certain angles it may appear brown with golden gloss; fringe grey. Legs predominantly pale brown with golden gloss and with some dark grey-brown scales speckled on upper side.

Male genitalia (Figs 12–21). Genital capsule approximately 505 µm long and 260 µm wide. Uncus (Figs 12, 13, 18) composed of two lateral lobes wide only at the base and slender along most of their length, appearing almost rod-like (Fig. 18). Socii (Figs 15, 18) strongly modified: laterally membranous but medially sclerotized, forming pseudognathos (Fig. 14) covered with fine spines. Valva (Figs 15, 19) approximately 305 µm long (excluding basal process); inner lobe of valva distinctly bulged (Fig. 19). Vinculum medium large, triangular distally (Fig. 15), without lateral lobes. Phallus (Figs 15, 20) approximately 515 µm long (measured including lateral processes), 155 µm wide basally, only slightly constricted medially; equipped with long lateral processes bearing 3–4 large spines, and unusually long and slender carinae, which are bifid basally (Figs 16, 17, 20, 21).

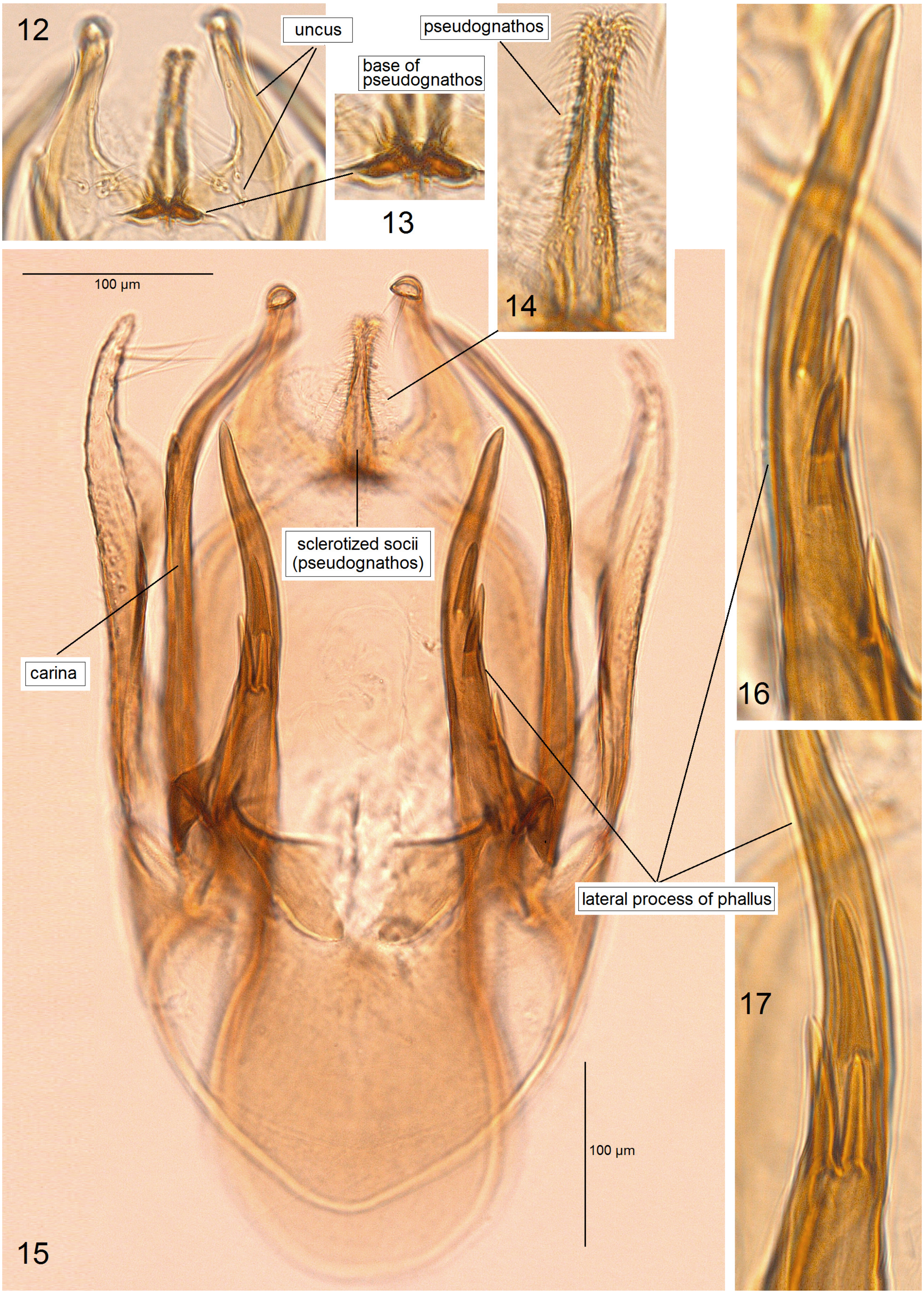
Female genitalia (Figs 22–28). Total length approximately 810 µm. Ovipositor lobes (Figs 22, 23) moderately large, rounded, and densely covered with peg-like setae; second pair of ovipositor lobes three to four times smaller, bearing numerous long setae; lateral lobes short, not reaching ovipositor lobes. Anterior and posterior apophyses of equal length (Fig. 22). Prela consists of two pairs of rod-like processes and one pair of lobular structures: transverse prela (Fig. 24) rod-like and relatively slender; median prela also rod-like and relatively long (Fig. 27); inner prela barely visible and lobular, with lobes widely separated (Fig. 27). Additionally, sclerotized thickenings occur caudal or ventral to the inner prela (Fig. 27). Corpus bursae without pectinations or signa (Figs 22, 25), but neck of corpus bursae hardened and with minute spines (Fig. 28). Ductus spermathecae with approximately 9–11 coils (predominantly small, with some being three times larger), and relatively large, rounded vesicle (Fig. 26).



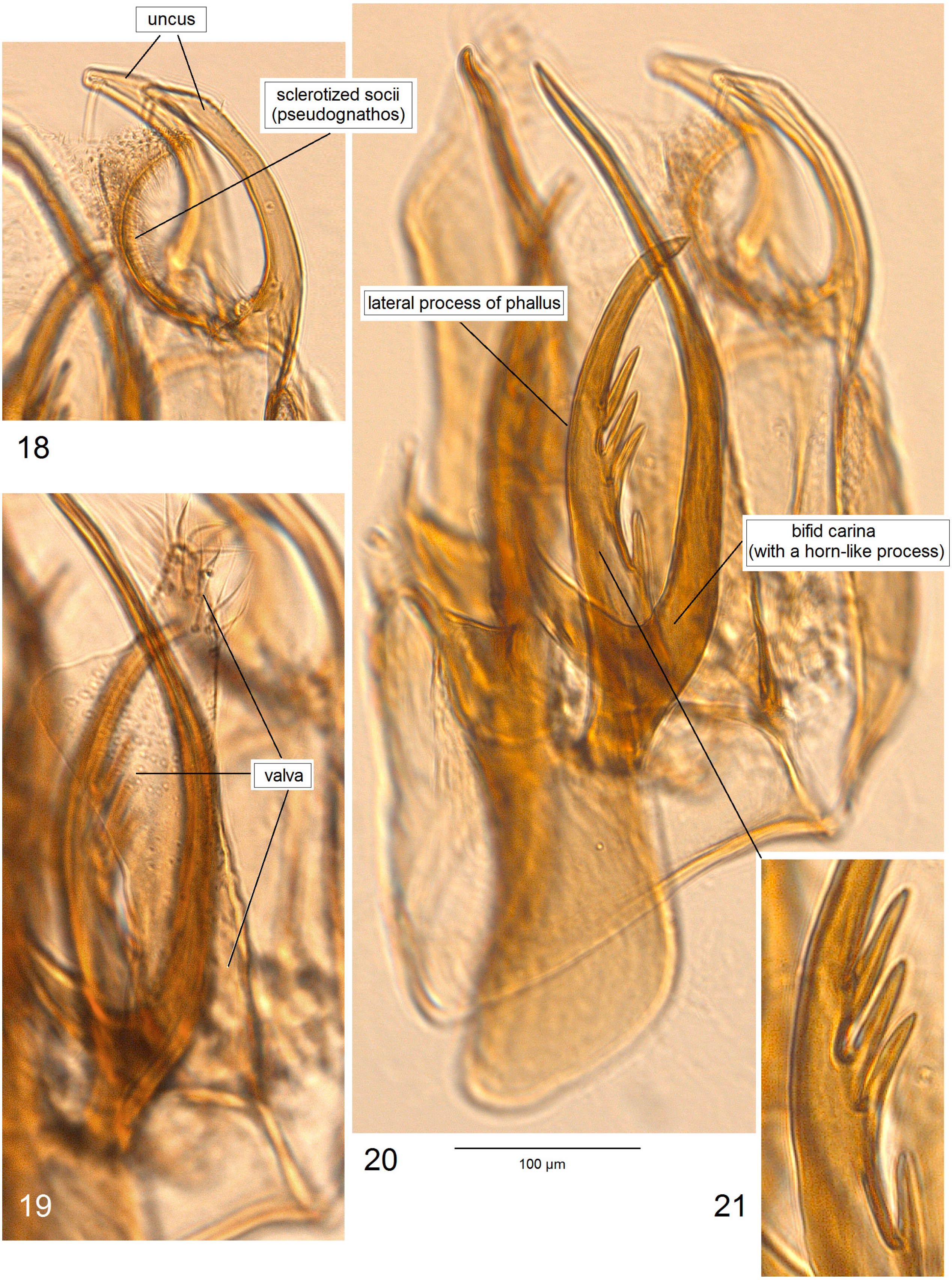
FIGURES 4–11. Female adult of *Dishkeya tarapotica* Diškus & Stonis, **sp. nov.**, paratype, Perú, San Martín Region, San Martín Department, Tarapoto, 6°26'57"S, 76°20'42"W, ex pupa 7.ii.2025 (MfN): 4, 5, general view under different angles of illumination; 6–11, details of morphology.

Bionomics (Figs 29–35). The host plant is *Gouania polygama* (Jacq.) Urb. (Rhamnaceae) (Figs 29–31). Larvae feed in late January to February and produce remarkably branched leaf mines (Figs 32, 33). When disturbed, the larvae cease feeding and retreat into the linear portion of the mine, often positioned along a leaf vein, making them barely visible. Pupation occurs within the leaf mine, along the leaf vein (Figs 34, 35), in an elongated white nidus that is not externally visible and requires dissection to detect. Based on rearing data, adults emerge and are active in February. The other aspects of the species' biology remain unknown.

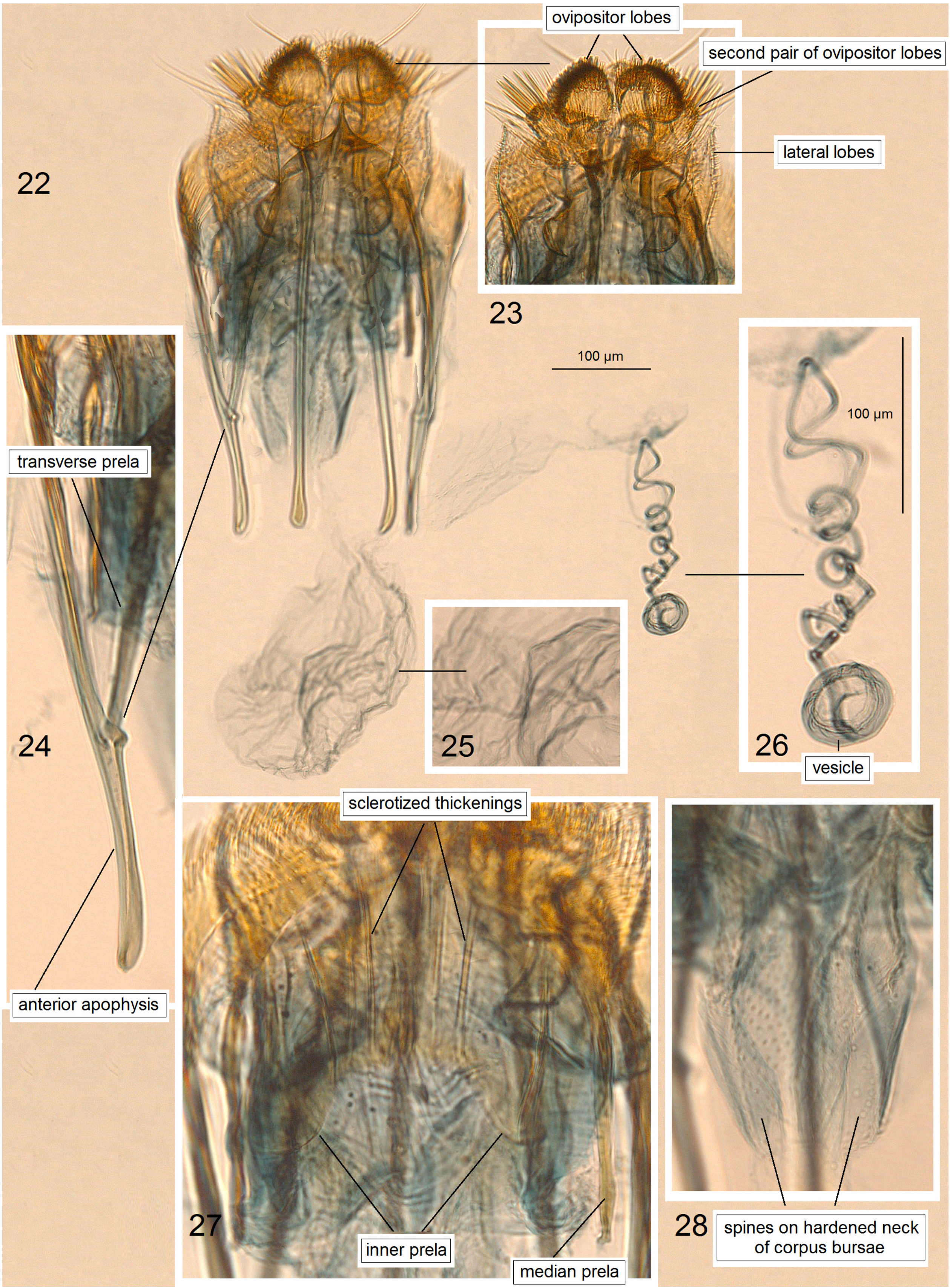
Distribution. This species is known only from a single locality in the Peruvian Amazon: Tarapoto, San Martín Department, Perú, at an elevation of approximately 520 m.



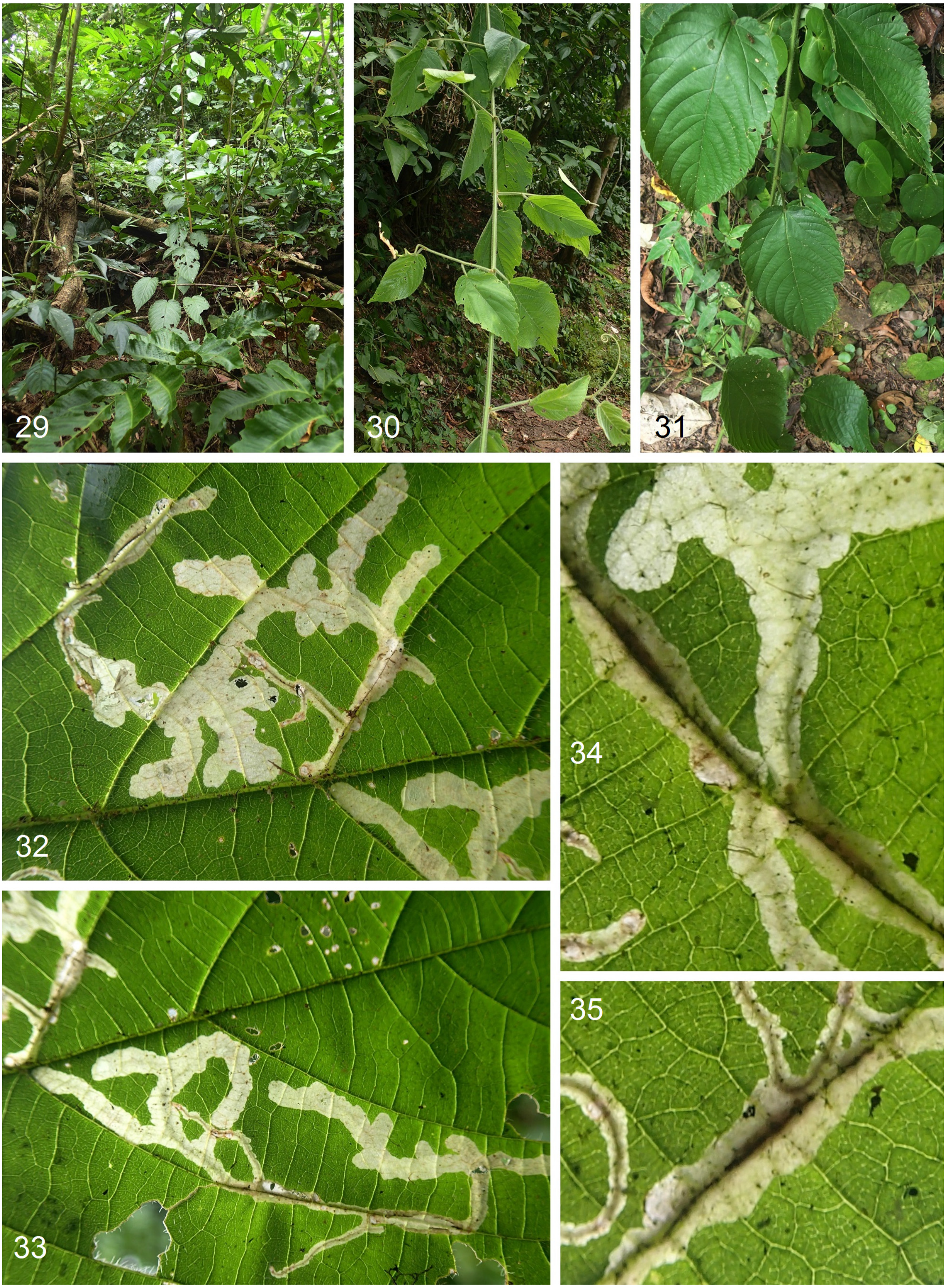
FIGURES 12–17. Ventral view of male genitalia of *Dishkeya tarapotica* Diškus & Stonis, **sp. nov.**, holotype, genitalia slide no. AD1225♂ (genitalia from adult in pupal skin, no pinned adult preserved) (MfN): 12–14, uncus and sclerotized socii forming pseudognathos; 15, general view; 16, 17, enlarged view of spines on lateral processes of phallus.



FIGURES 18–21. Lateral view of male genitalia of *Dishkeya tarapotica* Diškus & Stonis, **sp. nov.**, holotype, genitalia slide no. AD1225♂ (before permanent mounting in ventral view) (MfN): 18, uncus and chitinized socii forming a pseudognathos; 19, valva; 20, general view; 21, enlarged view of spines on lateral processes of phallus.



FIGURES 22–28. Ventral view of female genitalia of *Dishkeya tarapotica* Diškus & Stonis, **sp. nov.**, paratype, genitalia slide no. AD1233♀ (MfN): 22, general view; 23, ovipositor, focused on ovipositor lobes; 24, transverse prela and anterior apophysis; 25, an enlarged fragment of corpus bursae; 26, ductus spermathecae; 27, inner prela and sclerotized thickenings caudal and ventral to the inner prela; 28, spines on hardened neck of corpus bursae.



FIGURES 29–35. Bionomics of *Dishkeya tarapotica* Diškus & Stonis, **sp. nov.**: 29, habitat, Perú, San Martín Region, San Martin Province, Tarapoto, 6°26'57"S, 76°20'42"W, elevation 520 m; 30, 31, host plant *Gouania polygama* (Jacq.) Urb. (Rhamnaceae); 32, 33, leaf mines on *G. polygama*, 3.ii.2025; 34, 35, pupation within leaf mine, along leaf vein.

Etymology. The species is named after the locality of Tarapoto, where it was discovered. A noun in the nominative singular standing in apposition to the generic name.

Remarks. The external description of the species is largely based on the examined female paratype, as the holotype (a single male adult) was available only as a specimen within the pupal skin. Based on our observations—within the limits allowed by the preserved material—the male in the pupal skin closely resembled the female paratype. However, the entire male body, except genitalia, was used for DNA extraction; therefore, no pinned adult male was preserved.

Mitochondrial DNA sequences of *Dishkeya tarapotica* sp. nov. in the context of the genus and its phylogenetic position

Until now, *Dishkeya bifurcata* and *D. gouaniae* were the only two barcoded species of the recently erected genus *Dishkeya*. Here, we provide the 657 bp-long mtDNA CO1-5' sequences of a third species, *D. tarapotica* **sp. nov.** In our phylogenetic tree, all three *Dishkeya* species consistently formed a well-supported monophyletic clade (ML bootstrap value = 97%, Bayesian posterior probability = 100%), clearly separated from other genera included in the analysis (Fig. 36), thereby supporting the distinctiveness of the *Dishkeya* taxon.

Within the genus, the divergence of all three species—*D. bifurcata*, *D. gouaniae*, and *D. tarapotica* **sp. nov.**—was supported by the applied bPTP species delimitation method. However, the phylogenetic position of *D. tarapotica* **sp. nov.** in relation to the other *Dishkeya* species remains uncertain: according to the ML analysis, it clustered as a sister clade to *D. gouaniae*, but this was not supported by the Bayesian inference. Unlike *D. gouaniae* specimens, whose sequences were all identical, *D. tarapotica* **sp. nov.** is characterized by sequences representing different mitotypes. This finding suggests a potentially higher intraspecific variability in *D. tarapotica* **sp. nov.**, which may be confirmed by future research.

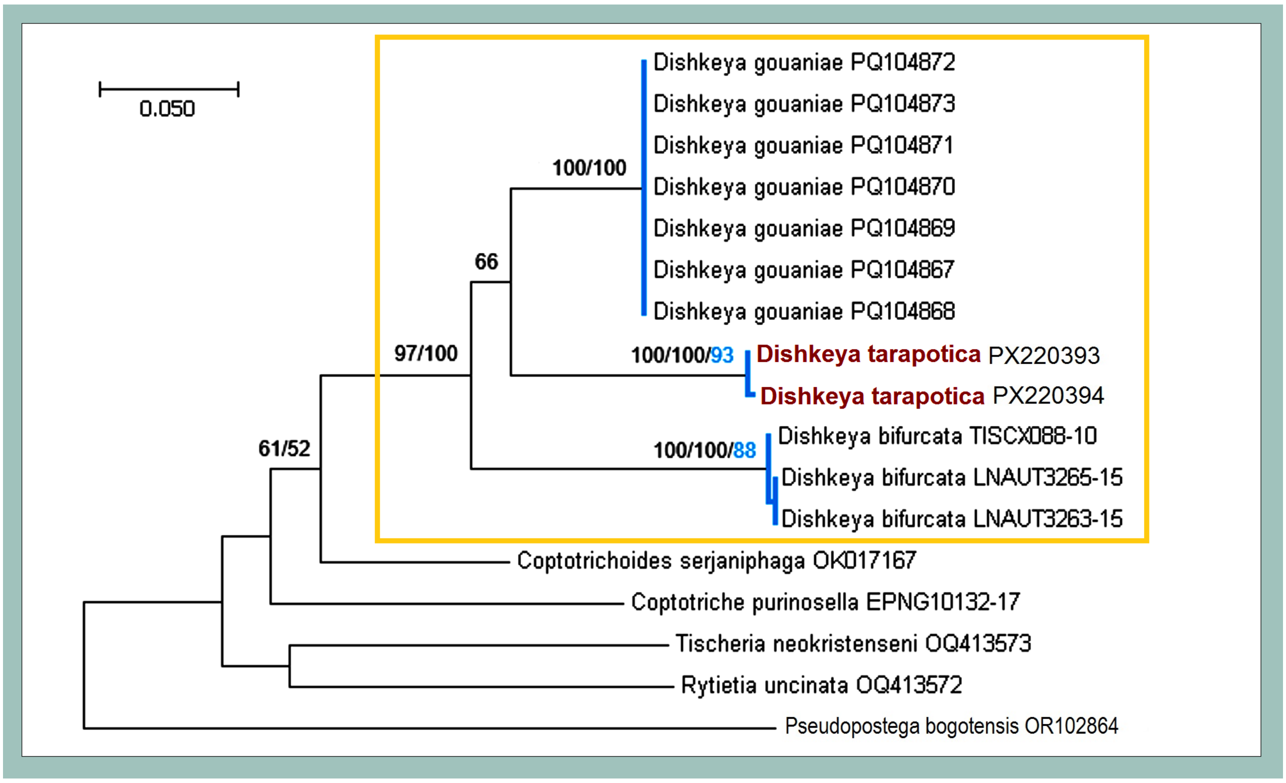


FIGURE 36. The phylogenetic relationships of *Dishkeya tarapotica* Diškus & Stonis, **sp. nov.** and the related species and genera based on the 657 bp-long mtDNA CO1-5′ sequences and the GTR+G+I evolution model; numbers represent the bootstrap values in % obtained by Maximum Likelihood (10,000 replicates) (black) / Bayesian inference (10,000,000 generations) (black) / bPTP (blue) (values below 50 are not shown); *Pseudopostega bogotensis* Vargas (Opostegidae) was included as an outgroup.

The phylogenetic tree presented here (Fig. 36) closely matches the topology of the diagram we previously published in a study on the Honduran findings of *D. gouaniae* (Stonis *et al.* 2024). With the inclusion of new sequences of *D. tarapotica* **sp. nov.** and additional sequences of *D. bifurcata*, the current analysis further supports the distinctiveness of the genus *Dishkeya* and enhances our understanding of its molecular characteristics. In both the previous and current trees, the closest taxon to *Dishkeya* appears to be *Coptotrichoides*; however, support for this relationship is moderate and not yet conclusive (ML bootstrap value = 61%, Bayesian posterior probability = 52%). To clarify the phylogenetic relationships among these and other related genera (*Coptotriche*, *Rytietia*, *Tischeria*), a more comprehensive analysis is needed, incorporating additional DNA sequences from a broader range of species.

Comparative insight into *Dishkeya tarapotica* sp. nov. and other *Gouania*-feeding *Dishkeya* species

In this study, in addition to describing and naming a new *Dishkeya* species, we compared *D. tarapotica* **sp. nov.** with the congeners that also utilize *Gouania* host plants—*D. gouaniae*, the type species of the genus from the tropical humid forests of Belize and Honduras, and *D. gothica* Diškus & Stonis from the Yungas forests of Bolivia (Figs 37–42).

When comparing the male genitalia, *D. gouaniae* appears most similar to *D. tarapotica* **sp. nov.** (Figs 38, 40). Nevertheless, even between these two species, the genital structures differ significantly enough to allow clear differentiation. The most prominent distinguishing features lie in the shape and length of the phallic lateral processes and the carinae.

When comparing the leaf mines of *D. gouaniae* and *D. tarapotica* **sp. nov.** (Figs 37, 39), no clear diagnostic differences were found, except that the mines of *D. tarapotica* **sp. nov.** are even more extensively and remarkably branched. Regarding the leaf mines of *D. gothica*, they were previously unknown. In this paper, we publish for the first time a sample of a *D. gothica* mine collected in Coroico, Bolivia (Fig. 41). These mines are also branched, but the branches are wider and often merge with each other, forming a more irregular, blotch-like pattern. As a result, they are less distinctly branched and differ noticeably from the mines of the other *Gouania*-feeding *Dishkeya* species.

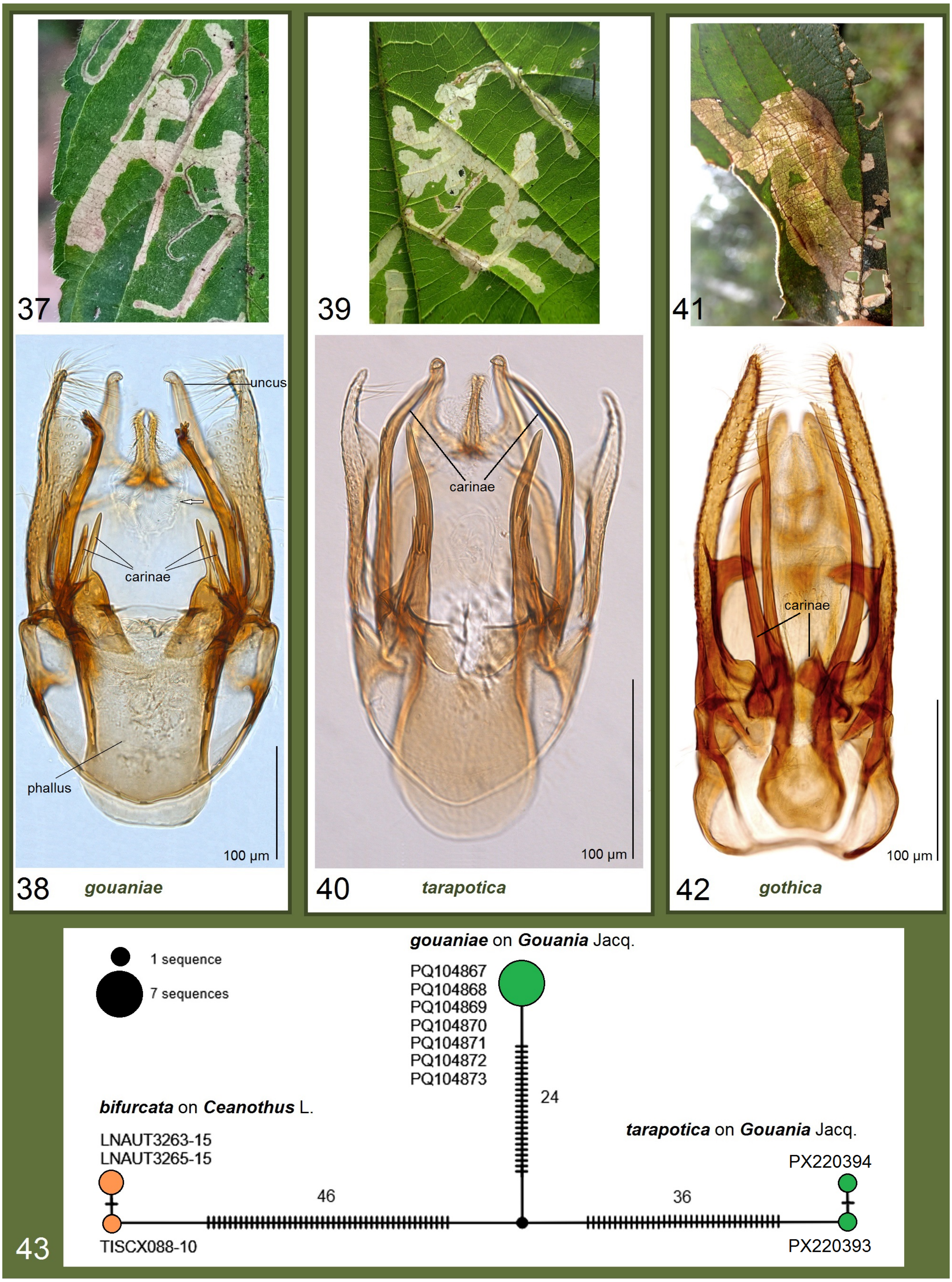
Interestingly, the observed differences in mining patterns between *D. gothica* and *D. gouaniae* + *D. tarapotica* **sp. nov.** correspond with morphological differences in the male genitalia, as well as differences in the specific *Gouania* host species utilized.

Gouania polygama (Jacq.) Urb., the host plant of both *D. gouaniae* and *D. tarapotica* **sp. nov.**, is a climbing shrub or liana with a native range extending from Mexico through tropical America to Paraguay, according to the database “Plants of the World Online” maintained by the Royal Botanic Gardens, Kew (POWO 2025b). It remains an open question whether the leaf-mining species *D. gouaniae* (recorded from Central America) and *D. tarapotica* **sp. nov.** (found in the Amazon Basin) are allopatric, or if their divergence is instead driven by other factors.

Meanwhile, *D. gothica* is trophically associated with *Gouania lupuloides* (L.) Urb., another climbing shrub or liana with a similar and largely overlapping native range, extending from Mexico and Florida to northeastern Argentina and southeastern Brazil.

The host plant of the Colombian species *D. ursipedella* remains unknown (Stonis *et al.* 2022). Additionally, in the northern part of the *Dishkeya* range (California and Arizona, USA), *D. bifurcata* is trophically associated with several species of the genus *Ceanothus* L. (Rhamnaceae), including *C. arboreus* Greene (Braun 1972). The genus *Ceanothus* is widespread throughout North America, from Canada to Panamá, although *C. arboreus* is native only to California (USA) and the Mexican Pacific islands (POWO 2025a).

To further investigate the *Gouania*-feeding *D. tarapotica* **sp. nov.** and its relationship to other *Gouania*- and *Ceanothus*-feeding species, we constructed a mitotype network. The network, based on three *Dishkeya* species (Fig. 43), was designed to visualize intraspecific variability and evolutionary distances among them. Surprisingly, it revealed relatively large distances, ranging from 60 mutational steps between *D. gouaniae* and *D. tarapotica* **sp. nov.**, to 84 steps between *D. bifurcata* and *D. tarapotica* **sp. nov**. These results indicate clear genetic divergence among the species and align well with the findings of both the phylogenetic analysis and the bPTP species delimitation (Fig. 36).



FIGURES 37–43. Comparison of *Dishkeya* species based on host-plant associations: 37, 38, leaf mines and male genitalia, *D. gouaniae* (Stonis & Diškus); 39, 40, same, *D. tarapotica* Diškus & Stonis, **sp. nov.**; 41, 42, same, *D. gothica* Diškus & Stonis; 43, mitotype network of the 657 bp-long mtDNA CO1-5' *Dishkeya* Stonis sequences, constructed using the TCS Network algorithm; the smaller, unnamed circle represents the mitotype predicted to exist but not sampled in the study; the dashes on the connecting lines indicate hypothesized mutational steps.

However, it should be noted that this divergence (in contrast to the morphological data), is not substantial enough to clearly separate the *Gouania*-feeding species (*D. gouaniae* and *D. tarapotica* **sp. nov.**) from *D. bifurcata*, which feeds on *Ceanothus*. The inclusion of additional species such as *D. gothica*—also associated with *Gouania*—could potentially reshape the current network, offering new insights and revealing unexpected patterns. Unfortunately, DNA sequences for *D. gothica* are not yet available.

Dishkeya can currently be considered an endemic genus to the Americas. However, Rhamnaceae plants (including *Gouania*) are well known outside of the Americas, while Tischeriidae species are considerably undersampled in most tropical regions globally. This raises the question of whether *Dishkeya* species feeding on Rhamnaceae might eventually be discovered outside of the Americas. Nevertheless, it remains a distinct possibility that the genus *Dishkeya* may not be present in tropical regions beyond the Americas. To illustrate, although plants of the family Asteraceae have a broad global distribution, the genus *Astrotischeria* Puplesis & Diškus, 2003—which is both species-rich and specialized in feeding on Asteraceae—is documented exclusively within the Americas. This example highlights that even widely distributed host plants do not necessarily imply a corresponding global distribution for their associated insect taxa.

Characteristics of *Dishkeya tarapotica* sp. nov. expanding and refining the taxonomic concept of the genus *Dishkeya*

It has long been known that one of the striking characteristics of Tischeriidae is the presence of long antennal sensilla (s. trichodea) in males, usually about three times the diameter of the flagellum but sometimes reaching up to four to five times its diameter. In contrast, such elongated sensilla have not been reported in females of this family. We measured the female sensilla of *Dishkeya tarapotica* **sp. nov.** and found that these sensilla are 3.5 times longer than the width of the flagellum. The discovery of long sensilla trichodea in females of this newly described species represents a remarkable phenomenon not previously documented within Tischeriidae. This naturally raises the question: are these long female sensilla a unique feature of the new species, or might they represent an overlooked diagnostic trait of the genus *Dishkeya*? To investigate this, comparative material from the type species *D. gouaniae* (collected in Honduras) and additional specimens of other species were examined. The results showed that females of *D. gouaniae* also possess sensilla that are 2–2.5 times longer than the width of the flagellum—that is, relatively long sensilla, though shorter than those in *D. tarapotica* **sp. nov.** Meanwhile, males of *D. gouaniae* have sensilla measuring 4–4.5 times the width of the flagellum. For comparison, sensilla trichodea in males of *Astrotischeria andina* Diškus & Stonis are equal in length to the width of the flagellum; in males of *Tischeria neokristenseni* Diškus & Stonis, they are 4.8 times longer; in males of *Coptotriche asiana* Diškus & Stonis, 2.5–3 times longer; in males of *Gnathitischeria atitlani* Diškus & Stonis, 3.5–3.7 times longer; and in males of *Rytietia uncinata* Diškus, Xu & Dai, 1–1.3 times longer. However, females of none of these species have sensilla trichodea on their smoothly scaled antennae—at least not ones prominent enough to be noticed without descaling the flagellum. Unfortunately, females of *Dishkeya gothica* and *D. ursipedella* (with males of the latter possessing sensilla trichodea approximately 3.5 times the width of the flagellum) have not yet been discovered, and females of *D. bifurcata* from the USA were not available for examination in this study.

Nevertheless, the presence of sensilla trichodea in females of two *Dishkeya* species (out of the five currently known)—especially the exceptionally long sensilla in the newly described *D. tarapotica* **sp. nov.**—allows us to suggest that long sensilla trichodea in females, although an atypical character for most Tischeriidae, represent an important diagnostic character of the genus *Dishkeya*.

Regarding the female genitalia, *D. tarapotica* **sp. nov.** exhibits relatively large ovipositor lobes. Therefore, we update the diagnosis of the genus *Dishkeya* by stating that ovipositor lobes range from small to medium-large, rather than only small as previously described. The concept of the genus *Dishkeya* can also be expanded based on novel traits observed in *D. tarapotica* **sp. nov.**: i) the female genitalia possess thickenings caudal or ventral to the inner prela; ii) the male genitalia exhibit variously developed carinae, which can be extremely long—exceeding the lateral processes of the phallus in *D. tarapotica* **sp. nov.**—and iii) the lateral processes of the phallus can bear large spines. On the other hand, both female and male genital structures—specifically the pseudognathos formed by modified socii, the uncus with slender lateral processes, the elaborated valva, the relatively large vinculum, and the wide phallus with well-developed lateral processes and prominent, long carinae, as well as the distinctly short corpus bursae in the female genitalia with a hardened and spinose neck—conform well to the established concept of *Dishkeya*. The strikingly branched leaf mines of *D. tarapotica* **sp. nov.** also remain fully consistent with the genus, clearly fitting its diagnostic description: unlike the majority of Tischeriidae, which possess round, irregular, or elongated blotch-like leaf mines, *Dishkeya* species have mines ranging from linear-slightly branched to greatly branched.

Conclusions

1. Despite the similarity of leaf mines between *Dishkeya tarapotica* **sp. nov.** and *D. gouaniae*, the new species is a distinct taxon, clearly differing from other *Dishkeya* species—including those feeding on the same host-plant genus, *Gouania* Jacq. (Rhamnaceae)—due to notable differences in genital morphology.

2. The morphological characters of both male and female genitalia—such as the small female corpus bursae with a hardened and spinose neck, the wide male phallus with strongly developed lateral processes and prominent carinae, the elaborately shaped valva, and most notably, the sclerotized socii transformed into a structure here termed the pseudognathos—support the current concept of the genus *Dishkeya*.

3. The presence of long antennal sensilla trichodea in females—especially the exceptionally long sensilla in *D. tarapotica* **sp. nov.**—represents an atypical trait within the family Tischeriidae. Along with other novel characters discovered in *D. tarapotica* **sp. nov.**, these sensilla contribute to the diagnostic characteristics of the genus *Dishkeya*.

4. Molecular analysis of *Dishkeya* species, incorporating newly generated sequences of *D. tarapotica* **sp. nov.**, confirmed the distinctiveness of the new species and, although not with sufficient reliability, further highlighted its possible phylogenetic relationships with the genera *Coptotrichoides* and *Coptotriche* rather than with *Tischeria*—a placement that might have been incorrectly assumed without detailed examination of genital structures.

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