BIOLOGIJA
2024. Vol. 70. No. 2–3. P. 116–129

ISSN 1392-0146 eISSN 2029-0578
DOI: https://doi.org/10.6001/biologija.2024.70.2–3.4

Discovery of the genus *Dishkeya* (Lepidoptera: Tischeriidae) in Honduras: unveiling the unique traits of *D. gouaniae*

Jonas R. Stonis[1*],

Arūnas Diškus[2],

Svetlana Orlovskytė[1]

[1] *State Research Institute Nature Research Centre, Akademijos g. 2, Vilnius 08412, Lithuania*

[2] *Vytautas Magnus University, K. Donelaičio g. 58, Kaunas 44248, Lithuania*

This publication records the endemic genus *Dishkeya* Stonis in Honduras and, for the first time, documents the female genitalia and leaf mines of *D. gouaniae* (Stonis & Diškus). Additionally, it provides the first barcodes and molecular considerations regarding the genus *Dishkeya*. The article is illustrated with 34 figures, including photographs of male and female genitalia, leaf mines, exuviae, and a molecular tree based on mtDNA COI sequences.

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

INTRODUCTION

The Tischeriidae, or trumpet moths, is a nearly cosmopolitan family found worldwide, with the exception of Australia, the southern part of South America, and Antarctica. Currently, the family comprises 192 species globally, but this number is constantly rising. The dynamics of the descriptions of the Tischeriidae species, from the first described species in 1795 to 2021, were analysed by Dobrynina et al. (2022). The first detailed and contemporary general characterisation of the family was provided by Braun (1972), followed three decades later by Davis (1999), and more exhaustively by Diškus & Puplesis (2003). Recently, various new taxonomical, morphological, ecological, and distribution data have been unveiled and published in numerous papers; almost all of these are cited in the most recent generic revision of Tischeriidae of the world fauna by Stonis et al. (2023).

According to Stonis & Solis (2020), the genus *Dishkeya* was originally named in honour of Dr Arūnas Diškus (Vilnius, Lithuania) in recognition of his outstanding contributions to Tischeriidae and Nepticulidae research as well as his remarkable enthusiasm for Tischeriidae inventories, especially in tropical countries. This naming was a congratulatory gesture on his 50th birthday and the achievement of 30 years in the field of leaf miner research (Stonis & Solis, 2020).

In contrast to the large genera *Tischeria* Zeller, *Manitischeria* Diškus & Stonis, *Astrotischeria* Puplesis & Diškus, and the particularly speciose *Coptotriche* Walsingham, *Dishkeya* Stonis is a small endemic genus of Tischeriidae recently described from the Americas in Stonis & Solis (2020).

* Corresponding author. Email: stonis.biotaxonomy@gmail.com

According to Stonis & Solis (2020), external features, including the forewing pattern or characters of wing venation, are not informative and are insufficient for generic differentiation in most Tischeriidae, including *Dishkeya*. However, the male genitalia of *Dishkeya* exhibit unique characteristics such as the presence of a pseudognathos derived from socii, greatly developed lateral processes of the phallus, and the presence of carinae. These features distinguish this genus from all remaining ten genera of Tischeriidae (for a comprehensive generic review, see the recent monograph by Stonis et al., 2023).

All currently ecologically investigated *Dishkeya* species are trophically associated with host plants from the Rhamnaceae family. At the time of its primary description, the genus comprised only three species: *Dishkeya bifurcata* (Braun, 1915) from the United States (California and Arizona), *D. gouaniae* (Stonis & Diškus, 2007) from Belize (Cayo District: Chiquibul Forest Reserve), and *D. gothica* Diškus & Stonis (in Stonis & Solis, 2020) from Bolivia (Nor Yungas Province: Coroico). Recently, with the discovery and description of *D. ursipedella* Diškus, Mey & Stonis, the genus was also reported from Colombia (Stonis et al., 2022a). Despite the genus being known from geographically separated areas, it was anticipated that *Dishkeya* might have a nearly continuous distribution across the Americas (Stonis & Solis, 2020).

Addressing this knowledge gap, we conducted targeted fieldwork in Honduras in 2023 and 2024, marking the first focused study of Tischeriidae in this country. During the fieldwork, along with a few other Tischeriidae species, we discovered leaf mines of *D. gouaniae* and successfully reared male and female adults from mining larvae. It should be noted that previously, leaf mines of *D. gouaniae* had never been documented, and females were completely unknown (including their genitalia, which had not been examined or illustrated). Additionally, there were no molecular data available for the species.

The goal of this study was to document *D. gouaniae* from Honduras (a new distribution record for the species and for *Dishkeya* as a genus). To achieve this, we barcoded *D. gouaniae* for the first time and provided photographic documentation of the previously unknown female genitalia and the morphology of the leaf mines.

We believe this study contributes to a better understanding of the enigmatic *Dishkeya* and, hopefully, will encourage further research on the fascinating Neotropical Tischeriidae.

MATERIALS AND METHODS

The new material for this paper was collected by the first author. Since 2023, Prof. Dr Jonas R. Stonis, Senior Researcher at Nature Research Centre (NRC), has been visiting the Delegation of the European Union to Honduras and conducting voluntary research on the biological diversity of Honduran forests. During this mission, he sampled leaf-mining Lepidoptera. This initiative was part of two long-term programmes between the European Union and Honduras: the Memorandum of Understanding between the Republic of Honduras and the European Union ('Forest Partnership') and the Multiannual Indicative Programme of the European Union for Honduras for 2021–2024, which includes 'Sustainable Management of Natural Resources and Climate Change' as Priority Area 1, with the participation of the Honduran Institute of Forest Conservation, Protected Areas, and Wildlife (ICF).

The new material from Honduras used in this paper will be deposited in the collection of the Museum für Naturkunde (MfN), Berlin, Germany, following publication. The type series from Belize has already been deposited in the Natural History Museum (NHMUK), London, United Kingdom.

Material examined. 2 ♂, 2 ♀ (non-type series specimens), HONDURAS, Atlántida Department, Tela, close to Las Palmas, 30 m, 15°46'64"N, 87°23'15.50"W, feeding larvae on *Gouania polygama*, Rhamnaceae, 13–19.iv.2024, leg. J. R. Stonis, genitalia slide nos AD1183♂, AD1192♂, AD1198♀ (MfN); 4 ♂ (holotype and paratypes), BELIZE, Cayo Distr., Chiquibul Forest Reserve, Las Cuevas, 16°43'53"N, 88°59'11"W, 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♂, 010316202♂ (NHMUK).

Collecting methods. Collecting adults using a light trap yielded no results; all adult specimens were reared from mining larvae. Adapted specifically for Tischeriidae, the method of rearing larvae has been detailed by Stonis et al. (2016).

Leaf mines were collected through thorough inspection of host plants or potential host plants. Tischeriidae mines were always found in leaves, with each mine typically containing only one larva. However, in cases of very abundant mining, two or more mines occasionally merged. Typically, a leaf would have one, sometimes two to three leaf mines, but instances of numerous mines on a single leaf were also recorded. For *Dishkeya gouaniae*, larvae were visible from the upper side of the leaf.

Determining whether the mines contained living or dead larvae, or pupae, was challenging with the naked eye or even with a magnifying glass by holding a damaged leaf against the light. Therefore, all found leaf mines were removed from the field for laboratory examination. Mining larvae are mobile and usually pale to bright green or pale to bright yellow in colour (Stonis et al., 2016). Dead larvae, by contrast, were motionless (even when disturbed with a magnifying glass or under a stereoscopic microscope) and usually dark throughout or spotty. The Tischeriidae larvae experience high levels of mortality from parasitoid wasps, and larvae or pupae of these parasitoids are often found within the mines (Lewis et al., 2002). Sometimes, especially in early larval instars, larvae were difficult to see even under a stereoscopic microscope as they were hidden in the initial part of the leaf mine. When slightly disturbed, feeding mature larvae would quickly move to the initial part of the leaf mine to hide.

Although rearing adults from mining larvae can be conducted both in natural environments and indoors, during our study, adults were reared indoors under conditions with temperatures around +26–30°C and humidity levels similar to outdoor conditions. The mined parts of the host plant, along with live larvae or pupae, were not cut out as is typically done for Nepticulidae rearing (Diškus & Stonis, 2022). Instead, the entire leaf, twig, or shoot was collected.

The most suitable leaf mines for rearing adults contained large, late instar larvae or, preferably, pupae, as these were less likely to result in plant material drying out or becoming mouldy before adult emergence. Collected larvae were allowed to feed for a few days to 1–2 weeks until pupation. Only medium or large plastic rearing containers were used, as test tubes and Petri dishes were found to be inconvenient for larval rearing.

Larvae pupate inside the leaf mines, so leaf mines were regularly examined for the presence of pupae under a stereoscopic microscope. Dark pupae were typically visible in the slender gallery of the leaf mine under transmitted light. Each pupa was carefully cut out with a 3–4 cm section of the leaf and placed in a smaller transparent container. The parts of the cut leaf were spaced apart to prevent the formation and spread of mould. In some cases, to prevent drying out of the live material, fresh leaves or a wet cotton wad was added to a corner of the rearing container. If there was excess moisture or condensation on the container cover, it was ventilated (Stonis et al., 2016). Containers were kept in a shaded or dark place, away from direct sunlight, at room temperature until adults emerged. Each hatched adult was captured in a small individual glass tube and killed by briefly immersing the tube with the moth in hot water for 1–2 seconds.

Specimen dissection and documentation. Methods and protocols for specimen dissection, species identification, and description followed earlier published monographs (Puplesis & Diškus, 2003; Stonis et al., 2022b). During dissection, the male genital capsule and the female genitalia were extracted after macerating the abdomen in 10% potassium hydroxide. These were then cleaned and mounted with the ventral side facing up. In many cases, the phallus was dissected from the genital capsule and mounted under a separate cover slip on the same microscope slide. Abdominal pelts were consistently preserved throughout this study.

Permanent preparations on microscope slides were photographed and examined using a Leica DM2500 microscope equipped with a Leica DFC420 digital camera. Exuviae were examined using a Lomo MBS-10 stereoscopic microscope, with images captured using a Leica S6D stereoscopic microscope paired with a Leica DFC290 digital camera. Specimens were illuminated using a Ring Light LED 60, directly attached to the stereo microscope lens. This light source provided adjustable illumination intensity and a colour temperature range of 7000 to 11000 K, delivering 8000 Lux illumination at a distance of 100 mm.

Most leaf mines were photographed in the wild using a smartphone with good photography capabilities (Samsung Galaxy). Some photographs of leaf mines were taken with an Olympus DP-26 camera.

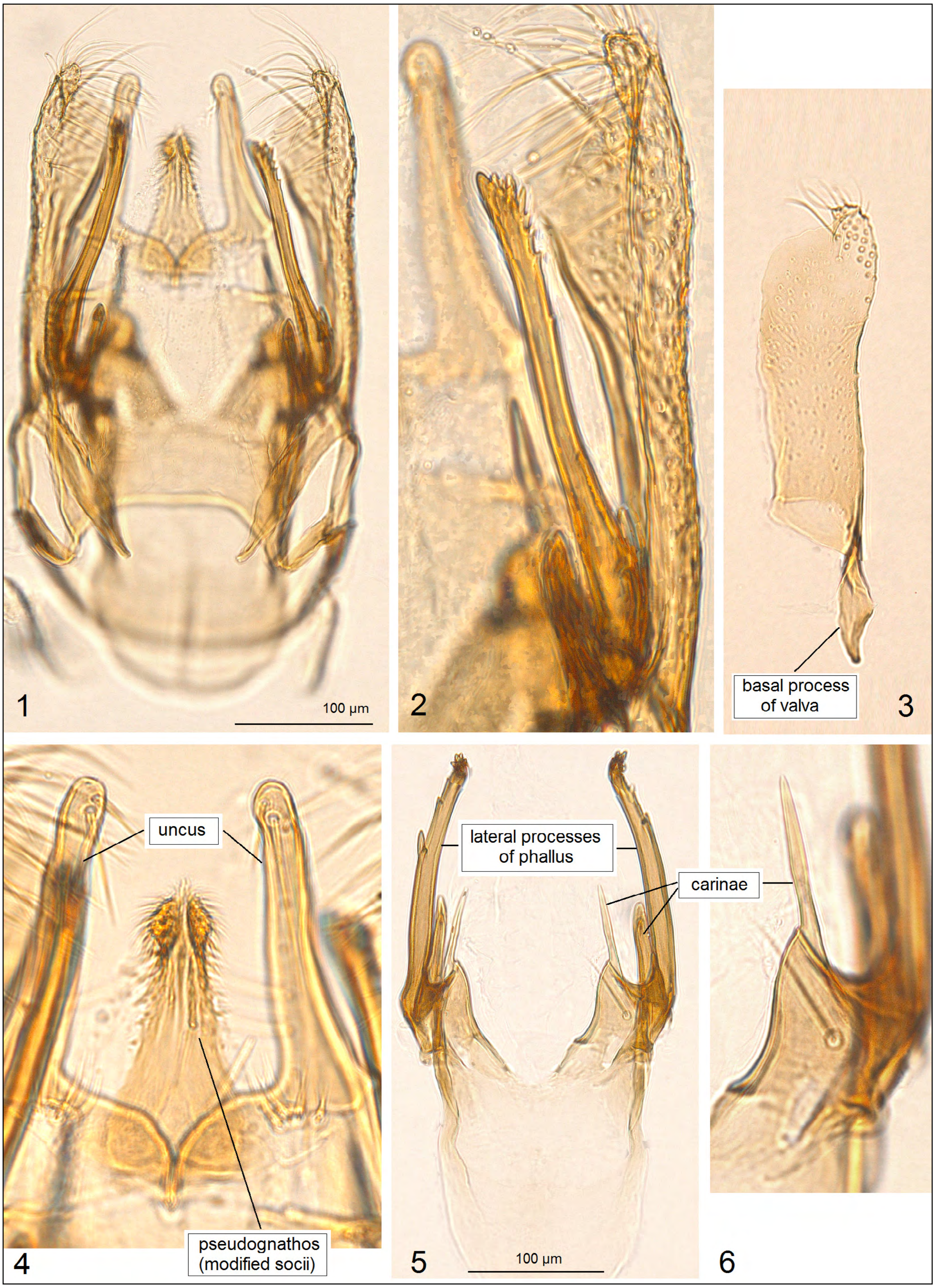
Molecular analysis. The air-dried or 96% ethanol-preserved larvae and pupae were used for the DNA extraction with the GeneJet Genomic DNA Purification Kit (Thermo Fisher Scientific Baltics). During the polymerase chain reaction (PCR), the partial cytochrome c oxidase subunit 1 of the mitochondrial DNA (COI) was amplified with the primers LCO1490 (5'-ggtcaacaaatcataaagatattgg-3') and HCO2198 (5'-taaacttcagggtgaccaaaaaatca-3') (European and Mediterranean Plant Protection Organization, 2016). The PCR mixture contained 12.5 μL of 2 × DreamTaq PCR Master Mix (Thermo Fisher Scientific Baltics), 2 μL of 10 pmol/μL of each primer, 2 μL of extracted DNA, and deionised water up to a volume of 25 μL. The PCR conditions were as follows: 5 min at 95°C; 45 cycles (40 s at 94°C, 40 s at 45°C, 1 min at 72°C), 5 min at 72°C. The PCR products were run on 1.5% agarose gel (Thermo Fisher Scientific Baltics) using the Roti Gelstain Red (Karl Roth) dye and purified with the exonuclease I and FastAP thermosensitive alkaline phosphatase (Thermo Fisher Scientific Baltics) enzymes. The sequencing was performed in BaseClear B. V. (Leiden, The Netherlands) by the ABI 3730xl 96-capillary DNA analyzer (Applied Biosystems). The BioEdit v.7.2.5 program (Hall, 1999) was used for the alignment of the obtained sequences, followed by the submission of them to the NCBI GenBank database (Benson et al., 2013) under the accession numbers PQ104867–OQ104873. The related species, genera, and outgroup previously acquired by the authors (Orlovskytė et al., 2023; Stonis et al., 2023) were included in the analysis; they are available at Genbank or the BOLD platform (Ratnasingham & Hebert, 2007). The MEGA v.7 program (Kumar et al., 2016) was used to construct the Neighbor-Joining (NJ) (TN93+I model, 10,000 bootstrap replicates) and the Maximum Likelihood (ML) (GTR+G+I model, 10,000 bootstrap replicates) trees. The Bayesian phylogenetic analysis was performed with the GTR+G+I model and run for 15 million generations using the MrBayes v.3.2.3 software (Ronquist & Huelsenbeck, 2003). Bayesian trees were designed with FigTree v.1.4.4 (Rambaut, 2018).

Abbreviation for institutions. 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 – the Natural History Museum, London, United Kingdom; NRC – the State Research Institute Nature Research Centre (NRC), Vilnius, Lithuania.

RESULTS

Examination of the genitalia armature of *Dishkeya gouaniae* (Stonis & Diškus), including the first documentation of the female genitalia

Male genitalia: updated considerations (Figs 1–6). Originally described as *Tischeria gouaniae* in Stonis & Diškus (2007: 1287–1291), the species was initially documented using black-and-white schematic drawings of the male genitalia. This was followed by



Figs 1–6. Male genitalia of *Dishkeya gouaniae* (Stonis & Diškus) discovered in Honduras (a new distribution record); 1, 2, 4 – genitalia slide no. AD1192; 3, 5, 6 – genitalia slide no. AD1183 (MfN)

Stonis et al. (2020) in their report on the exceptional diversity of Tischeriidae in the tropical forests of Belize, and by Stonis & Solis (2020) in the primary description of the genus *Dishkeya*. Both papers provided various photographs of the same paratype (genitalia slide no. 010316202, NHMUK).

The genital capsule of the type-series specimens is 438–500 μm long (Stonis & Diškus, 2007). In the Honduran material, however, it was found to be slightly smaller, approximately 400 μm in length. The uncus features long, very slender lateral lobes that are widened proximally (Figs 1, 4). The socii are significantly modified, thickened at the middle, and form a bifid and distally curved pseudognathos (Fig. 4). The tegumen is both wide and long. The valva of the type-series specimens measures 333–355 μm long (Stonis & Diškus, 2007). In the Honduran material (Figs 1–3), it was found to be slightly shorter, around 260 μm in length (excluding the basal processes). The small ventral lobe in the apical third of the valva appears larger in its natural position (Fig. 2) compared to when it is dissected out and slightly squashed (Fig. 3). The transtilla and anellus are absent. The vinculum is very wide but relatively short, with a wide anterior rounding.

The phallus (Figs 5–6) is notably wide, with long spinose lateral processes and two pairs of spine-like carinae. One pair of carinae is nearly merged with the lateral processes of the phallus proximally (Fig. 5), while the other pair arises from a triangular lobe (Fig. 6). Contrary to the description in Stonis & Diškus (2007), the juxta is absent; the shorter processes mentioned in the primary description are actually part of the carinae, while the longer processes represent the lateral processes of the phallus.

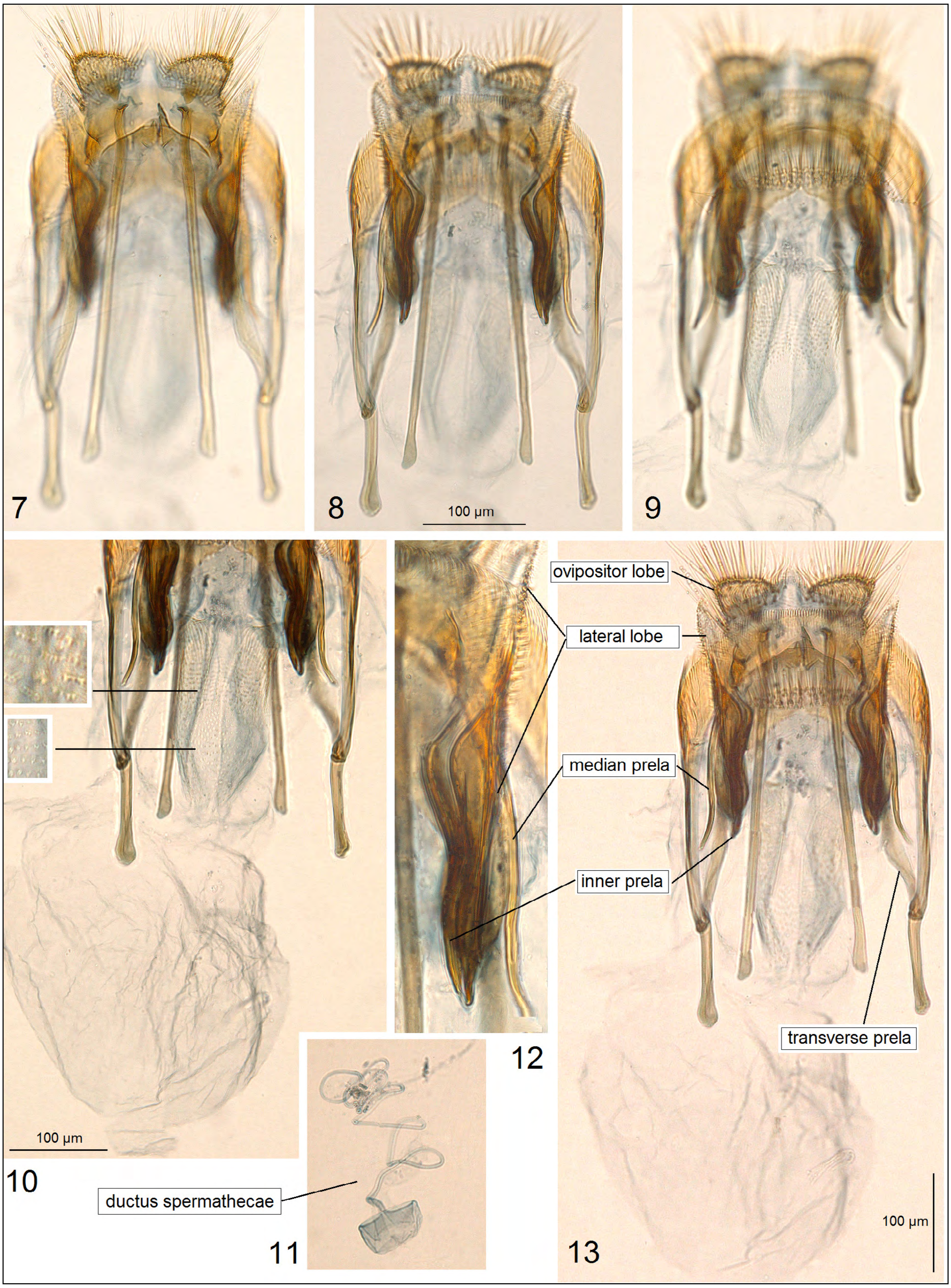
First examination and documentation of the female genitalia (Figs 7–13). Previously, females of *D. gouaniae* were unknown, and no female genitalia of any other *Dishkeya* species had been examined or documented, except for the apophyses and prela of *D. bifurcata* (Braun), which were only schematically illustrated by Braun (1972: Fig. 146). The accuracy of this sketch has not been re-examined (see Discussion).

Our current examination of the female genitalia of *D. gouaniae* from Honduras revealed the following, mostly unique characteristics. The total length of the female genitalia is 800 μm. The ovipositor lobes are large and angular (Figs 7, 8, 13), rather than rounded as is typical in Tischeriidae. The gap between the ovipositor lobes is relatively wide with a weakly sclerotized caudal bulge. Unlike other Tischeriidae genera, the second pair of ovipositor lobes, which are lateral and anterior to the main ovipositor lobes, are absent (Fig. 13). The lateral lobes are relatively long, triangular, and spinose laterally, with heavy sclerotization proximally that supports the inner prela (Fig. 12).

Generally, the prela of Tischeriidae consist of three rod-like or plate-like projections, which are likely modified 8th and 9th sternites, collectively referred to as prela by Braun (1972). In *D. gouaniae*, the prela are comprised of three distinctive pairs of unique rod-like projections: transverse, median, and inner prela. Notably, the inner prela in *D. gouaniae* are uniquely parallel and supported by the sclerotized proximal part of the lateral lobe (Fig. 13). The anterior apophyses are slightly longer than the posterior apophyses. The vestibulum is heavily covered with pectination (tiny spines) (Fig. 10). The corpus bursae is short (380 μm) and wide (310 μm), lacking a long and slender 'neck' and any pectination or signum. The accessory sac is absent. The ductus spermathecae is medium in length, very slender, with about 6–7 large and smaller coils, and features a relatively large, unique, bell-shaped vesicle (Fig. 11).

Updated distribution and biology (Figs 14–33). Currently, *D. gouaniae* is known from tropical humid forests in Belize (Chiquibul Forest Reserve, Las Cuevas, at an elevation of about 550 m) and is now recorded from tropical humid forests in Honduras (Atlántida Department, Tela, Las Palmas, at an elevation of about 30 m) (Figs 14, 15). This represents a new distribution record for the species.

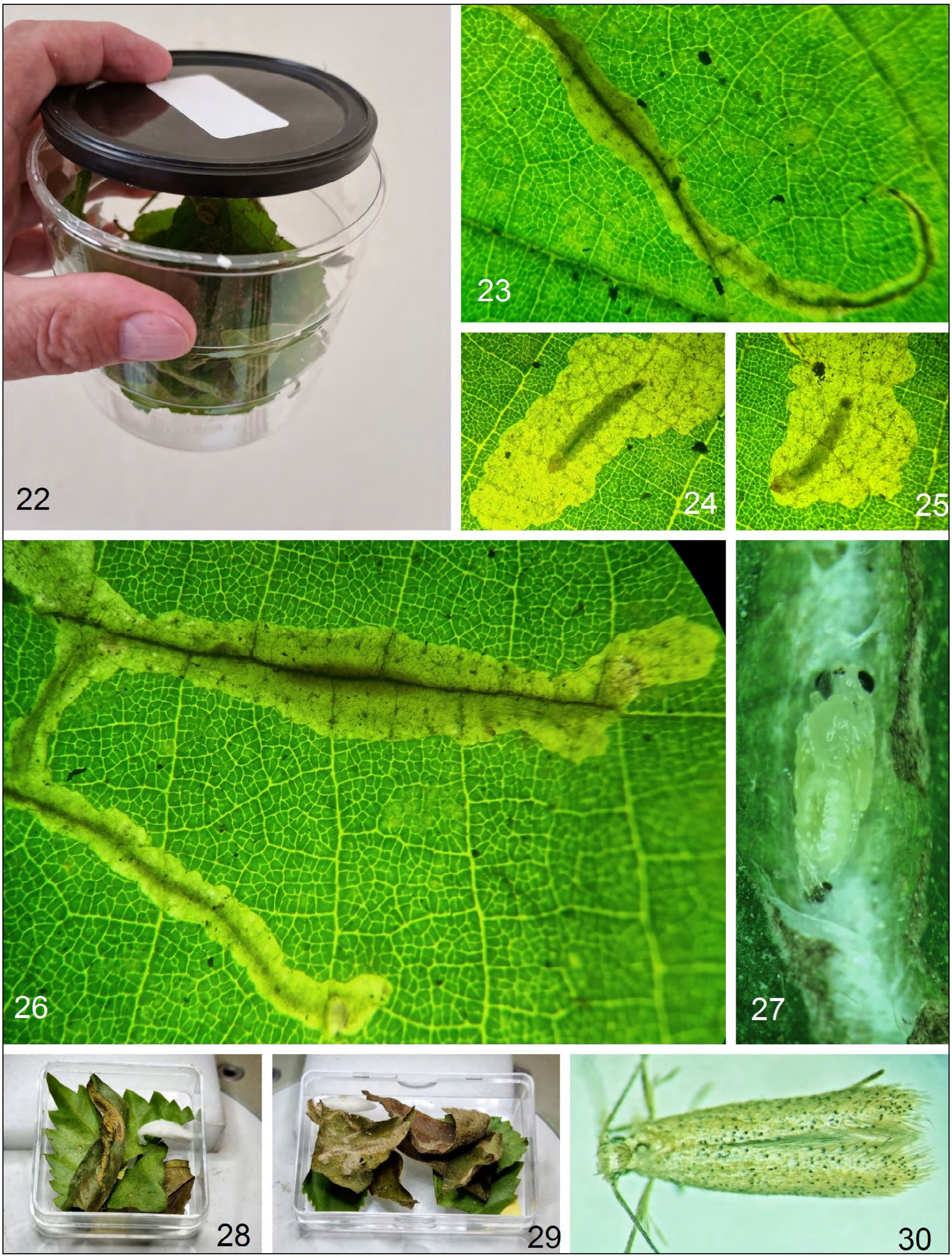
The host plant is *Gouania polygama* (Jacq.) Urb., Rhamnaceae (Fig. 16). The larvae mine the leaves. In Honduras, leaf mines with mining



Figs 7–13. The first documentation of the female genitalia of *Dishkeya gouaniae* (Stonis & Diškus), genitalia slide no. AD1198 (MfN); 7–9 – ovipositor lobes, apophyses, and prela at different focal depths; 10–12 – detailed views of the genitalia; 13 – a general view of the female genitalia

Figs 14–21. Bionomics of *Dishkeya gouaniae* (Stonis & Diškus); 14, 15 – the habitat, the remnants of a highly disturbed tropical humid forest along a river, surrounded by generally agricultural landscape (palm plantations), Honduras, Atlántida Department, Tela, Las Palmas, at an elevation of about 30 m); 16 – a shoot of the host plant *Gouania polygama* (Jacq.) Urb., Rhamnaceae; 17–21 – leaf mines

larvae were found in April. Previous research in Belize recorded larval mining in March–July and September (Lewis et al., 2002; Stonis et al., 2020). While it was previously known that the leaf mines have a linear or corridor shape, the mines were not preserved or studied in detail. The leaf mines examined in Honduras exhibited some variation (Figs 7–21).



Figs 22–30. Bionomics of *Dishkeya gouaniae* (Stonis & Diškus); 22 – leaf mines with feeding larvae in a rearing container; 23, 26 – larvae hiding in the slender initial gallery of the leaf mine, either completely invisible or only barely visible; 24, 25 – larvae; 27 – pupa of a parasitoid wasp in the leaf mine, with the upper epidermis opened during examination; 28, 29 – cut pieces of the leaf mines with pupae (note the addition of cotton wad and green leaves to prevent drying out of live material); 30 – an emerged female adult

Each mine typically contains only one larva; however, in cases of abundant mining, numerous mines can occur on a single leaf (Figs 18, 19). Feeding larvae are pale green with a bright green intestine and a brown head (24, 25). The larvae are very mobile and quickly retreat to the slender initial gallery of the leaf mine when disturbed by movement or even a change in lighting (Figs 23, 26). Successful development of the larvae was observed at temperatures between 26–30°C. The larvae pupate inside the leaf mine, in a nidus, typically in a slender gallery of the leaf mine. Compared to the round nidus of other Tischeriidae, the nidus of *D. gouaniae* is unique–slender and long, made of white silken threads, and hardly visible or invisible through the epidermis of the leaf mine (Figs 26, 27). Initially, the pupae are pale green, later turning brown-black. During our study, leaf mines with feeding larvae of various instars collected during 14–19 of April 'produced' adults in 21–29 of the same month. Therefore, we supposed that adults emerge about 7–8 days after pupation (Fig. 30). Based on reared specimens from Belize, adults are active from March to September, while the flying period for the Honduran sample was detected in late April and early May.

Initially, *D. gouaniae* was listed as '*Tischeria* sp. 12' by Lewis et al. (2002), who reported that 14 out of 25 living mines (56%) reared were infected with parasitoids (Lewis et al., 2002; Stonis et al., 2020). In Honduras, approximately

Figs 31–33. Bionomics of *Dishkeya gouaniae* (Stonis & Diškus); 31 – pupa, lateral view; 32, 33 – exuviae, lateral and dorsal views

35% of larvae in the sample collected in mid-April were infected with parasitoids, while in the sample collected at the same site a week later, about 50% of larvae were infected (Fig. 27). Incidentally, fixation in alcohol of a larva with a parasite inside allowed our molecular study to confirm Braconidae with 97.79% accuracy. Additionally, many collected larvae were lost due to unknown reasons, raising the average larval mortality to 70–75%.

MOLECULAR CONSIDERATIONS

In this study, the seven 657 base pair (bp) long partial sequences of the COI, used as the universal markers to barcode animal species (Hebert et al., 2003), were successfully obtained for *Dishkeya gouaniae* for the first time.

In order to find out its systematic status, the samples from the related genera *Coptotriche* Walsingham, *Coptotrichoides* Diškus & Stonis, *Rytietia* Diškus, Xu & Dai and *Tischeria* Zeller were included in the analysis. The constructed tree showed that the seven *Dishkeya gouaniae* sequences reliably separated from *D. bifurcata* (Braun) by all three applied methods (NJ probability, ML bootstrap value and Bayesian posterior probability were equal 100% each), thus proving the uniqueness of this species (Fig. 34). Together these two species formed a well supported monophyletic clade (NJ – 93%, ML – 95%, Bayesian probability – 100%), which confirmed one more time (Stonis et al., 2023) the status and distinctness of the recently described genus *Dishkeya* Stonis, 2020.

Unless not well supported, the topology of the genera corresponded to the arrangement in Stonis et al. (2023: Fig. 62): *Dishkeya*, *Coptotrichoides* and *Coptotriche* genera branched together and formed a sister taxon to *Rytietia* and *Tischeria*.

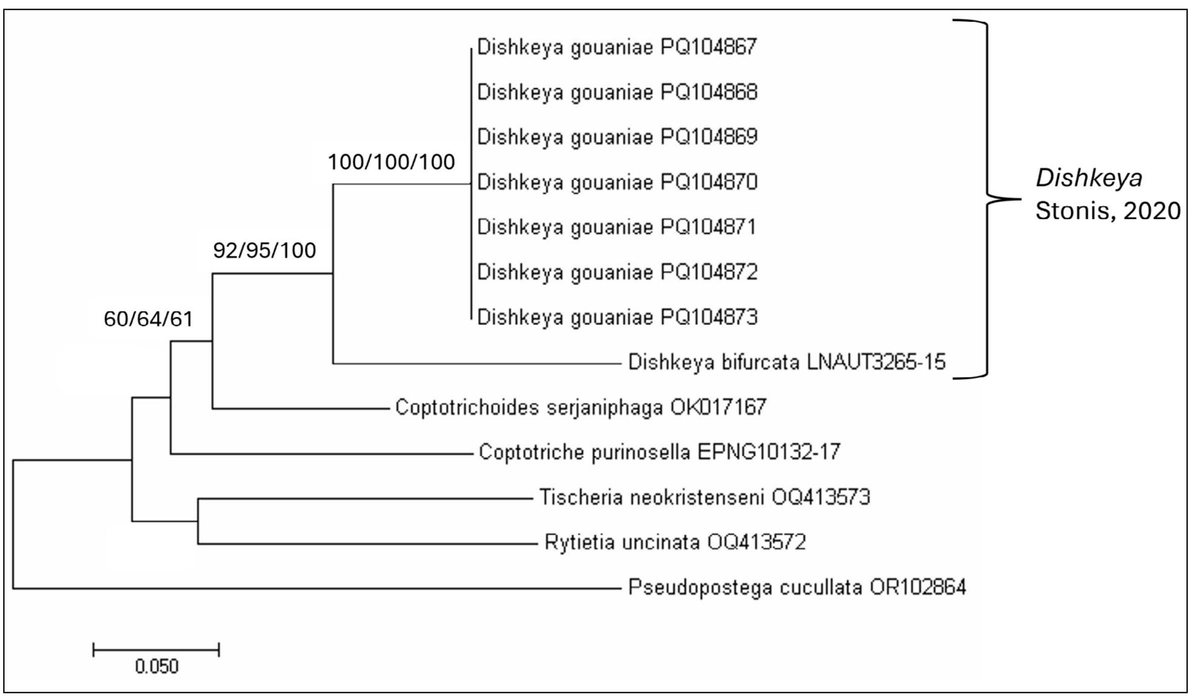


Fig. 34. The phylogenetic relationships of *Dishkeya gouaniae* (Stonis & Diškus) and the related species and genera reconstructed based on the 657 bp long COI sequences. Numbers of branches represent the bootstrap values obtained for Neighbor-Joining (T93+I, 10,000 replicates), Maximum Likelihood (GTR+G+I, 10,000 replicates), and Bayesian (GTR+G+I, 15,000,000 generations) probabilities in %. Bootstrap values below 50 are not shown. *Pseudopostega cucullata* Stonis & Vargas was included as an outgroup

DISCUSSION: WHY IS *DISHKEYA GOUANIAE* SPECIAL?

The genus *Dishkeya* is particularly intriguing due to its enigmatic nature and the numerous taxonomic, morphological, and biological characteristics that remain to be explored. As the type species of this genus, *D. gouaniae* plays an exceptionally important role in our understanding of *Dishkeya*'s peculiarities. The recent discovery of *D. gouaniae* in Honduras has expanded its known geographical range and underscored its association with tropical humid forests in both Belize and Honduras.

The male genitalia of *D. gouaniae* exhibit notably complex and unique structures, especially the well-developed carinae and lateral arms of the phallus. The documentation of female genitalia for the first time has provided valuable insights. Our findings suggest that the absence of a second pair of ovipositor lobes and the presence of the atypical inner prela, supported by the elaborately sclerotized lateral lobes, may not only be diagnostic for *D. gouaniae* but could potentially be characteristic of the genus *Dishkeya* as a whole. This is in contrast to the schematic illustration of the apophyses and prela of *D. bifurcata* (Braun, 1972), which appears less realistic and requires re-examination.

The leaf mines of *D. gouaniae*, documented for the first time, are morphologically distinct from the predominantly blotch-like leaf mines observed in the majority of other Tischeriidae, thus shedding more light on the biology of the genus.

Additionally, while *D. bifurcata* was the only species from *Dishkeya* previously barcoded, the inclusion of *D. gouaniae* with seven mtDNA COI sequences has furthered our understanding of molecular characteristics of the genus and reinforced its uniqueness.

ACKNOWLEDGEMENTS

We greatly appreciate the two long-term programmes in which the Republic of Honduras and the European Union act as partners: (1) El Memorando de Entendimiento entre la República de Honduras y la Unión Europea ('Partenariado Forestal'), signed at the 28th Conference of the Parties to the United Nations Framework Convention on Climate Change on 2 December 2023 in Dubai, and (2) the Multiannual Indicative Programme (MIP) of the European Union for Honduras for 2021–2024, which includes Priority Area 1: 'Sustainable Management of Natural Resources and Climate Change', with the participation of el Instituto de Conservación Forestal, Áreas Protegidas y Vida Silvestre de Honduras (ICF). The field sampling and subsequent investigation of plant-mining Lepidoptera were voluntary initiatives within the framework and goals of these aforementioned programmes in which international cooperation and understanding of the importance of bioinventory could contribute to a better evaluation of Honduran biodiversity and, hopefully, its consequent protection.

We also extend our gratitude to Professor Dr Owen T. Lewis (Department of Zoology, University of Oxford, United Kingdom) for providing valuable material collected in Belize (Las Cuevas) for our studies.

Received 6 August 2024
Accepted 20 August 2024

References

1. Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. GenBank. Nucleic Acids Res. 2013;41:36–42. https://doi.org/10.1093/nar/gks1195
2. Braun AF. Notes on some species of *Tischeria*, with descriptions of new species (Lep.). Entomol News. 1915;26(6):271–73.
3. Braun AF. Tischeriidae of America North of Mexico (Microlepidoptera). Mem Am Entomol Soc. 1972;28:1–148.
4. Davis DR. The Monotrysian Heteroneura. In: Kristensen NP, editor. Lepidoptera: Moths and Butterflies, 35. Evolution, Systematics, and Biogeography, 1. Handbook of Zoology, 4. Berlin: Walter de Gruyter; 1999. p. 65–90.

5. Diškus A, Puplesis R. Nepticuloidea & Tischerioidea – the world context. In: Puplesis R, Diškus A, editors. The Nepticuloidea & Tischerioidea (Lepidoptera) – a global review, with strategic regional revisions. Kaunas: Lututė Publishers; 2003. p. 38–175.

6. Diškus A, Stonis JR. Rearing of Nepticulidae adults from mining larvae. In: Stonis JR, Remeikis A, Diškus A, editors. Neotropical Nepticulidae: a pictorial monograph introducing an electronic identification tool. Vilnius: Nature Research Centre; 2022. p. 66–70.

7. Dobrynina V, Stonis JR, Diškus A, Solis MA, Baryshnikova SV, Young-Min S. Global Nepticulidae, Opostegidae, and Tischeriidae (Lepidoptera): temporal dynamics of species descriptions and their authors. Zootaxa. 2022;5099(4):450–74. https://doi.org/10.11646/zootaxa.5099.4.2

8. European and Mediterranean Plant Protection Organization. PM 7/129 (1) DNA barcoding as an identification tool for a number of regulated pests. EPPO Bulletin. 2016;46(3):501–37. https://doi.org/10.1111/epp.12344

9. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. 1999;41:95–8.

10. Hebert PDN, Cywinska A, Ball SL, de Waard JR. Biological identifications through DNA barcodes. Proc R Soc Lond B. 2003;270(1512):313–21. https://doi.org/10.1098/rspb.2002.2218

11. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33(7):1870–74. https://doi.org/10.1093/molbev/msw054

12. Lewis OT, Memmott J, Lasalle J, Lyal CHC, Whitefoord C, Godfray HCJ. Structure of a diverse tropical forest insect–parasitoid community. J Anim Ecol. 2002;71(5):855–73. http://dx.doi.org/10.1046/j.1365-2656.2002.00651.x

13. Orlovskytė S, Dobrynina V, Stonis JR. Unexpected mitotype diversity of *Simplimorpha promissa* (Lepidoptera: Nepticulidae) in Ukraine and Armenia revealing a possible cryptic taxon. Zootaxa. 2023;5336(1):113–24. https://doi.org/10.11646/zootaxa.5336.1.5

14. Puplesis R, Diškus A. The Nepticuloidea & Tischerioidea (Lepidoptera) – a global review, with strategic regional revisions. Kaunas: Lututė Publishers; 2003. 512 p.

15. Rambaut A. FigTree v1.4.4. 2018 [cited 2024 Aug 2]. Available at: http://tree.bio.ed.ac.uk/software/figtree/

16. Ratnasingham S, Hebert PDN. BOLD: The Barcode of Life Data system. Mol Ecol Notes. 2007;7:355–64. https://doi.org/10.1111/j.1471-8286.2007.01678.x

17. Ronquist F, Huelsenbeck JP. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics. 2003;19(12):1572–74. https://doi.org/10.1093/bioinformatics/btg180

18. Stonis JR, Diškus A. Distribution of *Tischeria gouaniae* sp. n. from the tropical forest of Belize – an exotic new addition to the American fauna of *Tischeria* (Insecta: Lepidoptera: Tischeriidae). Zool Sci. 2007;24(12):1286–91. https://doi.org/10.2108/zsj.24.1286

19. Stonis JR, Diškus A, Carvalho Filho F, Lewis OT. American Asteraceae-feeding *Astrotischeria* species with a highly modified, three-lobed valva in the male genitalia (Lepidoptera, Tischeriidae). Zootaxa. 2018;4469(1):1–69. https://doi.org/10.11646/zootaxa.4469.1.1

20. Stonis JR, Diškus A, Mey W. *Dishkeya*, a recently described endemic Tischeriidae genus, now discovered in Colombia. Zootaxa. 2022a;5214(2):285–93. https://doi.org/10.11646/zootaxa.5214.2.8

21. Stonis JR, Diškus A, Remeikis A. Specimen documentation and micro-mounts of genitalia structures adopted for the minute Lepidoptera. In: Stonis JR, Remeikis A, Diškus A, editors. Neotropical Nepticulidae: a pictorial monograph introducing an electronic identification tool. Vilnius: Nature Research Centre; 2022b. p. 71–5. https://www.researchgate.net/publication/361649792_Neotropical_Nepticulidae

22. Stonis JR, Diškus A, Remeikis A, Lewis OT. Exceptional diversity of Tischeriidae (Lepidoptera) from a single tropical forest site in Belize, Central America. Eur J Taxon. 2020;723:33–76. https://doi.org/10.5852/ejt.2020.723.1143
23. Stonis JR, Diškus A, Remeikis A, Orlovskytė S, Solis MA, Paulavičiūtė B, Xu J, Dai X. Genera of Tischeriidae (Lepidoptera): a review of the global fauna, with descriptions of new taxa. Zootaxa. 2023;5333(1):1–131. https://doi.org/10.11646/zootaxa.5333.1.1
24. Stonis JR, Solis MA. *Dishkeya* gen. nov., a New World endemic genus of leaf-mining Tischeriidae (Lepidoptera), transferred from *Tischeria* Zeller. Biologija. 2020;66(3):123–35. https://doi.org/10.6001/biologija.v66i3.4307

Jonas R. Stonis, Arūnas Diškus, Svetlana Orlovskytė

ENDEMINĖS GENTIES *DISHKEYA* (LEPIDOPTERA: TISCHERIIDAE) ATRADIMAS HONDŪRE: KOKIAS UNIKALIAS MORFOLOGIJOS IR BIOLOGIJOS YPATYBES ATSKLEIDĖ *D. GOUANIAE* TYRIMAI

Santrauka

Šioje publikacijoje pateikiami duomenys apie endeminės *Dishkeya* Stonis genties aptikimą Hondūre (Centrinė Amerika) ir pirmą kartą dokumentuojamos tipinės rūšies *D. gouaniae* (Stonis & Diškus) patelių genitalinės struktūros. Straipsnyje daroma prielaida, kad antrosios kiaušdėtės skiaučių poros nebuvimas ir neįprasta vidinė prela, palaikoma stipriai chitinizuotų šoninių skiaučių, gali būti ne tik diagnostinis *D. gouaniae* požymis, bet ir morfologinė adaptacija, būdinga visai *Dishkeya* genčiai. Pirmą kartą ištyrus *D. gouaniae* biologiją, pateikiami duomenys apie šios rūšies minas, gyvybinius ciklus ir lervų mirtingumą. Be to, remiantis pirmą kartą gautomis *D. gouaniae* COI sekomis, pateikiamos molekulinės įžvalgos apie *Dishkeya* gentį. Septynerių *D. gouaniae* COI sekų fragmentų įtraukimas į Tischeriidae analizę pagilino mūsų supratimą apie genties molekulines savybes ir atskleidė taksono unikalumą. Straipsnyje pateikiami 34 paveikslai nuodugniai dokumentuoja *D. gouaniae* patinų ir patelių genitalijų morfologiją, specifines lapų minas ir lėliukių išnaras (egzuvijus).

Raktažodžiai: lapų minos, mažieji šeriuotaūsiai, neotropinė fauna, vabzdžiai minuotojai