BIOLOGIJA. 2019. Vol. 65. No. 4. P. 223–235

Discovery of a new species of *Ozadelpha* van Nieukerken sheds more light on the diagnostics of this controversial genus of Nepticulidae (Lepidoptera)

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We describe a new species, *Ozadelpha nigella* Diškus & Stonis, sp. nov., a leaf miner on *Symplocos* Jacq. (Ericales: Symplocaceae), from the Yungas biogeographical province, South America. We briefly discuss the issues of diagnostics of the recently erected genus *Ozadelpha* van Nieukerken. The genus is now comprised of four named species having shared characters useful for efficient identification and genus recognition. The new species is illustrated with photographs of the adults, male genitalia and leaf mines.

Keywords: leaf mines, *Ozadelpha nigella,* pygmy moths, *Symplocos*

INTRODUCTION

The family of Nepticulidae, or pygmy moths ("pygmies"), comprises the world's smallest moths the larvae of which are leaf miners during all stages of their development and make tunnels (leaf mines) in assimilatory tissues of wild or cultivated plants. The history of documentation of the Nepticulidae fauna of the Neotropics counts about 133 years with the description of the first species from Colombia (see Puplesis, Robinson, 2000), but only during the recent decade it has become really active. A series of taxonomic publications on Nepticulidae covering Central and South America were published recently, notably, Remeikis et al., 2014; Stonis et al., 2013, 2014b, 2015, 2016a, 2016b, 2016c, 2016d, 2017a, 2017b, 2017c, 2018a, 2018b, 2018c, 2018d, 2019; Stonis,

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Remeikis, 2015; van Nieukerken et al., 2016a, 2016b; Stonis, Diškus, 2018, etc.

Here, we continue to document the Nepticulidae fauna of the Neotropics and describe one more species, *Ozadelpha nigella* Diškus & Stonis, sp. nov., feeding on *Symplocos* Jacq. (Symplocaceae) from the Yungas biogeographical province, Bolivia. This particular species belongs to the genus some aspects of diagnostics of which appeared controversial previously.

MATERIALS AND METHODS

The description of the new species is based on the material deposited in the collection of the Zoological Museum, Natural History Museum of Denmark, Copenhagen (ZMUC). In addition, some material of non-type series of *Stigmella ovata* Puplesis & Robinson was available from the collection of Biosystematics Research Group, Vilnius, Lithuania (BRG, recently transferred to ZMUC).

Collecting methods and protocols for species identification and description are outlined in Puplesis (1994); Johansson et al. (1990); Puplesis, Diškus (2003); and Stonis et al. (2014a). Permanent preparations on microscope slides were photographed and studied with a Leica DM2500 microscope and Leica DFC420 digital camera. Adults were photographed using a Leica S6D stereoscopic microscope with the attached Leica DFC290 digital camera. Molecular methods used in this paper are described in Stonis et al. (2019).

DESCRIPTION OF *OZADELPHA NIGELLA* DIŠKUS & STONIS, SP. NOV.

Type material. Holotype: ♀, BOLIVIA, Nor Yungas Province, Coroico, 16°12′25″S, 67°43′53″W, elevation 1660 m, leaf-mining larvae on *Symplocos* sp., Symplocaceae 15.vi.2018, ex pupa vii.2018, field card no. 5265, leg. Diškus and Stonis, genitalia slide no. AD877♀ (ZMUC). Paratype: 1 ♂, same label data as holotype, genitalia slide no. AD878♂ (from the adult in pupal skin) (ZMUC).

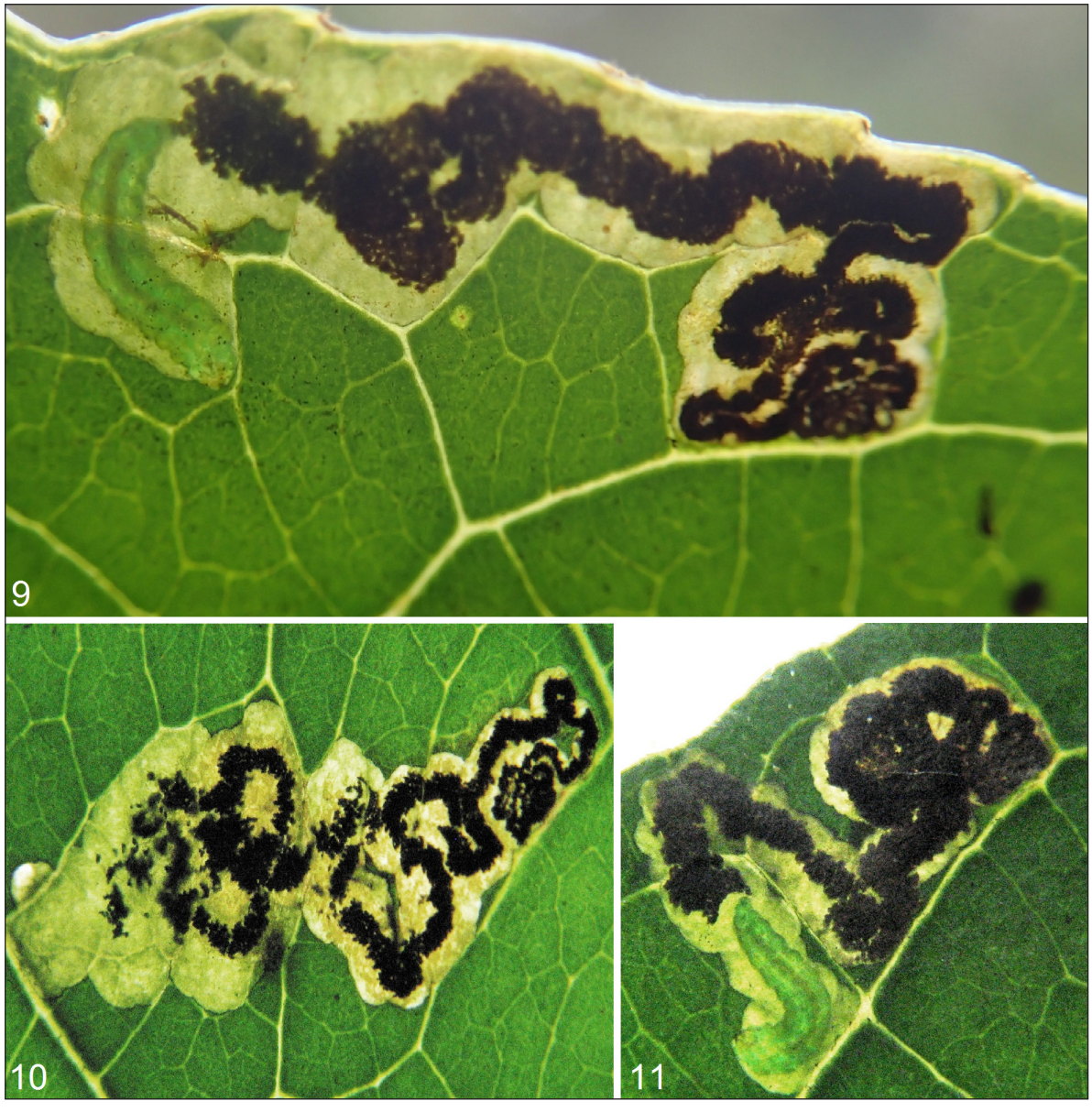
Diagnosis. *Ozadelpha nigella* sp. nov. differs from all congeneric species in the silvery shiny spots on the forewing and dark silvery shiny forewing base and thorax. In the male genitalia, the new species differs in the unique-shaped valva (Figs. 23, 24), wide gnathos (Fig. 21), and the unique set of conuti of the phallus (Fig. 27). In the female genitalia, *O. nigella* sp. nov. differs in the combination of a small, folded corpus bursae, ductus spermathecae without distinctive coils, slender anterior apophyses, and very long posterior ones. The host plant, *Symplocos*, also makes this species distinctive because no other *Ozadelpha* species are known to feed on Symplocaceae.

Male. Known from an adult in pupal skin; externally resembles female; for external characters see the female description.

Female (Figs. 12–15). Forewing length about 1.7 mm; wingspan about 4.4 mm. Head: palpi and frons (face) yellow-silvery glossy; frontal tuft (Fig. 13) orange-ochre or orange-brown; collar blackish brown to dark orange-brown, comprised of very slender scales; scape yellow-silvery glossy; antenna shorter than one half the length of forewing; flagellum with about 21 segments, black with some purple iridescence on upper side, black-grey on underside. Thorax and tegula dark silvery shiny to silvery shiny depending on the angle of view. Forewing (Figs. 12, 15) dark silvery shiny basally, but brownish black to black with purple iridescence in apical 2/3; spots only on apical part of forewing, very distinctive, comprised of uplifted, silvery shiny scales, some of them white-tipped (Fig. 14); fringe black, silvery shiny; fringe line absent; underside of forewing blackish brown, with purple and coppery gloss, without spots. Hindwing and fringe grey-black on the upper side and underside. Legs glossy, grey with purple iridescence, except yellow, silvery shiny femur of the foreleg, tibia of midleg, and tarsi of the hind leg. Abdomen grey-black with some purple iridescence on the upper side, whitish grey with metallic gloss on the underside; abdominal apex pointed distally, without tufts.

Male genitalia (Figs. 19–27). Capsule significantly longer (330–355 μm) than wide

Figs. 1–8. Bionomics of *Ozadelpha nigella* Diškus & Stonis, sp. nov.; 1, 2 – type locality and habitat, the Yungas, Coroico, Bolivia, at an elevation of about 1660 m; 3, 4 – host plant, *Symplocos* Jacq. (probably *S. arechea* L'Hér.), Symplocaceae (identified by Dr. Alfredo F. Fuentes, Universidad Mayor de San Andres, Bolivia); 5, 6 – leaf mines; 7, 8 – cocoon

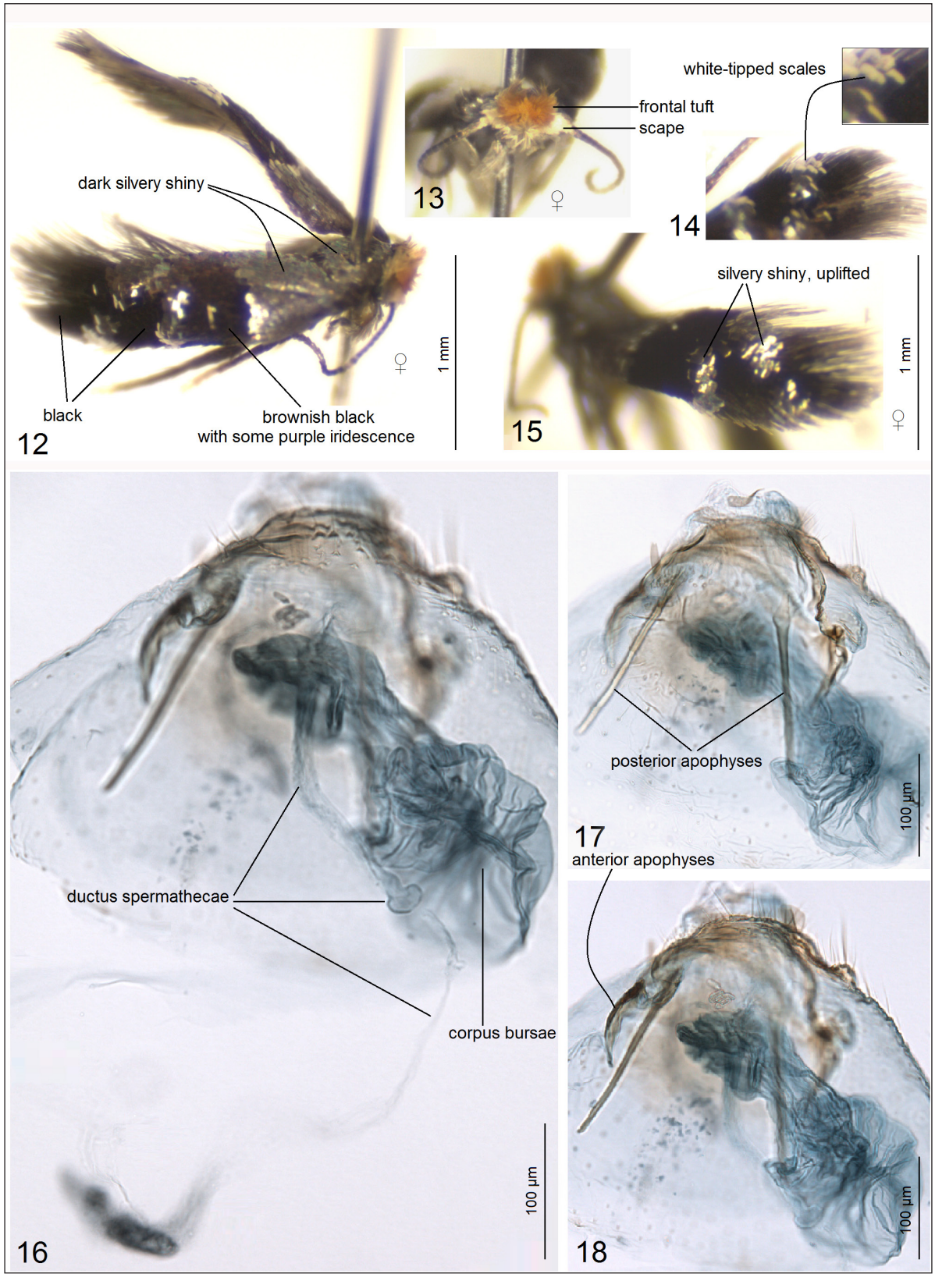


Figs. 9–11. Documentation of leaf mines and larva of *Ozadelpha nigella* Diškus & Stonis, sp. nov. on *Symplocos* sp., Symplocaceae (note the contorted beginning of the gallery in all samples)

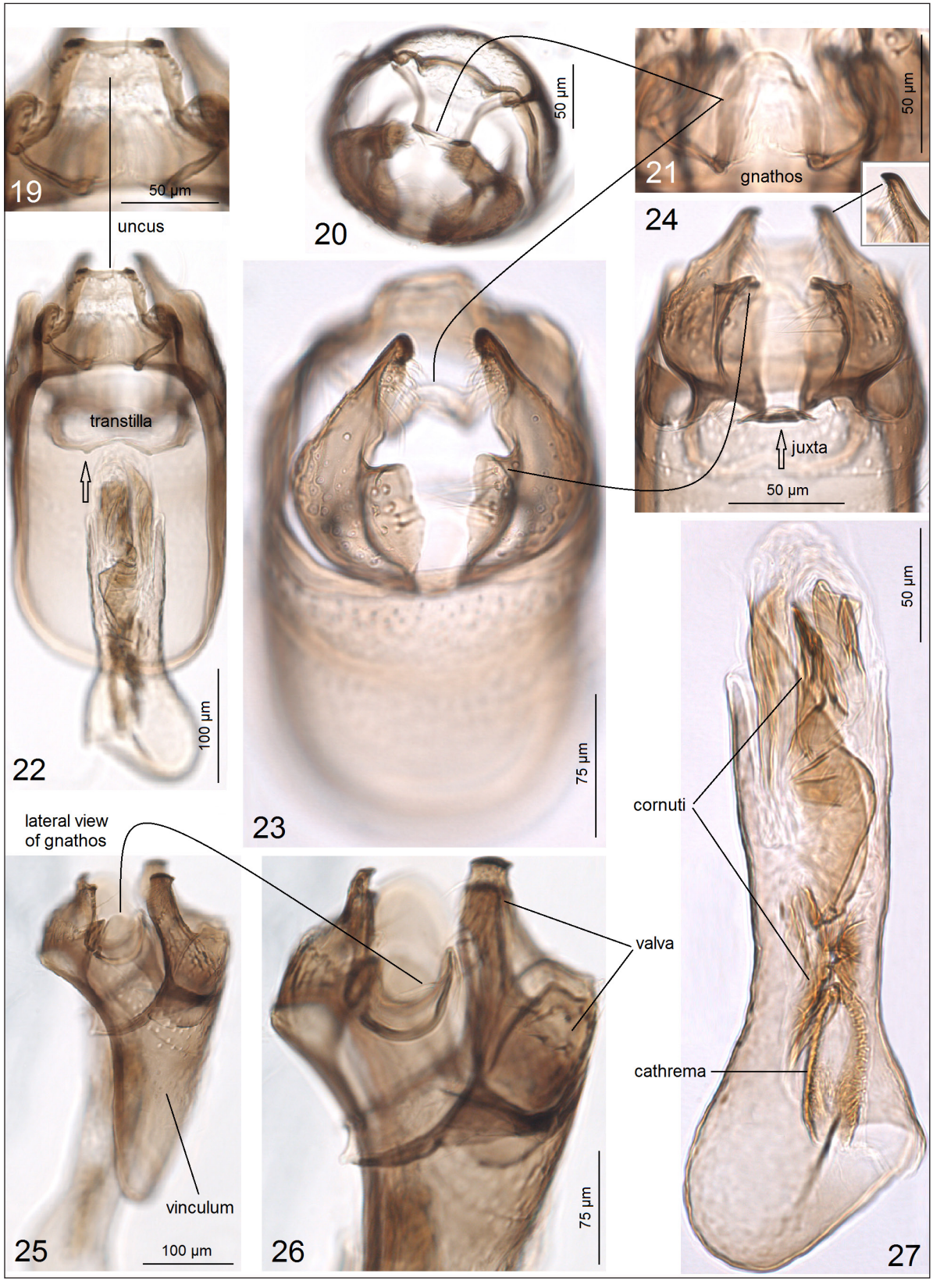
(180 µm). Pseuduncus absent. Uncus (Fig. 19) wide, truncated distally. Gnathos (Figs. 21, 26) with wide, rounded central element and slender arms. Valva (Figs. 23, 24, 26) 140 µm long, 60–65 µm wide, with an inner lobe basally (Fig. 23) and slender apical process (Fig. 24); transtilla with an elaborated transverse bar (Fig. 22), without sublateral processes. Juxta (Fig. 24) small, sometimes inconspicuous. Vinculum very large, without lateral lobes, widely rounded (Figs. 22, 23). Phallus (Fig. 27) 305 µm long, 60 µm wide, but widened basally (90 µm wide); vesica with a distinctive cathrema (Fig. 27) and unique set of mostly triangular, plate-like cornuti.

Female genitalia (Figs. 16–18). Total length about 320 µm. Corpus bursae small and heavily folded. Ductus spermathecae with indistinctive coils.

Bionomics (Figs. 1–11). Host plant is *Symplocos* Jacq. (Figs. 3, 4), probably *S. arechea* L'Hér., Symplocaceae (identified on the basis of photographs by Dr. Alfredo F. Fuentes,



Figs. 12–18. Female of *Ozadelpha nigella* Diškus & Stonis, sp. nov., holotype; 12–15 – adult; 16–18 – female genitalia, overview at different focal length, slide no. AD877 (ZMUC)



Figs. 19–27. Male genitalia of *Ozadelpha nigella* Diškus & Stonis, sp. nov., paratype, slide no. AD878 (ZMUC); 19–26 – genitalia capsule at a different angle of view (22, 25 – with phallus); 27 – phallus

Universidad Mayor de San Andres, Bolivia). Egg laid singly on the underside of the leaf; egg case very small, oval-shaped, silvery glossy, black when filled with frass. Larvae mine leaves in June; voltinism unknown. Larva (Figs. 9, 11) bright green with a slender, pale brown intestine and pale brown head. Leaf mine (Figs. 5, 6, 9–11) starts as a slender but contorted gallery filled with black frass; further on, the gallery gradually widens, with a wide central line of black frass (Figs. 9–11). Pupation outside the leaf mine, possibly in debris or litter because no cocoons were observed on the host plants. Exit slit on the upper side of the leaf. Cocoon (Figs. 7, 8) beige, without a distinct flat rim around the main body. Adults emerged in late July. Otherwise, biology is unknown.

DNA barcode. We barcoded the holotype of the new species; the sequence is available at GenBank under voucher/sample ID MN813061.

Distribution. Currently known from a single locality in Bolivia (Nor Yungas: Coroico), at an elevation of about 1660 m (Figs. 1, 2).

Etymology. The species name derived from the Latin *nigellus* (blackish, dark), in reference to the brownish black forewing of the new species (not after Nigel P. Farage, U.K.).

RE-DEPOSITION OF THE TYPE SERIES OF *OZADELPHA GUAJAVAE* PUPLESIS & DIŠKUS, 2002

During the preparation of the current paper, the entire series of the type material of *Ozadelpha guajavae* Puplesis & Diškus, described in Puplesis et al., 2002 was transferred to ZMUC.

Type material: 1 ♂ (holotype), Ecuador, 80 km E Guayaquil, Bucay, western foothills of Andes, 700 m, premontane tropical forest and orchards, 16–19.i.2001, leg. Puplesis and Hill, genitalia slide no. AD0340; 5 ♂, 2 ♀ (paratypes), same label as holotype, slide nos. AD0341♂, AD0342♂, AD0343♀, AD0344♀, AD0346 (forewing venation), RA1035 (forewing venation).

Remarks. The following additional material (2 ♂, 1 ♀, non-type series, collected in Ecuador, Loja province, Vilcabamba, 4°15′44″S, 79°13′53″W, elevation ca. 1800 m, on *Psidium guajava*, 16–18.ii.2014, leg. Remeikis and Stonis, genitalia slide nos. RA611♂, RA 612♀) was deposited at ZMUC earlier.

DISCUSSION

The controversy of *Ozadelpha*. The genus is among the most recently described genera of Nepticulidae (van Nieukerken et al., 2016b). The primary description was based on three named species: *Ozadelpha conostegiae* van Nieukerken & Nishida (the type species from Costa Rica), the Argentinian *O. ovata* (Puplesis & Robinson), published in Puplesis, Robinson, 2000, and the Ecuadorian *O. guajavae* (Puplesis & Diškus, 2002) published in Puplesis et al., 2002. Now, with the description of the Bolivian *O. nigella* sp. nov., this small genus contains four named species and one unnamed species, "specimen EvN4680", which was documented on a female only (van Nieukerken et al., 2016b). It is interesting that, at first look, some species of *Ozadelpha* possess characters somehow resembling those in other genera. For example, the rounded thickening in the female genitalia of *O. conostegiae* resembles that in the Australian *Pectinivalva brevipalpa* Hoare, and the blunt anterior apophyses are similar to the apophyses of some Australian and South American *Simplimorpha* (*sensu lato*). The large rounded vinculum and basally widened valva of all *Ozadelpha* resemble the analogous structures in *Simplimorpha*. In the female genitalia, the short corpus bursae of *O. guajavae* and *O. nigella* also have some similarity with the corpus bursae of *Simplimorpha*. On the other hand, the *Ozadelpha* species vary significantly from species to species. It made the genus and its diagnostics controversial, and previously we, the first three authors, had not been sure about the monophyly of the taxon. In the process of the current study, we observed several issues that need to be discussed.

Collar in *Ozadelpha*. It was stated in the original differential diagnosis of the genus (van Nieukerken et al., 2016b) that the genus was recognised by the collar with lamellar scales. We

found that only *O. conostegiae* possessed a collar of lamellar scales. The collar of *O. ovata* is comprised of distinctly piliform scales, and the collar of *O. guajavae*, and especially of *O. nigella*, represents an intermediate state: in *O. guajavae*, lamellar scales are long and slender and, at first look, resemble a collar of piliform scales; in *O. nigella*, lamellar scales are short and very slender and also resemble a collar of piliform scales.

Forewing venation in *Ozadelpha* (Figs. 28–31). Contrary to the diagnosis provided in the primary description, we believe that *O. conostegiae* possesses a closed cell (Fig. 28) formed by a cross vein, which was expected to be a staining artefact (van Nieukerken et al., 2016b). During the current study we also re-examined the forewing venation of *O. guajavae* and confirmed that the absence of CuA in *O. guajavae* in the earlier published study (Puplesis et al., 2002: Fig. 2) is not entirely an artefact, but represents a case of strong vein reduction (Figs. 30, 31). Thus it also confirms that the forewing venation in the genus differs from species to species (Figs. 28–31). Since the wing venation is strongly reduced in the Nepticulidae in general, the observed variation of venation in *Ozadelpha* makes this character less convenient for the genus recognition.

Host-plant relationships. By describing *O. nigella*, we introduce a new host plant genus (*Symplocos*) and a new host-plant family (Symplocaceae), both previously unknown for Nepticulidae. This is also important for another reason: earlier it was believed that *Ozadelpha* species were feeders on Myrtales (rosids), while in the case of *O. nigella*, the discovered host plant belongs to Ericales (asterids!). It certainly added more controversy to the understanding and diagnostics of *Ozadelpha*.

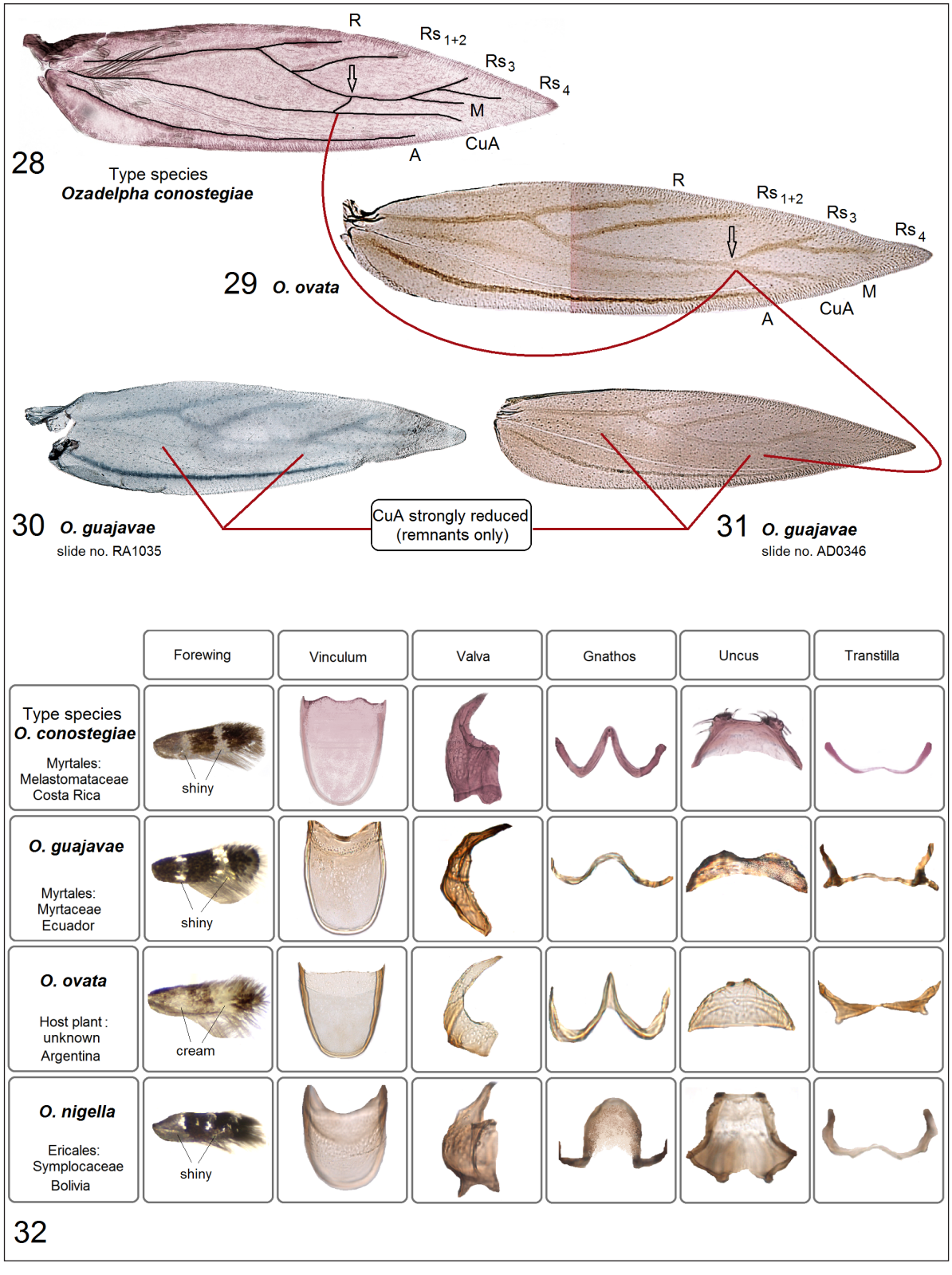
Diagnostics of the genus. We found that despite the differences in the collar, wing venation, and host-plant preferences, there were some shared external or internal characters of the *Ozadelpha* species (Fig. 32) making the genus recognition rather easy. We do not know which of these characters may represent synapomorphies and which, plesiomorphies. In addition to the characters presented in our pictorial tool (Fig. 32), the following features were also useful in diagnostics of the genus: in the male genitalia, the phallus with rather wide, triangularly shaped, plate-like cornuti (except for *O. ovata*, which possesses triangular but slender cornuti) and in the female genitalia, corpus bursae small, except for *O. conostegiae*.

Ozadelpha differs from the Gondwanan *Simplimorpha* (*sensu lato*) in the fully developed uncus and gnathos; these structures are reduced (absent) in *Simplimorpha* (for other apomorphies of *Simplimorpha sensu lato*, see Stonis et al., 2018d). From the Australian *Pectinivalva*, the *Ozadelpha* genus differs in many characters, including the gnathos in a shape of inverted V, very long and rounded vinculum, different shape of valva, presence of transtilla, etc.

Phylogenetical position of *Ozadelpha*. In earlier molecular studies (Doorenweerd et al., 2016), *Ozadelpha* was always grouped together with the Australian *Roscidotoga* (van Nieukerken et al., 2016b); *Roscidotoga* was recently recognized as a subgenus of the Gondwanan *Simplimorpha* (Stonis et al., 2018d) and it also feeds on Myrtaceae in Australia and South America.

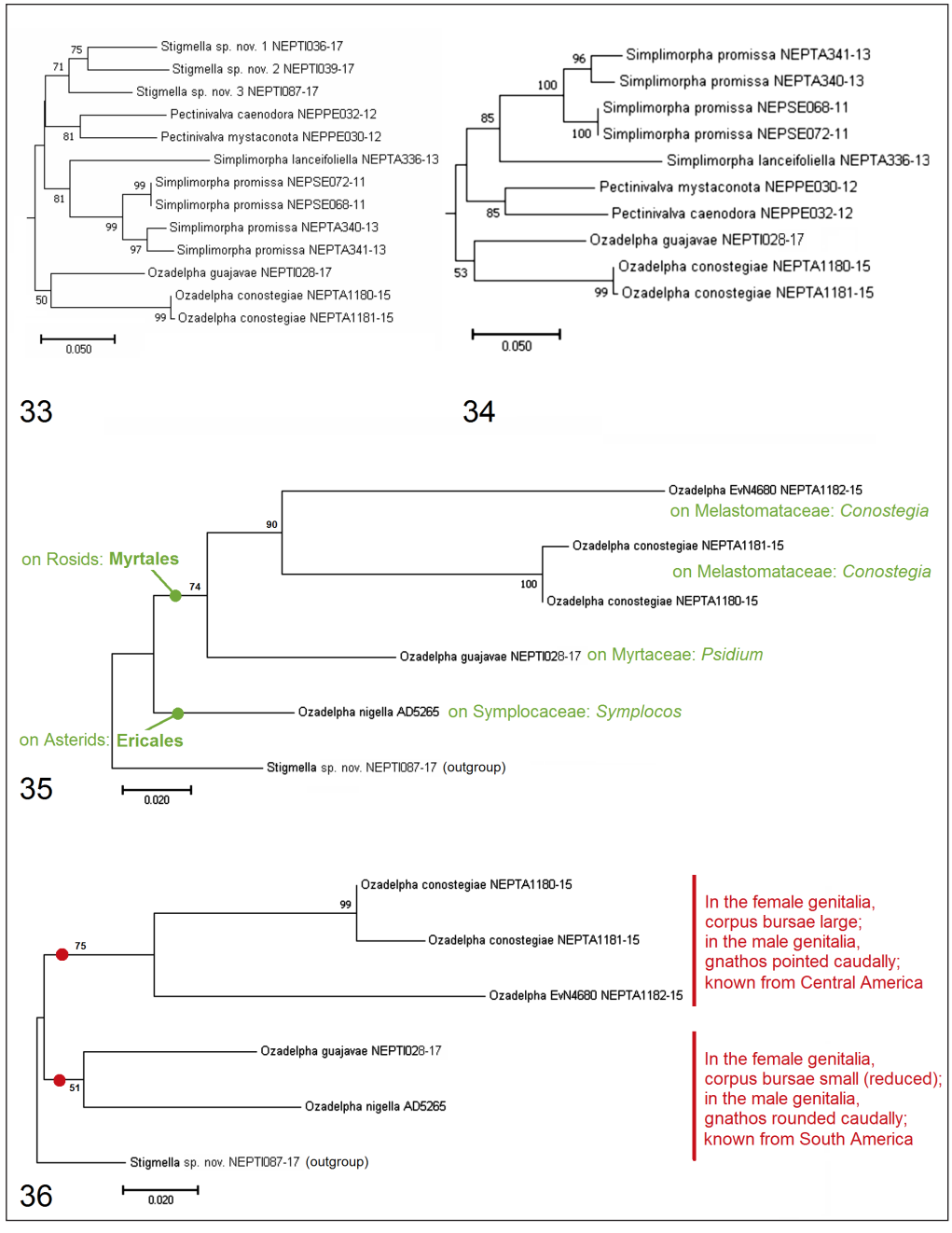
We barcoded *O. nigella* (the sequence is available at GenBank under voucher/sample ID MN813061) and *O. guajavae* (the sequence is available at at the BOLD database under voucher/sample ID ADH4024). Some barcodes of *O. conostegiae*, *O.* specimen EvN46680, *O. ovata*, and of some species of *Simplimorpha* and *Pectinivalva* are available at GenBank thanks to the earlier research by our colleagues Erik van Nieukerken, Camiel Doorenweerd, and Robert Hoare.

Because of fragmented and short sequences, we had experienced difficulties in putting *O. nigella* with *O. ovata* together on the same tree (*O. ovata* was often erroneously appearing at the very base of the branching, separately from the remaining *Ozadelpha*). Therefore, for Figs. 33 and 34, we used only *O. guajavae* and *O. conostegiae*. Once again, it was interesting to find how molecular phylogenies could be contradictive and even useless because of different sets of available barcodes or the length of sequences, etc. Within *Ozadelpha*, the species also



Figs. 28–32. Diagnostics of *Ozadelpha*; 28 – forewing venation, *O. conostegiae* van Nieukerken & Nishida (after van Nieukerken et al., 2016b); 29 – same, *O. ovata* (Puplesis & Robinson, 2000); 30, 31 – same, *O. guajavae* (Puplesis & Diškus, 2002); 32 – diagnostics of *Ozadelpha* spp. showing the shared characters

clustered in at least two different ways. In our best-resolved tree (Fig. 35), the Ericales feeder (*O. nigella*) turned out to be a sister group to the Myrtales feeders (*O. conostegiae*, *O. specimen* EvN4680, and *O. guajavae*). Our alternative tree is different (Fig. 36): in this case, *O. guajavae* and *O. nigella* clustered together, as a sister group to *O. conostegiae* + *O. specimen* EvN4680; it finds support in the male and female morphology, but little support in the host-plant relationships.



Figs. 33–36. Major versions of the Neighbour-Joining tree of *Ozadelpha*; 33, 34 – fragments, both showing *Ozadelpha* as a basal clade (the Neotropical *Pseudopostega* sp., Opostegidae were used an outgroup for full tree); 35, 36 – possible relationships of *Ozadelpha* spp. The divergence was calculated using the Kimura 2-parameter model based on 657 (for Figs. 33–35) and 219 bp mtDNA COI sequences (for Fig. 36). Bootstrap values lower than 50 are not shown

CONCLUSIONS

Drawing upon the shared characters, we conclude that diagnostics of *Ozadelpha* is possible and the genus most likely represents a separate and probably a monophyletic entity; molecular data do not contradict it. However, the exact phylogenetic position of *Ozadelpha* remains unclear.

ACKNOWLEDGEMENTS

We are indebted to our Ecuadorian scientific partners, including those who participated in the initial project in 1999 by R. Puplesis and S. R. Hill, with Professor Giovanni Onore, a former professor at the Pontifical Catholic University of Ecuador, Quito, Ecuador. J. R. Stonis thanks the Environmental Programme at the Andes Office of NGO DAR Peru for permission to provide training courses and fieldwork within the framework of the project "Rapid assessment of biodiversity plots of critical value in the provinces Chanchamayo and Satipo, Peru, and Bolivia", in cooperation with the Baltic-American Biotaxonomy Institute in 2017–2018.

The identification of *Symplocos*, a host-plant genus of *Ozadelpha nigella* by Dr Alfredo F. Fuentes (Universidad Mayor de San Andres, La Paz, Bolivia) was greatly appreciated. We are also grateful to the botanists who confirmed the identification: Dr José Luis Fernández-Alonso (Real Jardín Botanico, Madrid, Spain), Dr Arvind Singh (Banaras Hindu University, Varanasi, India), and Rajeev Kumar Singh (Botanical Survey of India, Kolkata, India).

This research was partially funded by a grant (S-MIP-19-30, "DiagnoStics") from the Research Council of Lithuania. Andrius Remeikis is grateful to the Research Council of Lithuania for the research stipend he was awarded in 2019.

Received 28 October 2019
Accepted 4 November 2019

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NAUJA MOKSLUI MAŽŲJŲ GAUBTAGALVIŲ (LEPIDOPTERA) RŪŠIS, PRIKLAUSANTI *OZADELPHA* VAN NIEUKERKEN GENČIAI

Santrauka

Straipsnyje aprašoma nauja mokslui *Ozadelpha nigella* rūšis, aptikta Jungo miškų provincijoje, Bolivijoje (Pietų Amerika). Jos duomenys papildo šios kontroversiškos genties diagnostiką. Straipsnyje pateikiama išsami *O. nigella* suaugėlių, patino ir patelių genitalijų bei minų morfologijos dokumentacija. Aptariami *Ozadelpha* genties diagnostikos klausimai, išskiriami požymiai, leidžiantys identifikuoti taksoną.

Raktažodžiai: lapų minos, mažieji gaubtagalviai, *Ozadelpha nigella*, *Symplocos*