Do leaf-mining Nepticulidae occur in the natural but so threatened Andean *Polylepis* forests?

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Despite the fact that Polylepis forests constitute the natural but threatened vegetation in much of the high Andes and are very important for their ecological functions, no leaf-mining Nepticulidae (Insecta: Lepidoptera, Nepticuloidea) associated with Polylepis Ruiz & Pav. as a host-plant have been recorded previously. In this paper, for the first time, we report on four discoveries of Polylepis-feeding Nepticulidae species in Ecuador and Peru. From the high Andes of Peru, we describe a new species Stigmella polylepiella Diškus & Stonis, sp. nov., whose larvae throughout all instars are leaf-miners in leaves of Polylepis racemosa Ruiz & Pav. and spin a unique shaped cocoon inside the leaf-mine. We also provide illustrations and short descriptions of male genitalia and leaf-mines of two other new Stigmella Schrank species, whose larvae are leaf-miners on Polylepis pauta Hieron. in Ecuador; these two taxa are documented but left unnamed because they are described from dissected developed pupae, not emerged adults. Additionally, we document leaf-mines of an unknown Nepticulidae taxon associated with Polylepis racemosa in the Peruvian Andes.

Key words: *Polylepis*, Nepticulidae, *Stigmella* Schrank, new species, leaf-mines, Peru, Ecuador

INTRODUCTION

Polylepis Ruiz & Pav. is the only arborescent genus that occurs naturally at high elevations of the Andes (Harling, 1979; Kessler, 2002; Andean Páramo, 2016) (Figs. 1–6). After the glacial period the highlands of the Andes were open to colonization by *Polylepis* trees, and *Polylepis* forests constituted the natural vegetation in much of the high Andes (Clapperton, 1983; Hooghiemstra, Cleef, 1995; Fjeldså, Kessler, 1996).

At altitudes between 3500 and 4000 m, *Polylepis* is the only resource of wood in a zone where other tree species are unable to grow (Fjeldså, Kessler, 1996). Some species of *Polylepis* even occur at an altitude of 4850 m (Braun, 1997). However, nowadays, mainly because of heavy deforestation, remnants of the high Andean forests, which have been conserved to present time, mostly either inhabit inaccessible steep slopes, deep canyons and ravines, places among rocks or the remaining patches of *Polylepis* forests are spread out in an open landscape but separated by huge areas of grass páramo (Ridbäck, 2008).

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Figs. 1-6. Polylepis forest habitat, elevation about 3800 m, near Papallacta Pass, Napo Province, Ecuador

Within the family Rosaceae, i. e. the most common host-plant family of Nepticulidae in boreal regions, the genus *Polylepis* belongs to the tribe Sanguisorbeceae (the section Elongatae) (Simpson, 1986). It is interesting and phylogenetically important that the Andean endemic *Polylepis* is most closely related to the shrubby genus *Acaena* which comprises about a hundred species mainly from New Zealand, Australia and South Africa. The wide geographical distribution of *Acaena* and its less specialized morphology suggest that it is a more primitive

group and that *Polylepis* evolved from it (Simpson, 1986).

All currently recognized 20 (or 28) Polylepis species are endemic to the mid- and high-elevation areas of the equatorial and sub-equatorial Andes. All Polylepis species are bushes and trees of usually gnarled shape (Figs. 2, 3); only seldom they are trees with thick trunks and up to 20 m tall. The foliage is evergreen, with dense small leaves (Figs. 6, 8, 9, 35) and often large amounts of dead twigs hanging down from the underside of the canopy (Figs. 1–6). Polylepis is well adapted to the harsh climate in the high Andean mountains, having reduced flowers and leaves covered with woolly hairs (Fjeldså, Kessler, 1996). Polylepis species (as the tribe Sanguisorbae) are unique in the Rosaceae family in showing a tendency towards wind-polination - an useful evolutionary event in the adaptation to the highland where insects are much scarcer than in warmer climates.

The forests of Polylepis have a unique biological diversity (Fjeldså, Kessler, 1996). The trees harbour many species of epiphytic vascular plants, mosses and lichens, as well as animals. In a landscape dominated by open páramos, the forests give shelter, nesting sites, and food to many mammals and birds (Fjeldså, Kessler, 1996; Ridbäck, 2008). The Polylepis forests have some very important ecological functions. Forests of mountain ridges with persistent winddriven fog comb moisture out of the atmosphere. The forests may also act like a sponge, storing large amounts of water in the vegetation and in the organic soil that accumulate, releasing it gradually during the dry season (Fjeldså, Kessler, 1996).

Deforestation in the Andes has a long history: different ethnic groups inhabited the Andean highlands many generations before the Spanish conquistadors arrived (Ridbäck, 2008). Since humans entered this region the forests have been used as a source for building material and fuel (Fjeldså, Kessler, 1996). The practice to burn large areas to create farmlands or improve pastures in the Andes has reduced *Polylepis* forests to a few percentage of their original extent (Ridbäck, 2008). Thus, human activities during several thousands of years have considerably changed the forests of *Polylepis* in the central Andes in Ecuador (Ridbäck, 2008) as well as in Peru or Bolivia. Moreover, these activities in the Andean páramo have increased drastically over the last two-three decades: there are strong scientific evidences that these activities have a dramatic impact on the integrity of the ecosystem (Cuesta, De Bievre, 2008).

Being native to the Andes (Hamen, Cleef, 1986; Kessler, 2002), *Polylepis* is of great value for reforestation programs. Considering its limited distribution in the Andean highlands, the *Polylepis* forests as such are worth conserving as a unique habitat and a harbour for very many species; they also protect the soil from erosion (Fjeldså, Kessler 1996).

No leaf-mining Nepticulidae associated with *Polylepis* have been recorded previously. During our fielwork conducted as part of *Polylepis Expedition* in 1987, and during other collecting in Peru (2008) and Ecuador (2001, 2005, and 2007), certain attention has been paid to Nepticulidae which would be associated with *Polylepis* as a host-plant. In this paper we report on the first discoveries of *Polylepis*-feeding Nepticulidae species in the high Andes of Ecuador and Peru (Fig. 7).

MATERIALS AND METHODS

Description or documentation of new species is based on material deposited in the collection of the Zoological Museum, University of Copenhagen, Denmark. Collecting methods and protocols for species identification and description are outlined in Puplesis (1994), Puplesis, Robinson (2000), and Puplesis, Diškus (2003). After maceration of the abdomen in 10% KOH and subsequent cleaning, male genital capsules were removed from the abdomen and mounted ventral side uppermost. The phallus was removed and mounted in Euparal separately but on the same genitalia slide. Abdominal pelts (sometimes also genital capsules) were stained with Chlorazol Black (Direct Black 38/Azo Black) (see Stonis et al., 2014).

Permanent slides were photographed and studied using a Leica DM2500 microscope and Leica DFC420 digital camera. The descriptive terminology of morphological structures follows Puplesis, Robinson (2000), except for the term "aedeagus", which is referred to here as "phallus", and the term "cilia", which is referred to here as "fringe".

Institutional abbreviations used in the text: ZMUC – Zoological Museum, University of Copenhagen, Denmark.

RESULTS

Description of *Stigmella polylepiella* Diškus & Stonis, sp. nov., on *Polylepis racemosa* from Peru

Type material. Holotype: ♂, PERU, 60 km NW of Cuzco, Ollantaytambo, 13°15'31"S, 72°15'54"W, elevation 2851 m, mining larvae

on *Polylepis racemosa* Ruiz & Pav., 21.x.2008, field card no. 4948, A. Diškus, genitalia slide no. AD739 (ZMUC). Paratype: 1♂, same label data as holotype, genitalia slide no. AD741 (ZMUC).

Diagnosis. The combination of the densely speckled forewing with some golden gloss and purple ireidescence, closely juxtaposed processes of gnathos, three-lobed uncus, and unique set of cornuti in the phallus distinguishes *S. polylepiella* sp. nov. from all other *Stigmella* species; the host-plant *Polylepis racemosa* (Rosaceae) also makes this species distinctive.

Male (Figs. 14–17). Forewing length about 2.6 mm; wingspan 5.7–5.8 mm. Head: palpi cream to greyish cream; frontal tuft ferruginous; collar and scape cream; antenna distinct-ly longer than half the length of forewing; flagellum with 44–47 segments, dark grey, glossy. Thorax, tegula and forewing densely speckled



Fig. 7. Map with currently known Nepticulidae records on Polylepis

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Figs. 8–13. Bionomics of *Stigmella polylepiella* Diškus & Stonis, sp. nov. 8, 9, host-plant *Polylepis racemosa* Ruiz & Pav., Rosaceae (with an infested bush in photograph 9); 10–13, leaf-mines, Ollantaytambo, 60 km NW of Cuzco, Peru, 13°15'31"S, 72°15'54"W, at elevation about 2850 m

with dark grey scales with coppery golden gloss and purple iridescence. Forewing without fascia; fringe dark grey; underside of forewing fuscous with little purple iridescence; no spots or androconia. Hindwing grey on upper side and underside; its fringe dark grey. Legs grey, silvery shining, darkened with dark grey on upper side. Abdomen dark grey to fuscous on upper side, grey cream on underside.

Female. Unknown.

Male genitalia (Figs. 22–26). Capsule longer (235–240 μm) than wide (130 μm). Uncus very



Figs. 14–21. *Stigmella polylepiella* Diškus & Stonis, sp. nov. 14, holotype, male adult; 15, same, descaled head; 16, same, fragment of descaled flagellum; 17, same, descaled head with flagellum; 18, 19, cocoon; 20, 21, leaf-mine with cocoon inside

wide, with three very short caudal lobes. Gnathos with two juxta-posed caudal processes, narrow central plate and extended corners. Valva 145–150 μ m long, 35–45 μ m wide, with two apical processes; inner lobe slightly bulged; transtilla without sublateral processes. Juxta membranous, indistinct or absent. Vinculum with triangular lateral lobes. Phallus (Figs. 24,



Figs. 22–26. Male genitalia of *Stigmella polylepiella* Diškus & Stonis, sp. nov. 22, caudal part of capsule, holotype, genitalia slide AD739; 23, capsule with phallus removed, paratype, genitalia slide AD741; 24, phallus, holotype, genitalia slide AD739; 25, same, paratype, genitalia slide AD741; 26, dorsal view of capsule, paratype, genitalia slide AD741 (ZMUC)

25) 190–195 μ m long, 50–65 μ m wide; vesica with unique set of cornuti; two apical cornuti strongly bent (well visible in slide AD741, Fig. 25; little visible but bent in slide AD739, Fig. 24).

Bionomics (Figs. 8–13, 20, 21). Larvae mine in leaves in October. Host-plant: Polylepis racemosa Ruiz & Pav. (Rosaceae) (Figs. 8, 9). Egg beige cream, mat (lustreless), oval-shaped, flattened dorso-ventrally, attached (not glued) on the leaf under side. Leaf-mine starts as a narrow gallery filled with black frass; later it develops abruptly to a large blotch with frass irregularly scattered but most of it remains accumulated in basal part of the blotch (Figs. 10, 11). Larva spins its cocoon inside the mine; the mine swells and becomes blisterlike at this stage (Figs. 12, 13, 20, 21). Cocoon purplish brown to purplish dark brown; shape of the cocoon unusual, narrow; length 2.0-2.4 mm, maximal width 0.8–0.83 mm. Exit slit on upper side of the leaf. After the 'Formula of Evaluation of Abundance and Occurrence of Leaf-miners' (see Diškus, Stonis 2012: 52–54), Stigmella polylepiella is extremely abundant in the type locality: a mass mining of the new species were observed (sometime with a few leaf-mines on a single leaf); more than 300 leaf-mines with larvae were collected at a single site in Ollantaytambo, Peru (Fig. 9).

Distribution (Fig. 7). This species occurs in the Andes (Peru: NW of Cuzco) at an altitude of about 2850 m.

Etymology. The species is named after the host-plant genus *Polylepis* Ruiz & Pav.

Documentation of *Stigmella* species 763 on *Polylepis pauta* from Ecuador

Material examined. 1∂ [dissected from completely developed pupa within the pupal case], ECUADOR: Chimborazo Province, ca. 30 km NE Pallatanga, 1°52'41"S, 78°54'11"W, elevation about 3325–3400 m, 8 mining larvae on *Polylepis pauta* Hieron. (Rosaceae), 24.i.2005, field card 4829, leg. A. Diškus & J. R. Stonis, genitalia slide no. AD763 (ZMUC); 1 mining larva on *Polylepis pauta*, same locality, 21.ii.2007, field card 4881, leg. A. Diškus. **Diagnosis.** The combination of the unique set of cornuti in the phallus, specific shape of uncus, very short lateral lobes of vinculum, and two apical processes of valva distinguishes *S.* species 763 from all other *Stigmella* species; the host-plant *Polylepis pauta* Hieron. (Rosaceae) also makes this species distinctive.

Male. Unknown (the species was documented from dissected, completely developed pupa within the pupal case).

Female. Unknown.

Male genitalia (Figs. 38–40). Capsule longer (260 μ m) than wide (180 μ m). Uncus wide, with short caudal lobes. Gnathos with two rather short caudal processes, wide central plate and extended corners. Valva 150 μ m long, 70–80 μ m wide, with two distinct apical processes; transtilla with very small sublateral processes. Juxta membranous (indistinct) or absent. Vinculum with very small rounded lateral lobes. Phallus (Fig. 40) 180 μ m long, 90–95 μ m wide; vesica with the unique set of cornuti: five strongly thickened horn-like cornuti and numerous, little thickened spine-like cornuti.

Bionomics (Figs. 33–37). Larvae mine in leaves in January. Host-plant: *Polylepis pauta* Hieron. (Rosaceae) (Fig. 35). Egg unknown. Larva brownish yellow (see Remarks on *Stigmella* species 764). Leaf-mine starts as a narrow gallery almost entirely filled with black-brown frass; later gallery develops abruptly to a large blotch with black-brown frass accumulated (irregularly scattered) in basal part of the blotch (see Remarks on *Stigmella* species 764) (Fig. 36). Larva spins its cocoon outside the mine; the mine turns brown when older or vacant, only little transparent. Cocoon yellowish brown, oval shaped. Exit slit on upper side of the leaf.

Distribution (Fig. 7). Known from a single locality in the Equatorial Andes (Chimborazo Province, Ecuador). The habitat is montane moist mixed forest with *Polylepis pauta* (Figs. 33, 34).

Remarks. This very remarkable and undoubtedly new species is documented but not named pending the availability of additional

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Figs. 27–32. Documentation of leaf-mines on *Polylepis racemosa* Ruiz Lopez & Pavon (Rosaceae), Peru: Junin Province, Ondores (collecting site "7" by O. Karsholt), elevation 4100 m, leg. O. Karsholt / *Polylepis Expedition* of 1987 (ZMUC)

material. The documented but unnamed taxon is distinguished by the number of the corresponding genitalia slide, i. e., Stigmella species 763 is a taxon exemplified by the pupa specimen from which male genitalia slide AD763 (ZMUC collection) was made.



Figs. 33–37. Bionomics of *Stigmella* species 763 and species 764. 33, 34, habitat, montane moist forest with *Polylepis pauta*, Pangor Canyon, 30 km NE Pallatanga, Ecuador, 1°52'41"S, 78°54'11"W, elevation 3025 m; 35, host-plant *Polylepis pauta* Hieron.; 36, 37, leaf-mines from which two different new species on *Polylepis (Stigmella* species 763 and species 764) were reared (see Remarks on *Stigmella* species 764)

Documentation of *Stigmella* species 764 on *Polylepis pauta* from Ecuador

Material examined. 1♂ [dissected from completely developed pupa within the pupal case], ECUADOR: Chimborazo Province, ca. 30 km NE Pallatanga, 1°52'41"S, 78°54'11"W, elevation about 3325–3400 m, 1 mining larva on *Polylepis pauta* Hieron. (Rosaceae), 24.i.2005, field card 4829, leg. A. Diškus & J. R. Stonis, genitalia slide no. AD764 (ZMUC).

Diagnosis. The combination of the membranous phallus with small group of spine-like cornuti, specific shape of widely lobate uncus, extremely very short ventral plate of vinculum with very short lateral lobes, membranous transtilla, and large lobate central plate of gnathos distinguishes *S.* species 764 from all other *Stigmella* species; the host-plant *Polylepis pauta* Hieron. (Rosaceae) also makes this species distinctive.

Male. Unknown (the species was documented from dissected, completely developed pupa within the pupal case).

Female. Unknown.

Male genitalia (Figs. 41–43). Capsule slightly longer (250 μ m) than wide (235 μ m). Uncus very wide, divided into two caudal lobes. Gnathos with two slender and long caudal processes and wide lobate central plate. Valva 200–205 μ m long, 65–75 μ m wide (see Remarks), without distinct apical processes; transtilla membranous, without sublateral processes. Juxta invisible or absent. Vinculum with extremely very short ventral plate and very small lateral lobes. Phallus (Fig. 43) not thickened, almost membranous, 175 μ m long, 125 μ m wide; vesica with a small group of little thickened spine-like cornuti.

Bionomics (Figs. 33–37). Larvae mine in leaves in January. Host-plant: *Polylepis pauta* Hieron. (Rosaceae) (Fig. 35). Egg unknown. Leaf-mine as a gallery which develops to a large blotch (see Remarks). Larva spins its cocoon outside the mine. Cocoon yellowish brown, oval-shaped. Exit slit on upper side of the leaf. Otherwise unknown.

Distribution (Fig. 7). Known from a single locality in the Equatorial Andes (Chimbo-

razo Province, Ecuador). The habitat is montane moist mixed forest with *Polylepis pauta* (Figs. 33, 34).

Remarks. There are certain doubts about morphology of the leaf-mine of the species. Leaf-mine samples should be collected very carefully: samples might easily be mixed up if two different species mine at the same hostplant at the same time and produce closely similar or variable leaf-mines. Nevertheless, during our investigation there were instances of rearing adults of two different species from the same sample as in case of *Stigmella* species 763 and *S.* species 764. Therefore, now it is rather difficult to described precisely and attribute correctly the similar leaf-mines to the reared different species from the mixed sample.

In contradiction to A. Diškus, who made the dissection, it was suspected by J. R. Stonis and A. Remeikis that the valva of *Stigmella* species 764 (Fig. 42) possesses two wide but overlapped lobes: ventral and dorsal. However, in the permanent mount no. AD764 it is too difficult to see either the valva simple or widely bilobed.

This very remarkable and undoubtedly new species is documented but not named pending the availability of additional material. The documented but unnamed taxon is distinguished by the number of the corresponding genitalia slide, i.e., Stigmella species 764 is a taxon exemplified by the pupa specimen from which male genitalia slide AD764 (ZMUC collection) was made.

Documentation of leaf-mines of Nepticulidae on *Polylepis racemosa* from Peru

Leaf-mine samples examined. 12 leaf-mines (no larvae or adults), PERU: Junin Province, Ondores (collecting site "7" by O. Karsholt), elevation 4100 m, 29.i.–5.ii.1987, leg. O. Karsholt / *Polylepis Expedition* (ZMUC).

Documentation. Mines in leaves (Figs. 27, 28). Host-plant: *Polylepis racemosa* Ruiz & Pav. (Rosaceae). Egg glossy white, oval shaped (not flattened!), attached (but not glued) to the highly hairy leaf underside. Larva mine in January and February (judging on observed vacant



Figs. 38–43. Male genitalia of new species on *Polylepis* from Ecuador. 38, *Stigmella* species 763, genitalia slide AD763, valvae; 39, same, dorsal view of capsule; 40, same, phallus; 41, *Stigmella* species 764, genitalia slide AD764, valvae; 42, same, dorsal view of capsule; 43, same, phallus (ZMUC)

leaf-mines), also possibly in early April. Leafmine as a gradually widening but strongly contorted gallery (therefore it may resemble a blotch) (Figs. 29, 30, 32). Black frass forms an interrupted or continuous central line filling most of the gallery; the distal part of the gallery with wide areas without frass. Larval exit slit on upper side of the leaf. It occurs in the high Andes NE of Lima (Peru) at an altitude of about 4100 m.

Remarks. The documented leaf-mines on *Polylepis racemosa* belong to an unknown Nepticulidae species (possibly belonging to the genus *Stigmella* Schrank). However, no pupae or adults are available for further discussion and description.

DISCUSSION

1. Leaf-mining Nepticulidae species are associated with at least two species of *Polylepis* as a host-plant (including *Polylepis racemosa* and *P. pauta*) occurring in the high Andes of Peru and Ecuador and belong to the genus *Stigmella* Schrank, the biggest and worldwide widespread generic taxa within the family Nepticulidae. All discovered Nepticulidae taxa are new, but only *Stigmella polylepiella* sp. nov. was described and named; other taxa were documented but left unamed pending additional material (currently, only dissected pupae, not adults, are available).

2. *Stigmella polylepiella* sp. nov. was observed as a highly abundant species with mass mining of *Polylepis racemosa* in the Andes NW of Cuzco (literally infesting the host-plant), while the Ecuadorian *Stigmella* species 763 and *S*. species 764 on *Polylepis pauta* are probably rare because so far only a few leaf-mines were recorded. Mining seasons of *Polylepis*-feeding species occur in January (*Stigmella* species 763 and *S*. species 764) and in October (*Stigmella polylepiella* sp. nov.).

3. There usually is no difficulty with rearing adult Nepticulidae from mining larvae using the proper methods as described in Diškus, Stonis (2012). But in the case of Polylepis-feeding species occurring in the high Andes (páramo or puna), the rearing of adults from larvae can be a great challenge. During our fieldwork, even in the case of exceptionally large samples of up to few hundreds of mining larvae (Stigmella polylepiella sp. nov.), only a couple larvae developed to mature pupae and emerged. In case of Stigmella species 763 and S. species 764, pupae stopped their development and died within the pupal case. Causes of this phenomenon (Stonis et al., 2016) are unknown and certainly not related to parasitoids.

4. In contradiction to expectations, the first discoveries of leaf-mining Nepticulidae on *Polylepis* revealed that species feeding on *Polylepis* are not closely related but indicate unexpectedly great morphological diversity (more than usual in comparison to species feeding on the most

of other host-plant genera in South America, see Stonis et al., *in prep.*), and represent highly distinctive taxa among all known *Stigmella* worldwide. Apriori we can assume that these species (particularly *Stigmella* species 764) are rather a result of older evolutionary events than the recent speciation so commonly known in the Andean fauna (including the fauna of Nepticulidae).

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AR LAPUS MINUOJANTYS MAŽIEJI GAUBTAGALVIAI (NEPTICULIDAE) GYVENA NATŪRALIUOSE, BET NYKSTANČIUOSE ANDŲ KALNŲ POLYLEPIS MIŠKUOSE?

Santrauka

Aukštujų Andų kalnų Polylepis miškai yra natūrali, ekologijos ir biologinės įvairovės požiūriu itin svarbi, bet žmogaus smarkiai naikinama buveinė. Iki šiol nieko nebuvo žinoma apie mažuosius gaubtagalvius (Lepidoptera, Nepticulidae, Nepticuloidea), kurie būtų mitybiniais ryšiais susieti su Polylepis Ruiz & Pav. genties medžiais. Šiame straipsnyje pateikiami tyrimų rezultatai pirmą kartą byloja apie Ekvadore ir Peru Polylepis lapus minuojančius Nepticulidae. Aprašoma nauja mokslui rūšis - Stigmella polylepiella Diškus & Stonis, sp. nov., kuri aptikta aukštuosiuose Andų kalnuose Peru ir kurios visų ūgių vikšrai yra Polylepis racemosa Ruiz & Pav. lapų minuotojai. Be išskirtinių morfologinių struktūrų ypatybių, šiai naujai rūšiai būdingi ir neįprastos formos kokonai, susidarantys minos viduje, o ne miško paklotėje. Taip pat aprašomos dar dvi kitos Stigmella Schrank genties rūšys, aptiktos Ekvadore; jų vikšrai minuoja Polylepis pauta Hieron. lapus. Nors abi šios rūšys yra naujos, straipsnyje joms nebuvo suteikti lotyniški pavadinimai tikintis daugiau tyrimų ir kolekcinės medžiagos ateityje; šiuo metu neįvardytų rūšių (Stigmella species 763 ir S. species 764) yra išaiškintos ir preparuotos tik suaugusios lėliukės, o ne patys suaugėliai (imagai). Papildomai straipsnyje yra dokumentuotos iki šiol neaprašytos rūšies iš Peru minos ant Polylepis racemosa lapų.

Raktažodžiai: *Polylepis*, Nepticulidae, *Stigmella* Schrank, naujos rūšys, minos ant lapų, Peru, Ekvadoras