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Trifurcula (*Glaucolepis*) *lituanica* sp. nov., an unexpected new stem-miner on *Salvia pratensis* occurring in eastern Europe (Lepidoptera: Nepticulidae)

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Abstract

Trifurcula (*Glaucolepis*) *lituanica* Ivinskis & van Nieukerken, **sp. nov.**, is described from adults reared from stem-mining larvae on *Salvia pratensis* (Lamiaceae) from Lithuania and some specimens taken as adults in Austria, Slovenia and Greece. In addition the new species is recorded from Bulgaria, the Czech Republic and Romania. Superficially, it resembles *Trifurcula* (*Glaucolepis*) *headleyella* (Stainton, 1854), especially the male, but it differs by male genitalia with additional cornuti, a unique character for the subgenus, and the female genitalia differ by the larger number of convolutions in the ductus spermathecae. It is the only known *Trifurcula* to make its cocoon partially inside a stem-mine. The synonymy of *Nepticula dubiella* Hauder, 1912 with *T. headleyella* is confirmed, but some specimens recorded under this name from Austria belong to the new species. DNA barcodes are provided and compared with related species.

Key words: Taxonomy, new species, hostplants, Lamiaceae, DNA-barcodes, COI gene, Palearctic

Introduction

The nepticulid genus *Trifurcula* Zeller, 1848 comprises globally 68 described species, of which the large majority occurs in the Mediterranean region; only six named species and a few unnamed ones are known from North America, Asia (outside the Mediterranean region), South Africa and Australia (van Nieukerken 1986b; van Nieukerken 2010). The species occurring in the Mediterranean are specialized in feeding on shrubs and herbs, where they make stem-mines or leaf-mines. The subgenera *Trifurcula* (with 26 species) and *Levarchama* Beirne, 1945 (7 species) all feed on Fabaceae, the first making stem-mines, the latter leaf-mines, and the subgenus *Glaucolepis* Braun, 1917 (29 species) makes stem- or leaf-mines, or a combination of both, on plants belonging to families such as Lamiaceae, Apiaceae (only the genus *Bupleurum*), Plantaginaceae (genus *Globularia*) and a few others. In Europe the diversity of the genus sharply declines towards the north (van Nieukerken *et al.* 2010); only a few species of *Glaucolepis* are known from northern parts in Europe: *T. (Glaucolepis) headleyella* (Stainton, 1854) on *Prunella* goes as far north as southern Sweden, Finland and Estonia, *T. (G.) thymi* (Szőcs, 1965) on *Thymus* is occasionally encountered in extremely hot localities in Germany and Poland (van Nieukerken & Johansson 1990) and *T. melanoptera* Van Nieukerken & Puplesis, 1991 has recently been recorded from Germany (van Nieukerken *et al.* 2010). It was thus a surprise for the authors to discover an unknown species in Lithuania, where hitherto only *T. headleyella* had been recorded (Diškus 2003).

Stem-mines and cocoons attached to the stems, characteristic for Nepticulidae, were found on *Salvia pratensis* in a river valley. The emerging moths externally resembled those of *T. headleyella*. However, *T. headleyella* has a completely different life history, with mines going through several leaves, petioles and stem of *Prunella*. Two species of *Trifurcula* have hitherto been recorded as leafminers on *Salvia* species: *T.* (*G.*) *trilobella* Klimesch 1978, common in Greece and Turkey, on *Salvia fruticosa* and *T.* (*G.*) *salvifoliae* Z. Laštůvka & A. Laštůvka, 2007 from Spain on *Salvia lavandulifolia*.

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Because of the resemblance of the new species to *T. headleyella*, we checked again the identity of two synonyms of that species, especially because an undescribed species resembling *T. headleyella* had previously been recorded as "*Nepticula dubiella*" sensu Klimesch (van Nieukerken & Johansson 1990: 262). Indeed some specimens of the new species were found under material identified as "*Nepticula dubiella* Hauder", but re-examination of type material of that species confirmed that *N. dubiella* is a junior synonym of *T. headleyella*. Mines of the new species were also found in Romania in 2011 and Bulgaria in 2012, and we found that more material in collections, previously identified as *T. headleyella* or its synonyms, belongs to this new species.

The subgenus *Glaucolepis* is difficult to get a handle on: many very similar species occur in southern Europe, and several undescribed species are known. Previous treatments deal with a particular species group or geographic area only (Klimesch 1975, 1978; van Nieukerken & Puplesis 1991; Laštůvka & Laštůvka 1997; Laštůvka & Laštůvka 1998; Laštůvka & Laštůvka 2000; van Nieukerken *et al*. 2006; Laštůvka & Laštůvka 2007; van Nieukerken *et al.* 2010), and a summary and key are long overdue. The currently ongoing e-taxonomy website project "Nepticulidae and Opostegidae of the World" (van Nieukerken 2010) intends to bring this information together in the coming few years and thereby facilitate a holistic interpretation of the subgenus.

Glaucolepis species can be recognised by the apomorphies of the genus (1. M in hindwing with two branches (Rs+M trifurculate), 2. males with velvet patch on hindwing underside—with a few exceptions—and 3. male abdomen with paired tufts of hair scales on segments 6, 7 and 8) and by the subgeneric characters: 4. forewing with complete venation, including closed cell, 5. the male genitalia without a sclerotised transtilla and 6. female genitalia with the ductus spermathecae basally a long straight part, followed by a coiled part.

The new species is here described and compared with *T. headleyella*, including the DNA barcodes. These species differ from all other European *Trifurcula (Glaucolepis)* by the presence of two metallic spots on the forewings, rather than just one or no spot at all as in all other species.

Material and methods

Larvae and cocoons of the new species were collected in Lithuania in July from 2000 to 2008, in Romania in July 2011, and in Bulgaria in August 2012. For rearing, the cocoons were stored outdoors in glass containers for hibernation, emerging moths were collected from the containers in spring.

Additional material was borrowed from the following collections, which are in material lists indicated by the city name:

Biology Center, Oberösterreichische Landesmuseen, Linz, Austria
Natural History Museum, Vienna, Austria
The Bavarian State Collection of Zoology, Munich, Germany
Institute of Ecology, Nature Research Centre, Vilnius, Lithuania
Naturalis Biodiversity Center, Leiden, Netherlands
Zoological Museum, Kiev National Taras Shevchenko University, Ukraine
Zoological Museum, State National History Museum, Copenhagen, Denmark
Private collections of G. Baldizzone, G. Bassi, B. Å. Bengtsson, J. Junnilainen and A. Laštůvka.

For comparison we studied *T. headleyella* specimens; they are only cited in detail when particularly relevant for this paper, or concern unpublished records.

Genitalia preparations follow general methods as described e.g. by van Nieukerken (1985). For staining we used chlorazol black for females and sometimes male abdomens, and haemaluin according to Mayer or phenosafranine for males. Genitalia were either embedded in euparal (Leiden) or glycerol (Vilnius).

Measurements of genitalia and larvae were obtained from digital images, using Zeiss AxioVision, 20x objective for male genitalia and 10x or 20x for females.

DNA was extracted from caterpillars or from dry adult abdomens. DNA extraction from larvae was usually destructive; from abdomens and some larvae the non-destructive protocol by Knölke *et al.* (2005) was followed, allowing the preparation of the genitalia or larval skin as well. Details of methods are presented by van Nieukerken *et al.* (2012), we provide here the COI DNA barcode for the new species and several related species of the subgenus *Glaucolepis*, and as outgroup the type species of the genus, *T.* (*Trifurcula*) *pallidella* (Duponchel, 1843).

Details can be found on the Barcode of Life webpages (http://www.barcodinglife.com/views/login.php) under the project “Nepticulidae—*Trifurcula* Public records”.

Photographs of moths and genitalia slides were taken with a Zeiss AxioCam (HR or MR5) digital camera attached respectively to a Zeiss Stemi SV11 stereo-microscope and a Zeiss Axioskop H, using Carl Zeiss AxioVision software (version 4), for some photographs using the module “Extended focus”. Field photographs were made by the authors in Lithuania and Romania. Manipulation of photographs, using Adobe Photoshop® was kept to a minimum: disturbing conspicuous shades, protruding parts of pins, dust and air bubbles in slides were removed or obscured.

Systematic part

Trifurcula (Glaucolepis) lituanica Ivinskis & van Nieukerken sp. nov.

(Figs. 1, 2, 5–10, 14–18, 23–32)

Trifurcula lituanica Ivinskis sp. n. in litt.: Ivinskis 2004: 33. [Nomen nudum, no description].
Fedalmia dubiella; partim: Klimesch 1961: 764; Klimesch 1990: 44 [misidentification]

Type material. Holotype ♂: LITHUANIA, Ringovė, 55° 02’ 59.6”N 23° 31’ 18.1”E, stemmines on *Salvia pratensis*, 19.vii.1999, e.l. 21–27.iii.2000, P. Ivinskis, Genitalia slide EJvN4210, RMNH.INS.24210 (coll. Leiden).—**Paratypes**: **8♂, 4♀.** AUSTRIA: 1♂, Umgebung von Linz, Steyregg, 12.v.1947, J. Klimesch, genitalia slide RJ1539; 1♂, Linz, Pfenningb[er]g, 25.iv–5.v.1948, J. Klimesch, genitalia slide EJvN4269; 2♀, Umgebung von Linz, Pfbg [Pfenningberg], 3.v.1948, J. Klimesch, genitalia slide EJvN4267 (all coll. Munich).—GREECE: 1♂, Piería, Leptokaria, 15 km W, Olympos, 750 m, 21–23.v.2001, J. Junnilainen, genitalia slide EJvN3398 (coll. Junnilainen).—LITHUANIA: 4♂, 2♀, data as holotype, emerged 21.iii–12.iv.2010, Genitalia slides ♂ Tr.01, EJvN3721, ♀ BJ1530, EJvN4266 (coll. Vilnius and Leiden).—SLOVENIA: 1♂, Prje [recte Brje, Ajdovščina], 28.iv.2007, J. Junnilainen, genitalia slide EJvN4277 (coll. Junnilainen).

Non type material: BULGARIA: Blagoevgrad Province, Ilindentsi, 41°40’18,6”N 23°16’55,96”, 880 m, 7.viii.2012, stem-mines with larvae on *Salvia pratensis*, P.Ivinskis, N. Savenkov (coll. Vilnius).—CZECH REPUBLIC: 1♀, Moravia, Klentnice, 25.viii.1997, A. Laštůvka [watercolour and drawing of genitalia examined]; 9 larvae, 1♀, Moravia, Čelechovice na Hané, 5–14.vii.2012, emerged 14.vii.2012, A. Laštůvka; 1♂, Czech Republic, Moravia mer., Bořetice, Zázmoníky Nature Reserve, 21.iv.2011, J. Liška (all coll. Laštůvka) [data received from A. and Z. Laštůvka].—LITHUANIA: Kaunas, western part of city, southern valley of river Neris, 54° 54’ 50.0”N 23° 52’ 46.1”E, 15.vii. 2008, 2 stem-mines with larvae on *Salvia pratensis*, P. Ivinskis (coll. Vilnius).—ROMANIA: 2 larvae in ethanol 96% (RMNH.INS.18487, 18488), stem-mines, Sibiu prov., Brădeni, 3 km N, 563 m, 46.1073 N, 24.8408 E, limestone grassland on hill, 30.vii.2011, stem-mines on *Salvia pratensis*, E.J. van Nieukerken (coll. Leiden).

Diagnosis. *Trifurcula lituanica* resembles *T. headleyella* closely: males especially can hardly be separated externally, although *T. lituanica* has a more pronounced shining basal area of the forewing. The female does not differ externally from the male, in contrast to *T. headleyella*, where the female has a completely black head (ferruginous in *lituanica*), and a very strongly metallic basal area contrasting with almost black distal part, whereas the basal area in *T. lituanica* is not strongly metallic. The male genitalia are best recognized by the aedeagus (phallus), which has just one short cornutus in *T. headleyella*, but several cornuti in *T. lituanica*, of which the basal one is also longer than in *headleyella*. The valva is narrower than in *T. headleyella*. The female genitalia are easily recognised by the ductus spermathecae with 7 convolutions against only 2–3 in *T. headleyella*.

Description. Male (Fig. 1): Forewing length 2.2–2.4 mm, wingspan 4.7–5.0 mm. Head: frontal tuft pale fuscous, scape white, antenna with 37–43 segments. Thorax and forewing dark fuscous, basal third of forewing covered with grey shining scales, demarcated from darker outer part; just beyond middle two opposite silvery white metallic spots, separated by 2–3 dark scale rows; cilia line present, cilia grey, underside dark fuscous. Hindwing grey on both sides, underside in apical third with “velvet” patch of raised scales. Abdomen fuscous, with paired tufts on tergites 6–8.

Female (Fig. 2). Forewing length 2.0–2.4 mm, wingspan 4.6–5.0 mm, antenna with 31–34 segments. External characters as in male, velvet patch absent. Abdomen slightly pointed at tip.

Male genitalia (Figs. 5–10). Capsule length 265–315 µm. Vinculum narrowed anteriorly, with clear concavity (bilobed). Tegumen pointed. Uncus pointed. Gnathos with large triangular, rounded central element. Valva length 135–170 µm, approximately triangular, distal process straight, pointed. Transverse bar of transtilla absent. Aedeagus (phallus) 295–335 µm long, near phallotrema two lateral rows of about 7–10 spines (not cornuti, Fig. 9), vesica with long, slightly curved, basal cornutus of *ca*. 110–145 µm long, and group of ca 60–70 µm long needle like cornuti apically (Fig. 10), close to phallotrema spines.

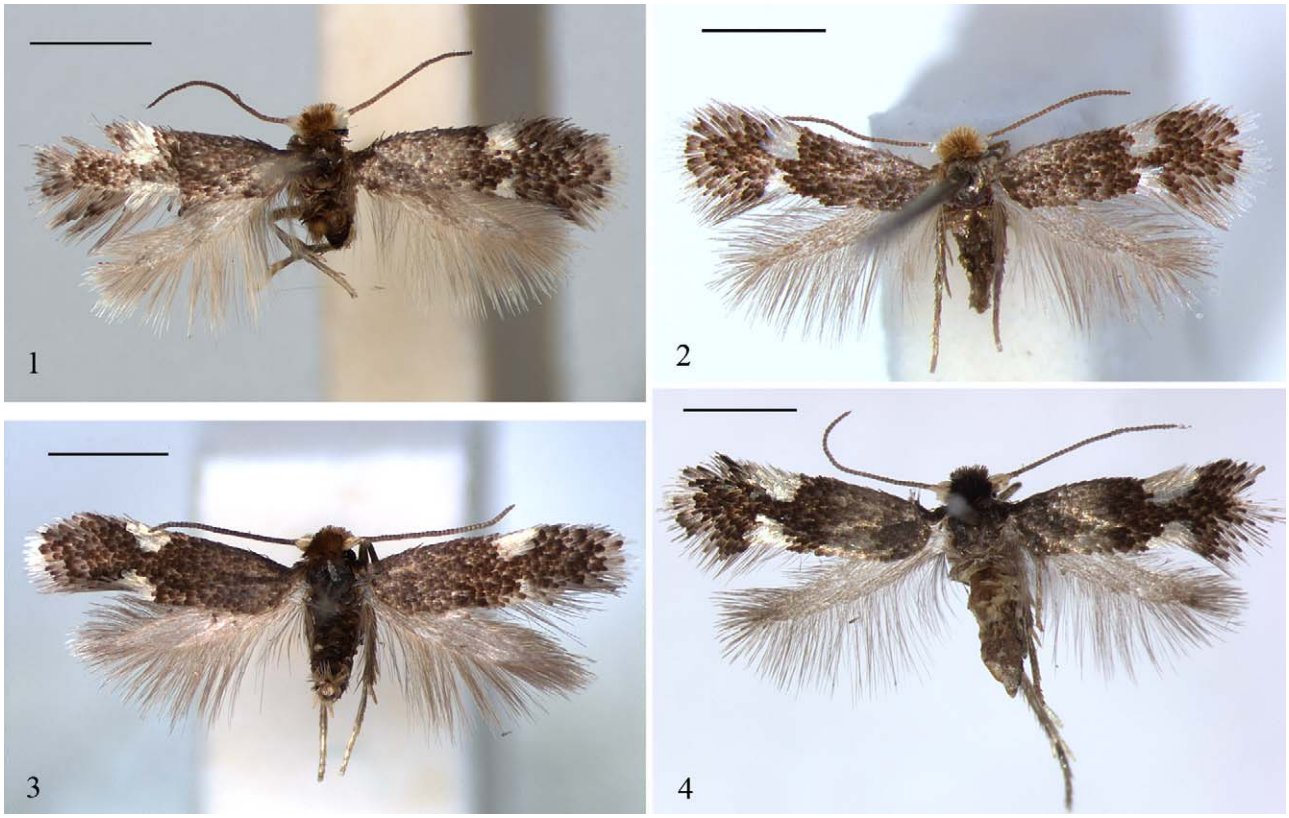
Female genitalia (Figs. 14–18). Total bursa length ca 790–870 µm. T8 with scales and ca. 6–7 setae at either side, anal papillae pointed, with 26–30 setae in total. Posterior apophyses about twice as long as anterior ones. Ductus spermathecae with relatively long straight part and ca. 6.5–7.5 convolutions. Corpus bursae covered with small pectinations, and indistinct paired reticulate signa of about 225–300 µm long.

Larva yellow, head capsule pale brown. Feeding with dorsum upwards, final instar about 5 mm long.

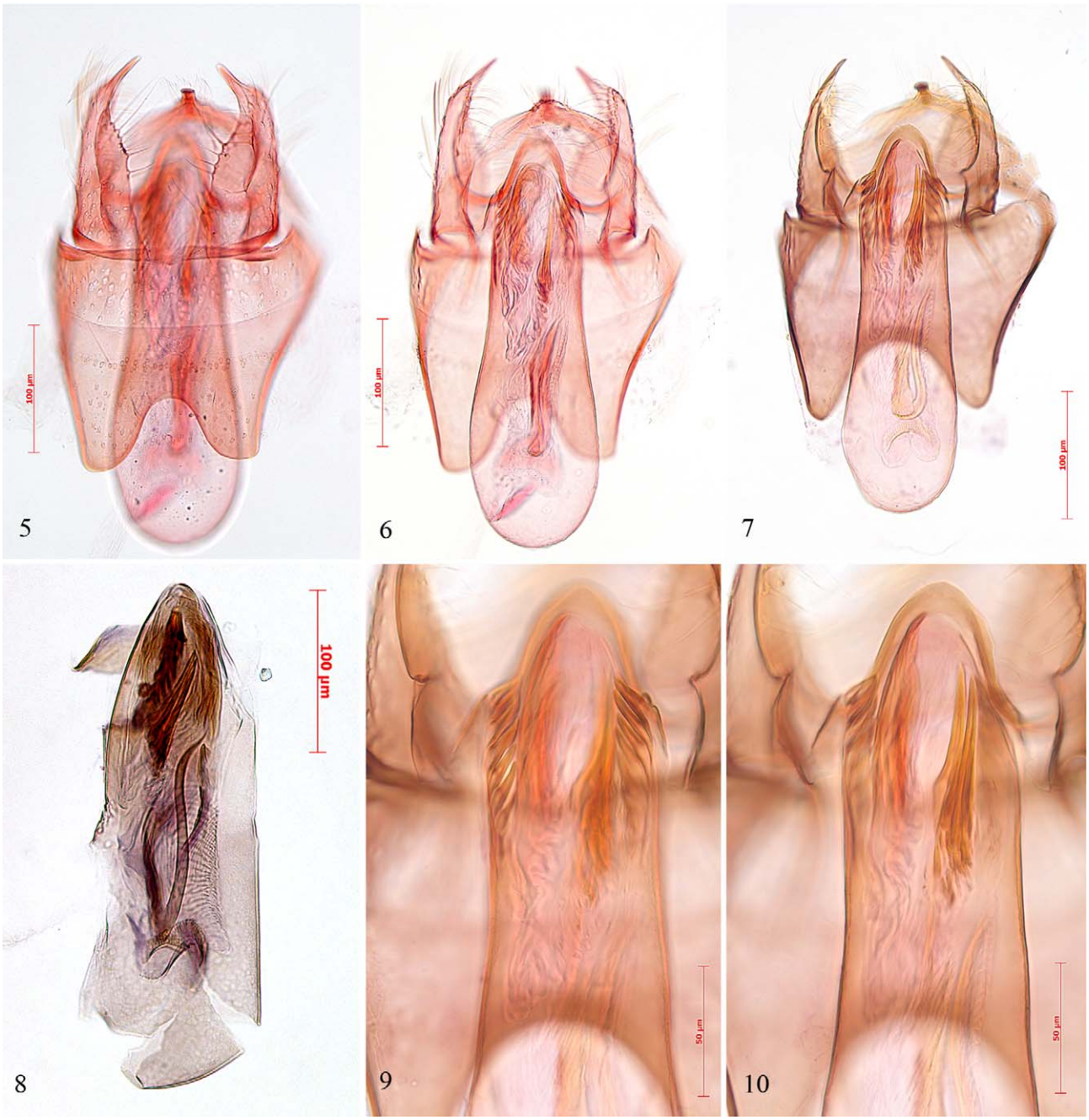
Cocoon (Figs. 24, 26–28). Dark brown, 4 x 2 mm. Upper side covered with plant epidermal tissue.

Biology. Host plant: *Salvia pratensis* L. (Lamiaceae). Egg deposited on the stem. Stem-mine (Figs 23–25, 29) most frequently in the lower parts of the stem, confined to one internode on one side of the stem, or running along a corner of the square stem. Mine a gallery with a total length of up to 23 cm, better visible on the green plant, much obscured in dried material. The mine starts usually upwards and goes back down after a u-turn. The frass is concentrated in the midline. The epidermis over the mine is elevated compared to the rest of the stem. The mine widens gradually in the second part and can occupy up to two thirds of the stem width. The larva eats out a rather wide semi-circular emergence slit, and then prepares the cocoon on the inner side of the epidermis covering the final part of the mine, near the slit. The cocoon is clearly visible within the epidermis. We found one to four mines per stem.

Voltinism. Univoltine. Larvae and cocoons found between 15 July and 7 August. From these larvae, adults emerged the next spring, but a single female in Moravia emerged immediately in July. Adults have been found in the wild from late April to mid May, usually earlier than *T. headleyella*, but another female in the Czech Republic was found late August, although the *Salvia* stems at that time are mostly dried out. These Czech females may indicate a partial second generation.



FIGURES 1–4. *Trifurcula (Glaucolepis)* species, adult habitus, dorsal. 1. *T. lituanica*, male holotype; 2. *T. lituanica*, female paratype, Austria; 3. *T. headleyella*, male, France, Génicourt-sur-Meuse, RMNH.INS.24212; 4. *T. headleyella*, female, Germany, Marmagen EvN95068. Scales 1 mm.



FIGURES 5–10. *Trifurcula (Glaucolepis) lituanica*, male genitalia in ventral view. 5–6. Holotype, RMNH.INS.24210; 8. Paratype Lithuania, RMNH.INS.23721. 7, 9–10. Paratype Slovenia, slide EJvN 4277, 9 showing phallotrema spines, 10 cornuti at slightly deeper level. Scales 100 µm (5–8), 50 µm (9–10).

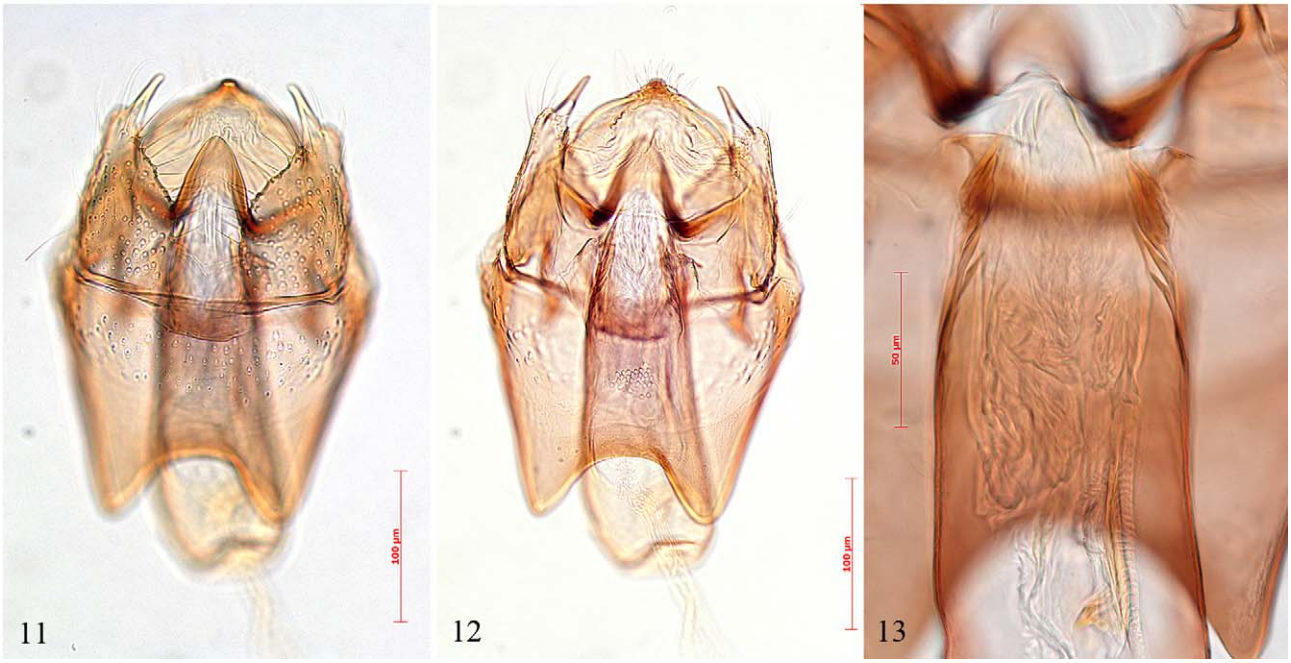
Distribution (Fig. 32)**.** Up to now isolated records are known from Lithuania (Kaunas district, two localities 40 km apart), the Czech republic (two localities in Moravia), Austria, Slovenia, Romania, Bulgaria and Greece. To be expected in a larger part of Europe with the host. *Salvia pratensis* occurs widely in Europe, with a northern border through the lowlands of the Netherlands, northern Germany and Poland to Russia (Hedge 1972). The Lithuanian locality is close to the northern border of the plant's distribution area.

Habitat and conservation (Figs. 30–31)**.** In Lithuania *T. lituanica* has been found only in the valley of the river Nemunas and its tributary Neris, where it occurs in flood plain meadows. *Salvia pratensis* occurs here on the slightly higher slopes of the floodplain. This plant is protected in Lithuania by law and included in the Red data book (Rašomavičius 2007). In Austria there is probably only one locality, the Pfenningberg in the municipality of Steyregg. The locality is on the western slopes of the Pfenningberg, close to the Danube (Klimesch 1990). In Romania the mines were found in a sunny limestone grassland on top of a hill. On the same plants of *Salvia* in

Romania, we observed different stem-mines nearer the tip of the stem, belonging to an agromyzid, probably *Ophiomyia labiatarum* Hering, 1937 (Hering 1957). The Greek locality (15 km W of Leptokaria) is an open mountain meadow with a rich vegetation including *Salvia pratensis*, and the Slovenian locality is a dry meadow with *Sanguisorba officinalis* in a river valley (Jari Junnilainen pers. comm.). Although the host is a widespread species, its occurrence in nutrient-poor grasslands in river valleys and on limestone make it vulnerable for habitat change: natural succession, afforestation, fertilizers and poor management are a risk for such vegetation types, and in general modernisation of agriculture in EU countries has often led to the loss of such habitats. Currently the species seems not to be threatened, but could become vulnerable when land management in eastern European countries changes too fast on a large scale.

DNA barcodes. For three specimens the DNA barcode has been analysed, two complete ones of one male paratype (RMNH.INS.23721, Genbank accession number JX261901) and a larva from Romania (RMNH.INS.18487, Genbank JX261899), differing only in one nucleotide, and an incomplete sequence of the holotype of 308 nucleotides (Genbank JX261900), identical to that part in the other two (see appendix)

Etymology. *Trifurcula lituanica*: an adjective, named after the country where the species' life history and hostplant were discovered.



FIGURES 11–13. *Trifurcula (Glaucolepis) headleyella*, male genitalia. 11–12. RMNH.INS.22293, Italy, Trento, Mte Maranza; 13. France, Génicourt-sur-Meuse, RMNH.INS.24212, detail of cornutus and phallotrema spines. Scales 100 µm, 50 µm (13).

***Trifurcula (Glaucolepis) headleyella* (Stainton)**

(Figs. 3, 4, 11–13, 19–22)

Nepticula headleyella Stainton 1854: 300. 2 Syntypes: [England, London]: Headley Lane, August, leg. Douglas [probably lost].

Nepticula argyrostigma Frey 1856: 261. Lectotype ♂ (designated by van Nieukerken & Johansson 1990: 262), Switzerland: Zürich, Frey coll., Genitalia slide 24089 (Natural History Museum London) (Synonymised by Frey 1880: 425) [examined].

Nepticula dubiella Hauder 1912: 273. Lectotype ♀ (designated by van Nieukerken & Johansson 1990: 262), Austria: Kirchdorf, 21.v.1900, leg. Hauder, genitalia slide EJvN2609 (see Fig. 22) (NMW) (Synonymised by Klimesch 1948: 76) [examined].

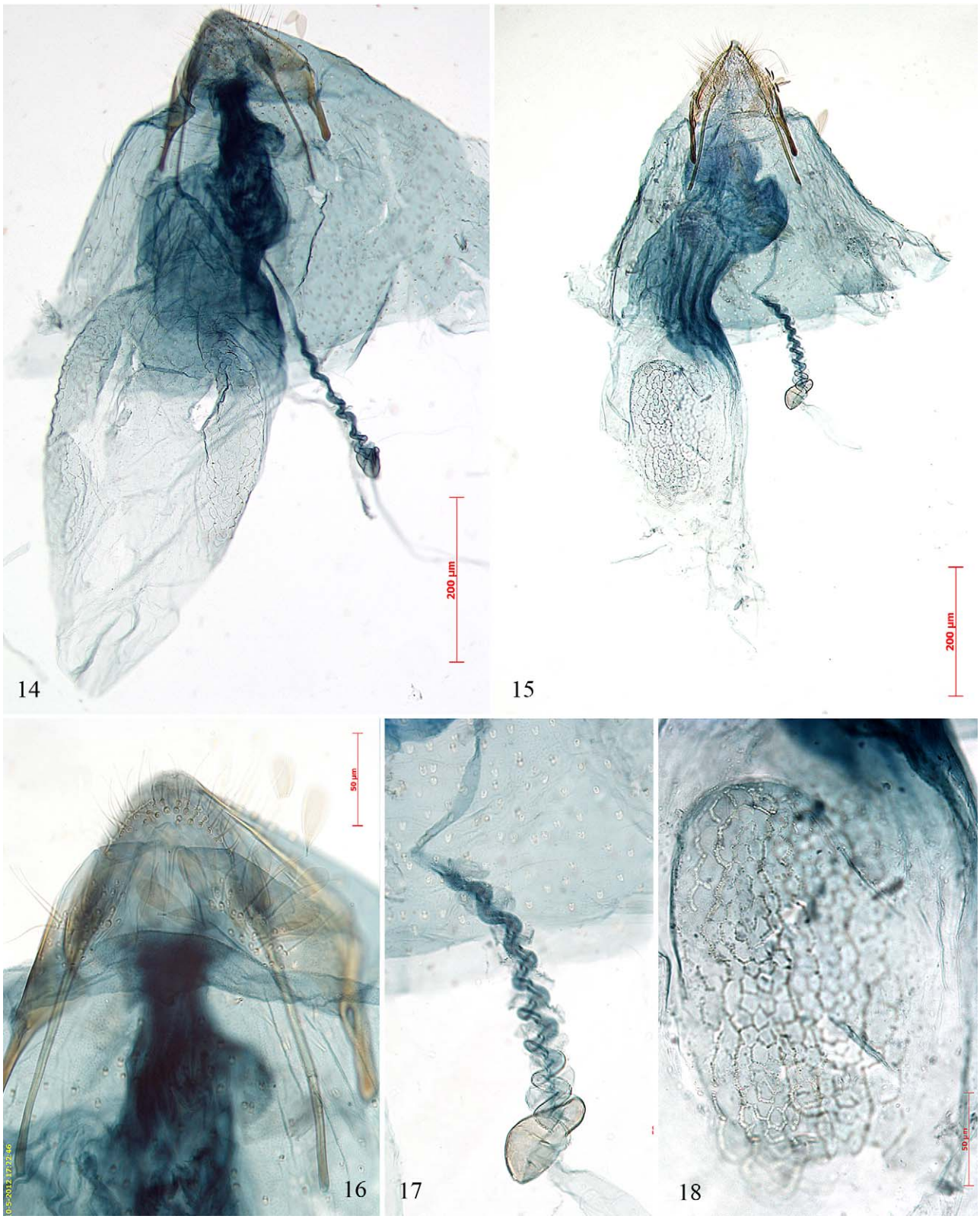
Trifurcula rodella Svensson 1982: 299. Holotype ♂, Sweden: Vg., Kinnekulle, 1–2.vii.1966, I. Svensson, Genitalia slide IS4550 (Lund) (Synonymised by van Nieukerken 1986a: 5) [examined].

Fedalmia headleyella; Beirne 1945: 207 [new genus, new combination]; Emmet 1976: 208 [redescription].

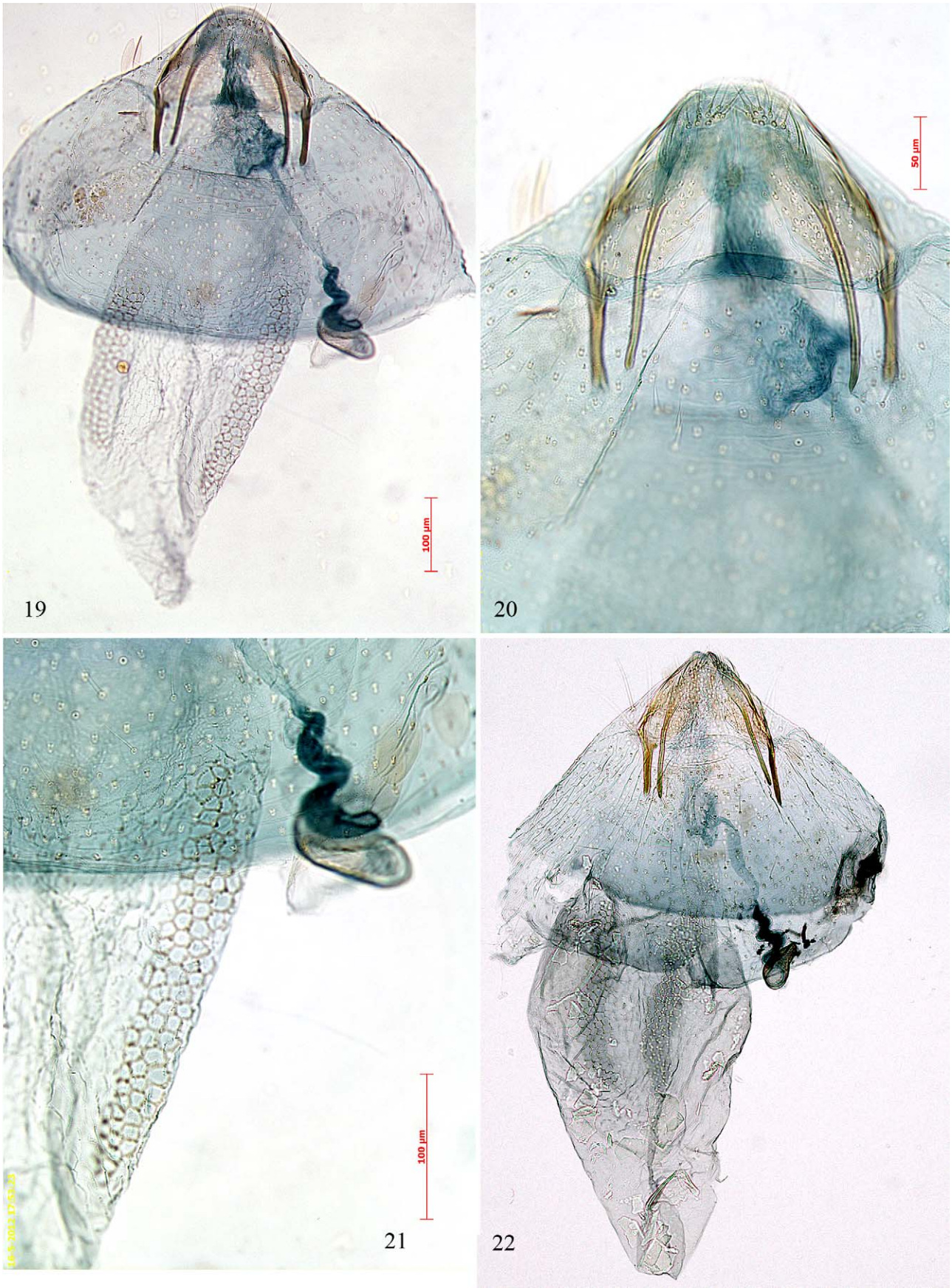
Trifurcula (*Fedalmia*) *headleyella*; Johansson 1971: 245. [new combination].

Trifurcula (*Glaucolepis*) *headleyella*; van Nieukerken 1986a: 15 [revised combination]; van Nieukerken & Johansson 1990: 261 [redescription]; Laštůvka & Laštůvka 1997: 125 [redescription]; Bengtsson *et al.* 2008: 234 [redescription].

Diagnosis. Trifurcula headleyella can be separated from other European Trifurcula, except T. lituanica (see there for differences), by the postmedial opposite metallic spots on the forewings in both sexes, and in the female (Fig. 4) in addition by the black head and strongly leaden shining basal third of the forewing. T. (Levarchama) eurema (Tutt, 1899), also has opposite spots, but never metallic, and often forming a fascia. Males of that species also possess a hairpencil on hindwing.



FIGURES 14–18. *Trifurcula (Glaucolepis) lituanica*, female genitalia. Paratypes, Lithuania, slide EJvN4266 (14, 16), Austria, slide EJvN4267 (15, 17–18). Scales 200 µm (14–15), 50 µm (16–18).



FIGURES 19–22. *Trifurcula (Glaucolepis) headleyella*, female genitalia. 19–21. Sweden, Klagshamn, RMNH.INS.22505; 22. Lectotype *Nepticula dubiella*, genitalia slide EJvN2609, photograph Sabine Gaal. Scales 100 µm (19, 21), 50 µm (20).

FIGURES 23–28. *Trifurcula (Glaucolepis) lituanica*, life history: stemmines and cocoons on *Salvia pratensis*. 23–24. Lithuania, Ringovė, 17.viii.2005; 25–28, Romania, Brădeni, 30.vii.2011 (photos taken later), in 25 the larva has been taken out of the mine. Scales 2 mm.

Descriptive notes. Male: Forewing length 1.8–2.5 mm, wingspan 4.1–5.7 mm, antenna with 35–41 segments (Bengtsson *et al.* 2008 give up to 45). Female: Forewing length 1.9–2.4 mm, wingspan 4.2–5.4 mm, antenna with 35–41 segments. *T. headleyella* is one of the few nepticulid species were the female has as many antennal segments as the male.

Male genitalia (Figs. 11–13): capsule 255–305 µm long, valva length 120–160 µm, aedeagus 220–275 µm long, cornutus 60–85 µm long.

FIGURE 29. *Trifurcula (Glaucolepis) lituanica*, life history.

Female genitalia (Figs. 19–22): total bursa length ca. 900 µm, signa 240–310 µm long, in some specimens very dissimilar, in others equally long. Anal papillae with ca. 9–10 setae, T8 with some setae and scales. Ductus spermathecae with 2.5–3 convolutions.

Biology. Hostplants: *Prunella vulgaris*, *P. grandiflora* and *P. laciniata* (Lamiaceae). Egg on upper surface of a leaf. The larva usually makes a short gallery in the first leaf and mines down the petiole, into the stem and then via the petioles into a second and often third leaf. The mine is linear, with linear frass, later becoming wider and more irregular, the frass often not in the middle. By mining the petiole the larva often partly cuts off the sap stream, leading to purple discoloration of one or more leaves (often those without mines!) (illustrated by Huisman *et al.* 2004). Discoloured leaves may be an indication for finding mines. In larger plants the larva may just use one leaf.

Distribution. Widespread, but very localised in many parts of Europe: southern England (Emmet 1976; Edmunds 2011), Denmark, southern Sweden and southern Finland (Bengtsson *et al.* 2008), *Netherlands (Huisman *et al.* 2004), Germany (van Nieukerken *et al.* 2010), *Poland (Borkowski 1975), France (Nieukerken *et al.* 2006), *Spain (van Nieukerken *et al.* 2004), Switzerland (SwissLepTeam 2010), *Austria*, *Italy*, Czech Republic, Slovakia (Laštůvka & Laštůvka 1994; Tokár *et al.* 2002), Hungary, *Croatia*, *Romania*, *Greece*, *Russia: Karelia (Kutenkova 1989), Estonia, Latvia, *Lithuania (Ivinskis 2004), **Ukraine* (general source: van Nieukerken 2011, other cited references contain detailed distribution information and/or maps). From countries with an asterisk just a single record or locality is known, for the country names in *italics*, detailed records are provided below.

Habitat. Considering how widespread and abundant the hostplant *Prunella* is, *T. headleyella* is remarkably local and rare. It is most frequently found in limestone grasslands, downland, dune slacks and alpine meadows. Where it occurs, it can be abundant, but still occurs very locally.

Remarks. Although the three synonyms and their types had been checked before (van Nieukerken & Johansson 1990), we re-examined the information on these types, including photographs of genitalia slides, in order to see whether one of these names could be the new species. Clearly all types show the characters of *T. headleyella*. The female lectotype of *Nepticula dubiella* (Fig. 22) can now be evaluated more accurately against the very different female genitalia of *T. lituanica*. In fact the complete type series of *Nepticula dubiella* and most other specimens under this name in the collections in Linz and Munich are after dissection shown to be *T. headleyella*, and only the four Klimesch specimens from Steyregg, Pfenningberg near Linz, cited above, belong to *T. lituanica*. Although Klimesch (Klimesch 1948) had synonymised *dubiella* with *headleyella*, he later changed his mind (Klimesch 1990), probably on the basis of the information that the single specimen dissected by Roland Johansson belonged to a different species. The confusion with *N. dubiella* lead EJvN (in Kasy 1985) to cite *T. headleyella* incorrectly as new for Austria.

FIGURES 30–31. *Trifurcula (Glaucolepis) lituanica*, habitat in Lithuania, Ringovė, 17.viii.2005.

Additional material examined. AUSTRIA: 1♂, Kärnthen, Wippach, 1.vi.1909, Polica (Vienna); 1♂, Niederösterreich, Buchberg, Spitz a. D., 8.v.1902, Preissecker (Vienna); 2♂, 1♀, Niederösterreich, Gramatneusiedl: Fischawiesen (Fürbachwiesen), larvae 2.x.1983 on *Prunella grandiflora*, emerged 25.iv–10.v.1984, E.J. van Nieukerken & J. Boomsma (Leiden); Oberösterreich: 1♂, Berg, 17.vii.1912, Wolfschläger (Linz); Hinterstoder, 10.v.1936, J. Klimesch (Munich); 2♂, Kirchdorf, 13.v.1893, 21.v.1900, Hauder (2 PLT *dubiella*, Linz); 1♂, Linz a. D., Prebenau, 17.viii.1910 (Linz); 1♂, Linz, Plesching, 25.v.1967, J. Klimesch (Munich); 1♂, Pregarten (Prägarten), 31.v.1909, Knitschke (PLT *dubiella*, Linz); 2♂, Prägarten, 26.v.1910, Hauder & [Knitschke] (2 PLT *dubiella*, Linz); 2♂, Puchenau [Puchenauer Graben], 17.viii.1910, [Frau Frazeni] (2 PLT *dubiella*, Linz); 2♂, Puchenau [Puchenauer

Graben], 31.v.1912, Hauder (labelled as type *dubiella*, but not cited in description, Linz, Vienna); 1♂, Niederösterreich, Buchberg, Spitz a. D., 8.v.1902, Preissecker (Vienna); 1♂, Ruefling Linz, v.1912 (Ende Mai 1912), Knitschke (PLT *dubiella*, Linz); 1♂, Steiermark, Altaussee, Seewiese, 1.vi.1969, J. Klimesch (Munich); 1♂, Wien, Haschberg, 21.v.1916 (Vienna). CROATIA: 2♂, 1♀, Slavonija, Banicevac, 7 km NE Cernik, larvae 17.x.1983 on *Prunella laciniata*, emerged 17.iv–8.v.1984, E.J. van Nieukerken & J. Boomsma (Leiden); 3♂, Dalmatia, Baska, Voda (Makarska), 18.v.1979, J. Klimesch (Munich). GREECE: 1♂, Ipiros, Peristrei Mts. S. Metsovo, 12–1900 m, 27–28.v.1994, O. Karsholt (Copenhagen); 1♂, Trikala, Oxinia, 8.vi.1999, A. & Z. Lastuvka (coll. A. Laštůvka). ITALY: 1♂, Cuneo, Pamparato, St. Gree, 25.vi.1987, G. Bassi (coll. Bassi); 1♂, Torino, Almese, Falde del Mt. Musiné, 450 m., 26.v.1983, U. Parenti (Leiden); 8♂, Verona, Monte Baldo, Rifuggio Novezzina, 1250–1350 m, 14.vii.1979, 24.vii.1984, 16.vii.1985, 16.vii.1987, U. Parenti (Leiden, Torino); Roma: Monti d. Tolfa, dint. Manziana, 300 m, 23–28.vi.1989, G. Baldizzone (coll. Baldizzone); 5♀, Trento: Mte Maranza, NW slopes, 4 km E Trento, 900 m, larvae 10.x.1983 on *Prunella grandiflora*, emerged 8–16.v.1984, E.J. van Nieukerken (Leiden); 1♂, 1♀, Veneto, Sappada, Passo Siera, 1600 m, 6.vii.1991, B.Å. Bengtsson (coll. Bengtsson). ROMANIA: larvae, leafmines, Covazna: Ozunca-Bai S, 770 m, *Prunella vulgaris*, E.J. van Nieukerken (Leiden). UKRAINE: 1♂, Karpaty, Iv.-Frankovskaya obl. s. Yaremche, 600 m, 28.vi.2003, A. Bidzilya (Kiev).

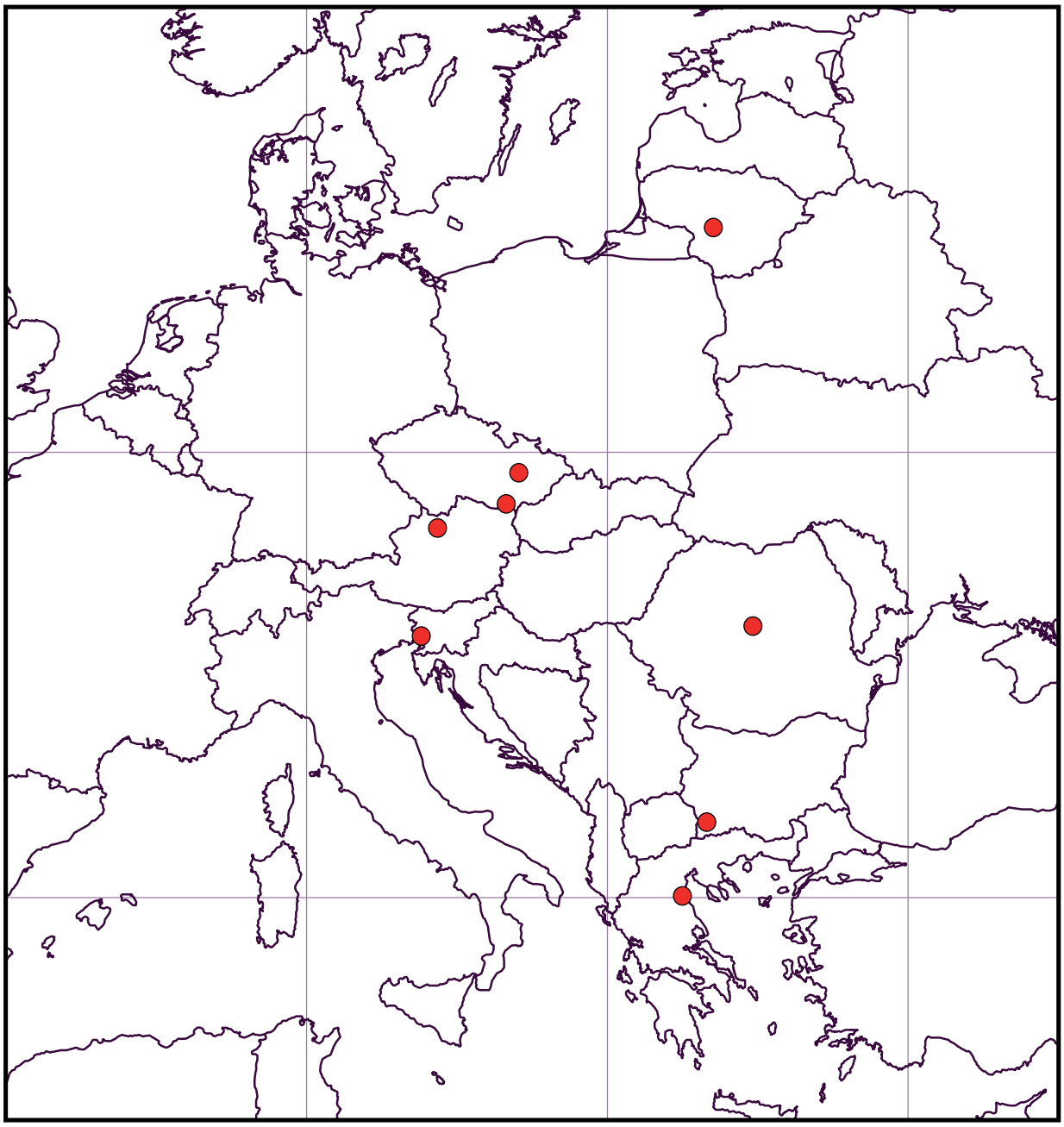


FIGURE 32. *Trifurcula (Glaucolepis) lituanica*, distribution.

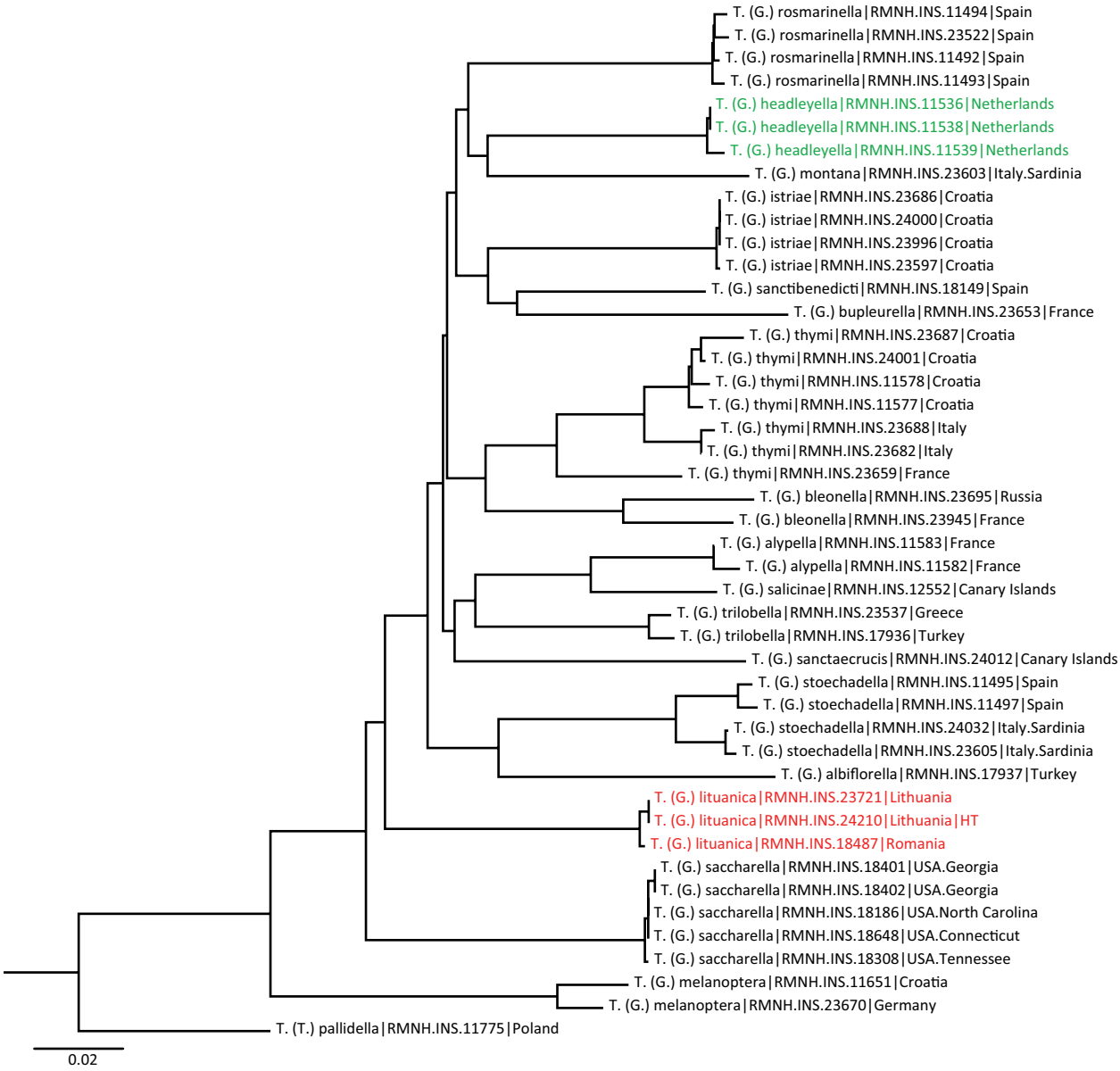


FIGURE 33. Neighbor Joining tree of DNA barcodes of *Trifurcula (Glaucolepis)* species, showing specimen registry numbers and country of origin. Outgroup is *Trifurcula (T.) pallidella* (Duponchel, 1843). *Trifurcula lituanica* sequences are indicated in red, those of *T. headleyella* in green. HT= holotype, short sequence.

Discussion

The DNA barcode of *Trifurcula lituanica* differs markedly from all other species of the subgenus *Glaucolepis* (Fig. 33), and bears no close resemblance to that of *T. headleyella*. The aberrant position of *T. lituanica* given by the COI gene is not repeated in other studied genes (unpublished information EJvN), so is probably not a good indication of true phylogenetic relationships. The species clearly shows all the characters of the subgenus *Glaucolepis*, but has two unique characters: the extra cornuti close to the phallotrema, not to be confused with the phallotrema spines, are not known from any other species; further it is the only *Glaucolepis* species that pupates inside the mine, and so far the first nepticulid that prepares its cocoon within a stem-mine. Until now only a few Nepticulidae are known that prepare their cocoon inside leafmines.

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APPENDIX.

Partial COI sequence (DNA barcode) of *Trifurcula lituanica*, based on Genbank sequences JX261901, JX261899 and JX261900. For the only one variable nucleotide the alternative base is shown in brackets.

TACATTATATTTTATTTTTGGAATTTGATCTGGAATAGTAGGTACATCTTTAAGCTTATTAATTCGAGCAG
AATTAGGAAATCCAGGATCTTTAATTGGAGATGATCAAATTTATAATAGTATTGTTACAGCTCATGCATT
TATTATAATTTTTTTTTATAGTTATGCCAATTATAATTGGAGGATTTGGAAATTGACTAGTCCCTCTAATACT
AGGAGCTCCTGATATAGCATTTCCTCGTCTTAATAATATAAGATTTTGGCTACTACCTCCTTCCTTATTAC
TTTTAATTTCAAGAAGAATTGTTGAAAATGGAGTTGGGACTGGATGAACAGTTTATCCTCCACTTTCA
GCCAATATTGCACACAGAGGAAGATCAGTAGATATAGCAATTTTTTCACTTCATTTAGCAGGAATTTCT
TCCATTTTAGGAGCAATTAATTTTATTACAACAGTAATTAATATACGAACTAATGGAATATCTTTTGATCA
AATACCTTTATTTGTATGAGCAGTAGCTATTACTGCTTTATTACTTCTTTTATCCCTTCCTGTATTAGCAG
GAGCTATTACAATATTATTAACTGACCGAAATTTAAATACTTCATTTTTTGATCCTG(A)CTGGAGGAGGA
GATCCTATTCTCTACCAACATTTATTC