A new species of Typhonia from the Ligurian Alps (Lepidoptera: Psychidae)

Edgardo BERTACCINI¹,*, Alberto ZILLI²

¹ Via del Canale 24, I-47122 Roncadello di Forlì (FC), Italy - edgardobertaccini@gmail.com
² Natural History Museum, Life Sciences DC2-2N - Cromwell Road, SW7 5BD London, UK - a.zilli@nhm.ac.uk

* Corresponding author

Abstract
Typhonia laitae sp. n. is described on basis of samples from the Ligurian Alps (NW Italy). The new species is a member of the Typhonia ciliaris-melana complex and though it seems closest to T. melana (= beatricis) by the thick antennae of the female, DNA-barcode analysis places it nearest to T. ciliaris.

Key words: taxonomy, distribution, DNA-barcoding, speciation.

Introduction
Research carried out over the last two decades in Western Liguria (NW Italy) enabled to assess presence of numerous species of Psychidae, some of which remarkable for their particularly specialised ecology or restricted distribution, a number of them turning also out as firstly recorded from Italy (Bertaccini 2009, 2011). Concerning the genus Typhonia Boisduval, 1834, two easily distinguishable species could be found. This genus encompasses about 150 species, mainly from the Afrotropical and Oriental regions, in the latter essentially in the Indian subregion (Sobczyk 2011).

Only three out of the ten species known from the Palaeartic occur in Europe, namely T. ciliaris (Ochsenheimer, 1810), T. melana (Frivaldszky, 1837) (= beatricis Hättenschwiler, 2000, cf. Arnscheid & Weidlich 2017) and T. christenseni (Hättenschwiler, 1990). The first two, the only occurring in Western Europe, show several diagnostic characters and also substantially differ in COI barcode (see below). Main differences are found in the male and female antennae, thin in T. ciliaris and thick in T. melana, morphology of wing scales (both on disc and fringe), and configuration of the genitalia. Our Ligurian samples could easily be sorted between T. ciliaris, already known from Western Liguria (Arnscheid 2000), and a second species. This, on account of habitus of the adults and its preference for lowland xerothermic biotopes, was initially identified as T. melana, present with certainty at least in the nearby Susa Valley (Western Piedmont) (Hellmann & Bertaccini 2004). Nevertheless, subsequent insights into the morphology of the few specimens initially available revealed different features with respect to T. melana, which thus prompted us to search for additional material. New visits to the area enabled then us to obtain a number of larvae and adults of this species, so that following more detailed morphological and genetical comparisons we are now able to conclude that it represents a new species, described herein.

Material and methods
Dried leg samples of three specimens of the taxon under investigation were submitted via Zoologische Staatssammlung München to the Canadian Centre for DNA Barcoding (CCDB, Biodiversity Institute of Ontario, University of Guelph) for DNA extraction, amplification and sequencing of the mitochondrial 5’ cytochrome oxidase gene, subunit 1 (COI) (Ivanova et al. 2006; deWaard et al. 2008). Sequences were uploaded in the Barcode of life Data Systems (BOLD) (Ratnasingham & Hebert 2007, 2013) and compared with other Typhonia samples from Europe. Neighbour-joining tree of DNA barcodes under the Kimura 2-Parameter distance model for nucleotide substitutions recovered both after BOLD functions and PhyML 3.0 (cf. http://www.atgc-montpellier.fr/phyml/) (Guindon et al. 2010) helped to figure out relationships between various Typhonia lineages, though despite the data they are based on are in the public domain, the trees
are omitted here in order not to interfere with any possible projects by other researchers. Features of covering scales refer to classes as coded by Hättenschwiler (1997), whereas nomenclature of wing veins follows Davis & Robinson (1999). Dissections were performed according to standard procedures for genitalia preparations (e.g. Robinson 1976). Breedings took place in cages placed outdoor (at Roncadello di Forlì, Italy) in order to reduce interference with normal life cycle by the moths.

**Taxonomic part**

*Typhonia laiaea* sp. n.

(Figs 1-3, 6, 10a, 11, 12, 15a, 17, 20, 23)

**Description**

**Male**

*Wingspan:* 23.5 mm.

*Head:* frons bulged; eye slightly convergent inferiorly with opposite; ocellus absent; antenna bipectinate, black with scattered white scales ventrally, reaching approximately half of forewing costa, flagellum with 37 antennomeres, rami cylindrical and finely ciliate; labial palpus greatly reduced, with barely discernible joints even under strong magnification.

*Thorax:* black; forewing dark blackish brown, with ten veins arising from discal cell, of which R3 and R4 with joint origin, fringe concolorous except in apical area, whitish; hindwing concolorous with forewing albeit darker at tornus and with a pale grey subcostal stripe, six veins from discal cell, fringe as in forewing; clothing of class 5-6 scales, these fairly elongated and distinctly toothed apically; legs black, distally white-tipped, protibia with long epiphysis, meso- and metatibiae with one and two pairs of spurs, respectively.

*Abdomen:* black, distally with slightly paler covering hair-like scales.

*Male genitalia:* tegumen bilobed, deeply notched distally, saccus markedly elongated, thin and sinuous; aedeagus tubular, slightly curved (Fig. 17).

**Female**

*Wingspan:* 24-26 mm.

*Head:* as in male except for smaller oval eye and filiform antenna, this thick, with 34-36 flagellomeres.

*Thorax:* as in male, with both wings deeper black colored, fringes too, and slightly more elongated covering scales; forewing veins R3 and R4 stalked (Fig. 3); legs entirely black. Abdomen: elongated, black, with off-white anal tuft.

*Female genitalia:* apparatus similar to that of congeners *ciliaris* and *melana*, spines of genital plate too, ovipositor long, partially protruding beyond anal tuft (Fig. 20).

**Preimaginal instars**

*Mature larva:* head shiny black; mouthparts well sclerotised, pale brown; ground colour of T1-T3 black as in *T. ciliaris* albeit more extended, with beige speckles (Fig. 2); legs black on outer side, paler on insides.

*Pupa:* length 12-15 mm; pale brown-colored; head capsule pale brown, similar to albeit less prominent than in *T. melana*, antennal sheat of female as in *T. melana* albeit shorter; abdomen with bundles of robust spines on A4-A8.

*Larval case:* length 26-29 mm, width 4.5-5.0 mm (n = 1♂, 7♀♀); configuration as in congeners, tubular, wholly covered with tiny mineral debris (sand grains and minute stones), whose colour depends on lithology of the collecting site (variation from grey to straw colored or brown observed).

**Material examined.** Syntypes: ♂, Italy: Liguria, Andagna (IM), 800 m, 22 Jul 2009, leg. E. Bertaccini, gen. praep. EB 890, in coll. E. Bertaccini (ZSM Lep 81560); ♀, Liguria, Pigna (IM), 350 m, 26 Jul 2011, leg. L. Fanti, in coll. E. Bertaccini (ZSM Lep 73495 & 73505); ♀, Liguria, Colle Drego (IM), 1100 m, 14 Jul 2006, leg. et coll. E. Bertaccini; ♀, *ibidem*, 25 May 2014, ex larva (emerged 16 Jul 2014), gen. praep. EB 907 (ZSM Lep 84809), leg. et coll. E. Bertaccini; 2 ♀♀, *ibidem*, ex larvis (emerged 03 Aug 2014), leg. E. Bertaccini, in coll. E. Bertaccini & Natural History Museum, London.

**Figs 1a-b** – *Typhonia laiaea* sp. n. from Ligurian Alps (syntypes): a, ♂, Liguria, Pigna; b, ♀, idem, Andagna.
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Distribution. At present the distribution of T. lailae appears to be restricted to the south-western section of the Ligurian Alps (Fig. 4), though its occurrence in nearby areas such as in the Maritime Alps cannot be excluded, as a number of accounts on the local fauna may suggest (e.g. Millière 1876; Turati & Verity 1912). As a matter of fact, a female specimen from Alpes Maritimes fairly close to the geographic delimitation between the two alpine districts, i.e. Vallée de Roubion (12-13 Juillet 1906, R. Powell [leg.], ex Oberthür Coll., in Natural History Museum, London) shows neatly thick antennae, and both covering and fringe scales fully corresponding to T. lailae, though we prefer examining also members of the male sex from this population before confirming attribution.
Bionomics. Xerothermophilous species of the sublittoral hilly-montane so far observed in Western Liguria at altitudes comprised between 350-1,100 m a.s.l. in garrigues, along rocky paths and edges of olive groves where several Mediterranean elements such as Gonepteryx cleopatra (Linnaeus, 1767), Satyrium esculi (Hübner, [1804]), Zygaena occitanica (de Villers, 1789) and Z. lavandulae (Esper, [1783]) do usually occur (Fig. 5). Larvae in captivity, despite being offered with a whole range of plants occurring in the collecting site of Drego, showed marked preference for Sedum album L. On 10 June 2014 a first larva fixed its case among stones in a breeding cage, and the resulting imago (female) emerged after 36 days; a further two larvae did on 26th and 28th June, and adults (females) emerged after 36-38 days. Emergences occurred around 18:00-19:00 GMT. Considering both specimens collected in the wild and bred ones, adults were thus on the wing at least between 14.VII-3.VIII. Univoltine, likewise congeners.

Molecular data. The ingroup average distance of T. lailae was 0.51% (n = 3) while the average distance with nearest neighbour turned out to be with a set of n = 12 specimens corresponding to T. ciliaris and equating to 2.59%, of same magnitude then to the distance between the latter and a set of n = 23 specimens corresponding to T. melana (= beatricis) Hättenschwiler (2000), i.e. 2.27%. Interestingly though, a specimen from Slovenia in BOLD (TIPSY287-12) preliminarily identified as T. ciliaris happens to cluster with T. lailae. Features of this specimen, a female, clearly exclude any possibility of conspecificity with the Ligurian entity, e.g. by the thin, comparatively short antennae and broad wings, but its relationships with T. ciliaris proper should be reassessed. In terms of percentage differences this specimen appears to be equally distant from T. ciliaris (distance 3.41%) and T. lailae (3.5%). To note that T. beatricis, described by Hättenschwiler (2000) from the German/Swiss border, was shown to fully embed in T. melana, which thus prompted its synonymisation with the latter by Arnscheid & Weidlich (2017).

Etymology. The new species is named after Laila Fanti, wife of the first author, who actively participated to fieldwork and found one of the female syntypes.

Diagnostic remarks. The new species is part of the same complex comprising Typhonia ciliaris and T. melana. Main diagnostic characters enabling separation of T. lailae sp. n. from the two allies are tabulated herein.

From Typhonia melana
Males
- antenna long and slender, that of melana shorter albeit with longer rami (Figs 10a-10b);
- genitalia with long, slender and sinuous saccus, that of melana shorter and broader-based (Figs 17-18);
- aedeagus tubular and slightly curved; slightly conical with subbasal constriction in melana.

Females
- antenna usually stouter (Figs 6, 8-9), although variation was observed in melana and antennae may occasionally approach in thickness those of lailae;
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- covering scales ending with sharper teeth;
- scales of fringe with 5-6 sharp teeth; more rounded with more smoothly rounded teeth in melana (Figs 11-14);
- sternum A1+2 flimsy and sinuous; stronger sclerotised and better defined in melana;
- pupa with neatly shorter antennal sheats (Figs 15a-15b);
- pupa with blunt, less prominent head capsule than in melana (Figs 16a-16b).

From Typhonia ciliaris

Males
- antenna slightly thinner;
- frons more bulged, that of ciliaris barely protruding and with paired incisions at sides;
- forewing veins R3-R4 with common origin on discal cell, contrary to split ones of ciliaris;
- genitalia with long, slender and sinuous saccus, that of ciliaris shorter and apically dilated;
- aedeagus tubular and slightly curved; stouter with sub-basal constriction in *ciliaris*.

Females
- wingspan little variable (24-26 mm), and at the lower end of the range of *ciliaris* (25-33 mm);
- antenna markedly stouter;
- fringe concolorous with wing; paler or even white in *ciliaris*;
- forewing veins R3-R4 stalked, contrary to free ones of *ciliaris*;
- covering scales more slender;
- sternum A1+2 flimsy with well separate anterior apodemes; stronger sclerotised with closer apodemes in *ciliaris*;
- larval cases shorter on average (26-29 vs 29-40 mm) and thinner (4.5-5.0 vs 4.5-7.5 mm);
- pupa with shorter antennal sheaths.

**Taxonomic remarks.** Bruand (1853, in 1850-1853) described *Typhonia phryganilugubrella* on the basis of a single female from Montpellier communicated by Pierre Mil-
lière and two larval cases with no real provenance, albeit likely from the surroundings of Fonscolombe, the living place of their collector (Mr Boyer). Despite the fact this name is currently sunk with *T. ciliaris* (cf. Sobiczyk 2011; Arnscheid & Weidlich 2017), it may either relate to *T. laiiae* sp. n. or *T. melana*, as the most important diagnostic feature is the thicker antenna with respect to *T. ciliaris*.

Unfortunately, the holotype of *T. phryganilugubrella* has to be considered as lost. In fact, research of this specimen in Muséum de la Citadelle (Besançon), where the Bruand collection is preserved, gave no results. Even in the case Bruand returned the specimen to Millière, most Psychidae of the latter were bought by F.J.M Heylaerts and eventually ended up in RMNH Leiden, where however there is no trace of that specimen. The circumstances that *T. melana* ranges from the Iberian Peninsula across Southern Europe eastwards to the Balkan Peninsula and we could locate three specimens (♀♀) referable to this species from Southern France (Cannes, Millière [leg.], in Natural History Museum, London) do not allow to clear up the original identity of *T. phryganilugubrella*, which must therefore be regarded as a *nomen dubium*.

**Discussion**

Largely unexpected, the presence of an additional species of the *Typhonia ciliaris-melana* complex in the SW Alps addresses to the remarkable orographic and ecological complexity of the area. On the one hand, this is a well-known suture zone between the atlanto- and adriato-mediterranean regions (sensu De Lattin, 1967), where the contact between western and central mediterranean biota led to countless intergradation phenomena; on the other hand, the orientation of the alpine axis, which in its southern portion is adjacent and sub-parallel to the coastline, originates strong ecological gradients developing also in a few hundred meters (cf. Zilli & Racheli 1992; Zilli 2002; Flanagan et al. 1999). Pleistocene climatic events further intervened on this scenario already promoting biotic diversity, so that divergence of populations was often enhanced after isolation in defined mountain groups and coastal micro-refugia. In such cases, the local distribution of various entities often retains a strong signal of the historical and ecological factors that affected them (Zilli 2002). Regarding the three species of *Typhonia* from the area, *T. laiiae*, linked to xerothermic biotopes of the sublittoral hilly-submontane belt of a restricted area in the Ligurian Alps, shows strong mediterranean preferences, and is therefore likely to have diversified during temporary isolation along coastal areas of this district. *Typhonia ciliaris*, with wider central-southern european distribution, is more orophilous, as it usually occurs in the Alps and central-northern Apennines between 1,700 and 2,200 m, rarely descending to lower altitudes. Finally, *T. melana* shows the widest geographic and ecological distribution out of the three, being a species ranging from the sea level in conditions not dissimilar from those of *T. laiiae* up to high altitude areas (e.g. Calabria, Aspromonte, 1,800 m; Sicily, Etna, 1,600–2,200 m) (Arnscheid & Weidlich 2017).

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**References**

Arnscheid W. 2000. Die Macrolepidopteren-Fauna Westliguriens (Riviera dei Fiori und Ligurische Alpen in Oberitalien). Neue entomologische Nachrichten, 47: 3–310.

Arnscheid W.R., Weidlich M. 2017. Psychidae. Microlepidoptera of Europe 8. Brill, Leiden & Boston, xiii + 423 pp.

Bertaccini E. 2009. *Ptilocephala silphella* (Millière, 1871) e *Pti-
locephala vesubiella (Millière, 1872) importanti conferme per la lepidottero fauna italiana (Insecta Lepidoptera Psychidae). Quaderno di Studi e Notizie di Storia naturale della Romagna, 28: 149–166.

Bertaccini E. 2011. Dalla Liguria occidentale una nuova sotto-specie di Ptilocephala muscella ([Denis & Schiffermüller], 1775): Ptilocephala muscella liguriensis n. sp. (Insecta Lepidoptera Psychidae). Quaderno di Studi e Notizie di Storia naturale della Romagna, 32: 165–186.

Bruand t., d’Uzelle 1850-1853. Essai monographique sur la tribu des Psychides. Comptes rendus de la Société libre d’Émulation du Doubs, 2/3 (1849): 23–68, (1853): 17–120.

Curò A. 1889. Aggiunte alla prima parte del Saggio di un catalogo dei Lepidotteri d’Italia. Bulletino della Società entomologica italiana, 21: 77–85.

Davis D.R., Robinson G.S. 1999. The Tineoidea and Gracillarioidea, pp. 91–117. In: Kristensen N.P. (ed.), Handbuch der Zoologie IV. Arthropoda: Insecta 35. Lepidoptera, Moths and Butterflies. Walter de Gruyter, Berlin 7 New York, x + 487 pp.

De Lattin G. 1967. Grundriss der Zoogeographie. VEB Gustav Fischer, Jena, 602 pp.

deWaard J.R., Ivanova N.V., Hajibabaei M., Hebert P.D.N. 2008. Assembling DNA barcodes: analytical protocols, pp. 275–293. In: Martin C.M. (ed.), Environmental genomics. Humana Press, Totowa, xii + 364 pp.

Flanagan N.S., Mason P.L., Gosálvez J., Hewitt G.M. 1999. Chromosomal differentiation through an Alpine hybrid zone in the grasshopper Chorthippus parallelus. Evolutionary Biology, 12: 577–585.

Guindon S., Dufayard J.F., Lefort V., Anisimova M., Hordijk W., Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic Biology, 59(3): 307–321.

Hättenschwiler P. 1997. Psychidae-Sackträger, pp. 165-308. In: Lepidopterologen-Arbeitsgruppe, Schmetterlinge und ihre Lebensräume 2: Arten-Gefährdung-Schutz. Pro Natura - Schweizerischer Bund für Naturschutz c/o Fotorotar AG, Egg.

Hättenschwiler P. 2000. Typhonia beatricis sp. n., eine möglicherweise aus dem östlichen Mittelmeerraum eingeschleppte Psychide (Lepidoptera, Psychidae). Mitteilungen der Entomologischen Gesellschaft Basel, 50: 2–17.

Hellmann F., Bertaccini E. 2004. I Macrolepidotteri della Valle di Susa. Italia Nord-occidentale (Alpi Cozie - Graie). Monografie del Museo Regionale di Scienze Naturali di Torino 40. Regione Piemonte, Torino, 389 pp., 16 pls.

Ivanova N.V., deWaard J., Hebert P.D.N. 2006. An inexpensive, automation-friendly protocol for recovering high-quality DNA. Molecular Ecology Notes, 6: 998–1002.

Millière P. (1876) Catalogue raisonné des Lépidoptères des Alpes-Maritimes. Troisième et dernière partie. H. Vidal, Cannes. 250–455 pp., 2 pls.

Ratnasingham S., Hebert P.D.N. 2007. BOLD: The Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology Notes, 7: 355–364.

Ratnasingham S., Hebert P.D.N. 2013. A DNA-based registry for all animal species: the Barcode Index Number (BIN) system. PLoS ONE 8: e66213.

Robinson G.S. 1976. The preparation of slides of lepidoptera genitalia with special reference to the microlepidoptera. The Entomologist’s Gazette, 27: 127–132.

Sobczyk T. 2011. Psychidae (Lepidoptera). World Catalogue of Insects 10. Apollo Books, Stenstrup, 467 pp.

Turati E., Verity R. 1912. Faunula Valderiensis nell’alta Valle del gesso (Alpi Maritiline). Heterocera. Bullettino della Società entomologica italiana, 43 (1911): 168–233

Zilli A. 2002. Clinal variation of a reproductive isolating mechanism in a sedentary moth from South-Western Alps (Lepidoptera: Heterogynidae). XIII European Congress of Lepidopterology, Korsor (Denmark) (Abstract): 64.

Zilli A., Racheli T. 1992. Gregariousness, apterism, matrivory, and the natural history of a moth (Lepidoptera, Heterogynidae). Animal & Human Biology, 2 (1989-1990): 7–40.