DNA barcoding of Tineidae from South Italian forests reveals faunistic novelties and potentially undescribed species (Lepidoptera, Tineoidea)

Sara La Cava[1,2], Giada Zucco[1,3], Teresa Bonacci[2], Stefano Scalercio[1,4]

1 *Council for Agricultural Research and Economics (CREA), Research Centre for Forestry and Wood, Via Settimio Severo, 83, 87036 Rende, Italy; sara.lacava@crea.gov.it*

2 *Department of Biology, Ecology and Earth Sciences, University of Calabria, Ponte Pietro Bucci, 87036 Rende, Italy; t.bonacci@unical.it*

3 *Department of AGRARIA, University "Mediterranea" of Reggio Calabria, Feo di Vito, Reggio Calabria, 89122, Italy; giada.zucco@crea.gov.it*

4 *NBFC, National Biodiversity Future Center, Piazza Marina, 61, Palermo 90133, Italy; stefano.scalercio@crea.gov.it*

https://zoobank.org/DDFEEDC7-49A5-4D0F-8A7D-D6F5889DEC0C

Received 13 February 2025; accepted 5 June 2025; published: 26 June 2025
Subject Editor: Théo Léger.

Abstract. Tineidae Latreille, 1810 includes about 119 species in Italy. Despite their economic and ecological importance, this family remains poorly studied in peninsular Italy, primarily due to taxonomic impediment. The aims of this paper were to improve molecular reference libraries to overcome the taxonomic impediment and to increase the knowledge of Tineidae in South Italian forests. The examined material was collected from the southernmost tip of peninsular Italy. A total of 85 specimens were DNA barcoded, recovering 74 sequences and 27 Barcode Index Numbers of which three are new to the Barcode of Life Data system. 66 specimens were assigned to 24 species. 67% of these identified species represent new records for parts of Italy or for the entire country. Notably, *Pelecystola fraudulentella* (Zeller, 1852) is new for Italy, while *Haplotinea insectella* (Fabricius, 1794), *Reisserita relicinella* (Herrich-Schäffer, 1853), *Stenoptinea cyaneimarmorella* (Millière, 1854), *Monopis neglecta* Šumpich & Liška, 2011, and *Triaxomera fulvimitrella* (Sodoffsky, 1830) are new for South Italy. Additionally, 10 species are new for the Calabria region. The most interesting cases concern eight specimens belonging to the genera *Nemapogon* Schrank, 1802 and *Neurothaumasia* Le Marchand, 1934. These specimens exhibited genetic distance ranging of 3.74% and 6.73% respectively from their nearest neighbors and morphology of genitalia cannot help to assign them to a known species. Further taxonomic studies are needed to ascertain their taxonomy.

Introduction

The family Tineidae Latreille, 1810 belongs to the superfamily Tineoidea Latreille, 1810 which includes a variable number of families depending on different systematic assessments (Mutanen et al. 2010; Sohn et al. 2013; Regier et al. 2015). European Tineidae includes approximately 285 species (Gaedike 2015, 2019), a number that continues to grow due to the ongoing description of new species, primarily in Mediterranean countries (Huemer et al. 2023). In Italy the first national checklist (Gaedike et al. 1995) listed 110 species, whereas the most recent European checklist (Gaedike 2015, 2019) includes 119 species, reflecting an increase of 9 species over a span of 25 years.

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In recent decades, the study of Tineidae in Italy has been limited, with most records included in comprehensive faunistic studies on Lepidoptera (Baldizzone 2004; Pinzari et al. 2010, 2013; Baldizzone et al. 2013; Huemer and Wieser 2023). The peninsular territory has been poorly investigated, with only 49 species recorded by Gaedike et al. (1995), and knowledge is even more limited for the southernmost regions of Italy. Although individual records of tineids are commonly found in applied research due to the economic importance of this family as pests of food storage, cultural heritage, and clothing (Plarre and Krüger-Carstensen 2011; Trematerra 2012; Trematerra and Pinniger 2018), taxonomic or faunistic studies specifically focused on Tineidae are rare. For instance, *Opogona sacchari* (Bojer, 1856), a crop pest not previously listed for Italy in the aforementioned checklists, was discovered in a greenhouse near Naples during preliminary observations on its sexual pheromone (Rotundo and Tremblay 1982). On the other hand, although few faunistic or taxonomic studies have included Tineidae records (Gaedike and Mally 2011; Bassi and Nel 2015; Bonelli et al. 2021), a one-week survey of microlepidoptera in the Aspromonte National Park and its surroundings was sufficient to identify nine species, some of which represented faunistic novelties at both regional and national levels (Baldizzone and Scalercio 2018).

The importance of Tineidae, beyond their well-known economic significance, also lies in their high potential as bioindicators of forested habitats (Jonsell and Nordlander 2002). Most of their larvae are trophically associated with fungi, lichens, bird nests, deadwood, and decaying organic matter in general (Gaedike 2015, 2019; Jaworski 2018), displaying strong functional relationships with microhabitats recognized as indicators of forest management (Asbeck et al. 2021). However, taxonomic impediments have significantly limited their use as indicators due to their small size and the similar habitus of several species.

The aims of this paper were: (1) to improve the basic knowledge of Tineidae in South Italian forests; (2) to highlight cases requiring further taxonomic studies; and (3) to improve molecular reference libraries to overcome the taxonomic impediment associated to Tineidae as bioindicators.

Materials and methods

The examined materials were primarily collected in the Calabria region, the southernmost tip of Peninsular Italy, during surveys conducted mainly in well-preserved mountainous forest habitats and highly fragmented plain forests. Specifically, the surveys that included the collection of Tineidae specimens targeted the following habitats (see references for further details): retrodunal areas (Bonelli et al. 2021), olive groves with remnants of Mediterranean shrubs and riparian forests (Zucco et al. 2024), urban and suburban areas (Zucco and Scalercio 2023), fragmented thermophilous oak woodlands (Scalercio 2022), chestnut woodlots (Greco et al. 2018), maple forests (Greco et al. 2019), mixed deciduous-conifers forests (Infusino et al. 2017a), pine forests (Scalercio and Greco 2018), mixed beech-pine forests (Scalercio 2020), and beech forests (Infusino and Scalercio 2018; Scalercio et al. 2022).

Standardised samplings were conducted using UV LED light traps (Infusino et al. 2017b), primarily targeting macromoths. However, voucher specimens of microlepidoptera morphospecies were also preserved for future studies. Additionally, some specimens were opportunistically collected from other light sources or during daytime observations. The collected Tineidae specimens were subsequently subjected to barcode analysis using SMRT Sequencing on PacBio Sequel platform and SPRI DNA Extraction at the Centre for Biodiversity Genomics, Canada. A total of 85 specimens were analyzed and stored in the Lepidoptera collection of the Research Centre for Forestry and Wood, Rende, Italy.

The resulting sequences were compared with those available in the Barcode of Life Data Systems (BOLD) database, utilising the identification engine and selecting the option "All barcode records on BOLD". As a result, an identification was provided along with a percentage of similarity to the deposited sequences. The specimens were named after dissection of genitalia and following a careful review of the tree-based identification (BOLD TaxonID tree), which was conducted using trees built with the Kimura-2 parameter as distance model and considering the hundred most similar sequences. Recovered sequences were automatically assigned to a Barcode Index Number (BIN), an online framework that clusters barcode sequences algorithmically, typically corresponding to a species (Hausmann et al. 2013). Additionally, a Nearest Neighbour search was conducted to identify the most similar taxonomic unit associated with a BIN, further enhancing the taxonomic assessment of our specimens.

Finally, a cross-check of wing patterns with available iconography (Gaedike 2015, 2019) was performed, and a list of taxa derived from molecular analysis and morphological validations was compiled, adhering to the nomenclature proposed by Gaedike (2015, 2019).

Results

Taxonomy

74 specimens were successfully DNA barcoded, yielding sequences with a length ranging from 648–672 base pairs. These sequences, along with images and collection data, are available in the public dataset DS-TINEITS on BOLD. (https://v4.boldsystems.org/index.php/MAS_Management_DataConsole?codes=DS-TINEITS). Specimens clustered into 27 BINs, corresponding to 24 identified and two unidentified species (Table 1, Fig. 1). Among named species, seven showed a high intraspecific DNA barcode divergence with two BINs each (Table 1) attributed to the same species by dissection of genitalia (Figs 2–10). The DNA barcoding library was improved by adding three BINs new to BOLD belonging to *Haplotinea insectella*, *Monopis obviella*, and *Reisserita relicinella* (Table 1).

Morphology of genitalia confirmed molecular identifications but were unable to identify two taxa belonging to the genera *Nemapogon* and *Neurothaumasia*. Their nearest neighbors showed distances of respectively 3.74 and 6.73% indicating the need of further taxonomic studies, potentially representing undescribed taxa.

Faunistics

All identified species are illustrated in Figs 11–34, 67% of which represent faunistic novelties for parts of Italy or for the entire country (Table 1). Specifically, *Pelecystola fraudulentella* (Zeller, 1852) is new for Italy, *Haplotinea insectella* (Fabricius, 1794), *Reisserita relicinella* (Herrich-Schäffer, 1853), *Stenoptinea cyaneimarmorella* (Millière, 1854), *Monopis neglecta* Šumpich & Liška, 2011, and *Triaxomera fulvimitrella* (Sodoffsky, 1830) are new for South Italy, and 10 additional species are newly recorded for the Calabria region.

Discussion

This paper represents the largest study ever carried out on South Italian Tineidae, integrating molecular and morphological techniques for their identification. We found 24 species, some of which of high faunistic interest, improving the DNA barcoding library of this family with molecular data gathered from an under-investigated European region.

Table 1. Summarised results of DNA barcoding of the analysed sample. BIN: Barcode Index Number; n: number of barcoded specimens in this study; N_{BIN}: number of records available in BOLD for a BIN (27th of December 2024); NN_{dist}: distance from the Nearest Neighbour; New for Italy: NI; New for South Italy: NSI; New for Calabria: NC; NA: not applicable.

BIN	n	N_{BIN}	Taxon	NN_{dist} (%)	Nearest Neighbour	Faunistic novelty
Unidentified taxa						
BOLD:ADQ6761	3	7	*Nemapogon* sp.	3.74	*Nemapogon hungaricus*	NA
BOLD:AAV6344	5	1	*Neurothaumasia* sp.	6.73	*Neurothaumasia cretica*	NA
Identified taxa						
BOLD:ACS5343	3	4	*Morophaga morellus*	6.05	*Morophaga choragella*	none
BOLD:AAF5407	1	25	*Morophaga choragella*	6.05	*Morophaga morellus*	none
BOLD:AAE8582	1	18	*Triaxomera fulvimitrella*	10.9	*Triaxomera puncticulata*	NSI
BOLD:AAD9379	1	20	*Triaxomera parasitella*	3.30	*Triaxomera parasitella*	NC
BOLD:AAF4848	1	7	*Triaxomera parasitella*	3.30	*Triaxomera parasitella*	NC
BOLD:AAX6122	4	2	*Nemapogon inconditella*	4.01	*Nemapogon reisseri*	NC
BOLD:ADR8751	3	17	*Nemapogon signatellus*	3.53	*Nemapogon reisseri*	NC
BOLD:AAI3059	7	69	*Nemapogon variatella*	5.57	*Nemapogon granella*	none
BOLD:AAU3216	2	8	*Infurcitinea finalis*	3.53	*Infurcitinea finalis*	none
BOLD:AAF3207	5	21	*Stenoptinea cyaneimarmorella*	4.36	*Stenoptinea cyaneimarmorella*	NSI
BOLD:AFT3285	1	0	*Haplotinea insectella*	3.21	*Haplotinea insectella*	NSI
BOLD:AFB7048	3	3	*Crassicornella crassicornella*	8.33	*Crassicornella* sp.	none
BOLD:AFR9903	1	0	*Reisserita relicinella*	3.21	*Reisserita relicinella*	NSI
BOLD:AAJ8384	2	13	*Trichophaga tapetzella*	9.72	*Trichophaga scandinaviella*	NC
BOLD:AAE7482	1	13	*Tinea dubiella*	5.94	*Tinea flavescentella*	NC
BOLD:AAQ3791	2	10	*Tinea basifasciella*	4.33	Tineidae specimen	NC
BOLD:AAD5562	1	38	*Tinea trinotella*	2.86	*Tinea trinotella*	NC
BOLD:AAF3430	2	60	*Niditinea fuscella*	6.17	*Niditinea truncicolella*	NC
BOLD:AAG3680	4	47	*Niditinea striolella*	6.17	*Tinea* sp.	none
BOLD:AAB6631	9	59	*Monopis laevigella*	4.23	*Monopis jussii*	none
BOLD:ABW5878	3	15	*Monopis neglecta*	9.27	*Monopis laevigella*	NSI
BOLD:AFT4096	1	0	*Monopis obviella*	2.72	*Monopis obviella*	NC
BOLD:AAC5209	1	46	*Monopis crocicapitella*	2.69	*Monopis* sp.	NC
BOLD:AAL3625	1	1	*Pelecystola fraudulentella*	4.17	*Pelecystola nearctica*	NI
BOLD:AAH4351	6	624	*Opogona omoscopa*	8.97	*Opogona sacchari*	none

Molecular libraries

Available molecular libraries allowed us to identify most of our specimens on a molecular basis. In the meantime, genitalia dissection enabled us to identify specimens for which molecular identification was not possible, as, although the species were already known, both sequence and the BINs were new to BOLD. Compared to other families, Tineidae showed a relatively high number of species having more than one BIN. In this study 30% of Tineidae species have more than one BIN, whilst in the Iberian Peninsula only the 2.5% of Erebidae (Ortiz et al. 2017) and in Austria 6.7% of Geometridae (Schattanek-Wiesmair et al. 2024) showed multiple BINs. Intraspecific variability is this high with mean interspecific divergence measuring 6.64% for the Tineidae in this study. However, the presence of several BINs under the same name could be due to the presence of cryptic diversity (Scalercio et al. 2021): such cases need further investigation using morphometrics and nuclear markers.

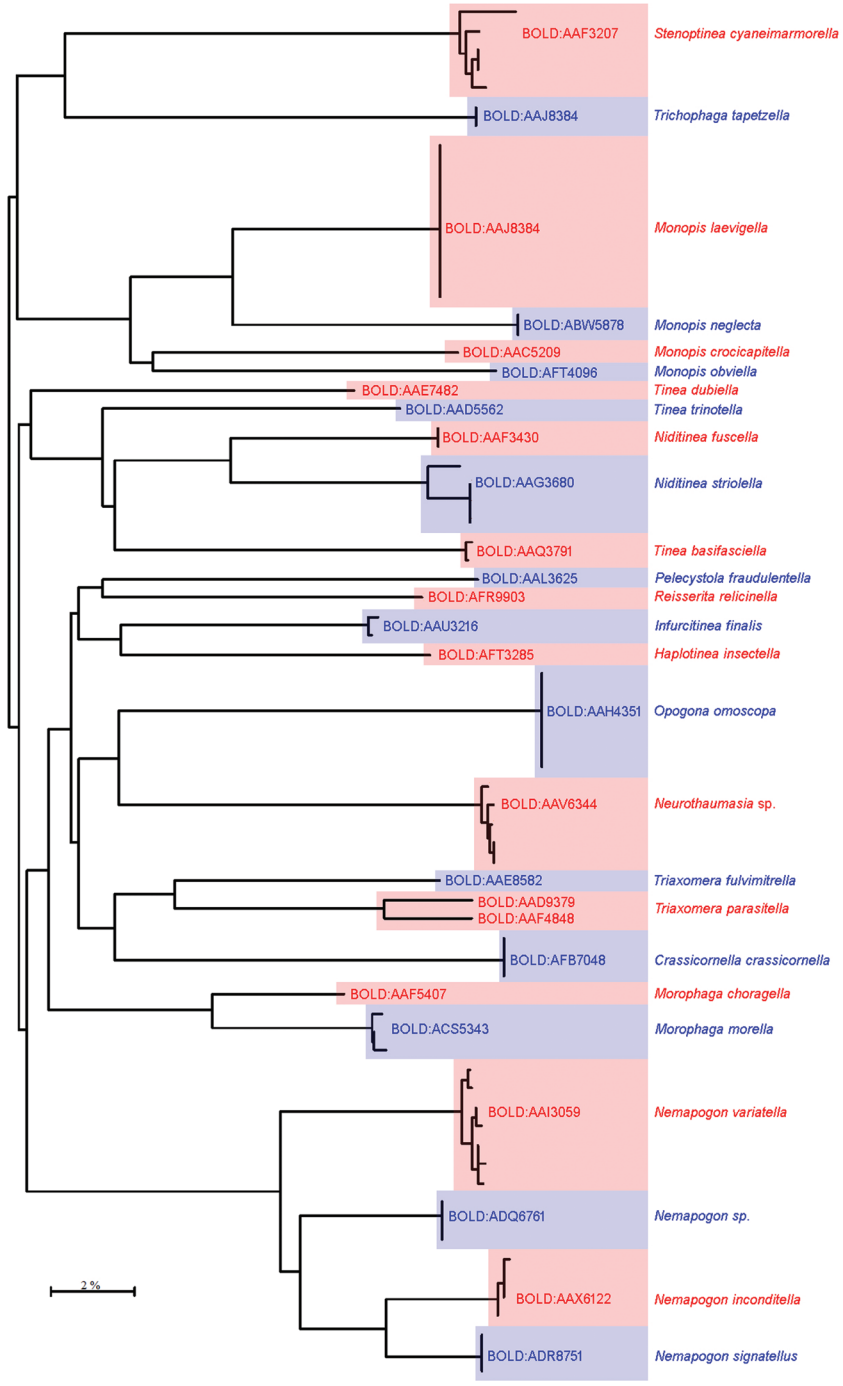
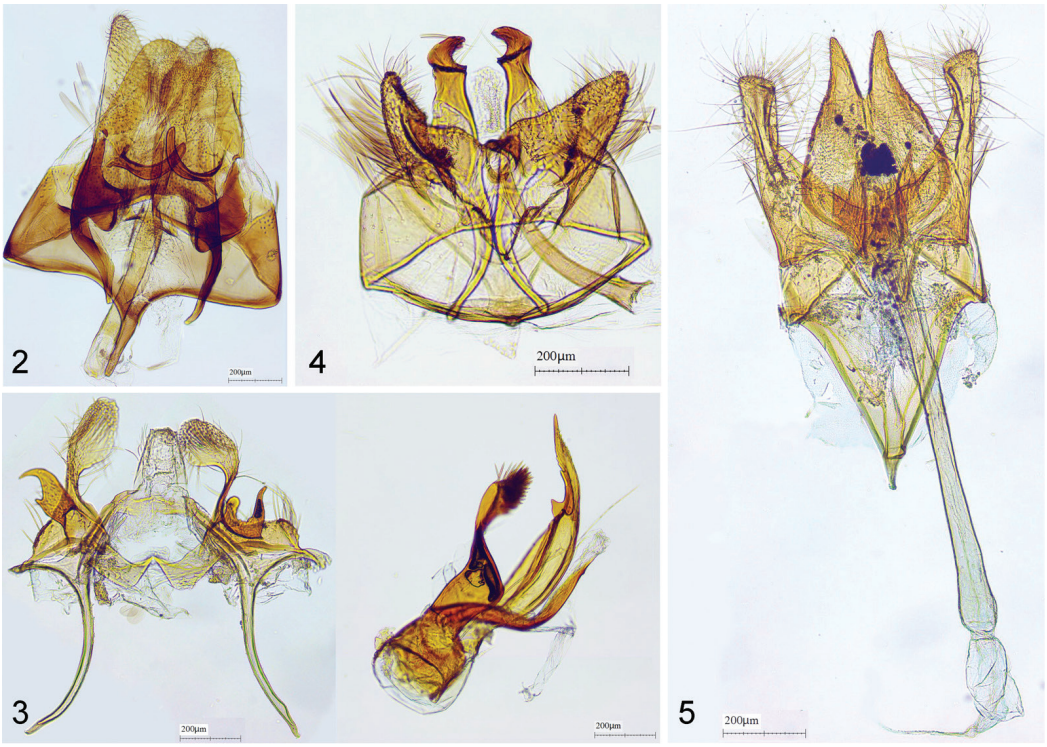


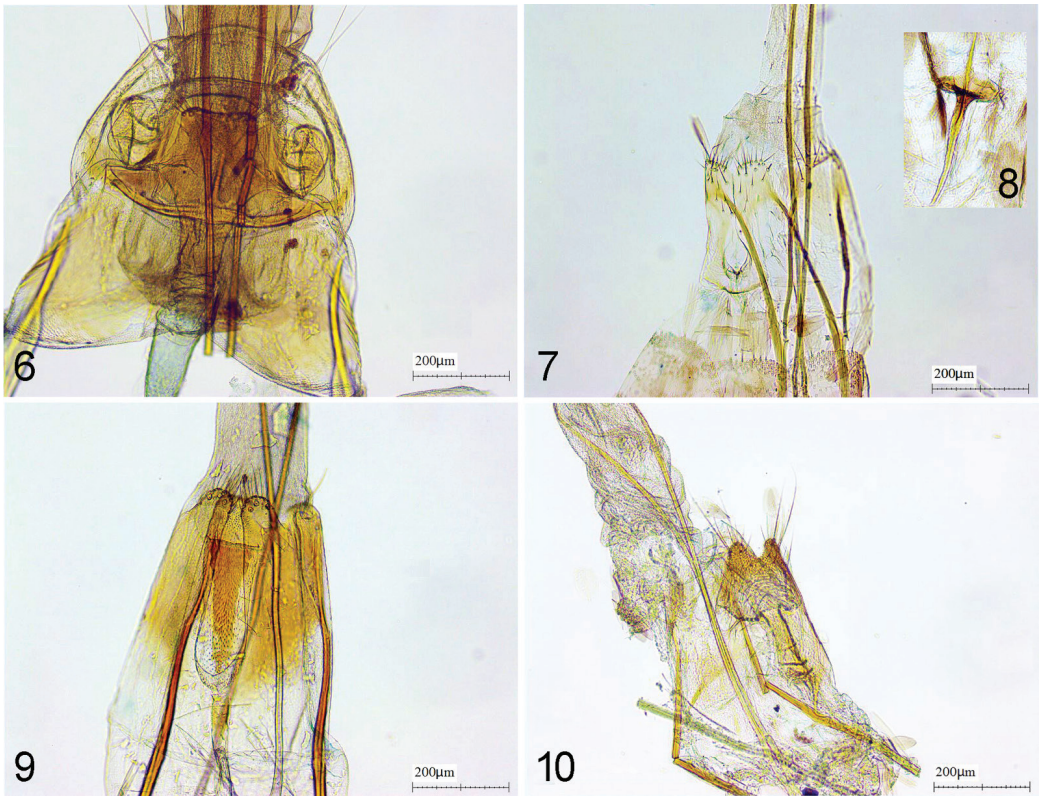
Figure 1. BOLD TaxonID Tree (distance model: Kimura 2 parameter) of Tineidae collected in South Italy. DNA barcodes belonging to a given taxon are highlighted alternately in red or blue. Barcode Index Numbers have also been reported.



Figures 2–5. Male genitalia of species with more than one BIN on BOLD. **2**. *Triaxomera parasitella*, BOLD:AAF4848, slide CREA-0288, Alessandria del Carretto; **3**. *Infurcitinea finalis*, BOLD:AAU3216, slide CREA-0290, Montalto Uffugo; **4**. *Haplotinea insectella*, BOLD:AFT3285, slide CREA-0297, Spezzano della Sila; **5**. *Reisserita relicinella*, BOLD:AFR9903, slide CREA-0296, Marcellinara.

The most interesting cases of our study concern the unidentified specimens belonging to the genera *Nemapogon* and *Neurothaumasia*. In the BOLD database, these genera are currently represented by 52 and five species, respectively. For *Nemapogon*, 39 species are described from Europe (Gaedike 2015; www.lepiforum.org), 25 of which are available in BOLD, corresponding to a coverage of 64.1%. For *Neurothaumasia*, six species are known from Europe including Canary Islands (www.lepiforum.org), five of which are available in BOLD, corresponding to a coverage of 83.3%. These values indicate a relatively good representation of both genera in the BOLD database. Our DNA barcoded specimens of these genera showed a distance (p-dist) of 3.74% (*Nemapogon*) and 6.73% (*Neurothaumasia*) from their respective Nearest Neighbors and may represent undescribed taxa. The recent paper describing *Neurothaumasia cretica* Huemer, Aarvik & Berggren, 2023 (Huemer et al. 2023) provides enough evidence that our *Neurothaumasia* should be an undescribed species.

The implementation of DNA barcoding libraries can also help to identify preimaginal stages (Hausmann and Scalercio 2016), most of which remain undescribed within the Tineidae (Gaedike 2015, 2019). This would help fill the gap in the biological knowledge of several species and to provide new bioindicators for evaluating forest ecosystem diversity.

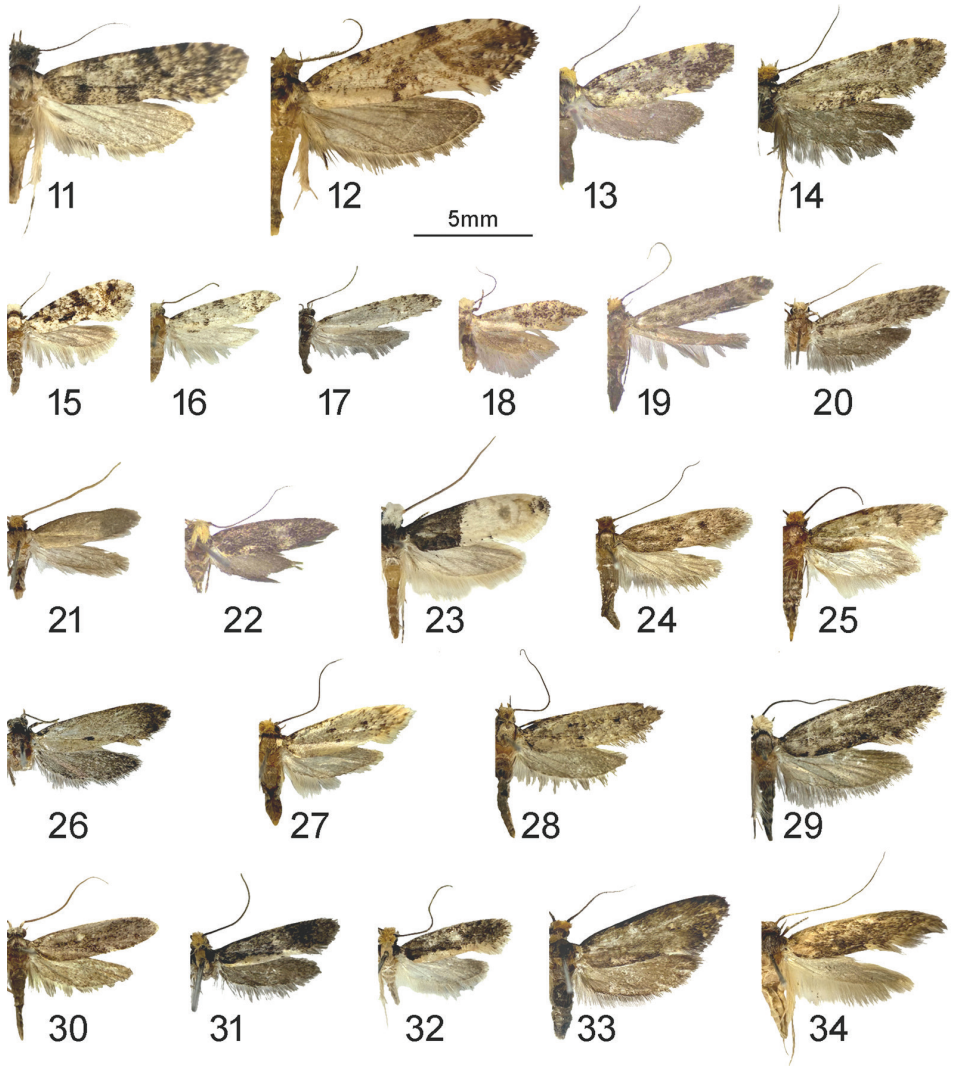


Figures 6–10. Details of female genitalia belonging with more than one BIN on BOLD. **6**. *Triaxomera parasitella*, BOLD:AAD9379, slide CREA-0289, Pedace; **7, 8**. *Stenoptinea cyaneimarmorella*, BOLD:AAF3207, slide CREA-0292, Acquaformosa; **9**. *Tinea trinotella*, BOLD:AAD5562, slide CREA-0294, Marcellinara; **10**. *Monopis obviella*, BOLD:AFT4096, slide CREA-0295, Spezzano della Sila.

Faunistic insights

The investigation of lepidopteran diversity in forest ecosystems allowed us to highlight the rich Tineidae fauna hosted by these habitats, revealing that many discoveries are yet to be made. Most of our specimens were collected from broadleaved forests, likely because they are rich in polypore fungi, one of the preferred larval foods of Tineidae, that grow predominantly in this forest type compared to conifer forests (Markkanen and Halme 2012). However, this aspect warrants further investigation as the available samples were randomly collected and cannot be rigorously used to confirm this apparent preference.

This research enabling us to enlarge the regional and national distribution of several species, among which the most interesting is *Pelecystola fraudulentella*, rarely recorded in Europe. It was first described from Slovenia, and its second record, after about 150 years, was published from Sweden (Lindeborg and Bengtsson 2008). Since then, it has also been found in Slovakia, Czech Republic, Hungary, Bulgaria, and Corsica (Gaedike, 2019). Our discovery supports Gaedike's (2019) expectation that further investigations would help clarify its distribution. We found it in a mountain beech forest in the Pollino Massif, a habitat very similar to those where it has been recorded in Central Europe.



Figures 11–34. Habitus of identified species. **11**. *Morophaga morellus*, LEP-SS-04478, Marcellinara; **12**. *Morophaga choragella*, LEP-SS-04474, Longobucco; **13**. *Triaxomera fulvimitrella*, LEP-SS-04557, Taverna; **14**. *Triaxomera parasitella*, LEP-SS-04483, Alessandria del Carretto; **15**. *Nemapogon inconditella*, LEP-SS-04533, Sangineto; **16**. *Nemapogon signatellus* (flipped), LEP-SS-04489, Sellia; **17**. *Nemapogon variatella*, LEP-SS-04500, Spezzano della Sila; **18**. *Infurcitinea finalis*, LEP-SS-05175, Spezzano della Sila; **19**. *Stenoptinea cyaneimarmorella*, LEP-SS-04687, San Giovanni in Fiore; **20**. *Haplotinea insectella*, LEP-SS-04532, Spezzano della Sila; **21**. *Crassicornella crassicornella*, LEP-SS-04502, Marcellinara; **22**. *Reisserita relicinella*, LEP-SS-04496, Marcellinara; **23**. *Trichophaga tapetzella*, LEP-SS-04491, Marcellinara; **24**. *Tinea dubiella*, LEP-SS-04507, Rende; **25**. *Tinea basifasciella*, LEP-SS-04517, Sellia; **26**. *Tinea trinotella*, LEP-SS-04524, Marcellinara; **27**. *Niditinea fuscella*, LEP-SS-04495, Sellia; **28**. *Niditinea striolella*, LEP-SS-04505, Saracena; **29**. *Monopis laevigella*, LEP-SS-04503, Spezzano della Sila; **30**. *Monopis neglecta*, LEP-SS-04545, Longobucco; **31**. *Monopis obviella* (flipped), LEP-SS-04535, Spezzano della Sila; **32**. *Monopis crocicapitella*, LEP-SS-04481, Cosenza; **33**. *Pelecystola fraudulentella*, LEP-SS-04514, Saracena; 34: *Opogona omoscopa*, LEP-SS-01431, Marcellinara.

Monopis neglecta was recently described from Central European countries with one of the paratypes collected near Lake Como (Šumpich 2011), and it remains the only specimen known from Italy. We found two specimens in different locations within the Pollino Massif, and a third specimen in the Sila Mountains. This suggests that *M. neglecta* is relatively common in the southern mountains.

Triaxomera fulvimitrella is primarily known from Central and northern Europe, with only a single record from northern Italy (www.boldsystems.org). The specimen we collected in the beech forest of the Sila Mountains represents a very disjunct record marking the southernmost known presence of this species in Europe.

Conclusions

The Tineidae records presented in this paper are by-catches of surveys aimed at describing macromoth communities of forests. As a result, the habitus of some specimens was not always perfectly preserved since most samples were collected using light traps, non-phototactic species were not sampled, and quantitative data are lacking. However, we discovered several faunistic novelties, and DNA barcoding not only facilitated the specimen identification, but also highlighted the presence of taxa that require further taxonomic investigation. The distribution of the sampling sites also shows that large areas of the region remain unsampled and deserve future investigation (Fig. 35). In the future, improving the taxonomy of this group could help to pave the way for using Tineidae as forest bioindicators.

Figure 35. Map of the sampling sites. Maps Data: Google, © 2025 SIO, NOAA, U.S. Navy, NGA, GEBCO Image Landsat / Copernicus.

Acknowledgments

We thank Carlo di Marco, Silvia Greco, and Marco Infusino for helping us in field activities. We also thank Pollino National Park and Sila National Park for the collecting permissions. This study was supported by the Societas Europaea Lepidopterologica (SEL), which covered the publication fee under its fee waiver program. We sincerely thank the SEL for this support. This work was funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU; Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP83D21014060006, Project title "National Biodiversity Future Center - NBFC", and Agritech National Research Center European Union NextGenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR)MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4—D·D 1032 17/06/2022, CN00000022).

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