

**Research article**Submitted: February 1<sup>st</sup>, 2018 - Accepted: May 4<sup>th</sup>, 2018 - Published: June 29<sup>th</sup>, 2018**A relict population of *Cymbalophora rivularis* on the Pollino Massif, southern Italy (Lepidoptera: Erebidae)**Silvia GRECO<sup>1,\*</sup>, Francesco Luigi LEONETTI<sup>1</sup>, Stefano SCALERCIO<sup>1</sup><sup>1</sup> Council for Agricultural Research and Economics, Research Centre for Forestry and Wood (CREA-FL) - Contrada Li Rocchi, 87036 Rende (CS), Italy - silvia.greco@crea.gov.it; francescoluigi.leonetti@virgilio.it; stefano.scalercio@crea.gov.it

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**Abstract**

The first record for South Italy of *Cymbalophora rivularis* (Ménétriès, 1832) is reported, until now recorded in Italy only for Central Apennine. Three specimens were collected during August-September 2017, in an *Acer* spp. forest located on Monte Sparviere, a Site of Community Importance within the Pollino National Park. Furthermore, this species was successfully barcoded for the first time. This finding reinforces the biogeographic importance of Pollino Massif as refuge area for relict populations of several animal and plant species.

**Key words:** *Acer* forest, Arctiinae, biodiversity, Calabria, DNA-barcoding, Natura 2000 network, Pollino National Park.

**Introduction**

The genus *Cymbalophora* Rambur, 1866 (family Erebidae, subfamily Arctiinae, tribe Arctiini, subtribe Arctiina) includes few species distributed in the western part of the Palearctic region. In Europe this genus is represented by *C. pudica* (Esper, 1785) and *C. rivularis* (Ménétriès, 1832), both present in Italy. These species can be easily distinguished as *C. rivularis* is smaller, has a yellowish abdomen, reddish in *C. pudica*, and exhibits three black spots on the inferior margin of forewings (four in *C. pudica*; Bertaccini et al. 1995). Furthermore, the female of *C. rivularis* is brachypterous, whilst the female of the congeneric species is fully winged (Bertaccini et al. 1995).

*Cymbalophora pudica* is distributed in North-West Africa, South Europe East to Balkans, Corsica, Sardinia, Sicily and Malta (Dubatolov 2010) and is largely distributed in Italy (Parenzan & Porcelli 2007), but it was never found in the same localities of *C. rivularis*. The whole range of *Cymbalophora rivularis* (Ménétriès, 1832) includes Central Italy, Albania, Macedonia, northern Greece, south-eastern Bulgaria, southern Ukraine, Turkey, north-western Iran, Armenia, western Azerbaijan, Chechnya and Dagestan (Dubatolov 2010; Karsholt & Nieuwerkerken 2013).

Described under the name *Chelonia rivularis* from Caucasus, “sur le bord du fleuve Terek”, probably in Chechnya (Dubatolov 2010), *C. rivularis* was discovered in Italy by Dannehl in the 1907 on the Majella Massif (Turati 1907). Populations from Central Italy were attributed to the sub-

species *dannheli* Turati, 1928, found also in Macedonia (Prola et al. 1978). Bang-Haas (1930) cited this subspecies underlying that its description was not published. Successively, De Freina & Witt (1984) noted that this subspecies has never been formally described by Turati and attributing the authorship to Dannhel (1929), which presented a detailed description of several forms of this variable species. Parenzan & Porcelli (2008) illustrated the history of the name of this subspecies concluding that it was never formally described by no one. In recent years, *Cymbalophora rivularis dannheli* was considered as a synonym of the nominal subspecies (De Freina & Witt 1987).

During a survey of moth diversity inhabiting *Acer*-dominated forests on the Pollino Massif, southern Italy, three males of *Cymbalophora rivularis* were surprisingly collected. This finding significantly enlarged the Italian range of this species, only known from mountainous areas of Umbria, Marche, Lazio and Abruzzo regions (Parenzan & Porcelli 2007). Moreover, it reinforces the strong biogeographic connections of the Pollino Massif with Central Apennine and South-East Europe, enhancing the importance of this massif as refuge area and diversity hotspot.

**Materials and Methods**

Moth surveys were carried out within the Site of Community Importance (SCI) Monte Sparviere, code IT9310019, Pollino National Park, southern Italy (Fig. 1). The aim of

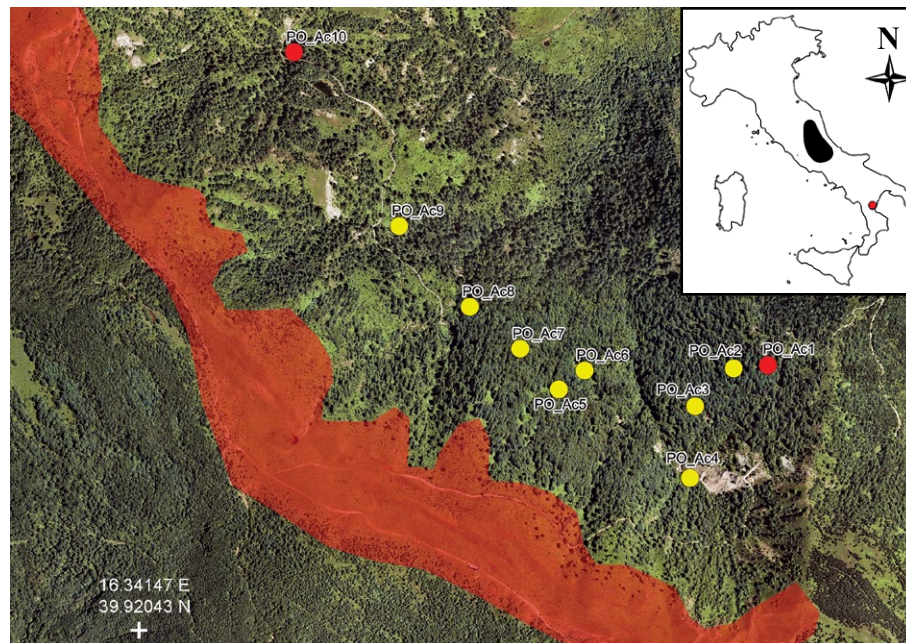
the survey was to compile a list of moth species inhabiting the *Acer*-dominated woodland that characterize this site. UV-LED light traps were used for moth monitoring (Infusino et al. 2017), set-up once per month from March to November, during nights favorable to moth activity, i.e. new moon, low wind speed, temperature near the mean

of the period or higher, no or low precipitations. Ten sites were sampled, representative of different vegetative and topographic aspect of this woodland (Fig. 2).

One male of *Cymbalophora rivularis* was collected the 17<sup>th</sup> August 2017, two additional males were collected the 18<sup>th</sup> September in a different locality. These specimens are



**Fig. 1** – Panoramic view of the Site of Community Importance (SCI) Monte Sparviere, code IT9310019, Pollino National Park, southern Italy (39.929686 N, 16.354392 E).



**Fig. 2** – Location of sampled sites. Red circles indicates the sites where *Cymbalophora rivularis* has been collected, yellow circles indicate the other sampled sites. The red area indicates the largest portion of suitable habitat for this species in the surrounding territory. On top right, the Italian distribution of *C. rivularis* from Bertaccini et al. (1994, modified) in black, and the newly discovered population in red.

preserved in the Lepidoptera collection of the Research Centre for Forestry and Wood, Council for Agricultural Research and Economics, Rende (CS), Italy (CREA-FL collection).

Tissue samples of two specimens (collection ID: LEP-SS-00648, LEP-SS-00730) collected in different sites and different dates were submitted to molecular barcoding analysis in order to explore inter- and intra-population genetic diversity. We followed the Canadian Centre for DNA Barcoding protocol for sequencing the mitochondrial 5' cytochrome oxidase gene, subunit 1 (COI), used as a standard marker for the identification of most animals. LepF1 and LepR1 were the primers used for PCR and sequencing (Hajibabaei et al. 2006). Sequences are deposited in the Barcoding Of Life Database (BOLD), accessible at [www.boldsystems.org](http://www.boldsystems.org).

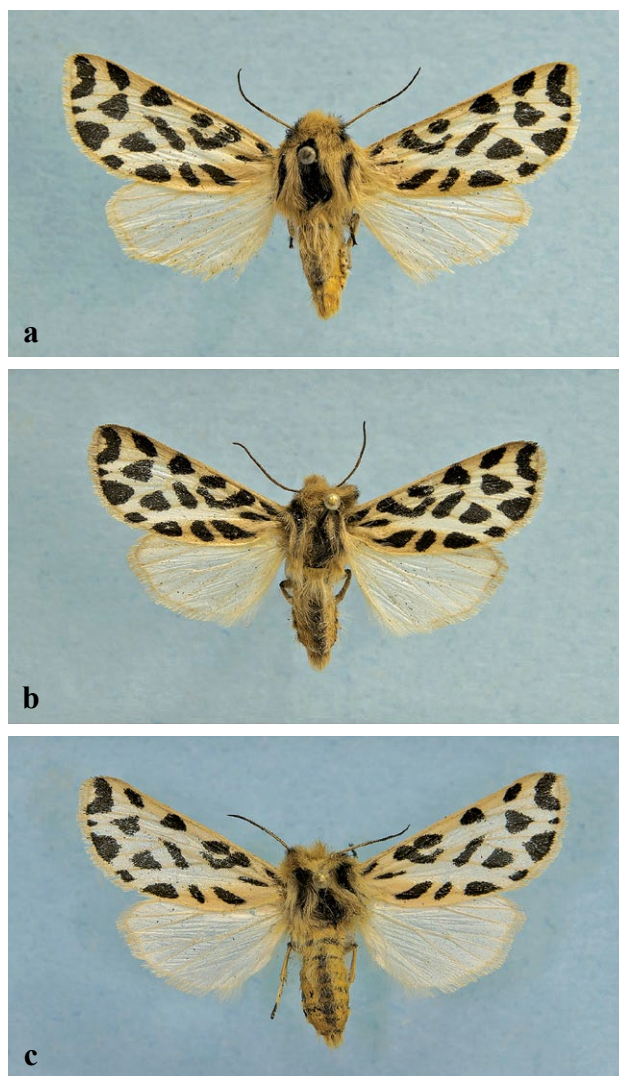
## Results

**Examined material. Italy:** Calabria, Balestrieri, Alessandria del Carretto (CS), 39.9275N, 16.3624E, 1371 m, 17 Aug 2017 (Infusino M. & Leonetti F. L. leg.), 1 ♂, wingspan 36mm, BOLD specimen ID: LEP-SS-00648, BOLD sequence ID: BCLEP198-17 (Fig. 3a), CREA-FL collection; Calabria, Tappaiolo, Alessandria del Carretto (CS), 39.9358N, 16.3471E, 1253 m, 18 Sep 2017 (Infusino M. & Greco S. leg.), 2 ♂, wingspan 30-34mm (Fig. 3b-c), BOLD specimen ID: LEP-SS-00730, BOLD sequence ID: BCLEP276-17, CREA-FL collection.

**Barcoding.** Two identical sequences, 658bp long, were recovered from the barcoded specimens (BCLEP198-17, BCLEP276-17 in the public BOLD dataset DS-CYMRIV), belonging to the BIN (BOLD Index Number) BOLD:ADK1292 (DOI: [dx.doi.org/10.5883/BOLD:ADK1292](https://doi.org/10.5883/BOLD:ADK1292)). Unfortunately, we cannot compare their sequences with those of other congeneric species as none specimens is available in public repositories. The nearest neighbor available was *Euplagia quadripunctaria* (Poda, 1761) (Erebidae, Arctiinae), with a distance of the 5.94%. These are the first sequences available for *Cymbalophora rivularis*, being not possible also to investigate the within-species genetic variability and the validity of the subspecies *Cymbalophora rivularis dannheli*.

**Habitat.** Males were found in two different forested habitats, representing the first and the last of the sequence of sampled sites (Fig. 2). The specimen found in August was collected in PO\_Ac1, an uneven aged *Acer* forest stand with individual trees older than 100 years and sparse trees of *Pyrus* sp. Trees exhibit a low density but offer a near total cover of the soil. Nevertheless, herbaceous cover is well developed, also due to occasional grazing favored by the mild topography (Fig. 4).

The specimens collected in September were found in



**Fig. 3** – Specimens of *Cymbalophora rivularis* collected on the Pollino National Park. **a**, ♂, wingspan 36 mm, 17 Aug 2017; **b**, ♂, wingspan 30 mm, 18 Sep 2017; **c**, ♂, wingspan 34 mm, 18 Sep 2017.

PO\_Ac10, a quite different habitat, being an *Alnus*-dominated forest, with 30-60 years old trees. Forest canopy covers near the 100% of the site. Herbaceous layer is very poor, with a high cover of *Urtica* sp. and the presence of large surfaces of bare soil. In addition, also topography is quite different, as the light trap was positioned near the bottom of a small valley with high gradient of slopes.

## Discussion

In this paper we reported the first finding of *Cymbalophora rivularis* in southern Italy, Calabria region, until now recognized only from Central Apennine mountains (Turati 1907; Teobaldelli 1976; Prola et al. 1978; Bertaccini et al. 1995; Grassi 2005; Parenzan & Porcelli 2007). The discovery of this species was particularly surprising as Lepi-



**Fig. 4** – The site (code: PO\_Ac1; Calabria, Balestrieri, Alessandria del Carretto (CS)) of the first finding of *Cymbalophora rivularis* in the Pollino National Park, southern Italy.

doptera of the Pollino Massif were intensively monitored in last decades, also during the flight period of this species (Parenzan 1977, 1982, 1995; Scalercio 2009; Infusino et al. 2016). It is probably very rare and with a small population present in only few localities of this Massif.

The lack of sequenced samples of this species in public repositories made impossible to explore inter-population diversity and to assess the taxonomic status of Italian populations, previously treated as a separate subspecies (*Cymbalophora rivularis dannheli*), now considered as a synonym of the nominal subspecies (De Freina & Witt 1987). Nevertheless, the perfect identity of the barcoded specimens, collected in different localities and at 31 days one from the other, can be an indication of low genetic intra-population variability that could increase the extinction risk of this population.

Calabrian specimens of *C. rivularis* (Fig. 3) are very similar to those collected in Central Italy, and their wingspan vary between 30 and 36mm, falling within the known wingspan range of this species (Dannhel 1929; De Freina & Witt 1984). Phenology was also the same, this species flying in Italy from August (rarely end of July), to mid-September in one generation per year (Bertaccini et al. 1995). Although no specific studies exist, available records supported the hypothesis that this species is proterandric, being females more often recorded in September.

*Cymbalophora rivularis* lives in xerothermophilous mountain prairies, from 750 to 2000 metres of altitude, but rarely below 1200 m (Bertaccini et al. 1995). Larvae of *C. rivularis* are polyphagous on herbaceous plants, especially Poaceae (De Freina & Witt 1987). Sampling sites in the Pollino Massif are located at similar elevation of those in

the Central Apennines, but the habitat of collecting sites, forest woodlots with low sunlight at soil, is quite different from those reported in literature as typical for this species. We can hypothesize that vagrant males has been collected by light traps and that their breeding habitat is, likely, located at higher altitude where the largest portion of their optimal habitat is present (Fig. 2). Small patches of suitable habitats also occur at lower elevation and at nearer places, but in any case this means that collected males were involved in dispersal flights when collected by traps. From a conservation point of view, it is important to underline that females of this species are brachypterous and not able to reach long distances, then species range can only be actively expanded by larvae. As a consequence, this species is more prone to local extinction in case of habitat changes such as natural reforestation.

The presence of a relict population of *Cymbalophora rivularis* on the Pollino Massif, reinforces the affinity of this mountainous area with those of Central Apennine, especially evident for the lepidopteran fauna associated with mountain prairies. There are several examples of species recorded in peninsular Italy only in Central Apennine and in the Pollino Massif, e.g. the Erebidae *Setina irrorella* (Linnaeus, 1758) (Parenzan & Scalercio 2001), the Nymphalidae *Erebia cassioides* (Reiner & Hohenwarth, 1792) (Scalercio et al. 2014), the Geometridae *Pareulype berberata* (Denis & Schiffermüller, 1775) and *Entephria flavicinctata* (Hübner, 1813), the Noctuidae *Epipsilia grisescens* (Fabricius, 1794) (Scalercio 2008), among others. Furthermore, also the affinity with the biota of South-East Europe is not new for the Pollino Massif, being the symbol of the Pollino National Park the Balcanic tree *Pinus hel-*

*dreichii* H.Christ (previously known as *Pinus leucodermis* Antoine).

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