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PENSOFT

Chrysaster ostensackenella (Fitch, 1859), a potentially invasive species newly recorded from Europe (Lepidoptera, Gracillariidae)

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Abstract

Chrysaster ostensackenella (Fitch, 1859), originally a New World species with recent records from East Asia, is newly reported from Europe (Province of Rieti, Italy). The potentially invasive species is a trophic leaf-mining specialist on *Robinia* L. Species identification is based on DNA barcoding and morphological characteristics.

Keywords

DNA barcode, New World, Italy, introduced species, *Robinia*

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Introduction

With more than 2000 species worldwide (De Prins and De Prins 2006–2018) and 263 species so far recorded from Europe, the Gracillariidae is one of the most diverse families of so-called microlepidoptera. However, even the European fauna seems insufficiently explored as shown by 21 potentially undescribed species (Lopez-Vaamonde et al. 2021). As well as these taxonomic shortcomings, 21 species of the European fauna are non-native and several of these taxa are considered as harmful invasive species most of which invaded the Continent during the last few decades. The majority of these introduced species feed as leaf-miners on ornamental trees and shrubs. Examples include the Horse-chestnut Leaf-miner *Cameraria ohridella* Deschka & Dimić, 1986, the Plane Leaf-miner *Phyllonorycter platani* (Staudinger, 1870), and the Firethorn Leaf-miner *P. leucographella* (Zeller, 1850), all of Mediterranean origin, but also species from Eastern Asia such as the Lime Leaf-miner *P. issikii* (Kumata, 1963), and from North America, the American Grape Leaf-miner *Phyllocnistis vitegenella* Clemens, 1859. The majority of these species have followed their original host-plants which are often cultivated in plantations, parks or gardens.

In the same way, the Black Locust *Robinia pseudoacacia* L., which originates from North America has subsequently been colonized in Europe by two gracillariids: the locust digitate leaf-miners *Parectopa robiniella* Clemens, 1863 and *Macrosaccus robiniella* (Clemens, 1859). Another New World gracillariid species associated to this host-plant, *Chrysaster ostensackenella* (Fitch, 1859), has been recently reported from China and South Korea, indicating an ongoing invasion into Asia. Here we report the unexpected collection of a single specimen of this species from mainland Italy.

Chrysaster ostensackenella belongs to a genus which has only two described species: *C. ostensackenella* from North America and the generic type species *C. hagicola* Kumata, 1961 from East Asia, including Korea (Kumata 1961). Both the reported species and genus are new for the European fauna. The establishment of this species in Europe needs confirmation and its potential invasion of Europe requires focused monitoring of the region .

Methods

A single unidentified male specimen of *Chrysaster* was collected at a UV light. This voucher was subsequently prepared for DNA barcoding, a method that allows accurate identification from a 658-base-pair-long segment of the mitochondrial cytochrome c oxidase subunit I (COI) gene. A tissue sample was prepared following the prescribed standards (deWaard et al. 2008) and successfully processed at the Canadian Centre for DNA Barcoding (CCDB, Biodiversity Institute of Ontario, University of Guelph). An additional eight public sequences >600 bp of *Chrysaster* (three *C. hagicola*, five *C. ostensackenella*) in the Barcode of Life Data Systems (BOLD; Ratnasingham and Hebert 2007; Ratnasingham 2018) were used as references for identification. Details including complete voucher data and images of these specimens can be accessed in the public dataset "*Chrysaster* sp." (https://doi.org/10.5883/ds-chryoste) in BOLD. Our sequences were submitted to GenBank.

Degrees of intra- and interspecific variation of DNA barcode fragments were calculated under the Kimura 2 parameter model of nucleotide substitution using analytical tools of BOLD systems v. 4.0. (https://www.boldsystems.org). Calculation of intraspecific distance was furthermore normalized with BOLD calculation tools to reduce bias in sampling at the species level. A neighbour-joining tree of DNA barcode data from the dataset was constructed using MEGA7 (Kumar et al. 2016) under the Kimura 2 parameter model for nucleotide substitutions.

Photographs of the adult were taken with an Olympus SZX 10 binocular microscope and an Olympus E 3 digital camera and developed using Helicon Focus 4.3, Adobe Photoshop CS4, and Lightroom 2.3. Genitalia photographs were taken with a Wild Heerbrugg microscope using a 10× objective and a 2.5× ocular. Photographs were edited using Helicon Focus 4.80 and Adobe Photoshop 6.0.

Results

***Chrysaster ostensackenella* (Fitch, 1859)**

Argyromiges ostensackenella Fitch 1859: 838.

Figures 2, 3

New record. ITALY – **Rieti** • Monte Terminillo Süd; 42°27′32″N, 012°55′19″E; 930 m alt.; 31.VII.2021; T. Mayr leg.; DNA Barcode ID TLMF Lep 31353; 1 ♂, genitalia slide GU 22/1539 P. Huemer, Research Collection T. Mayr.

Identification. The identification of the species was firstly based on DNA barcode reference sequences in BOLD (Fig. 1). Both species of *Chrysaster* cluster in different BINs (Ratnasingham and Hebert 2013) and can be easily separated by an interspecific *p*-distance of 7.85%. However, the intraspecific variation of *C. ostensackenella* is considerable with 1.19% average *p*-distance and a maximum of 3.44% and should be analysed for potential cryptic diversity (Kirichenko et al. 2017). Furthermore, adult morphology was studied (Figs. 2, 3) and compared with literature references. Detailed descriptions of the genitalia and external morphology have been published, for example, by Liu et al. (2015) and are not repeated here.

Distribution. The species is naturally distributed in North America (Braun 1908; De Prins and De Prins 2006–2018). It was recently introduced to China (Liu et al. 2015) and South Korea (Koo et al. 2019) (Fig. 4).

Biology. The larval ecology in Europe is unknown but probably corresponds to observations published from elsewhere. In North America larvae produce large, yellowish blotch mines of irregular shape on *Robinia* species (Fig. 5), including *R. hispida* L., *R. neomexicana* A.Gray, *R. pseudoacacia* L., and *R. viscosa* Michx. ex Vent. (De Prins and De Prins 2006–2018). Pupation is reported inside and outside the leaf-mine (Braun 1908). Adults occur in up to four generations per annum (Liu et al. 2015), but the number of generations probably varies regionally.

Habitat. The habitat at the Italian site is a south-facing quarry that has been abandoned for a long time. There are some open rocky places but most of the area is covered by a dense mixed forest with a few pines, but no detailed notes are available about the floristic composition. However, accompanying faunal elements clearly indicate thermophilous plant communities with, for example, several leaf-mining species from *Quercus* L.: *Stigmella basiguttella* (Heinemann, 1862), *S. szoecsiella* (Borkowski, 1972), *S. zangherii* (Klimesch, 1951), *Caloptilia alchimiella* (Scopoli, 1763), *C. robustella* Jäckh, 1972), and *Stigmella johanssonella* A. Laštůvka & Z. Laštůvka, 1997 from *Ostrya carpinifolia* or *Glaucolepis melanoptera* (van Nieukerken and Puplesis, 1991, which is suspected to feed on *Prunus spinosa*. Furthermore, mesophilous species such as *Zimmermannia liebwerdella* (Zimmermann, 1940) from *Fagus* and *Phyllonorycter coryli* (Nicelli, 1851) from *Corylus* have been recorded. Apart from *C. ostensackenella* no other leaf-mining species of *Robinia* was observed at the site.

Remarks. In contrast to *Macrosaccus robiniella*, the leaf mines are predominantly produced on the upper side of the leaflet and are yellowish and not white in colour. Adults are similar to some species of European *Phyllonorycter* and therefore may be easily overlooked.

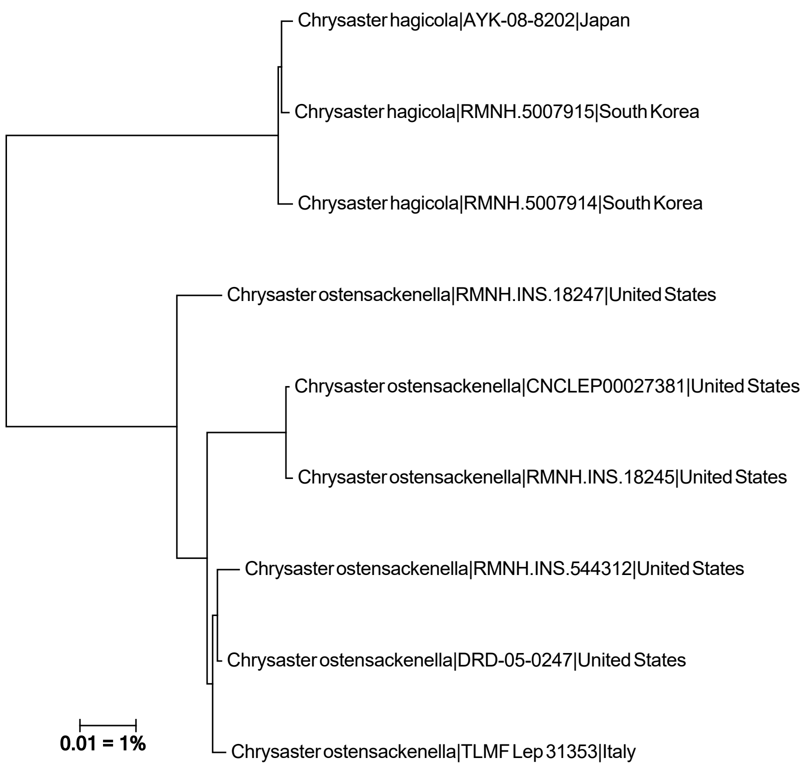

Figure 1. Neighbor-joining tree of *Chrysaster* (Kimura 2-parameter, built with MEGA7 (Kumar et al. 2016); Source: DNA Barcode data from BOLD (Barcode of Life Database; Ratnasingham 2018).

Figure 2. *Chrysaster ostensackenella*, adult male, Italy (scale bar = 1 mm).

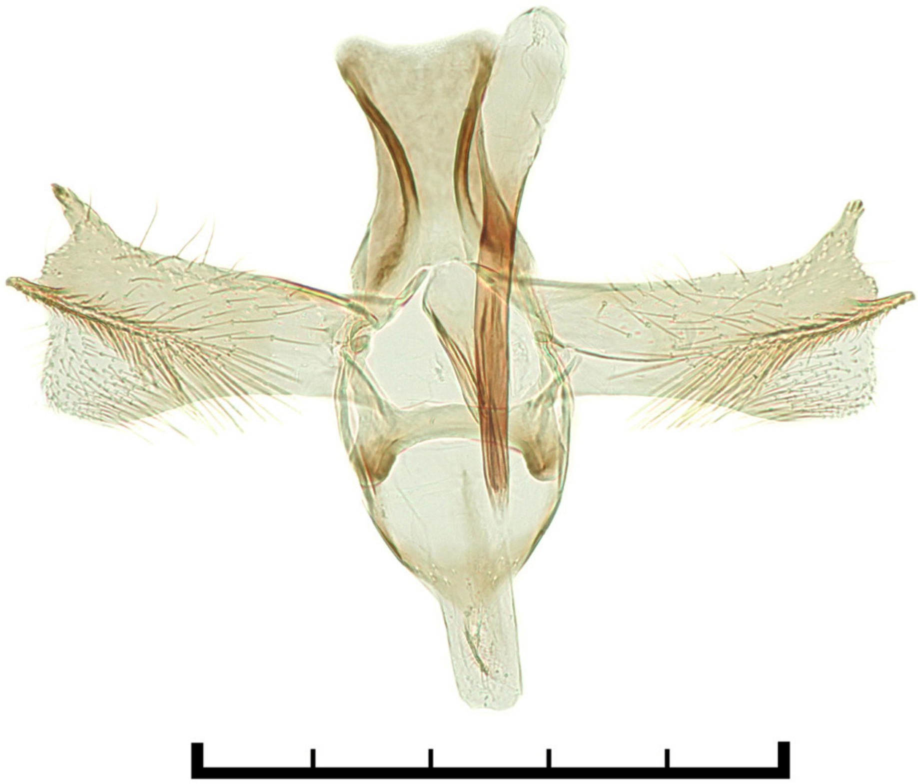

Figure 3. *Chrysaster ostensackenella*, male genitalia, Italy (scale bar = 100 µm).

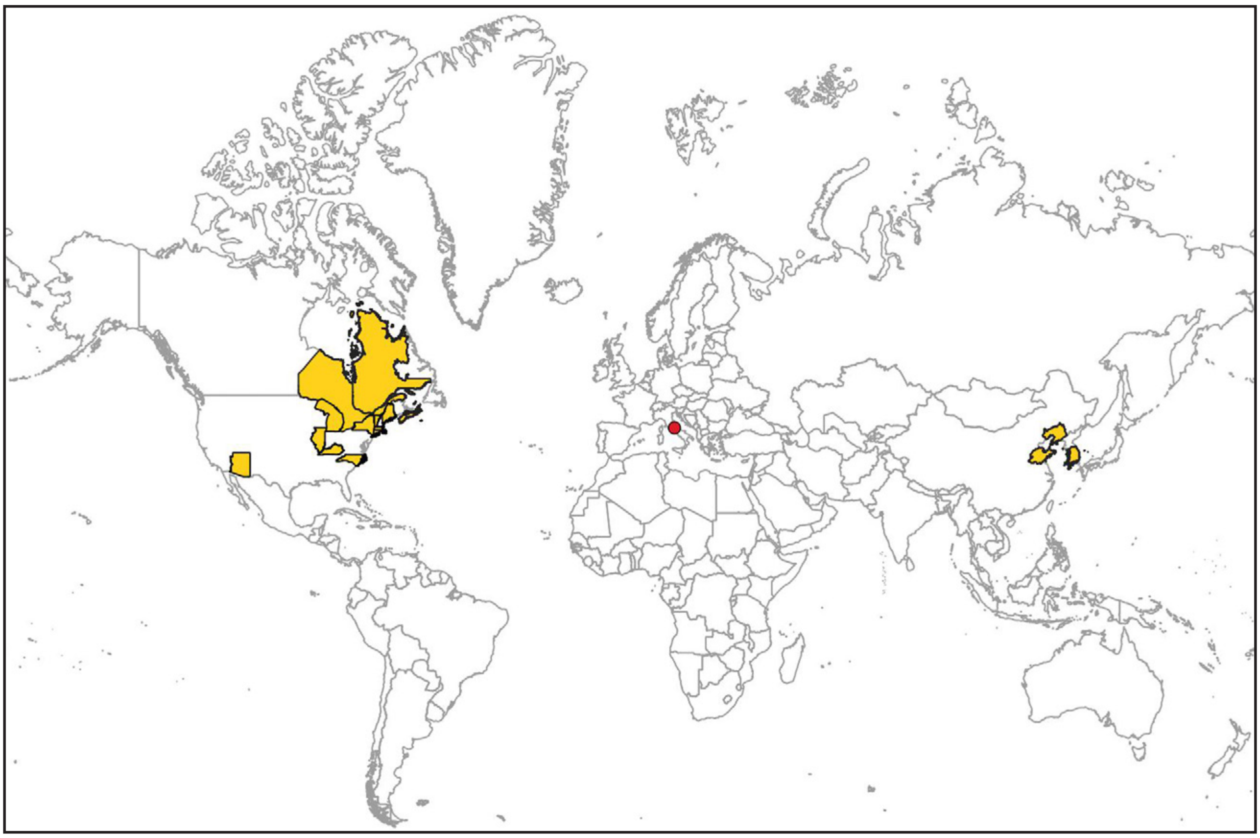

Figure 4. *Chrysaster ostensackenella*, actual distribution (in orange) and new record (red dot) (provincial/country data sources: De Prins and De Prins 2006–2018; Koo et al. 2019) (basic map: https://gadm.org/).

Figure 5. *Chrysaster ostensackenella*, leaf-mines, USA (© Jason J. Dombroskie, licensed under CC BY-NC).

Discussion

The unexpected, molecular based, discovery of a potentially new invasive species of gracillariid in Europe raises many questions that currently cannot be answered at all, or only incompletely. Such research questions include the origin of this species, time and place of introduction/immigration, current rate of spread, and questions about its biology and potential risks.

The first records of *Chrysaster ostensackenella* in East Asia, which are of recent origin, indicate an increase in distribution by anthropogenic means (Liu et al. 2015). In particular, the frequency of the species in the area of origin in North America would have led one to expect an introduction earlier.

The first European record may be directly related to newly established populations in the Far East, but it seems very unlikely that the species reached Europe by natural expansion of its range. In particular, the short time of only a few years since its discovery in China (Liu et al. 2015) and South Korea (Koo et al. 2019) contradicts such a scenario and also the lack of further records in Asia (Sinev 2019) is an argument against this theory. Instead, it is possible that the species reached Italy via the steadily intensified freight or passenger traffic between China and Europe, possibly in the pupal stage with dropped leaf mines. Alternatively, *C. ostensackenella* may have been introduced independently from North America to both East Asia and Europe, that is, with ship containers or even with living ornamental plants.

The newly discovered, isolated occurrence in mainland Italy gives no indication of possible immigration routes at first glance, especially as the site is located far from the nearest seaports. However, it is quite likely that the species is already much more widely distributed in central Italy and perhaps beyond. In particular, the considerable lack of experts on the systematics of Gracillariidae and other small moth families in the country means that even more conspicuous species have been and are still being overlooked (Huemer and Wieser 2020; Koster et al. 2019).

As there are up to four generations per year in China resulting in up to 90% of mined leaves (Liu et al. 2015) there may be a high risk of serious damage to *Robinia* trees with negative effects on honey production (Koo et al. 2019). This paper is therefore accompanied by an urgent appeal to fellow researchers, also from the applied field, to look for the presence of leaf mines on *Robinia* in order to be able to better estimate the directions of invasion and its extent and speed.

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Authors' Contributions

Conceptualization: PH. Formal analysis: PH. Investigation: PH. Methodology: PH. Project administration: PH. Resources: PH, TM. Supervision: PH. Validation: PH. Writing – original draft: PH. Writing – review and editing: PH, TM.

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