Bulletin of Insectology **77** *(1): 45-53, 2024*
ISSN 1721-8861 eISSN 2283-0332

Chrysaster ostensackenella, *Parectopa robiniella* and *Macrosaccus robiniella* (Lepidoptera Gracillariidae), three invasive leaf-miner of *Robinia pseudoacacia* in Italy

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Abstract

In the paper, we present the results carried out in central-southern Italy during the years 2022 and 2023 of a field survey conducted on the leaf-miners of the tree *Robinia pseudoacacia*. Three invasive alien species pests, *Chrysaster ostensackenella*, *Parectopa robiniella* and *Macrosaccus robiniella* (Lepidoptera Gracillariidae), were collected. Some ecological aspects and an illustration of their characteristics, supplemented with photographs of the three leaf-miners, emphasizing life history and diagnostic characteristics enabling their differentiation, are also provided.

Key words: *Chrysaster ostensackenella*, *Parectopa robiniella*, *Macrosaccus robiniella*, Italy, black locust, pests.

Introduction

The black locust, *Robinia pseudoacacia* L., which originates from North America and was introduced to the European continent in the 17th century, has subsequently been colonized in Europe by two Lepidoptera species of the family Gracillariidae, the locust digitate leaf-miner, *Parectopa robiniella* Clemens 1863, and the leaf blotch miner moth, *Macrosaccus robiniella* (Clemens 1859) (Vidano, 1970; Whitebread and Joos, 1986; Bolchi-Serini and Trematerra, 1989; Whitebread, 1990).

Another New World gracillariid species associated with the same host-plant, the leaf-miner moth *Chrysaster ostensackenella* (Fitch 1859), has recently been intercepted in central Italy (first record in Europe) as a single adult specimen (Huemer and Mayr, 2022).

Similar to other insects, these three species have followed their native host-plant, a tree species that is commonly cultivated in plantations, parks or gardens and has long been naturalized in Europe (Vítková *et al.*, 2016; Medzihorsky *et al*., 2023).

To increase knowledge on the subject, a survey was carried out in central-southern Italy to identify the damage on leaves of these three invasive leaf-miners and to study some ecological traits of the pests in the field.

In the present paper we illustrate, supplemented with photographs, the *Ch. ostensackenella* leaf miner, emphasizing life history and diagnostic characteristics that enable its differentiation from the two other North American gracillariid species associated with *R. pseudoacacia*, i.e., *P. robiniella* and *M. robiniella*.

Materials and methods

Several locations in five regions of central-southern Italy were visited: Latium, Abruzzo, Molise, Campania and Apulia (table 1). Mines made by gracillariids on *Robinia* leaves were observed directly in the field and collected from June to October 2022; the affected plants were distributed along the sides of commercial roads and in rural areas. The collected samples (mainly mined leaves) were taken to the laboratory to carry out further observations in a controlled environment at 27 °C and 70% relative humidity.

The mines were identified following the descriptions provided in North American and Chinese studies (Weaver and Dorsey, 1967; Liu *et al*., 2015). The identity of the mines was subsequently confirmed through emerged adult moths based on wing pattern and genitalia (Davis and De Prins, 2011; Huemer and Mayr, 2022).

To verify the presence and abundance of leaf-miners in the studied Italian locations, a fortnightly sampling was carried out in 2023 from the beginning of July to late October by checking each time 10 imparipinnate leaves per tree, as "sampling unit", from 10 black locust plants from each visited site (table 1).

Results

According to our study, the distribution of the three leaf-miners *Ch. ostensackenella*, *P. robiniella* and *M. robiniella*, in the considered localities of central-southern Italy is uneven. There are localities apparently free of their presence and localities heavily infested. Usually, the plants of black locust are mainly colonized at the lower branches and in bushy parts.

Table 1 reports the localities visited from July to October 2023, during the period when black locust is in leaf, with presence and the abundance estimate of the mines observed on sampled plants.

As an overall general consideration, referring to the presence of the three leaf-miners in central-southern Italy, the maximum number of mines on *R. pseudoacacia* leaves was registered in the II and III decades (10-day periods) of August and in the II decade of September 2022 and 2023. In particular, *P. robiniella* is present rather uniformly in most visited localities during our surveys. In contrast, *M. robiniella* was found mainly in Latium and in the hilly part of Molise region, whereas *Ch. ostensackenella* was found in Latium, in central Molise and mainly

Table 1. Localities visited in central-southern Italy during July-October 2023, with absence/presence and abundance of mines observed on *R. pseudoacacia* imparipinnate leaves, reported as: absence (-); low presence (1-2 mines +); medium presence (3-5 mines ++); high presence (more than 6 mines +++).

Localities visited		*Chrysaster ostensackenella*	*Parectopa robiniella*	*Macrosaccus robiniella*
Latium region				
Frosinone province	Paliano 450 m a.s.l.	++	+++	++
Abruzzo region				
Chieti province	Vasto 60 m a.s.l.	+	++	+
Molise region				
Campobasso province	Bojano 470 m a.s.l.	+	+++	+++
	Campobasso 701 m a.s.l.	+	++	++
	Campochiaro 480 m a.s.l.	+	++	+++
	Campomarino 30 m a.s.l.	+++	+++	+
	Ferrazzano 830 m a.s.l.	+	++	++
	Fossalto 520 m a.s.l.	-	+	+
	Guardialfiera 174 m a.s.l.	-	+	-
	Guardiaregia 490 m a.s.l.	+	+	+
	Guglionesi 275 m a.s.l.	-	+	-
	Larino 241 m a.s.l.	-	+	-
	Lucito 380 m a.s.l.	-	+	-
	San Polo Matese 480 m a.s.l.	-	++	++
	Termoli 30 m a.s.l.	+	++	+
	Vinchiaturo 510 m a.s.l.	-	+	-
Isernia province	Castelpetroso 850 m a.s.l.	-	+	-
	Isernia 423 m a.s.l.	-	+	+
	Monteroduni 450 m a.s.l.	-	++	-
	Venafro 222 m a.s.l.	-	++	+
Campania region				
Caserta province	San Pietro Infine 140 m a.s.l.	-	++	+
Apulia region				
Foggia province	Chieuti 40 m a.s.l.	+	++	-

Figure 1. Adults of *Chrysaster ostensackenella* (**A**), *Parectopa robiniella* (**B**), and *Macrosaccus robiniella* (**C**).

along the Adriatic strip of the Molise-Apulia regions, infesting plants located in the vicinity of the motorway and railway lines (table 1).

Hereafter, the examined material (mines and adults), distribution, morphological and ecological characteristics along with illustrations of some biological stages useful for species identification are reported (figures 1-6).

Chrysaster ostensackenella (Fitch 1859)
(figures 1A, 2A, 3A, 4A-L)

M a t e r i a l e x a m i n e d

Mines: few mines found during July 2022; several mines observed and collected from July to October 2023 in Latium, Abruzzo, Molise and Apulia regions.

Adults: 1 male, 27.VII.2022, Campomarino (Campobasso), 30 m a.s.l., ex mines of *R. pseudoacacia*, leg. P. Trematerra; 2 males and 1 female, 4.VIII.2023, Campomarino (Campobasso), 30 m a.s.l., ex mines of *R. pseudoacacia*, leg. P. Trematerra (coll. P. Trematerra). Additional uncatalogued material.

D i s t r i b u t i o n

The leaf-miner moth, *Ch. ostensackenella*, is naturally distributed in North America (USA and Canada) (De Prins and De Prins, 2022). In recent years, the species has been documented in China (Liaoning and Shandong provinces) in 2015 (Liu *et al*., 2015), South Korea (Gyeonggi, Chungbuk, Jeonnam provinces) in 2017 (Koo *et al*., 2019), Japan in 2021 (Sawada and Sakurai, 2022) and Primorsky Krai, Russia in 2022 (Kirichenko *et al*., 2023). Huemer and Mayr (2022) reported the capture in Italy of a single male collected at a UV light in Monte Terminillo (province of Rieti), Latium region, on 31.VII.2021. Our

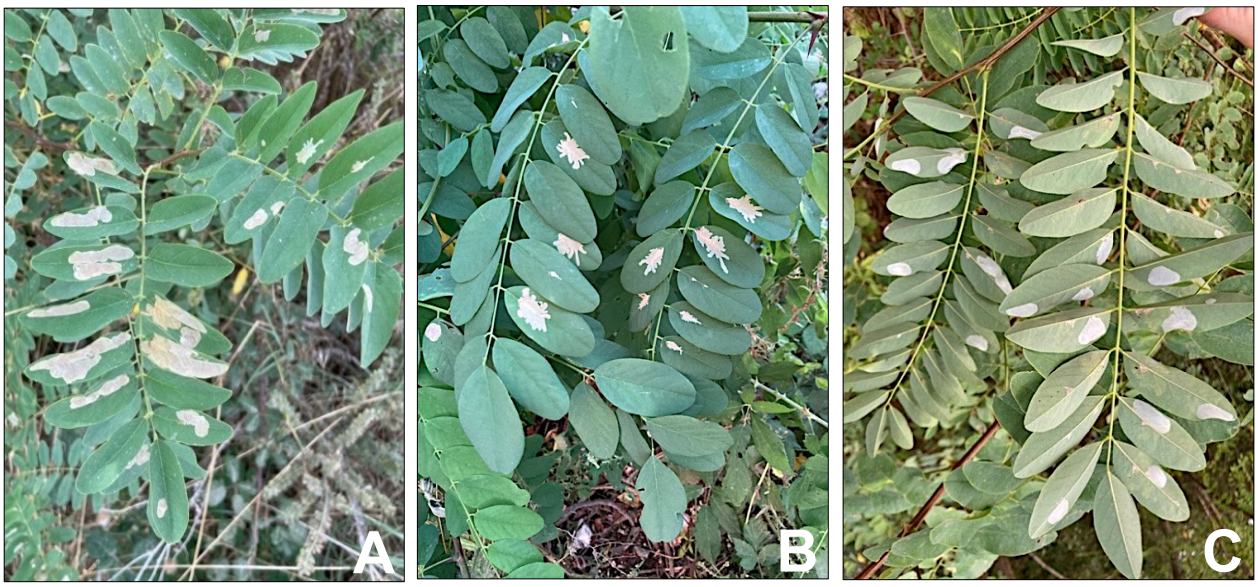

Figure 2. Host plant *Robinia pseudoacacia* and the damage by leaf-miners *Chrysaster ostensackenella* (**A**), *Parectopa robiniella* (**B**), and *Macrosaccus robiniella* (**C**).

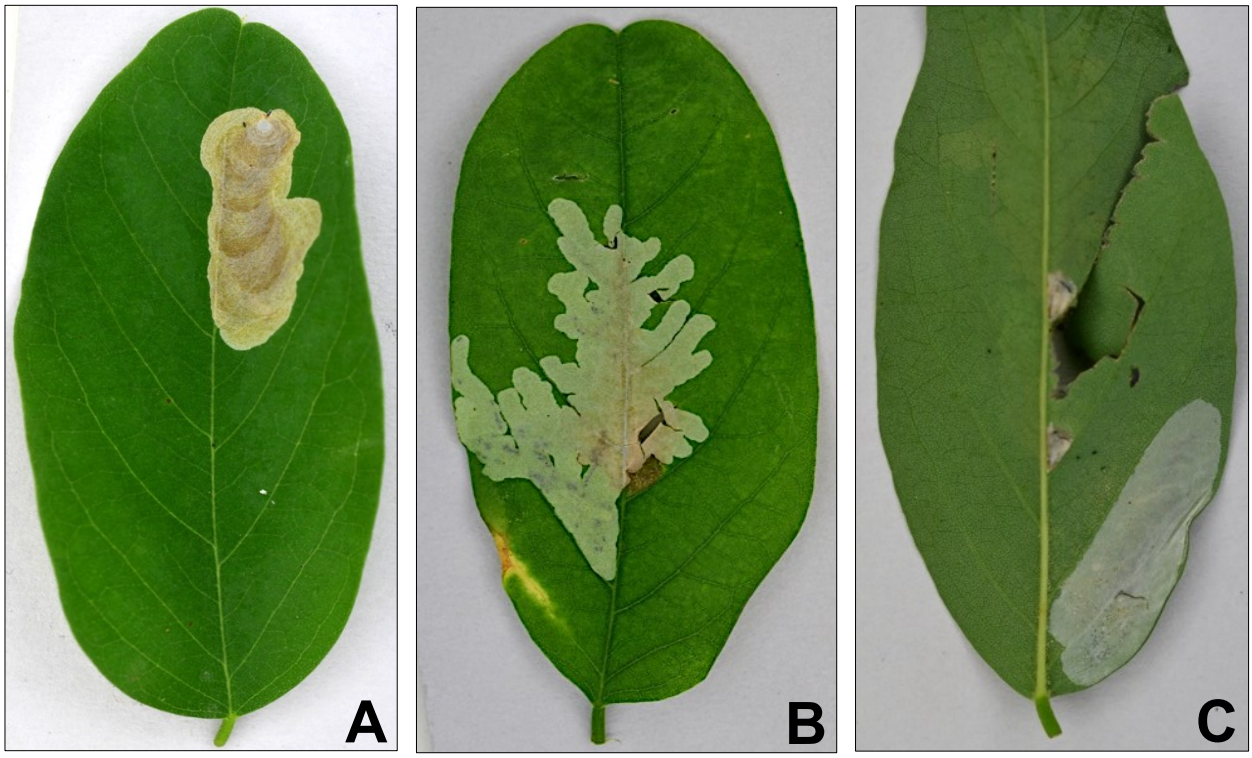

Figure 3. Leaf-mines of *Chrystaster ostensackenella* (**A**), *Parectopa robiniella* (**B**), and *Macrosaccus robiniella* (**C**).

data extend the Italian distribution of *Ch. ostensackenella* to the Latium, Abruzzo, Molise, and Apulia regions (table 1).

Adult morphology

The adult wingspan is about 5 to 6 mm; head with short blackish tuft; antenna dark brown, as long as forewing. Thorax with metallic gold-brown scales; abdomen metallic, dark brownish grey. Forewing ground colour orange, shiny, slightly darker basally, silvery white along their basal margins; four transverse fasciae silvery white outwardly, black inwardly; post-median and sub-terminal fasciae broken near middle. Termen black. Hindwings pale brownish, cilia tinged brownish-grey.

Biology and ecology

Ch. ostensackenella mines the leaves of *Robinia* species, including *R. hispida* L., *R. neomexicana* A. Gray, *R. pseudoacacia* and *R. viscosa* Vent. (De Prins and De Prins, 2022).

The mine has the form of a yellowish blotch, usually occurs on the upper side, but occasionally also on the

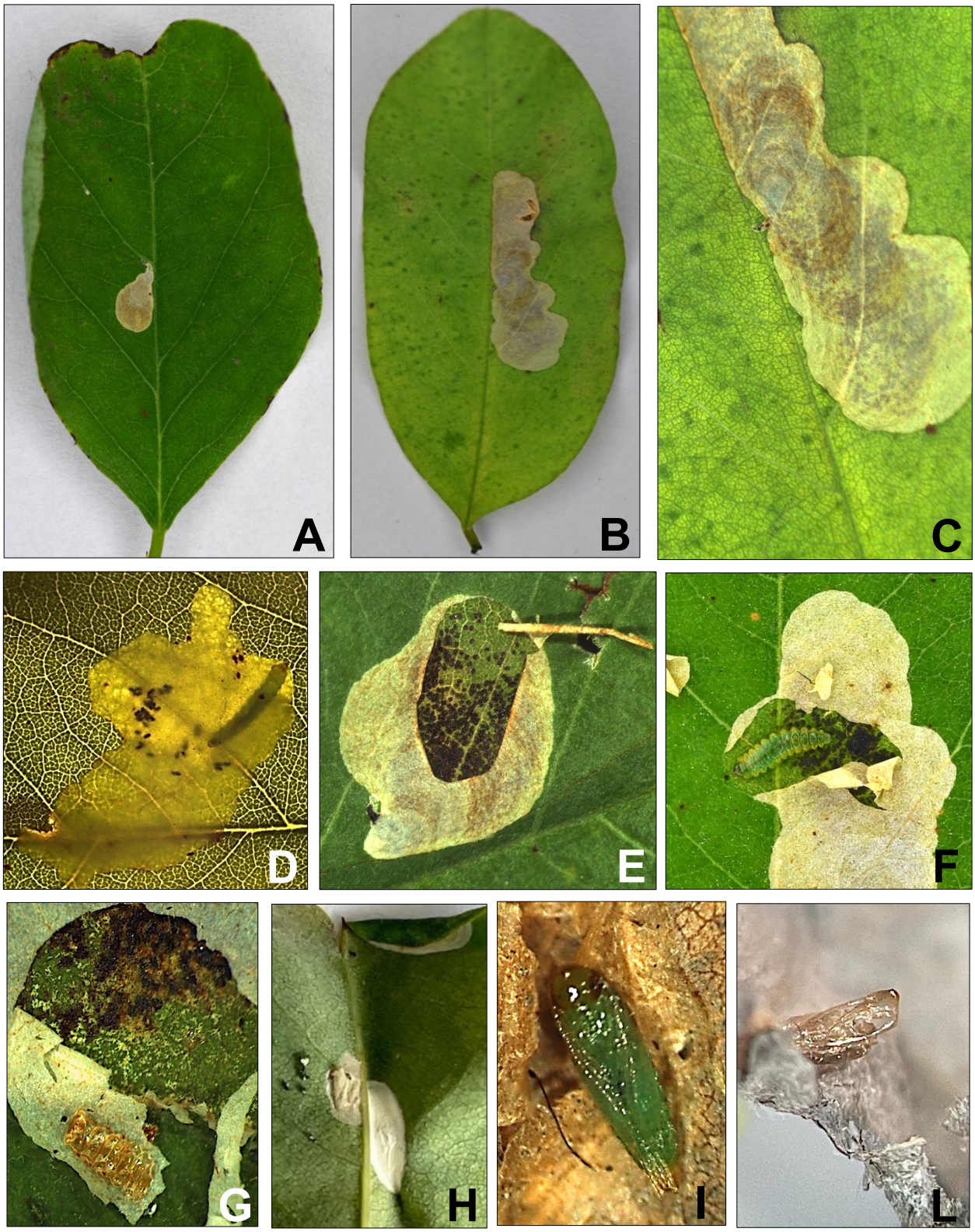

Figure 4. *Chrysaster ostensackenella*. Leaf-mine with a visible short tunnel preceding the blotch mine (**A**); leaf-blotch mines on the upper side of the leaves (**B**, **C**); leaf-blotch mine with first instar larva (**D**); opened leaf-mine with frass (**E**); dissected leaf-mine with larva (**F**); opened mine with larval exuvia (**G**); white silky cocoons on the lower side of the leaf (**H**); pupa (**I**); exuvia of pupa (**L**).

underside, of the leaflet. It is nearly circular in its early stages, but later in development, it becomes irregular and elongate in shape. The mines exhibit a colour change from yellow to light hazelnut; the frass is scattered in the mine and becomes more visible in gently pressed leaflets.

From the oviposition site, a short tunnel follows a secondary vein before suddenly widening into a blotch. The mines were primarily located next to the main vein on one of the halves of the leaflet. Based on our observations each mine contained a single larva. The late instar larva is yellow-greenish in colour. Prior to pupation, the larva creates a semi-circular cut in the upper epidermis near the margin of the mine as an exit. Sometimes the last larval exuvia is observed in the mine. Pupation occurs outside the leaf mine, inside a white tough cocoon on the lower side of the leaf at the slightly deformed or bent leaflet surface along the secondary vein, or hidden in the litter. The pupa is greenish dark brown in colour.

The number of generations per year that *Ch. ostensackenella* may develop in Italy is unclear, but is assumed to be three. In Primorsky Krai it may have two generations, in China it develops up to four generations per year (Kirichenko *et al*., 2023).

In central-southern Italy, *Ch. ostensackenella* is present mainly in the Adriatic part of Molise region, from the plains and hills up to 800 m a.s.l.

It is possible that this species has been introduced from North America with ship containers or even with living ornamental plants. Alternatively, it may have reached Italy through the steadily intensifying cargo or passenger traffic between China and Europe (e.g., through one of the several international airports in central Italy), perhaps at the pupal stage with fallen leaf mines.

Parectopa robiniella Clemens 1863
(figures 1B, 2B, 3B, 5A-H)

M a t e r i a l e x a m i n e d

Mines: several mines observed and collected from June to October of the years 2022 and 2023 in all investigated regions.

Adults: 1 male and 2 females, 7.VII.2022, Ferrazzano (Campobasso), 830 m a.s.l., ex mines of *R. pseudoacacia*, leg. P. Trematerra (coll. P. Trematerra). Additional uncatalogued material.

D i s t r i b u t i o n

The locust digitate leaf-miner, *P. robiniella*, is native to North America and was accidentally introduced to Italy, where it was first found close to Milan International Airport in 1970 (Vidano, 1970). From there, it gradually spread and was recorded extensively across Europe (Italy, France, Belgium, Germany, Lithuania, Slovenia, Croatia, Austria, Serbia, Slovakia, Romania, North Macedonia, Ukraine, Hungary, Southern Russia) (Whitebread, 1990; Trematerra and Zapparoli, 1994; De Prins and De Prins, 2022), reportedly moving mostly westward at an average rate of about 35 km/year (Mally *et al.*, 2021).

A d u l t m o r p h o l o g y

The adult wingspan is about 5 mm; dark brown head that is topped with elongated, white scale tufts. The forewings are dark brown with three oblique silvery costal streaks that alternate with three dorsal oval blotches or streaks. Near the tip of the wing, a transverse, curved silvery line is present from the costa to the tornus. There are also two converging dark brown to black lines at the base of the cilia that do not meet. Hindwings brown, cilia tinged brownish.

B i o l o g y a n d e c o l o g y

P. robiniella mines the leaves of the host plants *Amorpha fruticosa* L., *Desmodium* sp., *Galactia volubilis* L. Britton, *Meibomia* sp. and *Robinia* spp., including *R. hispida*, *R. pseudoacacia* and *R. viscosa* (Eiseman, 2019; De Prins and De Prins, 2022). The species makes distinctive digitate mines on the upper leaf surface after initially forming a mine on the lower surface. The common name is derived from "digitate", referring to the "finger-like" excavations all around the margins of the central blotch of the mine.

The female lays its eggs on the underside of the leaf of plants. The young larva causes a small irregular white mine in the angle of the midrib and a lateral vein, then works itself to the leaflet's upper surface and makes an upper-surface blotch on top of the midrib.

The greenish larva lives solitarily and defecates outside its mine; frass is ejected from the midrib and is partly arranged in an orderly row along one side of the mine.

The prepupa is formed in a cocoon in the place of pupation; pupation takes place outside the mine in a white cocoon attached to the deformed or bent leaf surface along the secondary vein, or hidden in the litter in the case of the autumnal generation. The overwintering stage is adult, hiding in shelters of various types.

In Italy there are currently 2 or 3 overlapping generations per year from June to September-October. The number of generations may vary depending on climatic conditions.

We observed that infestations are more abundant in the lowlands, with more mines per leaf, even together with other mining species. Damage is most evident in late summer, with early defoliation of the plant rarely occurring.

P. robiniella is widespread in all of Italy, especially along commercial traffic routes, from sea level up to the high hills.

Macrosaccus robiniella (Clemens 1859)
(figures 1C, 2C, 3C, 6A-I)

M a t e r i a l e x a m i n e d

Mines: several mines observed and collected from June to October 2022-2023 in Latium, Abruzzo, Molise, and Campania regions.

Adults*:* 1 male and 1 female, 18.VII.2022, Paliano (Frosinone), 450 m a.s.l., ex mines from *R. pseudoacacia*, leg. M. Colacci; 2 males and 1 female, 27.VIII.2023, Bojano (Campobasso), 470 m a.s.l., ex mines from *R. pseudoacacia*, leg. M. Colacci (coll. P. Trematerra). Additional uncatalogued material.

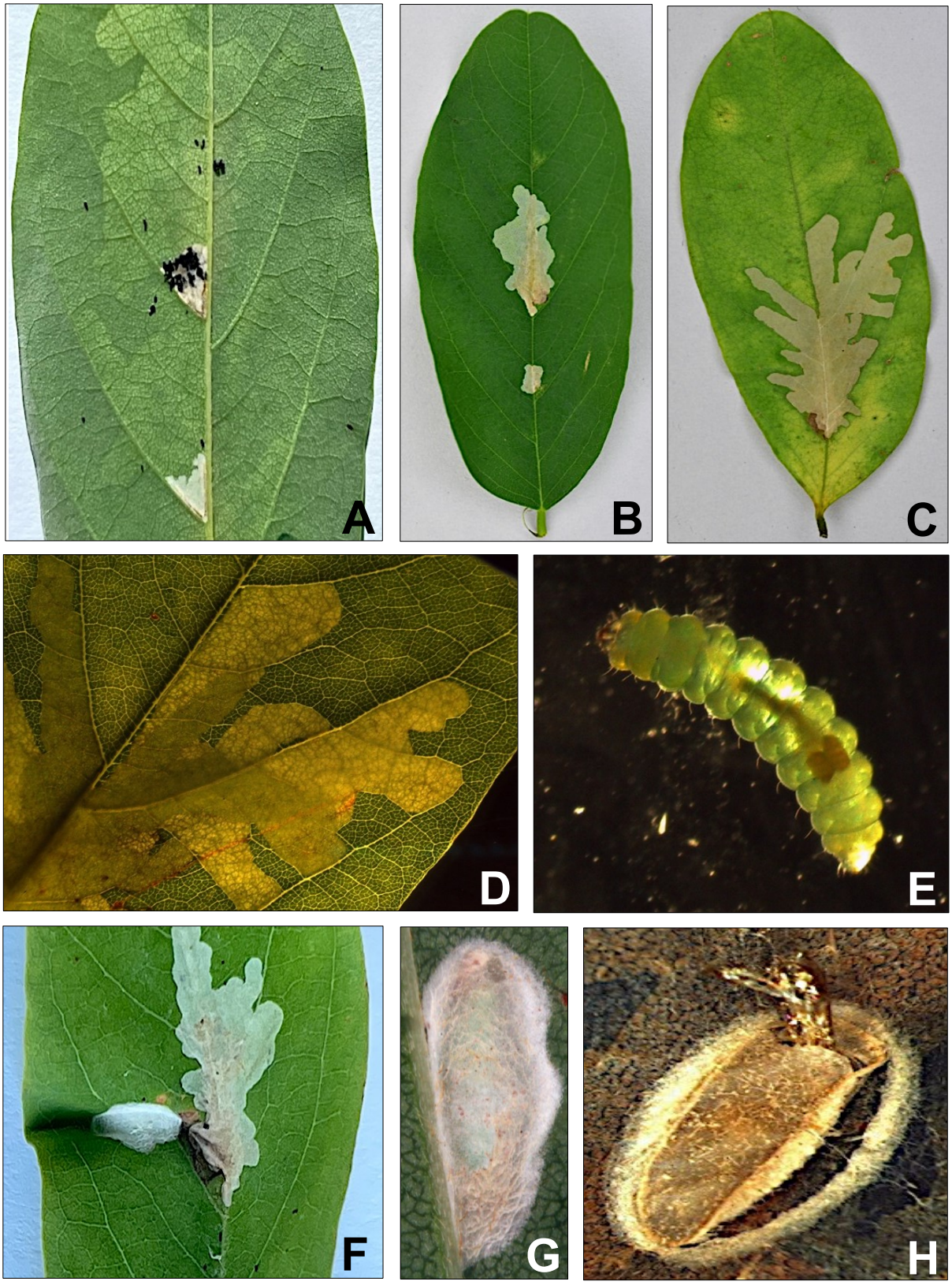

Figure 5. *Parectopa robiniella*. Lower surface of leaf-mine and midrib mine with frass (**A**); upper side early instar leaf-mines (**B**); upper side of digitate leaf-mine (**C**); lower side of digitate leaf-mine (**D**); mature larva (**E**); mina and pupa in a white cocoon (**F**); cocoon with pupa (**G**); detail of pupal exuvia out of the cocoon (**H**).

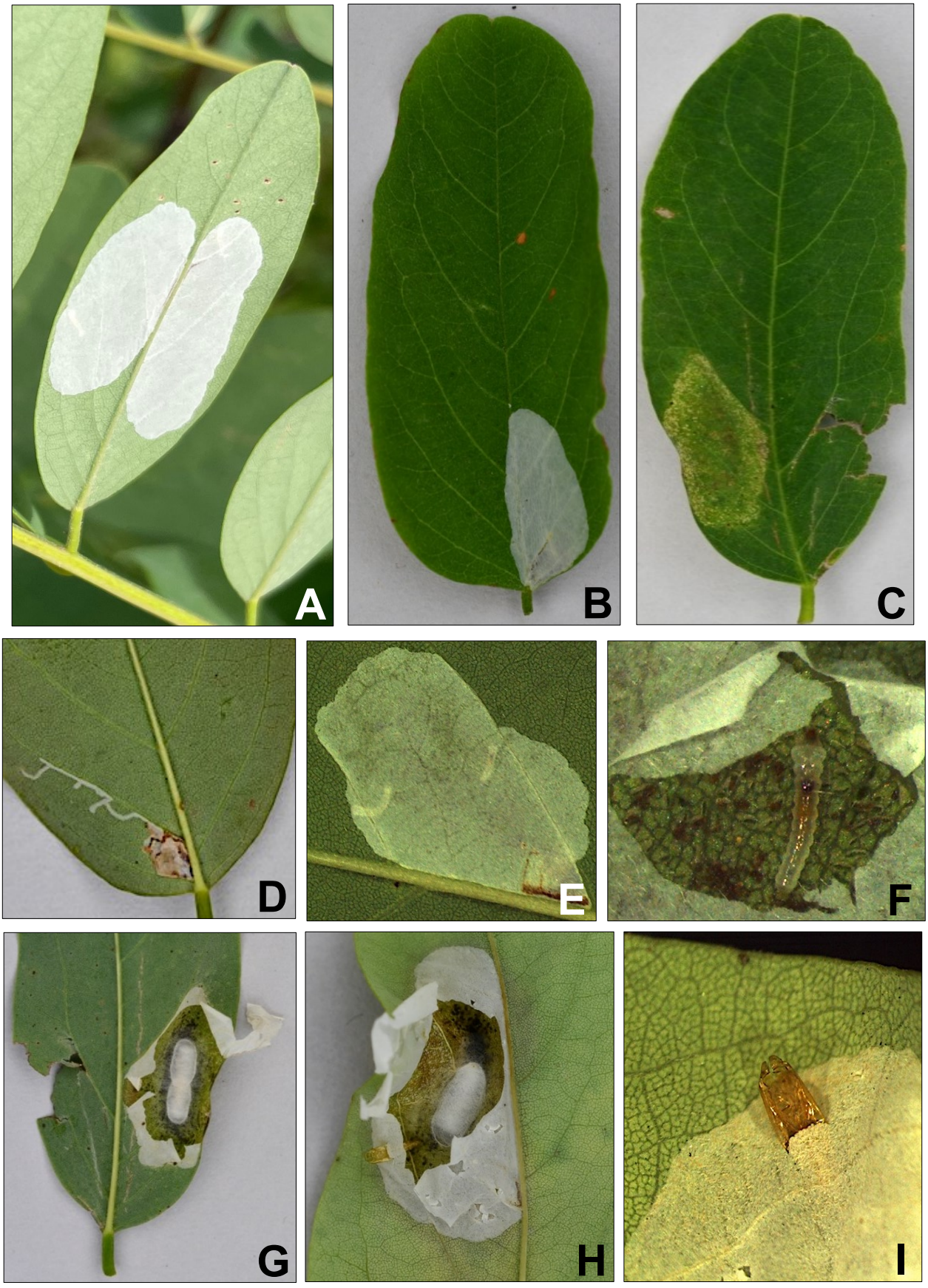

Figure 6. *Macrosaccus robiniella*. Lower side blotch leaf-mines (**A**); upper side leaf-mine (**B**); upper side blotch leaf-mine (**C**); early instar serpentine leaf-mine (**D**); blotch leaf-mine with two early instar larvae (**E**); larva inside open leaf-mine (**F**); open mines with two cocoons (**G**); leaf-mine with a cocoon and exuvia of pupa (**H**); detail of pupal exuvia (**I**).

D i s t r i b u t i o n

The leaf blotch miner moth, *M. robiniella*, formerly assigned to the genus *Phyllonorycter* Hubner, is native to and widely distributed in North America. It was accidentally introduced into Europe in 1983, where it was first found in France and Switzerland (Whitebread and Joos, 1986; Whitebread, 1990) before arriving in North Italy in 1988 (Bolchi-Serini and Trematerra, 1989; Trematerra and Bolchi-Serini, 1991) and has subsequently quickly invaded Europe, ranging from Spain to central-southern Russia (Davis and De Prins, 2011; Mally *et al.*, 2021; Kirichenko *et al*., 2023).

A d u l t m o r p h o l o g y

The adult wingspan is 5.5 to 6.5 mm; head tuft dark- or reddish-brown, mixed with white. Frons and labial palpi white. Thorax and abdomen dark grey to brown ferruginous. Forewings pattern complex, shining orange-brown, paler toward costa. Four white costal strigulae. Apex of forewing with a large black apical spot. Cilia pale greyish-brown. Hindwings pale grey, cilia tinged brownish-grey.

B i o l o g y a n d e c o l o g y

The larvae of *M. robiniella* feed on *Robinia neomexicana*, *R. hispida*, *R. pseudoacacia* and *R. viscosa* (De Prins and De Prins, 2022).

Moths lay their eggs on the lower surface of the leaves. The mine begins as an elongate serpentine track which enlarges to an elongate-oval, whitish blotch located on one side of the midrib. The mine becomes slightly tentiform due to the silk laid down by the later instar larvae. Many mines may be formed on a leaf and even on a single leaflet. The body colour of larvae is hyaline, pale green to white. There are five larval instars. Pupation takes place in a thick silky cocoon, which is clearly visible in the mine. The pupa is suspended by numerous silken threads attached to the roof of the mine. In high-density populations, multiple pupae can be found within a single mine. Very fine frass grains are loosely scattered throughout the mine, but more aggregated in the central part. The overwintering stage is unclear but it may be adults sheltering in ravines.

In Italy, *M. robiniella* completes 4-5 overlapping generations per year, from June to October, but the number may vary depending on climatic conditions. Mines are found on leaflets with maximum development, especially on the basal branches of the plants.

The species is present in north and central-southern Italy, especially along commercial traffic routes, from sea level up to the high hills at 1000 m a.s.l.

Comments

The characteristics of the blotch mines of *Ch. ostensackenella*, *P. robiniella* and *M. robiniella* can help distinguish between the different species. The leaf-mines of *P. robiniella* and *Ch. ostensackenella* are located on the upper side of the leaf, whereas the mine of *M. robiniella* is mostly found on the lower side of the leaf. Compared to the blotch mine of *P. robiniella*, the mine of *Ch. ostensackenella* is not strictly associated with the main vein and has a relatively smooth edge. The blotch mine of *Ch. ostensackenella* begins with a short tunnel, while the digitate mine of *P. robiniella* does not have a preceding tunnel. The mine of *M. robiniella* is typically found on one half of the leaflet and is not strictly associated with the main vein, like the mine of *P. robiniella*. The mine of *M. robiniella* is always white with the slightly folded epidermis covering the mine, giving it a somewhat voluminous tentiform appearance.

P. robiniella and *Ch. ostensackenella* pupate outside of the leaf mine, while *M. robiniella* larvae pupate inside the mine.

The three leaf-miners in central-southern Italy have been found at the same time and on the same plant and even on the same leaf, in the following combinations: *P. robiniella* + *Ch. ostensackenella* or *P. robiniella* + *M. robiniella*. We never observed mines of the three species together on the same plant.

In the future, it is necessary to observe if the simultaneous and combined feeding activity of the three leaf-miners on *Robinia* plants, with several insect generations per year, may result in high percentages of damaged leaves, which, in particular situations, could negatively impact plant flowering and subsequent honey production.

During our two-seasonal observations, parasitoid emergence from *Ch. ostensackenella*, *P. robiniella* and *M. robiniella* mines was only occasionally observed. In the time to come, it will be interesting to study what role beneficial insects and natural enemies will play in connection with these pests.

Acknowledgements

We are grateful to Stefano Maini (University of Bologna, Italy) for his information and helpful comments. We would like to thank reviewers, we appreciate all valuable comments and suggestions, which helped us in improving the quality of the manuscript.

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Received October 17, 2023. Accepted January 17, 2024.