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Characterization, distribution, biology and impact on Italian walnut orchards of the invasive North-American leafminer *Coptodisca lucifluella* (Lepidoptera: Heliozelidae)

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Abstract

The leafminer *Coptodisca* sp. (Lepidoptera: Heliozelidae), recently recorded for the first time in Europe on Italian black and common walnut trees, is shown to be the North-American *Coptodisca lucifluella* (Clemens) based on morphological (forewing pattern) and molecular (cytochrome oxidase c subunit I sequence) evidence. The phylogenetic relatedness of three species feeding on Juglandaceae suggests that *C. lucifluella* has likely shifted, within the same host plant family, from its original North-American hosts *Carya* spp. to *Juglans* spp. Over the few years since its detection, it has established in many regions in Italy and has become a widespread and dominant invasive species. The leafminer completes three to four generations per year, with the first adults emerging in April–May and mature larvae of the last generation starting hibernation in September–October. Although a high larval mortality was recorded in field observations (up to 74%), the impact of the pest was substantial with all leaves infested at the end of the last generation in all 3 years tested. The distribution of the leafminer in the canopy was homogeneous. The species is redescribed and illustrated, a lectotype is designated and a new synonymy is established.

Keywords: COI, damage, host-shift, *Juglans regia*, *Juglans nigra*, leafminer, phenology, phylogeny

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Introduction

In 2010 a leafminer of the genus *Coptodisca* Walsingham (Lepidoptera: Heliozelidae) was collected for the first time in Europe, both on black walnut (*Juglans nigra* L.) and common walnut (*Juglans regia* L.) (Juglandaceae) (Bernardo *et al.*, 2011, 2012). *Coptodisca* is a Nearctic genus that includes 18 described species of tiny leafminer moths (Lafontaine, 1974; Davis, 1983; 1998, online Supplementary material 3). Currently, there is only one dichotomous key for identifying the five *Coptodisca* species that feed on Ericaceae (Lafontaine, 1974). Species are described mostly on the basis of forewing colour pattern (Chambers, 1874; Busck, 1878; Braun, 1916; Dietz, 1921). The genitalia have been described or illustrated for only four species (Opler, 1971; Lafontaine, 1974) and these descriptions and illustrations are inadequate for taxonomic purposes. Therefore, identification of adults is nearly impossible when

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*Author for correspondence e-mail: bernardo@ipp.cnr.it the host plant is unknown. In addition, DNA sequences have thus been published for *Coptodisca kalmiella* Dietz (Pellmyr & Leebens-Mack, 1999; Wiegmann *et al.*, 2002); and *Coptodisca lucifluella* (Clemens) (as *C. ella*: van Nieukerken *et al.*, 2012), which limits the use of a molecular identification approach.

Adults of all *Coptodisca* species share a forewing that is whitish or metallic silver in the basal half and golden or yellow in the apical half, with two opposing white triangles at two-thirds of the length of the wing, and a black apical patch. Individuals of some species have a greater or lesser suffusion of dark-grey scaling on the basal part of the forewing (Davis, 1998).

As in the other Heliozelidae, *Coptodisca* females pierce the underside of a leaf using a greatly extensible, piercing ovipositor to lay eggs singly within the leaf (Davis, 1998). After hatching, the developing larva excavates a mine in the host leaf by eating the mesophyll. Once mature, the larva constructs a flat, oval case by cutting a disc from the upper and lower epidermis of the mine and joins them with silk to form a cocoon (Davis, 1998). In most instances, the larvae produce a silk strand to reach the ground, and there pupate among leaf litter or by attaching their cases to the bark or leaves of the host plant. Abandoned mines with small oval holes are characteristic of the leaf damage caused by these leafminers.

Coptodisca species have one or more generations annually (Slingerland & Crosby, 1914; Weiss & Beckwith, 1921; Hileman & Lieto, 1981; Maier, 1988; Davis, 1998), and overwinter as a larva inside a cocoon case (Braun, 1916; Weiss & Beckwith, 1921) or as an egg (in case of evergreen plants: Opler, 1971; Hileman & Lieto, 1981; Maier, 1988; Hespenheide, 1991; Rickman & Connor, 2003), with pupation and adult emergence occurring the following spring (Slingerland & Crosby, 1914; Davis, 1998). Hibernating diapausing pupae have been recorded only for *C. lucifluella* and an unidentified species (Brown & Eads, 1969; Heyerdahl & Dutcher, 1990). The pupal exuviae protrude from the case upon eclosion (Weiss & Beckwith, 1921; Payne *et al.*, 1972).

The only named species of *Coptodisca* known to attack walnut trees is the North-American walnut shield bearer *C. juglandiella* (Chambers, 1874), which has been recorded to feed only on the black walnut, *J. nigra* (Chambers, 1874). A precise characterization of the invasive species and a good knowledge of its current geographical distribution are the first steps in evaluating its potential hazard and defining management options. Therefore, the first aim of this paper was to provide a morphological and molecular characterization of the leafminer. Secondly, several different biological parameters were studied (cycle, number of generations, mortality, distribution on trees, etc.), as there was little known about the biology of this leafminer and its biology could be affected by association with different host plants and by its invasion into a new area. Finally, the level of infestation and the kind and the intensity of damage in walnut orchards were studied to evaluate the necessity of pest control.

Material and methods

Taxonomic characterization

Morphological analysis

To identify *Coptodisca*, we compared the specimens with the original descriptions of the genus *Coptodisca* (as *Aspidisca*: Clemens, 1860) and all of its species (checklist in online Supplementary material 3). In addition, we compared the specimens collected in Italy with available type material, with additional museum material borrowed from North-American collections, and with the specimens recently collected in North America by EJvN (see online Supplementary material 1 for details).

Morphological details were taken from mounted dry specimens or dissected specimens mounted on permanent slides in balsam–phenol or euparal. For morphological methods (dissection and photographing of genitalia and wings) and terminology see van Nieukerken *et al.* (2012). Male genitalia of *Coptodisca* are difficult to dissect and embed in a fixed position, because of their small size and almost cylindrical shape; we therefore studied genitalia first in glycerine. The number of antennal segments in adult moths was counted in preparations of descaled adults (see the species description below).

Molecular analysis

We used part of the mitochondrial cytochrome oxidase c subunit I (COI) gene, also known as the 'DNA barcode marker for animals'; a widely used marker suitable for systematics at the species level (Hebert *et al.*, 2004). Previously as phylogenetic reconstruction had not been attempted for the genus *Coptodisca*, and to have a robust inference we included eight species for which fresh material was available and that could be identified to species (*C. arbutiella* Busck, *C. juglandiella*, *C. kalmiella*, *C. lucifluella*, *C. negligens* Braun, *C. quercicolella* Braun, *C. saliciella* Clemens, *C. splendoriferella* Clemens) plus three specimens that could not be assigned to any known species (here indicated as *Coptodisca* sp. 1, 2 and 3). A total of 28 specimens were selected for molecular analyses (table 1). DNA from the abdomen of dry specimens or from fresh larvae killed and stored in alcohol was extracted using a Chelex–proteinase K-based protocol as in Gebiola *et al.* (2009) for Italian specimens and the Qiagen DNeasy Blood & Tissue Kit or a Macherey Nagel magnetic bead tissue kit on an automated KingFisher flex system for American specimens. Some extractions from the abdomen of adults or larvae were nondestructive, such that genitalia and larval pelts could be mounted in euparal on slides (Knölke *et al.*, 2005). COI barcodes were obtained using the primers LCO1490 and HCO2198 (Folmer *et al.*, 1994) or LepF and LepR (Hajibabaei *et al.*, 2006); the latter sometimes coupled with the internal primers MH-MR1 and MF1, respectively (Hajibabaei *et al.*, 2006). For American samples, universal tails (for example, M13 or a combination of T7 promotor and T3) were attached to the primers to increase yield and to facilitate higher throughput (Regier & Shi, 2005). PCR cycles were as in Gebiola *et al.* (2009). Amplicon size was checked on a 1.2% agarose gel, then the DNA was purified and sequenced at XiLin sequencing in Beijing, China (Italian specimens) or at Macrogen Europe or Baseclear, Leiden (American specimens). Ambiguous sections of chromatograms from Italian specimens were edited by eye using BioEdit (Hall, 1999), while Sequencher 4.2 (Gene Codes Corporation) or Geneious R6 (http://www.geneious.com) software was used to assemble the forward and reverse sequences of American specimens. A maximum likelihood tree was obtained using RAxML 7.0.4 (Stamatakis, 2006) after 1000 multiple inferences on the original alignments using the GTRCAT nucleotide model, starting from a random parsimonious tree, with the default initial rearrangement settings and the number of rate categories. Branch support was based on 1000 rapid bootstrap pseudoreplicates. *Incurvaria masculella* (Denis & Schiffermüller)

Table 1. Specimens used for molecular analyses, with host plant, collection localities and dates, geographical coordinates and Genbank accession numbers.

Code	Species	Host plant	Locality	Longitude	Latitude	Altitude (m a.s.l.)	Date	Collector	Genbank code	Stage
CAr01	*Coptodisca arbutiella*	*Arbutus menziesii*	West Vancouver, British Columbia, Canada	49°22′14″	−123°17′30″	5	12 April 2013	D.G. Holden	KJ426998	Larva
CAr02	*C. arbutiella*	*A. menziesii*	West Vancouver, British Columbia, Canada	49°22′14″	−123°17′30″	5	12 April 2013[1]	D.G. Holden	KJ426999	Adult
CJ01	*C. juglandiella*	*J. nigra*	Smokemont Campground, North Carolina	35°33′29″	−83°18′43″	675	29 September 2010	EJvN & C. Doorenweerd	KJ427001	Larva
CL01	*C. lucifluella*	*J. regia*	Palma Campania, Italy	40°49′56″	14°33′20″	16	31 July 2013	UB&SV	KJ427006	Adult
CL02	*C. lucifluella*	*J. regia*	Palma Campania, Italy	40°49′56″	14°33′20″	16	31 July 2013	UB&SV	KJ427005	Adult
CL03	*C. lucifluella*	*J. regia*	San Giorgio a Cremano, Italy	40°50′26″	14°21′08″	89	7 August 2013	UB&SV	KJ427004	Adult
CL04	*C. lucifluella*	*J. regia*	Palma Campania, Italy	40°49′56″	14°33′20″	16	10 October 2013	UB&SV	KJ427003	Larva
CL05	*C. lucifluella*	*J. regia*	Palma Campania, Italy	40°51′54″	14°32′57″	56	16 October 2013	UB&SV	KJ427002	Larva
CL06	*C. lucifluella*	*J. regia*	Grosseto, Italy	42°57′42″	11°05′14″	75	5 October 2013	F. Del Core	KJ427012	Larva
CL07	*C. lucifluella*	*J. regia*	Palma Campania, Italy	40°51′54″	14°32′57″	56	29 October 2013	UB&SV	KJ427011	Larva
CL08	*C. lucifluella*	*J. regia*	Bracciano, Italy	42°06′15″	12°10′35″	276	25 October 2013	R. Sasso	KJ427010	Larva
CL09	*C. lucifluella*	*J. regia*	Bracciano, Italy	42°06′15″	12°10′35″	276	25 October 2013	R. Sasso	KJ427009	Larva
CL10	*C. lucifluella*	*J. regia*	Bracciano, Italy	42°06′15″	12°10′35″	276	25 October 2013	R. Sasso	KJ427008	Larva
CL11	*C. lucifluella*	*J. regia*	Negrar, Italy	45°31′05″	10°55′42″	163	25 June 2012	P. Triberti	KJ427013	Adult
JQ412564	*C. lucifluella*[2]	*Carya glabra*	Cades Cove N., Tennessee	35°45′04″	−83°06′32″	570	1 October 2010	EJvN & C. Doorenweerd	JQ412564	Larva
CL12	*C. lucifluella*	*C. glabra*	Mansfield, Hunters Run, Connecticut	41°46′08″	−72°14′53″	178	8 September 2011	EJvN	KJ427014	Larva
CL13	*C. lucifluella*	*C. glabra*	Cades Cove N., Tennessee	35°45′04″	−83°06′32″	555	1 October 2010	EJvN & C. Doorenweerd	KJ427015	Larva
CL14	*C. lucifluella*	*J. regia*	Negrar, Italy	45°31′05″	10°55′42″	173	5 October 2012[1]	P. Triberti	KJ427007	Adult
CN01	*Coptodisca negligens*	*Vaccinium corymbosum* L.	Harvard Forest, Petersham, Massachusetts	42°32′02″	−72°11′24″	335	19 September 2011	EJvN	KJ427016	Larva
CN02	*C. negligens*	*V. corymbosum* L.	Connecticut College Arboretum, Connecticut	41°22′44″	−72°06′40″	60	10 September 2011	EJvN	KJ427017	Larva
CO01	*Coptodisca ostryaefoliella* Clemens	*Ostrya virginiana* (Mill.) K. Koch	Natchez Trace Parkway, Rock Spring, Alabama	34°51′29″	−87°54′22″	140	5 October 2010	EJvN & C. Doorenweerd	KJ427018	Larva
CQ01	*Coptodisca quercicolella*	*Quercus gambelii* Nutt	Spruce Tree Loop, Colorado	37°10′55″	−108°29′20″	1950	13 July 2012	EJvN	KJ427020	Larva

CSa01	*Coptodisca saliciella*	*Salix* sp. L.	Putah Creek, California	38°31′12″	−121°45′36″	17	28 October 2012	C. Eiseman	KJ427021	Larva
CSa02	*C. saliciella*	*Salix* sp. L.	Putah Creek, California	38°31′12″	−121°45′36″	17	28 October 2012	C. Eiseman	KJ427022	Larva
CSp01	*Coptodisca splendoriferella* (Clemens)	*Prunus serotina* Ehrh	Bay Springs Lake, Rec. Area W shore, Mississippi	34°32′06″	−88°20′20″	130	5 October 2010	EJvN & C. Doorenweerd	KJ427023	Larva
CSp02	*Coptodisca splendoriferella* (Clemens)	*P. serotina* Ehrh	Wallis Street, Mississippi	42°03′50″	−71°46′26″	210	19 September 2011	EJvN & C. Doorenweerd	KJ427024	Larva
CA01	*Coptodisca* sp. 1 ‘Amelanchier_USA’	*Amelanchier utahensis* Koehne	NP Black Canyon of the Gunnison, Sth Rim, Colorado	38°33′14″	−107°41′10″	2390	8 July 2012	EJvN	KJ426997	Larva
CC01	*Coptodisca* sp. 2 ‘Carya_Georgia’	*Carya* sp.	Cohutta Overlook, Georgia	34°47′06″	−84°37′37″	730	14 October 2010[1]	EJvN & C. Doorenweerd	KJ427000	Adult
CP01	*Coptodisca* sp. 3 ‘Populus_CA’	*Populus fremontii* S. Watson	Putah Creek, California	38°31′12″	−121°45′36″	17	26 October 2012	C. Eiseman	KJ427019	Larva
AF150907	*Coptodisca kalmiella* (Dietz)	–	Litchfield Co., Connecticut					D.L. Wagner	AF150907	
AF150926	*Incurvaria masculella* (Denis & Schiffermüller)	–	Sweden						AF150926	
JQ412568	*Heliozela sericiella* Haworth	–	Lemland, Finland	60°0′0″	20°27′36″		19 May 2007	M. Mutanen	JQ412568	Adult
JQ412555	*A. treitschkiella* (Fischer von Röslerstamm)	*Cornus mas* L.	Leiden West, Netherlands	52°10′12″	4°28′26″		17 October 2004	EJvN	JQ412555	Larva
JQ412542	*A. oinophylla*	*Vitis aestivalis* Michz.	Murray Co., Georgia	34°44′27″	−84°43′8″	523	14 October 2010	EJvN & C. Doorenweerd	JQ412542	Larva
JQ412572	*H. rivillei* (Stainton)	*Vitis vinifera* L.	Trento, Italy	45°44′10″	10°56′13″		18 January 2008	M. Baldessari	JQ412572	Adult

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[1] Date of collection of larva, the sequenced adult reared later from these larvae.

[2] Originally published under the name *Coptodisca ella*.

EJvN,E.J. van Nieukerken; UB&SV, Umberto Bernardo & Salvatore Vicidomini.

(Lepidoptera, Incurvariidae), *Heliozela sericiella* (Haworth), *Antispila treitschkiella* (Fischer von Röslerstamm), *Antispila oinophylla* (van Nieukerken & Wagner) and *Holocacista rivillei* (Stainton) (Lepidoptera, Heliozelidae) were used as outgroups to root the tree. Specimens used in this study are listed in table 1 along with GenBank accession numbers (KJ426997-KJ427024); sequence data are also available in the public BOLD dataset '*Coptodisca lucifluella* in Italy' **[dx.doi.org/10.5883/DS-COPIN]**. Uncorrected intra- and interspecific *p*-distances were calculated with Mega 4 (Tamura *et al.*, 2007).

Biological characterisation

The observations of life cycle, the level of damage and stage of invasion were conducted by sampling in agricultural and urban environments, in organically and chemically treated orchards (all trees of chosen fields were used to collect samples), in two Italian regions: Lazio and Campania. Further sampling was conducted in other Italian regions to evaluate the distribution of *C. lucifluella*. Geographical coordinates and altitude are presented in Online Resource 2. A site was suspected to be infested when mines and holes were present in the leaves of the sampled plants. The life cycle and the levels of infestation during the leafminers' last generation were studied from September 2010 throughout October 2013. Larval instars were recorded from 15 randomly collected leaves, twice monthly between June and September 2012 in Bracciano (RM, Lazio) and San Giorgio a Cremano (NA, Campania). In 2013, samplings were performed weekly, and extended to three other orchards (sites 1, 2, 3) in Palma Campania (NA) (online Supplementary material 2).

Individual leaflets were considered as sample units; however, the percentage of infestation was also calculated for compound leaves to give an estimate of distribution of infestation on the tree. The number of mines and holes per leaflet were recorded and the percentage of infestation was calculated as [infested leaflets (or leaves) × 100/sampled leaflets (or leaves)]. Furthermore, to have an estimate of leaves with greater damage due to the construction of the pupal cases, the percentage of leaflets with holes was calculated as [number of leaflets with holes × 100/sampled leaflets]. Lastly, the percentage of larval mortality from the first instar to mature larvae was calculated as [100−(number of holes per leaflets × 100/number of mines per leaflets)]. Living and dead larvae were singly isolated to evaluate the level of parasitization (study in progress) while the remaining part of the leaves were stored in plastic bags at 25°C in a climatic chamber, to evaluate the presence of eggs by checking for new mines after 1 week. Larval mortality in the pupal cases was not included in this study.

Sampling stopped when no more living larvae could be found in the sampled trees. The last sampling event of the year was the latest in which at least one living larva was recorded.

Samples collected in San Giorgio a Cremano were used to record the mean dimension of complete mines and pupal cases. They were measured with a stereomicroscope at a magnification of 25 ×. The following measurements were recorded: maximum length and width of mines, and length and width of pupal cases. The area of the oval pupal cases was calculated with the formula [$\text{width} \times \text{length} \times (2 \times \pi)^{-1}$]. A total of 44 adults collected in Italy (40 in San Giorgio a Cremano and 4 in Negrar) and 17 collected in the USA (online Supplementary material 1) were used to measure forewing length.

To evaluate the distribution in the canopy, an additional sampling in the Palma Campania orchards was made on 29 October 2013. The same number of leaves (15 compound leaves) was collected at 2 and 6 m from the ground by hand from low branches and with the aid of lopping shears from the medium–high canopy.

Statistical analyses

To assess whether the differences of infestation (of leaflets), the number of mines, the number of holes and the different larval mortality recorded in different years at the same locality, were significantly diverse, data satisfying conditions of normality and homoscedasticity, untransformed or after appropriate transformation, were analysed by analysis of variance (ANOVA). Means were subsequently separated at the 0.05 level of significance by a multiple range test (Tukey HSD or, in the case of unequal samples, Bonferroni). In all the other cases, a non-parametric test (Kruskal–Wallis) was used and medians were graphically separated by a Box-and-Whisker plot analysis (Statgraphics plus, 1997). Proportions of infested leaflets and leaves with holes between different years of sampling of the same field and the percentage of larval mortality (from the first instar to mature larvae) were compared by *G* test (Sokal & Rohlf, 1995). All data are presented non-transformed with their standard error (±SE).

Results

Taxonomic characterization

Morphological analysis

All the examined Italian specimens and the American specimens collected on *Carya* species, possess a forewing pattern characteristic of *C. lucifluella* Clemens (and present in the Lectotype, albeit faded due to age; see online Supplementary material 3), with a fuscous dark to almost black suffusion of the dorsal or posterior half of the wing tip, including the area proximal to the first silvery patches (fig. 1a, b). This is a good diagnostic character as all the other *Coptodisca* feeding on Juglandaceae and most other species have this area as pale as the costal region or just a shade darker (see online Supplementary material 3). However, two species feeding on different hosts have a similar forewing pattern: *C. quercicolella* (Braun, 1927; Opler, 1971) feeding on *Quercus* in the Western USA and *C. saliciella* feeding on *Salix* throughout the USA (for example, http://bugguide.net/node/view/849941/bgpage). These species can be diagnosed – apart from their host plants – by small differences in the genitalia (which need to be studied in more detail) and with different DNA barcodes. The American and Italian specimens that were examined also agree in the genitalia (fig. 2), but a lack of a taxonomic revision makes these characters presently less useful for diagnostic purposes.

Molecular analysis

COI barcodes were obtained for all the selected specimens (28) (table 1). No stop codons or frame shifts were detected. The phylogenetic analysis, which is the first for the genus *Coptodisca*, showed that the genus *Coptodisca* is monophyletic,

Fig. 1. Life stages and damage of walnut shield bearer, *Coptodisca lucifluella* on *Juglans regia* in Italy: (a) adult; (b) forewing; (c) larva in mine; (d) larva outside mine; (e) pupal cases on a leaf; (f) pupal cases under the bark; (g) damage on a leaf of *Juglans regia*; (h) mines with holes and pupal cases attached to the edge; (i) pupal exuvium protruding from the case upon eclosion.

although the basal relationships within the genus are not resolved. Among the American specimens, there are two distinct species feeding on *Carya: C. lucifluella* and an unknown, probably new, species from Georgia (*Coptodisca* sp. 2 (Cc01)), which had a COI distance of 11% (online Supplementary material 4). The phylogenetic tree also showed that all specimens collected in Italy (12) belong to the same clade as North American *C. lucifluella* (three specimens) (fig. 3) and that all sequenced *Coptodisca* feeding on Juglandaceae (*C. juglandiella*, *C. lucifluella* and *Coptodisca* sp. 2 (Cc01)) are closely related. Furthermore, the Italian specimens of *C. lucifluella* collected in seven different orchards (five localities) carried only two haplotypes (table 1), whereas each American specimen carried a distinct haplotype (fig. 3).

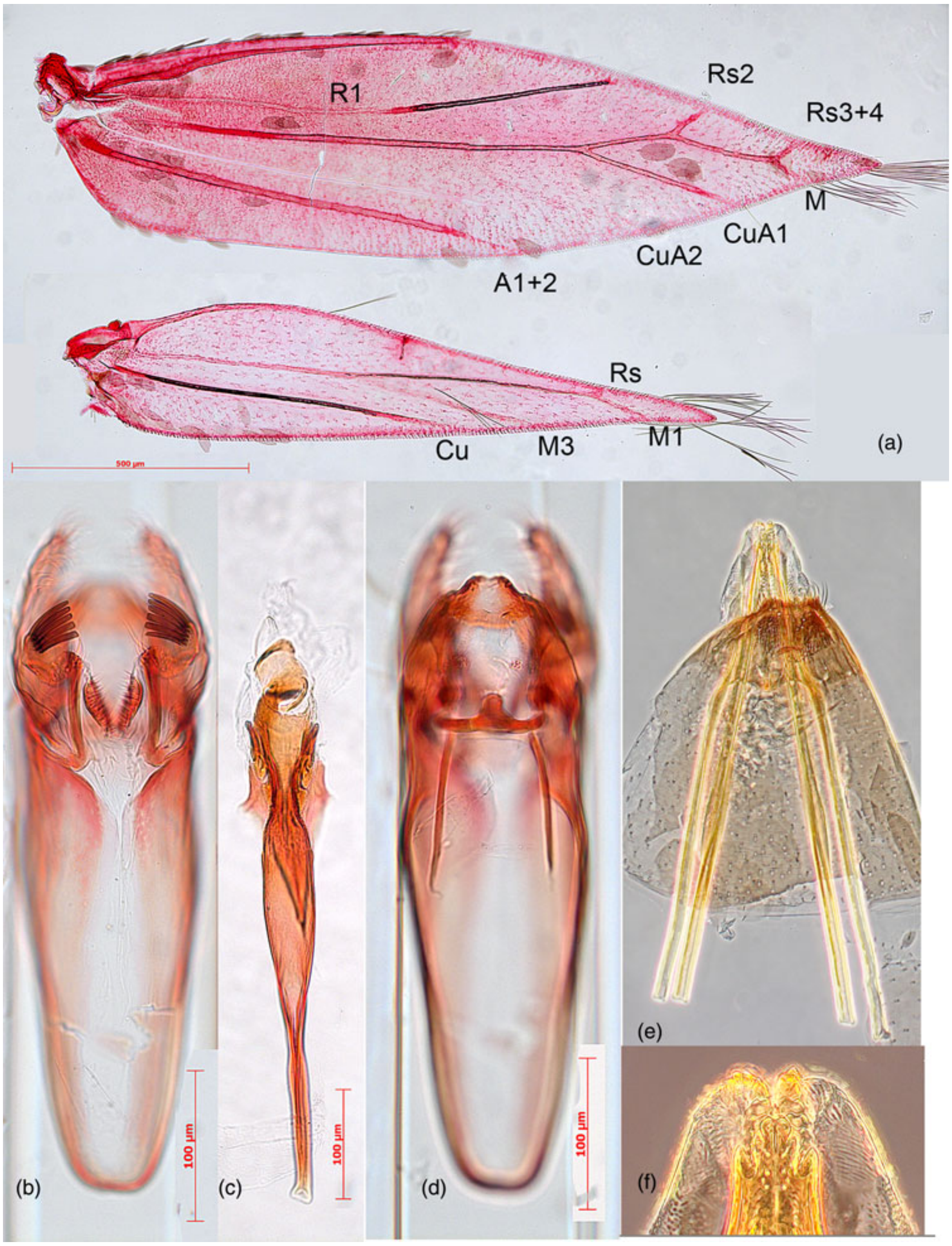

Fig. 2. *Coptodisca lucifluella*, morphology details: (a) wing venation with veins labelled (see text); (b) male genitalia (with phallus removed) in ventral view; (c) phallus in ventral view; (d) male genitalia in a more dorsal view; (e) terminal segments of female with oviscapt and apophyses; (f) detail of oviscapt tip with 5 teeth. a+ c slide EJvN4458, USA, Maryland; b, d slide EJvN4462, Italy, Negrar; e, f slide GV10Cl, Italy, San Giorgio a Cremano.

Species redescription

Based on the morphological and molecular evidence, we concluded that the species that has invaded Italian walnut orchards is *C. lucifluella*. Due to the poor original description (Clemens, 1860), the species is here redescribed using Italian and American material (online Supplementary material 1).

Coptodisca lucifluella (Clemens)

Aspidisca lucifluella Clemens, 1860: 209. **Lectotype** (here designated) ♀: [United States, Pennsylvania, Easton, larva September–October on hickory, imago emerged in June]. Label photographs see online Supplementary material 3.

Aspidisca ella Chambers, 1871: 224. Holotype [probably lost]: [United States, Kentucky, Covington, reared from case found on an Oak tree] **New synonymy**

Aspidisca lucifluella; Chambers (1871): 224.

C. lucifluella; Felt (1906): 717; Forbes (1923): 228; Payne *et al.* (1972): 74.

Coptodisca ella Forbes (1923): 228 [probably a synonym of *lucifluella*]; van Nieukerken *et al.*, 2012: 51.

Coptodisca sp.: Bernardo *et al.*, 2011: 638.

[*Coptodisca juglandiella*: van Nieukerken, 2013; Ellis, 2014. Misidentifications]

Male (fig. 1a, more photos in online Supplementary material 3). Head face and vertex covered with appressed, strongly metallic silvery-white scales, sometimes prominently raised in dried specimens (a drying artefact). Labial palps porrect, white. Maxillar palps small, 1-segmented. Antennae silvery grey with ca. 16 segments and each flagellomere with two rings of scales. Tongue as long as head.

Thorax and basal third of the forewing is silvery white (fig. 1b), usually becoming darker towards the forewing posterior edge. Forewing is from one-third yellow in the anterior (or costal) to two-thirds dark fuscous to almost black in the posterior (dorsal); in the middle along costa there is a triangular silver streak, edged with black on both sides; a second triangular spot, frontally edged black and distally ending against the black, terminal, fan-shaped patch (forming the darkest part of the forewing); posteriorly (on the dorsal edge) there is a triangular silvery streak in the fuscous area, opposite to the first costal streak; in the apical wing part there are two small silvery spots each comprising just a few scales, one spot just frontally of the fan-shaped spot and one just posterior of it; terminally there is a distinct fringe line of grey fringe interrupted by a few black hair-scales running straight from the fan-shaped spot; anteriorly the fringe becomes more yellowish. Hindwing grey, fringe grey but more anteriorly yellowish. Underside of the forewing almost black, anterior (costal) one-third yellowish. Abdomen dark grey.

Measurements: Wingspan 4.1 ± 0.1 mm (3.4–4.7, $n = 13$); forewing length 1.9 ± 0.2 mm (1.6–2.3, $n = 61$), no difference between sexes (1.9 ± 0.06, males $n = 10$, 1.9 ± 0.04, females $n = 11$) (ANOVA test d.f. = 1, $P = 0.94$, $F = 0.01$), nor between USA (1.9 ± 0.04, $n = 17$) and Italian specimens (1.9 ± 0.02, $n = 44$) (ANOVA test d.f. = 1, $P = 0.60$, $F = 0.28$).

Venation (Fig. 2a): Almost identical to that of *A. oinophylla* (van Nieukerken *et al.*, 2012). Forewing with Sc barely visible. R1 a separate vein, connected by persistent trachea to Rs+M stem. Rs+M terminating in five branches, interpreted as Rs2 (possibly with 1) to costa, Rs3 + 4 to costa just before apex, one M branch to dorsum just beyond apex, possibly CuA1 (earlier interpreted as M2 + 3) to dorsum and a weakly developed CuA2. A1 + 2 a strong, separate vein. Hindwing with Sc barely or not visible, Rs+M a strong vein, bifurcate from ca. one-quarter of upper vein ending in two branches: Rs and M1, lower vein single (M3); Cu separate, A absent.

Male genitalia: The general structure of male genitalia (fig. 2b, d) was very similar to all the checked congeneric species. Vinculum elongate and almost cylindrical, combined length of vinculum and tegumen ('capsule') ca. 395–570 µm ($n = 3$). Valva length ca. 130–175 µm; pectinifer with a comb of 5–7 teeth; the few specimens examined suggest that the number of teeth in the left valva is always one higher than in the right valva (fig. 2b). Phallus long, ca. 310–520 µm with lateral rows of ca. 5 annellar spines (fig. 2c).

Female: Colour as male. Antennal segments 14. Abdomen distally pointed. Anterior and posterior apophyses reaching anterior edge of segment V. Oviscapt tip with 5 teeth (fig. 2e, f).

Larvae pale yellow (fig. 1c, d); newly formed pupae pale yellow but darker with age. Pupal case oval (fig. 1e, f, i), mean length 2.92 ± 0.044 mm (2.20–4.18), mean width 1.74 ± 0.025 (1.32–2.42). Pupal case area 8.04 ± 0.216 mm^2 (4.75–14.63) ($n = 60$).

Comments

Probably the only surviving syntype from Clemens' collection, labelled 'Holotype', was examined and selected as the Lectotype (see details and photographs in online Supplementary material 3).

Counting antennal segments of adult moths appears to be difficult in dry specimens, because each antennal segment has two annuli of scales (as in other Adeloidea), a point that was previously overlooked (Davis, 1998; van Nieukerken *et al.*, 2012); in the descriptions of *A. oinophylla* (Lepidoptera: Heliozelidae) the numbers of antennal segments are therefore incorrect and ca. twice the real number. The finding of different numbers of teeth in the left and right valval pecten is interesting, and was also observed in several other *Coptodisca*.

Distribution

Coptodisca lucifluella is widespread in Eastern North America and has been recorded in NE Texas, Mississippi, Georgia, North Carolina, Maryland, Kentucky, Pennsylvania, Ohio, Wisconsin, New York and Connecticut (this study; Clemens, 1860; Chambers, 1871, 187; Payne *et al.*, 1972; Heyerdahl & Dutcher, 1985). It has also been recorded from pecan in New Mexico (Sutherland, 2011), probably introduced along with the host tree, as this state is outside the natural range of *Carya*.

Coptodisca lucifluella appears to be widespread throughout Italy. Since the first record in the Campania and Lazio regions (Bernardo *et al.*, 2012), it has been found from the Veneto (northern) to the Basilicata (southern) regions. It has been collected in all sampled localities, except for some isolated trees at high altitude (over 1200 m a.s.l.) in Montella (AV, Campania) (Bernardo *et al.*, 2012) (see fig. 4 and online Supplementary material 2 for more details).

Hostplants

Coptodisca lucifluella was collected in Italy on *J. regia* and *J. nigra*, but in North America on various *Carya* species: *C. glabra* Miller (pignut hickory), *C. illinoensis* (pecan) and *C. tomentosa* Sarg (mockernut hickory).

Life cycle

A total of approximately 1500 walnut leaves (9000 leaflets) were collected in the three sampling years and examined for the presence, the number and the life stage of *C. lucifluella*. The lifecycle of *C. lucifluella* was observed for other *Coptodisca* spp. (Davis, 1998) except for the following features: pupal cases were collected either from leaves, on bark or from leaf litter. However, the number of pupal cases that were attached to twigs and leaves (fig. 1e) with a silken thread decreased with the approach of winter, while the number of those between and under bark increased (fig. 1f). Developmental stages of the leafminer sampled during the years are reported in fig. 5.

The number of annual generations differed depending on the sampling year, but at least three (2011–2012) or four (2013) generations annually were recorded. Active mines (larva present and feeding) were continuously recorded in the period between June and September to October, resulting in overlapping generations. The first newly laid eggs were collected in June (2011) but egg-laying may start as early as in 2013 when some larval holes were already sampled in the first days of June. The duration of the first generation (egg to

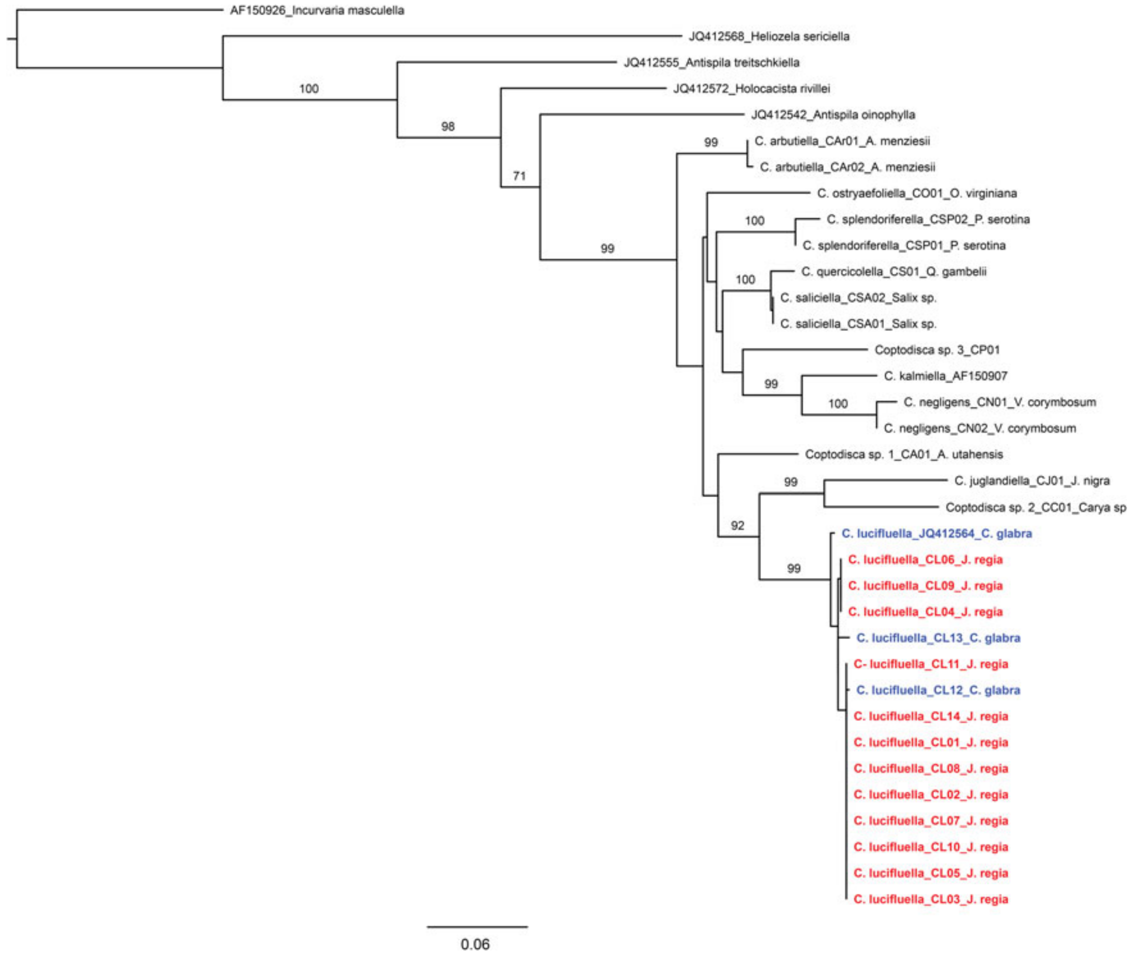

Fig. 3. Maximum likelihood tree for COI data. Bootstrap support >70% for taxonomically relevant splits are reported above branches. Specimens of *Coptodisca lucifluella* in red are from Italy, in blue are from the USA.

adult) in June was 18–20 days in 2011. The last larvae ready to hibernate inside the case on leaves were collected in September or October. Mature larvae hibernated in dormancy, mediated by quiescence. Adult emergence from pupal cases collected in November was forced in December by placing the cases at room temperature.

Level of damage

The mean number of mines per leaflet at 2 m of tree height (3.4 ± 0.20) and at 2 m (2.6 ± 0.13) did not differ significantly (d.f. = 1; $P = 0.126$; $n = 660$, $n = 332$ at 2 m and $n = 328$ at 6 m; Kruskal–Wallis test), and neither did the number of holes per leaflet at 2 m (1.2 ± 0.08) and 6 m (1.1 ± 0.08) (d.f. = 1; $P = 0.940$; $n = 660$, $n = 332$ at 2 m and $n = 328$ at 6 m; Kruskal–Wallis test). All sampled compound leaves of the last generation of each year, in September–October, were infested (table 2). However, the percentage of leaflet infestation changed with the sampling years, being higher in 2011 and 2013, and lower in 2012. The mean percentage of infested leaflets ranged between 52.4 ± 5.04 and 93.7 ± 2.08% (table 2) depending on year and locality. The mean percentage of infested leaflets with holes due to the preparation of pupal cases by larvae ranged from 24.2 ± 3.31 to 68.4 ± 6.45 (table 2).

No significant differences were recorded in the mean percentage of larval mortality over the years in either of the sampling localities (table 2). The mean number of mines per leaflet was variable, ranging between 1.1 ± 0.19 and 5.3 ± 0.42 (table 3), while the mean number of holes per leaflet ranged from 0.4 ± 1.03 to 1.5 ± 0.17 (table 3).

Data on the average number of mines and holes per leaflet showed the same trend in the two sampled areas; at each site differences were significant only between the data of 2012 and those of 2011 and 2013, and all numbers were lower in 2012 (ANOVA test d.f. = 2, $P < 0.01$, $F = 27.45$ for mines and Kruskal–Wallis d.f. = 2, $P < 0.01$ for holes at San Giorgio a Cremano; Kruskal–Wallis d.f. = 2, $P < 0.01$ for both mines and holes of Bracciano) (table 3). The mean length of the completed mines was 7.81 ± 0.211 mm (4.5–13.2) and the mean width was 3.48 ± 0.104 mm (1.98–5.94) ($n = 60$). An example of the annual trend in the mean number of mines and holes per leaflet is summarized in fig. 6 (2013, mean of the three orchards of Palma Campania, sites 1, 2, 3).

Discussion

Any new association between an indigenous plant and an exotic invasive species needs to be investigated, especially if the plant is of economic interest (Nentwig & Josefsson,

Fig. 4. Map showing the distribution of *Coptodisca lucifluella* in Italy. In red Bracciano, San Giorgio a Cremano and Palma Campania (the orchards of survey), in green the orchards where the leafminer was recorded and in blue the location where the leafminer was absent.

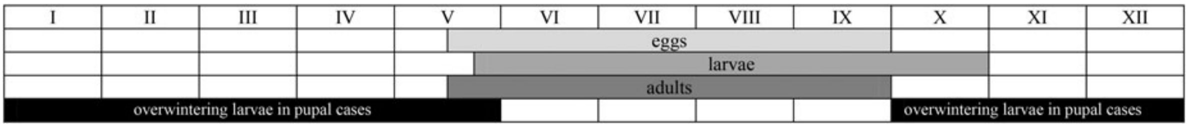

Fig. 5. Life cycle of *Coptodisca lucifluella* in Italy. Roman numbers denote months.

2010). For the majority of alien species, there is often a lack of knowledge about their biology and control, with *C. lucifluella* being no exception. Furthermore, when an invasive pest colonizes a new host plant in a new area, its impact can be devastating, eventually leading to the extinction of the plant species (Kenis *et al*., 2009).

The first important step when a species is found for the first time in a new habitat is its correct identification. The identification of *C. lucifluella* was challenging due to the unsatisfactory characterization of most species of *Coptodisca*, which necessitates a taxonomic revision of the genus. Nonetheless, the number of candidate species that could have matched the invasive Italian samples was greatly reduced by the evidence that in Italy and the rest of Europe there is only one other leafminer that attacks *J. regia*, *Caloptilia roscipennella* (Hübner) (Tomov *et al*., 2009; Lopez-Vaamonde *et al*., 2010). This species belongs to the family of Gracillariidae, thus is easily distinguishable from *Coptodisca*, for example, by the completely different shape of its mines (Patocka & Zach, 1995; Ellis, 2014).

Until the first record in Europe in 2010 (Bernardo *et al*., 2012), all *Coptodisca* species were exclusively found in the Nearctic and Neotropical regions (Davis, 1983, 1998; Hespenheide, 1991). In the Nearctic region, only *C. juglandiella*,

Table 2. Percentage of infestation of compound leaves and leaflets, percentage of leaflets with holes and of larval mortality in San Giorgio a Cremano and Bracciano after the last generation (September–October) over the last 3 years.

	San Giorgio a Cremano						Bracciano					
	2011		2012		2013		2011		2012		2013	
	Mean	Min–max	Mean	Min–max	Mean	Min–max	Mean	Min–max	Mean	Min–max	Mean	Min–max
% infestation of compound leaf	100		100		100		100		100		100	
% infestation of leaflet	89.6 ± 3.17^{a}	42.8–100	71.3 ± 4.71^{b}	33.3–100	93.7 ± 2.08^{a}	80–100	78.9 ± 4.14^{a}	40–100	52.4 ± 5.04^{b}	0–80	84.9 ± 5.14^{a}	33.3–100
			k	G	df	*P*			*k*	G	df	*P*
	2011–2012–2013		2	21.50	2	<0.01	2011–2012–2013		2	26.56	2	<0.01
	2011 versus 2013		2	1.14	1	<0.01	2011 versus 2013		2	0.59	1	<0.01
	2011 versus 2012		2	11.04	1	<0.01	2011 versus 2012		2	16.56	1	<0.01
% leaflet with holes	56.9 ± 5.36^{ab}	0–100	40.6 ± 6.21^{b}	0–83.3	68.4 ± 6.45^{a}	16.7–100	51.9 ± 3.66^{ab}	20–80	24.2 ± 3.31^{b}	0–50	57.3 ± 7.02^{a}	0–100
	2011–2013–2012		2	15.98	2	<0.01	2011–2012–2013		2	15.98	2	<0.01
	2011 versus 2013		2	2.82	1	<0.01	2011 versus 2013		2	2.82	1	<0.01
	2011 versus 2012		2	5.37	1	<0.01	2011 versus 2012		2	5.37	1	<0.01
% larval mortality	74.0 ± 2.32^{a} $n = 68$	0–100	63.8 ± 5.0^{a} $n = 66$	0–100	73.4 ± 2.69^{a} $n = 81$	0–100	68.0 ± 3.85^{a} $n = 73$	0–100	67.8 ± 6.96^{a} $n = 36$	0–100	67.5 ± 5.02^{a} $n = 48$	0–100
	2011–2012–2013		2	3.09	2	<0.05			2	0.06	2	<0.05

All data are presented non-transformed with their ±SE. Values with the same letter, in the same sampling locality, indicate no significant differences at the G test. A comparison between results recorded in different years of sampling, in the same locality, was performed. Fifteen compound leaves in each locality and year were sampled. *n* = number of replicates. Results of statistical analyses are shown on a grey background.

Table 3. Mean number of mines and mines with holes of *Coptodisca lucifluella* for leaflets in San Giorgio a Cremano and Bracciano after the last generation (September–October) in the last 3 years.

	San Giorgio a Cremano						Bracciano					
	2011		2012		2013		2011		2012		2013	
	Mean	Min–max	Mean	Min–max	Mean	Min–max	Mean	Min–max	Mean	Min–max	Mean	Min–max
Number of mines per leaflet	5.3 ± 0.42^a (n = 78)	0–29	2.0 ± 0.23^b (n = 92)	0–13	5.3 ± 0.46^a (n = 87)	0–21	4.2 ± 0.50^a (n = 94)	0–24	1.1 ± 0.19^b (n = 67)	0–8	3.6 ± 0.48^a (n = 56)	0–15
Number of holes per leaflet	1.4 ± 0.15^a (n = 78)	0–9	0.6 ± 0.083^b (n = 92)	0–3	1.5 ± 0.17^a (n = 87)	0–8	1.3 ± 0.20^a (n = 94)	0–8	0.4 ± 1.03^b (n = 94)	0–5	1.0 ± 1.32^a (n = 94)	0–7

All data are presented non-transformed with their ±SE and values with the same letter, in the same place of sampling, are not significantly different at the 5% ANOVA or Kruskal–Wallis test. A comparison between data recorded in different years of sampling, at the same locality, was performed. Fifteen compound leaves per locality per year were sampled, but they were composed by a different number of leaflets; n = number of leaflets).

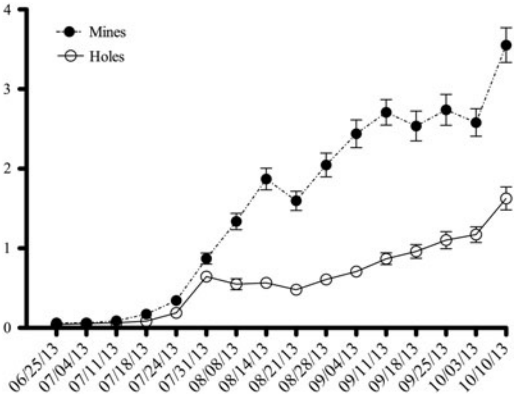

Fig. 6. Seasonal development of *Coptodisca lucifluella*, mean value of number of mines and holes per leaflet, in Palma Campania in 2013.

Stigmella juglandifoliella (Clemens) and *S. longisacca* Newton & Wilkinson (Nepticulidae) have been recorded to damage the genus *Juglans*, but the Nepticulidae produce completely differently shaped mines and the adults of these species are easily recognizable (Newton & Wilkinson, 1982). The first description of *C. lucifluella* was based on specimens reared from hickory in Pennsylvania by Clemens (1860). A few years after the first description, the very similar *C. ella* was described (Chambers, 1871). However, soon thereafter Chambers expressed doubts on the validity of this species, due to the morphological similarity with *C. lucifluella* and to it likely having the same host plant (he examined the residues of the leaf forming the pupal case) (Chambers, 1874). Synonymy between *C. ella* and *C. lucifluella* was also suggested by Forbes (1923). *Coptodisca ella* had unknown biology except that it had been reported from samples taken from the bark of oak trees (Chambers, 1871, 1874). No new records or information on *C. lucifluella* or *C. ella* have been published recently.

It is noteworthy that more unnamed species occur on Juglandaceae in North America, including a Californian species feeding on *Juglans californica* S. Wats, and one feeding on *Juglans microcarpa* Berlandier in the Guadalupe Mountains of Texas (D. Wagner, personal communication). Unfortunately, we were unable to get DNA sequences out of the material of these species that we examined (see online Supplementary material 1 and 3 for details and photos), but morphology shows that they are different from *C. lucifluella*.

Although the multiple lines of evidence here strongly suggest that the *Coptodisca* found in Italy is *C. lucifluella*, there are small inconsistencies with older literature (about American populations only) that should be evaluated, concerning: (a) the colour of larvae (previously described as light brown instead of yellow) (Payne *et al.*, 1972); (b) the initial shape of the mine (tortuous versus straight) (Payne *et al.*, 1972); (c) the shape of holes (most of the mined portion of leaflet leaf is contained in the pupal case versus only a small portion of the mine) (Payne *et al.*, 1972) (fig. 1g, h); and (d) the stage at which it overwinters (pupae versus mature larvae) (Heyerdahl & Dutcher, 1990). Notwithstanding the possibility of erroneous observations in earlier literature, such discrepancies have three possible explanations: (1) as we have shown that there are at least two species of *Coptodisca* on *Carya*, some of the previous data could refer to a species that is as yet unnamed; (2) differences could be associated with the host plants (*Carya* versus *Juglans*), for example, colour of larvae could be affected by plant substances (Yamasaki *et al.*, 2009). Similarly, both the shape of mines and holes could be affected by host plant condition, because a different thickness of the leaves influences the shape of mines, with smaller mines in thicker leaves and larger mines in thinner leaves; (3) the hibernating stage may be affected by the milder climate of Italy, with higher winter temperatures and hotter summers than the climatic conditions in the USA, Georgia (the state where Payne *et al.*, 1972 collected data), or by the existence of intraspecific variability in the hibernating life stage. Lastly, our observation that a large number of pupal cases can be found on walnut leaves during the first two generations – a behaviour previously reported only for *C. lucifluella* (Payne *et al.*, 1972) on pecan leaves – serves as confirmation of the identification.

Molecular analysis of the Italian specimens showed a low haplotype variability (only two); this could be due to the founder effect (the reduced genetic variation that occurs when a population is established by a single or a few specimens) (Gillespie & Roderick, 2014), suggesting that *C. lucifluella* arrived in Italy by a single introduction event and with few individuals. This is a common pattern recorded for invasive species (e.g. Rubinoff *et al.*, 2010; Cifuentes *et al.*, 2011). However, a wider knowledge of the haplotype variability in the native area of *C. lucifluella* and a finer scale analysis (for example, by microsatellites) are needed to obtain more information about the number of introductions.

Data collected over the last 3 years about *C. lucifluella*'s diffusion and density in Italy show that its invasiveness, based on the terminology of Colautti & MacIsaac (2004) ranged between the stages IVa (widespread but rare) and V (widespread and dominant). With the exception of Campania and Lazio Regions, the density of the leafminer is still quite low, having rarely been found at a density of more than two or three holes per leaflet. This suggests that either the introduction has happened in one of those two regions (Bernardo *et al.*, 2012) or that it is there where the leafminer finds better climatic conditions to develop. However, further investigations are needed to determine the locality and the source of the first introduction, especially considering that in recent years new plantations of pecan have been made in Italy.

Our biological data showed that *C. lucifluella* completes three to four generations annually in Central and Southern Italy. Our results are congruent with previous North-American observations, where at least four generations annually were hypothesized based on the increase in larval density per year on pecan trees (Heyerdahl & Dutcher, 1990). Active larvae were found throughout the period June to October and new mines were observed in almost all samples, matching data previously recorded in Georgia on another host (Heyerdahl & Dutcher, 1990). The large overlap between generations could lead to underestimation of the real number of generations. Our observations span 3 years, yet they are confined to Central and Southern Italy; hence, we cannot exclude the possibility that a smaller number of generations are completely in colder areas.

Our observations showed a homogeneous distribution of the population in the examined orchards (in the last sampling all of the sampled leaves were infested) and in the canopy. The damage, even with a high percentage of infestation, does not seem economically important, due to the small size of the insect and of its mines, confirming observations for several species of Heliozelidae recorded on cultivated plants in Italy over the last 20 years, which only episodically caused economic damage (Bernardo *et al.*, 2008; De Tomaso *et al.*, 2008; Baldessari *et al.*, 2009; van Nieukerken *et al.*, 2012). Except for occasional outbreaks, *Coptodisca* species are also not considered serious pests in their native countries (Slingerland & Crosby, 1914; Brown & Eads, 1969; Maier, 1988; Heyerdahl & Dutcher, 1990). However, the damage caused by the leafminer could be used by pathogens to introduce themselves inside the leaves. It has also been demonstrated that leaves of *Vaccinium macrocarpon* Aiton mined by *C. negligens* had an earlier abscission than unmined leaves, which lead to a reduction in the longevity of leaves (Maier, 1989, Hespenheide, 1991). This kind of damage, in the long run, could weaken a tree and therefore the real extent of the *C. lucifluella* damage on its host needs to be assessed over a longer time span.

Coptodisca lucifluella was described by Clemens in 1860 and hitherto collected only on hickory leaves (*Carya* sect. *Carya: Carya* spp.) and pecan (*Carya* sect. *Apocarya: Carya illinoensis* (Wang)) (Clemens, 1860; Chambers, 1871; Payne *et al.*, 1972). The association between *C. lucifluella* and *J. regia* and *J. nigra* is here reported for the first time. Recently, two other Nearctic insects feeding on *Juglans* invaded Italy: the walnut husk fly *Rhagoletis completa* Cresson (Diptera: Tephritidae) (Duso, 1991; Ciampolini & Trematerra, 1992; Eppo/Cabi, 1997; Benchi *et al.*, 2010) and *Pityophthorus juglandis* Blackman (Coleoptera: Scolytidaee) (Montecchio & Faccoli, 2014). In the case of *C. lucifluella*, we do not know if the actual host range in the native area also includes *Juglans* or whether the host shift occurred in Italy after its invasion. However, it is interesting to note that all *Coptodisca* feeding on Juglandaceae are phylogenetically close (fig. 2), following the general principle that related insects feed on related plants (Menken *et al.*, 2010).

The level of potential damage by *C. lucifluella* is greatly reduced by the high mortality of younger larval instars. This level of mortality is partly due to parasitization by indigenous parasitoids that are adapting to the new host (Bernardo *et al.*, in preparation). A conspicuous complex of parasitoids of *C. lucifluella* has also been recorded in the Nearctic region (Yoshimoto, 1973; Heyerdahl & Dutcher, 1985; Gates *et al.*, 2002). In the native area the parasitism rate of *C. lucifluella* has been reported to reach a maximum of 61% at the leafminer's peak density and to be host-density dependent. Both larvae and cocoons (with larvae and pupae) were parasitized but the highest percentage of parasitization was recorded for cocoons (Heyerdahl & Dutcher, 1985).

Concluding remarks

Due to its widespread distribution, we assume that *C. lucifluella* arrived in Italy several years ago. Its actual distribution is likely to be wider than recorded here, as it was collected in nearly every surveyed location. Considering its widespread presence and rapid reproduction, *C. lucifluella* might quickly increase its abundance and distribution in Italy and other European countries. Nevertheless, as also observed for other Heliozelidae and excluding the outbreaks, the level of damage does not seem to be worrisome. The possibility remains, however, that damage by *C. lucifluella*, if continued for a number of years, might lead to a progressive reduction in carbohydrate reserves and a gradual decline in tree health and performance. Based on the high mortality recorded and on the large number of parasitoids being collected, it would be preferable to encourage biological control rather than performing chemical treatments.

Supplementary material

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0007485314000947

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References

Baldessari, M., Angeli, G., Girolami, V., Mazzon, L., van Nieukerken, E.J. van & Duso, C. (2009) *Antispila* sp. minatore fogliare segnalato in Italia su vite. *L'Informatore Agrario* **15**, 68–70.

Benchi, D., Conelli, L. & Bernardo, U. (2010) La mosca delle noci minaccia le produzioni campane. *L'Informatore Agrario* **49**, 74–75.

Bernardo, U., Monti, M.M., Nappo, A.G., Gebiola, M., Russo, A., Pedata, P. & Viggiani, G. (2008) Species status of two populations of *Pnigalio soemius* (Hymenoptera: Eulophidae) reared from two different hosts: an integrative approach. *Biological Control* **46**, 393–403.

Bernardo, U., Sasso, R., Gebiola, M. & Viggiani, G. (2011) Minatrice fogliare segnalata in Italia su noce. *L'Informatore agrario* **46**, 64–65.

Bernardo, U., Sasso, R., Gebiola, M. & Viggiani, G. (2012) First record of a walnut shield bearer *Coptodisca* (Lepidoptera: Heliozelidae) in Europe. *Journal of Applied Entomology* **136**, 638–640.

Bold site: http://www.barcodinglife.com

Braun, A. (1916) New species of Microlepidoptera. *The Canadian Entomologist* **48**, 138–141.

Braun, A. (1927) Descriptions of new Microlepidoptera. *Transaction of the American Entomological Society* **53**, 191–199.

Brown, L. & Eads, C. (1969) Unnamed and little-known insects attacking cottonwood in Southern California. *Journal of Economic Entomology* **62**, 667–674.

Busck, A. (1878) A new species of moths of the superfamily *Tineina* from Florida. *Proceeding of the United States National Museum* **23**, 225–254.

Chambers, V.T. (1871) Micro-Lepidoptera. *The Canadian Entomologist* **3**, 221–224.

Chambers, V.T. (1874) Micro-Lepidoptera. *The Canadian Entomologist* **6**, 149–153.

Ciampolini, M. & Trematerra, P. (1992) Diffusa presenza di mosca delle noci (*Rhagoletis completa* Cresson) nel Nord Italia. *L'Informatore agrario* **48**, 52–56.

Cifuentes, D., Chynoweth, R. & Bielza, P. (2011) Genetic study of Mediterranean and South American populations of tomato leafminer *Tuta absoluta* (Povolny, 1994) (Lepidoptera: Gelechiidae) using ribosomal and mitochondrial markers. *Pest Management Science* **67**, 1155–1162.

Clemens, B. (1860) Contributions to American Lepidopterology. No. 3. *Proceedings of the Academy of Natural Sciences of Philadelphia* **12**, 4–48.

Colautti, R.I. & MacIsaac, H.J. (2004) A neutral terminology to define "invasive" species. *Diversity and Distributions* **10**, 135–141.

Davis, D.R. (1983) Heliozelidae. p 4 *in* Hodges, R.W., Dominick, T., Davis, D.R., Ferguson, D.C., Franclemont, J.G., Munroe, E. G. & Powell, J.A. (*Eds*) *Check list of the Lepidoptera of America north of Mexico, including Greenland*. London, Classey.

Davis, D.R. (1998) The monotrysian Heteroneura. pp 65–90 *in* Kristensen, N.P. (*Ed.*) *Lepidoptera, Moths and Butterflies, 1. Evolution, systematics and biogeography, vol 4. Handbuch der Zoologie/ Handbook of Zoology, vol 35*. Berlin, New York, De Gruyter.

De Tomaso, B., Romito, A., Nicoli Aldini, R. & Cravedi, P. (2008) Minatrice fogliare della vite segnalata in Puglia. *L'Informatore agrario* **31**, 59–61.

Dietz, W. (1921) A new species of *Coptodisca* (Lepid.). *The Canadian Entomologist* **53**, 44.

Duso, C. (1991) Sulla comparsa in Italia di un Tefritide neartico del noce *Rhagoletis completa* Cresson (Diptera: Tephritidae). *Bollettino di zoologia agraria e bachicoltura* **23**, 203–209.

Ellis, W.N. (2014) Bladmineerders van Europa/Leafminers of Europe. http://www.bladmineerders.nl/index.htm

EPPO/CABI (1997) Data Sheet on *Rhagoletis completa*. pp. 475–479 *in* Smith, I.M., McNamara, D.G., Scott, P.R., Holderness, M. (*Eds*) *Quarantine Pests for Europe*. 2nd edition. Wallingford, UK, CABI International.

Felt, E.P. (1906) *Insects Affecting Park and Woodland Trees, vol 8. Memoir – New York State Museum*. Albany, New York State Museum.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**, 294–299.

Forbes, W.T.M. (1923) The Lepidoptera of New York and neighboring states. Primitive forms Microlepidoptera, Pyraloids, Bombyces. *Memoir by Cornell University Agricultural Experiment Station* **68**, 1–792.

Gates M., Heraty J., Schauff M., Schauff M.E., Wagner D.L., Whitfield J.B., Wahlet D.B. (2002) Survey of the parasitic Hymenoptera on leafminers in California. *Journal of Hymenoptera Research* **11**, 213–270.

Gebiola, M., Bernardo, U., Monti, M.M., Navone, P. & Viggiani, G. (2009) *Pnigalio agraules* (Walker) and *Pnigalio mediterraneus* Ferrière and Delucchi (Hymenoptera: Eulophidae): two closely related valid species. *Journal of Natural History* **43**, 2465–2480. Geneious R6: http://www.geneious.com

Gillespie, R.G. & Roderick, G.K. (2014) Evolution: geology and climate drive diversification. *Nature* **509**, 297–298.

Hajibabaei, M., Smith, M.A., Janzen, D.H., Rodriguez, J.J., Whitfield, J.B. & Hebert, P.D.N. (2006) A minimalist barcode can identify a specimen whose DNA is degraded. *Molecular Ecology Notes* **6**, 959–964.

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. **41**, 95–98.

Hebert, P.D.N., Penton, E.H., Burns, J.M., Janzen, D.H. & Hallwachs, W. (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceeding of National Academy of Sciences of the United States of America* **101**, 14812–14817.

Hespenheide, H. (1991) Bionomics of leaf-mining insects. *Annual Review of Entomology* **36**, 535–560.

Heyerdahl, R. & Dutcher, J. (1985) Hymenopterous parasitoids of pecan leafminers. *Journal of Entomological Science* **20**, 411–421.

Heyerdahl, R. & Dutcher, J. (1990) Seasonal abundance and overwintering mortality in populations of lepidopterous leafminers of Pecan. *Journal of Entomological Science* **25**, 394–408.

Hileman, D.R. & Lieto, L.F. (1981) Mortality and area reduction in leaves of the Bog Shrub *Chamaedaphne calyculata* (Ericaceae) caused by the leaf miner *Coptodisca kalmiella* (Lepidoptera: Heliozelidae). *American Midland Naturalist* **106**, 180–188.

Kenis, M., Auger-Rozenberg, M., Roques, A., Timms, L., Péré, C., Cock, M., Settele, J., Augustin, S. & Lopez-Vaamonde, C. (2009) Ecological effects of invasive alien insects. *Biological Invasions* **11**, 21–45.

Knölke, S., Erlacher, S., Hausmann, A., Miller, M.A.& Segerer, A.H. (2005) A procedure for combined genitalia dissection and DNA extraction in Lepidoptera. *Insect Systematics and Evolution* **35**, 401–409.

Lafontaine, J.D. (1974) A new species of *Coptodisca* (Heliozelidae) from Mississippi on farkleberry (*Vaccinium arboreum*). *Journal of the Lepidopterists Society* **28**, 126–130.

Lopez-Vaamonde, C., Agassiz, D., Augustin, S., De Prins, J., De Prins, W., Gomboc, S., Ivinskis, P., Karsholt, O., Koutroumpas, A., Koutroumpa, F., Laštůvka, Z., Marabuto, E., Olivella, E., Przybylowicz, L., Roques, A., Ryrholm, N., Sefrova, H., Sima, P., Sims, I., Sinev, S., Skulev, B., Tomov, R., Zilli, A. & Leeset, D. (2010) Lepidoptera. Chapter 11. *BIORISK – Biodiversity and Ecosystem Risk Assessment* **4**, 603–668.

Maier, C. (1989) Accelerated abscission of cranberry leaves damaged by the leafminer, *Coptodisca negligens* (Lepidoptera: Heliozelidae). *Environmental Entomology* **18**, 773–777.

Maier, C.T. (1988) Life cycle of *Coptodisca negligens* (Lepidoptera: Heliozelidae) on Cranberry. *Journal of Economic Entomology* **81**, 497–500.

Menken, S.B.J., Boomsma, J.J. & Nieukerken, E.J. van (2010) Large-scale evolutionary patterns of host plant associations in the Lepidoptera. *Evolution* **64**, 1098–1119.

Montecchio, L. & Faccoli, M. (2014) First record of thousand cankers disease *Geosmithia morbida* and walnut twig beetle *Pityophthorus juglandis* on *Juglans nigra* in Europe. *Plant Disease* **98**, 696–696.

Nentwig, W. & Josefsson, M. (2010) Introduction. Chapter 1. In: Roques, A. *et al.*, (Eds) Alien terrestrial arthropods of Europe. *Biorisk* **4**, 5–9.

Newton, P.J. & Wilkinson, C. (1982) A taxonomic revision of the North American species of *Stigmella* (Lepidoptera: Nepticulidae). *Systematic Entomology* **7**, 367–463.

Nieukerken, E.J. van (2013). Fauna Europaea: Heliozelidae. *in* Karsholt, O. & Nieukerken, E.J. van (*Eds*) *Lepidoptera*. Fauna Europaea version 2.6.2. http://www.faunaeur.org/

Nieukerken, E.J. van, Wagner, D.L., Baldessari, M., Mazzon, L., Angeli, G., Girolami, V. & Doorenweerd, C.D.C. (2012) *Antispila oinophylla* new species (Lepidoptera, Heliozelidae), a new North American grapevine leafminer invading Italian vineyards: taxonomy, DNA barcodes and life cycle. *ZooKeys* **170**, 29–77.

Opler, P.A. (1971) Seven new Lepidopterous leafminers associated with *Quercus agrifolia* (Heliozelidae, Gracillariidae). *Journal of the Lepidopterists Society* **25**, 194–211.

Payne, J., Tedders, W., Cosgrove, G. & Foard, D. (1972) Larval mine characteristics of four species of leaf-mining Lepidoptera in Pecan. *Annals of the Entomological Society of America* **65**, 74–81.

Patocka, J. & Zach, P. (1995) The pupae of the central European *Caloptilia* (Lepidoptera: Gracillariidae). *European Journal of Entomology* **92**, 483–496.

Pellmyr, O. & Leebens-Mack, J. (1999) Forty million years of mutualism: evidence for Eocene origin of the yucca–yucca moth association. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 9178–9183.

Regier, J.C. & Shi, D. (2005) Increased yield of PCR product from degenerate primers with nondegenerate, nonhomologous 5′ tails. *Biotechniques* **38**, 34–38.

Rickman, J. & Connor, E. (2003) The effect of urbanization on the quality of remnant habitats for leaf-mining Lepidoptera on *Quercus agrifolia*. *Ecography* **26**, 777–787.

Rubinoff, D., Holland, B.S., Shibata, A., Messing, R.H. & Wright, M.G. (2010) Rapid invasion despite lack of genetic variation in the erythrina gall wasp (*Quadrastichus erythrinae* Kim). *Pacific Science* **64**, 23–31.

Slingerland, M.V. & Crosby, C. (1914) *Manual of Fruits Insects*. p. 503. New York, The Macmillan Company.

Sokal, R.R. & Rohlf, F.J. (1995) *Biometry: The Principles and Practice of Statistics in Biological Research*. (3rd edn. New York, USA, W.H. Freeman and Company.

Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**, 2688–2690.

Sutherland, C. (2011) Potential Pecan Pests to Ponder-Leafminers and Hickory Shuckworm. (pp. 8–12). Presented at the WPGA Pecan Show Pecan Food Fantasy and Pecan Trade and Equipment Show.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**, 1596–1599.

Tomov, R., Trencheva, K., Trenchev, G., Cota, E., Ramadhi, A., Ivanov, B., Naceski, S., Papazova-Anakleva, I. & Kenis, M. (2009) *Non-Indigenous Insects and their Threat to Biodiversity and Economy in Albania, Bulgaria and Republic of Macedonia*. pp. 1–57. Sofia–Moscow, Pensoft Publishers.

Weiss, H.B. & Beckwith, C.S. (1921) Notes on *Coptodisca kalmiella* Dietz, a leaf miner of *Kalmia angustifolia*. *The Canadian Entomologist* **53**, 44–45.

Wiegmann, B.M., Regier, J.C. & Mitter, C. (2002) Combined molecular and morphological evidence on phylogeny of the earliest lepidopteran lineages. *Zoologica Scripta* **31**, 67–81.

Yamasaki, A., Shimizu, K. & Eujisaki, K. (2009) Effect of host plant part on larval body-color polymorphism in *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America* **102**, 76–84.

Yoshimoto, C. (1973) Review of North American *Chrysocharis* (Kratochviliana) (Eulophidae: Chalcidoidea) North of Mexico, especially species attacking birch casebearer (Lepidoptera: Coleophoridae) and birch leafminer (Hymenoptera: Tenthredinidae). *The Canadian Entomologist* **105**, 1309–1349.