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“ REDIA „

GIORNALE DI ZOOLOGIA

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Consiglio per la ricerca in agricoltura e l’analisi dell’economia agraria
CREA Research Centre for Plant Protection and Certification

Volume CI
TERZA SERIE
101

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SEDIGHE AZIMI (*)

MORPHOLOGICAL AND MOLECULAR CHARACTERISATION OF *ECUMENICUS MONOHYSTERA* (NEMATODA DORYLAIMIDA QUDSIANEMATIDAE) AND ITS PHYLOGENETIC RELATIONS FROM IRAN

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Azimi S. – Morphological and molecular characterisation of *Ecumenicus monohystera* (Nematoda Dorylaimida Qudsianematidae) and its phylogenetic relations from Iran.

Ecumenicus monohystera was collected from the rhizosphere of faba bean (*Vicia faba* L.) fields in Khuzestan province, south-western Iran. Morphological and morphometric data are provided for this species. Additionally, sequence of the D2-D3 expansion segments of 28S rRNA gene for this species was also used for molecular phylogenetic analysis. The phylogenetic relationships of *E. monohystera* in relation to representatives of the order Dorylaimida, obtained from Bayesian inference (BI) analysis of the D2-D3 sequences, are presented and discussed.

KEY WORDS: 28S rRNA gene, *Ecumenicus*, morphology, morphometric, phylogeny.

INTRODUCTION

More than 80% of the most environmental stress-sensitive nematode families belong to the orders Mononchida Jairajpuri, 1969 and Dorylaimida Pearse, 1942 (HOLTERMAN *et al*., 2008). Dorylaims, the representatives of the nematode order Dorylaimida, are probably the most diverse taxon within the phylum Nematoda. The families Dorylaimidae de Man, 1876 (with 24 valid genera and 336 valid species) and Qudsianematidae Jairajpuri 1965 (with 31 valid genera and 402 valid species) are two important, highly diverse, free-living, dorylaimid taxa (PEÑA-SANTIAGO and ALVAREZ-ORTEGA, 2014).

The genus *Ecumenicus* was proposed by Thorne in 1974 to accommodate a cosmopolitan species, *E. monohystera* (de Man, 1880) Thorne, 1974, transferred from *Eudorylaimus* Andrássy, 1959. The new taxon was characterized by its mono-opisthodelphic female genital system, a rather rare feature among members of Dorylaimoidea (PEÑA-SANTIAGO and ABOLAFIA, 2007). DAREKAR & KHAN (1979) established a genus *Indokochinema*, with a single species, *I. conicauda*. This genus corresponds perfectly to *Ecumenicus* with ovary single, no prevulval uterin sac, oesophagus expanded posteriorly, amphids on lateral lips, spear small, tail short, conoid, so ANDRÁSSY (1991) synonymized *Indokochinema* with *Ecumenicus*. The second species, *Indokochinema ekramullahi* Jana & Baqri (1983), has been already synonymized by BAQRI & COOMANS (1985) with *Ecumenicus monohystera* (ANDRÁSSY, 1991).

PEÑA-SANTIAGO & ABOLAFIA (2007) studied the location of amphid aperture in *E. monohystera* with SEM and confirmed ANDRÁSSY'S (1991) action, regarding *Indokochinema* as a junior synonym of *Ecumenicus*. According to ANDRÁSSY (1991), the genus *Ecumenicus* includes four species. *E. monohystera* is a cosmopolitan species and has also been reported from many countries (PEÑA-SANTIAGO and ABOLAFIA, 2007).

The present study aims to characterize *E. monohystera* from the rhizosphere of faba bean in Iran using morphological and molecular data. Additionally, the phylogenetic relationships of this species is evaluated on the basis of the D2-D3 expansion segments of the 28S rRNA gene.

MATERIALS AND METHODS

NEMATODE SAMPLES

Soil samples were collected from faba bean (*Vicia faba* L.) fields in Khuzestan province, south-western Iran. The JENKINS'S (1964) method was used to extract the nematodes from soil samples. The collected specimens were killed by adding boiling formaldehyde solution (4%), transferred to anhydrous glycerin according to DE GRISSE'S (1969) method. Nematodes were mounted in a small drop of glycerin on permanent slides. Observations and measurements were done using a Leitz SM-LUX light microscope equipped with drawing tube. Some of the best-preserved specimens were photographed using an Olympus DP12 digital camera attached to an Olympus BX51 light microscope. Nematode species were identified based on morphological, morphometric and molecular characters. SIDDIQI, 2000 used for the abbreviations and ratios used in the morphological description.

DNA EXTRACTING, PCR AND SEQUENCING

For molecular analyses, a single female was picked out from samples, examined in drop of distilled water on a temporary slide under the light microscope, transferred to 7 µl of AE buffer (10 mM Tris-Cl, 0.5 mM EDTA; pH 9.0) on a clean slide, and then crushed using a cover slip. The suspension was collected by adding 20 µl AE buffer. Each DNA sample was stored at -20°C until used as a PCR template (PEDRAM *et al*., 2011). The D2-D3 expansion

– Received 25 August 2017 Accepted 19 December 2017

segments of the 28S rDNA was amplified using the forward D2A (5′-ACAAGTACCGTGAGGGAAAGTTG-3′) and reverse D3B (5′-TCGGAAGGAACCAGCTACTA-3′) primers (NUNN, 1992). PCR reactions of 25 µl were made with 14 µl of distilled water, 2.5 µl of 10 × PCR buffer , 0.5 µl of dNTP mixture, 1.5 µl of 50 mM MgCl2 , 1µl of each primer (10 pmol/µl), 0.5 µl of Taq polymerase (CinnaGen, Tehran, Iran, c. 5 U/µl), and 4 µl of DNA template. The thermal cycling program was as follows: initial denaturation at 95ºC for 6 min, followed by 35 cycles of denaturation at 94ºC for 30 s, annealing at 55ºC for 30 s and extension at 72ºC for 1 min. A final extension was performed at 72ºC for 10 min (PEDRAM *et al*., 2011). Amplification success was evaluated electro phoretically on 1% agarose gel. The PCR products were purified using the QIAquick PCR purification kit (Qiagen®) following the manufacturer's protocol and sequenced directly using the PCR primers with an ABI 3730XL sequencer (Bioneer Corporation, South Korea). The newly obtained sequence was deposited into the GenBank database (accession number MF667960).

PHYLOGENETIC ANALYSES

The newly obtained sequence of the D2D3 fragments of 28S rDNA and additional sequences of relevant taxa selected after a BlastN search, were aligned by Clustal X2 (http://www.clustal.org/) using the default parameters. The outgroup taxa were chosen according to a previous study (HOLTERMAN *et al*., 2008). Model of base substitution was selected using Mr Model test 2 (NYLANDER, 2004), and based on the Akaike criteria. A general time reversible model, including among-site rate heterogeneity and estimates of invariant sites (GTR + G + I), was selected for the phylogenetic analyses. Bayesian analysis was used to infer the phylogenetic tree on MrBayes v3.1.2 (RONQUIST & HUELSENBECK, 2003), running the chain for one million generations. After discarding burn-in samples and evaluating convergence, the remaining samples were retained for further analyses. The Markov chain Monte Carlo (MCMC) method within a Bayesian framework was used to determine equilibrium distribution and help estimate the posterior probabilities of the phylogenetic tree (LARGET & SIMON, 1999) using the 50% majority rule. The Bayesian posterior probabilities (BPP) higher than 50% were given on appropriate clades. The output file of the phylogenetic program was visualized using Dendroscope V.3.2.8 (HUSON & SCORNAVACCA 2012) and re-drawn in CorelDRAW software version 12.

RESULTS AND DISCUSSION

Ecumenicus monohystera **(de Man, 1880) Thorne, 1974**

(Figs I and II)
Measurements: Table 1.

DESCRIPTION

FEMALE: Body straight to slightly ventrally curved upon fixation; Medium-sized, 0.9-1.2 mm long. Cuticle very

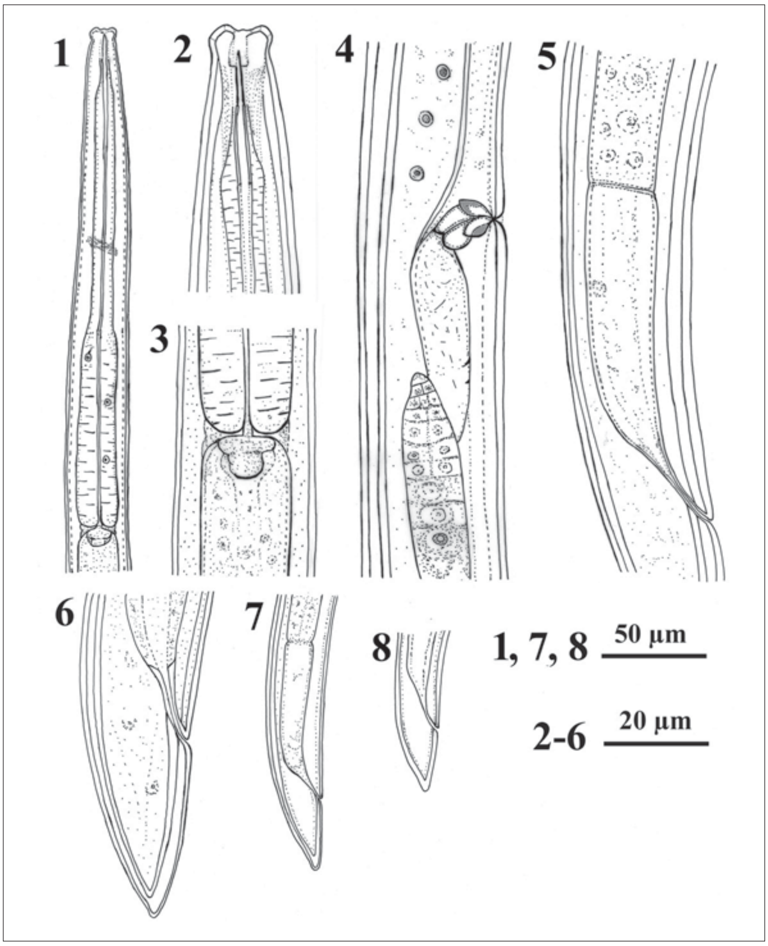

Fig. I – *Ecumenicus monohystera* - 1. Pharynx; 2. Lip region; 3. Pharyngeal cardia; 4. Vulval region; 5. Prerectum region; 6-8. Posterior region.

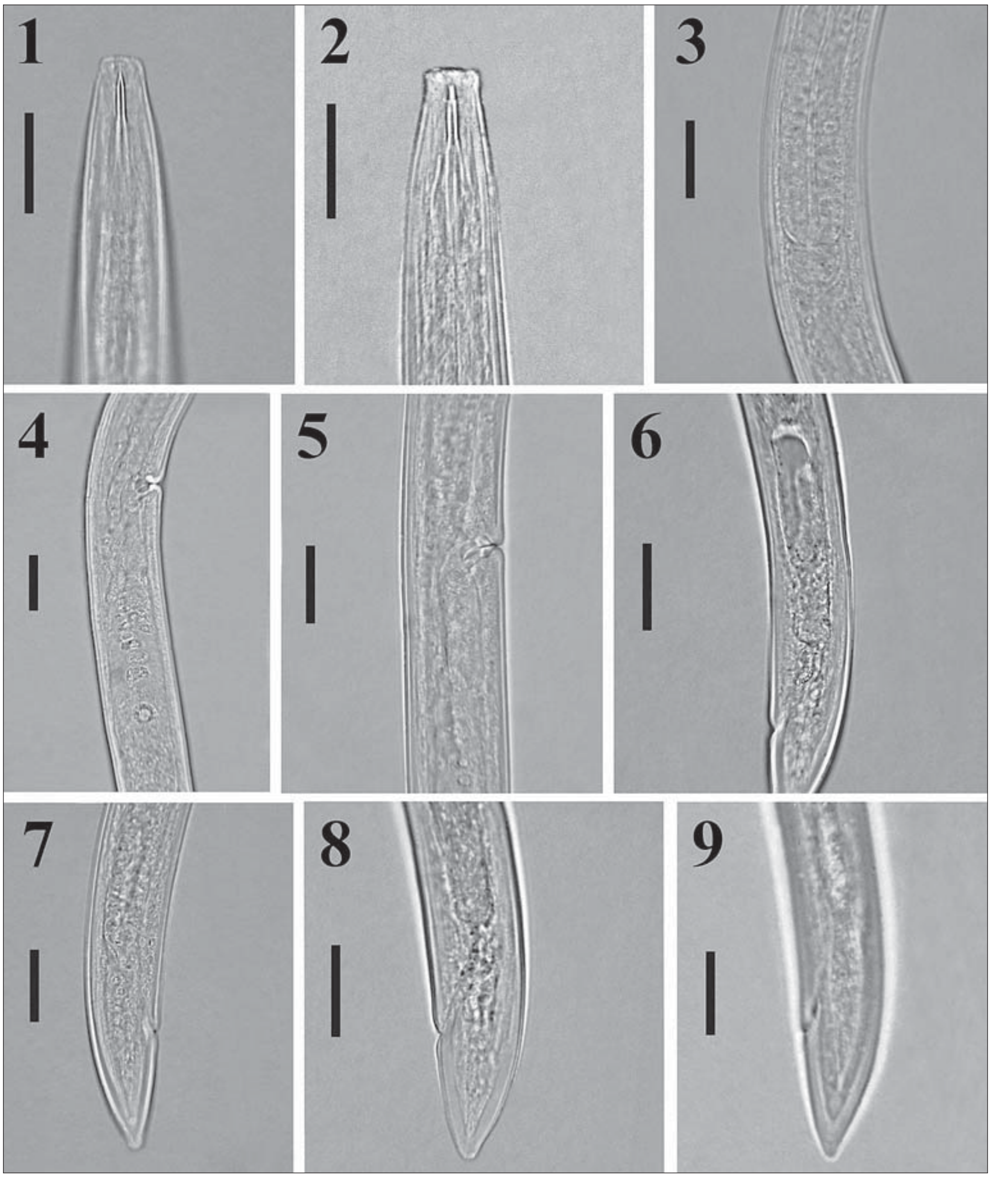

Fig. II – *Ecumenicus monohystera* – 1, 2. Lip region; 3. Pharyngeal cardia; 4, 5. Vulval region; 6. Prerectum region; 7-9. Posterior region. (Scale bars = 20 µm).

finely striated. Lip region set off by a slight depression, lips distinct. Odontostyle with aperture about one-third length, guiding ring single. Odontophore simple, rod-like. Pharynx enlarges more gradually and basal expansion part of pharynx occupying less than one-half of pharynx length. Dorsal oesophageal gland nucleus located a little bit behind to its orifice. Female genital system monodelphic-opistodelphic, Vulva a transverse slit, with slightly sclerotised labia. Vagina oblique. The length of rectum is slightly less than the anal body width. Tail conoid with straight ventral site and digitated rounded tip.

MALE: not found.

REMARKS: Iranian population of *E. monohystera* resembled more those described by PEÑA-SANTIAGO & ABOLAFIA (2007) than some other populations. Compared to Indian population (MUSHTAQ & AHMAD, 2007), the ratio c is slightly higher (30.6-40 *vs* 27.3-30.2), rectum and tail lengths are shorter (15.5-17.5 *vs* 22-29 µm and 29.0-34.5 *vs* 34-39 µm, respectively). Compared to Bulgarian population (Ilieva *et al*., 2017), the range of ratio V is higher (34-39.1 *vs* 29-34) and rectum length is shorter (15.5-17.5 *vs* 18-33 µm). These differences can be attributed to the intraspecific variation due to geographical differences.

E. monohystera is widely distributed in the world and has been reported from Iran by FADAEI-TEHRANI, 2008 (grapevine, Chaharmahal va Bakhtiari province), KASHI NAHANGI & KAREGAR BIDEH, 2010 (sugar beet, Hamadan) and HADI-ALIJANVAND & FADAEI-TEHRANI, 2013 (wheat, barley and clover, Chaharmahal va Bakhtiari province). In present study, this species was recovered from the rhizosphere of faba bean fields in the vicinity of Shushtar (GPS coordinates: 32°02′44′′N, 48°51′24′′E) city, Khuzestan province, south-western Iran. This is new record of *E. monohystera* for nematodes fauna in Khuzestan province.

Table 1 – Morphometrics of *Ecumenicus monohystera* collected from south-western Iran and their comparison with some other populations. All measurements are in μm and in the form: mean ± s.d. (range).

Character	Present study	Mushtaq& Ahmad (2007)	Peña-Santiago & Abolafia (2007)	Ilieva *et al.* (2017)
n	14	9	15	11
L	1136.9 ± 98.7 (949-1224)	1050 ± 50.0 (980-1120)	1003 ± 60.0 (910-1140)	1120 ± 70.0 (990-1180)
a	37.2 ± 3.4 (31.6-42.2)	33.4 ± 1.4 (31.6-35.0)	36.1 ± 4.4 (31.8-48.0)	37.0 ± 2.0 (34.0-39.0)
b	4.8 ± 0.1 (4.6-5.0)	4.6 ± 0.1 (4.3-4.8)	4.7 ± 0.2 (4.3-5.0)	4.6 ± 0.5 (3.9-5.1)
c	36.1 ± 3.0 (30.6-40.0)	29.1 ± 0.8 (27.3-30.2)	32.5 ± 2.6 (29.4-39.8)	32.0 ± 2.0 (31.0-36.0)
c′	1.5 ± 0.1 (1.4-1.7)	1.6 ± 0.1 (1.5-1.8)	1.6 ± 0.1 (1.3-1.8)	1.6 ± 0.1 (1.5-1.7)
V	36.1 ± 2.0 (34.0-39.1)	35.3 ± 0.5 (35.0-36.0)	35.4 ± 2.2 (32.0-39.0)	32.0 ± 2 (29-34)
Lip region width	10.9 ± 0.5 (10.0-11.5)	10.7 ± 0.4 (10.0-11.0)	10.3 ± 0.5 (10.0-11.0)	9.9 ± 0.2 (9.5- 10.0)
Odontostyle length	10.9 ± 0.0 (10.5-11.0)	12 ± 0.5 (11.0-13.0)	10.1 ± 0.5 (9.0-11.0)	10.0 ± 0.5 (10.0-11.0)
Neck length	236.5 ± 19.9 (198 -251)	228 ± 11.8 (210-246)	220.0 ± 12.0 (200-240)	245 ± 17.0 (225-261)
Body width at neck base	28.7 ± 1.7 (27.0-32.0)	30 ± 1.0 (28.0-32.0)	-	30.0 ± 2.0 (28.0-32.0)
Pharyngeal expansion length	87.5 ± 10.2 (71.0-95.0)	84.1 ± 3.7 (75.0-87.0)	82.2 ± 7.0 (70.0-90.0)	-
Body width at mid body	30.5 ± 1.7 (29.0-34.0)	31.5 ± 1.2 (29.0-33.0)	28.9 ± 3.3 (22.0-33.0)	31.0 ± 1.0 (29.0- 32.0)
Anal body width	19.9 ± 0.8 (19.0-21.0)	20.8 ± 0.7 (20.0-22.0)	20.3 ± 1.3 (18.0-22.0)	22.0 ± 2.0 (20.0- 24.0)
Prerectum length	46.9 ± 10.8 (24.3-58.0)	40.8 ± 6.7 (35.0-50.0)	35.8 ± 6.0 (21.0-45.0)	41.0 ± 9.0 (24.0-47.0)
Rectum length	16.5 ± 0.9 (15.5-17.5)	23.1 ± 1.5 (22.0-29.0)	-	24.0 ± 6.0 (18.0-33.0)
Tail length	31.5 ± 2.0 (29.0-34.5)	36.1 ± 1.4 (34.0-39.0)	31.9 ± 2.1 (27.0-35.0)	35.0 ± 2.4 (32.0-38.0)

MOLECULAR PHYLOGENETIC STATUS

The alignment of the D2D3 expansion fragments of 28S rRNA gene sequences of 32 taxa (including two out group taxa), yielded a data set with 1194 characters. The phylogenetic relationships between the Iranian population of *E. monohystera* and representatives of Dorylaimida, as inferred from the BI analysis, are presented in Figure III. Phylogenetic relationships among dorylaimid nematodes in this present are mostly congruent with those published by HOLTERMAN *et al.* (2008) and PEÑA -SANTIAGO & ALVAREZ-ORTEGA (2014). The BI analysis showed that Qudsianematidae are a polyphyletic taxon and some of its genera are closer to members of other families. This result is congruent with previous study focusing on the dorylaimid nematodes (PEÑA-SANTIAGO and ALVAREZ-ORTEGA, 2014). Three *Ecumenicus* sequences were grouped in a clade with high support (BI = 100). This is the first molecular study of *E. monohystera* in Iran. There were only two records in GenBank for sequences of D2-D3 expansion segments of 28S rRNA gene for the genus *Ecumenicus*.

In a study by HOLTERMAN *et al.* (2006) based on small subunit ribosomal DNA (SSU rDNA) gene, the phylogenetic relationships within the order Dorylaimida were fully unclear. Because of low diversity of the SSU rDNA within the order Dorylaimida, HOLTERMAN *et al.* (2008) used the large subunit ribosomal DNA (LSU rDNA) gene for this purpose. The LSU trees show a better resolution within the Dorylaimida, although a large basal polytomy still remained. The relatively high degree of variability of the LSU rDNA genes among nematodes reduces the chances of unwanted cross reactivity considerably (HOLTERMAN *et al.*, 2008).

PEÑA-SANTIAGO and ALVAREZ-ORTEGA (2014) studied the evolutionary relationships of rounded-tailed dorylaimid genera traditionally classified under the family Qudsianematidae. Evidences were provided that support a closer relationship of these genera with members of Dorylaimidae rather than with other representatives of Qudsianematidae. They believe that the rounded-tailed forms may evolutionary derive from long-tailed forms and transferred eight genera from Qudsianematidae to Dorylaimidae including *Labronema* Thorne, 1939, *Crassolabium* Yeates, 1967 (syn. *Thonus* Thorne, 1974) and *Labronemella* Andrássy, 1985. Postembryonic development and molecular data of most of these genera has not been studied yet.

Currently, a limited number of the genera among dorylaimid families have been sequenced. As a result, in order to clarify the relationships among genera of these families, a more comprehensive phylogenetic study is

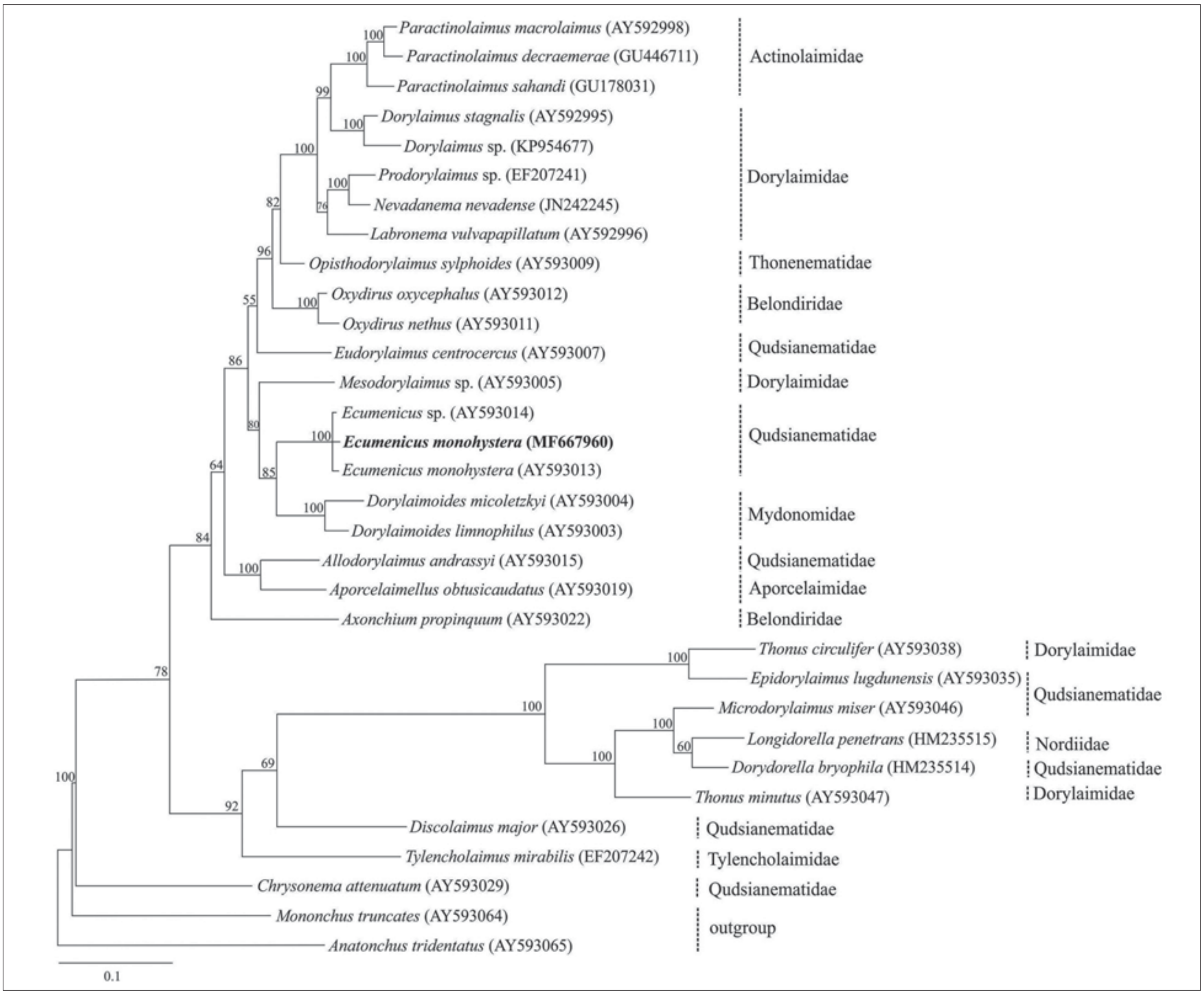

Fig. III – Bayesian 50% majority rule consensus tree inferred from analysis of the D2-D3 domains of the 28S rRNA gene under the GTR + G + I model. Bayesian posterior probability values more than 50% are given for appropriate clades. New sequence is indicated in bold.

needed, in particular on all available species of these genera from different geographical origins.

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DARIA CORCOS (*) - MASSIMILIANO CENTORAME (*) - PIERFILIPPO CERRETTI (*)

DNA BARCODES REVEAL A NEW HOST RECORD FOR *CARCELIA ATRICOSTA* HERTING (DIPTERA TACHINIDAE) IN ITALY

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Corresponding author: Daria Corcos (daria.corcos@uniroma1.it)

Corcos D., Centorame M., Cerretti P. – DNA barcodes reveal a new host record for *Carcelia atricosta* Herting (Diptera Tachinidae) in Italy

The parasitoid-host association between *Orgyia antiqua* (Linnaeus) (Lepidoptera: Lymantriidae) and *Carcelia atricosta* Herting (Diptera: Tachinidae) is recorded here for the first time in Italy. A single caterpillar of *O. antiqua* was collected in Northern Italy (Arzergrande, Padua, Veneto Region) in June 2015. After the specimen died, a single tachinid larva emerged and pupariated. The emerged parasitoid was identified using DNA barcoding, with DNA extracted from the tachinid pupa. This is the first distributional record of *C. atricosta* in Northern Italy and the second for Italy, only two other specimens having being recorded previously (Abruzzo Region, Central Italy).

KEY WORDS: Parasitoid, parasitoid-host association, host range, Lepidoptera, *Orgyia antiqua*, pupa.

INTRODUCTION

Tachinids are one of the largest and most diverse families of Diptera worldwide (STIREMAN *et al.,* 2006). The adults usually feed on nectar and pollen, while the larvae are parasitoids of arthropods, mostly exophytic caterpillars and other herbivorous insects. Compared to hymenopteran parasitoids, tachinids are generally considered to have a broad host range (STIREMAN *et al.,* 2006). However, recent studies show that their host range is extremely variable, with both polyphagous and specialized species (STIREMAN, 2016). Understanding the real extent of tachinid host ranges is extremely difficult because the family is taxonomically challenging, there are many undescribed and cryptic species, and rearing tachinids from all possible hosts poses practical problems (STIREMAN, 2005).

Orgyia antiqua (Linnaeus) (Lepidoptera: Lymantriidae), the rusty tussock moth, is a polyphagous moth native to Europe, but now introduced and widespread throughout Asia and North America. As with many other lymantriids, it can reach high population densities, becoming an important defoliator and pest of trees and cultivated plants. In Italy, *O. antiqua* is known to be parasitized by the tachinids *Compsilura concinnata* (Meigen) and *Exorista larvarum* (Linnaeus) (CERRETTI & TSCHORSNIG, 2010); in the Palaearctic Region as a whole, it is parasitized by an additional 15 species of Tachinidae (TSCHORSNIG, 2017).

As part of a sampling study aimed at understanding the parasitoid community of phytophagous insects in a managed forest near Arzergrande (Padua, Veneto Region, Northern Italy), a single caterpillar of *O. antiqua* was collected on a blackberry shrub (*Rubus* sp.; Rosaceae) in June 2015. The specimen was reared and died after a few days. A tachinid larva emerged and pupariated immediately after the caterpillar died. After one year of rearing, an adult tachinid had not eclosed, and the puparium was thus placed in ethanol for preservation. DNA barcoding was used to identify the parasitoid.

MATERIALS AND METHODS

LABORATORY ANALYSIS

Genomic DNA was extracted and isolated by cutting out a section of the tachinid pupa using standard proteinase K–phenol/chloroform method with ethanol precipitation. The pupa has died and dried inside the puparium. Remains of the puparium and pupa have been deposited in the Museum of Zoology, Sapienza University of Rome, in Rome, Italy. The mitochondrial DNA fragment Cytochrome c Oxidase subunit I (COI) was amplified using two primer pairs: M13F-LCO (5'-TGTAAAACGACGGCCAGTGGTCAACAAATCATAAAGATATTGG-3') and M13R-HCO (5'-CAGGAAACAGCTATGACTAAACTTCAGGGTGACCAAAAAATCA-3') (FOLMER *et al.*, 1994, modified). Amplification was carried out in 25 µl reaction volume containing 50mM of MgCl2, 10mM of dNTP, 25 pM of each primer, 0.75 U Taq of Polymerase (Bioline), 1X NH4 reaction Buffer and 50ng of DNA. Cycling parameters were as follows: initial denaturation (94°C, 5m), 35 cycles (94°C, 30s; 50°C, 30s; 72°C, 30s) and final extension (72°C, 10m). PCR products were purified using Exosap-IT (USB Corporation) and sequenced by Macrogen Inc.

PHYLOGENETIC RECONSTRUCTION

Consensus sequences were generated using Geneious R7.0.6 (Biomatters Inc.). Alignment was carried out using the ClustalW program in Geneious R7.0.6 with 26 sequences (Table 1). The Neighbor-joining clustering method was run with MEGA v.6 (KUMAR *et al.*, 2008), using the default parameters.

RESULTS

The COI DNA sequence of the tachinid pupa was deposited in GenBank (NCBI) and is available under accession number MF539618. It was compared with all the sequences of species belonging to the *Carcelia* genus

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Table 1 – Sequences used to run the analysis which results are showed in Fig. I. Our sequence was compared with all COI sequences of the species belonging to the genus *Carcelia* Robineau-Desvoidy available in GenBank (NCBI), excluding sequences without species identification. A minimum of three sequences for haplotype have been retained for each species.

ID in tree	Species	Acc. Num.
MF539618	*C. atricosta*	MF539618
GU142048.1	*C. flavirostris*	GU142048.1
GU142049.1	*C. flavirostris*	GU142049.1
KM960164.1	*C. reclinata*	KM960164.1
KP049202.1	*C. reclinata*	KP049202.1
KP189254.1	*C. formosa*	KP189254.1
KR390013.1	*C. atricosta*	KR390013.1
KR428353.1	*C. reclinata*	KR428353.1
KR435682.1	*C. reclinata*	KR435682.1
KX843832.1	*C. puberula*	KX843832.1
KX843855.1	*C. puberula*	KX843855.1
KX843862.1	*C. lucorum*	KX843862.1
KX843955.1	*C. lucorum*	KX843955.1
KX844011.1	*C. gnava*	KX844011.1
KX844177.1	*C. laxifrons*	KX844177.1
KX844236.1	*C. atricosta*	KX844236.1
KX844238.1	*C. tibialis*	KX844238.1
KX844339.1	*C. lucorum*	KX844339.1
KX844462.1	*C. atricosta*	KX844462.1
KX844500.1	*C. tibialis*	KX844500.1
KX844513.1	*C. tibialis*	KX844513.1
KX844522.1	*C. bombylans*	KX844522.1
KX844525.1	*C. bombylans*	KX844525.1
KX844543.1	*C. laxifrons*	KX844543.1
KX844474.1	*C. rasa*	KX844474.1
HQ548469.1	*Blepharipa* sp.	HQ548469.1

available in GenBank [July 2017], revealing a similarity of 100.0 % with *Carcelia atricosta* Herting (Diptera: Tachinidae) (Fig. I). Other than *C. atricosta*, eight additional species belonging to the same genus have been recorded in Italy: *Carcelia alpestris* Herting, *Carcelia bombylans* Robineau-Desvoidy, *Carcelia dubia* (Brauer & Bergenstamm), *Carcelia gnava* (Meigen), *Carcelia laxifrons* Villeneuve, *Carcelia lucorum* (Meigen), *Carcelia rasa* (Macquart), and *Carcelia rasella* Baranov. Among them, *C. gnava* and *C. rasa* are known as parasitoids of *O. antiqua* in the Palaearctic Region (TSCHORSNIG, 2017). Both species were included in the analysis.

Carcelia atricosta is scattered distributed throughout Europe from the Mediterranean to Norway (PAPE *et al.*, 2015). Only two specimens have been previously collected in Italy, both captured in Malaise traps in Central Italy (Collelongo site- Selva Piana (AQ), Abruzzo Region; 3-17 August 2004; lat. 41.8930°, long. 13.5968°; 1500 m; legit. M. Romano; collection P. Cerretti, Museum of Zoology, Sapienza University, Rome, Italy) (CERRETTI, 2010). This represents the first record for Northern Italy (Arzergrande site (PD), Veneto Region; 26 June 2015; lat. 45.2565°, long. 12.0551°, 8 m; legit. D. Corcos).

DISCUSSION

The association between *Orgyia antiqua* and *Carcelia atricosta* had already been reported for the Czech Republic, the Netherlands and the United Kingdom (TSCHORSNIG, 2017 and literature therein), but is here recorded for the first time for Italy. Other known lepidopteran hosts of *C. atricosta* are: *Orgyia recens* Hübner (Lymantriidae), *Malacosoma neustria* Linnaeus (Lasiocampidae) and *Acronicta psi* Linnaeus (Noctuidae) (TSCHORSNIG, 2017).

The importance of tachinids as natural enemies of phytophagous pest insects is well documented. However, tachinid-host associations are still poorly understood (STIREMAN, 2016), in part because of the difficulties in reproducing the optimal conditions for rearing specimens in the laboratory. The identification of tachinid larvae or puparia based on morphological characters is seldom possible. As an alternative to morphological identifications of these lesser known life stages, the increasing number of COI sequences of tachinids in molecular libraries are creating a growing inventory of data that allows for the rapid and affordable identification of taxa (POHJOISMAKI *et al.*, 2016). The use of molecular tools, as well as the availability of DNA sequences online, can dramatically improve our knowledge of parasitoid-host associations, especially in the case of rare or poorly-known species. Investigating the degree of tachinid host specificity and how widespread species conserve or change their host species in different regions, may help us to better understand the forces driving the diversification and evolution of these parasitoids (STIREMAN 2005). Also, because of their importance as enemies of pest insects, improving our knowledge of tachinid-host associations may be particularly useful in planning successful biological control programs (STIREMAN *et al.*, 2006).

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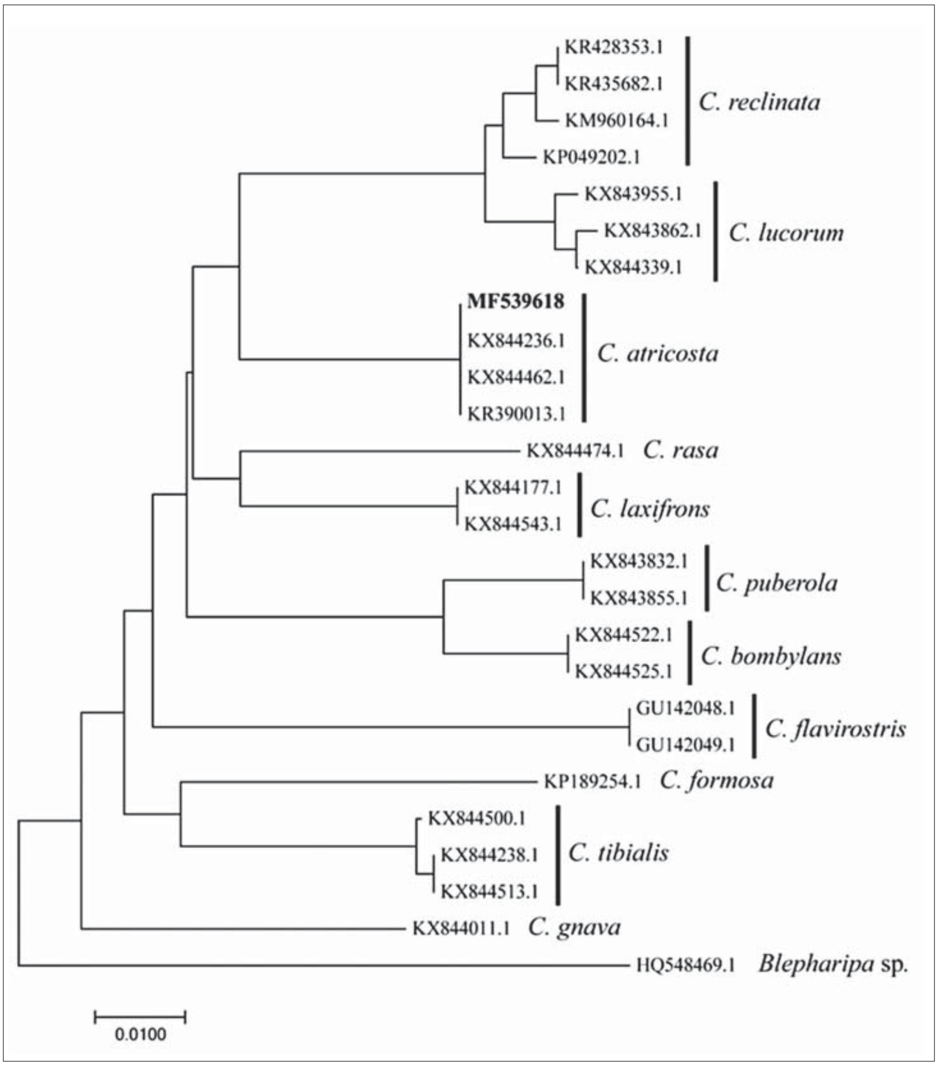

Fig. I – Phylogenetic tree based on 543bp of the COI gene built with the Neighbor Joining method. Our sequence (MF539618) clusters with available sequences for *C. atricosta* sharing the same haplotype.

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PASQUALE TREMATERRA (*) (°) - MARCO COLACCI (*)

MORPHOLOGY AND ETHOLOGY OF *THAUMETOPOEA HELLENICA* AND *THAUMETOPOEA MEDITERRANEA* (LEPIDOPTERA NOTODONTIDAE THAUMETOPOEINAE)

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Trematerra P., Colacci M. – Morphology and ethology of *Thaumetopoea hellenica* and *Thaumetopoea mediterranea* (Lepidoptera Notodontidae Thaumetopoeinae).

Thaumetopoea hellenica and *Thaumetopoea mediterranea* were recently described from Central Greece and Pantelleria Island (Italy), respectively. In this paper, we reported some information on their morphology, biological cycle and ecology. *T. hellenica* and *T. mediterranea* are closely related to *T. pityocampa* differing mainly in genetic characters. Morphologically, the three species resemble each other, and can be mainly differentiated by observing wing patterns, canthus, and the male and female genitalia. Some data on egg batches, mature larvae, pupae, flight period, host plants and colonized environments were reported.

Key Words: *Thaumetopoea hellenica*, *Thaumetopoea mediterranea*, Greece, Pantelleria Island, morphology, biological cycle, ecology.

INTRODUCTION

Thaumetopoea hellenica Trematerra and Scalercio, and *Thaumetopoea mediterranea* Trematerra and Scalercio, were recently described from central Greece and Pantelleria Island (Italy) respectively (Trematerra *et al.*, 2017). Specimens were identified by morphological characters and DNA barcoding analysis realized according to Hajibabei *et al.* (2006) and by Ratnasingham and Hebert (2007).

With the aim to provide a contribution to the knowledge of these two species we are reporting new data on their morphology, biological cycle and ecology. Additionally some remarks on morphological characters of the different stages, of the adults phenology, larval activity and food plants are reported.

Investigated localities for *T. hellenica* and field observations were in Goritsa (Volos) and Ktima Syggrou (Athens) during 2015 and 2016. Goritsa area is a suburban park covered by approximately 120 hectares of pine forest, mainly composed of *Pinus brutia* Tenore and secondarily of *Pinus halepensis* Miller. The urban park of Ktima Syggrou area is covered by approximately 65 hectares of pine trees, which in majority are *Pinus halepensis* (Fig. I).

Investigated localities for *T. mediterranea* and field observations were in Montagna Grande (Pantelleria Island) during 2016 and 2017 at a site with *Pinus pinaster* Aiton, and in Khaddiuggia site with plants of *Cedrus deodara* (Roxb.) G. Don. The National Park of Pantelleria extends for about 1,500 hectares. It is mostly covered by the maritime pine, *Pinus pinaster*. Also very common are reforestations of Aleppo pine, *Pinus halepensis* and *Pinus pinea* L. (Fig. VII).

The adults of *T. hellenica* were mostly found in seven G-trap pheromone funnel traps (SEDQ, Barcelona, Spain) baited with lures containing 1 mg of the synthetic sex pheromone component (*Z*)-13-hexadecen-11-ynyl acetate (Trécé Inc., Adair, OK, USA) (Athanassiou *et al.*, 2017). Data of *T. hellenica* were compared with the same data obtained in the monitoring of *Thaumetopoea pityocampa* (Den. & Shiff.), realized in Petacciato area (Central Italy) during 2015 and 2016 using data coming from four G-trap devices.

The adults of *T. mediterranea* were collected with three Mastrap funnel traps (Isagro, Milano, Italy), baited with lures containing 1 mg of the synthetic sex pheromone (*Z*)-13-hexadecen-11-ynyl acetate (Isagro, Milano, Italy). The data obtained in the study of this species were compared with the observations realized on *T. pityocampa* collected on Monte Etna, at site of Pitarrona (Sicily) during 2016 and 2017, using data coming from two Mastrap devices.

Additionally, several specimens of *T. hellenica* and *T. mediterranea* were collected using light traps (160 Watts mixed light).

To provide an overview of the available knowledge related to *T. hellenica* and *T. mediterranea*, some text already published by Trematerra *et al.* (2017) in the original description of the two species are reported.

Notes on ***Thaumetopoea hellenica***
Trematerra and Scalercio, 2017

Description – Adult (Fig. II, 1). The male has a wingspan of 30-35 mm, the female is bigger, with a wingspan of 43 mm. Pronounced “canthus” on the front. Wing whitish-grey with three dark-brown transverse bands: basal band small, median band interrupted in the middle and more evident in basal margin, distal band completely darker near costal margin; discocellular spot fairly vague, half-moon shaped, light brown-greyish in colour. Distal spot at apex of wing brown-greyish. Costal area brownish-grey. Cilia brown to light-brown, rather concolorous with markings, brown in basal part, alternate to whitish groups. Hindwings whitish-cream with vague anal spot; anal margin with a brownish

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Fig. I – Environments colonized by *Thaumetopoea hellenica* in Greece.

thin band; fringes whitish-cream. Tuft of hairs, light brown-cream on internal margin.

Frontal process of males (Fig. II, 2) – It has five teeth, one long and four small, and of these the first one is quite pronounced. In frontal view, the bottom four have a different shape. In front of the canthus a developed prominence is present.

Male genitalia (Fig. II, 3) – Uncus developed, slightly curved, projected forward; socii broad, subtriangular-shaped, twice as long as its width, sickle-shaped. Tegumen broad, constricting towards the apex. Valva subtriangular, elongated, about twice as long as its width, costal margin rather straight slightly concaved at apex; cucullus rounded; ventral edge of valva and sacculus rounded. Aedeagus amphora-shaped, longer than valva, slender, narrow at the distal part; vesica with tip, not margined medially, coecum penis enlarged; juxta subrectangular-shaped, longer than wide, proximal margin hollowed, distal margin rounded. Saccus rather long.

Female genitalia (Fig. II, 4) – Papillae anales small, apophyses short. Sterigma semi-circular shape narrow, evident and robust, with distal margin concave; subgenital sternite with dense thorns, wide and distally concave. Ductus bursae not sclerotized.

Egg batches (Fig. III) - The egg batches are light brown or greyish, ovum is spherical, whitish and covered with creamy scales. The 21 egg batches studied contained 4,753 eggs, the mean per batch is 226 (range 168-266) eggs. The number of egg-rows per batch varied between 7 and 14; a 1 cm egg-row contained a mean of 10 eggs. The length of the needles on which eggs were deposited varied between 41 and 112 mm and the length of the egg batches varied from 19 to 34 mm (mean 26 mm), with a diameter of 3.4-5.5 mm. Most of the egg batches were wrapped around one or two needles, or deposited over small twigs. The distance of the egg batches to the base of the needles varied. In all cases oviposition occurred from base to tip of the needles.

Wintering larvae and Nests (Fig. IV and Fig. V, 1 and 2) – The full-grown larva is 30–34 mm in length. The head capsule is black. In general, the integument is darker in colder areas and varies from dull bluish-grey to black. The lateral and ventral setae vary from white to yellowish. The dorsal setae range from yellowish white to orange and are borne on brown verrucae. The lower part of the body is from brownish to brown.

As in other *Thaumetopoea* species, also *T. hellenica* build winter nests on external parts of colonized trees. Nests are especially exposed to the South in the part that receives more sunlight during the winter.

In 2016, the first wintering migrant larvae of *T. hellenica* were observed during the first week of February in Ktima Syggrou (Athens) and two weeks later at Goritsa (Volos) when the temperature was high enough. The latest processions were observed on March 20 at Goritsa and on March 26 at Ktima Syggrou. In both localities the highest number of larvae was recorded from the end of February to mid-March (Colacci *et al.*, 2018).

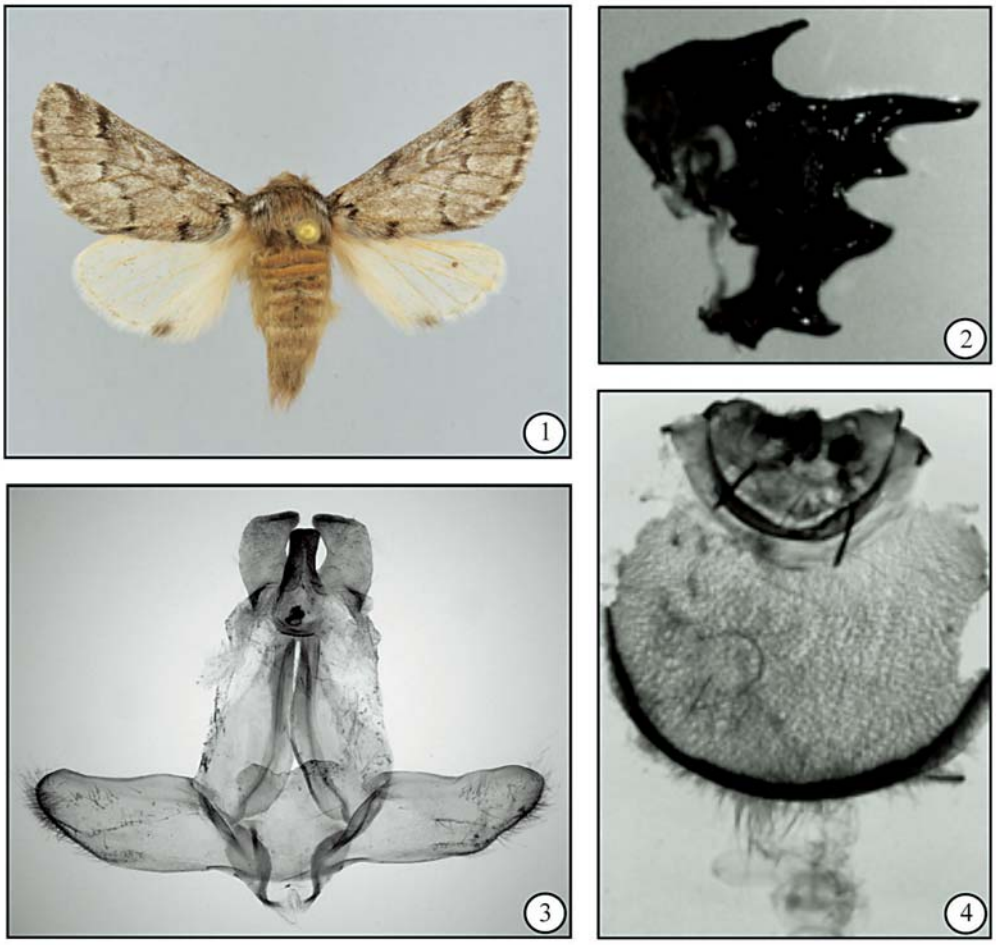

Fig. II – *Thaumetopoea hellenica*: adult (1); frontal process of male (canthus) (2); male genitalia (3); female genitalia (4).

Fig. III - *Thaumetopoea hellenica*: egg batches.

PUPA (Fig. V, 3 and 4) - The pupa is formed in an oval silk cocoon, below ground, and is of a brownish-white colour. The obtected pupae are about 15-19 mm in length, oval, and of a pale brownish-yellow colour that later changes to dark reddish-brown. The cremaster is bluntly rounded, with two curved spines.

FLIGHT ACTIVITY OF ADULTS (Fig. VI) - In Goritsa a total of 796 male adults were captured in the pheromone trap devices, during 2015. *T. hellenica* males started flying in early August (11 August) and ended in the fall in October. The highest number of adults was recorded during late August and mid-September. In Ktima Syggrou a total of 604 adults were captured in the pheromone trap, during 2015. The flight period of males was initiated in late August (25 August) and lasted until early November (10 November). The highest number of adults was recorded during early September, but captures were relatively high from early September and the following weeks till late September. In contrast, during October until the end of the monitoring period in November, captures were extremely low.

Fig. IV - *Thaumetopoea hellenica*: winter nests.

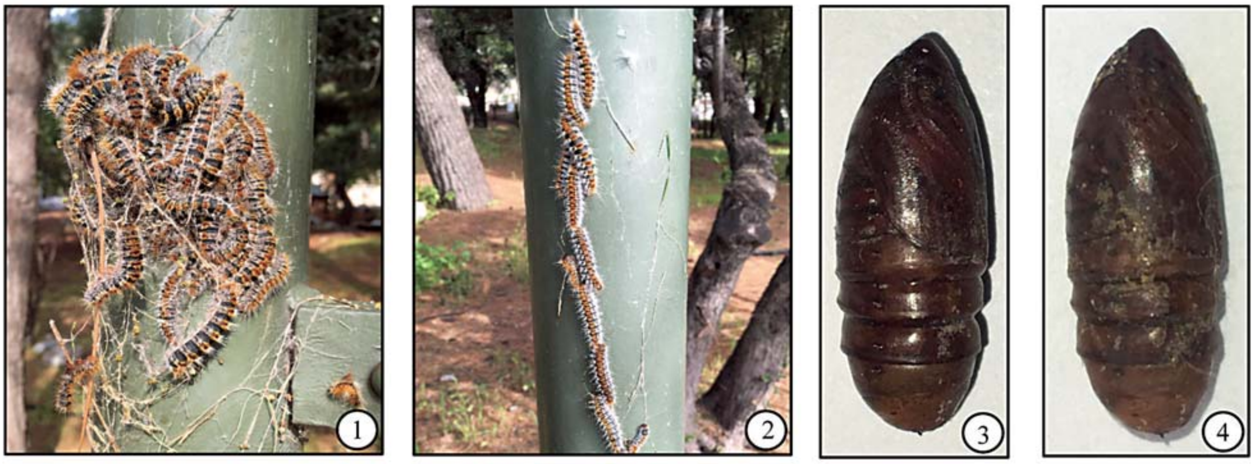

Fig. V - *Thaumetopoea hellenica*: wintering migrant larvae (1 and 2); pupae (3 and 4).

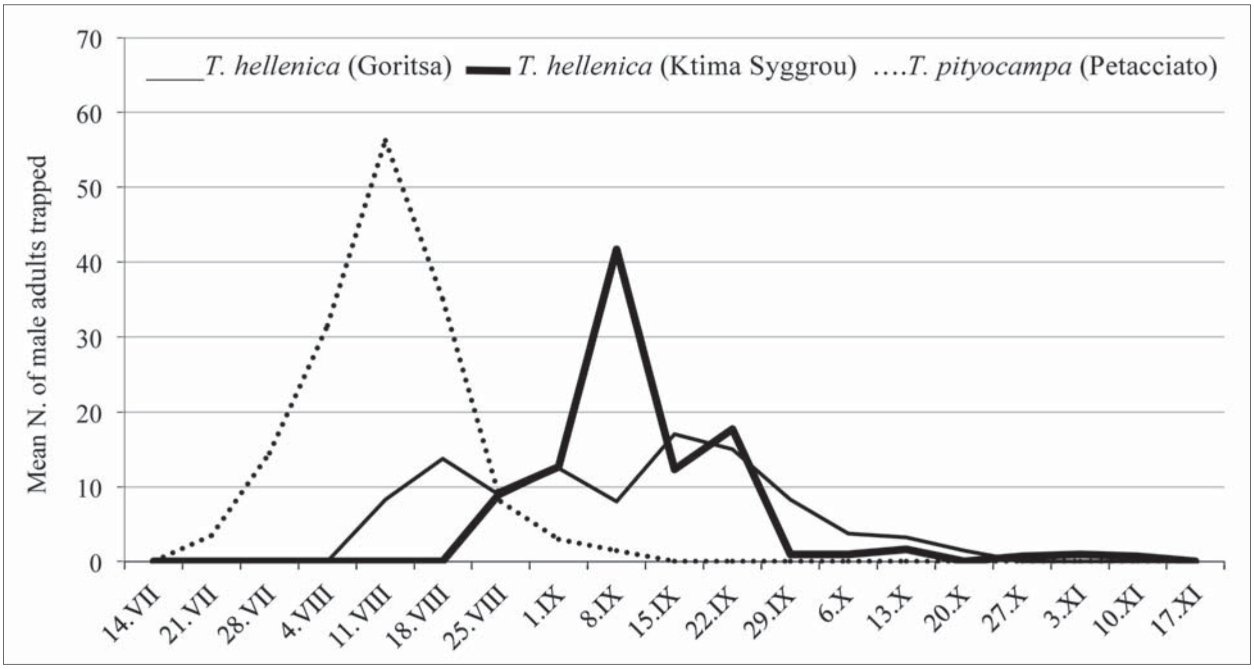

Fig. VI – Flight activity of male adults of *T. hellenica* in Goritsa and Ktima Syggrou (Greece); flight activity of male adults of *T. pityocampa* at Petacciato (Italy).

Fig. VII – Environments colonized by *Thaumetopoea mediterranea* in Pantelleria Island (Italy).

Notes on ***Thaumetopoea mediterranea***

Trematerra and Scalercio, 2017

Description - Adult (Fig. VIII, 1). The male moth has a wing-span of 30–35 mm, the female is bigger, with a wingspan of 41 mm. Scales on scapus and fronts, light brown; thorax blackish-brown, light-brown, with two lateral clear tufts; the abdomen is brushy and sharp, blakish-brown with light brown scales. Male and female both have a very pronounced canthus on the front. The antennae appear pectinate and bipectinate, light brown from the base to the tip. Wing basal colour whitish grey with three blackish transverse thin bands: basal band small, median band complete, distal band complete darker near costal and anal margins; discocellular spot evident, dark-brown/black, arrow shaped. Spot between basal band and median bands evident. Distal spot at apex of wing blackish-brown. Distal part of wings suffused of dark brown/black scales. Costal margin black, costal area blackish. Cilia dark-brown, rather concolorous with markings, alternate to whitish cilia. Wing veins dark-brown. Hindwings whitish-cream, suffused brownish in distal margin, evident black analspot; anal margin with a blackish band; fringes withish-cream, blackish at base. Wing veins of honey colour. Long tuft of hairs, light-brown-cream, on internal margin.

Frontal process of males (Fig. VIII, 2) – Frontal processes of males (canthus) of *T. mediterranea* is reported in figure VIII, 2. Front with a process having special appearance, it has six teeth one long and five small, of these the first is very small the four that follow the more pronounced have a similar shape, in frontal view as seen figure VIII, 2.

Male genitalia (Fig. VIII, 3) – Uncus developed, slightly rounded, projected forward, its top with two thorns; broad gnathos, subtrapezoidal-shaped, longer than wide, ear-shaped. Tegumen broad, constricting towards the apex. Valva subtrapezoidal, elongated, one half as long as its width, costal margin rather straight slightly concaved at apex; cucullus slightly rounded; ventral edge of valva and sacculus rounded with an angle. Aedeagus longer than valva, slender, narrow in the distal part; vesica with tip, immarginated medially, coecum penis enlarged; juxta subrectangular-shaped, wider than long, proximal margin hollowed, distal margin rounded. Saccus rather long.

Female genitalia (Fig. VIII, 4) – Papillae anales small, apophyses short. Sterigma large, broad, with well developed sinuous anteostial part; subgenital sternite is covered with

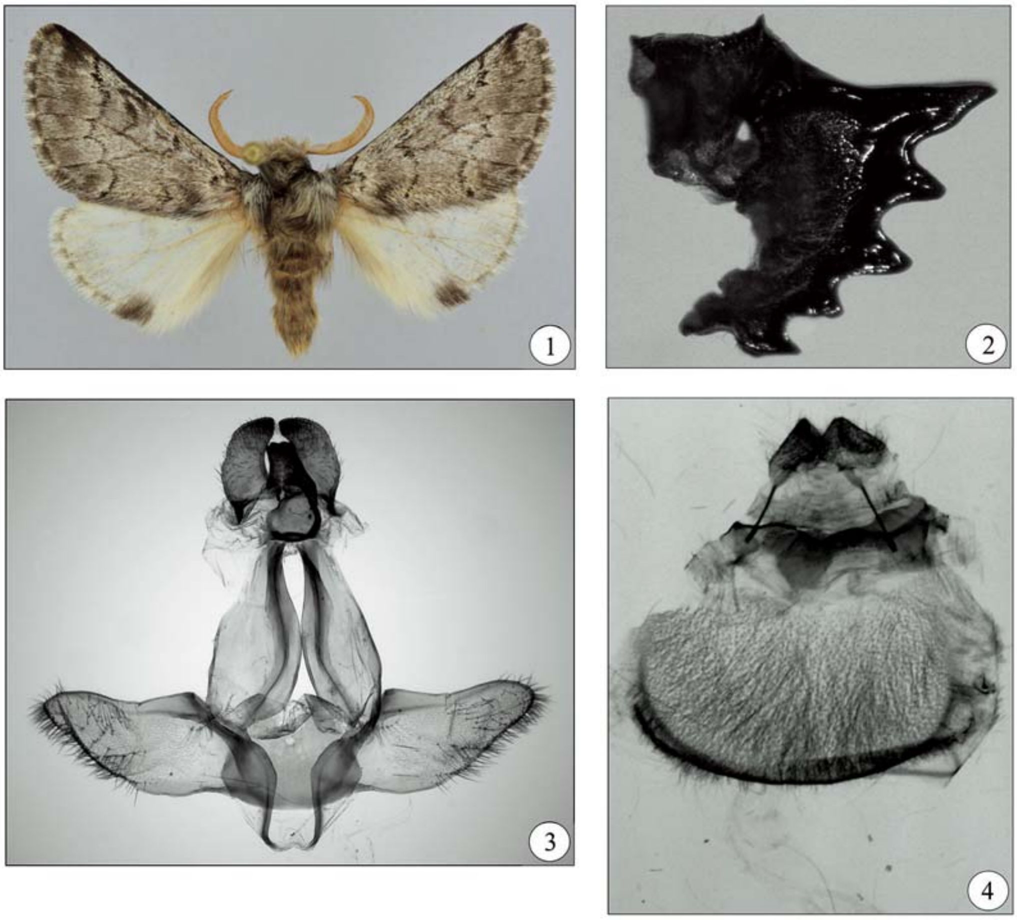

Fig. VIII – *Thaumetopoea mediterranea*: adult (1); frontal process of male (canthus) (2); male genitalia (3); female genitalia (4).

dense thorns and thin bristles, wide and distally concave. Ductus bursae not sclerotized.

Egg batches (Fig. IX, 1) – The egg batches are light brown or greyish, ovum is spherical, whitish and covered with scales. The 18 egg batches studied contained 3,525 eggs, the mean per batch being 195 (range 171-195) eggs. The number of egg-rows per batch varied between 7 and 14; a 1 cm egg-row contained a mean of 10 eggs. The length of the needles on which eggs were deposited varied between 62 and 105 mm and the length of the egg batches varied from 28 to 30 mm, with a diameter of 3.5–5 mm. Most of the egg batches were wrapped around one or two needles, or deposited over small twigs. The distance of the egg batches to the base of the needles varied.

Wintering larvae and Nests (Fig. IX, 2 and X) – The full-grown larva is 30-37 mm in length. The head capsule is black. In general, the integument is darker in colder areas and blackish in ground colour. The lateral band is whitish grey. The dorsal surface with tufts of whitish yellow to orange setae and red setae on reddish-brown verrucae. The lower part of the body is from brownish to brown.

As in other *Thaumetopoea* species, *T. mediterranea* also builds nests on external parts of colonized trees. Nests are especially exposed to the South in the part that receives more

Fig. IX - *Thaumetopoea mediterranea*: egg batches (1); mature larvae (2); cocoons (3); pupa (4).

Fig. X - *Thaumetopoea mediterranea*: winter nests.

sunlight during the autumn and winter. Nests are generally small (length 14.2 cm; width 5.5 cm) with 28-46 mature larvae inside. In winter larvae can be observed outside of nests during days with mild temperatures. In 2017, the first larvae emerging in procession were recorded during the second week of February, the latest migrant larvae were observed in late April. The highest number of larvae was recorded in late March.

Pupa (Fig. IX, 3 and 4) – The pupa is formed inside an oval silk cocoon below ground and is of a whitish-brown colour. The obtected pupae are about 18-20 mm in length, oval, and of a pale red-brownish colour that later changes to dark reddish-brown. The cremaster is bluntly rounded, with two robust curved spines.

Flight activity of adults (Fig. XI) – On Pantelleria Island a total of 327 male were captured in the pheromone trap devices, during 2016-2017. In 2017 the flight of *T. mediterranea* males started in the second week of July (14 July) and ended after the second week of October. The highest number of adults was recorded in the first week of September and mid-September.

DIAGNOSIS *T. pityocampa*, *T. hellenica* and *T. mediterranea*

As already stated, among *T. pityocampa*, *T. hellenica* and *T. mediterranea* there are genetic differences. Morphologically the three species are closed each other, but can be

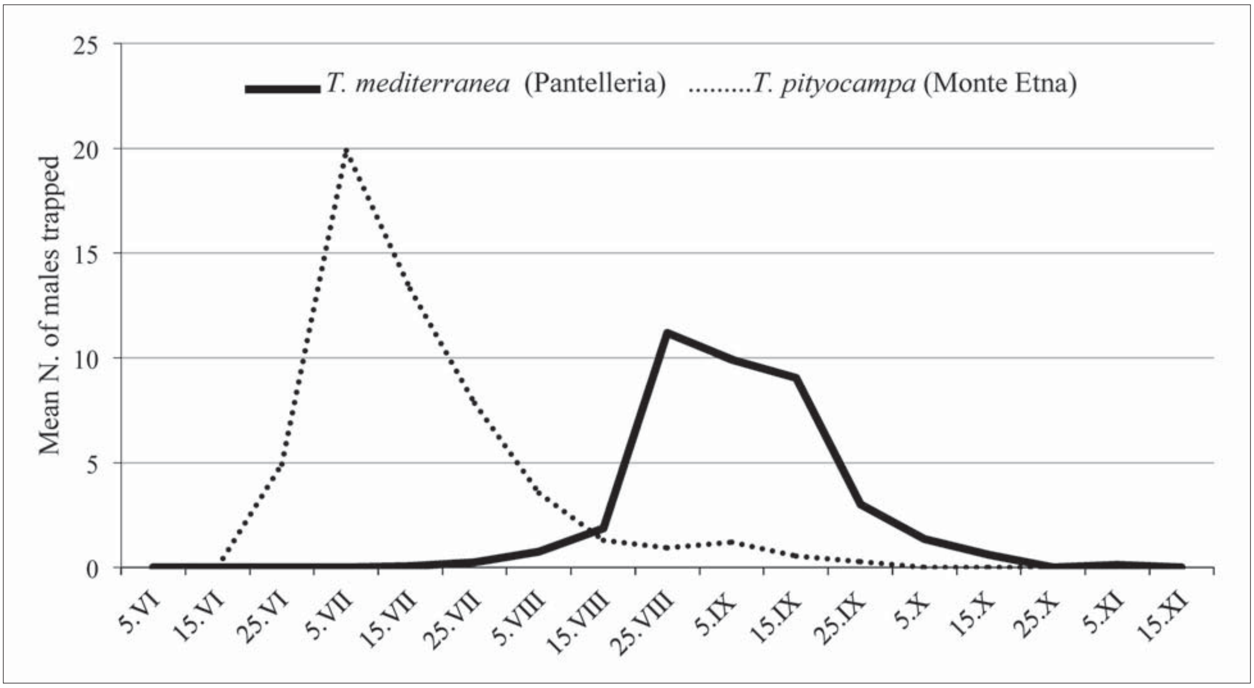

Fig. XI – Flight activity of male adults of *T. mediterranea* in Pantelleria Island (Italy); flight activity of male adults of *T. pityocampa* at Monte Etna (Sicily).

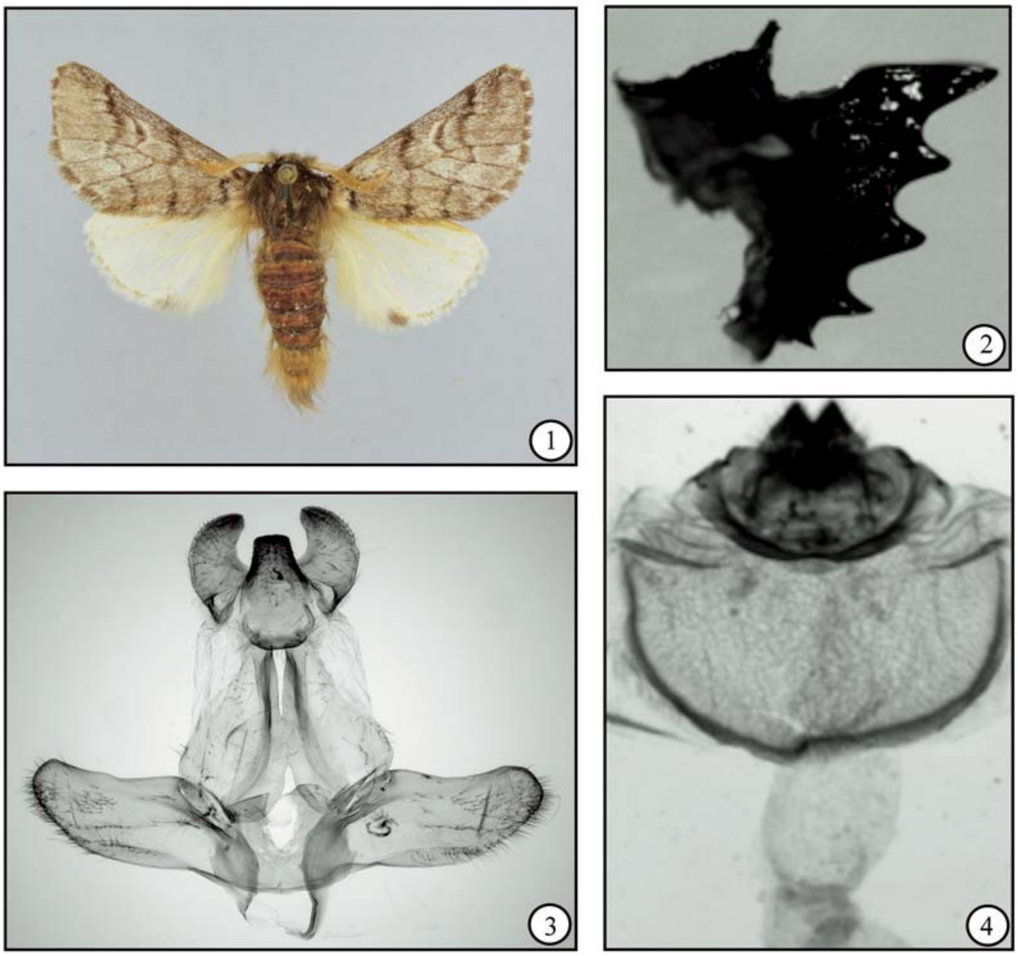

Fig. XII – *Thaumetopoea pityocampa*: adult (1); frontal process of male (canthus) (2); male genitalia (3); female genitalia (4).

differentiated by their wing patterns and the forms of the canthus, mainly in male genitalia (in shape of gnathos, valvae and juxta) and also in female genitalia (in sterigma and subgenital sternite).

Comparing the wing pattern of the three species it is possible to note that in *T. mediterranea* there is more marked and evident, black colour, with bigger anal spot on hindwings. Discocellular spot of *T. pityocampa* is more close to distal margin than in the other two species. Transverse bands are more thin in *T. mediterranea*. Median band is interrupt in *T. hellenica* and complete in *T. pityocampa* and *T. mediterranea* (Fig. II, 1; Fig. VIII, 1; Fig. XII, 1).

The frontal process of *T. pityocampa*, *T. hellenica* and *T. mediterranea* have five teeth, the upper one is smaller in *T. mediterranea* (Fig. II, 2; Fig. VIII, 2; Fig. XII, 3).

In male genitalia of *T. mediterranea,* the uncus is longer than gnathos; the gnathos in *T. mediterranea* is sub-triangular, ear-shaped and is longer than wide, while in *T. pityocampa* the gnathos is broad, bean-shaped or even semi-circular. Valva almost elongated and narrows in *T. hellenica*, and compared to *T. pityocampa* the valva in *T. mediterranea* is bigger. *T. mediterranea* has the aedeagus bigger and broader than that of *T. hellenica* and *T. pityocampa*. Shape of juxta is sub-rectangular, longer than wide, while in *T. hellenica*, it is subrectangular, wider than long in *T. mediterranea*. Juxta of *T. pityocampa* is sub-triangular. Proximal margin hollowed, in *T. hellenica* and *T. mediterranea,* less so in *T. pityocampa*. It is shield-shaped, with proximal margin stretched to the angles in *T. pityocampa* and *T. hellenica* (Fig. II, 3; Fig. VIII, 3; Fig. XII, 3).

Morphological characteristics of female genitalia in *T. pityocampa*, *T. hellenica* and *T. mediterranea* have not yet been illustrated and studied. In the three species the papillae anales are small and the apophyses short. The subgenital sternite has dense thorns and is covered with thin bristles especially in *T. mediterranea*. It is wide and distally concave in the three species, but in *T. pityocampa* two lateral transverse projections are present. Ductus bursae is not sclerotized. The sterigma in *T. mediterranea* is large, broad, with well-developed sinuous anteostial part, as opposed the semicircular shaped and narrow sterigma of *T. hellenica* and *T. pityocampa*. Characteristics of female genitalia need to be better considered in future researches (Fig. II, 4; Fig. VIII, 4; Fig. XII, 4).

In genetic characters, the distance of the *T. hellenica* Bin from that of *T. mediterranea* is equal to 3.05%; the distance from *T. hellenica* and the nearest sequence of *T. pityocampa* deposited in GenBank is quite higher, being equal to 7%. The distance from *T. mediterranea* and the nearest sequence of *T. pityocampa* deposited in GenBank is equal to 6% (Trematerra *et al*., 2017). The sequence of the holotype of the species *T. mediterranea* is identical to the sequence deposited in GenBank (accession number HE963113.1) belonging to the ENA clade of Simonato *et al.* (2013) (see also Avtzis *et al*., 2018).

Comparing egg batches, mature larvae and pupae those of *T. hellenica* are smaller than those of *T. pityocampa*. On the contrary, in *T. mediterranea* they are similar to those in *T. pityocampa*. In this respect, it is important to note that from the direct observations and data reported by various authors

Table 1 – Thaumetopoea species, distribution range and host plants. Data from: Agenjo (1941), Doganlar and Avci (2001), Doganlar *et al.* (2005), Simonato *et al.* (2013), Battisti *et al.* (2015), Kerdelhué *et al.* (2015), Basso *et al.* (2016), Hacker (2016), Trematerra *et al.* (2017).

Species	**Distribution**	**Host plants**
T. apologetica	Eastern Africa	*Maerua crassifolia*
T. bonjeani	North-Western Africa	*Cedrus atlantica*
T. cheela	Afghanistan, Pakistan, India	*Rhus cotinus*
T. dhofarensis	Arabian Peninsula	
T. hellenica	Greece	*Pinus halepensis, Pinus brutia* ?
T. herculeana	Europe, Middle East, India	*Cistus salviaefolius, Erodium moschatum, Erodium arborescens, Helianthemum vulgare, Helianthemum croceum*
T. ispartensis	Turkey	*Cedrus libani*
T. jordana	Northern Africa, Jordan valley	*Rhus tripartita, Schinus terebinthifolium*
T. libanotica	Lebanon	*Cedrus libani*
T. loxostigma	Yemen	
T. mediterranea	Algeria, Tunisia, Libya, Italy	*Cedrus deodara, Pinus pinea, Pinus pinaster*
T. pinivora	Europe	*Pinus* spp.
T. pityocampa	Europe, North Africa, Middle East	*Cedrus* spp., *Pinus* spp., *Pseudotsuga* spp.
T. processionea	Europe, Middle East	*Quercus* spp.
T. sedirica	Turkey	*Cedrus libani*
T. solitaria	Europe, Middle East	*Cupressus sempervirens, Fraxinus* spp., *Pistacia palaestina, Pistacia terebinthus, Pistacia atlantica, Pistacia vera, Rhus* spp., *Schinus* spp.
T. torosica	Turkey	*Pinus brutia*
T. wilkinsoni	Middle East	*Cedrus* spp., *Pinus* spp.

in different European locations, the data provided on *T. pityocampa* are variable (Mirchev *et al*., 2015).

Comparing flight period of *T. hellenica* (in Greece) and *T. pityocampa* (in Petacciato, Italy) it is noted that flight period of the first species is delayed, with adults of *T. hellenica* observed from early August to late October and early November, while *T. pityocampa* were found from late July-early August to mid September (Fig. VI). With regards to the flight of *T. mediterranea* (in Pantelleria Island) and *T. pityocampa* (in Pitarrona, on Monte Etna) it is noted that flight period of first species is delayed, with males of *T. mediterranea* trapped in the second week of July (14 July) and lasted after the second week of October. On Monte Etna, the flight period of *T. pityocampa* males started on 15 June and lasted until the fall in September (Fig. XI).

As in other *Thaumetopoea* species (Table 1), also *T. hellenica* and *T. mediterranea* build winter nests on external parts of trees. *T. mediterranea* nests are smaller than those of *T. pityocampa* and *T. hellenica*. *T. hellenica* colonize *Pinus halepensis* and probably also *P. brutia* plants; *T. mediterranea* colonize *Cedrus deodara, Pinus pinaster* and *Pinus pinea.*

Comparing *T. hellenica* (in Greece) and *T. pityocampa* (in Petacciato, Italy) winter migration of emerging larvae period, it is possible to note that migration period of *T. hellenica* larvae is advanced in comparison to *T. pityocampa*. Larvae of *T. hellenica* have been observed in February and in March, while *T. pityocampa* were found from the end of March till the last week of April. Comparing *T. mediterranea* (in the Pantelleria Island) and *T. pityocampa* (on the Monte Etna) migration larvae period, it is possible to note that migration period of *T. mediterranea* is advanced with larvae observed in February and in March, while *T. pityocampa* were found from late March to late April.

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REDIA, 101, 2018: 23-30 **SHORT REVIEW** http://dx.doi.org/10.19263/REDIA-101.18.04

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INSECTS AND FUNGI ON THE RELICT *ZELKOVA SICULA* (ROSALES, ULMACEAE) IN SICILY (ITALY): NEW RECORDS AND KNOWN SPECIES IN A SHORT REVIEW

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Campo G., Mazzeo G., Nucifora S., Perrotta G., Sidoti A., Bella S. – Insects and fungi on the relict *Zelkova sicula* (Rosales, Ulmaceae) in Sicily (Italy): new records and known species in a short review.

The Authors report the results of a study conducted with the purpose of increasing the knowledge about the insects and fungi living on *Zelkova sicula* Di Pasquale, Garfì & Quézel, 1992 (Rosales, Ulmaceae). The plant is a very rare relict of the Tertiary period, belonging to a genus of trees extinct in continental Europe. Only two small populations are known living in a restricted woodland in the province of Syracuse, Sicily. The results concern both the insects picked up during surveys that were carried out in 2014 and 2015, and the species of fungi and insects already known on *Zelkova sicula* from literature. As a result of surveys, two species of Buprestidae, two species of Cerambycidae, and two of Lepidoptera have been recognized. From literature, seven species of phytophagous insects are reported on *Zelkova sicula*, they belong to Hemiptera: Aphididae (1 sp.), Diaspididae (1 sp.), Coccidae (1 sp.); Coleoptera: Buprestidae (1 sp.), Cerambycidae (2 spp.); and Lepidoptera: Lasiocampidae (1 sp.). Five species of fungi are known from literature and they belong to: Botryosphaeriales (3 spp.), Pleosporales (1 sp.), and Diaporthales (1 sp.).

KEY WORDS: endemic plant, Sicilian Zelkova, insect hosts, tree conservation, phytosanitary status.

INTRODUCTION

As a consequence of paleogeographic events, Sicily is considered one of the most relevant hotspots of biodiversity in the Mediterranean area (BELLA, 2008; MASSA *et al.*, 2011; BELLA, 2014). The island has about 3,000 species of plants and more than 300 endemic taxa (BRULLO *et al.*, 1995; GIARDINA *et al.*, 2007; DOMINA *et al.*, 2012).

Zelkova (Rosales, Ulmaceae) is a small relict genus that dates back to the Tertiary period. The six extant species are distributed throughout western and eastern Asia (Caucasus: *Z. carpinifolia* (Pall.) Dippel), East Asia (*Z. serrata* (Thunb.) Makino) and China (*Z. schneideriana* Handel-Mazzetti and *Z. sinica* C.K. Schneider), except the two in the Mediterranean basin (Sicily: *Z. sicula* Di Pasq., Garfì & Quézel and Crete: *Z. abelicea* (Lam.) Boiss.) (GTC, 2017). Habitat loss, logging, increased periods of drought, and limited reproduction represent major threats for these species. The IUCN list evaluates this species as 'Critically Endangered' (KOZLOWSKI & GRATZFELD, 2013). Some species are threatened with extinction and others are still waiting assessment, so a global action plan 'Project Zelkova' has been developed (FINESCHI *et al.*, 2004; GARFÌ & BUORD, 2012).

The arthropod fauna living on the Sicilian endemic *Zelkova sicula* have been rarely studied, and only little data are currently available (CAMPO *et al.*, 2015), whereas fungal flora was studied in the past years and the results have been already published (GRANATA *et al.*, 2002; SIDOTI & GRANATA, 2005; TORTA *et al.*, 2008; SIDOTI *et al.*, 2016).

The aim of this study is to improve the knowledge both of the entomofauna and the fungal flora associated with this rare species and to group the currently known data on the phytosanitary issues that affect this plant.

MATERIALS AND METHOD

THE STUDY SITES

Samples were collected in the Iblei Mountains in southeastern Sicily, in the province of Syracuse (Fig. I):

- site ZS1: Buccheri, bosco Pisano, 450-550 m a.s.l., 37°10′18″ N - 14°51′ 37″ E (SIC: ITA 090022 "Bosco Pisano" – Buccheri, Francofonte and Vizzini, 1850,82 ha);
- site ZS2: Melilli, contrada Ciranna, 310-350 m a.s.l., 37°12′40″ N - 15°02′ 41″ E (SIC: ITA 090024 "Cozzo Ogliastri" – Melilli, 1338,16 ha).

– Received 19 December 2017 Accepted 19 February 2018

Fig. I – Presence of *Zelkova sicula* in southeastern Sicily (Iblei Mts.): sites ZS1 (red), and ZS2 (green).

THE PLANT

Endemic to Sicily, *Zelkova sicula* is considered one of the rarest and most endangered trees worldwide. It is a deciduous shrub or tree usually 2-3 m high. It was first discovered in 1991 on the northern slopes of the Iblei Mountains in southeastern Sicily, near the municipality of Buccheri, and was thought to exist only as a single population of 230 individuals over an area of 0.4 ha (DI PASQUALE *et al.*, 1992). A second population was discovered in the same mountain range in 2009, near the municipality of Melilli, and only includ 1,200 individuals covering an area of 0.8 ha. The two populations are 17 km apart (GTC, 2017). This species has anomalous pollen, which may explain why all seeds appear to be sterile; propagation is from root suckers. Studies have indicated that the remaining populations of *Zelkova sicula* may be traced to just one individual. This lack of genetic variation reduces the ability of the species to face environmental and/or biological changes (GARFÌ & BUORD, 2012). This species has been classified as “Critically Endangered” by the IUCN Red List because the areas in which it is found are very small, and both the areas and the number of individuals seem to be continuing to decline (MONTMOLLIN & STRAHM, 2005). *Zelkova sicula* has been the object of a conservation project since 2011 (LIFE10NAT/IT/000237).

SAMPLING METHODS AND SUMMARY OF THE BIBLIOGRAPHIC DATA

Field surveys were carried out in the years 2014-2015, in the spring-summer every 15 days. In both sites where *Zelkova sicula* grows, direct observations were made on plants for collecting phythophagous insects, meanwhile dead or suffering wooden samples were picked up for the possible presence of saproxylophagous insects. Samples, consisting of portions of green or woody organs, were taken, placed in containers and carried to the laboratory. Wood insects and phyllophagous species, were isolated in cages with standard temperature and relative humidity (25 °C and 65 % RH), and regularly inspected for collecting adults. In the case of phytomize species, they were observed at binoculars and prepared on a slide for species identification. Beetles have been prepared dry and identified by using dichotomous keys or by resorting to specialists.

Bibliographical search has been conducted on insects and fungi found on *Zelkova sicula* from its discovery to date in order to draw up the list of species already known.

ACRONYMS AND ABBREVIATIONS USED IN THE TEXT

IUCN: International Union for Conservation of Nature
SIC: site of community importance
ZS: *Zelkova sicula*
leg.: legit
loc.: locality
coll.: collected
emer.: emerged
ex./exx.: specimen/specimens

The following data are given for each species: chorotype, larval host plants, and data of collection. The systematics and nomenclature follow SAMA (2013) with the recent updates by BAVIERA *et al.* (2017) for the Cerambycidae family and KUBAN & BÍLÝ (2013) for the Buprestidae family. The rapid evolution of the taxonomy of fungi has led us to refer exclusively to the classification reported by the ‘Index fungorum bioscience database’ (CABI, 2017). The specimens of insects studied are stored in the collection to the UO S4.04 - Osservatorio per le Malattie delle Piante di Acireale.

RESULTS

NEW RECORDED TAXA

COLEOPTERA
Family BUPRESTIDAE Leach, 1815

Polycestinae Lacordaire, 1857
Acmaeoderini Kerremans, 1893
Acmaeoderella* (*Omphalothorax*) *adspersula adspersula
(Illiger, 1803)

CHOROTYPE: Olomediterranean.

MATERIAL EXAMINED: Buccheri, loc. Bosco Pisano, 1 ex., coll. 27.V.2015, emer. 2016. Melilli, loc. Ciranna, 1 ex., coll. 14.V.2014, emer. 2015.

Larval host plants: Aceraceae (*Acer*), Anacardiaceae (*Pistacia*, *Rhus*), Celtidaceae (*Celtis*), Cesalpinaceae (*Ceratonia*), Cistaceae (*Cistus*), Fabaceae (*Acacia*, *Cytisus*, *Genista*, *Retama*, *Spartium*), Fagaceae (*Castanea*, *Quercus*), Ephedraceae (*Ephedra*), Euphorbiaceae (*Euphorbia*), Moraceae (*Ficus*), Rosaceae (*Malus*, *Sorbus*), Salicaceae (*Populus*), Thymelaeaceae (*Thymelaea*), Ulmaceae (*Ulmus*), Vitaceae (*Vitis*), Zygophyllaceae (*Zygophyllum*) (Curletti, 1994; Mifsud & Barther, 2005).

Remarks: the larvae feed on wood of several plants and the adults are anthophilous (Luna, 2013; Ceccolini *et al.*, 2014).

Buprestinae Lacordaire, 1857
Anthaxiini Gory & Laporte, 1839
Anthaxia* (*Haplanthaxia*) *umbellatarum umbellatarum
(Fabricius, 1787)

Chorotype: Euro-Mediterranean.

Material examined: Melilli, loc. Ciranna, 1 ex., coll. 14.V.2014, emer. 2015; 1 ex., coll. 5.VIII.2014, emer. 2015; 5 exx., coll. 09.VII.2015, emer. 2016; 6 exx., coll. 25.IX.2015, emer. 2016.

Larval host plants: Anacardiaceae (*Pistacia*), Cesalpinaceae (*Cercis*, *Ceratonia*), Cupressaceae (*Cupressus*), Fabaceae (*Acacia*, *Cytisus*), Fagaceae (*Castanea*, *Quercus*), Moraceae (*Ficus*), Rosaceae (*Cydonia*, *Prunus*, *Pyrus*, *Rosa*), Salicaceae (*Salix*), Ulmaceae (*Ulmus*) (Curletti, 1994).

Remarks: the larvae of this polyphagous species feed both on deciduous plants and conifers, unlike the majority of Buprestidae (Curletti, 1981). The adults are anthophilous (Luna, 2013).

Family CERAMBYCIDAE Latreille, 1802

Cerambycinae Latreille, 1802
Graciliini Mulsant, 1839
Penichroa fasciata Dejean, 1839

Chorotype: Turanic-European.

Material examined: Melilli, loc. Ciranna, 2 exx., coll. 5.VIII.2014, emer. 2015; 16 exx., coll. 09.VII.2015, emer. 2016; 12 exx. 14.V.2014, emer. 2015.

Larval host plants: Anacardiaceae (*Pistacia*), Cesalpinaceae (*Ceratonia*, *Cercis*, *Cytisus*), Cupressaceae (*Thuya*), Fabaceae (*Glycyrrhiza*), Fagaceae (*Fagus*, *Quercus*), Moraceae (*Ficus*, *Morus*), Myrtaceae (*Eucalyptus*), Pinaceae (*Pinus*), Rosaceae (*Prunus*) (Baviera *et al.*, 2017).

Remarks: this species is extremely polyphagous mostly feeding on deciduous trees, sometimes on conifers (Sama *et al.*, 2010).

Gracilia minuta (Fabricius, 1781)

Chorotype: Cosmopolitan.

Material examined: 24 exx., coll. 27.V.2015, emer. 2016; 18 exx., coll. 09.VII.2015, emer. 2016; 6 exx., coll. 25.IX.2015, emer. 2016.

Larval host plants: Aceraceae (*Acer*), Anacardiaceae (*Pistacia*), Betulaceae (*Betula*), Celastraceae (*Euonymus*), Cesalpinaceae (*Ceratonia*), Corylaceae (*Corylus*), Fagaceae (*Castanea*, *Quercus*), Hippocastanaceae (*Aesculus*), Juglandaceae (*Juglans*), Moraceae (*Ficus*), Pinaceae (*Cedrus*, *Pinus*), Rhamnaceae (*Rhamnus*), Rosaceae (*Crataegus*, *Malus*, *Prunus*, *Rosa*, *Rubus*, *Sorbus*), Rutaceae (*Citrus*), Salicaceae (*Salix*), Ulmaceae (*Ulmus*) (Baviera *et al.*, 2017).

Remarks: this species affects thin twigs of deciduous trees and shrubs. Adults are xylophagous (Contarini, 2014).

LEPIDOPTERA
Family SATURNIIDAE Boisduval, 1837

Saturniinae Boisduval, 1837
Saturniini Boisduval, 1837
Saturnia* (*Eudia*) *pavoniella (Scopoli, 1763)

Chorotype: European.

Material examined: Buccheri, loc. Bosco Pisano, 1 ex., VI.2004.

Larval host plants: Betulaceae (*Carpinus*), Elaeagnaceae (*Hippophae*), Fagaceae (*Quercus*), Betulaceae (*Betula*), Salicaceae (*Salix*), Ericaceae (*Calluna*, *Erica*, *Spiraea*, *Vaccinium*), Lythraceae (*Lythrum*), Rosaceae (*Crataegus*, *Filipendula*, *Potentilla*, *Prunus*, *Pyrus*, *Rosa*, *Rubus*) (Mazzei *et al.*, 2017).

Remarks: young larvae generally feed on low vegetation and, after a period in which gather, they disperse and larger larvae tend to be found higher up on shrubs (Pittaway, 2018).

Family NYMPHALIDAE

Nymphalinae Swainson (1927)
Nymphalis polychloros (Linnaeus, 1758)

Chorotype: Centralasiatic-Euro-Mediterranean.

Material examined: Buccheri, loc. Bosco Pisano, 1 ex., VI.2004.

Larval host plants: Salicaceae (*Salix*), Rosaceae (*Crataegus*, *Prunus*, *Malus*, *Pyrus*, *Sorbus*), Salicaceae (*Populus*), Ulmaceae (*Ulmus*) (Mazzei *et al.*, 2017).

Remarks: this species, that is widespread in Europe, overwinters as adults which fly from March to April and are most active in summertime (Jurc *et al.,* 2016).

Taxa previously reported on Zelkova sicula

Hemiptera, Aphididae
Zelkovaphis trinacriae Barbagallo, 2002

This Eriosomatine aphid lives exclusively on *Zelkova sicula* protected inside ‘clustering type galls’ (Barbagallo, 2002). The aphid likely performs a dioic lifecycle, with adults from mid-April to early summer, whose secondary host plant remains yet unknown (Barbagallo & Cocuzza, 2008).

REMARKS: the aphid has been found in both stations, but is present with a more abundant population in the ZS1 site.

Hemiptera, Coccidae
***Parthenolecanium* sp.**

Some mature females have been recorded on the twigs of *Z. sicula* in the ZS1 site. The features of these females, characterized by a brown to reddish colour and convex body, seemed to lead to the genus *Parthenolecanium*.

REMARKS: further investigation are needed for the correct identification of the species (MAZZEO *et al.*, 2016).

Hemiptera, Diaspididae
Aspidiotus nerii Bouché, 1833

The oleander scale, a cosmopolitan species, is highly polyphagous, living on 325 genera in 120 families host plants. It is considered a pest of crops and ornamental plants (García Morales *et al.*, 2016).

REMARKS: the specimens, mostly females, were found in the ZS1 site, on the lower surface of leaves, where the presence of hairs caused a modification of the scale covers that showed an irregular outline (MAZZEO *et al.*, 2016).

Coleoptera, Buprestidae
Acmaeodera* (*Acmaeodera*) *pilosellae pilosellae
(Bonelli, 1812)

LARVAL HOST PLANTS: Aceraceae (*Acer*), Anacardiaceae (*Pistacia*), Corylaceae (*Corylus*), Fabaceae (*Colutea*), Fagaceae (*Quercus*), Juglandaceae (*Juglans*), Rosaceae (*Amygdalus*, *Crataegus*, *Prunus*) (CURLETTI, 1994).

REMARKS: this species was only found in the ZS1 site (LONGO & CAMPO, 2004).

Coleoptera, Cerambycidae
Chlorophorus* (*Perderomaculatus*) *sartor (Müller, 1766)

LARVAL HOST PLANTS: Anacardiaceae (*Pistacia*), Cesalpinaceae (*Ceratonia*, *Gleditsia*), Fabaceae (*Cytisus*, *Robinia*), Fagaceae (*Castanea*, *Fagus*, *Quercus*), Corylaceae (*Ostrya*), Moraceae (*Ficus*), Rhamnaceae (*Paliurus*), Rosaceae (*Crataegus*), Salicaceae (*Salix*), Ulmaceae (*Ulmus*) (BAVIERA *et al.*, 2017).

REMARKS: this species was found in the ZS2 site (SIDOTI *et al.*, 2016).

Niphona picticornis (Mulsant, 1839)

LARVAL HOST PLANTS: Anacardiaceae (*Pistacia*), Arecaceae (*Phoenix*), Caprifoliaceae (*Sambucus*), Cesalpinaceae (*Cercis*), Euphorbiaceae (*Euphorbia*), Fabaceae (*Calycotome*, *Genista*, *Robinia*, *Spartium*), Fagaceae (*Castanea*, *Quercus*), Lauraceae (*Laurus*), Moraceae (*Ficus*, *Morus*), Pinaceae (*Pinus*), Punicaceae (*Punica*), Rhamnaceae (*Rhamnus*), Rosaceae (*Prunus*), Ulmaceae (*Ulmus*) (BAVIERA *et al.*, 2017).

REMARKS: this cerambyx has been found in both stations ZS1 and ZS2 (SIDOTI *et al.*, 2016).

Lepidoptera, Lasiocampidae
Lasiocampa* (*Lasiocampa*) *quercus sicula
(Staudinger, 1861)

LARVAL HOST PLANTS: Betulaceae (*Alnus*, *Betula*), Caprifoliaceae (*Lonicera*), Salicaceae (*Populus*, *Salix*), Grossulariaceae (*Ribes*), Rosaceae (*Rubus*, *Spiraea*, *Malus*, *Sorbus*, *Prunus*), Fabaceae (*Trifolium*), Ericaceae (*Andromeda*, *Calluna*, *Ledum*, *Vaccinium*), Oleaceae (*Syringa*), Pinaceae (*Larix*) (Mazzei *et al.*, 2017).

REMARKS: generically reported as Lasiocampide sp. by LONGO & CAMPO (2004) in the ZS1 site.

FUNGI

Botryosphaeriales, Botryosphaeriaceae
Botryosphaeria sarmentorum
A.J.L. Phillips, Alves & Luque, 2005

Anamorph: *Dothiorella sarmentorum* (Fr.) Phillips, Alves & Luque, 2005 (= *Diplodia sarmentorum* (Fr.) Fries).
Botryosphaeria sarmentorum is common in Europe, where it is found in its anamorphic state on a wide range of woody hosts. It is not known if this species is pathogenic, but it is most likely a saprophyte because it is often seen associated with pathogens (PHILLIPS, 2017).

REMARKS: cankers on the bark of twigs and stems and necrotic woody tissues (pycnidia) in the ZS1 site (GRANATA *et al.*, 2002; SIDOTI & GRANATA, 2005; TORTA *et al.*, 2008).

Botryosphaeria iberica
A.J.L. Phillips, Luque & Alves, 2005

Anamorph: *Dothiorella iberica* A.J.L. Phillips, Luque & Alves, 2005.
This species is similar to *B. sarmentorum*. It is reported associated with the formation of canker on trunks and branches of *Malus* sp., *Quercus* sp., *Persea americana* Mill., *Vitis vinifera* L., and almond (PHILLIPS *et al.*, 2005; ESKALEN & MCDONALD, 2011; PITT *et al.*, 2008; DOLL *et al.*, 2015).

REMARKS: cankers on the bark of twigs and stems, and necrotic woody organs (pycnidia) in the ZS2 site (SIDOTI *et al.*, 2016)

Neofusicoccum ribis (Slippers, Crous & M.J. Wingf.)
Crous, Slippers & A.J.L. Phillips, 2006
(= *Fusicoccum aesculi* Corda, 1829)

Taxonomic reviews have given rise to *Neofusicoccum* gen. nov., in which were included *Fusicoccum* and *Diplodia* like synanamorphs as *F. aesculi* (CROUS *et al.*, 2006). More than 250 hosts are listed in FARR & ROSSMAN (2016) but many of the reports were published before the concept of *N. ribis* was clarified by SLIPPERS *et al.* (2004) and are thus not reliable. *N. ribis* was identified as the main cause of leaf blight disease in *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. in commercial plantations in Malaysia (NGOBISA *et al.*, 2013).

REMARKS: cankers on the bark of twigs and stems and necrotic woody tissues (pycnidia) in the ZS1 site (GRANATA *et al.*, 2002; SIDOTI & GRANATA, 2005; TORTA *et al.*, 2008).

Pleosporales, Didymosphaeriaceae

Didymosphaeria variabile (Riccioni, Damm, Verkley & Crous) Ariyawansa & K.D. Hyde, 2014
(= *Paraconiothyrium variabile* Riccioni, Damm, Verkley & Crous, 2008)

The Didymosphaeriaceae (= Montagnulaceae) family includes saprobes, endophytes, and pathogens associated with a wide variety of substrates worldwide (Ariyawansa *et al*., 2014). *D. variabile* (*Paraconiothyrium variabile*) has been isolated from discoloured tissues of various decaying woody host plants such as *Prunus persica* L., *P. salicina* Lindl. and *Malus* sp. in South Africa; *Actinidia chinensis* Planch. and *A. deliciosa* (A. Chev.) C.F. Liang & A.R. Ferguson in Italy; *Laurus nobilis* L. in Turkey (Damm *et al*., 2008; Cloete *et al*., 2011). Ligoxigakis *et al*. (2013) reported severe leaf spot on *Phoenix theophrasti* Greuter caused by *P. variabile* in Greece. This species is also endophytic, and laboratory research focused on secondary metabolites produced by endophytic fungi showed that, when co-cultured with *F. oxysporum*, it had an antagonistic effect on the growth of the phytopathogen and actively suppressed the production of beauvericin, a mycotoxin of *F. oxysporum* involved in virulence (Prado *et al*., 2015).

Remarks: cankers on the bark of twigs and stem, and necrotic woody organs (pycnidia) in the ZS2 site (Sidoti *et al*., 2016).

Diaporthales, Diaporthaceae

Diaporthe neotheicola A.J.L. Phillips & J.M. Santos, 2009

Anamorph: *Phomopsis theicola* Curzi 1927.

Diaporthe neotheicola has been reported as an agent of shoot blight and cankers and branch dieback on many cultivated plants such as *Diospyros kaki* L. (Golzar *et al*., 2012), *Olea europaea* L. (Frisullo *et al*., 2015), *Actinidia deliciosa* (Thomidis *et al*., 2013) and *Vitis vinifera* (Kaliterna *et al*., 2012). Udayanga *et al*. (2014) recently reviewed the taxonomy, based on molecular studies, of some species of the genus *Diaporthe* and considered *D. neotheicola* a synonym of *Diaporthe foeniculina* (Sacc.), basionym *Phoma foeniculina* Sacc., which includes a wider range of hosts and present in Argentina, USA (California), Europe (Portugal, Spain, Italy and Greece), South Africa, Australia and New Zealand.

Remarks: cankers on bark of twigs and stems, and necrotic woody organs (pycnidia) in the ZS2 site (Sidoti *et al*., 2016).

The results of field surveys and bibliographical search are summarized in Table 1.

CONCLUDING REMARKS

The relationship between insects and their host plants are very interesting, especially if the plants are endemic and relict and confined to a restricted area (Bella *et al*., 2006; Bella & Rapisarda, 2014). *Zelkova sicula*, in particular, have to be accurately monitored, due to its reproductive biology, the small number of living individuals and the severe environment where it grows, in order to avoid infestations by insects or diseases that could kill the plants (Garfì & Buord, 2012; Campo *et al*., 2015). The species we recorded in our surveys are apparently not harmful to *Zelkova* plants, but some of the insects collected are known

Table 1 – List of the newly recorded and known insects and fungi species reported on *Zelkova sicula* in Sicily.

ORDER	FAMILY	TAXON	REFERENCES
Insects			
HEMIPTERA	APHIDIDAE	***Zelkovaphis trinacriae*** Barbagallo, 2002	*Barbagallo, 2002; Barbagallo & Cocuzza, 2008*
	COCCIDAE	***Parthenolecanium*** sp.	*Mazzeo* et al., 2016
	DIASPIDIDAE	***Aspidiotus nerii*** Bouché, 1833	*Mazzeo* et al., 2016
COLEOPTERA	BUPRESTIDAE	***Acmaeodera (Acmaeodera) pilosellae pilosellae*** (Bonelli, 1812)	*Longo & Campo*, 2004
		Acmaeoderella (Omphalothorax) adspersula adspersula (Illiger, 1803)	*New record*
		Anthaxia (Haplanthaxia) umbellatarum umbellatarum (Fabricius, 1787)	*New record*
	CERAMBYCIDAE	***Penichroa fasciata*** Dejean, 1839	*New record*
		Gracilia minuta (Fabricius, 1781)	*New record*
		Chlorophorus (Perderomaculatus) sartor (Müller, 1766)	*Sidoti* et al., 2016
		Niphona picticornis (Mulsant, 1839)	*Sidoti* et al., 2016
LEPIDOPTERA	LASIOCAMPIDAE	***Lasiocampa (Lasiocampa) quercus sicula*** (Staudinger, 1861)	*Longo & Campo*, 2004
	SATURNIIDAE	***Saturnia (Eudia) pavoniella*** (Scopoli, 1763)	*New record*
	NYMPHALIDAE	***Nymphalis polychloros*** (Linnaeus, 1758)	*New record*
Fungi			
BOTRYOSPHAERIALES	BOTRYOSPHAERIACEAE	***Botryosphaeria sarmentorum*** A.J.L. Phillips, Alves & Luque, 2005	*Granata* et al. , 2002; *Sidoti & Granata* , 2005; *Torta* et al., 2008
		Botryosphaeria iberica A.J.L. Phillips, Luque & Alves, 2005	*Sidoti* et al., 2016
		Neofusicoccum ribis (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips, 2006	*Granata* et al. , 2002; *Sidoti & Granata* , 2005; *Torta* et al., 2008
PLEOSPORALES	DIDYMOSPHAERIACEAE	***Didymosphaeria variabile*** (Riccioni, Damm, Verkley & Crous) Ariyawansa & K.D. Hyde, 2014	*Sidoti* et al., 2016
DIAPORTHALES	DIAPORTHACEAE	***Diaporthe neotheicola*** A.J.L. Phillips & J.M. Santos, 2009	*Sidoti* et al., 2016

to be harmful, e.g. *Parthenolecanium* species or *Aspidiotus nerii* that are pests of cultivated trees and ornamentals.

Fungal species, that are agents of cankers on the bark of twigs and stems and on necrotic woody tissues are common. The *Botryosphaeriaceae* comprises endophytes, saprobes, and plant pathogens. Some taxa in Botryosphaeriaceae have recently undergone nomenclatural changes (DISSANAYAKE *et al*., 2016). *Botryosphaeria* is a species-rich genus with a cosmopolitan distribution commonly associated with dieback and cankers of woody plants. *Diaporthe* species and their *Phomopsis* anamorphs are endophytes and pathogens on a wide range of plant hosts and are responsible for several diseases, some of which are of economic importance (UDAYANGA *et al*., 2014).

The highly degraded ecological environment in which *Zelkova sicula* has long developed has undoubtedly represented the determining factor in the state of suffering of the population. In addition, the summer climatic conditions, often characterized by high temperatures and especially low rainfall, cause frequent leaf drop in the warmer months, a phenomenon now contained by rescue irrigation in ZS1. Plants in this weakened state are more prone to attack by fungal species and insects (CRIST & SHÖENEWEISS, 1975; LONGO & CAMPO, 2002; BELLA, 2013). Fungal species living in the plant in a latent endophytic state become under stress conditions favouring the decline of the population.

Our investigation and bibliographic data collection summarise the insect pest status and health issues related to this rare Sicilian tree, and other studies are still ongoing to increase knowledge in various fields for this interesting endemic species, especially, i.e. on the symbiotic relationships between insect and fungi.

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ADALGISA GUGLIELMINO (*) - MASSIMO OLMI (**) (°) - STEFANO SPERANZA (*)

DESCRIPTION OF *GONATOPUS XUI* SP. N. FROM INDIA (HYMENOPTERA DRYINIDAE)

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Guglielmino A., Olmi M., Speranza S. – Description of *Gonatopus xui* sp. n. from India (Hymenoptera Dryinidae).

A new species of Dryinidae (Hymenoptera: Chrysidoidea) is described from India: *Gonatopus xui* sp. n. It is the first species of Dryinidae reported from Himachal Pradesh. The new species belongs to group 7 of *Gonatopus*. The key to the females of the Oriental species of *Gonatopus* group 7 is modified to include the new taxon. The new species is named after the late Professor Zaifu Xu, from Guangzhou (China), well-known specialist of Chinese dryinids.

KEY WORDS: Gonatopodinae, Himachal Pradesh, Oriental region, taxonomy, keys.

INTRODUCTION

Dryinidae of the Oriental region were studied in recent years by XU *et al*. (2013). Their monograph was followed by supplements published by GUGLIELMINO & OLMI (2013), KIM *et al*. (2013), OLMI *et al*. (2013, 2014, 2015a, 2015b, 2015c, 2016), MITA & PHAM (2014), GUGLIELMINO *et al*. (2017), MITA *et al*. (2017).

In the Oriental region, the genus *Gonatopus* Ljungh, 1810, includes 59 species (XU *et al*., 2013; MITA *et al*., 2017), among which 20 are reported from India. In 2017 the authors have examined a further new species of *Gonatopus* collected in India. It is described below.

MATERIALS AND METHODS

The description follows the terminology used by OLMI (1984) and XU *et al*. (2013). The measurements reported are relative, except for the total length (head to abdominal tip, without the antennae), which is expressed in millimeters. In the descriptions POL is the distance between the inner edges of the lateral ocelli; OL is the distance between the inner edges of a lateral ocellus and the median ocellus; OOL is the distance from the outer edge of a lateral ocellus to the compound eye.

The term "metapectal-propodeal complex" is here used in the sense of KAWADA *et al*. (2015). It corresponds to the term "metathorax + propodeum" sensu OLMI (1984) and XU *et al*. (2013).

The types of all Oriental species of *Gonatopus* were examined. The material studied in this paper is deposited in the following collections:

TAMU: Department of Entomology, Texas A. & M. University, College Station, Texas, USA.

USNM: National Museum of Natural History, Washington, DC, USA.

RESULTS

Gonatopus xui sp. n.
(Fig. I, 1-2; Fig. II, 1)

MATERIAL EXAMINED

Holotype, 1 female: INDIA: Himachal Pradesh, Kothi Village and environs, IN-11, 32°18'N 77°11'E, 2300-2600 m, 29.V-8.VI.1999, Yu. M. Marusik leg. [TAMU (to be transferred to USNM)].

DESCRIPTION

Apterous female; length 4.2 mm. Head black, except mandible, clypeus, gena and anterior region of face testaceous; antenna brown, except antennomeres 1-2 testaceous; mesosoma black; metasoma brown; legs brown, except tarsi and part of trochanters testaceous. Antenna clavate; antennomeres in following proportions: 9:5:17:11:8:8:6:6:6:9. Head excavated, shiny, unsculptured; frontal line complete; occipital carina incomplete, only present behind ocellar triangle; POL = 1; OL = 2; OOL = 9. Palpal formula 5/3. Pronotum crossed by strong transverse impression, with anterior collar and disc shiny, punctate, unsculptured among punctures. Scutum shiny, sculptured by many longitudinal striae, laterally with two pointed apophyses (Fig. I, 1). Scutellum shiny, smooth, flat. Mesopleuron shiny, unsculptured. Metanotum slightly hollow behind scutellum (Fig. I, 2). Metapectal-propodeal complex shiny, with disc and anterior surface unsculptured; posterior surface transversely striate. Metapleuron with anterior half unsculptured and posterior half transversely striate. Meso-metapleural suture very thin, slightly distinct and complete. Protarsomeres in following proportions: 16:3:5:18:26. Enlarged claw (Fig. II, 1) with one small subapical tooth and one row of five peg-like setae, in addition to one bristle. Segment 5 of protarsus (Fig. II, 1) with one row of 20 lamellae; distal apex with about 10 lamellae. Tibial spurs 1/0/1.

Male: unknown.

DISTRIBUTION

Only known from the type locality (see above).

HOSTS

Unknown.

ETYMOLOGY

The species is named after the late Prof. Zaifu Xu, specialist of Chinese dryinids and author, with Prof. Junhua He, of the monograph on Dryinidae of the Fauna Sinica (HE & XU, 2002).

REMARKS

The female of the new species is apterous, with pronotum crossed by a strong transverse furrow, the enlarged claw

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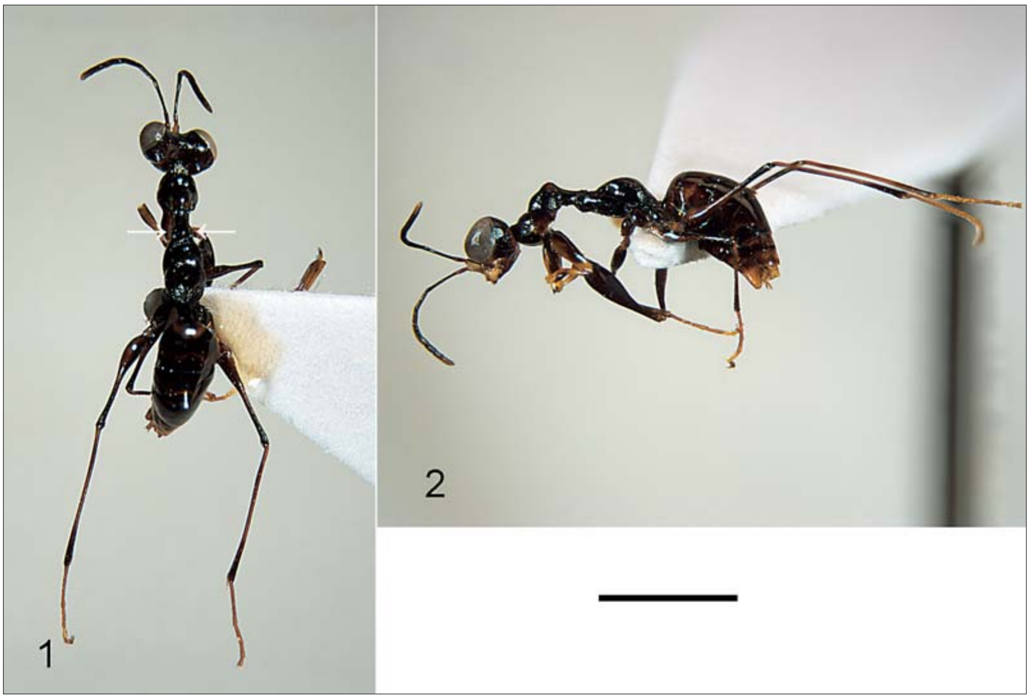

Fig. I – *Gonatopus xui* sp. n.: female holotype in dorsal (1) and lateral (2) view. Lateral apophyses of scutum indicated by arrows. Scale bar 1.83 mm.

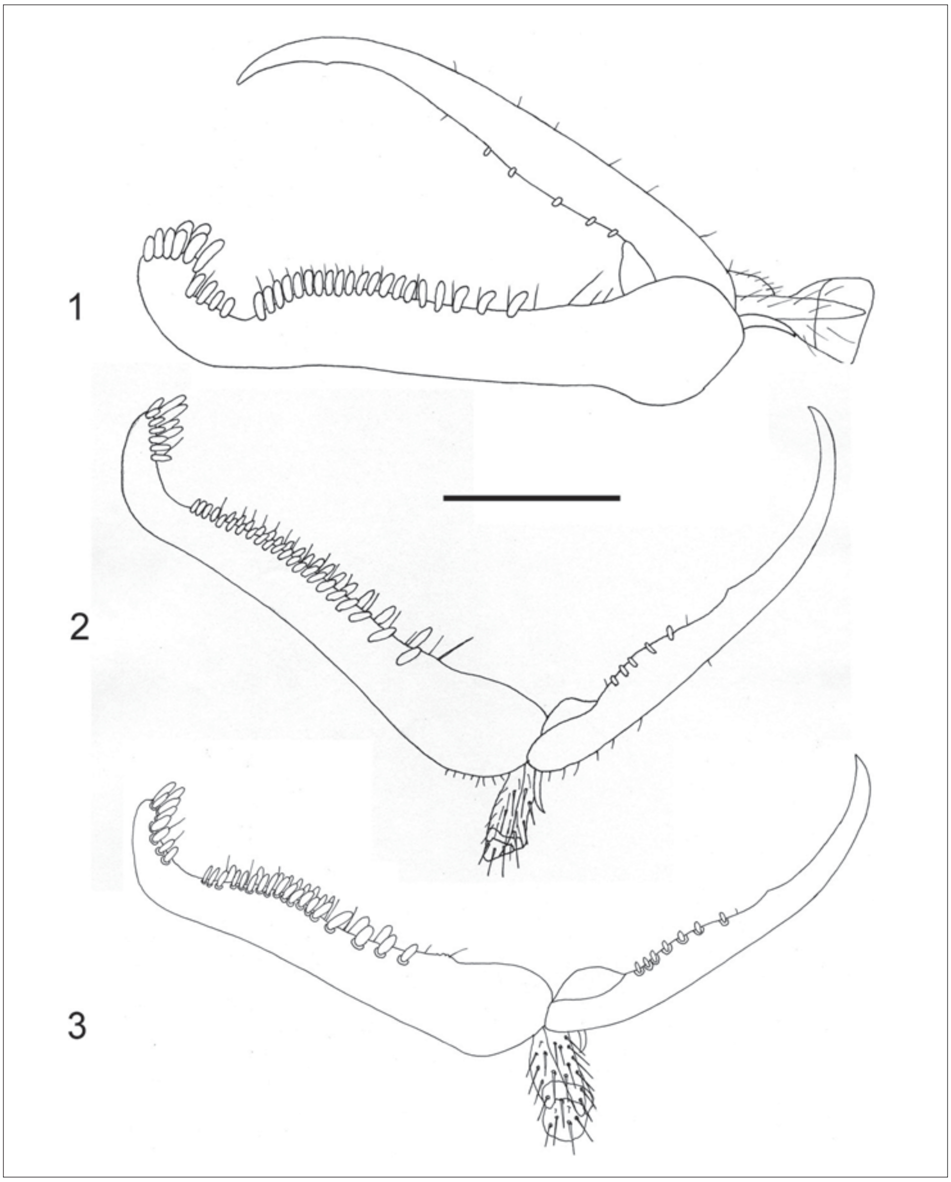

Fig. II – Chelae of holotypes of *Gonatopus xui* sp. n. (1), *G. lankanus* Olmi (2) and *G. hainanensis* Olmi (3). Scale bar 0.18 mm for 1, 0.26 mm for 2, 0.36 mm for 3.

provided of one small subapical tooth and the palpal formula 5/3. Because of these characters, *G. xui* belongs to group 7 of *Gonatopus*, according to the systematics proposed by Xu *et al*. (2013). The new species can be included in the key to the females of the Oriental species of *Gonatopus* presented by Xu *et al*. (2013) by replacing couplet 14 as follows:

14. Enlarged claw with subapical tooth situated nearer distal apex (Fig. II, 1) .... *G. xui* sp. n.
– Enlarged claw with subapical tooth farther from distal apex (Fig. II, 2-3) ....14'

14'. Head with face narrow and long [Fig. 128C in Xu *et al*. (2013)]; face and occiput sculptured by numerous parallel and longitudinal striae..*G. lankanus* Olmi
– Head with face shorter and more transverse (Fig. 127E in Xu *et al*. (2013)); face and occiput slightly granulated, not sculptured by parallel and longitudinal striae ....*G. hainanensis* Olmi

CONCLUSIONS

Species of *Gonatopus* from India are known mainly thanks to the monograph on Dryinidae of the Oriental region by Xu *et al*. (2013). The following 20 species were listed:

- Group 1: *G. nearcticus* (Fenton, 1927), known from Karnataka.
- Group 2: *G. nigricans* (Perkins, 1905): broadly spread in India and known from Bihar, Delhi, Orissa, Karnataka, Tamil Nadu and West Bengal; *G. nudus* (Perkins, 1912): known from Andhra Pradesh, Gujarat, Orissa and Tamil Nadu.
- Group 3: *G. bengalensis* Olmi, 1984: known from Assam; *G. pyrillae* (Mani, 1942): known from Punjab and Uttar Pradesh.
- Group 5: *G. flavifemur* (Esaki & Hashimoto, 1932): known from Andhra Pradesh, Orissa and Tamil Nadu.
- Group 7: *G. attenuatus* Olmi, 1984: known from Karnataka, Kerala and Mysore; *G. besucheti* Olmi, 1991: known from Uttar Pradesh; *G. daunus* Olmi, 1984: known from Mysore; *G. iarensis* Olmi, 2005: known from Delhi, Karnataka and Tamil Nadu; *G. javanus* (Perkins, 1912): known from Karnataka and Delhi; *G. lucidus* (Rohwer, 1920): known from Bihar; *G. mysorensis* Olmi, 1984: known from Karnataka and Mysore; *G. nivosus* Olmi, 1984: known from Kashmir and Delhi; *G. rufoniger* Olmi, 1993: known from Karnataka; *G. schenklingi* Strand, 1913: known from Karnataka; *G. superbus* Olmi, 1987: known from Karnataka and Orissa; *G. viraktamathi* Olmi, 1987: known from Karnataka; *G. yasumatsui* Olmi, 1984: known from Karnataka and Uttar Pradesh.
- Group 10: *G. indicus* (Olmi, 1987): known from Karnataka.

Following the above description of *G. xui*, the *Gonatopus* species known from India are now 21. *G. xui* is the first species of Dryinidae collected in Himachal Pradesh.

In Thailand, known *Gonatopus* species are 7 (Xu *et al*. 2013); in the Oriental region of China 24 (Xu *et al*., 2013); in Europe 50 (Olmi, 2013). The comparison with Europe shows that in general the knowledge of the *Gonatopus* species in all Asia and mainly in India is insufficient. Further research is necessary to fill the gap.

About the hosts, they are known only in ten of the 21 *Gonatopus* species recorded in India (Guglielmino *et al*., 2013): another gap to be bridged.

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ANGELA BOGGERO (*)

MACROINVERTEBRATES OF ITALIAN MOUNTAIN LAKES: A REVIEW

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Boggero A. – Macroinvertebrates of Italian mountain lakes: a review

The paper summarizes the state of knowledge and the evolution of the researches on benthic macroinvertebrates of Italian mountain lakes, and identifies key aspects that need to be further explored. Mountain lakes have been investigated since the end of the XIX century. In the beginning, studies focused mainly on their geography, geology, geomorphology and hydrology. Only lately, interests arise on their hydro-chemistry and biology, in relation to water acidification and, in rare cases, to eutrophication. In particular, in the 1920s, the studies dealt mainly on lake basins morphology and their genesis. Later, researches were driven mainly by the growing need of hydroelectric power plants, shifting the attention on their hydrological and geological aspects. Nonetheless, more detailed limno-biological studies began in confined areas. During the 1960s, attention was redirected to the alpine environment in terms of environmental awareness, for the creation of parks, and to analyse the alteration of water quality caused by the deposition of transported pollutants from the lowlands. This created the opportunity to combine observations on both the hydro-chemical and biological aspects, to reach a synoptic view of these environments. It is only in the mid-1990s that ISI journals came to power and, striving for higher visibility, strongly promoted the production of papers. In this decade, the application of paleo-limnology, the creation of a long term ecological research network, the never abandoned idea of implementing best practice management for freshwaters conservation purposes, and the growing attention on the global climatic change gave new impulse to the studies on mountain lakes. Since the 2000s, biodiversity preservation, biological recovery, environmental key-drivers (mainly nitrogen increase) have become the new focus of the current research activities.

KEY WORDS: Alps, Apennines, limnology, geographic distribution, historical perspective

To my father and to his passion for mountains

PREMISE

In Italy there are thousands of lakes, considering natural and artificial systems, placed at different altitudes: in particular, there are about 1100 natural lakes with an area greater than 0.01 km^2 and more than 4000 lakes, listed in the Alps, and characterized by small surfaces and high environmental value (NARDINI & SANSONI, 2006; TARTARI *et al.*, 2006). However, a comprehensive catalogue for these mountain lakes is still missing, because, at present, no universally accepted definition allows the clear separation among lakes, ponds, pools and wetlands (WHITAKER & AMLANER, 2012).

Mountain lakes are traditionally defined as lentic ecosystems located above the tree line (CATALAN *et al.*, 2009). This is the limit that separates the subalpine and the alpine areas, but its position depends on different climatic conditions that affect the growth of trees (KÖRNER, 1998). Currently, at a European level, the Water Framework Directive (EU, 2000) do not take into account this natural border, and strictly define mountain lakes as those systems located above 2000 m asl. Since conspicuous variations within the same mountain chain can affect the location of the tree line (besides altitudes, latitude, slope and other factors) (GRACE *et al.*, 2002), in this review I do not strictly respect these definitions, and I considered all the lakes above and close to the tree line.

Mountain lakes, characterized by harsh climatic and edaphic conditions, are frequently lacking of tributaries and are mainly fed by melting snowfields and glaciers. Although most of the mountain lakes originated during the last glacial retreat (approximatively 10,000 years ago), new ones are still forming due to global warming (SALERNO *et al.*, 2014). The main environmental characteristics of these systems are, in general, low depths (maximum depths, at mean water level, >1 m), small dimensions (surface areas > 0.01 km^2), cold water temperatures (< 20°C even in summer), long ice cover periods (up to 8 months), low buffering capacity (mainly in correspondence of acidic geo-lithology) and nutrient levels, extreme changes in light penetration between ice-covered and ice-free lake surface (SOMMARUGA, 2001). Moreover, frequent water mixing can occur because of their peculiar morphological features and wind exposure.

Because of these severe environmental conditions, these ecosystems host relatively simplified biological communities, almost homogeneous along the water column, but increasing in complexity and diversity along the littorals. Large animals are represented by few amphibians and fish, but, in most cases, vertebrates are totally and naturally lacking. The biological component is typically represented by phytoplankton and zooplankton (TOLOTTI *et al.*, 2006), macrophytes (occasionally, CHAMBERS *et al.*, 2008) and macroinvertebrates. The latter group include immature and adult stages of many different types of invertebrates that colonize all types of water bodies. Their distribution is mainly influenced by substrate, water depth, temperature, chemistry, food availability, and they act as a crucial link in the food webs by connecting organic matter resources with lower and higher trophic levels (HAUER & RESH, 2006).

Even if a direct anthropic pressure on mountain lakes is

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scarce (they are usually distant from urban areas and difficult to access), these systems can receive pollutants from regional and long-range atmospheric transport (KALLENBORN, 2006). Moreover, the reduced water renewal capacity and the presence of simplified food webs make these lakes extremely sensitive to environmental changes (PSENNER, 2002). For this reason, they are important sentinels of global climate changes (PARKER *et al.*, 2008; FENOGLIO *et al.*, 2010), and are among the most threatened surface systems in Italy. Currently, they are also among the less investigated ones. Notwithstanding their crucial importance, because of their small dimensions they are not included under the monitoring programs of the Water Framework Directive legislation.

From the beginning of 1900, macroinvertebrates were used in the biomonitoring programmes of many European lowland lakes that were showing serious symptoms of eutrophication (NAUMANN, 1921; LENZ, 1925; LUNDBECK, 1936; THIENEMANN, 1954; BRUNDIN, 1956). It was only some decades later that their employment in the ecological classification of lakes became popular (WIEDERHOLM, 1981; KANSANEN *et al.*, 1984; AAGAARD, 1986; ROSSARO *et al.*, 2010). Macroinvertebrates constitute one of the elements for the ecological evaluation of lakes. They are considered good indicators of freshwaters quality (ALLAN & CASTILLO, 2007), as their assemblage changes in relation with trophic condition, oxygen saturation, temperature and depth.

Macroinvertebrates sampling of mountain lakes constitutes a tricky debate among scientists. Mountain invertebrate sampling methods must comply with standards tailored to highlight the peculiar environmental conditions, through the analysis of their heterogeneous fauna found along the shores where sampling is easier (NIVA, 1987, 1995, 2010). Indeed, protection and management plans, and conservation efforts of mountain lakes cannot overlook a thorough understanding of the biological diversity of these environments, which still appears fragmented and limited to some biotic components or sectors of the Alps.

In this paper, a summary of the available knowledge on benthic macroinvertebrates of Italian mountain lakes, and of topics for future research is presented. In particular, this article reviews studies on benthic macroinvertebrates that were mainly carried out in mountain lakes at both the southern side of the Alps and at the higher altitudes of the Apennines. Mountain lakes from these two mountainous ranges, despite possible biogeographical differences, are considered together because they play a major role as essential environmental end ecological elements, they are important sources of water and touristic attraction, and they share similar threats at local and global scale.

SEEKING FOR BIBLIOGRAPHIC SOURCES

During the early preparation of this article, various Italian and foreign limnologists share their historical bibliography and advised on their field of expertise. A first search for old papers was carried out in the dusty catalogues, archives and on the online library of the CNR-Institute of Ecosystem Study, which hosts one of the largest and most comprehensive European collection of papers on limnology and hydrology. Papers that were not locally available were searched at academic or museum libraries. As for recent authors, Internet (mainly Google Scholar) provided bibliographic information, titles, authors and sources, speeding up time, and saving energy.

A total of 96 published papers were obtained for the period 1900-2017, including grey literature, educational, national and international scientific articles. The distribution of the different taxonomic groups among bibliographic resources is depicted in Fig. I. On the basis of the collected papers, the Table 1 (see Supplementary material 1) was prepared reporting information on latitude and longitude (WGS 84 data), altitude, maximum depth, and type of data found in each paper (presence/absence of species, families, or macroinvertebrates groups, relative abundances, absolute abundances, densities). Data are referred to different mountain ranges, different administrative regions, and different lakes. The geographic information reported in each paper was checked and corrected using either Google maps, the lake cadastre of the regions or provinces, or specific weblinks (for more detailed information see BOGGERO *et al.*, 2017). In the cases of changes in the name of a lake during its history or when the same lake has two different names, both names has been kept to avoid the loss of information. The list of species found in each lake and their updated taxonomy, divided per mountain range, per different administrative regions, per lake and per year of citation, is reported in BOGGERO *et al.* (2017) and, as a data set, is available at the CNR-ISE website (http://www.ise.cnr.it/it/products/datasets).

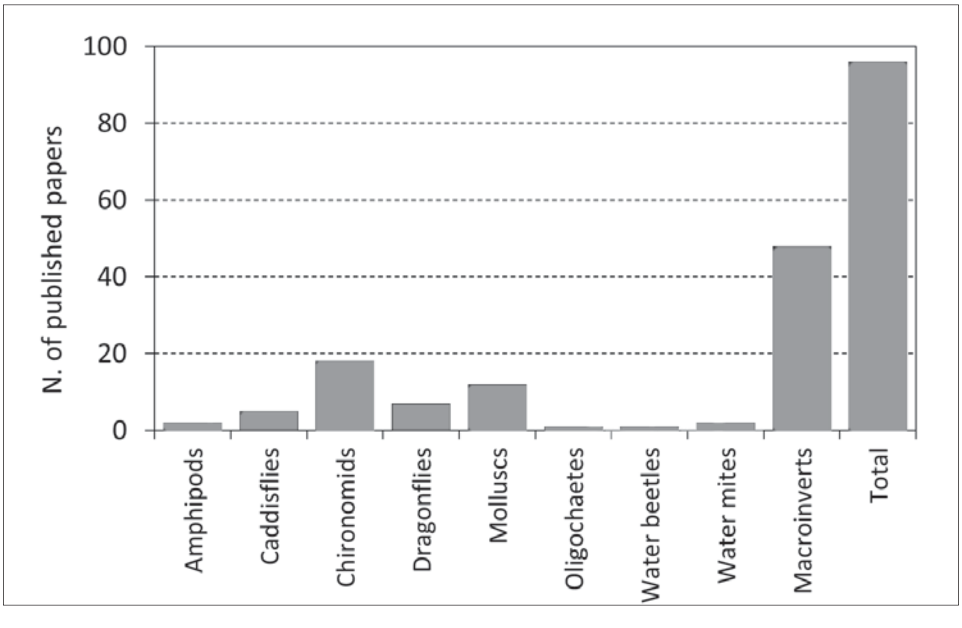

Fig. I – Papers published in the period 1900-2017 on the lacustrine macroinvertebrates in Italian mountain areas, divided per each taxonomic group.

THE EUROPEAN SCENARIO AT THE TURN OF THE XX CENTURY

Since the end of XIX century, aquatic environments of mountainous areas have received growing attention, because of their uniqueness, importance for water supplies, and vulnerability. In this period, pioneering studies started in European countries where mountains cover vast areas of the territory. This is the case of Italy (PERO, 1893; DE MARCHI, 1913a, b), Switzerland (ZSCHOKKE, 1894; BOURCART, 1906), and Austria (PESTA, 1912). These early researches considered mountain lakes as small and simplified systems to test and verify new hypothesis and theories. Furthermore, these lakes, characterised by a relatively simple fauna, were also captivating because of the high probability to find new species, frequently endemic. Thanks to these studies, a growing corpus of information on these water bodies, their origin and morphology, their chemical-physical structure, and their biological composition (mainly plankton and phyto-benthos) was acquired. Nonetheless, these early biological studies had a more taxonomical than a biogeographical or ecological perspective.

SEARCHING FOR ITALIAN MEMORIES (1900-1940)

In Italy, the first mentions of lacustrine benthos dates back to the beginning of the XX century: before World War I, the University of Padova carried out some sampling campaigns in Trentino aimed at characterizing the limnology of mountain lakes (BUFFA, 1902; LARGAIOLLI, 1907). In these early studies, researchers accidentally caught benthic macroinvertebrates through plankton hauls. After these early reports, no further study was carried out over a rather long period. This is mostly because of the logistic difficulties to sample high altitude freshwaters characterized by short ice-free periods in largely unexplored areas. In the 1930s, the Museum of Natural History of Milan launched a research project in the Gran Paradiso National Park to study the presence and the distribution of molluscs, including lentic species (GAMBETTA, 1932). Another pioneering work was performed by the University of Milan on the biological communities of Lake Valparola. Because of the peculiar geological composition of its catchment, this dolomitic lake drew the attention of Rina Monti, one of the most prominent Italian limnologist, which was surprised and satisfied by the discovery of an unusual community characterized by numerous Gammaridae typically associated with lower altitudes and running waters (MONTI, 1936).

THE ITALIAN MIDDLE AGE (1940-1990)

In the 1940s, Lake Tovel became one of the most studied Alpine lakes because the reddening of its waters attracted many researchers, including some benthologists (BALDI, 1941; MORETTI, 1942). Subsequently, researchers started to broaden their attention from single lakes to lakes grouped on entire mountain valleys (TONOLLI, 1949), mountain ranges (the Dolomites: MARCUZZI, 1956, 1961 or the entire Alps: MARCUZZI, 1988), provinces (BARBATO, 1984; BLESIO, 1985) or national parks (TORTONESE & ROSSI, 1954; PARISI *et al.*, 1968; GIANOTTI & DI GIOVANNI, 1971). They highlighted major species pattern of distribution, and the relation between community and lake morphological and chemical properties, focusing on the bio-limnological aspects in natural lakes (MARCHESONI & MORETTI, 1954) and reservoirs (SOMMANI, 1952; BAZZANTI & SEMINARA, 1987; MASTRANTUONO, 1987; BAZZANTI *et al.*, 1988). Some authors focused on boreo-alpine species (RUFFO, 1951) or on specific groups (e.g., TERZANI, 1977, and MASCAGNI & TERZANI, 1983 on dragonflies). Nonetheless, ecological studies were also realised, concerning, for example, the impact of fish predation on benthos occurrence and biomass (FERRARI & BELLAVERE, 1976). Afterwards, growing interests were directed towards the relationship between the macroinvertebrate assemblages and the environmental quality, in particular regarding trophic status (BONI *et al.*, 1983; BAZZANTI *et al.*, 1988; CASELLATO & ZANFEI, 1988; SEMINARA & BAZZANTI, 1988) and acidification (GARIBALDI *et al.*, 1987) of lakes. These studies were supported by the growing scientific, but also touristic and economic, importance of mountain lakes. In this context, macroinvertebrates became one of the crucial elements for evaluating the lacustrine environmental conditions and, at the end of the 1990s, macroinvertebrates, never considered before in national and European regulations, were finally incorporated in the Water Framework Directive (2000/60/EC - EU, 2000).

ITALIAN MODERN TIMES (1990-TO PRESENT)

The beginning of the 1990s was characterized by an increasing attention towards the ecology and distribution of trichopterans (CIANFICCONI & MORETTI, 1992) and a renewal of the studies on Lake Tovel (MORETTI & CORALLINI SORCETTI, 1991; PAGANELLI, 1992). At the same time, a significant revolution took place: due to the launch of broad-scale European projects on freshwater acidification impacts (ALPE, MOLAR, EMERGE), the number of papers regarding Italian mountain lakes grew conspicuously (Fig. II). Thanks to these projects and to the acknowledgement of the idea of an Italian network for long-term ecological research, a few high altitude lakes (upper and lower lakes Paione, Anterselva, Braies, Monticola, Tovel and lakes Santo and Scuro Parmense), representing the Alps and the Apennines, were chosen as part of the "Mountain Lakes" LTER macrosite (LTER Italy - http://www.lteritalia.it/). Lakes Paione underwent multidisciplinary studies also considering macroinvertebrates (MOSELLO *et al.*, 1993; BOGGERO & NOCENTINI, 1994; BOGGERO *et al.*, 1996; GUILIZZONI *et al.*, 1996).

In the second half of the nineties, to improve the correct management of freshwater habitats and to analyse water acidification processes, other high altitude lakes were considered, like those on the Gran Sasso (Central Italy – DI GIORGIO & ZUPPA, 1996), those lying in the Monte Avic Natural Park (Aosta Valley – NOVELLI *et al.*, 1997; FACCHINI & BADINO, 1998), and those in the Lake Maggiore watershed (Ossola Valley – BOGGERO, 1995; BOGGERO & NOBILI, 1998). In the year 2000, a study on Chironomidae of Italian lakes allowed, for the first time, to distinguish between oligotrophic mountain and eutrophic lowland lakes on the basis of the presence/absence of peculiar species (MIETTO *et al.*, 2000), and to propose indicator values for different midge taxa. In the same period, important findings on remote lakes were published at a Pan-European (FJELLHEIM *et al.*, 2000) and Alpine level (BOGGERO *et al.*, 2006; FÜREDER *et al.*, 2006), demonstrating the value of mountain lakes as indicators of local and global temperature changes. Then, a series of studies were carried out, mainly inside national parks, attempted to reconcile the traditional exploitation of mountain lakes as drinking trough for cattle with the conservation needs of these fragile freshwater ecosystems (RUGGIERO *et al.*, 2001, 2004; LENCIONI, 2001; BOGGERO *et al.*, 2005).

New works on the biogeographical distribution of molluscs were also carried out, highlighting threatened species and areas towards which conservation efforts were needed (DECET & FOSSA, 2001; EVANGELISTA, 2009; NARDI & CASTAGNOLO, 2009; NARDI, 2014). Research efforts were again directed towards Lakes Paione, showing the first signs of a biological recovery after twenty years of limnological studies (MARCHETTO *et al.*, 2004), and Lake Tovel, with a first study on the evolution of

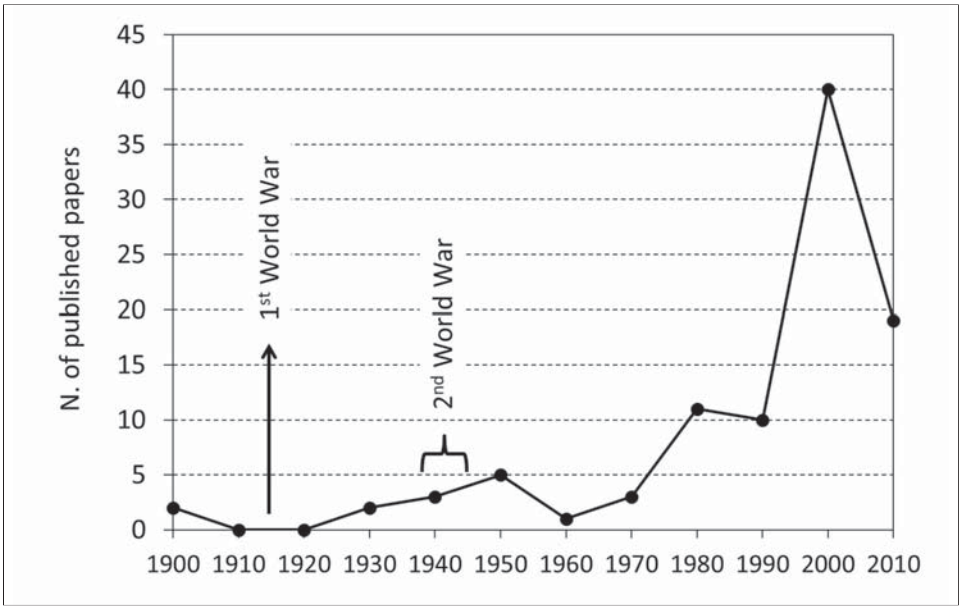

Fig. II – Decadal estimate of the number of published papers on macroinvertebrates of the Italian mountain lakes

the caddisfly fauna after the ending of the water reddening (CAPPELLETTI *et al.*, 2004). Others studies were focused on Chironomidae or, more in general, on invertebrates through neo- and paleo-ecological approaches (FERRARESE, 2002; FRANCESCHINI & LENCIONI, 2002; FERRARESE & LENCIONI, 2003; LENCIONI & LAZZARA, 2004; FILIPPI *et al.*, 2005a, 2005b; HEIRI *et al.*, 2005; MARZIALI *et al.*, 2005; BOGGERO & LENCIONI, 2006; LAZZARA *et al.*, 2006; DECET, 2007a, 2007b; MILLET *et al.*, 2007; MORABITO *et al.*, 2007-2008; CANTONATI *et al.*, 2014). Researches focused on more specific topics, such as the distribution of *Gammarus lacustris* (IANNILLI *et al.*, 2004), and the ecology of Hydrachnidia (DI SABATINO *et al.*, 2004;, MICCOLI *et al.*, 2005), Oligochaeta (DUMNICKA & BOGGERO, 2007, 2017), or Odonata (DECET, 2007c; DAL CORTIVO *et al.*, 2009) were carried out.

In 2009, two papers at a European level (CATALAN *et al.*, 2009; FJELLHEIM *et al.*, 2009) highlighted that four main lake features (size, tropho-dynamic status, acid-base balance and ice-cover duration) affect the macroinvertebrates assemblage in Alpine lakes. The same works pointed out, for the first time, that species inventory at high altitude was still incomplete due to critical problems in sampling designs. Meanwhile, other researches draw the attention on the growing impact of anthropogenic nitrogen inputs in the shaping of biological lake communities. So important that the biological lake communities could be used to estimate the water quality status not only in lowland (BOGGERO *et al.*, 2009), but even in mountain lakes (MARCHETTO *et al.*, 2009).

In the last decade, the SHARE Stelvio project (Stations at High Altitude for Research on the Environment) was launched. It was devoted to Alpine freshwaters as the last sources of uncontaminated waters, suitable to sustain biological communities poorly altered or unaltered by human impacts (LAMI *et al.*, 2011; BOGGERO *et al.*, 2012, 2018 submitted). In the very last years, interesting studies were also realized in the Gran Sasso and Gran Paradiso National Parks (OSELLA & PANNUNZIO, 2013; KHAMIS *et al.*, 2014), and in the Northern Apennines (ANSALONI *et al.*, 2015, 2016), areas acknowledged for their high conservation value. Even if some recent papers investigated the taxonomy and autecology of mountain lake macroinvertebrates (e.g., ROSSARO *et al.*, 2010, 2012), many aspects regarding the biology or the ecology of these organisms remain underestimated or even unknown in the entire mountain range (TIERNO DE FIGUEROA *et al.*, 2012).

In the last twenty years, in support to the mass diffusion of knowledge, a number of educational papers, pocket books, booklets and e-books regarding the Italian Alpine mountain lakes and their ecological importance, often focusing on macroinvertebrates, have been produced (BIANCOTTI *et al.,* 2001; BOGGERO & MOVALLI, 2001; MARCHETTO *et al.*, 2001; NARDI, 2005, 2010a, 2010b; NÖSSING & WINKLER WERTH, 2010; BOGGERO *et al.*, 2013; ROGORA *et al.*, 2014).

MACROINVERTEBRATES: HISTORICAL INFORMATION AND NEW RECORDS FROM ITALY

The first studies on mountain lakes provided very little information on their faunal assemblage, because researchers used to identify macroinvertebrates only as a group, or, seldom, to talk about few classes or families and to give only a general idea of their presence (BUFFA, 1902; LARGAIOLLI, 1907; GAMBETTA, 1932; MONTI, 1936; BALDI, 1941; MORETTI, 1942; RUFFO, 1951). Hydro-biological studies providing a differentiation between littoral and profundal faunal records date back to the 1950s (SOMMANI, 1952; MARCHESONI & MORETTI, 1954; TORTONESE & ROSSI, 1954). However, list of species were only provided starting from the 1980s (MASTRANTUONO, 1987; BAZZANTI *et al.*, 1988; CASELLATO & ZANFEI, 1988).

Central western Alps

Oligotrophic or ultra-oligotrophic lakes of the Central western Alps, characterized by acidic rocks with low buffering capacity and low ionic concentrations, host a low diversity, with assemblages dominated by Insecta and Oligochaeta, and minor abundances of Platyhelmintes and Mollusca (BOGGERO & NOCENTINI, 1994; BOGGERO, 1995; BOGGERO *et al.*, 1996; BOGGERO & NOBILI, 1998). In general, at higher altitudes where cold and extreme climate shape the environment, the taxonomic composition of Chironomidae (Diptera) is represented mainly by the subfamilies Orthocladiinae and Chironominae tribe Tanytarsini. On the contrary, lower altitude lakes are dominated by the subfamily Chironominae tribe Chironomini (BOGGERO *et al.*, 2006). Along the littorals, it is easy to find also the subfamilies Tanypodinae and Prodiamesinae. Orthocladiinae are represented by *Heterotrissocladius*, *Psectrocladius* and *Corynoneura*, Chironominae-Tanytarsini by *Micropsectra* and *Paratanytarsus*, Tanypodinae by *Zavrelimyia*, and Prodiamesinae by *Prodiamesa olivacea* (Meigen, 1818). Noticeable is the presence of rare species like *Acamptocladius reissi* Cranston and Saether 1982 (Orthocladiinae), recorded for the first time in Italy on the Central western Alps in 1993 and representing here an extension of its biogeographical distribution (BOGGERO, unpublished data). Another species, rare here and problematic to identify at the larval stage, is *Protanypus* sp.(Diamesinae) (ROSSARO *et al.*, 2012).

Oligochaeta followed in importance, usually representing about 10-20% of the macroinvertebrates assemblage, but rarely determined to species level (DUMNICKA & BOGGERO, 2007). Families that are frequently retrieved are Naididae [*Nais communis* Piguet, 1906 and *N. bretscheri* (Michaelsen, 1898)] and Enchytraeidae (*Henlea perpusilla* Friend, 1911, *Mesenchytreus armatus* (Levinsen, 1884), *Cernosvitoviella atrata* (Bretscher, 1903) and *C. microtheca* (Rota and Healy, 1999).

Then, Trichoptera Limnephilidae, Plecoptera Nemouridae, Coleoptera Dytiscidae, Hydrachnidia, and Mollusca Bivalvia Sphaeriidae (mainly *Pisidium casertanum* Poli, 1791) are taxa common at these altitudes (BOGGERO & NOCENTINI, 1994; BOGGERO *et al.*, 1996; NARDI, 2005).

Hydrachnidia, as Chironomidae, are mainly characterized by stenothermic rheobionts and crenobiontic species, since slow-flow conditions are present along the littorals of these lakes (DI SABATINO *et al.*, 2004, MICCOLI *et al.*, 2005). Within Hydrachnidia, new species for the Italian fauna were recorded, like *Atractides fissus* (Walter 1927) and *Arrenurus conicus* Piersig, 1894.

The deepest bottom of the same Alpine lakes, show limited animals usually very scarce and mainly represented by Chironomidae (*Procladius* and *Tanytarsus*), and Oligochaeta Naididae (Tubificinae, mainly *Tubifex tubifex*) (MOSELLO *et al.*, 1993).

All of the mentioned groups are known to be acid tolerant (RADDUM & FJELLHEIM, 1984; MERILAINEN & HYNYNEN, 1990) with the exception of Bivalvia which are more sensitive to acidification (RADDUM, 1980).

Central eastern Alps

On the eastern side of the Central Alps, more alkaline waters are found, with higher pH and alkalinity values, and a generally richer biodiversity (BOGGERO & LENCIONI, 2006). In these lakes, noteworthy is the finding of: *Lymnaea stagnalis* (Linnaeus, 1758) (Mollusca), reaching its maximum altitude at 1500 m asl (DECET, 2007a), *Niphargus strouhali* cfr. *alpinus* Schellenberg, 1933, which is usually present at altitudes in the range of 2000-2200 m asl, and found here up to 2700 m asl, and *Gammarus lacustris*, common inhabitant of high altitudes (1900-2300 m asl) (LENCIONI, 2001). The latter species is a post-glacial relict with a fragmented and scattered distribution in the Central eastern Alps (from Carnic to Orobie). It appears again in the northern Central Apennines (from Liguria up to Abruzzo at altitudes higher than 1500 m asl), with a more uneven distribution than in the Alps (IANNILLI & RUFFO, 2002). Like other boreo-alpine species of the Apennines, *G. lacustris* seems to have reached these areas from the north in a Quaternary glacial period, presumably during the Würm.

Chironomidae and molluscs are usually found, the former represented by Orthocladiinae (*Cricotopus* and *Eukiefferiella*) and Diamesinae (*Diamesa* spp.). Molluscs are mainly characterised by *Pisidium casertanum*, reaching here its highest altitude (2643 m asl), and by rare species like *P. hibernicum* Westerlund 1894 (NARDI & CASTAGNOLO, 2009; NARDI, 2010a, 2010b).

A richer fauna is also found in lakes where water level fluctuations are low because of their greater depths, and, consequently present more stable shores, macrophytes (providing food, substrate and refuge), and a higher organic matter content (BICHTELER *et al.*, 1998; FRANCESCHINI & LENCIONI, 2002; LAZZARA *et al.*, 2006).

The presence of an extended vegetation cover in the catchment related to a higher nitrogen atmospheric input, is also important for the presence and distribution of lake macroinvertebrates (FÜREDER *et al.*, 2006).

Since 2000, because of the increasing interest in mountain lakes and in Chironomidae, detailed analysis began and new or rare species were found in the southern side of the Central eastern Alps: *Acamptocladius reissi* Cranston and Saether 1982 (FERRARESE & LENCIONI, 2003), *Cricotopus (Cricotopus) pirifer* Hirvenoja, 1973, *Psectrocladius oligosetus* Wuelker,1956 among Orthocladiinae, *Paratanytarsus laccophilus* (Edwards, 1929), *Tanytarsus gibbosiceps* Kieffer, 1922, *T. sinuatus* Goetghebuer, 1936, and *T. mendax* Kieffer, 1925 among Chironominae (FERRARESE, 2002). Among Oligochaetes, notable is the presence of *Paranais litoralis* Müller, 1784 and *Pristinella idrensis* (Stephenson 1932), typical of organic matter enriched environments in relation with cattle grazing (BOGGERO *et al.*, 2012). In most of the cases, the presence of one or more inlets influences the lacustrine assemblage with strictly rheophilus species like: *Zavrelimyia punctatissima* (Goetghebuer 1934), *Pseudodiamesa branickii* (Nowicki, 1873), *Eukiefferiella minor* (Edwards 1929), *Eusimulium aureum* (Fries, 1824), *Baetis alpinus* (Pictet, 1843), and *Crenobia alpina* (FRANCESCHINI & LENCIONI, 2002, BOGGERO & LENCIONI, 2006).

Western Alps

In the oligotrophic waters of the Western Alps, the highly distributed groups are Coleoptera, Heteroptera, Diptera, Trichoptera, Ephemeroptera, Oligochaeta, and Platyhelmintes. *Crenobia alpina* (Dana, 1766) (Platyhelmintes) reaches here its maximum altitude (2850 m asl - TORTONESE & ROSSI, 1954). Interesting is the finding of *Gammarus lacustris* in Lake La Maddalena (1996 m asl - IANNILLI *et al.*, 2004), near the Italian-French border, where amphipods had never been found before. This site represents also the only site in which the species is present in the Western Alps.

Apennines

Biodiversity in mountain lakes with high alkalinity values is less documented, but on the Apennines, where karstic lakes are present, macroinvertebrate assemblages are richer (taxa reaching in some occasion 40-80 units) and more varied than those present in acidic lakes (< 25 taxa). Since Apennines lakes are small and shallow, frequently considered ponds, their naturalistic value equalises their fragility towards human intervention, as they are unable to mitigate the effects of any stress (RUGGIERO *et al.*, 2001). Thus, they are considered of exceptional value since they allow the survival of northern latitudinal species (widely distributed in the Alps and in central northern Europe), representing glacial relicts in Central Italy (DI GIORGIO & ZUPPA, 1996). Noteworthy examples are the Trichoptera *Sericostoma italicum* Moretti, 1978 and *Allogamus ausoniae* Moretti, 1991 endemic in the Apennines, and the Coleoptera *Agabus calchonatus* (actually *Ilybius chalconatus* Panzer, 1797), sporadic almost everywhere (DI GIORGIO & ZUPPA, 1996). In particular, *A. ausoniae* finds here its southern limit of distribution. All these aspects justified the inclusion of most mountain areas of central Italy (mainly, Lazio and Abruzzo) into the protection of national parks, and of other minor protected areas.

Mainly because of livestock grazing, eutrophic lakes on the Apennines are not unusual at these altitudes. Their assemblages are generally represented by the same taxonomic groups found on the Alpine lakes, but are characterized by higher mean annual densities due to higher nutrient content that favour the development of phytoplankton and periphytic algae along the shores during the ice-free period. Chironomidae, in particular, are well represented by *Natarsia* sp., *Paramerina divisa* (Walker, 1856), *Cricotopus (C.) tremulus* (Linnaeus, 1758), *Cricotopus (I.) trifasciatus* (Meigen, 1810), *Orthocladius consobrinus* (Holmgren, 1869), *Psectrocladius (Allop.) obvius* (Walker, 1856), *Dicrotendipes* gr. *tritomus* (Marchesoni & Moretti, 1954, Bazzanti & Seminara, 1987, Di Giorgio & Zuppa, 1996, Ruggiero *et al.*, 2001, 2004).

Finally, in the bottom area of reservoirs on the Apennines (Bazzanti *et al.*, 1988; Seminara & Bazzanti, 1988; Spitale *et al.*, 2015), studies conducted on the effects of hypo-limnetic draw-off and on the unnatural and variable water level draw-down showed that both impacts may favour the presence of active swimmers, instability- and pollutant-tolerant species like *Dero digitata* (Mueller, 1773) (Oligochaeta), *Procladius choreus* (Meigen, 1804) (Chironomidae) and *Chaoborus flavicans* (Meigen, 1830) (Chaoboridae), which avoid adverse environmental conditions by displacing actively following the water retreat. Ubiquitous sedentary mud-dwellers, like *Tubifex tubifex* (Müller, 1774), and *Potamotrix hammoniensis* (Michaelsen, 1901) (Oligochaeta), and *Chironomus plumosus* (Linnaeus, 1758) (Chironomidae), need more stable conditions to survive.

CONCLUDING REMARKS

In the 191 Italian mountain lakes considered in the paper, a total of 650 macroinvertebrate taxa were reported. Of these, only 354 were identified at the species level due to the presence of juveniles or poorly preserved specimens in the samples, or due to uncertainty of identification in presence of cryptic species and/or absence of a taxonomic expert. This emphasizes the huge taxonomic effort that still has to be undertaken to fully characterize these ecosystems. Nowadays, molecular approaches are becoming more and more important in providing methods for the faster identification of target species, or in monitoring these ecosystems. In particular, DNA barcoding, genomics and, to a lesser extent, proteomics of lacustrine macroinvertebrates seem to be promising as tools for taxonomic research and water quality assessment programs. Nonetheless, even if some DNA barcoding libraries already exist, these are still broadly incomplete, and not completely finalized to the national territory. Therefore, at present, morphological taxonomy cannot be totally replaced by the sole DNA analysis.

More generally, even if a scarce direct anthropic pressure is present, because most mountain lakes are distant from urban areas and difficult to access, these systems can receive pollutants from regional and long-range atmospheric transport, thus making them extremely sensitive to environmental changes damaging their beauty and their charms, and most of all, their food-web structures. Therefore:

- there is an urgent need of including mountain lakes within tailored monitoring programs, in view of the maintenance and the preservation of these small habitats as important non-perennial sources of biodiversity. Even if the continuous control of the ecological status of these systems is a time-consuming and expensive effort, the assessment of long-term trends is becoming pivotal in order to understand the effects of direct or indirect human impacts, thus ensuring the wealth of these unique ecosystems. In particular, they need to be included under the monitoring programs of the Water Framework Directive legislation, that at present takes into consideration only lakes with an area > 0.5 km^2;
- at this stage, every educational publication, even the shortest one, illustrating this often neglected branch of the scientific knowledge, is crucial. Increase the public awareness and attention is the only way we have to promote the protection of these systems and their fauna, and to stimulate political and conservational measures;
- improving advanced techniques such as remote sensing (image acquisition performed through sensors) undertaken concurrently to the traditional monitoring approach, both chemical and biological (phytoplankton and macroinvertebrates), will represent the frontier to acquire synoptic data on habitat conditions, especially in hardly accessible areas such as remote mountains;
- at the European level, the INSPIRE Directive (INfrastructure of SPatial InfoRmation in Europe - 2007/2/EC) promotes the creation of services that allow the storing, the availability and the sharing of data among different institutions, with the aim of ensuring that the future environmental policies will be based on big, easily accessible and interoperable data, ensuring their effectiveness. This must constitute another chance for studying and, therefore, developing further conservation strategies for such unstable and uneven environments.

Biodiversity is our natural heritage. It needs to be preserved for our and future generations. Freshwater ecosystems at high altitude are under threat. They are becoming prominent examples of the current global scale magnitude of species extinction (1000 times higher than the natural background rate). They provide ecosystem services constituting the basis of the economy of the European States that share with Italy the presence of mountains within their borders. Limit the biodiversity loss and the declining ecosystem services is thus the prominent challenge we have to deal in the next future.

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SUPPLEMENTARY MATERIAL TABLE 1 WITH LEGEND
(http://www.redia.it/images/stories/pdf2018/Boggero_review_per%20Redia_2018_Tab1.xlsx)

Table 1 – List of the Italian mountain lakes divided per mountain range and administrative regions. Geographic (latitude, longitude, altitude) and morphometric (max depth) information is also presented for each lake. The table also provides the list of published papers, and the type of frequency data format found in each paper. P = presence of a taxon; A = data expressed as total number of individuals; D = data expressed as density (ind m^{-2}); R = data expressed as relative abundances (%). The last column reports the number of lakes considered by each paper. Papers not reporting any species list were not considered in this table.

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MANAGEMENT OF THE ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA* ON TOMATO WITH DIFFERENT COMBINATIONS OF NEMATICIDES AND A RESISTANT ROOTSTOCK: PRELIMINARY DATA

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Landi S., d'Errico G., Roversi P.F., d'Errico F.P. – Management of the root-knot nematode *Meloidogyne incognita* on tomato with different combinations of nematicides and a resistant rootstock: preliminary data.

In south Italy, tomato growers commonly face severe root-knot nematode infestations. Alternative methods of control are required because of the high toxicity of current pesticides. Therefore, the potential of an integrated pest management strategy for the control of root-knot nematodes on tomato in greenhouse was investigated. The nematode susceptible tomato cv. Ikram, non-grafted or grafted onto the tomato rootstock cv. Armstrong, with intermediate resistance to the nematode, in combination with soil applications of the nematicides fosthiazate, oxamyl, and abamectin were tested. The resistant rootstock significantly reduced nematode soil population levels and root galling index until one month after transplanting, when soil temperature was below 28°C, but not by harvest due to increased soil temperature. Fosthiazate, abamectin and oxamyl increased tomato yield and reduced root galling caused by *Meloidogyne incognita*. The synergistic effect of the rootstock resistant to root-knot nematodes and soil treatments of fosthiazate in combination with abamectin or oxamyl could successfully be employed in integrated pest management programs to control *M. incognita* in tomato.

KEY WORDS: *Mi– 1* resistance gene; fosthiazate; abamectin; oxamyl

INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) are among the most damaging agricultural pests attacking a wide range of crops (SAHEBANI & HADAVI, 2008; D'ERRICO *et al*., 2014). Yield losses of 20.6% have been reported in tomato (RAVICHANDRA, 2014), although, this value could be underestimated because of the known negative synergistic interaction between fungi and nematodes (MANZANILLA & STARR, 2009; RAGOZZINO & D'ERRICO, 2011). Damages caused to tomato, mainly by *Meloidogyne incognita* (Kofoid & White) Chitw., are particularly severe in southern Italy (D'ERRICO *et al*., 2016). Therefore, the application of fumigant and non-fumigant nematicides is instrumental for satisfactory productions (NYCZEPIR & THOMAS, 2009). The withdrawal from market or restrictions in the use of several soil fumigants such as methyl bromide,chloropicrin, and 1,3-dichloropropene (1,3-D), able to reduce the soil population densities of the nematode to levels that could be tolerated by the following two crop cycles, have prompted researches on integrated control strategies suitable to the crop and the environmental sustainability (NYCZEPIR & THOMAS, 2009).

Among the various measurements for controlling root-knot nematodes on tomato, plant resistance is considered an effective, economic and environment friendly method. The *Mi-1* gene, identified in *Solanum peruvianum* L. and introgressed in many tomato cultivars, confers high level of resistance to *M. incognita*, *M. javanica* and *M. arenaria* (JACQUET *et al*., 2005), which are the most common species occurring all over the world, including southern Italy were they are often present as mixed populations. The same *Mi-1* gene governs the root-knot nematode resistance in several rootstocks used for grafting tomato. Unfortunately, both resistant cultivars and rootstocks may select for virulent populations of the nematode if used routinely (JARQUIN-BARBERENA *et al*., 1991; KALOSHIAN *et al*., 1996; VERDEJO-LUCAS *et al*., 2009). However, new resistant rootstocks derived from crosses between different wild *Solanum* species are being tested (CORTADA *et al*., 2008; 2009). The degree of resistance of the new rootstocks varies from high to intermediate, but some of them confer resistance also to virulent populations of these nematodes (WILLIAMSON & ROBERTS, 2009). To preserve the durability of the resistance and prevent the selection of virulent populations of these nematodes, resistant cultivars and rootstocks are suggested to be used within an Integrated Pest Management (IPM) context (VERDEJO-LUCAS & SORRIBAS, 2008).

Therefore, the objective of this investigation was to evaluate the potential of an IPM strategy for the control of root-knot nematodes in tomato in greenhouse. To achieve this goal, combinations of a resistant tomato rootstock and soil applications of the nematicides fosthiazate, oxamyl, and abamectin were tested.

MATERIALS AND METHODS

PLANT AND CHEMICALS

Tomato plants, *Solanum lycopersicum* L., cv. Ikram susceptible to *Meloidogyne* spp., grafted or non-grafted onto the resistant tomato rootstock cv. Armstrong were planted. Both cultivars were supplied by Syngenta. The nematicides tested were Fosthiazate 150 g L^{-1} (Nemathorin 150 EC, Syngenta), Oxamyl 100 g L^{-1} (Vydate 10L, DuPont) and Abamectin 20 g L^{-1} (Tervigo SC 1, Syngenta).

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FIELD SITE

The tomato trial was conducted in two contiguous commercial unheated plastic-houses, each of 630 m^2 (10 × 63 m), in the Nocera-Sarno area, southern Italy. The trial was established during summer of 2013. The plastic-houses had been under conventional tomato production for 30 years before the start of the experiment. The soil at the experimental site was sandy-loamy, with soil pH of 7.8, and uniformly and severely infested with *M. incognita*, as it was shown by the severe root galling and damage to lettuce cropped just before arranging the experiment. The mean temperatures in a nearby greenhouse in June, July, August and September were 26.5±3.0 (SD), 29.7±1.8, 30.8±1.6 and 26.4±4.1, respectively (Fig. 1).

CHEMICAL TREATMENTS

The nematicide treatments were: (a) fosthiazate, applied three days before transplanting by furrow at the dose of 10 L ha^{-1}; (b) fosthiazate, applied as above three days before transplanting at the dose of 10 L ha-^{1} followed by two applications of abamectin, each at 5 L ha^{-1}, 24 and 35 days after transplanting; (c) fosthiazate applied three days before transplanting as above at the dose of 10 L ha^{-1} followed by two applications of oxamyl, each at 10 L ha^{-1}, 24 and 35 days after transplant; (d) four applications of abamectin, each at the dose of 5 L ha^{-1}, every 10 days from transplanting; (e) four applications of oxamyl, each at the dose of 5 L ha^{-1}, every 10 days from transplanting; (f) untreated control.

LAYOUT OF THE EXPERIMENT

The greenhouses were divided in plots of 25m^2 (10 m × 2.5 m) each and the treatments were arranged according to a randomized block design with four replicates, two in each greenhouse. An individual plot consisted of 2 rows of plants in the centre of each plot; one row planted with the susceptible cv. Ikram and the other with cv. Ikram grafted onto the resistant rootstock cv. Armstrong. There were 21 non-grafted or 21grafted plants per individual row, spaced 50 cm along the row and 1 m between rows.

The application of the nematicides was made *via* drip irrigation by distributing 15.000 L water ha^{-1}. Besides the normal irrigation system, two sections of drip-line, as long as the plot row, were joined and transferred from time to time in the plot to be treated. The drip-line supplied with self-compensating drippers of a flow rate of 2 L/hour, had the hind-end closed by a cap and the fore-end connected to a pump (shoulder "ECHO") to distribute the solutions into the soil at the pressure of 2 atm. The untreated plots were irrigated by the same volume of water of the treated plots.

Six-week-old tomato seedlings were transplanted in each plot on June 22, 2013. Plants were staked and tied as needed during the season. Ordinary irrigation and fertilization were provided according to requirements of the crops. Insecticides, herbicides and fungicides were applied as recommended (MAYNARD *et al.*, 2003).

DATA COLLECTION

Soil samples were collected three days before transplanting (immediately after ploughing) and again 30 and 83 (end of the crop cycle) days after transplanting, and *M. incognita* second-stage juveniles (J2s) were extracted and counted. Ten soil core samples were taken from each plot, giving a total of about 0.35 kg soil per plot. The soil cores were collected uniformly on the plots before planting and along the row after transplanting. Each soil sample was thoroughly mixed and a 100 cm^3 sub-sample processed combining the Cobb's sieving and decanting method with a modified Baermann's funnel technique and the nematodes in the soil suspension counted under a stereomicroscope.

Root-knot nematode infestation was evaluated 30 and 83 days after transplanting on the base of the Root Galling Index (RGI) according to a 0–5 scale (LAMBERTI, 1971), were 0 = no galls – healthy plant, 1 = 1–5 galls per plant – very slight damage, 2 = 6-20 galls – moderate damage, 3 = more than 20 galls – medium damage, 4 = root system reduced and showing some large galls, 5 = root system completely destroyed or showing many large galls. Ten plants per plot (5 from the non-grafted and 5 from the grafted row) were uprooted at each evaluation date.

Plant vigour ratings of 10 plants per plot (5 per row) were estimated according to the weight (kg) of the whole plant fresh matter at 83 days after transplanting. Tomatoes of 10 plants per plot (5 per row) were harvested as they matured and total yields calculated by summing the weights of the six consecutive harvests (August 14, 21, 28 and September 3, 9, 13).

STATISTIC ANALYSIS

All data were submitted to analysis of variance (one way-ANOVA) and, when the F-test was significant at $P< 0.05$, treatment means were compared using the Student-Newman-Keuls test (Costat program). Factorial ANOVA was performed to test the effect of main factors (nematicide treatments, rootstock), and their interaction. If a main effect was significant, the ANOVA was followed by Student-Newman-Keuls test at $P< 0.05$.

RESULTS

At the beginning of the trial, the nematode soil infestation level (Table 1) was homogeneous among the different plots: 156.2±0.20 (SE) nematodes per 100 cm^3 of soil. After transplanting, the nematode population in soil decreased 30 days after transplanting, probably because of penetration of the nematode juveniles into the roots, but greatly increased 83 days after transplanting, because of the nematode reproduction, with significant differences among the treatments (Table 1)

Thirty days after transplanting (Table 1), the nematode population increased slightly on non-grafted tomatoes and remained at the same level on grafted tomatoes, probably because only a small proportion of the nematodes were already laying eggs of the first generation on grafted tomatoes. Also, there were no significant differences in soil population densities of the nematodes among plots treated and planted with grafted tomatoes or treated and planted with non-grafted tomatoes. However, the nematode soil population densities were significantly smaller along the row in the plots planted with grafted plants.

Instead, the nematode soil population densities greatly increased 83 days after transplanting (Table 1) in plots transplanted with both grafted and non-grafted tomatoes, when the nematode had probably completed about two generations (Table 1). At this date, compared with the control, in the non-grafted tomato rows the least nematode soil populations occurred in plots receiving combined treatments of fosthiazate and avermectin or oxamyl, while fostiazate and oxamyl alone gave intermediate results. Abamectin alone did not significantly reduce soil nematode density. In rows planted with grafted tomatoes, only the combined treatments of fosthiazate with abamectin or oxamyl reduced significantly the nematode soil population level and no differences occurred in plots planted with grafted or non-grafted tomatoes (Table 1).

Table 1 – Effects of the nematicides on second-stage juveniles of *Meloidogyne incognita* per 100 cm^3 of soil in plots planted with non-grafted susceptible tomato cv. Ikram and grafted onto the resistant rootstock cv. Armstrong 30 and 83 days after transplanting (DAT). Each data is the average of four replicated plots. Data on the same column sharing a common letter are not significantly different (P = 0.05) according to Student-Newman-Keuls test. * indicates statistical (P = 0.05) differences, between non-grafted and grafted plants according to Student-Newman-Keuls test. Average nematode population as affected by nematicide treatment (row) and grafting (column). A single representative experiment is shown.

Treatment	Before planting	30 DAT			83 DAT		
		Non-grafted	Grafted	Average per treatment	Non-grafted	Grafted	Average per treament
Fosthiazate	170 a	70.0 a	10.0 a *	40.0 a	4100.0 b	3760.0 ab	3930.0 bc
Fosthiazate + Abamectin	120 a	30.0 a	12.5 a *	21.3 a	2140.0 c	2900.0 b	2520.0 d
Fosthiazate + Oxamyl	190 a	40.0 a	10.0 a *	25.0 a	1990.0 c	2645.0 b	2317.0 d
Abamectin	130 a	60.0 a	35.0 a *	47.5 a	5137.5 ab	4580.0 ab	4858.8 b
Oxamyl	160 a	60.0 a	20.0 a *	40.0 a	4257.5 b	4050.0 ab	4153.8 c
Control	170 a	95.0 a	30.0 a*	62.5 a	6190.0 a	5590.0 a	5890.0 a
Average		59.2 a	19.6 b		3969.0 a	3920.8 a	

On non-grafted plants, root gall indices (Table 2) were low 30 days after transplanting and significantly higher in the control plots (1.5) compared to all nematicide treated plots (0.2-0.7) (Table 2). The RGI was largest (P<0.05) in the control plots (4.7) 83 days after transplanting, and least in those treated with fosthiazate alone (2.9) or combined with abamectin (2.7). Fosthiazate alone and combined with abamectin and oxamyl were the most effective treatments in reducing RGI on grafted tomatoes. From pairwise comparisons between non-grafted susceptible plants and grafted onto the resistant rootstock, for each treatment, significant differences were found only for J2s in soil and root galling index 30 days after transplanting (Tables 1 and 2) as the grafted plants were effective in controlling *M. incognita* infestation.

Only treatments of fosthiazate, alone or combined with abamectin or oxamyl, increased significantly the fresh weight of non-grafted plants, while these treatments and oxamyl increased the fresh weight of grafted plants. All nematicides greatly increased cumulated tomato yield of non-grafted and grafted plants compared to the untreated plots (Table 3). The greatest yield increases of 251-261% were obtained in plots treated with fosthiazate in combination with abamectin or oxamyl and planted with non-grafted or grafted tomatoes. The other treatments gave intermediate yield increases. The averages of plant weight and yield did not significantly differ between non-grafted and grafted tomato plants (Table 3).

The factorial ANOVA showed that treatment, rootstock resistant to the nematodes and their interactions had a significant effect on root galling index but only 30 days after transplanting (Table 4). Root galling indices were lower on nematicide treated than untreated plants and on the resistant rootstock than on susceptible plants. Moreover, the resistant rootstock resulted in a significant suppressing effect on nematode J2s in the soil, while the nematicide treatments had no significant difference effect (Tables 1 and 2). In contrast, 83 days after transplanting, the factorial analysis showed that treatment effect was significant for J2s in the soil, root galling index and crop yield (P=0.00001) (Table 4). Differences between non-grafted and grafted tomato plants were found only for RGI (P=0.016).

Table 2 – Effects of the nematicides on root galling index of the root-knot nematode, *Meloidogyne incognita*, in plots planted with the susceptible tomato cv. Ikram, non-grafted and grafted on the resistant root-stock cv. Armstrong, 30 and 83 days after transplanting (DAT). Each data is the average of four replicates. Data on the same column sharing a common letter are not significantly different (P = 0.05) according to Student-Newman-Keuls test. * indicates statistical significant (P = 0.05) difference, compared with the corresponding data in the previous column, according to Student-Newman-Keuls test. In column Average per treatment and in row Average per rootstock, root galling index as affected by treatment and rootstock at 30 and 83 DAT are reported.

Treatment	30 DAT			83 DAT		
	Non-grafted	Grafted	Average per treatment	Non-grafted	Grafted	Average per treatment
Fosthiazate	0.7 b	0.0 a *	0.4 b	2.9 c	2.3 c	2.6 c
Fosthiazate + Abamectin	0.5 b	0.0 a *	0.3 b	2.7 c	2.2 c	2.5 c
Fosthiazate + Oxamyl	0.2 b	0.3 a *	0.3 b	3.6 b	2.5 c *	3.0 c
Abamectin	0.7 b	0.0 a *	0.4 b	3.8 b	3.7 b	3.7 b
Oxamyl	0.7 b	0.0 a *	0.4 b	4.2 ab	3.9 b	4.1 b
Control	1.5 a	0.0 a *	0.9 a	4.7 a	4.6 a	4.7 a
Average	0.8 a	0.1 b		3.6 a	3.2 a	

Table 3 – Effects of the nematicides on plant weight, crop yield and increase of tomato compared to the control, in plots infested with the root-knot nematode, *Meloidogyne incognita*, and planted with the susceptible tomato cv. Ikram, non-grafted and grafted on the resistant root-stock cv. Armstrong. Each data is the average of four replicates. Data on the same column sharing a common letter are not significantly different (*P* = 0.05) according to Student-Newman-Keuls test. * indicates statistical significant (*P* = 0.05) difference, compared with the corresponding data in the previous column, according to Student-Newman-Keuls test. In column average per treatment and in row average per rootstock, plant weight and crop yield as affected by treatment and rootstock at 30 and 83 DAT are reported.

Treatment	**Plant weight (kg/plant)**			**Crop yield (kg/plant)**			**% crop yield increase over control**	
	Non-Grafted	**Grafted**	**Average per treatment**	**Non-grafted**	**Grafted**	**Average per treatment**	**Non-grafted**	**Grafted**
Fosthiazate	0.74 b	0.68 b	0.71 a	1.94 b	2.08 b	2.01 b	128.23	123.65
Fosthiazate + Abamectin	0.87 a	0.89 a	0.88 a	3.07 a	3.08 a	3.07 a	261.17	231.18
Fosthiazate + Oxamyl	0.92 a	0.90 a	0.91 a	2.98 a	3.12 a	3.04 a	250.59	235.48
Abamectin	0.55 c	0.53 c	0.54 a	1.35 c	1.34 c	1.35 cd	58.82	44.08
Oxamyl	0.65 bc	0.71 b	0.68 a	1.63 bc	1.69 bc	1.66 bc	91.76	81.72
Control	0.51 c	0.49 c	0.50 a	0.85 d	0.93 d	0.89 d		
Average	0.71 a	0.70 a		1.97 a	2.04 a			

Table 4 – Summary of the significant effects of treatment and root-stock on root-knot nematode population, *Meloidogyne incognita* and root galling index at 30 and 83 days after transplanting (DAT), plant weight and crop yield and their interaction at 83 DAT. Figures in bolt indicate high significance of main effects or interaction.

	30 DAT		**83 DAT**		**Plant weight (kg/plant)**	**Crop yield (kg/plant)**
	Nematodes 100 cm^3 soil	**RGI**	**Nematodes 100 cm^3 soil**	**RGI**		
Main effects:						
– Treatment	0.0561	**0.0002**	**0.00001**	**0.00001**	0.0708	**0.00001**
– Rootstock	**0.00001**	**0.00001**	0.4521	**0.0162**	0.09360	0.6703
Interaction: – Treatment – Rootstock	0.4436	**0.0016**	0.3114	0.4878	0.9990	0.9994

DISCUSSION

Grafting susceptible cultivars onto resistant rootstock is used successfully to control soil-borne pathogens in greenhouse in many countries (CORTADA *et al.*, 2008; 2009), including south Italy (SERGES & COLOMBO, 2005).

In this trial, the rootstock cv. Armstrong, with intermediate resistance, reduced nematode population levels and root galling index one month after transplanting, but later no positive effect of grafting was observed. Non-grafted and grafted tomatoes exhibited similar performance in terms of plant vigor and crop yield. This result could be partially attributed to the resistance breaking because the *Mi-1* gene is not effective at soils temperatures above 28°C (DROPKIN, 1969; KALOSHIAN *et al.*, 1996). In fact, the nematode densities on the resistant rootstock was low at low temperatures (June, 26.5±3.0) and increased with the rise of the temperature (July, 29.7±1.8; August, 30.8±1.6) (Fig. 1). Moreover, the repeated plantings of resistant tomatoes that may have occurred in past years may have selected resistance-breaking specimens of the nematode (VERDEJO-LUCAS *et al.*, 2009). In this case, using a resistant cultivar instead of a rootstock or vice versa may not be helpful because the resistance to root-knot nematodes present in the commercial varieties of tomato is conferred by the same *Mi-1* gene. The solution of this problem necessitates the incorporation of other resistance genes into cultivated tomato and the use in the rotation of resistant tomato with other crops for extending the durability of the effectiveness of the *Mi-1* gene (KALOSHIAN *et al.*, 1996). In this trial the resistance breaking may have been due to the high temperatures in July and August and because resistant tomato cultivars may have been included in the rotation in the cropping history of the farm. So far, chemical control measures are still needed to limit yield losses in tomato. In our study, soils treatment with fosthiazate, abamectin and oxamyl increased tomato yield and reduced root galling caused by *M. incognita*. Similar control results were obtained by other authors (GARABEDIAN & VAN GUNDY, 1983; PULLEN & FORTNUM, 1999; GUGINO *et al.*, 2006; SAAD *et al.*, 2011; QIAO *et al.*, 2012; D'ERRICO *et al.*, 2017). When these chemicals were used alone, their efficacy was lower than in combination. As suggested by OKA *et al.* (2012), nematicides taken up by roots prevent nematode attacks only in the early growth stage of tomato and, therefore, their use is effective only to suppress root-knot nematodes in short season crop. However, when fosthiazate was applied before transplanting and followed by post-plant applications of abamectin or oxamyl, plants were protected from nematode infestation much longer than with the pre-plant application. In addition, crop plants may have also benefited of the effect of abamectin and oxamyl against insect pests (PUTTER *et al.*, 1981). The results of this study suggest that the synergistic effect of rootstock resistant to root-knot nematodes and soil

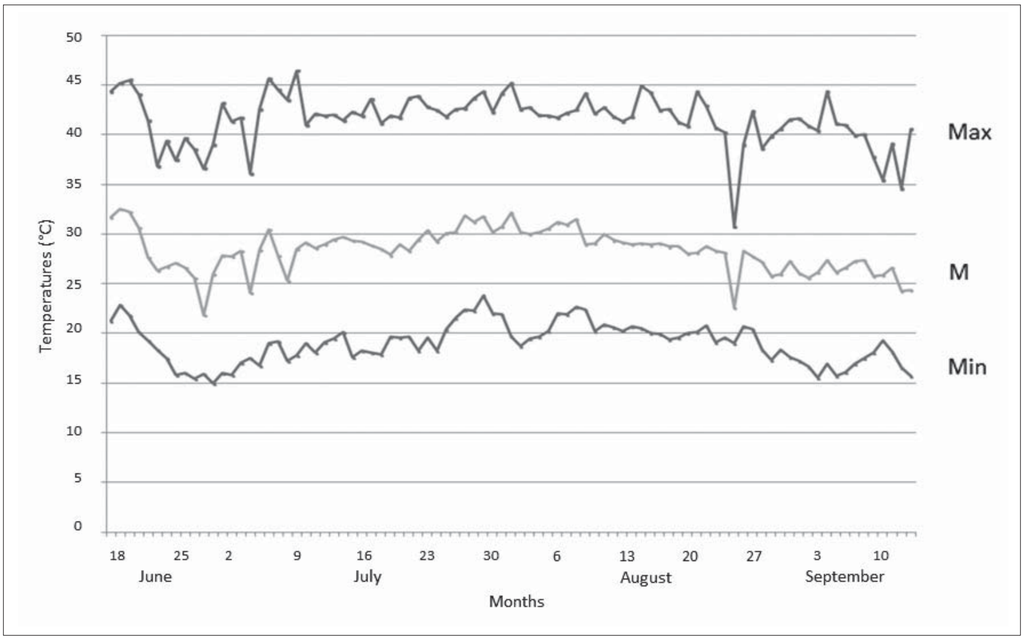

Fig. 1 – Average (M), minimum and maximum temperatures recorded in a greenhouse nearby those used for the experiment, from June to September 2013.

treatments with fosthiazate in combination with abamectin or oxamyl could successfully be employed in integrated pest management (IPM) programs to control *M. incognita* in tomato.

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ACTIVITY OF CHESTNUT TANNINS AGAINST THE SOUTHERN ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA*

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d'Errico G., Woo S.L., Lombardi N., Manganiello G., Roversi P.F. – Activity of chestnut tannins against the southern root-knot nematode *Meloidogyne incognita.*

Studies on the effects of tannins on plant-parasitic nematodes are few. A new formulation of a hydrolysable tannin extracted from chestnut (SaviotaN®) was tested for efficacy in controlling *Meloidogyne incognita*. Therefore, *in vitro* and pot experiments on tomato were performed to investigate the nematicidal activity of tannin aqueous solutions at different concentrations on *M. incognita.* In the *in vitro* experiment the following concentrations of tannin at 0.30, 0.40, 0.50, 0.75, 1.00, 1.25, 1.50 g L^{-1} were tested for their effect on the nematode. The second-stage juveniles (J2s) immobility increased with increasing concentration and exposure time. All tested tannin concentrations were effective to reduce viability from about 45 to 70% after 10 days of exposure, in comparison to the treated and untreated controls. The immobile J2s recovered their mobility over time after rinsing and transferring them in water, showing a nematostatic activity of tannins. In the pot experiment, tannins, as aqueous solutions at rates from 0.30 to 1.50 g L^{-1}, were applied to soil at three different application times (1: only at transplant; 2: at transplant, two weeks after transplant and repeated every seven days; 3: at transplant and two weeks later). The activity of tannins was compared to treated and untreated controls. Tested rates mostly repeated were effective to control nematode attack in comparison to untreated control. The height of treated plants was not significantly influenced by the different applied rates of tannins, whereas nematode population density and root galling index were affected by repeated application times. No visual symptoms of phytotoxicity were detected. The use of SaviotaN® appears promising for the control of *M. incognita* in sustainable agriculture of short-term crops and/or when nematode population densities are low and as a supplement to other chemical treatments.

KEY WORDS: Root-knot nematode; *Meloidogyne incognita*; Tomato; Hydrolyzable tannins; SaviotaN®

INTRODUCTION

Root-Knot Nematodes (RKN) are the most common and widespread group of nematodes in the world causing dramatic yield losses to a wide range of crops. The simultaneous presence of RKN and plant pathogenic fungi may cause synergistic damages (RAGOZZINO & D'ERRICO, 2011). The European Union has deeply restricted the use of pesticides on agricultural crops focusing the attention on enviromental safety, human and animal health (GHORBANI *et al.*, 2008). It has been estimated that the global damage caused by pesticides reaches $100 billion every year (KOUL *et al.*, 2008), and a significant percentage is represented by nematicides (RENČO *et al.*, 2014). Alternative strategies for the control of RKN are needed to limit their economic and environmental impacts. Various plant species or compounds extracted and exuded from plants (i.e. alkaloids, phenolic compounds, saponines, etc.) are able to control plant diseases or, at least, to shape the microbial rhizosphere in favour of beneficial microbes thus helping the plant to overcome stress conditions (SUKUL, 1992; CHITWOOD, 2002; SOPPELSA *et al.*, 2011; MOCALI *et al.*, 2015; LOMBARDI *et al.*, 2018). Many of the discovered biologically active phytochemicals are considered safer to humans and environment than conventional pesticides (CHITWOOD, 2002). Among them, tannins play a significant role in numerous ecological processes (KRAUS *et al.*, 2003), protect plants against herbivores (FEENY, 1976) and are toxic for numerous bacteria, fungi and yeasts (SCALBERT, 1991). Additionally, tannins are a group of water-soluble polyphenolic compounds that have the ability to precipitate proteins (BATE-SMITH & SWAIN, 1962). They are found in higher plants mainly grouped into two classes, termed condensed and hydrolyzable tannins. Both classes have been shown to possess nematicidal activity (MIAN & RODRIGUEZ-KABANA, 1982; MOHAMED *et al.*, 2000; NAZ *et al.*, 2013), and termed condensed have been shown to inhibit gastrointestinal nematodes (BUTTER *et al.*, 2001, ATHANASIADOU *et al.*, 2001; HOSTE *et al.*, 2006). Several studies demonstrated that tannins inhibit microbial activity (BALDWIN *et al.*, 1983; BENOIT & STARKEY, 1968a; b; BENOIT *et al.*, 1968; FIERER *et al.*, 2001; HARRISON, 1971; LEWIS & STARKEY, 1968; SCHIMEL *et al.*, 1996; 1998; SCHULZ *et al.*, 1992), whereas much more remains to be investigated about their effects on other soil biota such as plant parasitic nematodes (KRAUS *et al.*, 2003). HEWLETT *et al.* (1997) suggest that the behavioural response of different nematode species to tannic acid is variable. In their studies, tannic acid was attractive for *Meloidogyne arenaria* (Neal) Chitw. and *M. incognita* (Kofoid & White) Chitw., whereas it was repellent for *Radophulus similis* (Cobb) Thorne and no effects were observed on *Heterodera glycines* Ichinohe. In literature, tannins from chestnut have been reported to affect plant parasitic nematodes (BADRA & ELGINDI, 1979; HEWLET *et al.*, 1997; MAISTRELLO *et al.*, 2010). Soil treatments with tannic acid were found to control *M. arenaria* on squash (MIAN & RODRIGUEZ-KABANA, 1982). A previous

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formulation of tannins extracted from chestnut wood (brand name SaviotaN®) has been tested on *M. javanica* and on *Globodera rostochiensis* (Woll.) Skarbilovich at concentrations ranging from 0.32 to 20.48 g L^{-1} (MAISTRELLO *et al.*, 2010; RENČO *et al.*, 2012), and on eggs and juveniles of *M. incognita* at two concentrations (2 and 5 g L^{-1}) showing a nematostatic action and an inhibitory effect on eggs hatching (CARLETTI & MAISTRELLO, 2012). However, these authors have used a different formulation of SaviotaN® and higher concentrations of application therefore their results are not comparable to our data. The objective of the present work was to evaluate the effect of a new formulation of SaviotaN® nutraceutical for crops (Gruppo Mauro Saviola s.r.l., Viadana, Italy) in wet powder (WP) containing 75% of pure tannins on the RKN *Meloidogyne incognita* both *in vitro* and in pot experiments on tomato under controlled conditions.

MATERIAL AND METHODS

CHEMICALS

The chestnut tannin extract SaviotaN® was tested in seven doses ranging from 0.30 to 1.50 g L^{-1} as reported in Table 1. Fosthiazate 150 g L^{-1} (Nemathorin® 150 EC; Syngenta), abamectin 20 g L^{-1} (Tervigo® SC 1; Syngenta) and garlic extract, *Allium sativum* (L.), Allicin <1 ppm (Garland®; Omex Agriculture Ltd) were used for comparison at recommended doses.

Table 1 – Schedule treatments and codes.

Treatment codes	Active ingredient	Dose kg/ha^{-1}	Dose g/L^{-1} H_2O
T30	Tannin WP 75%	6 kg	0.30 g
T40	Tannin WP 75%	8 kg	0.40 g
T50	Tannin WP 75%	10 kg	0.50 g
T75	Tannin WP 75%	15 kg	0.75 g
T10	Tannin WP 75%	20 kg	1.00 g
T12	Tannin WP 75%	25 kg	1.25 g
T15	Tannin WP 75%	30 kg	1.50 g
Fost	Fosthiazate 150 g L^{-1}	10.0L	0.50 mL
Aba	Abamectin 20 g L^{-1}	5.0L	0.25 mL
Garl	Allicin < 1 ppm	8.0L	0.25 mL
Cont	Water		

IN VITRO EXPERIMENTS

Second-stage juveniles (J2s) were obtained from an Italian population of *M. incognita* reared on tomato *Solanum lycopersicum* (L.) Karst. ex Farw. cv. Naxos in the greenhouse of the University of Naples Federico II. The *Meloidogyne* species used in our experiments have been morphological and molecular identified previously (D'ERRICO *et al.*, 2014). Egg masses from infested tomato roots were collected and hatched by tap water to obtain J2s to use in the experiments. Freshly hatched J2s (24 hours old) were used. The irreversibility or reversibility of J2s mobility was determined by rinsing and shifting immobile nematodes to distilled water (GIACOMETTI *et al.*, 2010; D'ERRICO *et al.*, 2017a; D'ERRICO *et al.*, 2017b). Forty J2s were added to individual wells, each containing the solution under assessment, then wrapped with parafilm and stored at 25±1°C in the dark. The J2s exposed to water served as controls. Nematodes were considered paralyzed when no movement could be observed after poking them with a needle during an observation period of 10 s and their number was recorded every 24h for 28 days. Subsequently, immobile J2s were washed in distilled water to remove residuals and transferred, according to the different immobility times caused by each solution, in a new well containing only water under the above conditions. After exposure to water, the number of immobile J2s that became motile was also counted as previously described. J2s that did not recover their motility in water were considered dead. Mobility was observed using a stereomicroscope (Discovery V8; Zeiss, Germany). The experiment was performed twice and each treatment was replicated 4 times.

IN VIVO EXPERIMENTS

Pot experiments were done in a greenhouse located in Terlizzi (Bari, Italy), using 14 cm squared pots (height 10 cm and 3.6 L volume of soil) placed on soil surface covered with a polyethylene film. Pots were filled with uniformly and naturally infested soil by the same population of *M. incognita* used for *in vitro* experiments. This soil contains 4.0% clay, 52.0% silt and 44.0% sand (medium texture), and its texture is classified as silt loam (USDA) with pH of 7.8 and an organic matter content of 19 g kg^{-1}. The initial nematode population was extracted by cotton-wool filter method for 48 hours at 22±2°C and then counted (156 J2s per 10 cm^3 of soil). Certified seedlings, grown in polystyrene alveolate trays (ø 2 cm), were used. RKN susceptible tomato seedlings cv. Naxos, with two true leaves stage with an uniform growth, were transplanted in pots and, immediately, treated. Control pots received only water. Irrigation and fertilization were homogeneously supplied according to crop needs. The final nematode population in soil was counted as reported above. All applications were manually carried out using a Becher. Three groups of treatments were conducted concurrently. The first group included 7 treatments of tannin extract (T30, T40, T50, T75, T10, T25 and T15) applied at transplant. The second group included 7 treatments of tannin extract (T30a, T40a, T50a, T75a, T10a, T25a and T15a) applied at transplant and 14 days after and the treatment was repeated every 7 days. The third group included 2 treatments of tannin extract (T10b and T15b) applied at transplant and after 14 days. The tannin extract differently applied was compared to 4 additional treated and untreated control treatments (Fost, Aba, Garl and water) for a total of 20 treatments. Trial was performed for 60 days and treatments were arranged in a randomized complete block design with four replicates per treatment. The experiment was performed twice. The schedule treatments is described in Table 2.

DATA ANALYSIS

Data from the *in vitro* experiments were subjected to analysis of variance (ANOVA) and Fisher's least significant difference (LSD) tests using SPSS software (SPSS Statistics, v. 21 for Windows). For the *in vivo* experiments, plant heights were recorded at 30 days after transplanting (DAT) and 60 DAT, whereas root galling index (RGI) and final population density of nematodes were determined at 60 DAT from all plants. RGI was evaluated using the 0-10 scale where 0 = no galling visible, 1 = 10% of the root system galled, 2 = 20% of the roots galled, etc., and 10 = 100% galled roots (Bridge and Page, 1980). The effect of different treatments was examined using ANOVA and

Table 2 – Codes identifying pot treatments and effects of different concentrations of aqueous solutions of tannins and treated and untreated controls on the root-knot nematode *Meloidogyne incognita.*

Treatment codes	Dose at transplant (kg/ha^{-1})	Dose g/L^{-1} H_2O	Application time	Plant heights (cm)[a]		Nematodes 10 cm^3 soil[b]	RGI[c]
				30 DAT	60 DAT		
T30	6 kg	0.30 g	at transplant	45.69 a	71.38 b	607 a	5.25 a
T40	8 kg	0.40 g		47.81 a	71.56 b	595 a	5.13 a
T50	10 kg	0.50 g		45.56 a	70.63 b	597 a	5.13 a
T75	15 kg	0.75 g		46.13 a	71.88 b	596 a	5.19 a
T10	20 kg	1.00 g		45.94 a	71.94 b	586 a	5.13 a
T12	25 kg	1.25 g		47.63 a	71.13 b	590 a	5.19 a
T15	30 kg	1.50 g		47.19 a	71.81 b	585 a	5.06 a
T30a	6 kg	0.30 g	at transplant + 2 weeks later (6 kg/ha^{-1}) + every 1 week (6 kg/ha^{-1})	46.31 a	75.56 ab	458 c	4.69 ab
T40a	8 kg	0.40 g		46.25 a	76.75 ab	454 c	4.56 ab
T50a	10 kg	0.50 g		47.44 a	75.44 ab	457 c	4.50 ab
T75a	15 kg	0.75 g		48.38 a	77.56 ab	457 c	4.31 ab
T10a	20 kg	1.00 g		48.44 a	78.38 ab	451 c	4.50 ab
T12a	25 kg	1.25 g		48.19 a	79.38 ab	450 c	4.44 ab
T15a	30 kg	1.50 g		47.88 a	80.06 ab	448 c	4.38 ab
T10b	20 kg	1.00 g	at transplant + 2 weeks later (10 kg/ha^{-1})	45.06 a	78.06 ab	511 b	5.00 a
T15b	30 kg	1.50 g		45.19 a	78.69 ab	500 b	5.06 a
Fost	10.0L	0.50 mL	at transplant	46.50 a	89.75 a	371 d	3.50 b
Aba	5.0L	0.25 mL	at transplant + every 2 weeks (5 kg/ha^{-1})	47.25 a	80.31 ab	381 d	3.56 b
Garl	5.0L	0.25 mL	at transplant + every 2 weeks (11 kg/ha^{-1})	46.25 a	80.00 ab	390 d	3.94 ab
Cont	Water			46.81 a	69.69 b	621 a	5.25 a

[a] Plant heights were determined at 30 DAT and 60 DAT.

[b] Final nematode population density of Meloidogyne incognita (Kofoid & White) Chitwood in 10 cm^3 soil was extracted by cotton-wool filter method. [c]Nematode root galling index (RGI) was obtained using a 0-10 scale where 0 = no galls and 10 = 100% of roots galled. Data are arithmetic means of four replications and means separated with the Student-Newman-Keuls test ($P < 0.05$).

means were compared using Student-Newman-Keuls multiple comparison test. The level of significance was set at $P < 0.05$ in all the analyses.

RESULTS

IN VITRO EXPERIMENTS

The effect of each solution on J2s mobility is shown in Fig. I. Although the J2s mobility has been daily observed for 28 days, in Fig. I are reported only the most representative days. After 24 h, Fost demonstrated high nematotoxic activity with 90% of nematode immobility, Aba 25% of nematode immobility and three concentrations of tannin extract (T75, T12 and T15) immobilized 5% of J2s. Whereas no nematicidal activities in the other tannin extract concentrations (T30, T40, T50, T10) and Garl solution were observed. After 48 h of exposition to each solution tested, the nematicidal activity on J2s of Garl was up to 30%, Fost 92% and Aba 80% while the concentrations of tannin extract T12 and T15 were below to 15%, and T40, T75 and T10 were around 5%; no effects on J2s exposed to T30 and T50 were observed. After 72 h, Fost caused the highest immobility (100%) of J2s, Aba 90%, Garl 50% and still no effects of T30 on J2s mobility was observed, while immobile J2s exposed to all the other concentrations of tannin extract were ≤ to 20%. The immobilization of J2s exposed to tannin extract increased over time and about 50% in T30, T75, T10, T12 and T15 solutions of J2s were immobilized within 10 days, except for T50 (45%). The best performance at 10 days was shown by T40 (70%). Overall, during 10 days of experiment, all the solutions tested were statistically different compared to the untreated control (water). However, the nematode mobility in tannin extract at different concentrations was still lower than all the other solutions. During the first two weeks, viability of juveniles was suppressed in Garl solution and tannic extract at the highest concentration (T15). All concentrations with tannins from 0.30 to 1.2 g L^{-1} showed a nematode immobility in the range of 60-70%. A clear response of J2s to tannin extract was established from 15 to 20 days and a significant paralysis of J2s (up to 80%) was evident after 20 days of exposure. At 25 days, all the J2s exposed to T30, T40, T50, T10 and T12 were immobilized. Results showed that the viability of *M. incognita* J2s was significantly reduced ($P < 0.05$) over time by all concentrations of tannin extract throughout the experiment but slowly and in different ways. All the concentrations of tannin extract (0.30, 0.40, 0.50, 0.75, 1.00, 1.25, 1.50 g L^{-1}) had significant effects on J2 immobility compared to the water control. However, differences between some tannin extract concentrations were not found.

In the subsequent experiment (Fig. II), the immobile

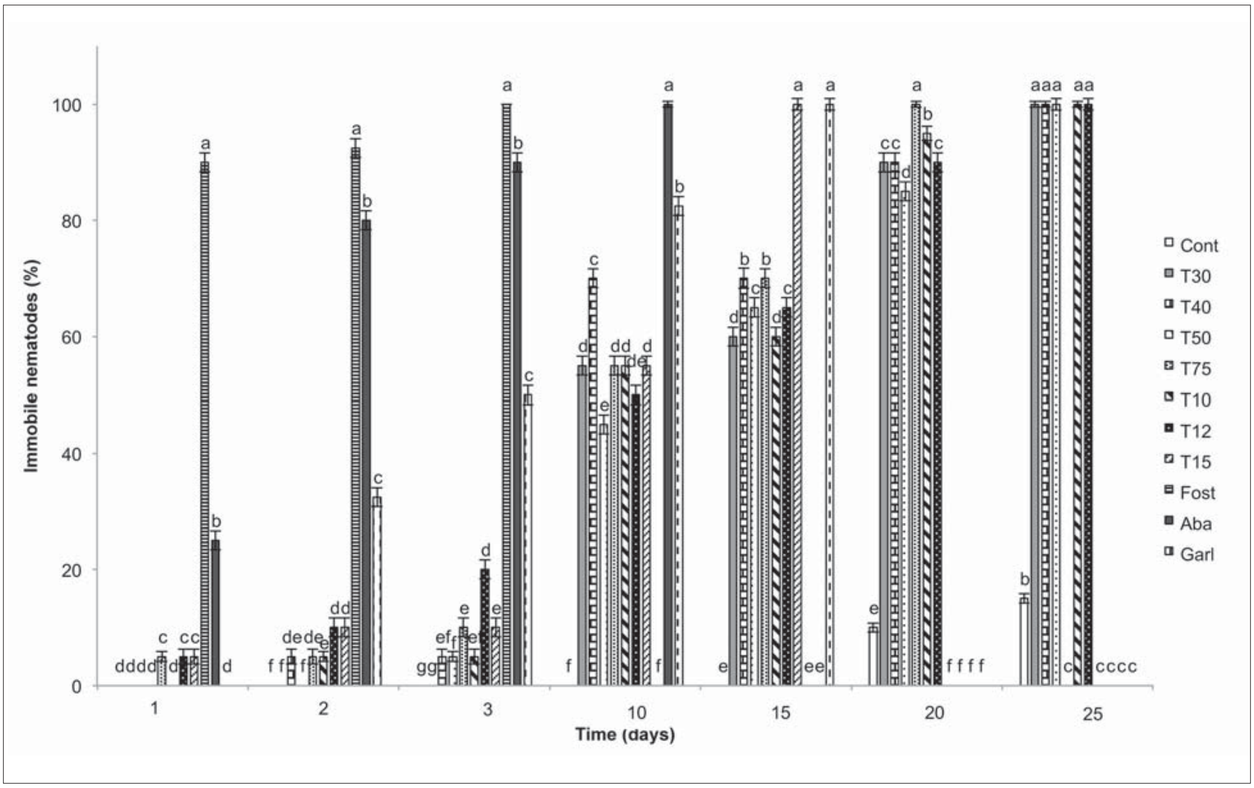

Fig. I – Percentage of immobile *Meloidogyne incognita* [(Kofoid & White) Chitwood] second-stage juveniles (J2s) after exposure to seven concentrations of tannin extract from 0.30 to 1.50 g L^{-1} (T30, T40, T50, T75, T10, T25 and T15) and treated (Fost, Aba and Garl) and untreated (Cont, water) controls over time (days). Values are means of four replicates ± SD.

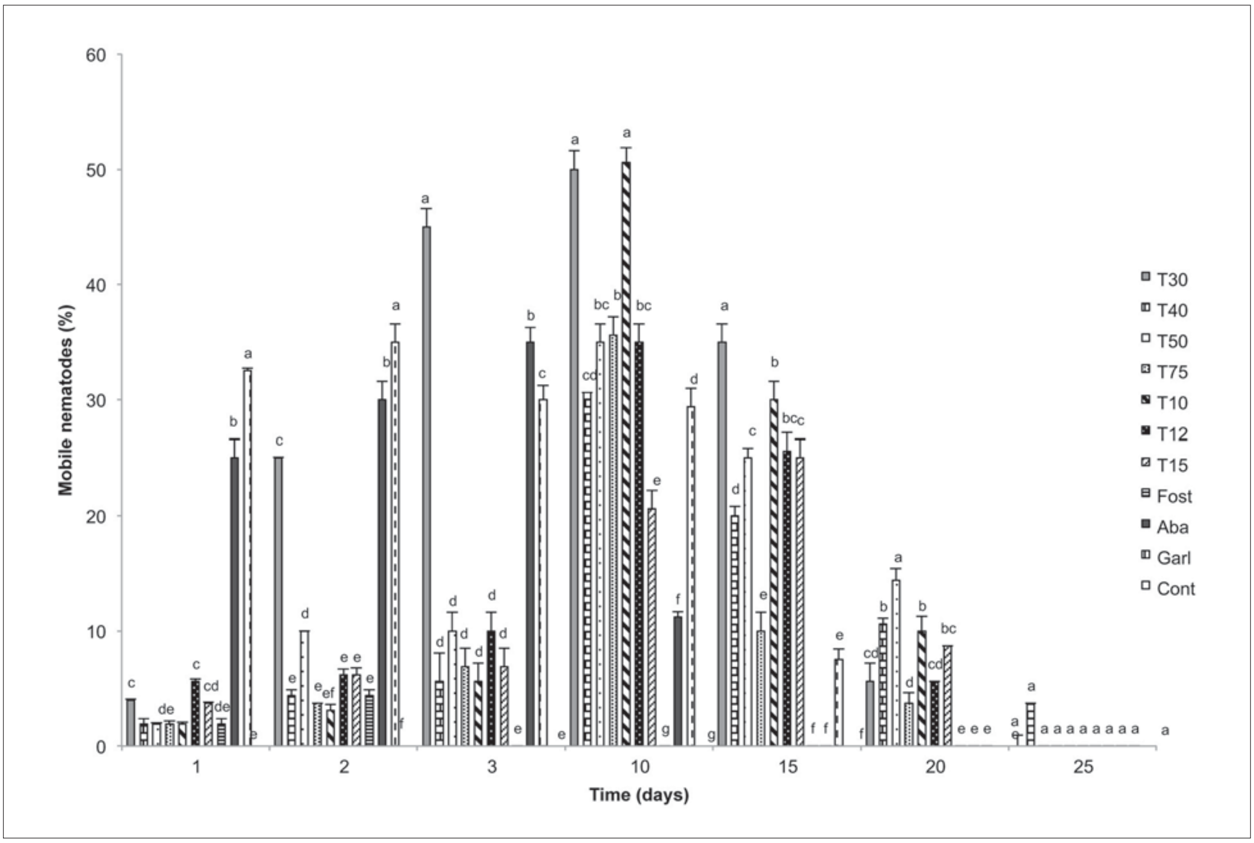

Fig. II – Cumulative percentage of mobile *Meloidogyne incognita* [(Kofoid & White) Chitwood] second-stage juveniles (J2s) incubated in distilled water after exposure to seven concentrations of tannin extract from 0.30 to 1.50 g L^{-1} (T30, T40, T50, T75, T10, T25 and T15) and treated (Fost, Aba and Garl) and untreated (Cont, water) controls over time (days). Data are averages of four replicates.

nematodes recovered their motility in water. This recovery was observed until J2s death. Generally, the mobility of J2s increased with exposure period to water. After exposure to the different concentrations of tannin extract, mobile J2s were in the range of 21% for T15 to about 50% for T30 and T10 after ten days rinse in water and these latter two concentrations (T30 and T10) were the highest peaks of recovery reached. Overall, the recovery in water of J2s previously immobilized by different concentrations of tannin extract (T30, T40, T50, T75,T10 and T15) was delayed in comparison with Aba and Garl and there were still alive J2s during an observation period of 25 days but it was not statistically significant. J2s immobilized by Garl also recovered their motility in water until death at 15 days. Only 4% of J2s, pre-exposed to Fost, recovered their motility just for two days; also, J2s pre-exposed to Aba recovered their motility but this recovery was shorter (until 10 days) than J2s pre-exposed to tannin extract and garlic extract. However, all J2s tested died gradually in time. The low percentage of J2s died in the control was considered physiological.

In vivo experiments

All 20 treatments did not significantly increased plant heights compared to the untreated control at 30 DAT (Table 2). Whereas, plant heights measured at 60 DAT were statistically different among treatments. The treated plants belonging to the first group (T30, T40, T50, T75, T10, T25 and T15) were not statistically different within them and in comparison to the untreated control (water). Also the treated plants belonging to the second group (T30a, T40a, T50a, T75a, T10a, T25a and T15a) were not statistically different within them but only when compared to the untreated control. The same results were shown by plants belonging to the third group (T10b and T15b). There were no statistically significant differences between the second and the third group when compared to Aba and Garl treated plants. The highest plants were recorded in pots treated with Fost.

Treatments with tannin extract applied only at transplant did not significantly reduced RGI in comparison to untreated control. The second group of tannin treated plants reduced RGI in comparison to untreated control, but RGI resulted significantly higher than that recorded in Fost and Aba treated plants. The plants treated with Garl had the same RGI recorded for the second group of plants treated with tannin extract. The third group of tannin treated plants (T10b and T15b) had the same RGI recorded for the first group of tannin treated plants and for untreated control. When tannin extract was applied only at transplant (first group) did not reduced the soil nematode population compared to the control. Applications of tannin extract at transplant and two weeks later (T10b and T15b) reduced the soil nematode population compared to the untreated control but less than the tannin extract treatments applied to the second group of plants and treated controls. However, the values of soil nematode population recorded in the second group of tannin treated plants were statistically higher than those in treated controls (Fost, Aba and Garl). No treatment showed any phytotoxic effects at the concentrations tested.

CONCLUSIONS

Phytochemicals can play an important role in the sustainable management of plant parasitic nematodes in organic and conventional systems (D'Addabbo *et al.*, 2014). The results of the present *in vitro* study revealed that the tannin extracts from *C. sativa* had effects on *M. incognita* J2s mobility. The immobility of nematodes increased with increasing concentration and longer exposure times. Likely, these factors were the main responsible for nematode control. The recovery of J2s mobility upon transfer to distilled water indicated that tannin extract caused, in the majority of cases, a reversible paralysis on *M. incognita*. However, although J2s may recover their mobility under laboratory conditions, likely they are too weak to locate host plant roots in the field (Haydock *et al.*, 2006).

In the pot experiment, the significant differences among the three application times demonstrated the positive effect of repeated tannin applications. Based on our results, the best treatment repetition regimen, irrespective of concentration, was the applications of tannin at transplant, 14 days after transplantation and then every 7 days, because we reached the highest nematicidal effect without phytotoxicity on tomato plants. Tannins applied once at transplant did not produced statistically different effects in comparison to the untreated control. However, repeated applications of tannins reduced disease parameters less than treated controls with exception of RGI in comparison to garlic extract. No effect of tannin extract on stimulation of tomato plant height was observed. Likely, the small differences detected on plant heights among treatments were due to the activity of nematodes that has contributed to malfunctioning of root system.

The tannins content in roots could be involved in passive plant defence working as chemical barriers in the roots for the nematode invasion (Taylor & Murant, 1966). It has been recognized that tannins may be chemical signals that *Meloidogyne* species utilize to recognize plant hosts and locate areas for root penetration (Ohri & Pannu, 2010). Application of tannins to soil, in pre-planting and at planting, could move up the feeding oriented-behavior of J2s and disorient them spreading "fake" chemical signals aimed to a reduction in the root searching efficiency of J2s that would either prevent or delay their attack; also post-planting applications, via drench or via drip irrigation, could have similar effects. According to other authors, we suppose that the observed nematostatic effect of tannins combined with their characteristics of attractants might represent a possible strategy to control RKN (Hewlett *et al.*, 1997; Maistrello *et al.*, 2010). Previous studies on the effects of tannins on plant parasitic nematodes reported a grave phytotoxicity to the host plant, instead we demonstrated that tannins at the tested concentrations are safe for tomato crop. However, the beneficial effect of natural phytochemicals is a promising area of nematode management (Chitwood, 2002) and tannins have great potential in environmentally friendly integrated pest management programs (Abid *et al.*, 1997). In conclusion, the use of SaviotaN® appears promising for the control of *M. incognita* in sustainable agriculture. The best performance may be obtained with short-term crops, low nematode population densities and to supplement other control strategies such as conventional treatments. However, further information is needed to investigate the effect of tannins in field experiments using different nematode-crop combinations and soil types.

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INSECT PESTS OF *EUCALYPTUS* PLANTATIONS IN SARDINIA (ITALY) ([1])

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Floris I., Cocco A., Buffa F., Mannu R., Satta A. – Insect pest of *Eucalyptus* plantations in Sardinia (Italy)

In Sardinia (Italy), *Eucalyptus* plantations cover approximately 23,000 hectares, above all in the southern part of the island. There is a complex of phytophagous insect pests that is threatening the health status of *Eucalyptus* trees. The most recent invasions include the sap-suckers, *Glycaspis brimblecombei*, *Blastopsylla occidentalis*, and the bronze bug, *Thaumastocoris peregrinus*. Other pest species have also been reported, including the gall wasps, *Ophelimus maskelli* and *Leptocybe invasa*, the longhorn beetles, *Phoracantha semipunctata* and *P. recurva*, and the weevils, *Gonipterus scutellatus* and *Polydrusus* (= *Metallites*) *parallelus*. Defoliation caused by the gypsy moth, *Lymantria dispar*, have also been frequently observed in various *Eucalyptus*-planted areas of Sardinia.

KEY WORDS: *Eucalyptus*, pests, Sardinia, monitoring, biological control.

INTRODUCTION

In Italy, the first introduction of a *Eucalyptus* (*E. robusta*) species dates to 1803 in the English garden of the royal palace at Caserta (Campania). A few years later, several other species were introduced and cultivated in the Botanical Gardens in Naples and in the *Hortus Camaldulensis*. The latter was an acclimatization garden created by the Count of Camaldoli, to whom Dehnhardt dedicated the species *E. camaldulensis* in 1832 (BRUNDU, 2017). In the early decades of the 19th century, *Eucalyptus* was only of ornamental interest and, according to some estimates, 78 different species were introduced for this purpose (BRUNDU, 2017). In 1869, the trappist monks of the Abbey of the Three Fountains in Rome started a *Eucalyptus* cultivation, believing that it had antimalarial properties due to both the presence of essential oils and its ability to reclaim wetlands. The seeds were brought from Australia by Monsignor Good, Bishop of Melbourne, who attended the First Vatican Council in Rome in December 1869.

At the beginning of the 20th century, extensive forestry programs were started, including wide plantations in land reclamation areas, along railway lines in Sicily, and for the production of the timber poles used in mines in Sardinia. Between 1930 and 1940, eucalypt species were widely used as windbreaks in the Agro Pontino area (Lazio region, central Italy) and in Sardinia (BRUNDU, 2017).

In the 1950s, extensive reforestation projects were carried out in Sicily and Sardinia, often without taking into account the ecological requirements of the species introduced. In the same period, Italy contributed to the knowledge of *Eucalyptus* species and played a prominent role in their diffusion; for example, in October 1956 in Rome the 1st World Congress on *Eucalyptus* was organized. The large number of species and the wide adaptability to different environments make it difficult to generalize the ecological characteristics and potential uses of the *Eucalyptus* species (BRUNDU, 2017).

Today, in addition to serving as windbreaks and as melliferous and polliniferous species, *Eucalyptus* species are also used to produce paper pulp, firewood, coal, and poles (DEIDDA *et al.,* 2016; BRUNDU, 2017).

In recent years, there has been a renewed interest in these species all over the world. For example, in 2015 Brazil authorized the use of transgenic *Eucalyptus* plants in reforestation. In Europe, *Eucalyptus* species are defined as exotic species by the European Regulation (EU) No. 1143/2014. Thus, both the introduction of new species and new plantations have to be carried out in compliance with legislation and good sustainable agricultural and forestry management practices. In Sardinia, *Eucalyptus* plantations are estimated to cover about 23,000 ha, excluding windbreaks and plantings smaller than one hectare, and are mainly located (90%) below 400 m a.s.l. in the southern part of the island (DEIDDA *et al.,* 2016).

The most widely cultivated species in the Mediterranean basin is the river red gum, *Eucalyptus camaldulensis* Dehnh., followed by *E. globulus* Labill. and *E. gomphocephala* DC.

Over the last few decades, a number of phytophagous insects and pathogenic fungi have threatened *Eucalyptus* species in the Mediterranean area. Among the most recent exotic invasive species, the red gum lerp psyllid, eucalyptus psyllid, and bronze bug have caused extensive damage to eucalypt plantations in consecutive years. In addition, gall wasps and some beetles, such as eucalyptus longhorned borers and weevils have also been reported. In some areas, the gypsy moth, *Lymantria dispar* (L.), has also caused defoliation.

This paper presents a comprehensive review of the

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Eucalyptus insect pests and their impacts on eucalypt plantations in a Mediterranean context (Sardinia, Italy).

THE MAIN PHYTOPHAGOUS INSECTS

PSYLLIDS

The invasive red gum lerp psyllid, *Glycaspis brimblecombei* Moore (Hemiptera: Psyllidae), is the most serious *Eucalyptus* pest in the Mediterranean area (Fig. I, 1) (MENDEL *et al.,* 2016), especially for *E. camaldulensis*, which has been shown to be extremely susceptible to *G. brimblecombei* infestations (DEIDDA *et al.,* 2016). The red gum lerp psyllid was recorded for the first time in Italy in 2010 (LAUDONIA and GARONNA, 2010) and spread rapidly in central and southern regions, including the islands. It was reported for the first time in Sardinia in spring 2010 (BUFFA, 2015).

Eggs are laid on the leaf surface, where nymphs develop under protective white conical coverings (lerps) composed of lipids, proteins and carbohydrates (Fig. I, 2) (MORGAN, 1984). Adults and nymphs are phloem feeders and produce large amounts of honeydew, thus promoting the development of sooty mold (MENDEL *et al.,* 2016; CUELLO *et al.,* 2018). The damage resulting from leaf feeding includes foliar discoloration, and, in cases of heavy infestations, dieback, early leaf fall and reduced plant growth (BRENNAN *et al.,* 1999, 2001; DAANE *et al.,* 2005, 2012). Defoliation in consecutive years can lead to the death of young plants or susceptible clones (DAANE *et al.,* 2005).

In March 2012, a monitoring program was started in Sardinia in 12 sites located in the main areas of *Eucalyptus* cultivation (BUFFA, 2015; DEIDDA *et al*., 2016; MANNU *et al.,* 2018). Adults and preimaginal stages were monitored by yellow sticky traps and leaf samples, respectively, in order to estimate the pest population density and natural mortality. Adult captures increased steeply from May-June and reached the highest values in late June-mid July, depending on the year, decreasing significantly from September onwards (MANNU *et al.,* 2018). Egg and nymph dynamics showed a similar pattern (Fig. II).

The specific koinobiont parasitoid *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae) (Fig. I, 3) has been effectively used in classical biological control programs in Mediterranean-climate areas (DAANE *et al.,* 2005, 2012; DAHLSTEN *et al*., 2005; FERREIRA FILHO *et al*., 2015). The parasitoid oviposits preferentially on third- and fourth-instar nymphs and develops inside late fourth and fifth instars (DAANE *et al.,* 2005; 2012). In 2015, the rate of *P. bliteus* parasitism was similar throughout the island, although an earlier peak was observed in July both in southern and central Sardinia (Fig. III).

The eucalyptus psyllid, *Blastopsylla occidentalis* Taylor (Hemiptera: Psyllidae) (Fig. I, 6), is native to Australia and was first reported in Italy in 2006 (LAUDONIA, 2006). However, *B. occidentalis* has established itself in other countries outside its native area, including New Zealand (TAYLOR, 1985), California (TAYLOR, 1985), Mexico (HODKINSON, 1991), Brazil (BURCKHARDT *et al*., 1999; SANTANA and BURCKHARDT, 2007), Chile (BURCKHARDT and ELGUETA, 2000), Argentina (BOUVET *et al.,* 2005), South Africa (ANONYMOUS, 2007), Cameroon (DZOKOU *et al.,* 2009), Turkey (AYTAR *et al.,* 2011), Spain and Portugal (PÉREZ OTERO *et al.,* 2011), and Uruguay (MARTÍNEZ *et al.,* 2014).

The adult females, which are generally darker than males and measure approximately 1.5 mm in length, can lay the eggs on shoots, leaves, flowers and twigs. As with other sap-sucking insects, the eggs are laid preferentially during the main periods of the flushing rhythm of trees (SOUFO and TAMESSE, 2015). The oviposition activity of *B. occidentalis* is closely related to the water conditions of host plants, as the psyllid population density decreased significantly at lower humidity levels in Cameroon (SOUFO and TAMESSE, 2015). Unlike red gum lerp psyllid juveniles, preimaginal stages of *B. occidentalis* are not characterized by lerps but produce a protective white waxy secretion, on which sooty mould can grow (TAYLOR, 1985; MENDEL *et al.,* 2016). The eucalyptus psyllid completes 5-6 generations under tropical conditions (SOUFO and TAMESSE, 2015).

In the regions where *Eucalyptus* trees are used as windbreaks and for fuelwood production, exploitation as a source of pollen and nectar for honeybees is also common. In such *Eucalyptus* plantations, *B. occidentalis* is considered one of the main concerns of eucalypt health (MENDEL *et al*., 2016). In fact, field observations carried out in the Mediterranean area have shown that the psyllid infested mainly young sprouts (Fig. I, 7) and inflorescences (Fig. I, 8) in formations of *E. camaldulensis* trees, causing a premature fall of flowers in the following spring period (BUFFA, 2015). Losses caused by *B. occidentalis* attacks can be exacerbated by the simultaneous presence of the red gum lerp psyllid infestations. Concomitant infestations of both psyllids could easily endanger the survival of the delicate floral structures of the *Eucalyptus*, with a subsequent impact on the production of the unifloral honey. In fact, joint attacks of the two psyllids led to a 85% decrease in eucalyptus honey production in Sardinia in the two years following *G. brimblecombei* introduction (BUFFA, 2015). Although a different pattern was not found along a latitudinal gradient, the mean abundance of *B. occidentalis* adults in Sardinia was lower in the southern part of the island, reaching a peak of approximately 20 adults per trap in late July (Fig. IV).

Other psyllid species belonging to *Ctenarytaina* have been introduced in the last few years in Europe: *C. eucaliptii* (Maskell), *C. spatulata* Taylor and *C. peregrina* Hodkinson.

THE BRONZE BUG

The bronze bug, *Thaumastocoris peregrinus* Carpintero & Dellapé (Hemiptera: Thaumastocoridae) (Fig. I, 4) is a sap-sucking pest infesting eucalyptus plantations. It originates from Australia (NOACK and ROSE, 2007; NADEL *et al.*, 2010), where it has been reported as a harmful species since 2002. The bronze bug is now widespread in many other areas: Africa (2003) (Zimbabwe, South Africa) (JACOBS and NESER, 2005; GILIOMEE, 2011), South America (2005) (Uruguay, Paraguay, Brazil, Chile, Argentina) (CARPINTERO and DELLAPÉ, 2006; NOACK and COVIELLA, 2006; MARTÍNEZ and BIANCHI, 2010; WILCKEN *et al.,* 2010; IDE *et al.,* 2011; SOLIMAN *et al.,* 2012), Europe (2011), and recently the Middle East (2014) and New Zealand (SOPOW *et al.,* 2012). The first report in Europe was recorded in Italy (2011) in the Lazio region (LAUDONIA and SASSO, 2012), followed by Portugal in 2012 (GARCIA *et al.,* 2013), Sicily in 2014 (SUMA *et al.,* 2014) and Sardinia in 2015 (DEIANA *et al.,* 2018), where the first record was reported in the south of the island on *E. camaldulensis*.

The bronze bug is known to attack at least 30 *Eucalyptus* species and three common commercial hybrids (JACOBS and NESER, 2005; CARPINTERO and DELLAPÉ, 2006; NOACK and COVIELLA, 2006). *E. urophylla* and *E. grandis* were found to be the most suitable host species, as nymph development

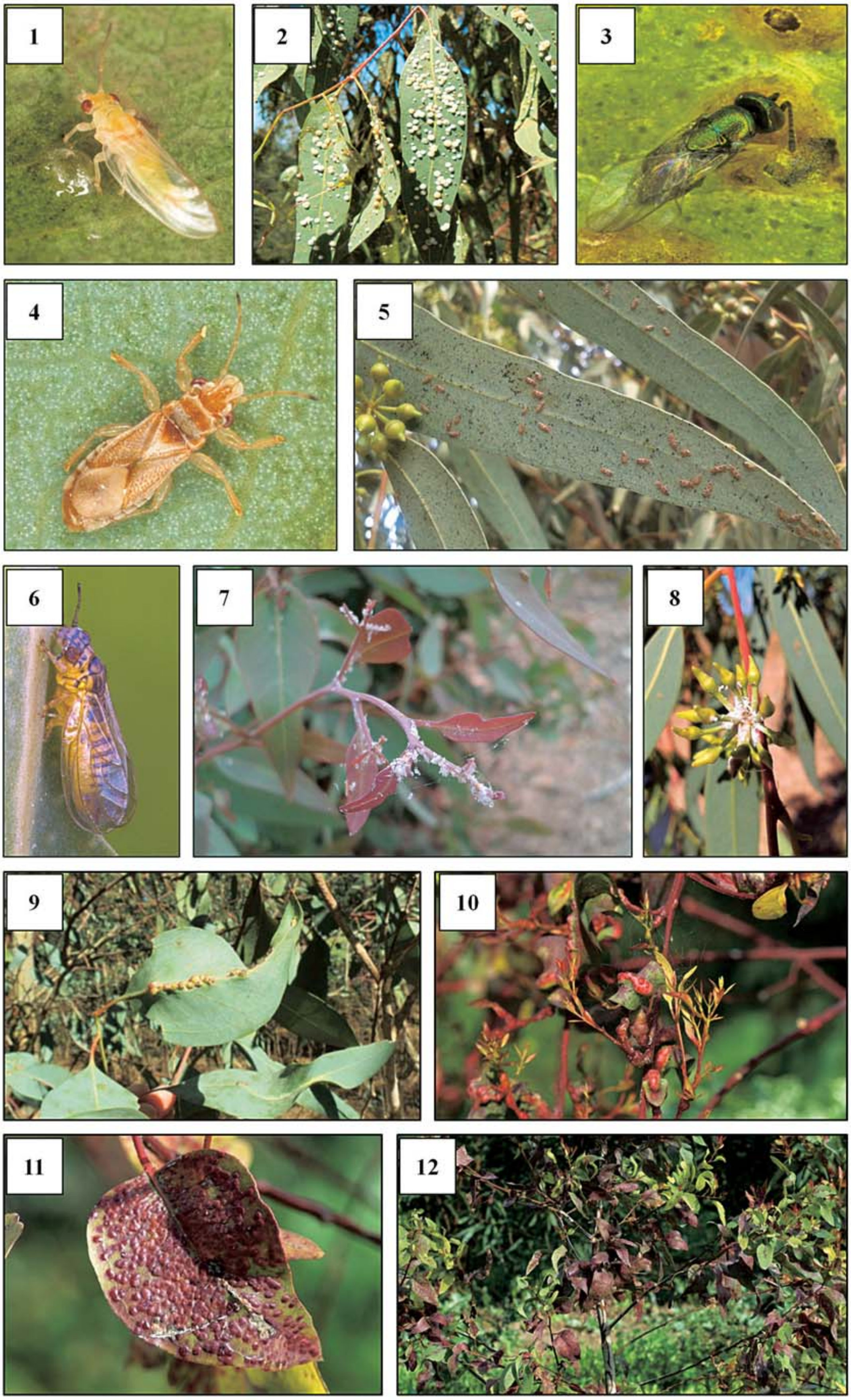

Fig. I – 1) adult male of *Glycaspis brimblecombei*; 2) leaves covered by lerps; 3) adult of *Psyllaephagus bliteus*; 4) adult of *Thaumastocoris peregrinus*; 5) grouping of *T. peregrinus* adults feeding on *Eucalyptus* leaves; 6) adult of *Blastopsylla occidentalis*; 7) *B. occidentalis* infestation on a young shoot; 8) *B. occidentalis* infestation on *Eucalyptus* flowers; 9) *Leptocybe invasa* galls on Eucalyptus leaves; 10) damage caused by *L. invasa* infestation; 11) *Ophelimus maskelli* galls on upper side of *Eucalyptus* leaf; 12) *Eucalyptus* young plant damaged by *O. maskelli* infestation.

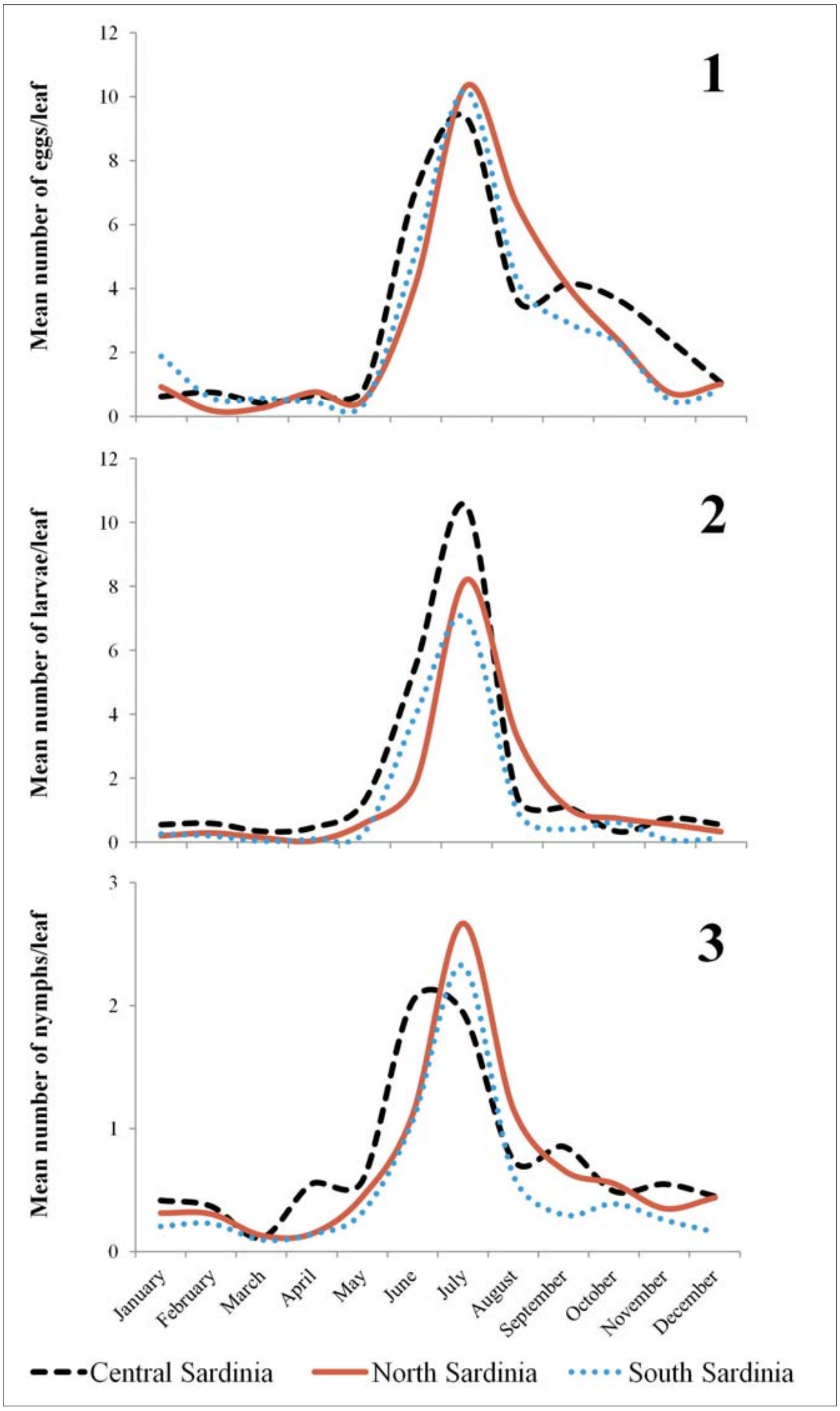

Fig. II – Mean number of *Glycaspis brimblecombei*: (1) eggs, (2) larvae, and (3) nymphs per leaf observed during 2015 in North, Central and South Sardinia.

was faster and female fecundity higher than on *E. camaldulensis* (Soliman *et al.,* 2012). Adults are light brown in color with darker spots, approximately 3 mm long, with a flattened body. Eggs are black, elliptical in shape and can be found individually or in patches on leaves and twigs. Nymphs develop through five instars to the adult stage in approximately 20 days, whereas adult longevity ranges from 14 to 42 days (Noack and Rose, 2007; Soliman *et al.,* 2012). The bronze bug completes multiple generations per year and all life stages can be observed at the same time on leaves.

The damage is caused by the feeding punctures of adults and preimaginal stages on expanded leaves (Fig. I, 5), causing silvering chlorosis that evolves to leaf bronzing and drying. The canopy turns progressively to a brownish-reddish discoloration, and thus has the common name of "winter bronzing" (Fig. I, 5). In addition to this symptom, early leaf fall and complete defoliation have been observed. Repeated infestations may cause branch dieback, reduce tree growth, and lead to the death of young weak plants (Soliman *et al.,* 2012).

In Sardinia, field surveys were carried out in *E. camaldulensis* plantations in order to study the seasonal occurrence of the pest in two consecutive years (2015: 12 monitoring sites; 2016: three sites). Adults were monitored by yellow sticky traps, whereas egg and nymph density was

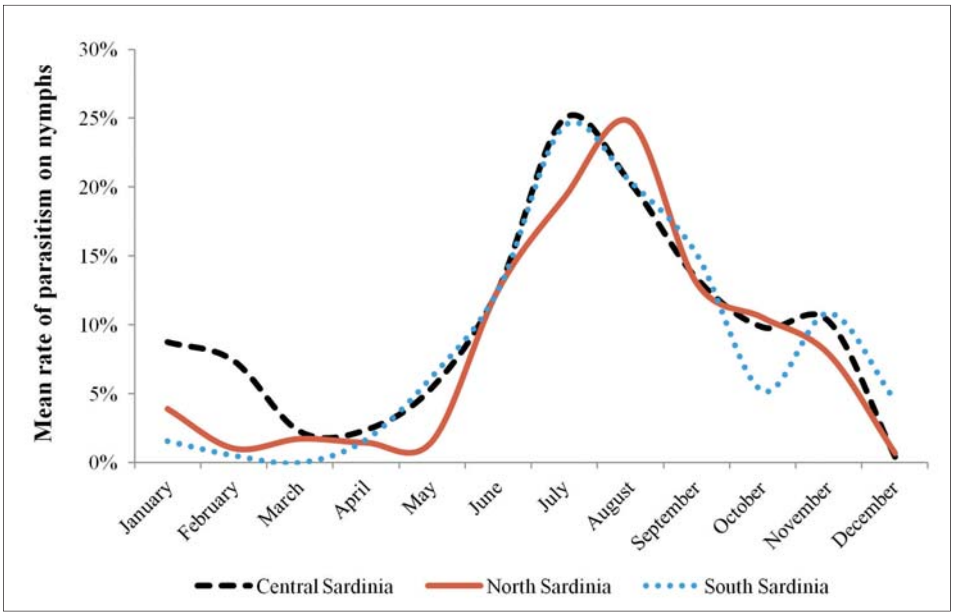

Fig. III – Mean rate of *Psyllaephagus bliteus* parasitism on *Glycaspis brimblecombei* nymphs in 2015 in North, Central and South Sardinia.

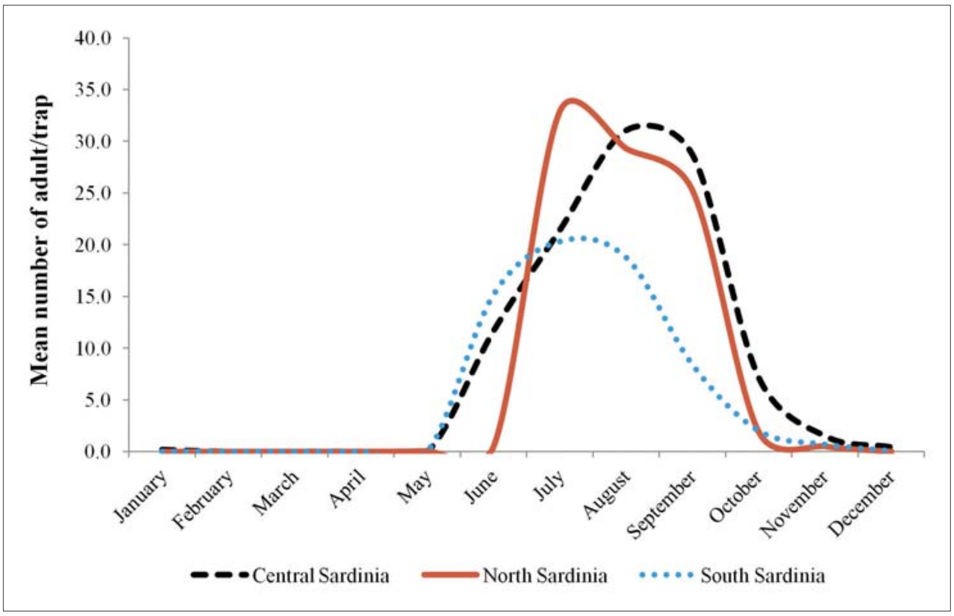

Fig. IV – Mean number of *Blastopsylla occidentalis* adults per trap captured in 2015 in North, Central and South Sardinia.

determined by sampling twigs (JIMÉNEZ-QUIROZ *et al*., 2016). The population dynamics in 2015 and 2016 showed high levels of adults in September, with a similar pattern for eggs and nymphs (DEIANA *et al.,* 2018). In addition, both the adults and the juvenile stages were also observed in the winter, suggesting that *T. peregrinus* can also be considered as being well established on the island (DEIANA *et al.,* 2018).

The most promising control strategies are currently based on biological control, in particular using the wasp, *Cleruchoides noackae* Lin & Hube (Hymenoptera: Mymaridae), an egg parasitoid that was recently confirmed as a potential biological control agent of *T. peregrinus* (BARBOSA *et al.,* 2017).

GALL WASPS

The gall wasps *Leptocybe invasa* Fisher & La Salle and *Ophelimus maskelli* (Ashmead) (Hymenoptera: Eulophidae) have been recently considered as serious pests of *Eucalyptus* trees (BRANCO *et al*., 2016). *L. invasa*, commonly known as blue-gum chalcid wasp, is native to Australia (MENDEL *et al.,* 2004), and its infestations can cause significant damage and production loss (MENDEL *et al.,* 2004; PETRO *et al.,* 2015; AHMED *et al.,* 2017).

As a rule, *L. invasa* reproduces by thelytoky (MENDEL *et al.,* 2004), although male adults have been found in Turkey (DOGANLAR, 2005) and India (AKHTAR *et al.,* 2012). Females lay eggs in the midrib and petiole of newly-developed leaves and in young stems and shoots, inducing a decrease in shoots and leaf growth of *Eucalyptus* (Fig. I, 9 and 10). The damage caused by the blue-chalcid gall wasp is probably due to changes in the leaf hydraulic architecture, as a reduction of both the development of stomata and a minor vein density have been observed in *L. invasa*-infested trees (TONG *et al.,* 2016). This insect has been reported as a serious pest of various *Eucalyptus* species worldwide (DITTRICH-SCHRÖDER *et al.,* 2012; PETRO *et al.,* 2015), as *E. camaldulensis* are one of the most suitable hosts for its development (BRANCO *et al.,* 2016).

O. maskelli also originates from Australia and was accidentally introduced into the Mediterranean region in 1999, where it was initially misidentified as *O. eucalypti* (ARZONE and ALMA, 2000; VIGGIANI and NICOTINA, 2001). After its first report in Italy as *O. eucalypti*, the wasp has been observed in other Mediterranean areas on *E. camaldulensis* trees, including Spain (PUJADE-VILLAR and RIBA-FLINCH, 2004), Greece (KAVALLIERATOS *et al.,* 2006), France (EPPO, 2006), Portugal (BRANCO *et al.,* 2006), and Tunisia (DHAHRI *et al.,* 2010). *O. maskelli* has also been reported in southern Asia (LAWSON *et al.,* 2012), South Africa (HURLEY, 2014), and California (BURKS *et al.,* 2015). Contrary to *L. invasa*, the galls induced by *O. maskelli* occur exclusively on the leaf blade of *Eucalyptus* leaves, forming typical round button-shaped galls (Fig. I, 11) (PROTASOV *et al.,* 2007). Under particular environmental conditions, the surface of the infested leaves can be entirely covered with galls (Fig. I, 12) (BRANCO *et al.,* 2016).

The populations of both gall wasps, as well as the presence of the specific parasitoid of *O. maskelli*, *Closterocerus chamaeleon* (Girault) (Hymenoptera: Eulophidae), were monitored from 2013 to 2014 in a Mediterranean area (BUFFA, 2015), showing a within-year pattern with two peaks of abundance for both *L. invasa* and *O. maskelli*. The highest number of galls per leaf occurred in the early spring and late summer for *O. maskelli* and *L. invasa*, respectively (BUFFA, 2015).

WEEVILS

The leaf-feeding beetle *Gonipterus scutellatus* Gyllenhal (Coleoptera: Curculionidae) (Fig. V, 1) is native to Australia and is probably the most studied weevil species infesting *Eucalyptus* trees worldwide. *G. scutellatus* species, which is also known as *Eucalyptus* weevil or *Eucalyptus* snout-beetle, belongs to a complex of cryptic species, whose revision is still in progress (NEWETE *et al.,* 2011; MAPONDERA *et al.,* 2012). Different species of the *G. scutellatus* complex have been accidentally introduced in Africa, America, Europe and New Zealand (NEWETE *et al.,* 2011; MAPONDERA *et al.,* 2012). *G. scutellatus* was first reported on *Eucalyptus* trees in Italy in 1975 (ARZONE, 1976).

G. scutellatus can cause severe damage to *Eucalyptus* trees mainly outside its native range, in which a rich community of predators and parasitoids has been shown to be able to control weevil infestations (TOOKE, 1953; LOCH and FLOYD, 2001; LOCH, 2008; LOCH and MATSUKI, 2010). In newly introduced areas, a different susceptibility of *Eucalyptus* species to *G. scutellatus* infestations and development has been observed (CORDERO RIVERA and SANTOLAMAZZA CARBONE, 2000; NEWETE *et al.,* 2011). *E. viminalis* is considered the most susceptible species to the snout beetle (TOOKE, 1953), although a variability in the weevil host preference has been reported in different countries (CLARKE *et al.,* 1998; HANKS *et al.,* 2000; LOCH 2006).

Adult females are polygynous and can lay more than 300 eggs during their lifetime (EPPO, 2005). The eggs are laid on leaves in capsules containing approximately 8-10 eggs. The weevil can complete up to four generations per year, even though only two generations have been recorded in Italy (EPPO, 2005). Larvae of *G. scutellatus* attack leaves and twigs (Fig. V, 2), whereas adults feed exclusively on leaf edges leading to a generalized dieback of shoot tips and the development of tufts of epicormic shoots (EPPO, 2005). Successive defoliations negatively affect the tree growth and a stag-headed appearance occurs in the presence of severe infestation. Occasionally, infested-trees may split and die.

The use of natural enemies and tolerant *Eucalyptus* species is the most sustainable alternative to control *G. scutellatus* infestations, whereas chemical treatments have a potential negative effect on various *Eucalyptus* pollinators (EPPO, 2005). The egg parasitoid *Anaphes* (*Patasson*) *nitens* (Girault) (Hymenoptera: Mymaridae) has been used in controlling snout beetle populations in Italy (ARZONE, 1985). Based on preliminary observations, no detectable damage caused by *G. scutellatus* has been observed in Sardinia.

Polydrusus (*Metallites*) *parallelus* Chevrolat (Coleoptera: Curculionidae) (Fig. V, 3) is an endemic species of Sardinia, Corsica and Elba which feeds on *Eucalyptus* trees in its distribution range. However, the biology of this insect is unknown. Adults are common in spring and summer, and larvae presumably live underground feeding on plant roots. Damage caused by the feeding activity of *P. parallelus* was detected in Sardinia in April and May 2013 on *Eucalyptus* shoots (Fig. V, 4). The presence of *P. parallelus* was also recorded in the spring of 2014 throughout the island (BUFFA 2015). Damage to *Eucalyptus*, which has never been reported before, appeared to be significant in young plants. Adults feeding on *Eucalyptus* caused annular erosions on young branches, thus determining the break and subsequent drying of the attacked branches (Fig. V, 5).

LONGHORN BEETLES

The two-closely related species of longhorn beetles, *Phoracantha semipunctata* (Fabricius) and *P. recurva* Newman (Coleoptera: Cerambycidae), are above all associated with *Eucalyptus*. *P. semipunctata* (Fig. V, 6) was first detected in Sardinia in the 1970s, representing the first detection of the pest in Europe (TASSI, 1969; PIRAS *et al.,* 1970). A few years after its detection, the species had spread throughout the surrounding area in which *Eucalyptus* trees were growing, particularly in southern Italy (ROMANO, 2007; LONGO, 2009). *P. semipunctata* adult females live one or more months and can produce up to 300 eggs, which are laid in groups of 3-30 under the loose bark of *Eucalyptus* trees.

The eggs hatch 7-14 days after oviposition, depending on the temperature. First-instar larvae can either chew a hole directly in the inner bark or mine the outer bark before making its way to the bark-cambium-xylem interface. Larval development lasts on average 90 days (MENDEL, 1985). Mature larvae excavate pupal chambers in the wood, sealing the chamber entrance with wood shavings and frass. After the pupation period, which lasts approximately 40 days in Mediterranean field conditions (MENDEL, 1985), adult beetles emerge from the same entry holes by chewing through the plugs of frass. The adult beetle can complete its life cycle in 3-4 or 9 months when eggs are laid during early spring or late summer, respectively (MENDEL, 1985). Hence, there are generally 2-3 generations per year.

The longhorn beetle *P. recurva* (Fig. V, 7) was discovered in Sardinia in 2006 (CILLO *et al.,* 2006), although it has been in Italy since 1992 (PALMERI and CAMPOLO, 2006). The life cycle of the insect differs somewhat from that of the other longhorn beetles that infest *Eucalyptus* trees, as *P. recurva* adults can emerge earlier than *P. semipunctata* (ROMANO, 2007). Today, *P. recurva* in Sardinia has almost completely displaced the congeneric species, which was very common until 2004, resulting in a greater number of *Eucalyptus* plantations infested exclusively by *P. recurva* (ROMANO, 2007).

The two longhorn beetle species can cause serious damage to young trees (Fig. V, 8), and are considered a potential threat to *Eucalyptus* reforestation (MENDEL, 1985; ROMANO, 2007). The presence of both *P. semipunctata* and

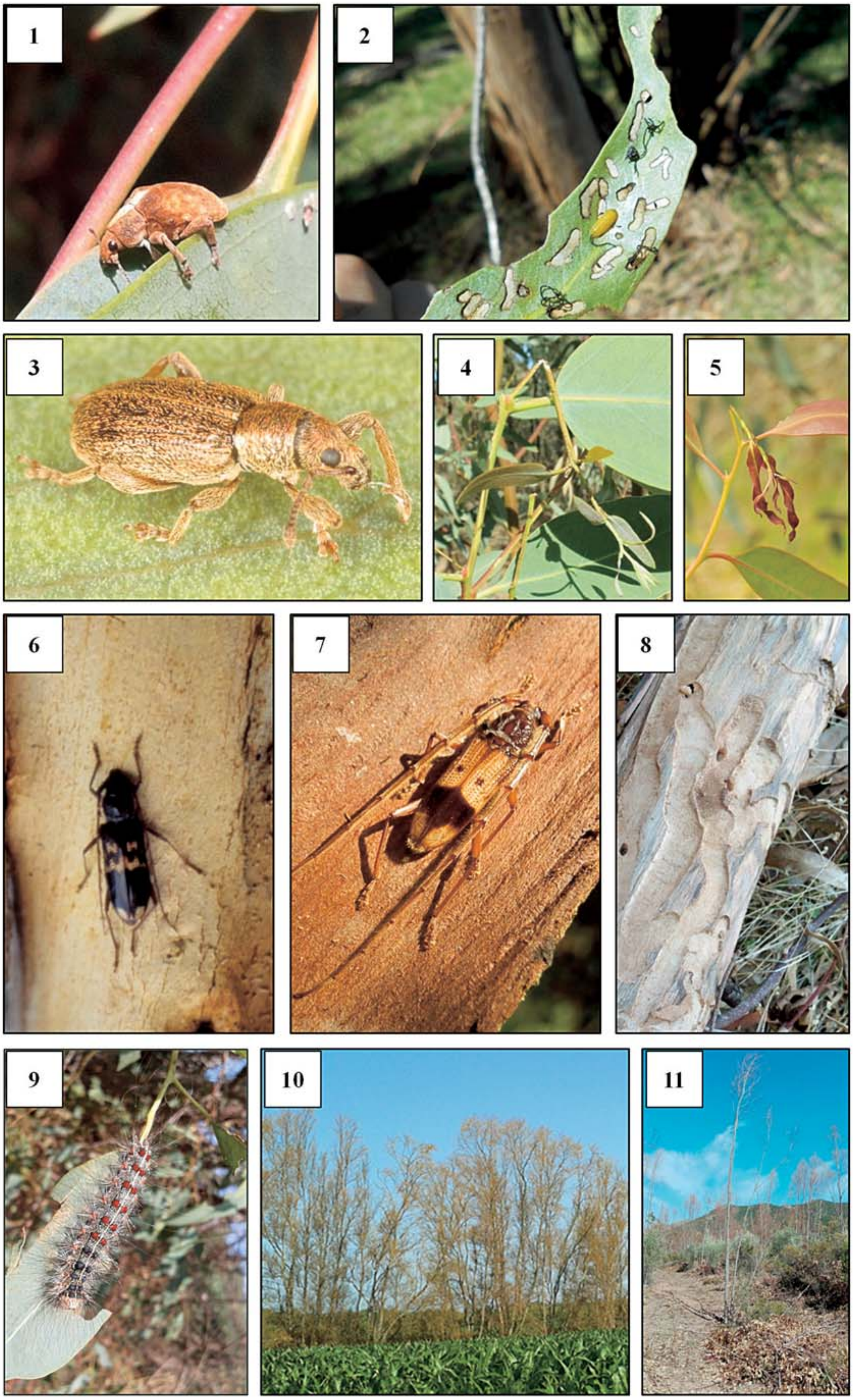

Fig. V – 1) adult of *Gonipterus scutellatus*; 2) leaf damaged by feeding activity of *G. scutellatus* larvae; 3) adult of *Polydrusus parallelus*; 4) and 5) young shoots damaged by *P. parallelus* infestations; 6) adult of *Phorachanta semipunctata*; 7) adult of *P. recurva*; 8) galleries of *Phorachanta* species on *Eucalyptus* tree; 9) mature larva of *Lymantria dispar*; 10) windbreak of *Eucalyptus* trees defoliated by *L. dispar*; 11) complete defoliation of *Eucalyptus* young trees caused by *L. dispar.*

P. recurva coupled with other pests threatens the sanitary status of *Eucalyptus* trees and makes the control of longhorn borers necessary, especially by enhancing the physiological status of *Eucalyptus* trees or using resistant *Eucalyptus* species (PAINE and MILLAR, 2003).

GYPSY MOTH

The gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Erebidae), is a univoltine pest species which is considered one of the main risks to oak woods worldwide (LUCIANO and PROTA, 1985; MCFADDEN and MCMANUS, 1991). The adult female gypsy moth is creamy white and flightless, whereas the adult male can fly several kilometers. Egg masses, which are laid on the trunks of trees during the summer, are covered by the abdominal hairs of the female which will protect the eggs until the following spring. Hence, larvae (Fig. V, 9) start feeding on young leaves after hatching, and pass through either five (male) or six (female) instars before the prepupal stage. Gypsy moth populations are characterized by cyclical fluctuations of their population density, which are commonly known as *gradations* (CAMPBELL, 1981). During the first period of gradation (i.e. latency phase), the population density is at a harmless level for several years. After this period, the population density increases gradually in 2-3 years (i.e. progradation), reaches a peak or *outbreak* (i.e. culmination), which can last for two consecutive years, and naturally decreases (i.e. retrogradation) until returning to a latency period. The complete defoliation of over 60,000 hectares of cork oak forests has been recorded in Sardinia during a gypsy moth outbreak (LUCIANO and PROTA, 1985).

Defoliation causes a significant reduction in photosynthetic activity and can negatively affect the tree growth, as well as compromise the health of forest stands when there are other abiotic (e.g. drought) or biotic stresses (e.g. fungal diseases). Moreover, defoliation during the summer tends to discourage tourism in infested areas, resulting in an additional economic loss.

Although in the Mediterranean area the gypsy moth is mainly associated with the *Quercus* tree species (COCCO *et al.,* 2010; MANNU *et al.,* 2017), it is a very polyphagous pest, capable of feeding on more than 300 host plants including some *Eucalyptus* species (LEONARD, 1981; MCFADDEN and MCMANUS, 1991; LIEBHOLD *et al.,* 1995). *L. dispar* larvae feeding on leaves of different *Eucalyptus* species under laboratory conditions have shown a high variability in development and survival, indicating both the variability and limited ability to process some secondary metabolites (MATSUKI *et al.,* 2011). However, the complete defoliation of *Eucalyptus* trees has recently been observed in Sardinia (Fig. V, 10, 11). These occasional infestations are more likely to be due to *L. dispar* populations spreading either actively or passively from close outbreak foci.

CONCLUSION

Sardinia is one of the most important areas of the Mediterranean basin for the cultivation of *Eucalyptus* tree species. In the last few years, several insect pests infesting *Eucalyptus* trees have been reported, including the leaf-feeding insects *G. brimblecombei*, *B. occidentalis* and *T. peregrinus*. These insects increase the complexity of *Eucalyptus* pest fauna already present throughout the entire area, such as gall wasps, longhorn beetles, and weevils. In addition, the defoliations caused by *L. dispar* and the symptoms of decay caused by the fungus *Neofusicoccum australe* have been recently found in some limited areas (DEIDDA *et al.,* 2016). The health status of *Eucalyptus* trees in Sardinia has severely deteriorated.

A monitoring program carried out from 2012 to 2016 as part of the project "Programma triennale di controllo biologico della Psylla lerp dell'eucalipto *Glycaspis brimblecombei* e monitoraggio delle problematiche fitosanitarie dell'eucalipto in Sardegna" financially supported by the "Regione Autonoma Sardegna - Assessorato Difesa Ambiente, Tavolo Tecnico Difesa Fitosanitaria Piante Forestali", investigated the health status of *Eucalyptus* in Sardinia (FLORIS and LUCIANO, 2017). The program also evaluated the economic impact of the most important pests on the quality and quantity of unifloral *Eucalyptus* honey, which represents more than 50% of the entire honey production in Sardinia (FLORIS *et al.,* 2016).

As a consequence of the increasing interest in *Eucalyptus* species as forestry and melliferous resources, a specific plan providing information on the most effective and sustainable management strategies for *Eucalyptus* plantations is needed. In line with international standards (IPPC/FAO, EPPO), a "collection field" of the *Eucalyptus* genus could be set up in order to: (1) evaluate the adaptation of different species, subspecies and hybrids to Mediterranean environmental conditions; (2) detect the presence of new pests and pathogens and assess the different susceptibilities of *Eucalyptus* species and hybrids; (3) evaluate the biological and ecological characteristics of different species, subspecies and hybrids of *Eucalyptus* in order to support their potential use as pollen resources for honey production; (4) increase ecological knowledge of plant-rhizosphere interactions focused on the naturalization of species and hybrids and their potential role as "biological invaders".

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THE PRESENT PEST STATUS OF *EUCALYPTUS* SAP-SUCKERS AND GALL WASPS IN CAMPANIA (ITALY) ([1])

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Garonna A.P., Bernardo U., Gualtieri L., Laudonia S., Nugnes F. – The present pest status of *Eucalyptus* sap-suckers and gall wasps in Campania

Sap-suckers and gall wasps may be a severe phytosanitary problem for several *Eucalyptus* species, particularly for the red gum *E. camaldulensis*, the most widely cultivated Australian native tree species worldwide. This paper reviewed the harmfulness of some of these invasive pests established in Campania, and provides new information about their pest status.

KEY WORDS: Red gum, bronze bug, Aphalaridae, Eulophidae, biological control.

Sap-suckers can be a serious phytosanitary problem of *Eucalyptus* species worldwide. During the last 30 years 4 allochthonous species of Aphalaridae (Psylloidea) and one species of Thaumastocoridae living on Myrtaceae and infesting mainly *Eucalyptus* spp. have been accidentally introduced in Italy. Chronologically listed the involved psyllids are, *Ctenarytaina eucalypti* (Maskell), *Ctenarytaina spatulata* Taylor, *Blastopsylla occidentalis* Taylor, and *Glycaspis brimblecombei* Moore, with the last two species widespread in Campania. In addition, the bronze bug, *Thaumastocoris peregrinus* Carpintero & Dellapé, must be added as the last established pest in the region (LAUDONIA and SASSO, 2012).

Among the psyllids, *G. brimblecombei,* which is commonly known as red gum lerp psyllid, is easily recognized by the nymphal stages protected by the conical crystalline white coverings (lerps) of secreted wax mixed with excreted honeydew (Fig. I, 1). The speed of its world invasion has few precedents. Native to Australia, the species is today considered a cosmopolitan pest and is recorded in 24 countries (EPPO, 2017). The first Italian record dated back to 2010, when huge populations were found spread over a large territory in Campania (LAUDONIA and GARONNA, 2010). The preferred host of *G. brimblecombei* is *E. camaldulensis*, largely distributed in the Mediterranean basin, where it is used as hardwood species in forestry and as ornamental species in urban greenery. The lerp psyllid (Fig. I, 2) became in few years a harmful pest detrimental to red gum trees with strong economic impact also involving the beekeeping sector. The heavy environmental and economic impact of chemical control (e.g. high cost and low effectiveness of pesticide applications due to overlapping generations) led to investigations on natural control realized by indigenous entomophagous species. Biological observations highlighted the limited role of generalist predators like anthocorids and ladybirds (*Coccinella septempunctata*, *Adalia* spp., *Oenopia* sp., *Scymnus* sp., *Chilocorus bipustulatus*), chrysopids (*Chrysopa* sp.), hoverflies larvae and predatory wasps (*Vespula* sp.) (LAUDONIA and GARONNA, 2010). Interestingly the wasps fed on honeydew and also on nymphal stages of the psyllid raising the lerp coverings. Among all recorded predatory species *Anthocoris nemoralis* Fabricius (Hemiptera: Anthocoridae) (Fig. I, 3), resulted the most efficient, preying adults and all preimaginal stages (GARONNA *et al.,* 2011).

The accidental introduction of the main parasitic species of the lerp psyllid in the Mediterranean basin, *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae), changed the biological control scenario. The encyrtid, already employed with success in biological control (BC) programmes in California, Brazil, Mexico e Chile, was first recorded in Campania during fall 2012 (Fig. I, 4). Nowadays the parasitoid controls the lerp psyllid in large part of the Italian territory. A recently published study about the host-parasitoid interaction showed that *P. bliteus,* even with low-density population of its own host, is strictly influenced by environmental factors (LAUDONIA *et al.,* 2013). In fact, population dynamics of host and parasitoid is affected by the seasonal trend of the climate, mainly during summer where high temperatures and limited rainfall can affect both species. Predictive modelling applied to this system highlighted a potential reduction of *G. brimblecombei* population density up to 64% due to *P. bliteus* activity (MARGIOTTA *et al*., 2017). In this scenario the pest species will persist in the environment reaching sometimes harmful densities detrimental to *E. camaldulensis*.

B. occidentalis, a species with negligible phytosanitary importance in Campania, preferably infests the apical trait of twigs. The eggs of the species can be found on shoots, leaf axils, small branches and young leaves. The pre-

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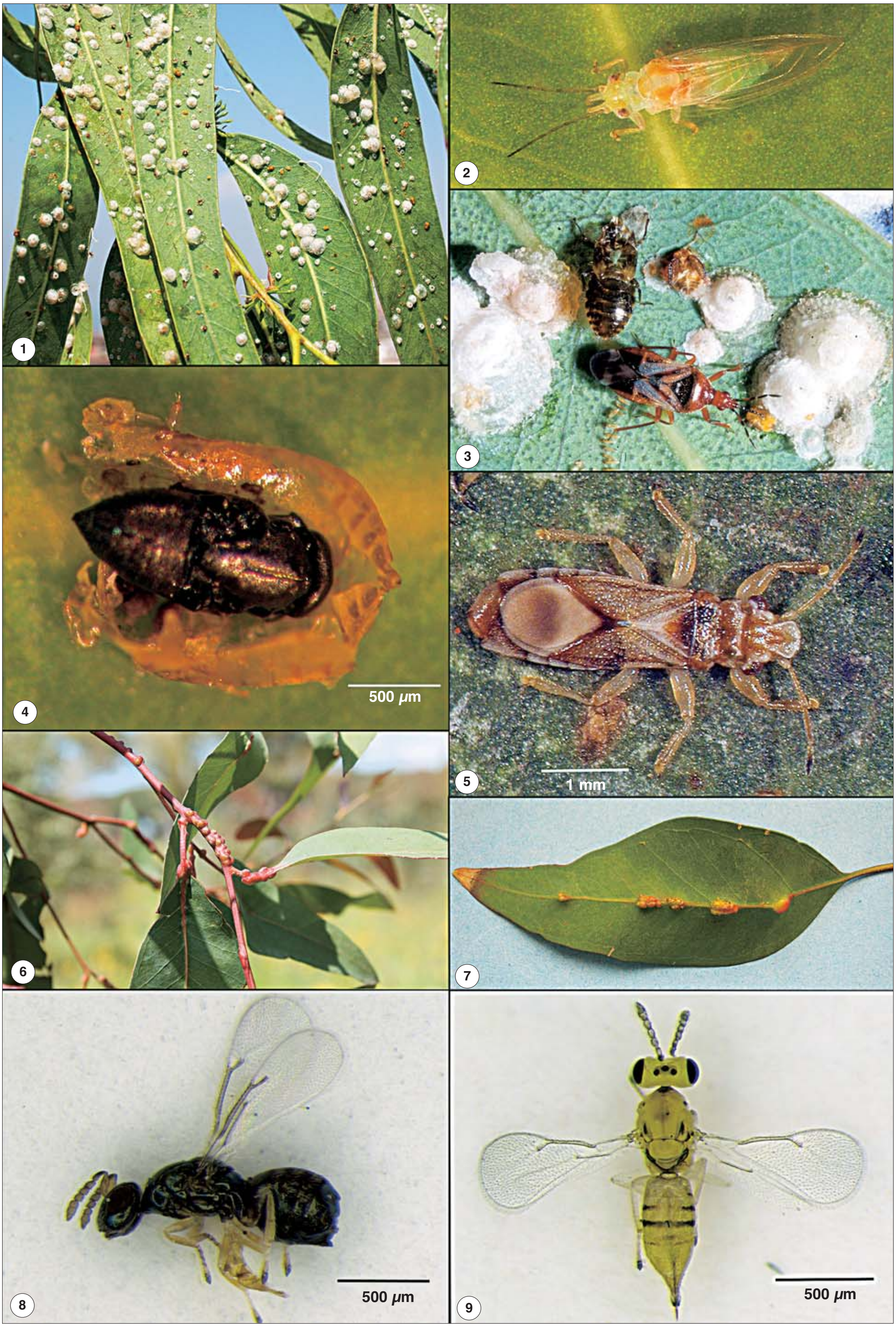

Fig. I – 1, colony of *Glycaspis brimblecombei*; 2, adult of lerp psyllid; 3, *Anthocoris nemoralis* feeding on lerp psyllids; 4, pupa of *Psyllaephagus bliteus*; 5, adult of *Thaumastocoris peregrinus*; 6, *Leptocybe invasa* galls on stem and petiole of *Eucalyptus* sp.; 7, *L. invasa* galls on eucalypt leaf main nerve; 8, female of *L. invasa*; 9, female of *Quadrastichus mendeli*.

imaginal stages are covered only by fluffy whitish wax and produce powdery globules of honeydew. Since its discovery in Campania (Laudonia, 2006) this psyllid has always been recorded at low density throughout the region. Scarce natural control is carried out by the same predatory species attacking the lerp psyllid. Up to now no parasitoid of *B. occidentalis* has been collected.

The bronze bug, *T. peregrinus* (Fig. I, 5), is native to Australia, where it feeds on a wide range of *Eucalyptus* species (Carpintero and Dellapé, 2006; Noack *et al.,* 2011; Soliman *et al*., 2012; Mutitu *et al*., 2013). It is also an invasive species that has spread to South Africa and Réunion (Jacobs and Neser, 2005; Streito *et al*., 2016), South and Nord America (Noack and Coviella, 2006; Wilcken *et al*., 2010; Martínez and Bianchi, 2010; Ide *et al*., 2011; Jiménez Quiroz *et al*., 2016; Arakelian, 2016) and New Zealand (Sopow and George, 2012). *T. peregrinus*, to date the only species belonging to thaumastocorids in the Fauna of Europe, has been reported for the first time for the Mediterranean basin in Italy (Laudonia and Sasso, 2012). Thereafter, the bronze bug has established in Portugal (Garcia *et al*., 2013), Spain (Vivas *et al*., 2015), Israel (Novoselsky and Freiberg, 2016) and Albania (Van Der Heyden, 2017). *T. peregrinus* causes leaf discoloration (bronzing, reddening, yellowing), early senescence and stunted growth. Heavy infestations can lead to severe defoliation, branch dieback, and in some cases, tree mortality (Laudonia and Sasso, 2012). The adults are light brown with a flattened body (2-3.5 mm). Eggs are typical dark, oval (0.5 mm long - 0.2 mm wide) with a sculptured chorion often laid in clusters on leaves and twigs. The eggs, the 5 pre-imaginal stages and the adults can be present on the same leaf. The life cycle is rather short, approximately 35 days in laboratory conditions (20 days at 17-20°C), and in the same condition, a female can lay approximately 60 eggs during its lifespan. However, it is difficult to study the timing and phenology of population fluctuations in the field (Noack and Rose, 2007; Jacobs and Neser, 2005; Bouvet and Vaccaro, 2007**)**. Data collected in Italy confirm that *T. peregrinus* has not any diapause period remaining active during all seasons, even though sometimes in very low numbers (Nadel *et al*., 2015; Laudonia *et al*., 2016**)**.

After a first steady trend during January-April 2015, at very low density (<0.05 specimens/leaf), a significant increase of the bronze bug population has been recorded in Campania from April to October (Fig. II). In particular, an exponential growth from the end of August of the same year has been observed (Laudonia *et al.,* 2016). Finally, it can be asserted that, at our latitudes and in field, the species is limited by unfavourable temperatures below 11 °C and above 30 °C (Laudonia *et al*., 2016).

In some countries the chemical control option for *T. peregrinus* is Imidacloprid, with systemic trunk injection also. Chemical control is not effective and at the same time unfriendly to the environment; moreover, it is both time consuming and costly (Wilcken *et al*., 2010; Zanuncio *et al*., 2010). In Italy, like in other areas of the world, there are no registered chemical options against *T. peregrinus*. Until now, no European indigenous enemy has been reported associated to the Bronze bug. In the native area, the most effective natural enemy is the egg parasitoid *Cleruchoides noackae* Lin and Huber (Hymenoptera: Mymaridae) (Lin *et al*., 2007). Host specificity tests established that *C. noackae* is able to parasitize other species of Thaumastocoridae, but

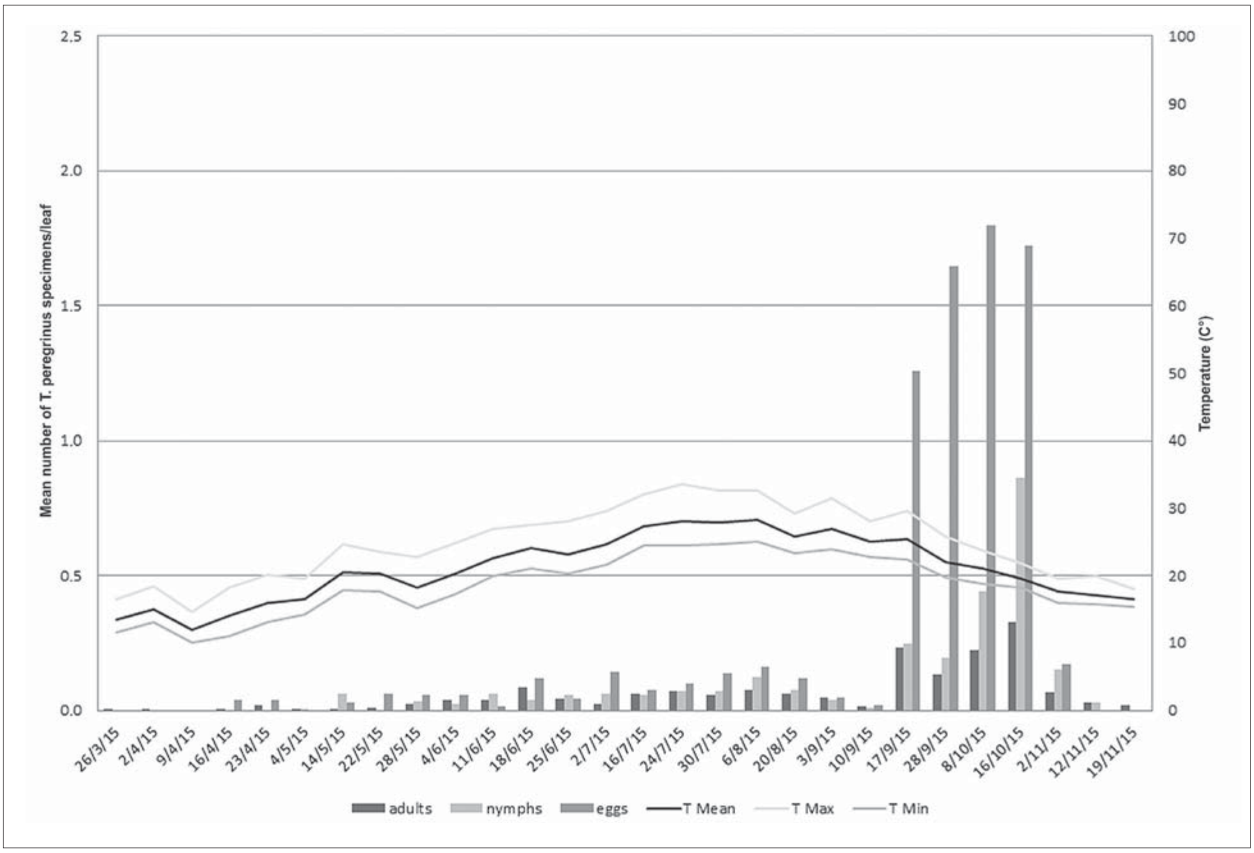

Fig. II – Mean values of *T. peregrinus* specimens per sampled leaf in Italy related to temperature (°C).

no attacks against potential hosts outside of this family have been recorded. This specificity allowed the release of *C. noackae* as a biological control agent of the Bronze bug in Chile, Brazil and South Africa (Nyeko, 2011; Barbosa *et al.*, 2017). So far, data on effectiveness of these realized biological control programs are not yet available in the literature.

The following two gall wasps (Hymenoptera: Eulophidae) in Campania may coexist without signs of interspecific competition since they occupy different ecological niches.

Ophelimus maskelli (Ashmead) has been the first eulophid gall-wasp recorded in Campania region during 2001 and misidentified as *O. eucalypti* (Gahan) (Viggiani and Nicotina, 2001) up to Protasov *et al.* (2007a). The wasp reproduces by thelytokous parthenogenesis and induces numerous small pimple-like, greenish-yellow to reddish, nearly round galls as the result of the oviposition and development of the larval stages (Raman and Withers, 2003; Laudonia and Viggiani, 2004). The galls are visible on both sides of the red gum tree leaves. The wasp attacks mainly the lower canopy developing three generations per year with a clear tendency to oviposit in developed, immature leaves. *O. maskelli* causes severe injury on *E. camaldulensis* determining dramatic leaf dieback and premature phylloptosis in few years both in urban and forest environments.

The harmfulness of this species, widespread in the Mediterranean basin, induced the organization of a classical biological control program on a large scale. Italy, too, was involved in this BC program. During 2006 the main parasitoid of the gall wasp, the eulophid *Closterocerus chamaeleon* (Girault), which was reared and distributed in Israel first (Mendel *et al.*, 2007), was introduced in Campania and in the southernmost regions of the country (Sasso *et al.*, 2008; Caleca *et al.*, 2011).

The highly efficient parasitoid species established in few weeks in all release sites and spread over a wide area in the same year, with recorded parasitization rates up to 60% (Sasso *et al.*, 2008). The continuous and long-distance dispersal mechanisms typical of *C. chameleon*, which was assessed in Italy, enhanced its effectiveness as a biological control agent (Caleca *et al.*, 2011). The success in the whole Mediterranean basin of this classical BC program realized against *O. maskelli* is one of the most powerful applications of this low impact control method. In about three years, populations of the harmful gall wasp have been significantly reduced by *C. chamaeleon* with positive impact for *E. camaldulensis* in all its growing areas (Mendel *et al.*, 2017). The introduction of a second parasitoid, belonging to the genus *Stethinium* (Chalcidoidea: Mymaridae) has been realized in the same year (2006) but without success (Sasso *et al.*, 2008). The present status of *O. maskelli* on *E. camaldulensis* in Campania has been reduced to an occasional secondary pest.

A second species of eulophid gall wasp infesting *E. camaldulensis* was recorded in Campania in the same year of *O. maskelli* (2001) and considered as a new species belonging to the genus *Aprostocetus* (Viggiani *et al.*, 2001). However, later the wasp was described as a new genus and species native to Australia and named *Leptocybe invasa* Fisher et La Salle (Mendel *et al.*, 2004). Rapid and harmful pest invasions are already widely-known, but *L. invasa* manifested an uncommon high spreading effectiveness and speed during its world invasion. Thanks to its high ecological plasticity, the blue-gum chalcid in 15 years has invaded the five continents spreading to about 40 countries and becoming a serious pest for eucalypt plantations in the world (Nugnes *et al.*, 2015; Mendel *et al.*, 2004).

L. invasa attacks tissues both of the stems and the leaf main nerve and petioles of new shoots where it lays eggs inducing the formation of typical bump-shaped galls (Fig. I, 6-7). The gall development eventually results in leaf-curling and premature aging of the leaves and severe attacks might cause death of juvenile shoots and leaves fall, resulting in stunted growth and tree weakening (Mendel *et al.*, 2004).

In addition to its ecological plasticity, the blue-gum chalcid has been facilitated in spreading by its particular reproductive modality. *Rickettsia* symbiont in fact is the causal agent of thelytokous parthenogenesis in *L. invasa* (Nugnes *et al.*, 2015) allowing a few females (Fig. I, 8) to infest a new territory in a very short time.

Recent integrative characterization highlighted that the population of *L. invasa* and its own *Rickettsia* symbiont recovered in Campania share the same molecular sequences with other populations recovered all over the Mediterranean region and South America. This population has a female biased sex-ratio and is characterized by rare occurrences of males.

Since the first finding in Campania, *L. invasa* has built up dense populations and spread all over the region territory. This behaviour was clearly in contrast to the small populations found in the native country, where *L. invasa* is limited by the presence of indigenous natural enemies (Kim *et al.*, 2008; Nugnes *et al.*, 2016; Mendel *et al.,* 2017).

In native and invaded countries, from *L. invasa* galls, were sporadically reared *Megastigmus* spp. (Torymidae), *Aprostocetus* sp. (Eulophidae: Tetrastichinae), *Parallelaptera* sp. (Mymaridae), and *Telenomus* sp. (Platygasteridae) (Viggiani *et al.*, 2001b; Protasov *et al.*, 2008; Doğanlar and Hassan, 2010; Vastrad *et al.*, 2010; Doğanlar *et al.*, 2013; Sangtongpraow and Charernsom, 2013) but their effectiveness in the control of blue-gum chalcid has not been already ascertained. However, the most effective natural enemies resulted to be the Tetrastichinae wasps *Selitrichodes krycery* Kim et La Salle (Kim *et al.*, 2008), *S. neseri* Kelly et La Salle (Kelly *et al.*, 2012), and *Quadrastichus mendeli* Kim et La Salle (Kim *et al.*, 2008) (Fig. I, 9).

Starting from 2007, *Q. mendeli* and *S. krycery* were released in Israel in BC program against blue-gum chalcid. Although *Q. mendeli* was never officially released in Italy, since 2013 it was unexpectedly collected for the first time in Campania and in following years also in neighbouring regions of Central and Southern Italy (Nugnes *et al.*, 2016). In Campania, samplings carried out on *L. invasa* infested trees showed a parasitization rate by *Q. mendeli* ranging from 27.3% to 100%.

Since its first finding in Campania, the activity of the parasitoid became stronger year after year and decisive for the containment of the blue-gum chalcid. *L. invasa* galls have almost completely disappeared, in several sampled places (Nugnes *et al.*, 2016). Likely, the short life-cycle of *Q. mendeli* [usually 30 days from egg to adults (Kim *et sal.*, 2008)] and its thelytokous reproduction, mediated by *Rickettsia* symbiont (Gualtieri *et al.*, 2017), have a synergistic effect on its effectiveness in the control of *L. invasa*.

In conclusion, field investigations carried out in recent years report that only few insect species can be considered real pests of *Eucalyptus* in Campania. While the impact of the gall-wasps has been strongly reduced by the spread of specific natural enemies, two sap-suckers, the lerp psyllid *G. brimblecombei* and the bronze bug *T. peregrinus*, are still able to develop harmful populations mainly in urban environment due to incomplete or lack of natural control. Further surveys can lead to a more exhaustive frame of the pest status of the bronze bug on *Eucalyptus* species, which may be essential to the development of control tools and strategies to reduce the harmfulness of this pest.

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A REVIEW ON INTRODUCED ALIEN INSECT PESTS AND THEIR ASSOCIATED PARASITOIDS ON *EUCALYPTUS* TREES IN SICILY ([1])

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Suma P., Nucifora S., Caleca V., Lo Verde G., Tortorici F., Rapisarda C., Bella S. – A review on introduced alien insect pests and their associated parasitoids on *Eucalyptus* trees in Sicily.

A review is reported in the present paper on invasive alien insects introduced in Sicily on *Eucalyptus* trees, together with unpublished results from recent surveys. As to the latter ones, observations were conducted especially on *Thaumastocoris peregrinus* (Carpintero & Dellapé) (Hemiptera, Thaumastocoridae), the most recently introduced species. Overall, eight alien insect pests have been accidentally introduced in the island on *Eucalyptus*, belonging to the orders Hemiptera (Aphalaridae, 2 spp.; Thaumastocoridae, 1 sp.), Coleoptera (Cerambycidae, 2 spp.; Curculionidae, 1 sp.) and Hymenoptera (Eulophidae, 2 spp.). Two encyrtid parasitoids, *Avetianella longoi* Siscaro and *Psyllaephagus bliteus* Riek, obtained from *Phoracantha* spp. and *Glycaspis brimblecombei* Moore respectively, and *Closterocerus chamaeleon* (Girault) (Hymenoptera, Eulophidae) attacking *Ophelimus maskelli* (Ashmead), are also reported. Details on current distribution, host plants, morphological and biological remarks are given for each species.

KEY WORDS: Alien insects; eucalypts; South Italy; psyllids; gall wasps; bronze bug.

INTRODUCTION

Eucalyptus trees are the most widely planted hardwood trees in the world, representing one of the main sources of biomass (TEULIERES & MARQUE, 2007). Native to the Australian continent, these species are widely diffused in the whole Mediterranean region not only as ornamental but also as timber trees. According to the Regional Forest Agency of the Sicily administrative region (South Italy), eucalyptus trees cover about 35,000 ha in this island (SAPORITO, 2006).

Despite continuous efforts to limit the accidental introduction of exotic plant and animal pests, a few alien insects feeding on *Eucalyptus* have been detected in Sicily so far. Although for most of them the mode of introduction has not been established, from the analysis of the different temporal and spatial patterns it is possible to assume that their occurrence is mainly linked with the increased trade of these plant species. In detail, eight species of insect pests have been recorded to infest eucalyptus trees in Sicily since mid of the 70's (Tab. 1); three of them belong to the order Hemiptera, three to the order Coleoptera and two are Hymenoptera. Some notes about their presence in Sicily are reported herein, pointing out their impact on the host plant.

HEMIPTERA

Among insects, the order Hemiptera account for many of the economically significant eucalyptus pests, damaging plants by feeding on phloem sap. Psyllids and true bugs are worth to be mentioned here within this insect group.

FAMILY APHALARIDAE

Plants of the genus *Eucalyptus* L'Hér. are known to host more than 250 species of Psylloidea worldwide, of which the greatest part in the family Aphalaridae and a few remaining ones in the Triozidae and Psyllidae (OUVRARD, 2017). Almost all of these species are native to Australia; some of them have been following the artificial diffusion of their host plants over the last decades and have been consequently spread all over the world, occasionally becoming invasive pests in *Eucalyptus* production areas of many continents (BURCKHARDT *et al.*, 1999).

In Italy, five species of psyllids have been recorded till now on *Eucalyptus*, all of them belonging to the family Aphalaridae. The first report dates back to about 35 years ago and concerns *Ctenarytaina eucalypti* (Maskell) (CAVALCASELLE, 1982), which remained for almost 20 years the only species known to feed on *Eucalyptus* spp. in Italy. Recently, over the last fifteen years, four other species have been reported almost at the average rate of one every four years: *Ctenarytaina spatulata* Taylor (COSTANZI *et al.*, 2003), *Blastopsylla occidentalis* Taylor (LAUDONIA, 2006), *Ctenarytaina peregrina* Hodkinson (MIFSUD *et al.*, 2010, who report unpublished data by

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Tab. 1 – Alien insects detected in Sicily on Eucalyptus trees during the last 40 years.

PESTS	ITALY	SICILY
Phoracantha semipunctata (F.) (Coleoptera: Cerambycidae)	Tassi, 1969	Romano & Carapezza, 1975
Ctenarytaina eucalypti (Maskell) (Hemiptera: Aphalaridae)	Cavalcaselle, 1982	Rapisarda, 1998
Leptocybe invasa Fisher & La Salle (Hymenoptera: Eulophidae)	Viggiani *et al.*, 2002	Bella & Lo Verde, 2002
Ophelimus maskelli (Ashmead) (Hymenoptera: Eulophidae)	Arzone & Alma, 2000	Bella & Lo Verde, 2002
Phoracantha recurva Newman (Coleoptera: Cerambycidae)	Sama & Bocchini, 2003	Mazzeo & Siscaro, 2007
Glycaspis brimblecombei Moore (Hemiptera: Aphalaridae)	Laudonia & Garonna, 2010	Lo Verde *et al.,* 2011
Thaumastocoris peregrinus Carpintero & Dellapé (Hemiptera: Thaumastocoridae)	Laudonia & Sasso, 2012	Suma *et al.*, 2014
Gonipterus scutellatus Gyllenhal (Coleoptera: Curculionidae)	Arzone, 1976; Sampò, 1976	Mazza *et al.*, 2015
PARASITOIDS	**HOST**	
Avetianella longoi Siscaro (Hymenoptera: Encyrtidae)	*P. semipunctata*	Siscaro, 1992
Closterocerus chamaeleon (Girault) (Hymenoptera: Eulophidae)	*O. maskelli*	Rizzo *et al.*, 2006
Psyllaephagus bliteus Riek (Hymenoptera: Encyrtidae)	*G. brimblecombei*	Caleca *et al.*, 2011*a*

Cocquempot & Costanzi) and *Glycaspis brimblecombei* Moore (Laudonia & Garonna, 2010).

Apart from its native areas in Australia, the "blue gum psyllid", *C. eucalypti*, occurs also in New Zealand, North and South America, Papua New Guinea, South Africa, Sri Lanka and is widely diffused in Europe (Conci *et al.*, 1996; Burckhardt, 1998; Rapisarda, 1998; Hollis, 2004; De Queiroz Santana & Burckhardt, 2007; Mifsud *et al.*, 2010; Burckhardt & Queiroz, 2012; Ouvrard *et al.*, 2015). Among the *Eucalyptus*-feeding psyllids, it is the one known for the longest time as pest outside its native regions. It is efficiently controlled by the parasitoid *Psyllaephagus pilosus* Noyes (Hymenoptera: Encyrtidae), which is successfully used in biocontrol programs of the psyllid (Hodkinson, 1999; Chauzat *et al.*, 2002).

An almost similar but rather limited geographical distribution is shown by *C. spatulata*, which is recorded from New Zealand, North and South America, Europe, in addition to its native areas of Australia (Brennan *et al.*, 1999; Costanzi *et al.*, 2003; Hollis, 2004; Valente *et al.*, 2004; De Queiroz Santana & Burckhardt, 2007; Bouvet & Burckhardt, 2008; Mifsud *et al.*, 2010; O'Connor & Malumphy, 2011; Burckhardt & Queiroz, 2012; Greenslade, 2016). In Italy, no records of this species are available till now out from the North-Western region Liguria, thus confirming its limited dissemination capacity and the rather secondary pest role that this species plays on *Eucalyptus* plantations throughout its distribution area. Unlike the previous two species, the eucalypt shoot psyllid *B. occidentalis* is recorded also from Africa, Middle East, Hawaii Islands and China (Hollis, 2004; Aytar, 2007; De Queiroz Santana & Burckhardt, 2007; Dzokou *et al.*, 2009; Spodek *et al.*, 2015), apart from Australia, New Zealand, America, and Europe (Halbert *et al.*, 2001; Bouvet *et al.*, 2005; Pérez-Otero *et al.*, 2011; Martinez *et al.*, 2014; Ouvrard, 2017). Such as all other species of the genus *Blastopsylla* Taylor, this psyllid feed on young leaves and growing tips of the host plants, producing a copious amount of flocculent wax that allows the insect presence to be easily detected on infested trees. *C. peregrina,* known till now to live only on *Eucalyptus parvula* L.A.S. Johnson & K.D. Hill, has been reported only from Great Britain and Ireland, where it has been described by Hodkinson (2007), and also (though almost informally and with no geographical specification) from France and Italy (Mifsud *et al.*, 2010); compared to the previous species, it seems to be still poorly known and in need of further investigation aimed at better understanding its geographical origin and distribution.

The last arrived, the red gum lerp psyllid *G. brimblecombei*, is the only *Eucalyptus*-feeding species presently occurring in Italy whose nymphs form the so called "lerp", a sort of characteristic shield-like conical white waxy covering, probably used by the nymphs for protection against natural enemies. It has a wide diffusion in many continents: Australia, Madagascar, Mauritius, North and South America, South Africa (Ouvrard, 2017). In Europe, it first appeared in the Iberian Peninsula about ten years ago (Hurtado & Reina, 2008; Valente & Hodkinson, 2009) and very rapidly (and almost contemporaneously) it has been recorded during the following years in Italy (Laudonia & Garonna, 2010) and in other countries of the Mediterranean Basin (Bella, 2013; Bella & Rapisarda, 2013; Malumphy *et al.*, 2013; Reguia & Peris-Felipo, 2013; Bella, 2014; Ben Attia & Rapisarda, 2014; Dhahri *et al.*, 2014). The first findings of this species in Sicily and the circum-Sicilian islands date back to spring 2011 (Lo Verde *et al.*, 2011). A fast mapping of the psyllid's rapid spread in this territory has been easily allowed by the visibility of its infestations, due to the presence of the mentioned white lerps. These follicles gave the possibility to ascertain how in a few months, and already in the summer of 2011, the psyllid was widely present in all Sicilian provinces, allowing also to hypothesize an important role played by the passive component in its dissemination.

Family Thaumastocoridae

Another exotic sap-sucking insect pest feeding on eucalyptus leaves was recently intercepted in Sicily, namely *Thaumastocoris peregrinus* (Carpintero & Dellapé) (Hemiptera: Thaumastocoridae). This species, native to Australia like its host plant, quickly invaded new areas in about ten years (Noack & Rose, 2007; Noack *et al.*, 2009) and it is by now considered a serious pest in Australia, South Africa, South America and New Zealand (Jacobs &

Neser, 2005; Noack & Coviella, 2006; Martinez-Crosa, 2008; Wilcken *et al.*, 2010; Giliomee, 2011; Ide *et al.*, 2011; Soliman *et al.*, 2012; Sopow *et al.*, 2012). In late 2011, it was recorded for the first time in Europe (Laudonia & Sasso, 2012) infesting several species of *Eucalyptus* in Italy (Latium region); later on, it was reported also from Portugal (Garcia *et al.*, 2013) and, in winter 2014, from Sicily (Suma *et al.*, 2014). The leaf damage is due to the bug feeding, that induces silvering chlorosis followed by the bronzing and drying of leaves. Severe infestations lead to leaves drop and severe canopy thinning, up to the branch dieback. Since its discovery in Sicily and in order to estimate its diffusion in the region, an accurate monitoring activity has been carried out. Currently, its presence is well documented in three main areas of this region: two in the East, in an urban area of the town of Catania and in the coastal zone of the Messina province, and one in the West side of the island, in a suburban area of the town of Palermo. Contrariwise, it has not been detected in the inland till now. In each prospected site, and as an "active collecting method" (*sensu* Grootaert *et al.*, 2010), an average of 10 *E. camaldulensis* trees were sampled from March 2014 to April 2015, collecting 10 twigs/tree, approximately 40 cm in length, that were subsequently examined in the lab under the microscope. From late June until January, all the bug instars were simultaneously present along the twigs and especially on the leaves from the previous years (i.e. not in the younger ones); mature copulating adults were often observed. During our survey, the infestation by the bronze bug was often recorded in association with that one by the red gum lerp psyllid *G. brimblecombei* (Fig. I). Clusters of black egg capsules of the bronze bug are often laid in the proximity of the leaf depressions (e.g. those associated with the infestations by the gall wasp *Leptocybe invasa* Fischer & La Salle) and singly, between the valves of *E. camaldulensis* fruits (Figs II and III). The infested trees showed only a slight discoloration of leaves caused by the feeding activity of *T. peregrinus* (Fig. IV) and, considering that the same plantations were regularly monitored from 2009 in the

Fig I – Adults of *T. peregrinus* on leaf of *E. camaldulensis* in association with the red gum lerp psyllid *G. brimblecombei.*

Fig II – Clusters of black egg capsules of *T. peregrinus* laid in proximity of the leaf depressions caused by the gall wasp *L. invasa.*

Fig III – Egg capsules of *T. peregrinus* laid between the valves of *E. camaldulensis* fruits.

Fig IV – Effect of the feeding activity of *T. peregrinus* on the leaves of *E. camaldulensis***.**

framework of the National Research Programme "GEISCA", it is highly assumable that the bug just arrived at that time. During the same prospecting period, yellow sticky traps (15x15 cm), as "passive collecting method", were also installed in the main infested areas at approximately 2.00 m height, in order to investigate the usefulness of this tool within a monitoring activity. Starting from late May, it was possible to detect adults of the bug stuck on the traps, whereas juvenile instars, although at a lesser quantity, were always detected starting from late June.

COLEOPTERA

Among this Insects order, both longhorn beetles and weevils (respectively living on wood and as defoliators) have been introduced in Sicily during recent years.

FAMILY CERAMBYCIDAE

The first alien insect species recorded on eucalyptus trees in Sicily is the wood-borer beetle *Phoracantha semipunctata* (Fabricius), a longhorn beetle whose spread was noticed in many Sicilian eucalyptus woods by the mid of the 70' (ROMANO & CARAPEZZA, 1975). It was first introduced from Australia into South Africa in 1890s, in wooden railway sleepers, and in 1917 it colonized eucalyptus plantations in Argentina, from where it arrived later in Uruguay, Chile and Peru. Probably during the Second World War, it reached some Mediterranean countries (e.g. Israel, Lebanon, Egypt, Tunisia, etc.) and its first findings in Europe occurred in Sardinia (TASSI, 1969; PIRAS *et al.*, 1970). During the 80's it was reported from Portugal, Spain, South France and Corsica (CADAHIA, 1980; CADAHIA & RUPEREZ, 1980; OROUSSET, 1984).

The congeneric species, *P. recurva* Newman, native to Australia and Papua - New Guinea (WANG, 1995), was first recorded in Mediterranean Basin in a Spanish territory of the north-western Maghreb (i.e. Ceuta) (RUIZ & BARRANCO, 1998); in Italy, it was then reported in 2003 (SAMA & BOCCHINI, 2003), even if the correct identification of specimens of *P. recurva* collected in Calabria and misidentified as *P. semipunctata* allowed to ascertain the occurrence of the species from 1992 (PALMERI & CAMPOLO, 2006). More recently, *P. recurva* was recorded also in Sardinia (CILLO *et al.*, 2006) and Sicily (MAZZEO & SISCARO, 2007; ROMANO, 2007).

Although the two *Phoracantha* Newman species share the same ecological niche and have an almost similar morphology, they can be differentiated based on the stains pattern on the elytrae (mostly cream to yellowish in *P. recurva*, with dark brown areas primarily limited to the rear third of the elytra, but dark brown in *P. semipunctata*, with a cream area in the middle), the chaetotaxy of antennal segments (which have dense setae in *P. recurva* adults but only a few or none in *P. semipunctata*), and the dense spines which occur on the front dorsal side of hind femora in *P. recurva* (WANG, 1995; PALMERI & CAMPOLO, 2006). *P. semipunctata* larvae have temples with a broad ferruginous cuneiform band behind the base of antennae and a uniform setose area, with distinctly central setae at each median lobe, at the 10th abdominal tergite; in *P. recurva*, the cuneiform band is absent and the 10th abdominal tergite has only sparse hairs and a pair of long setae on the median lobe.

The biology of the two *Phoracantha* species is subtly different. In California, four years after its introduction, *P. recurva* has largely replaced *P. semipunctata*, increasing from 0.1% of the total *Phoracanta* spp. population in 1995 to 74% in 1997 (PAINE *et al.*, 2000). Adults of these species attack large stems and branches that are dying or have died recently; attacks may also occur on living *Eucalyptus* trees, especially when they are stressed by drought, or on freshly cut logs. The life cycle is annual but can also involve two partially overlapping generations. Adults emerge in early spring and after mating, females lay their egg under loose bark or in bark crevices or branch stubs (WANG, 1995; PAINE *et al.*, 2000). Eggs are laid in clusters of 10-40 each and hatch in 6 to 15 days, depending on the ambient temperature and the larvae bore through or under the bark and mine along the cambium of the attacked trees. At maturity, they construct a vertical pupal chamber into the heartwood and an exit hole that is plugged with frass. Adults live for up to 3 months, they are nocturnal and, during the day they hide under the bark. Yellowing of the crown, apical dieback, resination, debarking and production of oval emergence holes, which appear through the bark, are the main symptoms of the attack by the two longhorn beetles. The egg parasitoid *Avetianella longoi* Siscaro (Hymenoptera: Encyrtidae), native to Australia, is the most important parasitic wasp used to control the two species of *Phoracantha*, and this is up to now the sole natural enemy of these beetles recorded in Sicily. However, results obtained in California and South Africa, indicate that the parasitoid is less effective against *P. recurva* than *P. semipunctata* (PAINE *et al.*, 2000; TRIBE 2003).

FAMILY CURCULIONIDAE

The third coleopteran species recorded on eucalyptus trees in Sicily is the snout beetle *Gonipterus scutellatus* Gyllenhal. It is a species native to Australia, which feeds on a wide range of host trees, mainly belonging to the genus *Eucalyptus*, and the ornamental ones are considered to be most at risk (CADAHIA, 1986). It is an important defoliator of eucalyptus in several parts of the world but, fortunately, biological control by using the egg parasitoid *Anaphes nitens* (Girault) (Hymenoptera: Mymaridae), claimed in France, Italy and Spain, had successfully reduced its attacks (CADAHIA, 1986). Eggs of *G. scutellatus* are laid attached to the leaves, on both surfaces, in greyish capsules containing about 8-10 eggs each. The larvae, yellowish-green with black marks, are more easily distinguished visually than the adults. The latter ones are grey-brown weevil, with a light, transverse band on each elytra, not readily discernible visually from *G. gibberus* Boisduval, though distinctive details of the genitalia can allow their discrimination (ROSADO-NETO & MARQUES, 1996). Although this weevil does not seem to be a threat for the Sicilian eucalyptus trees, some infested individuals of *E. globulus* were recently recorded in the province of Catania, where defoliations probably caused by this insect have been visible since 2012 (MAZZA *et al.*, 2015). Signs of its presence on infested trees can be detected from May to September, when it is possible to observe the damaged leaves as well as the egg capsules and larvae of the insect. These last ones can only eat on the leaf surface, leaving characteristic tracks, while adults chew the leave edges giving them a ragged, scalloped appearance.

HYMENOPTERA

FAMILY EULOPHIDAE

Two *Eucalyptus* gall wasps, *Leptocybe invasa* Fisher & La Salle and *Ophelimus maskelli* (Ashmead), are currently spread in several countries of the Mediterranean Basin and were detected on eucalypts in Sicily, about fifteen years ago (Bella & Lo Verde, 2002). They have been reported also from Africa, Asia and North America (Mendel *et al.*, 2017), whereas *L. invasa* distribution includes also South America (Costa *et al.*, 2008; Aquino *et al.*, 2011). Recently, molecular analyses suggested that *L. invasa* is a complex of two cryptic species (Nugnes *et al.*, 2015): the first one being recovered in the Mediterranean region and South America, the second from China (Mendel *et al.*, 2017).

Ophelimus maskelli galls cause serious defoliation to *E. camaldulensis* both on adult trees and on young plantations, whose growth rate is negatively affected (Mendel *et al.*, 2004; Protasov *et al.*, 2007). *Leptocybe invasa* induces plurilocular galls on the stems, shoots, petioles and mid-rib of the leaves, high infestations can cause heavy defoliation (Mendel *et al.*, 2004; Karunaratne *et al.*, 2010).

Several studies have shown how both gall wasps currently appear to be successfully controlled by various biological control agents, especially hymenopteran parasitoids. Among them, three species were in fact released in Israel for the biological control of *O. maskelli*: *Closterocerus chamaeleon* (Girault) (Eulophidae), *Stethynium ophelimi* Huber and *S. breviovipositor* Huber (Mymaridae) (Mendel *et al.*, 2007) and the first one was also released in Italy (Laudonia *et al.*, 2006; Rizzo *et al.*, 2006, 2015). As regards *L. invasa* biological control, four species of parasitoids were released in Israel as well: *Megastigmus zvimendeli* Doĝanlar, *M. lawsoni* Doĝanlar (Torymidae), *Selitrichodes kryceri* Kim & La Salle and *Quadrastichus mendeli* Kim & La Salle (Eulophidae) (Protasov *et al.*, 2008) but no information is available till now about their presence in Sicily.

DISCUSSION

In general, no substantial damage has been inflicted so far to eucalyptus trees by the insect pests recorded in Sicily. Certainly, some of them require constant supervision and monitoring in order to avoid they become exceedingly injurious. In particular, over their whole distribution area, all five psyllids recovered in Italy till now are oligophagous on various plant species of the genus *Eucalyptus*. Unlike what has been reported in other areas of the world, in most cases attacks by *G. brimblecombei* in Sicily have not produced serious problems to infested plants, apart from the aesthetic damage and some dieback; especially, no obvious signs of decay have been noted. By sure, the relatively low impact of this alien psyllid on Sicilian *Eucalyptus* plantations is largely linked to the spontaneous introduction and naturalization of its parasitoid, *P. bliteus*, found in almost all Sicilian sites where the psyllid has been detected at only a very short (or none) time distance from the first records of the pest (Caleca *et al.*, 2011a; Margiotta *et al.*, 2017). In this context, the importance shown by this parasitoid during the last years in reducing infestations by the psyllid in Sicily has stimulated a study on the effects of some ecological parameters (especially altitude and climate) on the distribution and density of the encyrtid wasp and on its parasitic efficacy (Caleca *et al.*, 2018).

As regards to the presence of the bronze bug in Sicily, it seems to be premature to claim about its harmfulness for the host plant. Up to now, no severe infestations were recorded in the main surveyed areas of the island and the fortuitous introduction and successful establishment in Sicily of its main biocontrol agent, the ooparasitoid *Cleruchoides noackae* Lin & Huber (Hymenoptera: Mymaridae), would be highly desirable. Interestingly, although deeper investigations are still in progress, by comparing data obtained from both the monitoring methods adopted, it is assumable that sticky traps have the potential to satisfactorily estimate the bug populations and thus they can be effectively employed as a time-efficient method for sampling *T. peregrinus* infestations.

About the wood-boring beetle species, although both *P. recurva* and *P. semipunctata* have been included by Wang (1995) among the *Phoracantha* species living on dead or declining trees, *P. recurva* has been found abundant also on living plants in Spain (Bercedo Paramo & Bahillo de la Puebla, 1999), showing how this species could have a huge impact on its host plant health. It is important to highlight as in some Mediterranean countries (Italy and Portugal) the oophagous encyrtid parasitoid *A. longoi* was detected in association with *P. recurva* and its presence seems to play an important role in maintaining beetle populations stable (Longo *et al.*, 1993).

Finally, in relation to the two *Eucalyptus* gall wasps, recent studies carried out after the release of the parasitoid *C. chamaeleon* in Sicily showed its high efficacy in reducing *O. maskelli* populations. Its establishment, spreading speed and the observed discontinuous pattern of dispersal showed the occurrence of both long and short distance dispersal mechanisms (Caleca *et al.*, 2011b).

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ENVIRONMENTAL FACTORS IMPACT AND INCIDENCE OF PARASITISM OF *PSYLLAEPHAGUS BLITEUS* RIEK (HYMENOPTERA ENCYRTIDAE) ON POPULATIONS OF *GLYCASPIS BRIMBLECOMBEI* MOORE (HEMIPTERA APHALARIDAE) IN MEDITERRANEAN CLIMATIC AREAS (1)

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Caleca V., Bella S., La Pergola A., Lombardo A., Lo Verde G., Maltese M., Nucifora S., Rizzo R., Tortorici F., Suma P., Rapisarda C. – Environmental factors impact and incidence of parasitism of *Psyllaephagus bliteus* Riek (Hymenoptera Encyrtidae) on populations of *Glycaspis brimblecombei* Moore (Hemiptera Aphalaridae) in Mediterranean climatic areas.

The red gum lerp psyllid, *Glycaspis brimblecombei* Moore (Hemiptera, Aphalaridae), is an Australian native sap-sucking insect pest of eucalypts that has been first reported for the West Palaearctic Region in 2008 and, in 2010, it has been found also in Italy. Subsequently its primary parasitoid, *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae), was also detected within the main European and North African infested areas, where no release of the parasitoid was ever performed. This study, carried out in 30 *Eucalyptus camaldulensis* plantations located along the coast, on the hills and the mountains in Mediterranean climatic areas of Sicily (Italy), aimed to determine the influence of environmental parameters on the incidence of both, the psyllid infestation level and the parasitization activity. *P. bliteus* reached highest average levels in summer samplings and resulted widespread in Sicily at all detected altitudes without statistically significant differences. *P. bliteus* parasitization is the main factor lowering *G. brimblecombei* infestation; this result, together with the accidental and contemporaneous arrival of the host and its parasitoid, could explain the absence of high damage level on eucalypts in Sicily. The most significant metric factors positively influencing *G. brimblecombei* infestation are the percentage of daily hours above 80% of relative humidity and the average maximum temperature, obviously related to other, but less significant climatic factors. The altitude affects both infestation and parasitization, but single sites could explain significantly more, so that the local conditions where the samplings were carried out have to be considered as the main responsibles for the variability in the obtained results. In any sampled Sicilian site, from sea level to 540 m a.s.l., both the psyllid and its parasitoids show a good adaptation to climatic conditions, confirming that areas fitting for *E. camaldulensis* growth fit also for *P. bliteus* activity, and proving that Mediterranean climate, differently from some inland areas of California, does not obstacle its parasitic activity.

KEY WORDS: Red gum lerp psyllid, Sicily, General Linear Model, Relative Humidity, Temperature.

INTRODUCTION

Glycaspis brimblecombei Moore (Hemiptera, Aphalaridae), commonly known as the red gum lerp psyllid, is an Australian native pest of *Eucalyptus* trees, which in the last years spread in many other regions.

It was detected outside Australia for the first time in California (USA), in 1998 (GILL, 1998; BRENNAN *et al.*, 1999); afterwards it was found in Hawaii (NAGAMINE and HEU, 2001), Central and South America (Mexico, CIBRIÁN *et al.,* 2001), Chile (SANDOVAL and ROTHMANN, 2002), Brazil (WICKEN *et al.*, 2003), Argentina (BOUVET *et al.*, 2005), Ecuador (ONORE and GARA, 2007), Venezuela (ROSALES *et al.*, 2008), Peru (BURCKHARDT *et al.*, 2008), El Salvador (JIMENEZ, 2013), Colombia (RODAS *et al.*, 2014) Uruguay (BALDINI *et al.*, 2006), Canary Islands (MALUMPHY, 2010), Morocco (BAMI, 2011, IBNELAZYZ, 2011), Algeria (REGUIA and PERIS-FELIPO, 2013), Tunisia (BEN ATTIA and RAPISARDA, 2014; DHAHRI *et al.*, 2014), Mauritius (SOOKAR *et al.*, 2013), and Madagascar (HOLLIS, 2004). From 2008 the psyllid was found in some European countries: Spain and Portugal (HURTADO and REINA, 2008;), France (COCQUEMPOT

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et al., 2012), Montenegro (MALUMPHY *et al.*, 2013), Greece (BELLA and RAPISARDA, 2013; REGUIA and PERIS-FELIPO, 2013). In Italy it has been recorded for the first time in Southern and Central regions in 2010 (LAUDONIA and GARONNA, 2010) and new information about pest spread were given by GARONNA *et al.* (2011) and PERIS-FELIPO *et al.* (2011). In 2011 it was recorded in Sicily (LO VERDE *et al.*, 2011), and few months after it was also detected in Sardinia (EPPO, 2011); now it is widespread in the whole country in areas where *Eucalyptus camaldulensis* Dehnh. is planted.

The host plant, like other species of the genus *Eucalyptus*, is very common in Italy as ornamental and forest species. As a consequence, serious damage was recorded on eucalyptus trees in parks, urban areas and plantations, and also the Italian beekeepers worried because of the dramatically lower quality and quantity of eucalyptus honey productions (GARONNA *et al.*, 2011; PIBIRI, 2011).

G. brimblecombei is a very aggressive exploiter of resources and for this reason its damage is important; outbreaks have been reported from many countries of South America were the species can cause the death of infested trees, resulting in serious timber production losses (DE QUEIROZ *et al.*, 2012).

The psyllid can be easily detected observing the presence on leaf surfaces of the characteristic white cones (lerps) produced by the nymphs which also serve as shelter until they reach adulthood (HALBERT *et al.*, 2001; BELLA, 2013). In California high population levels are reported to cause leaf fall, a decrease in growth rate, and in some cases death of the infested plant (GILL, 1998; DAHLSTEN *et al.,* 2005); eucalypt mortality rates can reach 15% in the first year of attack and up to 40% in the second year if efforts are not made to control the pest (GILL, 1998).

Biological control is a major component of psyllid IPM; nowadays the red gum lerp psyllid seems to be under control after the introduction of the parasitoid wasp *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae). In the native areas the endemic *Psyllaephagus* species attack nymphs of Psylloidea, and a few are reported as hyperparasitoids attacking other *Psyllaephagus* species (RIEK, 1962; NOYES and HANSON, 1996). *P. bliteus* parasitizes *G. brimblecombei* and other psyllids as: *Boreioglycaspis melaleucae* Moore on *Melaleuca quinquenervia* (Cav.) S.T. Blake (Myrtaceae); *Creiis costatus* (Froggatt), *Ctenarytaina eucalypti* (Maskell), *Glycaspis granulata* (Froggatt) and *Glycaspis* sp. on *Eucalyptus* spp. (RIEK, 1962; HERTING, 1972; WITHERS, 2001; DAHLSTEN *et al.,* 2002; DAANE *et al.*, 2005; BERRY, 2007). The first classical biological control attempt against *G. brimblecombei* was performed in California collecting *P. bliteus* from its native area (i.e. Australia), then releasing it from the end of 1999 to 2003 (PAINE *et al.,* 2000; DAHLSTEN *et al.*, 2005). *P. bliteus* has also been deliberately introduced for classical biological control against *G. brimblecombei* into Mexico (PLASCENCIA *et al.,* 2005) and Chile (IDE *et al.*, 2006).

The arrival of *P. bliteus* in Italy (Sicily: CALECA *et al.*, 2011a; Italian peninsula: LAUDONIA *et al.*, 2014) is due to an accidental introduction, probably together with its host, as also happened in New Zealand, Brazil, Spain, Morocco, Greece, Tunisia, Portugal and Turkey (WITHERS, 2001; BERTI-FILHO *et al.*, 2003; BERRY, 2007; PEREZ-OTERO *et al.*, 2011; BAMI, 2011; BELLA and RAPISARDA, 2013; BELLA, 2014; DHAHRI *et al.*, 2014; KARACA *et al.*, 2015).

In the framework of an IPM programme, the monitoring activity results highly important to determine the moment of pest population peak, the occurrence of natural enemies and to estimate other factors that can affect the population dynamic.

As it is known, the climatic factors can affect both the pest population density and parasitization level (SANTANA *et al.*, 2003b; BELLA and RAPISARDA, 2014; FERREIRA FILHO *et al.*, 2015).

Studies conducted in California showed that the pest population suppression by the encyrtids performed better in coastal sites than in inner arid areas, due to the higher summer temperatures herein recorded that reduced the efficacy of the parasitoid activity; still, as psyllid numbers have dropped, the defoliation and death of E*ucalyptus* trees due to the psyllid have been reduced (DAANE *et al.*, 2005, 2012). In Brazil, a decrease of *G. brimblecombei* population was recorded in the late spring and early summer in function of the rainfall frequency or intensity (DE QUEIROZ *et al.*, 2012).

In Portugal DHAHRI *et al.* (2014) report how the infestation levels by *G. brimblecombei* were not significantly affected by the different latitudes and longitudes of the studied sites recording also a relatively low parasitism rate.

Recently in Italy, in areas where *P. bliteus* was absent*,* it has been suggested that *G. brimblecombei* population size in the new area of colonization is negatively affected by low winter temperatures, but also by high temperatures in the absence of rainfall (LAUDONIA *et al.*, 2014).

In the present paper the distribution and activity of *G. brimblecombei* and *P. bliteus* in a Mediterranean area, *E. camaldulensis* plantations distributed in all Sicily, were studied in function of some environmental factors (i.e. altitude and climatic conditions) discussing their importance from an applied point of view.

MATERIALS AND METHODS

PRELIMINARY SAMPLINGS ON *P. BLITEUS* DISTRIBUTION IN SICILY

A first preliminary survey on the distribution of *P. bliteus* in Sicily was performed from December 2011 to February 2012, in order to ascertain the presence of the parasitoid in the whole island. Totally 20 sites have been investigated, most of which along the coast due to the cold period of sampling. Each sample consisted of leaves of *E. camaldulensis* bearing a total number of at least 100 fully developed lerps. Lerps were analyzed to detect the exit hole of the parasitoid. In the laboratory lerps without a hole were removed counting the number of underlying clearly parasitized hosts (mummies). Psyllid nymphs found on the leaves have been reared at room temperature (for about two weeks) until adults emerged.

SEASONAL SAMPLINGS

After this preliminary survey that ascertained the presence of *P. bliteus* also in eastern Sicily (Fig. I), widening its distribution in the western areas of the island already stated by CALECA *et al.* (2011a), data regarding *G. brimblecombei* infestation level and *P. bliteus* parasitization level have been collected in Sicilian *E. camaldulensis* plantations located in areas that fits with the good development of this tree. Thirty sampling sites were chosen from the coast to the interior zones at three altitudinal ranges, as following: 3-52 m a.s.l. (Coast), 101-356 m a.s.l. (Hill) and 418-542 m a.s.l. (Mountain), corresponding to an average yearly temperature in 1965-1994 of 18-19°C, 17-18°C and 16-17°C respectively (Fig. II, Tab. 1).

Seven samplings were performed in each site: two in spring 2012 (18-28 Apr.; 30 May-6 Jun.), two in the following summer (12-24 Jul.; 28 Aug.-6 Sep.), two in the following autumn (11-18 Oct.; 27 Nov.-19 Dec.) and the last one in winter 2013 (1-19 Feb.).

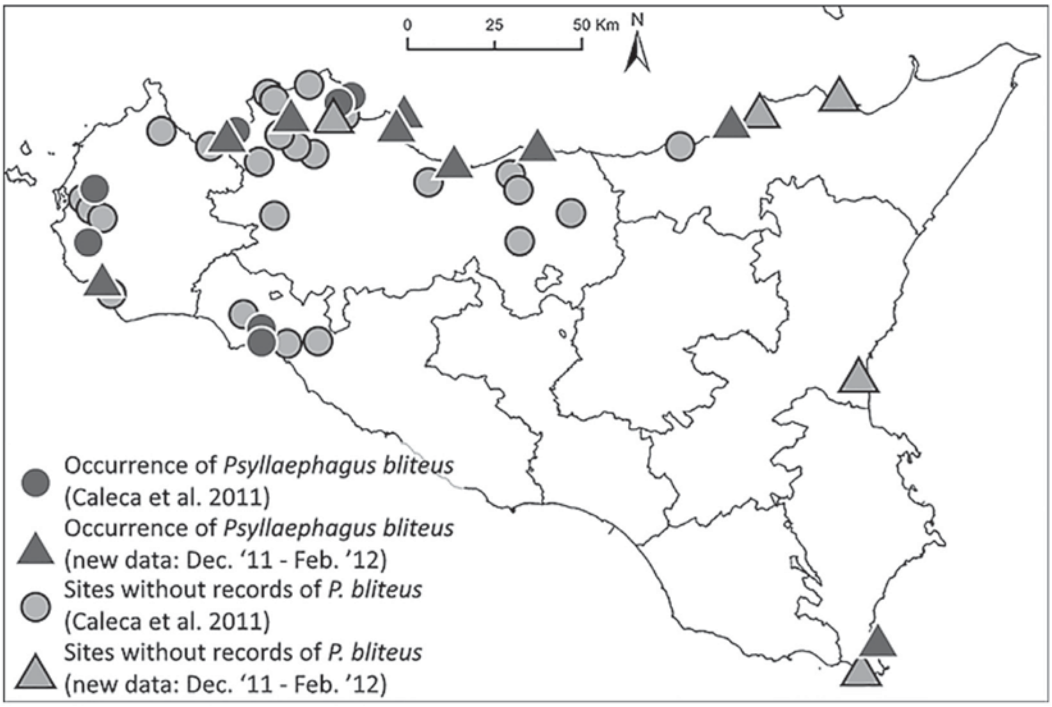


Fig. I – Occurrence of *Psyllaephagus bliteus* in Sicily, recorded from September 2011 to February 2012 (after CALECA *et al.*, 2011a, modified by addition of new data).

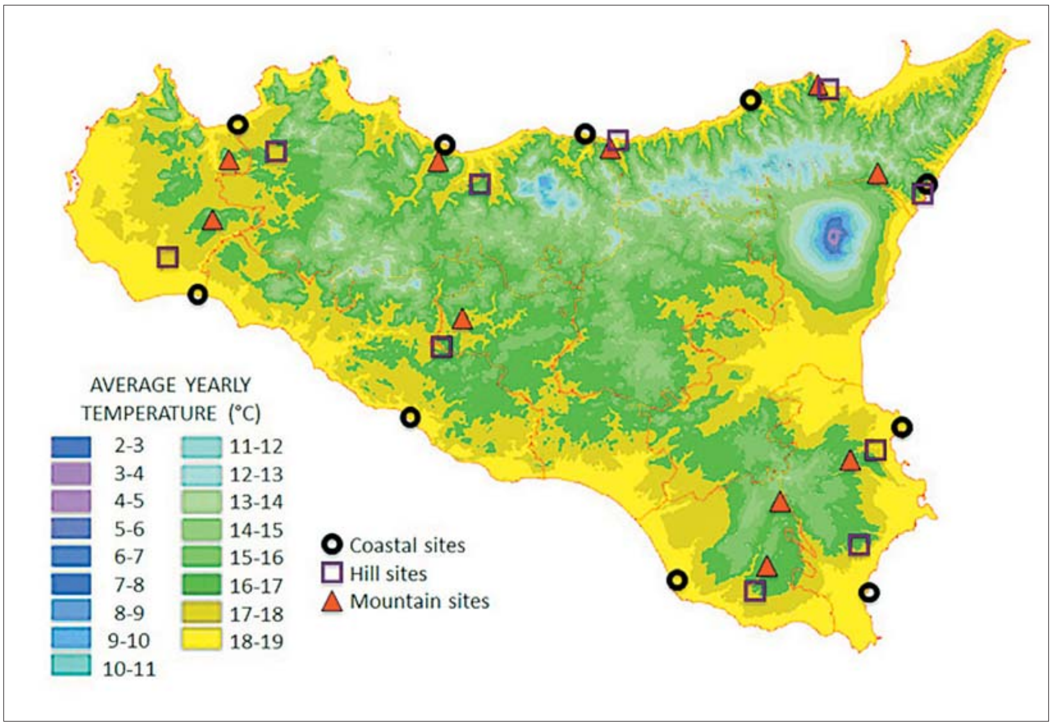


Fig. II – Average yearly air temperature in the period 1965-1994, based on data from the Sicilian Regional Hydrographic Service (after DRAGO, 2005), and localization of the 30 sampled sites.

At each sample date, in each site 20 leaves have been randomly collected from 5 trees, at man height in all four cardinal directions, reaching a total of 100 leaves. In this case the sampling time to collect 100 leaves has been set in 2 minutes. In order to have a larger set of data, if a total of 100 full mature lerps was not reached with the former sample, an additional sample was performed, collecting leaves bearing mature lerps directly from eucalyptus trees, recording the sampling time. In the laboratory the number of live 4th-5th instar nymphs, mummies and pierced mummies on each leaf has been counted. The final measure of the infestation was calculated as the number of live 4th-5th instar nymphs (including live mummies) collected per minute by one sampler in the field.

Regarding *P. bliteus* parasitization, all sampled leaves, after counting psyllid live instars, were put in glass jars,

Table 1 – List of all sampling sites, their latitude, longitude, altitude and cumulative rainfall (mm) recorded in 45 days preceding each sampling.

Site Nr.	Site name	Latitude N	Longitude E	Group of sites	Altitude (m a.s.l.)	Rainfall of 45 days preceding the sampling						
						Spring samples		Summer samples		Autumn samples		Winter sample
						1st	2nd	3rd	4th	5th	6th	7th
1	Augusta	37°14’36.35”	15°12’16.20”	Coast	3	111	13	0	48	19	139	31
2	Vendicari	36°48’15.70”	15°05’34.10”	Coast	5	61	2	1	0	22	101	75
3	Selinunte	37°35’11.26”	12°51’42.04”	Coast	11	55	18	5	7	115	194	125
4	Randello	36°50’25.00”	14°27’38.80”	Coast	15	113	3	0	0	119	137	74
5	Rocca di Caprileone	38°06’40.30”	14°42’33.46”	Coast	16	95	84	0	14	69	151	181
6	San Leone	37°16’08.92”	13°34’38.10”	Coast	17	82	5	0	15	59	166	81
7	Termini Imerese	37°59’19.10”	13°41’11.56”	Coast	29	50	16	1	24	49	151	169
8	Letojanni	37°52’55.25”	15°18’09.55”	Coast	30	107	32	6	9	38	145	48
9	Pollina	38°01’21.61”	14°09’11.77”	Coast	33	50	39	0	1	33	106	167
10	Balestrate	38° 02’25.80”	12°59’20.35”	Coast	52	104	28	6	12	81	159	262
11	Patti	38°08’47.34”	14°57’43.28”	Hill	101	69	38	0	6	46	84	236
12	Tusa bassa	38°00’08.56”	14°15’49.60”	Hill	125	40	25	0	21	30	111	162
13	Lago Trinità	37°41’08.33”	12°45’36.52”	Hill	168	79	27	4	13	90	202	169
14	Partinico	37°58’16.39”	13°07’04.59”	Hill	195	95	39	4	21	81	168	223
15	Scicli	36°48’45.00”	14°42’57.00”	Hill	200	142	1	0	6	92	131	104
16	Grotte	37°27’22.77”	13°40’43.54”	Hill	242	83	17	2	15	146	216	131
17	Taormina	37°51’42.00”	15°17’01.10”	Hill	250	No climatic datum						
18	Noto	36°55’55.00”	15°03’32.90”	Hill	320	155	12	0	6	52	130	104
19	Melilli	37°10’49.60”	15°06’49.60”	Hill	350	140	7	0	3	45	124	106
20	Cerda	37°53’13.63”	13°48’09.95”	Hill	356	57	27	2	19	53	212	227
21	Caccamo	37°57’02.70”	13°40’10.22”	Mountain	418	No climatic datum						
22	Francavilla di Sicilia	37°54’36.65”	15°07’42.60”	Mountain	425	178	92	0	12	53	122	131
23	Alcamo	37°56’45.46”	12°57’42.83”	Mountain	439	93	54	2	14	92	176	234
24	Sortino	37°09’35.80”	15°01’57.70”	Mountain	450	No climatic datum						
25	Modica	36°52’38.70”	14°45’12.70”	Mountain	460	No climatic datum						
26	Sorrentini	38°09’05.36”	14°56’20.77”	Mountain	478	74	64	0	16	78	176	334
27	Sutera	37°31’49.11”	13°44’42.34”	Mountain	501	135	30	2	11	47	116	147
28	S. Ninfa	37°47’05.12”	12°54’38.63”	Mountain	508	103	49	6	8	71	143	173
29	Giarratana	37°02’53.50”	14°47’58.00”	Mountain	520	309	13	0	38	83	76	105
30	Tusa	37°58’58.03”	14°14’20.21”	Mountain	542	63	42	0	27	16	114	179
Average coastal sites					21	83	24	2	13	60	145	121
Average hill sites					231	96	22	1	12	71	153	162
Average mountain sites					474	136	49	1	18	63	132	186

topped with a cotton cloth, to avoid excess of moisture, and maintained at room temperature for at least 15 days to allow the emergence of both adults of the psyllid and the parasitoid. Parasitism rate was calculated as ratio between emerged parasitoids and all emerged adults (parasitoids + psyllids).

The climatic data of each site refer to the ones deriving from the closest available fitting station of the Sicilian Agrometeorological Information Service (SIAS) of the Sicilian Region that kindly provided the following weather recordings: rainfall (total amount, number of events, intensity), relative humidity (daily percentage of RH>80% and of RH<40%), air temperature (daily maximum, minimum and average). Rainfall intensity rates refer to the classification by the World Meteorological Organization (WMO, 2014) for precipitation in the form of liquid water drops that have diameters greater than 0.5 mm:

– light rain, <0.41 mm/10 min;
– moderate rain, 0.41-1.6 mm/10 min;
– heavy rain, >1.6 mm/10 min.

These daily data have been grouped and referred to the 45 days before each sampling date. Climatic data were not available for four out of thirty sampling sites (one hill site and three mountain sites), because no SIAS station fits with them (Tabb. 1-2).

STATISTICAL ANALYSIS

Data were analysed using the General Linear Model, in which the response variable is "y" and the input variables are categorical and metric.

The first analysis regards *G. brimblecombei* infestation expressed as number of live 4th-5th nymphs collected per minute by one sampler (Inf/min). In order to apply the most common parametric statistics, the assumption of normality has to be satisfied. The original set of data was far from this distribution, but a simple transformation has been able to normalize data, y = log (1+Inf/min).

Input variables, categorical and metric:

- Sampling period, 1 (18-28 Apr. 2012), 2 (30 May-6 Jun. 2012), 3 (12-24 Jul. 2012), 4 (27 Aug.-6 Sep. 2012), 5 (11-18 Oct. 2012), 6 (27 Nov.-6 Dec. 2012), 7 (1-19 Feb. 2013).
- Site (see Tab. 1).
- Altitude, this metric variable has been used in alternative to Site.
- Parasitization (calculated as above described).
- Parasitization at the previous sampling.
- Light rain events (n) <0.41 mm/10 min, recorded in 45 days preceding the sampling.
- Moderate rain events (n) 0.41–1.6 mm/10 min, recorded in 45 days preceding the sampling.
- Heavy rain events (n) >1.6 mm/10 min, recorded in 45 days preceding the sampling.
- Total light rain (mm) <0.41 mm/10 min, recorded in 45 days preceding the sampling.
- Total moderate rain (mm) 0.41–1.6 mm/10 min, recorded in 45 days preceding the sampling.
- Total heavy rain (mm) >1.6 mm/10 min, recorded in 45 days preceding the sampling.
- Total rainfall (mm), recorded in 45 days preceding the sampling.
- Average maximum temperature of 45 days preceding the sampling.
- Average temperature of 45 days preceding the sampling.
- Average minimum temperature of 45 days preceding the sampling.
- Percentage of daily hours with Relative Humidity >80%, recorded in 45 days preceding the sampling.

Table 2 – Average air temperature (°C) recorded in 45 days preceding each sampling.

Site number	**Site name**	**Spring samples**		**Summer samples**		**Autumn samples**		**Winter sample**	**Average 1st-7th samples**
		1st	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**	
1	Augusta	12.7	17.1	25.5	26.7	23.5	17.9	10.5	19.1
2	Vendicari	14.0	17.9	24.5	27.4	24.7	19.2	12.4	20.0
3	Selinunte	13.0	17.3	25.2	26.1	21.7	17.7	11.3	18.9
4	Randello	15.2	18.1	24.9	27.7	24.0	18.6	11.6	20.0
5	Rocca di Caprileone	14.7	17.3	25.0	26.8	24.4	18.9	11.6	19.8
6	San Leone	13.4	17.3	24.6	26.0	22.9	18.3	11.5	19.2
7	Termini Imerese	14.2	18.2	24.5	25.4	22.7	14.5	10.4	18.6
8	Letojanni	12.9	16.4	23.2	26.4	22.8	17.7	11.6	18.7
9	Pollina	14.2	16.7	24.4	26.0	23.2	16.7	10.4	18.8
10	Balestrate	14.2	18.0	25.1	26.3	23.0	17.1	11.0	19.2
11	Patti	13.4	16.1	24.0	25.6	23.1	16.2	9.9	18.3
12	Tusa bassa	14.8	16.9	25.0	26.5	23.7	17.0	10.3	19.2
13	Lago Trinità	12.8	17.2	25.6	26.4	21.9	16.9	9.9	18.7
14	Partinico	13.4	17.5	24.2	25.1	21.9	15.2	9.7	18.2
15	Scicli	13.2	17.1	25.2	27.7	23.0	18.1	10.4	19.2
16	Grotte	11.7	18.2	27.4	27.9	22.7	16.2	9.0	19.0
18	Noto	12.4	17.1	26.5	27.7	23.5	18.1	10.2	19.4
19	Melilli	12.4	17.1	25.2	27.7	23.0	18.1	10.6	19.2
20	Cerda	13.1	18.0	24.2	25.2	22.4	13.7	9.4	18.0
22	Francavilla di Sicilia	12.1	15.9	24.0	26.9	22.4	13.9	9.4	17.8
23	Alcamo	11.3	15.3	24.7	24.8	20.5	13.2	7.6	16.8
26	Sorrentini	13.9	15.9	24.6	25.9	22.6	15.4	9.1	18.2
27	Sutera	10.6	15.1	24.4	24.9	20.0	14.6	7.4	16.7
28	S. Ninfa	11.9	16.0	25.7	26.0	21.7	16.3	8.8	18.1
29	Giarratana	11.7	15.4	24.2	26.7	21.6	15.1	8.0	17.5
30	Tusa	13.5	15.5	24.0	25.5	22.4	15.9	8.6	17.9
Average coastal sites		13.9	17.4	24.7	26.5	23.3	17.6	11.2	19.2
Average hill sites		13.0	17.3	25.3	26.7	22.8	16.6	9.9	18.8
Average mountain sites		12.2	15.6	24.5	25.8	21.6	14.9	8.4	17.6

- Percentage of daily hours with Relative Humidity <40%, recorded in 45 days preceding the sampling.

Output variable:
y= log (1+Inf/min)

In order to single out factors influencing *P. bliteus* parasitization, all variables have been taken into account, including the infestation level (this time used as input variable) and the lagged infestation level referred to the previous period.

Input variables, categorical and metric:

- Sampling period, 1 (18-28 Apr. 2012), 2 (30 May-6 Jun. 2012), 3 (12-24 Jul. 2012), 4 (27Aug.-6 Sep. 2012), 5 (11-18 Oct. 2012), 6 (27 Nov.-6 Dec. 2012), 7 (1-19 Feb. 2013).
- Site (see Tab. 1).
- Altitude, this metric variable has been used in alternative to Site.
- Infestation= log (1+Inf/min).
- Infestation of the previous sampling.
- Light rain events (n) <0.41 mm/10 min, recorded in 45 days preceding the sampling.
- Moderate rain events (n) 0.41–1.6 mm/10 min, recorded in 45 days preceding the sampling.
- Heavy rain events (n) >1.6 mm/10 min, recorded in 45 days preceding the sampling.
- Total light rain (mm) <0.41 mm/10 min, recorded in 45 days preceding the sampling.
- Total moderate rain (mm) 0.41–1.6 mm/10 min, recorded in 45 days preceding the sampling.
- Total heavy rain (mm) >1.6 mm/10 min, recorded in 45 days preceding the sampling.
- Total rainfall (mm), recorded in 45 days preceding the sampling.
- Average maximum temperature of 45 days preceding the sampling.
- Average temperature of 45 days preceding the sampling.
- Average minimum temperature of 45 days preceding the sampling.
- Percentage of daily hours with Relative Humidity >80%, recorded in 45 days preceding the sampling.
- Percentage of daily hours with Relative Humidity <40%, recorded in 45 days preceding the sampling.

Output variable:
y= Parasitization

In order to single out the best model, a progressive elimination of variables has been performed, dropping out variables one by one as they show the lowest significance level (higher P-value). In this way we can obtain the model showing the highest global significance with the lowest number of variables.

For what concerns weather conditions, as the variables are closely correlated with each other, it is reasonable that only a few of them have been selected in the final model.

As regards the influence of altitude, an alternative model has been tested; here only the altitude of the site is taken into account instead of the different sites. This model is more parsimonious, because it uses just one degree of freedom instead of 25.

RESULTS

Rainfall and average temperature recorded in the sampling period are shown in Fig. III and Tabb. 1, 2; these records are close to those recorded in 1965-1994 (Drago, 2005).

P. bliteus was the only parasitoid emerged from *G. brimblecombei*; it has been recovered in all 30 sampled sites, confirming data from our preliminary survey performed in winter 2011-12 and stating that the parasitoid is widespread all over Sicily.

G. brimblecombei infestation and *P. bliteus* parasitization levels recorded in all seasonal samplings are shown in Figs IV-VII, while the average levels recorded in each altitudinal

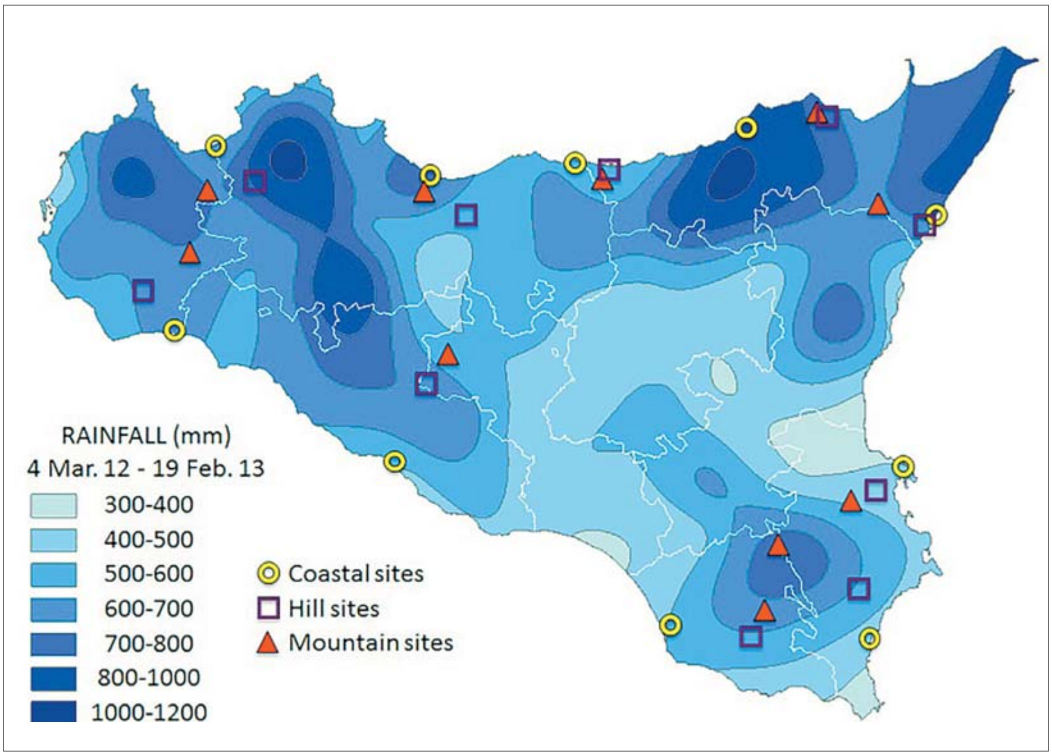
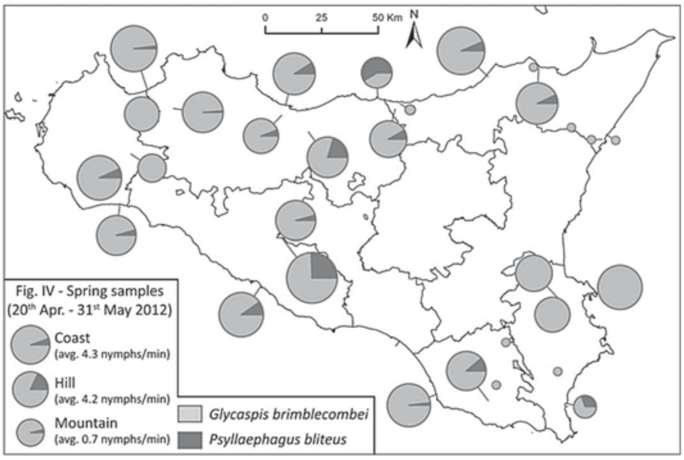
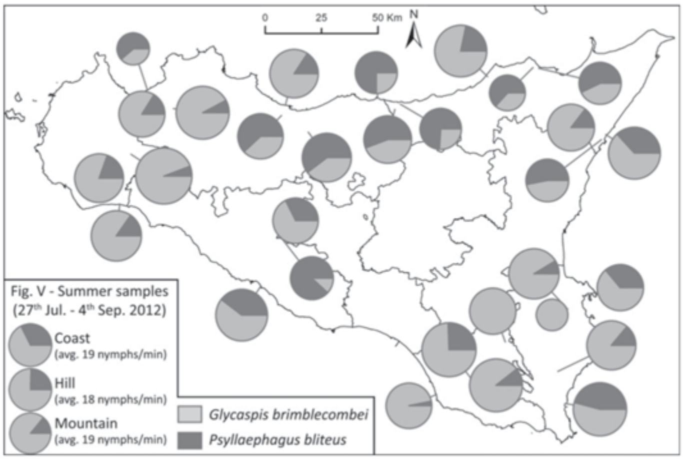
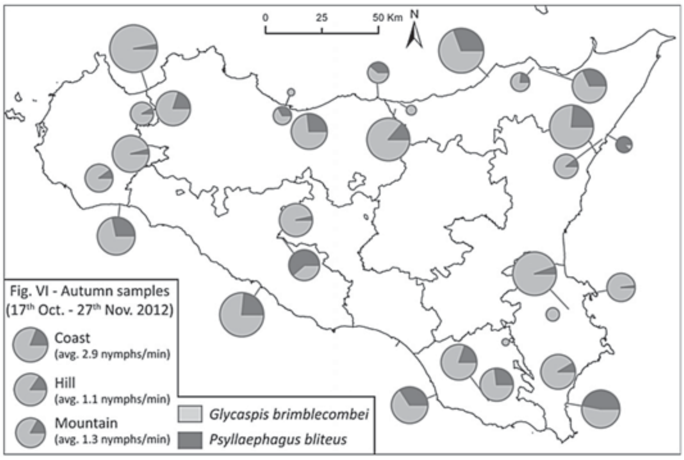
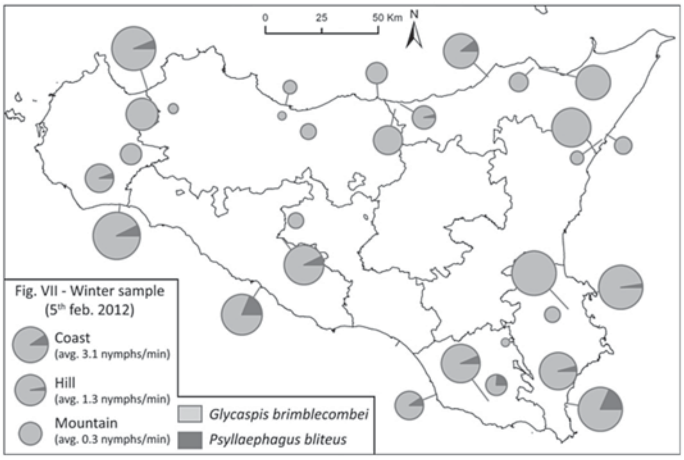


Fig. III – Sampled sites and map of rainfall recorded in Sicily during the sampling period, 4 Mar. 2012-19 Feb. 2013 (map kindly drawn by Luigi Pasotti using all daily data coming from SIAS stations).









Figs – IV-VII – *Glycaspis brimblecombei* infestation (4th-5th nymphs collected per minute per person) and *P. bliteus* parasitization (%) recorded by seasonal samplings in all 30 Sicilian sites; pie chart variation size is normalized by log.

group of sites is shown in Fig. VIII. In spite of some differences detectable in these figures, infestation and parasitization levels recorded at three different ranges of altitude resulted not statistically different; as shown in Figs IV-VII the variability of both infestation and parasitization levels is very high.

In all three altitudinal ranges infestation by *G. brimblecombei* reached a clear peak during the first summer sampling performed in July (Fig. VIII, 1), while the peak of parasitization due to *P. bliteus* was a little delayed maintaining high percentages also in the second summer sampling and the first one of autumn (Fig. VIII, 2).

The results of statistical analysis related to the infestation, measured as number of live 4th-5th nymphs collected per minute per person, are reported in the final model (Tab. 3).

As already described in materials and methods, Tab. 3 shows the input variables resulted as the most significant ones, obtaining the model having the highest global significance with the lowest number of variables.

The variable with the highest impact on infestation levels is *P. bliteus* parasitization (see Adjusted MS and F in Tab. 3). Although theoretical model contemplates that the present infestation depends on the previous parasitization, at unknown lag, evidently such lag is closer to the present date of sampling than to the previous sampling, which is around 45 days before the present date. This could be the reason why between the two covariate, parasitization and parasitization at the previous sampling period, the first one results significant, while the second one can be eliminated. Obviously, the parasitization enters the model with a negative slope (see coefficient in Tab. 3) that is an increment of the parasitization lowers the infestation.

As the variables of weather conditions are closely correlated one to each other, the average percentage of daily hours with RH > 80% was selected in the final model as the most significant one; for temperature, the chosen one is the average maximum temperature of the 45 preceding days, while rainfall is not present among the most significant variables, probably because it is linked to the relative humidity. Both of selected climatic variables (RH> 80% and average maximum temperature) enter the model with a positive slope (see coefficient in Tab. 3).

Beside the weather conditions, also the sampling period is significant; the site, although significant, has lower impact (Tab. 3).

An alternative model has been tested; there, in place of the different sites, only the altitude of the site was taken into account. This model is more parsimonious because it uses just one degree of freedom instead of 25; unfortunately, it explains a significantly smaller part of variance; this means that the altitude is an important factor, although many other local factors, typical of the site, are also influent, but the variability of the sites is very high.

Also in the statistical analysis regarding the factors influencing the parasitization, the lagged variable (infestation of the previous period) has resulted to be not significant, probably due to the same reasons expressed in the previous analysis regarding the infestation. The infestation has a negative impact, and this could be considered reasonable, because when there is an outbreak of infestation, the parasitization has a delay in the rise. In Tab. 4, the sampling period is the main factor influencing the parasitization, followed by the infestation level (with negative influence, as already written) and the percentage of daily hours with relative humidity> 80% (positively influencing). The altitude is an important factor too, but single sites explain significantly

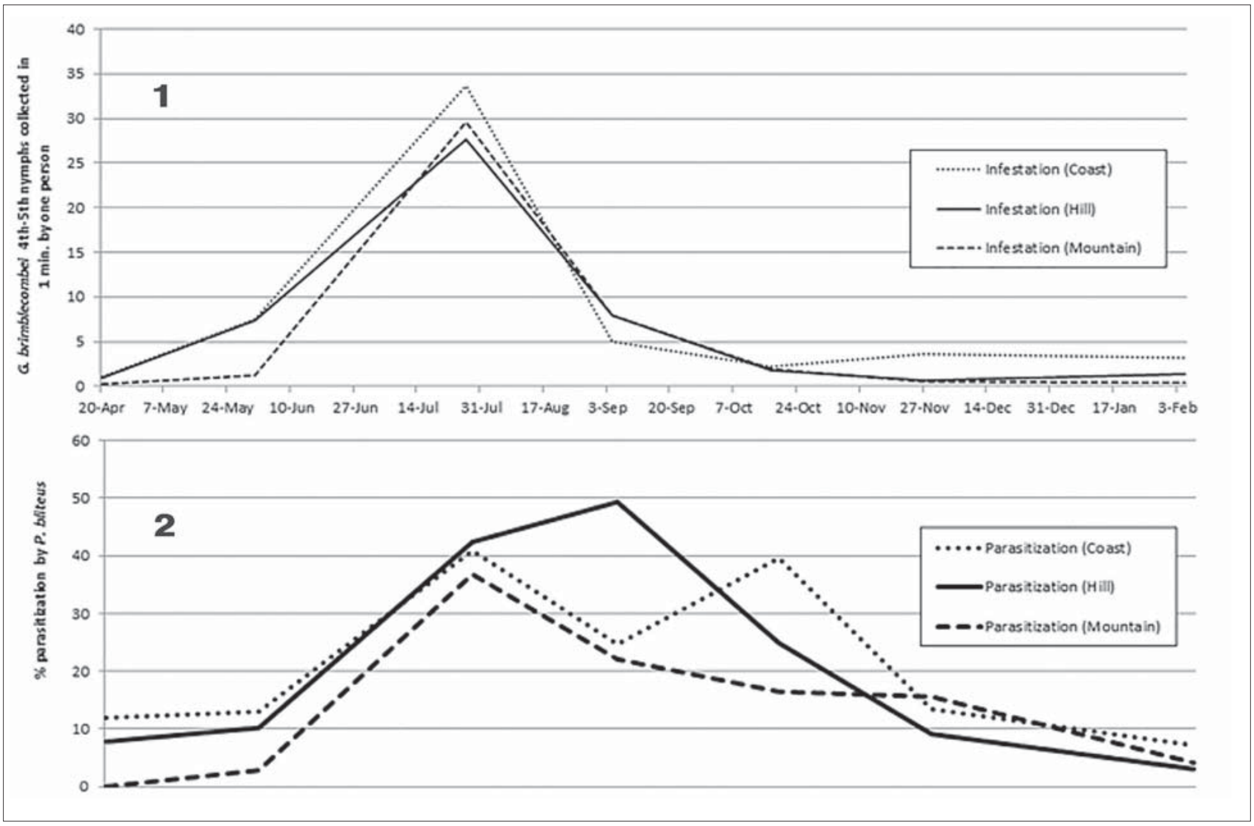


Fig. VIII – Trend of average infestation level of *G. brimblecombei* (1) and percentage of parasitization by *P. bliteus* (2) recorded in all groups of sites (Coast, Hill and Mountain).

Table 3 – Analysis of Variance for *G. brimblecombei* infestation, log(1+Infestation/min), using Adjusted SS for Tests.

Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**	**Coefficient (metric variable)**
P. bliteus parasitization	1	0.051	8.940	8.940	28.21	0.000	-1.317
Percentage of daily hours with Relative Humidity >80%	1	2.544	4.121	4.121	13.01	0.000	0.020
Average maximum temperature	1	9.197	2.944	2.944	9.29	0.003	0.144
Sampling period	6	17.143	17.965	2.994	9.45	0.000	
Site	25	37.090	37.090	2.994	4.68	0.000	
Error	118	37.390	37.390	0.317			
Total	152	103.415					
S = 0.562908, R-Sq = 63.84%, R-Sq(adj) = 53.43%							

Table 4 – Analysis of Variance for *P. bliteus* parasitization, using Adjusted SS for Tests.

Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**	**Coefficient (metric variables)**
Sampling period	6	7.370	9.396	1.566	44.32	0.000	
log(1+Infestation/min)	1	0.009	0.952	0.952	26.95	0.000	-0.138
Percentage of daily hours with Relative Humidity >80%	1	1.170	0.561	0.561	15.88	0.000	0.007
Site	25	6.387	6.387	0.255	7.23	0.000	
Error	119	4.204	4.204	0.035			
Total	152	19.140					
S = 0.187961, R-Sq = 78.04%, R-Sq(adj) = 71.94%							

more; so that we can say – as previously seen – that there are local conditions that cannot be reduced to a simple altitudinal factor.

DISCUSSION

After the first record of *P. bliteus* in Sicily, its spread all over the region occurred in a short time, showing the high dispersal capacity. Similarly, *Closterocerus chamaeleon* (Girault), a parasitoid released in Sicily to control the eucalypt gall wasp *Ophelimus maskelli* (Ashmead) (Hymenoptera, Eulophidae) (Rizzo *et al.*, 2015), rapidly spread through short and long distance dispersal mechanism, being widely distributed in the region after 18 months from its release in 2006 (Caleca *et al.*, 2011b).

At present, *Psyllaephagus bliteus* is widespread in Sicily and has been recorded at all detected altitudes without statistically significant differences.

Mediterranean areas fits with the good development of *Eucalyptus camaldulensis*, *Glycaspis brimblecombei* and its specific parasitoid *P. bliteus*, differently from what recorded in the arid climatic areas of inner California, where *E. camaldulensis* and *P. bliteus* showed clear difficulties in their development (Daane *et al.,* 2012).

P. bliteus parasitization is the main factor negatively influencing (=lowering) *G. brimblecombei* infestation; this result, together with the accidental and contemporaneous introduction of the psyllid and its parasitoid (Caleca *et al.*, 2011a), could explain the absence of high damage level on eucalypts in Sicily, confirming results achieved by Margiotta *et al.* (2017) which state that *P. bliteus* parasitization causes a 64% reduction of the host population. In this context, it is to further investigate the influence that a strong attack of the invasive bronze bug *Thaumastocoris peregrinus* Carpintero & Dellapé (Heteroptera, Thaumastocoridae) can have against both the host and the parasitoid (Suma *et al.*, 2014, 2018).

The most significant metric factors positively influencing *G. brimblecombei* infestation are daily hours above 80% of relative humidity and the average maximum temperature, obviously related to other, but less significant climatic factors.

Sampled sites with their particular characteristics are factors influencing infestation and parasitization more than their altitude; therefore local conditions of each site cannot be reduced to a simple altitudinal factor.

P. bliteus parasitization on *G. brimblecombei* showed to be influenced by the sampling period, as well as by the host infestation level (negatively) and by the percentage of daily hours of relative humidity >80% (positively), recording the highest average levels in summer samplings.

The time interval of about 45 days between one sample and the next was not able to confirm that both *G. brimble-*

combei infestation and *P. bliteus* parasitization were influenced by previous recorded levels as stated by MARGIOTTA *et al.* (2017), because current levels are likely affected by previous levels reached in a moment closer to current sampling than to the previous one.

ACKNOWLEDGEMENTS

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POPULATION DYNAMICS AND SEASONAL ABUNDANCE OF *THAUMASTOCORIS PEREGRINUS* IN *EUCALYPTUS* PLANTATIONS IN SARDINIA (ITALY) ([1])

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Deiana V., Pinna C., Buffa F., Mannu R., Satta A., Floris I. – Population dynamics and seasonal abundance of *Thaumastocoris peregrinus* in *Eucalyptus* plantations in Sardinia (Italy)

Thaumastocoris peregrinus is a eucalyptus pest native to Australia. It is currently the only species belonging to the Thaumastocoridae family in Europe. In Italy, it was reported for the first time in Latium in 2011, whereas in Sardinia it was detected in January 2015 on a *Eucalyptus camaldulensis* plantation located in the south of the island. Our research team carried out a monitoring program for two-years in order to verify its geographical distribution, the main periods of infestation, the flight peaks of the adults and to also detect the presence of unhatched eggs on leaves. During the first year, the occurrence of adults of *T. peregrinus* was verified by yellow sticky traps placed in twelve areas distributed throughout the island where *Eucalyptus* plantations were located. In the second year, the population dynamics and the occurrence of eggs on leaves were evaluated only in three representative study areas, already included in the previous monitoring program, located in the north, centre and south of the island, respectively. Field surveys showed the presence of *T. peregrinus* in all the monitored locations. The highest adult population was observed from late summer to initial autumn, with a significant increase in adults caught by yellow sticky traps from August, which reached its peak in September and gradually decreased in the following months. The presence of eggs of *T. peregrinus* on leaves from June to December also indicates that the insect is currently well established in the region.

KEY WORDS: bronze bug, *Eucalyptus* pest, nonnative species, monitoring.

INTRODUCTION

The bronze bug *Thaumastocoris peregrinus* Carpintero et Dellapé (Hemiptera Thaumastocoridae) is a sap-feeding insect native to Australia, which infests plant species belonging to Myrtaceae (NOACK *et al.*, 2011). Its common name is due to the symptoms associated with infestation: adults and nymphs feeding on leaves lead to a decrease in the photosynthetic area and leaf discoloration, followed by leaf silvering and tanning (DIAS *et al.,* 2014).

Bronze bug infestations cause tree defoliation, branch dieback, and reduction in tree growth, as well as tree death when attacks are particularly severe and prolonged (JACOBS and NESER, 2005; NADEL *et al.,* 2015). *T. peregrinus* thus represents a potential key pest of different plant species, especially in areas where the insect has been newly introduced.

The bronze bug has been recently found in several areas outside its native range, such as South Africa (JACOBS and NESER, 2005), Argentina (NOACK and COVIELLA, 2006), Uruguay (MARTÍNEZ and BIANCHI, 2010), Brazil (WILCKEN *et al.,* 2010), Italy (LAUDONIA and SASSO, 2012), Portugal (GARCIA *et al.,* 2013), and Mexico (JIMENEZ-QUIROZ *et al.,* 2016). Mediterranean and subtropical areas are considered to be the most suitable for *T. peregrinus* worldwide, as already observed by the analysis of its potential global distribution (MONTEMAYOR *et al.,* 2015; SAAVEDRA *et al.,* 2015). Among all potential hosts, *T. peregrinus* has been generally associated with several *Eucalyptus* species (LAUDONIA and SASSO, 2012; SOLIMAN *et al.,* 2012; GARCIA *et al.,* 2013), and different development rates and levels of infestation have been observed among the plant species on which it develops (JACOBS and NESER, 2005; NOACK and COVIELLA, 2006; IDE *et al.,* 2011; SOLIMAN *et al.,* 2012; BARBOSA *et al.,* 2014; SANTADINO *et al.,* 2017).

SOLIMAN *et al.* (2012) showed that the longevity of bronze bug adults varies from 4 to 78 days, and males generally live longer than females. On average, *E. urophylla* and *E. grandis* were found to be the most suitable species for the development and reproduction of *T. peregrinus* under laboratory conditions (SOLIMAN *et al.,* 2012). However, *T. peregrinus* develops well on other *Eucalyptus* species, such as *E. camaldulensis* (LAUDONIA and SASSO, 2012; SOLIMAN *et al.,* 2012; GARCIA *et al.,* 2013), thus leading to severe infestations and serious damages (JACOBS and NESER, 2005; GARCIA *et al.,* 2013).

E. camaldulensis is the most important *Eucalyptus* species cultivated in Italy, and is used as a windbreak and as ornamental plants in parks and gardens, as well as being cultivated for biomass fuel. In Italy, *Eucalyptus* plantations are located above all in central-southern regions, including Sardinia and Sicily (DEIDDA *et al.,* 2016).

In Sardinia, *E. camaldulensis* was introduced at the beginning of the last century and primarily in land

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reclamation areas. Today, *Eucalyptus* plantations are predominantly at less than 400 m above sea level (DEIDDA *et al.,* 2016), covering approximately 23,000 hectares and representing one of the main regions for *Eucalyptus* plantations in Italy (GASPARINI and TABACCHI, 2011). *Eucalyptus* trees are commonly found in several agricultural and forest landscapes in Sardinia and their flowers are valuable sources of nectar and pollen (FLORIS *et al*., 2007). The presence of *T. peregrinus* in such areas has a negative impact on the phytosanitary status of *Eucalyptus*, which has already been affected by several phytophagous species such as psyllids, particularly the red gum lerp psyllid *Glycaspis brimblecombei* Moore (Hemiptera Aphalaridae) (DEIDDA *et al.,* 2016; MANNU *et al.,* 2018).

A monitoring network in Sardinia covering different *Eucalyptus* distribution areas was carried out to: 1) obtain information on the distribution of *T. peregrinus* in Sardinia; 2) evaluate the seasonal abundance and main periods of infestation of bronze bug under Mediterranean conditions. We had already conducted similar study for *G. brimblecombei* (FLORIS *et al.,* 2018; MANNU *et al.,* 2018).

MATERIALS AND METHODS

FIELD SURVEYS AND SAMPLING PROCEDURE

Field surveys were carried out in 2015 and 2016, and stations were selected to cover the northern, central and southern parts of Sardinia. In 2015, four stations for each macroarea for a total of 12 were monitored, and three stations (one for each macroarea) were surveyed in 2016 (Fig. I): "Santa Maria La Palma" (Northern), "Ottana" (Central) and "Uta" (Southern). In both years we monitored the "Uta" location as it was the nearest to the first observation site of *T. peregrinus* in Sardinia (DI LASCIO and NANNINI, 2016).

Adults of *T. peregrinus* were monitored in 2015 and 2016 using yellow sticky traps, which are considered to be one of the most effective methods for capturing adults (NADEL *et al.,* 2015). Four yellow sticky traps (20 × 20 cm) were placed in four different *Eucalyptus* trees randomly selected at each location. Traps were positioned on branches located in the middle of the canopy, which is the best position to capture *T. peregrinus* adults (MARTÍNEZ *et al.,* 2010; NADEL and NOACK, 2012; NADEL *et al.,* 2015).

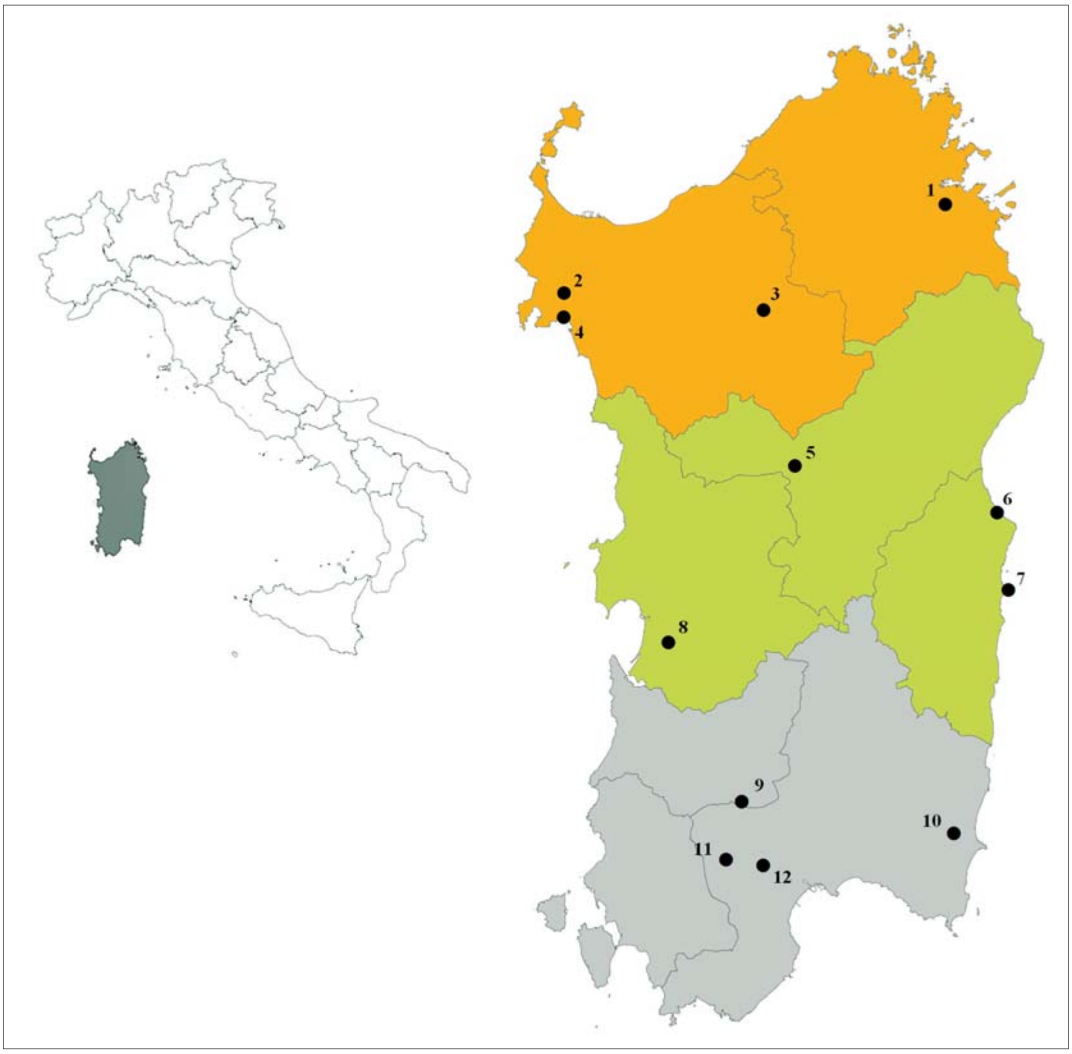

Fig. I – Distribution of locations constituting the monitoring network of *T. peregrinus* adults and eggs in Sardinia (Italy). Numbers from 1 to 12 indicating each location are sorted according to a North-South gradient (1="Olbia"; 2="Santa Maria La Palma"; 3="Ozieri"; 4="Alghero"; 5="Ottana"; 6="Siniscola"; 7="Arbatax"; 8="Arborea"; 9="Serramanna"; 10="San Vito"; 11="Siliqua"; 12="Uta"). Different colors in the map indicate different geographical areas (Orange=North Sardinia; Green=Central Sardinia; Grey=South Sardinia).

The traps were collected and replaced biweekly in the summer and monthly in other seasons. The traps collected in the field were then taken to the laboratory where the *T. peregrinus* adults were counted

Observations of eggs were conducted only in 2016. At each location and sampling date, four branchlets of approximately 40 cm in length were collected from the canopy of the four trees. Again, samplings were carried out from the middle of the canopy, as it is also recommended for assessing the density of bronze bug eggs (Martínez *et al.,* 2010; Nadel *et al.,* 2015; Jimenez-Quiroz *et al.,* 2016). Each branchlet was collected separately in a plastic bag and transferred to the laboratory, where the numbers of unhatched eggs were counted under a stereoscopic microscope on both sides of four mature leaves per branch.

Statistical analysis

All statistical analysis were performed using R software (R Core Team, 2016). Firstly, abundance data were log(x+1)-transformed to satisfy the normality assumption. In order to explore the effects of location on the abundance of *T. peregrinus* adults and eggs, a Linear Mixed Model (LMM) was considered for each year using the lmer function of the "lme4" package in R (Bates *et al.*, 2015). Location was considered as the fixed factor, whereas sampling dates and traps were the random factors. An LMM was fitted to the 2015 monitoring data to evaluate the effects of the different geographical areas on the abundance of *T. peregrinus* adults, considering the geographical areas as the fixed factor and sampling dates and traps as random factors. Analysis of variance (ANOVA) considering Type II Wald F tests with Kenward-Roger correction of degrees of freedom was used to test the significance of factors, followed by Tukey's post-hoc test at a significance level of $p<0.05$ for mean separation.

Finally, a linear regression model approach was used to evaluate the relationship between the average number of eggs and adults captured either at the same or different sampling times. The average monthly numbers of eggs observed in 2016 in all locations were thus compared to those of adults captured either in the same (*t*), or previous (*t*-1), or following (*t*+1) month, by linear regression models. ANOVA was performed to test the significance of the regression models and Pearson's correlation coefficient was calculated to evaluate the relationship between the variables.

RESULTS

Adults of *T. peregrinus* were captured in all monitored locations in 2015, and statistical differences among locations were found ($F_{11,40.257}=20.30$; $p<0.001$). "Serramanna", "Uta", "Olbia" and "Arbatax" were the most infested locations, which showed an average monthly number of adults greater than 10 individuals per trap (Table 1). However, five locations showed average values lower than one adult per month compared to all the monitored areas. Statistical differences in adult abundance were also found between geographical areas ($F_{2,73.158}=7.37$; $p<0.001$). The highest and the lowest average monthly number of adults captured by traps was observed in south (16.3 ± 3.1) and north (4.2 ± 1.7) Sardinia, respectively. The seasonal abundance of *T. peregrinus* in 2015 was similar in all the geographical areas (Fig. II). The population dynamics of adults was comparable in the three geographical areas, showing an increase in the average number of adults in August and a peak in population abundance from September to October in southern and central Sardinia. Although population abundance increased simultaneously in all areas, in northern Sardinia, the number of adults decreased immediately after September.

Significant differences between the three areas were also found in 2016 both for adults ($F_{2,9}=14.96$; $p<0.001$) and eggs ($F_{2,9}=14.60$; $p<0.001$). The "Ottana" (i.e. central Sardinia) location showed the highest average monthly

Table 1 – Average monthly number of adults per traps and eggs per branch of *T. peregrinus* captured at 12 and 3 locations distributed throughout Sardinia (Italy) during 2015 and 2016, respectively. Labels identify each location according to North-South gradient. Values are reported as mean ± standard error. Different letters in a column indicate significant differences in abundance among locations within same year (Tukey's post hoc test, p<0.05).

Label	Geographical area	Location	2015	2016	
			Adults	Adults	Eggs
1	North Sardinia	Olbia	12.45 ± 3.90 b	-	-
2	North Sardinia	S. M. La Palma	0.02 ± 0.02 a	8.36 ± 2.58 ab	0.58 ± 0.34 a
3	North Sardinia	Ozieri	0.09 ± 0.09 a	-	-
4	North Sardinia	Alghero	0.02 ± 0.02 a	-	-
5	Central Sardinia	Ottana	0.03 ± 0.02 a	20.54 ± 5.03 b	7.05 ± 1.81 b
6	Central Sardinia	Siniscola	6.38 ± 1.97 b	-	-
7	Central Sardinia	Arbatax	11.11 ± 4.00 b	-	-
8	Central Sardinia	Arborea	0.03 ± 0.02 a	-	-
9	South Sardinia	Serramanna	16.67 ± 5.51 b	-	-
10	South Sardinia	San Vito	8.72 ± 3.08 b	-	-
11	South Sardinia	Siliqua	7.16 ± 2.61 b	-	-
12	South Sardinia	Uta	16.48 ± 4.74 b	4.82 ± 1.28 a	0.21 ± 0.07 a

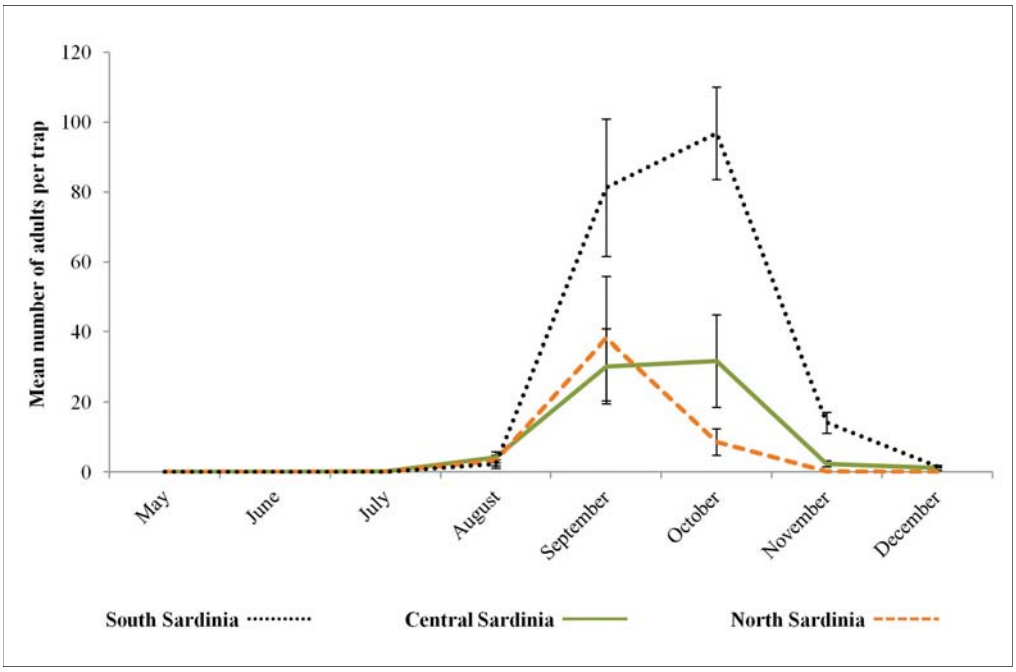

Fig. II – Average number of adults of *T. peregrinus* captured monthly during May-December 2015 in South, Central and North Sardinia (Italy). Error bars in graph indicate standard error of the average number of adults per month.

values of both adults and eggs compared to the other areas (Table 1). The seasonal abundance of eggs over time was comparable to that of adults in each location (Fig. III). Despite this, adults and egg abundance in "Ottana" reached their peak in August, whereas in other locations an increase in the population of both adults and eggs was observed only after the middle of September. In general, the seasonal abundance of eggs was comparable to that of adults in the same location, with an earlier abundance peak of eggs than of adults.

Finally, the average monthly number of eggs was significantly related to the average number of adults captured in the same month ($F_{1,29}$=29.73; p<0.001; Pearson's R=0.72) and in the following month ($F_{1,26}$=59.49; p<0.001; Pearson's R=0.84). On the other hand, no significant relationship was found between eggs and the average number of adults captured in the previous month ($F_{1,26}$=0.62; p=0.43; Pearson's R=0.16) (Fig. IV).

DISCUSSION

T. peregrinus is a serious pest of *Eucalyptus* species worldwide (LAUDONIA and SASSO, 2012; SOLIMAN *et al.,* 2012). The presence of the pest in all monitored locations throughout Sardinia ten months after its first recording confirms that the Mediterranean basin is one of the most suitable areas for its settlement (LAUDONIA and SASSO, 2012; MONTEMAYOR *et al.,* 2015; SAAVEDRA *et al.,* 2015). The rapid diffusion of *T. peregrinus* in Sardinia after its introduction may be due to both its high passive dispersal ability through wind and human transport (WYLIE and SPEIGHT 2012; SASSO *et al.,* 2014), and its capacity to remain active in the field during all seasons (SASSO *et al.,* 2014; NADEL *et al.,* 2015). In Sardinia, the highest population abundance of bronze bug was also observed during the seasonal period characterized by the highest presence of tourists (from August to September). which probably represents one of the most important phoretic means for its spread. However, the insect may also spread during other seasons, as no diapause period has been observed for *T. peregrinus* (NADEL *et al.,* 2015). The spread of *T. peregrinus* adults in the field on a local scale during all seasons, is also probably positively affected by the emission of different volatile compounds from uninfested trees, which are presumed to act as kairomones on mated females (CAMILA *et al.,* 2013).

The adult population dynamics observed during the first monitoring year in Sardinia was comparable to that reported for other infested regions in Italy (SASSO *et al.,* 2014). In 2015, the adult dynamics followed the same pattern in all three areas in Sardinia, despite the different environmental conditions of the monitored locations (DEIDDA *et al.,* 2016; MANNU *et al.,* 2018). However, a few differences in population dynamics, as well as seasonal abundances, were found from area to area in the second year. Although in southern Sardinia seasonal abundances decreased from the first to the second year, the adult population dynamics did not show a different pattern compared to 2015. In contrast to this, an increase in seasonal abundance was observed both in central and northern Sardinia, likely indicating the spatial expansion of *T. peregrinus* population throughout the Island, in line with the typical pattern of an invasive alien (non native) species (ANDOW *et al.,* 1990). In fact, biological invasions are always characterized by three time-steps (1) introduction of the invasive species, (2) its establishment, and (3) spatial expansion into new areas (ELTON, 1958).

In addition, the adult dynamics recorded in 2016 in central Sardinia was different to those of northern and southern Sardinia observed in the same and previous years. Population dynamics and seasonal abundance are generally affected by different factors, including management and environmental conditions, which both directly and indirectly influence other *Eucalyptus* pest species (LAUDONIA *et al.*, 2014; MANNU *et al.,* 2018). Of these, water availability, especially during the summer, is considered one of the most influencing factors as it allows a good foliage development which might influence sup-sucking insect feeding (LAUDONIA *et al.,* 2014).

Finally, the high correlations obtained between the average number of eggs and the average number of adults

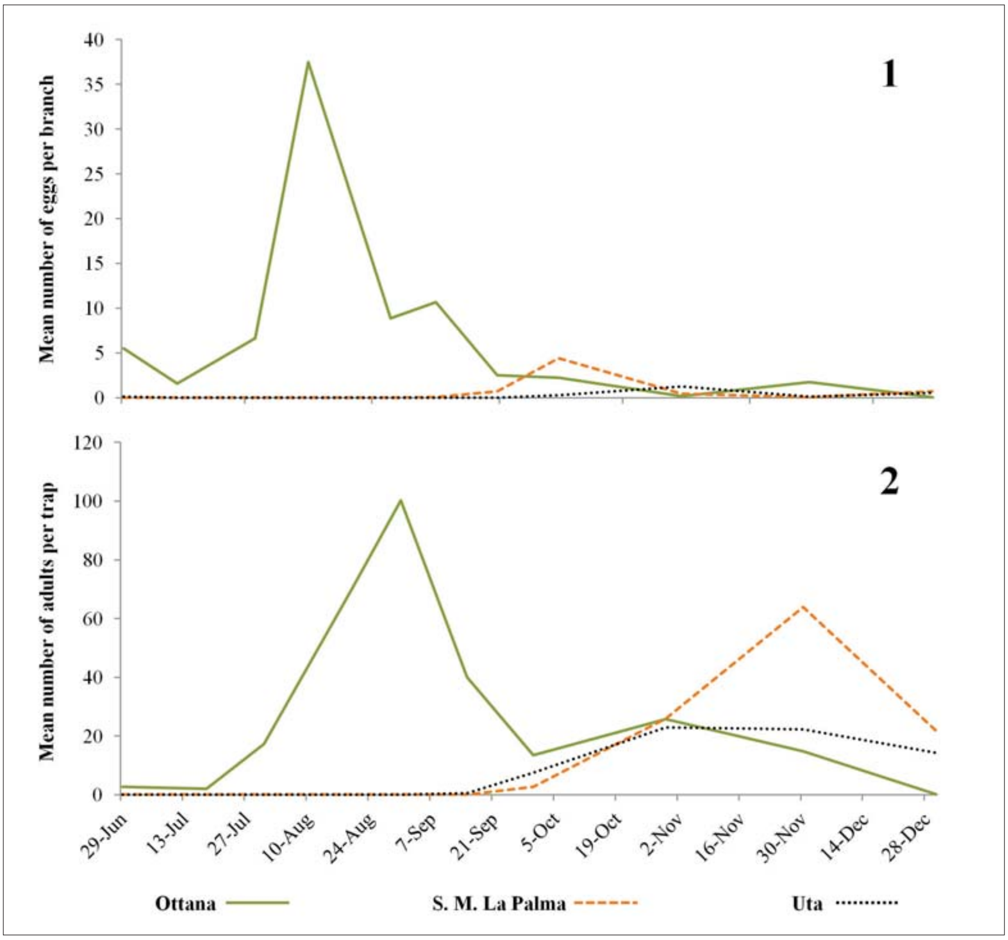

Fig. III – Average number of (1) unhatched eggs per branch and (2) adults per trap of *T. peregrinus* observed at each sampling date during June-December 2016 in Ottana (Central Sardinia), Santa Maria La Palma (Northern Sardinia), and Uta (South Sardinia), respectively.

could be particularly useful for improving monitoring activities. They could enable adult population abundance of the bronze bug to be predicted in the short-term, particularly from the perspective of biological control, e.g. using the specific egg parasitoid *Cleruchoides noackae* Lin and Huber (Hymenoptera: Mymaridae) (Barbosa *et al.,* 2017).

CONCLUSIONS

In Sardinia *T. peregrinus* is now well established throughout the island just two years after its introduction. The presence of bronze bug complicates the health of *Eucalyptus* plantations, which are already being affected by the presence of various phytophagous and several phytopathogens (Deidda *et al.,* 2016). This highlights the need to take phytosanitary measures to contain and limit the entry of other new species which could lead to irreversible ecological changes and economic losses. In accordance with Italian legislation, we are now evaluating the possibility of introducing the specific egg parasitoid *C. noackae*, due to its promising potential for the biological control of *T. peregrinus* (Barbosa *et al.,* 2017).

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PRELIMINARY RESULTS ON THE SPATIO-TEMPORAL VARIABILITY OF *GLYCASPIS BRIMBLECOMBEI* (HEMIPTERA PSYLLIDAE) POPULATIONS FROM A THREE-YEAR MONITORING PROGRAM IN SARDINIA (ITALY) ([1])

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Mannu R., Buffa F., Pinna C., Deiana V., Satta A., Floris I. – Preliminary results on the spatio-temporal variability of *Glycaspis brimblecombei* (Hemiptera Psyllidae) populations from a three-year monitoring program in Sardinia (Italy).

The red gum lerp psyllid *Glycaspis brimblecombei* is an invasive insect species worldwide. Prolonged attacks by this psyllid may cause both direct and indirect damages to *Eucalyptus* trees, such as plant weakening, developmental reductions and phylloptosis, resulting in death within 2-3 years. After the first report in Campania (Italy) in 2010 on *Eucalyptus camaldulensis* trees, it quickly spread to all surrounding central-southern regions of Italy. In Sardinia, *G. brimblecombei* was first recorded in 2011, and is currently found throughout the island. From 2013 to 2015 a monitoring program was carried out in 11 locations throughout Sardinia in order to estimate the density pattern of both its adults and preimaginal stages, as well as the general population trend of *G. brimblecombei* over the years. An analysis of *G. brimblecombei* population abundance showed an almost stable level of infestation over the years, and a seasonal pattern with a population peak in the summer. However, an earlier peak of population abundance was detected in 2015 compared to the previous years. A spatial heterogeneity of the level of infestation was observed throughout the island, highlighting the potential effects of environmental conditions in regulating the populations of both *G. brimblecombei* and its natural enemies.

KEY WORDS: *Eucalyptus*, psyllid populations, pests, monitoring.

INTRODUCTION

Glycaspis brimblecombei Moore (Hemiptera: Psyllidae), also known as red gum lerp psyllid, is a phloem-sucking insect native to Australia. The psyllid can infest several *Eucalyptus* species, particularly *E. camaldulensis* Dehnh (BRENNAN *et al.*, 1999; 2001). The insect feeds on leaves, completing its development underneath white conical shelters secreted by the nymphs, commonly called lerps. *G. brimblecombei* is a multivoltine species able to complete from two to four generations per year in its native range (MORGAN, 1984), although up to six generations have been estimated under neotropical environmental conditions (FIRMINO-WINCKLER *et al.*, 2009; CUELLO *et al.*, 2018). Temperature and relative humidity have been shown to be the main factors affecting *G. brimblecombei* populations (CUELLO *et al.*, 2018; MARGIOTTA *et al.*, 2017). In neotropical areas, the combination of low temperatures and high relative humidity negatively influences psyllid abundance, whereas positive effects on population abundance have been observed in relation to high temperatures and low relative humidity (LIMA DA SILVA *et al.*, 2013; CUELLO *et al.*, 2018).

The parasitoid *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae) is considered the most important biotic control factor of the red gum lerp psyllid, reaching a parasitism rate of 30% under laboratory conditions (DAANE *et al.*, 2005). The parasitoid was voluntary introduced and released in several psyllid-infested areas of California (DAHLSTEN *et al.*, 2005) and Mexico (PLASCENCIA-GONZÁLEZ *et al.*, 2005), to control *G. brimblecombei* infestations. Despite its host specificity, the parasitism rate of *P. bliteus* seemed to be different in areas with different environmental conditions, since the insect is less tolerant to high temperatures than the red gum lerp psyllid (DAANE *et al.*, 2012).

Severe *G. brimblecombei* infestations can cause premature defoliation, thus affecting tree growth (BRENNAN *et al.*, 2001), and potentially resulting in death within 2-3 years (DAANE *et al.*, 2005). The general weakening of the infested plants also results in a strong reduction in flowering and nectar production, consequently causing considerable production losses in unifloral eucalyptus honey (BUFFA, 2015). Such losses are particularly worrying, as eucalyptus honey represents about 50% of the total annual honey production of some Mediterranean regions, including Sardinia (FLORIS *et al.*, 2007).

G. brimblecombei was first recorded outside its native area in 1998 in California (USA) (BRENNAN *et al.*, 1999), whereas in Europe it was detected for the first time in Portugal and Spain in 2007 (VALENTE and HODKINSON, 2008). Currently, *G. brimblecombei* is also present in almost all Mediterranean countries, such as Italy (LAUDONIA

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[1] Original scientific contribution presented and discussed at the National Symposium on "Health status of *Eucalyptus* plantations in Italy", Oristano (Centro Congressi Hotel Anfora, Tramatza) - Italy, March 24-25, 2017.

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and GARONNA, 2010; LAUDONIA *et al.*, 2014), France (COCQUEMPOT *et al.*, 2012), Morocco (MAATOUF and LUMARET, 2012), Algeria (REGUIA and PERIS-FELIPO, 2013), Montenegro (MALUMPHY *et al.*, 2013), Greece (BELLA and RAPISARDA, 2013), and Tunisia (BEN ATTIA and RAPISARDA, 2014).

In Italy, after having been first found in Lazio and Campania in 2010 (LAUDONIA and GARONNA, 2010), *G. brimblecombei* spread rapidly into regions where eucalyptus plantations have been historically established, such as Sicily (LO VERDE *et al.*, 2011), and Sardinia (BUFFA, 2015). However, during the following years the specific parasitoid *P. bliteus* was also recorded in some psyllid-infested areas of Italy, where it was probably introduced accidentally together with the psyllid (CALECA *et al.*, 2011; PANTALEONI *et al.*, 2012; LAUDONIA *et al.*, 2014; MARGIOTTA *et al.*, 2014).

Sardinia, where over 88% of the Italian *Eucalyptus* plantations are located (GASPARINI and TABACCHI, 2011), is the most damaged area in Italy by the psyllid infestations. In addition, as *G. brimblecombei* infestations are also associated with other parasites and diseases, the sanitary status of *Eucalyptus* plantations may be worsened, making control strategies more difficult to be managed (DEIDDA *et al.*, 2016; FLORIS *et al.*, 2018; DEIANA *et al.*, 2018).

Given the economic and social importance of *Eucalyptus* plantations in Sardinia and in other Mediterranean countries, a *G. brimblecombei* monitoring program was carried out from 2013 to 2015 covering several areas of the Island. In this work, we analyzed the data from the monitoring activity in order to evaluate (1) the general trend of *G. brimblecombei* populations both between and within the years of the monitoring period; (2) the seasonal pattern of the different development stages during the spring-summer period; (3) the regional variability of pest abundance.

MATERIALS AND METHODS

FIELD SAMPLING AND LABORATORY ANALYSIS

Field surveys were carried out from March 2013 to December 2015 in 11 locations selected throughout Sardinia (Table 1).

During these three years, both adults and preimaginal stages were monitored monthly in the winter, and every fifteen days in the spring, summer and autumn. The adult populations of red gum lerp psyllid were monitored using four yellow sticky traps (20 cm × 20 cm) in each study area. Traps were spaced at least 20 m from each other and positioned on a branch of a tree crown at a height of approximately 2 m above the ground. All traps were collected and subsequently replaced at each sampling date. Traps were placed on the same *Eucalyptus* trees during the three-year monitoring period.

In addition, for each location and date, four *Eucalyptus* trees were randomly selected in the field and one branch approximately 40 cm long was randomly sampled from each tree canopy. In the laboratory, four leaves from each sampled branch were randomly selected, for a total of 16 leaves/location/date. In the laboratory, *G. brimblecombei* unhatched eggs, larvae and nymphs were counted on the upper side of the leaves using a stereomicroscope. For the larvae and nymphs, only the number of unparasitized juvenile stages of *G. brimblecombei* were counted.

DATA ANALYSIS

All statistical analysis were performed using R software (R CORE TEAM, 2016).

The temporal variability of the *G. brimblecombei* population in Sardinia was analyzed for all the monitored stages following the protocol suggested by ZUCCHINI and NEMADIĆ (2011). Firstly, the average monthly abundance was calculated for each stage to obtain time series data, and a non-parametric regression technique was applied. The trends (T_t) were estimated through a decomposition of the time series (X_t) using local polynomial regression, whereas the seasonal component (S_t) and residuals were estimated by calculating the differences (X_t - T_t).

In order to reduce misleading information resulting from an excess of zero counts typical of the winter period, spatial variation was examined on data from May to September, corresponding to the period of higher abundance of *G. brimblecombei* under Mediterranean field conditions (LAUDONIA *et al.*, 2014). The effects of location on the density of adults, eggs, larvae and nymphs were evaluated separately for each stage, fitting a zero-inflated negative binomial (ZINB) regression model estimated by maximum likelihood using the zeroinfl function of the "pscl" package in R (ZEILEIS *et al.*, 2008). ZINB regression models are commonly used to analyze insect count data as they generally account for over-dispersion and excess zero counts (SILESHI, 2006). In all fitted models, the factor "year"

Table 1 – General information about *Eucalyptus camaldulensis* monitored locations.

Location	Latitude	Longitude	Elevation	Type of plantation
Alghero	40.5939	8.2903	7	Ornamental
Arbatax	39.9284	9.7084	13	Ornamental
Arborea	39.8018	8.6298	7	Plantation
Olbia	40.8707	9.5155	15	Windbreak
Ottana	40.2325	9.0317	185	Windbreak
Ozieri	40.6134	8.9305	390	Windbreak
S. M. la Palma	40.6527	8.2905	30	Windbreak
San Vito	39.3344	9.5309	13	Windbreak
Serramanna	39.4134	8.8628	30	Windbreak
Siliqua	39.2712	8.8146	66	Plantation
Uta	39.2569	8.9307	6	Plantation

was included. To address variability due to time of sampling within each monitoring year, year × sampling time interaction were also considered. Analysis of deviance was performed to assess the statistical significance of factors, and χ^2 statistics were computed. Finally, a Tukey post-hoc test was conducted with a significance level of $p < 0.05$.

RESULTS

TEMPORAL VARIABILITY

Decomposition of the time series revealed a similar seasonal pattern (within-year pattern) for all different stages. However, differences in the between-years pattern were particularly evident between eggs and other stages (Fig. I). In particular, a moderate increase in the average population abundance was observed for all stages from 2013 to early 2014. However, while egg abundance revealed a strong decline and a subsequent stable period in 2014 and 2015, respectively, adults, larvae and nymphs showed a stationary period in 2014 preceding an insignificant population decrease in 2015.

SEASONAL PATTERN

Although the dynamics of the adults captured by traps had approximately the same pattern every year, a peak of abundance was observed in mid-July in 2013 compared to the beginning of July and the end of June in 2014 and 2015, respectively (Fig. II). After the peak, adult populations decreased in all the monitored years, reaching values below 100 individuals/traps in mid-September. Both the dynamics and the shift in peak of abundance among years were also similar for preimaginal stages (Fig. II).

The number of eggs regularly increased from May to

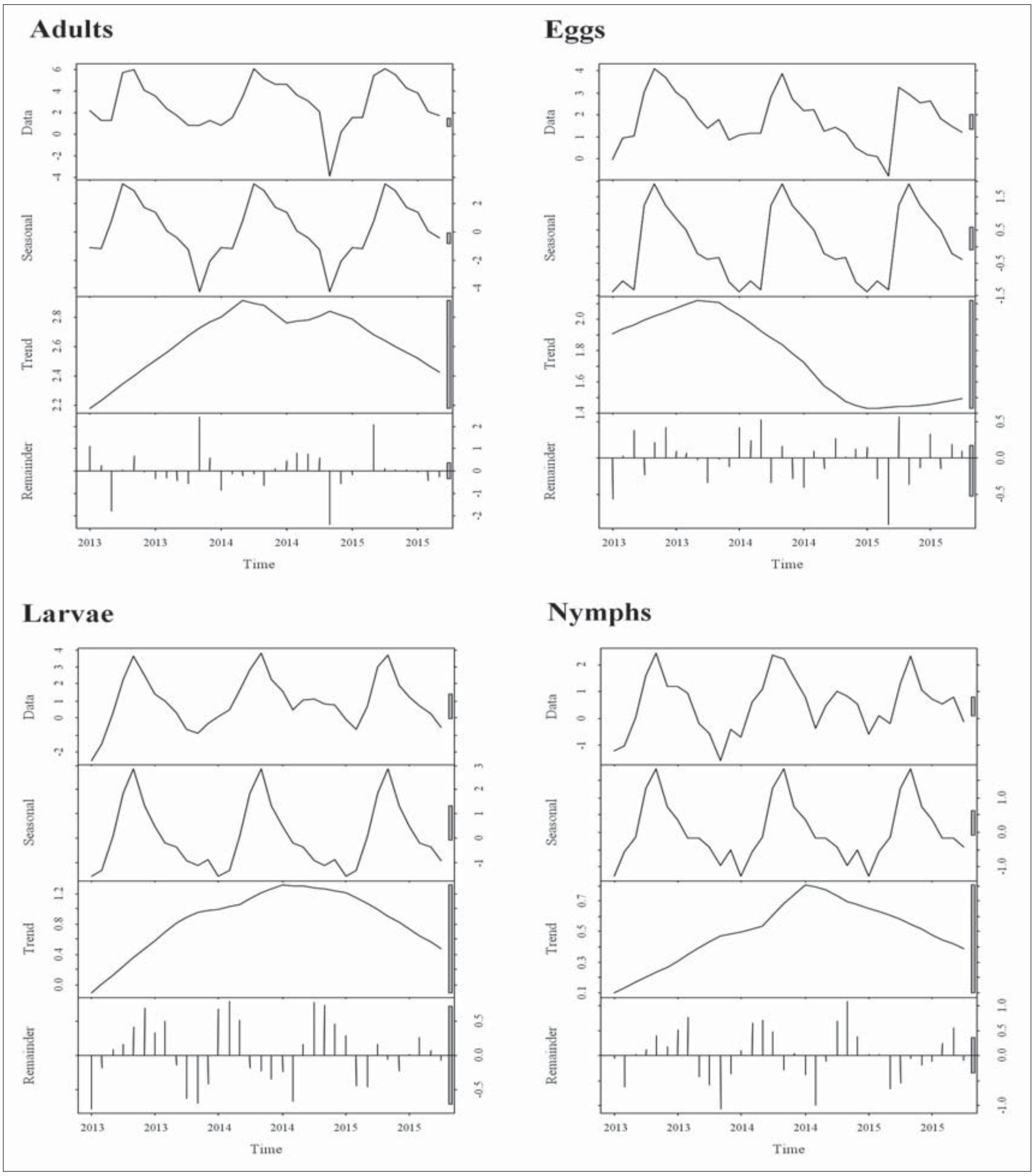

Fig. I – Seasonal decomposition of time series data for Adults, Eggs, Larvae, and Nymphs of *G. brimblecombei* from Sardinia (Italy) during 2013-2015. The original structure of time series data (Data), the within-year pattern (Seasonal), the between-year pattern (Trend), and the unexplained variance (Remainder) are reported for each stages.

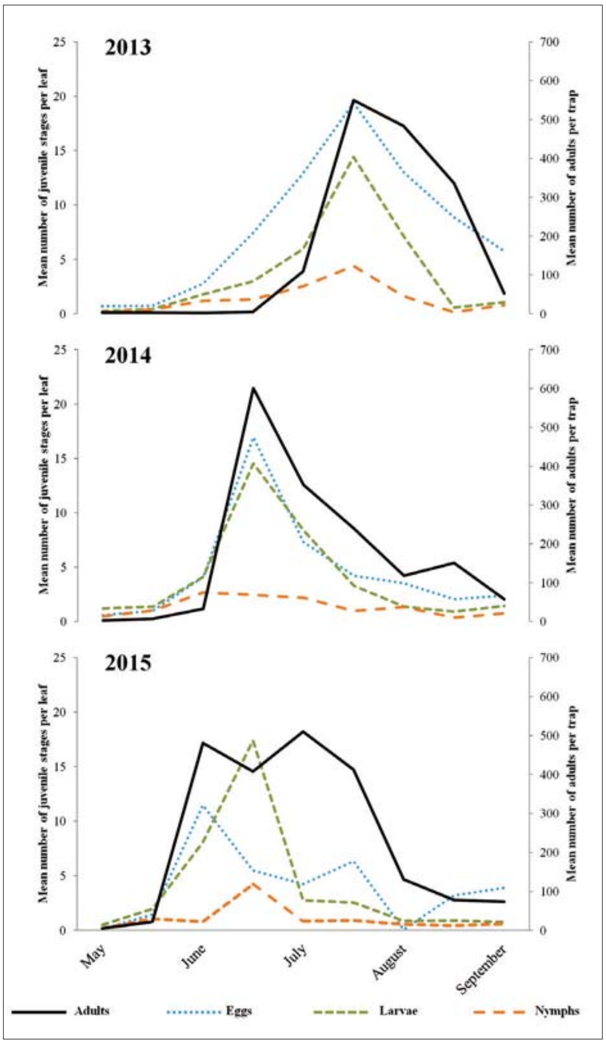

Fig. II – Activity pattern of *G. brimblecombei* adults, eggs, larvae and nymphs observed in late spring-summer period of 2013, 2014 and 2015, respectively. Each line indicates the average number of individuals captured during monitoring period.

June in 2013, reaching a peak of abundance in mid-July with 19.4 eggs/leaf, after which they decreased to the lowest level. However, in the following years, the highest total number of eggs was observed earlier in the season, as already shown for adult populations. In particular, a peak of population abundance of eggs in 2014 and 2015 was observed in early and mid-June with 16.9 and 11.5 eggs/leaf, respectively.

The analysis of the seasonal pattern of larvae also showed an earlier population peak in both 2014 and 2015 compared to 2013. Unlike the adults and other preimaginal stages, the larval populations reached the highest abundance in 2014 and 2015 with 14.5 and 17.4 larvae/leaf, respectively. Finally, the population dynamics of nymphs followed a pattern already observed for larvae, showing average values of 4.4, 2.7 and 4.2 nymphs/leaf with population peaks in 2013, 2014 and 2015, respectively.

SPATIAL VARIABILITY AT REGIONAL SCALE

Significant effects of location, as well as of year and their interaction, on *G. brimblecombei* adults and preimaginal stages were found (Table 2). During the three-year monitoring period, the numbers of adults captured by traps were significantly higher at the locations in "Serramanna" and "Ottana" ($p < 0.01$), whereas "Arbatax", "Siliqua" and "Uta" showed the lowest average values of 72, 78 and 81 individuals/trap, respectively (Table 3). However, different results at a regional level were observed for preimaginal stages. In fact, despite a low average values of adults captured by traps, "Arbatax" was more infested than the other monitored locations ($p < 0.01$), showing average egg, larval and nymph values of 3.9, 3.9, and 1.6 individuals per leaf, respectively. The location with significantly higher levels of infestation for almost all preimaginal stages was Ozieri, where mean values of 7.7, 4.9, and 2.1 were observed for eggs, larvae and nymphs, respectively. In general, a higher spatial heterogeneity of the infestation level was observed for eggs and nymphs, whereas the infestation level of larvae was relatively homogeneous across the different locations.

Table 2 – ANOVA table of ZINB regression results indicating the χ^2, degrees of freedom (df) and significance of year, location and their interaction on the abundance of *G. brimblecombei* juvenile stages and adults from May to September, respectively.

Preimaginal stage	Factor	χ^2	df	*p*
Eggs	Year	108.09	2	<0.01
	Location	54.64	10	<0.01
	Year × Location	50.37	20	<0.01
Larvae	Year	1.17	2	0.55
	Location	53.72	10	<0.01
	Year × Location	33.89	20	0.06
Nymphs	Year	15.94	2	<0.01
	Location	23.94	10	<0.01
	Year × Location	30.13	20	0.07
Adults	Year	5.28	2	0.07
	Location	135.51	10	<0.01
	Year × Location	34.11	20	<0.01

DISCUSSION

An examination of the time series revealed a similar within-year pattern among the various development stages. For each stage, the late-spring increase was followed by a peak in the summer and a subsequent decrease in the autumn-winter period. These results are in line with other studies carried out in the Mediterranean (LAUDONIA *et al.*, 2014). In particular, higher levels of infestation by *G. brimblecombei* eggs and juvenile stages have been observed in June and August in Campania and Lazio, respectively (LAUDONIA *et al.*, 2014). In addition, both adults and preimaginal stages of the red gum lerp psyllid were observed in winter. As reported for central Italy (LAUDONIA *et al.*, 2014), the presence of the different development stages in winter indicates that when favorable conditions occur *G. brimblecombei* can continue to develop even during the coldest period of the year, as recently observed in the Mediterranean environment (ISPRA, 2016).

A decreasing egg abundance over the monitoring years was particularly evident, whereas no reduction in the abundance of other stages was found. A reduction in the average egg abundance over the years could be related to

Table 3 – Mean ± standard error (SE) of adults, eggs, larvae and nymphs observed at 11 locations in Sardinia during May-September of 2013, 2014 and 2015, respectively. Different lowercase and capital letters indicate significant differences in abundance among years within same location and locations within same year, respectively (Tukey's test significance, p<0.05).

	Location	2013	2014	2015	Total
Adults	Arbatax	30 ± 12 aA	54 ± 13 abA	131 ± 30 bAB	72 ±12 A
	Siliqua	84 ± 29 aAB	61 ± 20 aAB	88 ± 29 aA	78 ±15 AB
	Uta	108 ± 31 aAB	75 ± 27 aAB	58 ± 21 aA	80 ±15 AB
	San Vito	120 ± 43 aAB	69 ± 20 aAB	80 ± 21 aA	90 ±17 AB
	Alghero	60 ± 22 aAB	88 ± 50 abAB	216 ± 54 bABC	121 ±26 ABC
	Olbia	95 ± 29 aAB	165 ± 65 aAB	180 ± 58 aABC	147 ±30 ABCD
	Arborea	149 ± 43 aAB	180 ± 48 aAB	162 ± 46 aAB	164 ±26 BCD
	S. M. la Palma	166 ± 56 aAB	305 ± 80 aB	256 ± 75 aABC	242 ±41 CDE
	Ozieri	163 ± 46 aAB	231 ± 60 abAB	408 ± 106 bBC	268 ±44 DE
	Ottana	381 ± 106 abAB	162 ± 40 aAB	486 ± 99 bC	343 ±51 E
	Serramanna	513 ± 215 aB	294 ± 98 aAB	446 ± 133 aBC	418 ±90 E
	Total	170 ± 24 ab	153 ± 16 a	228 ± 22 b	
Eggs	Alghero	6.3 ± 1.8 bA	3.0 ± 0.6 abAB	2.7 ± 0.9 aA	3.9 ± 0.7 A
	San Vito	6.0 ± 2.1 bA	4.1 ± 0.8 abABC	1.6 ± 0.5 aA	3.9 ± 0.8 AB
	Arbatax	5.2 ± 1.3 aA	4.3 ± 0.9 aABC	2.1 ± 0.5 aAB	3.9 ± 0.6 AB
	Olbia	7.7 ± 2.3 bA	2.5 ± 0.6 aA	3.6 ± 0.9 abAB	4.6 ± 0.9 AB
	Uta	4.8 ± 1.3 aA	5.5 ± 1.7 aABC	4.5 ± 1.7 aAB	4.9 ± 0.9 AB
	Siliqua	8.6 ± 1.8 bA	2.8 ± 0.7 aABC	4.1 ± 1.3 abAB	5.2 ± 0.8 ABC
	S. M. la Palma	7.7 ± 1.8 aA	5.0 ± 1.3 aABC	3.7 ± 0.8 aAB	5.5 ± 0.8 ABC
	Arborea	7.8 ± 2.3 bA	6.7 ± 2.0 abABC	4.1 ± 1.3 aAB	6.2 ± 1.1 ABC
	Serramanna	11.9 ± 2.3 bA	7.9 ± 2.5 bBC	3.5 ± 0.9 aAB	7.7 ± 1.2 BC
	Ozieri	9.6 ± 1.6 aA	6.8 ± 1.3 aC	6.7 ± 1.8 aAB	7.7 ± 0.9 C
	Ottana	11.6 ± 2.6 bA	3.9 ± 0.9 aABC	7.8 ± 1.6 abB	7.8 ± 1.1 C
	Total	7.9 ± 0.6 b	4.8 ± 0.4 a	4.0 ± 0.4 a	
Larvae	Siliqua	2.3 ± 0.7 aA	1.9 ± 0.5 aA	2.8 ± 0.7 aA	2.3 ± 0.3 A
	Alghero	3.0 ± 0.8 aA	2.6 ± 0.7 aA	2.2 ± 0.7 aA	2.6 ± 0.4 AB
	Ottana	3.2 ± 1.2 aA	1.6 ± 0.4 aA	4.1 ± 1.3 aA	3.0 ± 0.6 ABC
	S. M. la Palma	1.8 ± 0.4 aA	4.0 ± 0.9 aA	3.4 ± 1.0 aA	3.1 ± 0.5 ABC
	San Vito	4.3 ± 1.2 aA	2.6 ± 0.7 aA	2.8 ± 1.0 aA	3.2 ± 0.6 ABC
	Serramanna	3.7 ± 1.2 aA	2.9 ± 0.9 aA	3.2 ± 1.3 aA	3.3 ± 0.7 ABC
	Olbia	4.7 ± 1.5 aA	2.4 ± 0.5 aA	3.8 ± 1.2 aA	3.7 ± 0.7 ABC
	Arbatax	3.9 ± 0.8 aA	3.8 ± 0.8 aA	3.9 ± 0.9 aA	3.9 ± 0.5 ABC
	Uta	2.2 ± 0.6 aA	5.3 ± 1.8 aA	4.8 ± 1.8 aA	4.1 ± 0.9 ABC
	Ozieri	3.7 ± 0.8 aA	6.5 ± 1.2 aA	4.6 ± 1.2 aA	4.9 ± 0.6 ABC
	Arborea	6.4 ± 2.9 aA	8.2 ± 3.1 aA	4.1 ± 1.8 aA	6.2 ± 1.5 BC
	Total	3.6 ± 0.4 a	3.8 ± 0.4 a	3.6 ± 0.4 a	
Nymphs	Ottana	0.8 ± 0.2 aA	0.8 ± 0.1 aA	0.8 ± 0.2 aA	0.8 ± 0.1 A
	Siliqua	0.9 ± 0.3 aA	0.8 ± 0.2 aA	1.1 ± 0.2 aA	1.0 ± 0.1 A
	Olbia	0.9 ± 0.3 aA	1.0 ± 0.2 aA	1.0 ± 0.4 aA	1.0 ± 0.2 AB
	S. M. la Palma	0.8 ± 0.3 aA	1.6 ± 0.4 aA	0.8 ± 0.2 aA	1.1 ± 0.2 AB
	San Vito	1.3 ± 0.3 abA	1.5 ± 0.4 bA	0.5 ± 0.1 aA	1.1 ± 0.2 AB
	Alghero	1.6 ± 0.5 aA	1.0 ± 0.3 aA	1.0 ± 0.3 aA	1.2 ± 0.2 AB
	Arborea	1.6 ± 0.6 aA	1.4 ± 0.3 aA	0.7 ± 0.3 aA	1.2 ± 0.2 AB
	Uta	1.3 ± 0.5 aA	1.8 ± 0.4 aA	0.7 ± 0.2 aA	1.3 ± 0.2 AB
	Serramanna	1.9 ± 0.6 aA	0.7 ± 0.2 aA	1.3 ± 0.6 aA	1.3 ± 0.3 AB
	Arbatax	1.9 ± 0.5 aA	1.4 ± 0.3 aA	1.3 ± 0.3 aA	1.6 ± 0.2 AB
	Ozieri	2.5 ± 0.8 aA	1.7 ± 0.4 aA	2.1 ± 0.7 aA	2.1 ± 0.4 B
	Total	1.4 ± 0.1 b	1.2 ± 0.1 ab	1.0 ± 0.1 a	

the action of a generalist predatory complex during the whole egg-laying period. Observations carried out in Campania have highlighted the potential activity of ants, spiders, ladybugs and pirate bugs as general predators of different *G. brimblecombei* development stages (Laudonia *et al*., 2014), also before the detection of the specific parasitoid *P. bliteus* in the field (Laudonia and Garonna, 2010). An activity of the same predators was sporadically observed also in Sardinia during the monitoring period, though not quantified.

Contrary to our findings for eggs, there was no gradual downward trend over the years for larvae, nymphs and adults, apart from a slight decline from 2014 to 2015. These results could be related to the low effects of *P. bliteus* parasitoid activity on *G. brimblecombei* populations at a regional scale, especially during the first years of monitoring. Environmental conditions favoring *P. bliteus* parasitoid activity are different from those positively influencing *G. brimblecombei*, with a significant reduction in the parasitism rate at high temperatures (Firmino, 2004; Daane *et al*. 2005; 2012; Ferreira Filho *et al*., 2015; Cuello *et al*., 2017). Although environmental conditions are heterogeneous in space, we found a general increase in temperatures at regional scale throughout the monitoring years. In fact, despite the temperatures observed in Sardinia in 2013 being in line with those recorded during the previous years (Arpas, 2014), 2014 and 2015 were characterized by an anomalous increase in average annual temperatures, which were approximately 1 °C higher than historical temperatures (Arpas, 2015, 2016). This pattern of temperatures may have influenced *P. bliteus* activity within each monitoring year, thus affecting the development and survival on *G. brimblecombei* juvenile stages. Despite this, a positive effect of *P. bliteus* parasitism has been observed in Sardinia immediately after its accidental introduction, which caused a significant decrease in *G. brimblecombei* populations the following year (Buffa, 2015).

Within each development stage, the seasonal pattern based on abundance was similar among years. However, earlier population peaks were observed in 2014 and 2015 compared to the first year of monitoring. This was particularly evident for eggs, resulting in a regular shift of the *G. brimblecombei* seasonal pattern from the first to the last monitoring year. Changes in the pattern of *G. brimblecombei* dynamics over time might be mainly due to the effects of a general increase in mean temperatures recorded in the Mediterranean area over the last few years (Ispra, 2016). In particular, the increase in average monthly temperatures in Sardinia, which was significant from 2014 to 2015, was associated with a general decrease in the total annual rainfall (Arpas, 2014, 2015, 2016). Temperatures and relative humidity are the variables that most affect the abundance of *G. brimblecombei* (Firmino, 2004; Cuello *et al*., 2018). The development time of the red gum lerp psyllid is strongly associated with temperature, influencing the first instars more than the last ones when in the laboratory they were exposed to temperatures higher than 26 °C (Firmino, 2004). Temperatures above 30 °C limit both development and reproduction in laboratory conditions (Firmino, 2004). In Mediterranean field conditions, where temperatures above 30 °C also frequently occur in the summer, the reproductive and development rate is more likely to be affected, thus influencing both the abundance and the dynamics of *G. brimblecombei* populations in the field. Hence, the increase in temperatures may accelerate development during spring, while it slows down or stops development in summer.

Differences in abundance of both adults and preimaginal stages were found among locations. Locations where higher numbers of adults were collected did not necessarily show higher levels of egg infestation and juvenile stages in the same year.

Although the factors affecting *G. brimblecombei* infestations have not been accurately estimated, variability in space may be linked to the combination of both environment and local climatic conditions, such as water availability. In particular, ensuring optimal water conditions to *Eucalyptus* trees during summer and after a period of dryness can indirectly promote *G. brimblecombei* development, as a soft-tissue leaf favors sup-sucking insect feeding (Laudonia *et al*., 2014). However, maintaining tree vigor by hydration can also limit psyllid infestation (Paine and Hanlon, 2010).

CONCLUSIONS

The results of monitoring did not show a significant reduction in psyllid populations, nor did it show a regular spatial pattern at the regional level. This is probably due to variations in environmental climatic conditions (e.g. temperatures) in space and time during the monitoring period, which either positively or negatively affected the pest and its parasitoid. Based on these preliminary results, there is a clear need to improve monitoring activities in order to assess the evolution of *G. brimblecombei* population dynamics. Further control strategies, such as rearing and mass release of *P. bliteus*, should be set up to contain the infestations of red gum lerp psyllid.

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IMMUNOMODULATION OF HOUSE FLIES EXPOSED TO AZADIRACHTIN

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Mura M.E., Lentini A., Ruiu L. – Immunomodulation of house flies exposed to azadirachtin.

In this study the lethal and sub-lethal effects of azadirachtin on adult house flies, and the main variations in their immune-related gene expression levels were detected. Flies acquiring azadirachtin by ingestion showed different degrees of susceptibility depending on concentration and time of exposure to this compound, with LC_{50} value corresponding to 101.14 µg/ml, after five days of exposure to a dose of 7.5 µl/fly/day. Flies surviving ingestion of sub-lethal concentrations showed significant decrease in their lifespan and reproductive performance, including fecundity and percentage of egg hatching.

A significant immune-stimulation effect of lower azadirachtin concentrations (25 µg/ml), and a general immunosuppression of most AMPs (i.e. *attacin*, *cecropin*, *defensin*, *diptericin* and *muscin*) at higher concentration levels (100 µg/ml) were observed.

This study highlights the immunodeficiency potential of azadirachtin, providing new insights into understanding the physiological response of *Musca domestica* to this botanical compound at the molecular level.

KEY WORDS: *Musca domestica*, immune system, gene expression, botanical insecticide, immunosuppression.

INTRODUCTION

The triterpenoid compound azadirachtin represents the most important active substance present in seed kernels of the Indian neem tree, *Azadirachta indica* (A. Juss) (Meliaceae) (HUMMEL *et al*., 2012). Due to its well documented insecticidal properties, different azadirachtin-based biochemical formulations are available for pest management (ISMAN, 2015). Lethal and sublethal effects were observed on diverse insect species exposed to variable dosages of this compound and its derivatives (MORDUE and BLACKWELL, 1993). Whilst there are numerous studies reporting the interaction of azadirachtin with insect's physiological mechanisms (i.e. growth regulation, fecundity, and fitness) and feeding behavior, little is known on its potential as immune-modulator.

The innate immune defense system of insects typically relies on physical barriers, cellular mechanisms such as phagocytosis, encapsulation, or nodulation, and on a humoral response based on the production of antimicrobial peptides (AMPs) and other substances involved in stress tolerance like the copper-containing enzyme, Phenol oxidase (PO) and the heat-shock proteins (HSPs) (LEMAITRE and HOFFMAN, 2007; ROLFF and REYNOLDS, 2011; VALANNE *et al*., 2011, TANG *et al*., 2012). These immune functions are typically activated in response against invading microbial agents (MANACHINI *et al*., 2011; MASTORE *et al*., 2015), however immunity can be modulated as a result of insect exposure to sub-lethal doses of chemicals (SHAURUB, 2012). Accordingly, immunomodulation was observed after azadirachtin treatments, thus highlighting significant suppressive effects of this compound on the cellular mechanisms of defence. For instance, a significant reduction in total haemocyte count (THC) and changes in their morphology have been observed in some insect species (AZAMBUJIA *et al*., 1991; PETER and ANANTHAKRISHNAN, 1995; AYAAD *et al*., 2001; SHARMA *et al*., 2003; PANDEY *et al*., 2008). On the other hand, azadirachtin effects were reported to be immune-stimulatory on the freshwater teleost *Oreochromis mossambicus* (Peters) and on the goldfish *Carassius auratus* Linn. (LOGAMBAL and MICHAEL, 2000; KUMAR *et al*., 2013). Further studies are needed, especially to investigate the humoral immune response in azadirachtin-challenged insects. Such information would be very important to have a deeper understanding of the interaction of this biopesticide with the insect physiology. A better comprehension of the immune system implications deriving from the action of either botanical or synthetic insecticides would be very important for their optimal integration in pest management programs.

Several insect species show susceptibility to azadirachtin and, more in general, to neem based products. The house fly *Musca domestica* L. (Diptera: Muscidae), an ubiquitous and very common pest of medical and veterinary importance, can be controlled with azadirachtin (MILLER and CHAMBERLAIN, 1989; KHAN and AHMED, 2000; LARRAMENDY *et al*., 2004; RUIU *et al*., 2008, 2011). The effects of azadirachtin on the house fly immune system have not been investigated so far. Following the recent sequencing and annotation of *M. domestica* genome, several immune-related genes have been identified (SCOTT *et al*., 2014), which provides additional knowledge for investigations targeting the immune system of this species.

The main objectives of this study were 1) to determine the lethal and sub-lethal effects of azadirachtin on adult house flies and 2) to investigate its immunomodulatory properties at the transcriptional level.

MATERIALS AND METHODS

BIOASSAYS

Newly emerged *M. domestica* adults employed in bioassays were provided by the laboratory rearing facilities of the Dipartimento di Agraria of the University of Sassari (Italy) using the methods of RUIU *et al.* (2006). All experi-

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ments were carried out in a bioassay room at 25 °C and a photoperiod of L14:D10.

Azadirachtin [AZ] technical mixture (CAS N. 11141-17-6, Sigma Aldrich) was used for insect diet treatments in bioassays. Initial experiments were conducted with different azadirachtin concentrations in dose-response bioassays. Before starting experiments, flies were starved for 24 h, in order to force them to a rapid food intake. The experimental design involved groups of 10 flies maintained in plastic cages (10×15×5 cm) and fed daily 75 µl saccharose solution (30%) containing azadirachtin at variable concentration, administered through three capillary tubes (25 µl each). The control was just fed the saccharose solution (30%). Mortality was assessed consecutively for 5 days. The following range of concentrations was assayed to estimate the median lethal concentration (LC_{50}) value: 20, 40, 60, 80, 100, 150, 200 µg/ml. Each treated and control group involved four replications. The whole experiment was repeated three times with different batches of flies.

Supplementary observations were conducted to determine the sub-lethal effects caused by azadirachtin, in terms of fecundity, egg viability, and longevity of treated house fly adults in comparison with untreated controls. For this purpose, five replicated groups of 10 adults (5 males and 5 females), were maintained in transparent plastic boxes (10×15×5 cm) with a gauze covered window at the bottom (2×3 cm), which allowed females to lay eggs on a milk-soaked flock of cotton underneath. Flies were fed by capillary tubes (75 µl/cage/day) containing a saccharose solution (30%) with azadirachtin at two sub-lethal concentrations (40 and 80 µg/ml) for the first 5 days, and just the saccharose solution afterwards. A pinch of milk powder (1 g) was additionally provided to each cage to ensure an adequate source of protein and fat (Ruiu *et al.*, 2006). From the 6th day on, the numbers of laid eggs were recorded for a 3 weeks period. Fly mortality was assessed daily.

Egg viability was determined in three different days (10, 15 and 20) from adult emergence, by maintaining five groups of 20 eggs from each treatment in a growth chamber at 25 °C and 80% R.H. on wet filter papers inside Petri dishes (5 cm diameter). Egg hatching was verified under a stereomicroscope after 24 and 48 h.

Reverse transcription quantitative PCR (RT-qPCR) analysis

In total two different experiments were conducted, in order to determine the immune-related gene expression levels in flies exposed to diverse concentrations of azadirachtin for different time intervals. The general experimental design involved the analyses of house fly pools (10 flies per pool) fed by capillary tubes containing a 30% saccharose solution mixed with azadirachtin. Flies were administered a daily dose of 7.5 µl/fly. Pools of flies feeding only the saccharose solution were included as controls. Three independent biological replicates for each experiment were involved in analyses.

In the first experiment, analyses focused on the following genes: *attacin*, *cecropin*, *defensin*, *MdHSP-70*, and *lysozyme*. In this case flies were exposed to two different concentrations of azadirachtin (25 and 100 µg/ml) and processed after 24 h. A second experiment was conducted including a larger number of target genes (Table 1) to analyze fly pools exposed for 6, 12, and 24 h, to a unique azadirachtin concentration (100 µg / ml).

In all cases, total RNA was extracted from homogenized fly pools using TRIzol® Reagent (Life Technologies) according to manufacturer's protocol (Chomczynski and Sacchi, 1987). Retrotranscription and qPCR reactions were performed using the same procedures and conditions described earlier (Mura and Ruiu, 2017). The PCR efficiency of each primer set shown in Table 1 was preliminary tested by standard curve and dissociation curve analyses

Table 1 – Primer pair sequences used to amplify house fly immune-related genes.

Gene	Primer pair sequence	
	Forward	Reverse
Attacin	5'ATCGTTTTTTTAGCCACACTGGC3'	5'CCTAAAGTGTTCCCAGCTGC3'
Cecropin	5'CTGGATGGTTGAAAAAAATCGG3'	5'ACCCTTTAATGTGGCGGCAA3'
Defensin	5'GCTACTTGCGATTTGTTGAGC5'	5'TCAGTTACGGCAAACACAAAC3'
Diptericin	5'AAGTCACAGCCACCTCCACC3'	5'GACGACTATTGCCATAGGGTCCA3'
Domesticin	5'TTTTTCACACTCCTGGCTGCACT3'	5'CCTCCTGGCCTTCTAATTGGTGC3'
MdHSP-70	5'TACCCCCTTGTCTTTGGGTATTGAAACC3'	5'TCTGGGTTTGCTTAGTGGGGATGGTG3'
Lysozyme	5'TCCAACGGTTCCAACGATTACGGT3'	5'TCCAGCCTTGTTGGGACTTGATCT3'
Muscin	5'ATACTCGTGGTGCTGCTAAT3'	5'CTTCGTGTTAGCGTAATGTGG3'
Prophenoloxidase	5'CGGTCTTCGATGTTCCCGAC3'	5'GACGATGGCGTGGCACGAA3'
Tubulin	5'GCCTGCTGCATGTTGTATCGTGGT3'	5'CGAATTGAATGGTGCGCTTGGTCT3'

(Pfaffl, 2001). Tubulin was used after validation as a reference gene for immune-related genes expression normalization (Nayduch and Joyner, 2013).

Statistical analysis

Statistical analyses were performed with SAS software (version 9.1) with significance level set at $\alpha = 0.05$ (SAS, 2004). Linear regression analyses were used for analyzing the relationship between the azadirachtin concentration and fly mortality. Mortality data of dose-response bioassays were also analyzed by Probit model to determine the median lethal concentration (LC$_{50}$) (Finney, 1971). Data for longevity, fecundity, and egg viability were compared across treatments using one-way ANOVA, followed by LSD test to separate means in each sampling interval.

The relative expression of the target genes was analyzed using the comparative $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Fold changes in gene expression of azadirachtin-challenged house flies in different experiments were subjected to one-way ANOVA, followed by LSD test for post-hoc comparison of means.

RESULTS

Lethal and sub-lethal effects

A concentration-dependent acute toxicity of azadirachtin on house fly adults ingesting treated diets was observed in preliminary experiments (Fig. I). Mortality increased over time during bioassays achieving after five days an LC$_{50}$ (95% FL) of 101.14 (86.17-114.53) µg/ml (Slope ± SE = 4.92 ± 0.79 ; $\chi^2 = 39.05$; df = 1 ; $P < 0.0001$).

The effects of sub-lethal concentrations of azadirachtin administered by ingestion to adult flies are shown in Table 2. Longevity was significantly affected by treatments (Males: $F_{2,12} = 14.89$, $P = 0.0006$; Females: $F_{2,12} = 10.18$, $P = 0.0026$) that determined a significant reduction for the highest concentration assayed in both males (42.9 %) and females (42.1 %), and only in males (36.5 %) for the lowest concentration assayed in comparison with control. Fecundity was significantly influenced by treatments with different azadirachtin concentrations ($F_{2,12} = 27.74$, $P < 0.0001$), with a decrease reaching around 50 % in the case of the highest concentration assayed compared with control. Besides, a significant reduction in the percentage of egg hatching was determined by treatments with a diet containing an azadirachtin concentration of 80 µg/ml ($F_{2,33} = 30.99$, $P < 0.0001$).

Table 2 – Means (± SE) of longevity, fecundity, and egg viability of house flies surviving exposure to sub-lethal concentrations of azadirachtin.

Azadirachtin concentration (µg/ml)[1]	Longevity (days)[2]		Fecundity (No. eggs/ female)	Egg hatching[3] %
	Male	Female		
80	13.6 ± 1.3 a[4]	16.0 ± 1.2 a	118.6 ± 7.2 a	80.0 ± 1.4 a
40	18.8 ± 1.3 b	21.4 ± 1.5 b	198.6 ± 9.7 b	90.8 ± 1.2 b
Control	23.8 ± 1.4 c	25.2 ± 1.6 b	233.4 ± 15.1 c	92.9 ± 1.1 b

[1] Azadirachtin was administered to *M. domestica* adults only during the first 5 days of the experiment. From the 6th day on, flies were fed with just 30% sucrose solution, as the control.

[2] Days from adult emergence to adult death.

[3] Egg hatching was evaluated after 10, 15 and 20 days from adult emergence. Mean values are presented.

[4] Means in each column followed by different letters, are significantly different (ANOVA followed by LSD test, $P < 0.05$)

Relative expression of immune-related genes

Relative expressions of *attacin*, *cecropin defensin*, *MdHSP-70*, and *lysozyme* genes in house flies exposed for 24 h to two different concentrations of azadirachtin (25 and 100 µg / ml) are shown in Fig. II. A significant increase in expression of *attacin* ($F_{1,16} = 15.17$, $P = 0.0013$), *cecropin* ($F_{1,16} = 5.22$, $P < 0.0363$), and *defensin* ($F_{1,16} = 5.89$, $P = 0.0274$) genes was observed in flies treated with the lowest azadirachtin concentration, while a decrease was recorded for the highest concentration assayed. Besides, non significant were the differences between azadirachtin treatments for *MdHSP-70* ($F_{1,16} = 4.29$, $P = 0.0548$) and *lysozyme* ($F_{1,16} = 1.83$, $P = 0.1947$) gene expression levels that didn't substantially differ from untreated controls.

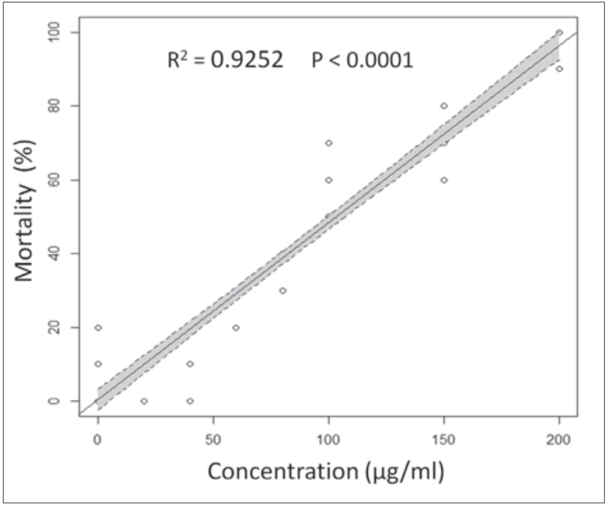

Fig. I – Linear regression plots with 95% confidence intervals (shaded areas) showing the predicted relationship between azadirachtin concentration and mortality of house flies exposed to a daily dose of 7.5 µl/fly/day.

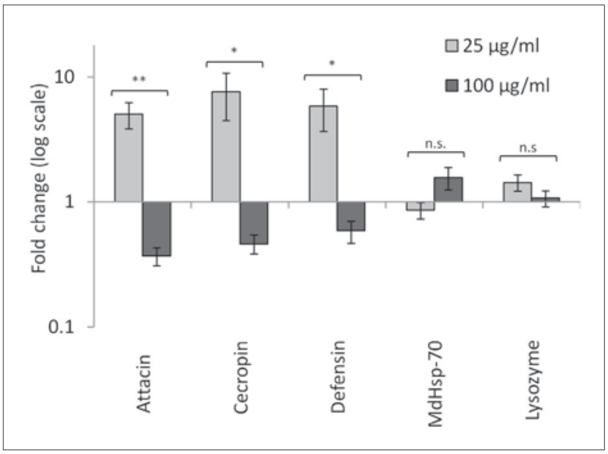

Fig. II – Comparison of relative expression fold change (mean ± SE) for selected genes of house flies exposed for 24 h to different concentrations (25 and 100 µg/ml) of azadirachtin. Statistical significance (1-way ANOVA followed by LSD test) of the relative expression ratio is indicated (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n.s. = non significant).

The relative expression (fold change) of *attacin*, *cecropin defensin*, *diptericin*, *domesticin*, *muscin*, *MdHSP-70*, *lysozyme*, and *prophenoloxidase* in house flies exposed to azadirachtin (100 µg / ml) for progressive time intervals (6, 12, and 24 h) are shown in Fig. III. A considerable over-expression after 6 h, followed by a significant under-expression after 12 and 24 h of exposure, compared with untreated controls, were observed for *attacin* ($F_{2,24} = 150.49$, $P < 0.0001$), *cecropin* ($F_{2,24} = 83.84$, $P < 0.0001$), *defensin* ($F_{2,24} = 54.12$, $P < 0.0001$), *muscin* ($F_{2,24} = 721.54$, $P < 0.0001$), and *prophenoloxidase* ($F_{2,24} = 34.19$, $P < 0.0001$). A similar initial over-expression, followed by a gradual decrease in expression was associated with *domesticin* ($F_{2,24} = 6.11$, $P = 0.0072$) and *lysozyme* ($F_{2,24} = 5.88$, $P = 0.0084$) genes, while more variable were variations in the expression of *diptericin* gene after successive time intervals ($F_{2,24} = 4.01$, $P = 0.0314$). Besides, non significant over time changes were associated with *MdHSP-70* gene ($F_{2,24} = 1.94$, $P = 0.1660$).

DISCUSSION

The action of azadirachtin on insects involves feeding deterrence, reduced fecundity, growth and moulting aberrations, and mortality (ASCHER, 1993). The potency of these lethal and sub-lethal effects is dose-dependent and varies with species. As expected, house fly adults assuming azadirachtin by ingestion showed different degrees of susceptibility depending on concentration and time of exposure to this compound, with an LC_{50} value corresponding to 101.14 µg/ml, after five days of exposure to a dose of 7.5 µl/fly/day. Flies surviving ingestion of sub-lethal concentrations showed significant decrease in their lifespan and reproductive performance, including fecundity and percentage of egg hatching.

These results are in line with previous studies reporting acute toxicity of azadirachtin on different house fly stages, with a considerably higher susceptibility of larvae developing on treated breeding media, as documented by larval LC_{50} values ranging between 10.45 µg/g and 24.53 µg/g (MILLER and CHAMBERLAIN, 1989; RUIU *et al*., 2008) depending on the azadirachtin formulation used and on the specific experimental conditions. Biocidal effects were observed as a result of direct treatments of *M. domestica* pupae with diluted neem oil solutions, which would support the penetration of the active substances through insect cuticle (DELEITO and MOYA BORJA, 2008). Beyond immature development inhibition, teratomorphic effects on house flies were occasionally observed (NAQVI *et al*., 2007). Detrimental consequences on fly fecundity have also been reported (SIRIWATTANARUNGSEE *et al*., 2008).

Similar effects have been observed on a variety of insect species in different orders (SCHMUTTERER, 1990; ABEDI *et al*., 2014a,b), even though the biochemical mechanisms, especially at cellular level, have not been clarified so far. It is known that azadirachtin may interact with insect corpus cardiacum (REMBOLD *et al*., 1989), interfering with the activity of the molting hormone that is known to play an important role in the regulation of house fly ovarian functions (ISHAAYA *et al*., 2007), which may partly explain the observed decrease in fecundity and egg viability (ADAMS *et al*., 1988). Some of these effects, might more broadly be related to the properties of azadirachtin to cause significant protein and lipid metabolism changes (HUANG *et al*., 2004; HUANG *et al*., 2012). At cellular level, oxidative stress induced by azadirachtin is related to mitochondrial dysfunctions and apoptosis in insect cell lines (HUANG *et al*., 2013).

Significant changes in the arthropod defense system are also reported as a consequence of azadirachtin challenges. This compound may affect insect immune reactivity as demonstrated by studies on *Rhodnius prolixus* Stal (Heteroptera: Reduviidae), wherein a significant decrease in the numbers of haemocytes and nodule formation following challenges with azadirachtin and the bacterium *Enterobacter cloacae* was observed (FEDER *et al*., 1997). Inhibition of nodule formation might be related to interference with the activities of endogenous mediators like eicosanoids, as reported for *Poekilocerus pictus* Fab. (Acrididae: Orthoptera) (SINGH *et al*., 2014). Cellular immune response impairment due to azadirachtin treatments has also been observed on the wolf spider, *Schizocosa episina* Gertsch & Wallace (Araneae: Lycosidae) (PUNZO, 1997) and the Greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) (ER *et al*., 2017). Moreover, azadirachtin antagonism with ecdysone has been reported to be a main cause of the reduction in haemocyte phagocytosis activity (FIGUEIREDO *et al*., 2006).

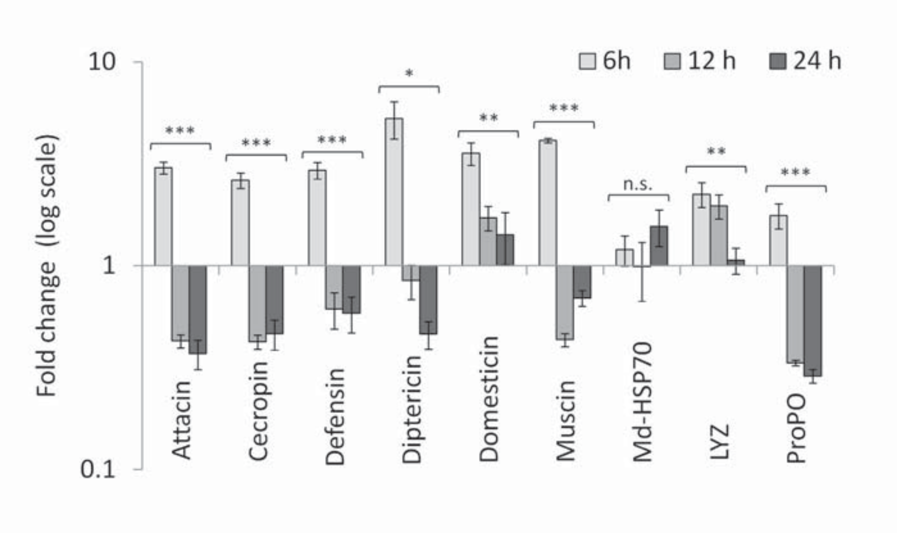

Fig. III – Comparison of relative expression fold change (mean ± SE) for different immune-related genes of house flies exposed for different time intervals (6, 12 and 24 h) to azadirachtin (100 µg/ml). Statistical significance (1-way ANOVA followed by LSD test) of the relative expression ratio is indicated (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n.s. = non significant).

All these findings are consistent with the results of studies revealing the immunomodulatory properties of various botanical and synthetic insecticides against diverse insect species. While the humoral response of insects to pathogens has recently been investigated (Kurata, 2010; Mastore *et al.*, 2015; He *et al.*, 2017; Taszlow *et al.*, 2017), the knowledge on the humoral immunity implications due to botanical extracts is still limited. Modulation of specific immune functions involving the production of heat shock proteins and the phenoloxidase cascade regulating the synthesis of oxidative species (ROS) have been associated with diverse insect growth regulators (IGRs), including buprofezin, flufenoxuron, and pyriproxifen (Salokhe *et al*. 2006; Nasr *et al*., 2010).

Overexpression of immune related genes *attacin*, *cecropin*, and *defensin* was detected after ingestion of lower concentrations of azadirachtin (25 µg/ml) by house fly adults. On the other hand, immunosuppression of the same genes after the same time interval (24 h) was noticed in flies feeding on a diet incorporated with a higher concentration of the terpenoid (100 µg/ml). In the same experimental conditions no significant changes were associated with *MdHSP-70* and *lysozyme* genes relative expression levels, in comparison with untreated controls. In a different experiment, overexpression of *attacin*, *cecropin*, *defensin*, *muscin*, *domesticin*, *lysozyme*, and *prophenoloxidase* genes was recorded after 6 h of exposure, followed by a rapid drop in the next hours, which resulted in under expression of *attacin*, *cecropin*, *defensin*, *muscin*, and *prophenoloxidase* genes, compared to controls. Even in this case, *MdHSP-70* gene relative expression in treated flies didn't change in respect to untreated control.

Our results highlight a significant immune-stimulation effect of low azadirachtin dosages, and a general immunosuppression of most AMPs at higher intake levels. The latter condition should be achieved either in the case of longer exposure to a lower concentration (first experiment) or when flies are exposed for a short time to a higher concentration (second experiment). The ability of azadirachtin to impair house fly immune system is confirmed by the results of our previous experiments showing that when flies were exposed to an adequate concentration of azadirachtin became more susceptible to the action of the entomopathogenic bacterium *Brevibacillus laterosporus* (Mura and Ruiu, 2017). This finding corroborates the additive acute toxicity previously detected in experiments involving combinations of azadirachtin and the biological control agent *Bacillus thuringiensis* Berliner against insect larvae (Abedi *et al*., 2014b; Trisyono and Whalon, 1999).

On the other hand, the immune-stimulatory effects of low azadirachtin dosages on a significant number of house fly AMP genes is not controversial, as the potential of appropriate dosages of this botanical substance to induce immune system improvements is well documented in the case of other organisms like diverse fish species (Logambal and Michael, 2000; Kumar *et al*., 2013). In the case of insects, changes in the expression level of genes related to development, stress, and immunity were noticed in larvae of the Indian meal moth, *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) feeding different azadirachtin doses (Kumar *et al*., 2013). These included up-regulation of *prophenoloxidase* gene that we observed only after a limited exposure of flies to this compound. In line with our results, these authors didn't find significant changes associated with the heat shock protein HSP 70, whose homologous represents an important component of house fly mechanisms of protection from damage under stress conditions (Tang *et al*., 2012). The up-regulation of *lysozyme* gene observed soon after fly exposure to higher azadirachtin concentrations, followed by a significant decrease in its expression level may represent another indicator of azadirachtin immunomodulatory properties. However, in the case of lysozyme, its availability is mostly related to a post-transcriptional mechanism of control (Nayduch and Joyner, 2013).

In conclusion, our study highlighted the immunodeficiency potential of azadirachtin when administered at appropriate dosage, and proved the involvement of house fly humoral defense mechanisms, thus providing new insights in understanding the physiological response of insects to this compound at the molecular level.

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EFFECT OF TEMPERATURE ON THE PATHOGENICITY OF MEDITERRANEAN NATIVE ENTOMOPATHOGENIC NEMATODES (STEINERNEMATIDAE AND HETERORHABDITIDAE) FROM NATURAL ECOSYSTEMS

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El Khoury Y., Oreste M., Noujeim E., Nemer N., Tarasco E. – Effect of temperature on the pathogenicity of Mediterranean native entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) from natural ecosystems.

Seven strains of entomopathogenic nematodes (EPNs) belonging to three species (*Steinernema feltiae, S. ichnusae* and *Heterorhabditis bacteriophora)* naturally isolated from Mediterranean countries (Southern Italy and Lebanon) were evaluated for their potential to infest greater wax moth (*Galleria mellonella*) larvae at different temperatures under laboratory conditions. The laboratory bioassay was conducted at six different temperatures ranging from 10°C to 35°C. Nematode Infective Juvenile (IJs) were put in contact with *G. mellonella* larvae in Petri dishes and mortality rates were recorded after 72 hours. The purpose of the study was to evaluate the temperature range in which the EPNs caused larval mortality; higher mortalities were recorded at 15°C and 20°C. All species failed at lower temperatures except for *S. ichnusae* ItS-SAR4, which caused 7% mortality. At 35°C *S. ichnusae* maintained its infectious activity (24%) along with *H. bacteriophora* ItH-LU1 (38%)*;* both were isolated from Italy and were more efficient at high temperatures than the remaining Lebanese isolates.

KEY WORDS: *Steinernema feltiae, Steinernema ichnusae, Heterorhabditis bacteriophora,* Mediterranean Habitats, temperature, bioassay.

INTRODUCTION

Entomopathogenic nematodes (EPNs) in the Steinernematidae and Heterorhabditidae families are obligate parasites to wide range of insect pests (EHLERS, 2001; LACEY *et al.,* 2015) but known as efficient biological control mostly for soil-dwelling insects (POINAR, 1990). Pathogenicity of EPNs is dependent on several biotic and abiotic conditions. Moreover, soil temperature can also affect the activity of entomopathogenic nematodes representing a barrier against their success as biocontrol agents. In fact, it may affect the ability of entomopathogenic nematodes to infest their host (GRIFFIN & DOWNES 1991; KUNG *et al.,* 1991; MOLYNEUX, 1985,1986; TARASCO, 1997; TARASCO *et al.,* 2015b) and to develop and reproduce (KAYA, 1977; DUNPHY & WEBSTER, 1986; ZERVOS *et al.,* 1991; GREWAL *et al.,* 1994). EPNs are naturally found in the soil and have a wide geographical distribution around the world. Their optimal temperatures for infection and reproduction may vary among nematode species and isolates (GREWAL *et al.,* 1994). In general, temperatures below 0°C and above 37°C are lethal to most of these entomopathogens (GREWAL *et al.,* 1994; GRIFFIN, 1993; ULU & SUSURLUK, 2014) while temperatures below 10-15°C can limit their mobility. However, despite the adaptation of some species to warm climate, others can maintain their pathogenicity also at low temperatures (WRIGHT, 1992; GREWAL *et al.,* 1994; BERRY *et al.*, 1997).

In order to enhance the efficiency of EPNs as biological control agents and ensure the success of the control, an adequate selection of strains according to their ability to infest under different temperatures is mandatory (YEO *et al.* 2003). Accordingly, the present study aims to evaluate the effect of different temperature on the pathogenicity of seven native Mediterranean EPNs strains isolated from natural ecosystems in Italy (TARASCO *et al.,* 2015a; TARASCO & TRIGGIANI, 1997) and Lebanon (NOUJEIM *et al.,* 2016) and to compare the pathogenicity of these isolates.

MATERIALS AND METHODS

ENTOMOPATHOGENIC NEMATODES

Bioassays were carried out with isolates of seven strains of EPNs belonging to: *S. feltiae* Filipjev, 1934 (4 strains from Lebanon: EHB1, EDA1, EHB5, EHB4); *S. ichnusae* Tarasco *et al.*, 2008 (one strain from Italy ItS-SAR4); *H. bacteriophora* Poinar, 1976 (Italian strain ItH-LU1) and *Heterorhabditis* sp. (Lebanese strain BAR8) (Table 1). EPNs were collected using the "*Galleria* baiting technique" (BEDDING , 1975) during a soil survey in different habitats in Italy (TARASCO *et al.,* 2015a; TARASCO & TRIGGIANI, 1997) and Lebanon (NOUJEIM *et al.,* 2016). To obtain fresh infective juveniles (IJs), nematodes were inoculated in last instar *Galleria mellonella* (Lepidoptera, Pyralidae) larvae at a temperature of 22±2 °C on a 100 x 10 mm Petri dish with one 90 mm filter treated with 2,000 IJs in 1,5 ml of water, as described by TARASCO *et al.,* (2015b). Dead last instar

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Table 1 – Characteristics of the locations of isolated Mediterranean EPNs

Strain	Locality	Altitude (m.a.s.l)	Ecosystem	Soil texture	Avg Temp (°C)
S. feltiae EDA1	Edde-Lebanon	200	Agriculture (Potatoes)	Sandy loamy	19.2
S. feltiae EHB5	Ehmej-Lebanon	1140	Cedars (rivers' border)	Sandy	16.3
S. feltiae EHB4	Ehmej-Lebanon	1140	Cedars (rivers' border)	Sandy	16.3
S. feltiae EHB1	Ehmej-Lebanon	1140	Cedars (rivers' border)	Sandy	16.3
S. ichnusae ItS-SAR4	Platamona (SS)-Italy	10	Sea coast	Sandy	19.9
H. bacteriophora ItH-LU1	Lucera (FG) Italy	70	Uncultivated land	Clay loamy	15.4
Heterorhabditis. sp. BAR8	Baskinta- Lebanon	1300	Pine	Loamy	13.8

m.a.s.l Metres above sea level; *FG* Foggia; *SS* Sassari; Avg Temp (°C) Average annual temperature obtained from www.wunderground.com

larvae were put on modified White traps (WHITE, 1927); juveniles emerging from *Galleria* cadavers were collected and used in bioassays within 24 hours.

INFECTIVITY BIOASSAYS AT DIFFERENT TEMPERATURES

The pathogenicity of *S. feltiae, S. ichnusae, Heterorhabditis* sp., and *H. bacteriophora* strains was tested under six temperatures ranging between 10°C to 35°C at intervals of 5°C. For every strain, plastic boxes (95 x 32 mm) filled with approximately 40 g of sterilized peat (75% degree of humidity) were inoculated with 1000 IJs in 1 ml of water each. Ten *G. mellonella* final instars larvae (100 IJs/larva) were enclosed in each box. For each treatment 3 replicates were considered and 3 boxes without nematodes were used as control for each species and temperature. The bioassays were repeated 3 times. Larval mortality was recorded after 72 hours of exposure to IJs. Cadavers, afterwards were removed from the boxes, rinsed in tap water and dissected to confirm the presence of nematodes.

STATISTICS

The statistical program used to perform the analysis was SPSS Statistics (version 22). Data were analyzed using a general linear model procedure (ANOVA - analysis of variance) and significant differences among means were separated by HSD Tukey's test. The minimum level of significance was taken as $p<0.05$.

RESULTS

Statistical analysis of mean larval mortality caused by EPNs at various temperatures showed that insect mortality was affected by temperature and strains. On the contrary, no mortality was recorded in the controls.

– **10°C**: *Steinernema ichnusae* gave the best result (7% of larval mortality) which was statistically different from all the remaining EPNs (F= 4; df= 7; P= 0.01), *S. feltiae* strains EHB1, EHB4, EHB5, EDA1 and *H. bacteriophora* ItH-LU1 and *Heterorhabditis* sp. BAR8 caused no mortality (0%) (Fig. I).

– **15°C**: *Steinernema feltiae* EDA1 produced the highest larval mortality percentage (100%) which was not significantly different from the larval mortalities scored by *S. feltiae* EHB1, EHB4, EHB5 and *Heterorhabditis* sp. BAR8 that ranged from 90% to 97% (F= 133.7; df= 7; P= 0.001). *Steinernema ichnusae* and *H. bacteriophora* had lower percentages, 27% and 47% respectively (Fig. II).

– **20°C**: All *Steinernema* strains caused high larval mortality percentages (> 95%) (F = 211.1; df = 7; P= 0.001), except for *S. ichnusae* ItS-SAR4 (77%) and *H. bacteriophora* (74%); mortality rates caused by ItS-SAR4 and ItH-LU1 were not significantly different (Fig. III).

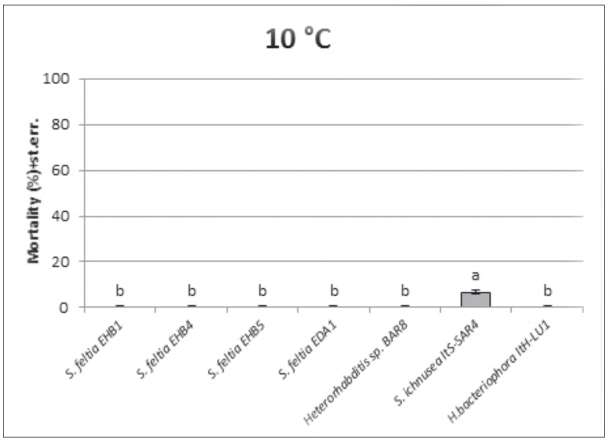

Fig. I – Pathogenicity comparison among seven native Mediterranean EPN strains: percentage mortality of *G. mellonella* larvae following 72 hrs of exposure to IJs at 10°C. Different letters above the bars indicate significant differences (P<0.05).

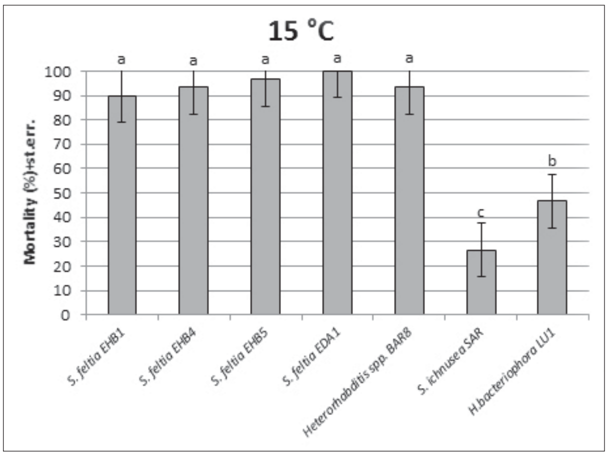

Fig. II – Pathogenicity comparison among 7 native Mediterranean EPN strains: percentage mortality of *G. mellonella* larvae following 72 hrs of exposure to IJs at 15°C. Different letters above the bars indicate significant differences (P<0.05).

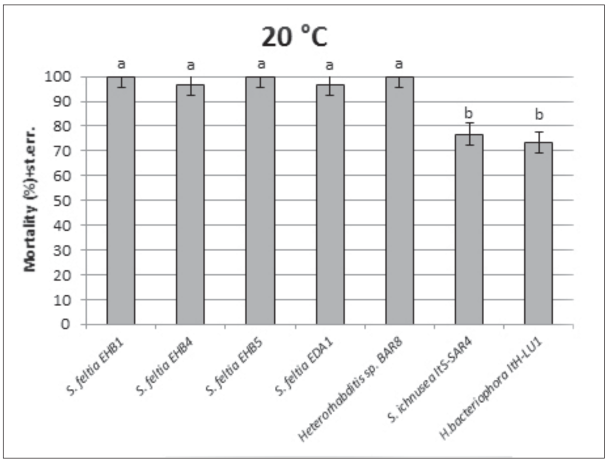

Fig. III – Pathogenicity comparison among 7 native Mediterranean EPN strains: percentage mortality of *G. mellonella* larvae following 72 hrs of exposure to IJs at 20°C. Different letters above the bars indicate significant differences (P<0.05).

– **25°C**: Almost all strains of *S. feltiae* killed about 90% of *Galleria* larvae except for EHB5 that caused 77% larval mortality, while *H. bacteriophora* caused 100% *Galleria* larvae mortality. *Steinernema ichnusae* and *Heterorhabditis* sp. BAR8 killed around 87% (F= 46.5; df= 7; P= 0.001) (Fig. IV).

– **30°C**: *Steinernema feltiae* strain EHB4 induced the highest mortality (97%) which was statistically different from the result given by the other *S. feltiae* strain EDA1 (57%); the remaining *S. feltiae* strains followed with lower larval mortality percentages 33% and 46% (F= 6; df= 7; P= 0.001). *Heterorhabditis bacteriophora* ItH-LU1 gave 74% larval mortality which was not statistically different from *S. ichnusae* (53%) and *Heterorhabditis* sp. BAR8 (60%) (Fig. V).

– **35°C**: *Heterorhabditis bacteriophora* ItH-LU1 presented the highest larval mortality percentage (37%) statistically different from *S. ichnusae* which induced mortality of 24%; no mortality was recorded for the remaining strains (F= 74; df= 7; P= 0.001) (Fig. VI).

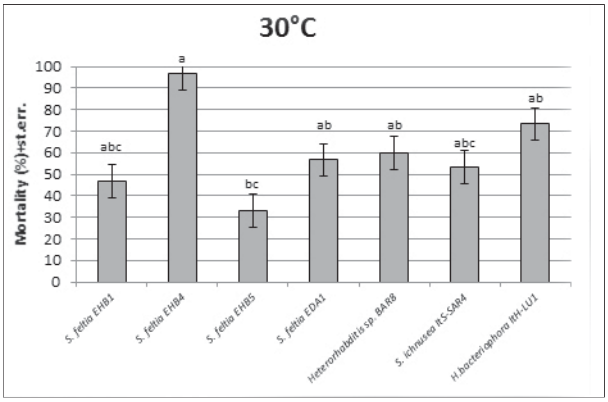

Fig. V – Pathogenicity comparison among 7 native Mediterranean EPN strains: percentage mortality of G. *mellonella* larvae following 72 hrs of exposure to IJs at 30°C. Different letters above the bars indicate significant differences (P<0.05).

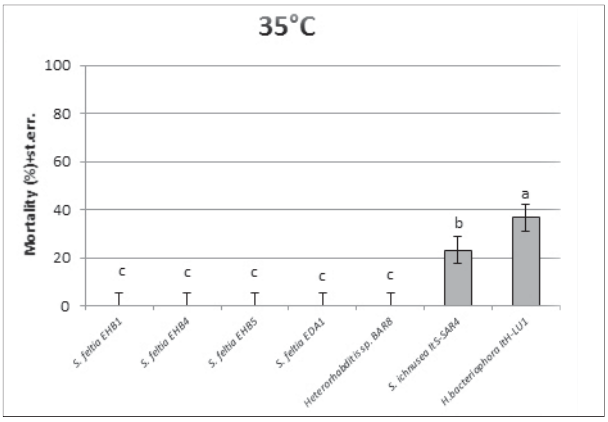

Fig. VI – Pathogenicity comparison among 7 native Mediterranean EPN strains: percentage mortality of *G. mellonella* larvae following 72 hrs of exposure to IJs at 35°C. Different letters above the bars indicate significant differences (P<0.05).

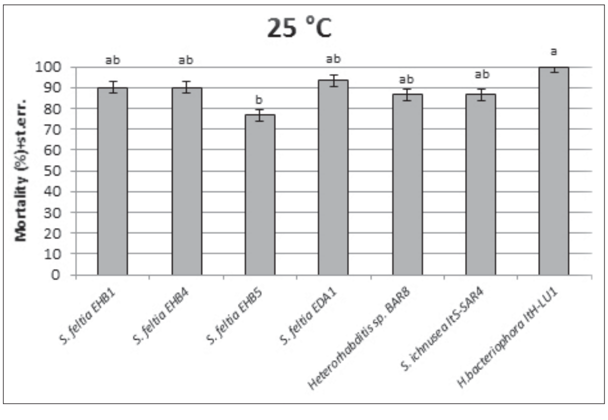

Fig. IV – Pathogenicity comparison among 7 native Mediterranean EPN strains: percentage mortality of *G. mellonella* larvae following 72 hrs of exposure to IJs at 25°C. Different letters above the bars indicate significant differences (P<0.05).

DISCUSSION AND CONCLUSION

Soil is the natural habitat of EPNs, it protects them from harmful environmental conditions such as extreme temperatures and low moisture levels (Kung *et al.,* 1991; Grewal *et al.,* 2001). Their failure as efficient and effective biocontrol agents may be due to the interaction of different factors affecting the performance of EPNs, such as ultraviolet radiation, extreme temperatures and low moisture resulting in desiccation (Rutherford *et al.,* 1987; Shapiro-Ilan *et al.*, 2006). The aim of this study was to determine the pathogenicity of Mediterranean native entomopathogenic nematode species and strains under different temperatures. All strains were able to kill *Galleria* larvae, but the pathogenicity of the strains differed significantly among different temperature regimes, and also among species. The infectivity of *S. ichnusae* to *G. mellonella* last-instar larvae increased with higher temperatures until 25°C. Our results are in line with those of Tarasco *et al.* (2015b), who tested isolated strains and found an advantageous higher mortality at 10°C, and the results of Shaurub *et al.* (2015) who studied the effects of ultraviolet (UV) light, temperature, soil type (texture), and soil moisture level on the infectivity of four EPNs used against late third instars of *Ceratitis capitata* (Wiedemann) where a temperature of 25°C gave the highest efficiency of nematodes, while low mortality rates were associated with low temperatures.

The current study demonstrated that *S. feltiae* isolates from Lebanon performed poorly at 10°C, although mortality at similarly low temperatures were recorded in different experiments (GREWAL *et al.*, 1994; TARASCO *et al.*, 2015b) where *Steinernema* spp. were able to cause mortality on *Galleria* larvae between 10 ºC and 32 ºC. One possible explanation could be that 72 hours were insufficient for *S. feltiae* to kill its host at that relatively low temperature. Higher infection rates might have been obtained by inoculation of EPNs for a longer period as previously shown in other studies (HAZIR *et al.,* 2001; RADOVÁ & TRNKOVÁ, 2010). However, rapid infection is critical and necessary when it comes to control a relatively dangerous pest. In our study, the highest mean mortality for the tested Lebanese isolates was achieved at 20°C, while 25°C was considered the optimum infestation temperature for the Italian strains. Significant differences between strains of the same species EHB5 (96%) and EHB4 (33%) isolated from close geographical areas were also recorded with Lebanese *S. feltiae* strains at 30°C; similar results were obtained by TARASCO (1997) who tested seven *S. feltiae* strains isolated from various Southern Italian regions. No mortality was recorded at 35°C except for *S. ichnusae* ItS-SAR4 and *H. bacteriophora* ItH-LU1 (isolated from Italy), which were 23% and 37% respectively. However, the absence of mortality caused by *Heterorhabditis* sp. BAR8 at 35°C is not consistent with what reported in published literature showing satisfactory efficiency at high temperatures (SHAURUB *et al*., 2015), although *H. bacteriophora* ItH-LU1 and *S. ichnusae* ItS-SAR4 were able to tolerate moderately the relatively high temperature and caused 37% and 23% respectively larval mortality.

These differences in survival and pathogenicity may be attributed to the climatic origins or the soil habitats of these nematode species (ULU & SUSURLUK, 2014). This could be correct in the case of *S. ichnusae* ItS-SAR4 and *H. bacteriophora* ItH-LU1 whose natural habitat is the sea coast and which reached at 30°C 53% and 74% mortality respectively. However our results with *S. feltiae* EHB5, EHB4, EHB1 isolated from mountains in Lebanon does not agree with this model inducing no mortality at a relatively low temperature (10°C). It can be hypothesised that a variation of 5°C could be significant in the micro-environment where the Lebanese *S. feltiae* strains EHB5, EHB4, EHB1 were isolated; consequently they caused almost total mortality at 15°C. Moreover, MUKUKA *et al.* (2010) showed that the strain's original habitat and environmental conditions do not affect the heat tolerance of EPNs, referring to the minimal fluctuation between soil temperatures. From a different perspective, CHEN *et al.* (2003) suggested that temperature affects the interaction between the nematode and the host insect, claiming that host cues are not emitted or detected equally at different temperatures. Although the thermal niche of the two families Steinernematidae and Heterorhabditidae have been previously studied, with the first well adapted to cool climates and the second to warmer environments, further studies are necessary. In fact the EPN-host relationship is believed to be affected by temperature and could be critical in determining the real mechanisms involved in the effect of temperature. Therefore a better investigation on this interaction might improve the likelihood of success of EPNs.

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ARNALDO BORDONI (*)

NEW DATA ON THE XANTHOLININI FROM THE ORIENTAL REGION.
43. NEW SPECIES AND NEW RECORDS
(COLEOPTERA STAPHYLINIDAE)
273th Contribution to the knowledge of the Staphylinidae

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Bordoni A. – New data on the Xantholinini from the Oriental Region. 43. New species and new records (Coleoptera Staphylinidae). 273th Contribution to the knowledge of the Staphylinidae

The following new species for the listed countries are described and their diagnostic structures are figured: *Pachycorynus insularis* sp. n. (Sri Lanka), *Metolinus sibayak* sp. n. (Sumatra), *Erymus tujuh* sp. n. (Sumatra), *Atopolinus tanarata* sp. n. (Malaysia), and *Atopolinus nigellus* sp. n. (Vietnam). The following species are new records for the named countries: *Zeteotomus bedugulensis* Bordoni, 2002 (Java), *Spaniolinus rubidus* Bordoni, 2002 (Malaysia), *Thyreocephalus annulatus* (Fauvel, 1895) (Tioman Isl., Malaysia), *Phacophallus japonicus* (Cameron, 1933) (Cambodia), *Emathidis humerosa* (Bernhauer, 1923) (Vietnam), and *Atopolinus ovaliceps* (Scheerpeltz, 1965) (Vietnam). New distribution records are given for several species.

Key Words: Coleoptera, Staphylinidae, Xantholinini, new species, *Pachycorynus*, *Metolinus*, *Erymus*, *Atopolinus*, new records, Oriental Region.

INTRODUCTION

Since the revision of the Xantholinini of the Oriental Region (Bordoni, 2002), I have dedicated 42 contributions to the knowledge of this tribe from this area. In this contribution I give the results of the study of numerous specimens received from some institutions and private collections. In these pages I describe five new species from various countries and list new records.

Acronyms

cB	coll. Bordoni, Firenze, Italy
cH	coll. Hayashi, Kawanishi, Japan
cI	coll. Ito, Kyoto, Japan
cS	coll. Schülke, Berlin, Germany
ISBD	Institute of Systematic Biology, Daugavpils, Latvia
MMUE	Manchester Museum, Manchester, UK
MNB	Naturhistorisches Museum, Berlin, Germany
MZF	Museo Zoologico "La Specola", Firenze, Italy
NHMB	Naturhistorisches Museum, Basel, Switzerland
NMW	Naturhistorisches Museum, Wien, Austria

TAXONOMY
(in systematic order)

Pachycorynus balux Bordoni, 2002

Material examined – N Sumatra, Brastagi, Gn. Sibayak, 1450-1900 m, Bocak & Bocakova 19-23.II.1991, 1 ex. (NHMB), 1 ex. (cB).

Distribution – The species seems endemic to Sumatra (Bordoni, 2002). This is the first record since the description of the species.

Pachycorynus alseus Bordoni, 2002

Material examined – Sumatra, Si Rambè, E. Modigliani XII.1890-III.1891, 1 ex. (NMW).

Distribution – The species seems endemic to Sumatra (Bordoni, 2002). This is the first record since the description of the species.

Remarks – The specimen bears the labels "*trigonocephalus* Epp." (handwritten by Eppelsheim), "c. Eppelsh./ Steind. d.", "Typus" (printed on red label) (*in litt.*).

Pachycorynus dimidiatus Motschulsky, 1858

Material examined – Engano, Malaconni, Modigliani 1891, 1 ex. (NMQ); Nias, Jordan, 10 exx. (NMW); Myanmar, Bhamò, Fea VIII.1885, 2 exx. (NMW).

Distribution – The species is very widespread in the Oriental Region, from India to Borneo and Philippines (Bordoni, 2002).

Pachycorynus selangorensis Cameron, 1936

Material examined – W Malaysia, Pahang, Cameron Highl., Tanah Rata, 1500-1700 m, 04.28N, 101.21E, R. & H. Fouquè 8-17.VII.2004, 1 ♂ (cH).

Distribution – The species is known only from Malaysia (Bordoni, 2002; 2006).

– Received 8 March 2018 Accepted 9 May 2018

Pachycorynus insularis sp. n.

Material examined – Holotype ♂: Ceylan (NMW).

Description – Length of body 4.5 mm; from anterior margin of head to posterior margin of elytra: 2.5 mm. Body yellowish-brown. Head sub-quadrangular, moderately dilated anteriad, with narrowly rounded posterior angles. Eyes small, scarcely protruding. Surface of head with lateral groove, polygonal micro-reticulation and scattered punctation. Pronotum narrower than head, as long as it, dilated anteriad. Surface with dorsal series of 6 punctures and lateral series of 3 anterior punctures. Elytra longer and wider than pronotum, dilated posteriad, with marked humeral angles. Surface with fine, superficial punctation, arranged in numerous series. Abdomen with transverse micro-striation and fine, sparse punctation.

Tergite and sternite of the male genital segment as in Fig. I, 1-2. Aedeagus (Fig. I, 3) 0.33 mm long, small, partially membranous, with short and narrow parameres; inner sac apparently not visible; distal sclerite very small.

Etymology – The specific epithet is the Latin *insularis-e* (insular).

Distribution – The species is known only from Sri Lanka.

Remarks – The specimen, labelled "*Holisomorphus*/ *ceylanensis* Kr./ Typ. Ceylan/ ded. Kraatz", "Cotypus" (on red label), "c. Eppelsh./ Steind. d.", was part of the series of *Holisomorphus ceylanensis* Kraatz, 1859, synonym of *Pachycorynus dimidiatus* Mot., from which the new species differs by smaller size, lighter color and aedeagus.

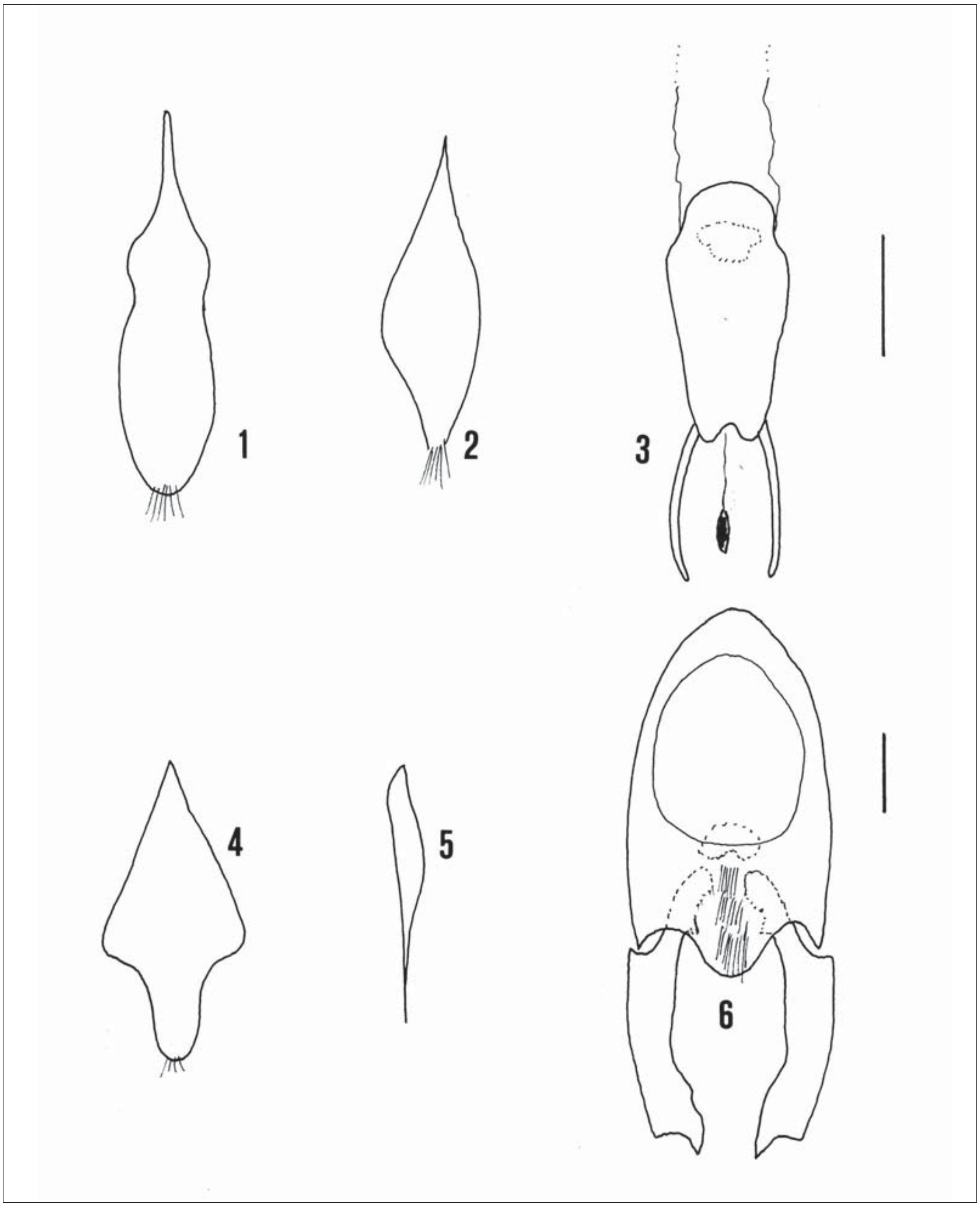

Fig. I – 1. Tergite, 2. sternite, 3. distal portion of aedeagus of *Pachycorynus insularis* sp. n.; 4. tergite of the male genital segment, 5. sternite of the same, 6. aedeagus of *Erymus tujuh* sp. n. (bar scale: 0.1 mm).

Neoxantholinus piraticus Bordoni, 2002

Material examined – W Malaysia, Pahang, Cameron Highl., Tanah Rata, 1500-1700 m, 04.28N, 101.21E, R. & H. Fouquè 8-17.VII.2004, 1 ex. (cH).

Distribution – The species is known from Malaysia and Sumatra (Bordoni, 2002). This is the first record since the description of the species.

Zeteotomus bedugulensis Bordoni, 2002

Material examined – E Java, Ijen Nat. Park, 12 km W Sodong, 1000 m, loc. coll. V.2011, 1 ex. (cB).

Distribution – This species is known only from Bali (Bordoni, 2002). New record for Java. This is the first record since the description of the species.

Spaniolinus rubidus Bordoni, 2002

Material examined – W Malaysia, Pahang, Cameron Highl., Tanah Rata, H. Ashida 8.VIII.1997, 1 ex. (cI).

Distribution – The species was known only from the Philippines (Bordoni, 2002). New record for Malaysia.

Achmonia laosiana Bordoni, 2016

Material examined – Vietnam, Hoalg Lien Son, Sa Pa, P. Pacholatko 11-15.V.1990, 1 ex. (cB).

Distribution – The species was described from NE Laos and cited from Vietnam (Bordoni, 2017) and Thailand (Bordoni, 2017a).

Thyreocephalus tonkinensis Bordoni, 2002

Material examined – "Indochine", Vanyen, Riv. Voire, Dussault 192-1924, 1 ex. (NHMB).

Distribution – The species is known from Vietnam, Laos and South China (Bordoni, 2002), Thailand (Bordoni, 2017a).

Thyreocephalus annulatus (Fauvel, 1895)

Material examined – Malaysia, Tioman Isl., Kampong Tekek, R. Hergovits 4-26.XI.2000, 8 exx. (cH); same data, K. Juara, 400 m, 2.48N, 104.11E, Dembicky & Pacholatko 9.III.1998, 1 ex. (NHMB); Malaysia, Benom Mts, 15 km E Kampong Dong, 700 m, 3.53N, 102.01E, Dembicky & Pacholatko 1.IV.1998, 4 exx. (NHMB), 1 ex. (cB); Malaysia, W Perak, 25 km NE Ipoh, 1200 m, Banjaran Titi Wangsa Mts, Korbu, P. Pacholatko 11-16.I.1999, 1 ex. (cI); S Sumatra, SW coast Ranau Lake, 1200 m, Bolm 1-4.VI.2001, 2 exx. (NHMB); Sumatra, Sumater Utara prov., Tangkahan, 100 m, 02°40'59''N, 098°04'22''E, R. Cibulskis 5.II.2011, 2 exx. (ISBD); same data, Gunung Leuser Nat. Park, 100 m, 03°40'59''N, 098°04'22''E, R. Cibulskis 4.II.2011, 1 ex. (ISBD).

Distribution – From Myanmar to Lombok (Bordoni, 2002), Sulawesi and New Guinea (Bordoni, 2012). New record from Tioman Island.

Thyreocephalus lorquini (Fauvel, 1877)

Material examined – Celebes, Bowonglangi, Drs. Sarasin, 5 exx. (NHMB), 2 exx. (cB); N Celebes, Mararang, Drs. Sarasin, 2 exx. (NHMB).

Distribution – Sulawesi, Moluccas (Bordoni, 2002), Australia (Bordoni, 2015), New Guinea (Bordoni, 2010).

Thyreocephalus indicus (Fauvel, 1895)

Material examined – Malaysia, Kedambe prov., 400 m, 03°40'48''N, 097°39'40''E, R. Cibulskis, V. Vahrusevs & D. Volkov 16.I.2011, 1 ex. (ISND).

Distribution – Indo-Malay subregion (Bordoni, 2002).

Thyreocephalus perakensis Bordoni, 2004

Material examined – Malaysia, Benom Mts, 15 km 15 km E Kampong Dong, 700 m, 3.53N, 102.01E, Dembicky & Pacholatko 1.IV.1998, 1 ex. (NHMB), 1 ex. (cB); Malaysia, Pahang. Cameron Highl., Tanah Rata, 1500-1700 m, P. Pacholatko 23-31.I.2003, 1 ex. (NHMB).

Distribution – The species was described from Malaysia (Perak). These are the first records since the description of the species.

Thyreocephalus nigricantis Bordoni, 2009

Material examined – Malaysia, Benom Mts, 15 km 15 km E Kampong Dong, 700 m, 3.53N, 102.01E, Dembicky & Pacholatko 1.IV.1998, 1 ex. (NHMB), 1 ex. (cB).

Distribution – This species was described from Malaysia (Johor).

Metolinus modiglianii Bordoni, 2002

Material examined – N Sumatra, Brastagi, 1450-1900 m, Bocak & Bocakova 19-23.II.1991, 2 exx. (NHMB), 1 ex. (cB).

Distribution – The species is known from Sumatra (Bordoni, 2002), Java and Mentawei (Bordoni, 2009a).

Metolinus perakensis (Cameron, 1950)

Material examined – N Sumatra, Brastagi, Gn. Sibayak, 1450-1900 m, Bocak & Bocakova 19-23.II.1991, 1 ♂ (NHMB).

Distribution – This specie is known from Sumatra and Malaysia (Bordoni, 2002).

Metolinus sibayak sp. n.

Material examined – Holotype ♀: N Sumatra, Brastagi, Gn. Sibayak, 1450-1900 m, Bocak & Bocakova 19-27.II.1991 (NHMB); paratype: same data, 1 ♀ (cB).

Description – Length of body 4.3 mm; from anterior margin of head to posterior margin of elytra: 2.2 mm. Body shiny, brownish-black, with posterior margin of elytra narrowly pale yellowish; antennae brown, legs brown with yellowish tarsi. Head sub-quadrangular, with sub-rectilinear sides and narrowly rounded posterior angles. Eyes medium-sized and scarcely protruding. Surface of head with traces of transverse micro-striation and scattered punctures. Pronotum sub-rectangular, longer than head, as wide as it, with almost rectilinear sides. Surface with dorsal series of 3 widely punctures and lateral series of 3 median punctures. Elytra sub-rectangular, moderately dilated posteriad, longer and wider than pronotum, with rounded humeral angles. Surface with very fine and scattered punctation, arranged in three series, one juxtasutural, one median and one lateral. Abdomen with traces of transverse micro-striation and very scattered, fine punctures.

Male unknown.

Etymology – The specific epithet refers to the type locality, as a noun in apposition.

Distribution – The species is known only from the type locality.

Remarks – This is the only species of *Metolinus* that I know with the uniformly dark body with narrowly yellowish pale posterior margin of elytra. Probably related to *marginalis*-group (Bordoni, 2002).

Talliella laosiana Bordoni, 2013

Material examined – N Laos, Phongsali prov., Boun Tai, 10 km SE, 1300-1500 m, Lao 16-25.V.2004, 2 exx. (cH), 1 ex. (cB).

Distribution – The species was described from the same locality of Laos (Phongsali).

Sulawesina brendelli Bordoni, 2002

Material examined – Sulawesi, Sulawesi Utara, Dumoga Bone N. P., P. Hornabrook XI.1985, 7 exx. (MMUE), 2 exx. (cB).

Distribution – The genus *Sulawesina* Bordoni, 2002 and the cited species seems endemic to Sulawesi (Bordoni, *l. c.).*

Remarks – The specimens bear the label “*Mitomorphus hornabrooki* Last” (in litt.).

Phacophallus flavipennis (Kraatz, 1859)

Material examined – Sri Lanka, Dumballa env., 300 m, J. Kolibac 19.IV-9.V.1991, 1 ex. (NHMB); Malaysia, Cameron Highl., Y. Kiyoyama 24.II.1954, 1 ex. (cH).

Distribution – Indian, Indochinese and Indomalay subregions (Bordoni, 2002).

Phacophallus japonicus (Cameron, 1933)

Material examined – Cambodia, Pailin, 200 m, S. Murzin 11-16.V.2009, 2 exx. (cS), 1 ex. (cB); W Sumatra, Talu, Simpangempat, Sukaminanti, W. Ullrich VII.1992, 4 exx. (NHMB), 1 ex. (cB).

Distribution – From Thailand to Bali and South China (Bordoni, 2002). New record for Cambodia.

Erymus gracilis (Fauvel, 1895)

Material examined – N Laos, Phongsali prov., Boun Tai, 10 km SE, 1300-1500 m, Lao 16-25.V.2004, 1 ex. (cH); N Laos, 20 km NW Louang, Namtha, 21°09.2N, 101° 18.7E, 900± 100 m, E. Jendek & O. Sausa 5-11.V.1997, 2 exx. (cH), 1 ex. (cB); Taiwan, Tai Chung Co., An Ma Shanm, 2 km Chun Lin, Li 24-26.VI.2003, 12 exx. (MNB), 2 exx. (cB).

Distribution – Widespread species, known from the Caspian area and from India to Sumba and South China (Bordoni, 2002), and Taiwan (Bordoni, 2011).

Erymus tujuh sp. n.

Material examined – Holotype ♂: Sumatra, (Jambi), Gunung Tujuh, Kerinci N. P., 1700-1900 m, Bocak & Bucakova 8.III.1991 (NHMB).

Description – Length of body 4.6 mm; from anterior margin of head to posterior margin of elytra: 2.7 mm. Body shiny, reddish brown with slightly darker head. Head ovoid, dilated anteriad, with rounded sides from eyes to the neck. Eyes medium-sized, very protruding. Surface of head with widely spaced, fine punctation. Pronotum dilated anteriad, moderately longer and narrower than head, with oblique anterior margins, rounded anterior angles and sinuate sides. Surface with dorsal series of 6 irregular punctures and lateral series of 3 anterior punctures. Elytra long, longer and wider than pronotum, dilated posteriad, with rounded humeral angles. Surface with superficial, spaced punctation, arranged in three series, one juxtasutural, one median and one lateral. Abdomen with fine punctation, arranged in two series on each segment.

Tergite (of peculiar shape) and sternite of the male genital segment as in Fig. I, 4-5. Aedeagus (Fig. I, 6) 0.77 mm long, ovoid elongate; parameres of peculiar shape; inner sac with distal portion covered with fine spinulae.

Etymology – The specific epithet refers to the type locality, as a noun in apposition.

Distribution – The species is known only from the type locality.

Remarks – This species differs from the congeners by the shape of tergite of the male genital segment, by the shape of parameres and by the inner sac of the aedeagus.

Gyrohypnus maximus Bordoni, 2002

Material examined – Taiwan, Tai Chung Co., An Ma Shanm, 2 km Chun Lin, Li 24-26.VI.2003, 1 ex. (MNB).

Distribution – The species seems endemic to Taiwan (Bordoni, 2002; 2011).

Megalinus metallicus (Fauvel, 1895)

Material examined – "Indochine", Jing Chay, Dassault 16.08, 1 ♂ (NHMB).

Distribution – In the Mountain areas from Pakistan to China and Taiwan (Bordoni, 2002).

Megalinus oculatus (Bordoni, 2002)

Material examined – Taiwan, Tai Chung Co., An Ma Shan, 2 km Chun Lin, Li 24-26.VI.2003, 1 ex. (MNB).

Distribution – This species seems endemic to Taiwan (Bordoni, 2002).

Emathidis humerosa (Bernhauer, 1934)

Material examined – C Vietnam, Tua Thien Hue prov., Bach Ma Nat. Park, surr. Hotel Morin, 1350-1400 m, 16.2N, 107.85E, L. Bartolozzi, G. Chelazzi, A. Bandinelli, S. Bambi & F. Fabiani 23-28.V.2014, 1 ♂ (MZF).

Distribution – This uncommon species was known until now only from China, Sichuan (Bordoni, 2003). The genus *Emathidis* Bordoni, 2007 (= *Cibyra* Bordoni, 2002, nom. preocc.) was described for the species *dilucida* Bordoni, 2002 from S Vietnam (Bao Loc) that differs from *E. humerosa* for the external characters and aedeagus. New record for Vietnam.

Atopolinus ovaliceps (Scheerpeltz, 1965)

Material examined – C Vietnam, Lam Dong prov., Lac Duong Distr., Bidoup Nuiba N. P., surr. Giamg Ly Ranger Station, 1420-1460 m, L. Bartolozzi, G. Chelazzi, S. Bambi, F. Fabiani, E. Orbach & V. Sbordoni 16-21.VI.2015, 2 exx. (MZF), 1 ex. (cB).

Distribution – Distribution. Bhutan, North Myanmar, North Thailand, Malaysia, Yunnan and Guizhou (Bordoni, 2002; 2006). New record for Vietnam.

Atopolinus phongsali Bordoni, 2013

Material examined -. N Laos, Phongsali env., 1300-1500 m, Lao 1-15.V.2004, 6 exx. (cH), 4 exx. (cB); Phongsali prov., Boun Tai, 10 km SE, 1100-1300 m, Lao 16-25.V.2004, 1 ex. (cB).

Distribution – This species is known only from Laos.

Atopolinus tanahrata sp. n.

Material examined – Holotype ♂: Malaysia, W Pahang, Cameron Highl., Tanah Rata, 1500-1800 m, P. Pacholatko 2-26.III.2004 (NHMB).

Description – Length of body 9 mm; from anterior margin of head to posterior margin of elytra: 6 mm. Body shiny, reddish brown with yellowish humeral angles; antennae brown; legs pale yellowish. Head ovoid, with rounded sides from the eyes to the neck. Eyes medium-sized and scarcely protruding. Surface of head with very sparse punctation on the sides. Pronotum moderately longer and evidently wider than head, with very oblique anterior margins, obsolete anterior angles and sinuate sides. Surface with dorsal series of 7-8 fine punctures and lateral series of 4-5 widely, fine punctures. Elytra subquadrangular, a little dilated posteriad, slightly longer and evidently wider than pronotum, with rounded humeral angles. Surface with a series of broad, deep punctures near the suture, one median and one lateral. Abdomen with fine, sparse punctation on the sides.

Sixth visible male abdominal tergite (Fig. II, 1) and sternite (Fig. II, 2) with posterior median lobule; male genital segment (Fig. II, 3) with very small tergite; sternite

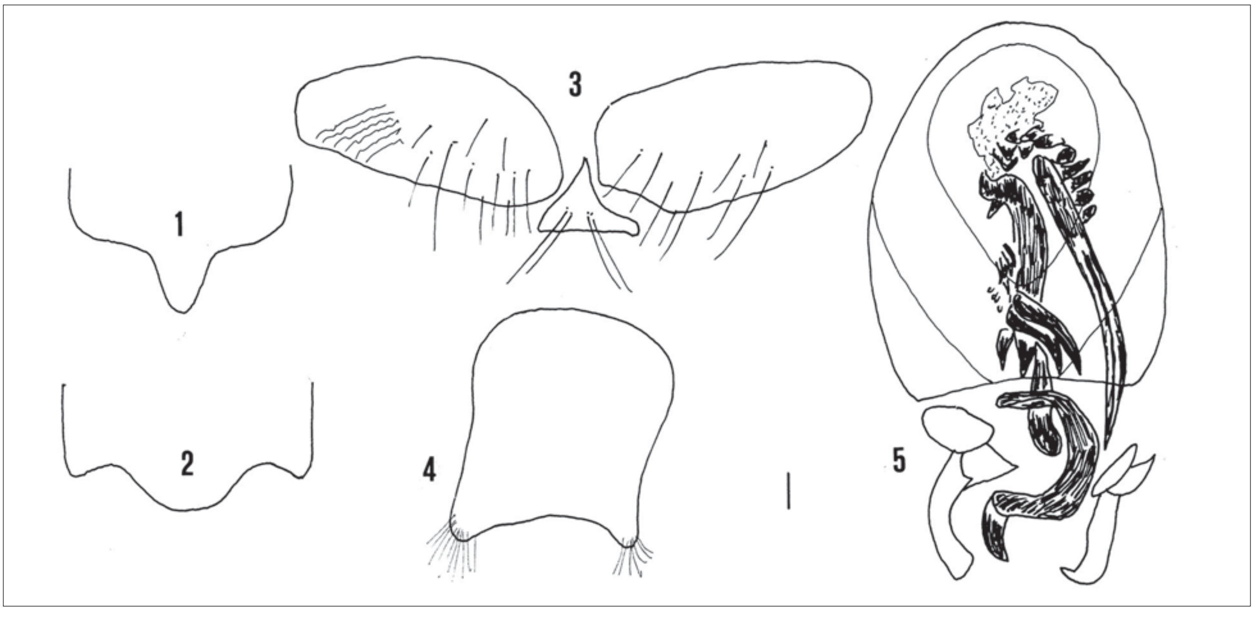

Fig. II – 1. Sixth male tergite, 2. 6th male sternite, 3. male genital segment (enlarged), 4. sternite of the same, 5. aedeagus of *Atopolinus tanahrata* sp. n. (bar scale: 0.1 mm).

of the same type as in Fig. II, 4. Aedeagus (Fig. II, 5) 1.88 mm long, sub-ovoid, distally dilated, with asymmetrical, partially membranous pseudoparameres; inner sac with some large spines.

ETYMOLOGY – The specific epithet refers to the type locality, as a noun in apposition.

DISTRIBUTION – The species is known only from the type locality.

REMARKS – The new species differs from the congeners by the colour of the body and humeral angles, and especially by the structure of pseudoparameres and inner sac of the aedeagus.

Atopolinus nigellus sp. n.

MATERIAL EXAMINED – Holotype ♂: N Vietnam, Vietnam-Laos border area, Quang Binh prov., 1 km N Cha Lo, 400 m, 17°41’22”N, 105°45’45”E, L. Dembicky 11-24.IV.2010 (NHMB); paratypes: same data, 3 ♂, 9 ♀ (NHMB), 3 ♂, 5 ♀ (cB).

DESCRIPTION – Length of body 8.5 mm; from anterior margin of head to posterior margin of elytra: 5 mm. Body shiny, black with yellowish tarsi; antennae brown, long, slender, with 2nd and 3rd segments very long; the following short and narrow. Head sub-rectangular, moderately narrow anteriad, with rounded sides and broadly rounded posterior angles. Eyes small and almost flat. Surface of head with sparse punctation. Pronotum narrow, slender, much narrower and longer than head, with oblique anterior margins and strongly sinuate sides. Surface with dorsal series of 7 deep, fine punctures and lateral series of 4-5 finer punctures. Elytra broad, dilated posteriad, longer and much wider than pronotum, with rounded humeral angles. Surface with fine, not dense punctation, arranged in 5-6 series on elytra, apart the lateral margin without punctation. Abdomen with evident, long, yellowish pubescence and fine, dense punctation on the sides.

Sixth visible male tergite (Fig. III, 1) with short, median, posterior protrusion; 6th visible sternite with concave posterior margin (Fig. III, 2); male genital segment with pleurae laterally provided with a long denticulation (Fig. III, 3); sternite of the same as in Fig. III, 4. Aedeagus (Fig. III, 5) 2.5 mm long, with asymmetrical, short pseudoparameres; inner sac with a series of short spines and two longer spines.

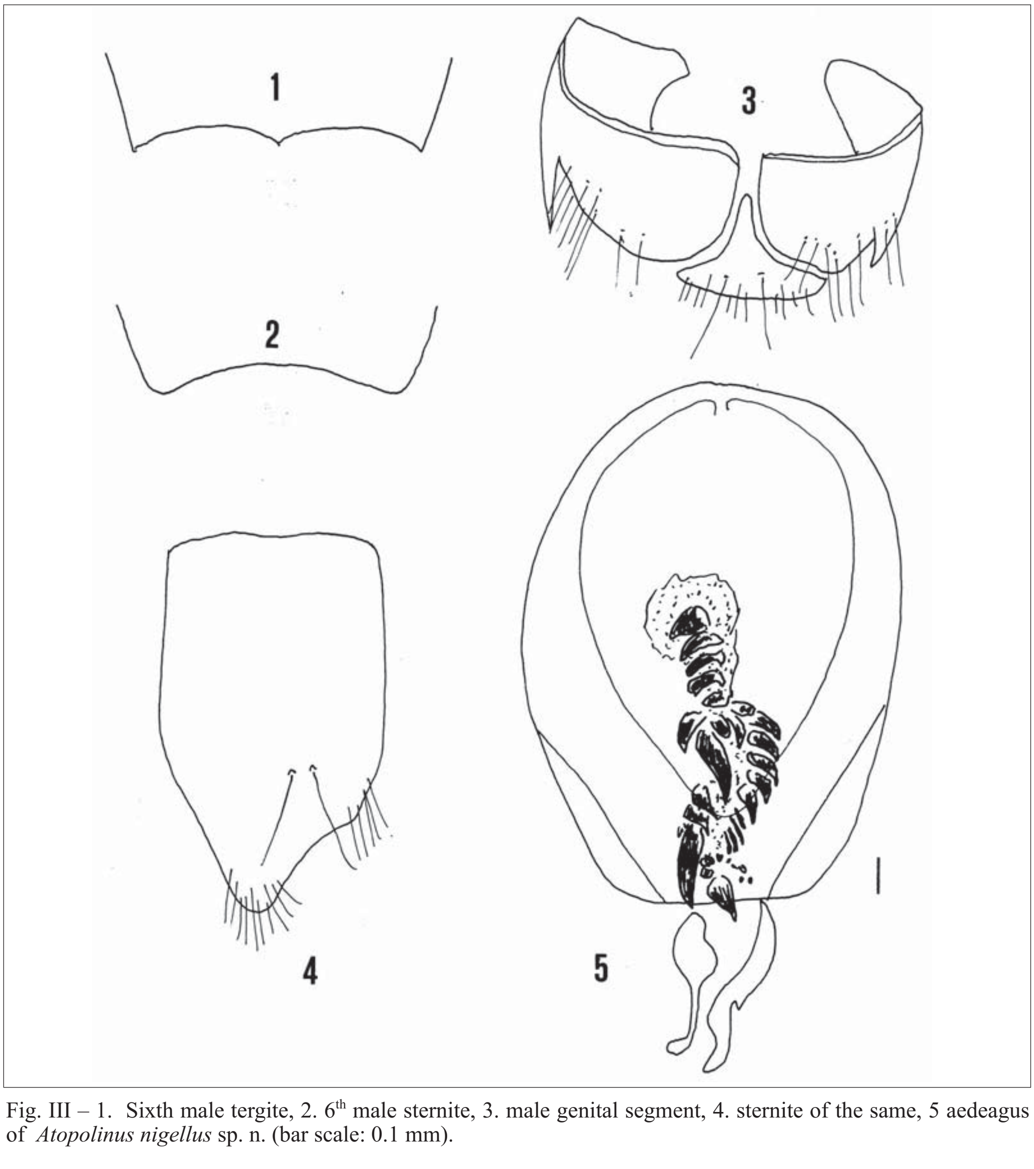

Fig. III – 1. Sixth male tergite, 2. 6th male sternite, 3. male genital segment, 4. sternite of the same, 5 aedeagus of *Atopolinus nigellus* sp. n. (bar scale: 0.1 mm).

Etymology – The specific epithet is the Latin *nigellus-a- um* (black).

Distribution – The species is known only from the type locality.

Remarks – The new species differs from the congeners by the color of body and tarsi, by the shape of the 2nd-3rd antennomeres and especially by the genitalia.

ACKNOWLEDGEMENTS

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CIPRIANO FOXI (*) (°) - CARLO CONTINI (**) - GAVINO DELRIO (*)

CONTRIBUTION TO THE KNOWLEDGE OF BITING MIDGES (DIPTERA CERATOPOGONIDAE) OF SARDINIA, ITALY

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Foxi C., Contini C., Delrio G. – Contribution to the knowledge of biting midges (Diptera Ceratopogonidae) of Sardinia, Italy.

The ceratopogonid fauna of northern Sardinia (Italy) was investigated using black light traps. The study was carried out in six localities from June to August 2015. A total of 3,879 ceratopogonids were collected and 40 species belonging to 8 genera were identified. The most abundant genus was *Culicoides* (96%) followed by *Forcipomyia* (1.5%), *Bezzia* (1.2%) and *Dasyhelea*, *Atrichopogon*, *Alluaudomyia*, *Stilobezzia* and *Brachypogon* (each <1%). Seven new species for Italian fauna were recorded: *Forcipomyia bipunctata*, *Forcipomyia psilonota*, *Culicoides achrayi*, *Culicoides cameroni*, *Culicoides corsicus* and *Bezzia pygmaea*. *Stilobezzia sahariensis* was reported for the first time in Europe.

KEY WORDS: Ceratopogonidae, *Forcipomyia*, *Culicoides*, *Bezzia*, *Stilobezzia*.

INTRODUCTION

Biting midges are small nematoceran dipterans belonging to the Ceratopogonidae family. They are closely related to chironomids and are separated from the latter for the different morphology and biology of adults and for the morphology of the immature stages. The Ceratopogonidae family is divided in 4 subfamilies: Ceratopogoninae, Dasyheleinae, Forcipomyiinae and Leptoconopinae. Over 6,000 species and 110 genera are known worldwide. The most diverse genus is *Culicoides* with over 1,300 species, followed by *Forcipomyia* with 1,150 species (BORKENT, 2014).

Ceratopogonidae larvae are present in many aquatic and semiaquatic habitats and, depending on the species, are phytophagous, saprophagous or zoophagous. Adult males are nectariphagous, while the feeding habits of the females change according to the species. Only the females of *Leptoconops*, *Culicoides* and those belonging to the subgenus *Lasiohelea* of the genus *Forcipomyia* are haematophagous, biting mainly mammals and birds. Some species of these genera are troublesome pests for humans. The more studied genus is *Culicoides*, which includes vectors of several arboviruses of veterinary importance such as Bluetongue Virus, Schmallenberg Virus, African Horse Sickness Virus and Bovine Ephemeral Fever Virus.

BOORMAN *et al*. (1995) in his review of the ceratopogonids checklist of Italy, reported the presence of 99 species of 13 genera. However, 13 species were of uncertain presence and 19 required their regional distribution to be specified. The most diverse genus is *Culicoides*, with 57 species, followed by *Dasyhelea* with 10 species, *Forcipomyia* (6), *Leptoconops*, *Atrichopogon* and *Palpomyia* (5), *Bezzia* (3), *Alluaudomyia* and *Sphaeromias* (2) and *Serromyia*, *Stilobezzia*, *Mallochohelea* and *Probezzia* (1). More recently, SZADZIEWSKI *et al*. (2017), taxonomic specialists of the European Fauna Database, reported 106 species and 12 genera for the Ceratopogonidae Italian fauna.

In Italy, very few surveys were carried out about ceratopogonids, most of which regarding the genus *Culicoides*, in particular those proven and potential vectors of Bluetongue virus, and *Leptoconops*. In Sardinia, only *Culicoides circumscriptus* (COLUZZI and KREMER, 1964) was reported until 2000, when the first epidemics of Bluetongue occurred. Subsequent studies updated the checklist of *Culicoides* reported in Sardinia to 45 species (DELRIO *et al*., 2002; PILI *et al*., 2002; FOXI and DELRIO, 2010, FOXI *et al*., 2011). PILI *et al*. (2002), in a study carried out in southern Sardinia, reported the presence of 15 genera, but identified only *Culicoides* to the species level. Therefore, no species of the Ceratopogonidae genera, except for *Culicoides*, is reported at present.

The main aim of the present study is to collect new data and update the Ceratopogonidae fauna in Sardinia.

MATERIALS AND METHODS

STUDY AREA

The survey was carried out in north-western Sardinia in the province of Sassari, in two areas named Meilogu and Goceano (Fig. I). The former is hilly-mountainous with peaks over 1,200 m. The municipalities involved in the research were: Anela (40°26’ N - 9°03′ E, 446 m slm), Bono (40°24′ N - 9°01′ E, 540 m slm) and Burgos (40°23′ N - 8°59′ E, 561 m slm). The second area has a hilly territory. The three municipalities where the study was conducted are Bonorva (40°25′N - 8°46′E, 508 m slm), Thiesi (40°31′ N - 8°42′ E, 461 m slm) and Torralba (40°31′ N - 8°46′ E, 435 m slm).

CERATOPOGONID SAMPLING

Adults of ceratopogonids were sampled using a miniature CDC black-light trap (G.Z. International, Migliarino, Ferrara, Italy) fitted with a blacklight tube (Philips TL 4W/08) and a

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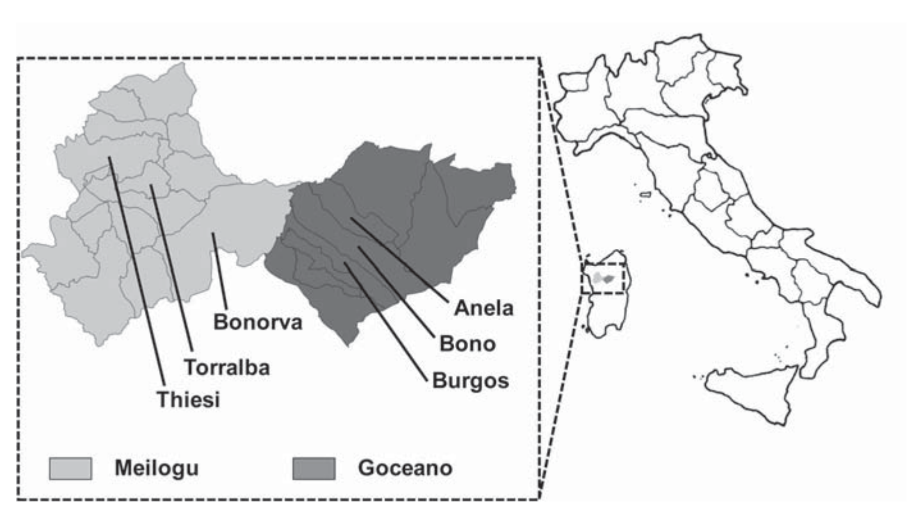

Fig. I – Map of Italy showing the two study areas, Meilogu and Goceano, and the collecting sites in the island of Sardinia.

down-draught suction motor. A 12 V battery was used in the absence of 220 V mains power supply. The CDC black-light trap is light and can be powered by a battery pack, making it very manageable to deploy in remote areas. This trap was preferred to the Ondersterpoort black-light trap much more effective at collecting *Culicoides*.

Black-light traps were set outdoors and operated during the night from dusk until morning.

Sampling was carried out from 15 June to 15 August 2015. The selected sampling sites were mostly farms, animal shelters (sheep and cattle) and orchards close to the towns. Adult ceratopogonids were captured in 36 sampling nights: 7 in Anela, 5 in Bono and 6 in Burgos (Meilogu area), 5 in Bonorva, 5 in Thiesi and 8 in Torralba (Goceano area).

In the laboratory, specimens of biting midges were separated from other insects and preserved in 2% formalin. Ceratopogonids were identified at the genus level with the keys proposed by BOORMAN & ROWLAND (1988) and WIRTH & GROGAN (1988). Subsequently, for a more accurate diagnosis, specimens difficult to classify were mounted on slides in phenol-balsam according to the method of BOORMAN & ROWLAND (1988) and observed under a microscope. The species of *Culicoides* were identified using the keys proposed by KREMER (1965) and DELECOLLE (1985), whereas several keys were used to identify other ceratopogonid species (HAVELKA 1976, 1977, CLASTRIER 1978, SZADZIEWSKI 1992, GHONAIM *et al*. 2001).

RESULTS

A total of 3,879 ceratopogonids were collected during 36 nights, belonging to the following genera: *Alluaudomyia*, *Atrichopogon*, *Bezzia*, *Brachypogon*, *Culicoides*, *Dasyhelea*, *Forcipomyia* and *Stilobezzia*. The number of each species caught is shown in Table 1. Some specimens belonging to *Atrichopogon*, *Dasyhelea* and *Brachypogon* were damaged and impossible to identify at species level.

The most abundant genus was *Culicoides*, with 96% of total adults captured (3,724 specimens) and 30 identified species. The most abundant species of *Culicoides* were: *Culicoides newsteadi* (1,291 adults), *Culicoides brunnicans* (453) and *Culicoides jumineri* (449).

The second most abundant genus was *Forcipomyia*, with 58 specimens belonging to four species. The genus *Bezzia* was represented by two species, while just one species of *Atrichopogon*, *Dasyhelea*, *Alluaudomyia* and *Stilobezzia* was identified.

Seven new species for Italian fauna were found: *Forcipomyia* (*Forcipomyia*) *bipunctata* (Linnaeus) 1767, *Forcipomyia* (*Euprojoannisia*) *psilonota* (Kieffer) 1911, *Culicoides* (*Silvaticulicoides*) *achrayi* Kettle and Lawson 1955, *Culicoides* (*Wirthomyia*) *cameroni* Campbell and Pelham-Clinton 1960, *Culicoides* (*Oecacta*) *corsicus* Kremer, Leberre and Beaucournu-Saguez 1971, *Bezzia pygmaea* Goetghebuer 1920, and *Stilobezzia* (*Acanthohelea*) *sahariensis* Kieffer 1923. Male genitalia and female wing and spermathecae of *Stilobezzia sahariensis* are reported in Fig. II.

DISCUSSION

Excluding the genus *Culicoides*, this survey represent the first study aiming to investigate the species composition of Ceratopogonidae family in Sardinia. An important finding of this survey is the large number of genera found, eight of the 12 reported in Italy. Although the black light trap is the most attractive tool to catch adults of *Culicoides*, both in terms of numbers and species, this type of trap seems to be useful to collect other adult ceratopogonids with nocturnal and/or crepuscular activity. In a similar study on the diversity of Ceratopogonidae family conducted in Spain, GONZALEZ *et al*. (2014) found 15 genera and the *Culicoides* genus represented up to 99% of the total biting midges captured.

With regard to the two new *Forcipomyia* spp. recovered during this study, *F. bipunctata* is certainly the most widespread species, being reported in many European countries including Germany, France, Britain, Spain, Portugal, Switzerland, the Netherlands, whereas *F. psilonota* has been reported in Spain, Andorra, Canary Island and Azores (SZADZIEWSKI *et al*., 2017).

BOORMAN *et al*. (1995) reported 10 species of the genus *Dasyhelea* in Italy, with their distribution needing to be specified. SANNINO & ESPINOSA (2004) reported *D*. (*Dasyhelea*) *bilineata* Goetghebuer, 1920 (= *tecticola* Remmert) updating the checklist to 11 species. The recovery of *D. modesta* (Winnertz) 1852, already reported

Table 1 – Species of ceratopogonids trapped in Goceano and Meilogu areas in Sardinia, Italy, in June-August 2015. Number of collections are in brackets.
§ Denotes new records to Europe. ‡ Denotes new records to Italy and Sardinia. * Denotes new records to Sardinia, although it had been previously found in mainland Italy.

Subfamily	**Tribe**	**Species**	**Anela (7)**	**Bono (5)**	**Burgos (6)**	**Bonorva (5)**	**Thiesi (5)**	**Torralba (8)**	**Total**
Forcipomyiinae		*Atrichopogon brunnipes* *		2		2	1		**5**
		Atrichopogon spp.				4	1	1	**6**
		Forcipomyia bipunctata ‡	2	1	1	1	5	6	**16**
		Forcipomyia brevipennis *	3	2		1		2	**8**
		Forcipomyia nigra *		3	3	3	7	3	**19**
		Forcipomyia psilonota ‡	1		3	1	4	6	**15**
Dasyheleinae		*Dasyhelea modesta* *	11	1	2	2	2		**18**
		Dasyhelea spp.	5	2		1		2	**10**
Ceratopogoninae	Culicoidini	*Culicoides achrayi* ‡				2	6	5	**13**
		Culicoides begueti					4		**4**
		Culicoides brunnicans	453						**453**
		Culicoides cameroni ‡					1		**1**
		Culicoides cataneii	45	78	3	3	47	25	**201**
		Culicoides circumscriptus	45	81	159	3	59	25	**372**
		Culicoides corsicus ‡	2						**2**
		Culicoides derisor					16		**16**
		Culicoides festivipennis	1	38		1	11	14	**65**
		Culicoides gejgelensis		1		1	5	8	**15**
		Culicoides haranti	1	4					**5**
		Culicoides imicola	2	14	32		2		**50**
		Culicoides jumineri	104	142	64	8	114	17	**449**
		Culicoides kibunensis	2	2			2	1	**7**
		Culicoides kurensis	5	1	15	3	48	17	**89**
		Culicoides longipennis			3	1	22	4	**30**
		Culicoides lupicaris						1	**1**
		Culicoides newsteadi	222	46	134	202	427	260	**1291**
		Culicoides obsoletus	22	30	16	4	17	9	**98**
		Culicoides odiatus	1	18	1		32	3	**55**
		Culicoides paolae		1		2	11	4	**18**
		Culicoides parroti				2		5	**7**
		Culicoides pulicaris	6	7	39	21	6	63	**142**
		Culicoides punctatus	9	31	34	32	24	67	**197**
		Culicoides puncticollis		1			19	30	**50**
		Culicoides sahariensis		60		1	15		**76**
		Culicoides scoticus			2			1	**3**
		Culicoides subfagineus			7				**7**
		Culicoides subfasciipennis	5				1		**6**
		Culicoides truncorum			1				**1**
	Ceratopogonini	*Alluaudomyia meridiana* *			3	4		2	**9**
		Brachypogon spp.					1		**1**
		Stilobezzia sahariensis §		2					**2**
	Palpomyiini	*Bezzia ornata* *	2	2	6	5		1	**16**
		Bezzia pygmaea ‡	1	5		5	8	11	**30**
		Total	**950**	**575**	**528**	**315**	**918**	**593**	**3879**

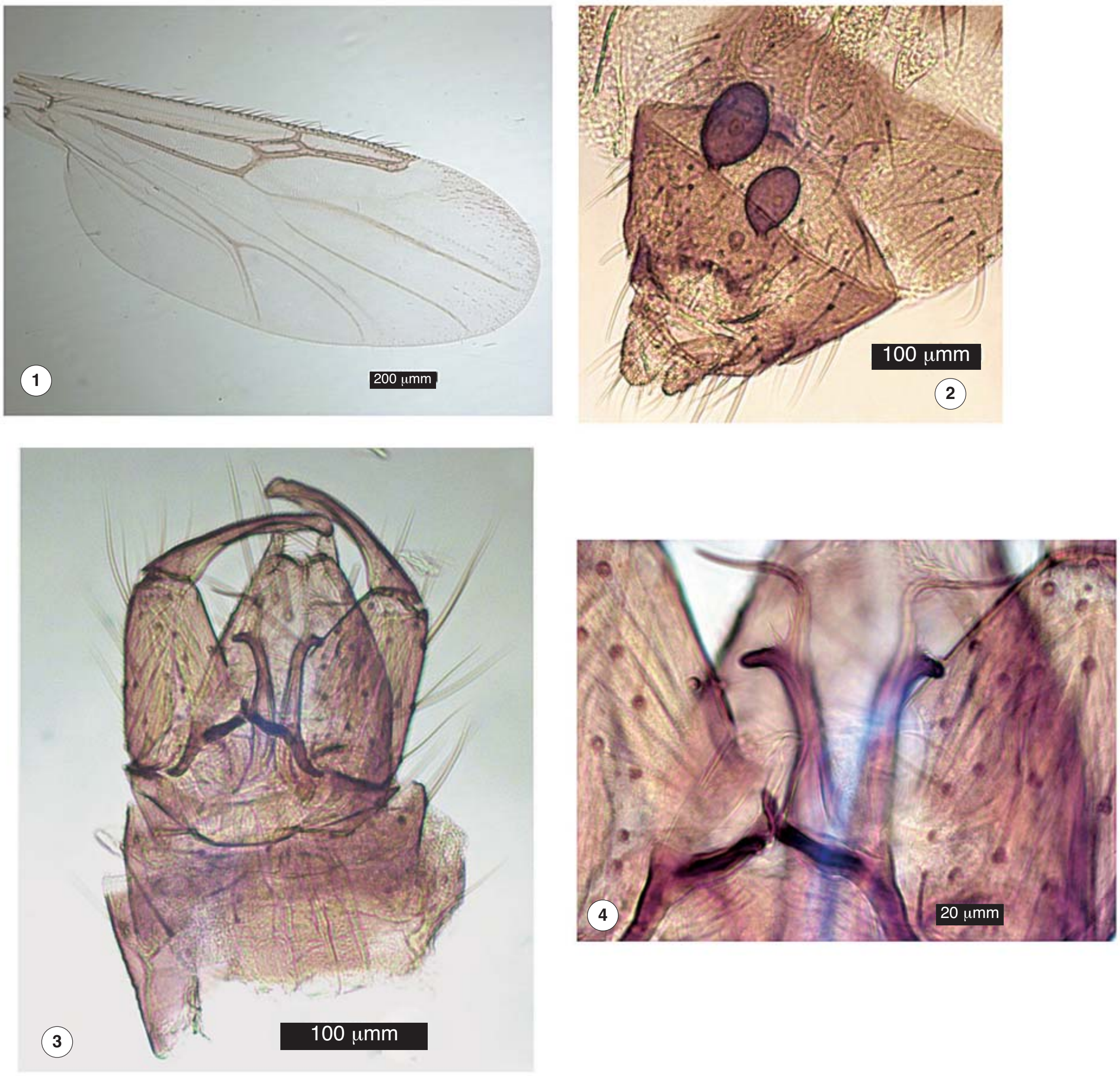

Fig. II – *Stilobezzia sahariensis*. Female (1, 2): 1 - wing, 2 - spermathecae. Male (3, 4): 3 - ventral view of male genitalia, 4 - aedeagus and parameres.

in Italy as *D. aestiva* (Winnertz) 1852, confirms its presence in Sardinia and Italy.

The genus *Culicoides* is a well-known group of Diptera including species of medical and veterinary importance. The females are haematophagous and some *Culicoides* are important vectors of viruses, protozoa and filariae that cause major diseases in mammals such as sheep, cattle and horses and even humans (BRAVERMAN and GALUN, 1973). The Italian checklist of *Culicoides* reported 57 species (BOORMAN *et al*., 1995) and has been recently updated to 66 following the studies of FOXI & DELRIO (2010) and FOXI *et al*. (2011) in Sardinia. The three species recovered in this study bring the number of *Culicoides* species in the Italian and Sardinian fauna to a total of 69 and 48, respectively.

The genus *Alluaudomyia* and *Brachypogon* are poorly known. The first genus is present in Italy with three species: *A. bertrandi* Harant & Cellier 1949, *A. hygropetrica* Vaillant 1954 and *A. meridiana* Clastrier 1978 (SZADZIEWSKI *et al*., 2017); the latter species is the only one found during our observations. None species belonging to the *Brachypogon* genus is reported in Italy. PILI *et al*. (2002) reported the presence of this genus in Sardinia without specifying the species. The lack of adequate identification keys makes the determination impossible at the species level.

The Italian fauna reports only one species of *Stilobezzia*: *S. flavirostris* (Winnertz) 1852. The species found during this survey (*S. sahariensis*), a species from North Africa, is reported for the first time in Europe. In Italy, the genus *Bezzia* is represented by four species. During our observations, we recovered two species, one of which, *B. pygmaea*, new for Italy.

This study represents a first significant contribution to the knowledge of Ceratopogonidae in Sardinia. Despite the biting midges were collected only during a short period in summer and using only a kind of trap, the number of specimens (3,879) and species (40) was relatively high. Future surveys will also employ other type of traps, such as CO_2-baited trap and Malaise trap, very efficient in collecting biting midges with diurnal activity, and emergence and white light trap.

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DISCOVERY OF *ALLONYX QUADRIMACULATUS* (SCHALLER, 1783) (COLEOPTERA CLERIDAE CLERINAE) IN RUSSIA

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Ruchin A.B., Egorov L.V. – Discovery of *Allonyx quadrimaculatus* (Schaller, 1783) (Coleoptera Cleridae Clerinae) in Russia.

The paper presents data on a record of *Allonyx quadrimaculatus* (Schaller) in the Republic of Mordovia (Russia, Central European Territory) and also the whole known information about specimens of the species stored in Russian museums. We discuss the species' distribution which is considered as intermittent. Data on biology of *Allonyx quadrimaculatus* and description of its newly found habitat are presented.

KEY WORDS*:* Coleoptera, Cleridae, Clerinae, *Allonyx quadrimaculatus*, new record, Mordovia, Russia.

Cleridae is a family of predatory beetles with a cosmopolitan distribution (GERSTMEIER & EBERLE, 2011; OPITZ, 2010). Most are predatory as adults and larvae, including some (e.g., *Thanasimus* Latreille, *Enoclerus* Gahan) that are important in controlling outbreaks of forest pests such as bark beetles (Curculionidae: Scolytinae) (GAYLORD *et al.,* 2006; BOUCHARD *et al.,* 2017). The world fauna of the Cleridae contains over 3400 valid species and subspecies (BOUCHARD *et al.,* 2017). The Palearctic fauna of the family is presented by about 215 species (GERSTMEIER, 1998; LÖBL *et al.,* 2007; GERSTMEIER, 2014). In the fauna of Russia there are no less 35 species from 19 genera (RICHTER, 1961; LÖBL *et al.,* 2007).

Allonyx Jacquelin du Val is a monotypic clerid genus, belonging to the subfamily Clerinae; the single species being *A. quadrimaculatus* (Schaller). Prior to its discovery, RICHTER (1961) had supposed that *A. quadrimaculatus* might occur in the south-western regions of USSR adjoining Europe, however, the first Russian records of *A. quadrimaculatus* were presented by specimens collected in the Bor and Vyksa districts of the Nizhniy Novgorod region (ANUFRIEV, 2004; MOKROUSOV, 2008). This species has been included in the Red Data Book of the Nizhniy Novgorod region (2014).

During our entomological research in 2017, a new location of the species in Russia was discovered in the Mordovia State Nature Reserve; its specimens are stored in the collections of Zoological Institute of the Russian Academy of Sciences (St. Petersburg, Russia) (ZIN) and of Zoological Museum of Lomonosov Moscow State University (Moscow, Russia) (ZMMU).

Allonyx quadrimaculatus (Schaller, 1783)

MATERIAL EXAMINED – Russia: Nizhniy Novgorod region, Vyksa district, Vyksa town, Vyksunskiy forestry, lower warehouse, 20.VI.2001, 1 ex., collected by Kozin, Mokrousov (ZMMU). Republic of Mordovia, Temnikov district, 17 km N Temnikov city, 54°45.65′ N, 43°08.18′ E, Mordovia State Nature Reserve, quarter 354, 20.V.2017, 1 ex., collected by A.B. Ruchin (stored in ZIN) (Fig. 1). One exemplar collected in the Nizhniy Novgorod region is stored in ZIN collection. Greece, 1 ex., without any else data (ZMMU). Moravia (Brno), 1 ex., Formanek, without any else data (ZMMU).

DISTRIBUTION (Fig. II) – Austria, Belgium, Croatia, Cyprus, Czech Republic, France, Germany, Greece, Hungary, Italy, Macedonia, Netherlands, Poland, Portugal, Romania, Slovakia, Spain, Switzerland, Turkey (SCHMIDL, 1997; MAJZLAN *et al.,* 1999; BAHILLO DE LA PUEBLA & LÓPEZ-COLÓN, 2006; LÖBL *et al.,* 2007; KURZELUK, 2012; SARIKAYA & IBIS, 2016), Russia (Central European Territory).

The range of *A. quadrimaculatus* shows a fragmented distribution with two main continuous part of the species' range located in Western and Central Europe. The Romanian record might represent an isolated surviving population within what might constitute its earlier range across most of Europe. In contrast, the range of species within Russia has a local character, and to date it is limited to the central part of European Russia.

Over the last 30 years, publication of range extension records for *A. quadrimaculatus* throughout Europe has increased in frequency (SCHMIDL, 1997; MAJZLAN *et al.,* 1999; JAŁOSZYŃSKI *et al.,* 2005; BAHILLO DE LA PUEBLA & LÓPEZ-COLÓN, 2006; SANCHEZ *et al.,* 2015; LÓPEZ VERGARA *et al.,* 2017). According to the map presented by KURZELUK (2012), the range of *A. quadrimaculatus* includes Slovenia, although we did not find any confirmation of this information. KURZELUK (2012) did also not include Greece in the species' range despite earlier inclusion of Greece in the species' range by LÖBL *et al.* (2007), which we confirmed by specimens deposited in the Zoological Museum of Lomonosov Moscow State University. Thus, to date there are three locations of this species in Russia: two records from the Nizhniy Novgorod region and one in the Republic of Mordovia. We predict that the distribution of *A. quadrimaculatus* in Russia may

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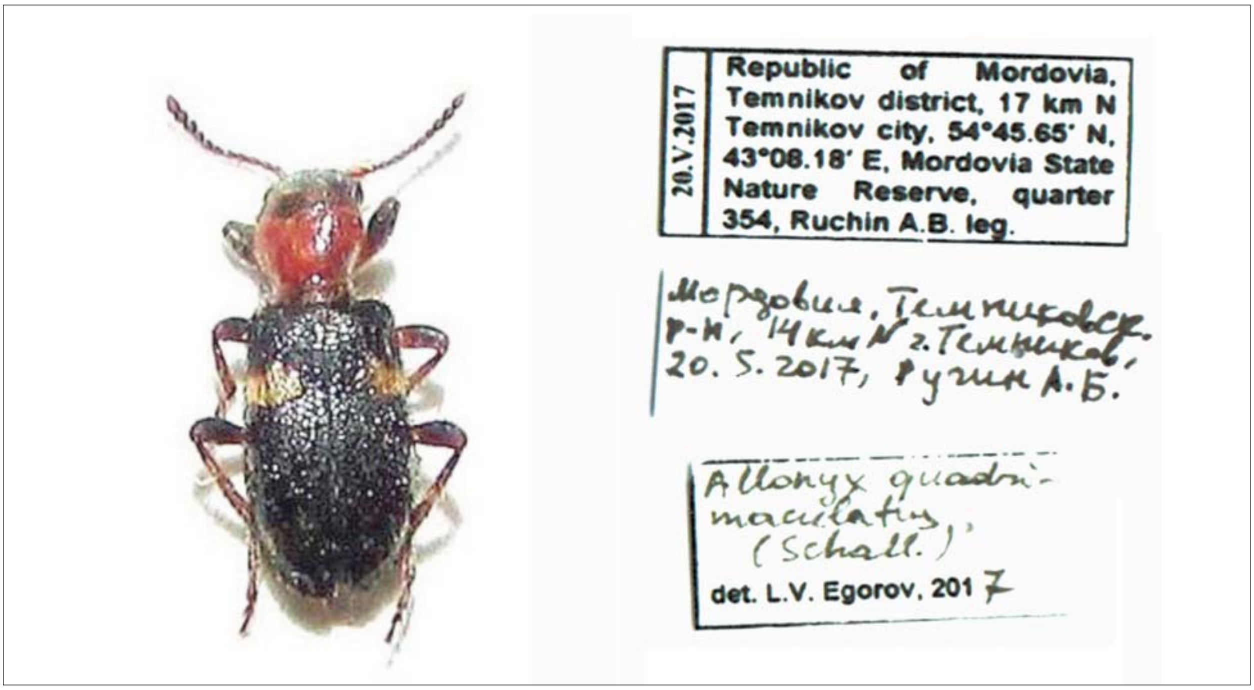

20.V.2017

Republic of Mordovia, Temnikov district, 17 km N Temnikov city, 54°45.65' N, 43°08.18' E, Mordovia State Nature Reserve, quarter 354, Ruchin A.B. leg.

Мордовия, Темниковск. р-н, 14 км N г. Темников, 20. 5. 2017, Ручин А.Б.

Allonyx quadrimaculatus, (Schall.)
det. L.V. Egorov, 2017

Fig. I – *Allonyx quadrimaculatus* and labels of the collected specimen.

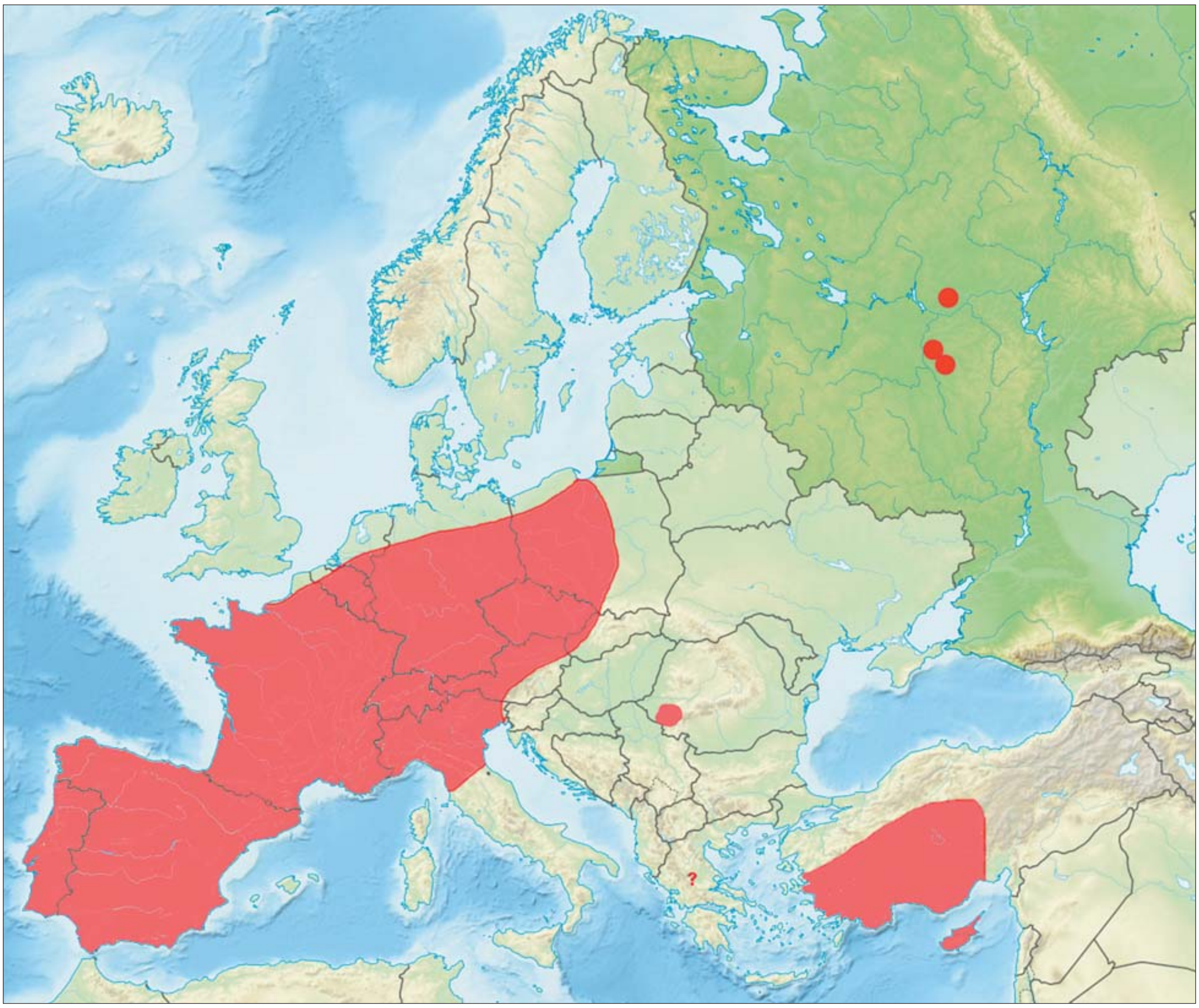

Fig. II – Map of the *Allonyx quadrimaculatus* distribution in Europe.

include the Kaliningrad region, bordering Poland, and likely also in regions adjacent to the Nizhniy Novgorod region and the Republic of Mordovia.

Biotopes – The habitat of the collection sites of *A. quadrimaculatus* was significantly damaged by wildfire in 2010, and moderately vigorous surface fire has been observed in the area since. Despite extensive damage to the habitat, some of mature pine trees have remained. Originally the floristical composition of the newly discovered site was pine forest plant community (*Pinus sylvestris* L.) with green mosses (*Pleurozium schreberi* Mitt., *Climacium dendroides* (Hedw.) F. Weber & D. Mohr, and less commonly *Dicranum polysetum* Sw.), in the ground layer. Most shrub, herb and ground layer plants were damaged by wildfire during in 2010 (Khapugin *et al.,* 2016a). In 2017 an undergrowth of secondary growth trees (*Betula pendula* Roth, *Populus tremula* L.) covered most of the study site area. Among other trees and shrubs *Acer platanoides* L., *Tilia cordata* Mill., *Sorbus aucuparia* L., *Quercus robur* L., *Rubus nessensis* Hall were represented by six-year plants or their stool shoots. *Pteridium aquilinum* (L.) Kuhn (about 15%), *Viola rupestris* F.W. Schmidt (about 10%) and *Convallaria majalis* L. (about 5%) were the most abundant herb layer plants at the study site. Other species of the herb layer included *Chamaecytisus ruthenicus* (Fischer ex Woloszczak) Klásk., *Genista tinctoria* L., *Calamagrostis epigejos* (L.) Roth, *Rumex acetosella* L., *Epilobium angustifolium* L., *Stellaria media* (L.) Vill., *Erigeron canadensis* L., *Milium effusum* L., *Rubus saxatilis* L., *Chelidonium majus* L., *Solidago virgaurea* L., *Lactuca serriola* L., *Hieracium umbellatum* L., *Polygonatum odoratum* (Mill.) Druce., *Melampyrum pratense* L. (Shugaev *et al.,* 2015; Khapugin *et al.,* 2016b).

Bionomics – *A. quadrimaculatus* is a thermophilous species inhabiting trunks and bark of *Pinus* and *Picea* trees (Kühnel & Mai, 1985; Mokrousov, 2008; Peres-Otero *et al.,* 2009; Sarikaya & Ibis, 2016; López Vergara *et al.,* 2017). The chorology (Taglianti *et al.,* 1992) (European-Mediterranean chorotype) and the range limits of *A. quadrimaculatus* in the Western Palaearctic coincides with those of *Pinus* but the clerid species can be found only in the parts of the tree species range where the mean annual temperatures approaches to sub-Mediterranean values. In Spain, the species is known in mesophilous habitats (Gómez De Dios *et al.,* 2015). *Allonyx quadrimaculatus* is a predator of various larvae and imago of xylophagous insects plus flatbugs of the genus *Aradus* Fabricius (Richter, 1961; Majzlan *et al.,* 1999; Peres-Otero *et al.*, 2009; Sanchez *et al.,* 2015).

The newly found location of *A. quadrimaculatus* in the Republic of Mordovia is not a first unique record of a Coleopteran species in the Mordovia State Nature Reserve. As a result of active investigations of this protected area in recent years, the ranges of a number of Coleoptera species have been specified (Legalov *et al.,* 2014; Egorov & Shapovalov, 2017; Ruchin & Egorov, 2018; Tomaszewska *et al.,* 2018). Such numerous and interesting findings indicate and underline the uniqueness of the forest massif of the Mordovia State Nature Reserve.

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NEW RECORDS OF MOTHS ELUCIDATE THE IMPORTANCE OF FORESTS AS BIODIVERSITY HOT-SPOTS IN CENTRAL MEDITERRANEAN LANDSCAPES (LEPIDOPTERA)

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Greco S., Ienco A., Infusino M., Leonetti F.L., Scalercio S. – New records of moths elucidate the importance of forests as biodiversity hot-spots in central Mediterranean landscapes (Lepidoptera).

In this paper we report new faunistic findings concerning 15 moth species collected in forested habitats of Calabria and Sicily regions, South Italy. Most interesting records concerned *Eupithecia trisignaria* and *Orectis proboscidata,* both recorded for the first time in southern Italy. Species with larval biology linked to the forest cover were locally common, whilst species linked to herbaceous plants and shrubs were often collected as singletons or in one locality, showing smaller populations. A study of a 658bp long sequence of the mitochondrial 5' cytochrome oxidase gene, subunit 1 (COI) (barcoding analysis) was performed for five species, two of them showing a divergence from closest populations near to 1%, one a 2% divergence from northern populations, and two other species a perfect identity with European populations. This study reinforced the role of forest habitats as biodiversity hot-spots in the Mediterranean Basin and the importance of the Italian peninsula for biodiversity conservation at European scale as an increased number of endemic or sub-endemic taxa and populations with endemic genetic lineages are recognized, underlining the existence of ongoing evolutionary processes. In the light of these results, faunistic surveys in forest ecosystems are strongly needed to define sustainable management strategies.

KEY WORDS: barcoding, faunistic inventories, forest management, protected areas, Italy.

INTRODUCTION

Ecological sustainability of forests is nowadays one of the main goal of silvicultural management, as they provide several ecosystem services among which biodiversity conservation is increasingly emphasized (LINDENMAYER *et al.*, 2000). Forests represent the climax stage of vegetation for the largest portion of Italian peninsula, but human activities largely fragmented their cover for thousands of years, increasing the proportion of non-forested habitats. Recently, human activities in interior areas were abandoned and, as a consequence, forested surfaces increased on large territories with consequences on biodiversity (MARULL *et al.*, 2015) and on the economic profitability due to an increased wood availability. Then, it is very important in this phase to take into account both conservation and economic interests in order to identify areas with different vocations and to favour a sustainable management of forests. Timber production is nowadays mostly concentrated within mountain forests, particularly vulnerable ecosystems as the increasing frequency of extreme climatic events due to climatic changes can strongly affect tree physiology (KRAMER *et al.*, 2000; LINDNER *et al.*, 2010). Furthermore, recent studies underlined the importance of Mediterranean forest as biodiversity reservoirs (WALENTOWSKI *et al.*, 2014; INFUSINO *et al.*, 2016; 2017a), but how really important they are is not clear enough as several forest types and their latitudinal variations are not systematically investigated. Under these perspectives, biodiversity inventories become a basic data for identifying areas with a prominent conservation interest and areas that can be designed primarily for timber production.

Indicator taxa are useful tools for exploring biodiversity patterns saving time and money. Among them, the order of Lepidoptera was often used as a bioindicator of forested habitats as it represents a large portion of the total animal biomass and diversity, occupying many crucial ecological niches (SUMMERVILLE *et al.*, 2004; SUMMERVILLE and CRIST, 2008). In Italy, biodiversity inventories for Lepidoptera are now available for several areas of conservation interest, e.g. the Parco Naturale delle Capanne di Marcarolo in Piedmont (BALDIZZONE *et al.*, 2013), the Tenuta Presidenziale di Castelporziano in Latium (ZILLI *et al.*, 2001), the silver fir forest of Rosello between Abruzzi and Molise regions (SCIARRETTA and ZAHM, 2002), the silver fir forest of Serra San Bruno and the Vivaio Sbanditi and in Calabria (INFUSINO and SCALERCIO, 2017; INFUSINO *et al.*, 2017b) and many more (see ZILLI, 2014; 2018). Unfortunately, methods and sampling efforts were usually not comparable across studies and only in few cases exact locations of sampled sites were provided, making it difficult to compare quantitative results and to repeat the monitoring over time. Nevertheless, faunistic lists provided by these inventories were enough to demonstrate that Mediterranean forests, especially mountain ones, are particularly important from a conservation point of view as they represent very important biodiversity hot-spots for Lepidoptera, inhabiting endemic species, relict populations of Euro-Asiatic species, populations with unique genetic lineages (SCALERCIO *et al.*, 2016; INFUSINO *et al.*, 2016, 2018).

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This paper would be a further contribution toward a better understanding of the role of forest ecosystems as biodiversity reservoirs. We reported the presence of species with high biogeographic and conservation interest noted during surveys carried out systematically in mountain forests and occasionally in other habitats of Calabria and Sicily, the southernmost Italian regions positioned in the middle of the Mediterranean Basin. The presence of these species was discussed from biogeographic and conservation perspectives, and the importance of a sustainable forest management was also underlined.

MATERIAL AND METHODS

Results showed in this paper were collected from systematic and occasional surveys of moths in different forested habitat of Calabrian region, southern Italy.

Systematic surveys were devoted to characterize moth community of a given forest type in order to assess functional relationships of forest attributes, such as tree species composition, management, age, with moth diversity and abundance. This kind of survey was carried out by the authors in several forest type during last years (Greco *et al.*, 2016; Infusino *et al.*, 2016; 2017a). In this paper we synthesized the most interesting biogeographic results obtained during the last year of monitoring within beech forests and *Acer*-dominated forests included in the Pollino National Park, Calabrian black pine forests and *Alnus*-dominated woodlots included in the Sila National Park, and mixed forests of both protected areas. Samplings were carried out using UV LED light traps (Infusino *et al.*, 2017c) powered by portable batteries. Traps were settled up one night per month through the entire flight season of moth at mountain altitude, under weather conditions favourable to moth activity and trap efficiency, i.e. low wind speed, temperatures near the average of a given period, low or absent moonlight, low or absent rain.

Occasional surveys interested Calabria and Sicily regions. In Calabria we surveyed a clearing within broadleaved forests in the Pollino National Park by diurnal visual census and a sparse Mediterranean maquis along the Ionian coast using a 160W mercury vapor lamp as light source for a moth trap. In Sicily we surveyed a *Taxus baccata*-dominated forest on the Nebrodi Mountains using a 160W mercury vapor lamp reflecting on a white sheet for attracting moth.

Detailed data concerning sites and nights of both kind of surveys were reported in the List of species section.

Identification was carried out using specialised literature concerning Lasiocampidae (Bertaccini *et al.*, 1995), Geometridae (Hausmann, 2001, 2004; Mironov, 2003; Hausmann and Viidalepp, 2012), Erebidae (Berio, 1991; Bertaccini *et al.*, 1995, 1997), and Noctuidae (Berio, 1985). Identification of most difficult species was carried out after dissection of genitalia. Nomenclature followed the Fauna Europaea available online at www.faunaeur.org. Specimens and slides of genitalia were preserved in the Lepidoptera collection of the Research Centre for Forestry and Wood, Rende, Italy.

Few specimens were submitted to barcoding, an analysis of the 658bp long sequence of the mitochondrial 5' cytochrome oxidase gene, subunit 1 (COI), submitting tissue samples to the standard procedures of the Canadian Centre for DNA Barcoding (CCDB). This sequence, considered a standard marker for species identification of most animals, allowed us to search for genetic divergences of southern Italy population of analysed species from other European populations, by comparing sequences deposited in the database of Barcoding of Life Database (BOLD) data systems (Ratnasingham and Hebert, 2007). For any barcoded specimen we reported the following genetic data: BOLD specimen ID, BOLD sequence ID, Barcode Index Number (BIN), sequence length, genetic similarity with the nearest sequence deposited in BOLD and its collecting site.

RESULTS

A total of 15 species of biogeographic interest has been collected during our study: seven Geometridae, one Lasiocampidae, three Erebidae, four Noctuidae (Fig. I). Among them, eight species were collected as singletons and further two in only one locality, whilst the others were collected in more than one locality. The *Acer*-dominated forest provided nine species new for the Calabria region, whilst beech, Calabrian black pine, *Alnus glutinosa*, *Taxus baccata*, and mixed forests provided one species each new for the regional fauna. Remaining species were collected in a clearing within broadleaved forests and in a sparse Mediterranean maquis.

Five specimens belonging to five species were submitted to DNA barcoding analyses, recovering four full-length sequences (658bp) and a somewhat shorter one (630bp).

List of species

Poecilocampa populi (Linnaeus, 1758) (Lasiocampidae) (Fig. I, 1)

Habitat – Beech forest of the Pollino National Park.

Records – Serrapaolo, Saracena (CS), 990m, 39.8225°N-16.0911°E, 18.XI.2015 (1m) (dissected, slide CREA-0182, Fig. II, 1); Serra Ambruna, Saracena (CS), 1035m, 39.8234°N-16.0768°E, 23. XI.2017 (1m); Timpone Magara, Saracena (CS), 1460m, 39.7914°N-16.0503°E, 23.XI.2017 (1f); idem, 1465m, 39.7939°N-16.0520°E, 23.XI.2017 (1f).

Alsophila aceraria (Denis & Schiffermüller, 1775) (Geometridae) (Fig. I, 2)

Habitat – Calabrian black pine forest of the Sila National Park.

Records – Serra Cannile, Spezzano Sila (CS), 1433m, 39.3465°N-16.4091°E, 24.XI.2017 (1m).

Emmiltis pygmaearia (Hübner, 1809) (Geometridae) (Fig. I, 3)

Habitat – Clearings in broadleaved forests of the Pollino National Park.

Records – Timpone Montillo, Alessandria del Carretto (CS), 960m, 39.9578°N-16.3754°E, 19.V.2017 (1m, 1f).

Cyclophora albiocellaria (Hübner, 1789) (Geometridae) (Fig. I, 4)

Habitat – *Acer* forest of the Pollino National Park.

Records – Balestrieri, Alessandria del Carretto (CS), 1371m, 39.9275°N-16.3624°E, 21.VI.2017 (1m), 17.VIII.2017 (1f); Difesa di Privitera, Alessandria del

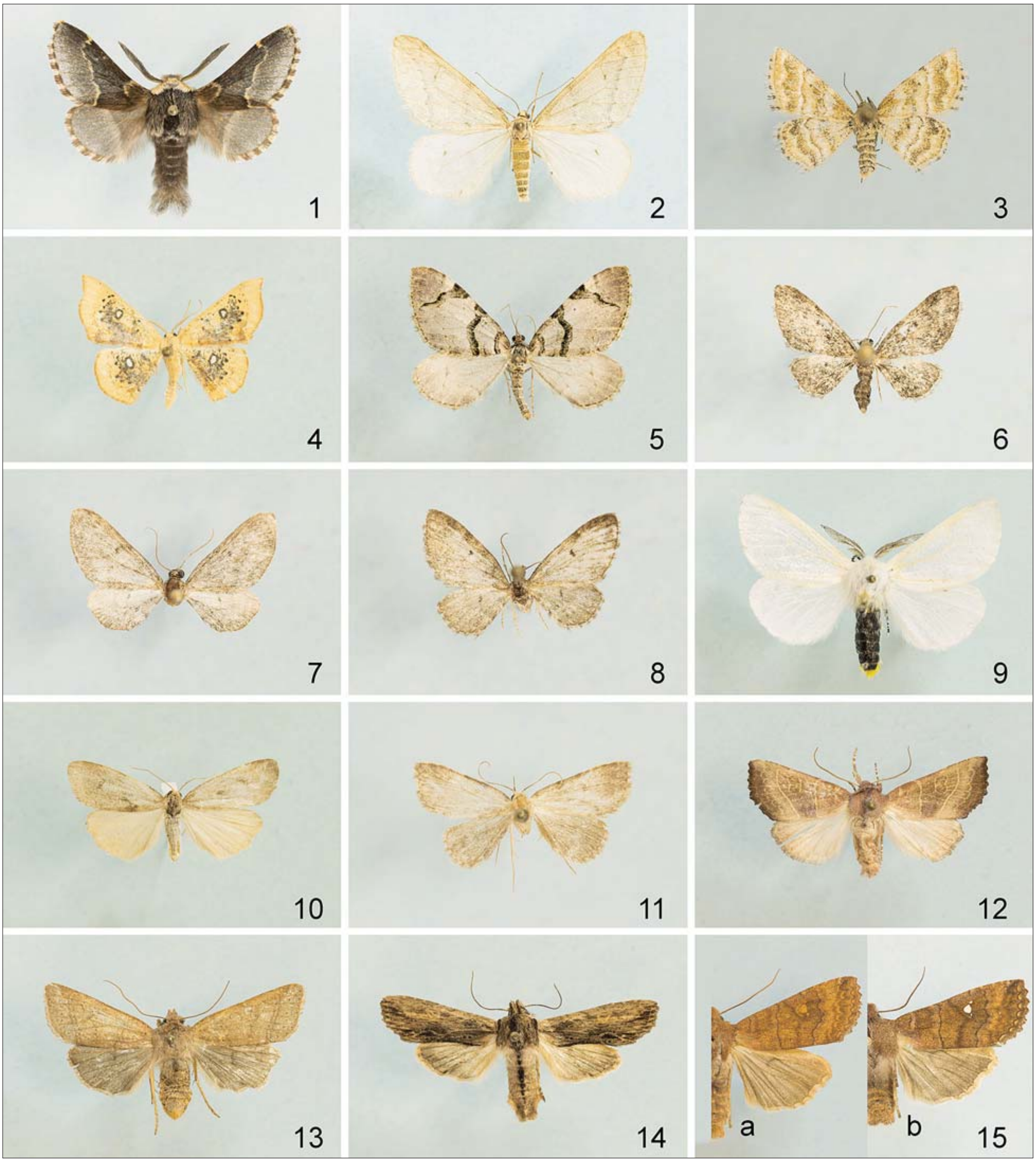

Fig. I – Habitus of species: *Poecilocampa populi* (Linnaeus, 1758), Serra Ambruna (1); *Alsophila aceraria* (Denis & Schiffermüller, 1775), Serra Cannile (2); *Emmiltis pygmaearia* (Hübner, 1809), Timpone Montillo (3); *Cyclophora albiocellaria* (Hübner, 1789), Sciortaglie (4); *Anticlea derivata* (Denis & Schiffermüller, 1775), Sciortaglie (5); *Eupithecia inturbata* (Hübner, 1817), Difesa di Privitera (6); *Eupithecia denotata* (Hübner, 1813), Vallone Lupara (7); *Eupithecia trisignaria* Herrich-Schäffer, 1848, Epicate (8); *Leucoma salicis* (Linnaeus, 1758), Sciortaglie (9); *Apaidia rufeola* (Rambur, 1832), Palizzi Marina (10); *Orectis proboscidata* (Herrich-Schäffer, 1851), Vallone Lupara (11); *Atethmia ambusta* (Denis & Schiffermüller, 1775), Difesa di Privitera (12); *Agrochola orejoni* Agenjo, 1951, Balestrieri (13); *Lithophane semibrunnea* (Haworth, 1809), Sciortaglie (14); *Eupsilia transversa* (Hufnagel, 1766), Bosco della Tassita (15a), Balestrieri (15b).

Carretto (CS), 1314m, 39.9269°N-16.3563°E, 17.VIII.2017 (2m); idem, 1285m, 39.9274°N-16.3572°E, 17.VIII.2017 (1m); Sciortaglie, Alessandria del Carretto (CS), 1253m, 39.9291°N-16.3532°E, 17.VIII.2017 (1m, 1f); idem, 1246m, 39.9313°N-16.3508°E, 17.VIII.2017 (1m, 2f); Tappaiolo, Alessandria del Carretto (CS), 1253m, 39.9358°N-16.3471°E, 17.VIII.2017 (1m).

Anticlea derivata (Denis & Schiffermüller, 1775)
(Geometridae) (Fig. I, 5)

Habitat – *Acer* forest of the Pollino National Park.

Records – Sciortaglie, Alessandria del Carretto (CS), 1246m, 39.9313°N-16.3508°E, 18.IV.2017 (1m).

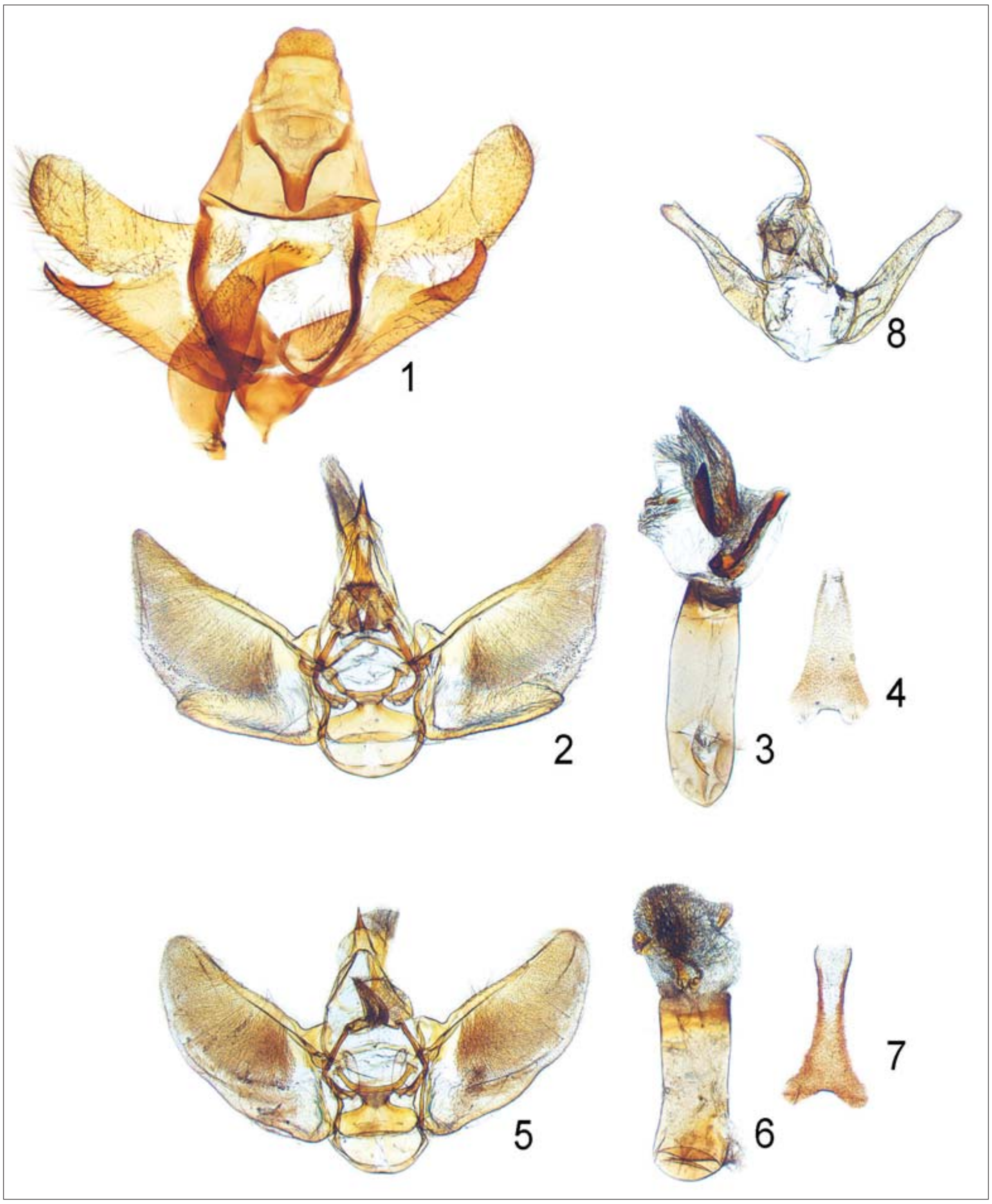

Fig. II – Male genitalia: (1) *Poecilocampa populi* (Linnaeus, 1758), slide CREA-0182; (2) *Eupithecia denotata* (Hübner, 1813) with (3) phallus and (4) sternum A8, slide CREA-0201; (5) *Eupithecia trisignaria* Herrich-Schäffer, 1848 with (6) phallus and (7) sternum A8, slide CREA-0190; (8) *Orectis proboscidata* (Herrich-Schäffer, 1851), slide CREA-0174.

Genetic data – LEP-SS-00607, BCLEP157-17, BOLD:AAD2706, 658bp, 99.08% of similarity with a specimen collected in Bavaria, Germany.

Eupithecia inturbata (Hübner, 1817) (Geometridae)
(Fig. I, 6)

Habitat – *Acer* forest of the Pollino National Park.

Records – Vallone Lupara, Alessandria del Carretto (CS), 1305m, 39.9259°N-16.3603°E, 19.VII.2017 (2f) (1 dissected); Difesa di Privitera, Alessandria del Carretto (CS), 1285m, 39.9274°N-16.3572°E, 19.VII.2017 (1m, 2f) (1 barcoded); idem, 1291m, 39.9274°N-16.3572°E, 17.VIII.2017 (1f) (dissected); Sciortaglie, Alessandria del Carretto (CS), 1253m, 39.9291°N-16.3532°E, 17.VIII.2017 (2f) (1 dissected); idem, 1246m, 39.9313°N-16.3508°E, 18.IX.2017 (1f) (dissected).

Genetic data – LEP-SS-00667, BCLEP217-17, BOLD: AAB89169, 630bp, 99.68% of similarity with a specimen collected in Carinthia, Austria.

Eupithecia denotata (Hübner, 1813) (Geometridae)
(Fig. I, 7)

Habitat – *Acer* forest of the Pollino National Park.

Records – Vallone Lupara, Alessandria del Carretto (CS), 1305m, 39.9259°N-16.3603°E, 19.VII.2017 (1m) (dissected, slide CREA-0201, Fig. II, 2).

Eupithecia trisignaria Herrich-Schäffer, 1848 (Geometridae) (Fig. I, 8)

Habitat – *Alnus glutinosa* forest of the Sila National Park.
Records – Epicate, San Giovanni in Fiore (CS), 1250m, 39.3349°N-16.6146°E, 28.VI.2017 (3m) (3 dissected, 1 slide CREA-0190, Fig. II, 3).

Leucoma salicis (Linnaeus, 1758) (Erebidae) (Fig. I, 9)

Habitat – Mixed forests of Pollino and Sila National Parks.

Records – Sciortaglie, Alessandria del Carretto (CS), 1253m, 39.9291°N-16.3532°E, 21.VI.2017 (1m); Lago Cecita, Spezzano della Sila (CS), 1176m, 39.3864°N-16.5516°E, 30.VII.2013 (1 m); Montagna Grande, San Giovanni in Fiore (CS), 1355m, 39.2717°N-16.6062°E, 5.VII.2016 (1m).

Apaidia rufeola (Rambur, 1832) (Erebidae) (Fig. I, 10)

Habitat – Sparse Mediterranean maquis.

Records – Palizzi Marina (RC), 3m, 37.9193°N-16.006°E, 15.V.2015 (1m).

Orectis proboscidata (Herrich-Schäffer, 1851) (Erebidae) (Fig. I, 11)

Habitat – *Acer* forest of the Pollino National Park.

Records – Vallone Lupara, Alessandria del Carretto (CS), 1345m, 39.9245°N-16.3609°E, 17.VIII.2017 (1m) (dissected, slide CREA-0174, Fig. II, 4).

Genetic data – LEP-SS-00720, BCLEP267-17, BOLD: AAP3890, 658bp, 99.05% of similarity with a specimen collected in Sicily, Italy.

Atethmia ambusta (Denis & Schiffermüller, 1775) (Noctuidae) (Fig. I, 12)

Habitat – *Acer* forest of the Pollino National Park.

Records – Difesa di Privitera, Alessandria del Carretto (CS), 1285m, 39.9274°N-16.3572°E, 17.VIII.2017 (1m).

Agrochola orejoni Agenjo, 1951 (Noctuidae) (Fig. I, 13)

Habitat – *Acer* forest of the Pollino National Park.

Records – Balestrieri, Alessandria del Carretto (CS), 1371m, 39.9275°N-16.3624°E, 12.XI.2017 (1f).

Lithophane semibrunnea (Haworth, 1809) (Noctuidae) (Fig. I, 14)

Habitat – *Acer* forest of the Pollino National Park.

Records – Sciortaglie, Alessandria del Carretto (CS), 1253m, 39.9291°N-16.3532°E, 17.VIII.2017 (1m).

Genetic data – LEP-SS-00645, BCLEP195-17, BOLD: AAJ2393, 658bp, 100% of similarity with specimens collected in several European countries.

Eupsilia transversa (Hufnagel, 1766) (Noctuidae) (Fig. I, 15a, b)

Habitat – *Acer* forest of the Pollino National Park and *Taxus baccata* forest of the Nebrodi Mountains.

Records – Balestrieri, Alessandria del Carretto (CS), 1371m, 39.9275°N-16.3624°E, 12.X.2017 (1f); Vallone Lupara, Alessandria del Carretto (CS), 1305m, 39.9259°N-16.3603°E, 18.IX.2017 (1f); Difesa di Privitera, Alessandria del Carretto (CS), 1285m, 39.9274°N-16.3572°E, 23.III.2017 (1m) (barcoded); Tappaiolo, Alessandria del Carretto (CS), 1253m, 39.9358°N-16.3471°E, 18.IX.2017 (1f); Bosco della Tassita, Caronia (ME), 1430m, 37.90°N-14.50°E, 13.X.2009 (3f).

Genetic data – LEP-SS-00629, BCLEP179-17, BOLD: AAC7414, 658bp, 100% of similarity with specimens collected in several European countries.

DISCUSSION

In this paper we report 15 new records of species that significantly enlarged their known range or confirmed their presence in southern Italy, elucidating the role of Mediterranean forests as biodiversity hot-spots.

Species perspectives

Most interesting findings certainly concerned the Euro-Siberian *Eupithecia trisignaria* and the South-East Euro-Anatolian *Orectis proboscidata* which had never found before in southern Italy.

Eupithecia trisignaria is widespread in Central and Northern Europe, with scattered populations towards the south. In Italy it is known from the Alps and for very few localities in the northern and central Apennines (Parenzan and Porcelli, 2007). The nearest known population to those found in the Sila Massif is about 370 km northward, at 1250 metres of altitude but authors did not provide any habitat description (Flamigni *et al.*, 2002). Recently, the moth fauna of Sila Massif was intensively investigated (Scalercio *et al.*, 2008; Infusino *et al.*, 2016; 2017a,b), but this species was never found before. This is probably due to the small population size, likely being restricted in its local range to the optimal habitat, here represented by a humid forest of *Alnus glutinosa*.

Orectis proboscidata has isolated populations in his range, found in Italy in the Alps, in one locality from central Apennines about one century ago (Dannehl, 1927), and in a few Sicilian localities (Bella and Fibiger, 2009). The specimen we found is particularly important as it could re-open a question about the taxonomy of southernmost Italian populations. Turati (1908) described *Orectis barteli* from Sicily, successively synonymized under *O. proboscidata* (Zilli, 1996). Based on DNA barcoding analysis, Sicilian and Calabrian populations show a genetic divergence of about 2% from Alpine and Balcanic populations, suggesting the need of further investigation on the taxonomy of southern Italian populations (Fig. III).

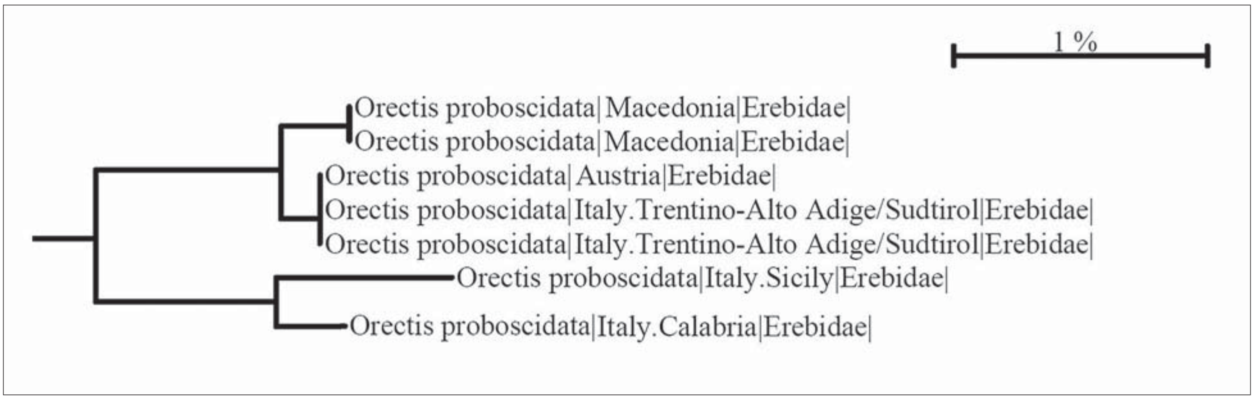

Fig. III – Neighbor joining trees (Kimura 2-parameter distance model for COI-5P marker) of European *Orectis proboscidata* specimens built using sequences deposited in BOLD.

Very interesting was also the second finding of *Agrochola orejoni* in Italy, a species having a very disjunct range being present in Spain and in the Pollino Massif, Italy, where an endemic subspecies was described by PARENZAN (1982a), *A. orejoni terranovae*.

Other species represent new findings for the Calabrian region and *Eupsilia transversa* was new also from Sicily, all enlarging their range southward. *Alsophila aceraria* was erroneously reported from Calabria by FLAMIGNI *et al.* (2001), as FLAMIGNI and BASTIA (2003) clarified that it was a mistake in data transcription. Our data confirmed its presence in Calabria, where it was found in the Sila Massif. *Lithophane semibrunnea* was also recorded from Calabria in a previous paper (INFUSINO *et al.*, 2017a), but it was due to a misidentification as the used reference literature (BERIO, 1985) shows vice-versa exchanged images for *L. semibrunnea* and *L. socia*. Our record is the first correctly attributed to *L. semibrunnea* in this region. Although *Poecilocampa populi* is known from the Pollino Massif since more than three decades (PARENZAN, 1982b), our finding in new localities of the same massif confirmed its presence with a population about 400 km apart from the nearest.

As discussed above, DNA barcoding analysis identified the population of *O. proboscidata* as the most diverging from Central and South-Eastern European populations. Other barcoded specimens showed a COI divergence varying from 0.92% for *A. derivata* to 0 for *L. semibrunnea* and Calabrian *E. transversa*, whilst *E. inturbata* diverged by 0.32%. We can hypothesize a more recent colonization of the Pollino Massif for less divergent species, and likely a longer isolation history for the most divergent.

HABITAT PERSPECTIVES

Systematic surveys carried out in the *Acer*-dominated forest provided most of the new records discussed in this paper. The main factor determining the relevance of this forest type was the lack of previous surveys in this peculiar forest, characterizing the Site of Community Importance IT9310019 Monte Sparviere, Pollino National Park. Five species of *Acer* are present in this forest (ROMA-MARZIO *et al.*, 2016), thanks mainly to the local microclimate, soil and geomorphology. These factors favor the presence of well-established populations of *Cyclophora albiocellaria* and *Eupithecia inturbata,* species having monophagous larvae on this tree genus (MIRONOV, 2003; HAUSMANN, 2004). The establishment of these populations is attributable not only to the presence of hostplants but also to the microclimatic conditions which characterize this area and to the biogeographic history of the Pollino Massif. In addition to the two above mentioned, most species found in this habitat have larvae feeding on deciduous trees, namely *Eupsilia transversa*, *Atethmia ambusta*, *Lithophane semibrunnea*, and *Leucoma salicis*, found also in mixed forests of the Sila Massif. Only *Eupithecia denotata*, oligophagous mainly on Campanulaceae, and *Orectis proboscidata*, with larvae feeding on vegetal detritus, are not linked to trees for their larval development. *Agrochola orejoni* is known to be a forest species, but larval biology is unknown.

Other species linked to deciduous trees found in systematic surveys were *Poecilocampa populi*, found in a beech forest, and *Alsophila aceraria*, found in a forest dominated by Calabrian black pine, whilst *Eupithecia trisignaria*, the only species of interest found in *Alnus glutinosa* woods, is oligophagous on Apiaceae (MIRONOV, 2003).

Finally, occasional findings were not strictly linked to forests, as *Emmiltis pygmaearia* inhabit xerothermophilous grasslands (HAUSMANN, 2004) and *Apaidia rufeola* coastal areas (BERTACCINI *et al.*, 1995). Only the Sicilian finding of *Eupsilia transversa* is attributable to a woody plant feeder.

INFUSINO *et al.* (2016) found mid and late successional stages of forests more important than early successional stages of forests in sustaining lepidopteran diversity in managed mountain forests. This occurred also in the highly exploited chestnut forest, in which mature coppices inhabit many species of biogeographic and conservation interest. The results showed in this paper were consistent with those of INFUSINO *et al.* (2016), as they were mostly obtained in forests included in protected areas submitted to low perturbations. Nevertheless, some considerations concerning forest management can be carried out. In particular, the role of undergrowth flora is underlined by the presence of isolated populations of important species linked to this forest layer. The species composition related to the undergrowth is also determined by microclimatic conditions favored by forest cover. Most ecological-oriented management strategies should take into account the effect of changes in forest cover on diversity and composition of understory flora as a key factor for sustaining biodiversity.

CONCLUSIONS

In this paper we report new finding concerning 15 moth species that clearly elucidate the role of forest habitats as biodiversity hot-spots, especially in the Mediterranean Basin. Most records enlarged the known species ranges southward, in a very significantly manner for *Eupithecia trisignaria*. As underlined by recent papers, the importance of Italian peninsula for biodiversity conservation at European scale is strategic as an increased number of endemic or sub-endemic taxa are recognized (SCALERCIO *et al.*, 2016; INFUSINO *et al.*, 2018), most of which trophically linked to trees. Furthermore, some species show endemic genetic lineages in southern Italy (TREMATERRA *et al.*, 2017; INFUSINO *et al.*, 2017a), underlying the existence of ongoing evolutionary processes. In the light of these results, an extensive study of biodiversity hosted by forest ecosystems, accompanied by barcoding analyses, are strongly needed to define the biodiversity hosted within Mediterranean forest ecosystems in order to refine conservation strategies towards adequate management regimes.

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NOTES ON THE BIOLOGY AND ECOLOGY OF *LABIDOSTOMMA* (ACARI PROSTIGMATA LABIDOSTOMMIDAE) IN POLAND

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Błoszyk J., Adamski Z., Napierała A. – Notes on the biology and ecology of *Labidostomma* (Acari Prostigmata Labidostommidae) in Poland.

The current article is a brief summary of results obtained from long-term observations (for over 67 years) of Labidostommatidae mites in Poland. The study is based on 21,470 samples collected in different environments and different locations, and each of them had a different protection status. The analysis focuses on the frequency of occurrence and habitat preferences of *Labidostomma luteum* Kramer, 1879, *Labidostomma denticulatum* (Schrank, 1776) and *Labidostomma cornutum* (G. Canestrini & Fanzago, 1878) in Poland. The results of the analysis show that *L. luteum* was the most frequent species found in the analyzed material, whereas specimens of *L. cornutum* were very rare. Moreover, the long-term research conducted in Poland since 1950 has allowed to analyze changes in the average abundance of the examined species in this area. The results of the analysis has revealed a decrease in the abundance of *L. luteum*, which is evident especially after 1990. Finally, the comparison of the abundance of *Labidostomma* in legally protected areas and those without any legal protection shows that the mites definitely prefer areas with a low anthropogenic pressure.

KEY WORDS: *Labidostomma*, habitat preferences, spatial distribution, protected areas, changes in abundance.

INTRODUCTION

There are three species belonging to the family Labidostommatidae which occur in Poland, i.e. *Labidostomma luteum* Kramer, 1879, *Labidostomma denticulatum* (Schrank, 1776) and *Labidostomma cornutum* (G. Canestrini & Fanzago, 1878). In 1980 Błoszyk published an article in which he showed for the first time that the geographical range and ecological niches of species from the cohort Labidostommatina can be different. Later studies has also confirmed that these mites are most common in natural biotopes, and their populations disappear due to increasing anthropogenic pressure (BŁOSZYK *et al.,* 1988, 2007; BŁOSZYK and CZARNOTA, 1998a, 1998b).

However, most of the studies mentioned above are based on small samples number, e.g. 718 samples were used in BŁOSZYK (1980). This study compares the habitat requirements of the three *Labidostomma* species occurring in Poland based on new data and over 7,000 samples. To confirm that the mites prefer habitats with a low anthropogenic pressure (BŁOSZYK *et al*., 1988, 2007; BŁOSZYK and CZARNOTA, 1998a, 1998b), we compared the occurrence of the three *Labidostomma* species in legally protected areas, such as national parks and reserves, as well as areas with no legal protection. Furthermore, the long-term research has also allowed analyzing the changes in the abundance of *Labidostomma* species since the end of the 50's until 2017. This study, beside the work by BŁOSZYK *et al*. (2017), who discuss the vertical and geographical distribution, as well as the post-glacial migration of mites from the genus *Labidostomma* in Europe, is a summary of the current knowledge in the biology, ecology, and distribution of these mites in Poland.

MATERIAL AND METHODS

The study presents an analysis of samples which were collected in Poland between 1950 and 2017 by different researchers. The total analyzed sample material contained 21,470 soil samples and samples from unstable microhabitats collected from protected (national parks and nature reserves) and unprotected areas (7,528 qualitative and quantitative 13,942 samples). On the basis of these samples a map showing the distribution of *Labidostomma* in Poland has been drawn (Fig. I). The material analyzed in this study contained only qualitative samples, which contained all necessary information about the environment and legal protection status of the area in which they were collected.

Sampling was done by sieving soil and forest litter, whereas the samples which were not sieved had been collected in open habitats, such as meadows, dead wood, and tree holes. The samples from dead wood were collected with plastic container and had a volume from 0.5-0.8 l. All the samples were extracted with Tullgren funnels for 4-6 days (depending on the level of moisture) just after the material collection. The extracted mites were preserved in 75% ethyl alcohol. The specimens found in the samples were identified with an Olympus SZ30 stereomicroscope. Due to the fact that *Labidostomma luteum*, *L. denticulatum*, and *L. cornutum* had been described in earlier studies, and

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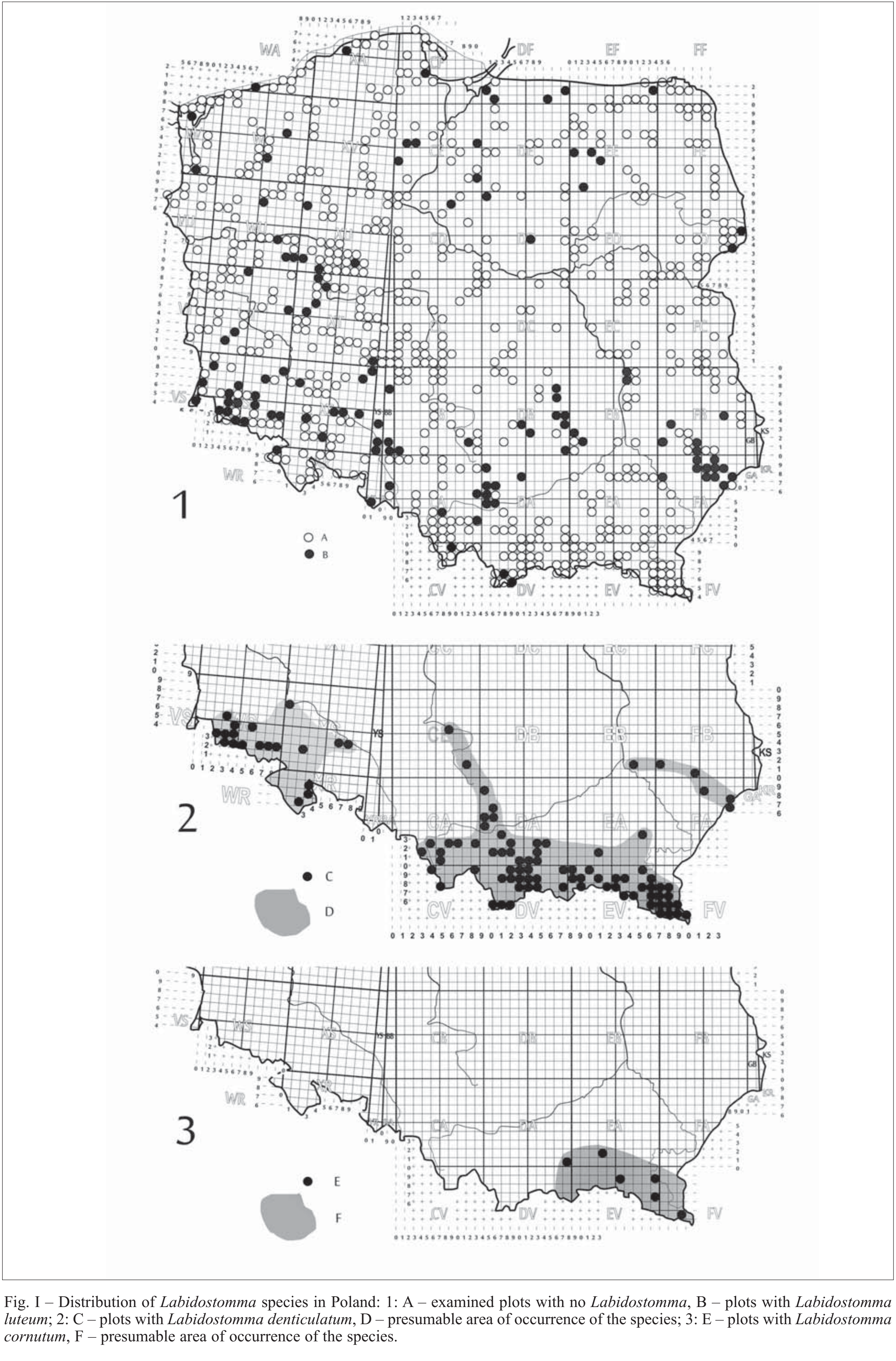

Fig. I – Distribution of *Labidostomma* species in Poland: 1: A – examined plots with no *Labidostomma*, B – plots with *Labidostomma luteum*; 2: C – plots with *Labidostomma denticulatum*, D – presumable area of occurrence of the species; 3: E – plots with *Labidostomma cornutum*, F – presumable area of occurrence of the species.

the descriptions provided by Štorkan (1939) and Grandjean (1942a, 1942b) seem to be very thorough, this study primarily focuses on the ecology and distribution of the species in question. The mites were classified on the basis of the original descriptions (Štorkan, 1939; Grandjean 1942a, 1942b, Pfliegler & Bertand, 2011). The examined specimens are stored in the *Invertebrate Database*, which is a part of the *Natural History Collection*, housed in the Faculty of Biology at Adam Mickiewicz University in Poznań.

The map showing the distribution of the mites in Poland (Fig. I) was drawn on the basis of the UTM grid (10×10 km). An analysis of long-term changes in the average number of *Labidostomma* species was carried out between 1950 and 2017. The only exception was the first research period (1950-1969) which covers 20 years due to the a low number of samples collected at the first stage of the project. In addition, we believe that anthropogenic changes of the environment were fairly slow at that time. In order to estimate the statistical significance of differences between average abundance of *L. luteum* and *L. denticulatum* in protected and unprotected areas, Student's T-test was used.

RESULTS

Frequency of the three *Labidostomma* species in Poland

The three species were found in 721 samples (9.58% frequency) out of all 7,528 qualitative samples collected in the whole area of Poland. The samples contained 3,160 specimens of mites from this group [*L. luteum* (1,631), *L. denticulatum* (1,506) and *L. cornutum* (23) specimens]. *L. luteum* occurred in 42.3% of the positive samples, *L. denticulatum* in 56.2%, and *L. cornutum* only in 1.5% of the samples. The specimens of the latter species were apparently rare – the average number of specimens in each of these positive samples was merely 4. The highest number of specimens in one sample was recorded for *L. luteum,* 57 specimens, in the case of *L. denticulatum,* 24 specimens, whereas *L. cornutum* was represented only by 6 specimens.

Changes of abundance and frequency of three *Labidostomma* species in Poland

The average number of the *Labidostomma* species in the positive samples in the local populations of *L. denticulatum* and *L. cornutum* in the examined areas is not decreasing over time, and their abundance is rather stable (Table 1). The situation is different in the case of *L. luteum*, the abundance of which decreased considerably after 1990.

The frequency of the two most common species *L. luteum* and *L. denticulatum*, was quite stable in the first three research periods (Table 2). A considerable decrease of this parameter has been observed after 1990 in the case of *L. luteum*. On the other hand, the data for *L. denticulatum* show that this species was the most frequent between 1990 and 1999 (Table 2).

Habitat preferences of the three species

The habitat and microhabitat preferences in the three species from genus *Labidostomma* were established on the basis of the qualitative samples. The habitats, in which the analyzed specimens were collected, can be classified into three major groups: (1) open, non-wooded areas, (2) woods and shrubs and (3) unstable microhabitats (merocenoses). A detailed list of the habitats with statistical data is given below in Table 3.

In Poland, species of *Labidostomma* are the most common in woods and shrubs (Table 3). The three species were found in hornbeam forests, mixed deciduous forests, beech-woods in the mountains, mixed forests (with spruce), and brushwoods. Only one species - *L. luteum*, inhabits beech-woods of lowlands, pine forests, spruce forests in lowlands, and larch stands. On the other hand, *L. denticulatum* can be found only in areas with dwarf pines. The three species are rare in open habitats. *L. luteum* clearly prefers xerophilous grasses, but both species are apparently frequent in rock grasses on limestones. *L. luteum* and *L. denticulatum*, were the most frequent in unstable microhabitats, like rotten trunks and tree holes. *L. cornutum* occurred in mixed forests (with spruce trees), brushwood, beech-woods in the mountains, and in hornbeam forests.

Table 1 – Changes in the average number of specimens (X) in positive samples observed in Poland from 1950 to 2017: Ps – positive samples, N – number of specimens, Max – maximum number of specimens in one sample.

Study period	**Number of soil samples**	***L. luteum***				***L. denticulatum***				***L. cornutum***			
		Ps	**N**	**X**	**Max**	**Ps**	**N**	**X**	**Max**	**Ps**	**N**	**X**	**Max**
1950-1969	286	11	30	2.7 ± 2.8 SD	10	25	118	4.7 ± 4.7 SD	17	4	7	1.7 ± 0.9 SD	3
1970-1979	1,295	110	612	5.6 ± 6.9 SD	45	115	405	3.5 ± 3.8 SD	24	3	6	2.0 ± 1.7 SD	4
1980-1989	1,788	85	452	5.3 ± 6.9 SD	46	47	115	2.4 ± 1.9 SD	12	–	–	–	–
1990-1999	1,294	23	98	4.3 ± 4.2 SD	18	197	834	4.2 ± 3.7 SD	24	–	–	–	–
2000-2010	2,074	41	245	6.0 ± 9.9 SD	57	2	2	1 ±	1	–	–	–	–
2011-2017	791	35	194	5.5 ± 5.9 SD	22	19	32	1.7 ± 1.1 SD	5	4	10	2.5 ± 2.4 SD	6

Table 2 – Frequency of *Labidostomma* species in Poland between 1950 and 2017.

Study period	***L. luteum***	***L. denticulatum***	***L. cornutum***
1950-1969	3.85	8.74	1.40
1970-1979	8.49	8.88	0.23
1980-1989	4.75	2.63	-
1990-1999	1.78	15.22	-
2000-2010	1.98	0.10	-
2011-2017	4.42	2.40	0.51

Occurrence of *Labidostomma* in protected and unprotected areas

In this study, 5,427 samples that were collected in habitats preferred by the mites from this group have been

Table 3 – Habitat preference of two species of *Labidostomma* in Poland: N - number of qualitative samples; F - frequency (%); Ns - number of specimens; x - mean of specimens/per sample, SD - standard deviation.

Habitat	**Species**	**N**	**F**	**Ns**	**X±SD**
Open habitat					
Xerophilous grasses	*L. luteum*	78	7.69	65	10.8±17.7
Sandhills		25			
Rock grasses (non-calcareous)	*L. denticulatum* *L. luteum*	168	2.98 1.19	8 6	1.6±0.9 3.0±1.4
Rock grasses on limestone	*L. denticulatum* *L. luteum*	103	8.74 9.71	25 40	2.8±2.0 4.0±2.8
Meadows	*L. luteum*	578	0.17	1	1.0
Peat-bogs	*L. denticulatum* *L. luteum*	82	1.22 4.88	1 11	2.7±2.1 1.0
Sedgelands	*L. luteum*	25	4.00	1	1.0
Forest and shrubs					
Alder forest	*L. denticulatum* *L. luteum*	142	0.70 2.82	3 70	3.0 17.5±26.6
Marshy forest	*L. denticulatum* *L. luteum*	213	0.47 1.41	3 16	3.0 5.3±3.2
Oak-hornbeam forest	*L. cornutum* *L. denticulatum* *L. luteum*	497	0.60 3.82 10.66	9 88 304	3.1±1.0 4.6±4.3 5.7±7.7
Mixed deciduous forest	*L. cornutum* *L. denticulatum* *L. luteum*	265	0.21 4.64 5.70	1 68 222	1.0 3.1±2.7 7.4±6.6
Beech-wood in lowlands	*L. luteum*	95	14.74	55	3.9±5.7
Beech-wood in the mountains	*L. cornutum* *L. denticulatum* *L. luteum*	499	0.20 36.67 5.21	1 770 84	1.0 4.2±3.6 3.2±4.2
Oak-wood	*L. denticulatum* *L. luteum*	58	1.72 12.07	1 54	1.0 7.7±7.0
Pine artyfical tree stands and pine forest	*L. denticulatum* *L. luteum*	585	0.34 3.76	2 87	1.0 4.0±3.7
Spruce forest in the mountains	*L. denticulatum* *L. luteum*	309	8.41 0.97	87 9	3.0±3.7 3.0±2.0
Spruce forest in lowland	*L. luteum*	49	6.12	10	3.3±1.2
Fir forest	*L. denticulatum* *L. luteum*	126	37.30 2.38	143 7	3.0±2.9 2.3±1.2
Larch stand	*L. luteum*	30	13.33	20	5.0±2.2
Yew-tree stand	*L. luteum*	90	2.22	2	1.0
Fir-beech forest	*L. denticulatum* *L. luteum*	66	36.36 12.12	89 19	3.7±3.1 2.4±2.4
Mixed forest (with pine)	*L. denticulatum* *L. luteum*	232	0.43 6.47	2 122	3.7±3.1 8.1±8.8
Mixed forest (with spruce)	*L. cornutum* *L. denticulatum* *L. luteum*	90	1.11 13.33 8.89	1 76 46	1.0 6.3±7.3 6.0±6.5
Dwarf pine	*L. denticulatum*	55	3.64	7	3.5±2.1
Bushwood	*L. cornutum* *L. denticulatum* *L. luteum*	120	0.83 7.50 4.17	1 26 36	1.0 2.9±3.3 7.2±5.6
Parks	*L. denticulatum* *L. luteum*	171	0.58 5.26	2 48	2.0 5.3±4.6
Merocenoses					
Ant-hills		42			
Nests of small mammals	*L. luteum*	233	0.45	1	1.0
Nests of birds		821			
Rotten trunks	*L. denticulatum* *L. luteum*	1,224	1.55 3.19	57 150	3.0±3.9 3.8±4.7
Tree hollows	*L. denticulatum* *L. luteum*	193	1.73 1.73	9 39	3.0±2.6 13.0±12.1
Total		**7,264**		**3,005**	

*Reported on 30.11.2017

analyzed; out of which 1,809 samples (33.3%) were collected in nature reserves and national parks. The other 3,618 samples were collected in areas with no legal protection status. Despite the higher number of samples collected in unprotected areas, over half of the found specimens (55.7%) come from the samples collected in reserves and national parks (Table 4). Also the frequency of the three species was higher in the samples collected in legally protected areas (Fig. II).

The data show that *Labidostomma* species are more abundant in protected areas. The average number of the two most common species in a positive sample (i.e. a sample containing a given species) was higher in protected areas (Fig. III). However, this difference is statistically significant only in the case of *L. denticulatum* [t 6,02 df 1098 p= 0,00 N 550], whereas the differences in the average abundance of *L. luteum* in protected and unprotected areas is not statistically significant [t 1,83 df 1098 p= 0,07 N 550].

Table 4 – Number of specimens (N) and positive samples (PS) for *Labidostomma* species in protected and unprotected areas; NSp – total number of specimens, Nsa – total number of samples.

Species	**Protected**		**Unprotected**		
	N	**PS**	**N**	**PS**	**NSp**
L. luteum	517	90	504	108	1,021
L. denticulatum	790	206	523	351	1,313
L. cornutum	1	1	12	6	13
Nsa	1,308	1,809	1,039	3,618	5,427

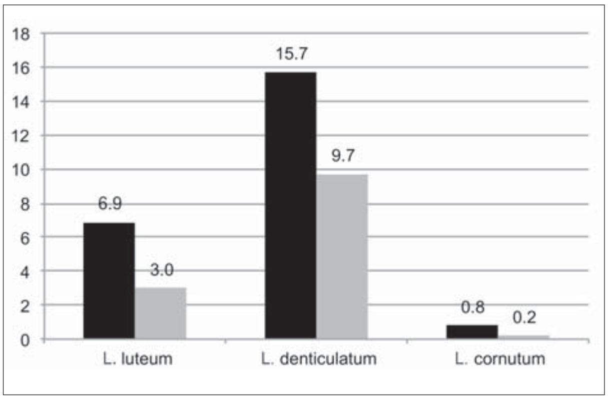

Fig. II – Frequency (%) of *Labidostomma* species in samples from protected (black) and unprotected (gray) areas.

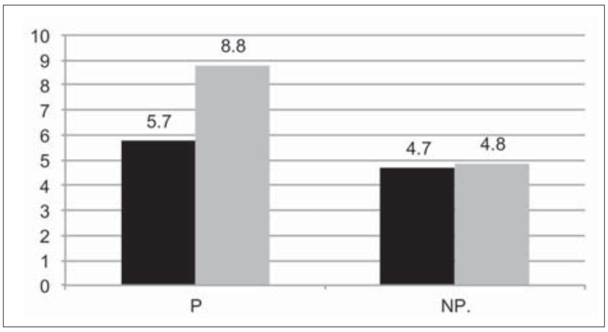

Fig. III – Average number of specimens per positive sample in protected (P) and not protected areas (NP). Black – *L. luteum*, gray – *L. denticulatum*.

DISCUSSION

Despite the fact that it has been 38 years since Błoszyk (1980) published his first study on mites from the cohort Labidostommatina in Poland, which was based on material containing a relatively low number of samples (718 samples), the current knowledge of the distribution and habitat preferences of these species has not changed much since that time. Our study is therefore a significant contribution to the research into *Labidostomma* mites in Poland, especially the data on the habitat preferences and changes in the abundance of the mites between the 50's and 2017. The analysis presented in this study has been carried out on a large number of qualitative samples, and in most cases the results corroborate the earlier observations on the habitat preferences of the discussed species. Similarly to the earlier studies, our results also show that the three *Labidostomma* species occurred most frequently in oak-hornbeam forests, beechwood forests, both in lowlands and highlands, in mixed forests, as well as in mixed forests with spruce, and in brushwoods (Table 2). Besides this, *L. luteum* occurred also in larch stands and in oak-woods, which has not been recorded in the earlier study by Błoszyk (1980).

The data presented in this study also provide new valuable information for example, the occurrence of *Labidostomma* species in legally protected areas and those with no legal protection status. The obtained results have confirmed that the three species definitely prefer areas with low anthropogenic pressure (Błoszyk, 1980). Despite the fact that the number of samples collected in protected areas was considerably lower, the frequency of the three species was much higher than in those collected in areas that were not protected. Also the abundance of *L. luteum* and *L. denticulatum*, which are the two most common *Labidostomma* species in Poland, was higher. This is especially noticeable for *L. denticulatum*. Moreover, the frequency of this species was also higher in legally protected areas. A similar dependency has been also observed in the case of mites from the suborder Uropodina (Napierała, 2008, 2009). The research conducted in Wielkopolska (Greater Poland) shows that 80% of all species of Uropodina found so far in Wielkopolska occurred mainly in legally protected areas (nature reserves and the Wielkopolski National Park), though protected areas constitute only 2% of the whole area of the region.

Another important problem that has not been discussed previously is the changes in the abundance and frequency over time of the three species of *Labidostomma*. The frequency of particular species found in the samples collected in the consecutive time periods fluctuated depending on the number of samples and place where the samples were collected. This probably stems from the fact that distribution of these mites is geographically restricted to a given area and is different for each species (Błoszyk, 1980; Błoszyk *et al*., 2017). The high frequency of *L. denticulatum* in the period 1990-1999 was probably caused by the great intensity of the research carried out at that time in the Gorce Mountains, where most of the analyzed samples were collected (Table 2). The changes of the average number of specimens in the positive samples show that the local populations of *L. denticulatum* and *L. cornutum* in the examined areas are not diminishing and their abundance is fairly stable (Table 1). The situation is different in the case of *L. luteum*, the abundance of which considerably decreased after 1990. This in turn probably stems from the fact that in this period the area of Wielkopolska (Greater Poland), where much of the analysed material was collected, suffered from a low rainfall and severe cold winters with no snow cover. Further research should provide more data which will show whether this situation has become a definite trend.

A similar decrease of abundance has also been observed in the case of Uropodina in two nature reserves, i.e. in Jakubowo and Las Grądowy (Greater Poland), which have been under survey for 36 years (Napierała *et al.*, 2014). During this very long period of time extensive research was conducted in these reserves, and also abundance of a small population of *L. luteum* has been monitored in Jakubowo reserve since the 80's. The observations show that the abundance of this species considerably decreased after 1990. The decrease was caused probably not only by the climate conditions, but also by the extensive successive changes in the vegetation of the observed area. It is also noteworthy that during this period, one species of mites from the suborder Uropodina, i.e. *Trachytes lamda* (Berlese, 1903) has became extinct in this reserve. This is a rare mite species, which is very sensitive to unfavorable environmental changes (Błoszyk, 1999). The extinction of this rare Uropodina and a decrease in the abundance of *L. luteum* in this area imply that there have been some deteriorating changes in the environment conditions in the area of the reserve. This also supports the hypothesis that *Labidostomma* species prefer areas with a low anthropogenic pressure, and therefore, these species can be good bioindicators of natural soil health.

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EFFECTIVENESS OF SPINOSAD AND MINERAL OIL BASED COMMERCIAL PRODUCTS ON OVIPOSITION AND EGG HATCHING OF *GRAPHOLITA FUNEBRANA* TREITSCHKE

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Rizzo R., Caleca V., Lombardo A., Lo Verde G. – Effectiveness of spinosad and mineral oil based commercial products on oviposition and egg hatching of *Grapholita funebrana* Treitschke.

Laboratory trials were performed to evaluate the action of spinosad and mineral oil on eggs of *Grapholita funebrana* Treitschke, the key pest in plum orchards. Fruits of cultivars Angeleno, President and Stanley were used in the trials. The first set of tests was carried out by introducing two mated females of *G. funebrana* into a cage together with fruits of a single cultivar. The second set of trials tested the three cultivars simultaneously. In all trials, one third of the fruits of each cultivar was treated with mineral oil, another third with spinosad and the final third was left untreated. Treatments were carried out before introducing mated females into the cages. The number of fruits with eggs, the number of eggs laid on each fruit and the number of hatched eggs were recorded. The number of eggs per fruit recorded in all trials was the same, indicating that plum moth females, after choosing the fruits for oviposition on the basis of the cultivar or the applied product, tend to use all suitable fruits in the same way. Oviposition in terms of number of fruits was significantly lower in mineral oil and spinosad treatments in all trials, compared to the control. Differences between the two products were found in trials carried out with Angeleno alone and with the three cultivars together. The percentage of egg hatching on the fruits was always significantly lower with spinosad treatment compared to the mineral-oil treatment and the control. In the trials carried out using the three cultivars, the number of infested fruits was significantly higher for Angeleno and no differences in the hatching percentages were found among the cultivars. The ovicidal and antiovideponent activities of commercial products containing mineral oils or spinosad could represent an interesting tool to reduce *G. funebrana* damage, both in organic and conventional plum orchards.

KEY WORDS: organic plum orchards, oviposition, egg hatching, plum moth, Lepidoptera, Tortricidae.

INTRODUCTION

The plum moth, *Grapholita funebrana* Treitschke, is considered the most important pest in plum orchards. The damage is caused by larvae feeding into the fruit, causing changes in terms of the colour, early ripening, rotting and fruit fall. Moreover, infested fruits bear gum-associated penetration holes made by neonate larvae and exit holes made by mature larvae leaving the fruits (ALFORD, 1987).

In conventional farming, synthetic insecticides are commonly used to control plum moth infestations, and the timing of application is often critical (BUTTURINI *et al.*, 2000). In recent years, there has been an increase in the use of the mating disruption method for the control of several pests, among them the plum fruit moth. Nevertheless, in many areas, the use of this method is currently limited because of environmental factors (wind, temperature), orchard characteristics (extent, shape, isolation, etc.) and cost (PFEIFFER and KILLIAN, 1988; RICE and KIRSCH, 1990; THOMSON *et al.*, 1999; GUT *et al.*, 2004).

In organic farming, according to European Union Regulations (EC) No 889/2008, synthetic products are not permitted; synthetic pheromones cannot be sprayed for mating disruption, but they are allowed in traps or dispensers. For organic plum orchards, both mineral oil and spinosad are included in the list of allowed products (Annex II, EC Regulation 889/2008).

Mineral oil has been used to control a large number of pest species on a variety of crops for many years (DAVIDSON *et al.*, 1991; AGNELLO, 2002). Trials carried out on several insect species, among them the tortricids *Grapholita molesta* (Busck) and *Cydia pomonella* (L.), showed that mineral oil has ovicidal activity (SMITH and PEARCE, 1948; RIEDL *et al.*, 1995) and may prevent oviposition (ZWICK and WESTIGARD, 1978; RIEDL *et al.*, 1995; FERNANDEZ *et al.*, 2001). Nevertheless, in both field and laboratory studies, oil treatment failed to reduce damage caused by *C. pomonella* to acceptable commercial levels (FERNANDEZ *et al.*, 2005, 2006; RIEDL *et al.*, 1995). Other laboratory studies on *Choristoneura rosaceana* (Harris) indicated that mineral oil significantly reduces the number of eggs laid as well as the level of egg survival (WINS-PURDYA *et al.*, 2009).

Spinosad is a mixture of spinosyns A and D, which is obtained from the fermentation of the actinomycete bacterium *Saccharopolyspora spinosa* Mertz and Yao. It is considered an effective insecticide against tortricids, like *G. molesta*, and other Lepidoptera (DOERR *et al.*, 2004; SMIRLE *et al.*, 2003), as well as against several species of Diptera, Coleoptera, Thysanoptera and Hymenoptera (BRET *et al.*, 1997; DUTTON *et al.*, 2003; THOMPSON and HUTCHINS, 1999).

The ovicidal and antiovipositional activity of commercial products containing mineral oils or spinosad could represent an interesting tool to control *G. funebrana* in organic plum

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orchards. In the present research, laboratory trials were carried out with mineral oil and spinosad based products in order to determine whether they reduce oviposition trough repelling *G. funebrana* females, and whether they reduce the hatching percentage of *G. funebrana* eggs.

MATERIALS AND METHODS

Adults of *G. funebrana* used in laboratory trials emerged from pre-imaginal instars that developed in infested fruits, which were collected from untreated trees in an organic plum orchard in San Giuseppe Jato (37°59′50″ N, 13°12′45″E, Palermo Province, Italy). The pupae were sexed (Beeke and De Jong, 1991) and kept separately in plastic cages (30 × 20 × 30 cm). As the flight performance of *G. molesta* is greater in adults fed before flight (Dorn *et al.*, 2001), *G. funebrana* adults were supplied with a 10% honey–water solution (g/ml). Mated females were obtained by placing two males and two females ($\leq$24 h old) in the same cage for 2 days before each test. Rearing was carried out with L16:D8 photoperiod, at constant temperature (26°C) and relative humidity (75%). The moths were not exposed to plant-related odour sources before the trials and were used only once. Moreover, the mated females used in the laboratory trials were removed from the cages after 2 days and then dissected to confirm their mating status, recording the presence of one or more spermatophores in the bursa copulatrix (Knight, 2006).

Uninfested fruits collected in the same San Giuseppe Jato organic orchard were used in all trials. Fruits of the same size were picked 12-24 hours before starting the trials and were handled by their pedicels to prevent contamination of the fruit surface.

In the first set of trials (“Single Cultivar”), two mated females were introduced into a cage (constructed of wood and glass), containing 18 fruits of the Angeleno cultivar. Six of the fruits had been sprayed with a spinosad based product, another six had been sprayed with a mineral oil product and six had not been sprayed. Success, Dow AgroSciences, was the commercial product containing 120 g per litre of spinosad; UFO, Bio-Intrachem, was the commercial product containing 96.9 g per 100 g of mineral oil. Success and UFO were used at concentrations of 120 ml in 100 l of water and 150 ml in 100 l of water, respectively, as suggested by the manufacturers. The entire surface of each fruit was sprayed 5–6 hours before the fruits were randomly located in the cage. The same trial was performed using President and Stanley cultivars. Each “Single Cultivar” trial was repeated eight times.

A second set of trials (“Three Cultivars”) tested the three cultivars simultaneously. In these trials, nine fruits of each cultivar were placed in a cage (27 fruits per cage) and two mated females were introduced. Three of the nine fruits of each cultivar were left unsprayed, three were sprayed with Success and three were sprayed with UFO; the commercial products and doses were the same as described for the previous assays. This trial was repeated eight times.

The number of fruits with eggs and the number of eggs laid on each fruit were counted after removal of the females (2 days) and the number of hatched eggs was counted daily over the following 7 days. All trials were carried out under the same rearing conditions described above. Moreover, the females used in the laboratory trials were removed from the cages after 2 days and then dissected to confirm their mating status (Knight, 2006).

STATISTICAL ANALYSIS

As the number of fruits with eggs and the number of hatched eggs are countable data (presence/absence of eggs on fruits and hatched/unhatched eggs), the binary logistic regression was performed. This method of statistical analysis uses the odds ratio (ratio of percentages) to compare one or several treatment percentages (product and cultivar) with the percentage under the reference conditions (control), when one or more of the input factors is changed. For the “Single Cultivar” laboratory trials, *Treatment* was the only factor to be considered. In the “Three Cultivars” laboratory trials, the independent variables were *Cultivar* and *Treatment.* Moreover, data on the number of eggs per infested fruit were normalised by means of a Box–Cox transformation and then analysed using ANOVA and Tukey’s post-hoc test.

MINITAB software (Minitab, Inc., State College, PA) was used for all statistical analyses.

RESULTS

The statistical analysis of infested/uninfested fruits in the “Single Cultivar” tests shows that, in all of the cultivars, the number of infested fruits was significantly higher in the control than in UFO and Success treatments (Tables 1-3). The infestation level recorded on fruits treated with UFO and Success resulted significantly different only on the Angeleno cultivar.

With regards to the hatching percentage, in the Angeleno cultivar, none of the eggs hatched in the Success treatment; therefore, it was only possible to compare the control and mineral-oil treatment, and no significant difference was shown between them (Table 1). In the President cultivar, the hatching percentage on Success treated fruits was significantly lower than on the control and mineral oil treated fruits. The latter two treatments did not result in a significant difference (Table 2). In the Stanley cultivar, no statistical differences were found in the hatching percentage recorded in UFO and Success treatments. Both of them showed a hatching level significantly lower than the control (Table 3).

In the second set of trials (“Three Cultivars”), significant differences were found in the percentages of fruits with eggs among both the different treatments and cultivars. The risk of infestation recorded on Angeleno was significantly higher than on President or Stanley, and there was no statistical difference between the latter two (Table 4). The egg hatching percentages recorded on the control as well as the UFO and Success treatments provided significantly different results, but no differences were found among the three cultivars (Table 4).

In all trials, the number of eggs on each infested fruit ranged from 1 to 4, without significant differences among the treatments in the “Single Cultivar” trials or among the cultivars and treatments in the “Three Cultivars” trials (ANOVA, $p<0.05$).

DISCUSSION

The antiovideponent effect of mineral oil has been demonstrated for several insect species belonging to Homoptera, Coleoptera and Lepidoptera (Powell *et al.*, 1998; Liang and Liu, 2002; Treacy, 1991), the latter including *C. pomonella* and *G. molesta* (Riedl *et al.*, 1995;

Table 1 – Binary logistic regression statistics applied to the infested fruits and the number of hatched eggs, in the "Single Cultivar" trials carried out on the Angeleno cultivar. The reference level is the control.

Angeleno								
Predictor	%	Coef.	Coef. SE	*Z*	*P*	Odd Ratio	Confidence Interval Lower	Confidence Interval Upper
Fruits with eggs								
Mineral oil	29.2	–1.22	0.43	–2.83	0.01	0.29	0.13	0.69
Spinosad	8.3	–2.73	0.60	–4.57	0.00	0.06	0.02	0.21
Control	58.3							
	Log-Likelihood = –75.344 Test that all slopes are zero: *G* = 29.730, *DF* = 2, *P* = 0.000							
Hatched eggs	% (No.)							
Mineral oil	79.2 (44)	–0.61	0.64	–0.95	0.34	0.54	0.15	1.92
Spinosad	0 (6)							
Control	87.5 (56)							
	Log-Likelihood = –33.381 Test that all slopes are zero: *G* = 0.872, *DF* = 1, *P* = 0.350							

Table 2 – Binary logistic regression statistics applied to the infested fruits and the number of hatched eggs in the "Single Cultivar" trials carried out on the President cultivar. The reference level is the control.

President								
Predictor	%	Coef.	Coef. SE	*Z*	*P*	Odd Ratio	Confidence Interval Lower	Confidence Interval Upper
Fruit with eggs								
Mineral oil	25	–1.10	0.44	–2.49	0.01	0.33	0.14	0.79
Spinosad	16.7	–1.61	0.48	–3.33	0.00	0.20	0.08	0.52
Control	50							
	Log-Likelihood = –81.890 Test that all slopes are zero: *G* = 13.483, *DF* = 2, *P* = 0.001							
Hatched eggs	% (No.)							
Mineral oil	64.3 (14)	–1.20	0.71	–1.69	0.09	0.30	0.07	1.21
Spinosad	11.1 (9)	–3.87	1.15	–3.37	0.00	0.02	0.00	0.20
Control	85.7 (42)							
	Log-Likelihood = –29.489 Test that all slopes are zero: *G* = 19.569, *DF* = 2, *P* = 0.000							

Fernandez *et al.*, 2001, 2005, 2006). In contrast, research on the antiovideponent action of spinosad products is very limited.

Both the "Single Cultivar" and "Three Cultivars" trials in this study showed that the application of the two products containing spinosad (Success) and mineral oil (UFO) significantly reduced the probability of oviposition by *G. funebrana* in comparison with untreated fruits. Moreover, as the "Three Cultivars" trials show, the antiovideponent action of the two products does not seem to be affected by the cultivar, despite the clear preference of *G. funebrana* females for Angeleno fruits. The antiovideponent action of Success was higher than UFO, as shown by the significant differences recorded when *G. funebrana* females were exposed to Angeleno alone and to the three cultivars together. Nevertheless, the better performance of Success in the laboratory could be subverted in the field, owing to its shorter persistence in comparison with UFO. In addition, analyses of the number of eggs laid on the infested fruits seem to indicate that plum moth females, after choosing the

Table 3 – Binary logistic regression statistics applied to the infested fruits and the number of hatched eggs, in the “Single Cultivar” trials carried out on the Stanley cultivar. The reference level is the control.

Stanley

Predictor	%	Coef.	Coef. SE	*Z*	*P*	Odd	Confidence Interval	
						Ratio	Lower	Upper
Fruit with eggs								
Mineral oil	18.7	–1.47	0.47	–3.13	0.00	0.23	0.09	0.58
Spinosad	6.3	–2.71	0.66	–4.09	0.00	0.07	0.02	0.24
Control	50.0							

Log-Likelihood = –67.657 Test that all slopes are zero: *G* = 26.639, *DF* = 2, *P* = 0.000

Hatched eggs	% (No.)							
Mineral oil	50.0 (14)	–1.36	0.65	–2.08	0.04	0.26	0.07	0.92
Spinosad	25.0 (4)	–2.46	1.21	–2.02	0.04	0.09	0.01	0.93
Control	79.5 (44)							

Log-Likelihood = –34.246 Test that all slopes are zero: *G* = 7.922, *DF* = 2, *P* = 0.019

Table 4 – Binary logistic regression statistics applied to plum moth infestation on fruits and egg hatchings in the “Three Cultivars” trials, in which the three cultivars and the three treatments were tested together. The reference level for the treatments is the control and for the cultivars is the Angeleno.

Fruit with eggs

Predictor	%	Coef.	Coef. SE	*Z*	*P*	Odd	Confidence Interval	
						Ratio	Lower	Upper
Treatment								
Mineral oil	27.8	–1.13	0.39	–2.90	0.00	0.32	0.15	0.69
Spinosad	12.5	–2.23	0.47	–4.79	0.00	0.11	0.04	0.27
Control	50.0							
Cultivar								
President	26.4	–1.22	0.39	–3.09	0.00	0.30	0.14	0.64
Stanley	13.9	–2.11	0.45	–4.65	0.00	0.12	0.05	0.29
Angeleno	50.0							

Log-Likelihood = –106.251 Test that all slopes are zero: *G* = 51.730, *DF* = 4, *P* = 0.000

Hatched eggs

Predictor	% (No.)	Coef.	Coef. SE	*Z*	*P*	Odd	Confidence Interval	
						Ratio	Lower	Upper
Treatment								
Mineral oil	64.0 (25)	–2.87	0.91	–3.15	0.00	0.06	0.01	0.34
Spinosad	9.1 (11)	–5.88	1.37	–4.29	0.00	0.00	0.00	0.04
Control	96.5 (54)							
Cultivar								
President	80.0 (20)	–0.24	0.83	–0.29	0.77	0.78	0.15	4.01
Stanley	86.7 (17)	–0.97	1.08	–0.90	0.37	0.38	0.05	3.15
Angeleno	71.7 (53)							

Log-Likelihood = –27.763 Test that all slopes are zero: *G* = 41.187, *DF* = 4, *P* = 0.000

fruits for oviposition on the basis of the cultivar or the applied product, tend to use all suitable fruits in the same way, always laying a similar number of eggs per fruit.

In general, the insecticidal effect of mineral oil and spinosad has been widely studied on a large number of insects, both pests and natural enemies (Davidson *et al.*, 1991; Williams *et al.*, 2003; Biondi *et al.*, 2012a, 2012b). On the other hand, the egg hatching failure due to the product sprayed before oviposition, has been reported for mineral oil products against the eggs of *Trialeurodes vaporariorum* (Westwood), *Liriomyza trifolii* (Burgess) (Zwick and Westigard, 1978; Larew, 1988) and *C. pomonella* (Riedl *et al.*, 1995), but this effect has not been previously investigated for spinosad based products. Our results indicate that UFO exhibits a clear negative effect on egg hatch in the "Single Cultivar" trials carried out on Stanley fruits as well as in the "Three Cultivar" trials.

Success pre-oviposition sprays showed to significantly reduce the percentage of eggs that hatched on plum fruits in all trials. Given the present results, it is reasonable to infer that control of *G. funebrana* by UFO and Success may result from their combined effects on oviposition and hatching.

Field experiments carried out in an organic plum orchard in Sicily showed that, at the end of July, the mineral-oil treatments on Angeleno caused a reduction in *G. funebrana* infestation by 36% relative to the untreated control (Rizzo *et al.*, 2012b). This result cannot be considered economically sustainable, which is similar to the results obtained on *C. pomonella* by Fernandez *et al.* (2005) and Riedl *et al.* (1995). In these cases, a significant suppression of tortricid pests by the mineral oil would require more frequent or higher rates of application (Fernandez *et al.*, 2005), but this procedure is inadvisable because of phytotoxicity, cost and regulatory limitations. Nonetheless, mineral oil is frequently recommended to prevent the development of resistance, because it is one of the few pesticide groups for which resistance has never been documented (Willett and Westigard, 1988).

Owing to its natural origin, to its very low mammalian toxicity (Breslin *et al.*, 2000) and rapid breakdown in the environment (Cleveland *et al.*, 2002; Zhao *et al.*, 2002), spinosad represents an important tool alternative to synthetic pesticides in organic farming as well as in conventional farming, especially in terms of farm workers and consumer safety (Williams *et al.*, 2003). On the other hand, spinosad has been demonstrated to induce resistance in several insect pests (Zhao *et al.*, 2002; Hsu and Feng, 2006; Wang *et al.*, 2006) and to have harmful side effects on beneficial arthropods such as bees and some natural enemies (Williams *et al.*, 2003). For these reasons, policies restricting the use of spinosad have been adopted in many countries, and the alternation between spinosad and other products is often suggested. The reliability of alternating the application of spinosad and mineral-oil products was confirmed by field results obtained on *G. funebrana* by Rizzo et al. (2012b). Furthermore, a reduced application of spinosad may result in an increase in the abundance and diversity of harmless arthropods, as found in pear and apple orchards, where more selective interventions for major arthropod pests, including mating disruption for codling moth, are adopted (Gut and Brunner, 1998; Knight *et al.*, 1997; Miliczky *et al.*, 2000).

An interesting finding of this study is that the tested cultivars differed in terms of their susceptibility to the plum moth. Susceptibility to the plum moth could depend on the fruit properties, as has been demonstrated for the olive fruit fly (Rizzo *et al.*, 2012a). Additional research on the effect of different population levels of *G. funebrana* on these cultivars could lead to a more targeted and integrated pest-management strategy and consequently to a further reduction in the use of chemicals for plum moth control.

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LEPIDOPTERA TORTRICIDAE FROM CALABRIA (SOUTHERN ITALY)

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Trematerra P., Goglia L., Scalercio S., Colacci M. – Lepidoptera Tortricidae from Calabria (southern Italy).

A list of 181 species of Lepidoptera Tortricidae recorded during several expeditions realized in Calabria (southern Italy) is reported. Fifty-nine species are mentioned for the first time in Calabria. Of particular interest are 23 species that have as a southern limit of their distribution area the Calabria territory.

KEY WORDS: Lepidoptera Tortricidae, fauna, Calabria, southern Italy.

Over time, the Lepidoptera Tortricidae from the Calabria territory (southern Italy) have received sporadic attention by both local and foreign entomologists. The only specific works refer to some catches taken on the southern slope of the Pollino Massif by TREMATERRA (1990 and 1991a) and in a chestnut area of the Sila Mountains by TREMATERRA *et al.* (1994).

In the present paper, we reported a tortricid list as updated and complete as possible for Calabria. For this purpose, in addition to our specimens collected from 2013 to 2018, we included in this list both the species found by P. Trematerra in the course of previous entomological researches in that region and the species reported in literature; these last species were collected in an unreported number of samples by light and pheromone traps (TREMATERRA, 1991a,b and 2003; TREMATERRA *et al.*, 1994 and 1997). In the list, the species from literature are marked with an asterisk.

Our specimens were caught mainly by attraction to light at night-time, mostly using UV-LED light traps (INFUSINO *et al.*, 2017), but also by butterfly net during day-time. The material was identified morphologically; genitalia were prepared using standard methods, the abdomen was macerated in 10% KOH and dissected under a stereoscopic microscope, the genitalia were separated and mounted in euparal on a glass slide. All specimens and slides were deposited in the Trematerra Collection (at the University of Molise, Campobasso, Italy). From 2013 to 2018 the localities visited were especially in the northern and central part of the Calabrian territory, as Pollino Massif, Sila Mountains and the bordering areas from sea level up to 1500 m of altitude (Figures I and II; Table 1).

In the compilation of this inventory we followed the systematic arrangement adopted by RAZOWSKI (2002 and 2003) with some modifications suggested by BROWN (2005) and by GILLIGAN *et al.* (2014). Distribution of the species was largely taken from RAZOWSKI (1970), TREMATERRA (2003) and AARVIK (2013), with new data from Trematerra's personal catalogue (unpubl. data). Chorotype of the species was taken from VIGNA TAGLIANTI *et al.* (1992) and PARENZAN (1994).

LIST OF TAXA

FAMILY TORTRICIDAE

SUBFAMILY TORTRICINAE

Tribe Tortricini

Tortrix viridana Linnaeus, 1758
Material examined: 2 males, Rende (Cosenza), 205 m, 14.V.2014, leg. Scalercio.
Chorotype: W-Palaearctic.

Aleimma loeflingianum (Linnaeus, 1758)
Material examined: 2 males, Montalto Uffugo (Cosenza), 230 m, 24.V.2014, leg. Scalercio; 1 male, San Fili (Cosenza), 831 m, 25.V.2017, leg. Colacci, Goglia and Scalercio.
Chorotype: W-Palaearctic.

Acleris abietana (Hübner, [1819-1822])
Material examined: 1 male, Vivaio Sbanditi (Cosenza), 1350 m, 11.V.2014, leg. Scalercio and Infusino; 1 female, S.S. Bruno (Vibo Valentia), 917 m, 6.IV.2016, leg. Scalercio and Infusino.
Chorotype: Sibiric-European.

Acleris sparsana ([Denis and Schiffermüller], 1775)
Material examined: 1 female, Vivaio Sbanditi (Cosenza), 1350 m, 27.X.2014, leg. Scalercio; 1 female, Lungro (Cosenza), 1270 m, 26.X.2016, leg. Scalercio and Infusino.
Chorotype: European.

Acleris rhombana ([Denis and Schiffermüller], 1775)
Material examined: 1 male, Vivaio Sbanditi (Cosenza), 1350 m, 13.X.2014, leg. Scalercio; 1 male, Rende (Cosenza), 205 m, 4.XI.2014, leg. Scalercio; 1 male, Saracena (Cosenza), 1010 m, 14.X.2015, leg. Scalercio and Infusino; 1 male, Alessandria del Carretto (Cosenza), 1285 m, 18.IX.2017, leg. Scalercio and Infusino.
Chorotype: Turanic-European.

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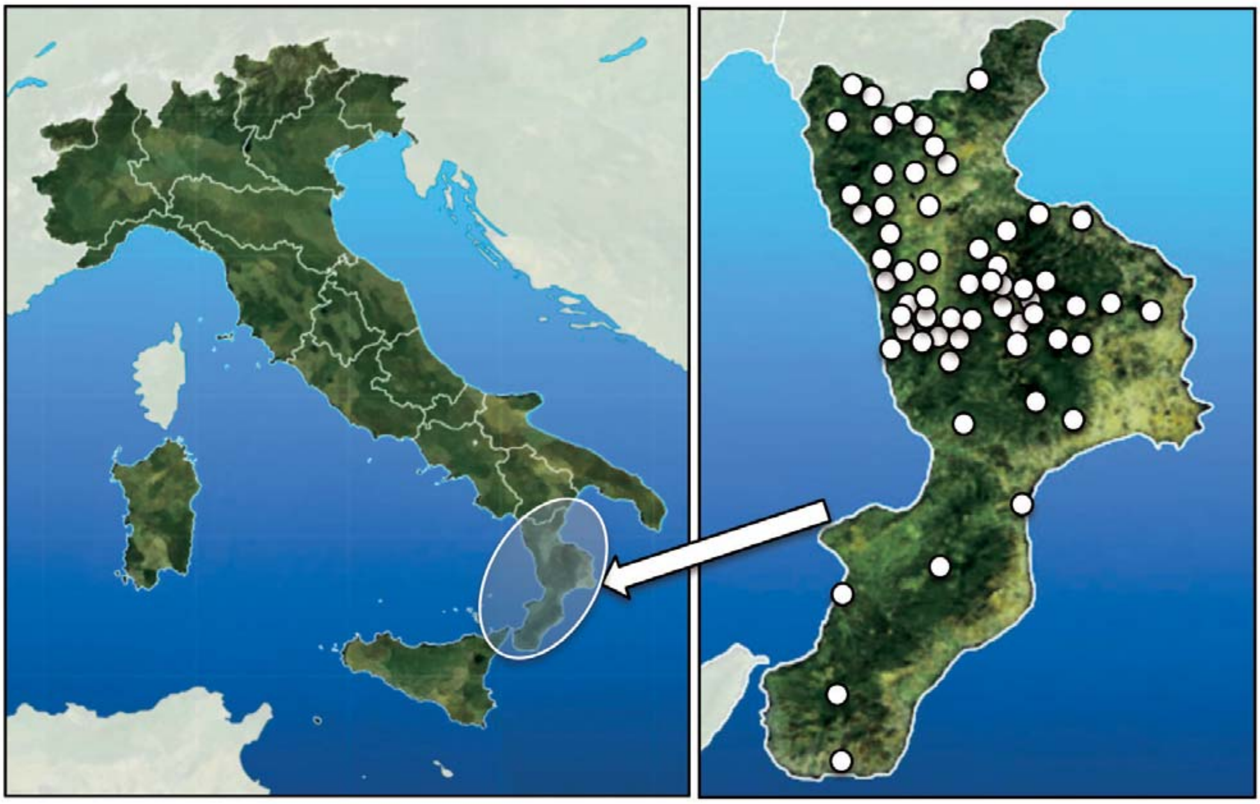

Fig. I – Calabria territory (southern Italy) (on left) and trapping localities (white circles) (on right).

Acleris schalleriana Linnaeus, 1761

Material examined: 1 female, Saracena (Cosenza), 1010 m, 19.VI.2015, leg. Scalercio.

Chorotype: Holarctic.

Acleris cristana ([Denis and Schiffermüller], 1775)

Material examined: 1 male, Aprigliano (Cosenza), 1310 m, 18.V.2015, leg. Scalercio and Infusino; 1 male, Saracena (Cosenza), 1465 m, 28.VII.2015, leg. Scalercio and Infusino; 1 male, Alessandria del Carretto (Cosenza), 1345 m, 17.VIII.2017, leg. Scalercio and Infusino.

Chorotype: Asiatic-European.

Acleris variegana ([Denis and Schiffermüller], 1775)

Material examined: 1 male, Lago Cecita (Cosenza), 1170 m, 15.XI.2013, leg. Scalercio; 1 male, Palizzi Marina (Reggio Calabria), 3 m, 15.V.2015, leg. Urso; 1 female, Fiego di San Fili (Cosenza), 740 m, 28.V.2015, leg. Scalercio and Infusino; 1 male, Saracena (Cosenza), 1010 m, 19.VI.2015, leg. Scalercio and Infusino; 1 female, S.S. Bruno (Vibo Valentia), 1120 m, 22.VI.2015, leg. Scalercio and Infusino; 1 male, Saracena (Cosenza), 1035 m, 23.IX.2015, leg. Scalercio and Infusino; 1 male, Montagna Grande (Cosenza), 1325 m, 4.XI.2015, leg. Scalercio and Infusino; 1 female, S.B. Ullano (Cosenza), 810 m, 1.VI.2016, leg. Scalercio and Infusino; 1 female, Lungro (Cosenza), 1270 m, 6.VI.2016, leg. Scalercio and Infusino.

Chorotype: Holarctic.

Acleris aspersana (Wood, 1839)

Material examined: 1 male, Righio (Cosenza), 1330 m, 24.VII.2017, leg. Scalercio and Infusino.

Chorotype: Centro Asiatic-European.

Acleris hastiana (Linnaeus, 1758)

Material examined: 1 female, S.S. Bruno (Vibo Valentia), 860 m, 7.VII.2016, leg. Scalercio and Infusino.

Chorotype: Holarctic.

Acleris notana (Dunovan, 1806)

Material examined: 1 female, M.te Pettinascura (Cosenza), 1300 m, 11.VI.1991, leg. Trematerra.

Chorotype: Palaearctic.

Acleris ferrugana ([Denis and Schiffermüller], 1775)

Material examined: 1 female, S.S. Bruno (Vibo Valentia), 830 m, 6.IV.2016, leg. Scalercio and Infusino.

Chorotype: Palaearctic.

Remarks: collected on early April, previously reported in TREMATERRA (2003) from June to November.

Acleris quercinana (Zeller, 1849)

Material examined: 1 female, Montalto Uffugo (Cosenza), 545 m, 1.IV.2016, leg. Scalercio and Infusino.

Chorotype: Turanic-European.

*** *Acleris boscanoides*** Razowski, 1959

Records: Strongoli (Crotone), 14.VI.1985 (in TREMATERRA, 2003; Pettersson, pers. comm.).

Chorotype: Turanic-European.

Acleris literana (Linnaeus, 1758)

Material examined: 2 females, Alessandria del Carretto (Cosenza), 1345 m, 17.VIII.2017, leg. Scalercio and Infusino.

Chorotype: European.

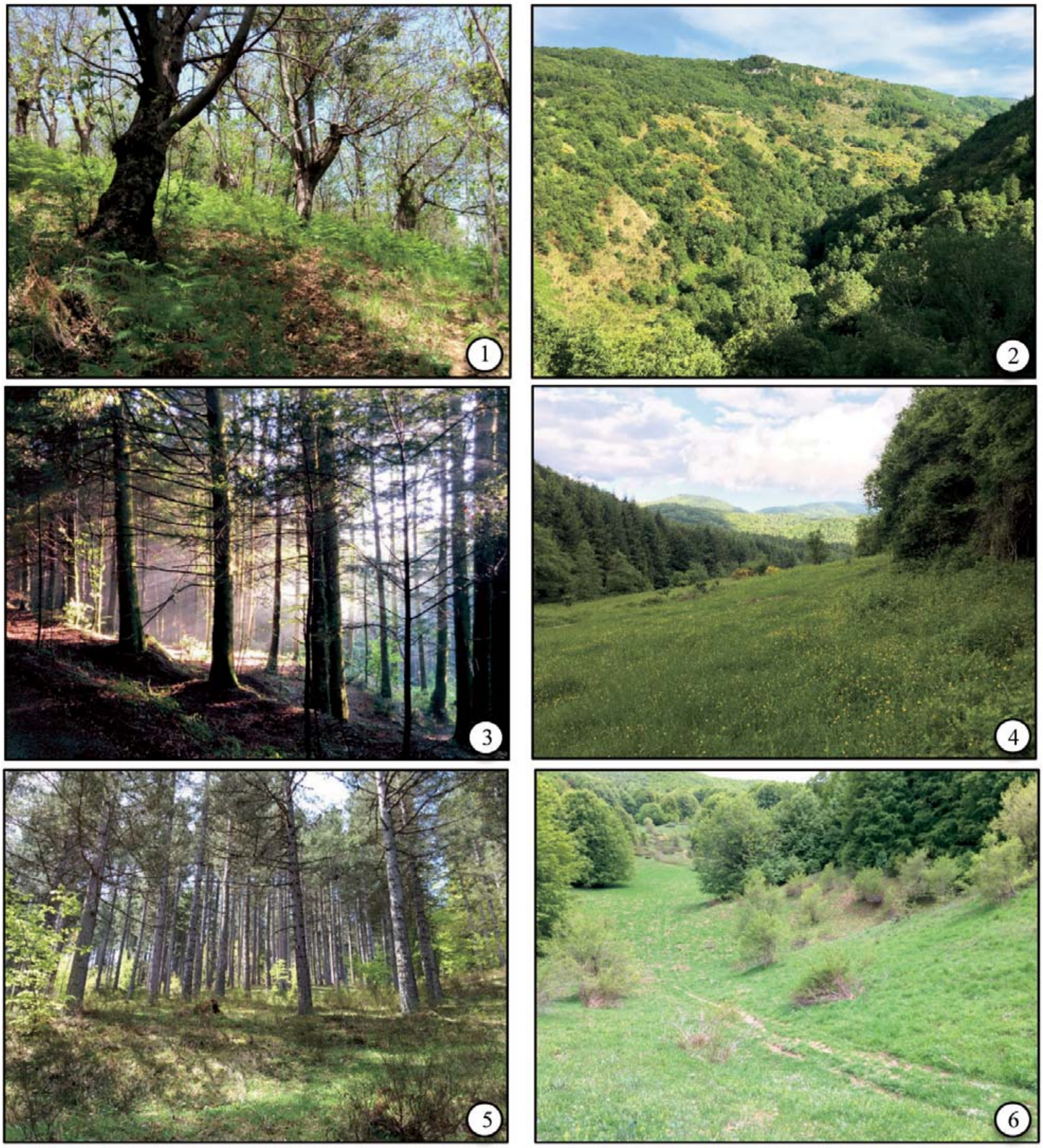

Fig. II – Some habitats visited during entomological expeditions in Calabria territory (southern Italy): Coastal chain of Paola 541 m a.s.l. (1); Carolei (Coastal chain) 558 m a.s.l. (2); Abetina of Serra San Bruno 917 m a.s.l. (3); Falconara Albanese (Coastal chain) 998 m a.s.l. (4); Sila National Park 1300 m a.s.l. (5); Orsomarso mountains, Pollino National Park 1470 m a.s.l. (6).

Tribe Cochylini

Phtheochroa purana (Guenée, 1846)

Material examined: 1 male, Vallone Colloreto (Cosenza), 800 m, 6.VI.1981, leg. Scalercio.

Chorotype: S-European.

Phtheochroa duponchelana (Duponchel, 1843)

Material examined: 2 males, Copanello (Catanzaro), 70 m, 9.V.1996, leg. Scalercio; 1 male, Copanello (Catanzaro), 80 m, 24.V.1996, leg. Gatti.

Chorotype: Mediterranean.

Phtheochroa rugosana (Hübner, [1769-1799])

Material examined: 1 female, S.B. Ullano (Cosenza), 893 m, 28.VI.2016, leg. Scalercio and Infusino.

Chorotype: Turanic-European-Mediterranean.

Cochylimorpha sparsana (Staudinger, 1880)

Material examined: 2 males, Vivaio Sbanditi (Cosenza), 1351 m, 18.V.2015, leg. Scalercio and Infusino; 1 male, Pedace (Cosenza), 1450 m, 18.V.2015, leg. Scalercio and Infusino; 1 male, Vivaio Sbanditi (Cosenza), 1351 m, 25.V.2015, leg. Scalercio and Infusino; 1 male, Montagna Grande (Cosenza), 1355 m, 18.V.2016, leg. Scalercio and Infusino.

Chorotype: Mediterranean.

Table 1 – List of trapping localities grouped by provinces of Cosenza, Crotone, Catanzaro, Vibo Valentia and Reggio Calabria (Calabria region, southern Italy).

Province of Cosenza	Mormanno	Spezzano della Sila, loc. Lago Cecita
Acquaformosa	Mormanno, loc. Monte Cerviero	Spezzano della Sila, loc. Vivaio Sbanditi
Aieta	Orsomarso	Spezzano Piccolo, loc. Righio
Alessandria del Carretto	Orsomarso, loc. Fiume Argentino	
Aprigliano	Paola	**Province of Crotone**
Calopezzati	Pedace	Savelli
Camigliatello	Rende	Strongoli
Camigliatello, loc. Monte Scuro	Rossano, loc. Rossano lido	
Campotenese, loc. Cozzi dell'Anticristo	San Benedetto Ullano	**Province of Catanzaro**
Carolei	San Bernardo in Fiore, loc. San Bernardo	Copanello
Castrovillari	San Fili	Perticaro
Cosenza, loc. Donnici	San Fili, loc. Fiego di San Fili	Sersale
Cotronei, loc. Lago Ampollino	San Giovanni in Fiore	Soveria Mannelli
Dipignano, loc. Pianette di Dipignano	San Giovanni in Fiore, loc. Mangiatoie	Taverna, loc. Monte Gariglione
Falconara Albanese	San Giovanni in Fiore, loc. Montagna Grande	
Laino Borgo, loc. Fiume Lao	San Giovanni in Fiore, loc. Monte Pettinascura	**Province of Vibo Valentia**
Longobucco	San Giovanni in Fiore, loc. Rovale	Serra San Bruno
Longobucco, loc. La Fossiata	San Lucido	
Lungro	San Nicola Silano	**Province of Reggio Calabria**
Mendicino	Sangineto	Palizzi Marina
Montalto Uffugo	Saracena	Roccaforte del Greco, loc. Diga del Menta
Morano Calabro, loc. Colloreto	Spezzano della Sila	San Ferdinando
Morano Calabro, loc. Vallone Colloreto	Spezzano della Sila, loc. Fallistro	

Phalonidia gilvicomana (Zeller, 1847)

Material examined: 1 male, S.S. Bruno (Vibo Valentia), 830 m, 30.V.2016, leg. Scalercio and Infusino.

Chorotype: European.

Phalonidia manniana (Fischer von Röslerstamm, 1839)

Material examined: 2 males, Orsomarso (Cosenza), 160 m, 27.VIII.2014, leg. Scalercio.

Chorotype: Asiatic-European.

Agapeta hamana (Linnaeus, 1758)

Material examined: 2 males, San Fili (Cosenza), 831 m, 25.V.2017, leg. Colacci, Goglia and Scalercio; 1 male; Falconara Albanese (Cosenza), 921 m, 25.V.2017, leg. Colacci, Goglia and Scalercio.

Chorotype: Centro Asiatic-European.

Agapeta zoegana (Linnaeus, 1767)

Material examined: 1 male, Pianette di Dipignano (Cosenza), 765 m, 29.VII.2013, leg. Scalercio; 1 male, La Fossiata (Cosenza), 1300 m, 13.VIII.2013, leg. Scalercio; 1 female, Spezzano della Sila (Cosenza), 1350 m, 8.VII.2014, leg. Scalercio; 2 males, Mangiatoie (Cosenza), 1275 m, 25.VII.2016, leg. Scalercio and Infusino.

Chorotype: Turanic-European.

Eupoecilia angustana (Hübner, [1796-1799])

Material examined: 1 female, Alessandria del Carretto (Cosenza), 1337 m, 19.VII.2017, leg. Scalercio and Infusino.

Chorotype: Asiatic-European.

Aethes piercei Obraztsov, 1952

Material examined: 1 male, Cozzi dell'Anticristo (Cosenza), 1300 m, 28.V.1989, leg. Trematerra; 1 female, M.te Crasta (Cosenza), 1200 m, 28.V.1989, leg. Trematerra; 2 females, S. Coppola di Paola (Cosenza), 1400 m, 13.VI.1991, leg. Trematerra.

Chorotype: European.

Aethes williana (Brahm, 1791)

Material examined: 1 male, San Ferdinando (Reggio Calabria), 5 m, 15.VII.2017, leg. Bonelli.

Chorotype: Centro Asiatic-European.

Aethes moribundana (Staudinger, 1859)

Material examined: 5 males, Saracena (Cosenza), 990 m, 20.V.2015, leg. Scalercio and Infusino.

Chorotype: Centro Asiatic-European-Mediterranean.

Aethes tesserana ([Denis and Schiffermüller], 1775)

Material examined: 1 male, Pedace (Cosenza), 1440 m, 15.VI.2015, leg. Scalercio and Infusino; 2 males, San Fili (Cosenza), 831 m, 25.V.2017, leg. Colacci, Goglia and Scalercio.

Chorotype: Centro Asiatic-European.

*** *Aethes kasyi*** Razowski, 1962

Records: Cozzi dell'Anticristo (Cosenza), 30.VII.1990, leg. Trematerra (in TREMATERRA *et al.*, 1994).

Chorotype: Turanic-European.

*** *Aethes francillana*** (Fabricius, 1794)
Records: 1 male, Castrovillari (CS), 350 m, 10.VI.1978 (in TREMATERRA, 2003; Parenzan, pers. comm.).
Chorotype: W-Palaearctic.

Aethes bilbaensis (Rössler, 1877)
Material examined: 1 male, Donnici (Cosenza), 550 m, 23.VII.2013, leg. Scalercio and Infusino; 1 male, Montagna Grande (Cosenza), 1355 m, 5.VII.2016, leg. Scalercio and Infusino; 1 male, Montalto Uffugo (Cosenza), 550 m, 27.VII.2016, leg. Scalercio and Infusino; 1 male, San Ferdinando (Reggio Calabria), 5 m, 11.VII.2017, leg. Bonelli.
Chorotype: Centro Asiatic-European-Mediterranean.

Aethes rubiginana (Walsingham, 1903)
Material examined: 2 males, Copanello (Catanzaro), 70 m, 10.V.1996, leg. Scalercio; 1 female, Copanello (Catanzaro), 70 m, 10.V.1996, leg. Gatti; 1 female, Copanello (Catanzaro), 70 m, 25.VI.1996, leg. Gatti.
Chorotype: NW-African (extension S-Apenninic-Sicilian).

Aethes mauritanica (Walsingham, 1898)
Material examined: 1 female, Rossano lido (Cosenza), 8.V.1981, leg. Scalercio.
Chorotype: Mediterranean.

Aethes cnicana (Westwood, 1854)
Material examined: 2 males, S.S. Bruno (Vibo Valentia), 925 m, 7.VII.2016, leg. Scalercio and Infusino.
Chorotype: Asiatic-European.

*** *Cochylis hybridella*** (Hübner, [1810-1813])
Records: 3 males, Camigliatello (Cosenza), 3.VIII.1921 (in TREMATERRA, 1991b).
Chorotype: Asiatic-European.

Cochylis salebrana (Mann, 1862)
Material examined: 1 male, Pianette di Dipignano (Cosenza), 765 m, 5.VIII.2013, leg. Scalercio; 1 male, Montalto Uffugo (Cosenza), 550 m, 25.VI.2015, leg. Scalercio and Infusino.
Chorotype: S-European.

Cochylis molliculana Zeller, 1847
Material examined: 1 male, Rende (Cosenza), 204 m, 24.V.2017, leg. Colacci and Goglia.
Chorotype: S-European.

Cochylis posterana Zeller, 1847
Material examined: 1 male, Lago Cecita (Cosenza), 1176 m, 2.IX.2013, leg. Scalercio; 2 males, San Fili (Cosenza), 630 m, 25.VI.2015, leg. Scalercio and Infusino; 1 male, Fiego di San Fili (Cosenza), 720 m, 25.VI.2015, leg. Scalercio and Infusino; 1 male, Fiego di San Fili (Cosenza), 740 m, 25.VI.2015, leg. Scalercio and Infusino; 1 female, San Bernardo (Cosenza), 1380 m, 11.V.2016, leg. Scalercio and Infusino; 1 male, Saracena (Cosenza), 1420 m, 6.VI.2016, leg. Scalercio and Infusino; 1 female, San Bernardo (Cosenza), 1380 m, 25.VII.2016, leg. Scalercio and Infusino; 1 female, Saracena (Cosenza), 1475 m, 1.VIII.2016, leg. Scalercio and Infusino; 1 female, Montalto Uffugo (Cosenza), 545 m, 29.VIII.2016, leg. Scalercio and Infusino; 1 male, Alessandria del Carretto (Cosenza), 1291 m, 21.VI.2017, leg. Scalercio and Infusino; 1 male, Alessandria del Carretto (Cosenza), 1291 m, 19.VII.2017, leg. Scalercio and Infusino.
Chorotype: W-Palaearctic.

Tribe Cnephasiini

*** *Propiromorpha rhodophana*** (Herrich-Schäffer, 1849)
Records: Rossano Lido (Cosenza), 5 m, 20.VII.1981, leg. Parenzan (in TREMATERRA *et al.*, 1997).
Chorotype: Palaearctic.

Eana penziana (Hübner, [1796-1799])
Material examined: 1 male, Saracena (Cosenza), 1035 m, 15.VI.2015, leg. Scalercio and Infusino; 1 male, S.S. Bruno (Vibo Valentia), 860 m, 22.VI.2015, leg. Scalercio and Infusino; 2 males, San Bernardo (Cosenza), 1383 m, 7.VI.2016, leg. Scalercio and Infusino.
Chorotype: Centro Asiatic-European.

Eana italica (Obraztsov, 1950)
Material examined: 1 female, Saracena (Cosenza), 1475 m, 1.VII.2016, leg. Scalercio and Infusino.
Chorotype: Apenninic-Dinaric.

Archicnephasia hartigi Razowski, 1983
Material examined: 1 male, Alessandria del Carretto (Cosenza), 1345 m, 12.XI.2017, leg. Scalercio and Infusino.
Chorotype: S-Apenninic.

Cnephasia incertana (Treitschke, 1835)
Material examined: 5 males, Falconara Albanese (Cosenza), 921 m, 25.V.2017, leg. Colacci, Goglia and Scalercio; 5 males, San Fili (Cosenza), 831 m, 25.V.2017, leg. Colacci, Goglia and Scalercio.
Chorotype: W-Palaearctic.

Cnephasia stephensiana (Doubleday, 1850)
Material examined: 1 female, Spezzano della Sila (Cosenza), 1299 m, 24.VII.2017, leg. Scalercio and Infusino; 1 female, Fallistro (Cosenza), 1376 m, 24.VII.2017, leg. Scalercio and Infusino.
Chorotype: Palaearctic.

*** *Cnephasia asseclana*** ([Denis and Schiffermüller], 1775)
Records: Sersale (Catanzaro), 6-20.VII.1986 (in TREMATERRA *et al.*, 1994).
Chorotype: Holarctic.

Cnephasia zangheriana Trematerra, 1991
Material examined: 1 male, Vivaio Sbanditi (Cosenza), 1350 m, 26.VI.2014, leg. Scalercio; 1 male, Saracena (Cosenza), 1370 m, 20.V.2015, leg. Scalercio and Infusino; 2 males, Saracena (Cosenza), 1431 m, 6.VI.2016, leg. Scalercio and Infusino; 3 males, San Nicola Silano (Cosenza), 1435 m, 17.V.2018, leg. Colacci and Goglia.
Chorotype: Apenninic.

Cnephasia pasiuana (Hübner, [1796-1799])
Material examined: 1 female, Palizzi Marina (Reggio Calabria), 3 m, 15.V.2015, leg. Urso.
Chorotype: W-Palaearctic.

Cnephasia communana (Herrich-Schäffer, 1847)
Material examined: 1 female, Pedace (Cosenza), 1450 m, 15.VII.2015, leg. Scalercio and Infusino; 1 male, Acquaformosa (Cosenza), 1357 m, 15.IV.2016, leg. Scalercio and Infusino; 1 male, Montalto Uffugo (Cosenza), 565 m, 1.VI.2016, leg. Scalercio and Infusino.
Chorotype: Centro Asiatic-European-Mediterranean.

Cnephasia cupressivorana (Staudinger, 1871)
Material examined: 1 male, Alessandria del Carretto (Cosenza), 1253 m, 18.IV.2017, leg. Scalercio and Infusino.
Chorotype: S-European.

Cnephasia abrasana (Duponchel, 1843)

Material examined: 1 male, Righio (CS), 1341 m, 24.V.2017, leg. Scalercio and Infusino; 1 male, Fallistro (Cosenza), 1376 m, 31.V.2017, leg. Scalercio and Infusino.

Chorotype: European.

*** *Cnephasia chrysantheana*** (Duponchel, 1842)

Records: Sersale (Catanzaro), 15.VI-20.VII.1985; Sersale (Catanzaro), 15.VI-20.VII.1986 (in Trematerra *et al.*, 1994).

Chorotype: W-Palaearctic.

Cnephasia fulturata Rebel, 1940

Material examined: 1 male, Palizzi Marina (Reggio Calabria), 3 m, 15.V.2015, leg. Urso.

Chorotype: Turanic-European-Mediterranean.

Cnephasia hellenica Obraztsov, 1956

Material examined: 1 male, Fiego di San Fili (Cosenza), 740 m, 25.VI.2015, leg. Scalercio and Infusino.

Chorotype: Turanic-European.

Cnephasia ecullyana Réal, 1951

Material examined: 1 female, Rende (Cosenza), 205 m, 14.V.2014, leg. Scalercio.

Chorotype: S-European.

Tribe Archipini

Batodes angustiorana (Haworth, [1811])

Material examined: 1 male, Montalto Uffugo (Cosenza), 565 m, 1.VI.2016, leg. Scalercio and Infusino.

Chorotype: W-Palaearctic.

Epagoge grotiana (Fabricius, 1781)

Material examined: 1 female, S.B. Ullano (Cosenza), 880 m, 28.VI.2016, leg. Scalercio and Infusino.

Chorotype: W-Palaearctic.

Paramesia gnomana (Clerck, 1759)

Material examined: 1 male, Cozzi dell'Anticristo (Cosenza), 1300 m, 30.VII.1990, leg. Trematerra.

Chorotype: European.

Paramesia diffusana (Kennel, 1899)

Material examined: 2 males, Lago Cecita (Cosenza), 1163 m, 13.VIII.2013, leg. Scalercio; 1 male, Vivaio Sbanditi (Cosenza), 1350 m, 5.VIII.2014, leg. Scalercio; 1 male, Vivaio Sbanditi (Cosenza), 1350 m, 13.VIII.2014, leg. Scalercio; 1 female, S.S. Bruno (Vibo Valentia), 924 m, 6.IV.2016, leg. Scalercio and Infusino; 1 male, Saracena (Cosenza), 1460 m, 1.VIII.2016, leg. Scalercio and Infusino.

Chorotype: W-Mediterranean.

Archips podanus (Scopoli, 1763)

Material examined: 2 males, Orsomarso (Cosenza), 160 m, 27.VIII.2014, leg. Scalercio; 1 male, S.S. Bruno (Vibo Valentia), 840 m, 22.VI.2015, leg. Scalercio and Infusino; 1 male, S.S. Bruno (Vibo Valentia), 830 m, 30.V.2016, leg. Scalercio and Infusino.

Chorotype: Asiatic-European.

Archips crataeganus (Hübner, [1796-1799])

Material examined: 1 male, Montalto Uffugo (Cosenza), 230 m, 24.V.2014, leg. Scalercio.

Chorotype: Palaearctic.

Archips rosanus (Linnaeus, 1758)

Material examined: 1 male, Fiego di San Fili (Cosenza), 720 m, 25.VI.2015, leg. Scalercio and Infusino.

Chorotype: Palaearctic.

Choristoneura diversana (Hübner, [1814-1817])

Material examined: 1 male, S.S. Bruno (Vibo Valentia), 840 m, 22.VI.2015, leg. Scalercio and Infusino.

Chorotype: Asiatic-European.

*** *Choristoneura murinana*** (Hübner, [1796-1799])

Records: Pollino, 10.VII.1989, leg. Trematerra (in Trematerra *et al.*, 1994).

Chorotype: Asiatic-European.

Argyrotaenia ljungiana (Thunberg, 1797)

Material examined: 1 male, Montalto Uffugo (Cosenza), 545 m, 25.VI.2015, leg. Scalercio and Infusino.

Chorotype: Holarctic.

Ptycoloma lecheana (Linnaeus, 1758)

Material examined: 1 male, Alessandria del Carretto (Cosenza), 1285 m, 21.VI.2017, leg. Scalercio and Infusino.

Chorotype: Asiatic-European.

Pandemis cerasana (Hübner, 1786)

Material examined: 1 male, Vivaio Sbanditi (Cosenza), 1350 m, 26.VI.2014, leg. Scalercio; 1 male, Vivaio Sbanditi (Cosenza), 1350 m, 29.VII.2014, leg. Scalercio.

Chorotype: Asiatic-European.

Pandemis heparana ([Denis and Schiffermüller], 1775)

Material examined: 1 female, Lago Cecita (Cosenza), 1163 m, 30.VII.2013, leg. Scalercio; 2 males, La Fossiata (Cosenza), 1300 m, 13.VIII.2013, leg. Scalercio; 1 female, Vivaio Sbanditi (Cosenza), 1350 m, 29.VII.2014, leg. Scalercio; 2 males, Lago Cecita (Cosenza), 1163 m, 13.VIII.2014, leg. Scalercio; 2 males, Orsomarso (Cosenza), 160 m, 27.VIII.2014, leg. Scalercio.

Chorotype: Asiatic-European.

Syndemis musculana (Hübner, [1796-1799])

Material examined: 1 male, Vivaio Sbanditi (Cosenza), 1350 m, 26.VI.2014, leg. Scalercio; 1 male, Vivaio Sbanditi (Cosenza), 1350 m, 20.X.2014, leg. Scalercio; 1 female, S.S. Bruno (Vibo Valentia), 830 m, 30.V.2016, leg. Scalercio and Infusino; 1 male, S.S. Bruno (Vibo Valentia), 835 m, 30.V.2016, leg. Scalercio and Infusino; 1 male, S.S. Bruno (Vibo Valentia), 883 m, 30.V.2016, leg. Scalercio and Infusino; 1 male, S.S. Bruno (Vibo Valentia), 917 m, 30.V.2016, leg. Scalercio and Infusino; 1 male, S.S. Bruno (Vibo Valentia), 924 m, 30.V.2016, leg. Scalercio and Infusino; 1 male, S.B. Ullano (Cosenza), 810 m, 1.VI.2016, leg. Scalercio and Infusino; 1 female, S.B. Ullano (Cosenza), 880 m, 1.VI.2016, leg. Scalercio and Infusino; 1 female, Saracena (Cosenza), 1431 m, 6.VI.2016, leg. Scalercio and Infusino; 1 male, San Giovanni in Fiore (Cosenza), 1250 m, 24.V.2017, leg. Scalercio and Infusino; 2 males, San Fili (Cosenza), 831 m, 25.V.2017, leg. Colacci, Goglia and Scalercio; 1 male, Rovale (Cosenza), 1440 m, 17.V.2018, leg. Colacci and Goglia.

Chorotype: Asiatic-European.

Cacoecimorpha pronubana (Hübner, [1796-1799])

Material examined: 1 male, Montalto Uffugo (Cosenza),

550 m, 27.VII.2016, leg. Scalercio and Infusino; 1 female, San Ferdinando (Reggio Calabria), 5 m, 15.VII.2017, leg. Bonelli.
Chorotype: European-Mediterranean.

*** *Aphelia ochreana*** (Hübner, [1797-1799])
Records: Cozzo San Lorenzo (Cosenza), 29.V.1990 (in TREMATERRA *et al.*, 1994).
Chorotype: Turanic-European.

Dichelia histrionana (Frölich, 1828)
Material examined: 1 male, Vivaio Sbanditi (Cosenza), 1355 m, 18.VI.2014, leg. Scalercio; 2 males, Vivaio Sbanditi (Cosenza), 1350 m, 20.VII.2014, leg. Scalercio; 1 male, Vivaio Sbanditi (Cosenza), 1351 m, 27.VII.2015, leg. Scalercio and Infusino.
Chorotype: European.

*** *Clepsis spectrana*** (Treitschke, 1830)
Records: Rossano (Cosenza), 2 m, 8.V.1981, leg. Parenzan (in TREMATERRA *et al.*, 1997).
Chorotype: European.

*** *Clepsis pallidana*** (Fabricius, 1776)
Records: Rossano (Cosenza), 2 m, 8.V.1981, leg. Parenzan (in TREMATERRA *et al.*, 1997).
Chorotype: Asiatic-European.

Clepsis consimilana (Hübner, [1814-1817])
Material examined: 1 female, Donnici (Cosenza), 550 m, 23.VII.2013, leg. Scalercio; 1 male, Pianette di Dipignano (Cosenza), 765 m, 5.VIII.2013, leg. Scalercio; 1 male, Rende (Cosenza), 205 m, 14.V.2014, leg. Scalercio; 1 male, Sangineto (Cosenza), 50 m, 10.VIII.2014, leg. Scalercio; 1 male, Palizzi Marina (Reggio Calabria), 3 m, 15.V.2015, leg. Urso; 1 male, Montalto Uffugo (Cosenza), 565 m, 28.V.2015, leg. Scalercio and Infusino; 1 male, Rende (Cosenza), 204 m, 24.V.2017, leg. Colacci and Goglia; 4 males, Carolei (Cosenza), 558 m, 24.V.2017, leg. Colacci, Goglia and Scalercio; 1 male and 1 female, Paola (Cosenza), 2 m, 25.V.2017, leg. Colacci, Goglia and Scalercio; 3 males, San Fili (Cosenza), 831 m, 25.V.2017, leg. Colacci, Goglia and Scalercio.
Chorotype: W-Palaearctic.

Tribe Euliini

*** *Eulia ministrana*** (Linnaeus, 1758)
Records: Sersale (Catanzaro), 29.VI-3.VIII.1985; Sersale (Catanzaro), 29.VI-3.VIII.1986 (in TREMATERRA *et al.*, 1994).
Chorotype: Holarctic.

SUBFAMILY CHLIDANOTINAE

Tribe Polyorthini

Olindia schumacherana (Fabricius, 1787)
Material examined: 1 female, S.S. Bruno (Vibo Valentia), 860 m, 22.VI.2015, leg. Scalercio and Infusino; 2 males, S.S. Bruno (Vibo Valentia), 924 m, 30.V.2016, leg. Scalercio and Infusino.
Chorotype: European.

Isotrias rectifasciana (Haworth, [1811])
Material examined: 1 female, Lago Cecita (Cosenza), 1163 m, 30.VII.2013, leg. Scalercio.
Chorotype: European.

*** *Isotrias joannisana*** (Turati, 1921)
Records: Cozzi dell'Anticristo (Cosenza), 28.V.1989; Cozzi dell'Anticristo (Cosenza), 28.V.1990 (in TREMATERRA *et al.*, 1994).
Chorotype: Apenninic.

Isotrias martelliana Trematerra, 1990
Material examined: 1 male and 1 female, Cozzi dell'Anticristo (Cosenza), 1300 m, 28.V.1989, leg. Trematerra.
Chorotype: S-Apenninic.

SUBFAMILY OLETHREUTINAE

Tribe Bactrini

Bactra bactrana (Kennel, 1901)
Material examined: 1 male, Righio (Cosenza), 1355 m, 23.V.2014, leg. Scalercio; 1 male, S.S. Bruno (Vibo Valentia), 830 m, 10.V.2016, leg. Scalercio and Infusino.
Chorotype: Subcosmopolitan.

Bactra lancealana (Hübner, [1796-1799])
Material examined: 1 female, Orsomarso (Cosenza), 160 m, 27.VIII.2014, leg. Scalercio.
Chorotype: Subcosmopolitan.

Bactra venosana (Zeller, 1847)
Material examined: 1 male, Paola (Cosenza), 20 m, 9.VI.2017, leg. Scalercio.
Chorotype: Subcosmopolitan.

Tribe Olethreutini

Endothenia gentianaeana (Hübner, [1796-1799])
Material examined: 1 male and 1 female, Orsomarso (Cosenza), 160 m, 27.VIII.2014, leg. Scalercio.
Chorotype: Asiatic-European.

*** *Endothenia marginana*** (Haworth, [1811])
Records: 1 male, Aieta (Cosenza), 500 m, 12.VII.1989, leg. Hausmann (in TREMATERRA, 2003; unpubl. data).
Chorotype: Palaearctic.

Endothenia lapideana (Herrich-Schäffer, 1848)
Material examined: 1 male, Aieta (Cosenza), 500 m, 12.VII.1989, leg. Hausmann.
Chorotype: European.

Endothenia nigricostana (Haworth, [1811])
Material examined: 1 male, Vivaio Sbanditi (Cosenza), 1350 m, 13.VIII.2014, leg. Scalercio.
Chorotype: Asiatic-European.

Endothenia pullana (Haworth, [1811])
Material examined: 1 female, Lago Cecita (Cosenza), 1163 m, 13.VIII.2014, leg. Scalercio.
Chorotype: Sibiric-European.

Lobesia botrana ([Denis and Schiffermüller], 1775)
Material examined: 1 male, Calopezzati (Cosenza), 10 m, 25.IV.2015, leg. Scalercio.
Chorotype: Holarctic.

**** Lobesia bicinctana*** (Duponchel, 1844)
Records: 1 male, Aieta (Cosenza), 300 m, 11.VII.1989, leg. Hausmann (in TREMATERRA, 2003; unpubl. data).
Chorotype: Holarctic.

Eudemis profundana ([Denis and Schiffermüller], 1775)
Material examined: 1 male, Donnici (Cosenza), 550 m, 23.VII.2013, leg. Scalercio.
Chorotype: European.

**** Hedya salicella*** (Linnaeus, 1758)
Records: 1 female, Lao river (Cosenza), 50 m, 8.VI.1990, leg. Hausmann (in TREMATERRA, 2003; unpubl. data).
Chorotype: Asiatic-European.

Hedya nubiferana (Haworth, [1811])
Material examined: 1 male, Alessandria del Carretto (Cosenza), 1285 m, 21.VI.2017, leg. Scalercio and Infusino.
Chorotype: Centro Asiatic-European.

**** Hedya ochroleucana*** (Frölich, 1828)
Records: Sersale (Catanzaro), 15.VI-10.VIII.1985 (in TREMATERRA *et al.*, 1994).
Chorotype: Holarctic.

Metendothenia atropunctana (Zetterstedt, 1839)
Material examined: 1 male, Saracena (Cosenza), 1340 m, 20.V.2015, leg. Scalercio and Infusino; 1 male, Vivaio Sbanditi (Cosenza), 1351 m, 25.V.2015, leg. Scalercio and Infusino; 1 male, S.S. Bruno (Vibo Valentia), 970 m, 26.V.2015, leg. Scalercio and Infusino; 1 male, Vivaio Sbanditi (Cosenza), 1352 m, 15.VI.2015, leg. Scalercio and Infusino; 1 male, Acquaformosa (Cosenza), 1357 m, 6.VI.2016, leg. Scalercio and Infusino; 1 female, Saracena (Cosenza), 1431 m, 6.VI.2016, leg. Scalercio and Infusino; 1 male, Saracena (Cosenza), 1010 m, leg. Scalercio and Infusino; 1 male, San Nicola Silano (Cosenza), 1435 m, 17.V.2018, leg. Colacci and Goglia.
Chorotype: Holarctic.

Piniphila bifasciana (Haworth, [1811])
Material examined: 1 female, Lago Cecita (Cosenza), 1163 m, 30.VII.2013, leg. Scalercio; 1 male, La Fossiata (Cosenza), 1300 m, 13.VIII.2013, leg. Scalercio; 1 male, Vivaio Sbanditi (Cosenza), 1350 m, 5.VIII.2014, leg. Scalercio; 3 males, Montagna Grande (Cosenza), 1325 m, 25.VII.2016, leg. Scalercio and Infusino.
Chorotype: Asiatic-European.

Olethreutes arcuellus (Clerck, 1759)
Material examined: 2 males, Vivaio Sbanditi (Cosenza), 1350 m, 18.VI.2014, leg. Scalercio; 4 males, San Fili (Cosenza), 831 m, 25.V.2017, leg. Colacci, Goglia and Scalercio; 4 males, Falconara Albanese (Cosenza), 921 m, 25.V.2017, leg. Colacci, Goglia and Scalercio; 1 male, San Nicola Silano (Cosenza), 1435 m, 17.V.2018, leg. Colacci and Goglia.
Chorotype: Centro Asiatic-European.

Celypha cespitana (Hübner, [1814-1817])
Material examined: 1 male, San Bernardo (Cosenza), 1380 m, 25.VII.2016, leg. Scalercio and Infusino.
Chorotype: Holarctic.

Syricoris lacunana ([Denis and Schiffermüller], 1775)
Material examined: 1 male, Donnici (Cosenza), 550 m, 7.VIII.2013, leg. Scalercio; 2 males, Sila (Cosenza), 1300 m, 18.VI.2014, leg. Scalercio; 1 male, Vivaio Sbanditi (Cosenza), 1350 m, 26.VI.2014, leg. Scalercio; 2 males, Vivaio Sbanditi (Cosenza), 1350 m, 17.VII.2014, leg. Scalercio; 2 males, Montalto Uffugo (Cosenza), 540 m, 28.V.2015, leg. Scalercio and Infusino; 1 male, Montagna Grande (Cosenza), 1370 m, 15.VI.2015, leg. Scalercio and Infusino; 2 males, Saracena (Cosenza), 1475 m, 19.VI.2015, leg. Scalercio and Infusino; 2 males, S.S. Bruno (Vibo Valentia), 840 m, 22.VI.2015, leg. Scalercio and Infusino; 1 male, S.S. Bruno (Vibo Valentia), 830 m, 30.V.2016, leg. Scalercio and Infusino; 2 males, S.S. Bruno (Vibo Valentia), 924 m, 30.V.2016, leg. Scalercio and Infusino; 1 male, Lungro (Cosenza), 1270 m, 6.VI.2016, leg. Scalercio and Infusino; 1 male, Acquaformosa (Cosenza), 1366 m, 6.VI.2016, leg. Scalercio and Infusino; 2 males and 1 female, Carolei (Cosenza), 558 m, 24.V.2017, leg. Colacci, Goglia and Scalercio; 9 males and 3 females, San Fili (Cosenza), 831 m, 25.V.2017, leg. Colacci, Goglia and Scalercio; 12 males, Falconara Albanese (Cosenza), 921 m, 25.V.2017, leg. Colacci, Goglia and Scalercio; 1 male, Alessandria del Carretto (Cosenza), 1285 m, 21.VI.2017, leg. Scalercio and Infusino.
Chorotype: Asiatic-European.

Syricoris rivulana (Scopoli, 1763)
Material examined: 1 male, S.S. Bruno (Vibo Valentia), 835 m, 30.V.2016, leg. Scalercio and Infusino.
Chorotype: Asiatic-European.

Tribe Enarmoniini

Ancylis laetana (Fabricius, 1775)
Material examined: 1 male, Soveria Mannelli (Catanzaro), 12.VI.1991, leg. Trematerra.
Chorotype: Sibiric-European.

Ancylis comptana (Frölich, 1828)
Material examined: 1 male, Saracena (Cosenza), 1315 m, 14.V.2015, leg. Scalercio and Infusino.
Chorotype: Holarctic.

Ancylis paludana (Barrett, 1871)
Material examined: 3 males, San Fili (Cosenza), 831 m, 25.V.2017, leg. Colacci, Goglia and Scalercio.
Chorotype: Holarctic.

Ancylis badiana ([Denis and Schiffermüller], 1775)
Material examined: 1 female, Vivaio Sbanditi (Cosenza), 1350 m, 26.VI.2014, leg. Scalercio; 1 male, Montagna Grande (Cosenza), 1370 m, 15.VI.2015, leg. Scalercio and Infusino.
Chorotype: Asiatic-European.

Ancylis mitterbacheriana ([Denis and Schiffermüller], 1775)
Material examined: 1 male, S.B. Ullano (Cosenza), 893 m, 1.VI.2016, leg. Scalercio and Infusino; 1 male, Saracena (Cosenza), 1431 m, 6.VI.2016, leg. Scalercio and Infusino.
Chorotype: European.

Tribe Eucosmini

**** Thiodia major*** (Rebel, 1903)
Records: Mendicino (Cosenza), 15.VI-6.VII.1989 (in TREMATERRA *et al.*, 1994).
Chorotype: S-European.

Thiodia trochilana (Frölich, 1828)

Material examined: 2 males, Saracena (Cosenza), 1035 m, 15.VI.2015, leg. Scalercio and Infusino.

Chorotype: Turanic-European-Mediterranean.

Rhopobota stagnana ([Denis and Schiffermüller], 1775)

Material examined: 2 males, Cozzi dell'Anticristo (Cosenza), 1300 m, 28.V.1989, leg. Trematerra.

Chorotype: European.

Rhopobota naevana (Hübner, [1814-1817])

Material examined: 1 female, S.S. Bruno (Vibo Valentia), 886 m, 30.V.2016, leg. Scalercio and Infusino.

Chorotype: Subcosmopolitan.

Spilonota ocellana ([Denis and Schiffermüller], 1775)

Material examined: 1 female, Lago Cecita (Cosenza), 1163 m, 30.VII.2013, leg. Scalercio; 1 male and 1 female, Vivaio Sbanditi (Cosenza), 1350 m, 29.VII.2014, leg. Scalercio; 1 male, Mormanno (Cosenza), 970 m, 1.VIII.2016, leg. Scalercio and Infusino; 1 female, Acquaformosa (Cosenza), 1357 m, 1.VIII.2016, leg. Scalercio and Infusino; 1 male, S.S. Bruno (Vibo Valentia), 883 m, 3.VIII.2016, leg. Scalercio and Infusino; 1 male, S.S. Bruno (Vibo Valentia), 925 m, 3.VIII.2016, leg. Scalercio and Infusino; 1 male, Alessandria del Carretto (Cosenza), 1253 m, 13.VII.2017, leg. Scalercio and Infusino; 1 male, Alessandria del Carretto (Cosenza), 1345 m, 19.VII.2017, leg. Scalercio and Infusino.

Chorotype: Holarctic.

*** *Spilonota laricana*** (Heinemann, 1863)

Records: Sersale (Catanzaro), 20.VII.1985 (in TREMATERRA *et al.*, 1994).

Chorotype: Holarctic.

Epinotia festivana (Hübner, 1799)

Material examined: 1 female, Donnici (Cosenza), 550 m, 23.VII.2013, leg. Scalercio; 1 male, Montalto Uffugo (Cosenza), 550 m, 25.VI.2015, leg. Scalercio and Infusino; 1 male, Montalto Uffugo (Cosenza), 545 m, 1.VI.2016, leg. Scalercio and Infusino.

Chorotype: Holarctic.

Epinotia thapsiana (Zeller, 1847)

Material examined: 1 female, Palizzi Marina (Reggio Calabria), 3 m, 15.V.2015, leg. Urso.

Chorotype: Centro Asiatic-European-Mediterranean.

Epinotia sordidana (Hübner, [1823-1824])

Material examined: 1 male, Vivaio Sbanditi (Cosenza), 1350 m, 13.X.2014, leg. Scalercio; 1 male, Alessandria del Carretto (Cosenza), 1285 m, 18.IX.2017, leg. Scalercio and Infusino; 1 male, Alessandria del Carretto (Cosenza), 1345 m, 18.IX.2017, leg. Scalercio and Infusino; 1 female, Alessandria del Carretto (Cosenza), 1305 m, 12.XI.2017, leg. Scalercio and Infusino; 1 female, Alessandria del Carretto (Cosenza), 1345 m, 12.XI.2017, leg. Scalercio and Infusino.

Chorotype: European.

Epinotia solandriana (Linnaeus, 1758)

Material examined: 1 male, Vivaio Sbanditi (Cosenza), 1350 m, 13.X.2014, leg. Scalercio.

Chorotype: Holarctic.

Remarks: collected in middle October, previously reported in TREMATERRA (2003) from June to August.

Epinotia signatana (Douglas, 1845)

Material examined: 2 females, Cozzi dell'Anticristo (Cosenza), 1000 m, 13.VI.1991, leg. Trematerra.

Chorotype: Asiatic-European.

Epinotia immundana (Ficher von Röslerstamm, 1839)

Material examined: 1 male, Donnici (Cosenza), 550 m, 23.VII.2013, leg. Scalercio.

Chorotype: Turanic-European.

Epinotia subocellana (Donovan, [1806])

Material examined: 1 male, Rovale (Cosenza), 1440 m, 17.V.2018, leg. Colacci and Goglia.

Chorotype: Sibiric-European.

*** *Epinotia nigricana*** (Herrich-Schäffer, 1847)

Records: Sila varie località (Cosenza), 13.VI.1991 (in TREMATERRA *et al.*, 1994).

Chorotype: European.

Epinotia tenerana ([Denis and Schiffermüller], 1775)

Material examined: 2 males, Vivaio Sbanditi (Cosenza), 1350 m, 17.VII.2014, leg. Scalercio; 1 male, Vivaio Sbanditi (Cosenza), 1350 m, 29.VII.2014, leg. Scalercio; 1 female, Vivaio Sbanditi (Cosenza), 1350 m, 13.VIII.2014, leg. Scalercio; 1 male, Vivaio Sbanditi (Cosenza), 1351 m, 6.VII.2015, leg. Scalercio and Infusino; 1 female, Vivaio Sbanditi (Cosenza), 1351 m, 27.VII.2015, leg. Scalercio and Infusino; 1 female, S.S. Bruno (Vibo Valentia), 917 m, 3.VIII.2016, leg. Scalercio and Infusino; 3 females, Lungro (Cosenza), 1270 m, 31.VIII.2016, leg. Scalercio and Infusino; 1 male, San Fili (Cosenza), 831 m, 25.V.2017, leg. Colacci, Goglia and Scalercio; 1 male, Spezzano della Sila (Cosenza), 1324 m, 24.VII.2017, leg. Scalercio and Infusino.

Chorotype: Cosmopolitan.

Epinotia fraternana (Haworth, [1811])

Material examined: 1 female, San Fili (Cosenza), 831 m, 25.V.2017, leg. Colacci, Goglia and Scalercio.

Chorotype: European.

Crocidosema plebejana Zeller, 1847

Material examined: 1 male and 1 female, Montescuro (Cosenza), 1610 m, 6.III.2016, leg. Scalercio; 1 male, Montalto Uffugo (Cosenza), 550 m, 1.VI.2016, leg. Scalercio and Infusino; 1 male, S.B. Ullano (Cosenza), 825 m, 1.VI.2016, leg. Scalercio and Infusino; 1 male, S.B. Ullano (Cosenza), 845 m, 1.VI.2016, leg. Scalercio and Infusino; 1 female, Paola (Cosenza), 20 m, 9.VI.2017, leg. Scalercio; 1 male, Alessandria del Carretto (Cosenza), 1314 m, 21.VI.2017, leg. Scalercio and Infusino.

Chorotype: Cosmopolitan.

Remarks: collected also on early March, previously reported in TREMATERRA (2003) from April to November and in TREMATERRA and COLACCI (2016) from May to November.

Pelochrista mollitana (Zeller, 1847)

Material examined: 2 males, Palizzi Marina (Reggio Calabria), 3 m, 15.V.2015, leg. Urso; 1 male, S.S. Bruno (Vibo Valentia), 883 m, 3.VIII.2016, leg. Scalercio and Infusino.

Chorotype: Turanic-European-Mediterranean.

Pelochrista agrestana (Treitschke, 1830)

Material examined: 1 male, Savelli (Crotone), 1000 m, 12.VIII.1998, leg. Sciarretta.

Chorotype: S-European.

*** *Pelochrista bleuseana*** (Oberthür, 1888)
Records: Sersale (Catanzaro), 6.VII.1985; Sersale (Catanzaro), 22.IX.1985; Perticaro (Catanzaro), 6.VII.1988; Mendicino (Cosenza), 4-18.VII.1988; Perticaro (Catanzaro) 18.VIII.1988; Mendicino (Cosenza), 19.VI.1989; Mendicino (Cosenza), 3.X.1989 (in Trematerra *et al.*, 1994).
Chorotype: W-Mediterranean.

Pelochrista subtiliana (Jäckh, 1960)
Material examined: 1 male, San Fili (Cosenza), 831 m, 25.V.2017, leg. Colacci, Goglia and Scalercio; 1 male, Falconara Albanese (Cosenza), 921 m, 25.V.2017, leg. Colacci, Goglia and Scalercio.
Chorotype: Central-European.

Eucosma cana (Haworth, [1811])
Material examined: 1 male, Fossiata (Cosenza), 1300 m, 13.VIII.2013, leg. Scalercio; 1 male, Vivaio Sbanditi (Cosenza), 1350 m, 29.VII.2014, leg. Scalercio; 1 male, S.S. Bruno (Vibo Valentia), 940 m, 19.VII.2015, leg. Scalercio and Infusino; 1 male, Spezzano della Sila, 1299 m, 24.VII.2017, leg. Scalercio and Infusino.
Chorotype: Asiatic-European.

Eucosma hohenwartiana ([Denis and Schiffermüller], 1775)
Material examined: 2 males, Pianette di Dipignano (Cosenza), 765 m, 5.VIII.2013, leg. Scalercio.
Chorotype: Centro Asiatic-European-Mediterranean.

Eucosma balatonana (Osthelder, 1937)
Material examined: 1 male, Montagna Grande (Cosenza), 1370 m, 15.VI.2015, leg. Scalercio and Infusino; 1 female, Montalto Uffugo (Cosenza), 545 m, 25.VI.2015, leg. Scalercio and Infusino; 1 male, Vivaio Sbanditi (Cosenza), 1351 m, 6.VII.2015, leg. Scalercio and Infusino.
Chorotype: Centro Asiatic-European.

Eucosma conterminana (Guenée, 1845)
Material examined: 1 male, Cozzi dell'Anticristo (Cosenza), 1000 m, 13.VI.1992, leg. Trematerra.
Chorotype: Asiatic-European.

Eucosma albidulana (Herrich-Schäffer, 1848)
Material examined: 1 male, Pianette di Dipignano (Cosenza), 765 m, 5.VIII.2013, leg. Scalercio; 1 male, Aprigliano (Cosenza), 1310 m, 15.VI.2015, leg. Scalercio and Infusino.
Chorotype: Centro Asiatic-European-Mediterranean.

Gypsonoma minutana (Hübner, [1796-1799])
Material examined: 1 male, San Fili (Cosenza), 831 m, 25.V.2017, leg. Colacci, Goglia and Scalercio.
Chorotype: Palaearctic.

Gypsonoma sociana (Haworth, 1811)
Material examined: 1 male, Cozzi dell'Anticristo, 1300 m, 28.V.1990, leg. Trematerra.
Chorotype: Asiatic-European.

Epiblema sticticanum (Fabricius, 1794)
Material examined: 1 male, San Lucido (Cosenza), 950 m, 25.V.2017, leg. Colacci, Goglia and Scalercio.
Chorotype: Asiatic-European.

*** *Epiblema scutulanum*** ([Denis and Schiffermüller], 1775)
Records: Sersale (Catanzaro), 3-17.VIII.1985; Sersale (Catanzaro), 3-17.VIII.1986; Soveria Mannelli (Catanzaro), 4.VI-27.VII.1987; Cozzi dell'Anticristo (Cosenza), 28.V.1989; Monte Gariglione (Catanzaro), 12.VI.1991 (in Trematerra *et al.*, 1994).
Chorotype: Palaearctic.

Epiblema foenellum (Linnaeus, 1758)
Material examined: 1 male, Fiego di San Fili (Cosenza), 720 m, 22.VII.2015, leg. Scalercio and Infusino; 2 males, Montalto Uffugo (Cosenza), 545 m, 28.VI.2016, leg. Scalercio and Infusino; 2 females, Montalto Uffugo (Cosenza), 545 m, 29.VIII.2016, leg. Scalercio and Infusino.
Chorotype: Asiatic-European.

Epiblema costipunctana (Haworth, [1811])
Material examined: 1 male, S. Coppola di Paola (Cosenza), 1300 m, 28.V.1990, leg. Trematerra.
Chorotype: European.

Epiblema graphana (Treitschke, 1835)
Material examined: 2 males and 1 female, M.te Gariglione (Catanzaro), 1600 m, 12.VI.1991, leg. Trematerra.
Chorotype: Centro Asiatic-European.

Notocelia cynosbatella (Linnaeus, 1758)
Material examined: 1 female, Vivaio Sbanditi (Cosenza), 1350 m, 26.VI.2014, leg. Scalercio; 1 male, Vivaio Sbanditi (Cosenza), 1350 m, 29.VII.2014, leg. Scalercio; 1 male, Vivaio Sbanditi (Cosenza), 1353 m, 15.VI.2015, leg. Scalercio and Infusino; 1 male, Montagna Grande (Cosenza), 1370 m, 15.VI.2015, leg. Scalercio and Infusino; 1 male, Mangiatoie (Cosenza), 1270 m, 11.V.2016, leg. Scalercio and Infusino; 1 male, Saracena (Cosenza), 1433 m, 6.VI.2016, leg. Scalercio and Infusino; 1 male, Saracena (Cosenza), 1431 m, 6.VI.2016, leg. Scalercio and Infusino; 3 males, San Fili (Cosenza), 831 m, 25.V.2017, leg. Colacci, Goglia and Scalercio; 1 male, Alessandria del Carretto (Cosenza), 1285 m, 21.VI.2017, leg. Scalercio and Infusino; 1 male, San Nicola Silano (Cosenza), 1435 m, 17.V.2018, leg. Colacci and Goglia.
Chorotype: Asiatic-European.

Notocelia uddmanniana (Linnaeus, 1758)
Material examined: 1 male, Vivaio Sbanditi (Cosenza), 1350 m, 24.VII.2014, leg. Scalercio; 1 male, San Fili (Cosenza), 630 m, 28.V.2015, leg. Scalercio and Infusino; 1 male, Fiego di San Fili (Cosenza), 720 m, 28.V.2015, leg. Scalercio and Infusino; 1 male, Aprigliano (Cosenza), 1310 m, 15.VI.2015, leg. Scalercio and Infusino; 1 male, S.S. Bruno (Vibo Valentia), 1080 m, 22.VI.2015, leg. Scalercio and Infusino; 1 male, Fiego di San Fili (Cosenza), 720 m, 25.VI.2015, leg. Scalercio and Infusino; 1 male, S.S. Bruno (Vibo Valentia), 879 m, 10.V.2016, leg. Scalercio and Infusino; 1 male, S.B. Ullano (Cosenza), 825 m, 27.VII.2016, leg. Scalercio and Infusino; 1 female, San Fili (Cosenza), 831 m, 25.V.2017, leg. Colacci, Goglia and Scalercio.
Chorotype: W-Palaearctic.

Notocelia aquana (Hübner, [1796-1799])
Material examined: 1 male, Montagna Grande (Cosenza), 1235 m, 25.VII.2016, leg. Scalercio and Infusino.
Chorotype: Palaearctic.

Notocelia rosaecolana (Doubleday, 1850)
Material examined: 1 male, Saracena (Cosenza), 1010 m, 20.V.2015, leg. Scalercio and Infusino; 1 male, Aprigliano (Cosenza), 1310 m, 15.VI.2015, leg. Scalercio and Infusino;

1 male, Saracena (Cosenza), 1010 m, 19.VI.2015, leg. Scalercio and Infusino; 1 male, Vivaio Sbanditi (Cosenza), 1351 m, 6.VII.2015, leg. Scalercio and Infusino.

Chorotype: Asiatic-European.

Notocelia trimaculana (Haworth, [1811])

Material examined: 2 males, Alessandria del Carretto (Cosenza), 1337 m, 21.VI.2016, leg. Scalercio and Infusino.

Chorotype: W-Palaearctic.

Blastesthia tessulatana (Staudinger, 1871)

Material examined: 3 males, Rende (Cosenza), 205 m, 14.V.2014, leg. Scalercio.

Chorotype: S-European-Mediterranean.

Rhyacionia buoliana ([Denis and Schiffermüller], 1775)

Material examined: 1 female, Aieta (Cosenza), 500 m, 12.VII.1989, leg. Hausmann.

Chorotype: Holarctic.

Rhyacionia pinicolana (Doubleday, 1849)

Material examined: 1 male, Lago Cecita (Cosenza), 1163 m, 30.VII.2013, leg. Scalercio; 2 females, Fossiata (Cosenza), 1300 m, 13.VIII.2013, leg. Scalercio; 1 female, Lago Cecita (Cosenza), 1163 m, 13.VIII.2014, leg. Scalercio; 1 male, Montagna Grande (Cosenza), 1235 m, 25.VII.2016, leg. Scalercio and Infusino.

Chorotype: Asiatic-European.

Rhyacionia pinivorana (Lienig and Zeller, 1846)

Material examined: 1 male, Vivaio Sbanditi (Cosenza), 1354 m, 5.VI.2015, leg. Scalercio and Infusino; 1 male, Aprigliano (Cosenza), 1310 m, 15.VI.2015, leg. Scalercio and Infusino; 1 male, Mangiatoie (Cosenza), 1275 m, 11.V.2016, leg. Scalercio and Infusino; 1 male, Montagna Grande (Cosenza), 1370 m, 11.V.2016, leg. Scalercio and Infusino; 1 male, Mangiatoie (Cosenza), 1275 m, 7.VI.2016, leg. Scalercio and Infusino; 1 male, San Bernardo (Cosenza), 1383 m, 7.VI.2016, leg. Scalercio and Infusino.

Chorotype: Asiatic-European.

Clavigesta sylvestrana (Curtis, 1850)

Material examined: 2 males, Lago Cecita (Cosenza), 1163 m, 13.VIII.2013, leg. Scalercio; 1 male, Fossiata (Cosenza), 1300 m, 13.VIII.2013, leg. Scalercio; 8 males, Lago Cecita (Cosenza), 1176 m, 2.IX.2013, leg. Scalercio; 5 males and 2 females, Lago Cecita (Cosenza), 1163 m, 13.VIII.2014, leg. Scalercio; 1 male, Diga del Menta (Reggio Calabria), 1380 m, 26.VIII.2016, leg. Scalercio.

Chorotype: European.

Tribe Grapholitini

*** *Cydia semicinctana*** (Kennel, 1901)

Records: Sersale (Catanzaro), 1.VI.1985; Sersale (Catanzaro), 31.VIII.1985; Sersale (Catanzaro), 10.V.1986; Sersale (Catanzaro), 16.VIII.1986; Perticaro (Catanzaro), 6.VII-3.VIII.1988; Mendicino (Cosenza), 1-15.VIII.1988 (in Trematerra *et al.*, 1994).

Chorotype: Turanic-European.

Cydia succedana ([Denis and Schiffermüller], 1775)

Material examined: 1 male, Righio (Cosenza), 1355 m, 23.V.2014, leg. Scalercio; 1 male, Vivaio Sbanditi (Cosenza), 1350 m, 26.VI.2014, leg. Scalercio; 1 male, Montagna Grande (Cosenza), 1355 m, 11.V.2015, leg. Scalercio and Infusino; 1 male, Montagna Grande (Cosenza), 1355 m, 18.V.2015, leg. Scalercio and Infusino; 1 male, S.S. Bruno (Vibo Valentia), 940 m, 26.V.2015, leg. Scalercio and Infusino; 2 males, S.S. Bruno (Vibo Valentia), 1040 m, 26.V.2015, leg. Scalercio and Infusino; 1 male, Fiego di San Fili (Cosenza), 740 m, 28.V.2015, leg. Scalercio and Infusino; 1 male, Vivaio Sbanditi (Cosenza), 1351 m, 15.VI.2015, leg. Scalercio and Infusino; 1 male, Pedace (Cosenza), 1440 m, 15.VI.2015, leg. Scalercio and Infusino; 1 male, S.S. Bruno (Vibo Valentia), 830 m, 10.V.2016, leg. Scalercio and Infusino; 1 male, S.S. Bruno (Vibo Valentia), 840 m, 10.V.2016, leg. Scalercio and Infusino; 1 male, Mangiatoie (Cosenza), 1270 m, 11.V.2016, leg. Scalercio and Infusino; 1 male, S.S. Bruno (Vibo Valentia), 840 m, 30.V.2016, leg. Scalercio and Infusino; 1 male, S.S. Bruno (Vibo Valentia), 925 m, 30.V.2016, leg. Scalercio and Infusino; 4 males, San Fili (Cosenza), 831 m, 25.V.2017, leg. Colacci, Goglia and Scalercio; 6 males and 1 female, San Nicola Silano (Cosenza), 1435 m, 17.V.2018, leg. Colacci and Goglia; 1 female, Rovale (Cosenza), 1440 m, 17.V.2018, leg. Colacci and Goglia.

Chorotype: Centro Asiatic-European-Mediterranean.

Cydia ulicetana (Haworth, [1811])

Material examined: 1 male, San Fili (Cosenza), 831 m, 25.V.2017, leg. Colacci, Goglia and Scalercio.

Chorotype: European-Mediterranean.

Cydia trogodana Pröse, 1988

Material examined: 1 male, Palizzi Marina (Reggio Calabria), 3 m, 15.V.2015, leg. Urso.

Chorotype: E-Mediterranean.

*** *Cydia coniferana*** (Saxesen, 1840)

Records: Sersale (Catanzaro), 15.VI-31.VIII.1985; Sersale (Catanzaro), 15.VI-31.VIII.1986 (in Trematerra *et al.*, 1994).

Chorotype: Sibiric-European.

Cydia pomonella (Linnaeus, 1758)

Material examined: 1 male, Pianette di Dipignano (Cosenza), 765 m, 29.VII.2013, leg. Scalercio; 2 males and 1 female, Pianette di Dipignano (Cosenza), 765 m, 5.VIII.2013, leg. Scalercio; 1 male, Montalto Uffugo (Cosenza), 230 m, 24.V.2014, leg. Scalercio.

Chorotype: W-Palaearctic.

Cydia triangulella (Goeze, 1783)

Material examined: 1 female, S.B. Ullano (Cosenza), 893 m, 29.VIII.2016, leg. Scalercio and Infusino.

Chorotype: Palaearctic.

Cydia fagiglandana (Zeller, 1841)

Material examined: 1 male, Pianette di Dipignano (Cosenza), 765 m, 5.VIII.2013, leg. Scalercio; 1 male, Donnici (Cosenza), 550 m, 7.VIII.2013, leg. Scalercio; 1 female, Lago Cecita (Cosenza), 1163 m, 13.VIII.2013, leg. Scalercio; 1 female, Vivaio Sbanditi (Cosenza), 1350 m, 26.VI.2014, leg. Scalercio; 1 male, Lago Cecita (Cosenza), 1163 m, 13.VIII.2014, leg. Scalercio; 1 female, Saracena (Cosenza), 1010 m, 20.V.2015, leg. Scalercio and Infusino; 1 male, Saracena (Cosenza), 1010 m, 19.VI.2015, leg. Scalercio and Infusino; 1 male, Saracena (Cosenza), 1460 m, 20.VII.2015, leg. Scalercio and Infusino; 2 females, Montagna Grande (Cosenza), 1355 m, 25.VII.2016, leg.

Scalercio and Infusino; 1 female, Lungro (Cosenza), 1270 m, 1.VIII.2016, leg. Scalercio and Infusino; 1 female, Acquaformosa (Cosenza), 1357 m, 1.VIII.2016, leg. Scalercio and Infusino; 1 female, Montalto Uffugo (Cosenza), 545 m, 29.VIII.2016, leg. Scalercio and Infusino; 1 female, S.B. Ullano (Cosenza), 731 m, 29.VIII.2016, leg. Scalercio and Infusino.

Chorotype: W-Palaearctic.

Cydia amplana (Hübner, [1796-1799])

Material examined: 1 female, f. Argentino (Cosenza), 250 m, 4.IX.1991, leg. Hausmann.

Chorotype: Turanic-European.

Lathronympha strigana (Fabricius, 1775)

Material examined: 1 male, Aprigliano (Cosenza), 1310 m, 15.VI.2015, leg. Scalercio and Infusino; 1 male, Alessandria del Carretto (Cosenza), 1291 m, 21.VI.2017, leg. Scalercio and Infusino.

Chorotype: Sibiric-European.

Selania leplastriana (Curtis, 1831)

Material examined: 1 female, Sangineto (Cosenza), 50 m, 10.VIII.2014, leg. Scalercio.

Chorotype: European-Mediterranean.

Selania capparidana (Zeller, 1847)

Material examined: 4 males and 4 females, Copanello (Catanzaro), 70 m, 25.VII.1996, leg. ?; 4 males and 4 females, Copanello (Catanzaro), 70 m, 10.VIII.1996, leg. Trematerra.

Chorotype: Mediterranean.

Grapholita fissana (Frölich, 1828)

Material examined: 1 male, S.B. Ullano (Cosenza), 893 m, 1.VI.2016, leg. Scalercio and Infusino.

Chorotype: European.

Grapholita difficilana (Walsingam, 1900)

Material examined: 1 male, Cozzi dell'Anticristo (Cosenza), 1300 m, 28.V.1990, leg. Trematerra; 1 female, lago Ampollino (Cosenza), 1300 m, 11.VI.1991, leg. Trematerra; 5 females, Cozzi dell'Anticristo (Cosenza), 1300 m, 13.VI.1991, leg. Trematerra.

Chorotype: European-Mediterranean.

Grapholita lunulana ([Denis and Schiffermüller], 1775)

Material examined: 1 female, Donnici (Cosenza), 550 m, 18.V.2014, leg. Scalercio.

Chorotype: Centro Asiatic-European-Mediterranean.

*** *Grapholita orobana*** Treitschke, 1830

Records: Sersale (Catanzaro), 15.VI-6.VII.1985; Sersale (Catanzaro), 15.VI-6.VII.1986; Soveria Mannelli (Catanzaro), 4.VI-23.VII.1987; Soveria Mannelli (Catanzaro), 4.VI-23.VII.1988; Mendicino (Cosenza), 6-20.VII.1987; Mendicino (Cosenza), 27.VI-4.VII.1988; Monte Cerviero (Cosenza), 28.V.1989 (in TREMATERRA *et al.*, 1994).

Chorotype: Sibiric-European.

*** *Grapholita gemmiferana*** Treitschke, 1835

Records: Sersale (Catanzaro), 28.V-22.VI.1985; Sersale (Catanzaro), 10.V.1986; Sersale (Catanzaro), 6.VII.1986; Soveria Mannelli (Catanzaro), 4-25.VI.1987 (in TREMATERRA *et al.*, 1994).

Chorotype: Centro Asiatic-European.

Grapholita lathyrana (Hübner, [1811-1813])

Material examined: 1 male, Gariglione (Catanzaro), 1600 m, 12.VI.1991, leg. Trematerra.

Chorotype: European.

*** *Grapholita funebrana*** Treitschke, 1835

Records: Sersale (Catanzaro), 22.VI-14.IX.1985; Sersale (Catanzaro), 17.V-16.VIII.1986; Soveria Mannelli (Catanzaro), 4.VI-10.IX.1987; Mendicino (Cosenza), 22.VI-21.IX.1987; Soveria Mannelli (Catanzaro), 4.VI-10.IX.1988; Mendicino (Cosenza), 22.VI-21.IX.1988; Perticaro (Catanzaro), 24.VI-20.VIII.1988; Mendicino (Cosenza), 22.VI-21.IX.1989 (in TREMATERRA *et al.*, 1994).

Chorotype: Palaearctic.

*** *Grapholita janthinana*** (Duponchel, 1835)

Records: Sersale (Catanzaro), 12.VII-2.VIII.1986; Mendicino (Cosenza), 20.VII.1987 (in TREMATERRA *et al.*, 1994).

Chorotype: Turanic-European.

*** *Grapholita tenebrosana*** Duponchel, 1843

Records: Sersale (Catanzaro), 15.VI-27.VII.1985; Sersale (Catanzaro), 15.VI-27.VII.1986; Soveria Mannelli (Catanzaro), 4.VI-2.VII.1987; Soveria Mannelli (Catanzaro), 4.VI-2.VII.1988; Mendicino (Cosenza), 29.VI.1989 (in TREMATERRA *et al.*, 1994).

Chorotype: Asiatic-European.

Pammene amygdalana (Duponchel, 1842)

Material examined: 1 male, Pianette di Dipignano (Cosenza), 765 m, 29.VII.2013, leg. Scalercio.

Chorotype: European-Mediterranean.

Pammene querceti Gozmány, 1957

Material: Sersale (Catanzaro), 25.V.1985; Sersale (Catanzaro), 10.VIII.1985; Sersale (Catanzaro), 10.V-7.VI.1986 (in TREMATERRA *et al.*, 1994).

Chorotype: S-European.

Pammene fasciana (Linnaeus, 1761)

Material examined: 1 male, Fiego di San Fili (Cosenza), 740 m, 25.VI.2015, leg. Scalercio and Infusino; 2 females, S.B. Ullano (Cosenza), 825 m, 27.VII.2016, leg. Scalercio and Infusino; 2 males, Montalto Uffugo (Cosenza), 550 m, 29.VIII.2016, leg. Scalercio and Infusino.

Chorotype: Turanic-European.

*** *Pammene argyrana*** (Hübner, 1799)

Records: Sersale (Catanzaro) 13.VII-17.VIII.1985 (in TREMATERRA *et al.*, 1994).

Chorotype: European.

Remarks: collected in July-August, previously reported in TREMATERRA (2003) from April to June.

*** *Pammene albuginana*** (Guenée, 1845)

Records: Sersale (Catanzaro), 14.IX.1985; Sersale (Catanzaro), 17.V.1986; Soveria Mannelli (Catanzaro), 4.VI.1987; Mendicino (Cosenza), 28.IX-19.X.1987; Soveria Mannelli (Catanzaro), 1.X.1987 (in TREMATERRA *et al.*, 1994).

Chorotype: European.

*** *Pammene gallicolana*** (Lienig and Zeller, 1846)

Records: Sersale (Catanzaro), 29.VI-5.X.1985; Sersale (Catanzaro), 10.V-2.VIII.1986; Mendicino (Cosenza), 1.VI-12.X.1987; Soveria Mannelli (Catanzaro), 4.VI-8.X.1987;

Mendicino (Cosenza), 1.VI-12.X.1988; Perticaro (Catanzaro), 20.VII.1988; Mendicino (Cosenza), 1.VI-12.X.1989 (in Trematerra *et al.*, 1994).
Chorotype: European.

*** *Pammene spiniana*** (Duponchel, 1843)
Records: Soveria Mannelli (Catanzaro), 20.VIII-8.X.1987; Mendicino (Cosenza), 21.IX-19.X.1987 (in Trematerra *et al.*, 1994).
Chorotype: European-Mediterranean.

*** *Pammene blockiana*** (Herrich-Schäffer, 1851)
Records: Sersale (Catanzaro), 19.VII-2.VIII.1986 (in Trematerra *et al.*, 1994).
Chorotype: S-European.

*** *Pammene aurana*** (Fabricius, 1775)
Records: Sersale (Catanzaro), 20.VII.1985; Soveria Mannelli (Catanzaro), 17.IX.1987 (in Trematerra *et al.*, 1994).
Chorotype: Sibiric-European.

Strophedra nitidana (Fabricius, 1794)
Material examined: 1 male, S.S. Bruno (Vibo Valentia), 830 m, 30.V.2016, leg. Scalercio and Infusino.
Chorotype: Asiatic-European.

*** *Dichrorampha acuminatana*** (Lienig and Zeller, 1846)
Records: Sersale (Catanzaro), 29.VI.1985; Copanello (Catanzaro), 24.IV.1992, leg. Trematerra (in Trematerra *et al.*, 1994).
Chorotype: European (extension Anatolian-Maghrebian).

Dichrorampha letarfensis Gibeaux, 1983
Material examined: 4 males and 5 females, Copanello (Catanzaro), 20 m, 24.IV.1992, leg. Trematerra.
Chorotype: W-Mediterranean.

Dichrorampha gemellana (Zeller, 1847)
Material examined: 1 male, Lago Cecita (Cosenza), 1176 m, 2.IX.2013, leg. Scalercio.
Chorotype: Tirrenic.

CONSIDERATIONS ON THE TORTRICIDAE FAUNA OF CALABRIA

During our entomological expeditions realized from 2013 to 2018 in Calabria region, 123 species of Lepidoptera Tortricidae were caught, to which we added 22 species deposited in the Trematerra Collection and other 36 species reported by Trematerra in the course of entomological researches carried out in the past in the Calabrian territory, up to a total of 181 taxa of the family.

Species belonging to all the tribes cited in the Italiana fauna (Trematerra, 2003) have been found in Calabria, with the exception of the members of the Sparganothini tribe (subfamily Tortricinae), the two most numerous tortricids subfamilies recorded are the Tortricinae and the Olethreutinae, which has 75 species and 102 species, respectively; the subfamily Chlidanotinae is represented by 4 taxa.

A biogeographic study on the Tortricidae found in Calabria, using chorological analysis, are reported in Table 2. It can be noted that there are few Cosmopolitan species (1.10%), Subcosmopolitan (2.21%) and Holartic (9.94%). More than 59% of the species show a wide Palaearctic distribution. In this grouping, the species with the chorotypes Asiatic-European (17.68%), W-Palaearctic (7.73%), Palaearctic (6.63%) and Turanic-European (6.63%) are well represented. Fewer are the Centro Asiatic-European (5.52%), Centro Asiatic-European-Mediterranean (4.42%), Sibiric-European (4.42%), European-Mediterranean (3.31%), Turanic-European-Mediterranean (2.22%) and European (with extension Anatolian-Maghrebian) (0.55%).

Table 2 – Chorological categories, number of species and percentages of Lepidoptera Tortricidae collected in Calabria (southern Italy).

CHOROTYPE	N. OF SPECIES	%
Cosmopolitan and Subcosmopolitan distribution	**6**	**3.31**
Cosmopolitan	2	1.10
Subcosmopolitan	4	2.21
Holarctic distribution	**18**	**9.94**
Holarctic	18	9.94
Wide Palaearctic distribution	**107**	**59.12**
Palaearctic	12	6.63
W-Palaearctic	14	7.73
Asiatic-European	32	17.68
Sibiric-European	8	4.42
Centro Asiatic-European-Mediterranean	8	4.42
Centro Asiatic-European	10	5.52
Turanic-European-Mediterranean	4	2.22
Turanic-European	12	6.63
European-Mediterranean	6	3.31
European (extension Anatolian-Maghrebian)	1	0.55
European distribution	**34**	**18.78**
European	24	13.26
Central European	1	0.55
S-European	9	4.97
Mediterranean distribution	**12**	**6.63**
Mediterranean	4	2.21
S-European-Mediterranean	1	0.55
W-Mediterranean	3	1.66
E-Mediterranean	1	0.55
Tirrenic	1	0.55
Apenninic-Dinaric	1	0.55
NW-African (extension S-Apenninic-Sicilian)	1	0.55
Endemic Italian species	**4**	**2.21**
Apenninic	2	1.10
S-Apenninic	2	1.10

More than 18% of the collected taxa are tortricids with European distribution; the elements belonging to this chorotype show European (13.26%), S-European (4.97%) and Central European (0.55%) distribution. The Mediterranean distribution taxa is shown only by 6.63% of the recorded species including Mediterranean (2.21%), W-

Mediterranean (1.66%) and, all with 0.55%, S-European-Mediterranean, E-Mediterranean, Tirrenic, Apenninic-Dinaric, NW-African (with extension S-Apenninic-Sicilian) elements. Finally, the presence of two species with Apennine chorotype and two with S-Apennines chorotype is reported.

COMMENTS

The fauna of Lepidoptera Tortricidae in the Calabrian study areas is dominated by forest elements, on the contrary, only few are agrarian elements. Many species are related to arboreal and uncultivated plants; the latter have been expanding on lands due to the abandonment of the fields and the continuous deforestation. The increase in plant cover favors the increase of lepidopteran populations (of all families) with mesophilic or nemoral behaviour.

Unlike what has occurred for other Italian regions, the tortricids present in the southern areas of the country have received little or sporadic attention from Italian and foreign entomologists. Overall information on this matter can be found in the Checklist of the Italian fauna (TREMATERRA, 1995) and in the "Catalogue of Lepidoptera Tortricidae of the Italian fauna" (TREMATERRA, 2003).

In the present report, 59 of the whole species (over 30% of the total) were found for the first time in the Calabria: *Aleimma loeflingianum*, *Acleris abietana*, *Acleris sparsana*, *Acleris rhombana*, *Acleris schalleriana*, *Acleris cristana*, *Acleris aspersana*, *Acleris hastiana*, *Acleris notana*, *Acleris ferrugana*, *Acleris quercinana*, *Acleris literana*, *Phtheochroa rugosana*, *Phalonidia gilvicomana*, *Phalonidia manniana*, *Agapeta hamana*, *Eupoecilia angustana*, *Aethes cnicana*, *Cochylis salebrana*, *Cochylis molliculana*, *Eana penziana*, *Archicnephasia hartigi*, *Cnephasia fulturata*, *Cnephasia hellenica*, *Batodes angustiorana*, *Paramesia diffusana*, *Archips crataeganus*, *Choristoneura diversana*, *Argyrotaenia ljungiana*, *Ptycoloma lecheana*, *Aphelia ferugana*, *Isotrias rectifasciana*, *Bactra lancealana*, *Bactra venosana*, *Endothenia nigricostana*, *Endothenia pullana*, *Piniphila bifasciana*, *Syricoris rivulana*, *Ancylis comptana*, *Ancylis paludana*, *Ancylis badiana*, *Ancylis mitterbacheriana*, *Rhopobota naevana*, *Epinotia sordidana*, *Epinotia solandriana*, *Epinotia fraternana*, *Pelochrista subtiliana*, *Eucosma hohenwartiana*, *Eucosma balatonana*, *Eucosma albidulana*, *Epiblema graphana*, *Notocelia aquana*, *Notocelia rosaecolana*, *Notocelia trimaculana*, *Rhyacionia pinivorana*, *Cydia trogodana*, *Grapholita fissana*, *Pammene amygdalana*, and *Strophedra nitidana*.

Our findings furthermore revealed 23 species that have as a southern limit of their distribution area the Calabrian territory: *Acleris notana*, *Phtheochroa purana*, *Eana italica*, *Archicnephasia hartigi, Cnephasia zangheriana, Choristoneura murinana, Dichelia histrionana, Olindia schumacherana*, *Endothenia lapideana*, *Endothenia pullana*, *Syricoris lacunana*, *Ancylis paludana*, *Spilonota laricana*, *Epinotia signatana*, *Epinotia nigricana*, *Pelochrista subtiliana*, *Eucosma balatonana*, *Gypsonoma sociana*, *Rhyacionia pinivorana*, *Cydia coniferana*, *Pammene querceti*, *Pammene albuginana*, and *Pammene aurana.*

Finally, the finding of the two Apennine endemisms, *Cnephasia zangheriana* and *Isotrias joannisana,* and two S-Apennines species, *Isotrias martelliana* and *Archicnephasia hartigi*, is of particular interest.

Overall it is possible to observe that a limited number of specimens belonging to entities potentially harmful to agricultural and forest plants was recorded in Calabria, especially in the northern and central part of the territory, this suggests that these areas may in good health and maintains a high degree of naturalness. However, the absence of tortricid pests could be also due to the particular natural habitats visited during the entomological expeditions.

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ENDOSYMBIONTS OF ENTOMOPATHOGENIC NEMATODES FROM SOUTH ITALY: A PHENOTYPIC STUDY

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Rappazzo G., Salvo E., Tarasco E., Petronio Petronio G., Buccheri M.A., Furneri P.M., Fuochi V., Clausi M. – Endosymbionts of Entomopathogenic Nematodes from South Italy: a phenotypic study.

We examined different *Xenorhabdus* strains (five of *X. bovienii* and two of *X. kozodoii*), obtained from EPN isolates belonging to the genus *Steinernema* (*S. feltiae, S. ichnusae, S. apuliae, S. vulcanicum*) of different geographic origin by both genotypic and phenotypic analysis. Common laboratory assays were done for traits such as antibiotic resistance, haemolytic activity, lactose utilisation, biofilm production, chosen as the least selectable traits for EPN life-cycle, and thus as (presumably) neutral traits. As selective marker, the activity of the endosymbiont's toxins was verified in an *in vivo* assay on *G. mellonella* larvae. Genotyping done by 16S partial sequencing was used for identification purposes. *Xenorhabdus bovienii* isolates showed a broad phenotypic spectrum; on the other hand, *X. kozodoii* showed a less degree of phenotypic variation, reduced ability of biofilm production and conspicuous β-galactosidase activity. However, all the strains were able to kill *G. mellonella* larvae with high efficiency.

KEY WORDS: symbiosis, *Steinernema*, *Xenorhabdus*, Italy.

INTRODUCTION

Entomopathogenic Nematodes (EPNs) rely on their endosymbionts for a number of processes which are finalized to kill the insect preys, mostly larvae. However, those endosymbionts are indeed autonomous micro-organisms, able to grow in synthetic media, and several have been characterized both biochemically and physiologically (POINAR, 1990). Furthermore, the existence of an evolutionary link, or coevolution, between the two organisms have been postulated, since each bacterial species is associated with a reduced number of EPN species (ADAMS *et al*., 2007). For example, *Xenorhabdus bovienii* was found to be associated with *Steinernema feltiae*, *S. kraussei*, *S. ichnusae* and with some other EPNs of the "*feltiae* group"; while *X. kozodoii* was found in association with a number of EPN species from the "*glaseri* group"; other endosymbionts are similarly specific to other groups of *Steinernema* (TAILLEZ *et al*., 2006). Because of this exclusive relationship, endosymbionts are expected to perform their life cycle only within their host, with little (if any) exchange with the environment and/or other organisms, excepting of course the EPN preys (CLAUSI *et al*., 2012). The presence of such a confined life cycle, if demonstrated, would be of considerable evolutionary interest.

In the present work, we analysed endosymbionts for a number of phenotypic traits which were apparently unrelated to EPNs life cycle and thus possibly of neutral value from an evolutionary point of view. Among them, antibiotic resistance/susceptibility, biofilm production, β-galactosidase activity seemed the most appropriate for our goal and are also easily performed in laboratory. On the other hand, endosymbionts produce a toxin, or a number of them, which are able to kill the EPN prey thus facilitating the development and the life-cycle of the EPN host (POINAR, 1979; CLAUSI *et al*., 2014). Toxin(s) production is to be considered as a trait of selective value, since it positively increases the fitness of EPN host (HINCHLIFFE *et al*., 2010). It is expected that neutral characters may broadly vary among samples, in particular as long as a closed life cycle takes place, while selective ones should be highly convergent.

To this purpose, five strains of *X. bovienii* and two of *X. kozodoii,* obtained from already described *Steinernema* isolates from South Italy (TARASCO *et al*., 2015), were grown *in vitro* and subjected to a number of biochemical assays originally developed for bacteria of the family Enterobacteriaceae.

MATERIALS AND METHODS

EPN ISOLATES AND THEIR ENDOSYMBIONTS

The origin of EPN isolates, all collected from South Italy, including Sardinia and Sicily, has been described (TARASCO *et al*., 2008; TARASCO *et al*., 2015; CLAUSI *et al*., 2011; DE LUCA *et al*., 2015). The relevant data are detailed in Table 1. EPNs were grown and collected as described in TARASCO *et al*. (2015).

ISOLATION OF ENDOSYMBIONTS FROM EPNs

Up to one hundred of freshly collected EPNs were sterilized in 1% Hyamine 1622 (Sigma-Aldrich) and homogenized. The resulting suspension was serially diluted and aliquots were plated on McConkey Agar (MC) (Oxoid limited) [Peptone 20 g/l; lactose 10 g/l; bile salts 5 g/l;

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Table 1 – EPN isolates and their endosymbionts used in the present study.

Isolate	EPN species	Area	ITS1 Acc. number	Endosymbiont
VE01	*S. feltiae*	South Sicily	HQ412835.1	*X. bovienii*
ESA	*S. feltiae*	East Sicily	GU599911.1	*X. bovienii*
CT036	*S. feltiae*	East Sicily	n.a.	*X. bovienii*
SAR6	*S. ichnusae*	Sardinia	EU421129	*X. bovienii*
MU1	*S. ichnusae*	Campania	HQ412841.1	*X. bovienii*
CS3	*S. apuliae*	Apulia	HQ416968.1	*X. kozodoii*
Esc1	*S. vulcanicum*	East Sicily	GU929442.1	*X. kozodoii*

sodium chloride 5 g/l; neutral red 0.075 g/l; agar 12 g/l; final pH 7.4 ± 0.2] plates. Growth was allowed at 28ºC for up to 48 hrs. Identification was routinely confirmed on NBTA agar plates (KOPPENHOFER, 2007).

ANTIBIOTIC SUSCEPTIBILITY

Patterns of antibiotic susceptibility to β-lactams (penicillin, amoxicillin, cephalothin, cefuroxime, and cefditoren), macrolides and lincosamides (erythromycin and clindamycin), tetracycline, aminoglycosides (gentamicin), fluoroquinolones (ciprofloxacin), glycopeptides (vancomycin) and phenicols (chloramphenicol) were determined by disc diffusion test according to CLSI guidelines (CLSI, 2017). Antibiotic disks were purchased from Oxoid limited.

HEMOLYTIC ACTIVITY, CATALASE ACTIVITY, OXIDASE ACTIVIY AND BIOFILM PRODUCTION

Hemolytic activity was investigated by using Columbia agar base (Oxoid limited) [special peptone 23.0 g/l; starch 1.0 g/l; sodium chloride 5 g/l; agar 10.0 g/l; final pH 7.3 ± 0.2 at 25°C] supplemented by 5 % defibrinated horse blood (Oxoid limited). All strains were incubated aerobically for 24 hrs and then observed for hemolysis production and results as recorded: alpha hemolysis (incomplete haemolytic activity with a not transparent halo), beta hemolysis (sharp and complete haemolytic activity around the colony), gamma hemolysis as no haemolytic activity.

Catalase activity was determined on colonies growth in MH agar. Briefly, by using a sterile plastic loop, one or two colonies were transferred on a surface of a clean and dry glass slide, then a drop of 3% hydrogen peroxide was added, and positivity was recorded as bubbling in at least 5 seconds.

Oxidase activity was assessed by using Oxidase Detection Strips (Oxoid limited) according to the procedure suggested by manufacturer.

The assay for *in vitro* biofilm formation was performed in polystyrene 96 wells microplates (GIUMMARRA *et al.*, 2010) after aerobic incubation for 18-24 hrs at 30°C. The biofilm index (B.I.) was calculated using the formula: OD570 / OD600 * 0.4. A cut-off value of 0.061 OD was used, and strains were classified as non-producing (OD <0.061), weak producer (0.061 <OD <0.120), medium-sized producers (0.121 <OD <0.300), strong producer (OD> 0.300). Tests were performed on Muller Hinton broth (MH) (BBL - BD) [Casein acid hydrolysate 1705 g/l; beef extract 3.0 g/l; starch 1.5 g/l; final pH 7.3 ± 0.2] alone or supplemented with 1% glucose (Sigma-Aldrich), mannose (Sigma-Aldrich) or sorbitol (Sigma-Aldrich). β-galactosidase assay was performed following Miller's protocol (MACKAY *et al*, 1970; FUOCHI *et al*., 2017).

PARTIAL PURIFICATION OF BACTERIAL TOXIN AND IN VIVO PATHOGENICITY ASSAY

The method devised by BRILLARD *et al.* (2001) was thoroughly followed in order to obtain a protein precipitate cointaining crude (pre-purified) toxins. The whole bacterial culture proteins (not only toxins) are precipitated with this method. *Xenorhabdus* strains were grown at saturation in LB [tryptone (BD) 10 g/l; yeast extract (BD) 5 g/l; NaCl (Sigma-Aldrich) 10 g/l; final pH 7.0] broth at 28ºC. The crude post-growth broth, containing the bacterial toxins, was concentrated by two-steps ammonium sulfate (Sigma-Aldrich) precipitation; the final pellet was resuspended in PBS (one hundredth of the original colture volume) and dialyzed overnight against phosphate buffered saline (PBS) [NaCl (Sigma-Aldrich) 8 g/l; KCl (Sigma-Aldrich) 0.2 g/l; Na_2HPO_4 (Sigma-Aldrich) 1.44 g/l; KH_2PO_4 (Sigma-Aldrich) 0.24 g/l; final pH 7.4 by using HCl (Sigma-Aldrich)]. Five µl of the so obtained crude toxins were injected into living *Galleria mellonella* larvae footpads using an Ethanol-sterilized Hamilton microsyringe. Four larvae were used for every bacterial strain; 8 control larvae were given PBS alone.

GENOMIC DNA EXTRACTION AND 16S RRNA GENE AMPLIFICATION

Genomic DNA was extracted and part of the 16S rRNA gene PCR amplified using primer pairs Xeno_F and Xeno_L (TAILLEZ *et al.,* 2006), using conditions described therein. For sequence analysis, the region around positions 950 – 990 of 16S rDNA, where diagnostic polymorphisms are found, was amplified with primers Xeno_888 (TGGAGCATGTGGTTTAATTCG) and Xeno_1016 (AACCCAACATTTCACAACACG). Sequence analysis was carried out with Big Dye Terminator 1.1 Sequencing Standard kit on a ABIPRISM 3130 (both from Applied Biosystems).

STATISTICAL ANALYSIS

Principal component analysis was performed using the software SIMCA v. 13. Cluster analysis was done using *dist* and *hclust* functions within the R package.

RESULTS

GENOTYPING

16S rDNA sequencing was used to confirm species' attribution, and no intraspecific variations were found with respect to known sequences.

PHENOTYPIC MARKERS

Xenorhabdus kozodoii grows well on MC plates, forming deep red colonies. In contrast, *X. bovienii* grows irregularly, giving rise to different kinds of red-brownish colonies. β-galactosidase activity was undetectable in *X. bovienii*, but significant in *X. kozodoii*, even if about a half than *E. coli* ATCC 35218 used as control. Growth on NTBA plates allowed easy differentiation of *X. bovienii* and *X. kozodoii* because of different use of the chromogenic substrate for glucosidase activity. All strains showed aerobic alpha-hemolysis on Columbia agar blood plates (Table 2).

The antibiotic susceptibility/resistance spectrum was very composite. All strains were found resistant to penicillins, macrolides, liconsamides glicopeptides, while susceptible to quinolones, tetracyclines, aminoglicosides. However, resistance to cephalosporins was strain dependent. All strains were resistant to cephalothin, while the susceptibility to cefuroxime was strain-dependent: all strains of *X. kozodoii* and one of *X. bovienii* (MU1) were susceptible, while the others were resistant. All strains tested were susceptible to ceftidoren (Table 3).

Biofilm production test showed different B.I. according to *Xenorhabdus* species and media tested (Fig. I). In MH, both *X. kozodoii* strains were almost non-producing (B.I. 0.041), while *X. bovienii* strains were overall medium-strong producers. Addition of sugars, as expected, caused marked B.I. increase, so that *X. kozodoii* showed a tenfold increase, while *X. bovienii* switched from small to moderate.

Toxicity of crude protein extract on *G. mellonella* larvae

The protein extract showed similar activity both on *X. bovienii* and on *X. kozodoii*, resulting in rapid (within 24 hrs) death of insect larvae at the dose of 5 μl; some dead even within 12 hrs (Table 4). At this time, no significant differences were found among strains or between *X. bovienii* and *X. kozodoii*. Interestingly, larvae became blackish in the same way as when infected by EPNs, even if, as expected, no massive bacterial infection was seen, but

Table 2 – Distinctive features of endosymbiont growth in selected conditions.

Strains	McConkey	Blood agar	Catalase	Oxidase	*β*-galactosidase
VE01	brown	α-hemolytic	Negative	negative	negative
ESA	brown	α-hemolytic	Negative	negative	negative
CT036	brown	α-hemolytic	Negative	negative	negative
SAR6	brown	α-hemolytic	Negative	negative	negative
MU1	brown	α-hemolytic	Negative	negative	negative
ESC1	red	α-hemolytic	Negative	negative	positive
CS3	red	α-hemolytic	Negative	negative	positive

Table 3 – Pattern of antibiotic resistance/susceptibility (inhibition halo in mm).

Strains	Penicillin	Amoxicillin	Cefuroxime	Erythromycin	Clindamycin	Tetracycline	Ciprofloxacin	Gentamycin	Chloramphenicol
VE01	n.h*	n.h*	n.h*	n.h*	n.h*	35	45	33	48
ESA	n.h*	n.h*	11	9	n.h*	33	40	25	37
CT036	n.h*	n.h*	12	n.h*	n.h*	34	40	23	43
SAR6	n.h*	n.h*	11	n.h*	n.h*	30	40	30	42
MU1	n.h*	n.h*	18	13	n.h*	32	51	30	46
ESC1	n.h*	n.h*	21	n.h*	n.h*	22	26	22	30
CS3	n.h*	n.h*	14	n.h*	n.h*	35	32	26	30

*n.h.: no inhibition halo reported

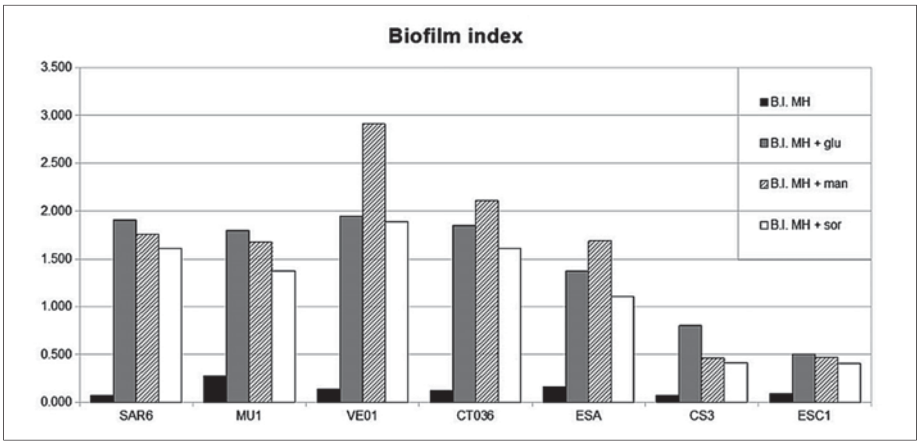

Fig. I – Biofilm index of different *Xenorhabdus* spp. Tested media are indicated on the right.

Table 4 – Toxicity of the crude toxin fraction at the time point indicated (n.rs of dead larvae).

Strain	Death at 12 hrs	Death at 24 hrs
CT036	75%	100%
ESA	25%	100%
SAR6	0	100%
VE01	0	100%
MU1	0	100%
ESC1	0	100%
CS3	0	100%
Control	0	0%

an important degeneration of internal organs was found (unpublished data).

PRINCIPAL COMPONENT ANALYSIS

Principal Component Analysis (PCA) provides an understanding of the relationships among the variables, i.e. which variables contribute similar information to the PCA model, and which provide unique information about the observations.

PCA was done on a dataset constructed in such a way as including data of antibiotic susceptibility/resistance, biofilm production, virulence at 12 hrs. The first component explained about 55% of the variance found, the second about 17%; taken together, more than 72% of the entire variance could be explained by combining both components. The Scatter Plot for the first components is shown in Fig. II. While *X. kozodoii* strains were found close to each other on the left, the other strains, belonging to *X. bovienii*, spread on the right, with no clear evidence of clustering or differentiation among SAR6 and MU1 (*S. ichnusae*) from the others (*S. feltiae*). The contribution plot (Fig. III) shows the weight of each variable to the observed distribution. It is apparent that distribution is mainly led by such variables as biofilm production induced by sugars. The effects of antibiotics, with the exception of chloramphenicol, and the pathogenicity (or virulence) seems to play a poorer role in strains' distribution. Finally, hierarchical clustering (Fig. IV) confirmed that strains of *X.*

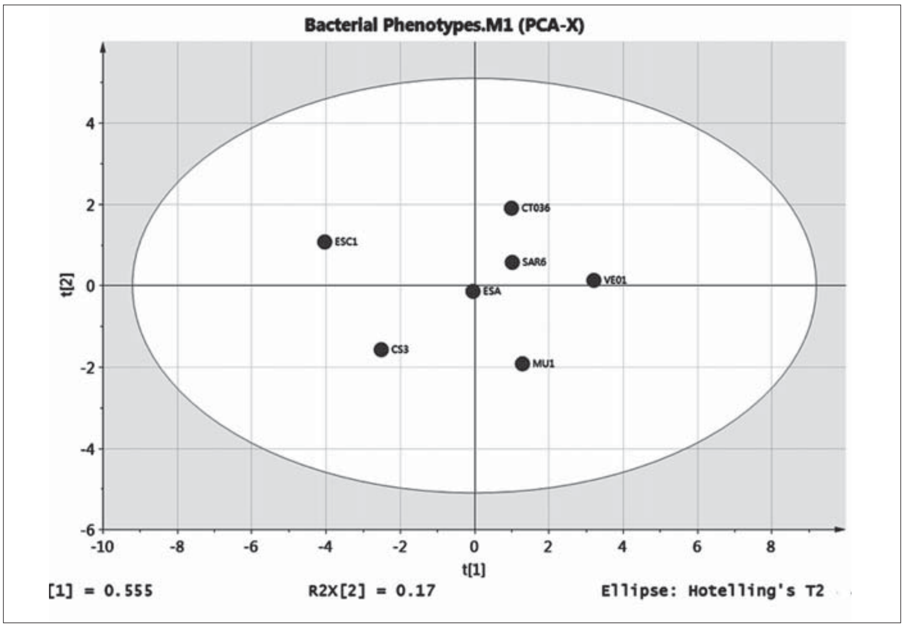

Fig. II – Scatter plot of the strains' variables displaying how the strains' variables are located, in a first-to-second components graph, with respect to each other.

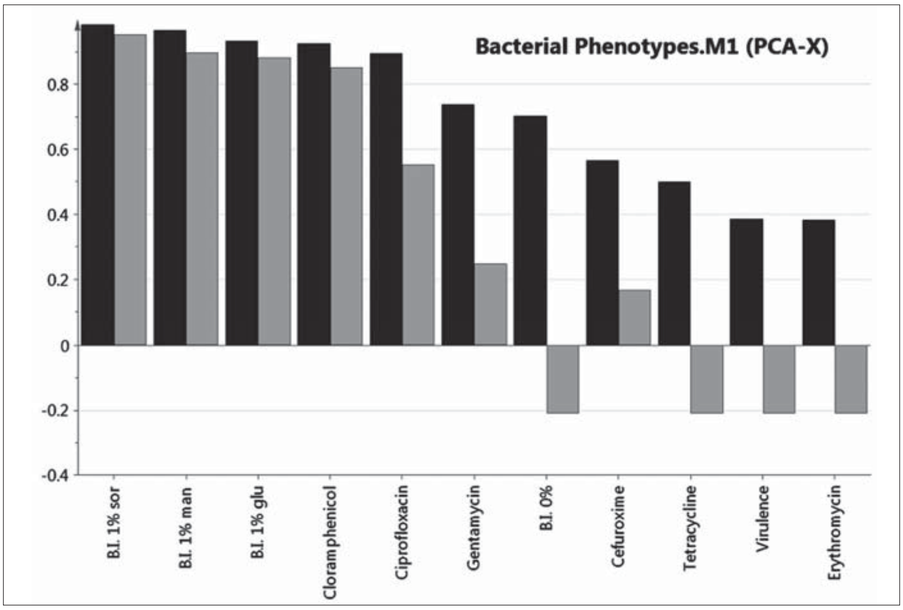

Fig. III – Cumulative R (black) and Q (grey) for each variable. R is a measure of fit, i.e. how well the model fits the data; Q explains how well the model predicts the variable. Acceptable values are >80%.

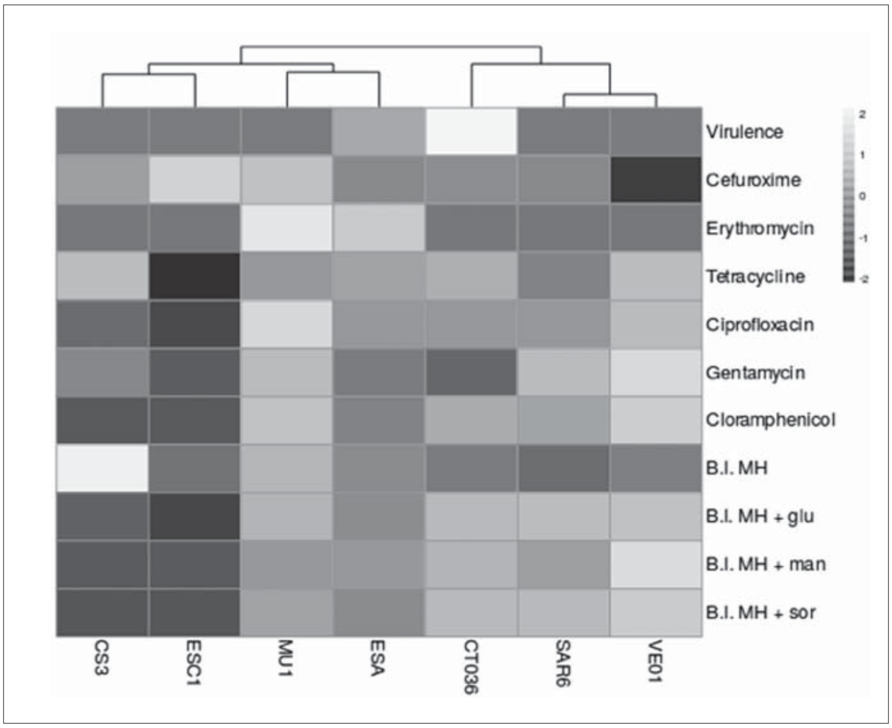

Fig. IV – Cluster analysis of the strains used for the study, based on the variables indicated on the right.

bovienii from *S. feltiae* were intermingled with those obtained from *S. ichnusae*, wile the strains of *X. kozodoii* cluster together. The graph shown in Fig. IV is representative of a number of different assays performed using several clustering algorithms; however, none of them was able to clusterize SAR6 with MU1 and differentiate them from *X. bovienii* belonging to *S. feltiae.*

DISCUSSION

Our results suggested that *Xenorhabdus* spp. may encode a plethora of functions not directly related to their role as endosymbionts of Steinernematidae, and a considerable phenotypic variability is present among strains of *X. bovienii* that could not be explained solely on the basis of guest-host association.

In fact those organisms are very similar to other Enterobacteriaceae, are able to grow in several media (both synthetic and natural) and might also exchange genetic material. Their genomes are large (4 to 5 Mb and may be more), suggesting that the association with *Steinernema* spp., although specific, has not hampered the full functionality of the genome. Congruent with this view is the pattern of biofilm production, which seems very heterogeneous in *X. bovienii* but more conserved in the *X. kozodoii* strains. For this reason, we raised the hypothesis of neutrality for most of the characters chosen for analysis.

The composite pattern of antibiotic resistance/susceptibility seems very puzzling. Resistance to ß-lactams, confirmed by our assays, was already described for almost all *Xenorhabdus* species. Genes contributing resistance to β-lactames have already been found in *X. bovienii* and *X. nematophila* genomes. However, the pattern of resistance to other antibiotics, in absence of any obvious exposure, is generally composite and suggests an acquisition of resistance genes from the environment. A suitable approach to answer that question will imply the identification of the resistance genes and the elucidation of the resistance mechanism(s). It has been shown that the genome of *X. bovienii* still contains mobilizing elements (Bisch *et al.*, 2016), suggesting those endosymbionts to be able to assume and mobilize DNA and/or genetic elements from and to the environment. More work is needed to elucidate the mechanisms behind such intriguing behaviour of endosymbionts.

It has been suggested that toxins produced by *Xenorhabdus* spp. should be considered as a selectable marker, because each strain competes with any other in case of co-infection (Adams *et al.*, 2007; Blouin *et al.*, 1999). Our results showed that this is the case even at the doses used; moreover, the toxin preparation was able to kill even larvae so large as those of *Rhynchophorus ferrugineus,* independently of the strain used (unpublished data). Our protocol was adapted from that of Brillard *et al.* (2001); those authors were able to purify a toxin from *X. nematofila*, which was later identified as the product of the *xaxAB* gene system (Vigneux *et al.*, 2007). However, since that genetic system is absent in the genome of *X. bovienii*, it is conceivable that other toxins, with similar physico-chemical properties, are produced by other *Xenorhabdus* species. Different toxins, or their proportions, might be responsible for the differential response of *G. mellonella* larvae in our experiments. However, because larval death was achieved within 24 hrs post injection regardless of the strain, our observations do not provide evidence of actual differences in virulence between strains. More experiments are needed to clarify the role of these bacterial endosymbionts in the biological control action of the entomopathogenic nematodes to which they are associated.

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ACCIDENTAL INTRODUCTION IN ITALY OF THE PARASITOID *SPATHIUS VULNIFICUS* WILKINSON (HYMENOPTERA BRACONIDAE DORYCTINAE)

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Jucker C., Loni A., Calzolari M., Belokobylskij S., Lupi D. – Accidental introduction in Italy of the parasitoid *Spathius vulnificus* Wilkinson (Hymenoptera Braconidae Doryctinae).

This paper reports the finding of the Hymenoptera Doryctinae *Spathius vulnificus* Wilkinson for the first time in Europe. This is an idiobiont ectoparasitoid attacking woodborer larvae of some Bostrichidae (Coleoptera) species. Such exotic parasitoid, mainly spread in the Oriental and southeastern part of the Palaeartic Region, has been intercepted in association with the lesser auger beetle *Heterobostrychus aequalis* (Waterhouse) (Coleoptera, Bostrichidae), imported from the Asian Far East in wood packaging material. This report represents an updating on the worldwide distribution of the parasitoid.

KEY WORDS: biological control, exotic species, new invasion, parasitoid.

INTRODUCTION

Expanding international trade, together with global warming and tourism, have been responsible for the unintentional introduction of many exotic insect pests and diseases, threatening native biological diversity and causing economic losses (PIMENTEL *et al.*, 2005; JUCKER & LUPI, 2011; LUPI *et al.*, 2013; BRADSHAW *et al.*, 2016). Numerous examples of introduced species (often pests) have become extremely invasive and caused serious environmental and economic damages to different biocenosis (ROQUES *et al.*, 2009; BRADSHAW *et al.*, 2016). The worldwide increase of trade in goods is mainly related with the use of solid wood packaging material (SWPM). However, wood for the construction of pallets can easily be infested by wood-boring beetles, and represent a pathway for their spread that augments the frequency of new introductions (HUMBLE, 2010; ROQUES, 2010). In the recent years, different xylophagous have been detected and established in new habitat thanks to SWPM. Some examples include the emerald ash borer (*Agrilus planipennis* Fairmaire) (POLAND & MCCULLOUGH, 2006; BARANCHIKOV *et al.*, 2008), and the Asian long-horned beetle (*Anoplophora glabripennis* (Motschulsky) (MACLEOD *et al.*, 2002; MASPERO *et al.*, 2007; FACCOLI *et al.*, 2015).

Many quarantine and inspection services act in a wide-scale aiming to limit the movement of invading pests, nevertheless introduction of exotic insects and pathogens remains an awkwardly challenge to manage. In particular, wood-boring insects have long-living preimaginal instars, making them extremely suited to survive long periods within dry wood along all the transport duration. Moreover, preimmaginal instars live hidden inside the wood, making them more difficult to detect. Due to numerous introductions of invasive xylophagous insects and other organisms in many Countries, International Standards for Phytosanitary Measures No. 15 (ISPM15) were developed in 2002 by the International Plant Protection Convention (IPPC) aiming to harmonize international regulations and phytosanitary treatments for SWPM used for trade.

Even if the main attention of inspection services focuses on insect pests, the finding of exotic parasitod insects that can be accidentally introduced with their host is not rare (BERRY & WALKER, 2004; KAUFMAN & WRIGHT, 2009). This is particularly permissible when the natural enemy is a concealed species, such as an endoparasitoid, or an ectoparasitoid living in hidden galleries excavated by larvae of wood boring insects (LONI *et al.*, 2012; LONI *et al.*, 2015).

In this paper we report the first detection of *Spathius vulnificus* Wilkinson (Hymenoptera Braconidae Doryctinae) in Italy, emerged from larvae of the lesser auger beetle, *Heterobostrychus aequalis* (Waterhouse, 1884) (Coleoptera, Bostrichidae).

MATERIAL AND METHODS

Four adults of a braconid wasp were detected in August 2017 in Medolla (MO), northern Italy (44°84'85''N; 11°07'09''E), in a factory importing goods from the far South of the Far East Asia. Samples were collected and the wasps were identified using the keys of WILKINSON (1931) and NIXON (1943). After this detection, an accurate monitoring in the same area has allowed the discovery of some wood packaging materials used to transport goods from Malaysia with evident signs of a massive infestation by xylophagous insects. Many exit holes due to xylophagous were observed and different life instars of the Coleoptera were collected, both alive and dead. Insects were between the stretch film and the cardboard that

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constitutes the boxes, and between the boxes themselves. Also these beetles have been sampled and classified.

RESULTS AND DISCUSSION

The collected females of parasitoids belonged to the braconid *Spathius vulnificus* Wilkinson, 1931 (Hymenoptera Braconidae). The xylophagous insects emerged from the infested wood material were all identified as *Heterobostrychus aequalis* (Waterhouse, 1884), (Coleoptera Bostrichidae), commonly known as the lesser auger beetle. This beetle species actively infests timber and wood products, especially if dry, and completes its entire cycle in these substrates. *H. aequalis* is native to the South East Asia, and it was intercepted in Italy and other European countries between 1987 and 2007, but was never established (AZMI *et al.*, 2011). This is the first record of *S. vulnificus* on the host *H. aequalis* in Italy and Europe.

Spathius Nees, 1818 is the largest genus in subfamily Doryctinae (Hymenoptera Braconidae). To date, more than 450 species are known in this genus (ZALDIVAR-RIVERON *et al.*, 2018), mainly widespread in the Oriental Region as well as in the Afrotropical and southeastern part of the Palaearctic Regions (BELOKOBYLSKIJ, 2003, 2009; BELOKOBYLSKIJ & MAETO, 2009; TANG *et al.*, 2015; YU *et al.*, 2016). As far as known, all species of *Spathius* are idiobiont ectoparasitoids of concealed woodborer Coleoptera larvae from the families Anobiidae, Bostrichidae, Buprestidae, Cerambycidae and Curculionidae (including Scolytinae) (SHENEFELT & MARSH, 1976; BELOKOBYLSKIJ, 1996a; 1996b; 2003; BELOKOBYLSKIJ & MAETO, 2009; YU *et al.*, 2016). However, some *Spathius* species were also found parasitizing lepidopteran larvae living in stems or wood, belonging to the families Sesiidae, Pyralidae, and Tortricidae, and also Hymenoptera larvae of the family Xiphydriidae (BELOKOBYLSKIJ, 2003).

Spathius vulnificus Wilkinson, 1931 (Fig. I, 1-8) was originally described from India (WILKINSON, 1931). NIXON (1943) in his World revision of this genus created the *S. vulnificus* species group together with Oriental *S. critolaus* Nixon, 1943 and *S. sul* Nixon, 1941; later *S. paracritolaus* Belokobylskij, 1996 described from Taiwan, was additionally added into this group (BELOKOBYLSKIJ, 1996a). The peculiar morphological distinguishing feature of this and related groups (*S. ocyroe* Nixon, *S. rusticulus* Wilkinson and *S. urios* Nixon; the last group was later united with the previous one: BELOKOBYLSKIJ & MAETO, 2009) is the "face having a satiny sheen due to a sculpture of excessively fine, absolutely even, transverse aciculation, like the surface of a gramophone record" (NIXON, 1943). Morphologically *S. vulnificus* is closely related to *S. critolaus*. In latter species the great majority of the specimens are micropterous forms while *S. vulnificus* as far as known has only macropterous (full-winged) forms. In case of macropterous specimens these two species can be easily distinguished for the coloration and sculpture of the second and third tergites surface; although reliable, this character can be rather variable in large series of specimens (NIXON, 1943). Other secondary features available for discrimination of these taxa are the length of the ovipositor sheet (shorter in *S. critolaus*) and the length of the second abscissa of radius (which is less the half of the third one in *S. vulnificus* and about two thirds in *S. critolaus*).

In nature in India, *S. vulnificus* has been commonly found parasitizing larvae of *Dinoderus brevis* Horn, 1878, *D. minutus* (Fabricius, 1775) and *D. ocellaris* Stephens, 1830 (Coleoptera: Bostrichidae) infesting bamboo stems of *Dendrocalamus strictus* (Roxb.) Nees (NIXON, 1943), but this species was also observed to live on the grubs of the cotton stem weevil *Pempherulus affinis* (Faust) and *Hypolixus truncatulus* (Fabricius) (Coleoptera, Curculionidae) under laboratory conditions (NIXON, 1943). *S. vulnificus* species was obtained for the first time from the lesser auger beetle in Israel (HALPERIN, 1986).

Our finding represents an interesting updating on the distribution of this wasp. Despite *H. aequalis* has still not established in Italy, *S. vulnivicus* could easily find other bostrichid hosts due to its capability to adapt to other species, affecting the biodiversity of indigenous communities. The role of exotic parasitoids in a new habitat is controversial and debated from long time as the establishment of an exotic species may alter food webs at different trophic levels (BENNET, 1993; KONOPKA *et al.*, 2016). Any contribution to the recording of new introduced species can enlarge the knowledge on the potential alterations of ecological relationships among native and exotic species and allow to perform a meta-analysis of the cumulated data across time.

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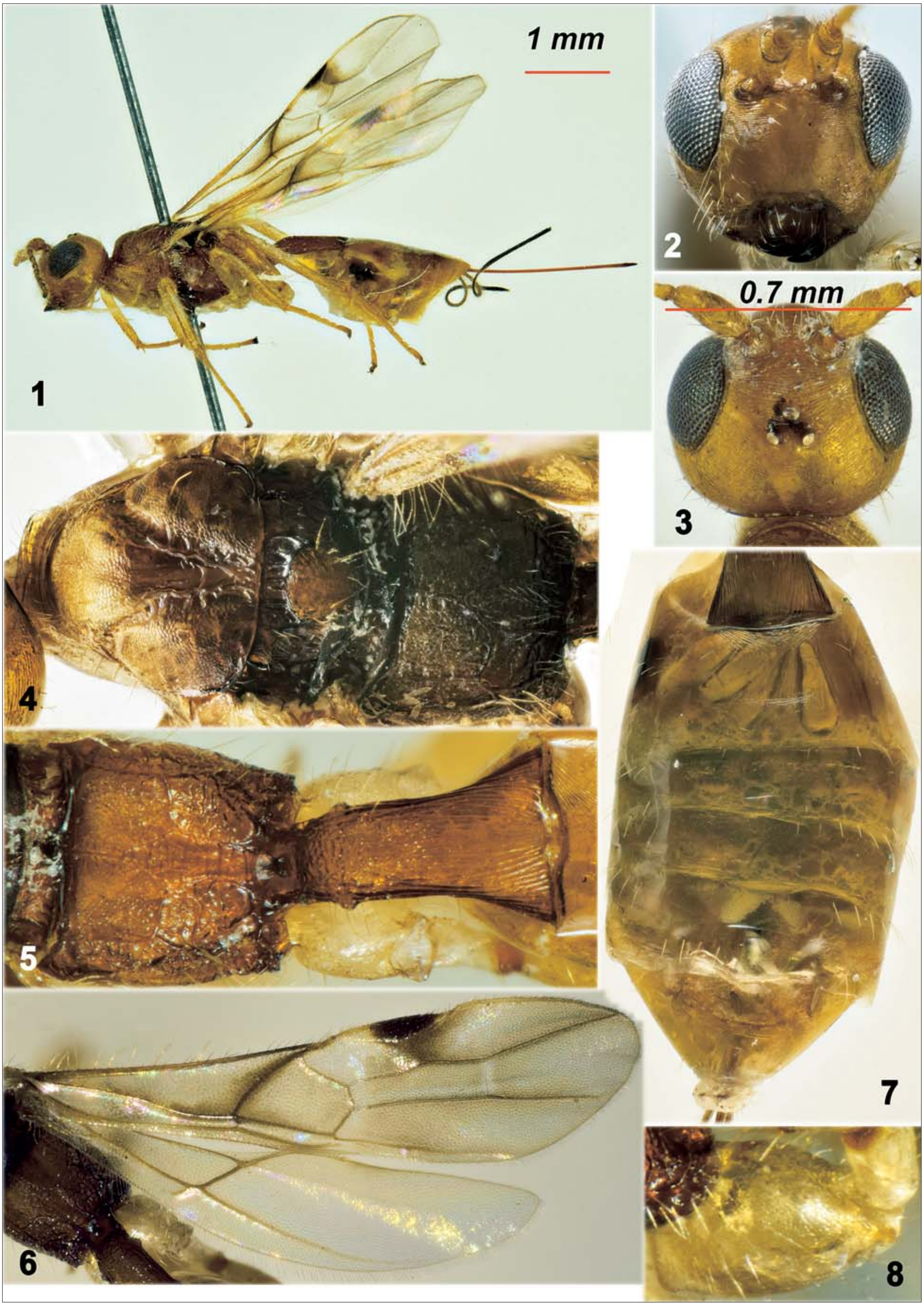

Fig. I – *Spathius vulnificus* Wilkinson (Italy). 1 - habitus, lateral view; 2 - head, front view; 3 - head, dorsal view; 4 - mesosoma, dorsal view; 5 - propodeum and petiole, dorsal view; 6 - wings; 7 - metasoma (without petiole), dorsal view; 8 - hind coxa, lateral view.

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REDIA, 101, 2018: 193-196 **SHORT COMMUNICATION** http://dx.doi.org/10.19263/REDIA-101.18.26

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SIGHTING OF SOUTHERN GREY SHRIKES PREYING ON RED PALM WEEVIL IN TWO COUNTRIES

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Fajardo M., Morales M., Fontenla E., Giordano C., Mori E., Mazza G. – Sighting of Southern Grey Shrikes preying on Red Palm Weevil in two countries.

Rhynchophorus ferrugineus, commonly known as Red Palm Weevil, is one of the most destructive and invasive palm pests' species in the world. The most important host species are coconut palms, oil palms, Canary Island palms and it is considered as a key pest in date palms crops. As the existing chemical – based treatments are not efficient, the eco-friendly biological control methods have now attracted high interest. In this note, we report for the first time the predation of the Red Palm Weevil by two subspecies of Southern Grey Shrike *Lanius meridionalis,* once in Spain and once in Saudi Arabia.

KEY WORDS: diet, *Lanius meridionalis*, palms, *Phoenix canariensis, Phoenix dactilifera, Rhynchophorus ferrugineus*, Saudi Arabia, Spain.

INTRODUCTION

The Red Palm Weevil (hereafter RPW), *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae), is an indigenous species to South East Asia which had recently spread worldwide mostly due to the movement of infested palms (e.g. FIABOE *et al*., 2012), and it is one of the most destructive and invasive pests of ornamental and economically important palms (e.g. DEMBILIO & JAQUES, 2015).

Currently, control methods against RPW mainly revolve around chemical treatments, but with inefficient results except for the case of Canary islands, where the RPW seems to be totally defeated (GIBLIN DAVIS *et al*., 2013). Moreover, chemical applications elicit serious concerns related to environmental pollution and insect resistance, in addition to human health impacts. For this reason, the eco-friendly biological control methods have now attracted high interest but they have to be developed or improved, in particular in an Integrated Pest Management point of view (e.g. GERLING *et al*., 2001; FRAVEL, 2005; DESNEUX *et al*., 2010; GIBLIN DAVIS *et al*., 2013).

Over 50 natural enemies have been reported to attack or negatively affect RPW (e.g. MAZZA *et al*., 2011; MAZZA *et al*. 2014) and besides the classic biocontrol agents such as bacteria, fungi and nematodes, some vertebrates (birds and mammals) are reported to feed on RPW. Some authors report that the role of these fortuitous predators against RPW is very limited and they are more a biological curiosity than a real opportunity of use (e.g. MAZZA *et al*., 2014). However, some recent works (BASHEER & THOMAS, 2012; ORIHUELA-TORRES *et al*., 2017) show that some birds, such as the Indian Rufous Treepie *Dendrocitta vagabunda parvula* and the Common Kestrel *Falco tinnunculus,* commonly feed on this invasive pest, in India and in Spain, respectively. The same authors suggest that these birds can be used as sentinel of the presence of this invasive beetle and that further research is needed to evaluate their role as a potential pest controller.

In this note, we report for the first time the predation of the RPW by two subspecies of Southern Grey Shrike *Lanius meridionalis* and we update the list of birds proven to feed on RPW (Table 1).

FIELD OBSERVATIONS

During a field-inspection (24/02/2009) for the control and eradication program of the RPW in the Canary Islands, performed in El Cotillo, a coastal town in the municipality of La Oliva (North of Fuerteventura, Canary Islands) and specifically in the Finca San Martin (N 28°39'29,49" and W 14°00'17,92"), an impaled female of RPW was found in one of the spines of a *Phoenix canariensis* palm tree (Fig. I, 1). Only 19 palms were attacked by the RPW out of 448 palms present in this area. The following days, some adults of the Canarian Southern Grey Shrike *Lanius meridionalis koenigi* were perched on a group of *Genista scorpius;* moreover, some pellets containing mainly the rest of beetles and some bones of vertebrates were found at the base of these bushes.

During a field inspection, another impaled female of RPW (Fig. I, 2) was found in Qassim (Saudi Arabia) (24/01/2018), in a highly RPW infested small farm- the specific infesting species of the area is *Phoenix dactilifera* (N 26°16.179 and E 43°35.791). Few adults of the Aucher's Southern Grey Shrike *Lanius meridionalis aucheri* were perched on the *Acacia* trees close to the site of collection. This Shrike may be easily recognized from other co-

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Table 1 – List of birds that fed on RPW. The RPW attacked stage/s and the location/s of record were reported in addition to the references.

Common name	**Species**	**Family**	**Attacked stage (s)**	**Location (s) of record**	**Reference**
Greater Coucal	*Centropus sinensis*	Cuculidae	Unknown	India	Faleiro (2006)
Common Kestrel	*Falco tinnunculus*	Falconidae	Adults	Spain	Orihuela-Torres *et al.* (2017)
Little Owl	*Athene noctua*	Strigidae	Adults	Italy	E. Mori, unpublished
Canarian Grey Shrike	*Lanius meridionalis koenigi*	Laniidae	Adults	Spain	this article
Arabian Grey Shrike	*Lanius meridionalis aucheri*	Laniidae	Adults	Saudi Arabia	this article
Rufous Treepie	*Dendrocitta vagabunda parvula*	Corvidae	Adults	India	Krishnakumar & Sudha (2002); Basheer & Thomas (2012)
Magpie	*Pica pica*	Corvidae	Unknown	Italy	Lo Verde *et al.* (2008)
Eurasian blackbird	*Turdus merula*	Turdidae	Unknown	Unknown	Ortega-García *et al.* (2017)

Fig. I – Impaled female of RPW found in (1) one of the spines of a *Phoenix canariensis* palm tree in the Finca San Martin (North of Fuerteventura, Canary Islands) and (2) Qassim (Saudi Arabia) in a *Phoenix dactilifera* spine.

occurring Shrikes, as it was the only Grey Shrike present in Qassim in winter, showing a deeper black face-mask and a paler beak with respect to the Mauryan Grey Shrike *L. m. pallidirostris* (Lefranc & Worfolk, 1997).

DISCUSSION

Contributions on the Southern Grey Shrike's diet ecology are scarce and restricted to Israel (Yosef *et al.*, 1991, Budden & Wright, 2000), Spain (e.g. Hernández *et al.*, 1993) and France (Lepley *et al.*, 2004). In the Canary Islands, the diet of this bird is represented mainly to beetles (Curculionidae and Tenebrionidae) and lizards and the rest consist of other arthropods and vertebrates (Padilla *et al.*, 2005). Beetles are reported in the diet in all seasons depending on their availability, with Curculionidae selected all year round and Tenebrionidae only in warm months. Padilla *et al.* (2005) also report that this subspecies endemic to Canary Islands may catch insects on shrubs in addition to the ground (Cramp & Perrins, 1993).

The habit of impaling prey on thorns or other sharp objects is a peculiar behavior of shrikes and it attests the presence of these birds in a certain area. Many types of thorns can be used to impale preys by these birds and, in two cases illustrated in the present communication (Table 1), the palm thorns were used to kill the RPW. The palm thorns are used by shrikes to impale ripe dates in North Africa (Beven & England, 1969) and lizards in Qatar (Cogălniceanu *et al.*, 2015). As reported by Panov (2011) insects are usually impaled through the thorax and can remain alive for a long time. Not all the caught preys are immediately utilized by shrikes (in particular vertebrates and toxic insects) and the use of the RPW for this bird could have several functions (e.g. food supply, mate attraction during the breeding season) but this aspect needs to be explored through further research.

Recent literature reviews have highlighted the important role of insectivorous birds feeding on pest populations in different agroecosystems (e.g. Maas *et al.*, 2015; Barbaro *et al.*, 2017) and the use of predatory birds as a viable alternative to pesticides or other chemical compounds to control pest insects is not a novel new (e.g. Franz, 1961). To support the evidence that shrikes may be excellent predator, the Rufous-Backed Shrike *Lanius schach* was introduced as biological control agent in 2011 in Indonesia, Sulawesi, in August 2011, for the management of the long horn grasshopper *Sexava nubile*. The operation, within 10 months after the release of the predators, showed a significant, marked downward trend concerning the insect populations and the leaf damage intensity (Lala *et al.*, 2014). As the present communication is based just on two recorded cases, a specific research to further explore this hypothesis is recommended, possibly using also molecular-based tools, in order to explore the diet variability among shrike species by collecting samples (e.g. faeces or pellets) at perching sites (see GALIMBERTI *et al*., 2013; GALIMBERTI *et al*., 2016). In fact, whether the predatory capacity of the Southern Grey Shrikes towards the RPW is confirmed, possible future uses of these birds in a biological control strategy might be conceived. In the areas where the Southern Grey Shrikes is already present, such as in the cited cases, it might be both used as a component of an integrated control program and as a control measure by itself.

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FIRST RECORD OF *RICANIA SPECULUM* (WALKER, 1851) (HEMIPTERA RICANIIDAE) FROM VENETO, PIEDMONT AND LATIUM REGIONS AND NEW HOST PLANTS

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Mazza G., Marraccini D., Lucchi A., Marianelli L., Sabbatini Peverieri G., Bosio G., Giacometto E., Rapa L., Cianferoni F., Roversi P.F., Gargani E. – First record of *Ricania speculum* (Walker, 1851) (Hemiptera: Ricaniidae) from Veneto, Piedmont and Latium regions and new host plants.

The distribution of *Ricania speculum* (Walker, 1851) (Hemiptera Fulgoromorpha Ricaniidae) in Italy was updated and the species was recorded for the first time in Veneto (2017), Piedmont (2018) and Latium (2018) regions. The occurrence of this species in Sardinia, previously recorded, is here excluded. Moreover, 33 new host plants belonging to 29 families resulted to be attacked by this alien invasive species confirming its outstanding polyphagy.

KEY WORDS: alien invasive species, Fulgoromorpha, Fulgoroidea, Italy, polyphagous insect.

INTRODUCTION

Ricania speculum (Walker, 1851) (Hemiptera: Fulgoromorpha: Ricaniidae) is an alien invasive species reported for the first time in Europe in 2009 (MAZZA *et al.*, 2014) and in a short time spread across Liguria (MAZZA *et al.*, 2014; ROSSI and LUCCHI, 2015) and Tuscany (SILVESTRI, 2017).

This univoltine species feeds and lays eggs on a wide range of host plants and its large polyphagy was immediately highlighted by MAZZA *et al.* (2014), ROSSI and LUCCHI (2015), ROSSI *et al.* (2015), LUCCHI and ROSSI (2016), LAUDONIA *et al.* (2017) and SILVESTRI (2017).

The host plants of this species in Italy include important crops (e.g. vines, citrus, olive and other fruit trees) and many ornamental plants (see references above). Sap suction is the main damage reported in addition to injuries, caused by the female which inserts the eggs into the plant tissues with its sharp ovipositor (e.g. ROSSI *et al.*, 2015; LUCCHI and ROSSI, 2016). This ovipositional behavior causes the withering of thin shoots or branch where the eggs were laid, as already mentioned in ROSSI *et al.* (2015). On the contrary, the honeydew production emitted from juveniles and adults seems to do not create damages on host plants (ROSSI *et al.*, 2015), but further studies are necessary in the light of the high number of individuals in the new areas of introduction (D. Marraccini, pers. obs.).

Since this exotic planthopper is a real pest for several crops in tropical and subtropical areas (reviewed in MAZZA *et al.*, 2014), the presence in Italy due to its polyphagy is noteworthy, representing a new possible threat for native species and human activities.

Moreover, since PILOTTI *et al.* (2014) found that the Banana-wilt associated phytoplasma (BWAP) was confirmed in two specimens of Ricaniidae collected from banana plants, the possible role of *R. speculum* in Italy as a vector of plant pathogens is pivotal and require further studies.

For these reasons, the aim of this note was to update the distribution of *R. speculum* and the list of host plants in Italy.

MATERIALS AND METHODS

Ricania speculum eggs, nymphs and adults were collected weekly from April to October 2018 in the field, particularly in the municipality of Viareggio (province of Lucca, Tuscany). During the monitoring the host plants and the developmental stages were recorded following ROSSI *et al.* (2015).

We collected data on this species concerning distribution and host plants also in some naturalistic forums on the web ("Forum Natura Mediterraneo", www.naturamediterraneo.com; "Forum Entomologi Italiani", www.entomologiitaliani.net; "iNaturalist", www.inaturalist.org).

Plant nomenclature followed the online nomenclatural database "The Plant List" (www.theplantlist.org). The datum for geographical coordinates is WGS84.

RESULTS AND DISCUSSION

We confirmed the widely spread of *Ricania speculum* in Tuscany, particularly in the provinces of Massa-Carrara and Lucca, as already highlighted by SILVESTRI (2017), and Pisa (A. Lucchi, pers. obs.).

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post in a naturalistic forum (Rossi and Lucchi, 2015) is, on the contrary, a misunderstanding, since F.C. interviewed directly the observer of the "Forum Entomologi Italiani" who explained that he lives in Sardinia, but the record of *R. speculum* regarded Liguria.

For the first time, we reported the presence of this species in Veneto and Piedmont regions. In particular, as regard Veneto, *R. speculum* was found in the Arcella neighborhood, province of Padua (an adult on an orchid plant in August 2017 and few adults inside a building in September 2018: 45°25'N 11°52'E) and in the province of Rovigo, where the species seems already well distributed (two sites in the center of Rovigo: 45°03'47.25"N 11°50'37.57"E; 45°04'19.25"N 11°49'33.13"E and one site in the municipality of Villadose: 45°04'N 11°53'E). In the Rovigo province several nymphs and adults were observed in July 2018, mainly on *Rosa* sp. and in September 2018 on *Magnolia grandiflora* L.

In Piedmont region the species was found in September 2018 on riparian plants around an artificial lake in the municipality of Settimo Torinese (45°08'37.6"N 7° 43'15.8"E). More than 50 specimens of this alien insect were collected on *Acer* sp., *Crataegus monogyna* Jacq., *Lythrum salicaria* L.*,* *Malvaviscus arboreus* Cav., *Quercus rubra* L., *Rosa* sp., *Rubus* sp., *Salix* sp., *Typha* sp. and *Ulmus* sp.

In Latium a specimen of *R. speculum*, attracted to lamp during a nocturnal monitoring, was photographed by Falvio Rocchi (www.inaturalist.org) in Rome in the "Parco Regionale dell'Appia Antica" (41°52'2.84"N 12°30'1.85"E).

Thirty-three new host plants, belonging to 29 families resulted to be attacked by this alien invasive species confirming its highly polyphagy (Table 1; Fig. I). The list of plants ranges from angiosperms to gymnosperms and, amongst the new host plants, we found several important vegetables, crops and ornamental plants in addiction to wild plants and trees (Table 1). Further investigations on how host plant species nutritional and defensive chemistry affect the subsequent host plant species selection, oviposition and fitness in *R. speculum,* are necessary.

Moreover, eggs of *R. speculum* were found also in a Chestnut tree stakes, *Castanea sativa* Mill. (Fig. II) in Castiglione Chiavarese (GE) in September 2018 and this could be another important pathway of introduction for this species. Since *R. speculum* lays eggs in several plants, the species could be accidentally introduced in several regions of Italy. Moreover, since Pistoia (Tuscany), the Italian center of nurseries and one of the three most important suppliers of ornamental trees and shrubs in Europe, is close to Lucca*,* *R. speculum* represents a concern and then needs to be carefully monitored for its possible expansion in all Europe, as already highlighted in Rossi *et al.* (2015).

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Table 1 – Development stages (E-eggs, N-nymphs and A-adults) of *Ricania speculum* in the new host plants.

Family	Genus, species and author	Development stage
Adoxaceae	- *Viburnum* sp.	- N
Altingiaceae	- *Liquidambar styraciflua* L.	- E, A
Anacardiaceae	- *Schinus molle* L.	- E, A
Apiaceae	- *Daucus carota* L.	- A
Apocynaceae	- *Hoya* sp. - *Trachelospermum jasminoides* (Lindl.) Lem.	- N - N, A
Bignoniaceae	- *Catalpa* sp.	- A
Cannabaceae	- *Cannabis sativa* L.	- E, A
Caricaceae	- *Carica × pentagona* Heilborn	- A
Compositae	- *Cirsium* sp.	- A
Convolvulaceae	- *Ipomoea* sp.	- N
Cucurbitaceae	- *Cucurbita pepo* L. - *Cucumis sativus* L.	- N - A
Cupressaceae	- *Juniperus oxycedrus* L.	- A
Cycadaceae	- *Cycas revoluta* Thunb.	- N, A
Ericaceae	- *Vaccinium* sp.	- N
Fagaceae	- *Quercus rubra* L.	- A
Iridaceae	- *Crocosmia* sp.	- N
Juglandaceae	- *Carya illinoinensis* (Wangenh.) K.Koch	- E, A
Lythraceae	- *Lythrum salicaria* L.	- A
Magnoliaceae	- *Magnolia grandiflora* L.	- A
Malvaceae	- *Abutilon pictum* (Gillies ex Hook.) Walp. - *Malvaviscus arboreus* Cav.	- E, A - A
Myrtaceae	- *Callistemon citrinus* (Curtis) Skeels	- A
Oleaceae	- *Fraxinus* sp.	- E, A
Poaceae	- *Hordeum marinum* Huds. - *Zea mays* L.	- N - A
Platanaceae	- *Platanus* sp.	- E, A
Solanaceae	- *Solanum melongena* L.	- N
Tamaricaceae	- *Tamarix* sp.	- A
Theaceae	- *Camellia* sp.	- N
Typhaceae	- *Typha* sp.	- A
Vitaceae	- *Parthenocissus quinquefolia* (L.) Planch.	- E, N, A

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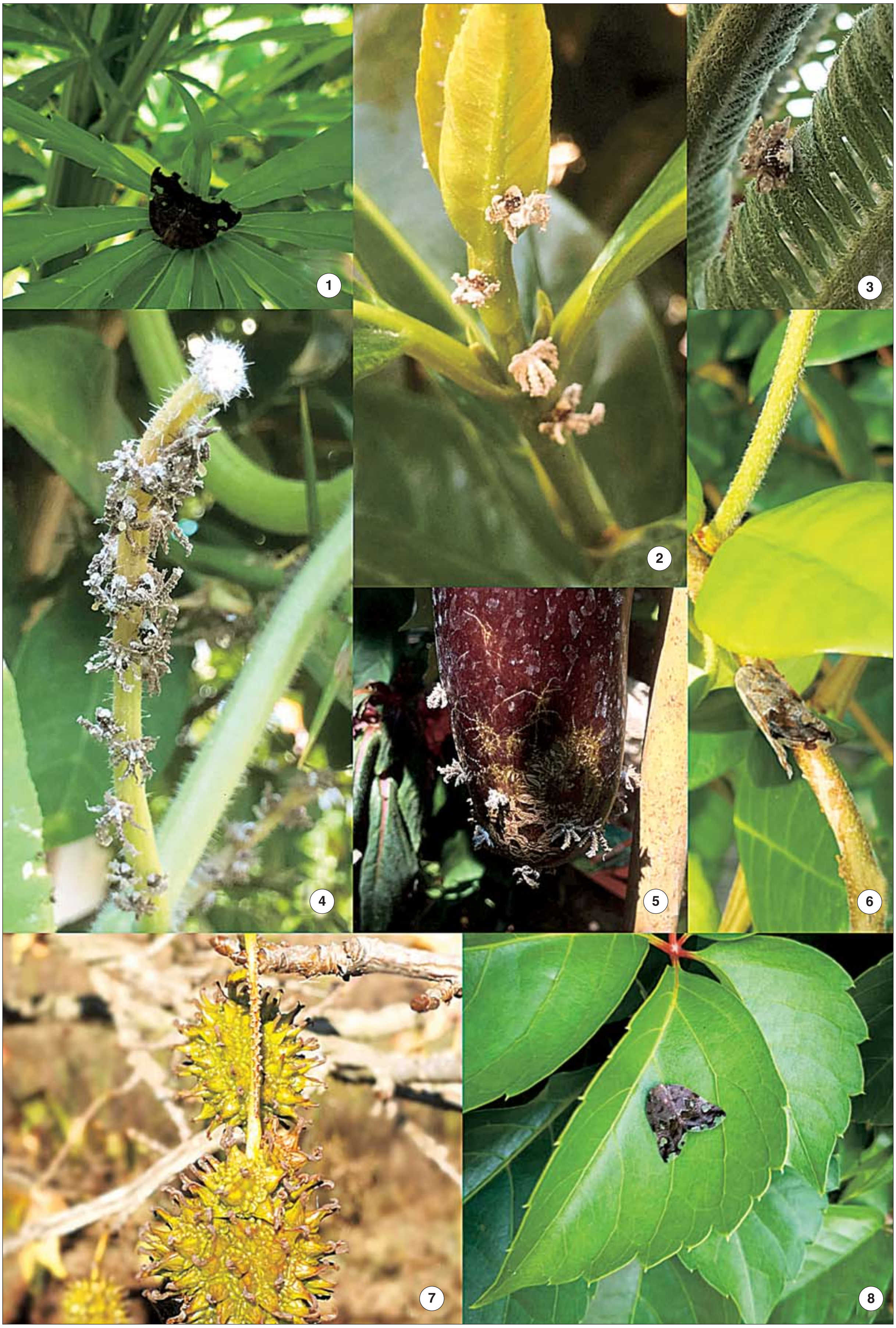

Fig. I – *Ricania speculum* on new host plants: 1) adult on *Cannabis sativa* L.; 2) nymphs on *Viburnum* sp.; 3) nymph on *Cycas revoluta* Thunb.; 4) several nymphs on *Cucurbita pepo* L.; 5) nymphs on *Solanum melongena* L.; 6) adult on *Trachelospermum jasminoides* (Lindl.) Lem.; 7) eggs inserted in a fruit of *Liquidambar styraciflua* L.; 8) adult on *Parthenocissus quinquefolia* (L.) Planch.

Fig. II – Eggs of *Ricania speculum* in a Chestnut tree stake.

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ACLEES CF. SP. *FOVEATUS* (COLEOPTERA CURCULIONIDAE), AN EXOTIC PEST OF *FICUS CARICA* IN ITALY: A SUSTAINABLE APPROACH TO DEFENCE BASED ON ALUMINOSILICATE MINERALS AS HOST PLANT MASKING SOLIDS

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Gargani E., Simoni S., Benvenuti C., Frosinini R., Barzanti G.P., Roversi P.F., Caselli A., Guidotti M. – *Aclees* cf. sp. *foveatus* (Coleoptera Curculionidae) an exotic pest of *Ficus carica* in Italy: a sustainable approach to defence based on aluminosilicate minerals as host plant masking solids.

The exceptionally frequent entries of alien pest are a major source of concern for the farmers who have to protect their crops from unknown insects, often without natural enemies in the new areas. A new pest belonging to the Molytinae family (Coleoptera: Curculionidae), tribe Hylobiini, reported as *Aclees* sp. cf. *foveatus* Voss, was recently introduced in Italy. The species is responsible for severe damages in many Italian fig nurseries and orchards, particularly in the Italian Central Northern regions, *i.e.* Tuscany, Ligury and Latium. Currently, no active ingredients are registered against this insect on fig crops. An innovative and eco-friendly approach for controlling this exotic weevil infestation was investigated, by using montmorillonite-based clays, either in their native state or containing copper(II) species, and clinoptilolite zeolites, in order to check the perception of the adults' weevil towards the different solid materials and, subsequently, to evaluate the capability of these innovative products to act as masking agent with respect to the host plant and/or as repellent upon contact. The formulations containing copper(II)-exchanged clay and clinoptilolite zeolite showed preliminary promising results in terms of efficacy and environmental sustainability.

Key Words: Asian fig weevil, alien pest, control, copper-containing clay, clinoptilolite zeolite, montmorillonite.

INTRODUCTION

The exponential rise in the movement of goods, food products and people are having, as a side consequence, an ever-growing number of exotic species (also referred as alien) introduced into new areas. Over the centuries, introductions of alien species have sometimes been intentional, especially in the field of organisms useful to humans, such as animals of zootechnical interest, beneficial insects, such as honey bees or different entomophagous insects, various crop plants (*e.g.* corn, potato, tomato, etc.) as well as ornamental species. However, often, in the case of arthropods, the entrances in new countries might have been unintentional, in particular for species, which resulted to be harmful to plants or, even, to humans. Despite complex international and national legislations aiming at hindering the diffusion of alien species from one country to another (see, for instance, EU Dir. 2000/29/CE; or Italian Law Decree 214/2005), the phenomenon is becoming increasingly topical. With regard to arthropods, it is estimated that the current rate of introduction of new insects in Italy is, at least, 6-7 new species a year (Inghilesi *et al*., 2013).

In 2005, a new pest belonging to the genus *Aclees* (Coleoptera: Curculionidae), was reported as the responsible of severe damages in fig nurseries in Tuscany (Central Italy). To date, the infestations have been spread across many fig orchards, particularly in the Italian Central Northern regions, *i.e.* Tuscany, Ligury and Latium, with considerable harvest yield losses and plant deaths (Gargani *et al*., 2016). The Curculionid, belonging to Molytinae family, tribe Hylobiini, is reported as *Aclees* sp. cf. *foveatus* Voss (Benelli *et al*., 2014b). The species, probably of Asian origin, is strictly related to *Ficus carica* L. Adults, indeed, feed on the epigeal part of the plants, while larvae are xylophagous during their whole development and can cause severe damages, destroying the wood tissues of the root and the trunk. Fig plants are infested in any season, adults show two different peaks of activity in spring and in summer. However, when climatic conditions are suitable, they can feed on the plants all year round. Up to now, no fig cultivars resistant to the attacks of this pest and very few control strategies capable of containing the adult infestations of this beetle, have been reported: treatments with entomopathogenic fungi, *Beauveria bassiana*, gave stimulating results (Gargani *et al*., 2016). Therefore, as the presence of the insect spreads from nurseries to scattered figs, if the problem will not be addressed systematically, the fig cultivation on the Italian territory might be likely decimated in a short time.

The study of eco-friendly control strategies against exotic

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insects is more and more important, not only in terms of sustainability, but also in view of the results. In fact, an efficient eradication of a newly introduced alien species, endangering plants, has never been achieved with traditional agrochemicals only. Furthermore, when a new pest is introduced in Italy, farmers have no authorized means to control the new alien species, since the current legislation requires that each agrochemical product is registered on a specific crop and also against a specific list of noxious insects.

For this reason, a round of experimental trials using a set of aluminosilicate solids to study innovative control strategies against this new pest of Italian fig was performed.

To this aim, three types of materials expected to have a detrimental effect on the ethology of *Aclees* cf. sp. *foveatus* were selected and prepared, *i.e.* 1) a montmorillonite-based clay from mineral origin, also known as bentonite, 2) a montmorillonite clay containing copper(II) species, obtained through cationic exchange, and 3) a clinoptilolite-type zeolite.

Clays and zeolite were here chosen as preferential materials for fig crop protection, thanks to their toxicological safety, environmental compatibility and particular physico-chemical characteristics, in terms of cation-exchange capability and sorption properties. Finely ground clays are indeed able to form a homogeneous particle film on plant leaves and tissues and this proved to be a viable strategy for controlling pests and diseases (GLENN *et al*., 2005; CHITU *et al*., 2009; SILVA *et al*., 2013; SHARMA *et al*., 2016). One of the most studied clays, kaolin, is well known for its effect against insect pests, due to its contact repellency action and disruption of feeding and oviposition (BENELLI *et al*., 2014a). Furthermore, in more recent study, the effects of kaolin-based particle films were evaluated on the emission of host plant volatile organic compounds, finding a positive correlation between the presence of this clay on plants and a lower attraction of the host towards the insects (GERMINARA *et al*., 2018). In addition, since clays have a layered porous structure and can accommodate and immobilise active ingredients within the layers of their phyllosilicate structure, clays can be efficiently used as a device for the controlled release of biocides, herbicides and/or fertilisers onto host plants (CHOY *et al*., 2007; SINGH *et al*., 2009). Micronized zeolites as well are attractive candidates as crop protection products, since they create uniform films, which do not interfere with the metabolic lifecycle of the plant and, on the contrary, enhance its resistance towards high temperatures and strong solar irradiation (DE SMEDT *et al*., 2015). Moreover, clay- or zeolite-based formulations typically contain chemically inert minerals and the thin particle films obtained from them can be easily removed from harvested fruits by a simple washing with water. In the present case, the aluminosilicate materials were used either without any further modification in their micronized powder form or, for the montmorillonite clay only, after a treatment of cationic exchange, in order to insert copper(II) into the interlayer spaces of the solid structure. Indeed, the beneficial use of copper-containing products for controlling insect pests has been already studied for *Bactrocera oleae*, the key pest of olive crops (BELCARI *et al*., 2005). Nevertheless, since the current trend is to avoid, or at least minimize, the use of copper-containing formulations (DAGOSTIN *et al*., 2011; KUEHNE *et al*., 2017), immobilization of Cu(II) ions within the montmorillonite framework might help in reducing the overall amount of active metal and in having a controlled, smooth release of Cu(II) species on the host plant and eventually into the environment (HU *et al*., 2006).

Then, laboratory-scale and preliminary field trials were carried out to verify their effectiveness in terms of masking the host plants and/or repellency upon contact towards the adults of *A.* cf. sp. *foveatus*. From ethological studies conducted so far, *Aclees* seems to be closely related to its host plant, *F. carica*. For this reason, we have studied the responses of weevil adults to plants treated with different formulations of solids covering the fig canopy.

MATERIALS AND METHODS

TEST INSECTS

The Asian fig weevils, *A.* cf. sp. *foveatus*, were obtained collecting adults in fig crops in Carmignano area (Prato province, Tuscany), a very well-known zone for fig production in Central Italy. The gathering step and, consequently, the laboratory trials were carried out in summer (July) and autumn (September). Before the experimental treatments, the adults were provided with fig fruits and leaves as food in entomological cages (100×60×60 cm) and were maintained under standard conditions. One pool of 10 adults, chosen randomly amongst the ones collected from the field, were used for each single trial. Twenty-four hours before the trial, the chosen adults were starved. The weevils were inserted into the experimental chamber and then their movements were monitored by an observer. Controls were carried out after 30 min, 1 h, 2 h, 3 h and 24 h, counting the number of adults in the different treated cages/plants (T1 -T5).

LABORATORY TESTS: EXPERIMENTAL APPARATUS

An all-plastic five-arm device was developed at CREA DC facilities. The system consists of a central plastic chamber (100 cm x 25 cm x 25 cm) covered by a screen tissue and having a surface suitable for the insects' walking, connected with five plastic tube (8 cm diameter) to five little entomological cages (30 cm x 20 cm x 20 cm), containing small fig plants (15 cm high) in pot (10 cm diameter). During the test, a group of ten individuals were put inside the central chamber, leaving them free to move and walk towards the cages. In the five lateral cages, three fig plants treated with the solid materials to be tested, one fig treated with Naturalis™, a well-known bioinsecticide, and a fig plant treated with tap water only, as control plant, were placed. Naturalis™ contains a naturally occurring soil born fungus. This fungal pathogen of insects begins working once it comes into contact with a target pest. Additionally, against certain pests, such as some types of flies, Naturalis™ has demonstrated repellent properties, when applied in preventive treatments (CUTHBERTSON *et al*., 2016).

CHEMICALS AND PREPARATION OF THE SOLID MATERIALS

Bentonite Globalfeed AR, hereafter Ben, is a montmorillonite-containing natural clay of mineral origin and was kindly obtained from Laviosa Chimica Mineraria SpA (Livorno, Italy).

ROTA Mining Zeolite, hereafter Zeo, is a clinoptilolite-containing natural clay of mineral origin and was kindly obtained from Biohelp Your Planet s.r.l. (Tarquinia, Italy).

Copper(II) nitrate hemipentahydrate (Sigma-Aldrich, 95%) was used as received.

Each batch of Ben and Zeo to be tested in laboratory and field trials was prepared as follows. A 240 g batch of Ben or Zeo was washed carefully with *ca.* 1 L of deionised water (10 MΩ•cm, Elix-70, Millipore-Merck purifying apparatus), in order to remove any water-soluble species. The solid was then dispersed in 7.5 L of tap water just prior to use, by vigorous shaking and a 3.1 wt.% dispersion of the solid in water was hence obtained.

The physico-chemical characteristics of Ben and Zeo and their appearance are reported in Table 1 and Fig. I, respectively. The metal composition, expressed as weight percentage of metal oxides, of the pristine materials was received as technical data from the manufacturer. For Si, Al, Fe and Ca, the oxide metal content was further confirmed by inductively coupled plasma optical emission spectroscopy, ICP-OES (ICAP 6300 Duo, Thermo Fisher Scientific) after mineralization of the sample with a 1:1 aqueous HF/HNO_3 mixture and quantification was obtained against genuine metal standard solutions (Fluka). The elemental analysis for total carbon and total nitrogen was performed on a PerkinElmer instrument (CHN 2400 Series II), equipped with a transistor-grade extrapure oxygen gas cylinder (SIAD, < 50 ppmv residual N_2 content) and connected to a Cahn C-30 Microbalance.

The initial Ben clay contained more than 90% of montmorillonite, the rest being a mixture of minor amounts of amorphous silicate and/or aluminate oxides. The initial Zeo contained at least 85% of crystalline clinoptilolite zeolite, the rest being a mixture of minor amounts of amorphous inorganic oxides. Semi-quantitative whole powder analysis (bulk mineralogy) was performed by powder X-ray Diffraction Method, XRD (Thermo ARL X'TRA-048 diffractometer with a Cu Kα (k = 1.54 Å) radiation).

Table 1 – Physico-chemical characteristics of Ben and Zeo aluminosilicate materials.

	Ben	Zeo
Na_2O^a	0.4[a]	0.1 [a]
MgO	2.3	1.1
Al_2O_3	14.2	11
SiO_2	72.3	70.1
P_2O_5	0.03	0.02
K_2O	1.9	3.0
CaO	0.9	3.1
TiO_2	0.3	n.d.[c]
MnO	0.03	0.1
Fe_2O_3	2.3	1.8
others minor oxides	5.1	9.2
C[b]	0.16	0.19
N[b]	0.08	0.27
most abundant particle size	25 μm[d]	20 μm
specific surface area[e]	114 m^2/g [f]	46 m^2/g
CEC[g]	140 meq/100g	173 meq/100g

a. Metal composition is expressed as metal oxide (expressed as wt.% over the dried form of the samples); b. obtained by C,H,N elemental analysis; c. not determined; d. with 97% of the particles below 53 μm; e. determined by Brunauer-Emmett-Teller equation; f. specific surface area for Cu-Ben = 121 m^2/g; g. cation exchange capacity according to the ammonium acetate method.

Fig. I – Appearance of the powders of Ben (left) and Zeo (right) materials.

The cation exchange capacity (CEC) of the two solids was determined by displacing the exchange sites using a 1 M solution of ammonium acetate.

N_2 physisorption measurements were carried out at 77 K in the relative pressure range from 1 x 10^{-6} to 1 P/P_0 (Quantachrome Autosorb1MP/TCD instrument). The specific surface area values were determined by using Brunauer-Emmett-Teller equation, in the relative pressure range from 0.01 to 0.1 P/P_0.

The copper-containing batch, Cu-Ben, on the contrary, was prepared from the pristine Ben material by cationic exchange with aqueous solution of copper(II) salt precursor. In detail, 72.5 g of $Cu(NO_3)_2.2.5H_2O$ were dissolved in 250 mL of deionised water. Then, 240 g of Ben was slowly added to the solution in small aliquots, under mechanical stirring at 250 rpm. After the addition, the total volume of the suspension was brought to 1 L by a further addition of deionised water and homogeneously stirred for 4 h. The suspension was then decanted and thoroughly rinsed with 1 L of fresh deionised water, in order to remove Cu(II) species in excess from the liquid phase. The rinsing step was repeated 8 times, so to obtain an almost colourless upper layer above the decanted solid. The concentrated suspension was finally diluted to 7.5 L, by addition of tap water just prior to use, by vigorous shaking and an overall 3.1 wt.% dispersion of the solid in water was hence obtained.

A small fraction of the Cu-Ben was withdrawn and dried. The content of Cu(II) in the solid was 3.43 wt.%, as obtained by indirect iodometric titration of Cu(II) species in the residual aqueous phase. The content of C and N, as from elemental analysis (*see above*), was 0.17 wt.% and 0.18 wt.%, respectively.

Naturalis™, the fourth treatment/thesis (Nat), is a commercial registered bio insecticide, based on living spores of naturally occurring strains of the entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv.) Vuillemin (Strain ATCC74040, 7.16 g, equal to 2.3 x 10^7 viable spores/mL): the product was use at label dose (Table 2).

FIELD TEST

At the end of July, the experimental solids (Table 2) were tested in preliminary field trials. In the fig production area of Carmignano (Prato province, Tuscany), three young fig plants/thesis were treated with the 3.1 wt.% aqueous suspensions, as described above. The solid-in-water fine suspension of the products was spread onto the crop trees by means of a backpack sprayer at normal volume. Controls, performed with visual observation on the damage on the fig plants, were carried out at the end of September. The visual inspection was carried out on the whole plant, counting the damaged and healthy shoots.

STATISTICAL ANALYSIS

The effect of collection period of the insects to be tested, of the check time and of the treatment were analysed by GLM (General Linear Model) and significance evaluated by Duncan test (P < 0.05).

Table 2 – Products tested in 2017 in laboratory and in field trials.

Treatment
T1 - Ben Globalfeed-AR clay Suspension 3.1 wt.%
T 2 - Cu-Ben Globalfeed-AR – Cu(II)-exchanged montmorillonite clay Suspension 3.1 wt.%
T 3 - Zeo Zeolite, natural clinoptilolite, Rota mining Suspension 3.1 wt.%
T4 - Nat Naturalis™, *Beauveria bassiana* 7.16 g (> 2.3 x 10^7 spores / mL) Coformulants up to 100 g
T 5 - Check (tap water treatment)

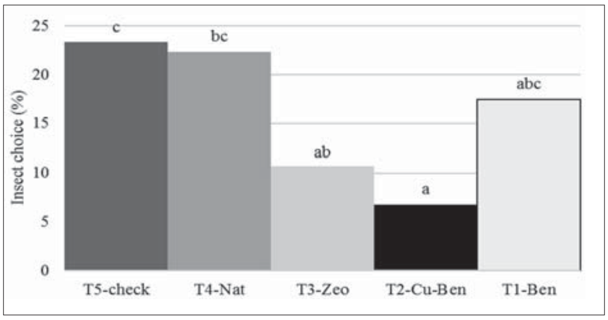

Fig. II – Insect choices registered at different intervals (Duncan test, P > 0.05). Different letters indicate significative differences.

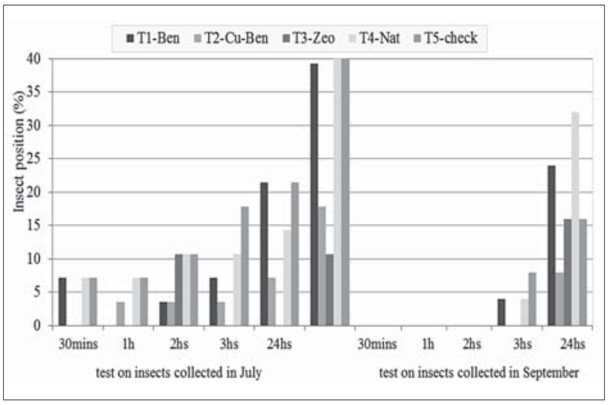

Fig. III – Different times of choice in summer and autumn *Aclees* adults.

RESULTS AND DISCUSSION

It is worth highlighting that the insects' choice is more stable after 24 hours from the start of the test: at this check time, we registered a consolidate selection for the plant/cage. During the laboratory trials, we observed that the insects' movement was affected by the treatment/thesis: they, in fact, differently colonized the five cages ($F_{1.5}$ =3.01; P=0.02). As shown in the graph, the formulations that the fig weevils chose less, were the Cu-Ben and Zeo samples (Fig. II). It is thus a first indication that these two solids could be active as masking substances on the host plants , likely confusing the insects in the search for them. The presence of a coating film of zeolite or metal-exchanged clay indeed proved to be able to modify the physico-chemical characteristics of the crop surface (in particular, the pH and the emission of volatile organics from fruits and leaves) and this may disorient the insect (LARENTZAKI *et al*., 2008; DE SMEDT *et al*., 2015; SHARMA *et al*., 2015). The insects, during their choosing activity, went directly to the fig plant and made a first contact with the leaves, drumming with antennas the surface. For the not selected thesis there was no contact with the plant. The slow release of Cu(II) soluble species, via leaching out of the clay structure, can additionally have a detrimental effect on the surface micro-organisms present on the target plant (WALTERS, 2006), thus causing a supplementary disorienting factor for the weevil.

The two different pools of insects, collected during summer or autumn, gave different results in terms of performance of their movements. The adults collected in summer were more mobile and made their choice amongst the cages more quickly (Fig. III). On the other hand, the adults collected in September showed the typical traits in approaching winter period.

With regard to the preliminary field trials, from a visual control on the whole plant and counting on the healthy and damaged fig sprouts, it was confirmed that the formulations containing the Cu(II)-modified montmorillonite clay, Cu-Ben, and the natural clinoptilolite zeolite, Zeo, showed good results, as they were less preferred. In fact, while the plants treated with the unmodified clay, Ben, and the check figs presented a high number of shoots with erosions (15 damaged sprouts out of 20 inspected for the Check, and 7 damaged out of 25 inspected for Ben), the plants of the two thesis treated with Cu-Ben and Zeo were healthy (no damaged sprouts).

As far as the preparation of the formulation is concerned, since the Zeo sample was used in its natural form, after a simple rinsing and suspension in water, without any further time-consuming ion-exchange procedures, it proved to be a viable and economically sustainable alternative approach for controlling the fig weevil. Moreover, the absence of added copper species in the Zeo formulation makes it more promising and environmentally sustainable than other currently available commercial products containing copper salts as active ingredients, whose use is being gradually reduced or, better, phased out, especially in organic and/or sustainable farming (CABÚS *et al*., 2017; KUEHNE *et al*., 2017).

CONCLUSIONS

Although the exotic weevil *Aclees* cf. sp. *foveatus* is not included in the list of quarantine species, the problems resulting from its infestations on the Italian production of the fig tree could be relevant. To date, its infestations are present in Tuscany, Latium and Ligury, but its population might reach Southern regions, where fig crops are an important economical resource. No products for the control of the insect populations have been registered so far. Furthermore, the ethology of the weevil, which spends the entire period of its preimaginal development inside the fig tree tissues, makes it very difficult to think of a possible use of the most common synthetic agrochemicals. Now, the new strategic approach presented in this study, testing innovative formulations that could have an impact on the selection of the target plant by the insect, by masking the chemical signals of the fig tree to the weevil, could be a successful strategy to solve the problem.

In this case, a Cu(II)-containing montmorillonite clay, Cu-Ben, and a clinoptilolite zeolite from mineral origin, Zeo, displayed the most promising results, both in laboratory-scale tests and in open-field trials. The application of the aqueous formulations containing one of these two solids onto the fig plants resulted in no evident attacks by *A.* cf. sp. *foveatus*. Furthermore, Zeo-based formulation was obtained via a simple and cheap preparation sequence, without any addition of copper(II) salt precursors. For these reasons, clinoptilolite zeolite Zeo, together with copper-exchanged clay, Cu-Ben, may be considered economically and environmentally sustainable innovative methods for controlling *Aclees* cf. sp. *foveatus*, as an alternative to other commercial insecticide products. Their efficacy can be attributed to a masking effect of the volatile organic compounds released by the host plant substances, thus affecting the insect's choice.

Even in the case of alien pests, an integrated and multidisciplinary approach to defence turns out to be the winning strategy and so, also the use of inorganic oxide zeolite- or phyllosilicates-based products could deserve further investigation, as an initial control strategy against alien insect species.

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