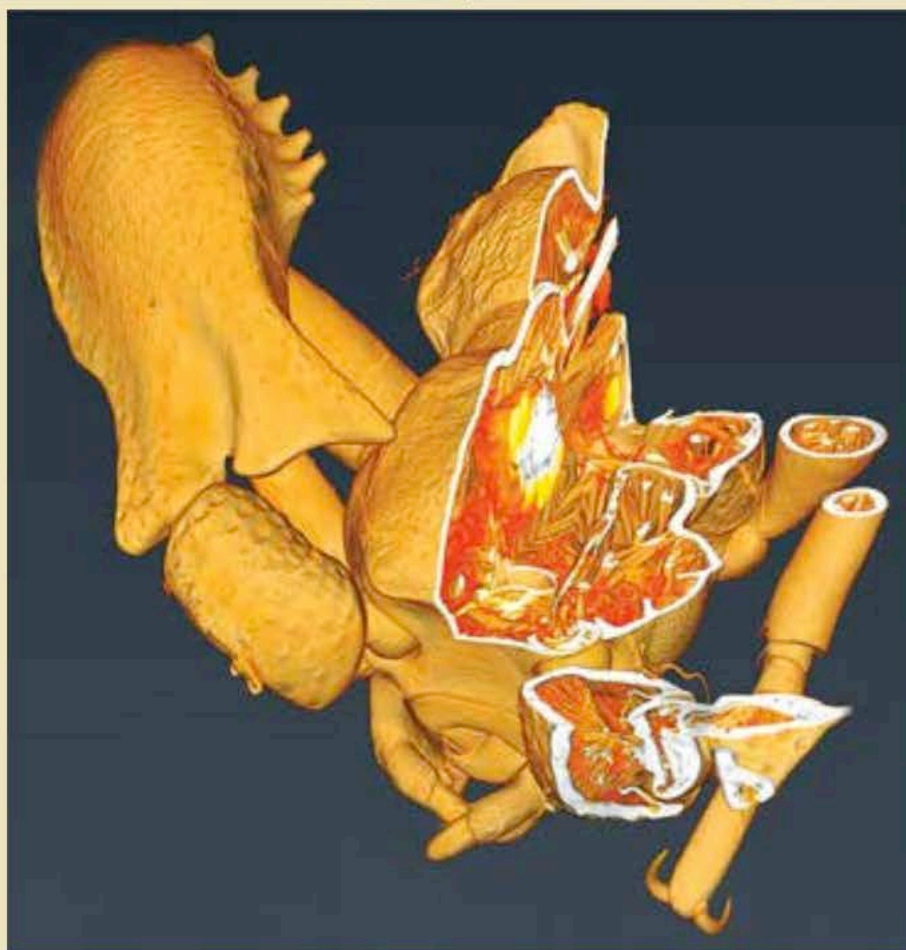


MONOGRAFIE

DELL'ASSOCIAZIONE ROMANA
DI ENTOMOLOGIA

1 - 2019



A.R.D.E. Fondata da Omero Castellani nel 1945

MARZIO ZAPPAROLI (*)

INSECTS AS FOOD AND FEED: A SOLUTION FOR FOOD EMERGENCY?

GLI INSETTI COME CIBO E MANGIME: UNA SOLUZIONE PER L'EMERGENZA ALIMENTARE?

Insects dominate terrestrial and freshwater habitats by species number and population size. They are fundamental in many ecosystem services, interacting with agricultural and forestry production and with human and domestic animal health. Apart from their use as models for the study of biological processes, some species play fundamental roles also by providing widely used products. Many species are also used as food. Insect consumption for food purposes has a very ancient history. Today, entomophagy is common in Central and South America, Africa, Asia, Australia. Coleoptera, Lepidoptera, Hymenoptera, Orthoptera, Isoptera, Odonata, Blattodea, Diptera and other arthropods are, in order, regularly used as food by two billion people in over 100 countries. Recently, entomophagy has caught the attention of the media, research institutes, legislators, food industries for the advantages it can offer in helping to solve the environmental problems that the growth of the world population and the increase in the demand for food are placing (Turillazzi & Giordana, 2015). According to the Food and Agriculture Organization of the United Nations (van Huis et al., 2013), the use of insects not only as food but also as feed in aquaculture and animal husbandry, could provide benefits for the environment, human health, economy and society.

According to the studies carried out so far, the conversion efficiency of insects is very high; the production of greenhouse gases (GHG) by enteric fermentation and ammonia is lower than in conventional livestock; breeding of insects has a low environmental impact also due to the lower consumption of soil. Insects can be fed with organic waste that can be transformed into quality proteins that can in turn be used for animal feed. Insects also use less water and provide proteins whose value is comparable to that of meat and fish. Many species also contain high amounts of fatty acids, fibers and micronutrients that can be used as food integrators. At the moment there are no known important consequences on human health following the consumption of insects. The collection and breeding of insects for food can provide entrepreneurial opportunities

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because they require a minimum investment. Collection, breeding, processing and sales can therefore be carried out by the weaker sections of the society. In addition to supplementing the diet, these activities can therefore also guarantee an income. On a vast scale, promising species for the production of feed that can supplement the traditional ones (soy, corn, cereals, fish meal), are *Hermetia illucens* (Linnaeus, 1758) (Diptera, Stratiomyidae), *Musca domestica* Linnaeus, 1758 (Diptera, Muscidae), *Tenebrio molitor* Linnaeus, 1758 (Coleoptera, Tenebrionidae). Other species are being investigated. In South Africa, the United States, China, Spain, there are specialized companies for the production of insects as feed in aquaculture and in the poultry industry. Insect breeding is technically feasible, but their production is now more expensive than conventional food and feed. There are still few companies that raise insects, but the initiatives to encourage their use feed & food are few. The breeding of insects takes place today mainly on a small scale and is aimed at niche markets and their production cannot compete with that of conventional food sources. However, if the costs for collection, production and transport also include the costs in terms of consumption of fossil fuels, water and GHG emissions, insects are a sustainable and economic alternative. Key elements for the growth of this sector are the improvement of techniques and the development of a regulatory framework that guarantees production and trade, while also guaranteeing nature conservation and preventing over-exploitation of species. Despite the advantages, in western countries one of the major obstacles to the use of insects as a source of protein is of a cultural nature. However, considering that eating habits can change rapidly, the food industry could play an important role in developing this alternative, guaranteeing quantity and quality of supply and developing suitable technologies. The use of insects as a source of protein could therefore be a valid solution to the problems posed by the food emergency but, in parallel, research and education must be encouraged, jurisprudence adapted, food safety and nature conservation guaranteed.

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FILIPPO MILANO (*) and MARCO ISAIA (*)

THE CONSERVATION OF SPIDERS IN ITALY
(Arachnida, Araneae)

LA CONSERVAZIONE DEI RAGNI IN ITALIA
(Arachnida, Araneae)

Despite their ecological importance and their diversity, spiders (Arachnida, Araneae) are still poorly considered in conservation policies and conservation biology, especially compared to other invertebrate groups. The existing international legislation on spider conservation is rather limited, with only one species listed in the Bern Convention and in the EU Habitat Directive, and 22 species listed in the Convention on International Trade in Endangered Species (CITES), none of which is naturally occurring in Europe (see Milano et al., 2017). In addition, the risk of extinction of 275 species has been assessed by the International Union of Conservation of Nature (IUCN), 66 of which occur in Europe (IUCN, 2019).

Among the 1670 species known to occur in Italy (Pantini & Isaia, 2019), one of the most species-rich countries in Europe for this group, only few ones are included in conservation acts. Here we provide a brief overview on spider conservation in Italy, with focus on the protected or red-listed species occurring in the country (Fig. 1).

Lacking a specific national law on spider conservation, there are only three species which are officially under protection in Italy, namely *Macrothele calpeiana* (Walckenaer, 1805), a species restricted to the southern Iberian Peninsula, but with isolated specimens recently reported far from their native range (e.g. Italy, Switzerland, Belgium and the Netherlands), and listed in the Bern Convention and therefore in the EU Habitat Directive; *Argyroneta aquatica* (Clerck, 1757), the only spider species known to conduct a wholly aquatic life, mentioned in the regional legislation of the Regione Lombardia; and *Dolomedes plantarius* (Clerck, 1757), closely associated with lowland wet habitats, and mentioned in the regional legislation of Lombardia as well, where it is listed among invertebrates of regional interest. It is interesting to note that the first one is an alien species and the last two are under the protection of regional – not national – legislation. None of the species listed in the CITES occur in Italy.

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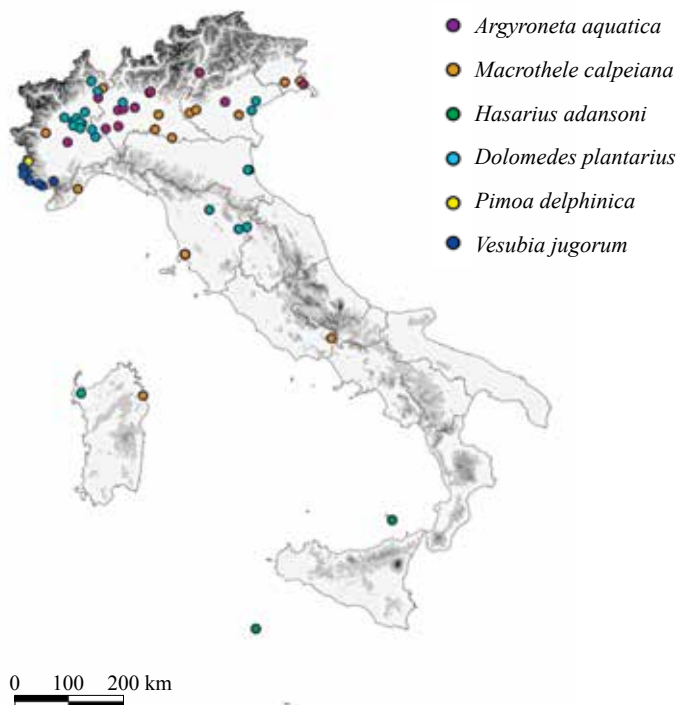


Fig. 1 – Distribution map of the species of conservation interest in Italy.

A first contribution to the conservation of the spiders in Italy was proposed by Groppali & Priano (1992), who listed 27 threatened spider species, with brief reference to their conservation status. Assessments were based on subjective criteria, with no reference to IUCN standards. More recently, in the framework of the Checklist of the Italian fauna (Ruffo & Stoch, 2007), the risk of extinction of 127 species belonging to the family Salticidae has been evaluated according to a simplified version of the IUCN criteria. At the regional level, the only Red List of threatened spiders available is that of the Province of Bolzano (Alto Adige, NE-Italy) (Noflatscher, 1994). This list sorts species among categories of threat, with reference to their main threats, biotopes and ecological requirements, without following the IUCN guidelines. Concerning the global IUCN Red List, only three species listed in it occur in Italy: *Dolomedes plantarius*, listed in the category Vulnerable, *Hasarius adansoni* (Audouin, 1826), Least Concern, and *Vesubia jugorum* (Simon, 1881), listed as Endangered. In addition, *Pimoa delphinica* Mammola, Hormiga & Isaia, 2016, is currently under assessment. Considering the peculiarity and the richness of the Italian spi-

der diversity, we conclude that efforts for conservation, even from the perspective of ongoing climate change, are far from being adequate. We point out the need to increase the efforts in defining the conservation status of the Italian spider species, in order to achieve baseline data towards realisation of a first Italian Red List based on IUCN criteria, which will become a primary tool for prioritising conservation actions and practical management, including role of research and monitoring, especially where data are lacking or inconclusive.

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DAVIDE DI GIUSEPPE (*), FRANCESCO BAINI (**), and MARZIO ZAPPAROLI (*)

EVALUATION OF FOREST SOILS BIOLOGICAL QUALITY SUBJECT
TO THE PASSAGE OF OFF-ROAD MOTORCYCLES

VALUTAZIONE DELLA QUALITÀ BIOLOGICA DEI SUOLI
FORESTALI INTERESSATI DAL PASSAGGIO DI MEZZI
MOTOCICLISTICI FUORISTRADA

Soil is one of the most important element for supporting life on Earth, Jeffery et al. (2010) and Menta (2012) highlight that processes that occur inside it, conduct ecosystemic functions which help to maintain life on the surface. Moreover, soil offers numerous services that start from food supply to water filtering. In the international strategies sphere that aim to conservation of biodiversity for migration of climate changes and for sustainable development, maintain soil quality is considerate a priority aim (De Groot, 2003). Antropic activities can negatively affect on soil quality since, beyond the impact on biogeochemical cycles and on population dynamics, can activate several degradation mechanisms, firstly for anything that concerns erosive and/or constipation processes (Piovesan & Zapparoli, 2018). It's believed that edaphic fauna is a useful indicator of soil quality because very sensitive to changes for administration of area, moreover, edaphic organisms are involved in several functions such as integration of soil phisic, chemical and microbiological characteristics and reflect general ecological changes Stork (1992). Purpose of this work is evaluate the effects that soil compaction cause on edaphic fauna, on terrain involved with the passage of off-road motorcycles, through QBS-ar index for analyse quantitative composition of microarthropod communities that create soil. This sample was made inside a training area for sport off-road competitions "Ceranesi Pro Park" in Genova. The material collected is represented by 22 taxa (Araneae, Pseudoscorpiones, Opiliones e Acari that belong to Subphylum Chelicerata; Isopoda that belongs to Subphylum Crustacea; other 17 groups belong to Subphylum Tracheata) that in the sampling of 2018 have contributed to detect the highest values of QBS-ar inside land involved in passage of motorcycle means, whereas in the sampling of 2019 the situation is the opposite, most probably to have analysed an area mainly affected by disturbance instead of the

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one made the previous year. Through a simple statistic elaboration of collected information has been evaluated the composition of quali-quantitative edaphic taxocenoses. Currently values of diversity and equitability identified involved areas by the widespread passage of competition motorcycles are often higher compared to the ones of natural areas indicating that the widespread and periodic passage of these means doesn't cause an actual compaction of soil.

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MORGAN AZZONI (*) and STEFANO VANIN (**)

THE INSECTS IN FORENSIC INVESTIGATIONS
(Insecta)

GLI INSETTI NELLE INDAGINI FORENSI
(Insecta)

The first evidence showing the relationship between insects and lifeless bodies dates back to a Mesopotamian seal of 5,000 years ago and the first evidence in the investigative field dates to the 13th century in China, in a murder case (Benecke, 2001). The relationship insect-corpse was already known in antiquity, reported in sacred texts and iconographies, and was differently interpreted according to culture. Forensic entomology proper originated at the end of 19th century with Mègnin, who in 1894 first codified the chronological sequence of cadaveric degradation caused by insect communities called “the workers of death”. Today, forensic entomology employs knowledge about arthropods in criminal investigations. In particular the temporal succession of necrophagous species on a body allows to ascertain with good approximation the time of death, especially in corpses that are in advanced state of decomposition. Furthermore, the estimation of the age of insects allows to establish with good precision the time of colonization of the corpse (minPMI: Minimum Post-Mortem Interval). The insects attracted by the volatiles released during decomposition of corpses are over 400 species, but only two orders play the main role in the process of decay, Diptera and Coleoptera (Reed, 1958). Forensic entomology can be divided in three main branches:

- urban (legal proceedings involving insects related to structures and human environment);
- store-products (insects that infest consumer goods);
- medico-legal (insect that colonized human bodies, mainly death but as well in cases of myases).

It is worth mentioning that the techniques and methods used in forensic entomology are also applied in the archaeological context. In this case the name of the discipline is funerary archeoentomology. It focus on the entomological findings from ancient remains and mummies and it is very useful to better un-

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derstand the funerary practices of the past. This discipline is the topic applied by the authors in a study of human remains from the skeletal collections of the Museum of Anthropology of the University of Bologna.

The authors are currently running an experimental study on carcasses of burnt piglets with accelerant (petrol) to simulate a corpse in a scene of fire or corpse concealment, and to understand if any accelerants may affect the life cycle of the associated insects.

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FRANCESCO CERINI (*), LUCA STELLATI (*) and LEONARDO VIGNOLI (*)

SEGREGATION STRUCTURE IN ODONATA ASSEMBLAGES
FOLLOWS THE LATITUDINAL GRADIENT

SCOPERTA DI UN GRADIENTE LATITUDINALE IN PATTERN DI
SEGREGAZIONE NELLE COMUNITÀ DI ODONATI

Latitude is known to deeply affect life with effects generalizable into ecological rules; the increasing species diversity toward tropics is the most paradigmatic (Pontarp et al., 2018). Several hypotheses tested patterns of biotic interactions' intensity along latitude. Negative interactions (i.e. competition and predation) are expected to be among the processes that produce checkerboard distribution of species (Presley et al., 2010). However, no relationship between checkerboard distribution and latitude has been uncovered. We tested Odonata assemblages worldwide for co-occurrence patterns by using a faunistic dataset (447 species arranged in 695 natural communities) spanning a wide latitudinal range (87°). We used co-occurrence analyses (C-score index and Standardized Effect Size) as an estimate of checkerboardness (Gotelli, 2000) then correlated the occurrence of segregation to latitude. Odonata followed the Latitudinal Diversity Gradient at the regional scale (i.e. country scale) within our analyzed assemblages spanning, whereas local richness (i.e. community scale) did not follow the same pattern. Odonata assemblages structured in with segregation followed a latitudinal gradient with a higher occurrence towards the tropics and were not influenced by local species richness. Despite our ability to mechanistically interpret species segregation is limited by the lack of theory to discriminate among the several factors potentially involved in producing similar

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FRANCESCO SIMONE MENSA (*), MAURIZIO MUZZI (*), FEDERICA SPANI (*)
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THE USE OF THREE-DIMENSIONAL MODELS FOR THE STUDY
OF PHENOTYPIC PLASTICITY AND FUNCTIONAL ANATOMY OF
BEETLES OF THE GENUS *PAUSSUS*
(Coleoptera, Carabidae, Paussinae)

UTILIZZO DI MODELLI TRIDIMENSIONALI PER LO STUDIO DELLA
PLASTICITÀ FENOTIPICA E DELL'ANATOMIA FUNZIONALE DEI
COLEOTTERI DEL GENERE *PAUSSUS*
(Coleoptera, Carabidae, Paussinae)

The genus *Paussus* Linnaeus, 1775 is a specialized, charismatic group of ground beetles (Carabidae) classified in the subfamily Paussinae. All species of *Paussus* are obligate myrmecophiles (associates of ants) (Figs 1–3). In this symbiosis the beetles provide the host ants with a rewarding chemical secretion and in exchange they receive a safe place for the development of their delicate larvae and a dependable source of high-protein food: that is ants and in particular the brood (Robertson & Moore, 2017). For this reason they are considered as “social parasites” of ants.

As with many other myrmecophilous or termitophilous beetles, *Paussus* have undergone phenotypic adaptations for life with ants, at the level of head, antennae, and prothorax. Most notably, the antennae of *Paussus* are strongly modified; all nine joints of the antennal flagellum are fused into a single piece, the ‘antennal club’. The basis for this astounding diversity of form remains unknown, but it is at least partially connected to (1) the amplified glandular function of the antennae (Di Giulio et al., 2009) in producing attracting chemicals for the ants, and (2) to different ways to spread, offer and store the antennal exudates (Di Giulio et al., 2012). Investigating anatomical structures have been fundamental to better understand living organisms, and their interplay with the surrounding environment, which could induce significant morphological variation. In the last few years, bio-imaging techniques paired with geometric morphometrics (GM) overcame the limits of traditional anatomical studies, becoming widely non-invasive and highly informative for both internal and external characters. The use of Computed Tomography (CT) scanners definitively allowed to advance in the knowledge of either known or neglected biological structures.

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Fig. 1 – *Paussus favieri* from Morocco (High Atlas Mountains, near Tizi-n-Test pass, May 2015) with its host, *Pheidole pallidula* (Nylander, 1849) (Photo: A. Di Giulio).



Fig. 2-3 – *Paussus howa* C.A. Dohrn, 1881 from Madagascar (Photo: F. S. Mensa).

For this project, we used X-ray micro-computed tomography (Micro-CT), in order to acquire 2D serial, cross-sections of various paussines samples, with a resolution below the micrometer.

CT is a three-dimensional (3D) X-ray imaging method for obtaining X-ray projection images at many angles of view around an axis through an object. Then, by applying a tomographic reconstruction algorithm is possible to obtain a stack of thin tomographic images of the object (Ritman, 2011).

This 2D images are then processed first with ImageJ for enhancing the contrast, reducing the noise, resizing, as well as for removing the slices without any useful information. After this process, we used Thermo Scientific™ Avizo™ Software for the reconstruction of 3D models.

With these models, we will be able to conduct a morphological study of the most variable parts in the body of the genus *Paussus* using 3D geometric morphometrics (3D GM). These integrative techniques allow to describe in a quantitative way even subtle differences between structures, using the Cartesian coordinates of anatomical landmarks, after the effects of non-shape variation



Fig. 4 – External and internal view of the head of *Paussus kannegieteri* Wasmann, 1896 from India (Photo: M. Muzzi).

have been mathematically held constant (Adams et al., 2013). With this technique we hope to be able to determine whether the striking diversity of phenotypes is caused by the host or by other factors, overlapping the results obtained with the molecular part of phylogeny.

We will perform a histological analysis of the head and its appendages in order to study the relationship between the muscles and mandibles, maxillary palps and labial palps. For the antennae and their muscles, we will try to determine the differences between non-myrmecophiles and myrmecophiles paussini from the point of view of antennal musculature. All of this will be done by using also the internal part of the 3D models (Fig. 4), in conjunction with histological methods.

These innovative practices help to deepen the meaning of shape in insect biology, from both structural and evolutionary views. They will allow to describe the relationship between phylogeny and functional morphology in the extremely variable species of the subfamily Paussinae.

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ETTORE PALUSCI (*), CRISTINA MANTONI (*) and SIMONE FATTORINI (*)

RESPONSES OF DUNG BEETLES TO FIRE DISTURBANCE
IN PASTURED AND FORESTED HABITATS IN
THE CENTRAL APENNINES (ITALY)
(Coleoptera, Scarabaeoidea)

RISPOSTA DEGLI SCARABEOIDEI COPROFAGI AL DISTURBO
DEL FUOCO IN HABITAT PRATIVI E FORESTALI
NEGLI APPENNINI CENTRALI (ITALIA)
(Coleoptera, Scarabaeoidea)

Fire represents an important factor of disturbance in Mediterranean ecosystems, especially at low elevations (Fattorini, 2010). In general, Mediterranean mountain ecosystems are less frequently and intensely affected by fire, but are profoundly altered by grazing. In Italy, anthropogenic pastures have replaced natural forests in most parts of Central Apennines. Although fire is a rare event in mountain areas, yet it represents here an important additional source of disturbance (López-Poma et al., 2014). However, the impacts of fire on the insect communities of these environments remain largely unknown.

Our research was aimed at investigating changes in dung beetle communities induced by fire in a high altitude pastured area in the Abruzzi region (Gran Sasso massif) at about 1500 m a.s.l. (Fig. 1). The study area was burnt by a fire that destroyed more than 300 ha of vegetation in August 2017. Dung beetles were collected one year after the fire by using pitfall traps (Fig. 2). Traps were baited with cattle dung and active for 48 hours each month (Da Silva & Medina-Hernández, 2015) from June to September 2018. Sampling was done in each of the three main vegetation types occurring in the study area: a pine-wood, a beechwood and a prairie resulting from grazing. For each habitat, we selected a burnt and an unburnt site, and in each site we placed three traps. We collected a total of 8510 dung beetles, belonging to 28 species. Namely, we collected 84 individuals in the beechwood, 279 individuals in the pinewood, and 8147 individuals in the prairie. We considered the following community structure parameters: total abundance, richness (Chao 1 non parametric estimator), diversity (Shannon-Wiener index), dominance (Simpson index), and equilib-

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Fig. 1 – Study area (Photo: C. Mantoni).

ity (Pielou index). Differences between vegetation type and the effect of fire were investigated using Analysis of Variance.

Dung beetles were more abundant in the prairie than in the two forest habitats. The proportion of captured individuals was similar in burnt and unburnt sites for the two forest habitats, whereas in the prairie we collected more individuals in the unburnt site (Fig 3). However, communities of burnt and unburnt sites did not differ in terms of richness, dominance and equitability. Shannon-Wiener diversity was higher in the burnt sites.

Our results indicate that fire may enhance dung beetle diversity, probably by converting forest habitats (which are not favoured by most dung beetles) into open habitats (which are more favoured). Because livestock rarely enters the forests, dung is rare there. By contrast, dung is abundant in the prairies (Allred et al., 2011), which are also more accessible to dung beetles (Smith et al., 2019), and can therefore support denser dung beetle populations. Because fire transforms woodlands into open habitats, burnt forests tend to resemble prairies and hence tend to become more favourable to dung beetles. Thus, our results indicate that, paradoxically, fire disturbance increase the abundance and diversity of high altitude communities of dung beetle.

Interestingly, we found that the pinewood was the habitat with the lowest diversity. This finding suggests that use of pines for reforestation might have negative impacts on dung beetles.



Fig. 2 – Type of pitfall traps used in this research. The trap is set into the ground (a), and covered with a grid (b); then the trap is filled with salt water and (c) a fixed amount of dung is placed on the grid (Photo: C. Mantoni).

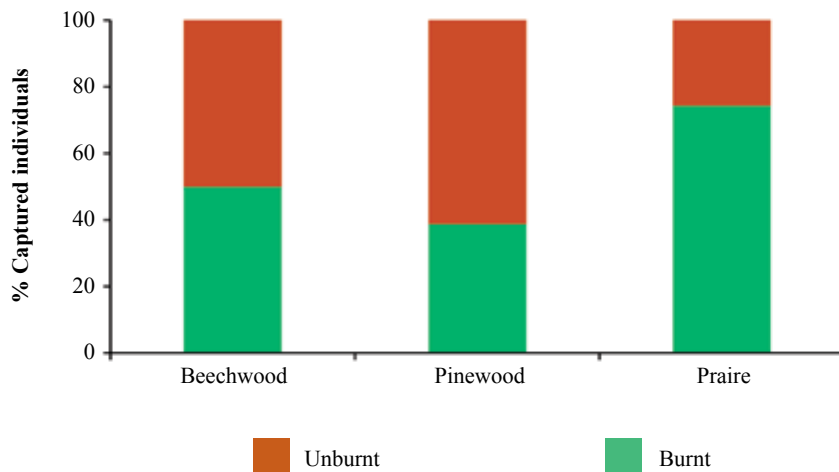


Fig. 3 – Abundance of dung beetles in burnt and unburnt sites for the three habitats.

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EDOARDO PULVIRENTI (*) and GIUSEPPE PLATIA (**)

UPDATED CATALOGUE OF CLICK-BEETLES OF ITALY
(Coleoptera, Elateridae)

CATALOGO AGGIORNATO DEGLI ELATERIDI D'ITALIA
(Coleoptera, Elateridae)

This work will contain an updated checklist and the distribution of the Elateridae of Italy. The data used in this work comes from literature, museums and private collections directly examined by the authors; however this work will not include the subfamily Lissomidae Laporte, 1835 and the Cebrionini Latreille, 1802 and Drilini Blanchard, 1845 tribes, previously considered independent families, because there aren't sufficient data. Since the publication of the Checklist of the Italian Fauna (Platia, 2005), 19 species have to be added to the list, 3 of them being allochthonous, as many of them were not described yet. So this work aims to be a summary of the actual situation of Elateridae in Italy. In Italy there are 253 species, and 42 of them are endemic while 9 of them are exclusive to Sicily and Sardinia.

The distribution in Italy will be reported at provincial level, while the world distribution will be at state level. In the end there will be a list of not yet certain, but probably present species on the Italian territory, one of wrongly reported species in the past, and various lists about synonyms of every species.

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Fig. 1 – One of the maps used in the work. The maps represent the provincial distribution of the species (in this case *Agrypnus murinus* (Linnaeus, 1758)). The numbers on the map are referenced to the provinces' acronyms and serves to recognize them.

ALESSANDRA RICCIERI (*), EMILIANO MANCINI (**), DANIELE SALVI (***)
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EVOLUTIONARY HISTORY OF *HYCLEUS*: PHYLOGENY AND
BIOGEOGRAPHY OF A HYPERDIVERSE GENUS
(Coleoptera, Meloidae)

STORIA EVOLUTIVA DI *HYCLEUS*: FILOGENESI E BIOGEOGRAFIA
DI UN GENERE IPERDIVERSO
(Coleoptera, Meloidae)

Hycleus Latreille, 1817 is a hyper-diverse genus of blister beetles including ~ 430 species widely distributed in the Old World, and with the highest diversity occurring in the Afrotropical Region (Bologna & Pinto, 2002). The phylogenetic relationships among the species and the biogeographic processes related to their diversification have never been investigated, but members of this genus were divided into three “sections” according to the mesosternal morphology (Pardo Alcaide, 1954, 1955), and some species groups were described according to additional morphological characters. Previous molecular studies (Bologna et al., 2008; Salvi et al., 2019) pointed out the close relation among *Hycleus* and both genera *Ceroctis* Marseul, 1870 (59 species) and *Paractenodia* Péringuey, 1904 (5 species), forming a complex of ca. 500 species.

In this study, we provide a first time-calibrated phylogenetic tree of *Hycleus*, using mitochondrial and nuclear DNA obtained from 125 species, in the attempt to study the phylogenetic relationships among the species of this mega-diverse taxon. Phylogenetic results were subsequently used for biogeographic inference carried out with the R package BioGeoBEARS (Matzke, 2013a, 2013b). Our results showed that the three *Hycleus* “sections” are polyphyletic and do not have a taxonomic value, whereas some of the groups morphologically described were confirmed. Furthermore, the monophyly of the complex *Hycleus-Ceroctis-Paractenodia* was verified, suggesting that a new taxonomic arrangement of these taxa is required. Moreover, the biogeographic results showed that the genus *Hycleus* likely originated in the Afrotropical Region in

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the Miocene (~20 Mya), and subsequently spread in the Saharo-Sindian Transitional Region, in the Palearctic Region and in the Oriental Region following different dispersal events through the Arabian Peninsula.

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DNA BARCODING OF *LONGITARSUS*: IDENTIFICATION EFFICIENCY IN
A TAXONOMICALLY COMPLEX GROUP
(Coleoptera, Chrysomelidae, Galerucinae, Alticini)

DNA BARCODING DI *LONGITARSUS*: EFFICIENZA DI IDENTIFICAZIONE
IN UN GRUPPO TASSONOMICAMENTE COMPLESSO
(Coleoptera, Chrysomelidae, Galerucinae, Alticini)

The use of molecular methods for the identification of species, such as DNA barcoding and species delimitation, has increased over the last few years (Goldstein & DeSalle, 2019). The large number of studies that used these methods to classify biodiversity has led to the increase of molecular data uploaded on large online databases such as GenBank and BOLD. However, this huge amount of sequence data, is often submitted to public repositories without an adequate quality control carried out by expert taxonomists. Moreover, there is no uniformity of the metadata associated to these sequences. This leads to the presence of identification errors that make molecular tools inaccurate.

In this work, we wanted to evaluate the quality of the molecular data set present on GenBank and BOLD of the leaf beetles of the genus *Longitarsus* Berthold, 1827 a taxonomic group that requires a high level of expertise for the species recognition based on morphological features.

Longitarsus is a very biodiverse and taxonomically complex genus with more than 700 species distributed in all zoogeographical regions (Salvi et al., 2019).

The aims of this study are: (i) to assess the quality of GenBank and BOLD molecular datasets for the genus *Longitarsus* using DNA barcoding gap analysis, clustering methods, and taxonomic validation; (ii) to identify and flag ambiguous and incorrect sequences; (iii) to describe a pipeline for a posteriori validation and rectification of BOLD data by taxonomist specialised in specific groups.

We produced 117 sequences of the standard barcode marker for animals, the mitochondrial *cox1* gene (658 bp). We analyse a final dataset of 1502 se-

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quences of 83 species of *Longitarsus* using the R library *spider* v1.5.0 (Brown et al., 2012). We identify 820 ambiguous and incorrect cases according to a barcoding gap analysis (Meyer & Paulay, 2005) and by comparing the *maximum intraspecific distance* and the *minimum interspecific distance* for each sequence. In order to confirm the results obtained from the barcoding gap analyses, a distance-based neighbour-joining tree was inferred using MEGA7 using the Kimura's two-parameter model of nucleotide substitution (1980), and 1000 bootstrap replicates. Once ambiguities and errors were identified, we optimized the threshold value for distance-based identifications with *localMinima* function of the R library *spider* v1.5.0 and assess whether the optimised threshold allowed improving the molecular identifications capacity of the dataset. This function is based on the concept of the barcoding gap, where a dip in the density of genetic distances indicates the transition between intra- and interspecific distances. The efficiency of molecular identification, before and after the threshold optimization, was estimated performing Best Close Match analyses (Meier et al., 2006) which compares each sequence of dataset with the others included in it and checks if the best matches (i.e., pairs of sequences with the lowest values of nucleotide distance) are between sequences of organisms morphologically identified as the same species. Process of threshold optimization led to a minimum improvement of molecular identification (from 1207 to 1214 correct identification).

A taxonomic review of all critical cases was carried out, such as polyphyletic species or multi-species clades, and this allowed finding errors of identification or taxonomy that have not yet been resolved. Nevertheless, in several cases the lack of morphological data did not allow identifying (and correcting) the source of error of incorrect or ambiguous sequences. To understand if the most complicated cases, such as the *pratensis* group or the *lycopi* group, are linked to recognition errors or to a lack of resolution of the molecular tool, it would be necessary the revision of all the material, where present, from which the DNA was extracted. This operation would take a long time, besides the fact that in very small specimens, during DNA extraction, the entire sample is sacrificed. In this study we tested a DNA extraction method that allows us to recover the entire sample when the lysis process was completed, and to reassemble it on an entomological card point, in order to preserve a reference sample of the sequence produced. In addition to preserving the reference sample, when the sequence are loaded on the online databases, in particular on BOLD which allows to associate metadata of the sample, it would be necessary to upload photos in which all the morphological characters useful for the identification of the species are present.

The quality of metadata and the maintenance of a reference collection would make a molecular tool, such as the DNA barcoding, more reliable and effective for species identification especially for taxonomically complex groups.

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GIULIA SCARPARO (*), FRANCESCA CASALE (*), MAURIZIO MUZZI (*)
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DESCRIPTION AND COMPARISON OF EXTERNAL MORPHOLOGY
AND ANATOMY OF FOUR EUROPEAN SPECIES OF *MICRODON*
GENUS: FROM THE EGG TO THE PUPA
(Diptera, Syrphidae, Microdontinae)

DESCRIZIONE E CONFRONTO DELLA MORFOLOGIA ESTERNA
E ANATOMIA DI QUATTRO SPECIE EUROPEE DEL GENERE
MICRODON: DALL'UOVO ALLA PUPA
(Diptera, Syrphidae, Microdontinae)

Social insects (wasps, bees, ants and termites) are among the most successful organisms which have evolved a multitude of adaptations to colonize almost entirely the terrestrial world. Among these, ants (Hymenoptera; Formicidae) are the best fitting example of eusocial organisms. All the about 13,000 species, to now known, (Lebas et al., 2016) are eusocial, and the ability to build within a limited time huge colony has allowed them to become the dominant taxa on the Earth in terms of biomass and colonized environments. Despite ants are very aggressive against the intruders, numerous inquilines live upon the colony, exploiting the resources of the nest.

These so-called “myrmecophiles”, obligate symbionts of ants at least in one stage of their life cycle, represent an extremely diverse assortment of taxa (Wilson, 1971), chiefly arthropods, that in parallel use very complex strategies to avoid the ant defences and coexist with their hosts. Most myrmecophiles are commensals or mutualists, but about 10,000 species (Thomas et al., 2005) are social parasites and inhabit principally the brood chambers where they feed upon immature ant instars (Akino et al., 1999) or trick the ant workers in order to be fed by trophallaxis (Cammaerts, 1995). One of the most striking examples of these parasites are the larvae of *Microdon* Meigen, 1803 hoverflies which are able to feed undisturbed upon the ant broods (Fig. 1). The study of these myrmecophiles is challenging in that they are rare, extremely localized in small patches of wetland areas, increasingly degraded and endangered, and live in concealed environments (the ant nests). To improve the knowledge about the scarcely known immature stages of *Microdon*, we analysed their functional morphology and anatomy, integrating optical and fluorescence microscopy

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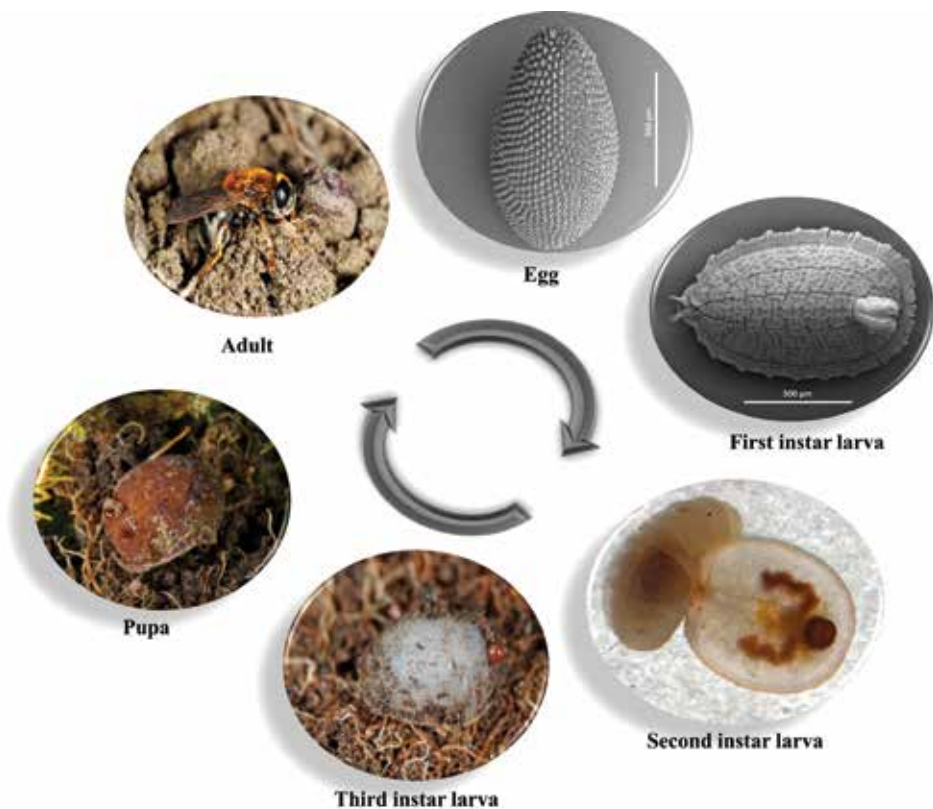


Fig. 1 – Lifecycle of *Microdon* spp. In early spring the free-living adults lay the eggs near the entrance of the ant host nest. First, second and third instar larvae remain inside the nest where they grow, feeding on brood ant, till they are ready to pupate near the surface of the ant nest. The adults emerge in spring from puparia, probably in the early hours of the days when the ants are less active and live for a few days without feeding (Photos: A. Di Giulio and G. Scarparo).

with focused ion beam-scanning electron microscopy (FIB/SEM) and synchrotron radiation micro computed tomography. We present here a detailed, comparative description of the different immature stages of four European *Microdon* species: *M. analis* (Macquart, 1842), *M. devius* (Linnaeus, 1761), *M. mutabilis* (Linnaeus, 1758) and *M. myrmicae* Schönrogge, Barr, Wardlaw, Napper, Gardner, Breen, Elmes & Thomas, 2002. With SEM microscopy, we illustrate differences and similarities between the analysed species. Furthermore, we present new diagnostic characters to distinguish *M. myrmicae* from its cryptic species *M. mutabilis*. During their development, *Microdon* immature stages face

huge structural changes showing a deep larval polymorphism. *Microdon* larvae develop a protective structure, characterized by a thick and multi-layered cuticle, retractile head, dome-shaped tergum and a flat and strongly adhesive “foot” (sternum). Furthermore, larval body shows numerous peculiar structures such as the marginal band, the dorsal reticulation and the “flower-like” sensilla. According to recent analyses, these sensilla seem to be mechanoreceptors, driving the larva inside the dark complexity of the ant nests. The study of these social parasites by integrating different techniques could help to clarify the evolution of this successful parasitic strategy, as well as their uncertain taxonomy, caused by the presence of numerous cryptic species, and to better understand their still partially obscure life cycle.

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SILVIA GISONDI (*), THOMAS PAPE (**), and PIERFILIPPO CERRETTI (*)

PHYLOGENY OF RHINOPHORIDAE AND POLLENIIDAE –
TOWARD THE EVOLUTION OF OESTROIDEA
(Diptera)

FILOGENESI DI RHINOPHORIDAE E POLLENIIDAE –
VERSO L'EVOLUZIONE DEGLI OESTROIDEA
(Diptera)

Calyptrates are a megadiverse, actively radiating group of dipterans, including the oestroid clade. Recent attempts at resolving the oestroid phylogeny (Kutty et al., 2019; Wiegmann et al., 2011; Winkler et al., 2015) employing few taxa seem converging in retrieving monophyly for most of the families and subfamilies, but deep relationships among these still have very low support. We mainly focused on two very interesting and relatively unexplored families of parasitoid flies: Rhinophoridae and Polleniidae.

Rhinophorids are the only insects exploiting crustaceans (Crustacea, Isopoda, Oniscidea) as hosts and their adult stages are difficult to separate from other oestroids, due to the lack of autapomorphies (Bedding, 1973).

Polleniids have traditionally been considered a subfamily of the Calliphoridae, but there is growing evidence supporting the view that this clade has evolved separately from “core” calliphorids and is the extant sister taxon to the Tachinidae (Cerretti et al., 2019).

A careful taxon sampling is employed for the first time in the current project: a good number of worldwide distributed samples are already present and stored in alcohol thanks to fieldwork (hand collecting and Malaise traps) and help from other research groups. Both Sanger sequencing using three informative nuclear markers (28S, CAD, MCS) and an anchored hybridisation approach will be employed together in order to shed light on the generic phylogeny of Rhinophoridae and Polleniidae. These molecular results will be analysed and compared with morphological data in a total evidence analysis and included in a more general context of calyptrate phylogeny.

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ALICE LENZI (*), ANDREA DI GIULIO (**) and PIERFILIPPO CERRETTI (*)

TACHINIDS FROM WEBSPINNERS, A CURIOUS
TROPHIC BEHAVIOUR
(Embioptera; Diptera, Tachinidae)

TACHINIDI DA EMBIOTTERI, UN CURIOSO
COMPORAMENTO TROFICO
(Embioptera; Diptera, Tachinidae)

Tachinidae (Fig. 1), a cosmopolite family of 8500 described species, are showy flies of different size and colours, parasitoids of 15 groups of arthropods (Cerretti, 2010). They are the focus of many systematic, evolutionary and applicative researches.

Embioptera (Fig. 2), commonly called webspinners, a small order of polyneopterans with 400 described species, are small and not really evident insects; they live in the soil and under bark in silk tunnels. Due to their biology and ephemeral life they are uncommon to see in nature, poorly investigated and their systematic is just now being solved (Miller, 2012; Edgerly, 2018).

Fifty years ago, twenty-two tachinid flies emerged from the big breeding of webspinners studied and collected in many different countries by the entomologist Edward Ross. Nowadays only three species are described in literature being parasitoids of webspinners (*Perumyia embiaphaga* Arnaud, 1963, *Rossimylops whiteheadi* Mesnil, 1953 and *R. exquisitus* Richter, 2001), so that these flies represent an important evidence of tachinid different trophic strategies. We analysed the material and we identified seven new species of three different tribes. We provide an official description of one new genus and seven new species, with a focus on *Rossimylops* Mesnil, 1953 making a revision of this genus (starting from Cerretti et al., 2009); we reviewed the biology and hosts of the tachinids developing on webspinners considering this host association as character evolved independently in family Tachinidae.

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Fig. 1 – Female of a new species of the genus *Perumyia* (Photo: A. Lenzi).



Fig. 2 – Webspinners (*Haploembia* sp.) in their nest under a stone. Monti della Tolfa, Lazio, Italy (Photo: L. Lenzini).

GIULIA BELLANTI (*), SILVIA GISONDI (*), MAURIZIO MEI (*),
ANDREA DI GIULIO (**) and PIERFILIPPO CERRETTI (*)

PELAMERA ATRA (RONDANI, 1861): A RARE PARASITOID FLY
OF THE EUROPEAN FAUNA
(Diptera, Tachinidae)

PELAMERA ATRA (RONDANI, 1861): UN RARO DITTERO
PARASSITOIDE DELLA FAUNA EUROPEA
(Diptera, Tachinidae)

Tachinidae, also known as ‘bristle flies’, is an extremely interesting family of oestroid dipterans, characterized by a peculiar biology: all the species in the family are in fact parasitoids on arthropods (Cerretti, 2010).

Due to their flashy appearance and great diversity, Tachinidae are relatively well studied and their phylogeny is rapidly improving thanks to many ongoing studies (see: Stireman et al., 2019).

Among bristle flies genera, one of the most poorly known monospecific genus is *Pelamera* Herting, 1969. Its type species is *Pelamera atra* (Rondani, 1861), originally described from Italy as *Myobia atra* and subsequently re-described and assigned to the genus *Pelamera* by Herting (1969). Based on the only four known females, the species was morphologically assigned to the tribe Brachymerini of the Tachininae subfamily.

From 1969, three more female specimens have been recorded (Spain and Switzerland in 1999, and Greece in 2003) but no male specimen was yet collected. The first male specimen was eventually found in Italy in 2017, making new morphological data and taxonomical perspectives available.

In this context, we made a description of the male complete with digital and SEM pictures, and drawings. Two nuclear protein-coding genes, CAD and MCS, were then sequenced and all these data were used to phylogenetically place the genus inside the world Tachinidae phylogeny (Stireman, 2019). So far, data seem converging in retrieving *Pelamera* in the Tachininae subfamily, as previously proposed, though inside a clade comprising Nemoraeni + Germariini, quite distant from the Brachymerini.

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Getting information about the biology of this poorly known genus would be of great importance for future research in this field.

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LORENZO GOGLIA (*) and MARCO COLACCI (*)

CONTRIBUTION TO THE KNOWLEDGE
OF THE TORTRICIDAE FAUNA OF SILA NATIONAL PARK
(Lepidoptera)

CONTRIBUTO ALLE CONOSCENZE DELLA FAUNA
A TORTRICIDAE NEL PARCO NAZIONALE DELLA SILA
(Lepidoptera)

The research reports a Lepidoptera Tortricidae list as updated and complete as possible for the Sila National Park (southern Italy, Calabria). For this purpose, in addition to specimens collected from 2013 to 2018, we also included species found by professor P. Trematerra (University of Molise, Italy) in the course of previous entomological researches done in that area.

The tortricids from southern Italy have received sporadic attention by both Italian and foreign entomologists. In particular, for the Calabrian territory there are only few data related to the southern slope of Pollino Massif and a chestnut area of Sila Mountains (Trematerra et al., 2018).

The localities visited by us were in the central part of the Calabrian territory, in the area of Sila National Park (Figs 1a–1b). This is a great upland above 1000 m of altitude; the highest peaks are Mount Botte Donato (1928 m/a.s.l.), in the Sila Grande, and Mount Gariglione (1764 m/a.s.l.) in the Sila Piccola. The National Park mainly made up of granite rocks and gneiss.

The climate is temperate-cold. The forests of Mount Sila are particularly sensitive to climate change. From the floristic point of view, lower altitudes are dominated by deciduous forests, whereas over 1100 m/a.s.l. dominate the black pine. The woodland formations are alternated by large grazing land of mainly secondary origin.

Our specimens were mostly caught by light at night-time and by butterfly net during day-time (Fig. 1c). The material was identified morphologically according to Razowski (2002, 2003); 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).

Before our contribution, there were 60 known species in the Sila Nation-

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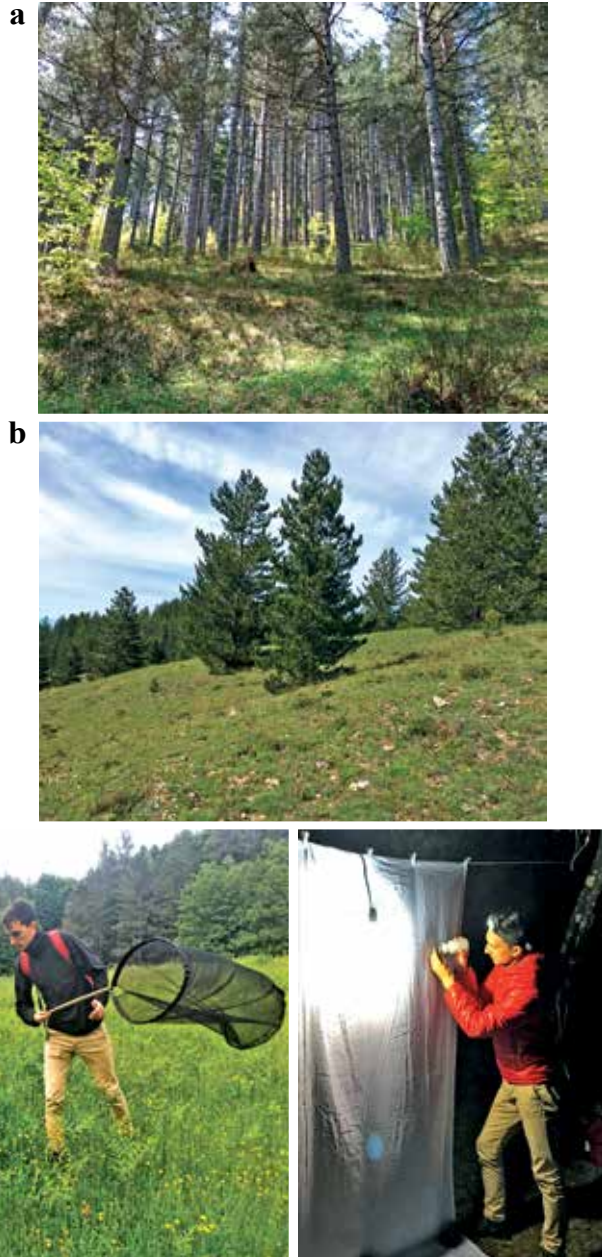


Fig. 1– Habitats visited during Entomological expeditions in Sila National Park (southern Italy) (a–b) (Photo: S. Scalercio); collection methods of the samples (c) (Photo: L. Goglia).

al Park. During our entomological expeditions 62 species were captured, of which 25 were new reports. With our data, the Lepidoptera Tortricidae known for the Sila National Park increased to 85 taxa (Trematerra et al., 2018).

Species belonging to all tribes cited in the Italian fauna have been found in the area (Trematerra, 2003), with the exception of the members of the Sparganothini tribe (subfamily Tortricinae), the most diverse subfamily is Olethreutinae with 57 species, followed by Tortricinae with 27 species, and the Chlidanotinae with 1 species.

A biogeographic study of Tortricidae reported for Sila National Park, using chorological analysis, was carried out. This study shows that the taxa with a Cosmopolitan (1.18%) or a Subcosmopolitan (1.18%) distribution are few; more than 57% of the taxa have a wider Palaearctic distribution. On the other hand, much fewer have a European (21.18%) and Mediterranean (4.71%) distribution. Of particular interest is the presence of *Cochylimorpha scalerciana* Trematerra, endemic species of the Sila National Park (Trematerra, 2019).

A limited number of specimens belonging to taxa, potentially harmful to agricultural and forest plants were recorded in Sila National Park, this shows that these areas are in good health and maintain a high degree of naturalness. However, the absence of tortricid pests could also be due to the particular natural habitats which are visited during the entomological expeditions.

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FLAMINIA MARIANI (*), ANDREA DI GIULIO (*) and SIMONA CESCHIN (*)

EXPERIMENTAL EVIDENCE OF THE CONSUMPTION OF THE
INVASIVE ALIEN *LEMNA MINUTA* (DUCKWEED) BY THE
HERBIVORE *CATACLYSTA LEMNATA*. ARE WE FACING A
POTENTIAL BIOCONTROLLER?
(Lepidoptera, Crambidae)

EVIDENZE SPERIMENTALI DEL CONSUMO DELL'ESOTICA
INVASIVA *LEMNA MINUTA* (LENTICCHIA D'ACQUA) DA PARTE
DELL'ERBIVORO *CATACLYSTA LEMNATA*. CI TROVIAMO DI
FRONTE A UN POTENZIALE BIOCONTROLORE?
(Lepidoptera, Crambidae)

Alien plants can become invasive due to a lack of their natural predators and competitors. One worrying example is the American duckweed *Lemna minuta* Kunth (Araceae) that, since its arrival in the 1940s, has rapidly and widely spread in Europe, becoming highly invasive in many countries (Ceschin et al., 2018).

The high growth rate allows *L. minuta* to rapidly colonize large water surfaces, resulting in floating multilayer mats that limit both light penetration in the underlying water column and gaseous exchanges in the air-water interface, creating highly limiting conditions for the survival of the aquatic flora and fauna. Moreover, *L. minuta* proves to be a strong competitor against native plant species that occupy similar habitat, such as the congeneric *L. minor*, which is partially or completely replacing. Controlling the growth of *L. minuta* has become a pressing issue and, since the using of chemical and physical methods for its removal are as risky for the environment health as only partially effective, it is necessary to explore the possibility of adopting some form of biological control. The Classical Biological Control (CBC), based on the introduction of natural enemies co-evolved with the alien species and coming from the same their home range, has been already successfully applied to some aquatic alien invasive plants (Gassman et al., 2006), but as regards *L. minuta*, cases of CBC and natural enemies of this species in its native home range are unknown.

The few existing studies differ from CBC and were performed testing the most common natural enemies of the native duckweeds, like the weevil *Tanysphyrus lemnae* (Paykull, 1792) (Coleoptera, Curculionoidea) and the shore fly

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Hydrellia albilabris (Meigen, 1830) (Diptera, Ephydriidae) which, however, turned out not to accept *L. minuta* as a host plant. *L. minuta* was found to be resistant to both these two European herbivores, probably highlighting the competitive advantage of the invasive species over native duckweeds in the absence of natural enemies, as expected by the “Enemy Release Hypothesis” (ERH) (Morrison & Hay, 2011). Basing on this hypothesis, plant species introduced outside their natural range should experience a decrease in regulation by herbivores and other native enemies, resulting in a rapid increase in their distribution and abundance.

Although the CBC is the most recognized accepted biological control method, the attempt to find a native herbivore acting as biocontrol agent can have relevance especially from an ecological viewpoint. In fact, controlling an alien species by introducing another alien species, as provided for by the CBC, could be risky because of possible unwanted negative effects on the ecological balances of the invaded ecosystem (Simberloff & Stiling, 1996). Considering this, we decided to look for a native herbivorous insect that could control the alien duckweed *L. minuta*.

Since the two herbivores known as natural enemies of the duckweeds in Europe were excluded as effective consumers of *L. minuta*, we chose the moth *Cataclysta lemnata* (Linnaeus, 1758) (Lepidoptera, Crambidae, Acentropinae) as a possible candidate for this role, since it is an herbivore with a diet including preferentially duckweeds, such as the native *Spirodela polyrhiza* (L.) Schleid. and *L. minor*. However, there was no previous evidence that *C. lemnata* feeds on the alien *L. minuta*.

Duckweed fronds are used by *C. lemnata* not only as trophic resource (Fig 1), but also as material for the construction of protective cases for larvae and



Fig. 1 – Larva of *Cataclysta lemnata* feeding on *Lemna minuta* fronds (Photo: F. Mariani).

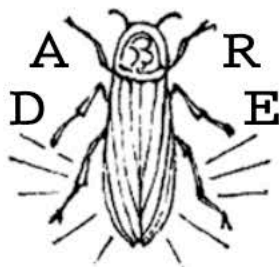
pupae against predation. We tested the effectiveness of larvae of three different instars in limiting the growth of *L. minuta* under laboratory conditions.

Our results show that larvae of the moth *Cataglyphis lemnae* feed on *L. minuta*. This is an important result since until now there was no direct evidence that this native insect consumed the alien duckweed.

In Italy, *C. lemnae* is a native species, but its density is locally too low to substantially reduce the alien duckweed populations, which have rapidly become extremely abundant and widespread (Ceschin et al., 2018). Thus, in order to achieve an effective herbivorous action against the alien plant, it would be necessary to use massive quantities of larvae from laboratory breeding, as typically done in augmentative biological control protocols, when a natural enemy is too scarce to control the invasive species (Hoy, 2008). The optimal number of larvae to be used should be calculated through preliminary indoor experiments. Our experimental study indicates that *C. lemnae* might be considered a potential candidate to be adopted as a biocontrol agent of *L. minuta*. However, further indoor experiments are needed to better test the host-specificity and the long-term effectiveness of this native insect in controlling populations of the invasive alien duckweed, especially in view of future tests to be carried out in the field.

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Pubblicato il 31 dicembre 2019