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XXIX CICLO DEL DOTTORATO DI RICERCA IN AMBIENTE E VITA

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LA NEOFITIZZAZIONE DEGLI HABITAT E LA SUA RIPERCUSSIONE SULLE COMPONENTI VEGETALI E SULL'ENTOMOFAUNA DEL CARSO DINARICO ITALIANO

Settore scientifico-disciplinare: BIO/03 Botanica ambientale e applicata

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Index

General Introduction

Colonization is a worldwide phenomenon considering the occupation of an habitat or a territory by a biological community, or the occupation of an ecological niche by a single population of a species (Onofri, 2011). Species can naturally colonize novel environments or they can expand their range, due to anthropogenic disturbances, into regions where they were not historically present. Moreover, human-mediated transport and human-activity allow more individuals and more species to reach new locations, more often and more quickly, ultimately resulting in colonization rates being greater than natural colonization (Blackburn et al., 2014; Hoffmann & Courchamp, 2016). These species, the so called exotic species (Sax, 2002a,b) or non-native species (Hejda et al., 2009), necessarily go through the same stages of introduction, establishment and spread of native species (Hoffmann & Courchamp, 2016). This happens because they experience the same barriers of survival, reproduction, dispersal and further range expansion (Hoffmann & Courchamp, 2016). Is noteworthy that biological invasions and natural colonization differ in processes and mechanisms in ways that are crucial for science, management, and policy (Wilson et al., 2016). Among introduced species, only a subset of them forms self-sustaining populations, becoming naturalized (Sax, 2002a, b).

The introduction of species beyond their native range, as a direct or indirect effect of human action, does not always causes changes in the ecosystems they are introduced to. Sometimes, these species are considered terrestrial habitats modifier (Schirmel & Buchholz, 2013), and their changes in ecosystems are dramatic and may negatively affect resident species (Hejda et al., 2009), with changes in natural faunal composition and food-webs. Moreover, they can cause extinction of native species, loss of biological diversity (i.e., genetic, species, and ecosystem diversity), radical changes in ecosystem functioning (Hejda et al., 2009), changes in natural habitats (Blackburn et al., 2014), in genetic composition of native populations, behavior patterns, species richness and abundances, phylogenetic and taxonomic diversity (Blackburn et al., 2014), and threat the well-being of humans (Schlaepfer et al., 2010). But for the vast majority of non-native species no quantitative information is available on the consequences of such introductions (Jeschke et al., 2014). In many cases, however, they can also provide conservation benefits. Schlaepfer (2010) examined the ways in which non-native species currently contribute to conservation objectives. These include, for example, providing habitat or food resources to rare species, serving as functional substitutes for extinct taxa, and providing desirable ecosystem functions. The greatest part of non-native species will continue to cause biological and economic damage, and substantial uncertainty surrounds the potential future effects of all non-native species (Schlaepfer et al., 2010). Nevertheless, the proportion of non-native species that are viewed as benign or even desirable, will

slowly increase over time as their potential contributions to society and to achieving conservation objectives become well recognized and realized (Schlaepfer et al, 2010). For this reason, ecological impacts of invasive species should be carefully evaluated before any definitive assertion of harmfulness (Motard et al., 2015).

Biological invasions and ecology of invasion are hot research topics, and the impact of species introduced outside their native range by humans is increasing in an era of globalization, also due to the global change that increases their tendency to spread over larger areas in close relationship with their triggering environmental driving force (Grigorescu, 2016). The study of non-native species became very popular in the last twenty years (Briggs, 2013; Díez et al., 2014), and recently this field has been undergoing an important shift in research priorities (Pyšek, 2008). Invasion biology has, over time, developed into the broader transdisciplinary field of invasion science. The heart of invasion science is the realization that biological invasions are not only a biological phenomenon: the human dimension of invasions is a fundamental component in the social-ecological systems, in which invasions need to be understood and managed (Wilson et al., 2016).

Invasive tree species often form mono-specific stands and they are major drivers of environmental change (Richardson, 2011). One of the most important colonizer tree is *Ailanthus altissima* (Mill.) Swingle (Motard et al., 2011). *A. altissima* is a deciduous tree native to China, belonging to the Simaroubaceae family, which was first introduced in Europe by the French missionary Pierre d'Incaville, who sent seeds from Nanking to Paris in the of mid-XVIII° century (Enescu et al., 2016). After this first episode, this tree was planted mainly as ornamental plant in all continents (except Antarctica). Thanks to its fast expansion, it is nowadays considered as invasive in the most part of Europe (Motard et al., 2011), and actually it is cited as a concern for biodiversity according to the Global Invasive Species Database (GISD, 2017). *A. altissima* arrived in Italy in 1760 at the Botanic Garden of Padova. The first reason of this tree success is the vegetative reproduction ability to resprout and develop root networks forming dense clonal stands; the seconds reason is the sexual reproduction with a high production of samaras, that can persist on the plant during winter and disperse over long distances. The species is common in disturbed urban and anthropic areas, for example along roadsides and railroads, in park areas and in old fields (Vila et al., 2006; McAvoy et al. 2012; Motard et al., 2011; Motard et al., 2015; Enescu et al., 2016). *A. altissima* produces allopathic compounds that can reduce seedling in artificial conditions; it also has an insecticidal activity (Tsao et al., 2002; Motard et al., 2011 and 2015; Enescu et al., 2016) and its pollen is allergenic (Ballero et al., 2003) .

It has been shown that this species can transform open ecosystems such as meadows or old fields

into closed forests (Kowarik & Böcker, 1984; Kowarik and Saümel, 2007). Moreover, it spreads and displaces native vegetation with a huge canopy cover, with a large amount of root suckers and thanks to allelopathy. The result is the impoverishment of biological diversity through competition (Motard et al., 2011). Invaded forest communities exhibit significantly lower floristic diversity, as evaluated by plant species richness, abundance and rarity indices, when compared to non-invaded adjacent zones (Motard et al., 2011). The species spontaneously invades not only disturbed microsites hardly colonized by other species, but also can penetrate forests through natural or artificial free space (Motard et al., 2011). An earlier study in forests colonized by *A. altissima* suggests that an increasing density of this plant is associated with a significantly lower floristic diversity (plant species richness, abundance and rarity indices), a lower soil microbial activity, with decreasing in abundance of Acari, Collembola, Coleoptera, and terrestrial Gastropoda. Contrary, increasing *A. altissima* density is linked to greater abundances of Lumbricidae and coprophagous Coleoptera (Motard et al., 2015). However, it is still unclear whether this impact is due to the direct action of *A. altissima* by phytotoxicity of the root system, or to the transformation of soil and litter fauna (Motard et al., 2011).

Nevertheless, a detailed knowledge of the biology and ecology of individual invasive species remains at the core of invasion ecology, and of its practical applications. Specific case studies are thus an important tool in the quest for a better understanding of invasion phenomena (Pyšek, 2008).

Due to the lack in information concerning the impact of *A.altissima* on terrestrial arthropods, we decided to study the impact of this tree on carabid beetles (Coleoptera, Carabidae) assemblages.

Invertebrates are ubiquitous, taxon-rich and dominant organisms throughout the world (Wilson, 1987), and recently there has been an increase in awareness of their importance as indicators in conservation planning (Kremen et al., 1993; McGeoch, 1998; Paoletti, 1999; Angelini et al., 2002; Andersen et al., 2002; Hodkinson, & Jackson, 2005; Gavinelli et al., 2017). Arthropods form the greatest part of biodiversity in each ecosystem (Duelli et al., 1999; Niemelä, 2000; Buchholz et al., 2012; Twardowski, 2017) and if we consider Insects, they are really useful bio-indicators due to their rapid response to environment changes (Lee & Albajes, 2016). The family Carabidae, the carabid beetles, is estimated to be the fourth largest group in Coleoptera, containing more than 40,000 described species classified into 86 tribes (Löwei & Sunderland, 1996). Most carabids are relatively long lived and many species do not show any strong seasonal fluctuations, allowing useful sampling to be carried out in relatively short periods (Lindroth, 1974; Ings & Hartley, 1999). According to Marinoni et al. (2001) and Marinoni (2001), carabids are included in the carnivore trophic group given their predatory character. However, many of them are specialized in feeding on

fruits and seeds (Paarmann et al. 2001, 2002; Brandmayr et al., 2005). Carabids are typical soil beetles with terrestrial habits, usually found on sandy terrain, under rocks and litter, but some groups inhabit trees and shrubs (Löwei et Sunderland, 1996; Koivula et al., 1999; Moraes et al., 2013).
Carabids are generally considered to be more bound to ecological requirements than to habitat features: they present structural features that may be suitable or adapted to perform in several different habitats (Ribera et al., 2001).

They are currently widely used because they can be sampled in an easy way and in enough large numbers using pitfall traps (Desender et al. 1994; Luff, 1975; Desender & Malfait, 1986; Lee & Albajes, 2016) and for these reasons they represent an important and suitable study group in ecological research, especially for what concerns standardized samplings (Lee & Albajes, 2016; Zhang et al., 2017). They are undoubtedly a taxonomically and ecologically well-known group, and they are increasingly being used as bio-indicators. Studies concerning carabids have addressed their diversity through distinct themes: indicators of environmental integrity (Taylor & Dorann, 2001); indicators of successional stages (Tyler 2008; Magura et al.2003; Humphrey et al.1999); fragmentation and urbanization (Lövei et al. 2003; Fujita et al.2008; Hartley et al.2007); disturbance gradients (da Silva et al.2008); responses to environmental characteristics (Uehara-Prado et al.2009; Schreiner & Irmler 2009; Latty et al.2006); regeneration in natural areas (Ings & Hartley 1999) and agroecosystems (Jukes et al.2001; Holland & Luff 2000); species traits and functional traits (Bargmann et al., 2016; Hanson et al., 2016; Ribeira et al., 2001; Brooks et al., 2012; Schirmel et Buchholz, 2013; Pizzolotto et al., 2016; Mazzei et al., 2015); distribution pattern (Judas et al., 2002); climate change effects (Buchholz et al., 2012); effects of alien species (Buchholz et al., 2015; Martínez et al., 2009). Some of these studies related carabid fauna to biotic and abiotic conditions (Rainio & Niemelä, 2003; Koivula, 2011) and to environmental variables such as temperature, rainfall, vegetation cover, soil moisture (Moraes et al., 2013; Ings & Hartley, 1999; Jukes et al. 2001; Perner & Malt 2003; Kotze et al. 2011; Lee & Albajes, 2016). Nevertheless, it is still not completely clear which of these variables best correlate to observed patterns of richness and abundance (Niemelä, 1996) and which are more informative at a certain scale and/or situation (e.g. preserved versus altered environments) (Moraes et al., 2013).

They reflect and are good indicators also of environmental modifications (e.g. Thiele; 1977; Ings & Hartley, 1999; Lövei & Sunderland 1996; Niemelä et al., 1993; Ribera et al. 2001; Villa-Castillo & Wagner 2002; Rainio & Niemelä, 2003; Kotze et al. 2011). Although heterogeneous environments have higher diversity and abundance (Fujita et al. 2008; Hartley et al. 2007; Magura et al. 2003;

Butterfield, 1997), when carabid fauna is compared between undisturbed and disturbed environments, richness (Latty et al., 2006) and in some cases also abundance (Uehara-Prado et al., 2009) can be higher in the latter category. This can be a consequence of generalist species emigrating from open areas, given the appropriate conditions created by disturbance (Latty et al., 2006). Carabid species composition changes under structural changes of the habitat (Scott & Anderson, 2003), being perhaps one of the most informative ways of studying faunal variations (Penev, 1996). Within habitat, small-scale influences (e.g. "patchiness"), are cited as determinant for the local diversity of Carabidae (Schreiner & Irmler, 2009).

With the aim of simplifying its reading, the thesis is organized in four chapters related to different aspects of carabid beetles communities in the North Adriatic Karst. In the first chapter we analyze the Carabid fauna describing the most common and abundant species in native and non-native habitats, and the beta diversity among all sampling sites, focusing in particular on the difference between native and non-native habitats. In the second chapter we consider the spatial and temporal assemblages and coexistence of carabid beetles in the study area, evidencing the different activity pattern of the more abundant species in the study area. In the fourth chapter there is a complex and detailed study on carabid beetles species traits and the functional diversity of the different habitats; more, there is an interesting comparison between traits and functional diversity between native and non-native sampling sites. In the last chapter, we consider the cross-taxon congruence and the analysis of all the environmental parameters (soil, vegetation structure, plants species) that can affect carabid beetles communities, arriving at the result that the plants can be considered as a surrogate group for carabid beetles analysis.

Since the study area and the carabid beetles sampling methods were always the same along all the chapters, they were described only in the first chapter.

At the end, in the Appendixes, there are the three still on-going project; then, the manuscript in preparation, the submitted papers, the papers in revision and the published papers.

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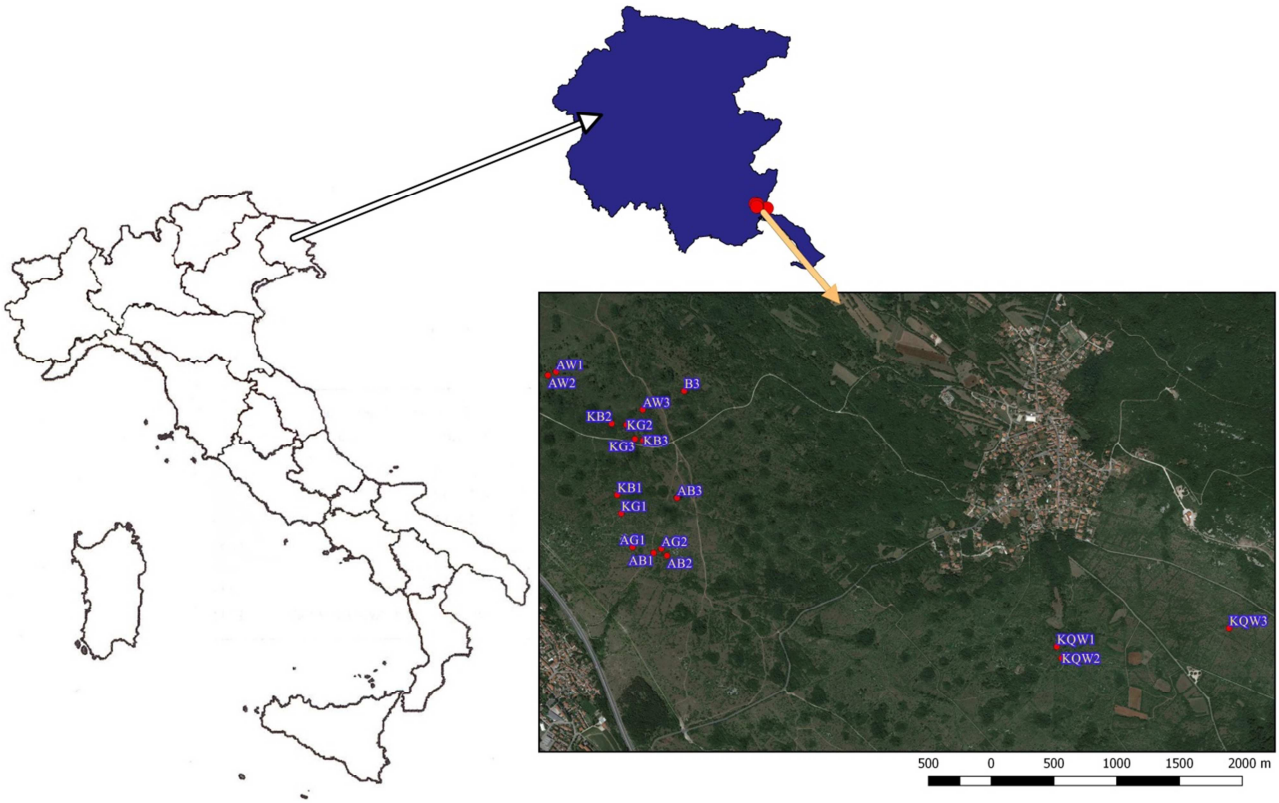
Study area



Indication of study plots. North-East Italy, Friuli Venezia Giulia: Ronchi dei Legionari, Doberdò del Lago (GO)

Checklist of the carabid fauna considered in this research

The species referred to in the following chapters are ordered by a taxonomic point of view.

Carabid beetles were determined to species level according to the taxonomy and nomenclature of Italian Carabidae based on Brandmayr et al. (2005) and Vigna Taglianti (2004; 2005), with a few recent updates.
Ecological, chorological, phenological and distributive aspects are not detailed here as they are widely dealt with in the following chapters.

Brachininae

Brachinini

Brachinina

Brachinus (*Brachynidius*) *explodens* Duftschmid, 1812

Carabinae

Carabini

Calosomatina

Calosoma (*Calosoma*) *sycophanta* (Linné, 1758)

Carabina

Carabus (*Carabus*) *granulatus interstitialis* Duftschmid, 1812

Carabus (*Eucarabus*) *catenulatus catenulatus* Scopoli, 1763

Carabus (*Tomocarabus*) *convexus dilatatus* Dejean, 1826

Carabus (*Megodontus*) *caelatus schreiberii* Kraatz, 1877

Carabus (*Procrustes*) *coriaceus coriaceus* Linné, 1758

Nebriinae

Nebriini

Leistus (*Pogonophorus*) *rufomarginatus* (Duftschmid, 1812)

Notiophilini

Notiophilus rufipes Curtis, 1829

Pterostichinae

Pterostichini

Poecilina

Poecilus (*Macropoecilus*) *koyi goricianus* (G. Müller, 1921)

Myadina

Myas (*Myas*) *chalybaeus* (Palliardi, 1825)

Molopina

Molops ovipennis istrianus G. Müller, 1916

Abax (*Abax*) *parallelepipedus subpunctatus* Dejean, 1828

Abax (*Abacopercus*) *carinatus sulcatus* A. Fiori, 1899

Licininae

Licinini

Licinus (*Neorescius*) *hoffmanseggii* (Panzer, 1803)

Harpalinae

Harpalini

Harpalina

Ophonus (*Hesperophonus*) *azureus* (Fabricius, 1775)

Pseudoophonus (*Pseudoophonus*) *rufipes* (De Geer, 1774)

Harpalus (*Harpalus*) *atratus* Latreille, 1804

Harpalus (*Harpalus*) *dimidiatus* (P. Rossi, 1790)

Harpalus (*Harpalus*) *rubripes* (Duftschmid, 1812)

Harpalus (*Harpalus*) *serripes* (Quensel in Schönherr, 1806)

Platyninae

Sphodrini

Synuchina

Synuchus vivalis (Illiger, 1798)

Calathina

Calathus (*Calathus*) *glabricollis* Dejean, 1828

Calathus (*Neocalathus*) *cinctus* Motschulsky, 1850

Calathus (*Neocalathus*) *melanocephalus* (Linné, 1758)

Sphodrina

Laemostenus (*Antisphodrus*) *cavicola* (Schaum, 1858)

Laemostenus (*Antisphodrus*) elongatus (Dejean, 1828)

Lebiinae

Dromiini

Dromiina

Philorhizus crucifer confusus Sciaky, 1991

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Vigna Taglianti, A., 2005. Appendice B - Checklist e corotipi delle specie di Carabidi della fauna italiana; pp. 186-225, in: Brandmayr, P., Zetto, T., Pizzolotto, R. (Eds). I Coleotteri Carabidi per la valutazione ambientale e la conservazione della biodiversità. Manuale operativo. APAT - Manuali e linee guida, 34.

Chapter 1

Carabid beetle assemblages in different native and non-native habitat types in the North Adriatic Karst (North East Italy)

1. Introduction

Comparisons among habitat types are very important to describe in detail the species' habitat requirements and the relative species diversity of the carabid assemblages in different habitats (Niemelä and Halme, 1992). In this study we used ground-beetles as monitoring tools along a gradient of vegetation dynamics (from grassland to wood) in a degraded area of the North Adriatic Karst, where the colonization of *Ailanthus altissima* is changing the landscape. Our aim was to test differences in species composition between native and non-native plots, and we predict low species richness in non-native plots, and a difference in beta diversity resulting in a more homogenization of *A. altissima* habitats. Beta diversity is the "extent of species replacement or biotic change along environmental gradients" (Whittaker, 1977) and represents the differentiation component of diversity as opposed to the inventory component, which describes the species composition of a single place (McKnight et al., 2007). As explained by Baiocchi et al. (2012), a large variation in beta diversity is the results of a series of sites influenced by the same environmental descriptors, which are not very similar in terms of species composition. In this case, local selection would promote species differentiation despite the same environmental conditions. On the other hand, in a group of sites that share the same environmental variables and that are also characterized by high similarity in species composition, the variation in those particular factors are responsible for the selection of species. In the last case, local selection would be able to select and/or favour, from a common pool of species, the same group of taxa under the same environmental conditions. Considering all habitats type, we suppose that *A. altissima* habitats are connected with low values of beta diversity, whereas native habitats present higher values of beta diversity.

This study was performed to examine the community composition of carabid beetles (Coleoptera: Carabidae) in native and non-native habitats in the North Adriatic Karst (North East Italy). More specifically we aimed to: (1) analyze the carabids community in the study area, (2) study the effects of some ecological variables (e.g. soil parameters and vegetation structure) on carabid assemblages, (3) compare the carabid beetles beta diversity of native and non-native habitat types, (4) find possible characteristic species for the non-native habitats. Specifically, we addressed the following

questions: (i) Does species beta diversity of carabid beetles decrease from native to non-native habitats? (ii) What factors modulate species composition of carabid beetles between native and non-native habitats? By answering these questions we aim to contribute to the comprehension of the value of the native environments.

2. Materials and methods

2.1. Study area

Fieldwork was carried out done in an area of the North Adriatic Karst (NAK) near Ronchi dei Legionari (GO), in the northeastern part of Italy, situated between 58-114 m a.s.l. The NAK is a well-known area for its geographical, geomorphological characteristics and speleological phenomena, positioned between the Adriatic Sea and the Alps. The study area lays in the westernmost part of the Italian "classical Karst", that consists of the limestone Karst plateau (Low Karst), 100-500 m a.l.m., characterized by the typical geomorphological karst phenomena (karst poljes, dolines, caves, etc.), and Red mediterranean soils (Poldini 2009; Kaligarič et al., 2006).

The Karst is known and traditionally recognized as a treeless, stony grassland landscape (with exceptions of many flysch-bedrock patches), where the strong and cold north-eastern bora wind affects vegetation causing desiccation and soil erosion. The climate is sub-mediterranean, transitional between Mediterranean and continental pre-Alpine types, with rainy cool winters and long and relatively dry summers; mean annual temperature on the Karst Plateau is 12 °C; average monthly temperature ranges from 4.7 °C (January) to ca. 24 °C (July), with the minimum ca. -15°C and the maximum ca. 34°C; average annual precipitation varies from ca. 1000-1100 mm along the coast area and at lower elevations to ca. 1500 mm in the higher parts of Karst plateau (Poldini, 2009; Kaligarič et al., 2006).

The dry calcareous grasslands of the NAK are known as one of the richest plant communities among all grasslands. Their diversity has been described, emphasized and partly explained in several works due to the specific biogeographic position of the area, since the NAK is in the transitional area where the Mediterranean basin joins the Dinaric mountains and Central Europe. The contingent of Euro-Mediterranean (sub-Mediterranean)-Illyrian species, particularly rich in biodiversity, in this area is accompanied by other large interesting contingents of species e.g., Mediterranean-Pontic or Mediterranean-Pannonian species (Pipenbaher et al., 2011). These grassland belong in the Natura 2000 habitat type 62A0 – Eastern sub-Mediterranean- grassland (Scorzoneretalia villosae of the Annex I of the 92/43/EEC "Habitats" Directive).

At present, most Karst landscape is dominated by mixed deciduous termophilous woodland of *Quercus pubescens*, *Ostrya carpinifolia* and *Fraxinus ornus,* belonging to the association *Aristolochio luteae-Quercetum pubescentis* (Horvat 1959) Poldini 2008 (class *Querco-Fagetea*, alliance *Quercion pubescenti-petraeae* Br.-Bl. 1932). This woodland community can be regarded as the potential vegetation on dry, calcareous soils of the Karst.

Karst dry grasslands are mainly secondary communities, being the product of human action on the deciduous mixed oak woodlands over centuries: deforestation and grazing caused their broad expansion in the past. Dry and semi-dry grasslands of the Karst area belong to the order *Scorzonero villosae-Chrysopogonetalia grylli* Horvatić & Horvat in Horvatić 1963 (class *Festuco-Brometea*). Different types of meadows and pastures are know from the plateau: the most xero-thermophilous karst grassland on less developed soils is represented by the association *Centaureo cristatae-Chrysopogonetum grylli* Ferlan & Giacomini 1955 (alliance *Saturejion subspicatae* (Horvat 1974) Horvatic 1975), typical of low altitudes and with great presence of Mediterranean species, widespread in the study area (Poldini, 2009).

In the study area we selected two different habitat series. The first one includes natural habitats representing the main seral stages of the dominating dynamic series on calcareous soils of the Karst plateau occurring in the study area, namely the karst dry grassland of the association *Centaureo cristatae-Chrysopogonetum grylli*, the *Cotinus coggygria* shrub land (association *Frangulo rupestris- Cotinetum coggygriae* Poldini & Vidali 2002), and the *Quercus pubescens* karst woodland in its most thermophilous aspect (*Aristolochio luteae-Quercetum pubescentis* (Horvat 1959) Poldini 2008 *pistacietosum terebinthi* Wraber (1954) 1960).

The second series includes NAK habitats altered by the massive presence of the non-native *A.altissima* in different phases of invasion.

Selected habitats are hereinafter briefly described, including the codes used in the text for sampling sites.them.

Sites dominated by the presence of native species, belonging to the downy oak edapho-xerophiluos series (*Aristolochio-Quercetum pubescentis* sigmetum) (Poldini, 2009) (4 habitat types) (1)-(4):

(1) Karst grassland: KG1, KG2, KG3. *Centaureo cristatae-Chrysopogonetum grylli* Ferlan & Giacomini 1955. Thermoxerophilous dry grassland, (*A.altissima* absent).

(2) Karst bushes (shrubland): KB1, KB2, KB3. *Frangulo rupestris-Cotinetum coggygriae* Poldini et Vidali 2002. Thermoxerophilous shrubs (*A.altissima* absent).

(3) Karst Wood in doline (dolines): KW1, KW2, KW3. Mixed deciduous oak woodland in shallow dolines (native>90%, *A.altissima*<1%).

(4) Karst Quercus wood: KQW1, KQW2, KQW3. *Aristolochio luteae-Quercetum pubescentis* (Horvat 1959) Poldini 2008. Mature woodland of *Q. pubescens* (*A.altissima* absent).
Sites dominated by the non-native *A. altissima* (3 habitat types):

(5) *A. altissima* on karst grasslands: AG1, AG2. First step of *A. altissima's* colonization on dry calcareous grassland (*A.altissima*>60%, native<20%).

(6) Shrub land with *A. altissima*: AB1, AB2, AB3. Phases of invasion of *A.altissima* in karst shrub lands and pre-woods (*A.altissima*>55%, native<35%).

(7) *A. altissima* Wood: AW1, AW2, AW3. Dense and monospecific *A.altissima* woods in shallow dolines (dolines) (*A.altissima*>90%, native<5%).

2.2. Ground beetles sampling

To evaluate the potential effects of *A. altissima* on local carabid biodiversity, eight native plots and 12 non-native plots were selected (see above). Three spatially distinct replicate plots were selected for each habitat, located at different elevation and at least 50 m away from neighboring plots. During the study, one sampling site of altered dry grassland (AG) was devastated by a fire, and it was not possible to replace it; therefore only two sampling sites are available for AG habitat. A total of 12 plots of natural habitats and 8 plots of habitats altered by the presence of *A. altissima* were selected.

In each plot, three pitfall traps were placed 10 m from adjoining traps in a straight line at the center of each plot. The pitfall method is considered an inexpensive and labour efficient way to collect carabids for statistical analysis (Capar, 2003). According to Brandmayr et al. (2005), Mazzei et al. (2015), Pizzolotto et al. (2016), Zhang et al. (2017) traps were made by plastic vessels with an upper diameter of 9 cm and a depth of 11 cm and provided with two small holes to avoid water filling and "aquaplaning effect" of beetles. Traps were filled up to two thirds of their depth with wine-vinegar saturated by sodium chloride as further preservation measure. Vinegar has been demonstrated to be a good collecting fluid, and compared to pure water it allows a collection of larger numbers of species and individuals by avoiding degradation of anatomical structures (Mazzei et al., 2015). Ground beetles were collected from 10th May 2014 until 12th January 2017 during all year with a monthly control and emptying of pitfall-traps. Since our field studies did not involve any protected species, no permits and approvals were required.

2.3. Vegetation structure analysis

In each plot where trees were higher than 1 m (Woodland and shrub land without or with *Ailanthus altissima*), vegetation structure was characterized by height, diameter, number of individuals, density and total percentage cover of woody plants in one randomly selected 10x10m plot (Tab.3 in Supplements). The diameter was calculated at breast height (DBH) using a metal tree caliper and the resulting measure was the arithmetic mean of the two readings, according with Clark et al. (2000). The estimate of the tree height was performed using a manual hypsometer, a precise and fast instrument (Rennie, 1979) using geometrical and trigonometric principles to determine tree height.

2.4. Soil parameters

A soil pit 10 X l0 X l0 cm was dug near the center of each plot. To characterize soil properties (Tab.3 in Supplements), one sample was randomly taken from the upper part of the mineral soil (0-7 cm in KG, AG, KB, 0-10 in KQW, 0-80 in AW, AB and KW) on each plot in October 2017. The soil bulk density is the mass to volume ratio of an oven-dried soil. It was determined in the 0-10 cm surface soil layer of all investigated locations, following the methods described by Grossman and Reinsch (2002). In non-gravelly soil surface strata it was determined with the core method by using a steel cylinder of 10 cm height and 0.724 l volume. The cylinder was inserted in the soil with the help of a steel hammer with nylon heads and dug out with a shovel. The soil sample was finally extracted from the cylinder and put in a plastic bag. When the content of rock fragments was high, an alternative excavation method was adopted. A plastic film with a central hole of 15 cm diameter was placed on a roughly plain soil surface. A soil sample was dug out of the hole with a knife, put on the plastic film and transferred in a plastic bag. Afterwards, the excavated hole was covered with a second plastic film and filled with water to the surface with the help of 250 ml graduated cylinder. The volume of the hole was given by volume of water added to the excavated hole. In both cases, samples were transported in the laboratory, oven-dried at 105°C and weighted. The bulk density was calculated as the mass to volume ratio of oven-dried cores and expressed as kg L-1. Total soil porosity was obtained by the equations:

$$\text{Total porosity} = 1 - \rho a / \rho s$$

where ρa is the bulk density, and ρs the particle density of the soil, which usually averages 2.65 Kg l-1.

Sampling site environmental characteristics (ordered by gradient of vegetation-from wood to grassland) are here reported (see Tab. 1 for topographic features).

Plot	AW1	AW2	AW3	KQW1	KQW2	KQW3	KW1	KW2	KW3	AB1	AB2	AB3	KB1	KB2	KB3	AG1	AG2	KG1	KG2	KG3
Vegetation_type	wood	wood	wood	wood	wood	wood	wood	wood	wood	bush	bush	bush	shrub	shrub	shrub	grass	grass	grass	grass	grass
Lat	45,85	45,85	45,84	45,82	45,82	45,83	45,84	45,83	45,84	45,83	45,83	45,84	45,84	45,84	45,84	45,83	45,83	45,84	45,84	45,84
Long	13,51	13,51	13,52	13,56	13,57	13,58	13,52	13,52	13,52	13,52	13,52	13,52	13,52	13,52	13,52	13,52	13,52	13,52	13,52	13,52
Slope	8,98	2,84	3,66	9,03	10,41	12,35	6,88	4,83	10,33	1,99	3,41	3,31	2,05	1,5	4,64	2,33	1,25	4,37	2,66	4,71
Elevation	111,47	115,25	96,72	25,29	31,70	79,12	63,34	60,87	85,06	52,86	51,12	73,59	70,76	101,42	88,11	53,40	50,94	63,96	100,24	89,74
Northness	0,6	0,88	-0,58	-0,97	-0,36	-0,85	-0,97	0,66	0,41	-0,46	-0,39	0,88	0,62	-0,75	-0,68	-0,51	0,15	0,97	0,34	-0,26
Eastness	-0,8	0,48	-0,81	0,24	-0,93	-0,53	0,23	0,75	0,91	-0,89	-0,92	-0,48	-0,79	-0,66	-0,73	-0,86	-0,99	0,26	0,94	0,97
Karstification	4	4	4	3	3	2	4	4	4	0	2	3	2	2	2	3	2	1	1	1
Sinkhole deep	3,278	0,155	1,879	0	0	0	3,704	4,396	6,75	0	1,996	0	0	0	0	0	0	0	0	0
Species richness_beetles	11	10	15	9	9	10	9	8	9	6	13	9	7	5	2	7	5	5	8	5
Species richenss_plants	27	29	22	21	27	28	30	27	29	33	29	23	15	17	11	31	38	45	45	44
TDAaT	1,8	1,94	2,05	1,43	3,15	1,21	0,98	2,13	0,88	0,21	0,69	0,68	0,92	1,71	1,59	0,94	0,68	0,99	1,45	0,88

Tab.1.Sample site's identification code: Vegetation structural types, main topographical features of the sampled sites ; degrees of karstification (following Forti, 1982); dolines depth; beetles species richness and abundance where TDAaT is the mean number of active individuals per trap in the standard period of 10 trapping days; line 10 plant species richness.

2.5. Data analysis

Carabid beetles data set was analyzed performing a Principal Coordinates Analysis (PCO) with the software PRIMER v.6 (Clarke and Warwick, 2001) to explore carabid-assemblage variation among sites. Data were arranged in Excel with plots as columns and abundances of carabid species as rows. Species data were transformed using square root options in the software and Bray-Curtis was used as similarity measure.

Poisson generalized model was used to study the response of carabid species richness to environmental variables, such as vegetation structures and soil parameters. In addition, we used redundancy analysis (RDA) to explore whether the variation in the beetle assemblages was related to the soil parameters variation.

To test whether the species compositions of native (Grassland, Shrub, Wood) and non-native (AG, AB) habitat types differ in their variances (beta diversity), we analyzed the multivariate dispersion based on the Bray-Curtis similarity of species (using the 'betadisper' function in the R package 'vegan' Oksanen et al., 2007) (Anderson et al., 2006).

A permutation ANOVA was used to test for differences between the multivariate homogeneity of dispersions of native and non-native sites.

All statistical analyses were performed using the free software environment R, version 2.11.1 (R Core Team, 2013).

3. Results

3.1. Abundances and distribution of carabidae in native and non-native habitats

We collected 3119 individuals belonging to 28 carabid species (Table 1). Between 2 and 15 species were collected per sampling site. The actual species richness is within confidence intervals, hence, sampling intensities was adequate (Fig. 1). Rarefaction curves showed a similar trend to the observed species richness, with KQW and AW having the highest rarefied species richness and KB reaching the lowest estimated value (Fig. 1). The total annual activity density (taAD) of carabid beetles had the higher value in the KQW2 (taAD =2.13) and the lowest in AG2 (taAD =0.21). Species aAD showed a wide range of values from the very low aAD of *Carabus granulatus interstitialis* (aAD=0.01 in AW3), *Harpalus serripes* (aAD=0.01 in KG3), *Ophonus azureus* (aAD=0.01 in AG2), *Brachinus explodens* (aAD=0.01 in AG1) to the maximum of *Carabus coriaceus coriaceus* (aAD=1.5 KB3), the species that contributes maximally to taAD.

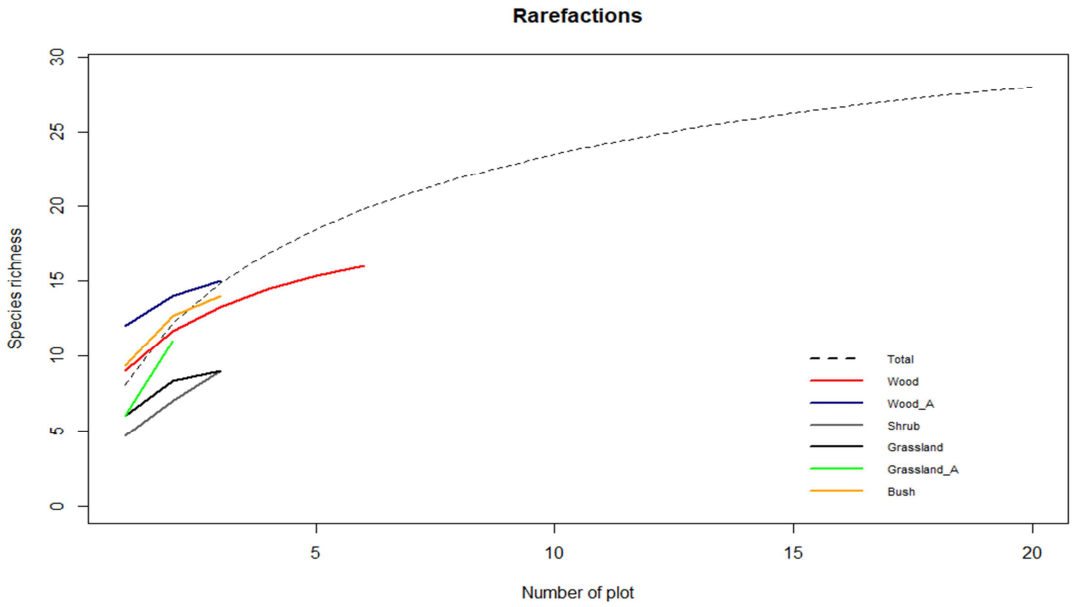

Fig.1. Rarefaction curves of carabid species richness in the different habitats types (Wood=KQW+KW; Wood_A=AW; Shrub=KB; Grassland=KG; Grassland_A=AG; Bush=KB).

Non-native wood is the habitat type were the highest number of species (see Tab.1) has been collected (15 species in AW3, 11 species in AW1), while a low number of species has been recorded in the native shrub (2 in KB3, 5 in KB2), non-native grassland (5 species) and native grassland (5 both in KG1 and KG3). Out of the 18 species sampled in the native and non-native wood habitats, *Laemostenus cavicola* and *Carabus granulatus interstitialis* were found only in the non-native plots, while *Abax parallelepipedus subpunctatus, Calosoma sycophanta, Notiophilus rufipes* were found exclusively in native woods. Among the other habitat typologies (KG, AG, KB,

AB) out of the 19 species sampled, 10 species were associated only with the habitats with *A.altissima: Licinus hoffmanseggii, Abax carinatus sulcatus, Carabus catenulatus catenulatus, Harpalus atratus, Leistus rufomarginatus, Harpalus rubripes, Ophonus azureus, Calathus melanocephalus, Brachinus explodens, Pseudoophonus rufipes*.

Principal Coordinate Analysis of carabid species data explained 49.1% of the variance in the first axis and 19.2% on the second axis (Fig.2). Sites tended to group according to vegetation structure (open-intermediate-close vegetation). The typical Karst wood (KQW) clustered together on the extreme left of the first PCO axis. Woods in dolines, native (KW) and non-native (AW), are closely grouped in the middle of the ordination, not far from non-native bushes (AB). Two plots (AG2 and KB3) were found grouped away from all other plots. Grassland_A (AG1) pulled closer to the KG karst habitats (KG and KB), that grouped closely on the right of the ordination. The typical Karst habitats (Wood, Shrub, Grassland) are at the extreme side of the ordination.

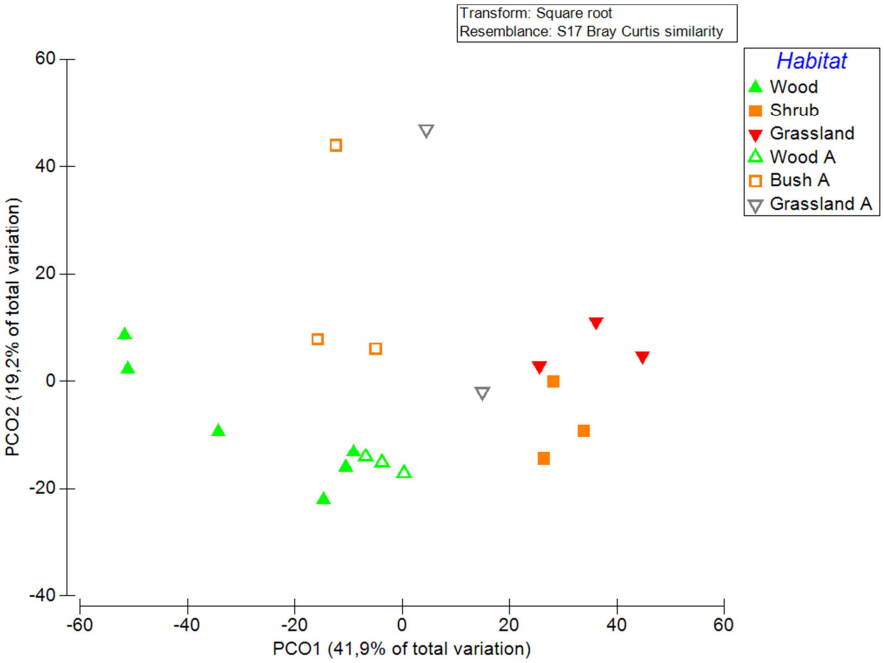

Fig.2. Sample sites ordination by means of PCO, where the 41.9% and the 19.2% of the total variance is represented by the first and second axis respectively. Habitat types (Wood=KQW+KW; Wood_A=AW; Shrub=KB; Grassland=KG; Grassland_A=AG; Bush=KB).

3.2. Environmental parameters

Poisson generalized linear model was applied on carabid beetles species richness, vegetation structure, plants species and soil structure. *A. altissima* mean Diameter (t=3.85, p-value<0.01), *A. altissima* mean Diameter (t=10.80, p-values<0.001) and soil porosity (z=6.88, p-value<0.001) were

positively correlated with species activity densities of carabids. In multivariate analysis soil parameters did not influence carabid beetles distribution within plots.

Redundancy Analysis (RDA) was used to examine the relationship between carabid beetle species and soil parameters (Fig.3). *Calathus melanocephalus, Ophonus azureus, Philorhizus crucifer confusus*, thermophilous and xerotolerant species characteristic of grasslands and grazing grasslands are located in the upper part of the diagram, where the soil mean weight diameter and the soil skeleton are high (karst grassland (KG) and shrub (KG and KB). The soil bulk density is inversely related to soil porosity and is higher (less pore in the soil) in the wood habitats (KW, KQW, AW, AB), where there are also the highest values of soil fine content. Thermophilous forest dwellers like *Synuchus vivalis, Harpalus atratus, Leistus rufomarginatus* and stenotopic *Querco-Fagetea* species like *Abax parallelepipedus subpunctatus, Molops ovipennis istrianus, Carabus catenulatus catenulatus, Carabus caelatus schreiberi* are located in the lower part of the diagram, in relationship to woody and bush habitats and to a finer soil texture.

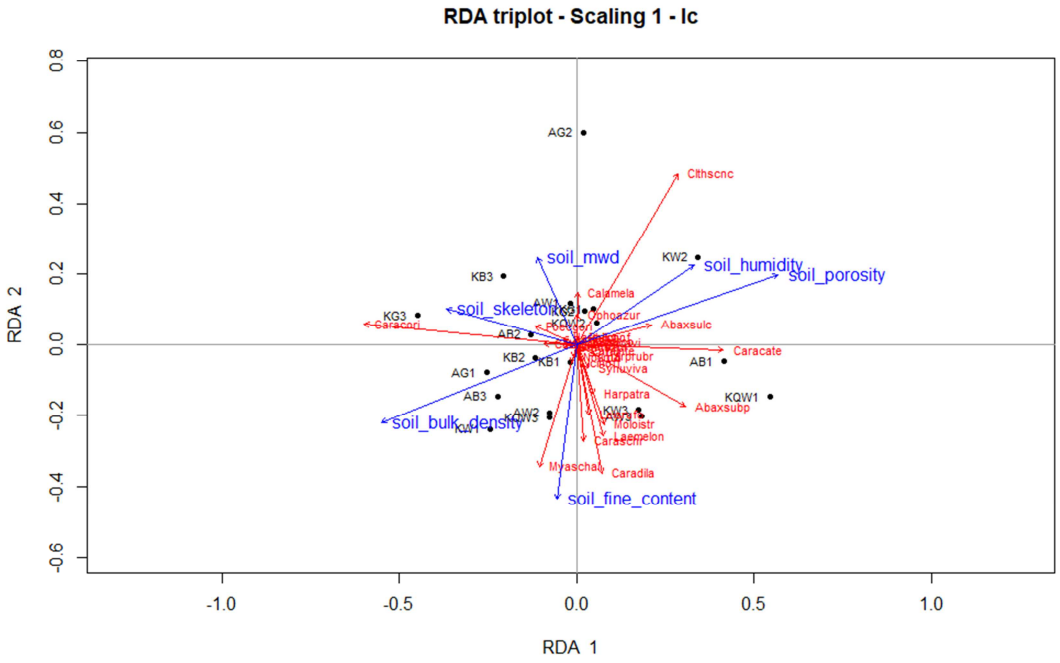

Fig. 3. Ordination triplot of the redundancy analysis (RDA). The blue arrows represent the soil parameters. The red dashed lines are the response variables. Sampling sites are labelled with the habitat code and the species names are abbreviated with the four first letter of the genus and the first four letter of the species.

3.3. Diversity indices of Carabidae species between native and non-native habitats

No significant differences were found in alpha-diversity measures between native and non-native habitats. Conversely, variances in carabids species composition between the habitats (beta diversity) differ significantly (F=6.1662, p-value=0.001) (Fig. 4). The habitats with greatest differences were Wood assemblages (KQ+KQW) and AW(p-value=0.0126464), KB and AG (p-value=0.0473369), KG and AG (p-value=0.0667913). Differences are found also within native habitats, as expected: Wood assemblage-KB (p-value=0.0445595), Wood assemblages-KG (p-value=0.0695083). The average distance to median showed that AG had the highest beta diversity (0.5849) and AW the lowest (0.1352).

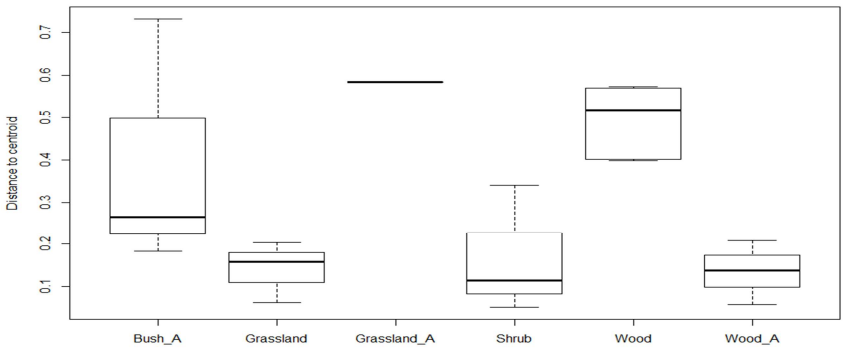

Fig.4. Variation in species composition (beta diversity) in Carabidae in native (Grassland, Shrub, Wood) and non-native habitats (Grassland_A, Bush, Wood_A). To test whether variances (multivariate dispersion) differed between the habitat types, the distances of group members to the group centroid were subjected to ANOVA (F=6.1662, p-value=0.001).

4. Discussion and conclusions

Following Buchholz et al. (2015), we decided to compare native plots to nearby non-native plots (using an already confirmed approach in invasion ecology), in order to assess invasion impacts on comparable pairs of native and non-native habitats in the same environmental matrix. We expected our study to explain effects of native versus non-native habitats on carabid beetles diversity in North Adriatic Karst. In the studied area the gradient of vegetation (native and non-native) is the first driver of carabid beetles assemblages among sites, with a clear division in open (thermophilous and grassland species), semi-open (sylvicolous thermophilous species), and close habitat type (sylvicolous and forest species), as already demonstrated in other studies where carabid beetles species composition presented differentiation in distribution across habitat types (Halme & Niemelä, 1993; Migliorini et al., 2002; Mazzei et al., 2005; Martínez et al., 2009; Brandmayr et al., 2005; Pizzolotto et al. 2016; Schirmel et Buchholz, 2011; Pizzolotto et al., 2016). When we consider successional vegetation stages, native wood, shrub and grassland are clearly separated

from non-native wood, grassland and bushes, that are grouped together. This fact highlights a high species turnover across the gradient of native vegetation and underline the importance to consider different stages of vegetation to maintain diversity (Schirmel and Buchholz, 2011). Going into a detail, the difference in species composition along the natural vegetation gradient shows an increasing of the species richness from grassland to forest, in contrast to what usually reported (Brandmayr et al., 1983; Elek and Tóthmérész, 2010). This trend is probably due to the low elevation of the region, that lays at the border of the Mediterranean garrigue, and to the lack of all the set of species typical of Central Europe grassland, Eurosibiric and Euroasiatic regions (Brandmayr et al., 2005).

In the study area the land-use was characterized by grazing activity until 1970; at the present day, the abandonment of this practice results in the missing of all the species linked with livestock activity, such as *Cymindis* ssp. or *Licinus* ssp (Brandmayr et al., 2005). In the grassland habitats, characterized by high values of soil skeleton and mean weight soil diameter, we found species normally connected to this habitat type, such as *Calathus glabricollis, Harpalus dimidiatus, Ophonus azureus, Poecilus koyi goricianus*. The encroachment of the Karst grassland favored the most sylvicolous species and drove a decrease in the more open-habitat species, due to the growing trees and bushes covering the soil. The greatest number of the sylvicolous species was found in the second richest habitat concerning species composition considered in this study, i.e. the non-native shrub land invaded by *A. altissima*. In this habitat we found a very exigent species, *Licinus hoffmannseggi*, a snails predator that is typical of *Fagetalia* mesophilous woods (*Asaro-Carpinetum*) (Brandmayr & Zetto Brandmayr, 1986; Brandmayr et al., 2005). The high number of species found can be explained with the so called "intermediate disturbance hypothesis (IDH)", that explains that diversity would be higher at the intermediate levels of disturbance, where the greatest landscape heterogeneity occurs and where the forest carabid beetles assemblages meet the open habitat species (da Silva et al., 2008; Halme et Niemelä,1993; Martínez et al., 2009). In mature karst wood, grassland species almost disappear and the sylvicolous species predominate, like *Abax parallelepipedus*, whose larva eats earthworms (Brandmayr et al., 2005), and *Carabus catenulatus,* a south-east alpine illyric species. The dolines woods, characterized by the higher values of soil fine content, were the most rich plots in sylvicolous and South-West european endemic species, resulting in a complex of well-structured carabid beetles communities. In this kind of habitat species such as *Laemostenus cavicola* and the two troglobious grasshoppers *Troglophilus cavicola* and *Troglophilus neglectus* clearly indicate high karstification processes.

A. altissima's habitats are usually associated with poorer floristic composition respect to native habitats (Motard et al., 2011) and high potential to overtop other species (Castro-Díez et al., 2014), but we did not found significance difference in alpha diversity. This can be compared with other studies, such as Buchholz et al. (2015) and Brown et al. (2001), suggesting that response to invasive species can change with the considered taxa, and considering that this impact is usually overestimated. Moreover, species richness will be relatively stable also if there is a change in vegetation composition or plant species. In our study, considering the environmental parameters that can affect carabid beetles biodiversity, the presence of *A. altissima* is in relationship with highest number of carabid beetles taxa, in accordance with many studies (da Silva et al., 2008), discovering more abundance and specific richness in degraded areas, due to the presence of generalist and opportunistic species (Moraes et al., 2013). Such species show a higher mobility and invasive ability that allow them to be more abundant in disturbed habitat, where prey availability is higher (da Silva et al., 2008; Brandmayr et al., 2005).

Finally, we also analyzed the difference in the pairwaise variation of carabid beetles assemblages (beta diversity) within the studied habitats, finding out that along the vegetation gradient, native and non-native grassland and native and non-native woods showed a high difference in beta diversity, that means that among these pairs of habitats (native versus non-native) there is a high difference in species composition. In native wood, the typical species of humid woods and *Querco-Fagetalia* woods (*Abax carinatus sulcatus, Molops ovipennis istrianus, Carabus catenulatus catenulatus*) were more abundant than in non-native wood, whereas *Carabus coriaceus coriaceus*, a typical generalist species, is more abundant in non-native wood respect to the native one. This result is different from what observed in Buchholz et al. (2015), where the comparison between *Robinia pseudoacacia* woods and native one, revealed no carabid beetles diversity.
We found also a high difference in beta diversity between native and non-native grassland, with different proportion of the typical grassland species. Considering the wood habitats, even if the number of species was higher in non-native woods, the homogenization of the habitat induced by the local selection of *A. altissima* created a strong community differentiation that promoted species repartition.

In conclusion, the study shows that carabid beetles in North Adriatic Karst are grouped mainly along two gradients: the first drives species composition from open to close habitats, following the gradient of vegetation, the second separates carabid beetles assemblages of native habitats from those of non-native habitats.

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6. Supplements

Habitat	AW1	AW2	AW3	KW1	KW2	KW3	KQW1	KQW2	KQW3	AB1	AB2	AB3	KB1	KB2	KB3	AG1	AG2	KG1	KG2	KG3
Laemostenus (Antisphodrus) cavicola	0,04	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Laemostenus (Antisphodrus) elongatus	0,13	0,21	0,19	0,01	0,00	0,09	0,02	0,02	0,04	0,00	0,01	0,00	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00
Licinus (Neorescius) hoffmanseggii	0,00	0,02	0,01	0,02	0,01	0,00	0,00	0,00	0,00	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Carabus (Carabus) granulatus interstitialis	0,00	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Abax (Abacopercus) carinatus sulcatus	0,00	0,00	0,01	0,00	1,31	0,29	0,00	0,00	0,00	0,00	0,04	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Abax (Abax) parallelepipedus subpunctatus	0,00	0,00	0,00	0,00	0,00	0,00	0,50	0,19	0,08	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Molops ovipennis istrianus	0,02	0,02	0,01	0,00	0,00	0,11	0,02	0,00	0,19	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Carabus (Eucarabus) catenulatus catenulatus	0,01	0,00	0,01	0,05	0,98	0,00	0,18	0,54	0,07	0,02	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Carabus (Megodontus) caelatus schreiberi	0,06	0,46	0,38	0,16	0,14	0,10	0,04	0,02	0,07	0,00	0,01	0,02	0,06	0,33	0,09	0,00	0,01	0,01	0,01	0,00
Synuchus vivalis	0,03	0,02	0,03	0,00	0,00	0,03	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Harpalus (Harpalus) atratus	0,00	0,00	0,01	0,13	0,00	0,00	0,00	0,00	0,00	0,03	0,01	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Calosoma (Calosoma) sycophanta	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,04	0,00	0,00	0,01	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Leistus (Pogonophorus) rufomarginatus	0,06	0,07	0,03	0,07	0,02	0,01	0,04	0,04	0,03	0,00	0,01	0,02	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Myas (Myas) chalybaeus	0,04	0,04	0,03	0,28	0,02	0,04	0,05	0,06	0,03	0,00	0,02	0,06	0,01	0,00	0,00	0,21	0,00	0,00	0,00	0,00
Carabus (Tomocarabus) convexus dilatatus	0,08	0,01	0,07	0,11	0,04	0,23	0,07	1,05	0,24	0,02	0,25	0,13	0,00	0,01	0,00	0,10	0,00	0,00	0,13	0,00
Carabus (Procrustes) coriaceus coriaceus	1,32	1,05	1,18	0,61	0,62	0,36	0,00	0,00	0,10	0,07	0,27	0,42	0,81	1,36	1,50	0,58	0,14	0,94	1,17	0,77
Calathus (Neocalathus) cinctus	0,02	0,05	0,06	0,00	0,00	0,00	0,07	0,17	0,00	0,05	0,03	0,01	0,01	0,00	0,00	0,00	0,49	0,01	0,01	0,00
Calathus (Calathus) glabricollis	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,02	0,01	0,00	0,02	0,00	0,00	0,02	0,02
Harpalus (Harpalus) rubripes	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Poecilus (Macropoecilus) koyi goricianus	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,00	0,00	0,01	0,00	0,01	0,10	0,07
Harpalus (Harpalus) dimidiatus	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,01
Harpalus (Harpalus) serripes	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01
Ophonus (Hesperophonus) azureus	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,00	0,00	0,00
Philorhizus crucifer confusus	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,01	0,00
Calathus (Neocalathus) melanocephalus	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,03	0,00	0,00	0,00
Brachinus (Brachynidius) explodens	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,00	0,00	0,00	0,00
Pseudoophonus (Pseudoophonus) rufipes	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,00	0,00	0,00	0,00	0,01	0,00	0,00	0,00	0,00
Notiophilus rufipes	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,02	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00

Tab. 1. Carabid species included in the analysis and their abundances (DaA).

Plot	n-n_H_mean	n-n_D_mean	n-n_density	n-n_N	n-n_C	n_H_mean	n_D_mean	n_density	n_N	n_C
AB1	2,58	2	0,52	52	3	5,00	4,17	0,03	3	3
AB2	6,8	5,63	0,84	84	3	4,20	3,00	0,05	5	2
AB3	2,58	2,47	0,61	61	4	5,83	4,92	0,12	12	2
AG1	3,29	2,62	0,38	38	3	4,20	3,35	0,33	33	2
AG2	2,19	2,22	0,98	98	4	4,75	16,25	0,02	2	1
AW1	8,55	7,98	1,23	123	5	9,83	8,33	0,03	3	3
AW2	5,37	5,7	1,98	198	5	4,00	6,85	0,10	10	2
AW3	7,23	5,67	0,75	75	5	6,99	6,47	0,39	39	3
KB1	NA	NA	NA	NA	NA	NA	NA	NA	NA	5
KB2	NA	NA	NA	NA	NA	NA	NA	NA	NA	5
KB3	NA	NA	NA	NA	NA	NA	NA	NA	NA	5
KG1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
KG2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
KG3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
KQW1	NA	NA	NA	NA	NA	8,02	13,14	0,31	31	5
KQW2	NA	NA	NA	NA	NA	5,85	11,29	0,25	25	5
KQW3	NA	NA	NA	NA	NA	14,28	10,39	0,31	31	5
KW1	2,45	1,14	0,45	45	0,1	5,31	8,88	0,21	21	5
KW2	3,76	3,43	0,53	53	0,1	5,07	4,84	0,27	27	5
KW3	NA	NA	NA	NA	NA	5,88	13,38	0,13	13	5

Tab. 2. Vegetation structure (n-n_H_mean=mean height of *A.altissima*'s tree (cm); n-n_D_mean=mean diameter of *A.altissima*'s trees (cm); n-n_density=density of *A.altissima*'s trees; n-n_N=number of *A.altissima*'s trees; n-n_C=canopy cover of *A.altissima*'s trees; n_H_mean= mean diameter of native trees (cm); n_D_mean=mean height of native trees (cm); n_density=density of native trees; n_N=number of native trees; n_C=canopy cover of native trees. NA=no data due to the lack of native/non-native trees in the selected plots.

Plot	oil_skeleto	_fine_con	oil_humidi	_bulk_den	oil_porosit	soil_mwd	soil_name
AB1	74,06	5,67	17,56	2,11	20,27	2,00	RED1
AB2	0,00	38,54	18,63	1,02	61,46	7,05	RED3
AB3	0,00	39,08	16,17	1,04	60,92	2,58	RED3
AG1	48,20	28,35	9,71	2,03	23,45	5,09	RED1
AG2	10,68	17,94	34,00	0,76	71,38	2,01	RED3
AW1	0,33	26,34	35,30	0,71	73,34	10,22	RED3
AW2	0,33	24,10	32,35	0,65	75,58	4,62	RED3
AW3	0,00	31,45	33,03	0,83	68,55	5,48	RED3
KB1	23,38	38,81	20,51	1,65	37,81	2,97	RED2
KB2	39,25	15,92	37,51	1,46	44,84	2,06	RED2
KB3	63,65	16,33	21,67	2,12	20,02	2,65	RED2
KG1	56,23	28,27	19,10	2,24	15,50	2,74	RED2
KG2	44,75	22,91	18,37	1,79	32,34	2,50	RED2
KG3	64,23	17,39	16,21	2,16	18,38	2,50	RED2
KQW1	0,00	37,76	26,86	0,70	62,24	10,33	RED1
KQW2	90,98	-19,13	55,46	2,74	28,15	2,47	RED1
KQW3	63,02	-20,68	31,59	1,12	57,66	8,68	RED1
KW1	0,00	27,64	32,56	0,73	72,36	6,63	RED3
KW2	0,30	29,14	34,17	0,78	70,56	6,47	RED3
KW3	8,58	40,94	20,17	1,31	50,47	5,59	RED2

Tab. 3. Soil parameters.

Chapter 2

Seasonal and spatial diversity of carabid beetles (Coleoptera: Carabidae) in North Adriatic Karst (North eastern Italy)

1. Introduction

Annual activity pattern are an important part of insect life cycles (Niemelä et al., 1989), and the study of these life cycles are important in both applied and theoretical biology (Matalin, 2007). Life cycles of carabid beetles are among topical problems of ecology, since their annual rhythms are adapted to the seasons (Timm, 2010) and their specific development reflect many environmental parameters and can serve as their markers (Khobrakova and Sharova, 2005).

The first classification of the reproductive rhythms of carabid beetles was elaborated in 1939 by Danish entomologist Sven Larsson: it was based on a careful analysis of museum collections, primarily representing the Danish fauna. Larsson studied the material of more than 270 carabid species caught by pitfall trapping. Using the three parameters of reproduction time, period of imaginal activity and developmental time, he established six groups among the ground beetles. The proportions of "spring" and "autumn breeders" were about three to one, while biennial development was established for two species only (Matalin, 2007). After him, Thiele (1977) recognized six types of annual reproduction rhythms in Carabidae: 1) spring breeders without larval dormancy either with obligatory dormancy in the adult (parapause), mainly governed by photoperiod or 2) facultative dormancy in the adult, governed by photoperiod (photoperiodic quiescence); 3) autumn breeders with larval hibernation, or parapause, either without dormancy in the course of adult development or 4) with adult photoperiodic aestivation or parapause; 5) species with unstable conditions of hibernation and potentially lacking dormancy; or 6) species requiring two years to develop (Matalin, 2007).

As later stated, the following subdivision of carabids into adult-overwinterers (hibernate as adults) and larval-overwinterers (hibernate mainly as larvae) is more appropriate (Paarman, 1979; Houston, 1981; Andersen, 1984; Loreau, 1985; Butterfield, 1986; Refseth, 1988; Niemelä et al., 1992; Sharova & Denisova, 1997). Paarman (1979) presented a model for the possible evolution of reproduction types. The low capacity of the larvae to survive periods of desiccation (e.g., Paarmann, 1966; Thiele, 1964) seems to be the main factor affecting reproduction types in different habitats. In the dry habitats of the temperate and boreal climate zone, autumn breeders with winter larvae are the predominant type (Timm, 2010). Moreover, not only methods but also sampling activity

throughout all the year are known to be essential in order to obtain a detailed spectrum of species and to obtain a comprehensive knowledge on the existing fauna, as a prerequisite for any nature conservation strategy (Timm, et al., 2008; Timm, 2010).

Following the methodology of Errouissi et al. (2009) in our study we wanted to investigate show seasonal pattern of carabid beetles in the North Adriatic Karst, in a mosaic of native and non-native (*Ailanthus altissima*) vegetation, by calculating and comparing monthly data for (1) species diversity (Shannon-Wiener index H'), (2) species turnover between monthly assemblages (Whittaker's β-diversity index: β_W), and (3) evenness (equitability, Pielou's index).

2. Materials and methods

For the study area and the sampling methods see the Chapter 1.

2.1. Data analysis

We investigated the numbers of carabid beetle species and their distribution patterns on different spatial and temporal scales. We evaluated the most abundant species based on the abundance of the species during the sampling period. We divided the species into four quartiles, based on the cumulative abundances caught. The main abundant species are defined as those with more than 25% of cumulative abundance among all of them.
The Pearson rank correlation test was used to test correlations between monthly Temperature (°C), Humidity (kg/m^3), Rainfall (mm) and Carabid beetles abundances. These data were extracted from weather station situated in the locality Doberdò del Lago (GO) (http://www.osmer.fvg.it/home.php).

To compare assemblages, the monthly variation (from April to November; December and January are pooled together in Winter) was described by parameters that include species richness, monthly changes in assemblages, composition and diversity. We used total-year catches with permanent traps, because they represent relative densities (Judas et al., 2002). The species diversity was calculated using the H' Shannon-Wiener information index (Pielou, 1975):

$$H' = -\sum_{i=1}^{s} P_i \ln P_i$$

where Pi is the proportion of the sample represented by i^{th} species (i = 1–S). Equitability was represented by $J=H'/H_{max}$ (Pielou, 1975). Species richness (S) represented the number of species belonging to each samples where one or more individuals were found (Pielou, 1975).

Species abundance (average per trap/months) was log(x+1) transformed.

Later, a cluster analysis, performed as in Kutasi et al. (2006), based on Jaccard coefficient and UPGMA clustering method was performed to determine the similarity between months (April-November) and carabid beetles abundances. Then, we performed a cluster analysis based on Bray-Curtis coefficient and UPGMA clustering method using carabid beetles abundances and sampling sites.

Spatial species turnover rates among sites and predefined groups of sample sites by UPGMA were estimated by Whittaker's β-diversity index (β_W; see Hammer (2012) for formulae). The beta diversity index of Whittaker expresses the change in the fauna (turnover) over time, i.e., between monthly assemblages (temporal scale):

$Ew= (S/D) – 1$

Where S is the total number of species in assemblages and D the average number of species observed within assemblages. Ew varies between 0 (identical species composition) and 1. The evenness or equitability E was calculated as below:

$E = H'/\log_2 N$

Where N is the number of species.

All statistical analyses were performed with SPSS Statistical package ver. 20.0 (SPSS inc., 1989, 2011) and program PAST (Palaeontological Statistics; Hammer 2012).

3. Results

The pooled sample for the trapping period (May 2014 – January 2017) included 3,119 carabid beetles belonging to 28 species (Fig. 1). Carabid beetles abundance were positively correlated only with Temperature (p-value=0.00664) in the study area, and not with Humidity and Rainfall.

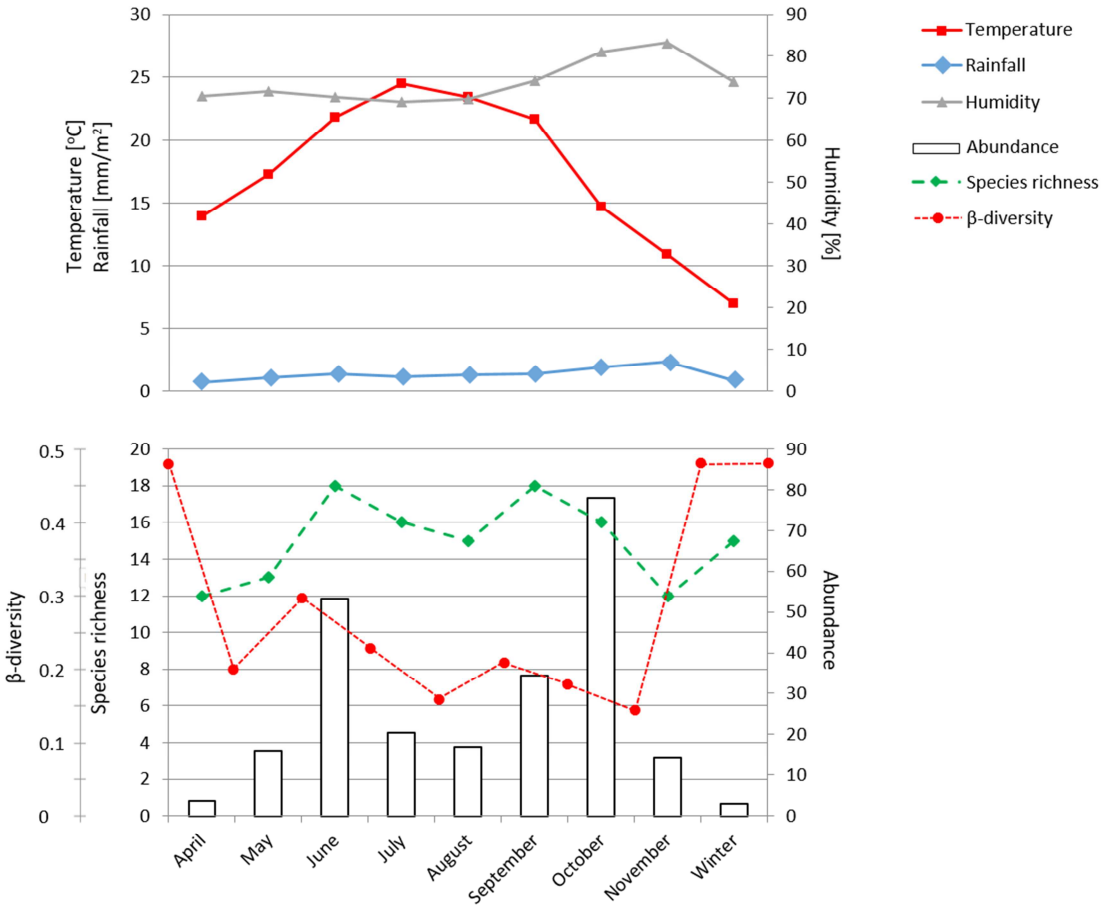


Fig. 1. A: Mean monthly temperature (°C), rainfall (mm), Humidity (%). B: Temporal variation in abundance, species richness and beta diversity of carabid beetles trapped during the course of the year.

3.1. Species assemblages

The lowest value of Diversity (H') and Evenness (E) indices were obtained for October, due to the absence of 12 species out of 28 and for the abundance of *Carabus coriaceus* dominating the species assemblages; the maximum of H' is for July and for E is April. The highest beta diversity values were found between May and June (0.29) and November-April (0.25).

3.2. Seasonality and temporal coexistence

The seasonal changes in abundance of the 17 most abundant species are shown in Fig. 2 (A and B) as relative frequency distributions.

Almost all (15) of the 17 more abundant species were univoltine, exceptions being *Carabus* (*Procrustes*) *coriaceus coriaceus* and *Calathus* (*Calathus*) *glabricollis* with two periods of emergence (spring and autumn) and *Laemostenus* (*Antisphodrus*) *elongates* and *Molops ovipennis istrianus*, mostly active from spring to autumn.

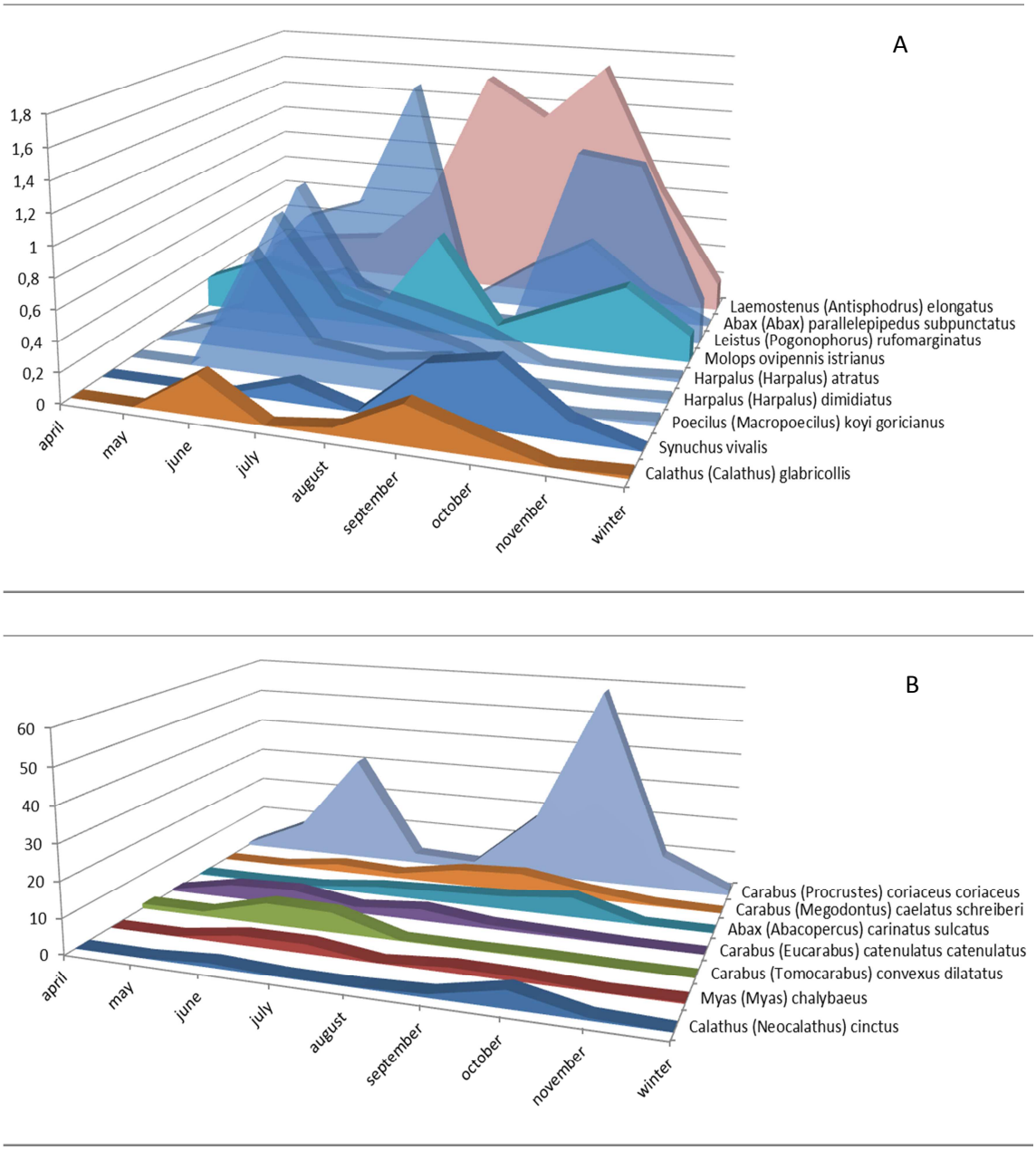
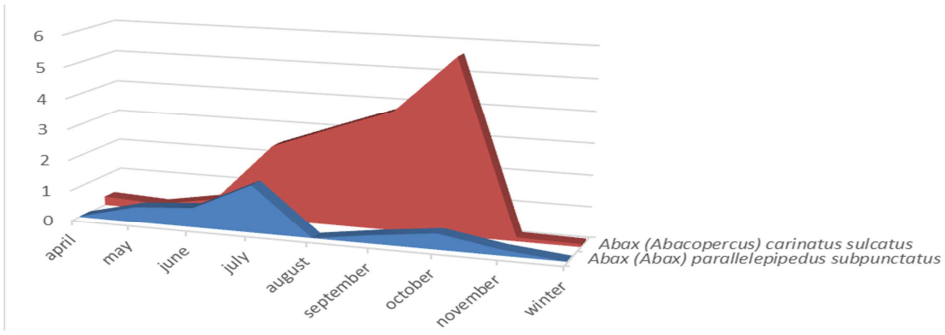
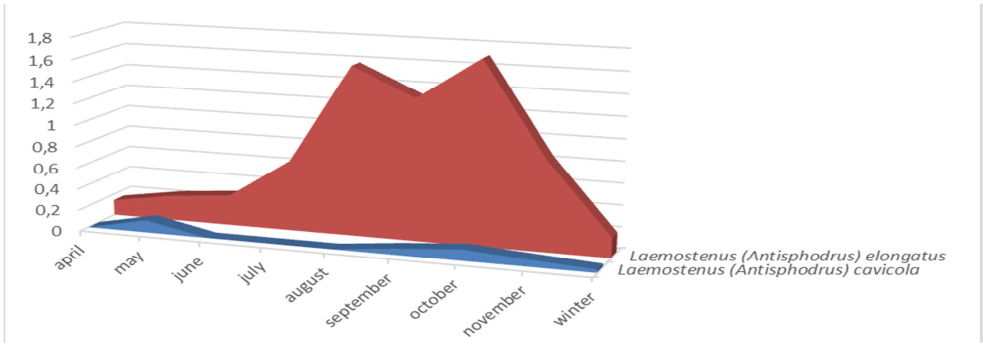
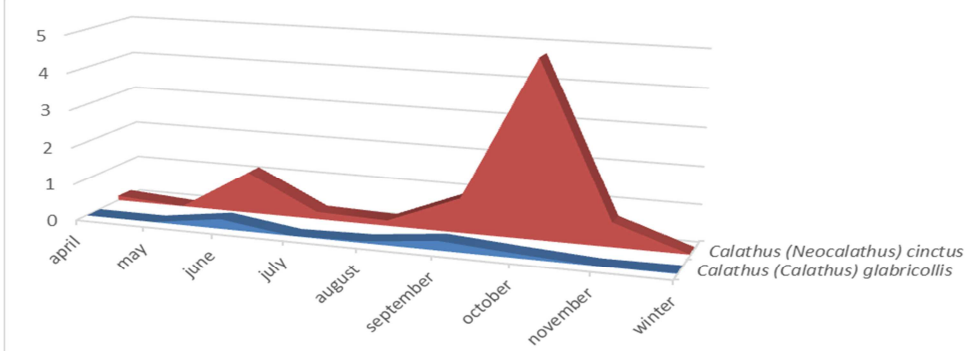


Fig. 3. A, B. Seasonal segregation of carabid beetles species. X-ordinate: time (months from April to November; December and January are pooled together in Winter); Y-ordinate: abundance of carabid beetles. Species are divided in two graphs (A) and (B) in relation to their abundance.

We select a subset of the species belonging to the same genus (Fig.4), in order to observe different seasonality in the species belonging to the genera *Carabus*, *Abax*, *Laemostenus*, *Calathus*, *Harpalus*. In particular, *C. coriaceus* shows two conspicuous peaks, one in spring and another in autumn, while *C. convexus dilatatus* and *C. catenulatus catenulatus* have one pick in spring, and *C. caelatus schreiberi* in late summer. *Abax carinatus sulcatus* was found with many individuals from June to November, with a pick in autumn, whereas *A. parallelepipedus subpunctatus* shows two peaks, in early summer and in autumn. *Laemostenus cavicola* shows two clear peaks in early spring and in autumn, even if only few individuals were collected among seasons, while the congener *L. elongatus* is mostly active from the beginning of the summer until the end of the season. *Calathus cintus* and *C. glabricollis* overlapped temporally, and the same happened for *Harpalus atratus*, *H. dimidiatus*, *H. serripes* and *H. rubripes* (these last two species were not considered as abundant species, but since it was possible to identify their abundance peaks, they were put together with the other two species).







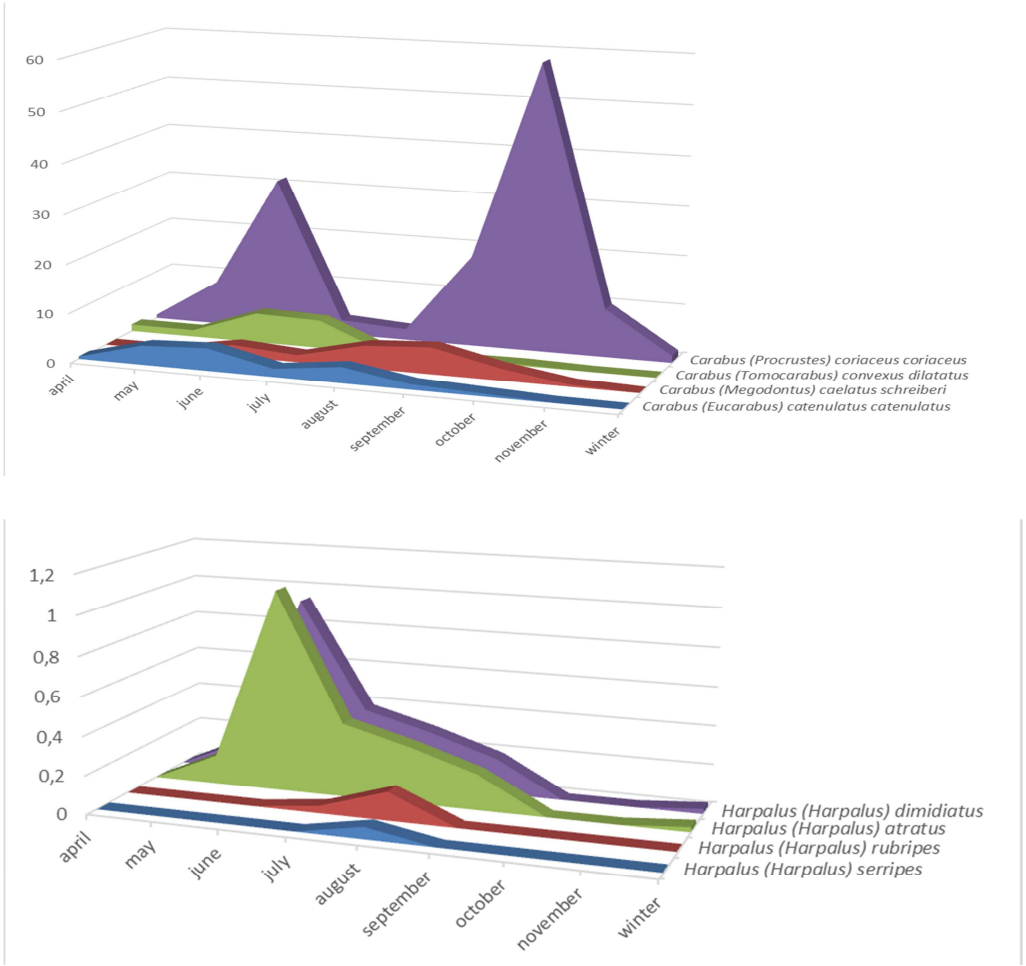
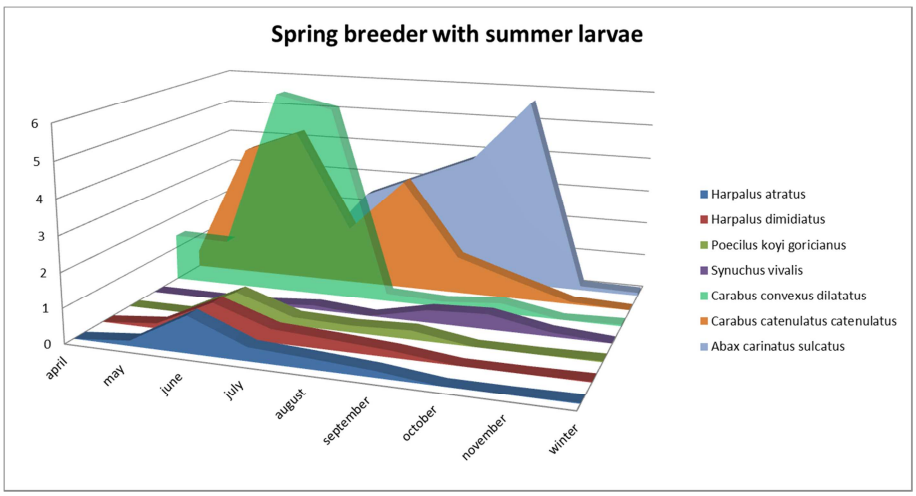


Fig. 4. Seasonal occurrence of the species belonging to the same genus. Species are divided in two graphs (A) and (B) in relation to their abundance. X-ordinate: time (months; from April to November; December and January are pooled together in Winter); Y-ordinate: abundance of carabid beetles.

In Fig. 5 it is possible to see the difference pattern in the "autumn breeder" and in the "spring breeder", with the major activity of the spring breeder concentrated in the first part of the graph (spring/early summer), whereas the major activity of the autumn breeder is summer/autumn.



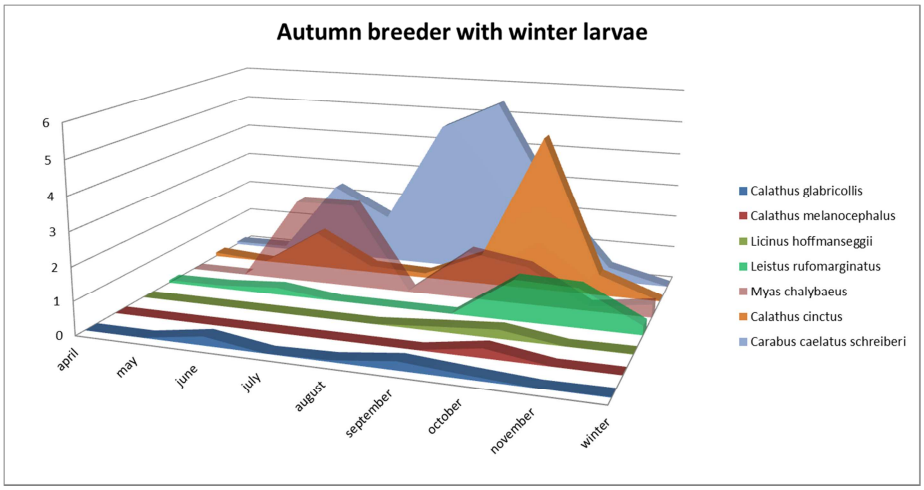


Fig. 5. Abundances of the Spring breeder and Autumn breeder. X-ordinate: time (months); Y-ordinate: abundance of carabid beetles.

Cluster analysis (Fig. 6) identified three distinct trends of carabid beetles abundance based on monthly changes of species abundances: (i) autumn-winter period (October-November); (ii) spring period (May-April); (iii)summer period (June-September).

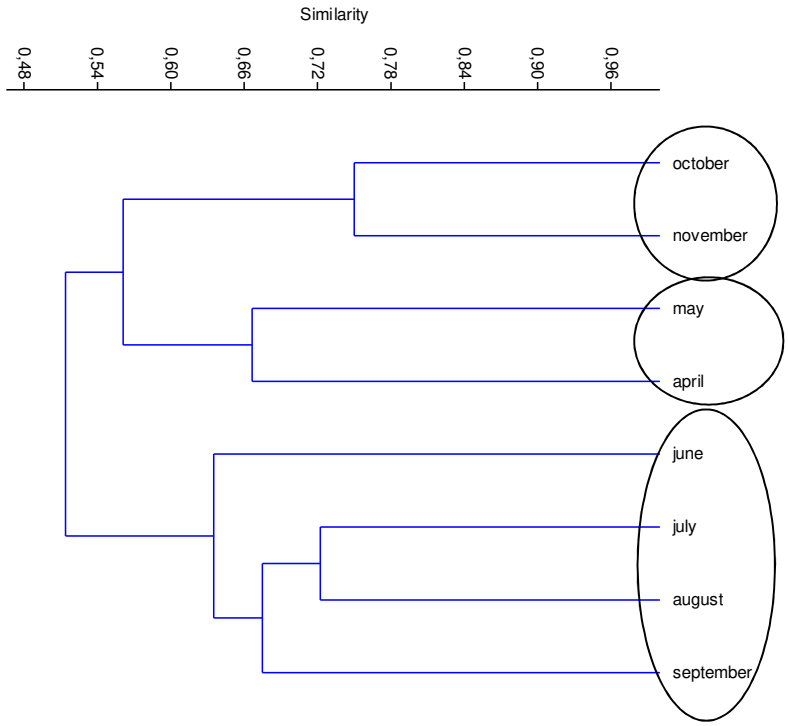


Fig. 6. Classification of the monthly abundance of species based on Jaccard index, showing three main groups.

3.3. Spatial diversity

Considering data referred to the different habitat types, the highest values of Diversity (H') and evenness (E) indices were obtained in native wood assemblages (KW, KQW) followed by non-native bushes and woods (AB, AW); the lowest value were found in native bushes (KB) and grassland (KG) (Fig. 7). The highest beta diversity values, indicating the change in the fauna during the months of the year in each investigated habitat, were found in native grassland (0.67) and the lowest value in non-native woods (0.11) compared with all the other habitats (Fig. 8).

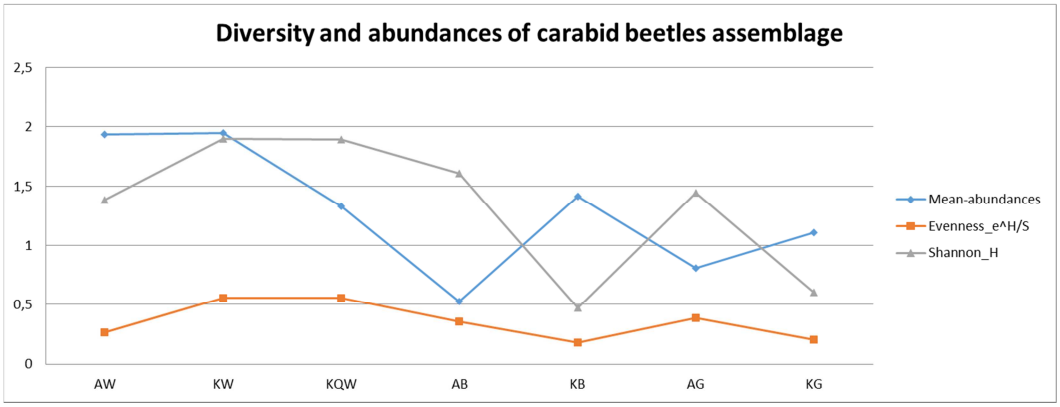


Fig. 7. Mean abundances and diversity indices of the carabid beetles assemblage in the study area. X-ordinate: habitats (plots); Y-ordinate: abundance.

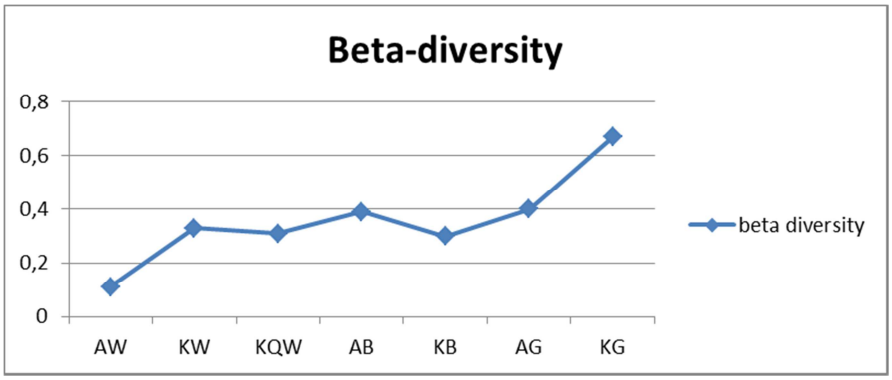


Fig. 8. Beta diversity indices for the different habitats.

As shown by the spatial distribution of the species (Fig.9), the highest turnover rates are found between grassland habitats (both native and non-native) and doline woods habitats (native and non-native) (KG-KW=0.71; KG-AW=0.67; AG-KW=0.65; AG-AW=0.62) and is the lowest between native dolines wood and non-native dolines wood (KW-AW=0.11). Native grassland and woods (KG-KQW=0.62; KG-KW=0.71) reveal almost the same spatial turnover of non-native grassland and woods (AG-AW=0.62); between native and non-native grassland there is also a spatial turnover (AG-KG=0.40) very similar to the one of native and non-native bushes (AB- KB =0.39). There is a

higher spatial turnover in native wood and non-native wood (AW-KQW =0.33) respect to native dolines wood and non-native wood (AW-KW=0.11), and this last value is the same than in native wood in dolines and native wood (KW-KQW=0.33).

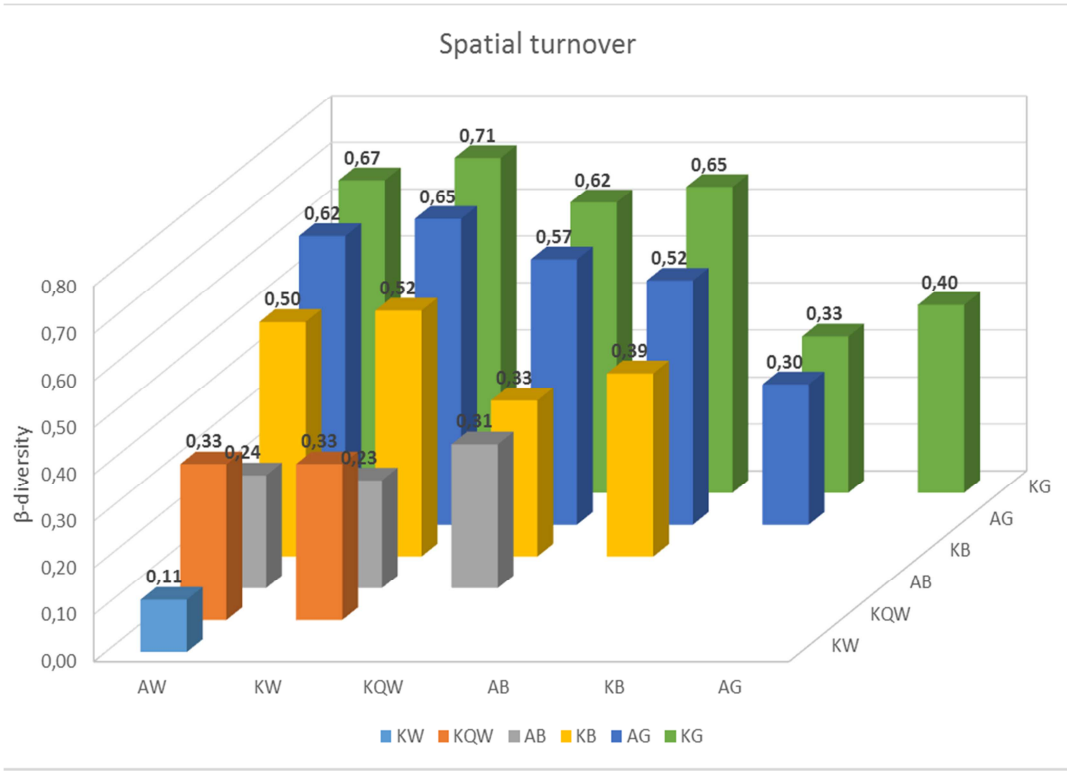


Fig. 9. Spatial species turnover (beta diversity) rates between sampling sites as defined by UPGMA carabid beetles.

Comparing the average beta diversity (Fig.10) in all investigated habitats, it is possible to see that native habitats (K) have always a higher beta diversity respect to the comparable non-native ones (A).

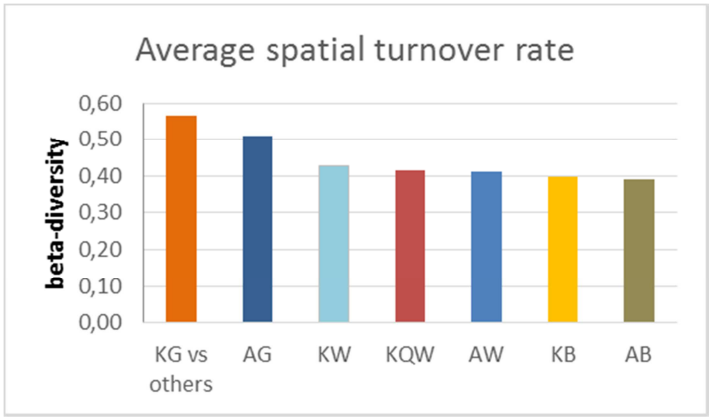


Fig. 10. Average spatial turnover rate between habitat type. For each habitat type the average of beta diversity values calculated versus the other habitats is reported.

Cluster analysis of the habitats based on ground beetles abundance (Fig.11) identified three distinct groups of habitats : (i) native and non-native doline woods (AW-KW); (ii) native mature woods (KQW); (iii) native and non-native bushes (AB-KB), native and non-native grasslands (AG-KG).

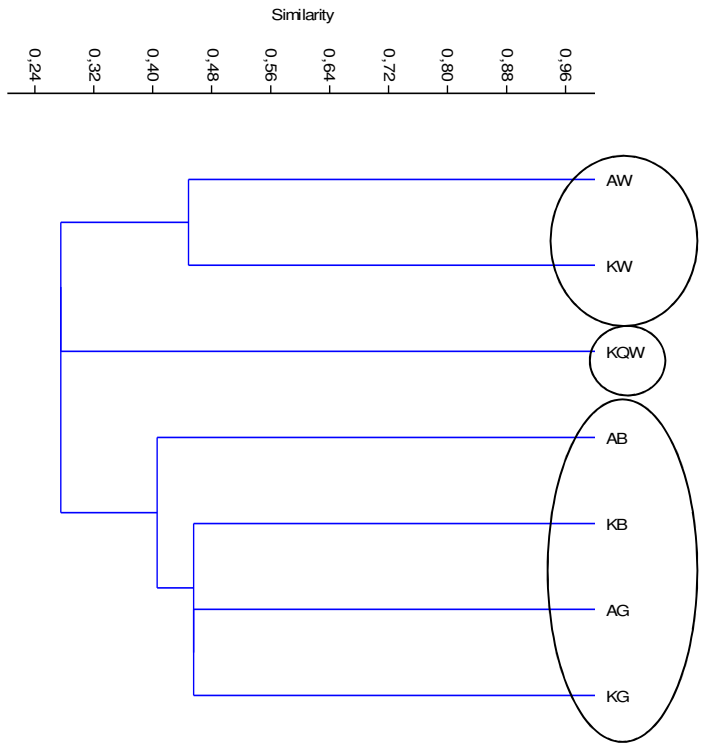


Fig. 11. Similarity of the habitat type abundances of species based on Bray-Curtis index, indicates four distinct groups: (i) native and non-native dolines woods (AW and KW); (ii) native mature woods (KQW); (iii) non-native and native bushes, native and non-native grasslands (AB, KB, AG, KG).

4. Discussion and conclusions

4.1. Species abundance and assemblages

Our study indicates that carabid species responses to habitat type with grouping together all the carabid assemblages belonging to the three woods habitats, that were more similar to each other respect to those in the semi-open and open habitats (Fig. 11). This result is consistent with the vegetation structure, microclimate and edaphic characteristics of the different habitat typology.

Closed habitats, i.e. woody and bush sites, supported more abundances and species diversity than open habitats.

Our results also indicates that native wood assemblages had higher diversity and equitability than non-native woods, whereas in bushes and grassland we found an opposite result, with native habitats showing less diversity and equitability than in the non-native ones. Considering native and non-native wood plots, it is likely that native woods had a higher structural and biotic heterogeneity than non-natural wood. This heterogeneity might contribute to the higher diversity of carabid assemblage, as found by Yu et al. (2006).

4.2. Temporal diversity

Seasonality is an important component of most biodiversity assessments. If the aim of a biodiversity study is a complete inventory of a specific higher taxon, a researcher should not only plan where (e.g. physical location, elevation, etc.) and how (e.g. collection methods) optimize the number of species sampled, but also when (e.g. time of year) collections should take place (Maavety et al., 2013). Resource availability (e.g., food, habitat requirements), temperature and rainfall are the three main factors that can influence seasonality in insects (Wolda, 1988), while phenological adaptations (e.g., diapause, adult longevity, breeding times, larval development and movements during lifecycles) during different times of the year is connected with the increasing number of species in an area (Rainio, 2013).

Factors regulating life cycles of carabid beetles species in extra-tropical regions are mostly regulated by temperature and photoperiodicism (Thiele, 1977; Sota, 1985; 1994; Lövei & Sunderland, 1996), as confirmed by our results on the distinctive seasonal activity peaks of carabids. In carabids, these peaks usually coincide with reproduction (Niemelä et al., 1989). In the present research, almost half of the species showed prominent abundance in spring or in autumn, in according with Niemelä (1989), that considers a single peak pattern (in spring or autumn) in Europe. Conversely, the two most abundant species found in our study, *C. coriaceus* and *C. glabricollis,* showed two peaks during the season. We found higher abundance of carabid beetles in October, as reported by Timm (2010) for Israel, with a smaller peak in May. This fact is in contrast with Paarmann (1970) that reported more abundances in April and May, and with Niemelä (1989) that reported a high decreasing in species abundances toward autumn. Niemelä et al. (1989) reveal that climate can cause differences among habitats and among geographic region influencing activity pattern, as was reported for the Finnish taiga, where they found flexible seasonal rhythm for several

dominant carabid species. The short growing season at high latitudes forces carabids to breed in early summer to ensure their offspring to have enough time to reach the adult stage before over-wintering (Niemelä et al., 1989; Sota 1994). The two-peaks activity (spring and autumn) in our study seems to be different to with Yu et al. (2006) that found a middle-season activity peak, probably due to optimal temperature and soil moisture found in their study area. In our study area we found a positive relationship between carabid beetles abundance and temperature (according to van der Drift, 1959; Niemelä, 1992; Judas et al., 2002) with the optimal conditions for carabids occurring in May and in September, as revealed also by the beta diversity. This fact is confirmed by the finding of almost the same proportion between winter and summer breeders in our plots. It is interesting to point out that in areas with low temperature excursion, development of spring breeders with summer larvae is accelerated by the increase of temperature (Thiele, 1979). Even if in October the species diversity was the lowest (almost half of the species were already absent), in this month there was the second assemblages peak, due to the most abundant species that showed higher abundances, as *C. coriaceus* that had its second peak .
Comparing temporal activity among the genus *Carabus*, the pattern of *C. coriaceus* and *C. convexus* were not corresponding completely respect with other studies: the start of the activity period for *C. convexus* was comparable with literature data, even if its reproduction in Hungary started earlier (mid-March) respect to elsewhere in Europe (Turin et al., 2003) and, as reported by Kádár et al. (2015), it is preceding *C. coriaceus.* The major activity of *C. convexus* in our study showed two peaks, the former in May and the latter in September, one months later than what observed in Kádár et al. (2015), where the second peak was in August, and conversely to the only one peak reported by Dülge (1994). We confirmed the "natural" fluctuation of *Abax parallelepipedus subpunctatus*, as already delineated by Andorkó & Kádár (2006). It was interesting to observe the synchrony in the peaks between *H. dimidiatus* and *H. atratus* and between *H. rubripes* and *H. serratus*, that are all phytophagous species.

4.3. Beta diversity turnover

Large variation in beta diversity within a series of sites influenced by the same ecologic descriptor indicates that habitats are not very similar in terms of species composition, so that local environmental pressures favored different groups of species (Baiocchi et al., 2012). Considering the micro-habitat quality indicated by differences in carabid beetles communities, non-native wood had less variation than native wood, which led us to hypothesize that in the former habitat carabid assemblages were more homogeneous than those of the latter. Evaluation of the similarity matrix between these habitat types supported this hypothesis, indicating the lowest beta diversity among

non-native wood. The same occurred for non-native grassland compared with native grassland, with the second habitat type showing more heterogeneity respect to the first one. It is possible to say that considering all the pairs of native and non-native habitats, the native ones show always a quite high beta diversity respect to the non-native.

4.4. Spatial diversity

Carabids responded to habitat structural differences in different ways depending on the spatial scale, and our catches demonstrate a succession of carabid assemblages along the vegetation gradient. As expected from earlier studies (Lindroth, 1961–1969; Thiele 1977), carabids were associated with broad habitat types, such as forest, shrub land (Yu et al., 2006) grassland habitats, and with mature or young forests (Szyszko 1990; Ings and Harley, 1999; Niemelä et al., 1996; Kinnunen, 1999; Koivula et al., 2002). Looking at the results of the cluster analysis, species could be divided into three groups according to their abundance in the different habitat type: closed-canopy species in dolines, closed-canopy species in mature wood, open and semi-open species in shrub land and grassland. Few of most 17 abundant species were common among habitats, as the generalist species *C. coriaceus coriaceus* and *C. convexus dilatatus*, whereas the majority of them was restricted to either forest and shrub sites or shrub and grassland. On a finer scale, our results also showed that forest fragments are often perceived as patchy environments for carabids, explaining the small-scale abundance variation of carabids (Koivula et al., 2002) as there were some species restricted to only one or two habitat types. Niemelä (1989) reported that carabid species may have abundance variation among different habitat types and that most of the species are habitat specialists in this sense, confirming our results. In fact, we found habitat selection among carabid species, as most of the carabids appeared to have forest, open or generalist habitat affinity.

The discovery of the highest species diversity in non-native woods and bushes can be explained, as already known for arthropod species richness in new habitats (Werling and Gratton, 2008), with the rule that when the species number tends to increase, then they level off with time as colonization and extinction processes drive it to equilibrium and local interactions that ‘‘sort’’ species (Price, 1997). The highest species abundances were found in woody habitats assemblages, where the species diversity reached also the highest values. Native bushes and grassland had always the lowest values of diversity. An interesting case is represented by the non-native bushes, that show quite high diversity, equitability and species number, comparable to wood habitats assemblages. This issue can be explained by the “edge effect” (Andorkó and Kádár, 2006) and the intermediate disturbance hypothesis (Connell, 1978) since this habitat typology is occupied by species

characteristic of open and woody habitats together. This reflect the typical condition of woodlands habitat. Desender et al. (2013) defined woodlands as a “disturbed habitats within a system” and in landscape represent buffered sites where the extremes of the environment have the least effect; for his reason they are richer in species, not because species do prefer them, but because individuals chose the least disturbed habitats. As already found in Bommarco and Fagan (2002) and in Andorkó and Kádár (2006), these habitats are important for the breeding period, the dispersal activity and the hibernating period of carabid beetles, and can contribute to the maintenance of the diversity of carabid communities (Andorkó and Kádár, 2006).

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Chapter 3

Ailanthus altissima affects trait composition and Functional Diversity of carabid beetles in a mosaic of native and non-native habitats in North Adriatic Karst

1. Introduction

The presence of a species in a given habitat is the result of the combination of habitat characteristics and of ecological traits deriving from the strategies that a species evolved to tackle its ecological constraints (Pizzolotto, 2009). These strategies are a fundamental part of evolutionary history of adaptation for a taxon (Ribeira et al., 2001). Considering species and their traits, the aim of research is to understand spatial and/or temporal phenomena affecting species distribution and abundances from community organization to patterns of biodiversity distribution and ecosystem functioning. Animal ecologists have a long tradition of species clustering based on ecological, rather than on taxonomic similarities (e.g. Root, 1967; Frenette-Dussault, 2013). In this sense, the trait-based approaches provide new perspectives to understand the diversity of forms and their functions within an ecosystem (Gravel et al., 2016), in community ecology (Litchman et al., 2007), in evolutionary, biological and biogeographical phenomena (Violle et al., 2007; Homburg et al., 2013). Thanks to the traits studies, it is possible to delineate the relative influence of abiotic and biotic drivers on species responses to disturbance pressures (Laliberté et al., 2010). Obtained results are not bound only to a specific study area but could be exported outside the investigated community (Bargmann et al., 2016). In applied ecology, functional traits are becoming the main ecological attributes that let different organisms and biological communities to influence ecosystem services (de Bello et al., 2010), and they represent a morpho-physio-phenological characteristic of an organism, directly connected to the organism's function, and with variation in the physical and biotic environment. In animals, these traits are connected with life-history, growth, reproduction, survival, behavioural and feeding habit traits (de Bello et al., 2010; Violle et al., 2007). Functional ecologists proposed the terms "functional traits" (Pizzolotto et al., 2016), and "functional diversity (FD)", that represents the range and distribution of functional traits values in a community (Lavorel et al., 2008). FD can be seen like a biodiversity component revealing functional interactions between organisms and their environment (Spake et al., 2015; Schirmel et Buchholz, 2013). FD is a very important parameter, since it goes beyond the traditional diversity concept of taxonomic richness of species communities, taking into account life-history and functional trait information and providing thus a useful approach to combine biodiversity research and ecosystem processes. In fact, functional diversity (FD) is a key driver of ecosystem processes (Hooper et al., 2005), ecosystem resilience to

environmental changes (Folke et al., 2004), and ecosystem services (Díaz et al., 2007; Laliberté, 2010).

Arthropods constitute the predominant, the most abundant and diverse faunal component of terrestrial habitats and for this reason they are considered good indicators of ecosystem integrity (Bargmann et al., 2016; Buchholz et al., 2012). They show a vast heterogeneity of functional traits, since they occupy a wide range of habitats and microhabitats, and they present several and different functional roles in ecosystem processes (Buchholz et al., 2012). Many ecological studies focused on carabid beetles (Coleoptera: Carabidae) as model organisms because they are the most diverse and abundant group of insects in most terrestrial ecosystems (Thiele, 1977).This taxon has a key role in many habitats, contributing to the ecosystem functioning with predation of other invertebrates and eating plant seeds (Kotze et al., 2011; Spake et al., 2015), and this ecological plasticity results in varied responses to anthropogenic disturbance (Zhang et al., 2017). Carabidae are also a well-known group from the taxonomic and ecologic point of view, suitable for standardized sampling, and sensitive to environmental change (Lövei & Sunderland, 1996; Rainio & Niemelä, 2003). For this reason, carabid beetles are often used to measure trait-environment interactions and traits sorting between habitats (Buchholz et al., 2012; Bargmann et al., 2016).

Many studies (Schirmel & Buchholz, 2013; Motard et al., 2015; Buchholz et al., 2015;) already tried to assess the impact of alien species on carabid beetles, focusing their attention on species assemblages, functional traits and functional diversity. In this work, we aimed to understand using FD how *Ailanthus altissima* colonization influences carabid beetles community composition along two environmental gradients: (1) habitat type measured as vegetation structure (grassland, shrubland, wood), and (2) *A. altissima* colonization on different vegetation type. The main target of this study was also to compare trait composition and FD of carabid beetle communities in native and non-native habitats, in order to evaluate differences among habitat types and to detect the key traits most affected by native and/or non-native vegetation. We expected to find less difference in FD among non-native habitats respect to native ones, and a more generalist species in non-native habitats respect to native ones.

2. Materials and methods

For study area and the carabid beetles sampling methods Chapter 1 of the thesis.

Trait selection criteria and classification

Carabid beetles were determined to species level according to the taxonomy and nomenclature of Italian Carabidae based on Vigna Taglianti (2004, 2005), with a few recent updates. We calculated Community-Weighted Means (CWMs) for 12 categorical traits (reproduction rhythm, chorology, wing morphology, flight ability, feeding preference, overwintering state, moisture preference, main period of activity, main period of emergence, adult locomotion, morpho-ecological larva types, adult habitat affinity) and one continuous trait (body length) to describe changes in trait composition (Hanson et al., 2016) depending on *A. altissima* colonization. CWMs for ground beetles were calculated using the following species coding:

i) fa = flight ability (poor = 1, good = 2, no flight = 3); species with poor flight ability included those with no or reduced wings, dimorphic species and those for which flight observations are missing or rare, while species with good flight ability included those with fully developed wings and those for which flight has been documented;
ii) diet = major diet main components (1 = collembola, 2 = earthworm, 3 = lepidoptera, 4 = shell-bearing snails, 5 = generalist predator, 6 = mixed/omnivore, 7 = mostly plant matter, seeds and phytophagous;
iii) ows =overwintering strategy (adult = 1, larvae = 2, both=3);
iv) mpe = main period of emergence (1 = spring, 2 = summer, 3 = autumn);
v) mpa = main period of activity (1 = spring, 2 = summer, 3 = autumn, 4 = whole year),
vi) LCD = life cycle (1 = 1 year, 2 = 2 years);
vii) moist = moisture level of preference (1 = hygrophilic, 2 = heterophilic, 3=xerophilic);
viii) aha= Adult habitat affinity = (Forest = 1, open = 2, generalist, eurytopic species = 3);
ix) loc = locomotion (1 = runner, 2 = pusher, 3 = digger, 4 = climber)

x) lmt = larval morpho-ecological type (1 = spermophagous, 2 = soil pore explorers, 3 = surface runners, 4 = surface walkers, 5 = parasitoid);
xi) repr = reproduction (1 = spring breeders with summer larvae (s), 2 = autumn breeders (w) with or without adult estivation, 3 = two year breeders (yy); 4 = aperiodic (ap);
xii) chor = chorology type (1 = Regional endemic species, 2 = Central-montane European species, 3 = European species, 4 = Euro-Asiatic, Euro-Siberian species, 5 = Palaearctic, Holoarctic,

Circumpolar species);
xiii) wing = wing morphology, a morphometric trait measured through metathoracic wing length (1 = macropterous, 2 = pteridimorphic, 3 = brachypterous);
xiv) dim = body length (2.75-35.5).

The functional trait categorization on each species identified within the study was based on information from Porta (1923-1934), Bargmann et al. (2016), Mazzei et al. (2015), Pizzolotto et al. (2016), Hanson et al. (2016), Lindroth (1992; 2007), Desender (1989), Luff (2007), Thiele (1977), Zetto Brandmayr et al. (1998); Brandmayr et al. (2005); Löser, 1970, Lampe (1975), within the constraints of data availability. Other traits that could have functional or ecological importance were not used due to lack of information for most of the species. Where information about the trait was missing, the NA code in the data matrix was used.

Community-weighted mean trait values are considered as the main predictors of ecosystem functioning (De Coster et al., 2015; Gagic, 2014) and have been shown to be useful to assess community dynamics and ecosystem properties (Garnier et al., 2007; Bargmann et al., 2016; Hanson et al., 2016; Ribera et al., 2001; Lavorel et al., 2008; Spake et al., 2016; Buchholz et al., 2012).

Prior to statistical analysis the data sets of each site were pooled over the season and the abundance data was used to calculate CWM traits and the resemblance matrix for species composition. For each trait, CWMs were calculated by summing the products of the species trait value and the corresponding species abundance, dividing the sum of the products by the total carabid beetles abundance. Species composition was analyzed based on un-transformed abundance values and Bray-Curtis similarity.

Permutation analysis of variance (PERMANOVA) (Anderson, 2001) was used as in Hanson et al. (2016) on selected trait composition and species assemblages of carabid beetle communities to test for the random factor “location” and the fixed factors “*Ailanthus*” and “habitat type” and their interactions. A multivariate model was used to identify model terms for follow-up tests of individual traits. The traits were then used to calculate a resemblance matrix based on Bray-Curtis distance in multivariate global test and Euclidean distances on single trait. All *p*-values are derived from 5000 residuals permutations under a reduced PERMANOVA model (Anderson, 2001). The following models for individual traits were then only performed for model terms being significant in the multivariate global test. For tests of individual trait variables, standardization across traits was not needed and raw traits were used. All other model specifications are identical to the multivariate

model. PERMANOVA post-hoc tests were performed to compare individual levels of the factor "*Ailanthus*" and "habitat type" where factors were significant in the main model. All statistical analyses were performed using the software Primer-E v6 (Clarke, 2006) and the PERMANOVA+ add-on (Anderson et al., 2008).

Non-metric multidimensional scaling (NMDS) was utilized to examine community composition of carabid beetles for the six habitat types (Wood=KW+KQW; Wood_A=AW; Shrub=KB; Bush_A=AB; Grassland=KG; Grassland_A=AG) using the software Primer-E v6 (Clarke, 2006). NMDS ordination is based on square root transformed data and Bray-Curtis similarity.

We calculated the functional diversity as Rao's quadratic entropy (FD_Q; Rao, 1982; Botta-Dukát, 2005), a multi-trait FD metric describing the variation of species trait composition within the community (Spake et al., 2016). It sums pairwise distances between species in a community weighted by their relative abundances. We calculated FD_Q for each site belonging to different habitats. Functional distances between species were calculated using Gower's distance metric, which allows for a mixture of continuous, ordinal, and categorical variables, and accommodates missing trait values (NAs) (Laliberte and Legendre, 2010; Sonnier et al., 2014). Continuous trait data were scaled by range to assign equal weighting among traits (Botta-Dukát, 2005). Tukey HDS test was used to compare the observed means of CWMs in the plots. FD_Q possesses all the necessary properties of a FD index including its representation of the range of character values and its ability to be relatively unaffected when a minor species with an extreme character value decreases in abundance (Botta-Dukát, 2005). It has widespread use and has been shown to successfully identify habitat filtering patterns (Spake et al., 2016).

Moreover, we evaluated carabid beetles community with regard to the functional diversity (De Coster et al., 2015): for each trait, we calculated the Bray–Curtis dissimilarity index on the sites-by-species matrix containing the trait values standardized by the species' abundances. We performed this analysis with Mantel tests on distance matrices of CWM, and beetles assemblages were used as non-parametric tests of association between single species and trait composition within sites (Legendre and Legendre, 1998). The method is based on resemblance matrices that contain all the pairwise distances between sampling locations. For species abundance data, Bray-Curtis distances was used to calculate distance matrices (Leduc et al., 1992). The statistical significance for Mantel tests was assessed by Spearman's rank correlation *rho* with 999 permutations (Barbaro et al., 2007).

A simple linear model was used to quantify the effects of environmental variables on carabid FD.

Two predictors were extracted from previous studies (e.g., Hanson et al., 2016) as the most important variables influencing carabid species richness: cover of native and non-native vegetation and soil porosity were include in this study as explanatory variables, tested as abiotic drivers of carabid beetles, included in the study.

All statistical analyses were computed in in R version 2.15.3 (R Core Team, 2013).

3. Results

Similarity relationships among ground beetle communities were visualized using two-dimensional NMDS ordination (Fig. 1). NMDS was calculated on a Bray-Curtis similarity matrix. Plots tended to group according to vegetation structure (open-intermediate-close vegetation). The typical Karst Wood (KQW) plots clustered together on the extreme left of the NMDS. Woods in doline, native (KW) and non-native (AW) woods, are closely grouped in the middle of the ordination, not far from non-native bushes (AB). Two plots (AG2 and KB3) were found grouped away from all other plots. Grassland with *A. altissima* (AG1) pulled closer to the Karst habitats (KG and KB), that grouped closely on the right of the ordination.

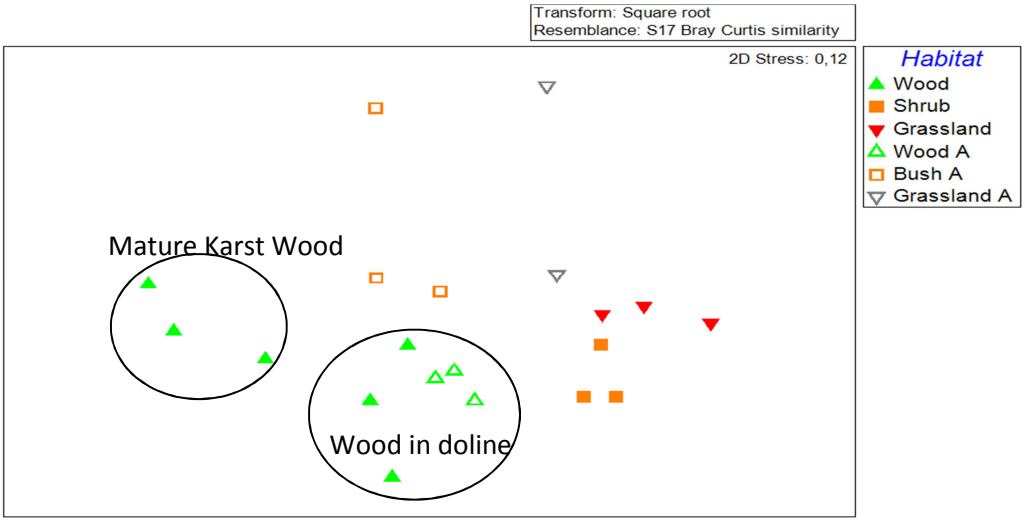

Fig. 1. NMDS. Diagram of non-metric multidimensional scaling (NMDS) performed on Bray-Curtis similarity matrix of ground beetle communities.

3.1. Functional traits and CWM diversity in carabid beetles between habitats

The species trait composition of carabid beetle communities (Appendix 1) differed significantly between the "habitat type" ($F_{2,20}$=5.8305, *p*-value=0.0014) and "*Ailanthus*" ($F_{1,20}$ =3.19, *p*-value=0.0252) without a significant interaction between both factors ($F_{2,20}$=1.6945, *p*-value=0.111).

Focusing on species traits (Table 1; for PERMANOVA results see Appendix 2), size trait had significant difference between grassland and wood, with small-sized taxa dominating in open areas ($F_{2,20}$=2824, *p*-value=0.0066), whereas large-size taxa dominated in closed areas ($F_{2,20}$=2.2361,p-value=0.0522). Generalist predator is the most common diet trait in the all sampled area, omnivorous and phytophagous traits were positively correlated to the presence of *A. altissima* (omnivorous, $F_{1,20}$=6.3602, p-value=0.0256; plant diet, $F_{1,20}$=4.6667, p-value=0.053). Hydrophilic and heterophilic moisture species were proportionally more abundant in wood habitat as compared to grassland (hydrophilic, $F_{2,20}$=8.5921, p-value=0.0046; heterophilic, $F_{2,20}$=14.151, $F_{2,20}$=0.0008), while xerophilic species traits were influenced by the presence of *A. altissima* ($F_{1,20}$=4.8678,p-value=0.045). There was a higher proportion of species with flight ability in grassland compared to wood ($F_{2,20}$=2.73806, p-value=0.021), and brachypterous species were more abundant in wood than in bushes ($F_{2,20}$=3.6604, p-value=0.003) and in wood respect to grassland ($F_{2,20}$=6.0133, p-value=0.0002). Species preferring forest habitats were proportionally more abundant in wood respect to bushes and grassland (wood-bush, $F_{2,20}$=3.5562, p-value=0.0038; wood-grassland, $F_{2,20}$=8.5056,p-value=0.0004), and there was a significant difference in generalist and open habitat preference traits in relation with the presence of *A. altissima* (generalist, $F_{1,20}$=4.1667, p-value=0.0602; open, $F_{1,20}$=5.5601, p-value=0.0358). The proportion of carabid beetles locomotion pusher was significantly higher in wood compared with bushes and with grassland ($F_{2,20}$=3.1024, p-value=0.0078; $F_{2,20}$= 6.2368, p-value=0.0006) and were influenced by the presence of *A. altissima* ($F_{1,20}$=5.0556,p-value=0.0398). Surface walkers larva morpho-ecological trait was more related with wood habitats than with grassland habitats ($F_{2,20}$=5.0556, p-value=0.0398), and soil pore explorer and surface runner were related with *A. altissima* presence from wood to grassland habitat (soil explorer, p-value=0.0036)(surface runner, p-value=0.0568). In Fig. 2 it is possible to see the trend of each trait among the different habitat typology.

	Factor 1	Factor 2	interaction
	Ailanthus altissima	habitat type	Ailanthus x habitat type
All traits	*	*	
Diet	°	*	
collembola		*	°
earthworms		°	
lepid			
snails			
predator generalist			
omn	°	°	
plants	*		*
Reproduction rythm	*	*	
w	*	*	
s			*
yy		*	
ap	*	*	
inst			
Wings morphology	°	*	
b		*	
d	*		
m			
Flight ability	*	*	
poor			
good	*	*	
not flight	*	*	
Moisture preference		*	
hydrophilic		*	
heterophilic		*	
xerophilic	*		
Habitat		*	°
forest		*	
open	*		*
generalist	*		°
Larva morphoecological type		*	
pusher			
digger			
climber	*	*	*
soil pore explorer		*	*
surface runner		*	°
surface walker		*	
parasitoid	°	°	°
Locomotion		*	
Pusher	*	*	
Size	*		
3		*	
4		*	
6	*	*	
7	*	*	
Chorology	*	*	
1		*	
3		*	
4	*	*	*
5	*	°	
*=p-value<0.05			
°=p-value<0.07			

Table 2. The trait composition of carabid beetle communities with the two factors (*Ailanthus altissima* and habitat type). Only significantly traits (p-value<0.07) were reported. *=p-value<0.05; °=p-value<0.07.

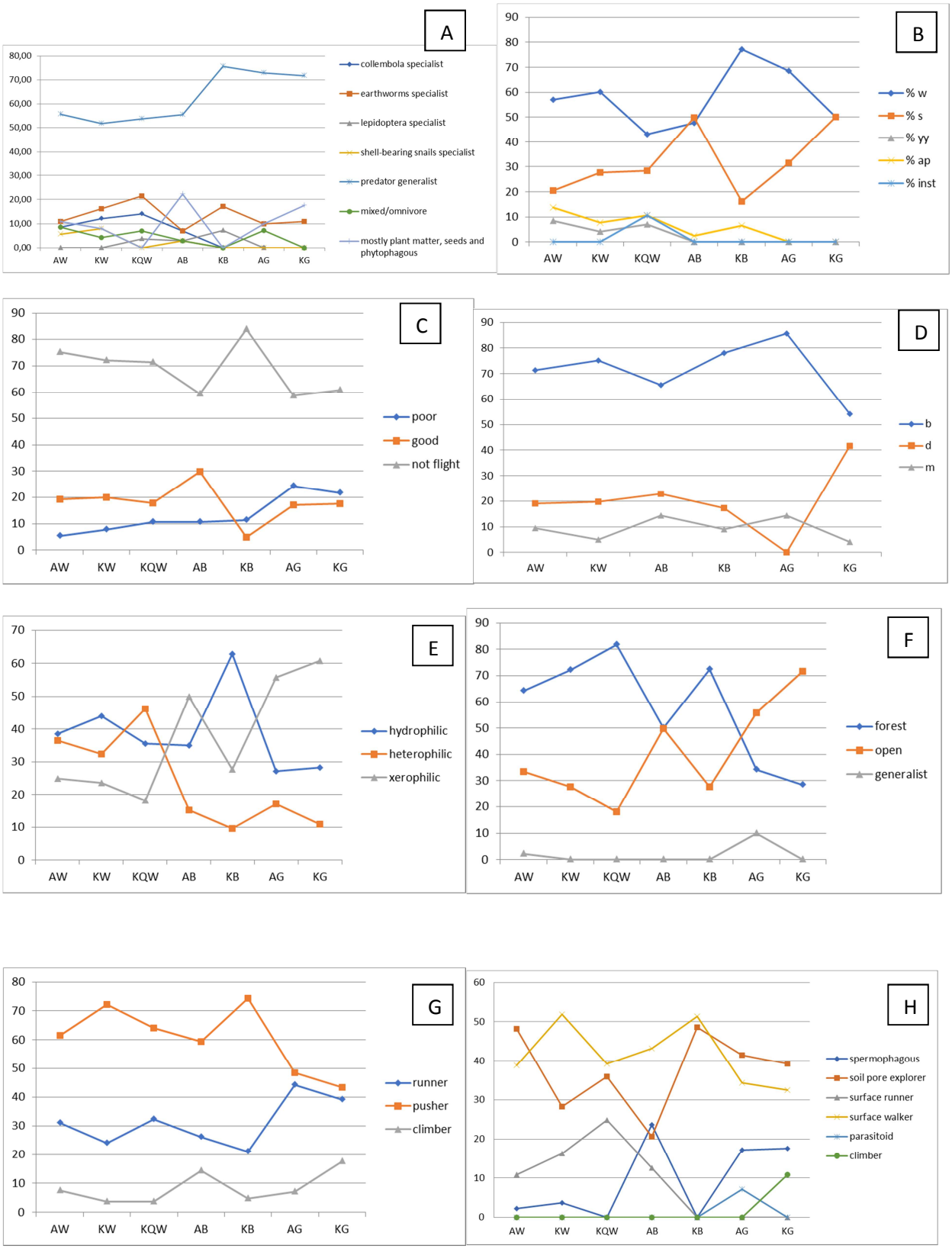

Fig. 2. Percentage of the selected species traits for each habitat (AW = *A. altissima* wood; KW = Karst wood in sinkhole; KQW = Karst wood in slope; AB = *A. altissima* bushes; KB = Karst bushes; AG = *A. altissima* grassland; KG = Karst grassland). In each plot there is the following functional trait: A=diet; B=reproduction; C=flight ability; D=; E=moisture preferences; F=habitat affinity; G=locomotion; H=larval morpho-ecological type.

3.2. Functional diversity (FD) among habitats

We analyzed functional trait diversity based on Rao's quadratic diversity (FD_Q) to evaluate the effects of habitats on the functional diversity of carabid beetles (Fig. 3). Significant differences in functional diversity (FD) among habitats were found between native grassland and native wood (p-value=0.0458251) and an almost significant difference was found between native bush and native wood (p-value=0.0823702). There was not significant difference in functional diversity among non-native habitats and within the comparison between native and non-native habitats. The highest FD was in Bush_A and the lowest in Grassland.

In Fig. 4 is represented the mean number of species detected in each habitat typology.

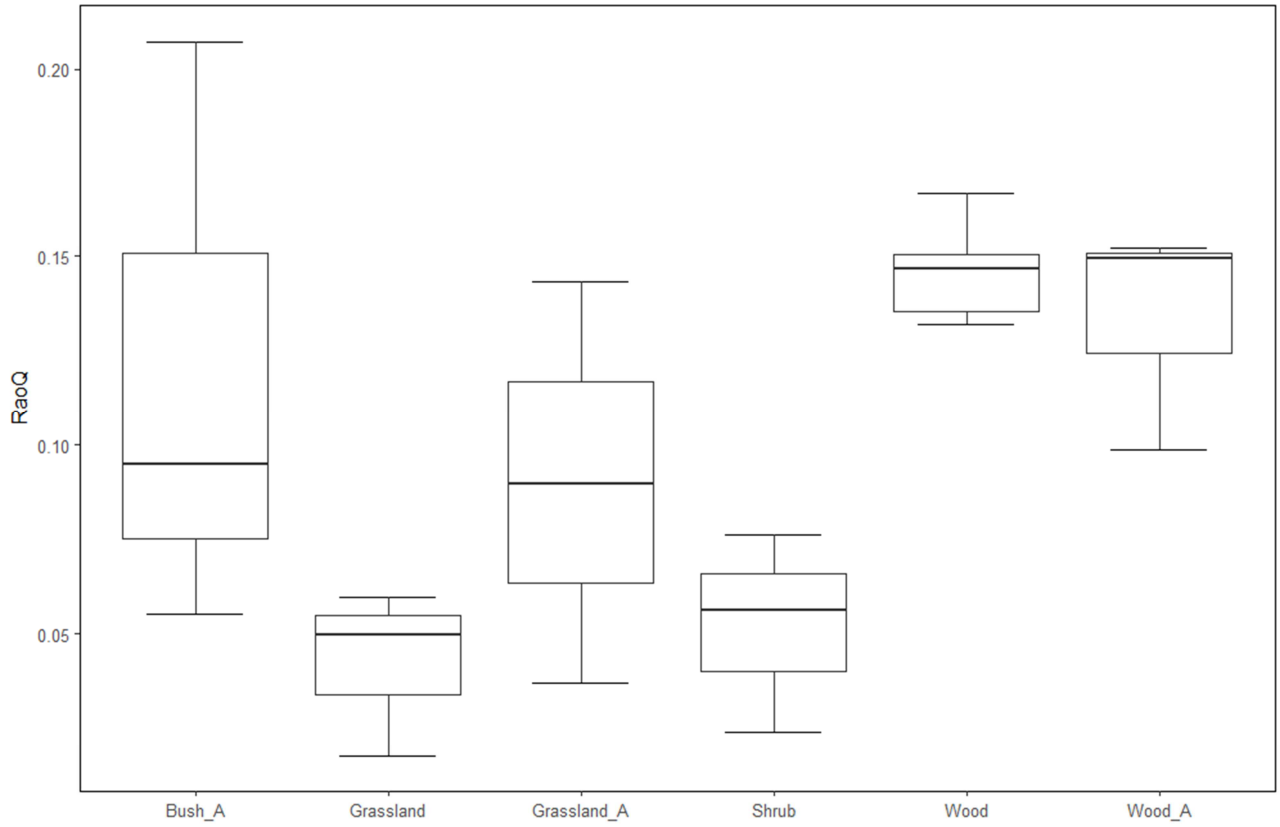

Fig. 3. Carabids functional diversity between native (Grassland (KG), Shrub (KB), Wood(KW+KQW)) and non-native habitats (Grassland_A (AG), Bush_A (AB), Wood_A (AW)) (F=3.567, p-value=0.0274).

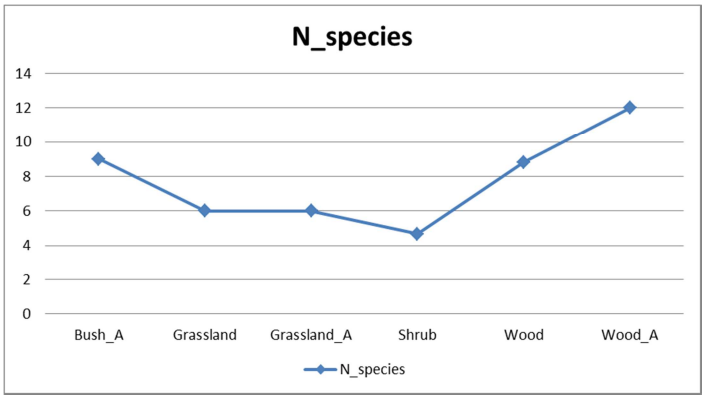

Fig. 4. Mean number (within plots) of species along the studied habitats.

3.3. Distance decay of similarity

We obtained a significantly positive correlation coefficient (r=0.7934, p-value=0.001) indicating a distance decay of similarity, that means that much more the carabid beetles communities are far away from each other (habitat typology), much more they have different in species compositions (taxonomic diversity). This reflects in functional diversity: more similar are the habitats in species composition, more similar they are also in functional diversity (Fig. 4).

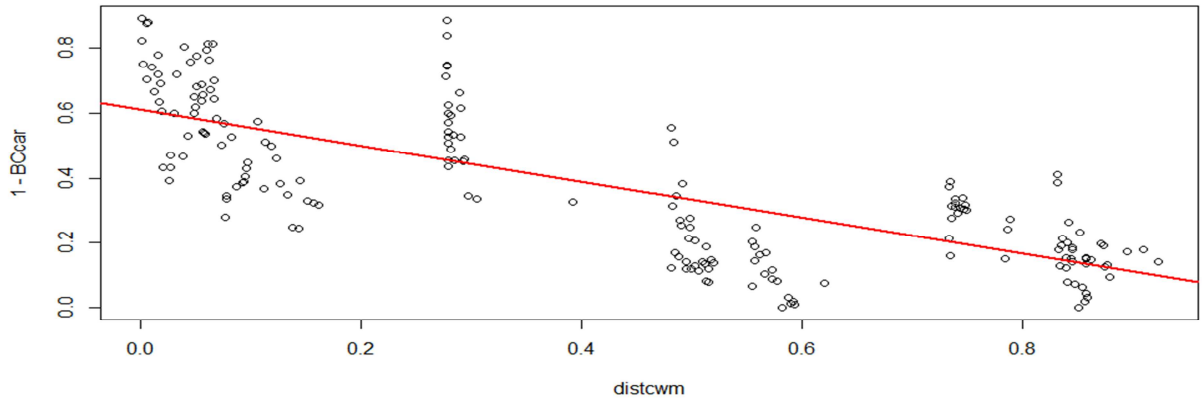

Fig. 4. Mantel correlation illustrating distance decay in taxonomic (Bray-Curtis on species abundances) and functional similarity (CWM) of carabid beetles communities (r=0.7934 p-value=0.001).

3.4. Environmental drivers of species trait distribution

Linear model selection suggested that among soil porosity and native and non-native canopy, only non-native canopy cover positively influences carabid beetles Functional Diversity (F=49.89, p-value=0.0001058) (Fig. 5).

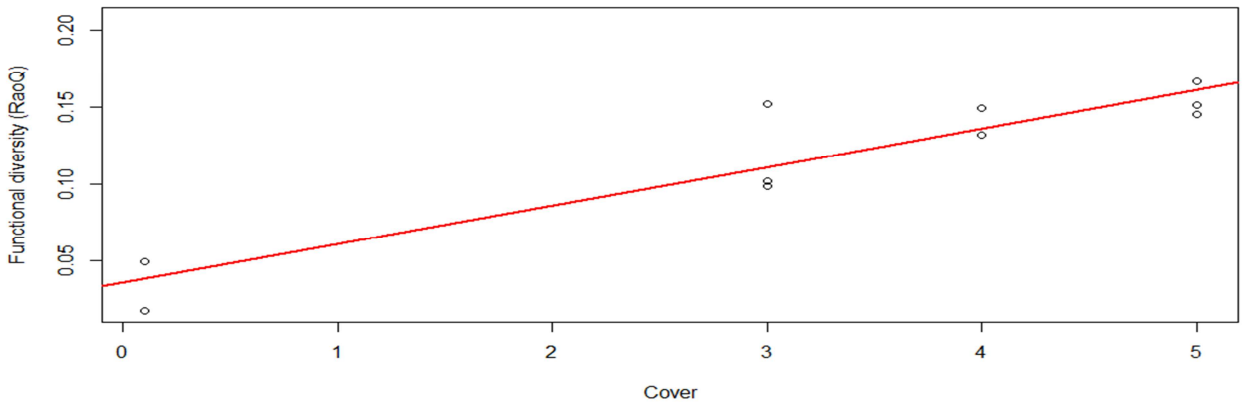

Fig. 5. Carabid functional diversity (FD) variation with non-native canopy (F=49.89, p-value=0.0001058).

4. Discussion and conclusions

The comparison between invaded and nearby non-invaded sites is a well-established approach in invasion ecology to assess invasion impacts when data on temporal changes are lacking, although results might be affected by pre-invasion differences between sites (Buchholz et al., 2015). In order to avoid this bias we compared pairs of native and non-native habitats in the same environmental context (same habitat type and land-use history). The aim of this study was to understand if the presence of *A. altissima* affects the trait composition and the functional diversity of carabid beetles in native *versus* non-native habitats. The first result was the finding that carabid beetles trait composition is affected by both *A. altissima* and habitat type (wood, bush, grassland).

Observing in detail the assemblage of trait compositions among habitats, it is possible to observe that small-sized taxa dominated in open areas where the majority of the species have a body length < 10 mm, whereas large-size taxa (>25mm) dominated in forest areas, especially were *A. altissima* is present. This fact confirms that large species are more connected with stable (Homburg et al., 2016) and less disturbed areas. Species belonging to the genus *Carabus*, for example, prefer undisturbed sites because of their lower reproductive output, longer larval stages and reduced dispersal power (as they tend to be flightless) (Ribera et al., 2001; Moraes et al., 2013; Mazzei et al., 2015; Alignier & Aviron, 2017; Zhang et al., 2017).

Considering the food preferences, the always prevalent feeding strategy was the generalist predator trait, that benefits of increasing disturbance (Hanson et al., 2016) and represented in all habitats more than 50% of the carabids diet traits.

The presence of Collembola-eater carabids are influenced by different habitat type, with higher amount of species in the forest, and a significant difference between native wood respect to non-native wood (less species number here); they are missing in grassland and bushes. Earthworm-eaters are also following the gradient of vegetation and are more present in woods than

in grassland; the shell-bearing snails specialist, like *Licinus hoffmanseggii* and *Carabus caelatus*, are almost the less common respect to the other feeding strategy traits in all habitats. It is important to underline that distribution of mixed and omnivorous carabid species is influenced by the presence of *A. altissima*, and these groups are proportionally more present in woods, bushes and grassland colonized by the non-native species, in accordance to Lövei (2008) that found omnivorous trait being the more abundant in urban areas. Even opportunistic species (Brandmayr et al., 2005) and phytophagous species, such as the representative of the genus *Ophonus* and *Harpalus*, that can be considered as good indicators of the availability of larger-seeded weeds (Brandmayr et al., 2005; Bukejs et al., 2008; Brooks et al., 2012), were influenced in their distribution along the plots by *A. altissima*, confirming the idea that *A. altissima*'s sites are the most disturbed ones in the study area.

Winter breeders with summer larvae carabid beetles are usually linked to agro-ecosystems and they show an increase in non-natural habitats respect to natural ones (Brandmayr et al., 2005). In the study area this is the most common reproductive strategy (around 50%), showing higher abundance in wood respect to grassland and in plots with *A. altissima*; only in native grassland they are equally distributed with spring breeders with summer larvae. However, in wood habitats (native and non-native) there are the most various set of reproductive rhythm, with the aperiodic *Laemostenus* ssp., the biennial *Molops ovipennis*, the instable *Abax parallelepipedus*.

Wing development is directly linked to dispersal ability and it is a relevant parameter for carabids (Homburg et al., 2013; Mazzei et al., 2015). Wingless species are more typical of permanent and stable habitats (Darlington, 1943; Brandmayr, 1983; 1991; 2005; Desender et al., 1989; Homburg et al., 2013; Bargmann et al., 2016), and winged species are more present in temporary habitats to allow dispersal to favorable sites when conditions turn difficult (Ribera et al., 2001; Hanson et al., 2016). Our finding confirm this issues, since brachypterous species were more common in wood habitats respect to bushes and grasslands, while pterodimorphic species, usually connected to degraded areas (Brandmayr et al., 2005), were linked only to *A. altissima* distribution along the plots. Hydrophilic and heterophilic traits were the more abundant moisture preference traits in the study area and were present in the same proportion within wood habitats, since xerophilic species were connected only to the presence of *A. altissima*.

Considering habitat affinity, many previous studies underlined that Carabidae can be divided according to their spatial ecological pattern into habitat generalist, forest species and open habitat species (Ings & Harley, 1999; Martínez et al., 2009; Mazzei et al., 2015). In this study, forest species were connected to all wood habitats and native bushes, whereas open habitat species were more present in the grassland plots. It is noteworthy that open habitat species were influenced in

their distribution by the presence of *A. altissima*¸ in fact their number decreased from non-native to native grassland and increased from native to non-native woods. Another important issue is that habitat generalists were only present in non-native habitats.

A. altissima increased also the presence of carabid beetles locomotion pusher trait, that was the most common trait in woody and bush plots, while runner/pusher traits were equally distributed in grassland.

Soil pore explorer larva trait, characterized by larvae of small and medium size that live in the litter or in the soil (genus *Pterostichus*, *Poecilus*, *Calathus*, *Laemostenus, Molops,* earthworm-eaters) (Brandmayr et al., 2005), was the more abundant trait in woods respect to grassland and bushes and especially in plots with the presence of *A. altissima.* We observed a similar pattern in the surface walker trait, typical of medium-large size larvae living on the soil surface, eating earthworms, snails and caterpillars (Brandmayr et al., 2005), that were more widespread in native wood where they were linked with the presence of *Calosoma sycophanta.* .

Parasitoid species were present only in non-native grassland, while spermophagous carabids (genus *Ophonus, Pseudophonus*, seed-eaters), were found in native and non-native grassland and non-native bushes. Surface runners carabids are characterized by species (genus *Notiophilus*, *Abax* in shady habitats) hunting on the soil surface (looking for springtails and earthworms) which in our study sites became more abundant moving from grassland to wood.

Considering the functional diversity (FD), we found a significant difference among the native habitats, in particular between grassland-wood and shrub-wood. Since the values of FD are considering relative species abundances into account (Laliberté & Legendre, 2010), functional diversity decrease with the increasing dominance of single species. As a consequence, higher values of functional diversity in non-native bushes and grassland reveal a decrease in dominant species. The comparison among native and non-native woods gives an opposite result, with a low functional diversity in non-native wood. This fact means that native sites exhibited an assemblage in which species were more scattered in trait space, and were less dominated by singles species, as already reported by Schirmel & Buchholz (2013). The comparison between FD and species richness is fundamental for the comprehension of the processes driving trait assemblages differences in habitats, and in particular for the observation of changes in the relationship between native and non-native habitats. Little diversity on functional diversity following an increase of species richness might be an indication of greater similarity in traits occurring in the assemblage and high functional similarity among species. Vice versa, if changes in functional diversity are greater than changes in species richness, traits are less similar. The latter situation might occur if functionally different species immigrate to a community (for example generalist and omnivorous species) - and therefore

add new traits to the system - while other species with similar traits (specialist species) disappear. In contrast, if functional similarity is high, habitat filtering might induce species sorting, which promotes distinct life-history traits (Schirmel & Buchholz, 2013).

Our results highlighted that carabid beetles had the same species richness both in native and non-native grassland but showed among these habitats great differences in functional diversity, probably due to generalist, omnivorous and spermatophagous species, colonizing disturbed (non-native) areas. On the other hand, an increasing of the species richness from native to non-native wood corresponded to high functional similarity among these two habitats, that can be explained with habitat filtering, as suggested by Schirmel & Buchholz (2013). This concept means that species occurring in sites with habitat filtering are particularly adapted to the predominant environmental characteristics and show more or less similar traits, which are the best adapted to that habitat features. In the case of native wood, it seems likely that many species were highly specialized to live in the Karst wood. After *A. altissima* invasion, even if the number of species is increasing, the functional diversity remained quite similar, meaning more homogeneity in traits respect to Karst wood.

The comparison among native and non-native bushes clearly pointed out that a higher species richness correspond to a higher functional diversity, and the difference between these two habitat type is due to the fact that non-native bushes grow in a more humid and less stony soil respect to Karst bushes, and these characteristics are more suitable for carabid beetles survival.

The above considerations are summarized by the distance decay of similarity between taxonomic and functional distances: habitats considerably different from each other (e.g. grassland and wood) comprehend a different set of species sharing different species traits. The connection between functional traits and taxonomical differences in species and plots are explained by diversity in species traits corresponding to different taxonomic richness. Synthetically, sites sharing the same species set, share also the same functional diversity.

Finally, to better understand how carabid beetles react to *A. altissima* landscape colonization it is essential to identify drivers that shape their community composition (Hanson et al., 2016); comparing native and non-native canopy, it seems to be that non-native ones drive the functional diversity in carabid beetles assemblages. This is also represented by the higher FD that we found in non-native bushes, where *A. altissima* create a more suitable habitat respect to Karst bushes, increasing soil cover and humidity.

In conclusion, *A. altissima* invasion induced changes in functional composition of carabid beetles by creating more heterogeneous community in grassland and more homogeneous community in

wood, but increasing in both habitats the number of generalist and omnivorous species, a sign that this alien tree creates more degraded and more disturbed areas.

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Appendix 1

Carabid beetles species	repr	corol	wings	size	fa	diet	ows	mpe	mpa	moist	aha	loc	lmt
Abax carinatus sulcatus	s	3	b	16	3	2				1	1	2	surface runner
Abax parallelepipedus subpunctatus	inst.	3	b	18,5	3	2	1	2		2	1	2	surface runner
Brachinus explodens	s	4	m	7	2	5		2	1	3	2	4	parasitoid
Calathus cinctus	w	4	d	7,5	1	5				3	2	1	soil pore explorer
Calathus glabricollis	w	1	b	12	3	5				3	2	1	soil pore explorer
Calathus melanocephalus	w	4	d	7	2	5	3	1		2	3	1	soil pore explorer
Calosoma sycophanta	s	5	m	27	2	3		2		2	1	4	surface walker
Carabus coriaceus coriaceus	w	3	b	36	3	5	3	2		2	3	2	surface walker
Carabus granulatus interstitialis	s	4	d	18	1	5	1	2	1	1	1	1	surface walker
Carabus caelatus schreiberi	w	1	b	35,5	3	2				1	1	2	surface walker
Carabus catenulatus catenulatus	s	1	b	24,9	3	5				1	1	2	surface walker
Carabus convexus dilatatus	s	4	b	18	3	5	1	3	2	3	2	2	surface walker
Harpalus serripes	s	4	m	10,5	2	7			1	3	2	4	spermophagous
Harpalus (Harpalus) atratus	s	3	d	11,5	2	7				3	2	2	spermophagous
Harpalus dimidiatus	s	3	m	13,5	2	7				3	2	2	spermophagous
Harpalus rubripes	s	4	m	10,5	2	7			2	3	2	4	spermophagous
Laemostenus cavicola	ap	1	b	15	3	5			3	1	1	4	soil pore explorer
Laemostenus elongatus	ap	1	b	15	3	5			3	1	1	2	soil pore explorer
Leistus rufomarginatus	w	3	d	8,5	2	1	3	2	3	2	1	1	surface runner
Licinus hoffmanseggii	w	2	b	13,5	3	4				3	2	2	surface walker
Molops ovipennis istrianus	yy	1	b	13	3	6				2	1	2	soil pore explorer
Myas chalybaeus	w	1	b	15	3	5				2	1	2	soil pore explorer
Notiophilus rufipes	s	3	m	6	2	1	1	3	2	2	1	1	surface runner
Ophonus azureus	s	4	d	8	1	7				3	2	1	spermophagous
Philorhizus crucifer confusus	s	3	d	2,75	1	5				2	2	4	climber
Poecilus koyi gorician us	s	3	b	13,5	3	5				3	2	1	soil pore explorer
Pseudoophonus rufipes	w	5	m	14,5	1	6				3	2	2	spermophagous
Synuchus vivalis	w	4	m	7,5	2	7	2	2	2	2	2	1	soil pore explorer

Tab. 1. Carabid species included in the analysis and their ecological attributes. I, Reproduction rhythm; II, geographical range (chorotype); III, dispersal power (wing morphology); IV, Size (body length) ; V, Flying ability; VI, Food of the adult; VII, Overwintering strategies; VIII, main period of emergence; IX, main period of activity; X, moisture preference; XI, habitat affinity; XII, locomotion of adult; XII, morpho-ecological types.

Appendix 2

PERMANOVA table of results – Fixed factor= etc

All traits

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	**1**	**1124.5**	**1124.5**	**3.19**	**0.0252**	**4985**	**0.029**
am	**2**	**4110.5**	**2055.2**	**5.8305**	**0.0014**	**4982**	**0.0018**
aixam	2	1194.6	597.32	1.6945	0.111	4990	0.141
Res	14	4935	352.5				
Total	19	11625					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**2.5658**	**0.0038**	**4976**	**0.0066**
1, 3	**3.78**	**0.0008**	**4973**	**0.0004**
2, 3	0.96453	0.4534	4739	0.418

Wings 1

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	1	1267.2	1267.2	*2.773*	*0.0646*	4988	0.0782
am	**2**	**2949.1**	**1474.5**	**3.2268**	**0.0276**	**4991**	**0.0322**
aixam	2	656.72	328.36	0.71857	0.6074	4981	0.59
Res	14	6397.5	456.96				
Total	19	11635					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	2.6465	**0.007**	4981	0.0094
1, 3	2.5063	**0.0042**	4976	0.0088
2, 3	Negative			

Wings 1–brachypterous

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	1	3.125	3.125	1.3462	0.272	4957	0.2602
am	**2**	**58.833**	**29.417**	**12.672**	**0.0014**	**4978**	**0.0008**
aixam	2	2.0106	1.0053	0.43306	0.6514	4990	0.6594
Res	14	32.5	2.3214				
Total	19	94.55					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	3.6604	**0.003**	4828	0.0044
1, 3	6.0133	**0.0002**	4708	0.0002
2, 3	0.92088	0.3904	3699	0.3788

Wings 1– Dimorphic

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	**1**	**6.7222**	**6.7222**	**7.4298**	**0.0156**	**4947**	**0.0156**
am	2	1.7872	0.89362	0.98768	0.3984	4989	0.393

aixam	2	3.1773	1.5887	1.7559	0.21	4990	0.2164
Res	14	12.667	0.90476				
Total	19	24.55					

Wings 2

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
ai	**1**	**943.56**	**943.56**	**2.9804**	**0.0508**	**4994**
am	**2**	**3488.6**	**1744.3**	**5.5097**	**0.0022**	**4987**
aixam	2	1120.6	560.29	1.7698	0.1368	4981
Res	14	4432.2	316.59			
Total	19	9775.6				

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms
1, 2	**2.4816**	**0.006**	**4984**
1, 3	**4.03**	**0.0004**	**4982**
2, 3	0.86877	0.5472	4709

Wings 2–Good flight ability

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	**1**	**4.5**	**4.5**	**11.813**	**0.0058**	**4835**	**0.003**
am	**2**	**3.0638**	**1.5319**	**4.0213**	**0.047**	**4987**	**0.0402**
aixam	**2**	**4.0851**	**2.0426**	**5.3617**	**0.0142**	**4983**	**0.0214**
Res	14	5.3333	0.38095				
Total	19	16.95					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	1.6818	0.117	3462	0.1164
1, 3	**2.7386**	**0.021**	**3603**	**0.0202**
2, 3	1.1832	0.2938	2077	0.2742

PAIR-WISE TESTS

Term 'aixam' for pairs of levels of factor 'ambiente'

Within level '1' of factor 'ailanto'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	0.70711	1	1	0.5198
1, 3	**3.0984**	**0.1024**	**4**	**0.054**
2, 3	3.873	0.1946	3	0.0266

Within level '2' of factor 'ailanto'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**3.5277**	**0.0364**	**6**	**0.0086**
1, 3	1.3663	0.3474	5	0.2248
2, 3	1	0.7098	3	0.3758

PAIR-WISE TESTS

Term 'aixam' for pairs of levels of factor 'ailanto'

Within level '1' of factor 'ambiente'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	1.7638	0.2528	4	0.1174

Within level '2' of factor 'ambiente'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	4.9497	0.0992	4	0.008

Wings2– Not flight ability

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	**1**	**10.125**	**10.125**	**4.7514**	**0.0452**	**4942**	**0.0512**
am	**2**	**58.833**	**29.417**	**13.804**	**0.0004**	**4977**	**0.0008**
aixam	2	6.1809	3.0904	1.4503	0.2664	4989	0.2714
Res	14	29.833	2.131				
Total	19	98.95					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**3.7095**	**0.004**	**4405**	**0.0028**
1, 3	**6.3259**	**0.0004**	**4583**	**0.0002**
2, 3	0.98674	0.3472	3452	0.363

Chorology

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	**1**	**2627.1**	**2627.1**	**7.4019**	**0.0004**	**4993**	**0.001**
am	**2**	**4264.8**	**2132.4**	**6.008**	**0.002**	**4989**	**0.0016**
aixam	2	1188.8	594.39	1.6747	0.1596	4981	0.1748
Res	14	4969	354.93				
Total	19	13303					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**2.2351**	**0.0116**	**4985**	**0.0208**
1, 3	**4.1471**	**0.0002**	**4962**	**0.0004**
2, 3	1.173	0.2774	4499	0.2898

Chorology– 1

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	1	0.88889	0.88889	0.88889	0.3636	4935	0.3654
am	**2**	**38.894**	**19.447**	**19.447**	**0.0006**	**4989**	**0.0002**
aixam	2	1.3901	0.69504	0.69504	0.5164	4988	0.5128
Res	14	14	1				
Total	19	53.8					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	4.1429	0.0022	4112	0.0016
1, 3	7.0711	0.0004	2651	0.0002
2, 3	1.3584	0.2166	2237	0.2244

Chorology– 3

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	1	0.88889	0.88889	1.3333	0.2726	4922	0.2828
am	2	3.0071	1.5035	2.2553	0.145	4989	0.1412
aixam	**2**	**5.4468**	**2.7234**	**4.0851**	**0.0444**	**4985**	**0.0376**
Res	14	9.3333	0.66667				
Total	19	18.8					

Within level '1' of factor 'ailanto'

Groups	t	P(perm)	Unique perms	P(MC)

1, 2	0.70711	1	1	0.514
1, 3	1.2439	0.608	4	0.3022
2, 3	1.9548	0.2912	5	0.144

Within level '2' of factor 'ailanto'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	3	0.077	4	0.02
1, 3	1.8524	0.1492	5	0.112
2, 3	2	0.3944	2	0.1132

Chorology– 4

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	**1**	**0.88889**	**0.88889**	**18.667**	**0.0002**	**2847**	**0.0012**
am	**2**	**1.5887**	**0.79433**	**16.681**	**0.0036**	**4956**	**0.0004**
aixam	**2**	**1.5887**	**0.79433**	**16.681**	**0.0002**	**4988**	**0.0004**
Res	14	0.66667	4.7619E-2				
Total	19	5.2					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	Denominator is 0			
1, 3	**4.4721**	**0.0038**	**655**	**0.002**
2, 3	**3.5277**	**0.0184**	**161**	**0.0098**

Within level '1' of factor 'ailanto'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	Denominator is 0			
1, 3	Denominator is 0			
2, 3	Denominator is 0			

Within level '2' of factor 'ailanto'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	Denominator is 0			
1, 3	**6.1101**	**0.0122**	**5**	**0.0006**
2, 3	4	0.1018	3	0.0156

Chorology– 5

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	**1**	**12.5**	**12.5**	**37.5**	**0.0002**	**4895**	**0.0002**
am	2	2.0426	1.0213	3.0638	0.0738	4978	0.0818
aixam	2	0.51064	0.25532	0.76596	0.4828	4990	0.496
Res	14	4.6667	0.33333				
Total	19	19.8					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**2.462**	**0.0322**	**1919**	**0.032**
1, 3	1.5	0.1678	2852	0.1646
2, 3	0.68313	0.5138	603	0.5162

Diet

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	1	1267.4	1267.4	2.6588	0.0586	4992	0.0682
am	2	3062.6	1531.3	3.2124	0.0096	4989	0.0162
aixam	2	1077	538.5	1.1297	0.3804	4986	0.3696
Res	14	6673.4	476.67				
Total	19	12461					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	2.1706	0.0082	4989	0.0124
1, 3	2.386	0.005	4986	0.0076
2, 3	0.77055	0.626	4697	0.5894

Diet- Collembola

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	1	0.125	0.125	1.1667	0.3208	4758	0.303
am	**2**	**4.0035**	**2.0018**	**18.683**	**0.0002**	**4987**	**0.0004**
aixam	2	0.62766	0.31383	2.9291	0.0718	4988	0.0894
Res	14	1.5	0.10714				
Total	19	6.8					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**3.7607**	**0.001**	**2737**	**0.0052**
1, 3	**6.5**	**0.0004**	**2218**	**0.0002**
2, 3	1.7638	0.1138	854	0.1258

Within level '1' of factor 'ailanto'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	1	1	1	0.3718
1, 3	Denominator is 0			
2, 3	1.5492	0.3375	2	0.2232

Within level '2' of factor 'ailanto'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**4.7819**	**0.0142**	**5**	**0.002**
1, 3	**4.7819**	**0.013**	**5**	**0.0028**
2, 3	Denominator is 0			

PAIR-WISE TESTS

Term 'aixam' for pairs of levels of factor 'ailanto'

Within level '1' of factor 'ambiente'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	0.68313	1	2	0.529

Within level '2' of factor 'ambiente'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	2	0.4	2	0.1158

Earthworms

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	1	0.125	0.125	0.33871	0.56	4901	0.5852
am	**2**	**2.6206**	**1.3103**	**3.5504**	**0.0558**	**4986**	**0.055**
aixam	2	9.5745E-2	4.7872E-2	0.12972	0.8762	4989	0.8778
Res	14	5.1667	0.36905				
Total	19	8.55					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	1.5353	0.1556	1862	0.1528
1, 3	**2.8214**	**0.0192**	**3631**	**0.0202**
2, 3	1.0116	0.3708	839	0.3466

Diet- Omnivorous

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	**1**	**0.88889**	**0.88889**	**4.6667**	**0.053**	**4794**	**0.0468**
am	**2**	**1.3901**	**0.69504**	**3.6489**	**0.0528**	**4988**	**0.0518**
aixam	**2**	**2.8369E-2**	**1.4184E-2**	**7.4468E-2**	**0.9294**	**4984**	**0.9272**
Res	14	2.6667	0.19048				
Total	19	4.8					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**2.4337**	**0.0358**	**228**	**0.0318**
1, 3	1.9365	0.0822	1546	0.0782
2, 3	0.33333	0.7346	1098	0.7598

PAIR-WISE TESTS

Term 'aixam' for pairs of levels of factor 'ailanto'

Within level '1' of factor 'ambiente'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	1.5275	0.4594	2	0.1732

Within level '2' of factor 'ambiente'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	1	1	1	0.3766

Diet- Generalist

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	1	6.125	6.125	3.2358	0.0976	4938	0.0972
am	2	10.55	5.2748	2.7867	0.0934	4983	0.0926
aixam	2	2.5213	1.2606	0.666	0.5296	4990	0.5202
Res	14	26.5	1.8929				
Total	19	44.2					

Diet- Plants

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	**1**	**2.3472**	**2.3472**	**6.3602**	**0.0256**	**4947**	**0.021**
am	**2**	**2.4823E-2**	**1.2411E-2**	**3.3631E-2**	**0.9712**	**4981**	**0.9656**
aixam	**2**	**3.2305**	**1.6152**	**4.3768**	**0.0342**	**4983**	**0.0368**
Res	**14**	**5.1667**	**0.36905**				
Total	19	11.75					

PAIR-WISE TESTS

Term 'aixam' for pairs of levels of factor 'ambiente'

Within level '1' of factor 'ailanto'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	0.70711	1	1	0.523
1, 3	1.4639	0.5974	2	0.2378
2, 3	2.0494	0.2984	4	0.1408

Within level '2' of factor 'ailanto'

Unique

Groups	t	P(perm)	perms	P(MC)
1, 2	1.0801	0.4952	3	0.3178
1, 3	1.3663	0.3452	5	0.2166
2, 3	1.7321	0.3966	2	0.1506

PAIR-WISE TESTS

Term 'aixam' for pairs of levels of factor 'ailanto'

Within level '1' of factor 'ambiente'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	2.6458	0.1128	3	0.0332

Within level '2' of factor 'ambiente'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	5	0.1024	3	0.012

Habitat

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	1	660.99	660.99	2.2072	0.135	4989	0.1408
am	**2**	**5829.3**	**2914.7**	**9.7325**	**0.0004**	**4983**	**0.0002**
aixam	**2**	**1442**	**721**	**2.4075**	**0.0648**	**4993**	**0.0822**
Res	14	4192.7	299.48				
Total	19	13145					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**2.858**	**0.0038**	**4975**	**0.0068**
1, 3	**5.4122**	**0.0002**	**4970**	**0.0002**
2, 3	1.4165	0.1392	4725	0.1542

Habitat type- Forest

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	1	5.0139	5.0139	2.1822	0.1608	4952	0.1744
am	**2**	**94.089**	**47.044**	**20.475**	**0.0008**	**4983**	**0.0002**
aixam	2	2.0603	1.0301	0.44835	0.6596	4981	0.6566
Res	14	32.167	2.2976				
Total	19	136.55					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**3.5562**	**0.0038**	**4577**	**0.0042**
1, 3	**8.5056**	**0.0004**	**4628**	**0.0002**
2, 3	**2.0592**	**0.0794**	**3167**	**0.077**

Habitat type- Generalist

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	**1**	**0.34722**	**0.34722**	**4.1667**	**0.0602**	**4904**	**0.0612**
am	2	0.18085	9.0426E-2	1.0851	0.3694	4957	0.3626
aixam	2	0.18085	9.0426E-2	1.0851	0.374	4984	0.3588
Res	14	1.1667	8.3333E-2				
Total	19	1.8					

Habitat type- Open

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	**1**	**7.3472**	**7.3472**	**5.5601**	**0.0358**	**4954**	**0.033**
am	2	3.0035	1.5018	1.1365	0.3446	4991	0.3442

aixam	**2**	**9.0745**	**4.5372**	**3.4336**	**0.0658**	**4990**	**0.0626**
Res	14	18.5	1.3214				
Total	19	43.8					

PAIR-WISE TESTS

Term 'aixam' for pairs of levels of factor 'ambiente'

Within level '1' of factor 'ailanto'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	0.5	1	2	0.6458
1, 3	0.3721	0.8008	6	0.7336
2, 3	0.69561	0.8994	3	0.5352

Within level '2' of factor 'ailanto'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	0.48305	0.7538	6	0.6476
1, 3	**3.3813**	**0.0218**	**9**	**0.014**
2, 3	2.1381	0.1972	5	0.1022

PAIR-WISE TESTS

Term 'aixam' for pairs of levels of factor 'ailanto'

Within level '1' of factor 'ambiente'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**3.7417**	**0.0248**	**5**	**0.0072**

Within level '2' of factor 'ambiente'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	2.8284	0.098	5	0.0502

Morpho-ecological larva types

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	1	372.76	372.76	1.0069	0.4012	4991	0.3854
am	**2**	**5834.2**	**2917.1**	**7.8801**	**0.0004**	**4986**	**0.0006**
aixam	2	1239.2	619.6	1.6738	0.1448	4987	0.1654
Res	14	5182.6	370.19				
Total	19	12643					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**2.5939**	**0.007**	**4987**	**0.0096**
1, 3	**4.517**	**0.0002**	**4982**	**0.0002**
2, 3	1.5142	0.099	4714	0.126

Larva- Climber

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	**1**	**0.22222**	**0.22222**	**4.6667**	**0.0462**	**4074**	**0.0468**
am	**2**	**0.39716**	**0.19858**	**4.1702**	**0.0378**	**4950**	**0.038**
aixam	**2**	**0.39716**	**0.19858**	**4.1702**	**0.0352**	**4984**	**0.0374**
Res	14	0.66667	4.7619E-2				
Total	19	1.8					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	Denominator is 0			
1, 3	**2.2361**	**0.05**	**1086**	**0.047**
2, 3	1.7638	0.129	120	0.1162

PAIR-WISE TESTS

Term 'aixam' for pairs of levels of factor 'ambiente'

Within level '1' of factor 'ailanto'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	Denominator is 0			
1, 3	Denominator is 0			
2, 3	Denominator is 0			

Within level '2' of factor 'ailanto'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	Denominator is 0			
1, 3	3.0551	0.0794	3	0.0212
2, 3	2	0.3902	2	0.1076

PAIR-WISE TESTS

Term 'aixam' for pairs of levels of factor 'ailanto'

Within level '1' of factor 'ambiente'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	Denominator is 0			

Within level '2' of factor 'ambiente'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	Denominator is 0			

Larva- Parasitoid

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	**1**	**0.125**	**0.125**	**3.5**	**0.0688**	**3953**	**0.0798**
am	**2**	**0.2234**	**0.1117**	**3.1277**	**0.0534**	**4913**	**0.0762**
aixam	**2**	**0.2234**	**0.1117**	**3.1277**	**0.0546**	**4980**	**0.0698**
Res	14	0.5	3.5714E-2				
Total	19	0.95					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	Denominator is 0			
1, 3	1.9365	0.072	999	0.0844
2, 3	1.5275	0.132	81	0.1658

PAIR-WISE TESTS

Term 'aixam' for pairs of levels of factor 'ambiente'

Within level '1' of factor 'ailanto'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	Denominator is 0			
1, 3	1.3416	0.4068	2	0.2602
2, 3	1.3416	0.3982	2	0.2812

Within level '2' of factor 'ailanto'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	Denominator is 0			
1, 3	Denominator is 0			
2, 3	Denominator is 0			

PAIR-WISE TESTS

Term 'aixam' for pairs of levels of factor 'ailanto'

Within level '1' of factor 'ambiente'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	Denominator is 0			

Within level '2' of factor 'ambiente'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	Denominator is 0			

Larva- Soil pore explorer

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	1	4.5	4.5	3.375	0.0908	4908	0.088
am	**2**	**20.057**	**10.028**	**7.5213**	**0.0044**	**4989**	**0.006**
aixam	**2**	**8.766**	**4.383**	**3.2872**	**0.0658**	**4990**	**0.068**
Res	14	18.667	1.3333				
Total	19	47.8					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**3.3031**	**0.0074**	**4336**	**0.0044**
1, 3	**3.411**	**0.0102**	**4045**	**0.0074**
2, 3	0.53872	0.607	2488	0.6082

PAIR-WISE TESTS

Term 'aixam' for pairs of levels of factor 'ambiente'

Within level '1' of factor 'ailanto'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**5.5**	**0.1006**	**5**	**0.0036**
1, 3	**5.5626**	**0.0972**	**6**	**0.0136**
2, 3	0.6	0.7056	4	0.587

Within level '2' of factor 'ailanto'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	0.80959	0.4706	10	0.4442
1, 3	0.68313	0.7526	4	0.5094
2, 3	0.27735	1	3	0.7958

PAIR-WISE TESTS

Term 'aixam' for pairs of levels of factor 'ailanto'

Within level '1' of factor 'ambiente'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**3.8711**	**0.0128**	**12**	**0.0052**

Within level '2' of factor 'ambiente'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	6.664E-9	1	4	1

Larva- Surface runners

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	1	0.34722	0.34722	0.78829	0.387	4939	0.3958
am	**2**	**7.9468**	**3.9734**	**9.0207**	**0.0028**	**4980**	**0.0026**
aixam	**2**	**2.9539**	**1.477**	**3.3531**	**0.0646**	**4981**	**0.062**
Res	14	6.1667	0.44048				

Total 19 18.95

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**2.2669**	**0.041**	**4101**	**0.0462**
1, 3	**4.6355**	**0.0014**	**4039**	**0.0014**
2, 3	1.7638	0.118	847	0.1208

PAIR-WISE TESTS

Term 'aixam' for pairs of levels of factor 'ambiente'

Within level '1' of factor 'ailanto'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	5.7712E-9	1	3	1
1, 3	**3.0984**	**0.0996**	**4**	**0.0568**
2, 3	1.5492	0.3305	2	0.2236

PAIR-WISE TESTS

Term 'aixam' for pairs of levels of factor 'ailanto'

Within level '1' of factor 'ambiente'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	1	0.634	3	0.357

Within level '2' of factor 'ambiente'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	2	0.4004	2	0.1222

Larva- Surface walker

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	1	2	2	1.8261	0.2038	4664	0.1868
am	**2**	**16.397**	**8.1986**	**7.4857**	**0.0056**	**4992**	**0.0064**
aixam	2	1.1915	0.59574	0.54394	0.5956	4989	0.5938
Res	14	15.333	1.0952				
Total	19	34.8					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	1.6818	0.1204	4561	0.1178
1, 3	**4.3412**	**0.0016**	**4521**	**0.0022**
2, 3	1.9568	0.0888	3387	0.095

Locomotion

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	1	418.89	418.89	1.475	0.2688	4993	0.2791
am	**2**	**5038.8**	**2519.4**	**8.8711**	**0.0012**	**4991**	**0.0022**
aixam	2	1199.3	599.63	2.1113	0.126	4989	0.1358
Res	14	3976	284				
Total	19	11073					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**2.8597**	**0.0048**	**4979**	**0.0076**

1, 3	**4.794**	**0.0006**	**4919**	**0.0002**
2, 3	1.1438	0.2894	4524	0.2991

Locomotion–Pusher

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	**1**	**9.3889**	**9.3889**	**5.0556**	**0.0398**	**4946**	**0.044**
am	**2**	**47.362**	**23.681**	**12.751**	**0.0012**	**4989**	**0.0012**
aixam	2	3.7305	1.8652	1.0044	0.3876	4987	0.3874
Res	14	26	1.8571				
Total	19	85.8					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**3.1024**	**0.0078**	**2497**	**0.0136**
1, 3	**6.2368**	**0.0006**	**4634**	**0.0004**
2, 3	1.4739	0.1812	1797	0.1806

Moisture preference

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	1	970.04	970.04	2.3397	0.106	4991	0.1154
am	**2**	**5984.2**	**2992.1**	**7.2166**	**0.0006**	**4989**	**0.0004**
aixam	2	680.95	340.48	0.8212	0.5492	4991	0.5356
Res	14	5804.5	414.61				
Total	19	13949					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**2.717**	**0.002**	**4983**	**0.0066**
1, 3	**3.9057**	**0.0004**	**4984**	**0.001**
2, 3	1.3139	0.198	4705	0.1944

Moisture preference– Heterophylic

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	1	1.6806	1.6806	1.0943	0.3058	4952	0.3162
am	**2**	**43.465**	**21.732**	**14.151**	**0.0008**	**4992**	**0.0006**
aixam	2	0.34397	0.17199	0.11199	0.8984	4985	0.895
Res	14	21.5	1.5357				
Total	19	68.55					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**3.9243**	**0.0028**	**3025**	**0.0028**
1, 3	**4.7294**	**0.0012**	**4739**	**0.0014**
2, 3	0.50918	0.6108	1912	0.633

Moisture preference– Hydrophilic

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	1	2.3472	2.3472	2.2663	0.1542	4954	0.1528
am	**2**	**17.798**	**8.8989**	**8.5921**	**0.0046**	**4988**	**0.0036**
aixam	2	1.7411	0.87057	0.84055	0.4522	4988	0.455
Res	14	14.5	1.0357				
Total	19	34.95					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**1.9621**	**0.082**	**119**	**0.0752**
1, 3	**4.3719**	**0.0012**	**4670**	**0.0022**
2, 3	2.1183	0.0768	1992	0.073

Moisture preference– Xerophilic

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	**1**	**6.7222**	**6.7222**	**4.8678**	**0.045**	**4935**	**0.0414**
am	2	4.1702	2.0851	1.5099	0.257	4988	0.2514
aixam	2	5.4184	2.7092	1.9618	0.174	4991	0.1724
Res	14	19.333	1.381				
Total	19	39.2					

Reproduction rhythm

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	**1**	**1111.2**	**1111.2**	**3.626**	**0.0362**	**4991**	**0.0442**
am	**2**	**3516.8**	**1758.4**	**5.738**	**0.0034**	**4987**	**0.0022**
aixam	**2**	**2135.8**	**1067.9**	**3.4849**	**0.011**	**4987**	**0.0228**
Res	14	4290.2	306.44				
Total	19	10934					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**2.5591**	**0.0054**	**4984**	**0.0122**
1, 3	**3.9709**	**0.0008**	**4984**	**0.001**
2, 3	0.84102	0.5466	4740	0.5183

Reproduction rhythm– Aperiodic

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	**1**	**89.667**	**89.667**	**3.4678**	**0.0332**	**4984**	**0.0298**
am	**2**	**423.23**	**211.62**	**8.1841**	**0.0002**	**4985**	**0.0008**
aixam	2	78.043	39.021	1.5091	0.1738	4980	0.1666
Res	14	362	25.857				
Total	19	953.6					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**2.8135**	**0.0024**	**4976**	**0.0044**
1, 3	**4.2231**	**0.0002**	**4975**	**0.0002**
2, 3	1.1373	0.2752	4706	0.2716

Reproduction rhythm– Summer

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	1	2.7222	2.7222	2.2867	0.1524	4954	0.155
am	2	9.9291E-2	4.9645E-2	4.1702E-2	0.9588	4989	0.9614
aixam	**2**	**14.794**	**7.3972**	**6.2136**	**0.0132**	**4990**	**0.0126**
Res	14	16.667	1.1905				
Total	19	34.55					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)

1, 2	**2.202**	**0.0436**	**4615**	**0.047**
1, 3	**5.5902**	**0.0006**	**4276**	**0.0002**
2, 3	0.4913	0.6342	3979	0.6408

PAIR-WISE TESTS

Term 'aixam' for pairs of levels of factor 'ambiente'

Within level '1' of factor 'ailanto'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	1.3363	0.4172	4	0.2584
1, 3	0.3873	0.906	7	0.7188
2, 3	2.7111	0.1028	6	0.0738

Within level '2' of factor 'ailanto'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	3.4157	0.0306	7	0.0092
1, 3	0.68313	0.6038	5	0.512
2, 3	2.4495	0.1936	4	0.0764

Reproduction rhythm– Winter

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	**1**	**10.125**	**10.125**	**6.593**	**0.0216**	**4950**	**0.0172**
am	**2**	**15.649**	**7.8245**	**5.095**	**0.0236**	**4990**	**0.0222**
aixam	2	1.1809	0.59043	0.38446	0.6822	4992	0.6866
Res	14	21.5	1.5357				
Total	19	46.8					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**2.202**	**0.0436**	**4615**	**0.047**
1, 3	**5.5902**	**0.0006**	**4276**	**0.0002**
2, 3	0.4913	0.6342	3979	0.6408

Reproduction rhythm– YY

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	1	0.125	0.125	1.1667	0.2974	4671	0.2926
am	**2**	**2.5851**	**1.2926**	**12.064**	**0.0018**	**4980**	**0.002**
aixam	2	0.28723	0.14362	1.3404	0.2832	4981	0.2948
Res	14	1.5	0.10714				
Total	19	4.2					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**3.7607**	**0.0078**	**818**	**0.0046**
1, 3	**3.3541**	**0.0042**	**2623**	**0.0086**
2, 3	Denominator is 0			

Size

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	**1**	**1848.8**	**1848.8**	**3.215**	**0.0318**	**4991**	**0.0402**
am	2	2255.8	1127.9	1.9613	0.0946	4988	0.1112
aixam	2	1902.1	951.03	1.6538	0.1702	4986	0.18
Res	14	8050.8	575.06				
Total	19	14265					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	2.1372	0.0068	4983	0.0158
1, 3	1.5244	0.1204	4987	0.1336
2, 3	0.6227	0.7344	4728	0.7142

Size= 3

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	1	0.125	0.125	0.80769	0.3806	4174	0.393
am	**2**	**1.7624**	**0.88121**	**5.6939**	**0.018**	**4988**	**0.0156**
aixam	2	0.62766	0.31383	2.0278	0.1736	4990	0.1638
Res	14	2.1667	0.15476				
Total	19	4.95					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	1.7384	0.114	2785	0.1198
1, 3	**3.3541**	**0.0124**	**2824**	**0.0066**
2, 3	1.7638	0.1212	861	0.1136

Size= 4

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	1	0.88889	0.88889	2.0741	0.168	4844	0.1714
am	**2**	**5.5887**	**2.7943**	**6.5201**	**0.006**	**4981**	**0.0076**
aixam	2	2.0142	1.0071	2.3499	0.1284	4987	0.1264
Res	14	6	0.42857				
Total	19	15.2					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**2.095**	**0.0542**	**3692**	**0.0616**
1, 3	**3.3806**	**0.0058**	**423**	**0.0104**
2, 3	1.594	0.1496	2650	0.1478

Size= 6

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	**1**	**8**	**8**	**21**	**0.0002**	**2779**	**0.001**
am	**2**	**6.7518**	**3.3759**	**8.8617**	**0.0036**	**4987**	**0.0044**
aixam	2	1.4988E-14	7.494E-15	1.9672E-14	1	3515	1
Res	14	5.3333	0.38095				
Total	19	18.95					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	**5.0143**	**0.0016**	**3777**	**0.0002**
1, 3	**2.7386**	**0.0276**	**3303**	**0.0218**
2, 3	0.72008	0.507	2820	0.5062

Size= 7

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
ai	**1**	**0.22222**	**0.22222**	**4.6667**	**0.0456**	**4013**	**0.0456**
am	**2**	**0.39716**	**0.19858**	**4.1702**	**0.0372**	**4936**	**0.0436**
aixam	2	0.39716	0.19858	4.1702	0.036	4990	0.0392
Res	14	0.66667	4.7619E-2				
Total	19	1.8					

PAIR-WISE TESTS

Term 'am'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	Denominator is 0			
1, 3	2.2361	0.0522	1097	0.0476
2, 3	1.7638	0.1206	121	0.1224

Within level '2' of factor 'ailanto'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	Denominator is 0			
1, 3	3.0551	0.084	3	0.0214
2, 3	2	0.3992	2	0.1212

Within level '1' of factor 'ailanto'

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	Denominator is 0			
1, 3	Denominator is 0			
2, 3	Denominator is 0			

Chapter 4

Effects of abiotic factors and biotic interactions on cross-taxon congruence in carabid beetles among habitats in North Adriatic Karst (North East Italy)

1. Introduction

In nature, the diversity of different taxa is influenced by spatial concordance and the strength of this association, named cross-taxon congruence, depends on the studied taxonomic groups and on the scale of analysis (Toranza andArim, 2010). In the strength of spatial taxonomic covariation, this scale dependence would be associated with changes in the factors determining species richness at different scales (Rahbeck, 2005). High cross-taxon congruence is connected with similarities in the response patterns of taxa to changes in environmental variables and in biotic interactions (Duan et al., 2016). However, the relative contributions of environmental abiotic drivers and biotic interactions towards cross-taxon congruence remain widely unknown (Gioria et al., 2011). The cross-taxon congruence analysis (Prendergast et al., 1993; Howard et al., 1998; Su et al., 2004) is used for the identification of surrogate taxa as potential biodiversity indicators (e.g. Margules & Pressey, 2000; Sætersdal et al., 2003). In this way, environmental parameters could be effectively used and potentially also be directly managed in the context of biodiversity conservation (Oertli et al., 2005; Su et al., 2004) to make biodiversity monitoring and conservation planning more efficient (Gioria et al., 2011). Thus surrogate taxa could be identified based on the direct biotic interactions such as trophic relationships between target and surrogate taxa (Westgate et al., 2014; Castagneyrol et al., 2012).

Rooney et al. (2015) found out that a the regional and disturbance-level dependence of cross-taxon congruence should be take into account when biodiversity surrogates across biogeographical regions are used. In fact, longer disturbance gradient can promote cross-taxon congruence by increasing the species pool characteristic of low or high disturbance levels. Moreover, regional context can influence (or even reverse) the relative strength of cross-taxon congruence in high and low disturbance sites, which may explain the inconsistent strength of cross-taxon congruence along the disturbance gradient.

Vascular plants are potentially known to be a good proxy for invertebrates and have been widely used for this purpose since their well-known ecology, their relatively ease in identification, and

sensitivity to environmental changes (e.g. Sætersdal et al., 2003; Schaffers et al., 2008). Also habitat type influences invertebrates ecology, and is the most important factor explaining the distribution patterns of carabid beetles at the regional and landscape scales (Dufrêne, 1992; Aviron et al., 2005; Eyre et al., 2005; Hartley et al., 2007); carabid assemblages are mainly affected by microhabitat variation and biotic interactions at smaller scales (Niemelä et al., 1992; Antvogel and Bonn, 2001; Thomas et al., 2001; Brose, 2003; Barbaro et al., 2007) and their community assemblages have been directly related to different forest stages (Riley et Browne, 2011), vegetation and litter (Niemelä et al. 1993; Niemelä and Spence 1994; Koivula 2001; Rainio and Niemelä 2003).

The aim of this study was to determine whether plants are a suitable surrogate group in North Adriatic Karst and the joint and independent effects of spatial location, environmental parameters, native and non-native vegetation structure, habitat type and soil variables on the distribution patterns of carabid beetles. Specifically, we aimed at: (1) assessing cross-taxon congruence in composition between carabid beetles and plants; (2) quantifying and comparing the capacity of vegetation structure and of environmental variables to predict ground beetle species composition; and (3) assessing and comparing the response of plant and beetle assemblages to environmental conditions.

2. Materials and methods

For ground beetles sampling, vegetation structure, soil parameters, see first chapter of the thesis.

Plants were sampled using the Braun-Blanquet method (Braun-Blanquet, 1932, 1964; Wikum et Shanholtzer, 1978).

2.1 Statistical analysis

Cross taxon congruence between plants and carabid beetles and their relationships with the other predictors were assessed using different statistical tools. Firstly, Mantel and partial Mantel test (Legendre and Legendre 2012; Frenett et al. 2013; Westgate et al. 2014; Larios et al. 2014) were used to assess the significance of the relationships between species composition matrix and predictor variables, Pearson's correlations and 999 permutations were used. Before performing Mantel and partial Mantel test; dissimilarity matrices for each group of variables were calculated as follows: 1) for environmental variables, soil variables and vegetation structure an Euclidean distance was computed. 2) For the vegetation, carabid beetles and plant communities a Bray-Curtis distance was calculated (Legendre and Legendre, 1998). Carabid communities was $\log_{10}(x+1)$

transformed prior to analysis**.** Later, Co-correspondence analysis (Co-CA, see ter Braak and Schaffers, 2004; Schaffers et al., 2008 for a full description of this method), was used to quantify the strength of plant community data in predicting the ground beetle species composition. Here we used the predictive version of Co-CA, which combines the maximization of weighted covariance between weighted averages of species scores and partial least squares methodology (PLS; see Martens and Naes, 1992). A leave-one-out cross-validatory fit percentage was estimated to select the minimal adequate predictive models. Schaffers et al. (2008) pointed out that, due to its predictive nature, any cross-validatory fit > 0 implicitly validates the model, indicating that prediction is better than that obtained under the null model.

At last, the pure and shared effect of spatial factors, soil and environment parameters, vegetation structure on plants on carabid communities was evaluated using a variance partitioning approach that allows the partitioning of the total variance (calculated as partial redundancy analysis) to be broken down into the contributions of each variable group (Borcard et al., 1992; Peres-Neto and Legendre, 2010).

3. Result

We tested the correlation between the dissimilarity matrices for plants and carabid beetles and each of the environment variables, e.g. soil variables, vegetation structures, habitat types distance matrices using Mantel tests (Tab. 1). First of all, Mantel Test showed a significant correlation between plants and carabid beetles (Pearson=30.74, p-value<0.001), plants and environmental variables (Pearson's r =0.3292, p < 0.001), plants and soil variables (Pearson=0.1843, p-value<0.001), plants and *A. altissima* presence (Pearson=0.2846, p-value<0.001), plants and native structure vegetation (Pearson=0.5019, p-value<0.001), plants and habitat type (Pearson=0.463, p-value<0.001). Partial Mantel Test correlated these two groups with the environmental variables (Pearson=0.2004, p-value=0.0093), the soil variables (Pearson=0.2585, p-value=0.002), *A. altissima* presence (Pearson=0.3568, p=<0.001) and habitat type (Pearson=0.1686, p-value=0.0257).

		Pearson	p-value	Spearman	p-value
Mantel Test	Plant ~ All Environment and Vegetation	**0.6139**	**<0.001**	0.5106	**<0.001**
	Plants ~ Environmental Variables	**0.3292**	**<0.001**	0.2448	0.008
	Plants ~ Soil Variables	0.1843	0.0178	0.2197	0.0123

	Plants ~ Ailanthus presence and structure	0.2846	0.0044	0.2216	0.0268
	Plants ~ Native Plant Structure	**0.5019**	**<0.001**	0.4158	**<0.001**
	Plants ~ Habitat Type	**0.463**	**<0.001**	0.449	**<0.001**
	Beetles ~ All Environment and Vegetation	**0.4914**	**<0.001**	0.4268	**<0.001**
	Beetles ~ Environmental Variables	**0.4092**	**<0.001**	0.4034	**<0.001**
	Beetles ~ Soil Variables	**0.4113**	**<0.001**	0.4289	**<0.001**
	Beetles ~ Ailanthus presence and structure	-0.1141	0.8498	-0.09093	0.7688
	Beetles ~ Native Plant Structure	**0.4225**	**<0.001**	0.3523	**<0.001**
	Beetles ~ Habitat Type	**0.3631**	**<0.001**	0.3392	0.0013
	Plants vs. Beetles	**0.3074**	**<0.001**	0.2196	0.0197
Partial Mantel Test	Plants vs. Beetles ~ All Environment and Vegetation	0.0083	0.4418***	0.00219	0.468***
	Plants vs. Beetles ~ Environmental Variables	0.2004	**0.0093**	0.1362	0.0849*
	Plants vs. Beetles ~ Soil Variables	**0.2585**	**0.002**	0.1423	0.074
	Plants vs. Beetles ~ Ailanthus presence and structure	**0.3568**	**<0.001**	**0.2469**	**0.010**
	Plants vs. Beetles ~ Native Plant Structure	0.1216	0.061*	0.0859	0.178***
	Plants vs. Beetles ~ Habitat Type	0.1686	**0.0257**	0.08009	0.1927***

Table 1. The Spearman and Pearson correlation coefficients of Mantel test and Partial Mantel tests between all environmental distance matrices and the dissimilarity matrix of ground beetles and plants (under one-tailed test of 999 random permutations). For Partial Mantel Test: *P<0.05; **P<0.01; ***P<0.001.

The prediction accuracies (cross-validatory % fit percentages) (Tab. 2) of plant species composition on beetles composition were above zero, indicating that the predictions of beetle species composition based on that variables were better than those expected under the null model of no relationship. Plotting the cross-validatory fit percentage for the compared predictive datasets against the number of axes showed the maximum prediction level obtained at three axis. In the model we therefore retained only the first axis.

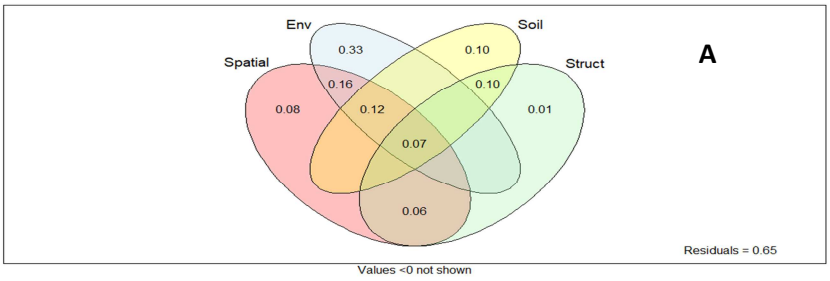
	Predictive Power (plants on beetles)	Cumulated Predictive Power	Cross-validatory %fit of plants	P-value
Axis 1	18.642	18.642	9.739	0.001
Axis 2	10.844	29.486	16.796	0.472
Axis 3	10.222	39.709	17.862	0.625

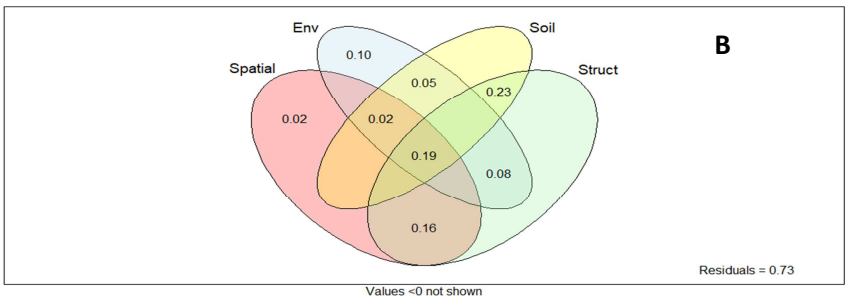
Axi□4	6.890	46.599	14.745	0.837

Table 2. Predictive power of Plants on Beetle Composition

3.1 Importance of environmental and spatial factors

The contribution of spatial, environmental, soil parameters and vegetation structure differed between carabid beetles and plants (Fig. 1). The relative contribution of spatial factors was similar between these two groups and environmental relative contribute was the most important factors in both, but influencing more beetles then plants. The relative contribution of the vegetation structure and soil factors were not coincident among the two groups. Soil parameters and vegetation structures were not important as factors to define the distribution of plant species in the study area, but combined together they contribute the most for the total plant distribution. Spatial and vegetation structure factors together determined the abundance and distribution of plant pattern almost like spatial, vegetation, environment and soil together. On one hand, soil parameters and vegetation structures together and on the other hand spatial, environment and soil structures together were the most important factors in defining patterns of carabid beetles species. Considering also *A. altissima* vegetation structure as relative factor, its presence explained carabid beetles distribution, but less than spatial and environment relative factors. Native vegetation considered as relative factor did not influence carabids species pattern, and very important remained the interaction between environment and spatial factors.





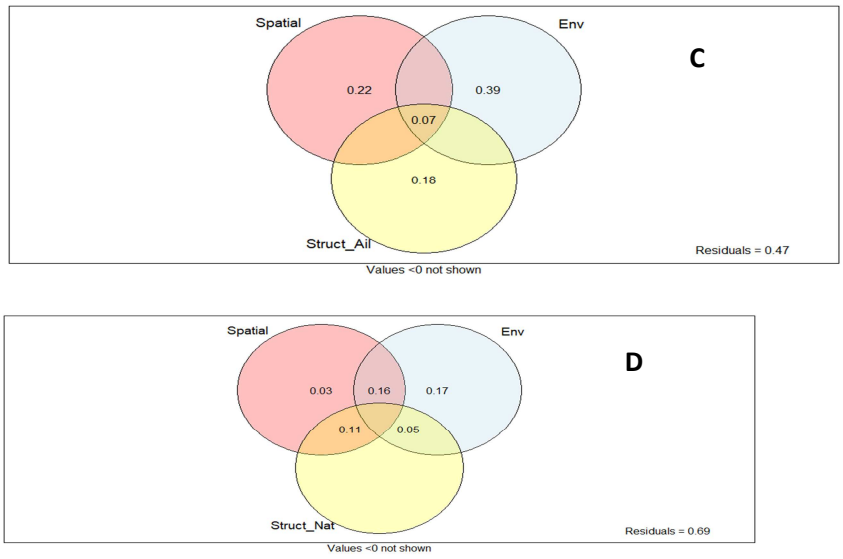


Fig. 1. Partial RDA to explain spatial, environmental (Env), soil and vegetation structure factors (Struct) on carabid beetles (A) and on plants (B). Spatial, environmental, *A. altissima* vegetation structure (Struct_Ail) factors (C) and spatial, environmental, native vegetation structure (Struc_Nat) factors (D) in detail on carabid beetles.

The ordination diagram of Co-CA analysis are presented in Fig. 2. A gradient based on vegetation structure (grassland, bushes, wood in sinkhole) could be identified along the first axis. This allowed the identification of thermophilous and xerotolerant beetle species, typical of open habitats such as *Harpalus serripes*, *Harpalus dimidiatus, Philorhizus crucifer confusus, Poecilus koyi goricianus*, on the bottom left of the diagram, associated with the characteristic grassland plant species as *Bupleurum veronense* and *Chrysopogon gryllus*. Native Quercus woods (KQW) were all grouped together (bottom right), and were associated to *Abax parallelepipedus subpunctatus*, *Notiophilus rufipes,* and with higher abundances of *Calosoma sycophanta, Molops ovipennis istrianus*, *Carabus catenulatus catenulatus.* These species are consistently associated with plant species typical of Karst mature wood (such as *Quercus pubescens, Quercus cerris, Asparagus tenuifolius*). In the upper part of the diagram species were connected with native and non-native woods in sinkhole: humid forest species, such as *Licinus hoffmanseggii* and *Abax carinatus sulcatus,* troglophilous and crevice dweller species like *Laemostenus cavicola* and *Laemostenus elongatus*, a species usually linked with swampy forest as *Carabus granulatus interstitialis,* and woodland thermophilous species as *Synuchus vivalis* and *Harpalus atratus*. In association with these carabid species there were *Lonicera caprifolium, Lamium orvala* and *Brachypodium sylvaticum*.

In Fig. 3 it is possible to distinguish between woods in sinkhole in the upper part of the diagram, corresponding also to a higher karstification values, higher soil porosity and soil fine content, and the Karst Quercus wood in the bottom right of the diagram, in connection to high values of soil skeleton and soil bulk density. The plots with the highest density of *A. altissima* are on the extreme

top of the diagram. Co-Correspondence Analysis Results showed the significance of all the tested variables (Tab. 3). It is important to underline that we found a strong correlation of carabid beetles with soil porosity (p-value=0.0053), karstification (p-value=0.002), native vegetation structure canopy cover (p-value=0.0052). Plants are strongly correlated with soil porosity (p-value=0.0058) and soil skeleton (p-value=0.0099), karstification (p-value=6e-04), native canopy cover (p-value=0.0008), native plants diameter (p-value=0.0059) and native plants height (p-value=0.0029), non-native diameter (p-value=0.0078) and non-native height (p-value=0.0049).

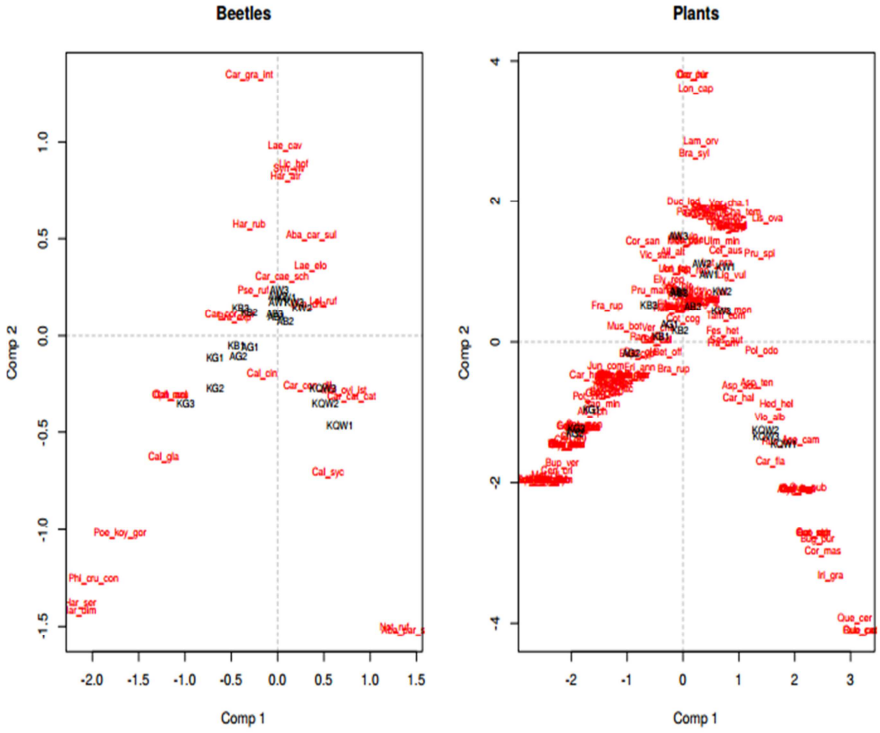
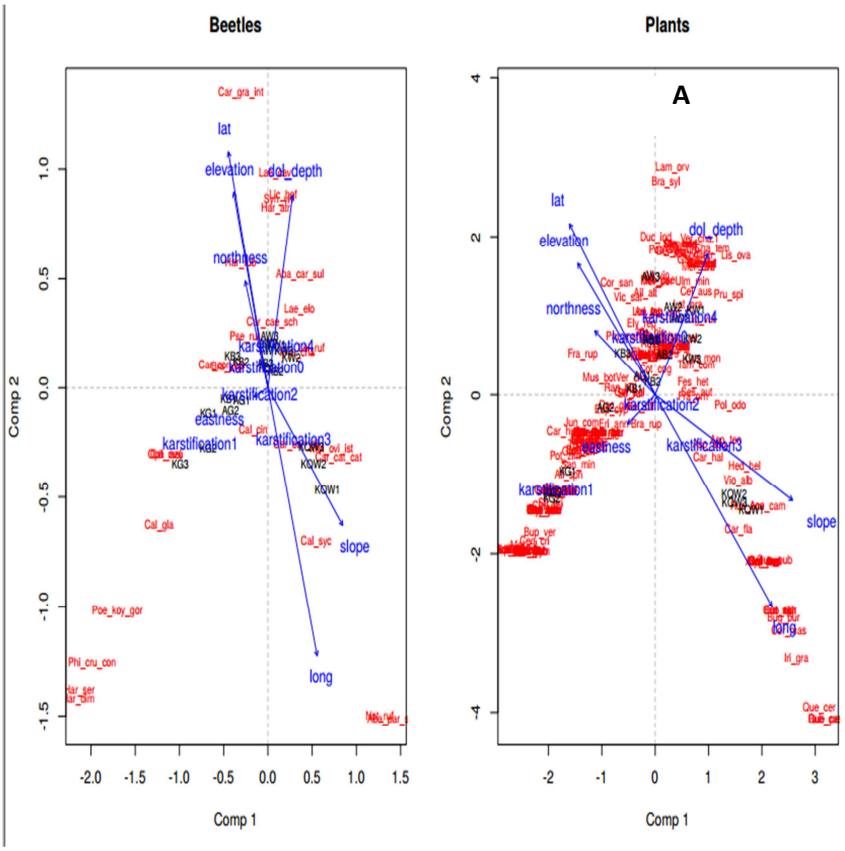
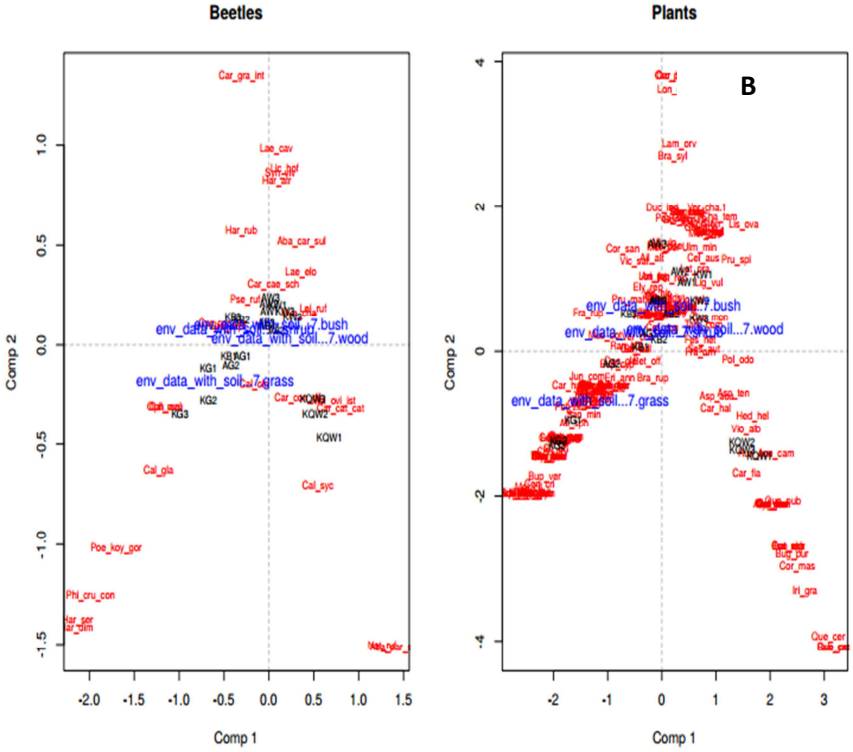


Fig. 2. Predictive Co-CA biplot of carabid beetle species composition (beetles, left) and plant species composition (plants, right) (A). In each plot, species are positioned according to their loadings with respect to normalized site scores derived from the plant composition data. The axes were rescaled to the same ranges so that sites occupy the same position in both plots. The full names of plant and beetle species are reported in Appendix A.


Beetles
Plants
A
Comp 1
Comp 2
lat
elevation
dol_depth
northness
eastness
karstification1
karstification2
karstification3
slope
long


Beetles
Plants
B
Comp 1
Comp 2
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env_data_with_soil...7.wood
env_data_with_soil...7.grass

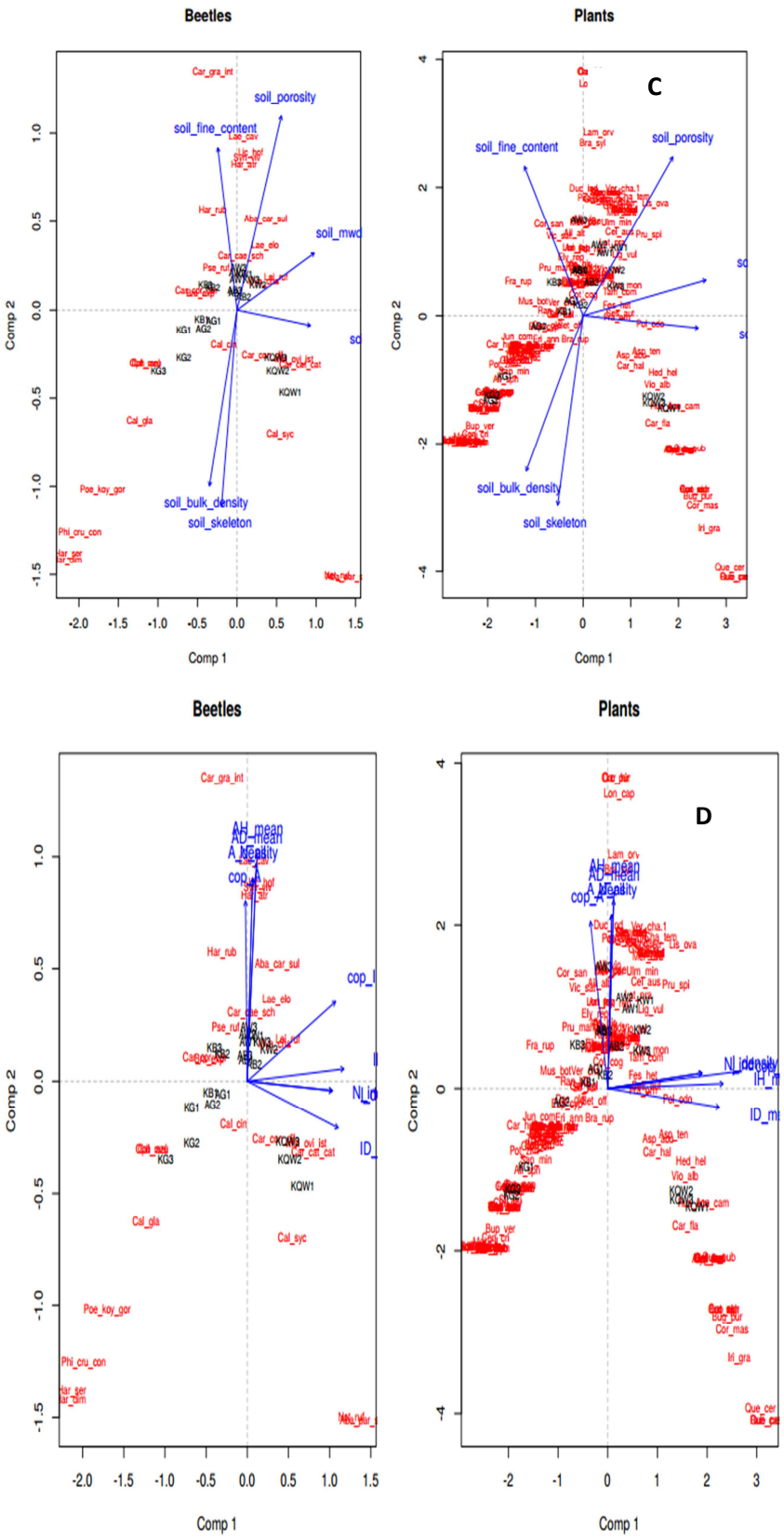

Fig. 3. Predictive Co-CA biplot of carabid beetle species composition (beetles, left) and plant species composition (plants, right) as in Fig. 1 combined with all Environmental Variables. In (A) Co-CA with Environmental, in (B) with habitat type, in (C) with soil variables, in (D) with vegetation structure. The full names of variables are reported in Appendix B.

Soil Variables	**Beetle**	**Beetle**	**Plant**	**Plant**
	R2	**P**	**R2**	**P**
Soil skeleton	0.3945	**0.0191***	0.4506	0.0099**
Soil fine content	0.2788	0.0647.	0.3456	0.0366*
Soil humidity	0.2697	0.0774.	0.2918	0.0655.
Soil bulk density	0.3461	0.0334*	0.3649	0.0300*
Soil porosity	0.4706	0.0053**	0.4811	0.0058**
Soil mwd	0.3283	0.0366*	0.3454	0.0358*
Env Variables	**Beetle**	**Beetle**	**Plant**	**Plant**
	R2	**P**	**R2**	**P**
Lat	0.6340	0.0002 ***	0.5248	0.0025**
Long	0.8428	0.0005***	0.8595	0.0003***
Slope	0.5173	0.0050**	0.6072	0.0021**
Elevation	0.4427	0.0075**	0.3519	0.0295*
Northness	0.1423	50.27.00	0.1400	51.20.00
Eastness	0.0121	150.39.00	0.0289	131.44.00
Dol_depth	0.3984	0.0220	0.3006	0.0648.
Karstification	96.46.00	0.002*	106.03.00	6e-04***
Vegetation Structure	**Beetle**	**Beetle**	**Plant**	**Plant**
	R2	**P**	**R2**	**P**
AH_mean	0.4037	0.0186 *	0.5398	0.0049 **
AD_mean	0.3716	0.0260 *	0.4906	0.0078 **
A_density	0.3199	0.0470 *	69.21.00	0.0171 *
N_ail	0.3199	0.0470 *	0.4161	0.0171 *
Cop_A	0.2491	0.0963 .	0.3958	0.0196 *
IH_mean	0.5352	0.0019 **	0.4956	0.0029 **
ID_mean	0.4891	0.0053 **	0.4681	0.0059 **
I_density	0.4150	0.0172 *	55.50.00	0.0430 *
N_indigene	0.4150	0.0172 *	0.3350	0.0430 *
Cop_I	0.4944	0.0052 **	0.6128	0.0008 ***
	Beetle	Beetle	Plant	Plant
	R2	**P**	**R2**	**P**
Environmental data with soil	97.15.00	2e-04 ***	0.4252	0.0083 **

Table 3 Co-correspondence with Environmental Variables (divided in groups for the Axis 1 and Axis 2 - See Triplots)

4. Discussion and conclusions

Our results showed a direct link between plant and carabid beetles communities. Carabid beetles assemblage composition was directly affected by environmental conditions combined with soil parameters and these two factors had an indirect effect on vegetation. The indirect effect of vegetation structure, in particular of the native canopy, was already reported to be an important factor conditioning carabid beetles assemblages (G□rdner, 1991; S□nder□□n et □l., 1995; Br□ndm□yr et □l., 2005; T□n□be et □l., 2007), because of greater shading and water uptake caused by larger trees, which in turn effects the soil moisture, humidity and the composition of the flora (Ings and Hartley, 1999). Shady humid conditions are associated with many species of carabid beetles especially in the early stages of their development (Thiele, 1977), whilst other species are associated with more dryer and open conditions. A good example of these indirect effects can be observed in the distributions of *Leistus rufomarginatus, Carabus catenulatus catenulatus, Carabus caelatus schreiberi, Molops ovipennis istrianus, Abax parallelepipedus subpunctatus, Calosoma sycophanta:* all these species were more present in the habitats with more abundance of old plants of *Quercus pubescens, Fraxinus ornus, Ostrya carpinifolia,* suggesting a preference for mature woodland (Lindroth, 1985). Ings and Hartley (1999) confirmed these observations and suggested that species are responding to the shady and humid microclimate found under large and old trees, rather than to the presence of mature trees *per se*. Furthermore, it is possible to observe that in our study the different wood habitats were grouped separately, differently respect to what found Tanabe et al. (2007), who did not report any distinction in carabid beetles living in conifer, oak and bamboo forests. We found different species communities: thermotolerant and eliophilous species (Brandmayr et al., 2005) (such as *Synuchus vivalis, Harpalus atratus, Leistus rufomarginatus*), species typical of more humid and fresh wood (*Licinus hoffmanseggii, Abax carinatus sulcatus*), species typical of mesophilous karst wood (*Carabus catenulatus catenulatus, Molops ovipennis istrianus*).

By the way, it is interesting to note that *Licinus hoffmanseggii, Synuchus vivalis, Harpalus atratus* are species more connected with non-native distribution, and this is confirmed by Brandmayr et al. (2005), describing *H. atratus* as a species connected to anthropic and disturbed areas. On the other hand, we found species connected exclusively to grassland habitats, such as *Harpalus dimidiatus, Harpalus serripes, Ophonus azureus,* and *Calathus cinctus,* a typical species of secondary ecosystems and degraded grasslands connected only with non-native grassland.

As already explained, the most important variables showing a determinant congruence across taxa (plants and carabid beetles) were the structure of native vegetation and habitat type (the gradient of vegetation, from grassland to wood). It is likely that when habitat type is changing, the native

structure of vegetation would change too, and as a consequence, also carabid beetles assemblages may change. At the contrary, if we consider non-native plots, in correspondence of habitat type change, the structure of non-native vegetation and carabid beetles assemblages remain almost the same. This fact confirm the known concept that carabid beetles show positive relationship with environmental parameters such as vegetation cover (Thiele, 1977; Gardner, 1991; Gardner et al., 1997; Sanderson et al., 1995; Eyre et al., 2001; Brandmayr et al., 2005; Deconde et al., 2013; Ings and Hartley, 1999; Alignier and Aviron, 2017), but it is noteworthy that this association is more clear in native respect to non-native habitats.

However, it is known that vegetation and soil factors influence the prey of the carabids rather than Carabidae themselves (Ings and Hartley, 1999). Despite Ings and Hartley (1999) did not found a strong effect of soil on carabid beetle communities, we found opposite results. The most important soil factors for both carabid beetles and plant species in our study area were the soil porosity and soil skeleton. Due to these two factors, studied habitats can be divided into two groups: soil porosity was connected with wood habitats, such as woods in sinkhole (native and non-native), and soil skeleton, on the other hand, was connected with grasslands. The first group of species (*Licinus hoffmanseggii*, *Synuchus vivalis, Harpalus atratus*) consisted of taxa more connected to thermophilous woods; the second group included open habitat species, such as *Harpalus atratus, Harpalus dimidiatus, Philorhizus crucifer confusus, Poecilus koyi goricianus.* Carabid beetles living in different kind of grassland are directly associated with soil factors such as soil organic matter content and soil moisture (Luff et al., 1989; Gardner, 1991; Gardner et al., 1997) and there are many studies showing how carabid fauna is useful to discriminate with fine resolution habitats such as grassland (e.g. Tanabe et al., 2007).

In this research, the highly complex interplay of environmental, soil and vegetation structure variables that explain the cross-taxon congruence of the investigated carabid beetles and plant communities, confirm the various biotic and abiotic factors (Judas et al., 2002; Samin et al., 2011) affecting carabid beetles in their distribution, both within and between habitats (Thiele, 1977; Thiele, 1979; Lövei and Sunderland, 1996). Our study area is localized, so that we were expecting less influence in environmental parameters (Duan et al., 2016), that usually become more important in large study area (Qian and Kissling, 2010; Toranza and Arim, 2010).

An in depth-analysis among the environmental variables, showed that plant and carabid beetle assemblages responded to almost the same variables, such as slope, elevation, karstification. Elevation is commonly considered as a whole array of environmental factors (Duan et al., 2016), and its crucial role as explanatory environmental factor for the species. The resulting congruence in cross-taxon is furthermore an evidence that elevation is strongly correlated not only with

intermediate and large study areas, but also with the small ones. Karstification is another important factor (more in plant than in carabid beetles distribution) that must be taken into account, and a proof is represented by two species, *Laemostenus elongatus* and *Laemostenus cavicola*, that in our study were present in relation only to doline forest plots. However, all the environmental variables combined with soil parameters are really significant for carabid beetles distribution.

In conclusion, our results suggest that spatial distribution patterns of carabid beetles species in the study area were mainly determined by environmental parameters rather than vegetation structure or true spatial dependence. Variance partitioning showed that 33% of the total explained variation for the ordination of carabid beetles assemblages was due to the independent effect of environment, whereas the pure effects of spatial variables, vegetation structure and soil parameters explained 8%, 10% and 1% and of this total variation, respectively. If we analyze independently the effect of non-native vegetation structure, it explained the 18% of the total variance, whereas the native vegetation was not informative. The combination of soil and vegetation structure together expressed the 10% of the total variation, and this is in accordance with the main known factors driving the distribution of carabid beetles, that are always related to micro- and mesoscale landscape (Barbaro et al., 2007), and that take into account microclimate, vegetation structure, prey density, predation, competition or localized oviposition sites (Niemelä et al., 1992; Antvogel and Bonn, 2001; Thomaset al., 2001; Magura, 2002; Brose, 2003). Finally, thanks to this similarity pattern in environmental variables -since plants respond to the same underlying main gradient and are at least sensitive to different conditions as carabid beetles (Gioria et al., 2009)-, plants best represent the diversity of carabid beetles in NAK region and can thus be defined as a surrogate group for carabid beetles, as already found by Lários et al. (2017) for Neotropical regions.

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General Conclusion

The results of this study show that the habitat type, i.e. the gradient of vegetation, is the first driver of carabid beetles assemblages among sites, with a clear division in open (thermophilous and grassland species), semi-open (sylvicolous thermophilous species), and close habitat type (sylvicolous and forest species). Going into a detail, the difference in species composition along the vegetation gradient shows an increasing of the species richness from grassland to forest, in contrast to what is usually reported from previous studies. This trend is probably due to the peculiar geographical position of the study area, that lays at low elevation in the warmer part of the Karst plateau at the North border of the Mediterranean zone.

The second driver of carabid beetles assemblages highlighted in the present research was connected with the presence of *A. altissima.* This species is correlated with sites with the highest number of carabid beetles observed in the study area, in accordance with many other studies describing more specific richness in degraded areas, due to the presence of generalist and opportunistic species. Such species show a higher mobility and invasive ability that allow them to be more abundant in disturbed habitat, where prey availability is higher. If we consider the species turnover, it is interesting to note that at this highest number of species correspond the lowest values of species beta diversity, that means that *A. altissima* promote a local selection on species assemblages that induce their homogenization.

Our results showed also a direct link between plant and carabid beetles communities, and the most important variables showing a determinant congruence across taxa (plants and carabid beetles) were again the habitat type and the structure of native vegetation. It is likely that when habitat type is changing, the native structure of vegetation would change too and, as a consequence, also carabid beetles assemblages change. On the contrary, if we consider non-native plots, in correspondence of habitat type change, the structure of non-native vegetation and carabid beetles assemblages remain almost the same. The effect of vegetation can be considered as an indirect effect, since it is intrinsically influenced by environmental conditions combined with soil parameters. Considering the soil, the most important soil parameters for carabid beetles were the soil porosity and soil skeleton. In this research, the highly complex interplay of environmental, soil and vegetation structure variables explain the cross-taxon congruence of the investigated carabid beetles and plant communities. Since we worked in a Karst area, it is interesting to note that also karstification can play an important role in carabid distribution.

The habitat type and the presence of *A. altissima* were the two most important factors related to the distribution of functional traits among plots. The influence of these two factors together was evident in diet, in the reproductive rhythm, in the flying ability, in the size and in the chorology traits. The habitat type was the most important factor determining the distribution of springtail-eater and earthworms-eater, brachypterous species, hydrophilic and heterophilic species, forest and open habitat affinity species, different larva morpho-ecological types, and small size species. The presence of *A. altissima* selected in its habitats more omnivorous and phytophagous species, dimorphic wings morphology species, xerophilic species and generalist species. Omnivorous, pterodimorphic, generalist species are known to be more abundant in disturbed and degraded areas.

Only among native habitats we found a consistent difference in Functional Diversity (FD), that means that there were heterogeneous assemblage of traits composition from grassland to wood. Combining species richness and functional diversity data, in *A. altissima* woods even if the number of species was increasing, the Functional Diversity remained quite similar, meaning homogeneity in traits composition, whereas in *A. altissima* grassland we found an opposite result. In conclusion, *A. altissima* invasion induced changes in functional composition of carabid beetles by creating more heterogeneous community in grassland and more homogeneous community in woodlands, but increasing in both habitats the number of generalist and omnivorous species.

APPENDIX 1

1- Preliminary results of on-going projects

A Dna Barcoding approach to identify plant species in multiflower honey in the North Adriatic Karst (North east Italy)

I started this research in collaboration with professor Alberto Pallavicini and the researcher Valentina Torboli. Using the DNA-barcoding I wanted to assess the floral composition of the Karst honey in order to detect the presence of the daisy *Senecio inaequidens*, an invasive widespread species coming from South Africa. This species contains pyrrolizidine alkaloids (Bicchi et al., 1985) that can cause cancer and hepatic diseases (Dimande et al., 2007; Eller and Chizzola, 2016). I collected 15 different Karst honey samples.

Abstract

The aim of this study was to test the ability of DNA barcoding to identify the plant richness and origin presented in the processed honey. Twelve multifloral plus two specific honeys (Santoreggia and Acacia) produced at different sites in a floristically rich area in the North Adriatic Karst were examined by using the ITS2 as barcode markers. An extensive reference database of barcode sequences was generated to determine the taxonomic composition of honey. One hundred and seven plant species were identified in the fourteen honey samples, each of which originated from a mix of common plants belonging to Castanea, Corylus, Quercus, Fagus, Salix and several herbaceous taxa. Interestingly, many typical Karst species were detected in the majority of the honey samples, providing a clear signature for the geographic identity of these products. DNA of the toxic plant *Senecio inaequidens* was detected in one sample, illustrating the usefulness of DNA barcoding for evaluating the safety of honey, as already reported by Bruni et al. (2015) for the toxic plant *Atropa belladonna*. More, we detected also the presence of *Zygosaccaromices mellis* and *Malassezia restricta*.

Keywords: Food traceability, Honey, Pollen identification, ITS2

2. Material and methods

2.1. Study area and honey sampling

In this study, 15 honey samples (Acacia_Peteano; Acacia_Piscianzi; Colombetta; Frusck_Medeazza; Gruden_Sgonico; Kaski_Gabrovica; Lombardo_Ronchi; Millefiori_Piscianzi; San Michele del Carso; Santoreggia; Settimi_Trebec; Silvan-Doberdò; Vilma_Pesek) produced in the North Adriatic Karst (North-eastern Italy), were selected to investigate their botanical composition through a DNA barcoding approach. Thirteen honeys were sold by the producers in the markets as 'multifloral honey', one as "Santoreggia honey", one as "Acacia honey" produced during the period of June - September 2016 by amateur beekeepers from fifteen different localities. For each sample an aliquot of 10 g was used for DNA extraction.

The North Adriatic Karst (NAK) is a well-known area for its geographical, geomorphological characteristics and speleological phenomena, positioned between the Adriatic Sea and the Alps. The study site is a typical Karstic area, that consist of limestone Karst plateau (Low Karst), 100-500 m s.l.m., with the typical geomorphological phenomena (i.e. karst poljes, sinkholes, caves, etc.), with characteristic Red mediterranean soils (Kaligarič et al., 2006). The Karst is known and traditionally recognized as a treeless, stony grassland landscape (with exceptions of manyflysch-bedrock patches), where the strong bora wind affects vegetation causing desiccation and soil. The climate is sub-mediterranean/transitional between Mediterranean and continental pre-Alpine, with rainy cool winters and long and dry summers; the mean temperature on the Karst Plateau is 12 °C, with the minimum around -15°C and the maximum about 34°C (Kaligarič et al., 2006).

The Karst grasslands of the NAK are known as one of the richest plant communities among all grasslands. Their diversity has been described, emphasized and partly explained in several works due to the specific biogeographic position of these grasslands, since the NAK is in the transitional area where the Mediterranean basin joins the Dinaric mountains and Central Europe. The contingent of Euro-Mediterranean (sub-Mediterranean)-Illyrian species, particularly rich in biodiversity, in this area is accompanied by other large interesting contingents of species e.g., Mediterranean-Pontic or Mediterranean-Pannonian species (Pipenbaher et al., 2011). The evergreen woodland, a North-Adriatic maquis, is developed as extrazonal vegetation only in very small patches: in this belt tree species such as *Quercus pubescens, Carpinus orientalis, Ostrya carpinifolia, Fraxinus ornus* and rarely *Acer monspessulanum* and *Pistacia therebinthus* constitute the present sub-Mediterranean vegetation in the NAK, below the beech forest zone (class *Festuco-Brometea*) (Kaligarič et al., 2006).

2.2 Reference DNA barcoding database

2.3 DNA extraction and purification

The Quick-DNA™ Fungal/Bacterial Miniprep Kit is designed for the simple and rapid isolation of DNA from tough-to-lyse fungi, bacteria, algae, protozoa, and pollen. The procedure is easy and can be completed in a few minutes: samples are rapidly and efficiently lysed with ultra-high density BashingBeads™. Fast-Spin column technology is then used to isolate the DNA that is ideal for downstream molecular-based applications including PCR, array, and NGS.

Honey (10 g which might contain pollen cells derived from plants) was dissolved in 50 ml sterile water and incubated at 65°C for 30 min, followed by centrifugation at 5000 rpm for 30 min. The supernatant was discarded, and the pellet was dissolved in 30 ml sterile water. Another centrifugation at 5000 rpm for 30 min follows this procedure. The pellet was dissolved in 10 ml sterile water and centrifuged at 5000 rpm for 30 min. The pellet, dried for 5 min at room temperature, was dissolved in 750 µl Lysis Solution provided with Quick-DNA™ Fungal/Bacterial Miniprep Kit Quick-DNA™ Fungal/Bacterial Miniprep Kit. DNA was extracted following the provided protocol. Concentration and quality of DNA were checked with NanoDrop 2000.

2.4. DNA Barcode region amplification and sequencing

The most common and recommended barcodes for Plantae recognition are the combination of two different DNA region from plastid genome, rbcL and matK. Nevertheless, plastid DNA is not always detectable in pollen (Corriveau et al., 1990) so ITS2 has been adopted as taxon barcode in this study. ITS (internal transcribed spacer of nuclear ribosomal DNA) or a part of it, specifically ITS2, is one of the most widely used DNA fragments in plant molecular systematics at the generic and species levels because of its potentially high resolution of inter- and intraspecific relationships (Yao et al., 2010; Claire-Iphanise et al., 2012). The amplification of the target barcode, the variable ITS region between sequence encoding for 5.8S and the sequence encoding for LSU (Large Subunit) of the ribosomal operon, was performed with primers ITS-u2_forward (ITS-u2 reversed, Cheng 2016) and ITS-p4, specific for Plantae (Cheng et al., 2016), with adapters. ITSu3-ITSp4 primers (Cheng, 2016) had been chosen previously, but the amplicon size was too large for Ion Torrent application. PCR amplification was carried out in 25 µl reactions containing 5X PCR buffer, 2 mM MgCl2, 0.5 µM of each primer, 0.2 uM dNTPs, 1 U Taq polymerase (KAPA HiFi DNA Polymerase). Amplification conditions were: 98°C for 1 minute, followed by 35 cycles of 95° for 10”, 57° for 15”, 72° for 30” and a step at 72°C for 3 minutes. Amplicons were checked on 2,5% agarose gel using TBE buffer. A second short PCR (5 cycles) was performed to generate a

library of DNA fragments flanked by the Ion Torrents adapters. Samples were quantified and pooled and are ready for Ion-Torrent sequencing.

3. Results

The library of ITS2 sequences was prepared. A first library was prepared using the universal primer system ITS-u3 and ITS-u4, developed by Cheng et al. (2016), which designed new universal PCR primers for amplifying the whole ITS region or a part of it (ITS1 or ITS2). An amplicon was obtained from a random sample and sequenced with Sanger chemistry to exclude the exclusive presence of bee DNA. We detected *Ascosphaera apis* (Maasen ex Claussen) L.S.Olive & Spiltoir (1955) ITS2 region. *A. apis* is a species of Fungi which causes chalkbrood diseases in honey bees (Wynns et al., 2012). In the future, it would be interesting to investigate the presence of *A. apis* in the hives of the Karst region.

After that, ITS-p3 and ITS-u4 PCR primer system specific for Plants, shown in Fig.1, were tested. Unfortunately, it was not possible to sequence the library with Ion Torrent technology due to the overly large size of the amplicons, which measured 529 bp with tails, adapters, and barcodes.

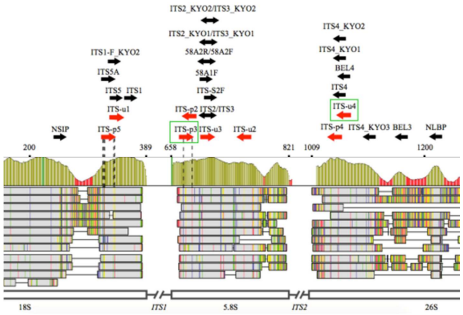


Fig. 1. Location of forward (right-pointing arrows) and reverse (left-pointing arrows) of primers developed in Cheng's study. In the green boxes, ITS-p3 and ITS-u4, used for the second version of the honey contents library.

Another new library was prepared using a Cheng's PCR primer system, modified *ad hoc* for this experiment, ITS-u2 reversed (the blue arrow in Fig.2) and ITS-p4 in order to shorten the amplicon as much as possible. The new amplicon measures 409 bp with tails, barcodes and adapters, suitable dimension for Ion Torrent sequencing.

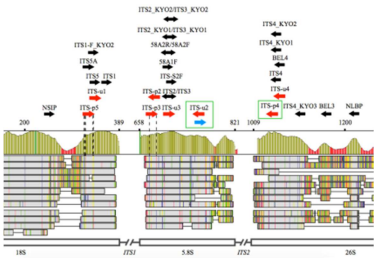
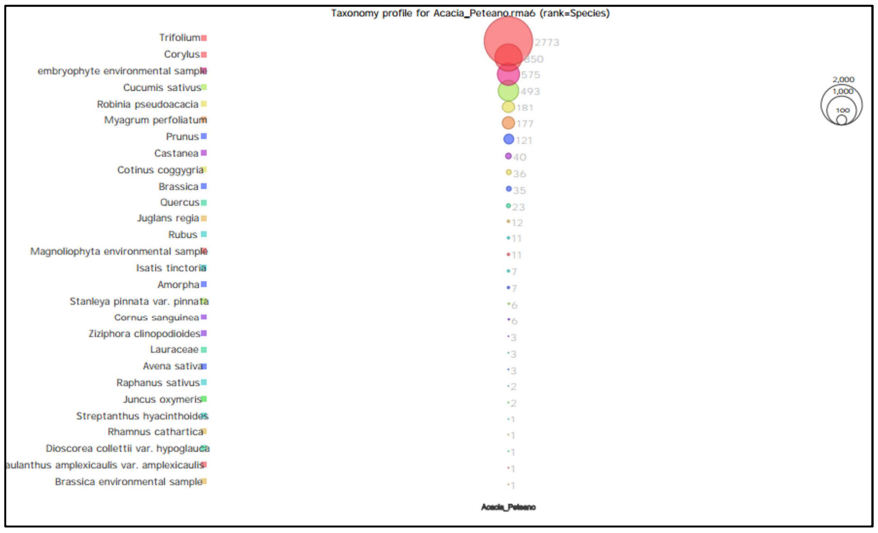
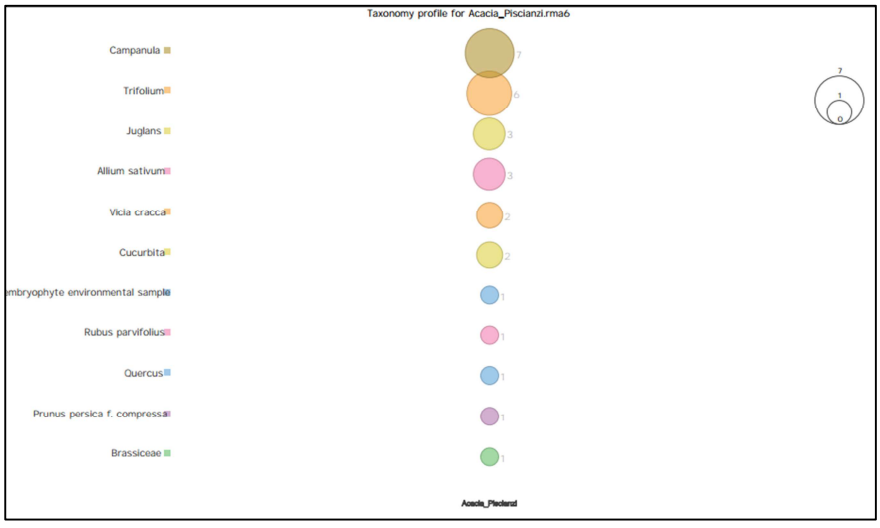


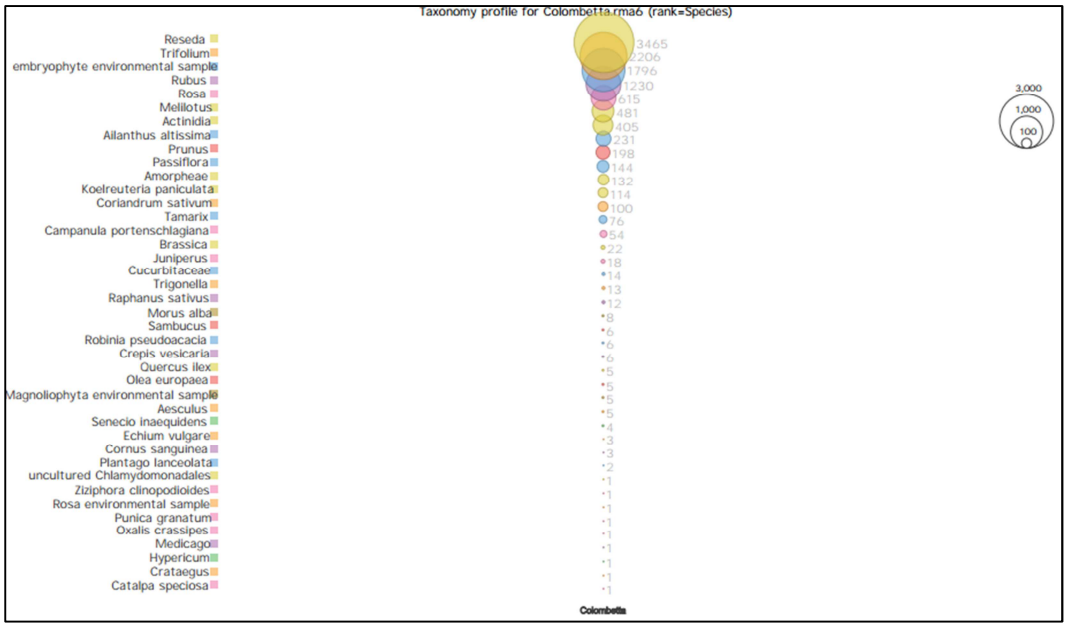
Fig. 2. Location of forward (right-pointing arrows) and reverse (left-pointing arrows) of primers developed in Cheng's study. In the green boxes, ITS-u2 and ITS-p4, used for the definitive version of the honey contents library. The blue arrow indicates that the original ITS-u2 was reversed for this study.

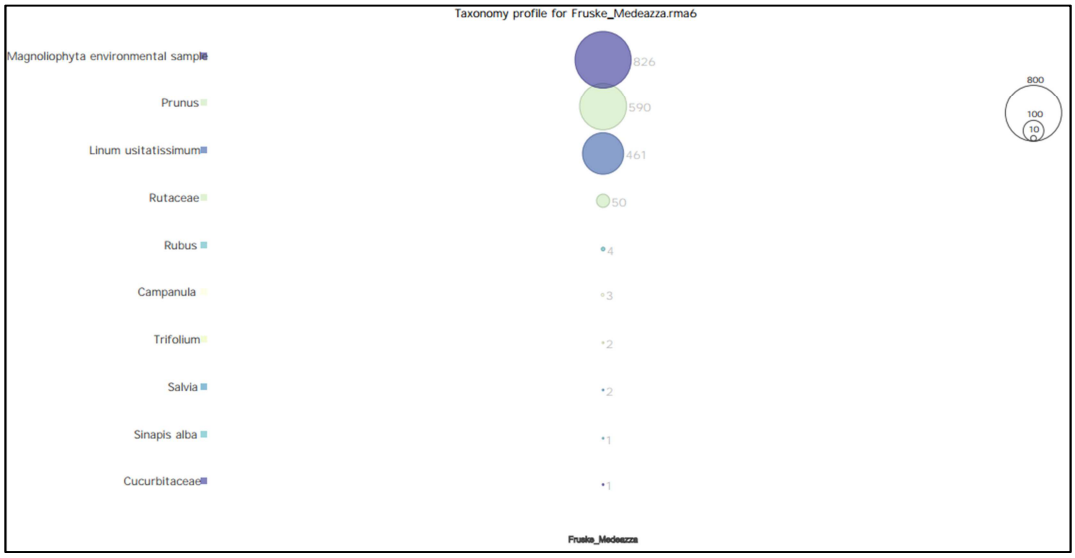
The library of ITS2 sequences of the floristic contents of honey is ready, already quantified, pooled and is waiting to be sequenced by Department of Life Sciences of the University of Trieste.

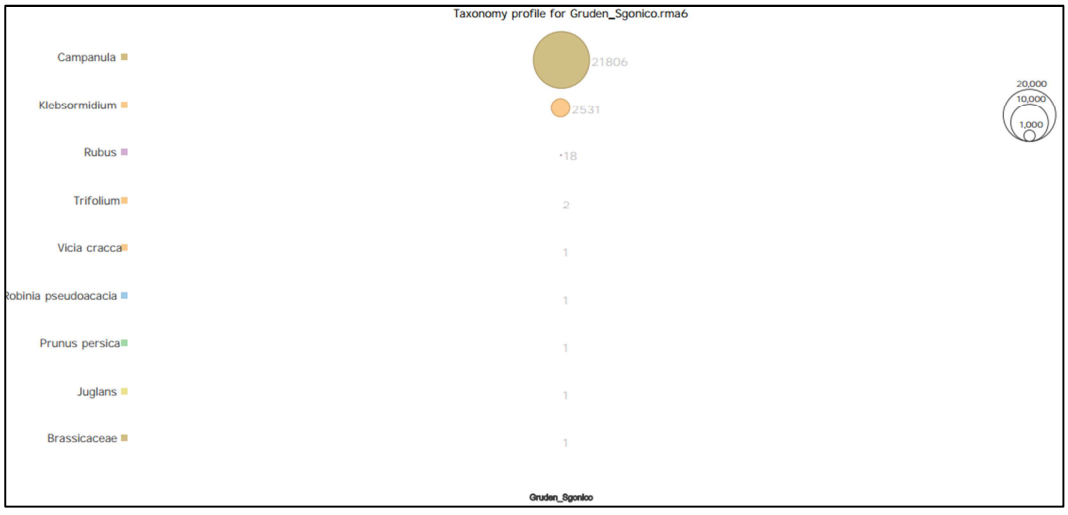
In the following images (Fig. 3) there are the different plant species found in each honey sample.

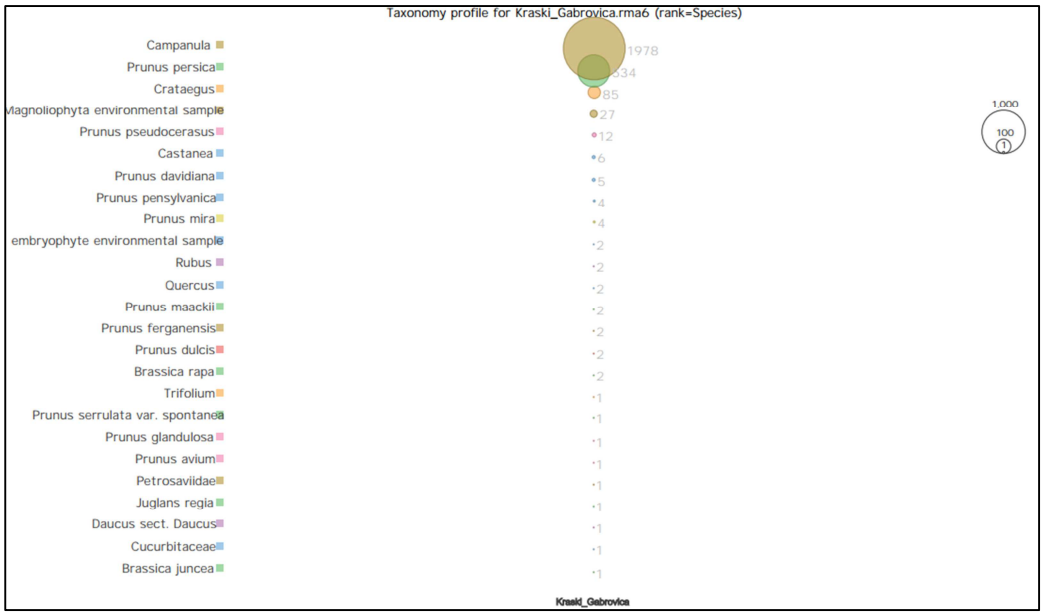





Taxonomy profile for Colombetta.rma6 (rank=Species)
Reseda
Trifolium
embryophyte environmental sample
Rubus
Rosa
Melilotus
Actinidia
Ailanthus altissima
Prunus
Passiflora
Amorpheae
Koelreuteria paniculata
Coriandrum sativum
Tamarix
Campanula portenschlagiana
Brassica
Juniperus
Cucurbitaceae
Trigonella
Raphanus sativus
Morus alba
Sambucus
Robinia pseudoacacia
Crepis vesicaria
Quercus ilex
Olea europaea
Magnoliophyta environmental sample
Aesculus
Senecio inaequidens
Echium vulgare
Cornus sanguinea
Plantago lanceolata
uncultured Chlamydomonadales
Ziziphora clinopodioides
Rosa environmental sample
Punica granatum
Oxalis crassipes
Medicago
Hypericum
Crataegus
Catalpa speciosa
3465
2206
1796
1230
615
481
405
231
198
144
132
114
100
76
54
22
18
14
13
12
8
6
6
5
5
5
4
3
3
2
1
1
1
1
1
1
1
1
1
3,000
1,000
100
Colombetta


Taxonomy profile for Fruske_Medeazza.rma6
Magnoliophyta environmental sample
Prunus
Linum usitatissimum
Rutaceae
Rubus
Campanula
Trifolium
Salvia
Sinapis alba
Cucurbitaceae
826
590
461
50
4
3
2
2
1
1
800
100
10
Fruska_Medeazza


Taxonomy profile for Gruden_Sgonico.rma6
Campanula
Klebsormidium
Rubus
Trifolium
Vicia cracca
Robinia pseudoacacia
Prunus persica
Juglans
Brassicaceae
21806
2531
18
2
1
1
1
1
1
20,000
10,000
1,000
Gruden_Sgonico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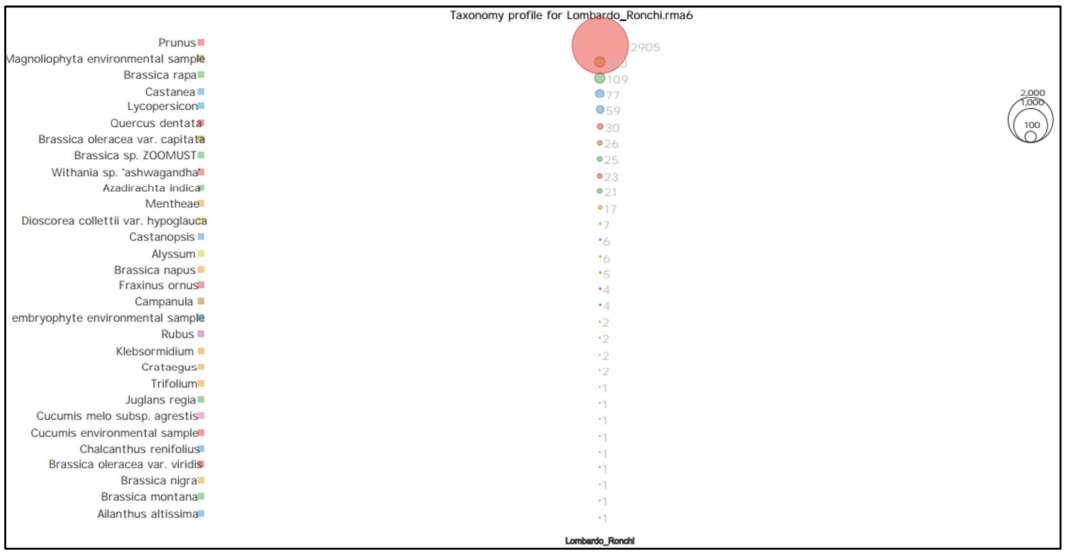
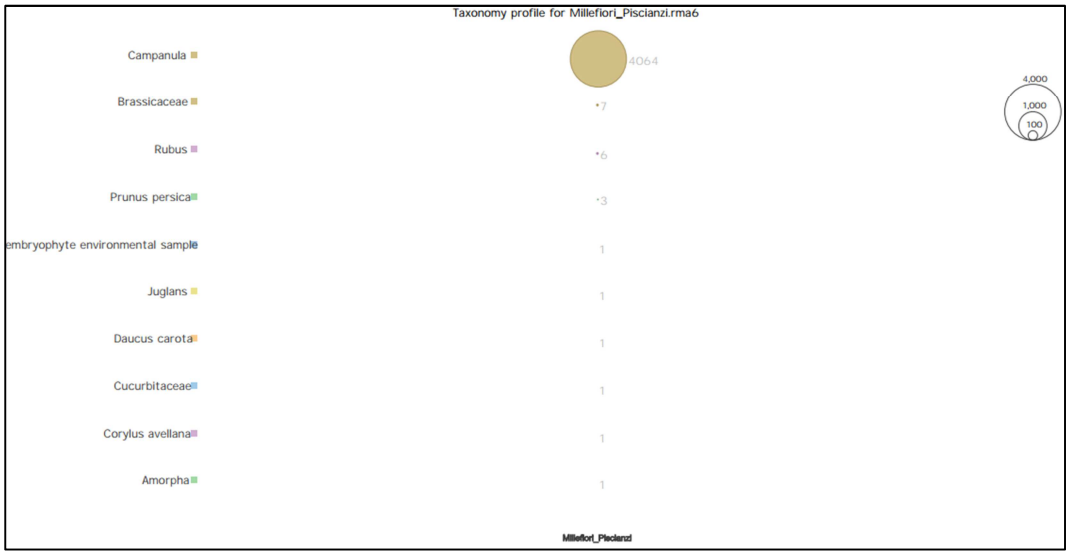
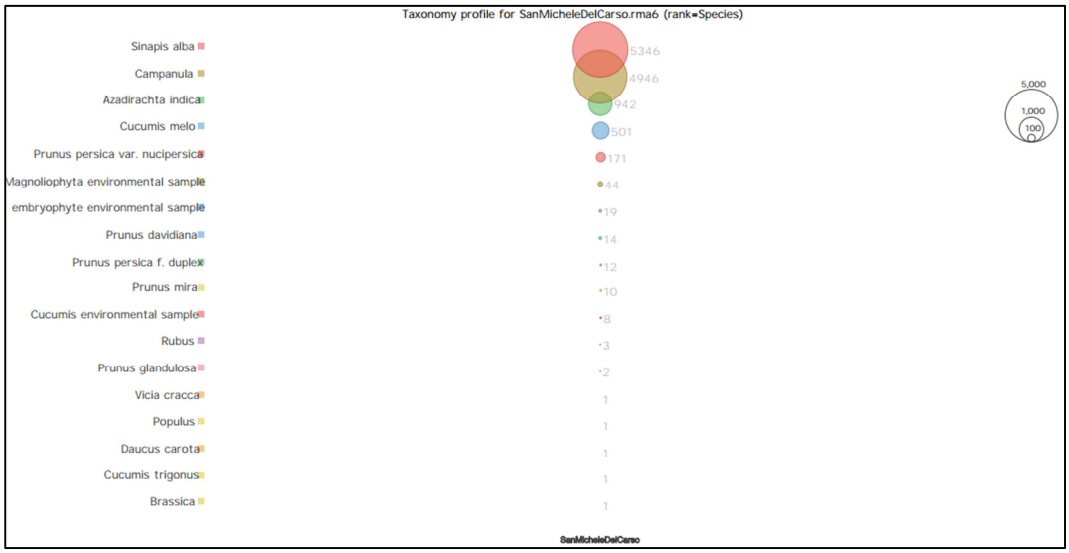
Campanula 1978
Prunus persica 234
Crataegus 85
Magnoliophyta environmental sample 27
Prunus pseudocerasus 12
Castanea 6
Prunus davidiana 5
Prunus pensylvanica 4
Prunus mira 4
embryophyte environmental sample 2
Rubus 2
Quercus 2
Prunus maackii 2
Prunus ferganensis 2
Prunus dulcis 2
Brassica rapa 2
Trifolium 1
Prunus serrulata var. spontanea 1
Prunus glandulosa 1
Prunus avium 1
Petrosaviidae 1
Juglans regia 1
Daucus sect. Daucus 1
Cucurbitaceae 1
Brassica juncea 1
1,000
100
1
Kraski_Gabrovica


Taxonomy profile for Lombardo_Ronchi.rma6
Prunus 2905
Magnoliophyta environmental sample 109
Brassica rapa 109
Castanea 77
Lycopersicon 59
Quercus dentata 30
Brassica oleracea var. capitata 26
Brassica sp. ZOOMUST 25
Withania sp. 'ashwagandha' 23
Azadirachta indica 21
Mentheae 17
Dioscorea collettii var. hypoglauca 7
Castanopsis 6
Alyssum 6
Brassica napus 5
Fraxinus ornus 4
Campanula 4
embryophyte environmental sample 2
Rubus 2
Klebsormidium 2
Crataegus 2
Trifolium 1
Juglans regia 1
Cucumis melo subsp. agrestis 1
Cucumis environmental sample 1
Chalcanthus renifolius 1
Brassica oleracea var. viridis 1
Brassica nigra 1
Brassica montana 1
Ailanthus altissima 1
2,000
1,000
100
Lombardo_Ronchi


Taxonomy profile for Milleflori_Piscianzi.rma6
Campanula 4064
Brassicaceae 7
Rubus 6
Prunus persica 3
embryophyte environmental sample 1
Juglans 1
Daucus carota 1
Cucurbitaceae 1
Corylus avellana 1
Amorpha 1
4,000
1,000
100
Milleflori_Piscianzi


Taxonomy profile for SanMicheleDelCarso.rma6 (rank=Species)
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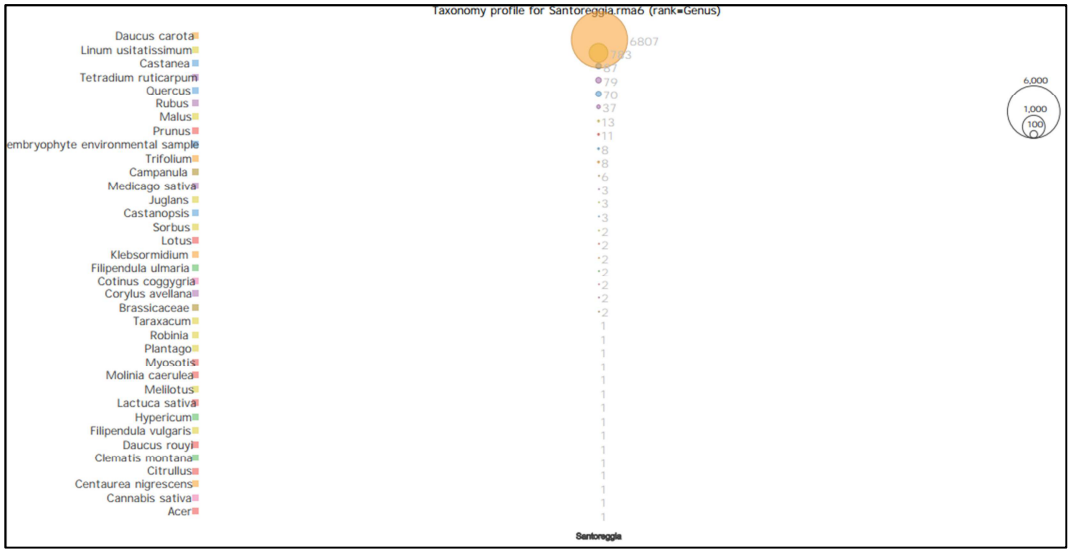
Campanula
Azadirachta indica
Cucumis melo
Prunus persica var. nucipersica
Magnoliophyta environmental sample
embryophyte environmental sample
Prunus davidiana
Prunus persica f. duplex
Prunus mira
Cucumis environmental sample
Rubus
Prunus glandulosa
Vicia cracca
Populus
Daucus carota
Cucumis trigonus
Brassica
5346
4946
942
501
171
44
19
14
12
10
8
3
2
1
1
1
1
1
5,000
1,000
100
SanMicheleDelCarso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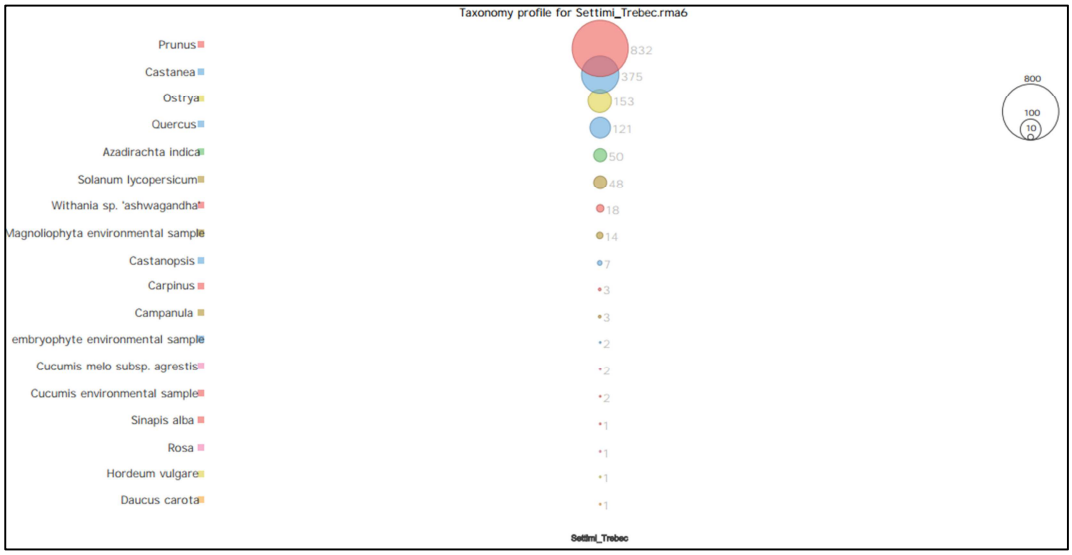
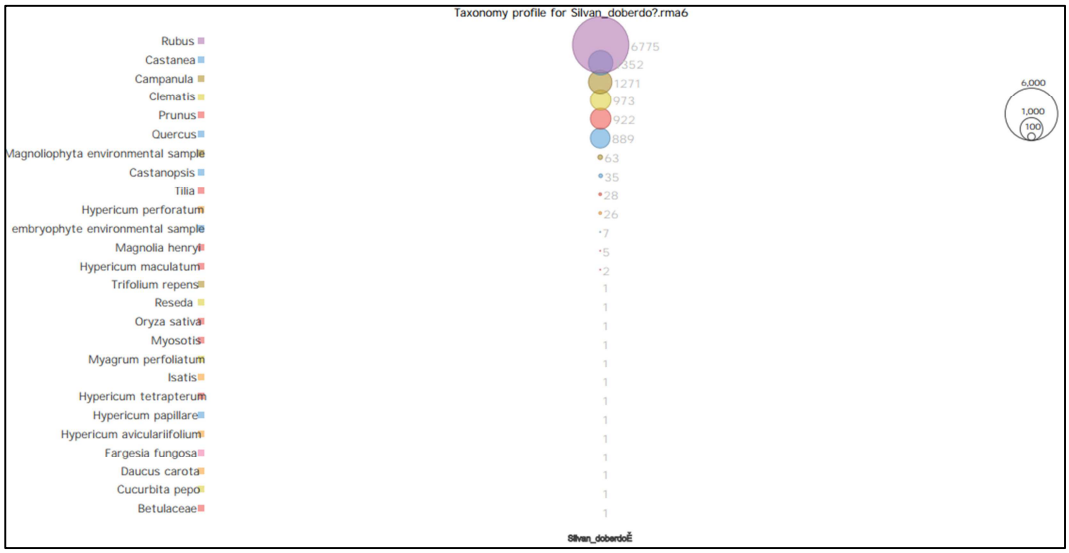
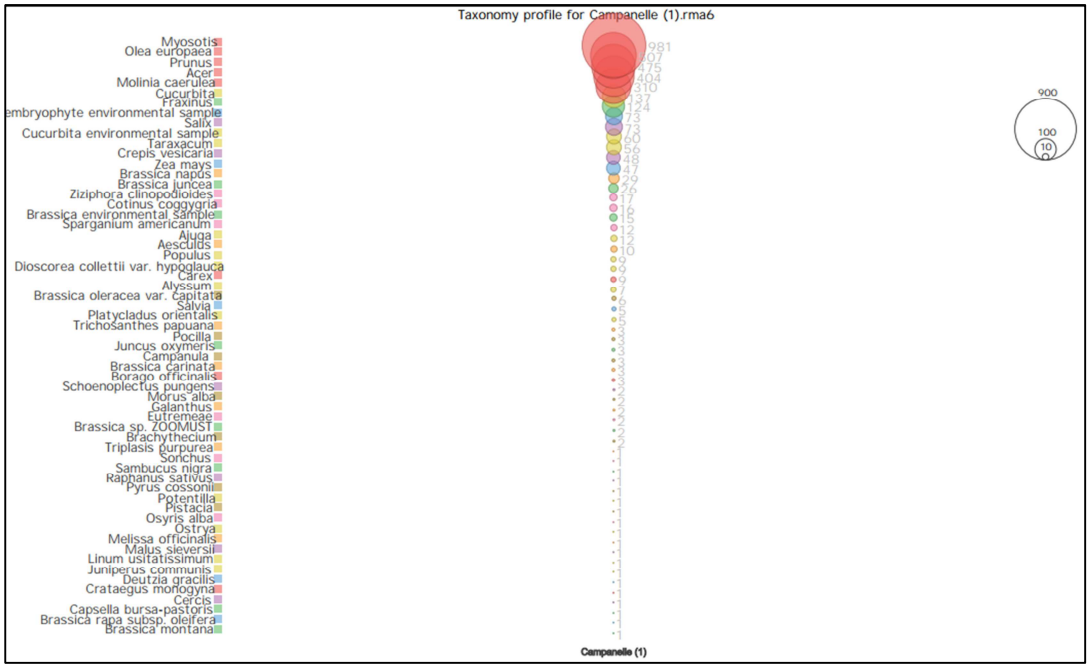
Daucus carota
Linum usitatissimum
Castanea
Tetradium ruticarpum
Quercus
Rubus
Malus
Prunus
embryophyte environmental sample
Trifolium
Campanula
Medicago sativa
Juglans
Castanopsis
Sorbus
Lotus
Klebsormidium
Filipendula ulmaria
Cotinus coggygria
Corylus avellana
Brassicaceae
Taraxacum
Robinia
Plantago
Myosotis
Molinia caerulea
Melilotus
Lactuca sativa
Hypericum
Filipendula vulgaris
Daucus rouyi
Clematis montana
Citrullus
Centaurea nigrescens
Cannabis sativa
Acer
6807
79
70
37
13
11
8
8
6
3
3
3
2
2
2
2
2
2
2
1
1
1
1
1
1
1
1
1
1
1
1
1
1
1
6,000
1,000
100
Santoreggia


Taxonomy profile for Settimi_Trebec.rma6
Prunus
Castanea
Ostrya
Quercus
Azadirachta indica
Solanum lycopersicum
Withania sp. 'ashwagandha'
Magnoliophyta environmental sample
Castanopsis
Carpinus
Campanula
embryophyte environmental sample
Cucumis melo subsp. agrestis
Cucumis environmental sample
Sinapis alba
Rosa
Hordeum vulgare
Daucus carota
832
375
153
121
50
48
18
14
7
3
3
2
2
2
1
1
1
1
800
100
10
Settimi_Trebec


Taxonomy profile for Silvan_doberdo?.rma6
Rubus
Castanea
Campanula
Clematis
Prunus
Quercus
Magnoliophyta environmental sample
Castanopsis
Tilia
Hypericum perforatum
embryophyte environmental sample
Magnolia henryi
Hypericum maculatum
Trifolium repens
Reseda
Oryza sativa
Myosotis
Myagrum perfoliatum
Isatis
Hypericum tetrapterum
Hypericum papillare
Hypericum avicularliifolium
Fargesia fungosa
Daucus carota
Cucurbita pepo
Betulaceae
6775
352
1271
973
922
889
63
35
28
26
7
5
2
1
6,000
1,000
100
Silvan_doberdoË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Myosotis
Olea europaea
Prunus
Acer
Molinia caerulea
Cucurbita
Fraxinus
embryophyte environmental sample
Salix
Cucurbita environmental sample
Taraxacum
Crepis vesicaria
Zea mays
Brassica napus
Brassica juncea
Ziziphora clinopodioides
Cotinus coggygria
Brassica environmental sample
Sparganium americanum
Ajuga
Aesculus
Populus
Dioscorea collettii var. hypoglauca
Carex
Alyssum
Brassica oleracea var. capitata
Salvia
Platycladus orientalis
Trichosanthes papuana
Pocilla
Juncus oxymeris
Campanula
Brassica carinata
Borago officinalis
Schoenoplectus pungens
Morus alba
Galanthus
Eutremeae
Brassica sp. ZOOMUST
Brachythecium
Triplasis purpurea
Sonchus
Sambucus nigra
Raphanus sativus
Pyrus cossonii
Potentilla
Pistacia
Osyris alba
Ostrya
Melissa officinalis
Malus sieversii
Linum usitatissimum
Juniperus communis
Deutzia gracilis
Crataegus monogyna
Cercis
Capsella bursa-pastoris
Brassica rapa subsp. oleifera
Brassica montana
981
907
475
404
310
137
124
73
73
60
56
48
47
29
26
17
16
15
12
12
10
9
9
9
7
7
6
5
5
3
3
3
3
3
3
2
2
2
2
2
2
1
1
1
1
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1
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1
900
100
10
Campanelle (1)

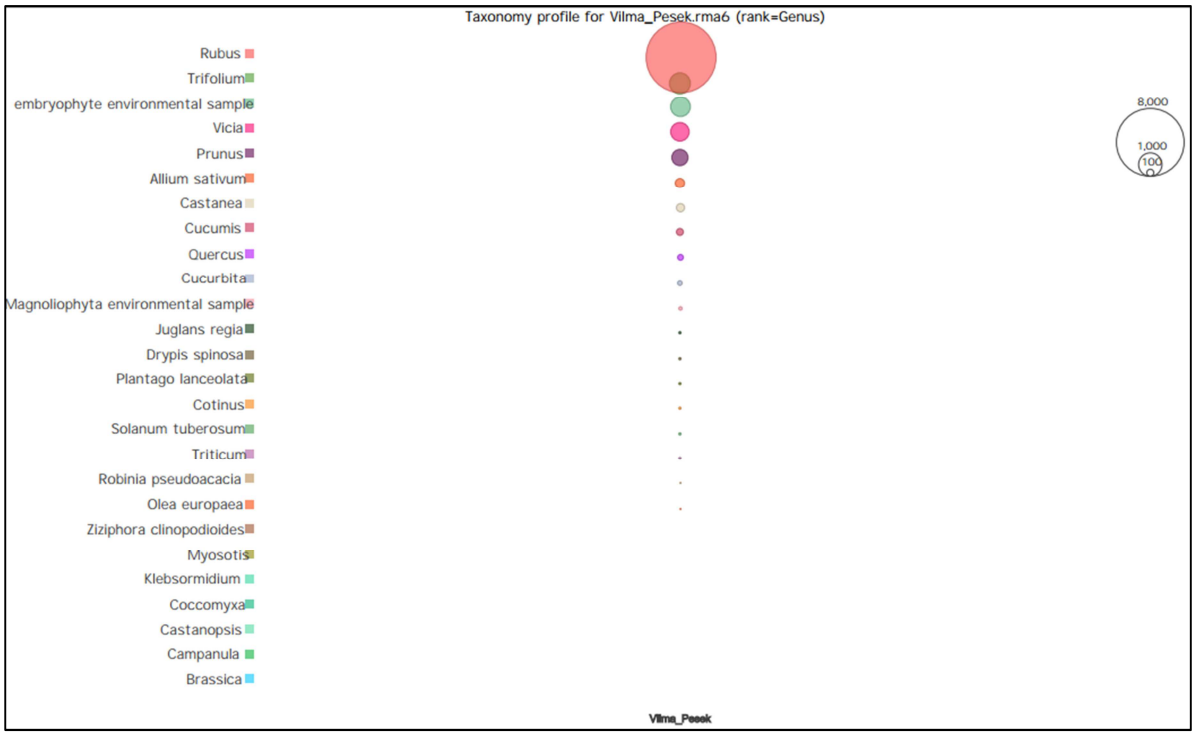

Fig. 3. Different honey flower composition.

It is importan to underline that in Acacia_Piscianzi, on 17313 reads, 16336 reads are attributed to Zygosaccaromices mellis; in Millefiori_Piscianzi on 11047 reads, 9648 reads are of Z. mellis; in Fruske_Medeazza on 8537 reads, 6957 are of Z. mellis and 1070 reads belong to Malassezia restricta.

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2-Preliminary results of ongoing projects

The utility of DNA metabarcoding for studying the response of arthropod diversity and composition to *Ailanthus altissima* invasion in the North Adriatic Karst (North East Italy)

1. Introduction

One limitation of metabarcoding is the efficiency of assigning taxonomy to molecular operational taxonomic units (MOTUs). Though the percentage of MOTUs assigned to order level is usually high, this is not the case for assignments at a lower taxonomic level (e.g. for insects; Order [96–99%], Family [17–37%], Genus [16–36%] and Species [16–35%] (Yu et al., 2012). This problem is not due to the metabarcoding pipeline used, but rather to the lack of comprehensive and taxonomically reliable barcode databases for most taxa (Yu et al., 2012; Glenn, 2014). To investigate the utility of DNA metabarcoding in a study of patterns of litter and soil arthropod diversity in different habitat type in North Adriatic Karst, we address the following questions:

1. Do clustered MOTUs indicate significant community differentiation among habitat typology environmental gradients?
2. Which arthropod groups show significant changes across habitats?

2. Material and methods

2.1. Study area

For the study area see the Chapter 1 of the thesis. We excluded in this part of the research the Grassland habitats (native and non-native).

2.1. Sample collection

Bulk litter arthropod samples were collected from 24 randomly selected points belonging to 8 different habitat type: three *A.altissima* woods (all three in sinkhole), two Karst woodlands in sinkhole, one mixed (*A.altissima* and native vegetation together) woodland, two Karst woods in slope. Plots were selected to be as similar as possible and spatially close to minimize confounding differences in environmental conditions (Beng et al., 2016). In each site, three quadrats of 1 m^2

were randomly selected (following Yang et al., 2014) and the entire amount of soil was collected using a hoe and was put in a PVC box and meshed; after that, 15 g were extracted and placed in a sterilized plastic tube. After every different habitat, the instruments were let in bleach for 20 minutes. All leaf litter and loose humus from within the frame area were collected into the box together with the soil and all the large leaf-litter materials were removed. The resulting 'siftate' was transported to the laboratory in polythene bags, where it was immediately freeze.

2.2 Soil sampling preparation

Soil samples from each site were prepared separately by pouring the contents of the collection tubes into a clean sterile petri dish. From each petri dish 1 g of soil was extracted and positioned in an Eppendorf. These samples were subsequently freezed. Genomic DNA was extracted using the E.Z.N.A.® Soil DNA Kit (Omega Biotek Store) according to the manufacturer's instructions.

2.3 DNA extraction

E.Z.N.A.® Soil DNA Kit (Omega Biotek Store) is formulated to isolate high purity cellular DNA from soil samples typically containing humic acid and other inhibitors of PCR. DNA was extracted from soil samples with E.Z.N.A.® Soil DNA Kit, after a bead-beating step. Bead-beating is a mechanical disruption method in which beads are added to the tube containing samples and the tube is then shaken, causing collisions between the beads and samples to improve the extraction efficiency. Concentration and quality of DNA were checked with NanoDrop 2000.

2.4 Barcode region amplification and sequencing

For the soil components analysis, COI has been adopted as taxon barcode. In fact, Oxidase subunit I gene (COI) is the most widely available sequence region in public reference libraries. The available versatile "universal" COI primers (Folmer, 1990) target the 658 barcoding region, whose size is considered too large for many NGS applications. So, the amplification of target region was performed with primers mlCOIintF/HCO2198 (Leray et al., 2013), with adapters, target a 313 barcode region. PCR amplification was carried out in 25 µl reactions containing 5X PCR buffer, 2 mM MgCl2, 0.5 µM of each primer, 0.2 µM dNTPs, 1 U Taq polymerase (KAPA HiFi DNA Polymerase). Amplification conditions were: 98°C for 1 minute, followed by 35 cycles of 95° for 10", 48° for 15", 72° for 30" and a step at 72°C for 3 minutes. Amplicons run on 2,5% agarose gel using TBE buffer. A second short PCR (5 cycles) was performed to generate a library of DNA fragments flanked by the Ion Torrents adapters. Samples were quantified and pooled and an

emulsion PCR was carried out with Ion PGM™ Hi-Q™ OT2 kit. They were sequenced with Ion PGM™ Hi-Q™ sequencing (Ion Torrent) on an Ion 314 V2 chip.

2.5 Data Analysis

We used site presence-absence data for diversity (alpha) and community composition analyses. Alpha (α)-diversity was estimated as the number of observed MOTUs per site. We used Pairwise differences (Table 1) in MOTU diversity among habitat types using the nonparametric multiple comparison function (dunn.test) implemented in the R package dunn.test 1.2.4. The dunn.test is equivalent to the Kruskall–Wallis and pair-wise Mann–Whitney post hoc tests with Bonferroni correction. These analyses were performed in R.

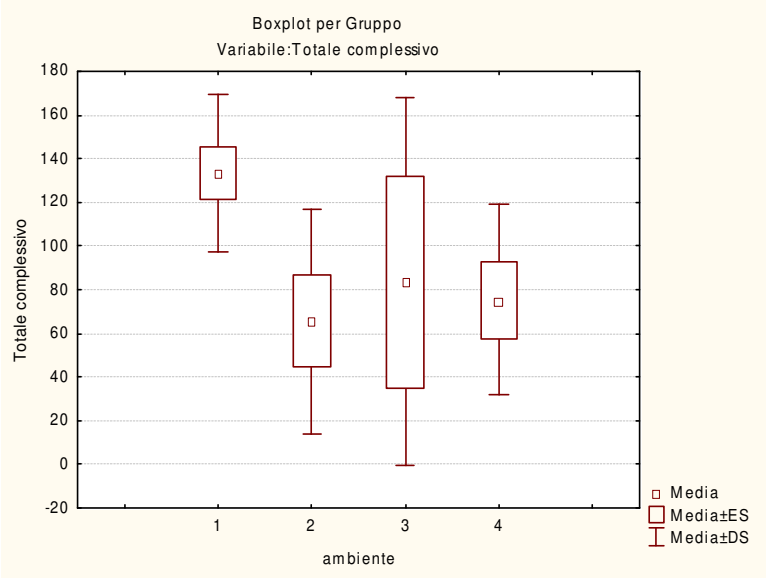
3. Results

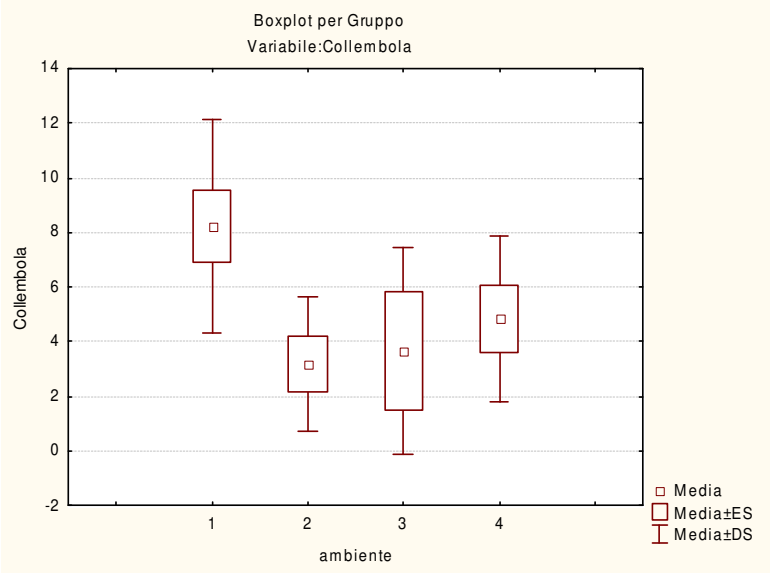
We tried to assign (BLAST) each sequences to a taxonomy using a reference database and we did the assignments at 97% of similarity. So we considered the species rank.

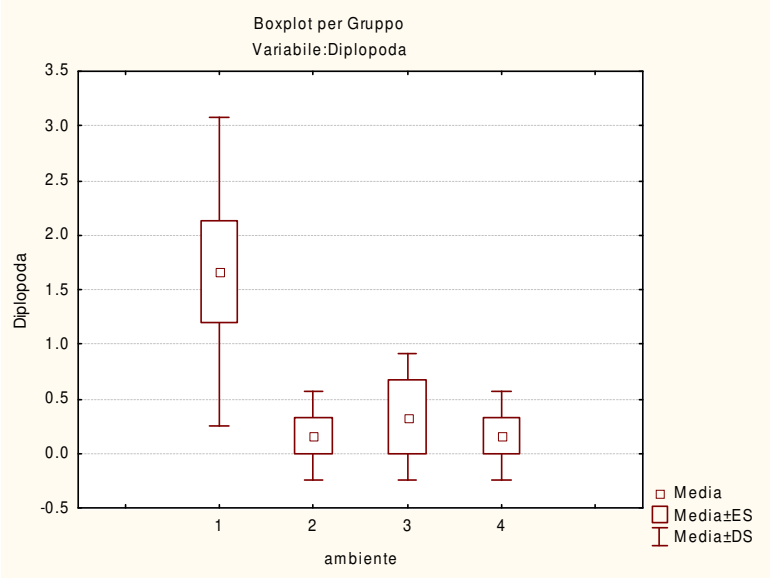
	Z^2	p-value	Pairwise comparison, only significant couple	H	p-value
All MOTUs	**7.11**	**0.06**	**Ns**		
Collembola	**7.53**	**0.05**	**Ailanthus vs Karst doline wood**	**2.48**	**0.07**
Diplopoda	**7.39**	**0.06**	**Ns**		
Diptera	**6.39**	**0.09**	**Ailanthus vs Karst doline wood**	**2.46**	**0.08**
Gastropoda	**8.66**	**0.03**	**Ailanthus vs Karst doline wood**	**2.66**	**0.04**
Hemiptera	**9.39**	**0.02**	**Ailanthus vs Karst doline wood**	**2.41**	**0.09**
	9.39	**0.02**	**Ailanthus vs mix woodland**	**2.43**	**0.08**
Hymenoptera	**6.95**	**0.07**	**Ns**		
Isopoda	**2.09**	**ns**	**Ns**		
Lepidoptera	**7.36**	**0.06**	**Ns**		
Protura	**7.66**	**0.05**	**Ns**		
Symphila	**6.27**	**0.09**	**Ns**		
Trombidoformes	**6.63**	**0.08**	**Ns**		
Araneae	**6.03**	**ns**	**Ns**		

Chilopoda	**1.47**	**ns**	**Ns**		

Table 1. Alpha diversity differences among the three main habitat types (*Ailanthus altissima* wood, Karst doline Wood, Mix Woodland) for all MOTUs combined and for individual arthropod groups. Differences are based on Kruskal-Wallis rank sum test and Mann-Whitney U test with Bonferroni correction



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Media
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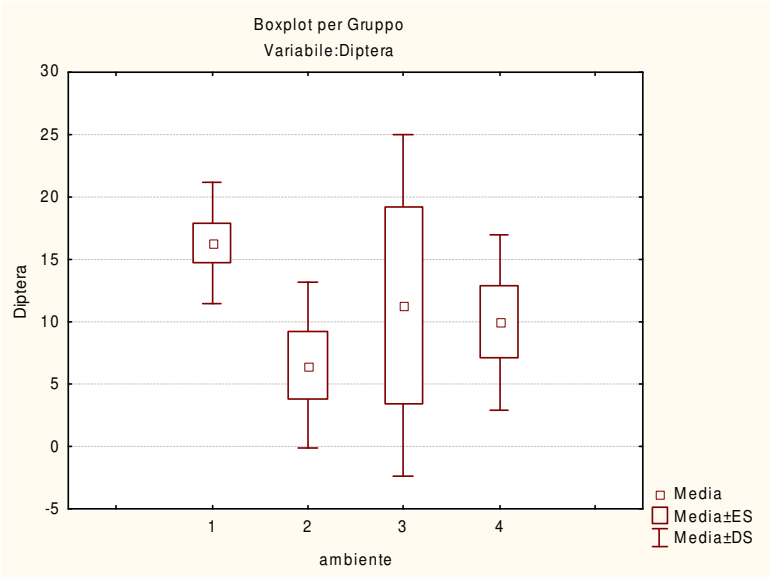
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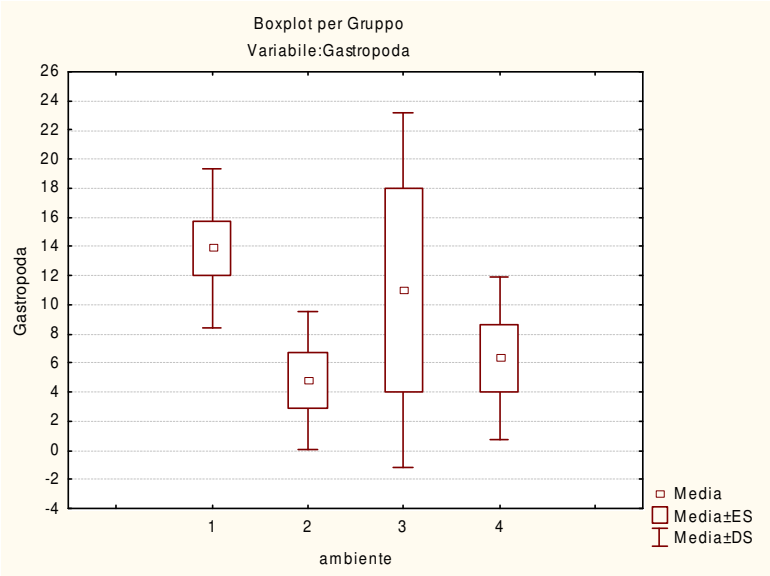
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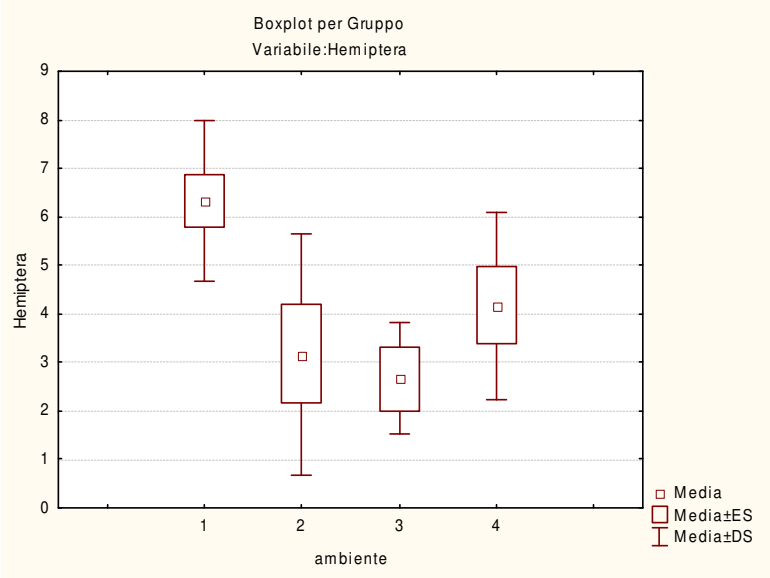
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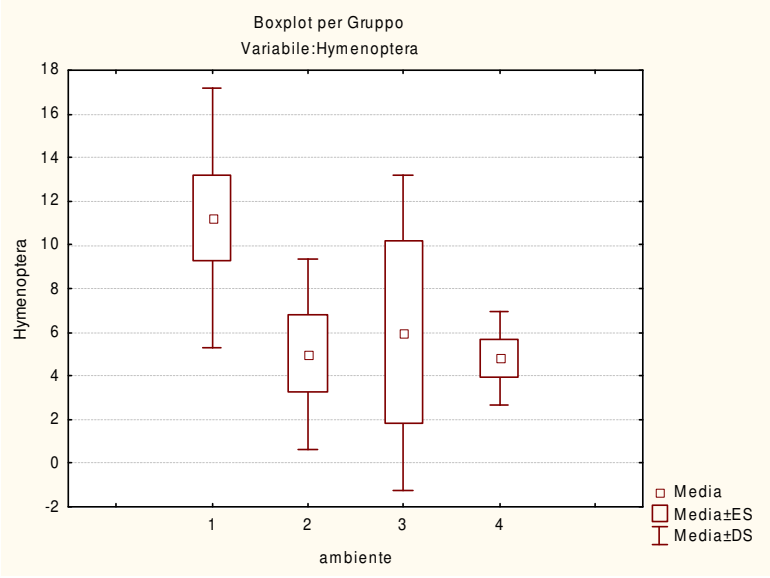
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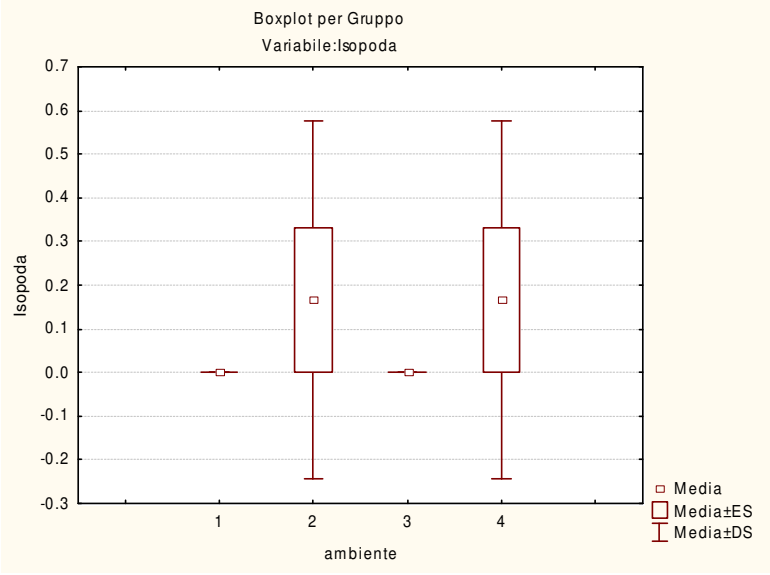
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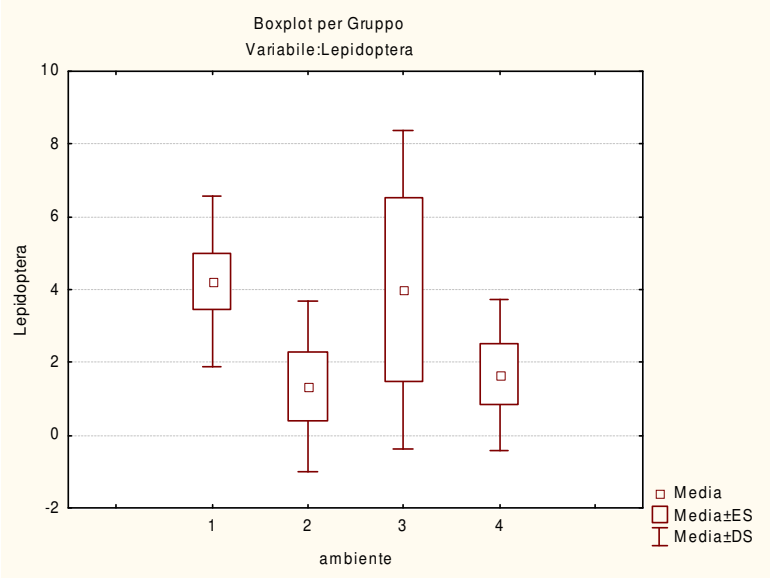
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ambiente
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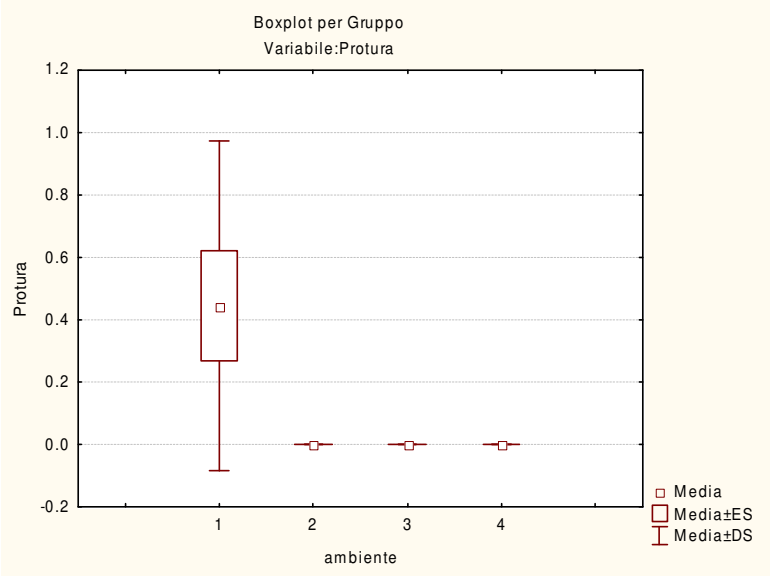
Media±DS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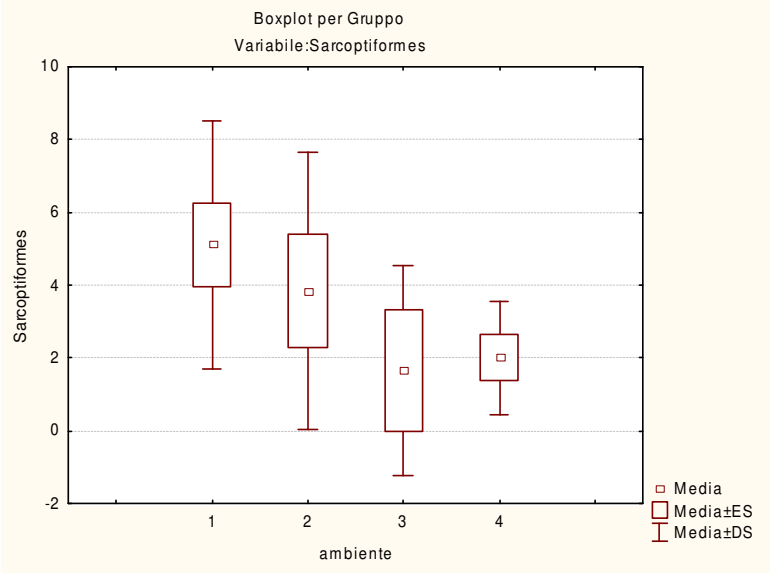
Media±DS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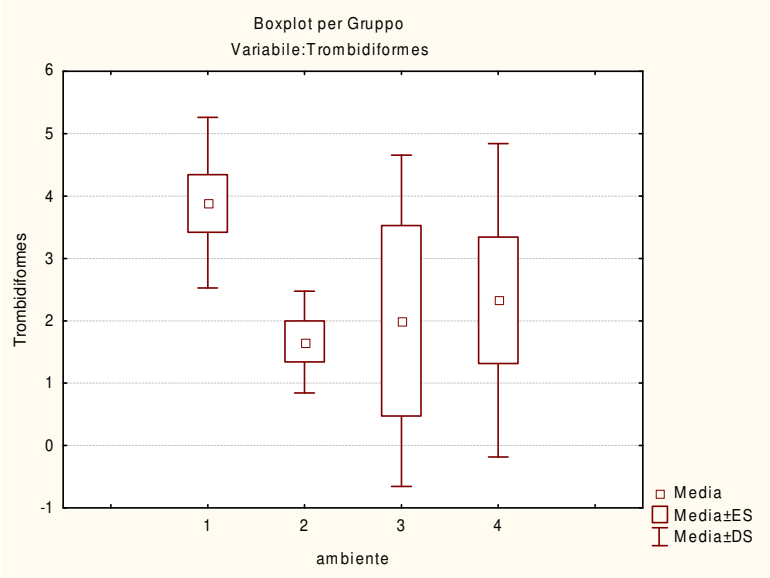

Figure 1. Comparison of α-diversity (mean ± s.e.m.) in matched habitats sites for all MOTUs and 13 arthropod orders. All tests are based on Kruskal–Wallis followed by Mann–Whitney post-hoc comparisons with Bonferroni correction. Significant differences between pairs are indicated with different lowercase characters. 1= *A.altissima* wood; 2= sinkhole wood; 3= wood in slope; 4= mixed wood.

3.1 Diversity and assemblage composition analysis

Alpha (α)-diversity was estimated as the number of observed MOTUs per site.

Pairwise differences in MOTU (α -) diversity varied considerably between habitats and across arthropod groups. Overall, MOTU α -diversity was significantly different among habitats, but not significantly higher comparing pairwise of plots (Fig. 1). Pairwise alpha diversity patterns of the main arthropod groups also differed across habitats, especially between *A.altissima* woods and sinkhole woods. There was a significantly difference in Collembola, Gastropoda, Hemiptera, Protura richness and a not significantly difference in Araneae, Isopoda, Chilopoda richness. Richness was always highest in *A.altissima* habitats, and more or less for all the taxa the groups with the major significantly differences were *A.altissima* woods and doline woods; only for Hemiptera there was a significantly difference also comparing *A.altissima* habitat with mixed woodland.

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APPENDIX 2

Collaboration with the Univeristy of Florence-Bachelor Degree of Dott. Federico Fondelli

Evaluation of the effects of *Ailanthus altissima* on ant communities in the North Adriatic Karst (North east Italy)

We analyzed the ant communities in the same sampling site of the Chapter 1 of the thesis.

The ant species are reported in the Table 3.1.

Lasius niger	*Crematogaster schmidti*
Lasius flavus	*Pheidole pallidula*
Lasius brunneus	*Temnothorax sp.1*
Lasius paralienus	*Solenopsis fugax*
Plagiolepis pigmea	*Messor capitatus*
Prenolepis nitens	*Myrmica sp.1*
Formica gagates	*Aphaenogaster epirotes*
Formica cunicularia	*Aphaenogaster subterranea*
Camponotus aetiops	*Mirmicina graminicola*
Camponotus lateralis	*Ponera testacea*
Camponotus piceus	*Tapinoma erraticum*
Camponotus truncatus	*Dolichoderus quadripunctatus*
Crematogaster scutellaris	*Ponera coarctata*

Tabella 3.1 Elenco delle specie riscontrate al termine dell'analisi dei campioni raccolti

From the MDS (Fig. 3.1 and 3.2) it is possible to see that the species grouped very well in the different habitats ().

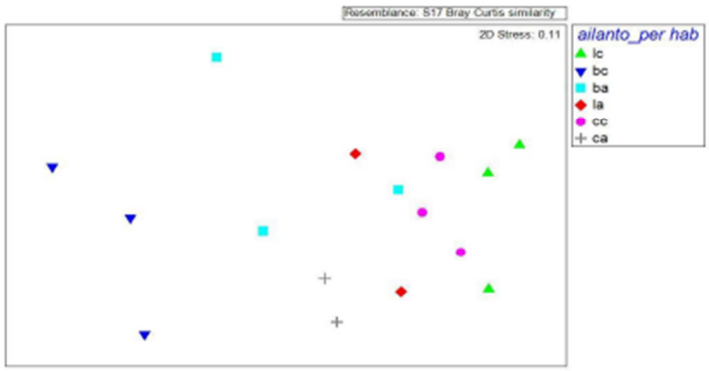

Fig. 3.1 Rappresentazione in MDS della distanza tra i diversi ambienti, relativamente alla composizione specifica, considerando *A. altissima* come fattore.

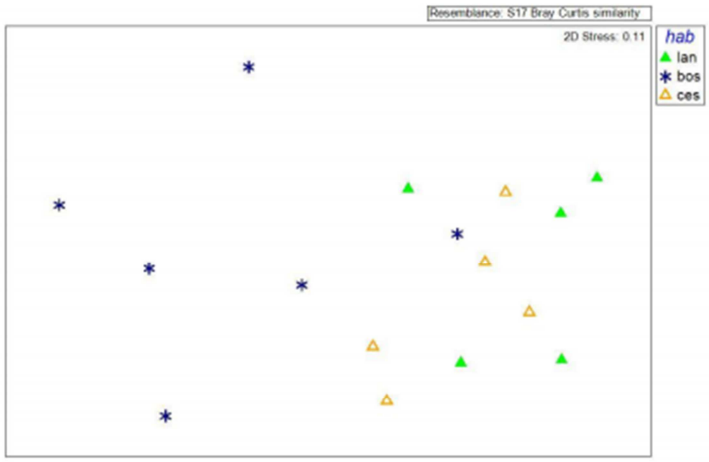

Fig. 3.2 Rappresentazione in MDS della distanza, relativamente alla composizione specifica, tra le diverse tipologie ambientali (Bosco, Landa, Cespuglieto).

24

lc=karst grassland; bc=karst wood; cc=karst bushes; la= *A.altissima* grassland; ba=*A.altissima* wood; ca=*A.altissima* bushes.

In Fig. 3.2 it is possible to see the results of the PERMANOVA with a p0.0047, that indicate a strong significant difference in the MDS analysis, confirming that *A.altissima* induce an influence on the ant communities in the study area.

PERMANOVA table of results:

Source	df	SS	MS	Pseudo-F	*P*
Habitat	2	5108.6	2554.3	1.7733	0.1799
Ailanto (Habitat)	3	4338.6	1446.2	3.0662	**0.0047**
Residual	10	4716.5	471.65		
Total	15	14591			

Tab 3.2 Risultati dell'analisi multivariata condotta sulle formiche catturate nei diversi ambienti dell'area di studio in relazione all'assenza/presenza dell'ailanto.

In Fig. 3.3 there is the difference between native and non-native plots on the ant communities. It is clear that *A.altissima* promote a more species number respect to the control plots.

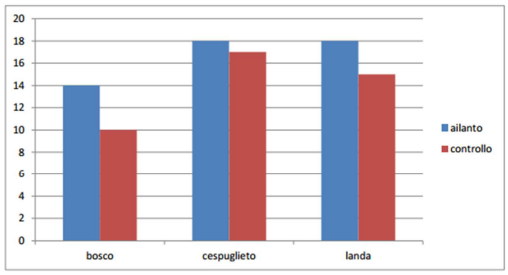

Fig.3.3 Differenze nel numero di specie negli ambienti controllo e in quelli in cui è presente l'ailanto.

APPENDIX 3

Manuscript in preparation

Dragonflies and Damselflies (Odonata) of the Natura 2000 Network site "Forest of the Cansiglio" (Veneto, Italy)

Uboni C., Borsato V., Dorigo L.

Introduction

The "Forest of the Cansiglio" is a site belonging to Natura 2000 Network (IT3230077) and it represents a priority area for the conservation of nature and biodiversity. In this context, forest, pastures, the surrounding hills (Monte Millifret 1581 m, Cimon del Cavallo 2551 m) and the ponds present all together a considerable number of ecological niches with a high number of plant and animal communities. Furthermore, the scarce anthropization of the area has allowed the preservation of the traditional landscape, one of the rarest in the entire Alps. This Pre-Alps plateau is situated at about 1000 m altitude and surrounded by reliefs with altitudes between 1400 and 1600 m; the Cansiglio area is located near the "pianura Veneto-friulana" and between the provinces of Treviso, Belluno (5000 ha) and Pordenone (1500 ha). It has a high naturalistic and cultural value, especially for the presence of a very extensive and compact forest and meadow-pastures, where it has been preserved the traditional alpine rural structure (pasture-pasture). The high biodiversity is guaranteed by the presence of Nature Reserves and forests where the natural balance is respected and the ecosystem dynamics are protected.

In this context, it is interesting to observe that no data are available for the Odonata group. Dragonflies are considered as good bio-indicators of habitat quality and they represent a well-known Insect group. For this reason, we decided to study dragonflies in 21 ponds situated in the Cansiglio plain.

2. Materials and methods

2.1 Study area

Fieldwork was conducted in 21 ponds situated in the North-eastern part of Italy, in an area called “Foresta del Cansiglio”, where no data for Odonata distribution were available.

This area belong to the SIC-ZPS "Forest of the Cansiglio-Veneto Region" (IT 3230077), an area of 5060 ha, that an average altitude of 1189 m s.l.m. (Natura 2000, Standard form) and is the westernmost portion of the Friulian-Friulian Prealps, part of the Cansiglio-Cavallo massif (Cancian et al., 1985). From the biogeographical point of view the entire surface of the site belongs to the Alpine biogeographical region bordering the Dinaric-Balkan one. From an administrative point of view it belongs to the provinces of Belluno (municipalities of Farra d'Alpago and Tambre) and Treviso (municipalities of Fregona and Vittorio Veneto) (Buffa and Lasen, 2010). The soil that lies under the wetland habitats belongs to the Cutanic Alisol (acidic pH) and Luvic Phaeozems (close to neutrality) types. These soils are mainly developed on the marly limestone type during the Cretaceous age, when the karstic phenomenon is was active (Garlato, Borsato, 2016). The plateau of Cansiglio has the shape of a large basin, a “polje”, resulting from the fusion of minor karst units (“uvala”): Pian Cansiglio, Pian di Valmenera, Pian di Cornesega, Pian delle Code. In this limestone plateau there are many dolines and ponors. The macroclimate of the study area is the same of the mountain regions, characterized by humid conditions and cold winter, and is affected by the phenomenon of thermal inversion (ARPAV data reprocessing, Meteorological Service, Data Validation Office and Climatology, 2013). In this plateau the most common vegetation is the beech-wood (*Dentario pentaphylli-Fagetum sylvaticae*) (Del Favero et al., 1998), but there are also many humid habitats represented by ponds, peat bogs and humid grasslands of secondary origin. The ponds, named “lame” or "lamarazzi", are located inside the dolines whose bottom is naturally waterproofed with the residue of the dissolution of the marl limestone (De Nardi, 1978) or with various materials used by humans to create reserves of water, useful for watering domestic and wild animals. The ponds have a variable water level, they are often of temporary origin, and usually they have a high content of nutrients due to the organic matter derived by the animals that use them for drinking. Their structure is varied, but basically attributable to the following structures: a mirror of water in the center, a riparian vegetation area and a humid edge, often characterized by the presence of *Deschampsia cespitosa*. When the ponds disappear, due to lack of water, they are incorporated into the surrounding grasslands. For a few of them evolution let them to become peat bogs. The peat bogs then, for natural or anthropogenic changes, become inactive bogs.

2.2 Sampling design

Samplings on Odonata were conducted almost monthly during the proper season (late spring-late summer) from May 2013 to 28th August 2015. At each pond, adults were searched along a predetermined transect at the ponds' banks of 30-40 m (respective to the surface of the ponds) in length, and 5 m of width and height; the adults were searched from 10 am to 6 pm during sunshine days when temperatures were higher than 20°C and with low wind speed (Buchwald, 1994). Specimens were caught with an entomological net, determined (Dijkstra & Lewington, 2006), photographed and then released.

2.3. Data analysis

For each pond defined by UPGMA, diversity of species on untransformed data abundances was shown by: (i) number of species, (ii) Shannon-Wiener index (H', here with ln), (iii) dominance (D = 1 – Simpson index) (Table 1). For the data analyses, MS Excel 2010, PAST (Palaeontological Statistics; Hammer 2012) and SPSS Statistical package ver. 20.0 (SPSS inc., 1989, 2011) were used. We defined the chorology of the species following the Ckeck-list of the Italian Fauna (http://www.faunaitalia.it/checklist/). The categories are:

N	Northern Italy, including: Friuli Venezia Giulia, Veneto, Trentino-Alto Adige, Lombardia, Val d'Aosta, Piemonte, Liguria, Emilia-Romagna
S	Peninsular Italy, including Toscana, Marche, Umbria, Lazio, Abruzzi, Molise, Campania, Puglia, Basilicata, Calabria
Si	Sicily and smaller islands
Sa	Sardinian and smaller islands

3. Results

In total, 1851 individuals belonging to 21 species of Odonata, were recorded at 21 ponds situated in the Cansiglio area (Table 2). It is possible to see in the UPGMA (Fig.1) that the ponds can be divided into 5 categories based on the recorded dragonflies species. The first one (A), on the extreme right, is characterized by the ponds that dried almost one time during the sampling period, for more than two weeks, and due to that no species were reported. In the second group (B), there are the ponds situated at the high altitude (>1000 m a.s.l.), and with the water surface completely covered by hydrophitic vegetation (LM and LCM) and *Typha latifolia* (ML5). To the third group (C) belong all the ponds that have shallow water (50 cm maximum) and are influenced by cow grazing. The (D) group put together the pond of small surface area. In (E) there are the pond where

the water level was higher respect to the other sites (7 m maximum) and where the water surface was colonized by eutrophic plants.

There is a notable difference in species numbers among the 21 ponds (see Table 1): GB showed the highest species richness, with 12 species, whereas LM5 and LCM had the lower species richness with only 1 species detected. Regarding the number of individuals, in ML4 we found the majority of individuals (393) and in LCM only 1 individual.

Concerning the diversity indices, the highest value of Shannon index was for GB and the lowest for LCM and ML5.

The total number of species represent the 22.34% of the Italian Odonata fauna.

The majority of the species (71%) are widespread in all Italy.

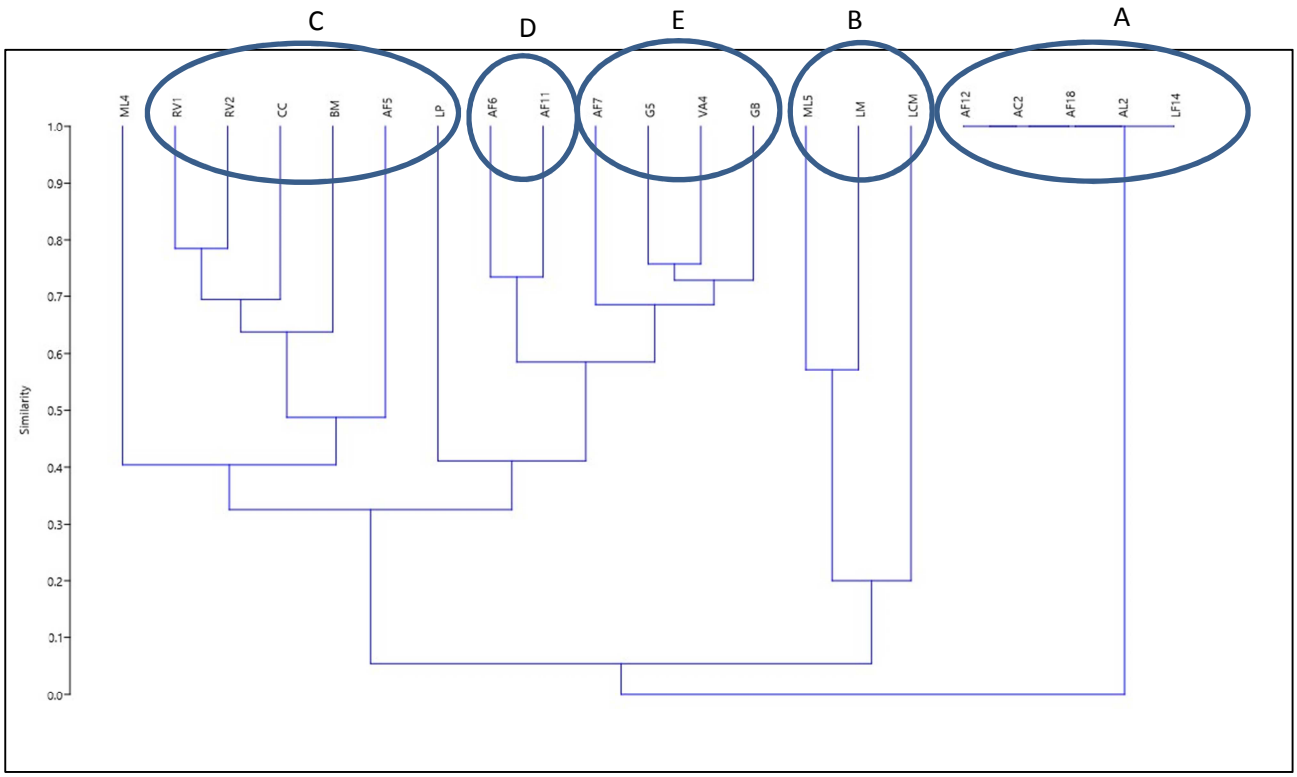

Fig. 1. UPGMA with Bray-Curtis similarity for the species distributed in the 21 sampling sites.

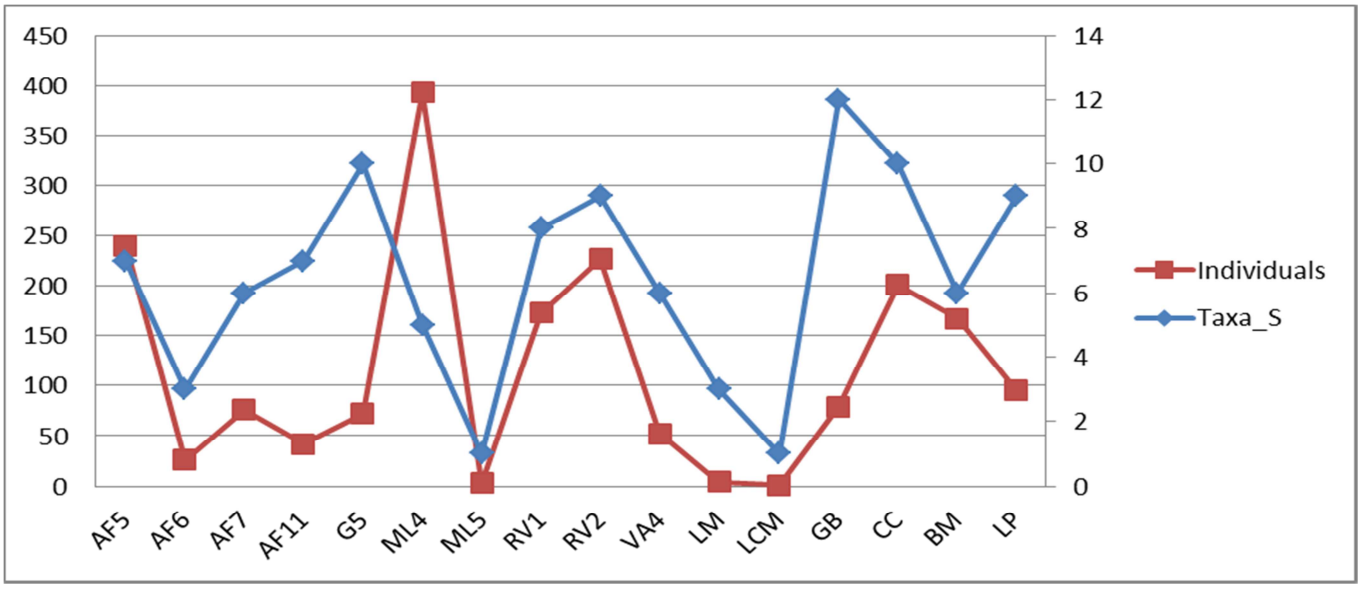

Fig. 2. Individuals and numbers of species detected in each ponds.

	AF5	AF6	AF7	AF11	G5	ML4	ML5	RV1	RV2	VA4	LM	LCM	GB	CC	BM	LP
Dominance_D	**0.3524**	**0.7308**	**0.5665**	**0.398**	**0.3681**	**0.9354**	**1**	**0.5081**	**0.3282**	**0.6087**	**0.375**	**1**	**0.2645**	**0.2855**	**0.8521**	**0.2773**
Shannon_H	**1254**	**0.5158**	**0.907**	**1287**	**1509**	**0.1807**	**0**	**0.9994**	**1426**	**0.8536**	**1.04**	**0**	**1859**	**1573**	**0.3834**	**1599**

Table 1. Dominance and Shannon Indices for each sampling site.

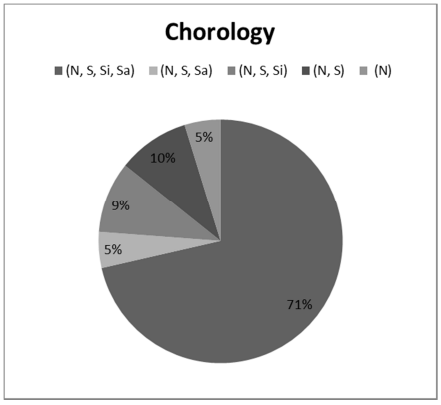

Fig. 2. Chorology spectrum of the investigated Dragonflies

5. References

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APPENDIX 4

Accepted manuscript on the "Bollettino della Società Entomolgica Italiana"

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First data on the reproduction of the Vagrant Emperor *Anax ephippiger* in North-Eastern Italy, Friuli Venezia Giulia (NE Italy) (Odonata Aeshnidae)

Abstract

The Vagrant Emperor, *Anax ephippiger* (Burmeister, 1839), is a migrant dragonfly species from Africa and Middle East; in Europe only summer generation are known, without evidence of overwintering larvae. In August 2010 a reproductive breeding site for this species was found in the in Friuli Venezia Giulia Region (north-eastern Italy). This discovery represents the first proof of reproduction for the species in north-eastern Italy. With the aim of increasing the knowledge on the species requirements, a study to delineate the emerging habitat was conducted: dragonfly community (adult and exuviae), vegetation, chemical and physical water parameters were sampled. This yielded data about larval tolerance toward salinity. This new data proves a northward move for the species, which may also have been facilitated by global warming.

Key words: Odonata, Aeshnidae, *Anax ephippiger*, Monfalcone, brackish water, global warming

Riassunto

Anax ephippiger (Burmeister, 1839) è una libellula migratrice che proviene dall'Africa e dal Medio Oriente e di cui in Europa sono conosciute solo generazioni estive e non vi sono evidenze di larve svernanti. Durante l'agosto 2010 in Friuli Venezia Giulia, Italia nord-orientale, è stato trovato un sito riproduttivo per questa specie, prima prova di riproduzione per la specie in Nord-Est d'Italia. Nello stagno in cui è stata accertata la riproduzione, con lo scopo di incrementare le conoscenze ecologiche sulla specie e delinearne l'habitat di riproduzione, sono stati rilevati: la comunità a odonati (adulti ed esuvie), la vegetazione e i parametri chimico-fisici dell'acqua (compresa la salinità dell'acqua, essendo lo stagno in comunicazione con il mare e non essendo disponibili informazioni dettagliate rispetto alla tolleranza delle larve a tale parametro). Questo nuovo ritrovamento faunistico, corredato da dati ecologici, si inserisce all'interno di una più vasta rete di avvistamenti per la specie, rappresentando la prova dell'ampliamento verso Nord dell'areale di *A. ehippiger* e avvalorando la tesi della sensibilità degli odonati al riscaldamento globale.

Parole chiave: Odonata, Aeshnidae, *Anax ephippiger*, Monfalcone, acqua salmastra, riscaldamento globale

Introduction

Anax ephippiger (Burmeister, 1839) is a species with strong migrational tendencies (Müller, 1974; Corbet, 1999; Silsby, 2001; Dijkstra & Lewington, 2006; Lambret & Boudot, 2013), that can often be seen in large numbers (Boudot *et al*., 2009). It is typical of arid parts of Africa, the Middle East and South-West Asia wandering to and from to breed after the rains, and it also breeds sporadically in southern Europe (Askew, 2004). However, localities with regular reproduction are strongly limited to regions with a hot climate in northern Africa and some European areas directly bordering the Mediterranean Sea, although a summer generation may also emerge in Central Europe during favourable years. The species has turned up various places in Europe, especially in the Mediterranean region but also in England (e.g. Cambridge and Leeds) (Askew, 2004) and even in Iceland (representing the only dragonfly ever recorded on Iceland) and the Faroe Islands (Jensen & Nielsen, 2012). The species is not uncommon in the East Mediterranean (e.g. Kos), Turkey, Iraq and along the East coast of the Black Sea (Askew, 2004). The timing of the species' occurrence in Europe is related to the life-cycle at the breeding ground in Africa: in May, after the wet season starts, eggs are laid in temporary pools and lakes. Larvae develop rapidly (60-90 days) and mass emergence occurs from September to November. Then the adults locally disperse and spend the winter as adults. The species flies following the rain-developing systems (monsoon) for several hundred kilometers until arriving in Europe. There they feed, mate, and lay eggs in newly filled water bodies (Edelaar *et al*., 1996; Resh & Cardé, 2009). Such long-distance migrations are accompanied by successful local breeding and appear to be mostly passive, largely due to strong winds blowing in the same direction for several days (Lambret & Boudot, 2013). Although far from the Mediterranean coast the species is usually seen only in autumn, emerging individuals and exuviae can be found during the summer period and not during the spring (Grand, 2009), with the exception of a female captured in Devonport (England) on 24th February (Mclachlan, 1903) and two exuviae and many fresh individuals found on 29th April in Camargue (Faton, 2003). In July 1983 several individuals were observed hawking along cliff tops near Novorossiysk on the Black Sea coast and in April specimens were seen flying over coastal dunes, arable land and heavily-grazed inland valleys on the island of Kos (Askew, 2004). In Iceland, the species was found in late September and at the beginning of October. After these records the species was found again in Iceland on October 29th and November 5th (Norling, 1967; Ólafsson, 1975). Even though winter deposition, starting in December, has been reported from northern Morocco, there is practically no evidence for development of a larval generation in the Mediterranean region during winter (Boudot *et al*., 2009). *A. ephippiger* breeds in small, shallow, warm (more than 35°C during summer) (Wildermuth *et al*., 2005) standing water bodies, sometimes of a temporary nature and sometimes brackish, in part sparsely overgrown (Gerken & Sternberg, 1999; Günther & Muersberger, 1999; Askew, 2004; Dijkstra *et al*., 2006) with abundant presence of vegetation, especially *Phragmites australis* and different species belonging to the following genus: *Juncus, Eleocharis, Carex, Schoenoplectus* (Bedjanic, 1999; Wildermuth *et al*., 2005). The water surface has to be partially covered by vegetation. Usually the dragonfly community living together with *A. ephippiger* is composed by 20-30 different dragonfly species (Wildermuth *et al*., 2005). Feeding occurs on emerged vegetation during the evening, when individuals form aggregations; in the same places males patrol and look for females (Günther & Muersberger, 1999). Oviposition occurs in tandem as in *Anax parthenope* (Selys, 1839) and in *Aeshna affinis* Vander Linden, 1820 (Askew, 2004) and eggs are laid down on leaves, dead or alive, or on the wet pond bank (Wildermuth *et al*., 2005).

The primary aim of this note is to describe the main ecological parameters of the first reproductive site of *A. ephippiger* in north-eastern Italy, in order to delineate the requirements for the species in Europe. The pond, despite its recent formation, is well naturalized and constitutes a unique habitat along the north-eastern Italian coastal system. The vegetation analysis and the water chemical and physical parameters allow to describe in detail the habitat and define how much tolerance toward salinity larvae show during their development.

Material and methods

Study area. The breeding site of *A. ephippiger* belongs to the brackish biotope "Zona del Lisert", an area that lies between the coastal region of Dalmatia, characterized by high mountains and rocky environments, and the Isonzo's river mouth, characterized by low altitude and sandy environments (Poldini, 2009). In the "Zona del Lisert" and in the neighboring areas the first human modifications date back to 1948-1950 (Michelutti *et al*., 2006), while the most recent one occurred in 2006, when an empty artificial area was created, filled with the extra material resulting from the port expansion and the drainage of the ship canals. After many years of work, a stop to this activity induced a spontaneous naturalization of the area, with the creation of many wet habitats. Despite the numerous modifications, nowadays the area displays a large biodiversity, with an interesting coastal habitat characterized by autochthonous flora and fauna species.

Chemical and physical parameters of the water (pH, Temperature (°C), Dissolved Oxygen (ppm), Conductivity (mS/cm) were measured every two weeks from 14th May 2010 to 23th April 2011 between 12 am and 2 pm, without rain and with low tides, necessary condition to have access to the area. Hanna Instruments Probes were used: pH and Temperature were measured with instrument HI 9025 (pH ± 0.01 and temperature ± 0.5 °C); Conductivity was measured with instrument HI 8633 (± 1% end to the scale); Dissolved Oxygen was measured with instrument HI 9143 (± 1.5% end to the scale). Since the pond is situated in proximity to the sea, chemical analyses of the water were conducted to correlate the high values of conductivity with salinity (presence of chloride ions); after that the conductivity value was converted to Practical Salinity Units (PSU), according to the international convention (U.N.E.S.C.O., 1985) that uses the practical salinity scale PSS-1978. Samplings on Odonata were conducted twice-monthly from 14th May 2010 to 29th September 2010 and from 15th March to 23th April 2011: adults were checked from 10 am to 6 pm during sunshine days when temperatures were higher than 20°C and with low wind speed (Buchwald, 1994); they were caught with an entomological net, photographed and then released. The vegetation in and around the pond was checked for exuviae, determined reaching the species rank (Gerken *et al*., 1999; Askew, 2004). Adults and exuviae were counted in three patches of 10 m^2 around the pond bank and classes of abundance were used to compare the data (Buchwald, 1990): 1: 1-4 specimens (or exuvie); 2: 5-10 specimens (or exuvie); 3: 11-20 specimens (or exuvie); 4: 21-40 specimens (or exuvie); 5: > 40 specimens (or exuvie). For data interpretation, 1=very few individuals; 2=poor population; 3=medium population; 4=dense population; 5=very big population, mass population (Buchwald, 1990). Furthermore, a phytosociological analysis on helophytic and hydrophytic plants in and around the pond was carried out, with particular attention to the perilacual vegetation. Each plant specimen was measured in height and the mean was annotated. The vegetation analysis was performed using the Braun-Blanquet method (1964); see also Reichelt and Wilmanns (1973), integrated with Pignatti (1953).

Results

A. ephippiger's exuvia was found on 1th August 2010. Thirteen species of Odonata (eleven of them confirmed by the presence of exuviae) were found in the pond together with *Anax ephippiger*: *Sympecma fusca* (Vander Linden, 1820), *Ischnura elegans* (Vander Linden, 1820), *Erythromma viridulum* (Charpentier, 1840), *Aeshna mixta* Latreille, 1805, *Aeshna affinis* Vander Linden, 1820, *Brachytron pratense* (Muller, 1764), *Orthetrum cancellatum* (Linnaeus, 1758), *Crocothemis erythraea* (Brullé, 1832), *Sympetrum fonscolombii* (Selys, 1840), *Sympetrum meridionale* (Selys, 1841), *Chalcolestes parvidens* (Artobolevski, 1929), *Anax imperator* Leach, 1815, *Lindenia tetraphylla* (Vander Linden, 1825). The most common species are: *Aeshna mixta, Crocothemis erythraea, Sympetrum fonscolombii, Sympetrum meridionale* (Table 2). The pond is subject to strong water fluctuations during the year with a maximum water level ranging from 60 cm to 74 cm; watering is pluvial and tidal due to the sea tidal waves. The phytosociological attribute of the helophytic vegetation was *Puccinellio festuciformis-Phragmitetum australis* (Pignatti 1953) Poldini and Vidali 2002 (Poldini *et al*., 1999) and consists of peripheral reed beds of *Phragmites australis s.l*. (200 cm height) with a brackish connotation and a massive presence of *Bolboschoenus maritimus*. The lakeside vegetation is *Puccinellio festuciformis-Scirpetum compacti* (Pignatti 1953) Gehu *et al*. 1984, while the phytosociological attribute of the hydrophytic vegetation is *Chaetomorpho-Ruppietum* Br.-Bl. 1952. The sporadic presence of *Tamarix africana* suggests the ephemeral nature of many basin zones. To better describe the characteristics of the pond, we obtained the following water chemical-physical parameters: pH average value was 8.65, T (°C) average value was 19.14°C; the Conductivity (mS) average value was 3.20 mS; the Oxygen Dyssolved (ppm) average value was 14.51 ppm; the PSU average value was 1.88 PSU.

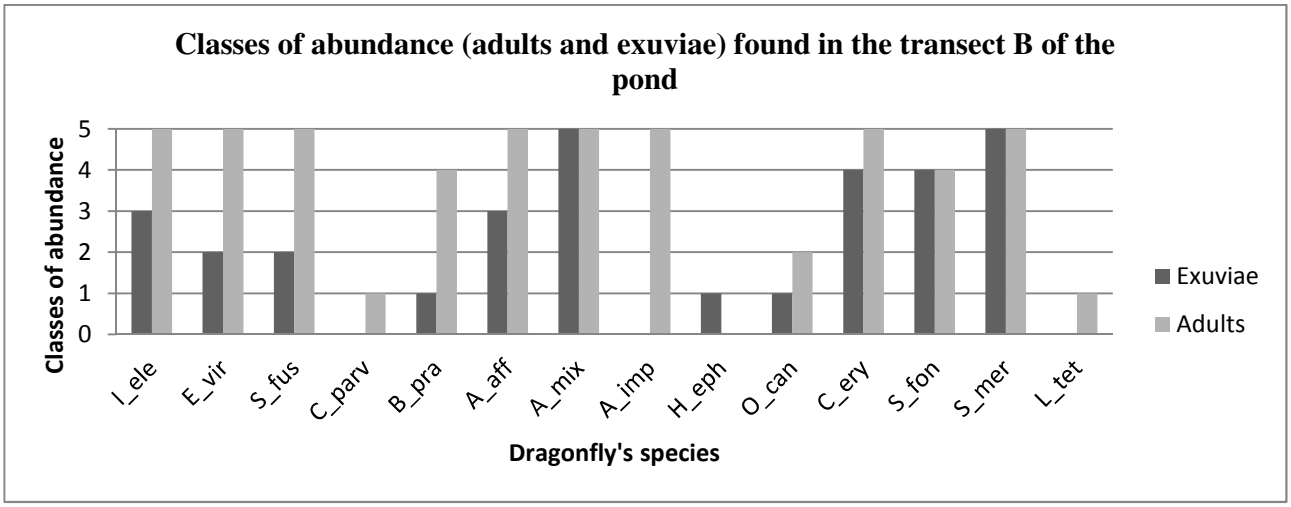

Table 2- Classes of abundance (see "Materials and methods") (adults and exuviae) found in the transect B of the pond. Species are abbreviated like follow: I_ele= *Ischnura elegans*; E_vir= *Erythromma viridulum*; S_fus= *Sympecma fusca*; C_par= *Chalcolestes parvidens*; B_par= *Brachytron pratense*; A_aff=*Aeshna affinis*; A_mix= *Aeshna mixta*; A_imp=*Anax imperator*; H_eph= *Anax ephippiger*; O_can= *Orthetrum cancellatum*; C_ery= *Crocothemis erythraea*; S_fon= *Sympetrum fonscolombei*; S_mer= *Sympetrum meridionale*).

Discussion and conclusions

The first Italian record of *A. ephippiger* goes back to 18th July 1867 when at around 7 pm Federico Craveri collected three individuals (sub *Anax mediterraneus* De Selys) flying in endless numbers over his garden in Bra (TO, Piedmont) (Ghiliani, 1867); on the 8th August a large number of specimens reached La Mandria (Venaria Reale, Torino) (Ghiliani, 1867) and at this place exactly a year later, a young female was

collected, as first evidence of the reproduction in Europe (Ghiliani, 1869). Actually, in Piedmont less is known about breeding populations of the species (Boano et al., 2007). One larva was found in Pieve (Macerata) and many oviposition were observed in Trapani (Sicily) (Utzeri *et al.,* 1987). In Sardinia two fresh and many adult specimens were observed in 1986, 1988 and 1989 (Leo, 1990). The data about the distribution of the species in Italy are reported in Riservato et al. (2014).

Considering the two countries bordering Friuli Venezia Giulia, the first record of *A. ephippiger* in Slovenia refers to 1995, when larvae and exuviae of this species were found at Proseniško, east of Celje (Pirnat, 1997). The species was later confirmed in 1998 near the city of Maribor (North-eastern Slovenia) with exuviae found in two intensive fish-ponds characterized by sparse emergent vegetation and shallow, warm and eutrophic water (Bedjanic, 1999). A few years later, 30 kilometers from the Italian border, in Secovlje Salina and Skocjan inlet (Koper), *A. ephippiger* individuals were observed many times during spring 2000, but proof of reproduction was not found. The habitat in Secovlje Salina is characterized by salt-pan channels and saltmarshes, and in Skocjan inlet by mudflats and abandoned places covered in rubble and overgrown with reeds (Geister, 2002). In Austria, the exuviae of the species were found in 1990 in a site situated nine kilometers north of the city of Salzburg, characterized by *Phalaris arundinacea* (where the exuviae were found) (Laister, 1991).

In Friuli Venezia Giulia, only scattered records of *A. ephippiger* exist: the first in Gorizia in September 1988 (Bognolo & Pecile 1995), then in Trieste in August 2007 (Uboni *et al*., 2008), at the Natural Reserve "Isonzo's river mouth" in 2008 (Boudot *et al*., 2009), in the town of Spilimbergo (PN) on 12th August 2017 (C. Uboni and L. Dorigo obs.).

The exuvia found on August 2010 at "Lisert" represents a first in north Italy for the species. The habitat, when investigated closely, resembles the prototypical habitat described for the species in the literature (Bedjanic, 1999; Gerken *et al*., 1999; (Günther & Muersberger, 1999; Askew, 2004; Wildermuth *et al*., 2005; Dijkstra *et al*., 2006): shallow ponds characterized by high temperature (more than 30°C during summer), weakly brackish water (3.41 as the maximum value of PSU) and the massive presence of emerged and submerged vegetation (*Puccinellio festuciformis-Phragmitetum australi*s, *Puccinellio festuciformis-Scirpetum compacti, Chaetomorpho-Ruppietum)*, and a species-rich odonata community living together with *A. ephippiger* (thirteen species in total). It is important to underline the observation of *Lindenia tetraphylla*, since this species is included in the annexes II and IV of the Directive 43/92 EEC and this finding represents the first data for the species at least in north-east Italy.

In conclusion, the new increasing data emerging from this study indicates that *A. ephippiger* is becoming more and more present in the north-eastern part of the Adriatic Sea, proving the trend of many African species moving to Europe probably due to climate changes and global warming (Walther *et al*., 2001; Ott, 2010; Grand, 2009).

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APPENDIX 5

Wetlands Ecology and Management

Community assemblages and biodiversity of damselfly and dragonfly (Insecta Odonata Zygoptera, Anisoptera) fauna in mixohaline coastal wetlands

--Manuscript Draft--

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Abstract:	Salinity can be considered as a stressor factor for many invertebrates, especially for organisms like Odonata that are typically considered freshwater insects. In Europe, only 15 Odonata species inhabit brackish water areas and only few detailed data on tolerance toward salinity are available. In order to study the brackish attitude of Odonata species, we investigated odonate fauna in 11 sampling stations chosen accordingly to a salinity gradient from freshwater through oligohaline, mesohaline to polyhaline environments in North-Eastern Italy. Adults and exuviae were sampled for one year, and the values of main chemical and physical water parameters were monitored. Of the total 25 recorded species, 17 species were detected as both adults and exuviae (final larval stage), 4 species were detected only as adults, and 4 only as exuviae. At larval stage, the fidelity to a certain environment was high: seven species were found exclusively to breed in freshwater sites and six species only in oligohaline environments, with no species strictly associated with mesohaline and polyhaline habitats. In total, 14 species (56 % of the whole sample) were able to complete their reproduction in brackish water environments. Among the adults, fidelity was weak: four species were found exclusively at freshwater habitats, no species seemed to be exclusively connected with oligohaline habitats, while one and three species were recorded at mesohaline and polyhaline sites, respectively. Our results underline the idea that coastal wetlands composed by a mosaic of different habitats support high biodivesity.
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Community assemblages and biodiversity of damselfly and dragonfly (Insecta: Odonata: Zygoptera, Anisoptera) fauna in mixohaline coastal wetlands

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Abstract

Salinity can be considered as a stressor factor for many invertebrates, especially for organisms like Odonata that are typically considered freshwater insects. In Europe, only 15 Odonata species inhabit brackish water areas and only few detailed data on tolerance toward salinity are available. In order to study the brackish attitude of Odonata species, we investigated odonate fauna in 11 sampling stations chosen accordingly to a salinity gradient from freshwater through oligohaline, mesohaline to polyhaline environments in North-Eastern Italy. Adults and exuviae (larval final instar) were sampled for one year, and the values of main chemical and physical water parameters were monitored. Of the total 25 recorded species, 17 species were detected as both adults and exuviae (final larval stage), 4 species were detected only as adults, and 4 only as exuviae. At larval stage, the fidelity to a certain environment was high: seven species were found exclusively to breed in freshwater sites and six species

only in oligohaline environments, with no species strictly associated with mesohaline and polyhaline habitats. In total, 14 species (56 % of the whole sample) were able to complete their reproduction in brackish water environments. Among the adults, fidelity was weak: four species were found exclusively at freshwater habitats, no species seemed to be exclusively connected with oligohaline habitats, while one and three species were recorded at mesohaline and polyhaline sites, respectively. Our results underline the idea that coastal wetlands composed by a mosaic of different habitats support high biodivesity.

Keywords: Odonata; brackish water; salinity; dragonfly species assemblage diversity.

Introduction

Odonata (Insecta: Zygoptera, Anisoptera) are considered to be freshwater-associated organisms (Smith and Smith 1996; Sühling et al. 2003; Zinchenko and Golovatyuk 2013) and a »flagship« indicator group (Sharma et al. 2006; Balzan 2012) due to their important role in the aquatic ecosystems (Balzan 2012). They inhabit both aquatic and terrestrial environments and, for this reason, they can be considered a mirror of habitat changes. The distribution of Odonata species in the environment is determined largely by the presence of suitable habitats to maintain their populations, even if individuals can sometimes be found in places where population cannot be sustained (McPeek 2008; Balzan 2012). Local abiotic factors such as temperature and water chemistry as well as biotic factors such as abundances of various food resources, predators, and parasites all affect the survival, growth, and fecundity of the individuals at particular sites. Many unanswered questions about the ecological regulation of odonates and autoecological factors limiting species distributions to particular habitats makes them a continually fascinating studing group (McPeek 2008; Balzan 2012). Although Odonata are not highlighted as being the saltmarshes' inhabitants (Cheng 1976), there is a "heterogenous assemblage of Zygoptera and Anisoptera that occupy brackish waters, usually of relatively low salinity compared with seawater" (Catling et al. 2006) and other species that are able to survive in waters of high salinity (Zinchenko and Golovatyuk 2013). Diptera, Coleoptera and Hemiptera dominate the majority of the saltmarsh insect fauna, while Odonata represents only ca. 3 % of this assemblage (Foster and Treherne 1976) and Corbet (1999) considered only one species like a truly marine dragonfly, *Erythrodiplax berenice* (Drury, 1773). Recently, Zinchenko and Golovatyuk (2013) published a review about the salinity tolerance of many Odonata species *Hemicordulia tau* (Selys, 1871) and some species of the genus *Ischnura* were collected in rivers with salt concentrations up to 2.24

g l^{-1}; larvae of *Anax* spp. colonize biotopes in the hyperhaline river upon its desalination to 3.5–6.8 g/L; Odonata belonging to the families Coenagrionidae, Aeschnidae, Gomphidae, Libellulidae, Hemicorduliidae, and Lestidae inhabit river waters with salinity of 5.9–40 g l^{-1}, and larvae of *Sympetrum sanguineum* (Muller, 1764), *Ischnura elegans* (Vander Linden, 1820) and *Aeshna* sp. have been found in mesohaline rivers at a salinity of 7.5–21.1 g l^{-1}. Experimental studies demonstrated a high tolerance towards water salinity for the larvae of *Libellula depressa* Linnaeus, 1758 and *Epitheca bimaculata* (Charpentier, 1825) , up to 6.3–8.1 g l^{-1} (Zinchenko and Golovatyuk 2013), and *Erythemis simplicicollis* (Say, 1839) showed a tolerance range between 7 and 18 ppt (Smith and Smith, 1996). The maximum salinity tolerance (25.8 g/L) were recorded for *Austrolestes annulosus* (Selys, 1862), belonging to the family Lestidae (Zinchenko and Golovatyuk 2013), and for *Aeshna mixta* Latreille, 1805 (27.8 g/L) (Aguesse, 1968) belonging to the family Aeshnidae.

There are still open questions concerning the use of saltmarshes by Odonata. Catling et al. (2006) showed that saltmarshes can be used by Odonata more than what was already known; in fact,these environments are exploited by Odonata not only as a food source, for example tabanid flies and mosquitoes (Diptera). The aim of the present study was to investigate (i) the structure of Odonata assemblages and diversity in different habitats along a gradient of salinity from freshwaters to polyhaline waters, and (ii) to connect species assemblages with the aquatic environment that determine their composition.

Materials and methods

Study area

Fieldwork was conducted at the northernmost coastal zone of the Adriatic Sea, between Monfalcone and Grado municipalities (Italy), within two areas: the brackish biotope called “Lisert Zone” and the Natural Reserve of the Isonzo River Mouth. The “Lisert Zone” lies between the northernmost coastal areas of the Balkan Peninsula (characterized by high mountains and rocky environments) and the Isonzo river mouth (characterized by low altitude and sandy environments (Poldini 2009). In the “Zona del Lisert” and in the neighboring areas the first human modifications date back to 1948–1950 (Michelutti et al. 2006), while the most recent one occurred in 2006. The last modification was the creation of an empty artificial area filled with extra material resulting from the Monfalcone port expansion and the drainage of the ship canals nearby. After the last modification, a spontaneous naturalization, resulting in new habitats occurred in the area and new habitats emerged, including many ponds. These wet zones are subject to strong water fluctuations during the year, with maximum water level

ranging from 60 cm to 74 cm (Uboni et al. 2011); water supply is partially due to rainfall, but it is also due to the sea tidal waves. Despite of the numerous modifications, nowadays the area displays a high biodiversity, with interesting coastal habitat characterized by autochthonous flora and fauna species.

The Natural Reserve of the Isonzo River Mouth was established in 1996 and consists of a complex lagoon structure situated in the easternmost side of the Po plain. It includes the last part of the high plain river areas, characterized by pebbly floods and the low valley areas with mainly muddy soils, where reclamation works were carried out during the 20th century. In this stretch, the remains of floodplain woods and canalized spring water courses are still present. The southernmost part of the Reserve lies in the Isonzo mouth and consisted of marshes; it is characterized by clayish floods, sandy sediments, and some islets (Perco et al. 2006).

Eight ponds were included in the study: four ponds from oligohaline to polyhaline characteristics (L1, L2, L3, L4: 45.778635 N, 13.575363 E) were located in the "Lisert Zone"; three freshwater ponds (C1, C2, C3: 45.754511 N, 13.500439 E) within the Natural Reserve of the Isonzo River Mouth; the last one mesohaline pond (C4: 45.724948 N, 13.541177 E) near the Isonzo mouth, in the southernmost portion of the Natural Reserve (Fig. 1). Pond areas extend from 40 (L1) to 5000 (L4) m^2. While all ponds with areas smaller than 1000 m^2 (L1–L3, C1–C3) had only one sampling station, ponds larger than 2500 m^2 had two or three sampling stations (C4 with sampling stations C4a and C4b, and L4 with sampling stations L4a, L4b and L4c; see Table 1 for details).

Information about physical parameters, chemical parameters, size of the ponds and vegetation around the ponds was retrieved from Uboni (2011, Table 1) and taken as follows: conductivity (mS cm^{-1}), pH, temperature (◦C) and dissolved oxygen (mg l^{-1}) were recorded using field meters (HI 8633 conductivity meter; HI 9025 pH and temperature meter; HI 9143 dissolved oxygen meter; all instruments are manufactured by Hanna Instruments Inc., Woonsocket, Rhode Island (USA). Measures were performed every two weeks from 14th May 2010 to 23th April 2011, between 12 am and 2 pm, without rain and with low tides. These conditions were necessary condition to have access to the area. Since many ponds are situated in the near proximity of the sea, chemical analysis of the water was conducted with the aim to correlate the high conductivity values with salinity (presence of chloride ions); conductivity values were converted to practical salinity units (PSU), referring to the international convention (U.N.E.S.C.O. 1985) that uses the practical salinity scale PSS-1978.

Table 1. Summary data on chemical and physical parameters from 11 stations at 8 ponds in years 2010–2011. Intervals show range of values (min–max). Information about presence of fish and vegetation type is also presented. Data retrieved from Uboni (2011).

Pond	C1	C2	C3	C4	C4	L1	L2	L3	L4	L4	L4
Sampling station	C1	C2	C3	C4a	C4b	L1	L2	L3	L4a	L4b	L4c
Chemical parametres											
pH	8.57–9.25	6.89–8.74	7.26–8.38	7.37–8.74	7.73–8.94	7.23–8.32	7.41–8.17	7.47–8.67	7.38–8.86	7.46–9.66	7.46–9.66
T [^{o}C]	5.6–30.9	4.0–30.7	6.3–30.9	7.2–35.9	6.1–32.5	6.6–32.1	6.8–34.5	8.7–31.7	3.7–26.3	5.1–32.3	5.1–32.3
O2 [mg l^{-1}]	5.36–15.02	2.93–11.59	3.44–12.88	7.19–14.54	7.66–19.28	4.56–20.41	3.2–15.41	2.98–20.95	3.23–11.11	8.28–21.95	8.28–21.95
Conductivity [mScm^{-1}]	0.226–0.306	0.336–0.606	0.391–0.673	8.690–25.600	8.040–27.100	8.860–36.400	14.680–40.400	12.770–37.600	2.010–6.270	2.170–6.320	2.170–6.320
PSU min-max	0–0	0–0	0–0	5.39–19.87	5.01–19.09	5.86–27.99	12.04–30.97	8.84–28.24	1.33–3.59	1.43–3.41	1.43–3.41
Physical parametres											
Pond surface [m^2]	172.7	376.8	219.8	2826	2826	37.68	942	339.12	5024	5024	5024
Water depth max	40	30	20	20	20	45	20	45	74	74	74
Water depth min	10	20	15	0	0	10	10	25	60	60	60
Influence of tidal waves	no	no	No	yes	yes	yes	yes	yes	yes	yes	Yes
Other data											
Presence of fish	no	no	No	yes	yes	no	no	no	yes	yes	Yes
Types of vegetation*	2	2	2	1	1	3	3	3	3	3	3

*grass (Poaceae) and grass like (Cyperaceae, Juncaceae) = 1; grass, grass-like and bushes = 2; grass, grass-like, bushes and forest = 3

Sampling design

The Odonata sampling campaigns were conducted twice-monthly from 14th May 2010 to 29th September 2010 and from 15th March to 23th April 2011. At each station, adults were searched along a predetermined transect at the ponds' banks of 30-40 m (respective to the surface of the ponds) in length, and 5 m of width and height; adults were searched from 10 am to 6 pm during sunny days, when temperatures were higher than 20°C, and with low wind speed (Buchwald 1994). Specimens were caught with an entomological net, determined (Dijkstra and Lewington 2006), photographed and then released. Transects for exuviae collection were located parallel to those for adults, but in shallow water with aquatic vegetation. The lengths were the same as before but they were half-narrower (2.5 m each). Height of the exuviae transects was determined by emergent water vegetation from where exuviae were collected by hands and stored. Each exuvia was later determined to the species rank (Gerken et al. 1999; Askew 2004) in the laboratory. Prior the data analysis, abundances of adults and exuviae per 100 m of length were recalculated per 100 m of length. After that abundance classes were used to compare the data, following indications reported by Buchwald (1990): 1: 1–4 specimens (or exuviae); 2: 5–10 specimens (or exuviae); 3: 11–20 specimens (or exuviae); 4: 21–40 specimens (or exuviae); 5: > 40 specimens (or exuviae). We interpreted the abundance classes as follows: 1 = very few individuals; 2 = poor population; 3 = medium population; 4 = dense population; 5 = very large population, mass population.

Data analysis

To investigate interconnections in species compositions among the sampling stations, species assemblages were inspected through Unweighted Pair Groups Method (UPGMA). The clusters of the sampling stations were interpreted according to the recorded environmental parameters (see Table 1). Distance matrix was computed using Bray-Curtis index for the abundance classes data.

For each pond and a group of ponds defined by UPGMA, diversity of species on untransformed data abundances was shown by: (i) number of species, (ii) Shannon-Wiener index (H', here with ln), (iii) Fishers's alpha (α), (iv) dominance (D = 1 – Simpson index), and (v) eveness (equality: E = H'/lnN; where N = number of species in the sample), (vi) spatial species turnover rates among pairs of ponds and predefined groups of ponds by UPGMA were estimated by Whittaker's β-diversity index (β_W; see Hammer 2012 for formulae).

For the data analyses, MS Excel 2010, PAST (Palaeontological Statistics; Hammer 2012) and SPSS Statistical package ver. 20.0 (SPSS inc., 1989, 2011) were used.

Results

In total, 25 species of Odonata (Zygoptera: 7 spp., Anisoptera: 18 spp.), were recorded at 11 sampling stations in ponds between Monfalcone and Grado (Table 2). According to the salinity gradient, the number of collected specis is notable different among the four habitats. Freshwater habitats suit best Odonata, as we detected there are not only the highest number of species as adults (16) but also the highest species number as exuviae (15), proving that these habitats are also suitable for their reproduction. Nevertheless, a complete lack of exuviae and only few species of adults (10) was detected in mesohaline environment. Except for the latter environment, at least some species managed to finish their reprodutive cycle in the remaining two habitats (14 in oligohaline and 5 in polyhaline environment) (Table 1).

Most individuals were counted in freswater (543 exuviae, 956 adults) and oligohaline (1208 exuviae, 1601 adults) environments, followed by polyhaline (156 exuviae, 1893 adults) and mesohaline (no exuviae, 513 adults) environments. The most common species in the investigated habitats were *Ischnura elegans* as imagos, with a total of 1802 sampled individual, and *S. meridionale* (Selys, 1841), with 867 collected exuviae.

Table 2 List of dragonfly species and their abundances (imagoes/exuviae) recorded in 11 sampling stations and 8 ponds between Monfalcone and Grado (NE Italy) in 2010–2011. Diversity indices for each sampling station and habitat (imagoes/exuviae) are given below.

Habitat	Freshwater			Mesohaline		Polyhaline			Oligohaline		
Pond	C1	C2	C3	C4	C4	L1	L2	L3	L4	L4	L4
Sampling station	C1	C2	C3	C4a	C4b	L1	L2	L3	L4a	L4b	L4c
Zygoptera											
Sympecma fusca	6	0/0	0/6	120/0	90/0	3/0	9/5	17/2	50/6	60/82	0/1
Lestes parvidens	0/0	40/45	0/0	0/0	0/0	0/0	40/0	30/0	10/0	0/0	0/0
Ischnura elegans	98/1	162/0	146/3	132/0	18/0	153/20	235/18	299/14	75/50	344/19	140/109
Ishnura pumilio	0/0	0/0	0/0	0/0	0/0	1/0	4/0	0/0	0/0	0/1	0/0
Coenagrion puella	199/0	10/0	10/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Coenagrion scitulum	0/0	0/0	0/5	0/0	0/0	0/0	0/0	10/0	0/0	0/0	0/0
Erythromma viridulum	12/1	12/0	0/5	10/0	0/0	0/0	0/2	1/2	10/0	45/8	3/52
Anisoptera											
Brachythron pretense	0/0	0/0	0/0	0/0	0/0	4/0	55/0	265/0	125/0	85/1	23/0
Aeshna affinis	1/0	1/0	0/0	0/0	0/0	23/0	46/0	65/0	3/31	37/18	7/0
Aeshna cyanea	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Aeshna isosceles	0/0	1/0	1/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Aeshna mixta	2/2	1/0	1/13	26/0	0/0	70/5	0/5	120/82	8/6	13/60	5/2
Anax imperator	4/4	0/0	1/1	2/0	0/0	0/0	1/0	0/0	2/2	8/0	2/36

Anax Parthenope	0/0	0/0	0/0	1/0	11/0	0/0	0/0	0/0	0/0	0/0	0/0
Hemianax ephippiger	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0
Lindenia tetraphylla	0/0	0/0	0/0	0/0	0/0	0/0	1/0	0/0	0/0	0/0	0/0
Libellula depressa	0/0	2/6	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Orthetrum albistylum	2/4	5/0	½	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Orthetrum cancellatum	0/0	1/0	0/0	19/0	12/0	0/0	5/0	0/0	1/1	7/2	3/4
Crocothemis erythraea	60/24	16/19	19/1	19/0	2/0	0/0	0/0	0/0	30/0	14/31	6/9
Sympetrum fonscolombii	0/0	0/0	1/0	11/0	35/0	0/0	0/0	0/0	35/1	10/24	0/1
Sympetrum meridionale	24/52	14/42	15/123	5/0	0/0	43/0	25/0	366/1	100/202	140/442	50/5
Sympetrum sanguineum	0/3	0/14	0/3	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0
Sympetrum striolatum	9/0	61/34	7/50	0/0	0/0	0/0	1/0	1/0	150/0	0/0	0/0
Sympetrum vulgatum	0/1	0/49	0/29	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Adults/exuviae per station (total)	12/9 (13)	13/8 (16)	10/12 (15)	10/0 (10)	6/0 (6)	7/2 (7)	11/4 (13)	10/5 (10)	13/9 (14)	11/12 (13)	9/9 (11)
Adults/exuviae per habitat (total)	16/15 (20)			10/0 (10)		14/5 (14)			13/14 (16)		
Shannon-Wiener (H')	1.56/1.29	1.60/1.81	1.05/1.53	1.57/0	1.32/0	1.28/0.50	1.45/1.08	1.65/0.64	2.01/1.05	1.72/1.30	1.29/1.37
Shannon-Wiener (H')	1.74/1.90			1.62/0		1.69/0.94			1.97/1.61		
Fishers's alpha (α)	2.29/2.47	3.71/1.65	2.21/2.66	1.93/0	1.22/0	1.29/0.51	2.07/1.24	1.50/1.11	2.34/1.75	1.82/2.06	1.85/1.89
Fishers's alpha (α)	2.73/2.86			1.76/0		2.05/0.99			1.94/2.22		
Dominance (D)	0.29/0.39	0.30/0.18	0.54/0.32	0.28/0	0.35/0	0.35/0.68	0.35/0.42	0.23/0.68	0.16/0.49	0.26/0.44	0.40/0.33
Dominance (D)	0.26/0.22			0.27/0		0.23/0.46			0.19/0.33		
Eveness (E)	0.40/0.40	0.38/0.76	0.28/0.38	0.48/0	0.62/0	0.52/0.82	0.39/0.74	0.52/0.38	0.57/0.32	0.51/0.31	0.40/0.44
Eveness (E)	0.36/0.45			0.51/0		0.39/0.51			0.55/0.36		

The UPGMA confirmed the separation of the species communities into four groups according to the salinity gradient, and the groups are the same regardless the used dataset (imagoes or exuviae; Fig. 2). Diversity indices were in most cases the highest in freshwater habitats (but note the highest H' = 1.97 for imagoes in oligohaline habitats) (imagoes: H' = 1.74, α = 2.73; larvae: H' = 1.90, α = 2.86) (Table 1). Regarding the exuviae, least diversified were mesohaline (H' = 0, α = 0) and polyhaline (H' = 0.94, α = 0.99) environments. Mesohaline environments were also least diversified by the adults (H' = 1.62, α = 1.76). The dominance values were highest in mesohaline environments for adults (D = 0.27), and polyhaline environments for exuviae (D = 0.46). Finally, the eveness values showed that species are most unequally distributed in oligohaline (adults, E = 0.55) and polyhaline (exuviae, E = 0.51) environments (Table 1).

Differences in species tolerance towards the salinity gradient was evident (Fig. 3). Imagoes of four species (*Coenagrion puella* (Linnaeus, 1758)*, Aeshna isoceles* (Muller, 1767)*, Orthetrum albistylum* (Selys, 1848)*, Libellula depressa* Linnaeus, 1758) were recorded at the banks of freshwater ponds exclusivelly, and for seven species (*Sympetrum vulgatum* (Linnaeus, 1758)*, Aeshna cyanea* (Muller, 1764)*, O. albistylum, Lestes parvidens* Artobolevsky, 1929*, Sympetrum striolatum* (Charpentier, 1840)*, Coenagrion scitulum* (Rambur, 1842), *L. depressa*) a succesful reproduction was recorded only there. On the other hand, imagoes of *C. scitulum, Ishnura pumillio* (Charpentier, 1825) and *Lindenia tetraphylla* (Vander Linden, 1825) were recorded only at polyhaline habitats, and *Anax parthenope* (Selys, 1839) adults were found only at mesohaline habitats. No exuviae were confirmed for the following four species: *C. puella*, *A. isosceles*, *L. tetraphylla* and *A. parthenope*. All other species of adults proved to be less connected with a certain water type as they were recorded at least at two different types of ponds. In the study area for another five species (*Hemianax ephippiger* (Burmeister, 1839)*, Brachytron pratense* (Muller, 1764)*, A. affinis* Vander Linden, 1820*, Orthetrum cancellatum* (Linnaeus, 1758)*, S. foscolomebii* (Selys, 1840)) reproduction was confirmed only in oligohaline ponds. Six species (*S. meridionale, Erythromma viridulum* (Charpentier, 1840)*, I. elegans, A. mixta, Sympecma fusca* (Vander Linden, 1820)*, I. pumilio*) are able to complete their life cycle in polyhaline ponds, and with an exception of *I. pumilio*, were the only ones that were proved to be able to reproduce in three different habitats (freshwater, oligohaline, and polyhaline). Exuviae of *I. pumillio* on the other hand were not confirmed at freshwater habitats. Finally, exuviae of three species (*S. sanguineum, Crocothemis erythraea* (Brullé, 1832)*, Anax imperator* Leach, 1815) were recorded either at freshwater or oligohaline stations. Interestingly, no species was recorded to sucessfully complete its life cycle in mesohaline habitats.

As shown by the spatial distribution of the species, adults are less connected to a certain habitat than larvae. This is reflected in values for species spatial turnover rates that in all compared pairs of habitats are higher for exuviae than for the adults (Fig. 4). Since no exuviae were recorded in mesohaline environments (C4), species turnover rates between this and any other habitat is 1 (i.e. 100 % turnover rate). Furthermore, freshwater habitats (C1C3) differ most from the remaining two habitats (C1C3-L1L3 (polyhaline) = 0.60; C1C3-L4 (mesohaline) = 0.50). There is also a high species turnover rate between oligohaline and polyhaline habitats (L4-L1L3 = 0.60).

Discussion

In the study area 25 species of Odonata were encoutered, almost half of the species present in Friuli Venezia Giulia region (62 species: Zandigiacomo et al. 2014). In all Europe, 15 dragonfly species are known to survive in habitats characterized by some degrees of salinity (Askew 2004; Dijkstra and Lewington 2006; Zinchenko and Golovatyuk 2013): *Sympecma fusca, Lestes barbarus* (Fabricius, 1798)*, Lestes macrostigma* (Eversmann, 1836)*, Ischnura elegans, Ischnura pumilio, Erythromma viridulum, Brachytron pratense, Aeshna affinis, Aeshna mixta, Hemianax ephippiger, Orthetrum cancellatum, Crocothemis erythraea, Sympetrum fonscolombii, Sympetrum sanguineum, Sympetrum striolatum*. Except for *L. barbarus* and *L. macrostigma*, *S. striolatum* (inthe study area was found only in the freshwater habitats) all the other 13 species were found in the study area with breeding populations. *L.barbarus* is common and widespread in southern Europe (Spain, Italy, South France) and show strong fluctuations in populations (Boudot et al. 2009; Clausnitzer 2009). The absence of this species in the study area in period 2010–2011 is not clear, even if high fluctuations in population sizes could be the reason. *L. macrostigma* is a very rare species with fluctuating population sizes in the most of its range. In Italy, only few populations in South Italy (Puglia and Sardinia) are known and records from Europe suggest an ongoing decline (Boudot et al. 2009; Kalkman 2014).

Thanks to this study two more species, *Anax imperator* and *Sympetrum meridionale* are recognised for the first time to be tolerant towards the brakish waters, since their reproduction is confirmed in at least oligohaline (both species) or even polyhaline (*S. meridionale*) habitats. The two most common species in the investigated area are *I. elegans* (imagoes) and *S. meridionale* (exuviae). *I. elegans* is a very widespread species in all over its range and one of the most present damselflies in Europe, which breeds in a wide variety of standing and slow flowing waters and it is common at eutrophic and mesotrophic sites (Dow 2010). *S. meridionale* (exuviae) is a species common and often abundant accross most of the southern parts of its range (Spain, France, Italy, the Balkans, the mediterranean islands) (Askew 2004), becoming scarce to rare towards the north, typical of unshaded, hot and often shallow standing waters which partially or totally dry up during summer (Kalkman 2014). On the other hand, we detected only one flying male of *L. tetraphylla,* a EU Habitat Directive species that in Italy is present with only few fragmented populations in Toscana, Campania and Sardinia (IUCN 2014) and with disjunct populations in the Balkans (Boudot 2013), where the population closest to the Italian border is in Pag Island in Croatia (Belančić et al. 2008) (Vilenica 2016). The habitat where the male was flying resembles almost perfectly the typical habitat of the species (Schorr et al. 1998), i.e. brackish, shallow and warm water with abundant presence of *Phragmites australis*. An exuvia of *H. ephippiger* (Burmeister, 1839), an obligate afro-tropical

migrant species, was found in the oligohaline habitat; in Italy the species is present in the mainland and in Sardinia and Sicily (Subramanian 2016). This exuvia is the first proof of the species reproduction in at least North-eastern part of Italy (Uboni et al. 2018).

The diversity indeces on imagoes show highest diversity for oligohaline and freshwater habitats with many individuals belonging to five species in oligohaline habitat (*Ischnura elegans, Sympetrum meridionale, Brachythron pratense, Sympetrum striolatum, Sympecma fusca*), and *Ischnura elegans* and *Coenagrion puella* like the two dominant species in freshwater habitat. The diversity indeces on exuviae show highest diversity in freshwater habitats, with many exuviae of *Sympetrum meridionale, Sympetrum striolatum, Sympetrum vulgatum, Lestes parvidens, Crocothemis erythraea,* while the lowest diversity was recorded in polyhaline habitats, with *Sympetrum meridionale* and *Ischnura elegans* like dominant breeding species. However, it must be noted that no species in juvenile stage was recorded in mesohaline habitats. It has been already reported that in Odonata sightings of the adults may not always accurately predict the distribution of the larvae (Painter 1998; Painter 1999; Balzan 2012; Corbet 1999). This difference is also clear if we compare the correspondence between the odonate species that were recorded as imagoes flying over certain water-bodies habitats and the exuviae that were associated with the same habitat (i.e. 75% of adults flying on oligohaline water used it for their breeding site; this value decreases to 65% in freshwater, and down to 23% and 0 % in polyhaline and mesohaline habitats, respectivelly), reitering the idea that the presence of imagoes along a water-body may not necessarily indicate the suitability of the habitat for larval stages (cf. McPeek 2008; Balzan 2012). Most of the species recorded during the sampling activity within the study area sucessfully reproduce in freshwater (15 species) and in oligohaline water (14 species), with 8 species in common between the habitats, while only a small amount of the freshwater-oligohaline species (5 species) that are able to complete their metamorphosis also in polyhaline waters. According to this species composition, the investigated fauna is composed of five euryoecious species (*S. fusca, I. elegans, A. mixta, E. viridulum, S. meridionale*) that are able to breed in different aquatic habitats along a fresh-polyhaline water continuum of habitat types and that are the most tollerant species toward salinity. Hence, they can complete their life cycle in water with a PSU range from at least 5.86 to 30.97. These species can be identified like »polyhaline« species since they can tolerate 30.97 PSU (*A. mixta* is the only species belonging to this group that emerges in higher numbers in polyhaline habitat than in fresh and oligohaline water-bodies); the »polyhaline« behaviour of *A. mixta* (27.8 g/L in Aguesse, 1968) and *I. elegans* (21.7 g/L in Zinchenko and Golovatyuk 2013) is confirmed in the present study, however we increase the values of tolerance for both species to the value of 30.97 g/L. Further, five species from the investigated area seem to be connected

only to oligohaline habitats (*A. affinis, B. pratense, H. ephippiger, O. cancellatum, S. fonscolombii)* and can breed with a PSU range from at least 1.33 to 3.59. These species can be named like pure »oligohaline« species. Seven species (*L. parvidens, C. scitulum, A. cyanea, L. depressa, O. albystilum, S. striolatum, S. vulgatum*) can be defined as stenoecious and seem to be stricly associated with freshwater habitats. It is important to underline that *L. depressa* in this study is present only in the freshwater habitats, despite the known laboratory tests that the larvae of this species were able to survive at salinity equal to 6.3–8.1 g/L (Zinchenko and Golovatyuk 2013). On the other hand, we are not able to confirm the tolerance range of 7.5–21.1 g/L (Zinchenko and Golovatyuk 2013) for larvae of *S. sanguineum* since we found the vast majority (95 %) of them in freshwater, and exceptionally (5 %) also in oligohaline water-bodies.

Therefore, salinity can be considered a stressor factor for aquatic invertebrates (Hauton 2016), and the number of detected species roughly decreases with the increased salinity (see Table 2). Nevertheless, the abundance of larvae is highest in hostile oligohaline habitats (63.3 % of all collected exuviae) than in freshwater-bodies (28.5 %) that are generally preferred by Odonata, only then followed by polyhaline (8.2 %) and mesohaline (0 %) environments. The latter result indicates that in environments with lower species diversity some species can become highly abundant (Brower and Zar 1977); in our case euryoecious *S. meridionale* with ca 55 % of individuals collected in oligohaline waters. In the mesohaline water the species assemblage in adults resembles a mixture between species that can breed in both freshwater and oligohaline habitats (*A. imperator*, *O. cancellatum, S. fonscolombii, C. erythraea*), and species that can be found along the whole salinity gradient (*S. fusca, I. elegans, E. viridulum, A. mixta, S. meridionale*). Since many species of larvae were found from one to another end of the salinity gradient, we assume that in investigated mesohaline habitat the level of salinity alone was not the most important reason causing the complete lack of exuviae. Instead we suggest that the wind exposure and the scarce vegetation structure strongly diminished or completelly prevented certain behaviour inevitably important for completion of their life cycle. Namely, presence of rich vegetation is an essential part of Odonata habitat because it represents possible places where individuals can copulate and warm up, and display their behaviours (e.g. territoriality). Further, vegetation represents a substrate for females to lay eggs. Submerged vegetation also represents the suitable habitat for the larvae, and when the vegation is emerging from the water also the substrate where individuals emerge (Buchwald 1990). Finally, exposure to strong winds also negativelly affect the diversity of imagoes since strong winds may create problems in flight and feeding.

Conclusions

Thanks to this study on the diversity and assemblage structure of damselfly and dragonfly fauna along the salinity gradient we find out the following conclusions.

(1) We identify a gradient of salinity and we prove that the salinity influences the occurrence and abundance of Odonata: freshwater is the most rich habitat in species composition (imagoes: 16 spp., exuviae: 15 spp.), while brackish water habitats are less diverse (imagoes: 10–14 spp., exuviae: 0–14 spp.). For 13 European species that are recorded in brakish environments during the present study we report the exact data of larval tolerance to the ione chloride.

(2) Species assemblages strongly differ along the salinity gradient. Although the gradient is not strictly the same for the adults and exuviae, there are obvious parallels between the juvenile and adult stage. We detect that almost 4/5 of the adults flying in the oligohaline habitats are connected to this habitat typology for reproduction, and that proportion is decreasing to 3/5 for the imagoes flying on freshwater and to 1/5 for the imagoes flying on polyhaline habitats. Even if many Odonata species are observed flying in the mesohaline habitats (7 in common between polyhaline and oligohaline, plus 2 oligohaline species) no exuviae are found, probably due to many other environmental parameters, strong winds and a lack of diverse vegetation that prevented the completion of their life cycle.

(3) Oligohaline water body represents a bridge habitat and appears to be a transition and connection between freshwater habitat and polyhaline habitat, with a mosaic of species belonging to all the different species combinations along the salinity gradient.

Finally, we proved one more time that coastal wetlands are very important in displaying large biodiversity and sustaining natural ecosystem functions (Camacho-Valdez et al. 2013), thanks to their structure and their inner ecological processes. These charcateristics highlight the importance of sustainable management strategies (Turner et al. 2000) for these habitat typology.

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Figures

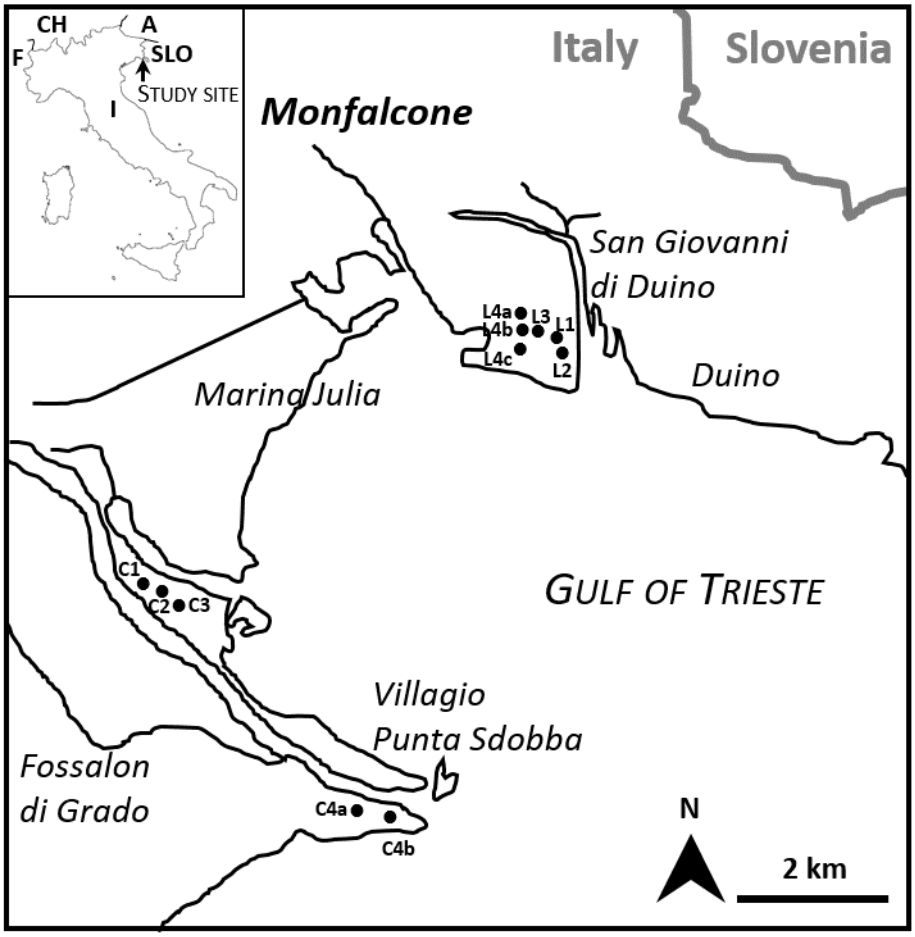

Fig. 1 Geographic position of the study site (inset) and of the eleven sampling stations at eight ponds (L1–L4, C1–C4) between Monfalcone and Grado, NE Italy. Letters denote different sampling stations at two largest ponds (L4a–c, C4a–b)

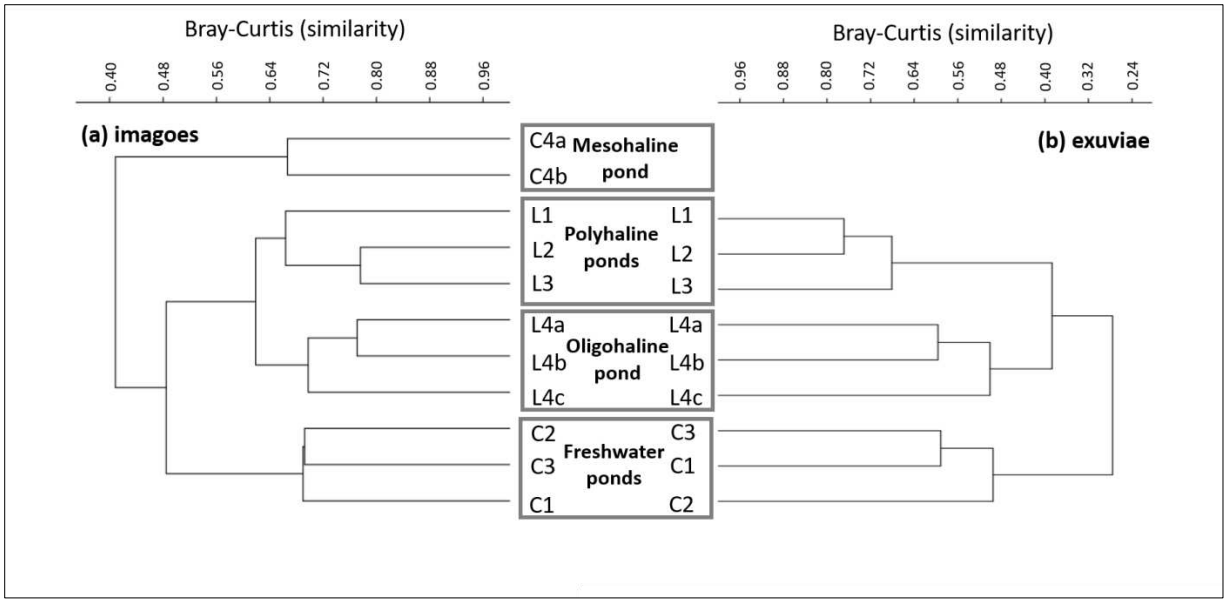

Fig. 2 Similarity in species composition (UPGMA; Bray-Curtis index) from eleven sampling stations at eight ponds Odonata for imagoes (a) and exuviae (b). No exuviae were recorded at stations C4a and C4b

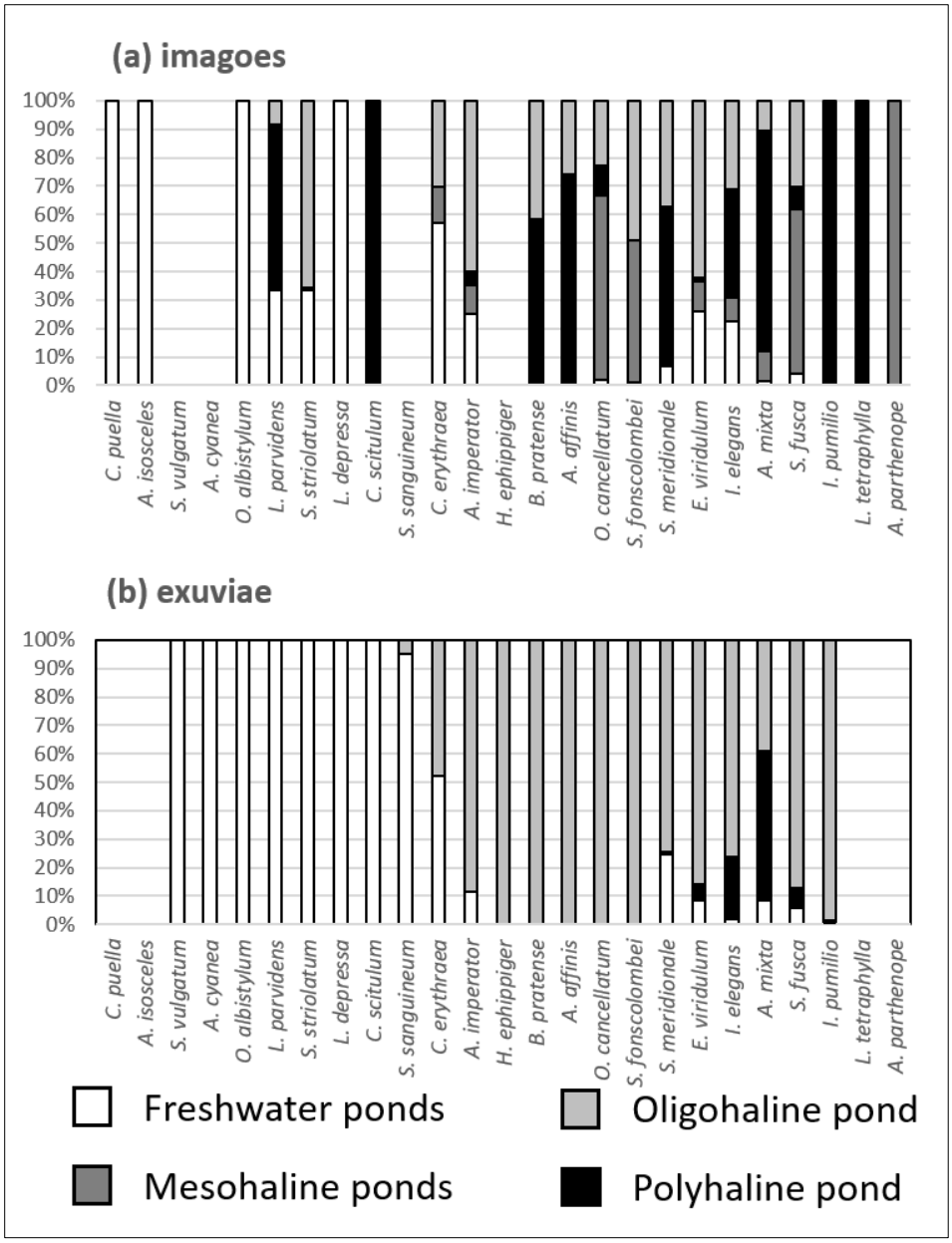

Fig. 3 Spatial distribution of Odonata species in four types of ponds, separated by imagoes and exuviae

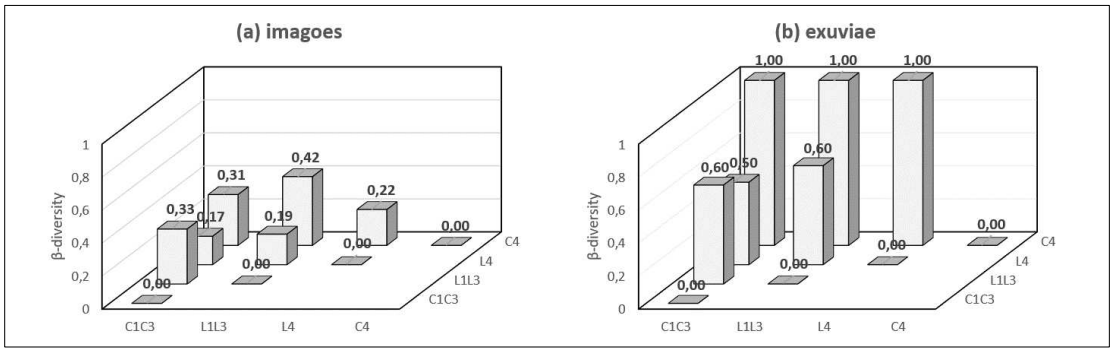

Fig. 4 Species turnover (β-diversity) rates between groups of sampling stations as defined by UPGMA for dragonfly adults (a) and exuviae (b)

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Demography of endangered Marsh fritillary butterfly, *Euphydryas aurinia* (Lepidoptera: Nymphalidae) in the Mediterranean dry meadows

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Demography of endangered Marsh fritillary butterfly, *Euphydryas aurinia* (Lepidoptera: Nymphalidae) in the Mediterranean dry meadows

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Key words: mark-release-recapture, *Euphydryas aurinia*, demography, movement, nectar plants, metapopulation

Abstract

We studied adult demography, movement ability, behaviour, and choice of nectaring plants in a Mediterranean population of *Euphydryas aurinia* inhabiting a system of loosely connected dry karst meadows. Migration, emigration and immigration rates were assessed for differently large patches. The study system comprised of four meadows spanning 2.84 ha. Total population size was estimated to be approximately 347 (95 % confidence interval: 262–432) and 326 (95 % confidence interval: 250–402) for males and females, respectively. The sex ratio was unbiased. The average lifespan was 6.3 and 8.6 days, with maximal observed lifespans of 18 and 21 days for males and females. Higher capture probability in males indicates their high activity as they spend most of their time on wings patrolling and searching for their mates. Daily population sizes followed a parabola with expressed protandry. High mobility of both sexes was detected, and proved occasional long distance movements over half a kilometre to be highly probable in both sexes. The spatial distribution of animals seemed to be connected to patch size and nectar sources, resulting in much higher densities of captures in the largest patch. The adult behaviour differed between the sexes, with more pronounced resting and less pronounced flying in females, and the opposite in males. Adults were confirmed to be opportunistic feeders, since as many as ten nectar sources were detected. Because the demographic parameters highly differ among regions and habitats, conservation aims should be planned in accordance to differences among them. In the Mediterranean Slovenia, mobility was high in comparison to some other populations throughout the species' distribution area. Although the studied population is apparently in good condition, there are threats that may hamper the long term persistence of the species in this area: overgrowing processes, intensive mowing and overgrazing.

Introduction

The marsh fritillary, *Euphydryas aurinia* (Rottemburg 1775) (Lepidoptera: Nymphalidae) is listed on the habitat Directive 92/43 EEC of the European Union, and included in the Red List of Butterflies (van Swaay et al., 2010) as of Least concern. In Slovenia, the species is classified as vulnerable in the Red List (Official Gazette of the Republic of Slovenia, 2010), and its populations are in general decline that is assessed to be around 20 % over the last 20 years (Verovnik et al., 2012). Declines, or even local extinctions, have been observed especially for populations inhabiting wet meadows in Eastern, but also Central Slovenia; even in protected areas and Natura 2000 sites (Čelik, 2015). Although the species is thoroughly studied throughout its entire distribution area that stretches all across Europe to temperate Asia (Casacci et al., 2015), estimated demographic parameters considerably differ among regions and parts of distribution area. The species inhabits a wide range of different habitats: e.g. pastures in UK (Asher et al., 2001), hay meadows in central Europe (Anthes et al., 2003; Konvicka et al., 2003), woodland clearings in Scandinavia (Wahlberg et al., 2002a) and it reaches high altitudes (*E. a. glaciegenita* up to cca. 2300 m a.s.l.: Casacci et al., 2015). Thus populations appear to be regionally host- and habitat-specialised (Warren, 1994; Anthes et al., 2003). In the light of conservation efforts it should be emphasized that the management adequate for one part of the distribution area would be impractical or even harmful in other areas (Konvicka et al., 2003). Most studies of European populations were conducted in nutrient poor grasslands and fens of central and northern Europe (Wahlberg et al., 2002a; Konvicka et al., 2003, 2005; Hula et al., 2004; Fric et al., 2010; Zimmermann et al., 2011a; Zimmermann et al., 2011b; Čelik, 2015), and rarely in Mediterranean or Alpine (Casacci et al., 2015) habitats.

In Slovenia the species' main distribution centres are in western and southwestern parts of the country (Verovnik et al., 2012). Populations differ regionally at morphological and ecological levels, and different parts of the country are occupied with one of the three ecotypes; the first living in marshes and nutrient-poor wet meadows, and the second on dry and thermophilic meadows (Čelik, 2015). The latter populations are also present in a wider area of our research system within a Mediterranean climate of southwestern (SW) Slovenia, where the species persists in a system of loosely connected habitat patches. Moreover, Alpine meadows are home to the alpine ecotype and morphotype (*E. aurinia* f. *debilis*) (Verovnik et al., 2012).

The knowledge on demographic patterns is inevitably necessary for a successful conservation of any species. Since butterflies have complex life cycles, detailed ecological studies of (i) metapopulations, consisted of spatially restricted colonies interconnected by dispersal of the adults (Warren, 1994; Hanski, 1999; Wahlberg et al., 2002a; Hula et al., 2004), (ii) nectar plants utilization and (iii) the butterflies' movement ability, are crucial for successful conservation actions and long-term survival of the species throughout different regions. The species' dispersal ability is important in natural habitats, as well as in increasingly fragmented landscapes that are a by-product of human activities (Zimmermann et al. 2011b). *E. aurinia* was traditionally recognised as a sedentary species, as there were only few movements demonstrated (Warren, 1994; Casacci et al., 2015). Zimmermann et al. (2011b) were first to systematically record the existence of long distance movements. Results of these studies are quite contrasting, so they could be the result of habitat and regional differences within the species.

During a one-season study of a population of *E. aurinia* inhabiting a system of fragmented patches in dry karst meadows in SW Slovenia we aimed to: (i) assess demographic parameters and movements among the patches, (ii) identify the habitat use through different types of behaviour, and (iii) assess utilization of nectar plants. Our goal was to estimate what are the prospects for a long term persistence of the allegedly largest Slovenian metapopulation, considering changes in land management during the last 100 years from traditional extensive grazing, to two notably opposite practices: intensification of agricultural practices (such as mowing and grazing) and abandonment leading to overgrowing.

Materials and methods

Study species

The Mediterranean populations of *E. aurinia* occur in the SW part of Slovenia, from the sea level to around 800 m of altitude (Verovnik et al., 2012). More particularly, the studied metapopulation inhabits a fragmented semi-natural grassland (Natura 2000 habitat type 62A0) with the following typical plant species: *Chrysopogon gryllus, Stipa eriocaulis, Danthonia alpina, Carex humilis, Festuca rupicola* (all *Festuco-Brometea*) and additional submediteranean-illyric species, such as *Scorzonera austriaca, Thesium divaricatum, Jurinea mollis, Muscari botryoides, Leontodon crispus, Plantago holosteum, Knautia illyrica* (*E. aurinia* hostplant), *Helianthemum ovatum* and *Serapias vomeracea* (Ogorelec, 2007). Lately these areas are being overgrown due to the abandonment of grazing and traditional mowing (Kaligarič et al., 2006; Jugovic et al., 2013).

At these habitats, this univoltine species undergoes its life cycle. Females lay the eggs in groups (up to 250, rarely more) on the lower side of host plant leafs (Wahlberg et al., 2002a; Smee et al., 2011; Nunner et al., 2013). Larvae hatch after three to four weeks (Nunner et al., 2013) and at first they live in a common nest. They feed on the host plant and are not very mobile as they stay close to the host plant, not more than 50 cm away (Liu et al., 2006; Tjørnløv et al., 2015), hence, they need fresh leaves of host plants nearby. They feed and sunbathe until autumn, when they start to overwinter in a common waterproof nest. In the beginning of spring larvae stay together for a while and continue to feed, but spread before pupating on leaves or stems of the nearby vegetation (Čelik, 2015).

Study system

The study system is located in SW Slovenia (WGS84: 45°28'51.83'' N, 13°56'38.08'' E; Fig. 3) near the village of Rakitovec (Municipality of Koper) at elevations between 500 and 620 m a.s.l. on the carbonate bedrock of the Karst Edge (part of the Slovenian Natura 2000 site named "Karst"). Four patches (A: 2.22 ha, B: 0.26 ha, C: 0.11 ha, D: 0.25 ha; total area: 2.84 ha) were selected in a system of dry meadows enclosed by primary forests of *Quercus pubescens* and *Ostrya carpinifolia*, mixed with secondary pioneer vegetation of *Pinus nigra*. In some places hedges, consisted mainly of *Prunus spinosa* and *Crataegus* spp., separate the habitat patches into subdivisions. In this area, dry meadows are secondary open habitats, mostly rocky and highly eroded; many of which have been abandoned and are now completely or partially overgrowing with scrubs of *Juniperus communis* or *Cotinus coggygria* (Jogan et al., 2004). The patches consist of fully or at least partially maintained meadows, where mowing is slowing down the overgrowing processes. Host plants in the area are predominately *Knautia illyrica* and *Scabiosa columbaria* (Verovnik et al., 2012).

Mark-release-recapture (MRR), population density and life expectancy

The study was conducted in 14 sampling occasions from 5th May to 5th June 2015 in one to four day intervals, weather permitting (Table 1). On each sampling occasion two people were searching and netting butterflies. The first day of sampling was unsuccessful, as we could not find any *E. aurinia*, therefore we omitted this occasion from further analyses. Each butterfly was individually marked with a

black permanent marker and released immediately after at the location of capture. Prior to netting, behaviour of each butterfly was recorded (flying, resting, feeding, copula or courtship). When feeding or resting, the plant species were recorded. We also collected data on butterfly individual marking, sex, exact locality and time (date, hour) of a capture (Garmin Oregon 200, precision ±5 metres).

Please insert Table 1.

We recorded some movements among all four sampling plots; therefore the data on capture were pooled for the study of demographic parameters in MARK 8.0 (White & Burnham, 1999). Within MARK, we used the POPAN module that indicates the existence of a superpopulation. This approach is suitable for the demographic study of butterfly populations, which are changing on a daily basis due to recruitment from pupae and deaths of imagoes, and is often applied in such research (Öckinger & Smith, 2008; Čelik et al., 2010; Čelik, 2012; Weyer & Schmitt, 2013; Pennekamp et al., 2014; Jugovic et al., 2017).

A two-step procedure (Schtickzelle et al., 2003; Čelik, 2012) was used in MARK to select the best models fitting the data. Initially, data were analysed within Cormack-Jolly-Seber (CJS) module, modelling for survival probability (φ) and capture probability (p). The best-fit models were then incorporated into the POPAN module. Within this formulation, we modelled for φ, p and recruitment rate (or probability of entrance; b), which resulted in an estimate of total population size (N), and derived parameters, such as daily population sizes (N_i) and daily recruitment (B_i). The best models were selected according to Akaike Information Criterion corrected for small sample size (AIC_c) (Burnham & Anderson, 2002), standardly choosing models with $AIC_c < 2$. A goodness-of-fit test for the global model ($\varphi_{(g*t)}\ p_{(g*t)}$) was also run in CJS module, using the median c-hat approach, incorporated in MARK. The resulting estimate of c-hat was then used to correct for overdispersion of the global model in the mark-recapture analysis.

In CJS, we modelled both φ and p in eight different ways, allowing them to be constant (.), to differ between groups (g), to be time-dependent (t), to show a linear trend (tlin), or being arranged in an additive ([g + t], [g + tlin]) or interactive way ([g x t], [g x tlin]), giving 64 different models in total. In POPAN the additional parameter of recruitment included eight basic and three additional structures ([tlin + $tlin^2$], [g + tlin + $tlin^2$], [g x [tlin + $tlin^2$]]). The best models were used to infer parameters of

interest (daily survival, capture probability, daily and total population sizes), applying the procedure of model averaging (weighted average of selected best models).

To detect possible influence of weather upon demographic parameters data on rainfall from the nearest precipitation station located in Rakitovec was collected from Slovenian Environmental Agency (National Meteorological Service, 2017).

We used χ^2-test of homogeneity ($p < 0.05$) for a possible difference in ratios of captures and recaptures between males and females.

Population densities (maximum daily number of specimens per hectare) were estimated on the days with peak abundance for males and females ($N_{i\ max}$/area). Moreover, total population densities (N/area) for males and females were also calculated. Densities of captures for males and females were calculated also for each of the patches to inspect for possible differences among them.

We assessed average lifetime expectancy following the method proposed by Nowicki et al. (2005): $e_{avg} = (1- \varphi)^{-1} - 0.5$. The formula is reported to be suitable for animals that emerge in groups in early morning hours (Nowicki et al., 2005).

Behaviour and nectar plants

We used χ^2-test of association to check for possible differences in behaviour and nectar plants choice between males and females. Since in some cases expected frequencies were lower than 1, likelihood ratio (LR) statistics was used. We compared the distribution frequencies of five behavioural types between the sexes, and accepted the difference at $p < 0.05$. Also the distribution frequencies for nectar plants were compared between males and females. In both cases, standardized residuals were used to assess the relative contributions of the cells to the overall χ^2 value. Significant contribution was accepted when the absolute value of the standardized residual for the cell was ≥ |2.0| (Čelik, 2013). Then we classified plant species used for nectar source into three categories: rarely (< 3 %), commonly (3–25 %) and frequently (> 25 %) selected nectar plants.

Movements

For each patch, we have counted and calculated the proportions of residential and non-residential males and females. All animals that were marked in one patch but later recaptured in at least one of the other patches were considered as non-residential. We distinguished transferring individuals from transferring events, the latter occurred every time when a single individual moved between two patches; however, a single individual may have transferred more than once. Due to a relatively small number of transfers, we calculated migration, emigration and immigration rates for the largest plot separately, and for the three smaller patches joined together; with merging the data we minimized the statistical error because of small number of capture events at these three patches (Hill et al., 1996). Migration was estimated as the difference between immigrating and emigrating animals in a given patch divided by all recaptures at a given patch. Emigration was the number of butterflies recorded for the first time at one, but later recaptured at any other patch, divided by the total number of recapture events of animals from this one patch anywhere in the system. Finally, immigration was calculated as the number of butterflies recorded for the first time at any other patch, but later recaptured at a given patch, divided by the total number of recapture events of the animals from any other patch recaptured at the given patch. We analysed both sexes together and expressed the results in percentages of migrating, immigrating and emigrating animals.

We calculated the cumulative distances between the first and the last capture of all the animals that were recaptured at least once, and compared the ability of movements between males and females in classes of 50 meter distances from the marking point. Average distances and standard deviations were calculated, and possible significant statistical difference between the sexes was tested by one-way T-test ($p < 0.05$).

We calculated cumulative proportions of individuals moving certain distances of 50 m classes and these proportions were then fitted to a negative exponential function, where probability of an individual (P) moving distance D (in km) is $P = e^{-kD}$ (k represents a dispersal constant describing the shape of the exponential function). We also calculated the expected distance (D') between the two consecutive captures expressed by $D' = 1/k$ (Hill et al., 1996). The same data were also fitted to an inverse-power function (IPF), where probability of moving a certain distance is given by $P = CD^{-n}$, with C and n representing two scaling constants (Hill et al., 1996). Both approaches were used to estimate the dispersing ability of a species within a landscape and give important information for the species conservation (Pennekamp et al., 2014). We then assessed the slopes and standard errors of the resulting fitted distributions by means of linear regression. The calculations were $\ln P = -kD$ in NEF and $\ln P = \ln C -$

n*lnD in IPF, respectively. T-test in regression was used to compare the slopes between males and females (Soper, 2016). The population size estimation derived from the MRR analysis was used to calculate the number of individuals capable of moving certain distances.

Statistical analyses were performed using MS Excel 2010 and SPSS statistical package ver. 20.0 (IBM SPSS Inc., 1989, 2011).

Results

Demographic parameters

During the study, we marked 379 butterflies (200 males and 179 females; Table 2). Altogether, 136 individuals (73 males and 63 females) were recaptured (35.9 %).

Please insert Table 2.

The median c-hat approach indicated a slight overdispersion of the data (c-hat = 1.3). This was corrected for in both modelling steps: CJS and POPAN. Six models with $\Delta QAIC_c < 2$ were derived from all tested models and were taken into consideration for model averaging (Table 3). Survival was shown to be constant but sex dependent through the duration of the study in all six proposed models, whereas probability of capture was only time dependent or time dependent with an additive interaction with the sexes. Recruitment rate was always linked to some type of linear change in time and differed for the two groups, while displaying additive or interactive combinations between them.

Please insert Table 3.

Average daily survival was 0.853 (95 % confidence interval 0.815-0.884) for males and 0.890 (95 % confidence interval 0.852–0.919) for females throughout the flying season. Average lifetime expectancy was estimated to 8.6 days and 6.3 days for females and males, respectively. Maximal observed lifespan in females surpassed the maximal observed lifespan in males for three days (longest living male recorded: 11th–29th May), and lasted for 21 days (longest living female recorded: 13th May–3rd June). None of the capture or recapture ratios were biased towards one of the sexes (males : females = 1.12 : 1; χ^2-test, $p > 0.05$, estimate from captured animals). Probability of capture was similar in males and females, with a decreasing pattern in time (Fig. 1a). An unusual and substantial fall of catchability on the 4th sampling occasion (16th May) was noticed, following two days of rainfall. Peak of male population (13th of May) was a week earlier than that of females (20th of May; Fig. 1c). Daily population sizes of males and females were approximately the same on 18th of May. Emergence of adult animals into the population or recruitment (*Bi*) was highest between the first two sampling occasions and declined towards the end of the season (Fig. 1b).

Please insert Figure 1.

In the study area, the total population size of males counted for 347 specimens (SE = 43; 95 % CI: 262–432), and for females, the total population size counted for 326 specimens (SE = 39; 95 % CI: 250–402). The estimated population density on the days with the peak abundances were 44 males/ha and 39 females/ha. Total population density was 120 males/ha and 112 females/ha. The density of captures was 110 males/ha and 98 females/ha. However, most of the animals were found on the largest of the three patches (see patch A, Table 2), where density of captures was 132 males and 116 females per hectare. The densities there were at least two times higher than in any other of the three patches (Table 2).

Behaviour and nectar plants

From the five recorded types of behaviour, flying was recorded in almost two thirds (62 %) of all cases (Fig. 2). In a quarter of cases we recorded resting behaviour (25 %), followed by feeding (11 %), copula and courtship (~1 % each). There was a significant difference in behaviour between males and females (LR = 17.502, df = 4, p = 0.002). While the observed frequencies for copula, courtship and feeding did not

significantly deviate from the expected frequencies (stand. res. < |0.6|), males were resting less (stand. res. = -2.2) and flying more (stand. res. = 1.7, close to significance) than expected. Behaviour of females was reversed; they were flying less (stand. res. = -1.8, close to significance) and resting more frequently (stand. res. = 2.3) than expected.

Altogether butterflies were recorded to feed on ten different plant taxa (Fig. 2). They were feeding most frequently on *Trifolium* sp. (27 %) and *Leucanthemum* sp. (25 %), while other common nectar sources were recorded in the following order: *Thymus* spp. (11 %), *Knautia illyricum*, *Scorzonera villosa* and *Lotus corniculatus*, each with 8.5 %, and *Vicia cracca* with 5.6 %. Feeding on *Polygala nicaensis*, *Potentilla* sp. and Orchidaceae was only occasional (< 3 % of all feeding occasions). No difference in choice of nectar plants was shown between the sexes (LR = 11.546, df = 9; p = 0.240).

Please insert Figure 2.

Movements

Twenty-one individuals (10 males, 11 females, 5.5 %) transferred between patches, with two males and two females transferring two times and one female transferring three times between patches (Fig. 3). Altogether we observed 27 transfer events between patches (12 transfers in males and 15 transfers in females). One transferring male moved in a direction from patch A to B and back, and another in a direction from A to D and back. One transferring female moved from A to B and back, another one from A to D and back, and a third one moved three times between the patches in a direction from A through B and C to B. Other moving males (8) and females (8) were detected to transfer between patches only once. All the other butterflies were not detected to move from the patch where they were marked (190 males and 168 females, counted for 95.0 % and 93.9 % in males and females, respectively).

The largest patch seemed to be a source patch for the others, since seven males and nine females (76.2 % of transferring animals) later recaptured in smaller patches were first observed and marked in patch A. Only three males (from D to A) and two females (from B and D to A) were moving in an opposite direction. Nevertheless, patch A had the lowest emigration and immigration rates since the total number (325: 174 males and 151 females) and proportion (85.8 %: 87.0 % in males and 84.4 % in females) of residential animals there were the highest. In patch C, 12 (7 males, 5 females) out of 15

294 animals were residential (80.0 %: 87.5 in males and 71.4 % in females), followed by patch B with 11 (5
295 males and 6 females) out of 15 animals were residential (61.1 %: 71.4 in males, 54.5 in females) and
296 patch D, where only 10 (5 males, 5 females) out of 22 animals were not detected to move from the
297 patch (45.5 %: 41.7 in males and 50.0 % in females). Absolute values of migration, emigration and
298 immigration rates were all smaller at the largest patch (migration/emigration/immigration: -0.9 %/-3.0
299 %/2.1 %) compared to the three smaller patches together (10.7 %/37.5 %/25.0%).

300

301 Please insert Figure 3.

302

303 Altogether, 73 males and 63 females were recaptured from one to five times. Regarding the cumulative
304 distances between the first and the last catch, the proportion of animals was decreasing with distance
305 (Fig. 4). No statistical difference (one tailed T-test, $p > 0.1$) was found in average distance in males (AVG
306 ± SD: 165 m ± 174.5 m) and females (135 m ± 124 m). Nevertheless, the longest distance that was
307 measured in a single male exceeded the longest distance in females for 2.5 times (1220 meters in males
308 and 480 m in females). Moreover, a higher proportion of females (27.7 % in females vs. 18.7 % in males)
309 were recorded in a distance less than 50 m from the marking point (Fig. 4).

310

311 Please insert Figure 4.

312

313 Both functions predicting probability of long distance movements (NEF and IPF) fitted well to the data in
314 males and females ($p < 0.001$). The NEF equations are $P = e^{-11.15D}$ for males and $P = e^{-12.6D}$ for females,
315 respectively (Fig. 5). The expected distance (D') between the consecutive captures were 90 m for males
316 and 79 m for females. The IPF prediction equals the formulae $P = 0.0071D^{-1.492}$ for males and $P =$
317 $0.0059D^{-1.48}$ for females, respectively. The regression slopes of males and females in both NEF ($t = 0.65$,
318 $df = 162$, $p = 0.515$) and IPF ($t = 0.15$, $df = 162$, $p = 0.878$) did not differ. The predictions for moving
319 certain distances are summarised in Appendix A. In general, higher probabilities of movements for
320 distances shorter than 300 m were estimated by NEF, and higher for longer distances by IPF.
321 Nevertheless, all equations predict that approx. 10 % or less individuals move distances longer than 200
322 m. Moreover, the IPF predicts less than 2 % of individuals being able to reach or move beyond 500 m,

and 1 % of individuals to reach 750 m. For males, NEF and IPF predicted a higher probability of movement of distances over 100 m and 500 m than for females.

Please insert Figure 5.

Discussion

Demographic parameters

Striking difference of our results compared to many other MRR studies for butterflies (Lewis & Hurford, 1997; Kuras et al., 2003; Čelik, 2012) is nearly the same population size estimate of males and females. This is a result of comparable numbers of caught butterflies of each sex and their similar probability of capture. In other studied populations of *E. aurinia*, many more males than females were present, and the probability of capture for the latter was always lower (Casacci et al., 2015; Fric et al., 2010; Wahlberg et al., 2002b; Zimmermann et al., 2011a; Zimmermann et al., 2011b). Sex ratio close to 1:1 is uncommon for many butterflies and other insect species. The more commonly reported male-sex bias (Ehrlich et al., 1975; Stoks, 2001a, 2001b; Nowicki et al., 2005; Hovestadt & Nowicki, 2008) is usually interpreted by three possible explanations (Ehrlich, 1984) – (i) increased mortality of immature females associated to their prolonged development, (ii) increased mortality of adult females and (iii) higher emigration rate in females. While the first two possible causes for (un)biased sex rate remain inconclusive in our case and would request a separate study to investigate them, the emigration rate of females (indicated by movements from the largest to smaller study patches) was small (5.02 %) and congruent with unbiased sex ratio.

Similarly to other studies, the probability of capture was time-dependant (Schtickzelle et al., 2002; Vlasanek et al., 2009; Zimmermann et al., 2011a) and most likely affected by weather; e.g. the most marked change in catchability (during the 5th sampling) occurred after heavy rainfall before that occasion. We can exclude sampling-effort as a cause of differential catchability (cf. Vlasanek et al., 2009), as this was consistent through all sampling occasions.

Difference in survival rates for *E. aurinia* males and females from SW Slovenia was somewhat different as in similar studies, where survival of males always surpassed that of females (Fric et al., 2010; Zimmermann et al., 2011a); the opposite results were drawn from our data (0.853 in males and 0.890 in

females). Nevertheless, the estimated constant survival suggests an unchangeable rate of loss of butterflies after the emergence from pupae, with apparently no effect of weather, age at marking or other circumstances on survival (Fric et al., 2010). We can conclude, similarly to Zimmerman et al. (2011a), that differently old animals are equally affected by different mortality causes.

Higher survival in females is reflected also in their higher average longevity that surpasses the lifespan in males for more than two days. These results contradict other studies (Wahlberg et al., 2002b; Zimmermann et al., 2011a), where the estimated longevity in males was always higher. In our case, males were more active and spending more time on wings than females, which could lead to higher energy loss and shorter average lifespan in males. Moreover, our estimates of 6.3 and 8.6 days of average lifespan in males and females, respectively, are lower than reported by Wahlberg et al. (2002a); 10.7 days for males and 8.9 days for females. However, high variability of survival among years and populations within a given year, has already been noticed in *E. aurinia* (Zimmermann et al., 2011a; Zimmermann et al., 2011b), possibly due to differing temperature conditions which affect the insects' metabolism and its lifespan (Wahlberg et al., 2002b). Wahlberg (2001) discussed on *E. aurinia* high lifetime expectancy as a result of the defensive chemical substances (iridoids) consumed by larvae, which have a positive effect also in imagoes. Our estimates are comparable or even lower than estimates for some other species of univoltine Melitaeini (e.g. *Melitaea athalia*, *M. cinxia*, *M. diamina*, *Euphydryas maturna*; see Wahlberg et al. 2002b; Konvicka et al., 2005). Contrary, Casacci et al. (2015) report on lower average lifespan for two populations of *E. aurinia* from North Italy than Wahlberg et al. (2002b); Casacci's results are closer to our estimates, especially for males in their Alpine population (males/females: 6.2 days/5.7 days), and similar for females in the Mediterranean population (males/females: 12.2 days/7.7 days). Their results for the shorter lifespan at higher altitudes indicate also the possible negative effect of harsh conditions in the Alps upon the longevity of animals. In our case, the investigated season 2015 was quite wet which could also negatively affect the longevity of the animals.

As in other studied populations of *E. aurinia* (Zimmermann et al., 2011a) and many other univoltine butterfly species (Fric et al., 2010; Pennekamp et al., 2014), recruitment of adults into the population differed between sexes. Recruitment in our populations was time dependent, being higher for males at the beginning, but lower at the end of the season. As a result, parabola-like shaped daily population sizes are apparent. At two occasions (16th May and 24th May) the N_i deviated from the expected course, preceded by longer periods (≥ 2 days) of rainfall (see Fig. 1). We consider that the whole flying season of

383 2015 was included in our analysis, since no *E. aurinia* were recorded before or after the analysed
384 interval.

385

386 *Behaviour, nectar plants and movements*

387 Although not the most frequent behaviour, feeding is important for restoring the energy that can be
388 invested in other activities. There were no exact data on nectar choice by *E. aurinia* so far, but the
389 species was referred to be an opportunistic feeder (Čelik, 2015). An important novel contribution of our
390 work is the detection of as many as ten different nectar sources that confirms this assumption. It was
391 also reported that the higher abundance of nectar sources can dampen the emigration rate (Casacci et
392 al., 2015), and is possibly also positively correlated with the density of the animals. The latter was
393 noticed also in our study system where density of captures at the largest patch was much higher than at
394 any other patches that were also poorer with the nectar sources (own observations). The estimated
395 density of animals derived from our MRR study (232 individuals/ha), was substantially higher than
396 reported for most Central European (Czech Republic) and Southern European (Italy) populations (Casacci
397 et al., 2014; Fric et al., 2010; Zimmermann et al., 2011a: 20–379 individuals/ha, always lower than 200
398 individuals/ha, with one exception).

399 The most striking difference in the behaviour between the sexes is the pronounced resting in females,
400 and a deficit of it in males. In the latter, this is probably due to their mate-locating behaviour (van
401 Helsdingen et al., 1996), that involves more movements and was reflected in a higher proportion of
402 flying behaviour in males. Consequently, the largest distance between the points of first and last capture
403 in males by far exceeded the largest distance in females. Nevertheless, no difference in average ability
404 of movements was detected between the sexes, similar to other studies (e.g. Casacci et al., 2015). While
405 some studied populations showed low ability of movements (Wahlberg et al., 2002a; Anthes et al., 2003;
406 Casacci et al., 2015), an extensive study of Zimmermann et al. (2011a) demonstrated that *E. aurinia* is
407 capable of long distance movements. Significant differences among different habitat types (Casacci et
408 al., 2015), with higher movement ability for Mediterranean compared to Alpine populations should
409 however be noted. Moreover, in our case long distance predictions of movements were proved in both
410 sexes. This was true even when better fitting ($R^2_{NEF} > R^2_{IPF}$, see Results) but often allegedly
411 underestimating NEF procedure (Baguette, 2003; Zimmermann et al., 2011a) was applied. Our study
412 system in the Karst edge lie in a well-documented fragmented landscape (Jugovic et al., 2013; Lužnik et

al., 2014) and in such environments the ability for long distance movements is crucial for long-term survival (*Euphydryas beckeri*: Junker & Schmitt, 2010). Nevertheless, results of movement abilities can be affected by the scale of the respective study area (28 ha (Zimmermann et al., 2011b), and our study 2.84 ha), habitat quality and differences (Schneider, 2003; Casacci et al., 2015) and are therefore very hard to estimate accurately. Even applying the conservative estimate with at least 3 % of animals moving a certain distance (Zimmermann et al., 2011a) however allows to conclude that currently the metapopulation around our study system (SW Slovenia, see Verovnik et al., 2012 for current data on distribution of *E. aurinia*) is quite large and can span at least 100–140 km^2 of loosely connected area.

Residence rates were highly different between the largest patch (A) and other three much smaller patches (B, C, and D). Despite most immigrants and emigrants were captured at patch A, this patch had the lowest migration, emigration and immigration rates. This corresponds to the highest abundance of flowering plants at the largest patch, as the lack of nectar plants can stimulate the emigration (Casacci et al., 2015). Besides that, it is always more probable that the animals encounter the patch boundary when the patch is smaller, hence also the higher emigration rate. Since it is impossible to assess the number of butterflies leaving the whole study system, the detected emigration rate (4.6 % of animals were recaptured outside the patch where they were marked) is probably underestimated (see also Hill et al., 1996). This is further supported by the longest movements recorded during the study and the high ability of occasional long distance movements indicated by NEF and IPF. Moreover, Zimmermann et al. (2011a) showed that *E. aurinia* is capable of flying even much longer distances (over 15 km).

Implications for conservation

Since this species lives in metapopulations (Wahlberg et al., 2002a; Anthes et al., 2003; Bulman et al., 2007), it is crucial to estimate its potential for movements between patches that should be close enough to allow migrations. In Mediterranean Slovenia, *E. aurinia* is currently still widespread and patches are apparently close enough to maintain connections among them. Nevertheless, there are some threats to the long term survival of the species in this area: (i) overgrowing processes that result in the loss of open habitats rich with nectar sources (Jugovic et al., 2013), (ii) mowing of whole habitat patches and (iii) where still present, overgrazing (van Swaay et al., 2012). The grazing animals change the structure of vegetation, reduce numbers and diversity of flowering plants and input nitrogen into the soil. Additionally, they can directly harm the population by consuming the eggs on host plants (Čelik, 2015).

Nonetheless, grazing pressure in Karst edge is not widespread, but where it does occur the main problem is its intensity (Jugovic et al., 2013). Mowing in late spring can directly harm larvae, pupae and adult butterflies and destroys feeding and nectaring plants. Immediate baling of the hay poses a further threat as developing stages of butterflies are directly taken from the area by this activity.

Given that high variability of nectar plants is used by imagoes, we argue that meadows with high plant richness are preferred and an optimal balance between abandonment and intervening actions of these grasslands (occasional mosaic mowing) should be maintained. As the studied area is part of the Natura 2000 network some general guidelines for habitats are established (Bandelj et al., 2014; Čelik, 2015). However, no systematic monitoring and management is currently in place.

The long term persistence of the species is maintained with relatively large areas (at least 70 ha) of suitable and continuous habitat (Bulman et al., 2007), yet even larger patches are preferred (100–600 ha; Tjørnløv et al., 2015). The comparably high movement ability and density of *E. aurinia* detected in our study may indicate a persistent population and a stronghold for the species at the regional scale. Thus, we argue that the minimum viable population criterion for the species (at least 1800 individuals, as proposed by Schtickzelle et al. (2005)), is reached in the wider area of the research (Schtickzelle et al., 2005). Finally, remarkable differences of population parameters estimated for populations of *E. aurinia* at different habitats across the distribution area (see references in previous sections) should be considered when implementing conservation measures.

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611 **Tables**

612 **Table 1.** Summary of the data collected on the numbers of males and females of *E. aurinia* caught in SW Slovenia for all
613 sampling occasions in May and June 2015.

Sampling occasion	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Date	5.05.	8.05.	11.05.	13.05.	16.05.	18.05.	20.05.	24.05.	25.05.	27.05.	29.05.	1.06.	3.06.	5.06.
Interval	3	3	2	3	2	2	4	1	2	2	3	2	2	
No. of males	0	29	44	42	13	53	31	30	23	23	11	10	9	1
No. of females	0	10	21	32	20	31	41	20	27	18	24	23	12	5
Total no.	0	39	65	74	33	84	72	50	60	41	35	33	21	6

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617 **Table 2.** Summary data for all sampling occasions and numbers of caught males and females of *E. aurinia* in SW Slovenia.
618 Densities of captures are shown for each patch. A–D: sampling patches, T: all patches together (total).

	No. of marked individuals	No. of recaptured individuals	% of recaptured individuals	No. of captures	No. of recaptures	% of recaptures	Density of captures (ha^{-1})				
							A	B	C	D	T
Males	200	73	36.5	318	118	37.1	132	29	56	48	110
Females	179	63	35.2	285	95	33.3	116	43	56	42	98
Total	379	136	35.9	603	213						

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622 **Table 3.** The best supported POPAN models according to the Akaike information criterion (with $\Delta AIC_c < 2$), number of
623 parameters (Np) and estimates of population size (*N*) for males (♂) and females (♀) with standard errors (SE) and 95 %
624 confidence intervals (CI).

Model	**$\Delta QAIC_c$**	**Np**	*N* ♂ (95 % CI)	*N* ♀ (95 % CI)
$\varphi_{(g)}\, p_{(t)}\, b_{(g*tlin)}\, N_{(g)}$	0	21	345 (260–429)	312 (257–366)
$\varphi_{(g)}\, p_{(t)}\, b_{(g*(tlin+tlin2))}\, N_{(g)}$	0.4771	22	340 (281–399)	338 (253–422)
$\varphi_{(g)}\, p_{(t)}\, b_{(g+tlin)}\, N_{(g)}$	0.8186	20	387 (303–471)	316 (260–372)
$\varphi_{(g)}\, p_{(g+t)}\, b_{(g*(tlin+tlin2))}\, N_{(g)}$	1.0158	23	328 (270–385)	354 (259–449)
$\varphi_{(g)}\, p_{(g+t)}\, b_{(g*tlin)}\, N_{(g)}$	1.5372	22	314 (267–360)	314 (260–368)
$\varphi_{(g)}\, p_{(g+t)}\, b_{(g+tlin)}\, N_{(g)}$	1.8323	21	366 (277–455)	326 (263–389)
Model averaging	-	-	347 (262–432)	326 (250–402)

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627 **Figures' legends**

628 **Figure 1**. Estimates and 95 % confidence intervals of (a) probability of capture, (b) daily recruitment, and (c) daily
629 population sizes for *E. aurinia* in a karst meadow system in SW Slovenia. Data were extracted from the averaging of
630 the first six models with $\Delta QAIC_c < 2$. Estimates in (a) and (c) are shown for sampling occasions, and for (b) during
631 intervals between occasions. Data on daily rainfall for the nearest rain station in Rakitovec is shown below (c).

632

633 **Figure 2**. (a) Behaviour of *E. aurinia* in a system of karst meadows in SW Slovenia (males inner circle, females outer
634 circle), and (b) chosen nectar plants (different shades of grey from the lightest to the darkest represent rarely (< 3
635 %), commonly (3–25 %) and frequently (> 25 %) selected nectar plants, respectively).

636

637 **Figure 3.** Geographic position and recorded movements of *E. aurinia* between the four study patches (A, B, C, D) in
638 a system of dry karst meadows in SW Slovenia.

639

640 **Figure 4.** Cumulative distances between first and last catch of recaptured males and females represented in 50-
641 meter distance classes.

642

643 **Figure 5.** Probability of moving a certain distance for males and females of *E. aurinia* derived from NEF and IPF
644 based on distances between consecutive captures (in the formula, P represents the probability of moving a certain
645 distance, D).

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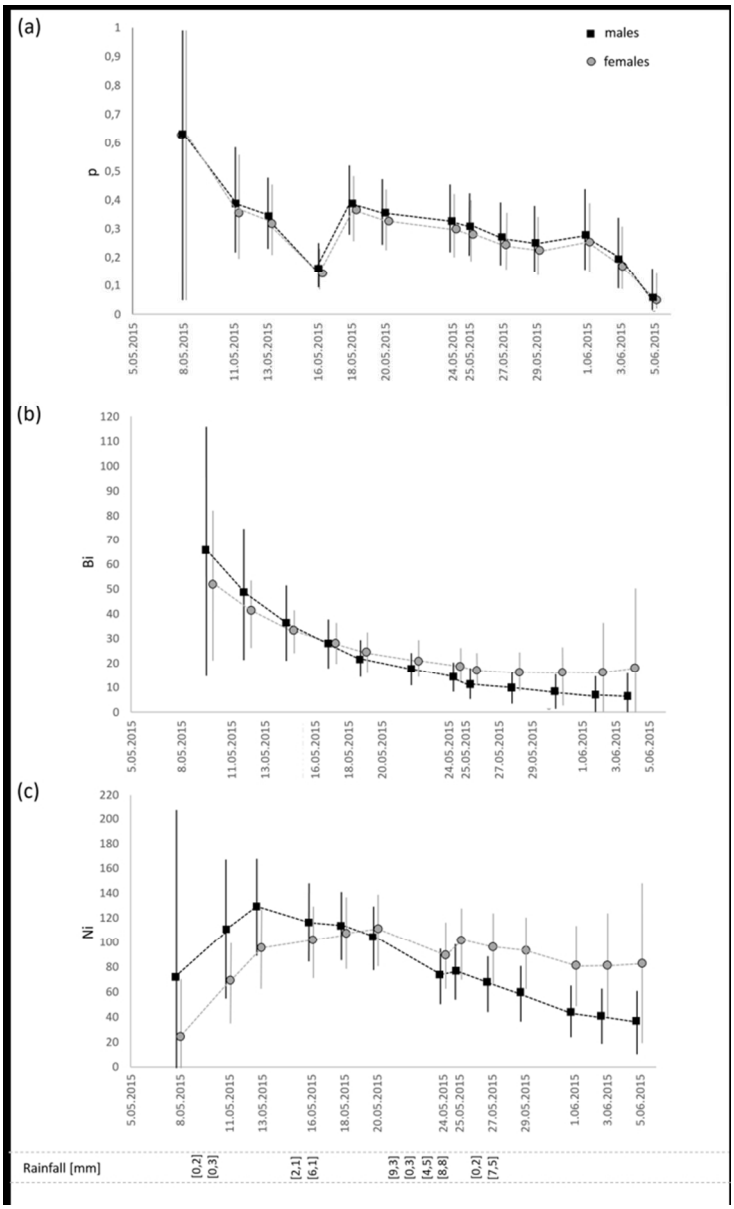
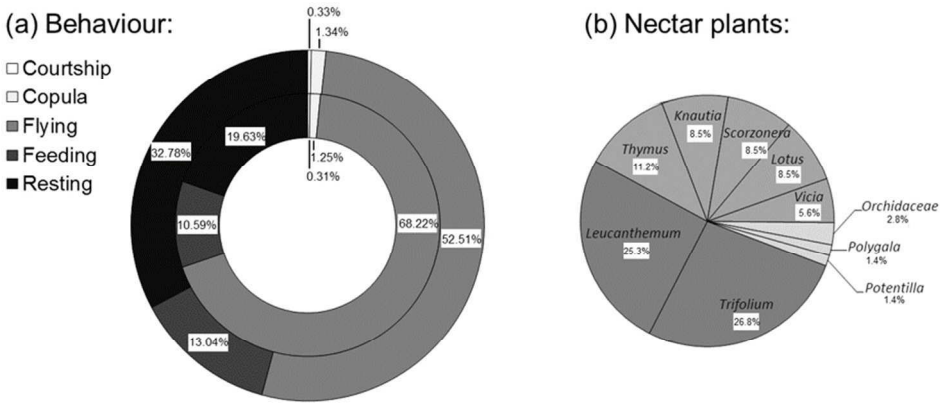
Appendix

Appendix A. Estimated proportion and number of *E. aurinia* individuals (NEF and IPF models), moving certain distances. Basis for individual measurements were estimates for population size of males (N = 347) and females (N = 326), with their 95 % confidence intervals (males: N^- –N^+ = 262–432; females: N^- –N^+ = 250–402).

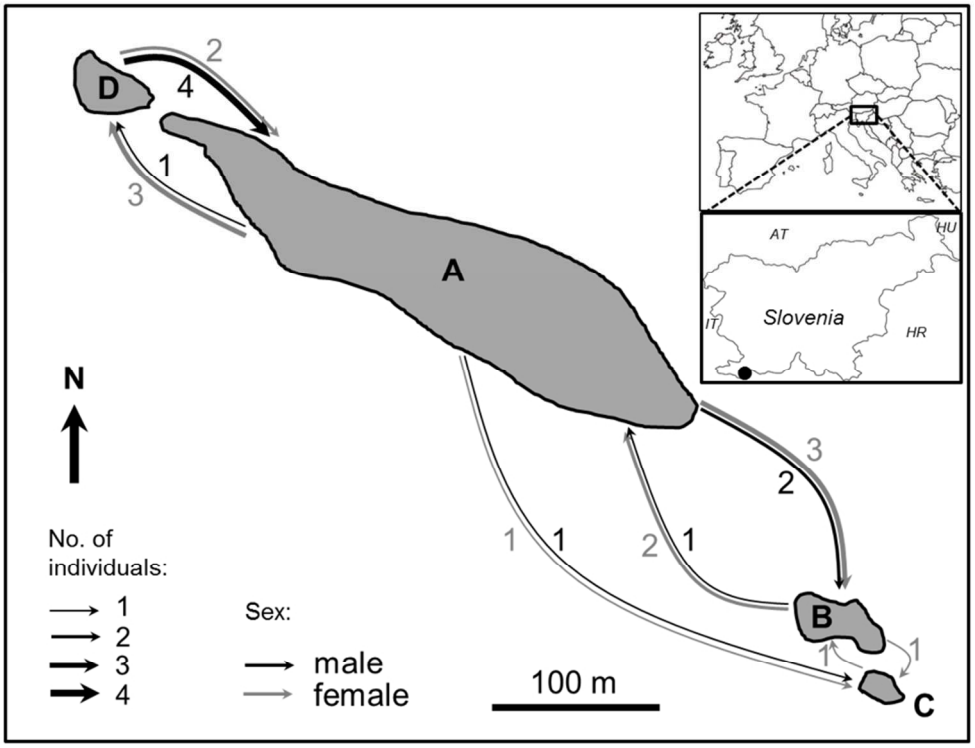
		Males				Females			
	Distance (km)	% individuals	N individuals	N^-	N^+	% individuals	N individuals	N^-	N^+
NEF	0.05	57.26	198.71	150.03	247.38	53.26	173.62	133.15	214.10
	0.1	32.79	113.79	85.91	141.66	28.37	92.47	70.91	114.03
	0.15	18.78	65.16	49.20	81.12	15.11	49.25	37.77	60.73
	0.2	10.75	37.31	28.17	46.45	8.05	26.23	20.11	32.34
	0.3	3.53	12.24	9.24	15.23	2.28	7.44	5.71	9.17
	0.4	1.16	4.01	3.03	4.99	0.65	2.11	1.62	2.60
	0.5	0.38	1.32	0.99	1.64	0.18	0.60	0.46	0.74
	1	$1.44\ 10^{-3}$	$4.99\ 10^{-3}$	$3.77\ 10^{-3}$	$6.21\ 10^{-3}$	$3.37\ 10^{-4}$	$1.10\ 10^{-3}$	$8.43\ 10^{-4}$	$1.36\ 10^{-3}$
	2	$2.07\ 10^{-8}$	$7.17\ 10^{-8}$	$5.41\ 10^{-8}$	$8.93\ 10^{-8}$	$1.14\ 10^{-9}$	$3.71\ 10^{-9}$	$2.84\ 10^{-9}$	$4.57\ 10^{-9}$
	3	$2.97\ 10^{-13}$	$1.03\ 10^{-12}$	$7.78\ 10^{-13}$	$1.28\ 10^{-12}$	$3.83\ 10^{-15}$	$1.25\ 10^{-14}$	$9.59\ 10^{-15}$	$1.54\ 10^{-14}$
	4	$4.27\ 10^{-18}$	$1.48\ 10^{-17}$	$1.12\ 10^{-17}$	$1.84\ 10^{-17}$	$1.29\ 10^{-20}$	$4.21\ 10^{-20}$	$3.23\ 10^{-20}$	$5.20\ 10^{-20}$
	5	$6.14\ 10^{-23}$	$2.13\ 10^{-22}$	$1.61\ 10^{-22}$	$2.65\ 10^{-22}$	$4.36\ 10^{-26}$	$1.42\ 10^{-25}$	$1.09\ 10^{-25}$	$1.75\ 10^{-25}$
	10	$3.77\ 10^{-47}$	$1.31\ 10^{-46}$	$9.87\ 10^{-47}$	$1.63\ 10^{-46}$	$1.90\ 10^{-53}$	$6.2\ 10^{-53}$	$4.75\ 10^{-53}$	$7.64\ 10^{-53}$
IPF	0.05	62.00	215.14	162.44	267.84	49.70	162.03	124.26	199.80
	0.1	22.04	76.49	57.75	95.22	17.82	58.09	44.54	71.63
	0.15	12.04	41.77	31.54	52.00	9.78	31.88	24.44	39.31
	0.2	7.84	27.19	20.53	33.85	6.39	20.82	15.97	25.68
	0.3	4.28	14.85	11.21	18.49	3.51	11.43	8.76	14.09
	0.4	2.79	9.67	7.30	12.04	2.29	7.46	5.72	9.21
	0.5	2.00	6.93	5.23	8.63	1.65	5.37	4.11	6.62
	1	0.71	2.46	1.86	3.07	0.59	1.92	1.48	2.37
	2	0.25	0.88	0.66	1.09	0.21	0.69	0.53	0.85
	3	0.14	0.48	0.36	0.60	0.12	0.38	0.29	0.47
	4	$8.97\ 10^{-2}$	0.31	0.24	0.39	$7.58\ 10^{-2}$	0.25	0.19	0.30
	5	$6.43\ 10^{-2}$	0.22	0.17	0.28	$5.45\ 10^{-2}$	0.18	0.14	0.22
	10	$2.29\ 10^{-2}$	0.08	0.06	0.10	$1.95\ 10^{-2}$	0.06	0.05	0.08



Estimates and 95 % confidence intervals of (a) probability of capture, (b) daily recruitment, and (c) daily population sizes for *E. aurinia* in a karst meadow system in SW Slovenia. Data were extracted from the averaging of the first six models with $\Delta QAIC_c < 2$. Estimates in (a) and (c) are shown for sampling occasions, and for (b) during intervals between occasions. Data on daily rainfall for the nearest rain station in Rakitovec is shown below (c).

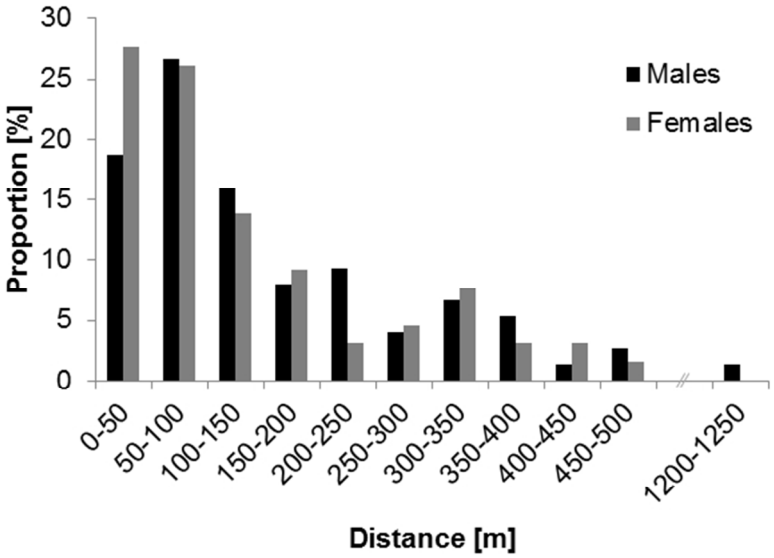


(a) Behaviour of *E. aurinia* in a system of karst meadows in SW Slovenia (males inner circle, females outer circle), and (b) chosen nectar plants (different shades of grey from the lightest to the darkest represent rarely (< 3 %), commonly (3–25 %) and frequently (> 25 %) selected nectar plants, respectively).



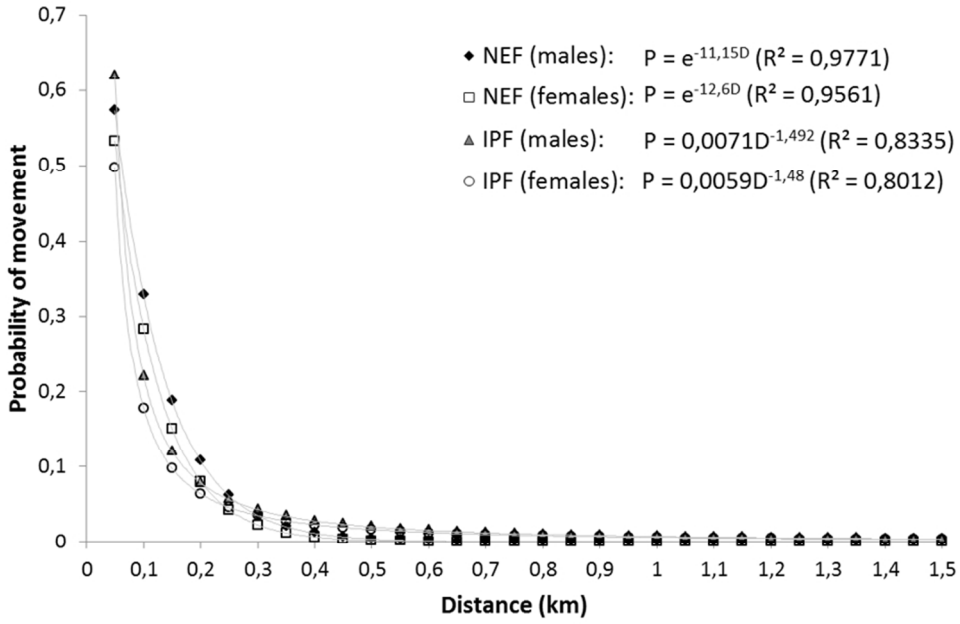
Geographic position and recorded movements of *E. aurinia* between the four study patches (A, B, C, D) in a system of dry karst meadows in SW Slovenia.

167x130mm (150 x 150 DPI)



Cumulative distances between first and last catch of recaptured males and females represented in 50-meter distance classes.

127x76mm (150 x 150 DPI)



Probability of moving a certain distance for males and females of *E. aurinia* derived from NEF and IPF based on distances between consecutive captures (in the formula, P represents the probability of moving a certain distance, D).

165x107mm (150 x 150 DPI)

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ODONATA OF FRIULI VENEZIA GIULIA: SECOND UPDATE OF CHECKLIST AND FURTHER REMARKS

ODONATI DEL FRIULI VENEZIA GIULIA: SECONDO AGGIORNAMENTO DELLA CHECKLIST E ULTERIORI OSSERVAZIONI

Abstract - Within the Project "Atlas of the Odonatofauna of the Friuli Venezia Giulia region", additional remarks of the Odonata of the region (North-eastern Italy) was carried out in the years 2010-2014. The new data have allowed us to enrich the regional Checklist of Odonata with five species: *Chalcolestes parvidens* (Artobolevsky, 1929) and *Lindenia tetraphylla* (Vander Linden, 1825) that have not been observed previously in the region and *Anax ephippiger* (Burmeister, 1839), *Gomphus vulgatissimus* (Linnaeus, 1758), and *Sympetrum flaveolum* (Linnaeus, 1758) that have been detected in previous years. In addition, knowledge of the distribution of twelve species that are rare or of natural interest has been improved. At the present time the Checklist of Odonata of Friuli Venezia Giulia includes 62 species representing 66% of the Italian fauna. Two species, *L. tetraphylla* and *Cordulegaster heros* Theischinger, 1979, are listed in the Annexes of the Habitats Directive. The list includes some species that have migratory tendencies and probably do not breed regularly in the region, such as *L. tetraphylla* and *A. ephippiger*. It is possible that in the near future other species might be found, some of which have already been reported for the region. Despite the considerable richness of species, we highlight a critical status for some species that are typical of mountain or alpine habitats, such as *Coenagrion hastulatum* (Charpentier, 1825), *C. heros*, *Somatochlora alpestris* (Selis 1840), *S. arctica* (Zetterstedt, 1840), *Sympetrum danae* (Sulzer, 1776) and *Leucorrhinia dubia* (Vander Linden, 1825). In addition, *Nehalennia speciosa* (Charpentier, 1840) is near extinction at the regional and national level due to the presence of just one breeding site located in a peat bog in the morainic hilly area.
Key words: Odonata, Damselflies, Dragonflies, Faunistic survey, Diversity, Conservation, North-eastern Italy.

Riassunto breve - *Nell'ambito del Progetto "Atlante degli Odonati del Friuli Venezia Giulia", negli anni 2010-2014 sono stati condotti ulteriori rilevamenti sulla fauna regionale a Odonati. Le osservazioni hanno permesso di aggiungere alla Checklist regionale degli Odonati cinque nuove specie:* Chalcolestes parvidens *(Artobolevsky, 1929) e* Lindenia tetraphylla *(Vander Linden, 1825) in precedenza non rilevate in regione, nonché* Anax ephippiger *(Burmeister, 1839),* Gomphus vulgatissimus *(Linnaeus, 1758) e* Sympetrum flaveolum *(Linnaeus, 1758) già osservate in anni precedenti. Inoltre, è migliorato il quadro delle conoscenze sulla distribuzione di dodici specie rare o di interesse naturalistico. Al momento attuale la Checklist degli Odonati del Friuli Venezia Giulia comprende 62 specie che rappresentano il 66% dell'odonatofauna italiana. Due specie,* L. tetraphylla *e* Cordulegaster heros *Theischinger 1979, sono incluse negli allegati della Direttiva Habitat. Nell'elenco sono comprese alcune specie migranti che, probabilmente, non si riproducono regolarmente sul territorio regionale, quali* L. tetraphylla *e* A. ephippiger. *È possibile che nel prossimo futuro possano essere rinvenute altre specie, alcune delle quali già segnalate in passato per la regione. Nonostante la rilevante ricchezza di specie, si segnalano situazioni critiche relative ad alcune di esse, in particolare di ambiente montano o alpino, come* Coenagrion hastulatum *(Charpentier, 1825),* C. heros, Somatochlora alpestris *(Selis, 1840),* S. arctica *(Zetterstedt, 1840),* Sympetrum danae *(Sulzer, 1776) e* Leucorrhinia dubia *(Vander Linden, 1825). Inoltre,* Nehalennia speciosa *(Charpentier, 1840) appare prossima all'estinzione sul territorio regionale e nazionale, in quanto già da alcuni anni risulta presente in un unico sito riproduttivo localizzato in una torbiera nell'area collinare morenica.*
Parole chiave: *Odonata, Damigelle, Dragoni, Indagine faunistica, Biodiversità, Conservazione, Italia nord-orientale.*

Introduction

At the end of 2011 an initial Checklist of the Odonata of the Friuli Venezia Giulia region (North-eastern Italy) was compiled on the basis of observations conducted in the years 2009-2011 by a team of observers operating in the Project "Atlas of the Odonata" in this region (Fiorenza et al. 2012). Fifty species were recorded, which was slightly lower than the number reported many years earlier, first by Kiauta (1969) and then by Pecile (1984). This fact can be attributed to a less rigorous observation or capture, but also to the difficulty of performing observations in the mountain sector in particular.

Additional samplings conducted in 2012 have allowed the observation of specimens belonging to new taxa for a total of 57 species (Fiorenza et al. 2013); moreover the very critical status of *Nehalennia speciosa* (Charpentier, 1840) was also noted (Chiandetti et al. 2014).

In the years 2013 and 2014 the survey was continued. In particular, new sites with the presence of the rare species *Cordulegaster heros* Theischinger, 1979 listed in the Habitats Directive (Council Directive 92/43/EEC) were identified in some areas of the Julian Pre-Alps (Chiandetti et al. 2013, 2015).

In this note we present the most important data collected during the last five years. In particular, the updated Checklist of Odonata species of the Friuli Venezia Giulia region is reported and the distribution of some rare species or those of natural interest is discussed.

Materials and methods

In the years 2010-2014 the observations concerning Odonata adults were carried out in different areas of the Friuli Venezia Giulia region, in particular in the mountain sector, as in the previous surveys (Fiorenza et al. 2012, 2013). Digital cameras were used extensively with the instructions to collaborators to photograph specific details useful for the identification of the different species. Capture tools for adults were used only in areas where no restrictive regulations were enacted. In the case of particularly interesting findings that, for example, involved species that had not been detected previously at sites considered new or unusual, further field research was carried out in order to confirm the results of the initial observations. Part of data collected during the years 2010-2012 were already reported in Fiorenza et al. (2012, 2013).

For the identification of adults we referred to various manuals, in particular to the volumes of Dijkstra & Lewington (2006), Grand & Boudot (2006), Bellmann (2013) and Galliani et al. (2014), in addition to constant contact with experts in Odonata identification. Moreover, as comparison material, specimens already identified and present in the collections of the Dipartimento di Scienze Agrarie e Ambientali, University of Udine, or the personal collections of a number of the authors were used.

The complete database of Odonata fauna recorded in the region is regularly updated by the authors of this work and some members of the Italian society for the study and conservation of dragonflies "Odonata.it".

The following abbreviations have been used in the text: CU = Costanza Uboni; GN = Gessica Nadalon; IC = Ivan Chiandetti; TF = Tiziano Fiorenza. Administrative provinces acronyms: GO = Gorizia; PN = Pordenone; TS = Trieste; UD = Udine. Other abbreviations: ad. = adult/adults, ex./exx. = specimen/specimens; exu. = exuvia/exuviae; det. = determinavit; leg. = legit; obs. = observavit/observaverunt.

Results and discussions

The updated Checklist of the Odonata of Friuli Venezia Giulia is compared with two previous lists compiled by other Authors in tab. I.

New species added to the Checklist, not previously observed in the region

Below we deal with two species new for the Checklist, which were not previously observed in the region.

Chalcolestes parvidens (Artobolevsky, 1929)

Records: some ♂♂ and ♀♀, 25.IX.2010, Isola della Cona (Staranzano, GO) (1 m a.s.l.), wet coastal area in the Nature Reserve of the Isonzo River Mouth, CU obs. (Uboni 2011); some ♂♂ and ♀♀, 29.IX.2011, Lisert (Monfalcone, GO) (1 m a.s.l.), wet coastal area, CU obs. (Uboni et al., in prep.); some ♂♂ and ♀♀, 29.VIII.2013, Isola della Cona (Staranzano, GO), same site as before, I. Maiorano obs.; 1 ♂, 04.X.2014, Precenicco (UD) (2 m a.s.l.), along the Stella River, IC obs.

Notes: Often the species is placed in the genus *Lestes* Fabricius. The identification in the field of *C. parvidens* is rather difficult because of the similarity with the allied species *C. viridis* (Vander Linden, 1825). Recent findings indicate the constant occurrence of the species in coastal and lowland areas of the region. *C. parvidens* is also present in several sites on the plains of the Veneto region (Riservato et al. 2014; Dalla Via & Zanetti 2015).

Lindenia tetraphylla (Vander Linden, 1825)

Records: 1 ♂, 2.VI.2010 Lisert (Monfalcone, GO) (1 m a.s.l.), wet coastal area, CU obs. (Uboni et al., in prep.).

Notes: The species was also observed along the West coast of Istria (Croatia) some decades ago (Kiauta 1963). The adult found in Friuli Venezia Giulia may be ascribed to a vagrant individual that arrived during a migration from the Balkan area. The species is not reported from the plains of the Veneto region (Dalla Via & Zanetti 2015). In Italy *L. tetraphylla* is localized in a few sites in some Central and Southern regions, and in Sardinia (Riservato et al. 2014). The species has migratory tendencies from its reproductive sites (Dijkstra & Lewington 2006; Boudot et al. 2009). *L. tetraphylla* is listed in Annex II and Annex IV of the Habitats Directive.

Species	Kiauta 1969	Pecile 1984	Remarks 2009-2014
ZYGOPTERA			
Calopteryx splendens (Harris, 1782)	X	X	X
Calopterix virgo (Linnaeus, 1758)	X	X	X
Sympecma fusca (Vander Linden, 1820)	X	X	X
Lestes barbarus (Fabricius, 1798)	X	X	X
Lestes dryas Kirby, 1890	X(1)		
Lestes sponsa (Hansemann, 1823)	X	X	X
Chalcolestes viridis (Vander Linden, 1825)	X	X	X
Chalcolestes parvidens (Artobolevsky, 1929)			X(3)
Platycnemis pennipes (Pallas, 1771)	X	X	X
Pyrrhosoma nymphula (Sulzer, 1776)	X	X	X
Ischnura elegans (Vander Linden, 1820)	X	X	X
Ischnura pumilio (Charpentier, 1825)	X	X	X
Enallagma cyathigerum (Charpentier, 1840)	X		X
Coenagrion hastulatum (Charpentier, 1825)			X(4)
Coenagrion ornatum (Selys, 1850)	X	X	
Coenagrion puella (Linnaeus, 1758)	X	X	X
Coenagrion pulchellum (Vander Linden, 1825)	X	X	X
Coenagrion scitulum (Rambur, 1842)	X	X	X
Erythromma najas (Hansemann, 1823)	X	X	X(4)
Erythromma viridulum (Charpentier, 1840)	X	X	X
Erythromma lindenii (Selys, 1840)	X	X	X
Ceriagrion tenellum (De Villers, 1789)		X	X
Nehalennia speciosa (Charpentier, 1840)		X	X(4)
ANISOPTERA			
Brachytron pratense (Müller, 1764)	X	X	X
Aeshna affinis Vander Linden, 1820	X	X	X
Aeshna cyanea (Müller, 1764)	X	X	X
Aeshna grandis (Linnaeus, 1758)		X	X(4)
Aeshna isoceles (Müller, 1767)	X	X	X
Aeshna juncea (Linnaeus, 1758)	X	X	X
Aeshna mixta Latreille, 1805	X	X	X
Anax ephippiger (Burmeister, 1839)			X(3)
Anax imperator Leach, 1815	X	X	X
Anax parthenope (Selys, 1839)	X	X	X
Gomphus vulgatissimum (Linnaeus, 1758)	X(2)	X(2)	X(3)
Onychogomphus forcipatus (Linnaeus, 1758)	X	X	X
Lindenia tetraphylla (Vander Linden, 1825)			X(3)
Cordulegaster bidentata Selys, 1843	X	X	X
Cordulegaster boltonii (Donovan, 1807)	X	X	X
Cordulegaster heros Theischinger, 1979			X(4)
Cordulia aenea (Linnaeus, 1758)	X	X	X
Somatochlora alpestris (Selys, 1840)		X	X(4)
Somatochlora arctica (Zetterstedt, 1840)		X	X(4)
Somatochlora flavomaculata (Vander Linden, 1825)	X	X	X
Somatochlora meridionalis Nielsen, 1935			X(4)
Somatochlora metallica (Vander Linden, 1825)	X	X	X
Libellula depressa Linnaeus, 1758	X	X	X
Libellula fulva Müller, 1764	X	X	X
Libellula quadrimaculata Linnaeus, 1758	X	X	X
Orthetrum albistylum (Selys, 1848)	X	X	X
Orthetrum brunneum (Fonscolombe, 1837)	X	X	X
Orthetrum cancellatum (Linnaeus, 1758)	X	X	X
Orthetrum coerulescens (Fabricius, 1798)	X	X	X
Crocothemis erythraea (Brullé, 1832)	X	X	X
Sympetrum danae (Sulzer, 1776)		X	X(4)
Sympetrum depressiusculum (Selys,1841)	X	X	X(4)
Sympetrum flaveolum (Linnaeus, 1758)	X	X	X(3)
Sympetrum fonscolombii (Selys, 1840)	X	X	X
Sympetrum meridionale (Selys, 1841)	X	X	X
Sympetrum pedemontanum (Allioni, 1766)	X	X	X
Sympetrum sanguineum (Müller, 1764)	X	X	X
Sympetrum striolatum (Charpentier, 1840)	X	X	X
Sympetrum vulgatum (Linnaeus, 1758)	X	X	X
Leucorrhinia dubia (Vander Linden, 1825)		X	X(4)
Leucorrhinia pectoralis (Charpentier, 1825)	X	X	
Selysiothemis nigra (Vander Linden, 1825)			X(4)
Total species	**52**	**55**	**62**

1) species listed by Kiauta (1969) on the basis of the old information of Lazzarini (1896), recorded by Tacconi (1906) and then by Bentivoglio (1908).
2) species listed by Kiauta (1969) and Pecile (1984) on the basis of the old information of Senna (1890), recorded by Lazzarini (1896) and then by Bentivoglio (1908), but questioned by Tacconi (1906).
3) new species found in 2013-2014 and included in the present Checklist.
4) new records of rare species or those of natural interest reported in this paper.

Tab. I - List of Odonata species of the Friuli Venezia Giulia region following Kiauta (1969), Pecile (1984), and more recent observations (2009-2014) of adults carried out within the Project "Atlas of Odonata of the Friuli Venezia Giulia region".
- *Elenco delle specie di Odonati rilevate sul territorio del Friuli Venezia Giulia sulla base di Kiauta (1969), Pecile (1984) e delle più recenti osservazioni (2009-2014) sugli adulti condotte nell'ambito del Progetto "Atlante degli Odonati del Friuli Venezia Giulia".*

New species added to Checklist, already observed in the region

Below we treat three species new for the Checklist and that had been detected in the region in previous years.

Anax ephippiger (Burmeister, 1839)

Records: 1 ex., 01.VIII.2010, Lisert (Monfalcone, GO) (1 m a.s.l.), wet coastal area, CU obs. (Uboni et al. in prep.).

Notes: Often the species is placed in the genus *Hemianax* Burmeister. Previously, the occurrence of *A. ephippiger* was also reported as occasional in the region: specimens were found near Castel Novo (Sagrado, GO) (in August 1988) and along the road between Ronchi dei Legionari and Doberdò del Lago (GO) (in September 1988) (Bognolo & Pecile 1995). Two specimens of *A. ephippiger*, collected by Pecile in 1988, are conserved in the collections of the Friulian Museum of Natural History in Udine (P. Glerean, pers. comm.). In June-July 2008 the species was observed in the Nature Reserve of the Isonzo River Mouth (Mekkes 2008). The species was also observed in the Veneto plain, in particular close to the border with the Friuli Venezia Giulia region (Riservato et al. 2014), but in recent years the species was not found in that area (Dalla Via & Zanetti 2015). In Italy *A. ephippiger* has been found in many regions, but in none of these it is present with stable populations (Riservato et al. 2014). The species is strongly migratory from the arid regions of Africa and Asia, and occasionally spreads to Central and Northern Europe (Dijkstra & Lewington 2006; Boudot 2009).

Gomphus vulgatissimus (Linnaeus, 1758) (fig. 1)

Records: 1 ♂, 21.V.2013, Rupa (Savogna d'Isonzo, GO) (49 m a.s.l.), near the Vipacco River, CU obs.; 2 ♂♂ and 1 ♀, 10.V.2014, Peci (Savogna d'Isonzo, GO) (40 m a.s.l.), along the Vipacco River, IC obs.; 4 ♂♂ and 2 ♀♀, 11.V.2014, Muzzana del Turgnano (UD) (3 m a.s.l.), along the Cormôr River, IC obs.; some exx., 24.V.2014, Precenicco (UD) (2 m a.s.l.), along the Stella River, TF obs.

Notes: A rather curious observation of one dead specimen of this species was made in 2003 on the body-work of a car that travelled a route in the Gorizia area (T. Zorzenon, pers. comm.). This species was reported for Lake Cavazzo and Lake Ragogna by Senna (1890), but subsequently individuals of this species have not been found in these areas, even during a detailed survey of Lake Ragogna (Pecile 1989). In May 1986 a female was collected along the Ospo stream near Trieste (Bognolo & Pecile 1995); the specimen is conserved in the collections of the Friulian Museum of Natural History in Udine (P. Glerean, pers. comm.).

Fig. 1 - Couple of *Gomphus vulgatissimus;* 10.V.2014, Muzzana del Turgnano (UD) (photo by I. Chiandetti).
- *Coppia di* Gomphus vulgatissimus*; 10.V.2014, Muzzana del Turgnano (UD) (foto I. Chiandetti).*

Fig. 2 - Male of *Sympetrum flaveolum*; 09.VIII.2014, Fusine (Tarvisio, UD) (photo by I. Chiandetti).
- *Maschio di* Sympetrum flaveolum*; 09.VIII.2014, Fusine (Tarvisio, UD) (foto I. Chiandetti).*

Fig. 3 - Couple of *Nehalennia speciosa*; 20.VI.2013, Torbiera di Lazzacco (Pagnacco, UD) (photo by I. Chiandetti).
- *Coppia di* Nehalennia speciosa; *20.VI.2013, Torbiera di Lazzacco (Pagnacco, UD) (foto I. Chiandetti).*

Sympetrum flaveolum (Linnaeus, 1758) (fig. 2)

Records: 2 ♂♂, 9.VIII.2014, Fusine (Tarvisio, UD) (847 m a.s.l.), wet meadow, IC obs.

Notes: The discovery of two individuals of *S. flaveolum* near Tarvisio in the Italian area at the Fusine pass is very interesting. The presence of adults in this site, as well as in wet grasslands in the nearby Ratece area (Slovenia), is worth further consideration in the future due to the migrations that characterize this species (Dijkstra & Lewington 2006). These new data confirm those of Conci (1956) (Tarvisio, 10.VII.1956, leg. C. Nielsen), Kiauta (1969) (Valbruna, VIII.1933, leg. B. Finzi) and Minelli (1977) for the area around Tarvisio. Three specimens of *S. flaveolum* collected by Minelli in July 1973 at Fusine are conserved in the collections of the Museum of Natural History in Verona (L. Latella, pers. comm.). Kiauta (1969) also reports specimens collected at Gemona (12.VII.1968, leg. B. Kiauta). This is notable because this species is actually extremely rare and localized in the Southern side of the Italian Alps. Recently it was reported in a limited number of alpine sites in the Piedmont and Valle d'Aosta regions (Riservato et al. 2014). There are also some reports from Slovenia (Kotarac 1997) and Carinthia (Austria) (Holzinger & Komposch 2012).

Rare species or those of natural interest confirmed in the Checklist

In the reference period, observations concerning specimens of species that are rather rare or of natural interest occurred in different sites of the region. These data confirm the inclusion of these species in the Checklist.

Coenagrion hastulatum (Charpentier, 1825)

Records: some exx., 08.VII.2012, Laghetto di Somdogna (Dogna, UD) (1442 m a.s.l.), mountain pond, TF obs.; some exx., 08.VII.2012, Passo Pramollo lake (Pontebba, UD) (1522 m a.s.l.), mountain lake, TF obs. (Fiorenza et al. 2013); 2 ♂♂, 30.VI.2013, Laghetto di Somdogna (Dogna, UD), same site as before, IC obs.; 2 ♂♂, 11.VIII. 2013, Passo Pramollo (Pontebba, UD) (1522 m a.s.l.), near a mountain lake, IC obs.; 1 ♂ and 1 ♀ (in tandem), 16.VIII.2013, Malga Lussari (Malborghetto-Valbruna, UD) (1573 m a.s.l.), near a mountain pond, TF obs.; 2 ♂♂, 29.VII.2014, Passo Pramollo lake (Pontebba, UD), same site as before, TF obs.

Notes: Previously, five males of the species were observed in July 1984 near Fusine (Tarvisio, UD) by Pecile (1991). In Italy, the species is fairly common in alpine areas of Trentino Alto Adige and Veneto (Riservato et al. 2014).

Erythromma najas (Hansemann, 1823)

Records: 1 ♂, 27.VII.2013, Rutte Piccolo (Tarvisio, UD) (800 m a.s.l.), artificial pond, IC obs.

Notes: The species was reported by Kiauta (1969) for the pond of Percedol (Monrupino, TS) in a karst area; this data was mentioned by Pecile (1984) but then questioned by Bognolo & Pecile (1995) because it was not possible to locate the collected specimens previously reported and also for the widespread presence of the allied species *E. viridulum* (Charpentier, 1840). For the area around the mouths of the Timavo River (Trieste) *E. najas* was also reported by Stammer (1932). In Italy, recent reports of the species are not numerous (Riservato et al. 2014).

Nehalennia speciosa (Charpentier, 1840) (fig. 3)

Records: 5 exx., 08.V.2013, Torbiera di Lazzacco (Pagnacco, UD) (189 m a.s.l.), peat bog in the morainic hilly area, TF obs.; 10 ♂♂ (of these, two exx. during emergence), 12.V.2013, same site as before, IC obs.; 10 ♂♂ (of these, one ex. during emergence), 25.V.2013, same site as before, IC obs.; 10 ♂♂ and 5 ♀♀, 20.VI.2013, same site as before, IC obs.; 1 ex., 12.V.2014, same site as before, TF obs.; 4 ♂♂ and 1 ♀, 07.VI.2014, same site as before, IC, CU and TF obs.; 2 ♀♀, 12.VII.2014, same site as before, IC obs.

Notes: The first discovery of *N. speciosa* in Friuli Venezia Giulia occurred in 1980; it refers to the peat bog of Brazzacco (Moruzzo, UD) (Pecile 1981), in the morainic hilly area. Later, a population of the species was found in the swamp of Cima Corso (Ampezzo, UD), in the Carnic Alps (Pecile 1991). In 2009, viable populations of the species were observed only at the Cima Corso swamp and the peat bog of Lazzacco (Pagnacco, UD) (Fiorenza & Pecile 2009). In the 2010-2014 period the only occurrence of the species in the region was from the peat bog of Lazzacco (Chiandetti et al. 2014). Therefore, at the present time the only viable population of *N. speciosa* present in Italy is that of the peat bog of Lazzacco; the populations recorded in the Lombardy region in the 1970s are considered extinct.

Aeshna grandis (Linnaeus, 1758)

Records: 1 ♂, 20.VIII.2011, Rifugio Nordio (Malborghetto Valbruna, UD) (1300 m a.s.l.), mountain area; IC obs.; 1 ♂, 24.VIII.2012, Val Bartolo (Tarvisio, UD) (1000 m a.s.l.), mountain area, IC obs.

Notes: The first report of *A. grandis* for Friuli Venezia Giulia was made by Kiauta (1971) who observed one male of the species on Mount Lussari (1790 m a.s.l.) (Tarvisio, UD); then the species was observed twice in 1984 by Pecile (1991) near Fusine (880 m a.s.l.) (Tarvisio, UD). The different observations of adults (all in the mountain Tarviso area) refer to vagrant individuals that probably do not breed in the Friuli Venezia Giulia region. The records of *A. grandis* relating to the coastal area of the region (Riservato et al. 2014) are likely misidentifications.

Cordulegaster heros Theischinger, 1979 (fig. 4)

Records: 4 ♂♂, 29.VI.2013, Savorgnano del Torre (Povoletto, UD) (200 m a.s.l.), along a small stream tributary of the Storto stream, IC obs.; 1 ♂, 03.VII.2013, Magnano in Riviera (UD) (210 m a.s.l.), along the Bosso stream, IC obs.; 2 ♂♂, 03.VII.2013, Tarcento (UD) (255 m a.s.l.), along the Rabagnolo stream, IC obs.; 2 ♂♂ and 1 ♀, Togliano (Torreano di Cividale, UD) (255 m a.s.l.), on the bottom of a small and narrow valley called Busa di Culigna, IC obs.; 3 ♂♂, 06.VII.2013, Attimis (UD) (260 m a.s.l.), along the Musil stream, IC obs.; 1 ♂, 15.VII.2013, Bosco di Plessiva (Cormons, UD) (90 m a.s.l.), near the Fidri stream, IC obs.; 3 ♂♂ and 1 ♀ (one ex. during emergence), 11.VII.2014, Savorgnano del Torre (UD), same site as before, IC obs.

Notes: The species was observed for the first time in the Friuli Venezia Giulia region (and the first in Italy) in three streams in the Isonzo River basin in the province of Gorizia (Bedjanič & Šalamun 2003). Later a population was found in a stream in the city of Trieste (Uboni et al. 2007). Recently, specimens of the species were observed in some streams in the basins of the Torre River, a tributary of the Isonzo River, and of the Tagliamento River (Chiandetti et al. 2013, 2015). *C. heros* is listed on Annex II and Annex IV of the Habitats Directive.

Somatochlora alpestris (Selys, 1840) (fig. 5)

Records: some exx., 01.VII.2012, Laghetto di Plotta (Paluzza, UD) (1950 m a.s.l.), mountain pond, IC obs.; 1 ♂ and 2 ♀♀, 07.VII.2012, torbiera of Piani di Lanza near the Attila's cave (Paularo, UD) (1750 m a.s.l.), peat bog, IC obs.; 1 ♂, 29.VII.2012, Laghetto di Malins (Prato Carnico, UD) (1699 m a.s.l.), mountain pond, IC obs.; 1 ♂, 05.VIII.2012, torbiera near Passo Zauf (Sauris, UD) (1878 m a.s.l.), peat bog, IC obs.; 1 ♂, 06.VII.2013, in the area of Passo Pura (Ampezzo, UD) (1400 m a.s.l.), mountain pond, S. Sava obs. (S. Hardersen det.; H. Wildermuth det.); 3 ♂♂, 11.VIII.2013, wet areas between the Monte Carnizza and the Monte Corona (Pontebba, UD) (1700 m a.s.l.), mountain/alpine ponds, IC obs.

Notes: The occurrence of this species in the region was reported by Pecile (1983) for some alpine sites in the Carnic Alps (Passo Pramollo, Cason di Lanza, Torbiera under the Zuc della Guardia).

Somatochlora arctica (Zetterstedt, 1840) (fig. 6)

Records: 2 ♂♂ and 1 F, 17.VI.2012, Palude di Cima Corso (Ampezzo, UD) (836 m a.s.l.), mountain pond, IC obs.; 1 ♂, 15.VIII.2012, Torbiera Scichizza near Fusine (Tarvisio, UD) (850 m a.s.l.), peat bog, IC obs.; 1 ♀, 09.IX.2012, same site as before, IC obs.; 1 ♂ and 1 ♀ (both exx. during emergence), 22.VI.2013, Palude di Cima Corso (Ampezzo, UD) (836 m a.s.l.), swamp area, IC obs.; 2 ♂♂ (both exx. during emergence), 09.VI.2013, Torbiera Scichizza near Fusine (Tarvisio, UD) (850 m a.s.l.), peat bog, IC obs.; 1 ♂ and 1 ♀, 23.VI.2013, same site as before, IC obs.; 1 ♂, 03.VIII.2014, same site as before, IC obs.; 1 ♂, 09.VIII.2014, same site as before, IC obs.

Fig. 4 - Male of *Cordulegaster heros*; 19.VII.2014, Savorgnano del Torre (UD) (photo by I. Chiandetti).
- *Maschio di* Cordulegaster heros*; 19.VII.2014, Savorgnano del Torre (UD) (foto I. Chiandetti).*

Fig. 5 - Male of *Somatochlora alpestris*; 05.VIII.2012, Peat bog near Passo Zauf (Sauris, UD) (photo by I. Chiandetti).
- *Maschio di* Somatochlora alpestris*; 05.VIII.2012, torbiera presso Passo Zauf (Sauris, UD) (foto I. Chiandetti).*

Notes: Adults of this species were observed in 1977-1981 by Pecile (1983) in the mountain area of Fusine (Tarvisio, UD) and in 1988 by Pecile (1991) at the Palude di Cima Corso (Ampezzo, UD). One female and one exuvia of *S. arctica* were found at the Palude di Cima Corso on 23.VI.2008 (S. Hardersen, pers. comm.). The sites Palude di Cima Corso (Carnic Alps) and Torbiera Scichizza (Julian Alps) are therefore important breeding sites for *S. arctica* in the mountain sector of Friuli Venezia Giulia.

Somatochlora meridionalis Nielsen, 1935

Records: 5 ♂♂ and 7 ♀♀, 30.VI.2015, Rio Ospo (Trieste), CU obs.; 1 ♀, 08.VII.2011, Colloredo di Monte Albano (UD) (200 m a.s.l.), along a stream in the morainic hilly area, IC obs.; 1♂, 16.VI.2012, Torbiera del Chialcinat (Moruzzo, UD), (189 m a.s.l.), along a stream in the morainic hilly area, IC obs.; some exx., 16.VIII.2012, Savorgnano del Torre (Povoletto, UD) (185 m a.s.l.), along a small stream tributary of the Storto stream in a prealpine area, IC obs.; some exx., 21.VIII.2012, Savorgnano del Torre (Povoletto, UD) (200 m a.s.l.), along a stream in a prealpine area, IC obs.; 1 ♂, Flagogna (Forgaria

Fig. 6 - Male of *Somatochlora arctica*; 09.VI.2013, Torbiera Scichizza (Tarvisio, UD) (photo by I. Chiandetti).
- *Maschio di* Somatochlora arctica*; 09.VI.2013, Torbiera Scichizza (Tarvisio, UD) (foto I. Chiandetti).*

Fig. 7 - Male of *Sympetrum danae*; 27.VII.2013, Torbiera Scichizza (Tarvisio, UD) (photo by I. Chiandetti).
- *Maschio di* Sympetrum danae*; 27.VII.2013, Torbiera Scichizza (Tarvisio, UD) (foto I. Chiandetti).*

nel Friuli, UD) (145 m a.s.l.), along a stream in a prealpine area, IC obs.; 2 ♂♂, Pian delle Farcadizze (Faedis, UD) (650 m a.s.l.), along a stream in a prealpine area, IC obs.; 1 ♂, 08.VI.2014, Colloredo di Monte Albano (UD) (193 m a.s.l.), same site as before, IC obs.; 1 ♂, 08.VI.2014, Cormons (GO) (73 m a.s.l.), near the Fidri stream in a prealpine area, IC obs.; 1 ♂, 02.VIII.2014, Montenars (UD) (358 m a.s.l.), along a small stream tributary of the Orvenco creek in a prealpine area, IC obs.; 1 ♂, 09.VIII.2014, Bonifica dei Quattroventi (Moruzzo, UD) (185 m a.s.l.), along a stream in the morainic hilly area, TF obs.

Notes: Adults of this species were previously observed in several sites by Bognolo & Pecile (1995) and Uboni (2008) in the karst area. *S. meridionalis* seems absent from the Veneto region (Riservato et al. 2014).

Sympetrum danae (Sulzer, 1776) (fig. 7)

Records: 4 ♂♂ (of these, two exx. during emergence), 27.VII.2013, Torbiera Scichizza near Fusine (Tarvisio, UD) (850 m a.s.l.), peat bog, IC obs.; 2 ♂♂, 03.VIII.2013, same site as before, IC obs.

Notes: This alpine species was reported by Minelli (1977) for the area of Fusine. One female of *S. danae*, collected by Minelli in July 1973 at Fusine, is conserved in the collections of the Museum of Natural History in Verona (L. Latella, pers. comm.).

Sympetrum depressiusculum (Selys, 1841) (fig. 8)

Records: 1 ♂ (immature), 17.VII.2011, Magredi di Basaldella near the Meduna River (with a typical dry gravel bed) (Vivaro, PN) (120 m a.s.l.), temporary pool, P.L. Taiariol obs.; some exx., 19.VIII.2011, Titiano (Precenicco, UD) (5 m a.s.l.), plain ponds, IC obs.; some exx., 01.X.2011, Flambro (Talmassons, UD) (30 m a.s.l.), wet area, IC obs.; some exx., 20.VII.2012, Stagno di Vivaro (Vivaro, PN) (138 m a.s.l.), plain pond, IC obs.; 1 ♂, 22.VIII.2012, Palude Vuarbis (Cavazzo Carnico, UD) (274 m a.s.l.), swamp area, IC obs.; 3 ♂♂ and 1 ♀, 23.VII.2014, Laghetti di Villanova (San Daniele del Friuli, UD) (111 m a.s.l.), ponds near the Tagliamento River, IC obs.; 2 ♂♂ and 1 ♀, 26.VII.2014, same site as before, IC obs.; 1 ♂, 31.VIII.2014, same site as before, IC obs.

Notes: This species was reported by Kiauta (1969) from an adult collected in 1937 (leg. G. Marcuzzi) in Staranzano (GO); the specimen is preserved in the collections of the Museum of Natural History in Trieste. The species was also found in 1982 at Lake Ragogna in a morainic hilly area (Pecile 1989).

Leucorrhinia dubia (Vander Linden, 1825)

Records: some ♂♂ and ♀♀ (also in tandem) and exu., 08.VII.2012, Laghetto di Somdogna (Dogna, UD) (1442 m a.s.l.), moutain pond, TF obs.; 3 ♂♂ and 1 ♀, 23.VI.2013, Laghetto di Somdogna (Dogna, UD), same site as before, IC obs.; 1 ♂ (probably vagrant), 27.VII.2013, Rutte Piccolo (Tarvisio, UD) (800 m a.s.l.), artificial pond, IC obs.; 1 ♂ (probably vagrant), 27.VII.2013, Fusine (Tarvisio, UD) (850 m a.s.l.), artificial pond, IC obs.; 1 ♂, 13.VIII.2013, Laghi di Festons (Sauris, UD) (1833 m a.s.l.), mountain ponds, IC obs.

Notes: This alpine species was found previously in a single site in the region: the surroundings of Fusine (Minelli 1977). Two specimens of *L. dubia*, collected by Minelli in July 1973 at Fusine, are conserved in the collections of the Museum of Natural History in Verona (L. Latella, pers. comm.); in the same Museum

Fig. 8 - Male of *Sympetrum depressiusculum*; 23.VII.2014, Laghetti di Villanova (S. Daniele del Friuli, UD) (photo by I. Chiandetti).
- *Maschio di* Sympetrum depressiusculum*; 23.VII.2014, Laghetti di Villanova (S. Daniele del Friuli, UD) (foto I. Chiandetti).*

Fig. 9 - Male of *Selysiothemis nigra*; 24.VI.2015, artificial pond in the Parco delle Dote (Azzano Decimo, PN) (photo by G. Nadalon).
- *Maschio di* Selysiothemis nigra*; 24.VI.2015, laghetto artificiale nel Parco delle Dote (Azzano Decimo, PN) (foto G. Nadalon).*

several males and females of *L. dubia*, collected by Minelli in July 1973 at Lake D'Antorno (1800 m a.s.l.) near Misurina (BL) (Veneto region), are conserved.

Selysiothemis nigra (Vander Linden, 1825) (fig. 9)

Records: numerous exx. and exu., 2013 and 2014, Azzano Decimo (PN), artificial pond in the "Parco delle Dote" (10 m a.s.l.) (adults observed also in tandem; GN obs.) (Uboni et al. 2015); 1 ex. 09.IX.2013, near Tauriano (Spilimbergo, PN) (171 m a.s.l.), artificial pond to provide water for wild fauna, TF obs.; some exx., 24.VI.2014, near Tauriano (Spilimbergo, PN), same site as before, TF obs.; 1 ex., 17.VIII.2014, Spilimbergo (PN) (132 m a.s.l.), a dead specimen near a window of Spilimbergo hospital, TF obs.

Notes: Notable is the occurrence of a reproductive population of *S. nigra* in the territory of the Pordenone district (Uboni et al. 2015), and the occurrence of adults north of the town of Tauriano (Spilimbergo, PN), collected inside an military area in an artificial pond created for watering wild ungulates. Adults of this species have been previously reported for the Veneto and Friuli Venezia Giulia regions (Zandigiacomo & Buian 2011). The northward extension of this species has been associated with climatic change (Groppali 2009; Ott 2009, 2010).

Species not found during this survey but already observed in the region

Four other species of Odonata have been reported in the past for the Friuli Venezia Giulia region (tab. II), but in the six years of the present Project (2009-2014) their occurrence in the region was not confirmed.

The species listed here have been frequently detected in neighbouring areas, such as Slovenia (Kotarac et al. 2004), Carinthia (Holzinger & Komposch 2012), Veneto (Bucciarelli 1978; Riservato et al. 2014) and Trentino Alto Adige (Festi 2012; Macagno et al. 2012). Therefore, it is possible that in the future specimens of these taxa will be found again in some sites of Friuli Venezia Giulia.

Final comments

Within the Project Atlas of the Odonata of Friuli Venezia Giulia, 62 species of Odonata were detected, considering *S. meridionalis* and *S. metallica* (Vander Linden, 1825) as separate species. The taxa mentioned in the present Checklist represent about 66% of the species currently known in Italy, which amount to 93 (Riservato et al. 2014). Two species, *L. tetraphylla* and *C. heros*, are listed in Annex II and Annex IV of the Habitats Directive. The list includes some species that have tendencies to migrate over long distances from their reproductive localities and probably do not breed regularly in the region, such as *L. tetraphylla* and *A. ephippiger*.

This rich faunal context could potentially increase, not only through the discovery of species already observed in the recent or more distant past, but also with other species detected in neighbouring areas and never reported in this region. Among the latter species we mention:

a) *Lestes macrostigma* (Eversmann, 1836), a Mediterranean-Pannonian species rare and localized in Europe that could live in coastal lagoon environments;

b) *Aeshna caerulea* (Ström, 1783), common in Boreal-Alpine areas of Central Europe and known from some localities of the Trentino and Alto Adige, and which could live in mountain peat bogs (Festi 2011; Riservato et al. 2014);

c) *Aeshna subarctica* Walker, 1908, with Circumboreal distribution, discovered in recent years in Trentino

Species	Habitat/Locality/Area	Year of observation	References
Lestes virens (Charpentier, 1825)	pond near Sagrado (Sgonico, TS) in a karst area	1985, 1986, 1989	Bognolo & Pecile 1995
	ponds near Lisert (Monfalcone, GO) in a coastal area	1991	Bognolo & Pecile 1995
Lestes dryas Kirby, 1890	along the Tagliamento river near S. Michele al T. (VE)	n.i.	Kiauta 1969
	pond near Sagrado (Sgonico, TS) in a karst area	1982	Bognolo & Pecile 1995
	pond near S. Lorenzo (Bagnoli della Rosandra, TS), in a karst area	1990, 1991	Bognolo & Pecile 1995
Coenagrion ornatum (Selys, 1850)	"*häufiger in den Lagunen von Monfalcone; Mai bis Juli*"	n.i.	Strobl 1906 (see also Kiauta 1969)
	Triest (J. Staudacher leg.)(1)	1944	Kiauta 1969; see also Bognolo & Pecile 1995
	near Fusine (Tarvisio, UD)	n.i.	Pecile 1984
Leucorrhinia pectoralis (Charpentier, 1825)	pond of Percedol (TS) (E. Stolfa leg.) in a karst area	1928	Kiauta 1969; Bognolo & Pecile 1995
	small lake at Ospedaletto (or Laghetto Minisini) (Gemona del Friuli, UD) (two exx. in tandem)	1982	Pecile 1983

1) One male, collected 27.VI.1944, is conserved in the collections of the Slovenian Museum of Natural History in Ljubljana.

Tab. II - Species not found in this survey, but already observed in the Friuli Venezia Giulia region (n.i. = year not indicated).
- *Specie non rilevate nella presente indagine, ma già osservate in Friuli Venezia Giulia (n.i. = anno non indicato).*

and Alto Adige (Festi 2011) and potentially present in some bogs of the Carnian mountain area.

It is possible that the valuable environments of water resurgence of the Friulian plain could include further species, such as *Gomphus flavipes* (Charpentier, 1825) and *Ophiogomphus cecilia* (Furcroy, 1785), known from plains habitats in several areas of the Po valley (Riservato et al. 2014).

However, some species are localized in very small areas, which are often at risk of substantial alterations for anthropic intervention or significant changes in the climate. In particular, some mountain species associated with wetlands, such as *C. hastulatum*, *S. alpestris*, *S. arctica*, *S. danae* and *L. dubia* seem endangered.

Some threats that may affect the presence and spread of *C. heros* have been described (Chiandetti et al. 2015). The species *N. speciosa*, close to extinction at the regional and national level, is currently known from a single reproductive site in the area of the morainic amphitheatre of the Tagliamento River, whose population can be estimated (in 2014) as a few dozen adults (Chiandetti et al. 2014).

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Evidence of breeding of *Selysiothemis nigra* in the regions of Friuli Venezia Giulia and Veneto, northeastern Italy (Odonata: Libellulidae)

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Abstract. Exuviae of *Selysiothemis nigra* collected at two artificial lakes in the regions of Friuli Venezia Giulia and Veneto on the northern Adriatic coast in Italy represent the northernmost evidence of breeding of the species worldwide. Basic information on the larval habitat is given and the recent apparent range expansion is outlined and discussed.

Key words. Exuviae, northernmost evidence of breeding, Adriatic coast, Italy

Introduction

Selysiothemis nigra (Vander Linden, 1825) is the type species of the monotypic genus *Selysiothemis* Ris, 1897 within Urothemistinae. It is confined to the western and central Palaearctic region ranging from the Taklamatan and Thar deserts of north-western China and India (Ris 1897; Fraser 1936: 451; Tyagi & Miller 1991) and adjacent Central Asia (Bartenev 1913: 179; Borisov & Haritonov 2007; Schröter 2010) and Pakistan in the east, across the steppe and desert belt of southern Russia (Yanybaeva et al. 2006; Popova & Haritonov 2008; Skvortsov & Kuvaev 2010), the Ukraine (Matushkina 2007; Tytar 2007; Khrokalo et al. 2009), the Caucasus region (Dumont 2004; Tailly & Tabarroni 2006), the Levant (Schneider 1986; Dumont 1991; Schneider et al. 2013), Iraq and the Arabian Peninsula (Morton 1919; Asahina 1973; Waterston 1984; Waterson & Pittaway 1991) to Spain, Portugal and Morocco in the west (Grand 1995; Lohr 2005; Boudot 2008; Juillerat & Monnerat 2009; Boudot & de Knijf 2012).

As an eremic species *S. nigra* is especially well adapted to desert and steppe conditions (Borisov 2005) and reproduces in a wide range of permanent and ephemeral habitats of fresh or brackish water (Wildermuth & Martens 2014: 653). Due to its highly nomadic and migrant behaviour generally only adults are observed and only a fraction of records includes evidence of reproduction (Compte Sart 1960; Borisov 2005; Lohr 2005; Dijkstra & Boudot 2010; Brochard & van der Ploeg 2013; de Knijf et al. 2013). In Iraq and south-western Tajikistan the species was found to be bivoltine (Morton 1919; Borisov 2005). However, from Europe generally comparatively little information on the species' ecology with re-

gards to larval habitats and life cycle is available (cf. Wildermuth & Martens 2014: 655).

Selysiothemis nigra is scarce throughout its range and seldom reported in large numbers (Morton 1919: 195; Compte Sart 1960; Fraser 1936: 451; Schneider 1981; Brochard & van der Ploeg 2013; De Knijf et al. 2013) and from large parts of its range only few scattered strong populations are known (cf. Boudot et al. 2009). In recent years however *S. nigra* has been found in several countries of Europe for the first time and in the context of this possible range extension the known latitudinal range border has also shifted northwards, resulting in records e.g. from Ukraine (Matushkina 2007), Bulgaria (Marinov 2000; Gashtarov & Beshkov 2010), Romania (Benstead 2013; Boscain 2014; Skolka 2014) and the very north of Spain (Boudot et al. 2009).

In the course of this study the continuous occurrence of *S. nigra* in north-eastern Italy as previously reported by Zandigiacomo & Buian (2010) was confirmed and supplemented by records of exuviae, representing the northernmost evidence of breeding in Europe and even worldwide.

Material and methods

Data were obtained between April and August of the years 2012 to 2014 at two public parks in the regions of Friuli Venezia Giulia and Veneto, Italy. Surroundings and embankments of two artificial lakes were sampled systematically for both adults and exuviae of Odonata. For determination of the exuviae Gerken & Sternberg (1999) was used. Exuviae were deposited in the collections of the Natural History Museum of Pordenone as well as in the private collection of the first author. Field surveys were carried out by CU and GN.

Localities sampled and referred to in the text

Locality **(1)** was situated in the park "Parco delle Dote", (45.8764°N, 12.715172°E), municipality of Azzano Decimo, province of Pordenone (Fig. 2). The park amounts to 5 ha and was created only recently (2007–2010). The lake had a maximum depth of 3.5 m and was situated in open landscape with meadows and isolated trees. The gravelly and stony banks were overgrown with low *Phragmites australis* and *Carex* cf. *riparia*, *Cyperus* sp., the neophytic grass *Paspalum distichum* and *Salix triandra*. The zone near the shore was covered with algae and sumberged hydrophytes such as *Veronica beccabunga, Myriophyllum* cf. *spicatum*, and *Potamogeton* sp.. A total of 20 Odonata species were recorded, of which the following nine reproduced syntopically: *Ischnura elegans*, *Coenagrion puella*, *Anax imperator*, *A. parthenope, Orthetrum cancellatum*, *Sympetrum fonscolombii*, *S. sanguineum*, *S. striolatum*, and *Crocothemis erythraea*. Other fauna recorded included the Edible frog *Pelophylax* kl. *esculentus*, terrapins *Emys orbicularis* and *Trachemys* sp. and Largemouth bass *Micropterus salmoides*. Locality **(2)** was in the park "Oasi Ca' del

Lago" (45.825722°N, 12.789472°E), in the province of Venice. The park stretches over 60 ha and is part of the nature reserve "Parco regionale di interesse locale dei fiumi Reghena, Lemene e dei Laghi di Cinto". The lake, a former gravelpit, was used for fish farming from 1974 to 1979 and converted into a more natural condition in 1996. It had a maximal depth of 6 m and an average depth of about 2.5 m. It was fed by groundwater and the gravely embankment was sparsely overgrown by stands of helophytes as *Thypha latifolia*, *Phragmites australis*, *Sparganium erectum*, *Carex elata*, *C. gracilis*, *C. pseudocyperus,* and *C. riparia.* Aquatic vegetation was well developed and composed of both submerged (*Myriophyllum spicatum*) and floating hydrophytes (*Nymphaea alba, Nymphoides peltata,* and *Nuphar luteum*). The odonate community reproducing syntopically included the species recorded at **(1)** and additionally *Lestes parvidens, Sympecma fusca*, *Calopteryx virgo* (spring-fed section)*, Coenagrion puella*, *Erythromma viridulum*, *Platycnemis pennipes*, *Aeshna affinis*, *Orthetrum albistylum*, *O. brunneum,* and *O. coerulescens.* Potential predators recorded were Yellow-bellied toad *Bombina variegata*, European green toad *Bufotes viridis*, Italian crested newt *Triturus carnifex*, Italian agile frog *Rana latastei*, as well as European pond turtle *Emys orbicularis* and several fish species such as Pigo *Rutilus pigus*, Marble trout *Salmo marmoratus*, and Italian barbel *Barbus plebejus*.

Results

At both localities numerous adults of *S. nigra* were observed, also *in copulae* and ovipositing. More than 400 exuviae of *S. nigra* (Fig. 1) were collected as follows.

(1) 24, 10-vi-2013; 34, 16-vii-2013; 96, 27-vii-2013; 10, 07-viii-2013; 2, 19-v-2014; 7, 21-v-2014; 26, 22-v-2014; 14, 29-v-2014; 2, 30-v-2014; 90, 31-v-2014;3, 01-vi-2014; 54, 02-vi-2014
(2) 5, 04-vii-2013; 27, 21-v-2014; 57, 04-vii-2014

Discussion

Northernmost European records of *S. nigra* were reported by Skvortsov & Kuvaev (2010) from deserts of the Kalmyk Republic (Республика Калмыкия; Хальмг Тангч), Russia, in the Aral-Caspian Depression at 46°42'N, from the Autonomous Republic of Crimea (Автономна Республіка Крим) at 45°00'N (Matushkina 2007) and from the nearby Mykolaiv Oblast (Миколаївська область) from at least 46°28'N (Tytar 2007), both Ukraine. According to current knowledge records of *S. nigra* above 50°N are confined to the steppe and desert belt of southern Russia and Central Asia. Records of adults from the Europe-Asia border area on the southern slopes of the Ural mountains near Uvildy Lake (Увильды озеро) at Chelyabinsk oblast (Челя́бинская о́бласть; 55.533056°N, 60.499722°N) and from the Bashkirskyi state reserve, Burzyansky District (Бурзя́нский райо́н; 53°30'N,

Fig. 1. Exuvia of *Selysiothemis nigra* collected at "Parco delle Dote", Pordenone county, Friuli Venezia Giulia, Italy (27-vii-2013). Photo: Luca Dorigo

Fig. 2. Breeding habitat of *Selysiothemis nigra* at "Parco delle Dote", Pordenone county, Friuli Venezia Giulia, Italy (18-vii-2013). Photo: GN

57°43'E), Republic of Bashkortostan (Респу́блика Башкортоста́н; Башҡортостан Республикаһы) given by Popova & Haritonov (2008) and Yanybaeva et al. (2006), respectively, represent the present northernmost records at all (depending on the definition the latter site might also be considered as belonging to Europe proper).

These records however referred to observations of adults only which is why data from **(1)** at 45.8764°N therefore represent the northernmost evidence of breeding *S. nigra* worldwide.

It is noteworthy that the larval habitats of *S. nigra* as decribed at **(1, 2)** in view of morphology and structure showed striking similarities to artificial lakes and reservoirs frequently colonised by *Lindenia tetraphylla* (Vander Linden, 1825). As Iranoeremian faunal elements both species exhibit the same distribution type and seem to share a common biogegraphical history (cf. Lohmann 1981; De Lattin 1967: 396). Based upon this, further commonalities are very similar habitat requirements and a highly mobile and nomadic behaviour typical for species adapted to desert or steppe environments with prevailing intermittent or temporary water bodies. Both species frequently co-occur and breed syntopically (e.g., Skvortsov & Kuvaev 2010; Gashtarov & Beshkov 2010; cf. Brochard & Van der Ploeg 2013 in combination with Stille et al. 2014; J.-P. Boudot pers. comm.) and were even found to migrate together in huge aggregated swarms (Fraser 1936; Schneider 1981). It is thus likely to be no coincidence that both species in recent years generally showed a positive population trend in Europe benefitting from the increasing number of suitable artificial habitats (cf. Popova 1997; Brochard & Van der Ploeg 2013; Boudot 2014; Stille et al. 2014). Such perennial and deep man made water bodies just as dam lakes and reservoirs strikingly deviate from shallow and often temporary brackish habitats as salt marshes and lagoons previously described as habitat for *S. nigra* (e.g., Morton 1919; Compte Sart 1960; Lohr 2005; Kalkman 2006; Kalkman & van Pelt 2006: 140). In the case of **(2)** even a deep former fishpond with well developed aquatic and riparian vegetation served as larval habitat suggesting a rather broader ecological valence of *S. nigra* than previously expected.

Whilst lakes and reservoirs recently colonised by *L. tetraphylla* were situated within the original species' range, several of the above mentioned recent observations of *S. nigra* constitute a considerable latitudinal expansion of its range. Based on the limited data available in the 1950s Compte Sart (1960) identified the 25°C isotherm as possible climatic border ruling the northern limit of occurrence of *S. nigra*. Although several of these records which recently came to known are situated clearly beyond the 25°C isotherm and rather coincide with the 24°C or 23°C isotherm (cf. Franz 1973), in the particular case of *S. nigra* only further research will shed light to the general question whether and to what extend the increasing number of records is due to real lasting range expansion to the north or rather reflect increasing faunistic research and hydro-engineering.

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