

**Notes on the biology of *Orientus ishidae* (Matsumura, 1902)
in Piedmont (Italy)
(Hemiptera: Cicadellidae: Deltocephalinae)**

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Summary: Some aspects of the biology of *Orientus ishidae* (Matsumura, 1902) (Hemiptera: Cicadellidae: Deltocephalinae) were studied in some protected areas of Piedmont (Northern Italy) in the years 2012-2014 and in 2016. A case of predacious attack by *Icius* spec. (juvenile) (Araneae: Salticidae) was observed. Cases of parasitism by Dryinidae (Hymenoptera: Chrysidoidea) and Erythraeidae (Acarina: Prostigmata) were also observed. The mite *Erythraeus jowitae* Haitlinger, 1987 is reported for the first time for the Italian fauna.

Key words: biological cycle, parasitism, predation, *Anteon fulviventris*, *Erythraeus jowitae*, *Charletonia cardinalis*, *Icius* spec.

1. Introduction

Orientus ishidae (Matsumura, 1902) (Hemiptera: Cicadellidae) is a leafhopper widespread in Japan, the USA, Canada, Switzerland, Austria, Slovenia, the United Kingdom, France, Italy, Germany, Hungary, the Czech Republic (EPPO 2015), the Netherlands (den Bieman & van Klink 2015) and Poland (Klejdzysz et al. 2017). It is probably present also in Belgium, Slovakia and Spain (EPPO 2015). In Italy *O. ishidae* was found for the first time in Lombardia in 1998 (Guglielmino 2005) and afterwards in Tuscany (Mazzoni 2005), Piedmont (Alma et al. 2009), Veneto and Emilia-Romagna (Nicoli Aldini & Piccolino 2014) (Fig. 1).

Its biology is poorly known. It inhabits a wide range of habitats, where it feeds on various host plants (Guglielmino 2005). In Japan it was found on *Malus* spp. (Ishihara, 1968), in North America on *Carpinus caroliniana* Walter, *Ostrya virginiana* (Mill.) K. Koch, *Betula* spp., *Crataegus* spp., *Acer* spp., *Quercus palustris* L., *Aralia spinosa* L., *Tilia cordata* Mill., *Buxus* spp., *Gleditsia triacanthos* L., *Prunus virginiana* L., *Salix babylonica* L., *Salix* spp., *Juglans nigra* L., *Malus sylvestris* Mill., *Corylus* spp., and weeds (Felt & Bromley 1941; Rosenberger & Jones 1978; Valley & Wheeler Jr. 1985; Hamilton 1985; Johnson & Freytag 2001).



Fig. 1: *Orientus ishidae* (Matsumura), ♂
(Photo: G. Kunz)

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In Europe it was found on *Salix* spp., *Salix X rubens*, *Salix babylonica* L., *Betula pendula* Roth, *Malus domestica* Borkhausen, *Prunus laurocerasus* L., *P. domestica* L., *Urtica dioica* L., *Salix alba* L., *Corylus avellana* L., *Fagus sylvatica* L., *Carpinus betulus* L., *Acer campestre* L., *Hedera helix* L., *Quercus* spp., *Viburnum tinus* L., *Vitis vinifera* L., *Ulmus minor* L., *Chelidonium majus* L., *Berberis* spp., *Ostrya carpinifolia* Scop., *Rubus fruticosus* L., *Rosa canina* L., *Buxus sempervirens* L., *Juglans nigra* L., *Cornus sanguinea* L., *Populus nigra* L. and *Crataegus oxyacantha* L. (Guglielmino, 2005; Mazzoni 2005; Nickel 2010; Koczor et al. 2013; Lessio et al. 2016). *O. ishidae* passes the winter in the egg stage (Lessio et al. 2016). Nymphs appear at the end of May and are present until the beginning of August; adults are present from the last days of June until the beginning of October (Garman & Townsend 1952; Lessio et al. 2016). In transmission tests, under laboratory conditions, this leafhopper was found to transmit the Peach X-disease, classified as '*Candidatus* Phytoplasma pruni' (Davis et al. 2013), to celery plants (Rosenberger & Jones 1978) and 16 SrV phytoplasmas to grapevine (grapevine's "flavescence dorée") (Lessio et al. 2016).

In 2012-2014 and in 2016, some aspects of the biology of *O. ishidae* were studied in protected areas of Piedmont (Northern Italy) with the aim to increase the knowledge about the host plant range, the temporal dynamics of the nymphs and adults and the presence of natural enemies. The results of this effort are presented below.

2. Material and methods

The research was conducted in the years 2012-2014 and in 2016 from May to October (first 10 days) in three protected areas of Piedmont: Po Morto Oasis (near Carignano, Turin Province,), Merlino Wood (near Caramagna, Cuneo Province) and Crociato Wood (near Pocapaglia, Cuneo Province) (Fig. 2).

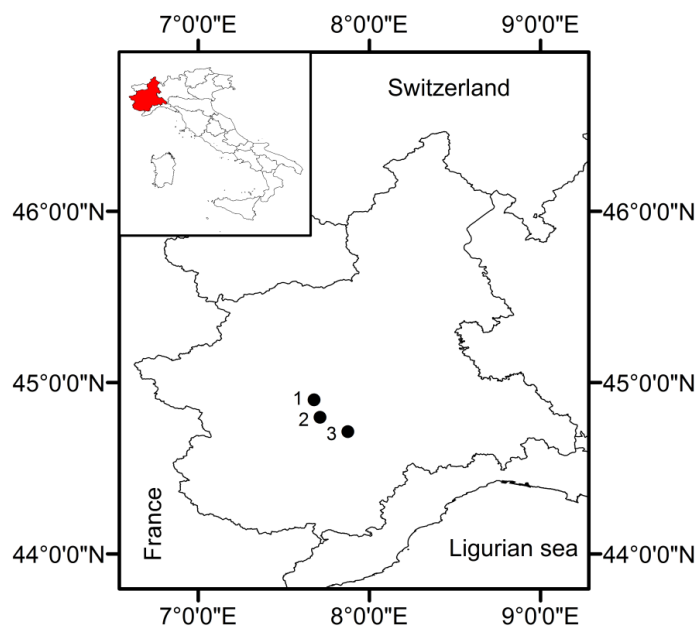


Fig. 2: Position of sampling sites of *Orientus ishidae* (Matsumura) in Piedmont, Northern Italy - 1: Po Morto Oasis; 2: Merlino Wood; 3: Crociato Wood.

Po Morto Oasis is a 490 hectares natural reserve which extends on abandoned meanders of Po River. It is protected by the European Union as reproductive area of *Pelobates fuscus* (Laurenti, 1768) (Amphibia: Pelobatidae), which is a species listed in IUCN red list (IUCN 2016). This oasis is characterized by a riparian vegetation, with a high incidence of *Robinia pseudo-acacia* L. and broad-leaved plants (Vai 1997). Merlino Wood (Fig. 3) is one of the last plain forests of South Piedmont. It extends about 50 hectares in width. It is characterized by a woodland vegetation with a prevalence of *Quercus robur* L., *Carpinus betulus* L. and *Corylus avellana* L. (Abbà 1982). This wood is recognized as Site of Community Importance (SCI) as reproductive area of *Rana latasei* (Boulanger, 1879) (Amphibia: Ranidae), which is a vulnerable species listed in IUCN red list (IUCN 2016). Crociato Wood is situated in "Rocche del Roero" region, a wide hill area of the Central Piedmont. This area was originated from the Tanaro river stream piracy about 250,000 years ago and is characterized by a high incidence of *Quercus pubescens* Wild., *Populus* spp. and *Tilia* spp. (Rota 2008).

Immature stages of *O. ishidae* were captured by vial aspirator from May to August. The adults were collected by TEMO-O-CID® 25x 40 cm yellow sticky traps and by beating tray (sheet opening 0.95 x 0.95 m) from June to the beginning of October. Samplings by sticky traps were conducted for three years from 2012 to 2014. The traps were placed in Po Morto Oasis and Merlino Wood in 2012, in Po Morto Oasis and Crociato Wood in 2013 and in Merlino and Crociato Wood in 2014. There was a trap in every sampling site (for two years) and the traps were replaced every two weeks. Both nymphs and adults were killed in 95% alcohol.

Five genitalia of *O. ishidae* ♂♂ were handled by the techniques proposed by Knight (1965) for leafhopper identification according to Guglielmino (2005). Mites were killed in 30% alcohol and sent to Prof. Ryszard Haitlinger (Wrocław University, Poland) for identification. Nymphs of *O. ishidae* parasitized by dryinids were reared in glass tubes with a layer of clay to permit the cocooning of dryinid larvae. Inside the tubes a Japanese quince leaf was also placed for leafhoppers feeding. It was changed every two days until the cocooning of dryinid larvae. After the emergence, the dryinid adults were killed in 95% alcohol and sent to Prof. Massimo Olmi (Tropical Entomology Research Center, Italy) for identification. The spider was killed in 70% alcohol and sent to Dr. Marco Isaia (Turin University, Italy) for identification.

Host plant range of *O. ishidae* was recorded by observing the feeding activity of nymphs causing symptomatic leaf alterations which consist of a uniform yellow patch evolving to brown followed by necrosis of the entire leaf. The leaf yellowing and necrosis follow a basipetal course (Fig. 4a and b) (Felt & Bromley 1941; Garman & Townsend 1952). On the other hand, adults were captured by beating directly on host plants.

3. Results

3.1 Surveys of immature stages and adults

Immature stages were present from mid May to early August. The adults were present from late June to late September; adult females with eggs were observed from early July to late September. Concerning traps, 170, 1176 and 107 specimens, resp., were collected in 2012, 2013 and 2014. The last captures were made in mid September. The flight peak of adults occurred in late July 2012 and in mid July in 2013 and 2014 (Fig. 5).



Fig. 3: Merlino Wood.

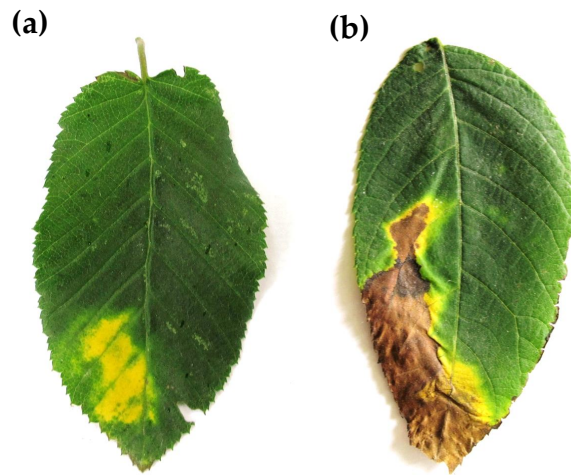


Fig. 4: (a) Early symptoms of feeding activity of *Orientus ishidae* (Mats.) on a leaf of *Carpinus betulus* L., (b) advanced symptoms of feeding activity of *O. ishidae* on a leaf of *Juglans nigra* L.

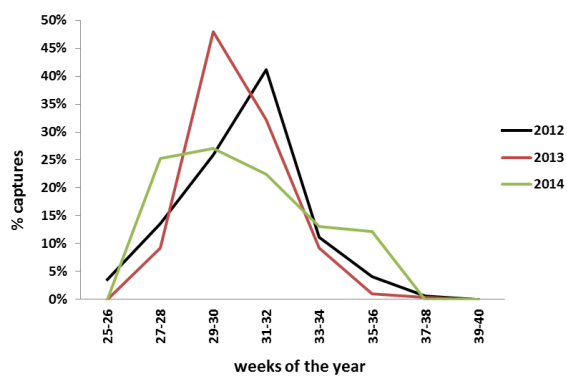


Fig. 5: Seasonal abundance of adults of *Orientus ishidae* (Mats.) in 2012, 2013, 2014.

3.2 Putative host plants

The nymphs were observed on 18 species of flowering plants belonging to 11 families (Table 1). The family more prevalent was Rosaceae (22%); the prevalent life-form was Phanerophytes caespitose (44%) (Fig. 6). The adults of *O. ishidae* were collected on 9 plant species belonging to 9 families (Table 1). The family more prevalent was Betulaceae (33%); both Phanerophytes caespitose and Phanerophytes scapose were the prevalent life-forms. On *Ostrya carpinifolia* the nymphs of first and second instar of *O. ishidae* were concentrated on leaves of water sprouts and on *Corylus avellana* on leaves of suckers.

3.3 Natural enemies

From the last decade of June to the last week of July parasitic activity by mites belonging to the family Erythraeidae was discovered. Larvae of *Charletonia cardinalis* (C.L. Koch, 1837) (Acarina: Erythraeidae: Callidosomatinae) were observed to parasitize nymphs and adults of *O. ishidae*. In addition, in 2014, larvae of *Erythraeus jowitae* (Haitlinger, 1987) (Acarina: Erythraeidae: Erythraeinae) were observed to parasitize nymphs.

Between the end of June and the first week of August, parasitic attacks of *Anteon fulviventre* (Haliday, 1828) (Hymenoptera: Chrysidoidea: Dryinidae) on nymphs of *O. ishidae* (Fig. 7) were observed (7 parasitized specimens in 2013, 9 in 2014) as well as a predaceous attack by a juvenile stage of *Icius* spec. (Araneae: Salticidae).

Table 1: Putative host plant life-forms of nymphs and adults of *O. ishidae* in the studied area. P lian: Phanerophytes liane, P caesp: Phanerophytes caespitose, P scap: Phanerophytes scapose, H scap: Hemipterophytes scapose, NP: Nanophanerophytes.

Species	Family	Life form	Nymphs	Adults
<i>Hedera helix</i> L.	Araliaceae	P lian	X	
<i>Carpinus betulus</i> L.	Betulaceae	P caesp	X	X
<i>Ostrya carpinifolia</i> Scop.	Betulaceae	P caesp	X	X
<i>Viburnum tinus</i> L.	Adoxaceae	P caesp	X	
<i>Corylus avellana</i> L.	Betulaceae	P caesp	X	X
<i>Robinia pseudoacacia</i> L.	Fabaceae	P caesp	X	
<i>Juglans regia</i> L.	Juglandaceae	P scap	X	X
<i>Juglans nigra</i> L.	Juglandaceae	P scap	X	X
<i>Forsythia viridissima</i> Lindl.	Oleaceae	P caesp	X	
<i>Fraxinus excelsior</i> L.	Oleaceae	P scap	X	
<i>Chelidonium majus</i> L.	Papaveraceae	H scap	X	X
<i>Chaenomeles speciosa</i> (Sweet) Nakai	Rosaceae	P caesp	X	
<i>Cydonia oblonga</i> Mill.	Rosaceae	P scap	X	X
<i>Prunus avium</i> L.	Rosaceae	P scap	X	X
<i>Rubus fruticosus</i> L.	Rosaceae	NP	X	
<i>Populus alba</i> L.	Salicaceae	P scap	X	
<i>Ribes uva-crispa</i> L.	Grossulariaceae	NP	X	
<i>Ulmus minor</i> Mill.	Ulmaceae	P caesp	X	X

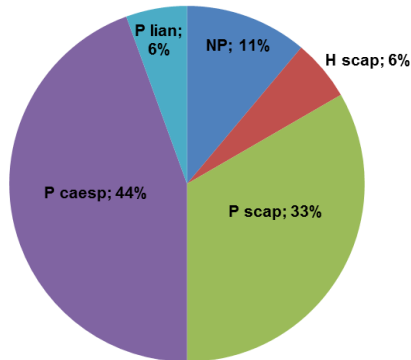


Fig. 7: Life-form spectrum of putative host plants of nymphs of *Orientus ishidae* (Mats.) in the studied area. P lian: Phanerophytes liane, P caesp: Phanerophytes caespitose, P scap: Phanerophytes scapose, H scap: Hemicryptophytes scapose, NP: Nanophanerophytes.

4. Discussion

According to the observations of Lessio et al. (2016) in Piedmont and the data here presented, *O. ishidae* seems to have a life cycle very similar to *Scaphoideus titanus* (Chuche & Thiery 2014) a leafhopper belonging to the same subfamily (Deltocephalinae) (Zahniser & Dietrich 2013) and a vector of 16 SrV phytoplasmas to grapevine. *O. ishidae* is a univoltine species present as immature stages from mid May until early August and as adult from early July until early October (Lessio et al. 2016). Under laboratory conditions *O. ishidae* laid eggs on branches of grapevine (Lessio et al. 2016) confirming that *O. ishidae* passes the winter in the egg stage. The ovipositing process, in the area studied, seems to begin in July and to finish at the beginning of October.

The polyphagy characterizes both nymphs and adults. *O. ishidae* seems to feed preferably broad-leaved shrubs and trees, more rarely herbaceous plants (Lessio et al. 2016, Nickel 2010). The broad range of putative host plants can explain the ability of this leafhopper to inhabit a wide range of biotopes (Guglielmino 2005; Nickel 2010; Lessio et al. 2016). Differences in the host range between nymphs and adults of *O. ishidae* can be explained with a moving of nymphs after the hatching (Lessio et al. 2016).

A predaceous attack by a juvenile stage of *Icius* spec. on this leafhopper is here reported for the first time. The genus *Icius* is present in the Italian fauna with four species of Mediterranean or western Mediterranean distribution (Pantini & Isaia 2016). These spiders are generalist predators with the capacity of capturing a wide variety of arboricolous arthropods (Dr. Marco Isaia, personal comm.).



Fig. 7: Nymph of *Orientus ishidae* (Mats.) with a thylacium of *Anteon fulviventre* (Haliday).

Hunting spiders, as *Icius* species, may be efficient in reducing and stabilizing prey densities (Symondson et al. 2002) and spiders may prey on leafhoppers more frequently than predaceous insects (Fournier et al., 2008). More observations are necessary to better evaluate the potential of this hunting spider as biocontrol agents of leafhoppers.

Parasitic activity of *Charletonia cardinalis* and *Erythraeus jowitae* on the nymphs of *O. ishidae* is here reported for the first time. *C. cardinalis* is widespread in Australia, Azerbaijan, Japan, Europe and the USA (Małol & Wohltmann 2012), where it is known as an ectoparasitic mite on insects belonging to Hemiptera, Coleoptera and Lepidoptera (Stroiński et al. 2013). Among the Hemiptera, the following hosts of *C. cardinalis* are known: *Javesella pellucida* (Fabricius, 1794); *Eurybregma nigrolineata* Scott, 1875; *Muellerianella brevipennis* (Boheman, 1847) (all Fulgoromorpha: Delphacidae) and *Cicadella viridis* (Linnaeus, 1758) (Cicadomorpha: Cicadellidae) (Małol & Wohltmann 2012; Stroiński et al. 2013). Other known hosts are *Phyllotreta undulata* (Kutschera, 1860) (Coleoptera: Chrysomelidae) (Haitlinger 1987b), *Simyra henrici* (Grote, 1873) (Lepidoptera: Noctuidae) and *Nerice bidentata* (Walker 1855) (Lepidoptera: Notodontidae) (Treat 1975, 1980). *E. jowitae* is a mite described from Poland (Haitlinger 1987a) and afterwards reported in France, Macedonia, Hungary, Sweden and Romania (Małol & Wohltmann 2012). No hosts of this mite were known so far. The record presented in this paper is the first for Italy. Generally very little is known about the potential of mites as biological control agents because of the limited knowledge of their biology and ecology (Muñoz-Cárdenas et al. 2015).

The parasitic activity of *Anteon fulviventre* on nymphs of *O. ishidae* is a new record. In Japan, where this leafhopper was originally described, there is only one record of an unidentified dryinid wasp attacking *O. ishidae* nymphs (Toshiharu Mita, personal comm.). In the USA Valley & Wheeler (1985) reported three *O. ishidae* nymphs parasitized by unidentified dryinid wasps of the subfamily Anteoninae. *A. fulviventre* is widespread in Europe, Morocco and Algeria (Olm 1999). Its hosts are Cicadellidae Deltocephalinae (Guglielmino et al. 2013). The new record of *A. fulviventre* as a parasitoid of *O. ishidae* nymphs highlights the potential of this dryinid wasp to fit a new exotic host.

5. Zusammenfassung

Einige Aspekte der Biologie von *Orientus ishidae* (Matsumura, 1902) (Hemiptera: Cicadellidae: Deltocephalinae) wurden in Schutzgebieten in Piemont (Norditalien) von 2012 bis 2014 und in 2016 untersucht. Eine räuberische Attacke durch *Icius* spec. (juvenil) (Araneae; Salticidae) und Fälle von Parasitismus durch Dryiniden (Hymenoptera: Chrysidoidea) und Erythraeiden

(Acarina: Prostigmata) wurden beobachtet. Eine Milbenart (*Erythraeus jowitae* Haitlinger, 1987) wurde zum ersten Mal für die italienische Fauna nachgewiesen.

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