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OCCURRENCE OF THE ANGULAR ROUGH SHARK, *OXYNOTUS CENTRINA* (CHONDRICHTHYES: OXYNOTIDAE) IN THE EASTERN MEDITERRANEAN

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ABSTRACT

Based on field surveys and available literature, 88 specimens of Oxynotus centrina *(Linnaeus, 1758), were recorded from Marmara, Aegean and Mediterranean seas, between the late 1800's and October 2012. Sixty-four specimens (72 %) were recorded in the Aegean Sea, followed by 19 records (21.5 %) from the Sea of Marmara and 5 records (5.6 %) from the Mediterranean Sea. Total length of the recorded specimens (sexes combined) ranged from 22.5 cm to 79 cm. Bottom-trawling was the main fishing gear (n = 64, 72 %), by which most angular rough sharks have been caught, followed by beam-trawl (n = 3, 3.3 %), gill-net (n = 3, 3.3 %) and long-line (n = 1, 1.1 %). Seven specimens (7.8 %) were recorded by means of visual sampling (underwater imaging), all of which were carried out in the Sea of Marmara.* O. centrina *is a rare and threatened deep-sea shark in the eastern Mediterranean and adjacent seas. Capture of pregnant females creates a significant threat to the survival of the species, thus, before the implementation of evidence-based measures for the conservation, and even a ban on the fishing of* O. centrina*, promoting fishermen to release live specimens, appears to be an urgent, feasible first step in the protection of this rare species.*

Key words: Elasmobranchii, *Oxynotus centrina*, mortality, survival, protection, Mediterranean Sea

PRESENZA DI PESCE PORCO, *OXYNOTUS CENTRINA* (CHONDRICHTHYES: OXYNOTIDAE), IN MEDITERRANEO ORIENTALE

SINTESI

In base alla letteratura disponibile ed ai rilevamenti effettuati in mare, la presenza di 88 esemplari di Oxynotus centrina *(Linnaeus, 1758) è stata confermata nel Mar di Marmara, nell'Egeo e nel Mediterraneo, tra la fine del 1800 e l'ottobre 2012. Sessantaquattro campioni (il 72 % del totale) provengono dall'Egeo, 19 campioni (ossia il 21,5 %) dal Mar di Marmara e solo 5 campioni (il 5,6 %) dalla restanti aree del Mediterraneo. La lunghezza totale degli individui campionati (di entrambi i sessi) è compresa tra i 22,5 cm e i 79 cm. L'attrezzo da pesca principale con il quale sono stati catturati gli esemplari di pesce porco è risultata la rete a strascico con divergenti (n = 64, pari al 72 %), seguita dai rapidi o ramponi (n = 3, pari al 3,3 %), dalla rete ad imbrocco (n = 3, pari al 3.3 %) e dal palangaro (n = 1, pari all'1,1 %). La presenza di sette individui (il 7,8 %) è stata confermata mediante campionatura visiva (immagini subacquee) effettuata nel Mar di Marmara. Il pesce porco è uno squalo di acque profonde raro e minacciato nella parte orientale del Mediterraneo e nei mari adiacenti. La cattura di femmine gravide è una minaccia significativa per la sopravvivenza della specie. Come primo passo nella protezione di questa specie rara risulta urgente e fattibile educare i pescatori a rilasciare gli esemplari vivi, ancor prima di mettere in atto linee guida per la conservazione o il divieto di pesca della specie in questione.*

Parole chiave: Elasmobranchi, *Oxynotus centrina*, mortalità, sopravvivenza, protezione, mare Mediterraneo

INTRODUCTION

The angular rough shark, *Oxynotus centrina* (Linnaeus, 1758), is a rare to uncommon deep sea shark throughout its range (Ebert & Stehmann, 2013). In the eastern Atlantic, its distribution range extends from Norway to Portugal, and extending southward to the South African coast (Ebert & Stehmann, 2013). *O. centrina* occurs in the entire Mediterranean Sea, from the Straits of Gibraltar to Israel (Serena, 2005), and extending northward to the Sea of Marmara (Kabasakal, 2010). It is a sluggish and harmless shark found over the continental shelf and upper slope from depths of 60 to 660 m, where it is an uncommon bycatch in Mediterranean deep demersal fisheries (Serena, 2005). A recent DESEAS survey in the western Ionian Sea demonstrated that, the lower limit of its depth distribution could extend to 800 m (Sion *et al.*, 2004).

Historical and contemporary occurrence of *O. centrina* in the Mediterranean Sea dates back to the 16th century, recorded both in general ichthyological (*e.g.,* Belon, 1553; Risso, 1810; Carus, 1889-1893; Ninni, 1912; Quéro, 1984; Akşıray, 1987; Papaconstantinou, 1988; Serena, 2005) and shark specific studies (*e.g.,* Tortonese, 1956; Quignard & Capapé, 1971; Capapé, 1977; Barrull *et al.*, 1999; Kabasakal, 2002; Cugini & De Maddalena, 2003; Kabasakal & Kabasakal, 2004; Lipej *et al.*, 2004; Storai, 2004). Although, several studies have been carried out to reveal the life history parameters of the angular rough shark (Calderwood, 1892; Geldiay & Mater, 1968; Capapé *et al.*, 1999; Barrull & Mate, 2001; Megalofonou & Damalas, 2004; Capapé, 2008; Dragičević *et al*. 2009; Kabasakal, 2009), there are still many gaps present in our understanding of the life history of *O. centrina*. Furthermore, the fragmentary nature of the eastern Mediterranean records of the species is a complicating factor in providing a complete picture of its distribution in the mentioned region.

Oldest known records on the eastern Mediterranean occurrence of *O. centrina* were reported by Carus (1889-1893) and Hoffman & Jordan (1892), from Greek waters, and the most recent records of the species from the mentioned region have been reported by Kousteni & Megalofonou (2012). The occurrence of *O. centrina* from the eastern Mediterranean and adjacent seas have also been reported by the following authors: Erazi (1942), Akyüz (1957), Geldiay & Mater (1968), Papaconstantinou & Tortonese (1980), Papaconstantinou & Tsimenidis (1985), Başusta *et al*. (1998), Karakulak *et al*. (2000), Eryilmaz (2003), Kabasakal (2003, 2010), Kabasakal & Kabasakal (2004), Megalofonou & Damalas (2004), Eryilmaz & Meriç (2005), Golani (2006), Hadjichristophoru (2006), Öziç & Yilmaz (2006), Bayhan *et al*. (2006), Saad *et al*. (2006), Damalas & Vassilopoulou (2009), Keskin & Eryilmaz (2010) and Moftah *et al*. (2011). The aim of this article is to provide new data on the occurrence of *O. centrina* in the eastern Mediterranean, as well as in the adjacent Aegean and Marmaric

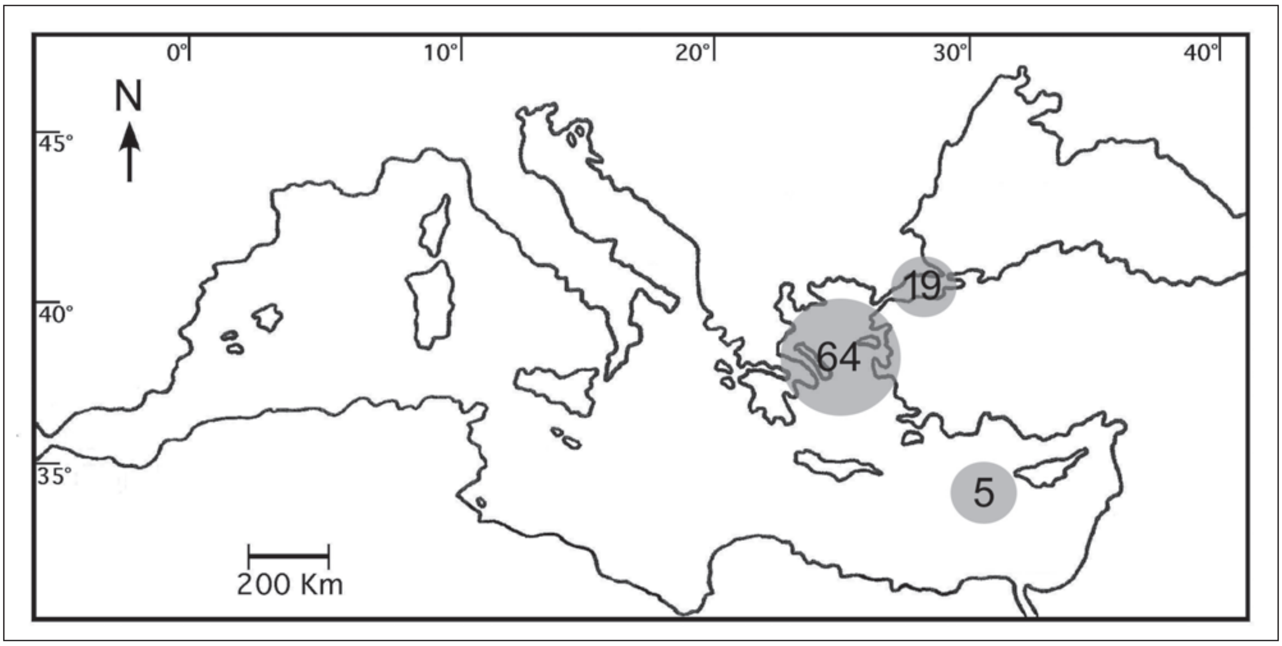

Fig. 1: Map showing the study area, where specimens of* Oxynotus centrina *recorded in eastern Mediterranean and adjacent waters. Numbers in shaded circles indicate the number of confirmed records in Marmara, Aegean and Mediterranean waters.
Sl. 1: Zemljevid obravnavanega območja, kjer so bili ugotovljeni primerki morskih prašičev (*Oxynotus centrina*). Številke v osenčenih krožcih kažejo število potrjenih zapisov v Marmarskem, Egejskem in Sredozemskem morju.

waters, with a brief review of historical and contemporary records, as well as the rarity of the species in the mentioned region.

MATERIAL AND METHODS

This study is part of an extensive research to provide up-to-date information on the status of shark species occurring in the seas of Turkey, which has been ongoing since 2000, as an initiative of the Ichthyological Research Society (IRS). A previous report of *Oxynotus centrina* substudy, which summarised the historical and contemporary records of the angular rough shark from Turkish waters, was recently published by Kabasakal (2010). Eastern Mediterranean, Aegean and Marmaric records of *O. centrina* were obtained from the following sources: (a) available ichthyological literature; (b) printed or electronic versions of popular media such as daily newspapers, fishing and/or diving magazines, with reliable photographic evidence; (c) field surveys; (d) specimens stored in museums; and (e) underwater photographs and/or video footage with information on locality of sight, date, depth, etc.

The following data were recorded, if conditions allowed for such measurements and observations: total length (TL), weight (w), sex, depth and date of sight or caught, and type of sampling. Total length data of specimens was extracted from the literature or measured on board. Since the underwater cameras of recreational scuba divers lack twin laser pointers, which can be used to create inwater lased reference points on specimens and utilized as a scale bar for further length measurements, TL data of the visually sampled angular rough sharks could not be recorded in most occasions. Total length is the horizontal line between the tip of the snout and the tip of the upper lobe of caudal fin, where the caudal fin depressed to body axis (Serena, 2005). Regarding the records based on popular media, the author visited the fishing ports indicated in the press releases to interview the fishermen and validate the shark species as *O. centrina*. Description of the species and taxonomic nomenclature follow Serena (2005). Raw data sheets and specimen photographs and/or video footage are stored in the archives of IRS and available for inspection on request.

REVIEW

General remarks on catch data and specimens

Based on field surveys and available literature, of which references are quoted in Table 1, 88 specimens of *Oxynotus centrina* were recorded from Marmara, Aegean and Mediterranean seas between the late 1800's and October 2012. Sixty-four specimens (72 %) were recorded in the Aegean Sea, followed by 19 records (21.5 %) from the Sea of Marmara and 5 records (5.6 %) from the Mediterranean Sea (Fig. 1). Total length of the recorded specimens (sexes combined) ranged from 22.5 cm to 79 cm. Bottom-trawling was the main fishing gear (n = 64, 72 %), by which most angular rough sharks have been caught, followed by beam-trawl (n = 3, 3.3 %), gill-net (n = 3, 3.3 %) and long-line (n = 1, 1.1 %) (Fig. 2). Seven specimens (7.8 %) were recorded by means of visual sampling (underwater imaging), all of which were carried out in the Sea of Marmara. No data was obtained on the type of sampling of 10 specimens (Fig. 2). Individual remarks of records and the relevant references are summarised in Table 1.

The following description of *O. centrina* is based on examined specimens: the body is robust with a short, blunt snout and large nostrils; high, sail-like dorsal fins with strong spines (Fig. 3d); no anal fin, first dorsal fin spine inclined forwards, high and thick. Cross-section of the body is triangular with strong dermal ridges extending from the base of the ventral fin to the front corner of the ventral fin base (Fig. 3d). Spiracles are large and vertically elongated, crescent or oval in shape (Fig. 3b). Teeth on upper and lower jaws dissimilar; upper teeth are lanceolate and lower teeth are bladelike. Colour is grey to greyish brown above and below, with darker blotches on head and sides; light horizontal lines separate the dark areas on head (Figs. 3a, b).

Notes on occurrence and rarity

As it can be seen in Table 1, the oldest known records of *O. centrina* from eastern Mediterranean and adjacent seas date back to the late 1800's (*e.g.*, Carus, 1889-1893; Hoffman & Jordan, 1892). In one of the pioneering studies on the fauna and flora of the Mediterranean Sea, Carus (1889-1893) recorded *O. centrina* off Pire in Greek waters of the Aegean Sea. Furthermore, Hoffman & Jordan (1892) reported on an angular rough shark, which they observed in the Athens market, with brief remarks.

Although eastern Mediterranean records of *O. centrina* date back to the late 1800's the species was not mentioned in the pioneering ichthyological studies of Turkey's waters by Ninni (1923), Deveciyan (1926) and Ayaşlı (1937). The oldest confirmed record of the species from Turkey's waters is based on a mid 20th century ichthyological inventory of Marmaric and Bosphoric waters by Erazi (1942).

Based on available literature and field surveys the most recent confirmed specimen of *O. centrina*, a discarded bycaught female, from the mentioned region was recorded on 21 October 2012 in the prebosphoric region of the Sea of Marmara (Tab. 1). The occurrence of only 88 confirmed records of *O. centrina* from the eastern Mediterranean and adjacent seas (Tab. 1) from the oldest confirmed records (Carus, 1889-1893; Hoffman & Jordan, 1892) to the most recent record on 21 October 2012 is proving the rarity of the species in the mentioned area.

Tab. 1: Historical and contemporary records of angular rough shark,* Oxynotus centrina*, in eastern Mediterranean and adjacent seas between 1800's and 2012. AE – Aegean Sea, BT – bottom trawling, BET – beam trawling, GN – gill netting, LL – longline, ME – Mediterranean Sea, SM – Sea of Marmara, VS – visual sampling

Tab. 1: Starejši in recentni podatki o pojavljanju morskega prašiča Oxynotus centrina v vzhodnem Sredozemlju med koncem 19. stoletja in letom 2012. AE – Egejsko morje, BT – pridnena koča, BET – vlečna mrežo z gredjo, GN – zabodna mreža, LL – parangal, ME – Sredozemsko morje, SM – Marmarsko morje, VS – opazovalni popis

No	Date	Locality	Region	TL (cm)	Sex	Depth (m)	References & Remarks
1	late 1800's	Pire, Greece	AE	?	?	?	Carus (1889-1893). One of the earliest records of *O. centrina* from eastern Mediterranean.
2	late 1800's	Athens, Greece	AE	?	?	?	Hoffman & Jordan (1892). One of the earliest records of *O. centrina* from eastern Mediterranean based on a specimen observed in the market at Athens by the authors.
3	1942	Sea of Marmara	SM	?	?	?	Erazi (1942)
4	1957	İskenderun Bay	ME	?	?	?	Akyüz (1957)
5	1966	İzmir Bay	AE	?	♀	35	Geldiay & Mater (1968), BT
6	1980	Thermaikos Gulf, Greece	AE	24.8	?	?	Papaconstantinou & Tortonese (1980)
7	1985	Pagassitikos Gulf, Greece	AE	?	?	?	Papaconstantinou & Tsimenidis (1985)
8	Nov. 1994	Yassıada	SM	40	♀	90	Kabasakal (2003), BT
9	Feb. 1996	Ekinlik Island	SM	35	♂	60	Kabasakal (2003), GN
10	Feb. 1996	Ekinlik Island	SM	41	♂	60	Kabasakal (2003), GN
11	Nov. 1998	Gökçeada	AE	65	♀	?	Kabasakal & Kabasakal (2004), BT
12	1994-1996	İskenderun Bay	ME	53.2	?	70-80	Başusta *et al*. (1998), BT
13	Feb. 1999	Kea Island	AE	69	♀	100-200	Megalofonou & Damalas (2004), BT. Gravid female with 15 embryos.
14	2003-2004	Gökova Bay	AE	?	?	80	Öziç & Yilmaz (2006), BT
15	2000	Yassıada	SM	ca. 50	♀	ca. 35	Kabasakal (2010), VS
16	2000	Turkey's Mediterranean coast	ME			?	Unpubl. data. Preserved in fishery collections at Samatya Fishing Harbour (Fig. 2d).
17	Aug. 2000	Sea of Marmara	SM	?	?	35-100	Karakulak *et al*. (2000), BT
18	Sept. 2000	Bozcaada	AE	22.5	?	60	Eryilmaz (2003), BT
19	Dec. 2000	SW Sea of Marmara	SM	36	?	42-86	Bayhan *et al*. (2006), BET
20	Mar. 2001	SW Sea of Marmara	SM	50	?	42-86	Bayhan *et al*. (2006), BET
21	June 2001	SW Sea of Marmara	SM	52	?	42-86	Bayhan *et al*. (2006), BET
22	19 June 2001	Turkey's Mediterranean coast	ME			?	Unpubl. data. Preserved in fishery collections at Samatya Fishing Harbour.
23	2004	Balıkçı Adası	SM	?	?	54	Unpubl. data, VS. Recorded by Cem Yıldırım.
24	2005	Sea of Marmara	SM	?	?	?	Eryilmaz & Meriç (2005)
25	2005	Sedef Island	SM	?	?	43	Unpubl. data, VS. Recorded by Serço Ekşiyan.
26	2006	Balıkçı Island	SM	?	?	56	Unpubl. data, VS. Recorded by Cem Yıldırım (Fig. 2b).
27	Oct. 2006	SW Sea of Marmara	SM	?	?	52-74	Keskin & Eryilmaz (2010), BT
28-78	1995-2006	Central Aegean Sea	AE	?	?	?	Damalas & Vassilopoulou (2009). A total of 51 specimens were caught in the BT haulings over 11 year period, which were 100 % discarded. Authors did not give individual details of the specimens.
79	Mar. 2007	SW Sea of Marmara	SM	?	?	38-45	Keskin & Eryilmaz (2010), BT
80	Oct. 2007	Psara, Greece	AE	53.3	?	130	Kousteni & Megalofonou (2012), BT
81	Oct. 2007	Psara, Greece	AE	56.5	?	130	Kousteni & Megalofonou (2012), BT
82	7 Mar. 2008	Sea of Marmara	SM	?	?	?	Unpubl. data, GN
83	25 May 2008	Yassıada	SM	?	♀	35	Unpubl. data, VS. Recorded by Polat İnce.
84	between May. and Nov. 2008	Off Alexandria, Egypt	ME	?	?	?	Moftah *et al*. (2011). Sampled in the fish market.
85	27 Sept. 2009	Balıkçı Island	SM	ca. 60	♀	35	Kabasakal (2010), VS (Fig. 2a).
86	June 2010	Korinthiakos Gulf, Greece	AE	79	?	180	Kousteni & Megalofonou (2012), LL
87	May 2011	Evia - Greece	AE	67.4	?	366-458	Kousteni & Megalofonou (2012), BT
88	21 Oct. 2012	Ahırkapı	SM	57	♀	7	Unpubl. data, VS. Discarded by fishermen off the coast (Fig. 2c).

The rarity of *O. centrina* in the entire Mediterranean Sea is a generally well-accepted fact, both in the historical and contemporary reports (Tortonese, 1956; Capapé, 1977; Papaconstantinou & Tortonese, 1980; Cugini & De Maddalena, 2003; Kabasakal & Kabasakal, 2004; Storai, 2004; Hadjichristophoru, 2006; Kabasakal, 2010; Moftah *et al*., 2011; Kousteni & Megalofonou, 2012). According to Cugini & De Maddalena (2003), the population of *O. centrina* in Italian waters declined constantly during 20th century. Kabasakal & Kabasakal (2004) recorded only one specimen of *O. centrina* during an extensive survey of sharks in the northern Aegean Sea between 1995 and 2004. During this survey, Kabasakal & Kabasakal (2004) recorded 1068 shark specimens representing 20 species and *O. centrina* represented 0.09 % of the total catch. Capapé *et al*. (1999) and Capapé (2008) carried out the most detailed investigations on the reproductive biology and diet of *O. centrina*, respectively, in the western and central Mediterranean Sea; in both studies total numbers of the examined specimens over the 20 year research period were 80 and 102, respectively. During a survey on bycatch sharks caught by commercial bottom-trawlers in the central Aegean Sea between 1995 and 2006 Damalas & Vassilopoulou (2009) recorded 51 specimens of *O. centrina* and stated that the angular rough shark is > 95 % discarded shark. Based on the results of a survey on the abundance of sharks in the central Mediterranean Sea, Ragonese *et al*. (2013) considered *O. centrina* as a rare shark. Results of the present study have confirmed the rarity of *O. centrina* in the eastern Mediterranean. According to Serena & Relini (2006), *O. centrina* is more common in the western Mediterranean.

Contrary to above statements, which suggest the rarity of *O. centrina* in the eastern Mediterranean, Golani (2006) postulated that the angular rough shark is a prevalent species off Israel's coast. Furthermore, Eryilmaz & Meriç (2005) also postulated that *O. centrina* is also prevalent in the Sea of Marmara. The low number of records of *O. centrina* from the eastern Mediterranean and adjacent seas (n = 88) contradicts with the suggestions of Eryilmaz & Meriç (2005) and Golani (2006), and provides solid evidence supporting the well-accepted rare status of *O. centrina* in the mentioned region. In a recent work on the distribution of demersal fishes in the eastern Levant basin, Keskin *et al*. (2011) did not record any specimens of *O. centrina* in the bottom-trawl haulings between the depths of 43 and 121 m. Regarding the depth distribution of the angular rough shark (60-660 m, and in one occasion 800 m) (Sion *et al*., 2004; Serena, 2005) it is obvious that further research should carried out in deeper waters in order to obtain more information on the occurrence of *O. centrina* on the bathyal grounds of the Levant Sea. Furthermore, in a recent update of Syrian sharks no comment was made on the abundance of *O. centrina* in the mentioned region (Saad *et al*., 2006).

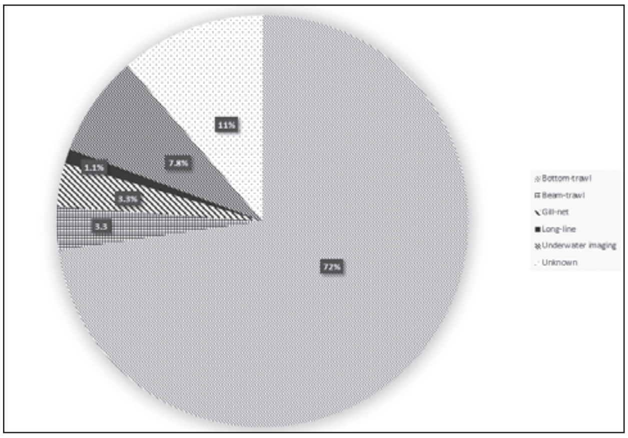

Fig. 2: Main fishing gears of* O. centrina *and proportion of visual sampling in overall records.
Sl. 2: Glavna ribolovna orodja, s katerimi so bili ujeti primerki morskih prašičev, in delež opaženih primerkov v opazovalnih popisih glede na celotno število zapisov

The angular rough shark is a bycatch of demersal fisheries, which is a well-recognized fact of *O. centrina* (Serena, 2005) and results of the present study have confirmed this fact. The majority of the present specimens (n = 71, 80 %) were caught by demersal fishing gears (Tab. 1, Fig. 2). Since the angular rough shark is not a commercially valuable species, it is generally discarded (*e.g*., > 95 % discarded in central Aegean Sea; Damalas & Vassilopoulou, 2009). However, in several occasions this harmless shark can be killed onboard before discarding (Fig. 3c). Not surprisingly, *O. centrina* is another shark species, stigmatized with superstitions; for instance, it is immediately returned to the sea by fisheries from Mazara, Sicily (Italian waters) because they think the angular rough shark brings bad luck (Ragonese *et al*., 2013). Thus, attempts to change this attitude of fishermen, who kill *O. centrina* before discarding, would increase the chance of survival of this rare deep-sea shark.

The Sea of Marmara, a small land-locked sea, appears to provide a shelter for the angular rough shark throughout its distribution range in the eastern Mediterranean. Recent research demonstrated that several rare or vulnerable deep-sea shark species (*e.g., Hexanchus griseus, Dalatias licha, Echinorhinus brucus, Centrophorus granulosus, Squalus blainvillei* and *O. centrina*) are the inhabitants of the deeper zones of Marmaric waters (Kabasakal, 2003, 2010, 2013; Kabasakal & Bilecenoğlu, 2014; Kabasakal & Kabasakal, 2014). A recent survey on the biology of *O. centrina* showed that the angular rough shark regularly occurs in the coastal waters off the Princes' Islands, northeastern Sea of Marmara (Kabasakal, 2009). Coastal occurrence of *O. centrina* in the mentioned region was visually documented by recreational divers (visual sampling in Table 1). Thus, this area offers remarkable opportunities for *O. centrina* research to collect data on behaviour and spatiotemporal distribution of the species on a 24h basis. Results of such research can provide vital information for the implementation of protective measures to minimize the fishing pressure on this threatened shark.

CONCLUSIONS

Oxynotus centrina is a rare deep-sea shark in the eastern Mediterranean and adjacent seas. According to Serena (2005), it is a threatened shark in the entire Mediterranean Sea. Based on the FAO classifications for the status of exploited species, *O. centrina* is placed in B1, which means, while it is an exploited species, its exploitation, vulnerability or decline status is not clear due to lack of data and therefore, it should be urgently investigated as recommended by IUCN (Serena, 2005). Because the fishing grounds of deep demersal fisheries overlap with the habitat of the angular rough shark, Ebert & Stehmann (2013) consider *O. centrina* as a vulnerable species. According to Ebert & Stehmann (2013), the most notable population decline of *O. centrina* may be inferred in the Mediterranean Sea. Given the low reproductive potential of *O. centrina* with up to 15 young per litter (Megalofonou & Damalas, 2004), capture of pregnant females create a significant threat to the survival of the species. Thus, before the implementation

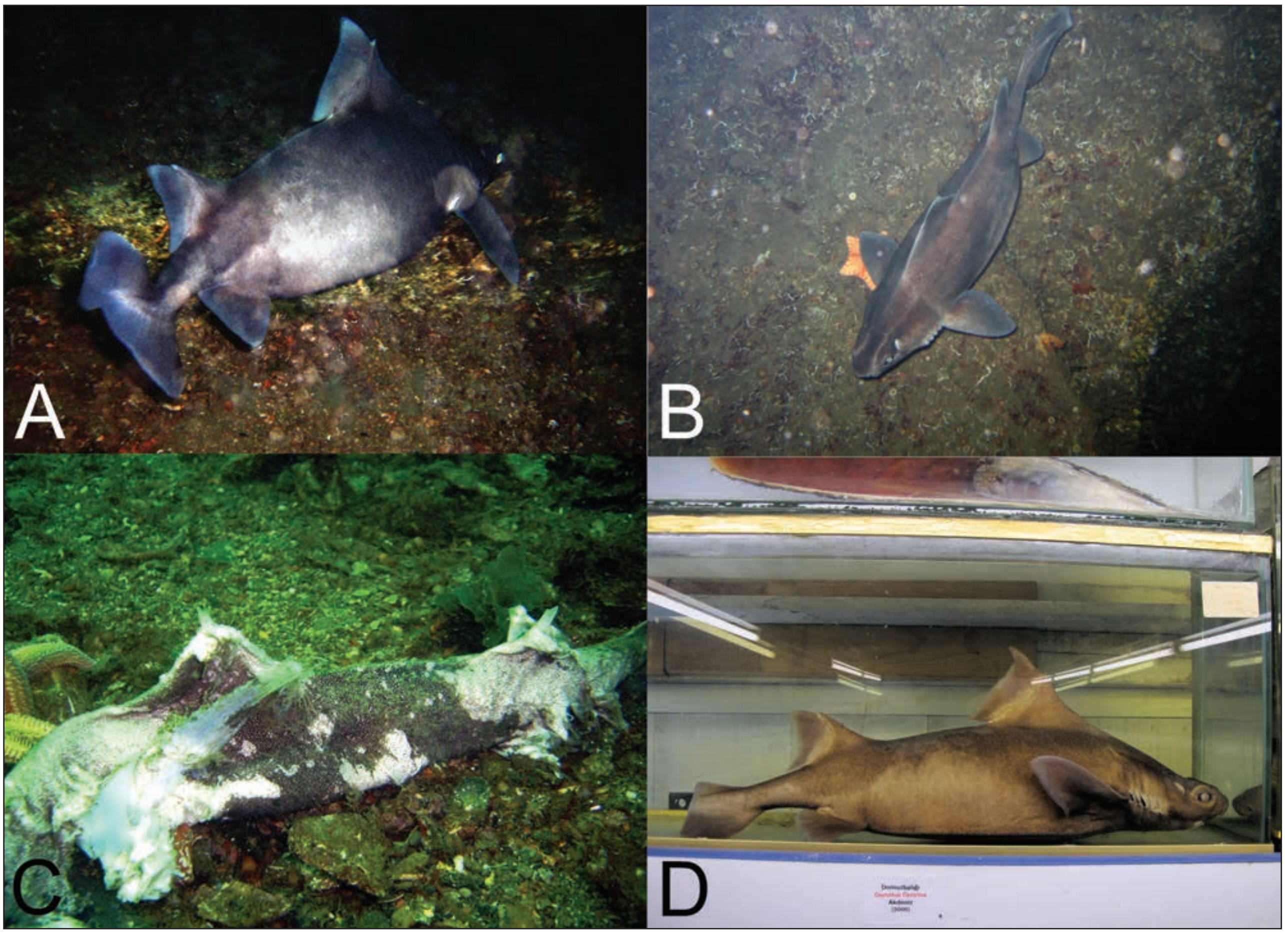

Fig. 3: Several specimens of *O. centrina* ***recorded in the seas of Turkey. (a) Specimen observed off Balıkçı Island (Sea of Marmara) on 27 September 2009 (sp No. 85, Tab. 1; photo: H. Kabasakal); (b) specimen observed off Balıkçı Island (Sea of Marmara) in 2006 (sp No. 26, Tab. 1; photo: C. Yıldırım); (c) specimen observed off Ahırkapı (Sea of Marmara) on 21 October 2012 (sp No. 88, Tab. 1; photo: H. Kabasakal); (d) specimen caught in Mediterranean off Turkey's coast in 2000 and stored in museum at Samatya Fishing Port (sp No. 16, Tab. 1; photo: H. Kabasakal).***
Sl. 3: Številni primerki morskega prašiča iz turških morij: (a) primerek, opažen 27. 9. 2009 pri otoku Balıkçı (Marmarsko morje) (primerek št. 85, Tab. 1; foto: H. Kabasakal), (b) primerek, opažen v letu 2006 pri otoku Balıkçı (Marmarsko morje) (primerek št. 23, Tab. 1; foto: C. Yıldırım), (c) primerek, opažen 21. 10. 2012 blizu lokalitete Ahırkapı (Marmarsko morje) (primerek št. 88, Tab. 1; foto: H. Kabasakal), (d) primerek, ujet na sredozemski turški obali leta 2000 in shranjen v muzeju Samatya Fishing Port (primerek št. 16, Tab. 1; foto: H. Kabasakal).

of evidence-based measures for the conservation, and even a ban on the fishing of *O. centrina*, promoting fishermen to release live specimens appears to be an urgent, feasible first step for the protection of this rare species.

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POJAVLJANJE MORSKEGA PRAŠIČA, *OXYNOTUS CENTRINA* (CHONDRICHTHYES: OXYNOTIDAE) V VZHODNEM SREDOZEMSKEM MORJU

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POVZETEK

Na temelju terenskih pregledov in razpoložljivih pisnih virov med koncem 19. stoletja in oktobrom 2012 je avtor pridobil podatke o pojavljanju 88 primerkov vrste Oxynotus centrina *(Linnaeus, 1758) iz Marmarskega, Egejskega in Sredozemskega morja. Štiriinšestdeset primerkov (72 %) izvira iz Egejskega morja, 19 (21,5 %) iz Marmarskega morja in 5 (5,6 %) iz Sredozemskega morja. Obravnavani primerki (obeh spolov) so merili od 22,5 do 79 cm telesne dolžine. Največkrat so se morski prašiči ujeli v pridneno kočo (n = 64, 72 %), nekajkrat v vlečno mrežo z gredjo (n = 3, 3,3 %) in zabodno mrežo (n = 3, 3,3 %) ter v enem primeru na parangal (1,1 %). Sedem primerkov so opazili pri opazovalnih cenzusih (podvodni popisi) v Marmarskem morju. Morski prašič je redka in ogrožena pridnena vrsta v vzhodnem Sredozemskem morju. Ulov oplojenih samic predstavlja znaten problem za preživetje te vrste. Pred implementacijo naravovarstvenih smernic ali celo prepovedjo lova na morske prašiče, bi bil še toliko bolj smiseln prvi korak k varovanju vrste; to je prepričevanje ribičev, da ujete primerke takoj po ulovu izpustijo.*

Ključne besede: Elasmobranchii, *Oxynotus centrina*, smrtnost, preživetje, varovanje, Sredozemsko morje

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RECENT RECORD OF THE GREAT WHITE SHARK, *CARCHARODON CARCHARIAS* (LINNAEUS, 1758), FROM CENTRAL AEGEAN SEA OFF TURKEY'S COAST

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ABSTRACT

On 19 September 2014, a great white shark was incidentally caught by a stationary net set off Yeni Foça (38° 46' 40" N, 26° 53' 40" E), which was deployed for lobster fishing. The great white shark measured approximately 200 cm and weighed 40 kg. It was a juvenile male with uncalcified claspers, which were shorter than the pelvic fins.

Key words: Great white shark, occurrence, eastern Mediterranean, juvenile, by-catch

RECENTI SEGNALAZIONI DEL GRANDE SQUALO BIANCO, *CARCHARODON CARCHARIAS* (LINNAEUS, 1758), NELL'EGEO CENTRALE AL LARGO DELLE COSTE DELLA TURCHIA

SINTESI

Il 19 settembre 2014 un grande squalo bianco è stato catturato accidentalmente da una rete da posta, utilizzata per la pesca dell'aragosta, al largo di Yeni Foça (38° 46' 40" N, 26° 53' 40" E). L'esemplare era lungo circa 200 cm e pesava 40 kg. Si trattava di un giovane maschio con i clasper (o pterigopodi) non calcificati e più corti delle pinne pelviche.

Parole chiave: grande squalo bianco, presenza, Mediterraneo orientale, stadio giovanile, catture accessorie

INTRODUCTION

The great white shark, *Carcharodon carcharias* (Linnaeus, 1758), has always been a point of human attraction since antiquity and a subject of research; therefore, significant data on the occurrence of the species has been collecting throughout the Mediterranean, of which the vast majority of data represents the specimens recorded in western and central parts of the basin.

In a recent inventory study, De Maddalena & Heim (2012) gave details of 596 records of *C. carcharias* from the entire Mediterranean and adjacent waters. Although some 20th century authors have reported on the presence of *C. carcharias* in Turkey's waters (Ninni, 1923; Deveciyan, 1926), a remarkable lack of knowledge has dominated the story of the great white shark in Turkey's seas for almost the entire 20th century. In a recent study, Kabasakal (2014) reported on the status of the great white shark in Turkey's waters. Based on available literature, the author provided up to date data on the historical and contemporary occurrence of 46 specimens of *C. carcharias*, which were either sighted or caught in Turkey's seas between 1881 and 2011.

Since the distributional map of this vulnerable shark in eastern Mediterranean has remarkable gaps, every record of the great white shark from the region, both in historical and contemporary perspectives, is a significant contribution to the understanding on different biological and ecological aspects of the species in the eastern Mediterranean. In the present article, the authors report on a recent record of *C. carcharias* off Turkey's coast of central Aegean Sea.

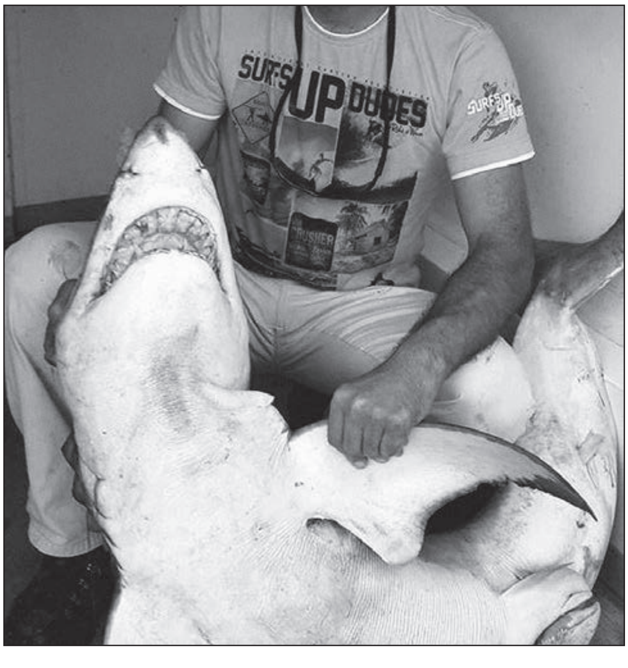

Fig. 1: Great white shark,* Carcharodon carcharias*, caught off Yeni Foça on 19 September 2014. Characteristical triangular teeth are seen on the upper jaw. (Photo: Ceyhun Gamze Ekinci, SAD)

Sl. 1: Beli morski volk,* Carcharodon carcharias*, ujet 19. septembra 2014 v vodah pri Yeni Foça. Značilni trikotni zobje so vidni v zgornji čeljusti. (Foto: Ceyhun Gamze Ekinci, SAD)

MATERIAL AND METHODS

On 19 September 2014, a great white shark (Fig. 1) was incidentally caught by a stationary net set off Yeni Foça (38°46′40″ N, 26°53′40″ E), which was deployed for lobster fishing. Total length of the great white shark was approximately 200 cm and weight was 40 kg. It was a juvenile male with uncalcified claspers, which were shorter than the pelvic fins. The photograph of the present specimen is kept in the archives of the Ichthyological Research Society.

RESULTS AND DISCUSSION

Species identification is based on the following descriptive characters (Bigelow & Schroeder, 1948): strong keels on both sides of caudal peduncle, strong and conical snout, triangular upper and lower teeth with regularly serrated edges, gill slits long but not encircling the head, upper and lower caudal lobe almost equal in length.

The occurrence of the great white shark in Turkey's Aegean waters date back to the mid-16th century (Belon, 1553). Although, Petri Belloni reported on the capture of a great white shark in waters off Izmir, a city on the Aegean seaboard of Turkey, with a short description of the specimen, its presence in the mentioned area has always been subject to debate, due to the lack of records from the region until the late 20th century (Kabasakal, 2014). Before the present record on a juvenile male, 13 specimens of *C. carcharias* were recorded off Turkey's coast of Aegean Sea, between 1991 and 2011 (Kabasakal, 2014). Eight of them were neonates and juveniles. Since 2008, 6 neonate great white sharks were incidentally captured in the waters of Edremit Bay (NE Aegean Sea), as well as 2 juveniles, which were entangled in commercial fishing gears in the vicinity of bay waters (Kabasakal, 2014). Based on the records of neonates and juveniles in the investigated region, the author suggested the area of Edremit Bay to be a possible nursery and breeding ground of *C. carcharias*. Thus, the recent capture of the juvenile great white shark in close vicinity to bay waters, provides new evidence supporting the presence of this suggested nursery ground.

Therefore, a monitoring program is required in order to figure out the seasonality of occurrences of neonates and juveniles in the studied region. At the very same time, the approximate borders of the nursery area should be defined. Since small scale coastal fishery continues all year round in Turkey's territorial waters, the proposed monitoring program is of critical importance to set regulations, such as banning coastal netting or long-lining inside the borders of this suggested nursery ground.

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NOVEJŠI ZAPIS O POJAVLJANJU VELIKEGA BELEGA VOLKA, *CARCHARODON CARCHARIAS* (LINNAEUS, 1758), IZ OSREDNJEGA EGEJSKEGA MORJA OB TURŠKI OBALI

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POVZETEK

Devetnajstega septembra 2014 je bil v stacionarno ribiško mrežo, ki se uporablja za lov jastogov, ujet primerek belega morskega volka v vodah ob Yeni Foça (38° 46′ 40″ N, 26° 53′ 40″ E). Beli morski volk je meril v dolžino približno 200 cm in tehtal 40 kg. Sodeč po nepoapnelih klasperjih, ki so bili krajši od trebušnih plavuti, je šlo za mladega samca.

Ključne besede: beli morski volk, pojavljanje, vzhodno Sredozemlje, mladostni primerek, prilov

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GROWTH OF THE INVASIVE COCKLE *FULVIA FRAGILIS* (MOLLUSCA: BIVALVIA) IN NORTHERN TUNISIA (CENTRAL MEDITERRANEAN)

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ABSTRACT

Fulvia fragilis *(Forsskål in Niebuhr, 1775), a non-indigenous species (NIS), has occurred in Tunisian coasts since 1994. Monthly and bimonthly sampling was conducted in marine and lagoon waters (of the Bay of Tunis and the Bizerte Lagoon, respectively) in Northern Tunisia. A total of 4,534 specimens were examined. Growth data were studied against environmental parameters (temperature, salinity, pH, dissolved oxygen and chlorophyll* a *concentration) and biotic parameters (reproductive cycle). This study showed significant differences between specimens from the two studied sites. Morphological differences were observed, with the specimens from the Bay of Tunis exhibiting a triangular shape as opposed to the elongated form featured in the specimens from the Bizerte Lagoon. Mass gain in the Bay of Tunis has been linked to the reproductive cycle and found at a disadvantage with respect to the shellfish length. In the Bizerte Lagoon, shell growth showed a reverse trend, probably due to pollution.*

Key words: *Fulvia fragilis*, growth, pollution, Bay of Tunis, Bizerte Lagoon

CRESCITA DEL CARDIDE INVASIVO *FULVIA FRAGILIS* (MOLLUSCA: BIVALVIA) NELLA TUNISIA SETTENTRIONALE (MEDITERRANEO CENTRALE)

SINTESI

Fulvia fragilis *(Forsskål in Niebuhr, 1775), specie non indigena (NIS), è presente lungo la costa tunisina dal 1994. Campionamenti mensili e bimestrali sono stati effettuati in acque marine (baia di Tunisi) e lagunari (Laguna di Biserta) nella Tunisia settentrionale. È stato esaminato un totale di 4534 individui. I dati riguardanti la crescita sono stati studiati in relazione ai parametri ambientali (temperatura, salinità, pH, ossigeno disciolto e concentrazione della clorofilla* a*) e a quelli biotici (ciclo riproduttivo). Questo studio ha evidenziato differenze significative tra gli individui dei due siti studiati. Tra le differenze morfologiche gli autori riportano una forma triangolare degli esemplari nella baia di Tunisi e una forma allungata di quelli nella Laguna di Biserta. L'aumento della massa nella baia di Tunisi è risultato legato al ciclo riproduttivo ed è stato svantaggiato rispetto alla lunghezza della conchiglia. Nella Laguna di Biserta, invece, la crescita della conchiglia ha mostrato un'inversione di tendenza, probabilmente a causa dell'inquinamento.*

Parole chiave: *Fulvia fragilis*, crescita, inquinamento, baia di Tunisi, Laguna di Biserta

INTRODUCTION

The invasive cockle *Fulvia fragilis* (Forsskål in Niebuhr, 1775), originating from the Indo-Pacific and commonly found in the Red Sea, has been observed in the Mediterranean since the beginning of the 20th century or, more precisely, since it was first collected in Port Said (Egypt) in 1939 (Moazzo, 1939). *F. fragilis* has been classified among the most widespread non-indigenous species (NIS) (Occhipinti-Ambrogi, 2014) and ranks among the worst invasive species (Galil *et al*., 2014) in the Mediterranean Sea. In Tunisia, the species was first discovered in the south, in the Gulf of Gabès (Passamonti, 1996), and has since spread northwards as far as the Bay of Tunis (Ben Souissi *et al*., 2003) and the Bizerte Lagoon (Zaouali, 2004).

The *F. fragilis*' relative growth was studied in the two mentioned sites, which are characterised by different ecological properties. The Bay of Tunis displays a satisfactory ecological status (Ayari & Afli, 2003), whereas the Bizerte Lagoon is heavily polluted (Yoshida *et al*., 2003; Trabelsi & Driss, 2009). The studies conducted both in the species' natural range and in its new habitats focussed on the biology of the species (Ozturk & Poutiers, 2005; Mohammad *et al.*, 2006; Rifi *et al*., 2011, 2012, *2015*), as well as its use as biomarker in the lagoon of Bizerte (Mahmoud et *al*., 2010). The morphometric variables identified have been correlated with endogenous (reproductive cycle) and exogenous (environmental parameters) factors at the two sampling sites.

This work was conducted in order to evaluate the acclimatization of this non-indigenous species in two different habitats (the Bay of Tunis and the Bizerte Lagoon) by studying its relative growth.

MATERIAL AND METHODS

Sampling

Preliminary investigations were carried out between 2004 and 2005 in 13 sites throughout the Tunisian coast (Fig. 1). Surveys conducted in shallow coastal waters (between 0 and 30 m of depth) were aimed at determining the distribution and the frequency of *Fulvia fragilis* in Tunisia. A seasonal density survey showed that *F. fragilis* was abundantly present throughout the year and therefore suitable for regular biological monitoring in two sites – the Bizerte Lagoon (site 3) and the Bay of Tunis (site 5) – and the two locations were then selected for this study.

The Bay of Tunis (Fig. 1) is located between 36° 42′ and 37° 10′ N, and 10° 17′ and 11° 37′ E, and represents the southern limit of the Strait of Sicily (Pérès, 1967). Also known as the 'small Gulf of Tunis', it covers a total area of 350 km² with an average depth of 15 m (Souissi *et al.,* 2000). Although the coastline of the

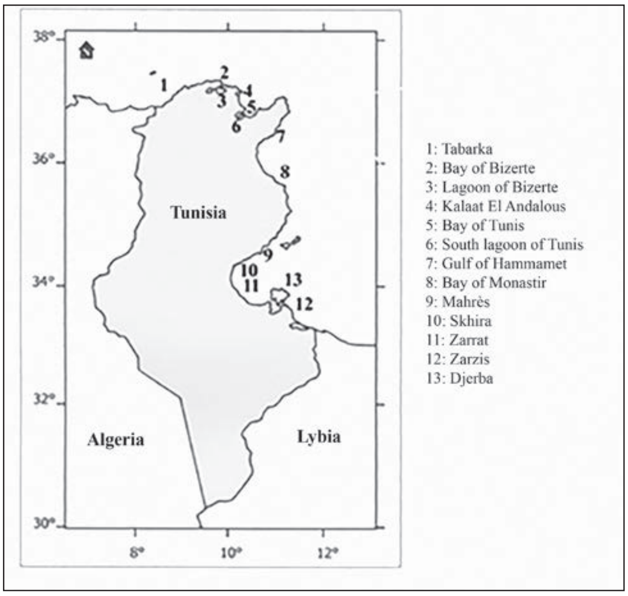

Fig. 1: Sampling sites
Sl. 1: Vzorčevalne postaje

Gulf of Tunis is exposed to anthropogenic pressures of various types (fishing, industry, urbanization, tourism, etc.), Ayari & Afli (2003), based on macrozoobenthos analysis, revealed it nevertheless remains a location of environmental value.

The Bizerte Lagoon (Fig. 1) is located in northern Tunisia between 37° 08′ and 37°14′ N and 9° 46′ and 9° 56′ E. This lagoon communicates with the Mediterranean Sea through an artificial channel, an elliptical depression of 128 km² with a maximum width of 11 km, a maximum length of 13 km and a 7 m average depth. It is surrounded by the cities of Bizerte, Zarzouna, Menzel Aberrahmen, Menzel Jemil and Menzel Bourguiba and is polluted because of several anthropogenic pressures. Organic pollution through chemical fertilizers and heavy metal contamination are mainly associated with disturbances to fauna and flora (Aissa, 1991) and biogeochemical cycles (Yoshida *et al*., 2002a, 2002b, 2003; Ben Garali *et al*., 2009, 2010, 2011).

In the Bay of Tunis, *F. fragilis* was sampled in the intertidal zone at very shallow depths (between 0.5 and 1.5 m) in sandy mud sediment. Samples were collected using dredges and scuba divers. Monthly and bimonthly samples were collected from January 2006 to October 2007, except in July, August and September 2006 due to summer mortality (Rifi *et al.,* 2012). A total of 2,893 specimens of *F. fragilis* were examined for the purpose of monitoring growth. In the Bizerte Lagoon, samples were collected in a muddy sediment at a depth of 5 m, employing experimental dredges hauled by a motorboat. Monthly and bimonthly surveys were conducted from June 2006 to September 2007, and 1,641 specimens were measured for the purpose of studying growth.

Environmental variables

The temperature of the sea surface was recorded by a thermometer with an accuracy of 0.1 °C and by a multi-type Lab (WTW, Multi/340i/SET) developed for measurements of other parameters, such as pH, salinity and dissolved oxygen. Seawater samples were kept in 1.5-litre opaque plastic bottles and transferred to the laboratory for a chlorophyll *a* (Chl *a*) concentration measurement employing the Aminot and Chaussepied method (Aminot & Chaussepied, 1983).

Relative growth

Specimens were measured for shell length (SL), shell height (SH) and shell width (SW) by a digital calliper to the nearest 0.01 mm. Moreover, total mass (TM), fresh meat mass (FMM) and dry meat mass (DMM) (obtained after oven drying at 60 °C for 72 hours) were measured with an electronic scale to the nearest 0.01 g.

Allometric relationships linking weight parameters to shell length were investigated. Linear regressions using the least-squares method with a logarithmic transformation were performed. The regression equation reads as follows:

$$\log(y) = \log(a) + b \log(x)$$

where y is a dependent variable representing the size or mass of some part or the entire cockle, x is an independent variable representing the reference parameter, a is the intercept and b is the slope.

To analyse the allometry, slope b was compared to the theoretical value of 1 when two linear measurements were analysed, and to the theoretical value of 3 when linear and mass parameters were considered.

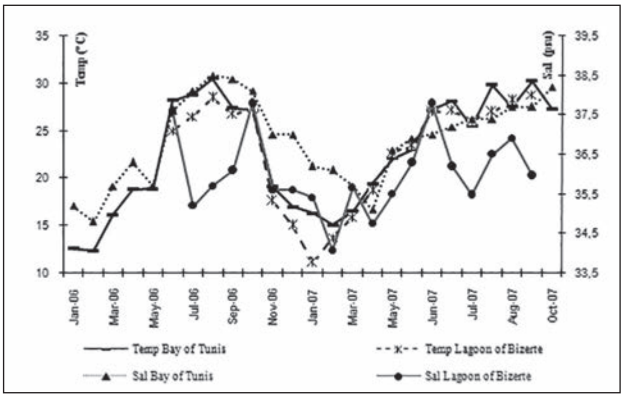

Fig. 2: Monthly evolution of the sea surface temperature (°C) and salinity (psu) in the Bay of Tunis (January 2006–October 2007) and in the Bizerte Lagoon (June 2006–September 2007).
Sl. 2: Površinska temperatura (°C) in slanost (psu) v Tuniškem zalivu (januar 2006–oktober 2007) in v Bizertski laguni (junij 2006–september 2007)

Data analysis

Stepwise regression was used to evaluate potential relationships between hydro-biological parameters (*i.e.*, temperature, Chl *a*, dissolved oxygen, salinity and pH) and the growth descriptors of *F. fragilis*. The differences in the environmental variables of the two sampling sites were tested by analysis of variance (ANOVA) ($p < 0.05$). The significance of the allometries was determined by a Student's *t*-test. All data analyses were carried out using Statgraphics Centurion software.

RESULTS

Environmental parameters

In the Bay of Tunis, the average sea surface temperature was 22.6±5.8 °C (between 12.4 °C and 30.5 °C). In the Bizerte Lagoon, this parameter ranged between 11.1 °C and 28.8 °C with an average of 23.0±5.7 °C. The mean salinity values were 36.4 ± 1.6 and 36±0.98 psu in the Bay of Tunis and in the Bizerte Lagoon, respectively (Fig. 2). Greater variations in salinity were observed in the Bizerte Lagoon due to high summer evaporation and freshwater inputs.

The recorded values of Chl *a* concentration in the Bay of Tunis were highly variable, with an average value of 3.03 μg/L and the lowest values registered in winter and early spring (Fig. 3). In the Bizerte Lagoon, this parameter varied less in the first months of the sampling, but from April 2007 onwards, there were significant increases, with a peak value (6.77 μg/L) reached in August 2007 (Fig. 3).

Average dissolved oxygen concentrations in the Bay of Tunis and the Bizerte Lagoon were 5.75 and 4.46 mg/L, respectively, with the lagoon being less oxygenated than the bay (Fig. 4). In the Bay of Tunis, the average pH was 8.20 (between 7.89 and 8.34), whereas in the Bizerte Lagoon this pollution indicator parameter ranged between 7.31 and 8.42, showing major environmental disruption (Fig. 4).

Statistical analysis showed significant differences between the salinity, the dissolved oxygen concentration and the pH of the two sampling sites, with a confidence level of 95 % (ANOVA, $p < 0.05$).

Relative growth

In the Bay of Tunis, fresh and dry meat mass were positively correlated with the Chl *a* concentration, while in the Bizerte Lagoon, the shell thickness and dry meat mass were negatively correlated with pH (Tab. 1).

The global allometry combining SH and SL was negative (b < 1) for the entire sample from the Bay of

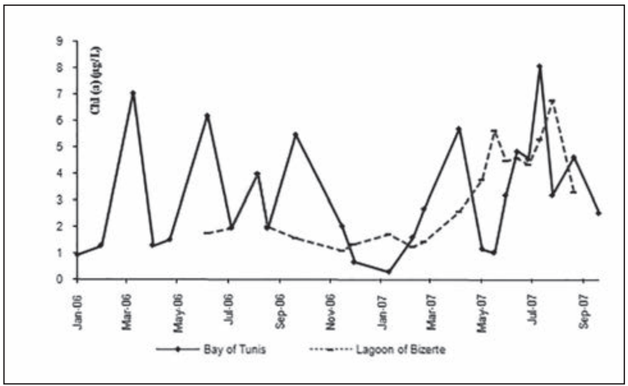

Fig. 3: Monthly evolution of Chl** a **concentration (µg/L) in the Bay of Tunis (January 2006–October 2007) and in the Bizerte Lagoon (June 2006–September 2007).
Sl. 3: Koncentracije klorofila** a **(µg/L) v Tuniškem zalivu (januar 2006–oktober 2007) in v Bizertski laguni (junij 2006–september 2007)

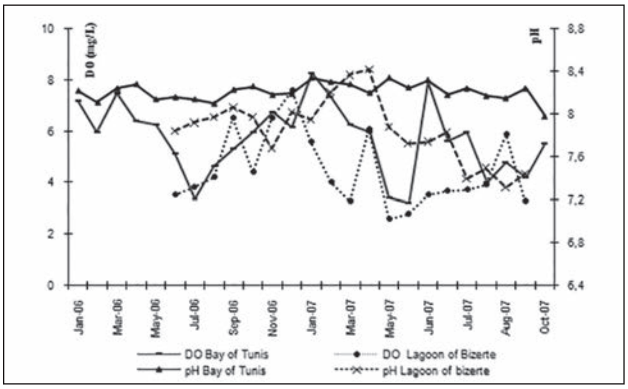

Fig. 4: Monthly variations of dissolved oxygen concentration (mg/L) and pH in the Bay of Tunis (January 2006–October 2007) and in the Bizerte Lagoon (June 2006–September 2007).
Sl. 4: Mesečna nihanja koncentracije raztopljenega kisika (mg/L) in pH v Tuniškem zalivu (januar 2006–oktober 2007) in v Bizertski laguni (junij 2006–september 2007)

Tunis and positive (b > 1) in the Bizerte Lagoon cockles. These results showed that the growth at the first site was generally faster in length, while at the second it was usually faster in height (Tab. 2). Regressions involving ST and SH to SL indicated that the shell grew more in length and height than in thickness in both sites (Tab. 2). The coefficient of determination R^2 for these three relationships displayed high values.

Tab. 1: Parameters of multilinear regression between morphometric parameters of** F. fragilis **and environmental variables in the two sampling sites (Bay of Tunis and Bizerte Lagoon). Legend: Chl** a **- chlorophyll** a **(µg/L), DMM - Dry Meat Mass (mg), FMM - Fresh Meat Mass (mg), DSM - Dry Shell Mass (mg), ST - Shell Thickness (mm), Temp - temperature (°C).
Tab. 1: Multilinearna regresija med morfometričnimi parametri školjke** F. fragilis **in okoljskimi spremenljivkami na dveh vzorčevalnih lokalitetah (Tuniški zaliv, Bizertska laguna). Legenda: Chl** a **- klorofil** a **(µg/L), DMM - suha masa mesa (mg), FMM - sveža masa mesa (mg), DSM - suha masa lupine (mg), ST - debelina lupine (mm), Temp - temperatura (°C).

Sampling site	Descriptor	Model
Bay of Tunis	FMM	FMM = cte + 0.27 Chl *a*
	DMM	DMM = cte + 0.04 Chl *a*
	DSM	DSM= cte + 0.15 Temp
Lagoon of Bizerte	ST	ST = cte - 4.00 pH
	DMM	DMM = cte - 0.14 pH
	DSM	DSM = cte - 2.14 pH

Regressions linking mass parameters to SL revealed negative allometries in the Bay of Tunis. Thus, *F. fragilis'* increase in shell length was less rapid than its mass gain (total and different parts) (Tab. 3). In the Bizerte Lagoon, the global regressions between TM, FMM, DMM and SL showed negative allometries (Tab. 3).

In cockles from the Bay of Tunis, the monthly regressions combining mass parameters to length presented mainly positive allometries reflecting a faster shell length growth than mass gain (total mass and different parts) (Tab. 4). The monthly coefficient of determination R^2 relating the total mass, the fresh and dry soft tissue mass to shell length showed low values in the months of high gonadal activity. It increased in months characterized by gametic emissions, especially in May and November 2006 (Rifi *et al*, 2011) (Tab. 4).

In the Bizerte Lagoon, monthly allometries between total mass and shell length suggested a faster SL growth than total mass gain (Tab. 5). Moreover, the allometries linking the soft tissue masses (fresh and dry) to shell length were generally positive, indicating a slower shell length growth than soft tissue mass gain (Tab. 5). The coefficient of determination R^2 was often relatively high, reflecting strong inter-individual variations (Tab. 5).

DISCUSSION

Growth is one of the most used measures of animal vitality. In this context, Dame (1972) suggested that '*Allometric relationships are often developed between shell parameters and body weight in order to nondestructively*

Tab. 2: Parameters of allometric regression models (Shell Height (SH) – Shell Length (SL), Shell Thickness (ST) – Shell Length (SL), Shell Thickness (ST) – Shell Height (SH) measured in mm) corresponding to the samples of* F. fragilis *collected from the Bay of Tunis and the Bizerte Lagoon. R^2: coefficient of determination;* t*-test, **: significant, NS: not significant for the confidence interval of 95 %.

Tab. 2: Modeli alometrične regresije (višina lupine (SH) – dolžina lupine (SL), debelina lupine (ST) – dolžina lupine (SL), debelina lupine (ST) – višina lupine (SH), izražene v mm) vzorcev školjke* F. fragilis *iz Tuniškega zaliva in Bizertske lagune. R^2: koeficient determinacije;* t*-test, **: značilna razlika, NS: razlika ni značilna na nivoju 95-% intervala zaupanja.

Sampling site	Relation	N	log(a)	b	R^2	t-test	Allometry
Bay of Tunis	SH-SL	2893	0.38	0.88	0.83	**	negative
	ST-SL		0.01	0.88	0.74	**	negative
	ST-SH		-0.08	0.91	0.75	**	negative
Lagoon of Bizerte	SH-SL	1641	-0.20	1.04	0.94	**	positive
	ST-SL		0.11	0.86	0.77	**	negative
	ST-SH		0.33	0.80	0.79	**	negative

estimate soft body biomass in living bivalves'. The study of the *Fulvia fragilis*' relative growth is crucial to understanding the growth of the different parts of the cockle, in relation to environmental variables (hydro-biological conditions, pollution, etc.) and during its reproductive cycle. Our results showed remarkable differences by site. This method has been used for many decades in bivalves because of its reproducibility that allows a comparison between multiple sampling sites (*e.g.*, Seed, 1973; Bejaoui, 1998; Gimin *et al.*, 2004; Aragon-Noriega *et al.*, 2007). Such results can contribute to the management of this potentially exploitable species and to a better understanding of its bioinvasion.

Morphometric allometries in *F. fragilis* at the two sampling sites attested that the cockle shell growth was different. The global regression of height-length was

Tab. 3: Parameters of allometric regression models (Total Mass (TM) – Shell Length (SL), Fresh Meat Mass (FMM – Shell Length (SL), Dry Meat Mass (DMM) – Shell Length (SL), Dry Shell Mass (DSM) – Shell Length (SL)) corresponding to the samples of* F.fragilis *collected from the Bay of Tunis and the Bizerte Lagoon. R^2: coefficient of determination;* t*-test, **: significant, NS: not significant for the confidence interval of 95 %.

Tab. 3: Modeli alometrične regresije (celokupna masa (TM) – dolžina lupine (SL), sveža masa mesa (FMM) – dolžina lupine (SL), suha masa mesa (DMM) – dolžina lupine (SL), suha masa lupine (DSM) – dolžina lupine (SL)) vzorcev školjke* F. fragilis *iz Tuniškega zaliva in Bizertske lagune. R^2: koeficient determinacije;* t*-test, **: značilna razlika, NS: razlika ni značilna na nivoju 95-% intervala zaupanja.

Sampling site	Biometric relationships	log(a)	b	R^2	t-test	Allometry
Bay of Tunis	TM-SL	-5.85	2.33	0.62	**	negative
	FMM-SL	-7.93	2.54	0.62	**	negative
	DMM-SL	-10.12	2.59	0.42	**	negative
	DSM-SL	-7.78	2.55	0.71	**	negative
Lagoon of Bizerte	TM-SL	-7.396	2.72	0.70	**	negative
	FMM-SL	-7.975	2.504	0.68	**	negative
	DMM-SL	-9.375	2.266	0.44	**	negative
	DSM-SL	-9.792	3.060	0.84	**	positive

negative in the Bay of Tunis and positive in the Bizerte Lagoon. *F. fragilis'* shell presents a triangular shape in the Bay of Tunis samples and an elongated form in the Bizerte Lagoon specimens. This morphological difference could be explained by endogenous (gametogenesis) and exogenous (nature of the sediment) factors. Previous work conducted by Rifi *et al*. (2011) confirmed that *F. fragilis* is a simultaneous hermaphroditic species with a diffuse gonad extending between the digestive gland and the pedal constriction. These authors described a maturation scale partitioned into eight stages: sexual rest (0), initiation of gametogenesis (1), advanced ga-

Tab. 4: Monthly parameters of allometric regression models (Total Mass (TM) – Shell Length (SL), Fresh Meat Mass (FMM) – Shell Length (SL), Dry Meat Mass (DMM) – Shell Length (SL)), Bay of Tunis, January 2006–October 2007. All morphometric parameter ranges are in mm and all masses are in mg. In bold are values, where b > 3. I: first half of the month; II: second half of the month; N: monthly individual number; R²: coefficient of determination; t-test, **: significant, NS: not significant for the confidence interval of 95 %.

Tab. 4: Mesečna nihanja modelov alometrične regresije (celokupna masa (TM) – dolžina lupine (SL), sveža masa mesa (FMM) – dolžina lupine (SL), suha masa mesa (DMM) – dolžina lupine (SL)) vzorcev školjke *F. fragilis* ***iz Tuniškega zaliva (januar 2006–oktober 2007). Vsi morfometrični parametri so v mm in vse mase v mg. Vrednosti b > 3 so v krepkem tekstu. I: prva polovica meseca; II: druga polovica meseca; N: število osebkov na mesec; R²: koeficient determinacije; t-test, **: značilna razlika, NS: razlika ni značilna na nivoju 95-% intervala zaupanja.***

Month	TM-SL				FMM-SL				DMM-SL			
	log(a)	b	R^2	*t*-test	log(a)	b	R^2	*t*-test	log(a)	b	R^2	*t*-test
Jan 06	-5.35	2.23	0.61	**	-4.90	1.70	0.49	**	-6.87	1.67	0.20	**
Feb 06	-6.93	2.64	0.60	**	-8.97	2.84	0.60	**	-8.89	2.18	0.38	**
Mar 06	-7.42	2.77	0.83	**	-7.66	2.53	0.81	**	-9.88	2.55	0.57	**
Apr 06	-5.97	2.30	0.31	**	-5.77	1.91	0.28	**	-6.63	1.54	0.10	**
May 06	-9.86	**3.42**	0.89	**	-12.5	**3.80**	0.92	**	-14.4	**3.72**	0.83	**
June 06	-4.71	2.02	0.29	**	-5.51	1.95	0.42	**	-9.49	2.48	0.31	**
Oct 06	-7.96	2.92	0.93	**	-10.8	**3.40**	0.96	**	-14.05	**3.79**	0.87	**
Nov 06	-7.839	2.82	0.89	**	-10.3	**3.25**	0.91	**	-13.58	**3.62**	0.80	**
Dec 06	-8.16	2.99	0.62	**	-11.04	**3.43**	0.83	**	-13.95	**3.66**	0.72	**
Jan 07	-5.35	2.23	0.61	**	-6.48	2.04	0.40	**	-8.95	2.15	0.40	**
Feb 07	-3.30	1.55	0.22	**	-8.97	2.84	0.60	**	-8.74	2.11	0.30	**
Mar 07	-6.104	2.40	0.63	**	-8.20	2.64	0.64	**	-9.88	2.55	0.57	**
Apr 07	-4.035	1.87	0.46	**	-5.08	1.83	0.40	**	-7.58	2.00	0.28	**
May I 07	-8.043	2.97	0.79	**	-10.2	**3.20**	0.67	**	-13.60	**3.58**	0.50	**
May II 07	-6.45	2.51	0.69	**	-9.15	2.88	0.58	**	-12.04	**3.14**	0.45	**
June I 07	-8.377	**3.03**	0.74	**	-10.7	**3.28**	0.68	**	-11.49	**3.03**	0.39	**
June II 07	-1.986	1.22	0.23	**	-3.30	1.27	0.19	**	-5.80	1.40	0.14	**
July I 07	-7.98	0.99	0.22	**	-2.18	0.97	0.20	**	-6.14	1.57	0.20	**
July II 07	0.100	0.78	0.27	**	-1.38	0.82	0.20	**	-3.97	1.00	0.17	**
Aug 07	-5.79	2.35	0.55	**	-8.15	2.60	0.49	**	-10.16	2.62	0.19	**
Sept 07	-6.78	2.52	0.52	**	-9.39	2.89	0.65	**	-2.22	0.39	0.11	N.S
Oct 07	-0.70	0.91	0.11	N.S	-1.00	0.69	0.32	N.S	-3.26	0.56	0.11	N.S

metogenesis (2), maturity (3A), partial spawning (3B1), advanced spawning (3B2), restoration (3C) and spent (3D). Moreover, the species breed differently in the two study sites. In the Bay of Tunis, where conditions were favourable, *F. fragilis* showed a continuous spawning activity, rare in winter and with seasonal peaks the rest of the year. Spring peaks were thus noted in May 2006 and May 2007, summer peaks in June 2006, June 2007 and August 2007, and an autumn peak in November 2006 (Rifi *et al.,* 2011, 2015). Conversely, in the Bizerte Lagoon, a very low gonadic activity with a prevalence of the sexual rest stage was observed (Rifi *et al.*, 2015). These reproductive abnormalities could be explained by the vast extent of pollution (Rifi *et al.*, 2015). Indeed, in the Bay of Tunis, this species displays a continuous reproductive activity and the position of the gonad in the animal's foot requires the shell to grow in length. The nature of the sediment could also explain this morphological difference between the two sampling sites. The sandy-mud sediment in the Bay of Tunis allows for the *F. fragilis* surface and sub-surface alimentation. Conversely, the muddy sediment of the Bizerte Lagoon explains the shell's height growth, which facilitates the animal's alimentation with its short siphons. Some authors had already highlighted similar morphological adaptations of some bivalves, such as *Donax trunculus* and *Panopea globosa*, to their biotopes (Gaspar *et al.*, 2002; Aragon-Noriega *et al.*, 2007).

In the Bay of Tunis, allometries linking soft tissue mass (fresh or dry) to shellfish length revealed a less

Tab. 5: Monthly parameters of allometric regression models (Total Mass (TM) – Shell Length (SL), Fresh Meat Mass (FMM) – Shell Length (SL), Dry Meat Mass (DMM) – Shell Length (SL)), Bizerte Lagoon, June 2006–September 2007. All morphometric parameter ranges are in mm and all masses are in mg. In bold are values, where b > 3. N: monthly individual number; R²: coefficient of determination; t-test, **: significant, NS: not significant for the confidence interval of 95 %.

Tab. 5: Mesečna nihanja modelov alometrične regresije (celokupna masa (TM) – dolžina lupine (SL), sveža masa mesa (FMM) – dolžina lupine (SL), suha masa mesa (DMM) – dolžina lupine (SL)) vzorcev školjke** F. fragilis **iz Bizertske lagune (junij 2006–september 2007). Vsi morfometrični parametri so v mm in vse mase v mg. Vrednosti b > 3 so v krepkem tekstu. N: število osebkov na mesec; R²: koeficient determinacije; t-test, **: značilna razlika, NS: razlika ni značilna na nivoju 95-% intervala zaupanja.

Month	TM-SL				FMM-SL				DMM-SL			
	log(a)	b	R²	t-test	log(a)	b	R²	t-test	log(a)	b	R²	t-test
June 06	-7.49	2.73	0.63	**	-7.06	2.20	0.43	**	-10.73	2.66	0.43	**
July 06	-4.22	1.82	0.36	**	-5.96	1.92	0.57	**	-5.21	1.80	0.46	**
Aug 06	-7.14	2.64	0.43	**	-10.66	**3.31**	0.73	**	-11.14	2.81	0.42	**
Sept 06	-8.99	**3.16**	0.69	**	-10.69	**3.28**	0.75	**	-12.04	**3.06**	0.60	**
Oct 06	-9.16	**3.23**	0.80	**	-10.25	**3.18**	0.71	**	-15.25	**3.96**	0.74	**
Nov 06	-8.68	**3.11**	0.82	**	-10.66	**3.28**	0.73	**	-12.50	**3.24**	0.58	**
Dec 06	-8.28	2.98	0.79	**	-11.54	**3.51**	0.72	**	-15.22	**3.90**	0.64	**
Jan 07	-9.35	**3.28**	0.65	**	-10.88	**3.30**	0.78	**	-15.48	**3.88**	0.58	**
Feb 07	-8.30	2.97	0.53	**	-10.77	**3.26**	0.73	**	-13.82	**3.41**	0.63	**
Mar 07	-7.14	2.65	0.60	**	-8.77	2.70	0.57	**	-10.02	2.39	0.49	**
Apr 07	-7.81	2.81	0.39	**	-10.04	**3.02**	0.62	**	-13.76	**3.40**	0.55	**
May 07	-8.75	**3.10**	0.60	**	-9.965	**3.02**	0.59	**	-9.53	2.30	0.27	**
June 07	-8.16	2.93	0.44	**	-11.14	**3.31**	0.49	**	-15.55	**3.88**	0.48	**
July 07	-9.18	**3.08**	0.61	**	-11.05	**3.25**	0.60	**	-17.44	**4.36**	0.45	**
Aug 07	-5.71	2.32	0.40	**	-8.58	2.74	0.58	**	-8.56	2.08	0.32	**
Sept 07	-5.29	2.16	0.70	**	-9.105	2.82	0.60	**	-9.12	2.19	0.36	**

important mass gain compared to shell length. However, an opposite trend coinciding with gamete production (at spawning stages, restoration and initiation of gametogenesis) and the sexual rest period was observed. It was demonstrated that mass increase in some bivalves was related to gonadic mass, which represents a major share of the visceral mass (Bayne & Worrall, 1980; Alunno-Bruscia *et al.*, 2001). Otherwise, in *F. fragilis*, during the sexual rest and especially in January and February 2007, the tissue growth probably resulted from gametes transforming to food reserves (Rifi *et al.*, 2011). This phenomenon has already been described in some bivalves (Thompson, 1979; D'Orange *et al.*, 1989; Rodríguez *et al.*, 2003). In addition, throughout our study period, except for winter months (from December to February), we recorded relatively high Chl *a* concentrations, which in part explained the growth of soft tissue. This observation was verified through stepwise regression and corroborated a positive correlation between flesh mass and Chl *a* concentration in samples from the Bay of Tunis. This phenomenon has already been described in other bivalve species (Ansell, 1974; Brown, 1988; Dridi *et al.*, 2008).

In the bay of Tunis, during gamete emission periods, *F. fragilis* has a synchronous growth in soft tissue and shell. In bivalves, the shell and flesh growth depend on different nutrient sources. The soft tissue growth depends on the seasonal nutrient cycle and reproduction strategies of storing food reserves, while the shell growth depends partially on metabolic carbon and occurs mainly during the deposition of materials in the water column (Borrero & Hilbish, 1988; Alunno-Bruscia *et al.,* 2001). In general, bivalves have a delayed growth of flesh and shell. Besides, in some species of bivalves (as *Mytilus edulis* and *Geukensia demissa*), the shell and flesh growths were not synchronous (Hilbish, 1986; Borrero & Hilbish, 1988; Alunno-Bruscia *et al.*, 2001). Hilbish (1986) reported that *M. edulis* shell growth preceded the flesh gain. Thompson (1979) explained that this phenomenon was an adaptive strategy to increase the volume in anticipation of the flesh growth.

The coefficient of determination R^2 between the fresh and dry flesh masses versus shell length has generally high values, indicating a strong relationship. However, this ratio decreased during spring and summer successive spawn periods extending from March 2007 till the end of August 2007 (Rifi *et al.*, 2011). The same trend has been described in *Crassostrea gigas* (Dridi *et al.*, 2008). High inter-individual variations were also observed in October 2007, probably due to high mortalities. The large inter-individual variation in *F. fragilis* was probably accentuated by its weakness after successive spawning episodes.

In the Bizerte Lagoon, regressions between flesh masses (fresh and dry) and shell length revealed that the *F. fragilis'* flesh mass usually developed faster than shellfish length. These results show that the growth of the non-native cockle at this location is directly opposed to that registered at the Bay of Tunis site. The differences in growth could be attributed to environmental factors; they are likely related to pH values being statistically lower than in the Bay of Tunis (ANOVA, $p < 0.05$). Indeed, the shellfish growth was probably affected by pollution. Several authors (Gazeau *et al.*, 2007; Kurihara *et al.*, 2007; Guinotte & Fabry, 2008; Portner, 2008) have shown that low pH values have several negative effects, particularly inhibition of shell calcification. In addition, in some species a decline in shell growth was explained by chemical pollution (Thain, 1984).

The monthly coefficients of determination on regressions between the total mass, fresh and dry flesh mass to shellfish length in the population of *F. fragilis* from the Bizerte Lagoon were relatively low in the warmer period, reflecting high inter-individual variations. These variations could be explained by increased levels of pollutants, especially chemical contaminants, and low dissolved oxygen concentrations. Several studies have showed that the availability of dissolved oxygen in water is a limiting factor for molluscs' growth (Baker & Mann, 1992; Harris *et al.*, 1999; McDowell *et al.*, 1999; Wilson & Burnett, 2000). McDowell *et al.* (1999) demonstrated that some bivalves have biological reactions, including allocation of nutrients and biosynthetic processes following exposure to certain contaminants.

To conclude, the growth of the allochthonous cockle *F. fragilis* at the two selected sites revealed major differences. The nature of the allometric regression associated with metric variables changing from one site to another demonstrates a more elongated shellfish in the Bizerte Lagoon. In specimens of *F. fragilis* collected in the Bay of Tunis, monthly relationships involving masses with shell length depend on the progress of gametogenesis. Indeed, the growth in mass is higher in the months of sexual activity. Moreover, the growth in shellfish and flesh masses was synchronous in favourable conditions. In the Bizerte Lagoon, the sampling site characterized by a very low reproductive activity, the fresh and dry flesh mass growth depended essentially on the availability of nutrients. In this lagoon, the increase in shellfish length could have been affected by the "acidification" of water. This phenomenon, caused by pollution, compromised the shell calcification (Gazeau *et al.*, 2007; Guinotte & Fabry, 2008; Kurihara, 2008; Portner, 2008).

In the Bay of Tunis and the Bizerte Lagoon, the high inter-individual variations in mass and length in *F. fragilis* implied by the low coefficients of determination occur in the months of high sexual activity (gametic production) and in warmer months, which correspond to peak pollution period, respectively.

These results imply that the mode of reproduction of *F. fragilis* facilitated its spreading beyond its geographical area, but this cockle shows signs of vulnerability especially in highly polluted environments, such as the Bizerte Lagoon. It is noteworthy that we were forced to stop our sampling in October 2007 in the Bay of Tunis and in September 2007 in the Bizerte Lagoon following massive mortality and scarcity of the studied species. Recent sampling has shown, on the one hand, a massive reappearance of this species in the Bay of Tunis, and on the other, its absence from the Bizerte Lagoon.

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RAST INVAZIVNE ŠKOLJKE *FULVIA FRAGILIS* (MOLLUSCA: BIVALVIA) IZ SEVERNE TUNIZIJE (OSREDNJI MEDITERAN)

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POVZETEK

Tujerodna vrsta školjke Fulvia fragilis *(Forsskål in Niebuhr, 1775) se vzdolž tunizijske obale pojavlja že od leta 1994. Mesečna in dvomesečna vzorčenja te vrste so bila opravljena v morskih (Tuniški zaliv) in lagunskih vodah (Bizerta) v severni Tuniziji. Avtorji so pregledali skupno 4.534 osebkov. Raziskovali so odnos med rastnimi podatki in okoljskimi parametri (temperatura, slanost, pH, raztopljeni kisik in koncentracija klorofila* a*) ter biotskimi parametri (razmnoževalni cikel). Pokazale so se značilne razlike med obema obravnavanima lokalitetama. Očitne so bile tudi morfološke razlike med obema lokalitetama, saj so bile lupine trikotne v tuniškem zalivu in podolgovate v Bizertski laguni. Biomasni prirastek v Tuniškem zalivu je bil povezan z razmnoževalnim ciklom in je bil nižji v primerjavi z dolžino lupine. V Bizertski laguni pa je bil obraten trend rasti školjk, najverjetneje zaradi onesnaževanja okolja.*

Ključne besede: *Fulvia fragilis*, rast, onesnaževanje, Tuniški zaliv, Bizertska laguna

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FIRST RECORD OF THE CHINESE MITTEN CRAB (*ERIOCHEIR SINENSIS*) IN THE LAGOON OF MARANO AND GRADO (NORTHERN ADRIATIC SEA)

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ABSTRACT

*The Chinese mitten crab (*Eriocheir sinensis*, H. Milne Edwards, 1853) is native to the Far East and represents one of the most invasive alien species. The aim of the paper is to report the first record of this species in the Lagoon of Marano and Grado, which is also the third record in the northern Adriatic, with a brief overview on the introduction, distribution and the life span of this species in the European waters.*

Key words: *Eriocheir sinensis*, alien species, Lagoon of Marano and Grado, northern Adriatic Sea

PRIMA SEGNALAZIONE DEL GRANCHIO CINESE (*ERIOCHEIR SINENSIS*) NELLA LAGUNA DI MARANO E GRADO (ALTO ADRIATICO)

SINTESI

*Il granchio cinese (*Eriocheir sinensis*, H. Milne Edwards, 1853) è originario dell'Estremo Oriente e rappresenta una delle specie aliene più invasive. Lo scopo del presente articolo è di riportare la prima segnalazione di questa specie nella Laguna di Marano e Grado, la quale è anche la terza segnalazione per l'area dell'Alto Adriatico, con un breve resoconto sull'introduzione, distribuzione e ciclo vitale di questa specie nelle acque europee.*

Parole chiave: *Eriocheir sinensis*, specie aliene, Laguna di Marano e Grado, Alto Adriatico

INTRODUCTION

The Chinese mitten crab (*Eriocheir sinensis*, H. Milne Edwards, 1853) is native to the Far East, where its range extends from Hong Kong to the border with North Korea (Hymanson *et al*., 1999). This species has two likely pathways of introduction: through ballast waters release (Peters, 1933; Cohen & Carlton, 1997) and escapes or deliberate releases associated with the transport of this valued aquaculture species (Herborg *et al*., 2005). The Chinese mitten crab supports a $1.25 billion per annum aquaculture industry in China, supplying local and international markets with live animals (Hymanson *et al*., 1999). *E. sinensis* is catadromous, spending most of its life in fresh-waters, and only returning to estuaries to reproduce, whereupon it dies (Herborg *et al*., 2005). The downstream migrations of sexually mature crabs are well known in the rivers Elbe and Weser (Germany) (Peters, 1933, 1938a) and in the River Thames (United Kingdom) (Robbins *et al*., 2000). The Chinese mitten crab is listed as one of "100 of the world's worst invasive alien species" (DAISIE, 2015). The aim of the paper is to report the first record of this species in the Lagoon of Marano and Grado, which is also the third record in the northern Adriatic, with a brief overview on the introduction, distribution and the life span of this species in the European waters.

MATERIAL AND METHODS

The Marano and Grado Lagoon (northern Adriatic Sea, Italy) is an extremely important wetland. It extends approximately 32 km reaching up to 5 km of width for a total area of 160 km^2, between the Tagliamento and Isonzo River deltas (Fontolan *et al*., 2012). The drainage basin (~1880 km^2) delivers important loads of both nutrients and pollutants (Covelli *et al*., 2012; Saccon *et al*., 2013). Since 1971, the Lagoon is protected by the Ramsar Convention. Following the implementation of the Habitats Directive (Council Directive 92/43/EEC), it was also identified as a Site of Community Importance (SCIs – IT3320037) within Natura 2000 network. The area hosts economic, tourism and industrial services, with fishing, clam harvesting (mainly the Manila clam, *Ruditapes philippinarum* Adams & Reeve, 1850) and fish-farming comprising the most important resources for local inhabitants (Bettoso *et al*., 2013).

Fig. 1: Map of the records of* E. sinensis *in the northern Adriatic Sea: (1) Mizzan (2005), (2) Fiorin* et al. *(2013), (3) Marano and Grado Lagoon (present study).
Sl. 1: Dosedanje najdbe vrste* E. sinensis *v severnem Jadranu: (1) Mizzan (2005), (2) Fiorin* et al. *(2013), (3) maranska in gradeška laguna (pričujoča študija)

One specimen of *Eriocheir sinensis* was caught on 20 November 2014, using the fyke nets, at a depth of approximately 1 m, on muddy bottom. The specimen was determined and measured to the nearest millimetre and weighed to the nearest gram. It was dry preserved and deposited in the Invertebrate Collection of the Museo Friulano di Storia Naturale (Udine), under accession number ZI-05279.

RESULTS AND DISCUSSION

The capture site of *Eriocheir sinensis* was located off the mouth of Stella river in the Lagoon of Marano and Grado (45° 43′ 29″ N, 013° 05′ 48″ E) (Fig. 1). The specimen caught was a male with a carapace width of 75 mm, carapace length 67 mm and the wet weight of 202 g (Fig. 2). This is similar to the previous record in the Venice Lagoon where in June 2013 a male with a carapace width of 61.1 mm was caught in the inner area of the northern lagoon (45° 32′ 07″ N, 012° 27′ 01″ E) (Fiorin *et al*., 2013). The square shaped carapace clearly distinguishes this alien species from other native European brachyuran crabs. It can reach a carapace width of 5 to 7cm with the maximum of approximately 10 cm (Czerniejewski *et al*., 2003). One key identification feature is the hair-like covering on the claws, which is especially well developed in male individuals. The colour varies from yellow to brown, rarely purple.

The first record in the Venice Lagoon arises to May 2005, when a specimen was collected in the central part of the basin (Mizzan, 2005), but its first appearance into the Mediterranean Sea arose to 1959 in the Narbonne littoral lagoons (France) (Petit, 1960).

The first report of a Chinese mitten crab in continental Europe was in a tributary of the river Weser in North West Germany in 1912 and in the river Elbe in 1914. No further range expansion along the European coast was reported until 1927. In the following 10 years the species dispersed rapidly in a westerly direction along the North Sea and English Channel coast and into northern France as far as St. Malo (1954) (André, 1954). There was an average rate of spread of 441 km per year during the peak period of 1927–1931. In 1954, *E. sinensis* arrived at the West coast of France and in

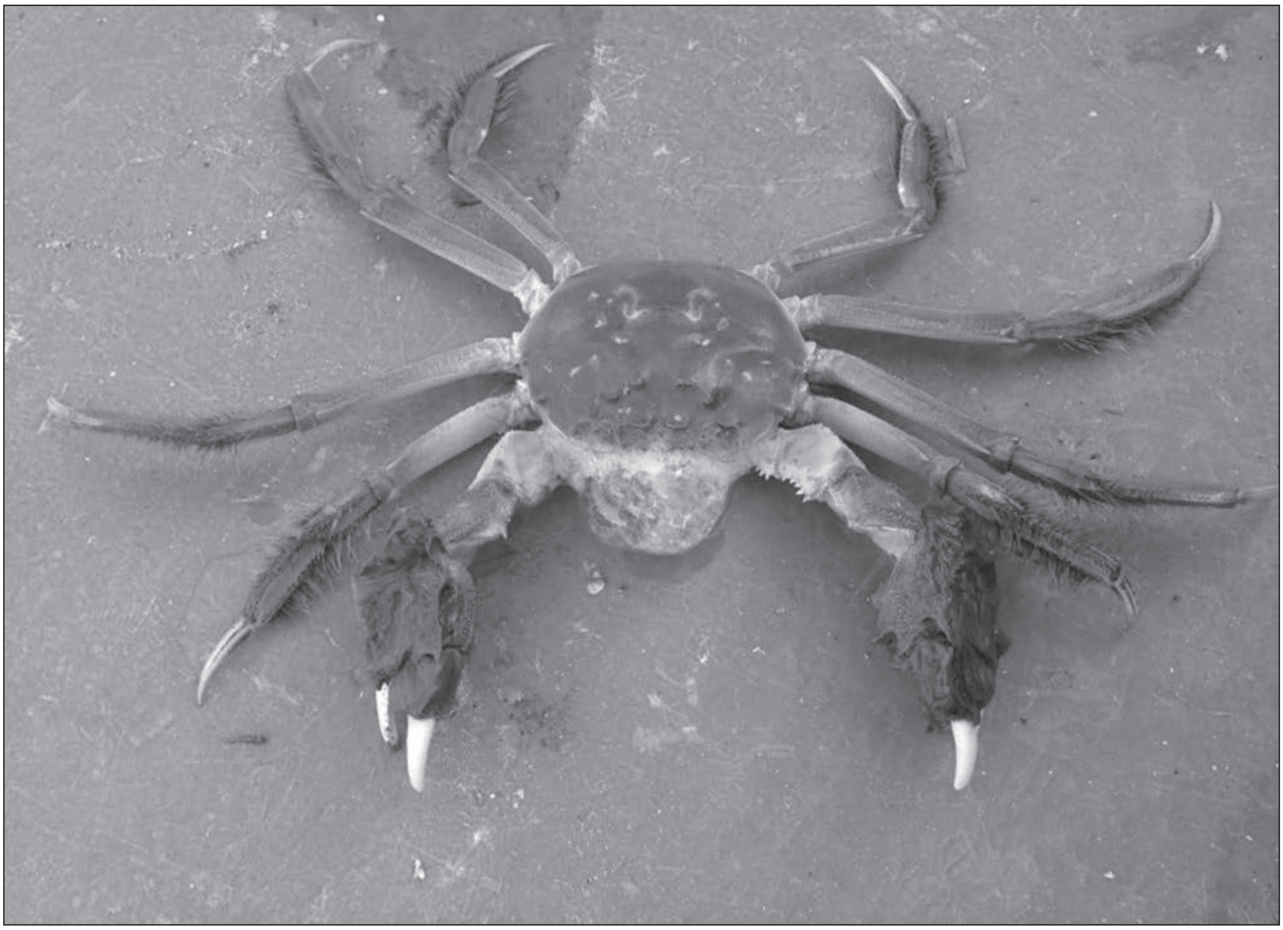

Fig. 2: Specimen of *Eriocheir sinensis* ***caught in the Marano and Grado Lagoon.***
Sl. 2: Primerek vrste *Eriocheir sinensis****, ujete v maranski in gradeški laguni***

1958 spread to Hendaye on the French-Spanish border (Hoestlandt, 1959). To the East, the mitten crab entered the Baltic Sea in 1928, spreading as far East as Vyborg in Finland by 1933, and as far North as Gävle in Sweden by 1934 (Peters, 1938b). During the period 1928–1935, *E. sinensis* spread at an average rate of 355 km per year in the Baltic Sea (Herborg *et al*., 2005).

The Chinese mitten crab was first reported in the United Kingdom in the Thames River in 1935, but became established only in 1973 (Ingle, 1986; Rainbow *et al*., 2003). In the late 1980s, crabs were found in the Tagus River in Portugal, where the species had become established by 1990 (Cabral & Costa, 1999). Since 1997, the crab has also established itself in the Guadalquivir estuary, near Seville's harbour in Spain (Cuesta *et al*., 2004, 2015). These last two records represent the southernmost occurrences to date of this species in Europe. Two Chinese mitten crabs were also collected in the Serbian section of the Danube River in June 1995 and November 2001 (Paunović *et al*., 2004), but after first records the occurrence of this species is still occasional (Škraba *et al*., 2013).

It is largely accepted that the worldwide spread of the Chinese mitten crab was due to human-mediated activities and was not the result of natural causes (Cohen & Carlton, 1997). The most probable unintentional vector for the introduction and transfer is the transport by ship ballast waters. Presumably, the uptake of pelagic larval stages would be responsible for the spread of the species via this vector (Cohen & Carlton, 1997). In fact, the Chinese mitten crab was one of the first species reported in ballast tanks and was used as a first piece of evidence for the transport of living aquatic alien species in ballast waters (Carlton, 1985). In northern Europe, ship traffic from China headed for Hamburg (Elbe River) and Bremen (Weser River) has been frequent since the end of the 19th century, which may have very likely contributed to a founder specimens introduction via incoming ships from China. The presence of the crab in southern France probably arose through shipping activities (Herborg *et al*., 2003).

E. sinensis is an euryhaline species characterized by a catadromous life cycle. It spends most of its life in fresh or brackish waters. Mature adults migrate downstream during the fall to reproduce in brackish or salt waters. Both sexes are thought to die following reproduction (Panning, 1938). Females brood the eggs and, upon hatching, larvae are planktonic for one to two months. During this marine free-swimming phase, larvae pass through a series of developmental stages: a brief non-feeding pre-zoea stage, five zoea stages and one megalopa stage (Anger, 1991; Montú *et al*., 1996). Following the megalopal stage, the larvae metamorphose into juvenile crabs that settle to the bottom, usually in late summer or early fall (Rudnick *et al*., 2005). The onset of this benthic life for the young crabs corresponds to the beginning of the active upstream migration into rivers to complete the life cycle in fresh waters.

The species can live between one and five years, depending on location. This variability in longevity is apparently related to the time needed to reach the maturity and reproductive activity, since the crab is believed to spawn only once (Panning, 1938). In northern Europe the time to maturity varies between four and five years, while in the warmer waters of the San Francisco Bay, the majority of spawning crabs are at least three years old (Rudnick *et al*., 2005; Veilleux & de Lafontaine, 2007). This geographic variation in age at maturity suggested that the achievement of maturity can be strongly dependent upon environmental conditions (Rudnick *et al*., 2005).

Nevertheless, after its appearance in the Narbonne littoral lagoons (Petit, 1960), the population disappeared after a dozen years from the Mediterranean Sea (Petit & Mizoule, 1973). Until the new record in the Venice Lagoon (Mizzan, 2005) there were no other records in the Mediterranean area, although the species was reported in the Ukrainian part of the Black Sea and in the adjacent Azov Sea, where they probably arrived from the Baltic Sea by active migration via canals and causeways (Murina & Antonovsky, 2001; Gomoiu *et al*., 2002).

The Chinese mitten crab interferes with recreational and commercial fishing (Ingle, 1986), causes river bank erosion through extensive burrowing (Herborg *et al*., 2005), and may compete for resources with native freshwater crustaceans (Clark *et al*., 1998). In this way the presence of *E. sinensis* in the Lagoon of Marano and Grado could represent a potential threat. A number of alien species from different phyla has been reported in the recent past from the North Adriatic lagoons (Froglia & Speranza, 1993; Mizzan, 1999; Occhipinti Ambrogi, 2000). With regard to exotic crustaceans, the last finding was an established population of the Oriental shrimp (*Palaemon macrodactylus* Rathbun, 1902) in the Lagoon of Venice (May 2012) (Cavraro *et al*., 2014), Lagoon of Marano and Grado (May 2013) and Sacca di Goro (July 2013) (Cuesta *et al*., 2014). Also in this case, ballast waters discharge seems to be the most likely vector of introduction (Cavraro *et al*., 2014; Cuesta *et al*., 2014).

Even if recorded with a specimen at present, it is recommended that the presence of the Chinese mitten crab and its eventual expansion within the lagoon and adjacent river systems will be monitored in the next few years, through directed monitoring programmes as well as in the artisanal fishery catches.

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PRVI ZAPIS O POJAVLJANJU KITAJSKE VOLNOKLEŠČE RAKOVICE *ERIOCHEIR SINENSIS* V MARANSKI IN GRADEŠKI LAGUNI (SEVERNI JADRAN)

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POVZETEK

Kitajska volnokleščа rakovica Eriocheir sinensis *H. Milne Edwards, 1853, ki izvira iz Daljnega vzhoda, je ena izmed najbolj invazivnih tujerodnih vrst. Namen pričujočega prispevka je podati prvi zapis o pojavljanju te vrste v maranski in gradeški laguni. Sicer je to tretji zapis o pojavljanju te tujerodne vrste za severni Jadran.*

Ključne besede: *Eriocheir sinensis*, tujerodna vrsta, maranska in gradeška laguna, severni Jadran

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DESCRIPTION, REPRODUCTIVE BIOLOGY AND ECOLOGY OF THE *SPHAEROMA WALKERI* (CRUSTACEA: ISOPODA) ALIEN SPECIES FROM THE TUNIS SOUTHERN LAGOON (NORTHERN TUNISIA, CENTRAL MEDITERRANEAN)

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ABSTRACT

Sphaeroma walkeri *Stebbing, 1905 is a non-indigenous species first recorded in the Tunis Southern Lagoon in 2002, where it is at present established as a result of the environmental restoration of this brackish area. Specimens of* S. walkeri *were collected monthly at 10 stations from February 2012 through January 2013. This species occurs in the Tunis Southern Lagoon throughout the year, but only reproduces during the warm period between April and October. From May 2012 onwards, the number of gravid females exceeded 50 % of the total sample, with a peak of 88.59 % in July 2012. Fecundity ranged between 13 and 26 eggs. Among the gravid females, a significant relationship between brood and total body length of specimens was only noted in the smallest (4.8–5.8 mm) and largest (8.8–9.8 mm) ovigerous females. In our sample, the females outnumbered the males. This isopod colonizes empty balanoid barnacle shells, ascidians and especially sponges.*

Keywords: bioinvasion, fecundity, habitat, Tunisian waters

DESCRIZIONE, BIOLOGIA RIPRODUTTIVA ED ECOLOGIA DELLA SPECIE ALIENA *SPHAEROMA WALKERI* (CRUSTACEA: ISOPODA) NELLA LAGUNA TUNISINA MERIDIONALE (TUNISIA SETTENTRIONALE, MEDITERRANEO CENTRALE)

SINTESI

Sphaeroma walkeri *Stebbing, 1905* è una specie *non indigena trovata per la prima volta nella laguna tunisina meridionale nel 2002. La specie vi si è stabilita in seguito al recupero ambientale di quest'area salmastra. Esemplari di* S. walkeri *sono stati raccolti mensilmente in 10 stazioni tra febbraio 2012 e gennaio 2013. Questa specie* è *trovata nella laguna meridionale tunisina durante tutto l'anno, ma vi si riproduce solo durante il periodo più caldo, da aprile a ottobre. Il numero di femmine gravide ha superato il 50 % del campione totale a partire da maggio, con un picco dell'88,59 % nel mese di luglio 2012. La fertilità* è variata tra *13 e 26 uova. Tra le femmine gravide, è stata trovata una relazione significativa tra covata e lunghezza totale del corpo degli esemplari solo per le femmine ovigere più piccole (4,8–5,8 mm) e quelle più grandi (8,8–9,8 mm). Nei campioni analizzati le femmine superavano in numero i maschi. Questo isopode colonizza il carapace vuoto dei cirripedi balani, ascidie e soprattutto spugne.*

Parole chiave: bioinvasione, comportamento, habitat, acque della Tunisia

INTRODUCTION

Sphaeroma walkeri is an isopod species commonly found in the Indian Ocean and the Red Sea among intertidal fouling communities, and reported in warm and warm-temperate waters (Galil, 2008). The species was first recorded in Tunisian waters, and the record took place in the northern brackish area, the Tunis Southern Lagoon (Ben Souissi *et al*., 2003).

While the original description by Stebbing (1905) was rather succinct, Jacobs (1987) represented *S. walkeri* anew and in much more detail. However, discrepancies found in the literature between several authors (Stebbing, 1910; Loyola e Silva, 1960; Mak *et al.*, 1985; Jacobs, 1987; Khalaji-Pirbalouty & Wägele, 2010) offer us an opportunity to provide a thorough description of the species in the present paper, based on the specimens collected from the Tunisian waters.

Isopods are considered as an important component of benthic communities and play a fundamental role in the ecosystems (Guarino *et al.,* 1993). Nevertheless, except for some morphological descriptions and checklists, there are no data concerning the biology and ecology of the species available. This paper represents the first contribution to enhancing the knowledge about the reproductive biology of *S. walkeri* and, consequently, about its behaviour in the Tunis Southern Lagoon. The results are useful to explain the establishment of this non-indigenous isopod in its new environment.

MATERIAL AND METHODS

Specimens of *Sphaeroma walkeri* were collected monthly in the Tunis Southern Lagoon. The lagoon adjoining the city of Tunis is located in the southwestern part of the Gulf of Tunis (36° 47′ N and 10° 17′ E) and divided into northern and southern areas by a navigation channel (Fig. 1). Both areas used to be heavily polluted (Zaouali, 1983; Ben Souissi, 2002), but have recently been rehabilitated with success (Jouini *et al*., 2005). Tunis Southern Lagoon extends over an area of 720 ha with a regular depth of about 2.1 m, except in restricted areas where it reaches a maximum of 4 m. It appears as an ellipse stretching in a SW-NE direction, between 36° 46′ 47″ and 36° 48′ 00″ N and 10° 12′ 22″ and 10° 16′ 41″ E. Its shores have been excavated and protected by large rocky stones.

The sampling conducted between February 2012 and January 2013, took place in ten stations (Fig. 1) situated in shallow, less than 3 m deep waters. In stations 1 through 6, characterized by muddy grounds, samples were obtained by dredging and scuba diving, whereas in stations 7 through 10, located in intertidal rocky shores covered by algae, the samples had to be collected manually from under the stones. Our investigations were conducted regularly between 2012 and 2013, at least twice a month for abiotic parameters, such as temperature, salinity, pH and transparency (measured with

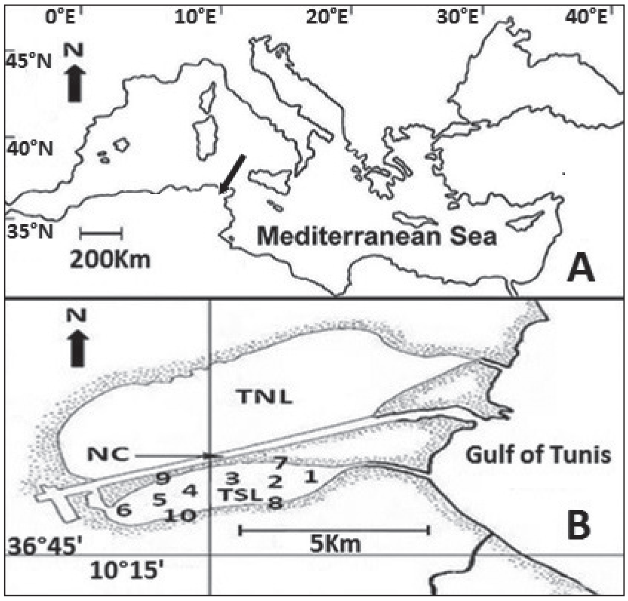

Fig. 1: (A) Map of the Mediterranean Sea, with the arrow pointing at the site of the Tunis Southern Lagoon, located in northern Tunisia. (B) Tunis Northern Lagoon (TNL), separated from the Tunis Southern Lagoon (TSL) by a navigation channel (NC). Numbers 1 to 10 denote sampling stations in TSL.

Sl. 1: (A) Zemljevid Sredozemskega morja z označeno Tuniško južno laguno v severni Tuniziji. (B) Tuniška severna laguna (TNL) je od Južne (TSL) razmejena s plovnim kanalom (NC). Številke od 1 do 10 označujejo vzorčevalne postaje v TSL.

a Secchi disk), and monthly or seasonally for benthic communities. Historical data (1995–1997) for abiotic and trophic variables before the ecological restoration of the lagoon are also available (Ben Souissi, 2002).

Vegetation and benthic communities were delivered to the laboratory, sorted and rinsed with seawater prior to being filtered through a 0.47 mm sieve. Identification of specimens belonging to *S. walkeri* was confirmed using a binocular microscope. Samples were stored in a 70 % ethanol solution. Of the 1191 specimens examined, 801 were females. Appendages were dissected, mounted on slides in a glycerine solution for observation and drawing under a camera Lucida. The total length of females was measured to the nearest 0.01 mm by means of a micrometre scale. The eggs in the marsupia of ovigerous females were removed and counted. Monthly sexual activity was determined as the value (in %) obtained by dividing the number of gravid females by the total number of females (Guarino *et al.,* 1993). The relationship between the total body length of ovigerous females by size classes and number of eggs was estimated. The size of the smallest gravid female was taken as the definition of the size at the first sexual maturity (Garcia-Guererro & Hendrickx, 2005).

The identification of specimens and the terminology adopted in this paper follow the description and illustrations provided by Jacobs (1987).

The differences in size classes were tested by analysis of variance (ANOVA, $p < 0.05$). To explain correlations between ovigerous females and different environmental variables the method of 'Analyses of the Principal Components' was used. Data analyses and treatment were conducted by means of the Statgraphics Centurion software.

RESULTS

Environmental parameters

Prior to the lagoon's ecological rehabilitation, the average monthly salinity had ranged between 30.9 and 48.9, with a peak value of 51.9 registered in 1995. After the rehabilitation, it has been registered between 28 and 38.8 with a monthly average of 37.15. There have been no significant changes in the monthly and annual temperature values before and after the restoration.

Abiotic parameters recorded simultaneously with *S. walkeri* sampling showed that the average temperature was 20.72 °C with a minimum of 11.75 °C and a maximum of 26.4 °C (observed in February 2012 and July 2012, respectively). The mean salinity value was 37.05 with a minimum of 36.1 measured in January 2013 and a maximum of 38 in August 2012. The average pH was 8.02 (7.74 in December 2012 and 8.29 in July 2012). The mean transparency was 1.7 m (1.1 m in December 2012 and 2.1 m in July 2012).

Description of Tunisian *Sphaeroma walkeri*

Sphaeroma walkeri is easily identified among its congeners by the presence of numerous prominent tubercles running the entire dorsal face of the carapace. Its length may exceed 10 mm. This isopod is characterized by a net sexual dimorphism. Males are larger than females (Fig. 2A); by contrast, females are more convex (Fig. 2B). The surface of pereonites 1 and 2 is smooth, but pereonites 3 and 4 each have two irregular rows of low tubercles. Pereonites 5 and 6 and the pleon show one row of prominent round tubercles, additionally, the posterior edge of the pleon presents a second row of small round tubercles. The pleotelson is long and tapers to a rounded point that is slightly upturned with four rows of 3–6 tubercles along the surface. The tubercles of pleotelson are less prominent in females (Fig. 2B).

Antenna (Fig. 2C). Antenna with a five-segmented peduncle and flagellum of 14 to 16 articles. Each article of the flagellum bears a fringe of smooth setae at the distal interior angle. Adult males with these setae are more abundant, the seta measure up to 2–3 times the length of the articles. In females, these setae are shorter and less dense (Fig. 2E).

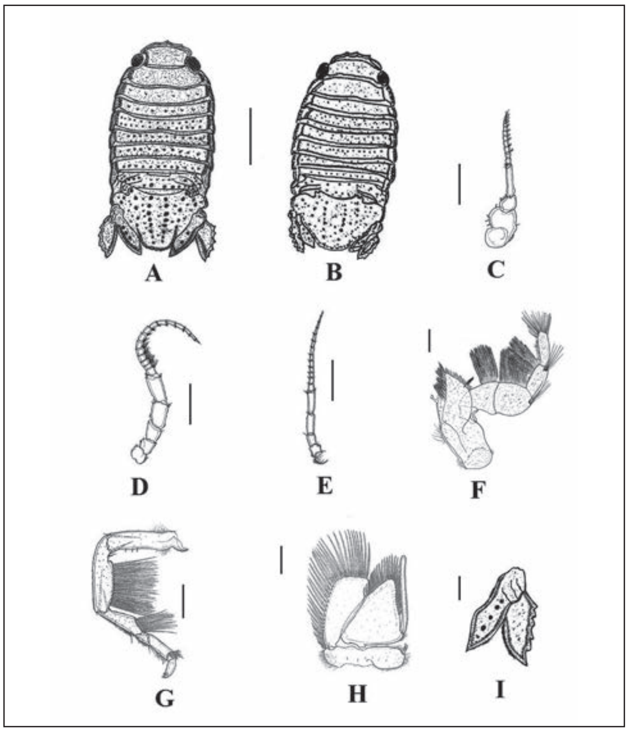

Fig. 2: *Sphaeroma walkeri* **Stebbing, 1905. *(A) Adult male in dorsal view, scale bar = 2 mm; (B) adult female, in dorsal view, scale bar = 2 mm; (C) male antenna, scale bar = 0.5 mm; (D) male antennules, scale bar = 0.5 mm; (E) female antenna, scale bar = 0.5 mm; (F) maxilliped, scale bar = 0.25 mm; (G) pereopod 1, scale bar = 0.5 mm; (H) male pleopod 2, scale bar = 0.5 mm; (I) male uropod, scale bar = 0.5 mm.***

Sl. 2: *Sphaeroma walkeri* **Stebbing, 1905. *(A) Odrasel samec s hrbtne strani, merilo = 2 mm; (B) odrasla samica s hrbtne strani, merilo = 2 mm; (C) samčeva antena, merilo = 0,5 mm; (D) samčeve antenule, merilo = 0,5 mm; € samičja antena, merilo = 0,5 mm; (F) maksiliped, merilo = 0,25 mm; (G) pereopod 1, merilo = 0,5 mm; (H) samčev pleopod 2, merilo = 0,5 mm; (I) samčev uropod, merilo = 0,5 mm.***

Antennule (Fig. 2D). With a three-segmented peduncle; article 3 is elongated, slender and about 3 times the length of article 2; flagellum 13-articled, articles 3-12 each bear aesthetascs extending to the posterior margin of pereonite 1.

Maxilliped (Fig. 2F). Endite wide distally, with some plumose setae set amongst fine simple setae in a semicircular distal margin, mesial margin with a single coupling hook, ventral surface with a row of about 19–20 long robust, plumose setae; palp articles 2–4 bearing dense fringes of long fine, plumose setae on superior margin, articles 3–4 with some slender fine, plumose setae on inferodistal angle.

Pereopods (Fig. 2G). Basis proximal superior margin with dense fine setae; ischium superior margin fringed

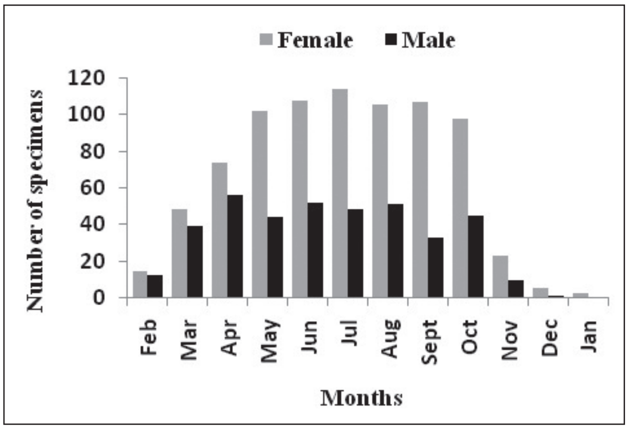

Fig. 3: Monthly distribution of male and female specimens of** S. walkeri **collected from the Tunis Southern Lagoon, February 2012–January 2013.
Sl. 3: Delež samcev in samic vrste** S. walkeri **iz Tuniške južne lagune po posameznih mesecih v obdobju februar 2012–januar 2013

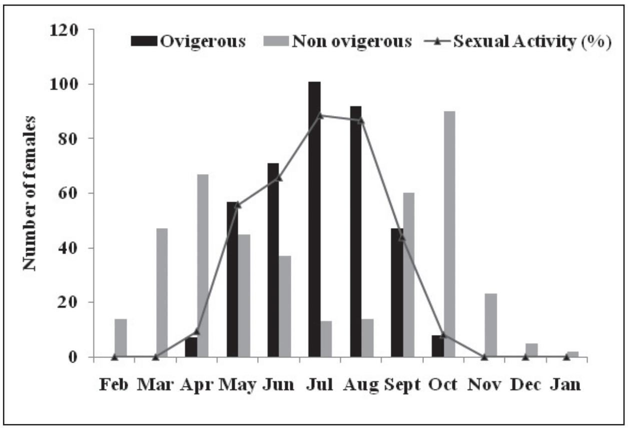

Fig. 4: Monthly distribution of ovigerous and non ovigerous females and sexual activity of** S. walkeri **collected from the Tunis Southern Lagoon, February 2012–January 2013.
Sl. 4: Delež ovigerih samic in samic brez jajc ter spolne aktivnosti vrste** S. walkeri **iz Tuniške južne lagune po posameznih mesecih v obdobju februar 2012–januar 2013

with numerous long, plumose setae, proximal superior corner with a single robust seta; merus, carpus and propodus inferior margins with a dense fringe of short setae; propodus of pereopod 1 with one serrated rostro-distal spine. Near rostro-distal spine transverse row of 10–15 long, plumose setae being inserted.

Pleopod 2 (Fig. 2H). With short sympodite bearing three lateral setae. Endopodite larger than exopodite, with a broad masculine appendix, laterally inserted with an acute tip, much longer than the endopodite itself. This pleopod also features long marginal plumose setae.

Uropods (Figs. 2A, B, I). Flattened and attached to the side of the pleotelson towards the front. Endopod rigidly fused, while exopod movable and larger or equal to the length of endopod. External margin of exopod has 5–6 pronounced teeth, mostly situated in the caudal region. Endopod margins fringed with dense simple setae, dorsal surface bearing 2–3 prominent tubercles. Uropods exceed the distal end of the pleotelson and are more developed in males than in females (Fig. 2I).

Based on the schemes presented above, the Tunisian specimens are morphologically similar to those from Africa (Jacobs, 1987) and those from the Persian Gulf (Khalaji-Pirbalouty & Wägele, 2010). These authors describe only 5–6 teeth and an acute apex. Conversely, Stebbing (1905) counted 6 or 7 teeth on the outer margin of the uropod.

Reproductive biology

Males and females were present throughout the year (Fig. 3) and the sex ratio showed that the females outnumbered the males from May 2012 to November 2012 (df = 2, $\chi^2 > 3.84$, $p > 0.05$) (see Table 1). *S. walkeri* presented a discontinuous spawning period throughout the year. However, no monthly sexual activity was observed during two periods: from February to March and from November to January. Ovigerous females were collected from April to October. The number of gravid females exceeded 50 % of the total sample from May to August, and two peaks were recorded in summer, 88.59 % in July 2012 and 86.79 % in August 2012. In autumn, the sexual activity decreased, reaching 43.92 % in September 2012, and showing an even more significant decline in October 2012 (about 8 %) (Fig. 4).

The size at the first sexual maturity observed in *S. walkeri* was 4.8 mm. The fecundity ranged between 13 and 26 eggs. Among the gravid females, a significant

Tab. 1: Monthly sex ratio of** S. walkeri **(Sr) and χ^2 test (significant if $\chi^2 > 3.84$).
Tab. 1: Delež samcev in samic vrste** S. walkeri **(Sr) in χ^2 test (značilen v primerih, ko je $\chi^2 > 3,84$)

Month	Feb-2012	Mar	Apr	May	Jun	July	Aug	Sept	Oct	Nov	Dec	Jan-2013
Sr	0.86	0.81	0.76	0.43	0.48	0.42	0.48	0.31	0.46	0.39	0.20	0.00
χ^2	0.15	0.93	2.49	23.04	19.60	26.89	19.27	39.11	19.64	6.13	2.67	2.00

relationship between the importance of brood and the total body length of specimen was only noted in the extreme size classes, in the smallest (4.85 mm) and largest (8.89 mm) ovigerous females, respectively (ANOVA, $p < 0.05$) (Fig. 5).

Monthly distribution in size classes of females

The largest females were observed during the warmest period of the year, from April to August. From February to April, the size of the specimens sampled ranged between 3 and 6.5 mm. The predominant size classes were 6–7 mm in the May to June period, and 7.5–8.5 mm in July and August. From October onwards, the big size classes were substituted by juveniles (2–3 mm). Figure 6 shows that the autumn and winter seasons coincide with the recruitment of juveniles in the area. To highlight a possible correlation between the ovigerous females and environmental variables we applied the principal component analysis (PCA). Two principal components explaining 86.33 % of the total inertia were retained. The number of ovigerous females was found to be positively correlated with all the studied abiotic parameters, but a highly significant correlation was observed particularly with transparency and temperature (Fig. 7).

DISCUSSION

This study was conducted in the Tunisian Southern Lagoon following the environmental rehabilitation of its ecosystem, as a significant improvement of ecological variables, such as water quality, and invertebrate species previously unknown in the lagoon, in the nearby Gulf of Tunis and along the Tunisian coasts had been recorded (Ben Souissi *et al.,* 2003; Rifi *et al.,* 2011; Rifi *et al.,* 2013).

Sphaeroma walkeri is present in Tunisian brackish waters throughout the year except in December and January. Mak *et al.* (1985) noted that the species was also found in India, but only during the warm period from April to August. This alien species has established in Tunisian waters and reproduces during the period oscillating between April and October, as corroborated by the presence of gravid females. In Hong Kong, *S. walkeri* breeds almost continuously throughout the year with peaks during spring and autumn (Morton, 1987). In our study, fecundity ranged between 13 and 26 eggs per female and the importance of the brood was positively correlated with extreme body size lengths. Similar results were recorded for *Paradella dianae* (Garcia-Guererro & Hendrickx, 2005). The monthly distribution of females by size classes in the Tunis Southern Lagoon showed that except for the spawning period, the largest females disappeared from October to March and were replaced by young specimens (juveniles). This period concerns the recruitment phase, where class sizes ranged between 2 and 6 mm, with the smallest specimen (2.2 mm) collected in November. The virtual disappearance of large specimens of this species in Tunisia during the mentioned period was probably due to intense predation by *Zeus faber* Linnaeus, 1758, which is particularly abundant in the lagoon in winter. A study of the stomach contents of Z. *faber* confirmed this hypothesis (Ben Souissi *et al.*, 2004). Reproductive effort can also induce and explain the mass mortalities observed. This phenomenon was observed in non-indigenous behaviour outside the native habitats of the species (Rifi *et al.,* 2011). According to Shafir & Field (1980) and Kroer (1989), dispersion could also explain the absence of adults. PCA showed that the reproductive biology of *S. walkeri* was positively correlated with environmental variables (salinity and pH) and highly linked to transparency and temperature. Similar patterns were recorded by Radhakrishnan *et al.* (1987) concerning salinity, temperature and suspended solids that affect the species' occurrence. These results corroborated the feeding behaviour of the species, *S. walkeri* being considered a voracious detritus-feeder (Guarino *et al.,* 1993).

S. walkeri was found in the lagoon in very shallow water, generally less than 3 m deep. All records are from intertidal zone except for one from India, where the isopod was collected in waters up to 46 m of depth (Carlton & Iverson, 1981). The species has the flexibility to occupy different habitats related to its distribution. In the studied area, this sphaeromatid species is a typical animal of biofouling found in high densities, mainly among benthic communities, algae, as well as under stones and among dead sea squirts, as in the present study. *S. walkeri* is able to burrow in mangrove roots

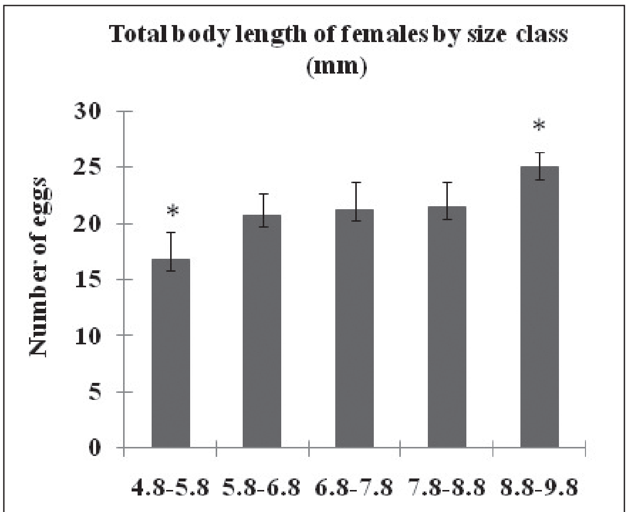

Fig. 5: Relationship between females' size classes and the number of eggs in the brood of *S. walkeri* ***specimens collected from the Tunis Southern Lagoon.***
Sl. 5: Odnos med velikostnimi razredi samic in številom jajc v leglu pri vrsti *S. walkeri* ***iz Tuniške južne lagune***

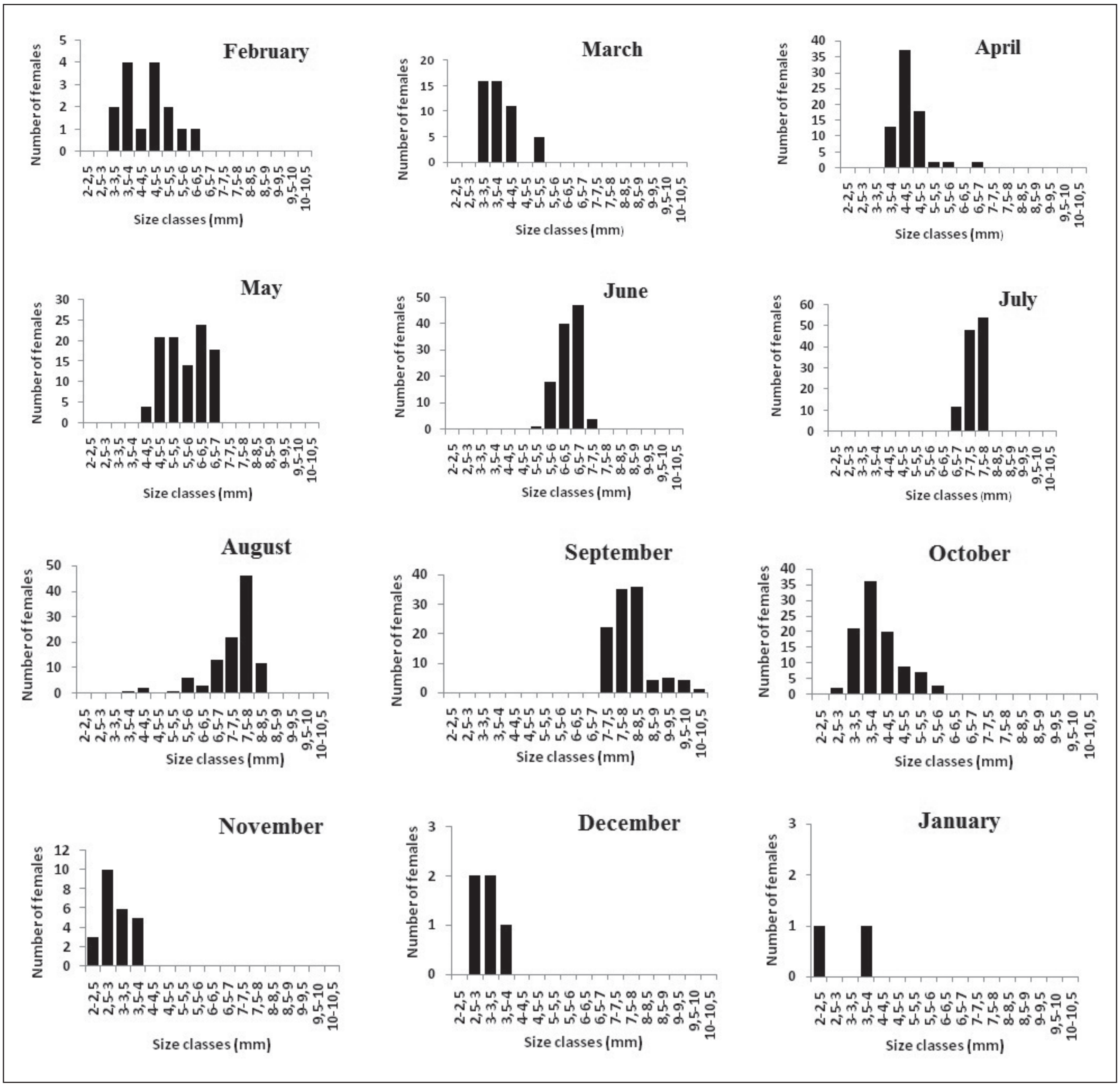

Fig. 6: Monthly size class distribution of *S. walkeri* ***females collected in the Tunis Southern Lagoon.***
Sl. 6: Velikostni razredi samic vrste *S. walkeri* ***iz Tuniške južne lagune po posameznih mesecih***

to live and breed, as described by Khalaji-Pirbalouty & Wägele (2010), who define the species as a wood-borer.

In the Tunis Southern Lagoon, *S. walkeri* inhabits different bottoms and is found among fouling communities. This isopod species colonizes empty barnacle shells of balanoids, such as *Balanus amphitrite amphitrite* Darwin, 1854, spaces between benthic fauna, oscula of sponges, especially *Ircinia* sp., *Chondrosia reniformis* (Nardo, 1847), and ascidians *Phallusia mammillata* (Cuvier, 1815), *Ecteinascidia turbinata* (Herdman, 1880), *Ascidiella aspersa*, (Müller, 1776) and *Ciona intestinalis* (Linnaeus, 1767). In this study, we observed that *S. walkeri* invades via oral and cloacal siphons, burrowing into the thick tunic of dead ascidians of the genus *Microcosmus*, to escape from predators and cope with large environmental variations in the lagoon ecosystem.

This species takes refuge among the dense herbaria of the lagoon constituted by *Gracilaria verrucosa* ((Hudson) Papenfuss, 1950) and *Cystoseira barbata* ((Stackhouse) C. Agardh, 1820) associated with two non-indigenous isopods *Paradella dianae* (Menzies, 1962) and *Paracerceis sculpta* (Holmes, 1904) and the native congeners

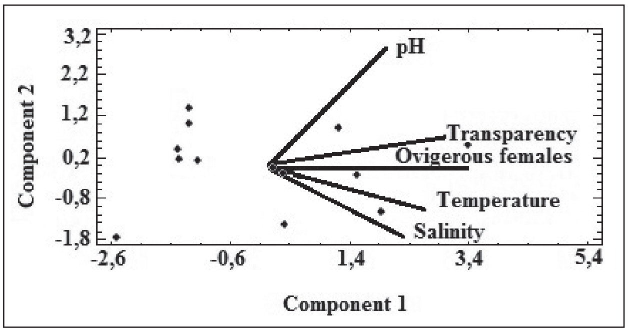

Fig. 7: Principal Component Analysis (PCA): environmental parameters and ovigerous females of *S. walkeri* ***specimens collected from the Tunis Southern Lagoon.***
Sl. 7: Analiza glavnih komponent (PCA): okoljski parametri in ovigere samice vrste *S. walkeri* ***iz Tuniške južne lagune***

S. serratum (Fabricius, 1787) and *Cymodoce truncata* (Leach, 1814). This non-native isopod prefers the habitat of sponges to that of ascidians. Ounifi Ben Amor *et al.* (2010) noted that the highest densities of *S. walkeri* were observed among sponges during spring (18 ind./m^2) and summer (23 ind./m^2) seasons. Conversely, the lowest densities of *S. walkeri* were among ascidians during autumn (3 ind./ m^2) and winter (2 ind./ m^2) seasons. In the Tunis Southern Lagoon, a significant decrease of the *S. serratum* density was observed subsequent to the environmental restoration. This, however, could also be explained by an interspecific competition pressure between *S. walkeri* and its congeneric species *S. serratum*. The improvement in the water quality in the lagoon after its rehabilitation, in its salinity and pH, for instance, is to the advantage of non-indigenous rather than native species. Indeed, *S. serratum* does not tolerate salinity changes, especially during determinate life stages (Charmantier & Charmantier-Daures, 1994).

CONCLUSION

Sphaeroma walkeri is a thermophilic isopod introduced a decade ago in the Tunis Southern Lagoon, where it rapidly spread throughout the restricted brackish area and colonized both muddy and rocky grounds. Specimens in all developmental stages were observed throughout the year. High densities of *S. walkeri* were recorded especially during spring and summer, showing that the species is substantially established in the Tunis Southern Lagoon. Such settlement is probably due to a successful rehabilitation of the area, which facilitated the introduction of species previously unknown in the region (Mejri *et al.*, 2004; Ben Souissi *et al.*, 2004, 2005). These results are useful to explain the establishment of this non-indigenous isopod in its new environment.

The establishment of *S. walkeri* in the Tunis Southern Lagoon constitutes one of the best examples of biological change that has affected Tunisian waters for the last four decades at least. New marine species are regularly and continuously recorded in Tunisian waters, incoming from the eastern tropical Atlantic through the Strait of Gibraltar, on the one hand, and from the Indo-Pacific and Red Sea via the Suez Canal, on the other (Spanier & Galil, 1991). Such changes are owed to an intensification of shipping activities (Galil, 2009) and a significant increase in the average water temperature throughout the Mediterranean Sea (Francour *et al.*, 1994), which further the introduction and establishment of several species belonging to different zoological groups.

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OPIS, RAZMNOŽEVALNA BIOLOGIJA IN EKOLOGIJA TUJERODNE MOKRICE *SPHAEROMA WALKERI* (CRUSTACEA: ISOPODA) IZ TUNIŠKE JUŽNE LAGUNE (SEVERNA TUNIZIJA, OSREDNJI MEDITERAN)

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POVZETEK

Sphaeroma walkeri *Stebbing, 1905, je tujerodna vrsta, ki je bila leta 2002 prvič potrjena v tuniški južni laguni, kjer je ustaljena vrsta, najverjetneje zaradi okoljske oživitve tega brakičnega okolja. Avtorji so primerke vrste* S. walkeri *zbirali v mesečnih intervalih na 10 postajah od februarja 2012 do januarja 2013. Ta vrsta se v Tuniški južni laguni pojavlja skozi vse leto, razmnožuje pa v toplem delu leta od aprila do oktobra. Število oplojenih samic je v maju bilo več kot 50 % celotnega vzorca, v juliju 2012 pa doseglo višek 88,59 %. Število jajc je bilo od 13 do 26. Pri oplojenih samicah je bil razviden značilni odnos med leglom in celotno dolžino telesa le pri najmanjših (4,8–5,8 mm) in največjih ovigerih samicah (8,8–9,8 mm). V vzorcu je bilo več samic kot samcev. Ta rak enakonožec naseljuje prazne lupine rakov vitičnjakov, kozolnjake in še posebej spužve.*

Ključne besede: bioinvazija, plodnost, habitat, tunizijske vode

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TWO SIBLING GREEN LACEWING SPECIES, *CHRYSOPA PALLENS* AND *CHRYSOPA GIBEAUXI* (INSECTA: NEUROPTERA: CHRYSOPIDAE) IN SLOVENIA AND WESTERN BALKAN COUNTRIES[1]

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ABSTRACT

Green lacewing species, Chrysopa gibeauxi *(Leraut, 1989), was recently reinstated as a valid species, and closely related to* Chrysopa pallens *(Rambur, 1838). Evaluation of morphological diagnostic characters of the two sibling species justified the separation of the two species. After re-examination the available insect material from Slovenia and western Balkan countries it was revealed that* Chrysopa pallens *is widely distributed in the area, whilst* Chrysopa gibeauxi *is a rare green lacewing, recorded in Slovenia, Croatia and Serbia (Kosovo). The distribution of the latter species is for sure larger than appearing. Some details concerning morphology and habitat of the species are provided. The erroneous citations given for Slovenia, assigned now to* Chrysopa gibeauxi*, are corrected.*

Key words: Neuroptera, *Chrysopa gibeauxi*, *Chrysopa pallens*, sibling species, new records, Slovenia, Balkan

DUE SPECIE GEMELLE DI CRISOPIDI, *CHRYSOPA PALLENS* E *CHRYSOPA GIBEAUXI* (INSECTA: NEUROPTERA: CHRYSOPIDAE) IN SLOVENIA E NEI PAESI DEI BALCANI OCCIDENTALI

SINTESI

Il crisopide Chrysopa gibeauxi *(Leraut, 1989) è stato recentemente ripristinato come specie valida, strettamente correlata a* Chrysopa pallens *(Rambur, 1838). La valutazione dei caratteri diagnostici morfologici delle specie gemelle ha giustificato la separazione delle due specie. Dopo un nuovo esame del materiale entomologico proveniente dalla Slovenia e dai paesi dei Balcani occidentali è stato concluso che* Chrysopa pallens *è una specie ampiamente diffusa nell'area in questione, mentre* Chrysopa gibeauxi *è un crisopide raro, trovato in Slovenia, Croazia e Serbia (Kosovo). La distribuzione della seconda specie è sicuramente più ampia di quanto al momento appaia. L'articolo fornisce alcuni dettagli riguardanti la morfologia e l'habitat delle specie considerate. Le citazioni erronee fornite per la Slovenia sono state corrette e ora assegnate a* Chrysopa gibeauxi.

Parole chiave: Neuroptera, *Chrysopa gibeauxi*, *Chrysopa pallens*, specie gemelle, nuove segnalazioni, Slovenia, Balcani

[1] Dedicated to the memory of Jan Carnelutti (1920-2012)

INTRODUCTION

Green lacewings (Chrysopidae) are neuropteran insects used as biological pest control agents of insect and mite pests in agriculture and gardens (*e.g.,* McEwen & Senior, 1998; Senior & McEwen, 2001; for reviews see Stelzl & Devetak, 1999; Canard, 2001; McEwen *et al.*, 2001). The family Chrysopidae comprises more than 1,200 described species in approximately 80 valid genera world-wide (Brooks & Barnard, 1990). Green lacewing genus *Chrysopa* Leach in Brewster, 1815 is distributed in Holarctic, with 11 valid species occurring in North America and approx. 50 in Europe, North Africa and Asia. Among them, 17 are recorded from Europe (Brooks & Barnard, 1990; Aspöck *et al*., 2001).

From specimens captured in the French Alps, Leraut (1989) described a new green lacewing subspecies, *Metachrysopa pallens* ssp. *gibeauxi* Leraut, 1989. It was later raised as *bona species* (Leraut, 1992), but in 2001 it was synonymized with *Chrysopa pallens* (Rambur, 1838) by Aspöck *et al*. (2001). A decade and a half later, the species *Chrysopa gibeauxi* (Leraut, 1989) was confirmed and its valid status reinstated in a comprehensive study by Tillier *et al.* (2014).

Tillier *et al.* (2014) provided diagnostic characters separating the two species. In *Ch. gibeauxi* is the pronotum covered with numerous thick black hairs, whilst in *Ch. pallens* there are slender blond and blackish brown hairs (Figs. 1, 2). In forewings of *Ch. gibeauxi* is first costal cross-veinlet black, and other costal cross-veinlets are fully black except in pterostigma, whilst in *Ch. pallens* is first costal cross-veinlet green, and other costal cross-veinlets are partly blackened, *i.e.* in the very distal part the veinlets are bright (Figs. 3, 4). On the head of *Ch. gibeauxi* seven black spots are larger and more intensively marked than in *Ch. pallens*. (Figs. 1, 2) (Tillier *et al.*, 2014). Furthermore, examination of the male genitalia of *Ch. pallens* from France and Romania, and of *Ch. gibeauxi* from France showed differences justifying the separation of the two species (Tillier *et al.*, 2014).

In the past, when the existence of *Ch. gibeauxi* has not yet been confirmed, *Chrysopa pallens sensu lato* was considered a common species in Slovenia and western

Figs. 1, 2: Dorsal view of the head and the prothorax: 1 – *Chrysopa pallens* **(Rambur) (Hrastovlje, Slovenia), 2 –** *Chrysopa gibeauxi* ***(Leraut) (Šempeter pri Gorici, Slovenia). (Photo: D. Devetak)***

Sl. 1, 2: Glava in predprsje dorzalno: 1 – *Chrysopa pallens* **(Rambur) (Hrastovlje, Slovenija), 2 –** *Chrysopa gibeauxi* ***(Leraut) (Šempeter pri Gorici, Slovenija) (Foto: D. Devetak)***

Balkan countries (reviews in Aspöck *et al.,* 1980, 2001; Devetak, 1992a).

As we now know, two closely related species occur in Europe (Tillier *et al.*, 2014), consequently it was necessary to re-examine material in the first author's collection to reinstate the knowledge of the occurrence of the both species in the area.

MATERIAL AND METHODS

Green lacewings were collected using a sweep net. Pinned individuals and lacewings preserved in alcohol are deposited in the first author's collection. Insects were photographed under a stereoscopic zoom microscope Nikon SMZ800 with a mounted digital camera Nikon DS-Fi1, and processed with NIS-Elements F 3.0 software. Digital images captured at different focal planes were assembled using the application Helicon Focus 4.62 Lite. A map of the studied area was created using ArcGIS 9.3 software (ESRI, 2010) and the free available DIVA-GIS database (http://www.diva-gis.org/gdata, accessed on 9.2.2015).

RESULTS

Distribution of *Chrysopa pallens* (Rambur) and *Ch. gibeauxi* (Leraut) in the studied area

***Chrysopa pallens* (Rambur, 1838)**

In older literature (e.g., Klapálek, 1898, 1899, 1900; Živojinović, 1950; Zelený, 1964; Devetak, 1984, 1991, 1992b), the species is cited as *Chrysopa septempunctata* Wesmael, 1841. After re-inspection the first author's collection the presence of the species was confirmed for Slovenia, Croatia, Montenegro, Serbia, Serbia: Kosovo, and Macedonia (=FYROM) (Fig. 5). Klapálek (1898, 1899, 1900) reported *Ch. pallens* in Bosnia and Herzegovina, Živojinović (1950) in Serbia, Zelený (1964) in Albania, and a series of papers in Croatia (for a review see Devetak, 1992b), but due to the lack of preserved individuals it was impossible to verify the identity of species cited in the papers for these countries.

Slovenia

Literature records

Verified citations: Devetak (1984): Bohinj; Črni Kal; Ljubljana; Maribor; Maribor: Meljski hrib; Sečovlje; Šempeter pri Gorici; Škocjan pri Divači. Erroneous citations – *Ch. gibeauxi* mistaken for *Ch. pallens* in the following collecting places: Devetak (1984): Dravograd; Maribor: Starše; Kidričevo; *Devetak (2011):* Predoslje: grad Brdo.

Re-examined individuals:

Bohinj, jezero, 9. VIII. 1975 1 ♂ 1 ♀, 1. VIII. 1976 1 ♂, D. Devetak leg.; Črni Kal, Predloka, 24. VI. 1990 1 ♀; Haloze: Belski vrh, 14. VII. 1986 1 ♂, F. Janžekovič leg.; Ljubljana, VI. 1968 1 ♀, VI. 1971 1 ♂ 1 ♀, 1.-23. IX. 1971 1 ♀, I. Sivec leg., 8. VIII. 1976 7 ♂ 1 ♀, D. Devetak leg.; Makole: Šega, 13. VII. 1984 1 ♂, C. Krušnik leg.; Maribor, 24. VII. 1975 1 ♂, 28. VIII. 1976 3 ♂ 1 ♀, 10. IX. 1986 1 ♀, 14. IX. 1986 1 ♂, D. Devetak leg.; Maribor: Kalvarija, 16. VIII 1991 1 ♂ 1 ♀, D. Devetak & Barbara Senegačnik leg.; Maribor: Kamnica, Kamniška Graba-Medič, 12. IX. 1992 1 ♂, Nataša Kočev leg.; Maribor, Meljski hrib, 22. VII. 1978 1 ♂, D. Devetak leg.; Nazarje, 23. VIII. 1986 1 ♀, D. Devetak leg.; Nova Gorica: Šempeter pri Gorici, 6. VII. 1973 1 ♀, 14. IX. 1973 1♂ 1♀, I. Sivec leg.; Obalno-kraška regija: Hrastovlje, 13. IX. 1980 1♀; 13. IX. 1990 1♂ 1 ♀, D. Devetak leg.; Ptuj: Gorišnica, 26. VIII. 1988 1♂, F. Janžekovič leg.; Rižana, izvir, 24. VII. 1990 1 ♀, C. Krušnik leg.; Sečovlje, VIII. 1973, 1 ♀, 19. VIII. 1983 1 ♂, D. Devetak leg.; Škocjan pri Divači, Škocjanske jame, 22. V. 1976 1 ♂, D. Devetak leg.

Croatia

Literature records

Verified citations: Devetak (1992b): Biograd na Moru; Istra, Premantura; O. Brač, Bol; O. Brač, Vidova gora; O. Rab, Lopar.

Re-examined individuals:

Biograd na Moru, 19. VIII. 1973 1 ♂, D. Devetak leg.; Istra, Premantura, 20. VII. 1984 1 ♀, D. Devetak leg.; O. Brač, Bol, 12. VII. 1987 1 ♂, 25.-30. VII. 1997 1 ♀, all D. Devetak leg.; O. Brač, Vidova gora, 9. VII. 1990 1 ♂, D. Devetak leg.; O. Rab, Lopar, 21. VI. 1976 2 ♀, D. Devetak leg.

Montenegro

Literature records

Verified citations: Devetak (1991): National Park Durmitor: Crno jezero: Čeline, Drenova gora, Komarnica – Nevidio, Mlinski potok, Tara – Tepca.

Re-examined individuals:

Budva, 25. VIII. 1988 1 ♀, F. Janžekovič leg.; National Park Durmitor, Crno jezero: Čeline, 11. VIII. 1988 1 ♂, P. Jakšić leg.; Durmitor, Drenova gora, 20. VIII. 1987 4 ♂ 3 ♀, F. Janžekovič leg.; Durmitor, Komarnica, Nevidio, 1100 m, 6. VII. 1986 1 ♂, P. Jakšić leg.; Durmitor, Mlinski potok, 17. VIII. 1987 1 ♂, F. Janžekovič leg.; Durmitor, Tara – Tepca, 4. VIII. 1984 1 ♀, 13. VIII. 1988 1 ♀, both P. Jakšić leg.; Tuzi, 5.-17. IX. 1982, 2 ♀, F. Janžekovič leg.

Serbia

Re-examined individuals:

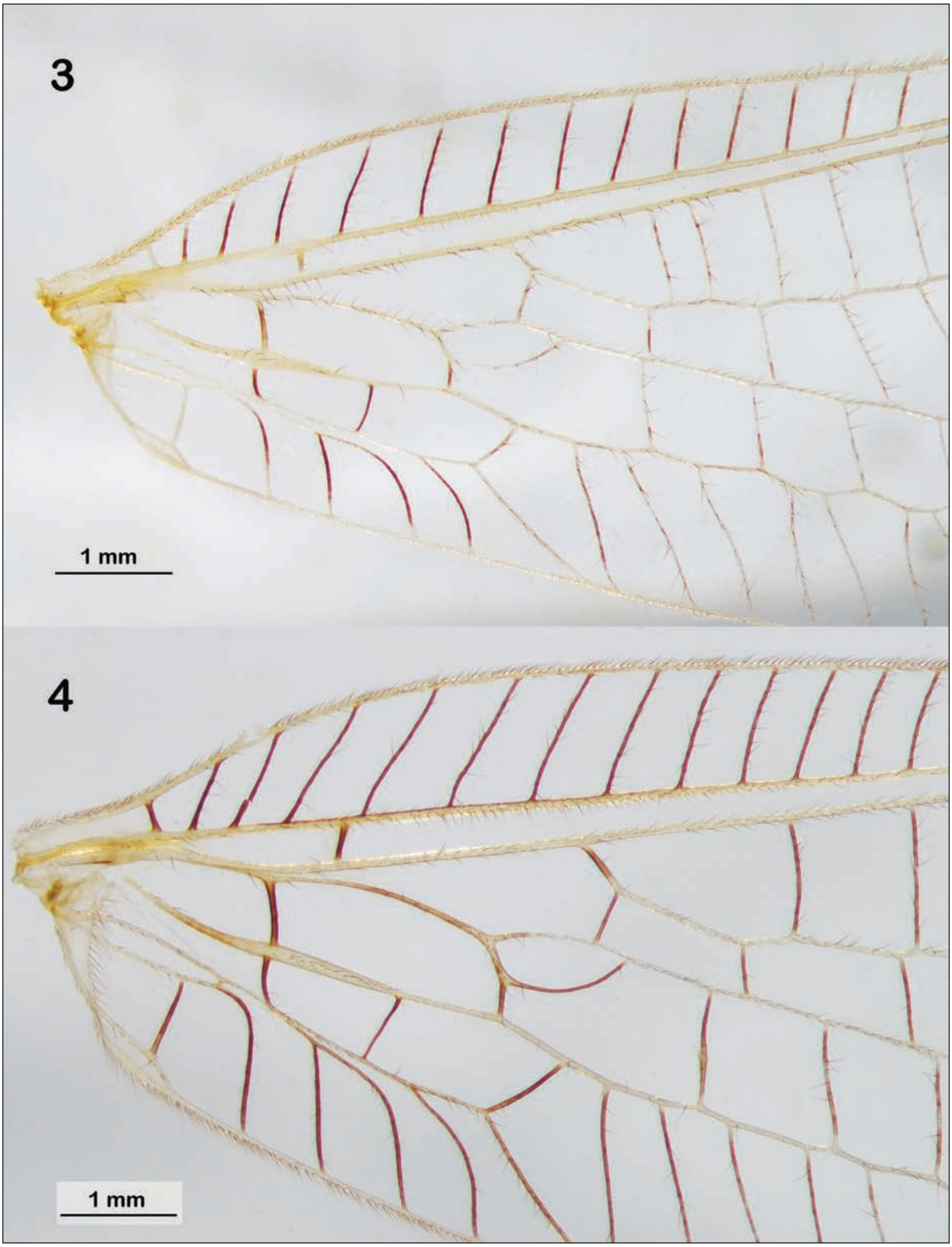
Kopaonik: Jošanička Banja, 6. VIII. 1986 5 ♂, P. Jakšić leg.; Raška: Raška, Razdolje, 500 m, 29. VII. 1987 1 ♀, P. Jakšić leg.

Serbia: Kosovo

Literature records

Verified citations: Devetak & Jakšić (2003):

Ibarska klisura: Košutovac, Košutovački potok; Mt. Grmija; Mt. Goleš; Mt. Šar planina, Prizrenska Bistrica; Priština; Prizren; Slatina.



Figs. 3, 4: The forewing of both species: 3 – *Ch. pallens* **(Hrastovlje, Slovenia),** ***4 –*** *Ch. gibeauxi* ***(Šempeter pri Gorici, Slovenia). (Photo: D. Devetak)***
Sl. 3, 4: Sprednje krilo: 3 – *Ch. pallens* **(Hrastovlje, Slovenija),** ***4 –*** *Ch. gibeauxi* (Šempeter pri Gorici, Slovenija)**.** ***(Foto: D. Devetak)***

Re-examined individuals:

Ibar (Ibër): Ibarska klisura: Košutovac, Košutovački potok, 510 m, 24. VII. 1987 1 ♂ 5 ♀, P. Jakšić leg.; Kosovska Mitrovica (Mitrovicë) /former: Titova Mitrovica/, Ribariče, 1. VII. 1987 1 ♀, P. Jakšić leg.; Mt. Goleš (Golesh), 5. VI. 1979 1 ♂, D. Devetak leg.; Mt. Grmija (Gërmia), 21. VII. 1979 1 ♂, D. Devetak leg.; Mt. Šar planina (Malet e Sharrit), Prizrenska Bistrica, 530 m, 22. VII. 1986 1 ♂, P. Jakšić leg.; Priština (Prishtinë), from V. to IX. 1979 41 ♂ 11 ♀, D. Devetak leg.; 21. VIII. 1980 5 ♂ 2 ♀, P. Jakšić leg.; Prizren (Prizren), 22. VII. 1986 1 ♀, P. Jakšić leg.; Slatina (Sllatinë), 25. VII. 1979 1 ♀, D. Devetak leg.

Macedonia (=FYROM)

Re-examined individuals:

Demir Kapija, 21. V. 1988 1 ♂, P. Jakšić leg.; Dojransko Ezero, N part, 7. VIII. 1978 6 ♂ 2 ♀, F. Janžekovič leg.; Sv. Naum, Ohridsko Ezero, 5. VIII. 1988 1 ♂, F. Janžekovič leg.; Zrnovci, Kochani, 8. VI. 1985 1 ♂, I. Sivec & M. Štangelj leg.

***Chrysopa gibeauxi* (Leraut, 1989)**

Distribution of re-examined individuals (Fig. 5):

Slovenia

Črni Kal, Predloka, 2. VII. 1990 1 ♀; Dravograd, 1. VII. – 6. VIII. 1975 38 ind.; Ig, Kremenica, 3. VIII. 1980 2 ♀, S. Brelih leg.; Kidričevo, 26. VI. 1983 1 ♀, D. Devetak leg.; Maribor, 4. VII. 1975 1 ♂, 16.-23. VII. 1975 5 ♂ 1 ♀, D. Devetak leg.; Mt. Pohorje, Pameče: Jesenkov vrh, 25. VII. 1985 1 ♀, M. Jež leg.; Nova Gorica: Šempeter pri Gorici, 6. VII. 1973 1 ♂, I. Sivec leg.; Predoslje: grad Brdo, 31. V. 2007 1 ♂, D. Devetak leg.; Starše, VIII. 1974, 2 ♀.

Croatia

Baranja: Ilok, 26. VIII. 2013 1 ♂, T. Koren leg.

Serbia: Kosovo

Priština (Prishtinë), 9. VIII. 1979 1 ♀, D. Devetak leg.

Diagnostic differences between the two species

Both sibling species observed in this study are - like Polish green lacewings studied by Tillier *et al.* (2014) - unambiguously separated. The key characters separating the two species are colouration of hairs on the pronotum (*Ch. pallens*: slender blond and blackish brown hairs, *Ch. gibeauxi*: thick black hairs) (Figs. 1, 2) and colouration of first costal cross-veinlet in forewings (*Ch. pallens*: green cross-veinlet, *Ch. gibeauxi*: black cross-veinlet) (Figs. 3, 4). Colouration of second antennal segment is not clearly visible in individuals preserved in alcohol, because, after a few years, it loses its colour. Most of the abdomen and the legs of *Ch. pallens* are covered only with blond (light) hairs, whilst in *Ch. gibeauxi* there are black hairs. Black spots on the head are in most individuals (but not in all) of *Ch. gibeauxi* larger than in *Ch. pallens*. In male genitalia, the gonocristae (central group) are smaller and more numerous in *Ch. pallens* than in *Ch. gibeauxi*.

Plant substrate specificity

The two sibling species differ according to their preference for plant substrate. Whilst *Ch. gibeauxi* was found on conifers (*Picea, Pinus*) or in coniferous forests, *Ch. pallens* occurred on a variety of deciduous trees. Twelve tree species were noted in Slovenia and western Balkan countries as plant substrates for *Ch. pallens* (Tab. 1).

Tab. 1: Plant substrate species for* Chrysopa pallens *in Slovenia and western Balkan countries.
Tab. 1: Rastlinske vrste, na katerih je bila najdena* Chrysopa pallens *v Sloveniji in deželah zahodnega Balkana

Plant substrate species	Country			
	Slovenia	Croatia	Serbia: Kosovo	Montenegro
Pinus nigra J.F. Arnold			X	
Acer monspessulanum L.		X		
Acer campestre L.	X			
Acer negundo L.			X	
Acer platanoides L.			X	
Acer pseudoplatanus L			X	
Fagus sylvatica L.	X			X
Prunus avium L.	X			
Quercus pubescens Willd.	X			
Quercus virgiliana (Ten.) Ten.	X			
Robinia pseudacacia L.			X	
Tilia cordata Mill.	X			

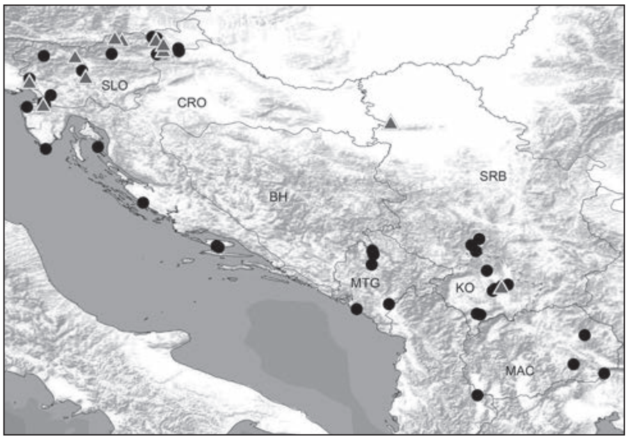


Fig. 5: Known distribution of* Ch. pallens *(circles) and* Ch. gibeauxi *(triangles) in Slovenia and western Balkan countries. BH – Bosnia and Herzegovina, CRO – Croatia, KO – Serbia: Kosovo, MAC – Macedonia, MTG – Montenegro, SLO – Slovenia, SRB – Serbia. (Author of the map: D. Ivajnšič)

Sl. 5: Znana razširjenost vrst* Ch. pallens *(krogi) in* Ch. gibeauxi *(trikotniki) v Sloveniji in deželah zahodnega Balkana. BH – Bosna in Hercegovina, CRO – Hrvaška, KO – Srbija: Kosovo, MAC – Makedonija, MTG –* Črna Gora*, SLO – Slovenija, SRB – Srbija. (Avtor risbe: D. Ivajnšič)

DISCUSSION

Species of the green lacewing taxon, *Ch. pallens sensu lato*, are known as beneficial predatory insects, feeding on crop pests, mainly small arthropods (*e.g.*, Grimal & Canard, 1990; Canard, 2001). The species play role in vegetables, forests, field, fruit and nut crops (Duelli, 2001; Szentkirályi, 2001a, b). In this study, the occurrence of *Ch. pallens* is confirmed for Slovenia and western Balkan countries, and the presence of *Ch. gibeauxi* is noted for the first time in Slovenia, Croatia and Serbia: Kosovo.

A series of papers containing data on the Balkan distribution of *Ch. pallens* published before 1989 exists, *e.g.*, for Serbia (Živojinović, 1950), Bosnia and Herzegovina (Klapálek, 1898, 1899, 1900), Albania (Zelený, 1964), and Croatia (Novak, 1891; Mocsáry, 1899; Klapálek, 1906; Esben-Petersen, 1925; Saure, 1989). However, these papers have no value in these particular cases, considering the fact that they were published before 1989, when *Ch. gibeauxi* has not yet been known.

When morphological characters are considered, the both sibling species are unambiguously separated, as was already pointed out by Tillier *et al.* (2014). The association of green lacewings with particular tree species in Europe was reviewed extensively by Monserrat & Marín (2001) and Szentkirályi (2001b). The two species in the studied area differ in their ecological preference – whilst *Ch. gibeauxi* is found mainly on conifers, *Ch. pallens* prefers deciduous trees (Tab. 1).

It may be possible that *Ch. gibeauxi* is in Europe more frequent than it appears until now (Tillier *et al.*, 2014) and is probably relatively common also in Balkan countries.

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VRSTI TENČIČARIC, *CHRYSOPA PALLENS* IN *CHRYSOPA GIBEAUXI* (INSECTA: NEUROPTERA: CHRYSOPIDAE), V SLOVENIJI IN DEŽELAH ZAHODNEGA BALKANA

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POVZETEK

Tenčičarico vrste Chrysopa gibeauxi *(Leraut, 1989) so v novejšem času ponovno ovrednotili kot veljavno vrsto, ozko sorodno vrsti* Chrysopa pallens *(Rambur, 1838). Na osnovi ovrednotenja morfoloških diagnostičnih znakov so utemeljili razlikovanje dveh sestrskih vrst. Ob ponovnem pregledu dostopnega materiala iz Slovenije in držav zahodnega Balkana smo ugotovili, da je vrsta* Chrysopa pallens *na območju splošno razširjena, medtem ko je* Chrysopa gibeauxi *redka, prvič zabeležena v Sloveniji, na Hrvaškem in Kosovem. Prav gotovo je slednja vrsta bolj razširjena, kot nakazujejo najdbe. Navajamo podatke o morfologiji in habitatu obeh vrst. Popravljeni so napačni citati za Slovenijo, ki se nanašajo na* Chrysopa gibeauxi.

Ključne besede: Neuroptera, *Chrysopa gibeauxi*, *Chrysopa pallens*, sestrske vrste, nove najdbe, Slovenija, Balkan

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SEASONAL DYNAMICS OF MACROZOOBENTHIC COMMUNITY IN THE WETLAND OF THE NATURAL REGIONAL RESERVE OF THE ISONZO RIVER MOUTH, NORTHEAST ITALY: A THREE-YEARS ANALYSIS

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ABSTRACT

This study was seasonally performed over a three year period in a large freshwater temporary pond to investigate (a) seasonal trends of the main abiotic factors, (b) the most important abiotic drivers in shaping macrobenthic invertebrate communities and (c) the structures of these communities among different years. As the study area was placed within a Natural Reserve, the leaf bag technique was used as non invasive sampling method in order to reduce disturbance. The results of chemical and physical investigations point out a clear seasonal trend, while macrobenthic communities differ significantly from year to year, and their main shape drivers were identified to be conductivity, temperature and dissolved oxygen.

Key words: macrozoobenthos, leaf bag, wetland, temporary pool, seasonal dynamics

DINAMICA STAGIONALE DELLE COMUNITÀ MACROZOOBENTONICHE NELLA RISERVA NATURALE REGIONALE DELLA FOCE DELL'ISONZO, NORDEST ITALIA: ANALISI TRIENNALE

SINTESI

Il presente studio triennale è stato condotto stagionalmente in un ampio stagno temporaneo d'acqua dolce, allo scopo di (a) verificare la presenza di gradienti stagionali per i principali fattori abiotici, (b) identificare quali tra questi fattori abbiano il maggior peso nel plasmare le comunità macrozoobentoniche e (c) indagare la struttura di dette comunità su scala pluriennale. Poiché l'area di studio è situata all'interno di una Riserva Naturale, per i campionamenti è stata utilizzata la tecnica dei pacchi fogliari, allo scopo minimizzare l'impatto dovuto alle operazioni di raccolta. Per quanto concerne i parametri abiotici è stato individuato un chiaro trend stagionale, mentre le comunità macrobentoniche sono risultate differire significativamente di anno in anno ed i principali fattori abiotici che le condizionano sono stati identificati nella conduttività, nella temperatura e nell'ossigeno disciolto.

Parole chiave: macrozoobenthos, pacchi fogliari, zone umide, stagni temporanei, dinamica stagionale

INTRODUCTION

Wetlands are one of the most biologically productive ecosystems (Dixion & Wood, 2003; Rolon & Maltchik, 2006; Mereta *et al*., 2012), which perform a wide variety of ecological functions, including nutrient cycling (Bunn *et al*., 1999) and carbon storage (Adhikari & Bajracharaya, 2009). They are also main breeding and feeding grounds for many birds and other wildlife (Williams, 2006). Some temporary wetlands in the Mediterranean Region are considered as priority habitat under the Habitat Directive 92/43/CEE, according to the Natura 2000 network of the European Union (Natura code 3170, 92/43/CEE, 21 May 1992), wetland restoration becoming increasingly important to reverse habitat degradation, recover ecosystem services and maintain biodiversity (Sebastián González & Green, 2014).

Macrobenthic invertebrates have a central role in these ecosystems as they cover all trophic functions (Cummins, 1974; Metcalfe Smith, 1994) and are trophic resources for many species of fishes and birds (Pizzul *et al*., 2008). Due to the severe conditions characterizing temporary habitats, morphological, physiological and/or behavioural adaptation is required for living organisms to survive (Wiggins *et al*., 1980). Alternation of dry and wet phases, which vary from year to year in the Mediterranean Region makes hydroperiod one of the main challenging factors for macrobenthic community structures (Wellborn *et al*., 1996; Spencer *et al*., 1999). Drought in particular, due to its unpredictability, represents a major constraint for organisms inhabiting temporary waters (Grillas & Roche, 1997). In fact, the macroinvertebrate assemblage structures are deeply affected by droughts (Acuña *et al*., 2004; Bonada *et al*., 2006), though the extent of changes depends on the biological adaptations of the species within the community (Boulton & Lake, 1992). Annual and seasonal variations of macroinvertebrate assemblages have been reported for temporary ponds in wetlands of Massachusetts (Brooks, 2000) or in intermittent streams in Victoria Australia (Boulton & Lake, 1992) and have been associated with parallel changes in environmental conditions during the wet phase. Jeffries (1994) found differences in the macroinvertebrate assemblages of the same ponds in three different years, including a low rainfall year in which ponds did not fill. Similar studies have been also recently carried out, also in the Mediterranean area (Florencio *et al*., 2009; Dìaz-Paniagua *et al*., 2010).

Our investigation was carried out in the Regional Reserve of the Isonzo River Mouth (Northeast Italy), a marshy area including mainly freshwater environments alongside with brackish. The Reserve represents the northernmost wetland in the Mediterranean area and it is included in a Site of Community Importance (SCI IT3330005) and in a Special Protection Area (SPA IT3330005). Several studies on the macrobenthic fauna were conducted in the Reserve, both in freshwater and salt marshes (Stoch, 1995; Pizzul *et al*., 2008; Boggero *et al*., 2011; Ruzič *et al*., 2013) to assess the taxonomic structure of the communities, but a long temporal scale (years) was yet never considered.

This study was performed within a large freshwater temporary pond over a three year period. Our aims were to investigate (a) the presence of seasonal trends for the main abiotic factors, (b) which are the most important chemical and physical drivers in shaping macrobenthic invertebrates communities, and (c) the presence of differences in macrobenthic invertebrate communities structure among different years in a temporary environment.

MATERIAL AND METHODS

Study area

The study was performed in an enclosed basin with a surface of about 30 ha (Fig. 1) and supplied mostly by rainwater and by an artesian well. The basin, enclosed by an embankment, looks like a large temporary pond, partially resulting from environmental recovery management. The western portion of this area is a damp pasture, while the eastern portion is a reed bed (*Phragmites*

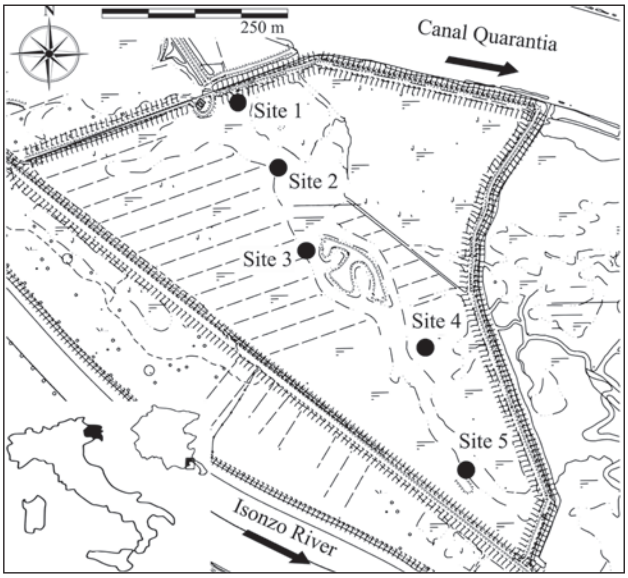

Fig. 1: Study area and sampling sites (UTM coordinates: site 1 N 33T5067699.47 – E 383832.23; site 2 N 33T5067605.64 – E 383897.48; site 3 N 33T5067474.89 – E 383955.56; site 4 N 33T5067349.38 – E 384063.44; site 5 N 33T5067140.24 – E 384187.07).

Sl. 1: Območje raziskave in vzorčna mesta (UTM koordinate: lokaliteta 1 N 33T5067699.47 – E 383832.23; lokaliteta 2 N 33T5067605.64 – E 383897.48; lokaliteta 3 N 33T5067474.89 – E 383955.56; lokaliteta 4 N 33T5067349.38 – E 384063.44; lokaliteta 5 N 33T5067140.24 – E 384187.07)

australis (Cav.) Trin ex Steud). The study pond was of autumnal origin (autumnal ponds, *sensu* Wiggins *et al*., 1980): the dry phase occurs in summer (from June to September) and the wet phase begins in early autumn. Basin waters are classified from limnic to oligohaline (Stoch, 1995) and maximum depth ranged from 1.7 to 2.0 m (Stoch, 1995; Perco *et al*., 2006).

As this area represents a wintering and stopover site for many bird species, management policy limits the human access only to the working personnel. However, some management actions are conducted: the vegetation growth control is performed both passively (grazing of Camargue horses and periodically cattle) and actively (using machines), while the water level is controlled only with a flap sluice gate placed at the northwest side of the basin. This gate is occasionally open in summer for a few days (usually late July/mid August, but not every year) to ease the up drying of the basin. The water level is controlled for ecological reasons (Pizzul *et al*., 2008), to avoid anoxic condition in the bottom sediments and to favour the nutrients remineralisation (Street, 1982).

Sampling design

The present study was carried out from October 2009 to July 2012 at five sampling sites (Fig. 1), which were chosen considering water depth, vegetation coverage on the bottom and presence of vegetation nearby. The first site was placed near the sluice side gate, the second beside an islet without vegetation and the third beside a wooded islet, the fourth site was placed at the centre of the basin and the fifth near the reeds, where vegetation cover was observed. Sampling campaigns were conducted in autumn, spring and early summer. Although planned, the winter campaigns were not performed, as the presence of ice prevented the access to the area.

Abiotic parameters

Depth was measured at each sampling time with a graduated rod. In water column, conductivity, temperature, dissolved oxygen (DO) and pH were recorded using field meters. Sediment samples were collected with a manual corer (6 cm internal diameter) in order to assess concentrations of NH_4^+, NO_2^-, and NO_3^- in the bottom interstitial water from October 2011 to July 2012. The corer was pushed into the surface sediment about 20-25 centimetres, then samples were frozen and brought to the laboratory, where concentrations of nutrients were measured using the methods reported by Solórzano (1969) and Presley (1971) for NH_4^+, IRSA CNR & APAT (2003) for NO_2^-, and APHA (1992) for NO_3^-. Seasonal rainfall data (mm of fallen rain) were obtained by a weather station placed nearby the Reserve.

Macrobenthic invertebrates

The leaf bag technique (Petersen & Cummins, 1974) was used to assess macrobenthic community structure.

Tab. 1: Mean seasonal values and standard deviations (in parenthesis) of the physical and chemical parameters for the whole study area.
Tab. 1: Srednje sezonske vrednosti in standardni odkloni (v oklepaju) fizikalnih in kemijskih parametrov za celotno območje raziskave

	2009-2010		2010-2011		2011-2012		
	Autumn	Spring	Autumn	Spring	Autumn	Spring	Summer
Depth (cm)	30.2 (9.2)	36.4 (11.1)	37.9 (11.4)	24.2 (14.6)	33.8 (10.4)	27.8 (10.7)	12.6 (11.5)
T (°C)	11.7 (3.4)	19.0 (4.2)	13.3 (1.9)	19.3 (2.5)	10.0 (5.2)	20.3 (2.6)	22.3 (1.6)
pH	7.2 (0.5)	7.8 (0.6)	7.7 (0.6)	8.5 (0.4)	7.7 (0.3)	7.8 (0.3)	8.3 (0.7)
DO (mg l^{-1})	4.5 (2.3)	4.0 (1.9)	5.1 (1.0)	2.3 (0.6)	8.5 (2.7)	2.4 (1.3)	3.2 (1.3)
Cond (mS cm^{-1})	2.6 (0.9)	2.7 (0.6)	2.1 (0.2)	3.0 (1.4)	3.7 (1.0)	4.9 (1.3)	6.5 (2.9)
Rainfall (mm)	185.3 (3.8)	62.4 (26.8)	172.2 (65.3)	51.8 (21.1)	96.9 (103.5)	104.6 (39.1)	31.5 (28.2)
NO_3^- (mg l^{-1})	-	-	-	-	0.74 (0.41)	0.55 (0.16)	0.52 (0.25)
NO_2^- (mg l^{-1})	-	-	-	-	0.06 (0.08)	1.11 (1.07)	1.38 (1.40)
NH_4^+ (mg l^{-1})	-	-	-	-	2.78 (1.85)	3.97 (1.91)	4.73 (1.93)

This method is commonly accepted as a quantitative approach to the study of both detritus processing and colonization by macrobenthic invertebrates and it was used in lotic environments (Robinson & Jolindon, 2005; Fenoglio *et al*., 2006) as well as in lentic (Pope *et al*., 1999) and transitional waters (Mancinelli *et al*., 2005; Sangiorgio *et al*., 2008). The technique was chosen in agreement with the management policies of the Reserve in order to reduce the disturbance and the impact of the sampling operations, because this method is less invasive than others (*e.g.* grabs and corers), it is faster to be performed on the field and therefore the disturbance to the present fauna (especially birds) was reduced. Leaves of *P. australis* were collected within the Reserve in early autumn 2009 and were air dried. The fragments of central leaf section (10 cm length) were oven dried to constant weight (60 °C for 72 h) and single lots of 3±0.001 g dry weight were placed in mesh bags (5×5 mm mesh size). Subsample units were set up connecting four leaf bags. Three subsamples were placed at each site in early October, early April and at the beginning of June for the collection in autumn, spring and summer, respectively. Subsamples were recovered after 15, 30 and 45 days of submersion. In the laboratory, leaf bags were opened and macrobenthic invertebrates were separated from the leaves and stored in a formaldehyde solution (final concentration 4%) until sorting operations. After washing, all invertebrates were counted and identified to family level. Chironomidae were identified until subfamily or tribe level. Percentage frequencies and seasonal mean number of observed taxa were calculated for each site.

Statistical analysis

Seasonal mean values of physical and chemical parameters were calculated for each sampling site and Principal Component Analysis (PCA) was performed using a three year data set to search for ecological gradients. Another PCA was carried out using only the third year data set, which includes also nutrients. Pearson's coefficient was used to seek correlation among abiotic variables and between variables and PCA axes. Two way ANOVA (factors: season, year) and LSD post hoc tests were performed to search for significant differences among the seasons and among same seasons of different years, while one way ANOVA was performed on the last year data set, to assess seasonal differences. Before analysis, all data were log(x+1) transformed.

Differences about community composition were investigated with two way PERMANOVA (factors year and season; 999 permutations) (Anderson, 2001) and SIMPER test, which were carried out on the similarity matrix based on the Bray-Curtis coefficient. Finally, Canonical Correspondence Analysis (CCA) was adopted to correlate abiotic factors and taxa relative abundances. Taxa present only in a subsample and with very low occurrences (1 individual) were considered rare and excluded from the analysis. Relations between biotic and abiotic data were also investigated with Mantel test (Mantel & Valand, 1970) performed on Bray Curtis based similarity matrix. The normality of all datasets was verified with the Kolmogorov-Smirnov test and variance homogeneity was checked with Brown-Forsythe test. We used STATISTICA 7.1 and PAST 3.2 (Hammer *et al*., 2001) for all analyses. Leaf bags for site 3 were lost in autumn 2009, because of the interference of animals living in the area (horses and/or coypus). Summer data were obtained only for the last year (summer 2012), but not for site 5 because it dries up before the end of the sampling operations. Therefore, data of sites 3 (autumn 2009) and 5 (summer 2012) were not included in the statistical analysis.

RESULTS

Abiotic parameters

Mean seasonal abiotic parameters are reported in Table 1. The first two components obtained with the PCA using the three year dataset, explain 70.2 % of the system variance (Fig. 2a): loadings of sampling stations groups indicate a seasonal gradient, identified by the first axis, which explains 53.0 % of the system variation. Total rainfall, dissolved oxygen, conductivity and temperature are correlated to the first axis. The second axis explains 17.2 % of variance and is correlated to depth and pH (Tab. 2) and likely identifies a spatial gradient. In particular, we observed that site 5 always displayed at the bottom of seasonal groups: this site shows lower depths than the others, it was the first site subjected to draining in late spring and the last site subjected to flooding in early autumn. Finally, it harbours rich cover vegetation during spring. Except for depth and pH, two way ANOVA showed significant differences for all abiotic parameters among seasons and confirmed the seasonal trend ($F_{5,23} > 3.13$, $p < 0.05$; LSD test: at least $p < 0.05$, except for dissolved oxygen and conductivity during the first year and for rainfall during the last year).

Temperature values differ significantly from year to year both in autumn and spring as dissolved oxygen levels do (except between first and second year in autumn) (LSD test: at least $p < 0.05$). Conductivity and rainfall values observed during the third year differ significantly from others (LSD test: at least $p < 0.05$). Positive correlations with rainfall were found for depth ($r = 0.42$, $p < 0.05$) and dissolved oxygen concentrations ($r = 0.45$, $p < 0.05$) while a negative correlation was found with water temperature ($r = -0.66$, $p < 0.05$), pH ($r = -0.46$, $p < 0.05$) and conductivity ($r = -0.54$, $p < 0.05$).

The seasonal trend was confirmed by the results of the PCA using the last year data set with an increased number of parameters (Fig. 2b). The first two components explain 71.3 % of the variability and the main seasonal gradient was identified by the first axis again: all

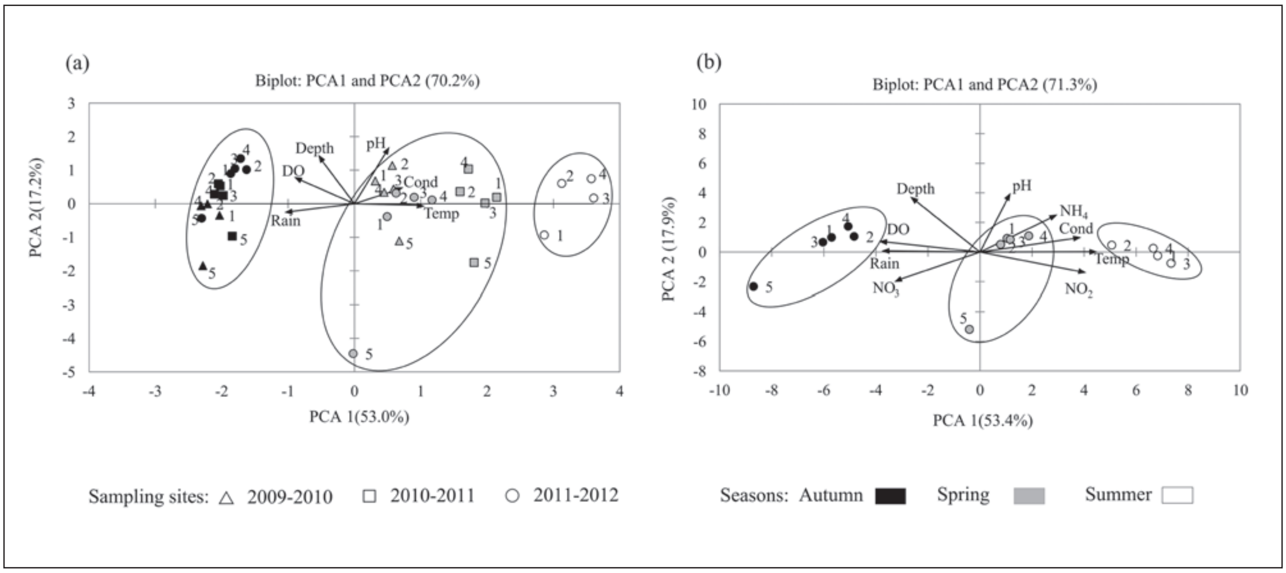

Fig. 2: (a) Principal Component Analysis (PCA) applied to the mean seasonal values of physical and chemical parameters for each sampling site to the three year data set; (b) PCA applied to the mean seasonal values of physical and chemical parameters (including nutrients) for each sampling site during the years 2011-2012. DO - dissolved oxygen, Cond - conductivity, Temp - temperature.
Sl. 2: (a) Analiza glavnih komponent (PCA) srednjih sezonskih vrednosti fizikalnih in kemijskih parametrov za vsako vzorčno mesto na triletnem podatkovnem nizu; (b) PCA analiza srednjih sezonskih vrednosti fizikalnih in kemijskih parametrov (vključno s hranili) za vsako vzorčno mesto v letih 2011 in 2012. DO - raztopljeni kisik, Cond - prevodnost, Temp - temperatura.

Tab. 2: Significant correlations (Pearson coefficient enlightened in bold, p < 0.001) among parameters and PCA factors (axes 1 and 2).
Tab. 2: Značilne korelacije (Pearsonov koeficient v krepkem tisku, p < 0,001) med parametri in PCA dejavniki (osi 1 in 2)

	PCA 1		**PCA 2**	
Axis	F1	F2	F1	F2
Depth	-0.445	**-0.664**	-0.538	**0.753**
Temperature	**0.879**	-0.022	**0.930**	0.004
pH	0.411	**-0.756**	0.235	-0.800
DO	**-0.724**	-0.359	**-0.776**	0.145
Cond	**0.602**	-0.220	**0.781**	0.198
Rainfall	**-0.903**	0.126	**-0.758**	0.015
NO_3^-	-	-	**-0.679**	-0.406
NO_2^-	-	-	**0.833**	-0.287
NH_4^+	-	-	**0.593**	0.502

monitored nutrients are correlated to the first axis (Tab. 2) and follow the seasonal trend with higher concentration of NO_3^- in autumn and higher concentrations of NO_2^- and NH_4^+ during the warmer seasons. One way ANOVA showed significant seasonal differences for depth, temperature, dissolved oxygen, conductivity and NO_2^- ($F_{5,23} > 4.66$, at least $p < 0.05$). Water temperature was positively correlated with NO_2^- and NH_4^+ concentrations ($r = 0.83$ and 0.59, respectively, $p < 0.05$) and negatively with NO_3^- ($r = -0.69$, $p < 0.05$).

Macrozoobenthic community

27,154 macrobenthic invertebrates, belonging to 23 taxa, were identified. Differences were detected among relative abundances during the three years but the organisms most frequently observed belonged to few taxonomical groups: Hexapoda, Oligochaeta, Ostracoda, Malacostraca and Nematoda (Tab. 3). Mean number of observed taxa was always significantly higher in spring (Fig. 3) ($F_{5,23} = 11.9$, $p < 0.001$; LSD test: $p < 0.001$ for all comparisons). The two way PERMANOVA (factors: year and season) was highly significant for both main effects (year: $F = 6.67$, $p < 0.001$; season: $F = 2.18$, $p < 0.001$) and also for the year × season interaction ($F = -0.48$, $p < 0.001$). SIMPER test showed that main contributes to the differences are related to Chironominae (30.8 %), Ostracoda (24.4 %), Nematoda (12.5 %) and

Tab. 3: Seasonal occurrence (%) of the observed taxa for all the study years. Codes used in the CCA analysis are reported for each taxon. Legend: Aut – autumn, Spr – spring, Sum – summer.
Tab. 3: Sezonsko pojavljanje (%) opazovanih taksonov v vseh letih raziskave. Za vsak takson je prikazana tudi koda, uporabljena pri CCA analizi. Legenda: Aut – jesen, Spr – pomlad, Sum – poletje.

Phylum	Class	Order	Family /Subfamily	CCA code	Aut 2009	Spr 2010	Aut 2010	Spr 2011	Aut 2011	Spr 2012	Sum 2012
Nematoda				Nem		1.61	0.87	5.00	32.76	11.94	1.49
Mollusca	Gastropoda	Pulmonata	Planorbidae	Pla	0.31	0.93	0.14	0.03		0.09	
			Physidae	Phy	0.10	0.03	1.92	0.13		0.03	
Anellida	Oligochaeta	Tubificida	Tubificidae	Tub	0.31	11.97	8.60	30.17	3.43	0.66	
			Naididae	Nai	0.31	1.83	6.40	16.89	0.53	0.02	
			Enchytraeidae	-				0.01			
		Opisthopora	Lumbricidae	-					0.20		
Arthropoda	Ostracoda			Ost	20.90	15.63	12.83	7.59	23.78	63.40	36.31
	Malacostraca		Asellidae	Ase	1.67	3.10	0.17	0.16	4.43	0.54	0.13
			Gammaridae	Gam	0.63	2.79	0.98	2.32	3.10	2.31	5.57
	Hexapoda	Odonata	Libellulidae	Lib		0.19		0.01			
			Corixidae	Cor	0.10					0.05	0.26
		Coleoptera	Haliplidae	Hal		0.16		0.03			
			Dytiscidae	Dyt		0.59	0.07	0.06		0.02	
			Hydrophilidae	Hyd	0.10	0.84	0.03	0.08		0.15	0.71
		Diptera	Ceratopogonidae	Cer	0.52	1.27		0.21	0.33	0.31	0.06
			Chironominae*	Chi	74.51	58.91	67.35	36.43	30.84	20.31	55.91
			Orthocladiinae*	Ort		0.03		0.09			
			Tanypodinae*	Tan	0.21	0.09	0.59	0.76	0.33	0.12	0.26
			Tabanidae	Tab	0.31	0.03	0.03	0.01	0.07	0.03	
			Dolichopodidae	-					0.07		
			Ephydridae	Eph					0.13	0.02	
			Muscidae	-				0.02			

* Subfamily

the Oligochaeta Tubificidae (12.4 %) and Naididae (7.1 %). Remaining taxa shows contributes less than 5 %. Diptera Chironomidae of the subfamily Chironominae (tribes Chironomini and Tanytarsini) represent the most abundant taxon (Tab. 3). The crustacean class Ostracoda showed percentage frequencies between 7.59 and 63.4 % and was the most abundant taxon during spring and summer of the third year (Tab. 3). Oligochaeta belonged almost exclusively to the families Tubificidae and Naididae and were more abundant in samples taken between spring 2010 and spring 2011 (Tab. 3), while a decline was observed during the third year. Finally, the Phylum Nematoda was one of the most abundant taxon in the last year, showing percentage frequencies significantly higher than in previous. Coleoptera (families Dytiscidae, Haliplidae, Hydrophilidae) and Gastropoda (families Planorbidae, Physidae) showed higher abundances in the sites near the reeds (sites 1 and 5).

Temperature, dissolved oxygen and conductivity were significantly correlated with biotic dataset (Mantel test: $p < 0.01$ for all cases). The relations among abiotic parameters and taxa are showed in the CCA graph (Fig. 4): the first two axes explain 74.8 % of the system variance; eigenvalues are reported in Table 4. All the sites

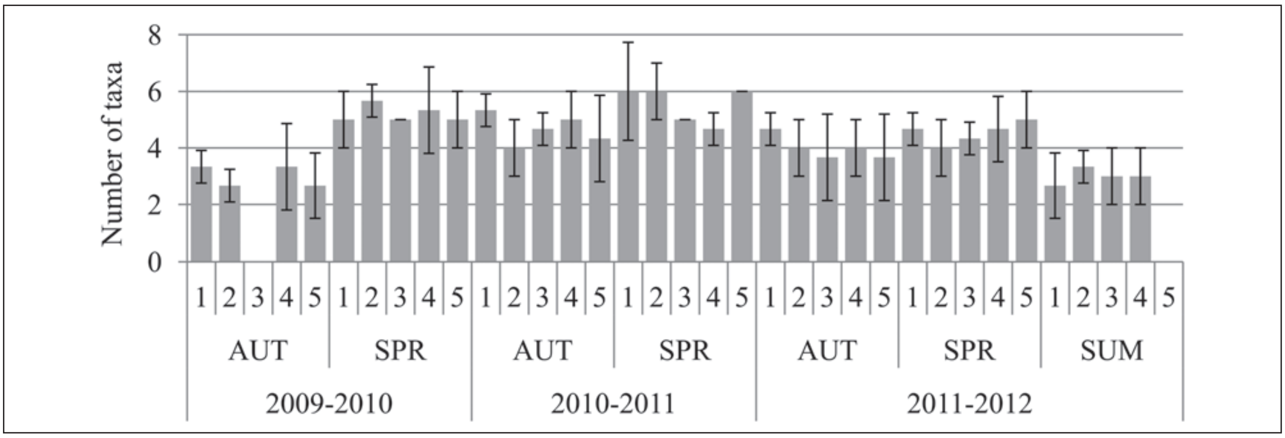

Fig. 3: Mean number of taxa ± SD observed in every site at each sampling season.
Sl. 3: Povprečno število taksonov ± SD za vsako vzorčno mesto, v vsaki sezoni vzorčenja

studied during the last year are placed at the left side of the graph, corresponding to significantly higher values of conductivity (Tab. 1) and to higher occurrences of Ostracoda (Tab. 3). Oligochaeta (families Tubificidae, Naididae) and Gastropoda (families Planorbidae and Physidae) showed negative preferences for higher conductivity values. Coleopterans Dytiscidae, Haliplidae and Hydrophilidae, as Gastropoda families Planorbidae and Physidae seem to be more related to the lower depths of site 5, where their occurrences were higher.

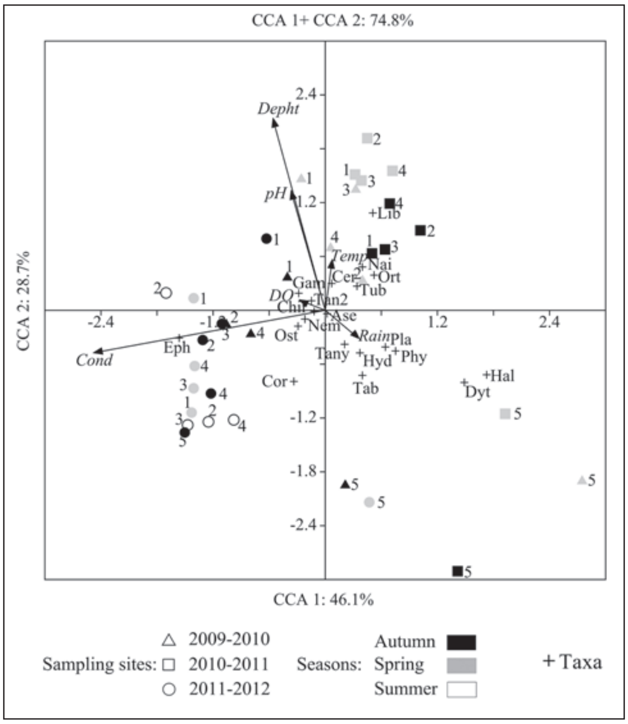

Fig. 4: Canonical Correspondence Analysis (CCA) biplot of environmental-taxa relationships. DO - dissolved oxygen, Cond - conductivity, Temp - temperature; taxa codes are reported in Table 3.
Sl. 4: Kanonična korespondenčna analiza (CCA): prikaz sestave taksonov v odnosu s spremenljivkami okolja. DO - raztopljeni kisik, Cond - prevodnost, Temp – temperatura; kratice taksonov so prikazane v tabeli 3.

DISCUSSION

The coupled effect of physical environmental factors and biotic interactions has been proposed as the mechanism for generating community structures in freshwater habitats (Wellborn *et al.*, 1996). Along a gradient from small ephemeral pools to larger semi-permanent and permanent ponds, periodic drying is recognized as a major constraint to invertebrate species composition (Jeffries, 1994; Schneider & Frost, 1996; Williams, 2006). The observed ranges for the values of dissolved oxygen concentrations, pH, water temperature and depth appear to be similar to what reported from other Authors in studies about Mediterranean temporary environments (Waterkeyn *et al.*, 2008; Bazzanti *et al.*, 2010; Dìaz-Paniagua *et al.*, 2010; Florencio *et al.*, 2013) and the results of chemical-physical investigations point out a seasonal trend, due to the partially natural and partially managed draining/flood cycle.

Conductivity shows a wide range among the years, probably because of infiltrations through the flap sluice gate but also because of the different rainfall observed. In fact, all the abiotic parameters appear to be related to rainfall, which can have great influence on many physical characteristics of temporary ponds, with important variation from dry to wet years. Consequently, macroinvertebrate assemblages may also differ among wet and dry periods (Jeffries, 1994).

Chemical and physical analyses point out a seasonal trend and the taxonomical richness (as number of taxa) was always significantly higher in spring. Nevertheless, the

Tab. 4: Summary of the CCA analysis performed on the observed taxa relative abundances and the physical-chemical parameters.

Tab. 4: Povzetek CCA analize, izvedene na podatkih o relativni številčnosti opazovanih taksonov in fizikalno-kemijskih parametrih

	Axis 1	Axis 2
Eigenvalue	0.109	0.068
Cumulative percentage variance of taxa-environment relationship	46.08	72.75
Significance to Montecarlo test (999 permutations), p	0.002	0.001

macrobenthic community was always dominated by few taxa which showed different occurrences from year to year.

Higher abundance of Ostracoda observed during the last year could be related to their wide ranges of ecological tolerance (Külköylüoğlu *et al.*, 2012). In particular, some species tolerate a wide range of salinity and/or temperature (Ghetti & Mc Kenzie, 1981). In addition a previous study within the study area (Stoch, 1995) reported the presence of *Cyclocypris ovum* and *Cypridopsis vidua*, able to resist to low concentrations of dissolved oxygen (respectively down to 2.0 and 1.6 mg l^{-1}) and to colonize both limnic and oligohaline environments. In this way temperature, dissolved oxygen and conductivity were found to be the main factors affecting the structure of macrobenthic invertebrates communities (Rossaro, 1991; Kagalou *et al.*, 2006; Gabriels *et al.*, 2007; Boets *et al.*, 2010), whereas the duration of the wet phase may have a particular impact on the number of present taxa (Schneider & Frost, 1996; Brooks, 2000; Batzer *et al.*, 2004; Della Bella *et al.*, 2005). The most abundant invertebrates collected during this study were Diptera Chironomidae (subfamily Chironominae), Oligochaeta, Ostracoda and Nematoda, which mainly contributed to the community differences among the years. The high abundance of these taxa is linked to their time of colonization strategies (Oter-

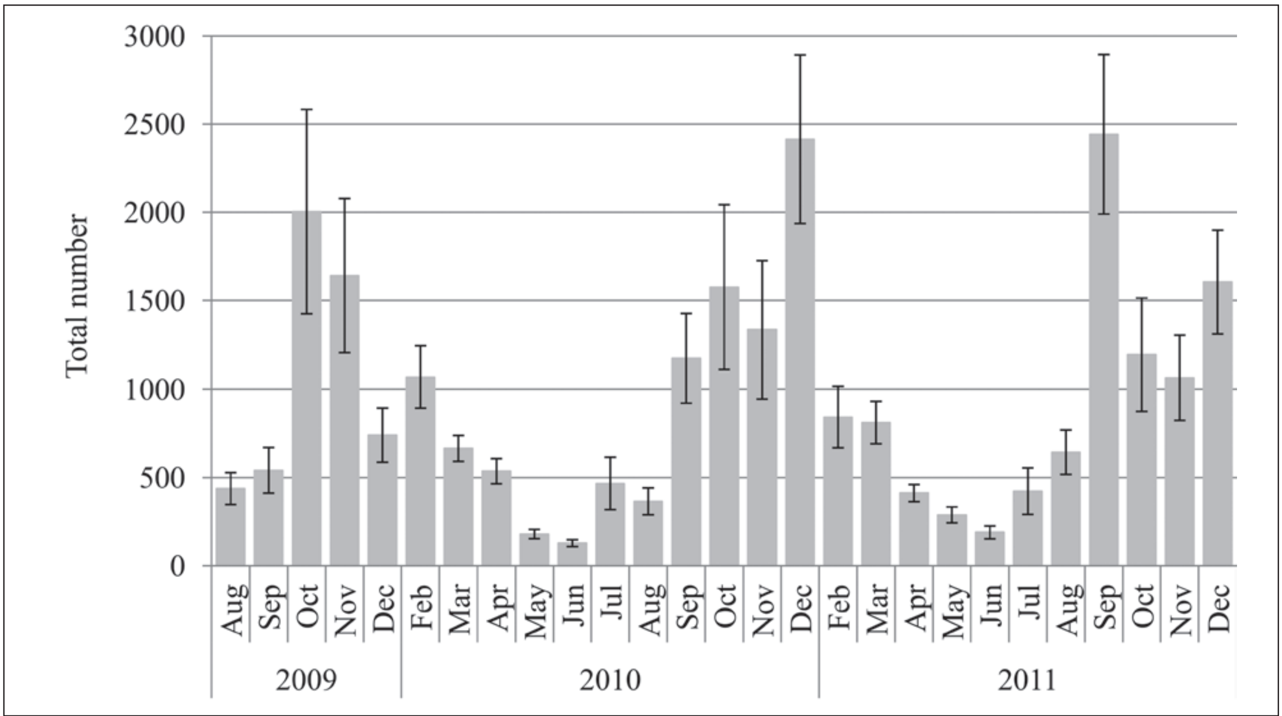

Fig. 5: Total number of bird specimens ± SD in the study area from August 2009 to December 2011. Considered species are: *Cygnus olor, Cygnus atratus, Anser fabalis, Anser albifrons, Anser erythropus, Anser anser, Tadorna tadorna, Anas penelope, Anas strepera, Anas crecca, Anas platyrhynchos, Anas acuta, Anas querquedula, Anas clypeata, Philomachus pugnax, Limosa limosa, Limosa lapponica, Anser indicus, Branta ruficollis, Branta leucopsis* ***and*** *Alopochen aegyptiaca* ***(data provided by the Biological Station of Cona Island).***

Sl. 5: Skupno število osebkov ptičjih vrst ± SD na območju raziskave med avgustom 2009 in decembrom 2011. Upoštevane vrste so: *Cygnus olor, Cygnus atratus, Anser fabalis, Anser albifrons, Anser erythropus, Anser anser, Tadorna tadorna, Anas penelope, Anas strepera, Anas crecca, Anas platyrhynchos, Anas acuta, Anas querquedula, Anas clypeata, Philomachus pugnax, Limosa limosa, Limosa lapponica, Anser indicus, Branta ruficollis, Branta leucopsis* ***in*** *Alopochen aegyptiaca* ***(podatke je posredovala Biološka postaja Isola della Cona).***

min *et al.*, 2002) and adaptations to overcome dry phases (Wiggins *et al.*, 1980). Furthermore, subfamily Chironominae can become numerically dominant in environments with features similar to those of the study area (Bazzanti *et al.*, 1997), whereas Oligochaeta and Nematoda can be found in the sediments, where they can feed on huge quantities of small sized organic matter (Heino, 2000). As observed by Ruzič *et al.* (2013), due to the water shallowness, the sediment probably greatly affects the water column processes. In fact, low concentrations of dissolved oxygen are likely influenced both by redox conditions and by deposition of an organic layer on the bottom sediment (Mereta *et al.*, 2012). In this work the nutrient load, in particular ammonium, was rather high and could be related to the massive presence of birds (Boros *et al.*, 2008). Indeed, during the study from August 2009 to December 2011 a higher number of individuals of main bird species always occurred between September and February (Fig. 5). Most likely, the sediment got a load of nutrients during autumn/winter whereas in summer the drainage of the area could have had an effect both on the substrate oxygenation and on the nutrients mineralization dynamics, which is dominated by aerobic processes (Reed *et al.*, 2011). Furthermore, also the management practices have to be considered: water level control can be implemented through the opening of the sluice gate during summer, which may have important consequences on the system, as observed, because dry phase enhances the mineralization of nutrients, avoiding hypoxia or anoxia conditions (Street, 1982; Pizzul *et al.*, 2008).

Finally, even though the leaf bag technique is known to be potentially selective for certain taxa (Basset *et al.*, 2006), for this long term study we preferred it instead of a quantitative approach with a box corer sampler, which was used by Boggero *et al.* (2011) in the same sites in a previous work. As shown by Quintino *et al.* (2011), box-corer and leaf bags could potentially lead to different results, but both techniques can disclose same ecological patterns in linkage between macrobenthic communities and abiotic descriptors. Furthermore, patterns of benthic communities variation present important similarities. As reported by Ruzič *et al.* (2013), data obtained for the spring communities appear to be comparable with those obtained by Boggero *et al.* (2011), and the impact of the sampling operations was considered greatly lower than a box-corer approach, which can be potentially unsustainable for a protected area, both in terms of human presence in the study site and environmental perturbation. Furthermore, the use of the box corer can be very time consuming.

The study area is included in a Natural Reserve, which is also a Site of Community Importance and a Special Protection Area and represent an example of ecological restoration, where biodiversity reaches high levels and where many microhabitats can be found (Perco *et al.*, 2006). The investigated system as the whole Natural Reserve could provide an excellent model to study succession and changes in macrobenthic invertebrate community structures (Boix *et al.*, 2012; Miguel Chinchilla *et al.*, 2014) and it represents a natural laboratory in a permanent re-colonization state (Matthaei *et al.*, 1996) in which the effect of conservation management strategies on communities of macrobenthic invertebrates could be directly observed (Ruzič *et al.*, 2013).

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SEZONSKA DINAMIKA MAKROZOOBENTOŠKIH SKUPNOSTI V REGIONALNEM NARAVNEM REZERVATU IZLIVA SOČE, SEVERNA ITALIJA: TRILETNA ANALIZA

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POVZETEK

Pričujoča triletna raziskava je bila izvedena sezonsko v veliki začasni sladkovodni mlaki, z namenom, da se: (a) preveri prisotnost sezonskih gradientov za glavne abiotske dejavnike, (b) ugotovi, kateri od teh dejavnikov imajo največjo težo pri oblikovanju makrobentoške skupnosti, in (c) razišče strukturo teh skupnosti v večletnem obdobju. Ker se območje raziskave nahaja v naravnem rezervatu, so avtorju uporabili tehniko »listnatih zavojev« za vzorčenje, da bi zmanjšali vpliv nabiranja vzorcev. Potrdili so sezonski trend abiotskih parametrov, medtem ko so se makrobentoške skupnosti bistveno razlikovale med leti. Avtorji so ugotovili, da med glavne abiotske dejavnike, ki vplivajo na makrobentoške skupnosti, lahko štejemo prevodnost, temperaturo in raztopljeni kisik.

Ključne besede: makrozoobentos, listnati zavoji, mokrišča, začasne mlake, sezonska dinamika

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LE ORCHIDACEAE DELLA PUGLIA (ITALIA MERIDIONALE)

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SINTESI

La Puglia è una regione dell'Italia meridionale con una superficie di 19.540,9 km² e vanta un patrimonio floristico che al termine del 2010 era stimato in 2352 taxa. Nel presente lavoro si riporta l'elenco floristico di tutte le entità di Orchidaceae segnalate compresi gli ibridi e si esegue l'analisi corologica. Nel complesso sono segnalati 102 tra specie e sottospecie cui si aggiungono 188 ibridi. A sua volta l'analisi corologica evidenzia la prevalenza degli elementi mediterranei e degli endemismi.

Parole chiave: Orchidaceae, checklist regionale, Puglia, Italia meridionale

THE ORCHIDS OF PUGLIA (SOUTHERN ITALY)

ABSTRACT

Apulia is a region in Southern Italy extending over an area of 19,540.9 km² and boasting a floral heritage of an estimated 2,352 taxa as of the end of 2010. This paper lists all the members of the Orchidaceae family reported, including hybrids, and provides a phytogeographical analysis. The results show a total of 102 reported species and subspecies, with an additional 188 hybrids, whereas the phytogeographical analysis indicates the predominance of Mediterranean elements and endemism.

Keywords: Orchidaceae, regional checklist, Apulia, Southern Italy

INTRODUZIONE

Il presente articolo è finalizzato, attraverso l'esame degli studi condotti nell'area d'interesse noti in letteratura, a compilare una *Checklist* comprendente le specie, le sottospecie e gli ibridi di Orchidaceae censiti e ricavare approfondimenti di carattere quantitativo e qualitativo.

Le ricerche floristiche di una certa importanza in Puglia iniziarono nel XIX secolo con Baselice (1812), Tenore (1830), Rigo (1876), Groves (1887), Martelli (1893), Palanza (1898) e Fiori (1899), che nei loro scritti segnalano il ritrovamento di varie Orchidaceae.

In particolare Baselice (1812) segnalò sul Gargano *Ophrys insectifera* L. mai più ritrovata, Groves (1887) segnalò diversi ritrovamenti nei dintorni di Otranto, Martelli (1893) segnalò *Orchis mascula*, recentemente riconfermata e Palanza (1898) segnalò il ritrovamento in Terra di Bari di *Serapias parviflora*. Qualche anno dopo Cortesi (1915) pubblicò uno studio monografico sulle orchidacee osservate nei pressi di Nardò (Le).

In seguito le segnalazioni si sono incrementate e nella flora d'Italia Pignatti (1982) riportava per la regione cinquanta diverse entità. Bianco *et al.* (1994) ne indicavano settantatré e, a sua volta Pezzetta (2011) ne riportava ottantacinque.

Poi sono seguite altre pubblicazioni su riviste scientifiche e studi monografici (Turco & Medagli, 2012; Turco *et al.,* 2012; Gennaio & Medagli, 2013; Perilli, 2013; Griebl, 2014; Rossini & Quitadamo, 2014; D'Alonzo, 2015; Gennaio & Medagli, 2015; Siletti & Medagli, 2015) che tenendo conto delle ricerche sul campo e delle novità tassonomiche hanno incrementato il patrimonio orchidologico regionale.

MATERIALI E METODI

La Puglia è una regione dell›Italia Meridionale con la superfice di 19.540,9 km² che oltre alla terraferma comprende: l'arcipelago delle Tremiti situate a nord-est del Gargano, le piccole isole Cheradi presso Taranto e l'isola di Sant'Andrea davanti a Gallipoli (Mola, 1997). Essa confina a nord-ovest con il Molise, a ovest con la Campania e la Basilicata, a est e nord con il mare Adriatico e a sud con il mar Ionio.

Com'è visibile nella Figura 1, l'ambito si può ripartire in 8 diverse subregioni: il Gargano, il Subappennino Dauno il Tavoliere, le Murge, la Terra di Bari, la Valle d'Itria, l'Arco ionico tarantino e il Salento. Il territorio è pianeggiante per il 53 %, collinare per il 45 % e montuoso solo per il 2 %. I monti più elevati si trovano nel Subappennino Dauno dove si toccano i 1152 m del Monte Cornacchia e sul Gargano con m 1056 del Monte Calvo. L'ambito collinare è suddiviso tra: 1) le Murge, un altopiano carsico ove si raggiunge la massima altitudine con il Monte Caccia (679 m); 2) le Serre Salentine che si trovano nella parte meridionale della provincia di Lecce e raggiungono l'altezza massina di 201 m a Serra dei Cianci. Le pianure sono costituite dal Tavoliere che occupa circa 3000 km² e dalla Pianura Salentina, che si estende in gran parte del brindisino e dalla parte settentrionale della provincia di Lecce sino alla parte meridionale della provincia di Taranto. Dal punto di vista geologico la Puglia è costituita per quasi l'80 % da rocce calcaree e dolomitiche.

La natura carsica del territorio e la scarsità di precipitazioni rendono la regione povera di corsi d'acqua superficiali. I fiumi sono caratterizzati da corsi brevi a carattere torrentizio tranne l'Ofanto e il Fortore, che hanno in regione solo parte del loro percorso. I laghi naturali, tutti costieri, sono separati dal mare Adriatico da stretti cordoni sabbiosi. Tra essi i più importanti sono: quelli di Lesina e di Varano sulla costa settentrionale del Gargano; l'area umida del lago Salso presso Manfredonia.

La Puglia è dominata dal clima mediterraneo che assume parametri diversi nei vari ambiti territoriali. Il regime pluviometrico, è caratterizzato da un massimo di precipitazioni in autunno inoltrato, un massimo secondario ad inizio primavera e valori minimi durante la stagione estiva. I valori di piovosità variano da oltre 1100 mm annui nella Foresta Umbra a 600-700 mm lungo i rilievi appenninici e scendono sino a 400 mm sul Tavoliere e nel Golfo di Taranto (Macchia *et al.,* 2000).

Sul Gargano, l'Appennino Dauno e l'altopiano delle Murge sono frequenti le nevicate in caso di correnti fredde provenienti da est. Le temperature medie annue oscillano tra 11 e 16 °C mentre le escursioni termiche tra estate e inverno sono notevolissime nelle aree interne. Infatti, nel Tavoliere si può passare da oltre 40 °C durante l›estate a -3 °C nella stagione invernale. Secondo Macchia *et al.* (2000) è possibile ripartire la regione in tre diverse aree climatiche caratterizzate da isoterme che vanno da 7-11 °C sino a 14-16 °C. Alle particolari tipologie climatiche e di morfologia territoriale si associano diverse fitocenosi vegetali in cui attecchiscono 2352 entità di piante vascolari (Peruzzi, 2010).

La Puglia rappresenta un'area di grande interesse dal punto di vista floristico e biogeografico. Infatti, a causa della sua storia geologica e della posizione costituisce un punto d'incontro tra la flora del mediterraneo orientale e quella del resto della penisola (Francini Corti, 1966, 1967; Pezzetta, 2010).

L'elenco floristico è stato realizzato tenendo conto delle ricerche sul campo dell'autore (condotte soprattutto sul Gargano), delle segnalazioni inedite di vari studiosi e dei dati ricavati dalle consultazioni bibliografiche. Esso comprende le specie, le sottospecie e gli ibridi ora noti mentre non si considerano le varietà cromatiche e morfologiche le cui citazioni non sono riportate neanche in bibliografia. A fianco di ogni taxon si riportano gli autori di segnalazioni recenti (dal 1985), eventuali diversi sinonimi utilizzati dagli studiosi e osservazioni e opinioni formulate sul rango tassonomico. Nelle citazioni delle segnalazioni fatte in più occasioni

Fig. 1: Suddivisione della Puglia in diverse sub regioni
Sl. 1: Razdelitev Apulije v posamezne podregije

dai medesimi autori, si è scelto di preferire i loro lavori riassuntivi e non riportare più volte gli stessi nomi.

Numerosi e vari sono stati gli studi botanici sia sulla flora regionale nel suo complesso e sulle Orchidaceae in particolare. Considerata la grande vastità delle pubblicazioni esistenti, in tale sede sono citati e inseriti in bibliografia quelle effettivamente consultate e recenti che ad avviso dello scrivente sono ritenute più importanti poiché segnalano per la prima volta il ritrovamento di un taxon, lo riconfermano o ne approfondiscono il rango tassonomico.

Per la nomenclatura si sono seguite le indicazioni riportate nel recente volume a cura di GIROS (2009). Per l'assegnazione dei tipi corologici si è tenuto conto di quanto riportato in Pignatti (1982) e Pezzetta (2011). Inoltre per varie entità, tenendo conto delle nuove segnalazioni e dell'attuale distribuzione, si è operata una ridefinizione del corotipo di appartenenza.

Tutte le segnalazioni di *Ophrys classica*, *Ophrys romolinii* e *Ophrys tenthredinifera* sono state ricondotte rispettivamente a *Ophrys sphegodes, Ophrys bertolonii* e *Ophrys neglecta*.

Nel corotipo Appennino-Balcanico sono stati inclusi i taxon che sono compresi entro i seguenti limiti territoriali: a) per quanto riguarda la penisola italiana tutte le isole e l'arco appenninico dalla Liguria all'Aspromonte; b) per quanto riguarda la penisola balcanica tutte le isole ionico-egeiche e il territorio continentale posto a sud dell'asse fluviale che va dalle sorgenti della Sava alle foci del Danubio e dal Mar Nero all'Adriatico-Ionio (Pezzetta, 2010).

RISULTATI E DISCUSSIONE

Le ricerche effettuate hanno portato alla realizzazione dell'elenco floristico che segue [1].

1. *Anacamptis collina* (Banks & Solander) R.M. Bateman, Pridgeon & M.W. Chase; Stenomediterraneo (A, B, D, E, Terzi *et al.,* 2010, K, M, N, Q, T)
2. *Anacamptis coriophora* (L.) R.M. Bateman, Pridgeon & M.W. Chase subsp. *fragrans* (Pollini); Eurimediterraneo (A, B, D, E, F, G, I, K, L, M, N, Q)
3. *Anacamptis laxiflora (*Lam.) R.M. Bateman, Pridgeon & M.W. Chase; Eurimediterraneo (D, E, K, L, Q)
4. *Anacamptis morio* (L.) R.M. Bateman, Pridgeon & M.W. Chase; Europeo-Caucasico (A, B, Dura, 2004a, D, E, F, G, I, K, L, M, N, Q, R)
5. *Anacamptis palustris* (Jacq.) R.M. Bateman, Pridgeon & M.W. Chase; Eurimediterraneo (Bianco *et al.,* 1989a, A, B, D, E, L, M)
6. *Anacamptis papilionacea* (L.) R.M. Bateman, Pridgeon & M.W. Chase; Eurimediterraneo (A, B, D, E, F, G, I, K, M, N, Q, R)
7. *Anacamptis pyramidalis* subsp. *pyramidalis* (L.) Rich.; Eurimediterraneo (A, B, Dura, 2004a, D, E, F, I, K, L, M, Q, R)
8. *Barlia robertiana* (Loisel.) Greuter; Stenomediterraneo (A, B, D, E, F, G, K, Gennaio *et al.,* 2014, M, N, Q, R, T)
9. *Cephalanthera damasonium* (Mill.) Druce; Eurimediterraneo (A, B, Dura, 2004a, D, F, K, M, N, P, Q, Q)
10. *Cephalanthera longifolia* (L.) Fritsch; Eurasiatico (A, B, D, F, M, N, P)
11. *Cephalanthera rubra* (L.) Rich.; Eurasiatico.(A, B, D, M, N)
12. *Coeloglossum viride* (L.) Hartm.; Circumboreale (A, B, D, Palladini & Russo, 2014, M, N)
13. *Dactylorhiza maculata* subsp. *saccifera* (Brongn.) Diklić; Paleotemperato (A, D, F, M, N, P). Del Fuoco (2003) segnala per il Gargano *Dactylorhiza maculata* (L) Soó senza specificare la sottospecie. Biondi *et al.* (2008) segnalano *Dactylorhiza maculata* subsp. *fuchsii* che non ha mai avuto altre conferme.
14. *Dactylorhiza romana* (Sebast.) Soó; Stenomediterraneo (A, D, E, G, K, M, N, Q)
15. *Dactylorhiza sambucina* (L.) Soó; Europeo (A, B, D, E, M, N)
16. *Epipactis helleborine* subsp. *aspromontana* (Bartolo, Pulvirenti & Robatsch) H. Baumann & R. Lorenz; Endemico (B, D, M)

[1] Nell'elenco floristico le lettere maiuscole riportate dopo ogni singolo taxon sono sigle che si riferiscono agli autori delle segnalazioni, sono state utilizzate al fine di evitare lunghe ripetizioni e hanno il seguente significato:
V florističnem seznamu so velike črke, ki se pojavljajo po vsakem taksonu, in kratice, ki se nanašajo na avtorje sporočil. Uporabljene so bile v izogib dolgim ponavljanjem in imajo naslednji pomen:
A: Lorenz & Gembardt, 1987; B: Del Fuoco, 2003; C: Souche, 2008; D: GIROS, 2009; E: Gennaio *et al.,* 2010; F: Santoro, 2006, 2010; G: Dura *et al.,* 2011; H: Romolini & Souche, 2012; I: Turco *et al.,* 2012; K: D'Alonzo, 2013; L: Lumare & Medagli, 2013a; M: Rossini & Quitadamo, 2014; N: Griebl, 2014; O: Souche Remy (*com. pers.*); P: Wagensommer *et al.,* 2014; Q: Siletti & Medagli, 2015; R: De Leo, 2015; S: D'Alonzo, 2015; T: Perilli, 2013

17. *Epipactis helleborine* subsp. *helleborine* (L.) Crantz; Paleotemperato (B, D, Dura, 2010, F, K, M, N)
18. *Epipactis helleborine* subsp. *schubertiorum* (Bartolo, Pulvirenti & Kreutz); Endemico (B, D, M, N)
19. *Epipactis leptochila* (Godfery) Godfery subsp. *neglecta* (Kümpel) Gévaudan; Centro-Europeo (D, M)
20. *Epipactis leptochila* (Godfery) Godfery subsp. *umbrae*; Endemico (Kreutz *et al.,* 2014, M). Bongiorni *et al.* (2015) ritengono che le piante assegnate a tale taxon siano da inquadrare nell'ambito della variabilità di *Epipactis neglecta,* che considerano specie tipica e non sottospecie *di Epipactis leptochila*.
21. *Epipactis meridionalis* H. Baumann & R. Lorenz; Endemico (A, B, Bartolo *et al.,* 2006, D, E, M, N)
22. *Epipactis microphylla* (Ehrh.) Sw.; Europeo-Caucasico (A, B, Dura, 2004b, Dura, 2010, De Matteis *et al.,* 2009, D, E, K, M, N)
23. *Epipactis muelleri* Godfery; Centro-Europeo (B, D, M, N)
24. *Epipactis palustris* (L.) Crantz; Circumboreale (Pantaleo, 1991, Del Fuoco & Scirocco, 2002, B, D, M, N)
25. *Epipactis persica* subsp. *gracilis* (B. Baumann & H. Baumann) W. Rossi; Sud-Est Europeo (M)
26. *Epipactis purpurata* Sm.; Subatlantico (M, N)
27. *Gymnadenia conopsea* (L.) R. Br. in W. T. Aiton; Eurasiatico (A, B, D, M, N)
28. *Himantoglossum hircinum* (L.) Spreng; Mediterraneo-Atlantico (A, B, D, K, M, P, Q, R)
29. *Limodorum abortivum* (L.) Sw.; Eurimediterraneo (A, B, Dura, 2004a, D, E, F, G, M, N, Q)
30. *Listera ovata* (L.) R. Br.; Eurasiatico (A, D, M, N)
31. *Neotinea lactea* (Poir.) R.M. Bateman, Pridgeon & M.W. Chase; Stenomediterraneo (A, Bianco *et al.,* 1989, B, D, E, K, M, N, Q)
32. *Neotinea maculata* (Desf.) Stearn; Mediterraneo-Atlantico (A, B, D, M, N)
33. *Neotinea tridentata* (Scop.) R.M. Bateman, Pridgeon & M.W. Chase; Eurimediterraneo (A, B, D, G, K, M, N, Q)
34. *Neotinea ustulata* (L.) R.M. Bateman, Pridgeon & M.W. Chase; Europeo-Caucasico (Kajan, 1987, A, Dura, 2002, B, K, M, N, Q)
35. *Neottia nidus-avis* (L.) Rich.; Eurasiatico (Sigismondi & Tedesco, 1990, B, Dura, 2004b, D, H, M, N)
36. *Ophrys apifera* Huds.; Eurimediterraneo (A, B, Dura, 2004a, D, E, F, G, K, M, N, P, Q)
37. *Ophrys argolica* subsp. *biscutella* (O. Danesch & E. Danesch) Kreutz; Appennino-Balcanico (A, B, D, H, M, N) (Fig. 2)
38. *Ophrys bertolonii* subsp. *bertolonii* Moretti; Appennino-Balcanico (A, B, D, E, F, G, H, I, K, L, M, N, P, Q, R, S)
39. *Ophrys bertolonii* subsp. *bertoloniiformis* (O. Danesch & E. Danesch) H. Sund.; Endemico (A, B, D, H, K, M, N, Q)
40. *Ophrys bombyliflora* Link; Stenomediterraneo (A, B, D, E, F, G, H, I, K, L, M, N, Q, R, S, T)
41. *Ophrys brutia* P. Delforge; Endemico (D, H, M)
42. *Ophrys calocaerina* Devillers-Tersch. & Devillers; Appennino-Balcanico (Wucherpfennig & Presser, 2005)
43. *Ophrys candica* (Nelson ex Soó) H. Baumann & Künkele; Mediterraneo Orientale (D, E, H, I)
44. *Ophrys cinnabarina* Romolini & Soca; Endemico (H, M, Q, T)
45. *Ophrys corsica* Soleirol ex G. Foelsche & W. Foelsche; Mediterraneo Orientale (H, K, M, N, S). Del Fuoco (2003) e GIROS (2009) segnalano per la regione *Ophrys lutea* subsp. *phryganae* (Devillers-Tersch. & Devillers) Melki con cui l'entità è da porre in sinonimia.
46. *Ophrys exaltata* subsp. *archipelagi* (Gölz & H.R. Reinhard) Del Prete; Appennino-Balcanico (A, B, D, H, M, N, T)
47. *Ophrys forestieri* (Rchb. f.) Lojacono; MediterraneoOccidentale (H, M). Secondo Delforge (2005) il taxon è un endemismo del Sud della Francia assente in Italia.
48. *Ophrys fusca* subsp. *fusca* Link; Mediterraneo-Atlantico (A, B, D, E, F, K, M)
49. *Ophrys fusca* subsp. *funerea* (Viv.) Arcang; Stenomediterraneo (B, D, F, K, M, N)
50. *Ophrys fusca* subsp. *lucana* (P. Delforge, Devillers-Tersch. & Devillers) Kreutz; Endemico (Perilli, 2010, F, G, H, M, P, Q)
51. *Ophrys fusca* subsp. *lupercalis* Devillers-Tersch. & Devillers; Mediterraneo Occidentale (E, Q)
52. *Ophrys gravinensis* D'Alonzo; Endemico (S)
53. *Ophrys holosericea* (Burm. f.) Greuter subsp. *apulica* (O. Danesch & E. Danesch) Buttler; Endemico (A, B, D, E, F, G, H, K, L, M, N, Q, R). Secondo Hertel & Presser (2009) il taxon è da mettere in sinonimia con *Ophrys pharia* P. Devillers & J. Devillers-Terschuren, presente sull'isola di Hvar (Croazia) e quindi va considerato Appennino-Balcanico. Tuttavia, in attesa di altri studi e ricerche, in tale sede si continua a considerarlo endemico mentre *Ophrys pharia* un suo vicariante geografico.
54. *Ophrys holosericea* (Burm. f.) Greuter subsp. *gracilis* (Büel, O. Danesch & E. Danesch) Büel, O. Danesch & E. Danesch; Endemico (Perilli, 2009, H, K, M, Q, T)
55. *Ophrys holosericea* (Burm. f.) Greuter subsp. *paolina* Liverani & Romolini; Endemico (Liverani V. & Romolini, 2010, H)
56. *Ophrys holosericea* (Burm. f.) Greuter subsp. *parvimaculata* (O. Danesch & E. Danesch) O.

Danesch & E. Danesch; Endemico (A, B, D, E, H, M, N)

57. *Ophrys incubacea* subsp. *incubacea* Bianca; Stenomediterraneo (A, B, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T)
58. *Ophrys iricolor* Desf. subsp. *eleonorae* (Devillers-Tersch. & Devillers) Pualus & Gack ex Kreutz; Subendemico (H, M, N) (Fig. 3)
59. *Ophrys iricolor* Desf. subsp. *lojaconoi* P. Delforge; Endemico (Delforge, 1995, D, H, K, M)
60. *Ophrys lacaitae* Lojac.; Appennino-Balcanico (A, Dekker, 1991, B, D, H, M, N)
61. *Ophrys lucifera* Devillers-Tersch. & Devillers; Endemico (D, H, K, M, N)
62. *Ophrys lutea* Cav. subsp. *lutea;* Stenomediterraneo (A, B, Dura, 2004a, D, E, F, G, H, K, L, M, Q, S)
63. *Ophrys lutea* subsp. *minor* (Tod.) O. Danesch & E. Danesch; Stenomediterraneo (A, B, D, E, G, H, K, L, M, N, Q, R, S)
64. *Ophrys mateolana* Medagli, D'Emerico, Bianco & Ruggiero; Endemico (Cillo, 2009, K, Q)
65. *Ophrys mattinatatae* P. Medagli, A. Rossini G. Quitadamo & A. Turco; Endemico (Medagli *et al.,* 2012, H, M). Secondo Medagli *et al.* (2012) vanno escluse dalla flora garganica e attribuite a questa nuova entità le segnalazioni precedenti di *Ophrys oestrifera* Cav. subsp. *bremifera* Auct. e di *Ophrys oestrifera* subsp. *oestrifera*.
66. *Ophrys minipassionis* Romolini & Soca; Endemico (D, H, K, M, T)
67. *Ophrys murgiana* Cillo, Medagli & Margherita; Endemico (Medagli & Cillo, 2009)
68. *Ophrys neglecta* Parl.; Subendemico; (A, B, D, E, F, G, H, I, K, L, M, N, P, Q, S, T). L'entità da diversi studiosi è segnalata con varie denominazioni tra cui *Ophrys tenthredinifera* Willd.
69. *Ophrys oestrifera* Steven in M-Bieb. subsp. *montis-gargani* B. Van de Vijver, W. Van Looken, G. Thiers & A. Cuypers; Endemico (Van de Vijver *et al.,* 2010, H, M). Al taxon vanno attribuite le segnalazioni precedenti di *Ophrys oestrifera* subsp. *oestrifera* Lorenz & Gembardt (1987) e *Ophrys oestrifera* subsp. *cornuta* (Del Fuoco, 2003).
70. *Ophrys oxyrrhinchos* Tod. subsp. *celiensis* O. Danesch & E. Danesch; Endemico (Gennaio, 2008, E, G, H, K, Q)
71. *Ophrys oxyrrhinchos* Tod. subsp. *ingrassiae* Dura, Turco, Gennaio & Medagli; Endemico (G)
72. *Ophrys passionis* subsp. *passionis* Sennen ex Devillers-Tersch. & Devillers; Mediterraneo Occidentale (A, B, E, F, K, L, M, N, Q, R, T)
73. *Ophrys peucetiae* Lozito, Turco, D'Emerico & Medagli; Endemico (I)
74. *Ophrys pinguis* Romolini & Soca; Endemico (Perilli, 2015). Le descrizioni di *O. cinnabarina* e *O. pinguis* portano all'esclusione dalla flora pugliese di *Ophrys holosericea* subsp. *holosericea* (Burm. f.) Greuter con cui le due entità sono da porre in sinonimia. Tuttavia la presenza in natura di piante del gruppo con caratteristiche intermedie di difficile classificazione e il fatto che le differenze morfologiche tra le specie talvolta sono minime dovrebbero condurre a una revisione tassonomica ed altri studi e ricerche.
75. *Ophrys promontorii* O. Danesch & E. Danesch; Endemico (A, Kalteisen & Reinhard, 1987, B, D, H, K, M, N)
76. *Ophrys pseudomelena* Turco, D'Emerico & Medagli; Endemico (I, M). Secondo Turco *et al.* (2012) sono da attribuire alla specie tutte le segnalazioni precedenti di *Ophrys melena* (Renz) Paulus & Gack.
77. *Ophrys scolopax* subsp. *conradiae* (Medki & Deschatres) H. Baumann, Giotta, Künkele, R. Lorenz & Piccitto; Subendemico (Del Fuoco, 2008, D'Alonzo, 2009, K, D, Campochiaro & Dura, 2011, H, M)
78. *Ophrys sipontensis* R. Lorenz & Gembardt; Endemico (A, B, D, H, K, M, N, Q, T) (Fig. 4)
79. *Ophrys speculum* Link; Stenomediterraneo (A, B, D, Dura, 2011, H, M, N, T)
80. *Ophrys sphegodes* Mill.; Eurimediterraneo (A, B, Dura, 2004a, E, F, I, K, R). Souche (2008), Romolini & Souche (2012), Perilli (2013), Rossini & Quitadamo (2014) e Siletti & Medagli (2015) segnalano per la Puglia *Ophrys classica*, un'entità controversa che secondo vari studiosi rientrerebbe nella variabilità di *Ophrys sphegodes*.
81. *Ophrys tardans* O. Danesch & E. Danesch; Endemico (D, E, H, K, Q, S)
82. *Ophrys tarentina* Gölz & H.R. Reinhard; Endemico (Gölz & Reinhard, 1982, D, E, H, Terzi *et al.,* 2010, K, Q)
83. *Ophrys tarquinia;* Endemico (Del Fuoco, 2008, H, M, T)
84. *Orchis anthropophora* (L.) All.; Mediterraneo-Atlantico (A, B, D, E, F, G, K, M, N, P, Q)
85. *Orchis italica* Poir.; Stenomediterraneo (A, B, D, E, F, G, K, M, N, P, Q, R)
86. *Orchis mascula* L. subsp. *mascula;* Centro Europeo (Palladini & Russo, 2014, N, P)
87. *Orchis pauciflora* Ten.; Stenomediterraneo (A, B, D, M, N)
88. *Orchis provincialis* Balb. Ex Lam.; Stenomediterraneo (A, B, D, F, M, N)
89. *Orchis purpurea* Huds.; Eurasiatico (A, B, D, F, K, M, N, P, Q)
90. *Orchis quadripunctata* Cirillo ex Ten.; Mediterraneo Orientale (A, B, D, K, M, N)
91. *Orchis simia* Lam.; Eurimediterraneo (Biscotti, 2002, D'Agostino & Liuzzi, 2010, K)
92. *Platanthera bifolia* (L.) Rchb.; Paleotemperato (Del Fuoco & Pantaleo, 2002, B, D, K)

93. *Platanthera chlorantha* (Custer) Rchb.; Eurosiberiano (A, B, Dura, 2004a, D, G, K D, M, N, Q)
94. *Serapias bergonii* E.G. Camus; Mediterraneo Orientale (A, D, E, G, K, L, M, N, Q)
95. *Serapias cordigera* L.; Stenomediterraneo (A, B, D, E, Terzi *et al.,* 2010, K, L, M, N)
96. *Serapias lingua* L.; Stenomediterraneo (A, B, D, E, I, Terzi *et al.,* 2010, K, L, M, N, Q)
97. *Serapias orientalis* Nelson subsp. *apulica;* Endemico (A, B, D, E, K, L, M, N, Q) (Fig. 5)
98. *Serapias parviflora* Parl.; Stenomediterraneo (A, B, D, E, G, I, F, K, L, M, N, Q, R)
99. *Serapias politisi* Renz; Mediterraneo Orientale (Bianco *et al.,* 1992, B, D, E, I, M)
100. *Serapias vomeracea* (Burm. f.) Briq. subsp. *longipetala* (Ten.) H. Baumann & Künkele; Mediterraneo Orientale (B, D, E, F, L, M, Q)
101. *Serapias vomeracea* (Burm. f.) Briq. subsp. *vomeracea;* Eurimediterraneo (A,B, D, E, F, G, I, K, L, M, N, P, Q, Lumare *et al.,* 2015, R)
102. *Spiranthes spiralis* (L.) Chevall; Europeo-Caucasico (A, B, D, E, F, G, K, M, N, Q)

Fig. 2/Sl. 2: *Ophrys argolica* **subsp.** *biscutella*

Ibridi

1. *Anacamptis collina* × *Anacamptis morio* (*Anacamptis ×semi-saccata* nsubsp. *murgiana* Medagli, D'Emerico, Ruggiero & Bianco) (Medagli *et al.,* 1993)
2. *Anacamptis collina* × *Anacamptis papilionacea* (*Anacamptis ×dülükae* Hautz.) (A, Kohlmüller, 1993, Medagli *et al.,* 1993, E, N)
3. *Anacamptis collina* × *Serapias parviflora* (× *Serapicamptis nelsoniana* Bianco, Medagli, D'Emerico & Ruggiero) (Bianco *et al.,* 1990, E)
4. *Anacamptis coriophora* subsp. *fragrans* × *Anacamptis laxiflora (Anacamptis ×bicknelli* E.G. Camus) (Lumare & Medagli, 2013b)
5. *Anacamptis coriophora* subsp. *fragrans* × *Anacamptis palustris* (*Anacamptis ×barlae* E.G. Camus) (Lumare & Medagli 2013b).
6. *Anacamptis laxiflora* × *Anacamptis palustris* (*Anacamptis ×lloydiana* Rouy) (E, L)
7. *Anacamptis laxiflora* × *Anacamptis papilionacea* (*Anacamptis ×caccabaria* Verguin) (E)
8. *Anacamptis laxiflora* × *Anacamptis morio* (*Anacamptis ×alata* [Fleury] H. Kretzschmar, Eccarius & H. Dietr.) (E)
9. *Anacamptis morio* × *Anacamptis papilionacea* [*Anacamptis ×gennarii* (Rchb. f.) H. Kretzschmar, Eccarius & H. Dietr.] (E, K, Q)
10. *Anacamptis morio* × *Anacamptis pyramidalis* (*Anacamptis ×laniccae* Br. Bl.) (E, L)
11. *Anacamptis morio* × *Orchis quadripunctata* (*Anacamptis ×adriatica* Soó) (M)
12. *Anacamptis papilionacea* × *Serapias lingua* (× *Serapicamptis barlae* E.G. Camus) (E)
13. *Cephalanthera damasonium* × *Cephalanthera longifolia* (*Cephalanthera ×schulzei* E.G. Camus, Bergon & A. Camus) (M)

Fig. 3/Sl. 3: *Ophrys iricolor* **subsp.** *eleonorae*

14. *Dactylorhiza maculata* subsp. *saccifera* × *Dactylorhiza romana* subsp. *romana* [*Dactylorhiza ×daunia* (Sebastiani) Soó] (M)
15. *Dactylorhiza romana* × *Dactylorhiza sambucina* [(*Dactylorhiza ×fascicolata* Sebastiani) Soó] (M, N)
16. *Neotinea maculata* × *Orchis anthropophora* (× *Neotiorchis mattinatae* Kohlmüller) (Kohlmüller, 1988, M, N)
17. *Neotinea tridentata* × *Neotinea ustulata* (*Neotinea ×dietrichiana* (Bogenh.) H. Kretzschmar, Eccarius & H. Dietr.) (M, N)
18. *Ophrys apifera* × *Ophrys bombyliflora* (*Ophrys ×circaea* W. Rossi & Prola) (E, O)
19. *Ophrys apifera* × *Ophrys candica* (*Ophrys ×morellensis* O. Danesch & E. Danesch) (E)
20. *Ophrys apifera* × *Ophrys holosericea* subsp. *apulica (Ophrys ×albertiana* E.G. Camus nothosubsp. *grovesii* Gennaio, Gargiulo & Medagli) (Gennaio *et al.,* 2013)
21. *Ophrys argolica* subsp. *biscutella* × *Ophrys bertolonii* (*Ophrys ×salvatoris* O. Danesch) (C, M, N)
22. *Ophrys argolica* subsp. *biscutella* × *Ophrys bertolonii* subsp. *bertoloniiformis* (*Ophrys ×permutata* O. Danesch & E. Danesch) (C)
23. *Ophrys argolica* subsp. *biscutella* × *Ophrys exaltata* subsp. *archipelagi (Ophrys ×garganensis* R. Soca) (Souche, 1997, C, N)
24. *Ophrys argolica* subsp. *biscutella* × *Ophrys holosericea* subsp. *apulica* (*Ophrys ×rossiniae* Medagli & Turco) (Medagli & Turco, 2011, M, N)
25. *Ophrys argolica* subsp. *biscutella* × *Ophrys holosericea* subsp. *gracilis* (Perilli, 2012, M)
26. *Ophrys argolica* subsp. *biscutella* × *Ophrys holosericea* subsp. *parvimaculata* (*Ophrys ×pizzulensis* Soca) (C, H, M, N)
27. *Ophrys argolica* subsp. *biscutella* × *Ophrys incubacea* (M, N)
28. *Ophrys argolica* subsp. *biscutella* × *Ophrys minipassionis* (T)
29. *Ophrys argolica* subsp. *biscutella* × *Ophrys neglecta* (*Ophrys ×montis-angeli* O. Danesch & E. Danesch) (C, M, N, T)
30. *Ophrys argolica* subsp. *biscutella* × *Ophrys oestrifera* subsp. *montis-gargani* (*Ophrys ×carpinensis* O. Danesch & E. Danesch) (N)
31. *Ophrys argolica* subsp. *biscutella* × *Ophrys passionis* subsp. *passionis* (B, H, M, N)
32. *Ophrys argolica* subsp. *biscutella* × *Ophrys promontorii* (*Ophrys ×vernonensis* O. Danesch & E. Danesch) (M, N)
33. *Ophrys argolica* subsp. *biscutella* × *Ophrys sipontensis* (*Ophrys ×cornelli* O. Danesch & E. Danesch) (M)
34. *Ophrys argolica* subsp. *biscutella* × *Ophrys sphegodes* (*Ophrys ×boscoquartensis* O. Danesch & E. Danesch) (M, N)
35. *Ophrys bertolonii* × *Ophrys bertolonii* subsp. *bertoloniiformis* (C, N)
36. *Ophrys bertolonii* × *Ophrys bombyliflora* (*Ophrys ×cataldii* Gölz) (C, N)
37. *Ophrys bertolonii* × *Ophrys brutia* (*Ophrys ×vernacchiae* Soca) (O)
38. *Ophrys bertolonii* × *Ophrys exaltata* subsp. *archipelagi* (N)
39. *Ophrys bertolonii* × *Ophrys candica* (E)
40. *Ophrys bertolonii* × *Ophrys corsica* (*Ophrys ×anxatina* R. Congedo) (Congedo, 2007, H)
41. *Ophrys bertolonii* × *Ophrys holosericea* subsp. *apulica* (*Ophrys ×degiorgii* Ruggiero, Bianco, Medagli, D'Emerico) (Ruggiero *et al.,* 1988, C, E, N, Q, T)
42. *Ophrys bertolonii* × *Ophrys holosericea* subsp. *parvimaculata* (*Ophrys ×gumprechtii* O. Danesch & E. Danesch) (C, E)
43. *Ophrys bertolonii* × *Ophrys incubacea* (*Ophrys ×lyrata* H. Fleischm.) (C, E, M, N, P, Q, T)
44. *Ophrys bertolonii* × *Ophrys lutea* subsp. *minor* (*Ophrys. ×anxantina* R. Congedo) (Congedo, 2007, E)
45. *Ophrys bertolonii* × *Ophrys mateolana (Ophrys ×gambettae* Siletti & Medagli) (Q)
46. *Ophrys bertolonii* × *Ophrys neglecta* (*Ophrys ×inzengae* (Tod.) Nyman) (C, M, N, Q, T)
47. *Ophrys bertolonii* × *Ophrys oxyrrhinchos* subsp. *celiensis* (C)
48. *Ophrys bertolonii* × *Ophrys passionis* subsp. *passionis* (*Ophrys. ×grottagliensis* Gölz & H.R. Reinhard) (C, E, N, Q)
49. *Ophrys bertolonii* × *Ophrys promontorii* (*Ophrys ×couloniana* P.Delforge & C. Delforge) (Delforge & Delforge, 1986, N, T)
50. *Ophrys bertolonii* × *Ophrys sipontensis* (*Ophrys ×perillii* Romolini & Soca) (Romolini & Soca, 2014, M, N, T)
51. *Ophrys bertolonii* × *sphegodes Ophrys ×saratoi* Camus (N, O)
52. *Ophrys bertolonii* × *Ophrys tardans* (H)
53. *Ophrys bertolonii* × *Ophrys tarentina* (*Ophrys ×monopolitana* H. Baumann & Künkele) (C, E, Q)
54. *Ophrys bertolonii* subsp. *bertoloniiformis* × *Ophrys bombyliflora* (C, H, N, T)
55. *Ophrys bertolonii* subsp. *bertoloniiformis* × *Ophrys holosericea* subsp. *apulica* (C, H, N)
56. *Ophrys bertolonii* subsp. *bertoloniiformis* × *Ophrys incubacea* (*Ophrys ×lorenzii* Soca) (C, M, N, T)
57. *Ophrys bertolonii* subsp. *bertoloniiformis* × *Ophrys lojaconoi* (T)
58. *Ophrys bertolonii* subsp. *bertoloniiformis* × *Ophrys neglecta* (*Ophrys ×lupiae* O. Danesch & E. Danesch) (C, H, M, N, T)
59. *Ophrys bertolonii* subsp. *bertoloniiformis* × *Ophrys passionis* subsp. *passionis* (*Ophrys ×manganaroi* Perilli & Soca) (C, M, N, T)

60. *Ophrys bertolonii* subsp. *bertoloniiformis* × *Ophrys promontorii* (*Ophrys* ×*azurea* H. Baumann & Künkele) (C, M, N)
61. *Ophrys bertolonii* subsp. *bertoloniiformis* × *Ophrys sphegodes* (C, M)
62. *Ophrys bertolonii* subsp. *bertoloniiformis* × *Ophrys sipontensis (Ophrys* ×*castellerana* P. Delforge & C. Delforge) (Delforge & Delforge,1986, C, N, T)
63. *Ophrys bertolonii* subsp. *bertoloniiformis* × *Ophrys sphegodes* (*Ophrys* ×*gelmii* Murr) (N)
64. *Ophrys bertolonii* subsp. *bertoloniiformis* × *Ophrys tarentina* (*Ophrys* ×*motolesensis* Soca) (C)
65. *Ophrys bombyliflora* × *Ophrys candica* (*Ophrys* ×*medaglii* Turco, Ruggiero, Gennaio & D'Emerico) (C, E, I)
66. *Ophrys bombyliflora* × *Ophrys exaltata* subsp. *archipelagi* (M, N)
67. *Ophrys bombyliflora* × *Ophrys eleonorae* (C, E)
68. *Ophrys bombyliflora* × *Ophrys holosericea* subsp. *apulica* (*Ophrys* ×*resurrecta* O. Danesch & E. Danesch) (E, H, N, Q)
69. *Ophrys bombyliflora* × *Ophrys holosericea* subsp. *parvimaculata* (*Ophrys* ×*ozantina* R. Gennaio) (Gennaio, 2009, E, N)
70. *Ophrys bombyliflora* × *Ophrys incubacea* (*Ophrys* ×*cosana* H. Baumann & Künkele) (E, I, M, N, Q, R)
71. *Ophrys bombyliflora* × *Ophrys lutea* subsp. *minor* (*Ophrys* ×*domitia* Del Prete) (N)
72. *Ophrys bombyliflora* × *Ophrys neglecta* (*Ophrys* ×*sommieri* Sommier) (C, E, L, M, N, P, T)
73. *Ophrys bombyliflora* × *Ophrys passionis* subsp. *passionis* (*Ophrys* ×*daunia* H. Baumann & Künkele) (C, E, M, N, R, T)
74. *Ophrys bombyliflora* × *Ophrys sipontensis* (M, N)
75. *Ophrys bombyliflora* × *Ophrys sphegodes* (*Ophrys* ×*hoeppneri* Ruppert) (N)
76. *Ophrys bombyliflora* × *Ophrys tarentina* (*Ophrys* ×*mannarica* P. Delforge & C. Delforge) (Delforge & Delforge,1986, C, Q)
77. *Ophrys bombyliflora* × *Ophrys tardans* (E)
78. *Ophrys brutia* × *Ophrys exaltata* subsp. *archipelagi* (H)
79. *Ophrys brutia* × *Ophrys holosericea* subsp. *apulica* (*Ophrys* ×*trazzonarae* Romolini & Soca) (Romolini & Soca, 2014)
80. *Ophrys brutia* × *Ophrys holosericea* subsp. *parvimaculata* (*Ophrys* ×*buffaloriae* Romolini & Soca) (Romolini & Soca, 2014)
81. *Ophrys candica* × *Ophrys holosericea* subsp. *apulica* (*Ophrys* ×*valdevariabilis* O. Danesch & E. Danesch) (C, E, H, I)
82. *Ophrys candica* × *Ophrys holosericea* subsp. *parvimaculata* (C, E)
83. *Ophrys cinnabarina* × *Ophrys holosericea* subsp. *gracilis* (O)
84. *Ophrys cinnabarina* × *Ophrys lacaitae* (H, M, T)
85. *Ophrys corsica* × *Ophrys exaltata* subsp. *archipelagi* (N)
86. *Ophrys corsica* × *Ophrys lutea* subsp. *lutea* (*Ophrys* ×*sulphurea* Gennaio & Medagli) (Gennaio & Medagli, 2015)
87. *Ophrys corsica* × *Ophrys lutea* subsp. *minor* (*Ophrys* ×*calchasii* Romolini & Soca) (Romolini & Soca, 2014)
88. *Ophrys exaltata* subsp. *archipelagi* × *Ophrys fusca* (*Ophrys* ×*turrium* Kohlmüller) (Kohlmüller, 1993)
89. *Ophrys exaltata* subsp. *archipelagi* × *Ophrys holosericea* subsp. *parvimaculata* (*Ophrys* ×*ingaranensis* Soca) (C, M, N)
90. *Ophrys exaltata* subsp. *archipelagi* × *Ophrys incubacea* (*Ophrys* ×*kelleri* Godfery) (C, Perilli, 2012, M, N)
91. *Ophrys exaltata* subsp. *archipelagi* × *Ophrys lojaconoi* (T)
92. *Ophrys exaltata* subsp. *archipelagi* × *Ophrys neglecta* (*Ophrys* ×*devillersiana* P. Delforge) (Delforge, 1988, M, N)
93. *Ophrys exaltata* subsp. *archipelagi* × *Ophrys oestrifera* subsp. *montis-gargani* (*Ophrys* ×*lewinii* Soca) (M)
94. *Ophrys exaltata* subsp. *archipelagi* × *Ophrys passionis* subsp. *passionis* (*Ophrys* ×*manacorensis* H. Baumann & Künkele) (C, N)
95. *Ophrys exaltata* subsp. *archipelagi* × *Ophrys promontorii* (C, M, N)
96. *Ophrys exaltata* subsp. *archipelagi* × *Ophrys sipontensis* (N)
97. *Ophrys exaltata* subsp. *archipelagi* × *Ophrys sphegodes* (*Ophrys* ×*trombettensis* Soca) (C, M)
98. *Ophrys forestieri* × *Ophrys sphegodes* (H, N)
99. *Ophrys fusca* s. l. × *Ophrys incubacea* (*Ophrys* ×*braunblanquetiana* Briq. ex Soó in G. Keller) (M)
100. *Ophrys fusca* s. l. × *Ophrys holosericea* subsp. *parvimaculata* (E)
101. *Ophrys fusca* s. l. × *Ophrys lutea* subsp. l*utea* (*Ophrys* ×*battandierii* E.G. Camus) (E)
102. *Ophrys fusca* s. l. × *Ophrys lutea* subsp. *minor* (*Ophrys* ×*fenarolii* Ferlan) (E, M)
103. *Ophrys fusca* s. l. × *Ophrys passionis* subsp. *passionis* (*Ophrys* ×*gumprechtiana* O. Danesch & E. Danesch) (M)
104. *Ophrys fusca* s. l. × *Ophrys sphegodes* Del Fuoco 2008
105. *Ophrys fusca* subsp. *lupercalis* × *Ophrys tarentina* (*Ophrys* ×*parenzani* Medagli, Ruggiero & D'Emerico) (Q)
106. *Ophrys holosericea* s. l. × *Ophrys incubacea* (N)
107. *Ophrys holosericea* s. l. × *Ophrys neglecta* (*Ophrys* ×*maremmae* O. Danesch & E. Danesch) (N)
108. *Ophrys holosericea* s. l. × *Ophrys promontorii* (*Ophrys* ×*aquilana* H. Baumann & Künkele) (N)

109. *Ophrys holosericea* s. l. × *Ophrys sphegodes* (*Ophrys* ×*obscura* Beck) (N)
110. *Ophrys holosericea* subsp. *apulica* × *Ophrys holosericea* subsp. *parvimaculata* (*Ophrys* ×*cosentiana* H. Baumann & Künkele) (Baumann & Künkele, 1986, Gennaio, 2005, C, E, H, M, N)
111. *Ophrys holosericea* subsp. *apulica* × *Ophrys incubacea* (*Ophrys* ×*franciniae* Bianco, Medagli, D'Emerico & Ruggiero) (Bianco *et al.,* 1988c, C, E, N, Q, T)
112. *Ophrys holosericea* subsp. *apulica* × *Ophrys lacaitae* (T)
113. *Ophrys holosericea* subsp. *apulica* × *Ophrys mateolana* (*Ophrys* ×*turcoi* Siletti & Medagli) (Q)
114. *Ophrys holosericea* subsp. *apulica* × *Ophrys neglecta* (*Ophrys* ×*salentina* O. Danesch & E. Danesch) (E, H, M, N, T)
115. *Ophrys holosericea* subsp. *apulica* × *Ophrys oestrifera* subsp. *montis-gargani* (*Ophrys* ×*turrica* R. Lorenz & Gembardt) (A, N)
116. *Ophrys holosericea* subsp. *apulica* × *Ophrys oxyrrhinchos* subsp. *celiensis* (*Ophrys* ×*caliandri* O. Danesch & E. Danesch) (C, H)
117. *Ophrys holosericea* subsp. *apulica* × *Ophrys passionis* subsp. *passionis* (*Ophrys* ×*coturii* Romolini & Soca) (Romolini & Soca, 2014, N, T)
118. *Ophrys holosericea* subsp. *apulica* × *Ophrys scolopax* subsp. *conradiae* (*Ophrys* ×*difesagran-deana* D'Alonzo) (D'Alonzo, 2014)
119. *Ophrys holosericea* subsp. *apulica* × *Ophrys sipontensis* (N)
120. *Ophrys holosericea* subsp. *apulica* × *Ophrys tarentina* (*Ophrys* ×*marinoscii* Ruggiero, Bianco, Medagli & D'Emerico) (Bianco *et al.,* 1988a, C, Q)
121. *Ophrys holosericea* subsp. *apulica* × *Ophrys tardans* (*Ophrys* ×*hydruntensis* O. Danesch & E. Danesch) (E)
122. *Ophrys holosericea* subsp. *gracilis* × *Ophrys incubacea* (M)
123. *Ophrys holosericea* subsp. *gracilis* × *Ophrys holosericea* subsp. *parvimaculata* (Perilli, 2012, M, T)
124. *Ophrys holosericea* subsp. *gracilis* × *Ophrys lacaitae (*T)
125. *Ophrys holosericea* subsp. *paolina* × *Ophrys neglecta* (O)
126. *Ophrys holosericea* subsp. *parvimaculata* × *Ophrys incubacea* (E)
127. *Ophrys holosericea* subsp. *parvimaculata* × *Ophrys oxyrrhinchos* subsp. *celiensis* (*Ophrys* ×*pugliana* H. Baumann & Künkele) (C)
128. *Ophrys holosericea* subsp. *parvimaculata* × *Ophrys neglecta* (*Ophrys* ×*laurentii* O. Danesch & E. Danesch) (E, M, T)
129. *Ophrys holosericea* Gennaio subsp. *parvimacu-lata* × *Ophrys passionis* subsp. *passionis* (*Ophrys* ×*coulotii* R. Soca) (Gennaio, 2006, E, N, Q)
130. *Ophrys holosericea* subsp. *parvimaculata* × *Ophrys sphegodes* (C)
131. *Ophrys holosericea* subsp. *parvimaculata* × *Ophrys neglecta* (C)
132. *Ophrys incubacea* × *Ophrys lucifera* (C)
133. *Ophrys incubacea* × *Ophrys lutea* subsp. *minor* (*Ophrys* ×*piscinica* nsubsp. *mattinatellae* R. Kohlmüller) (Kohlmüller, 1993, M, N, O, T)
134. *Ophrys incubacea* × *Ophrys mateolana* (*Ophrys* ×*nettii* Siletti & Medagli) (Q)
135. *Ophrys incubacea* × *Ophrys neglecta* (*Ophrys* ×*manfredoniae* O. Danesch & E. Danesch) (E, M, Q, T)
136. *Ophrys incubacea* × *Ophrys oxyrrhinchos* subsp. *celiensis* (*Ophrys* ×*gelana* H. Baumann & Künkele nsubspec. *murgettensis* Soca) (C)
137. *Ophrys incubacea* × *Ophrys passionis* subsp. *passionis* (*Ophrys* ×*celani* O. Danesch & E. Danesch) (C, E, I, L, N, Q)
138. *Ophrys incubacea* × *Ophrys promontorii* (*Ophrys* ×*angelensis* H. Baumann & Künkele) (M, N)
139. *Ophrys incubacea* × *Ophrys sipontensis* (C, M, N, T)
140. *Ophrys incubacea* × *Ophrys sphegodes* (*Ophrys* ×*todaroana* Macchiati) (E, I, M)
141. *Ophrys incubacea* × *Ophrys tarentina* (*Ophrys* ×*alberobellensis* H. Baumann & Künkele) (C, Q)
142. *Ophrys iricolor* subsp. *eleonorae* × *Ophrys lojaconoi* (*Ophrys* ×*quitadamoi* Medagli & Turco) (Medagli & Turco, 2011, M)
143. *Ophrys lojaconoi* × *Ophrys lutea* subsp. *minor* (T)
144. *Ophrys lojaconoi* × *Ophrys passionis* subsp. *passionis* (O, T)
145. *Ophrys lojaconoi* × *Ophrys sipontensis* (T)
146. *Ophrys* ×*lyrata* × *Ophrys passionis (Ophrys* ×*pescolusae* Gennaio & Medagli) (Gennaio & Medagli 2013, Q).
147. *Ophrys* ×*lyrata* × *Ophrys tarentina (Ophrys* ×*amatoi* Siletti & Medagli) (Q)
148. *Ophrys lucifera* × *Ophrys lutea* subsp. *lutea* (C)
149. *Ophrys lucifera* × *Ophrys passionis* subsp. *passionis* (C)
150. *Ophrys lutea* subsp. *lutea* × *Ophrys neglecta* (*Ophrys* ×*personei* Cortesi) (Bianco *et al.,* 1989b, E)
151. *Ophrys lutea* subsp. *lutea* × *Ophrys tarentina* (*Ophrys* ×*cyrciarium* Pellegrini, Bellusci & Musacchio) (Q)
152. *Ophrys lutea* subsp. *minor* × *Ophrys passionis* subsp. *passionis* (N)
153. *Ophrys lutea* subsp. *minor* × *Ophrys tarentina* (*Ophrys* ×*sansimonensis* R. Soca) (C, E)
154. *Ophrys mateolana* × *Ophrys passionis* (*Ophrys* ×*durae* Siletti & Medagli) (Q, T)
155. *Ophrys mateolana* × *Ophrys* ×*lyrata* (*Ophrys* ×*santerama* Siletti & Medagli) (Q)
156. *Ophrys mateolana* × *Ophrys tarentina* (*Ophrys* ×*margheritae* Siletti & Medagli) (Q)
157. *Ophrys minipassionis* × *Ophrys neglecta* (T)

158. *Ophrys neglecta* × *Ophrys mattinatatae (Ophrys ×messeniensis* Lorenz & Gembardt) (A, Gransinigh & Buono, 2005)
159. *Ophrys.neglecta* × *Ophrys oestrifera* (C)
160. *Ophrys neglecta* × *Ophrys passionis* subsp. *passionis* (*Ophrys ×surdii* O. Danesch & E. Danesch) (E, M, N,O, Q, T)
161. *Ophrys neglecta* × *Ophrys promontorii (Ophrys ×campolati* O. Danesch & E. Danesch) (C, N)
162. *Ophrys neglecta* × *Ophrys sipontensis* (*Ophrys ×bonniorum* Soca) (A, C, M, N)
163. *Ophrys neglecta* × *Ophrys sphegodes* (*Ophrys ×etrusca* Asch. & Graebner) (M, N, T)
164. *Ophrys.neglecta* × *Ophrys tarentina* (*Ophrys ×venusiana* H. Baumann & Künkele) (Bianco *et al.,* 1988, E, Q)
165. *Ophrys oestrifera* subsp. *montis-gargani* × *Ophrys sphegodes Ophrys ×calenae* (M)
166. *Ophrys oxyrrhinchos* subsp. *celiensis* × *Ophrys tarentina* (H)
167. *Ophrys passionis* subsp. *passionis* × *Ophrys promontorii* (M, N, O)
168. *Ophrys passionis* subsp. *passionis* × *Ophrys sipontensis* (*Ophrys ×japigia* Lorenz & Gembardt) (A, M, N, T)
169. *Ophrys passionis* subsp. *passionis* × *Ophrys sphegodes* (*Ophrys ×biancoae* P. Medagli, S. d'Emerico & L. Ruggiero) (M, N)
170. *Ophrys passionis* subsp. *passionis* × *Ophrys tarentina* (*Ophrys ×trullana* H. Baumann & Künkele) (C, Q)
171. *Ophrys promontorii* × *Ophrys sphegodes* (*Ophrys ×terrae-laboris* W. Rossi & F. Minutillo) (N)
172. *Ophrys sphegodes* × *Ophrys tarentina* (*Ophrys ×calabrica* H. Baumann & Künkele) (C)
173. *Orchis antropophora* × *Orchis italica* (*Orchis ×bivonae* Tod) (M, N, T)
174. *Orchis pauciflora* × *Orchis quadripunctata* (*Orchis ×pseudoanatolica* H. Fleischm.) (M, N, T)
175. *Serapias bergonii* × *Serapias cordigera* (*Serapias ×halacsyana* Renz & Soó) (N)
176. *Serapias bergonii* × *Serapias lingua* (*Serapias ×demadesii* Renz) (E, Lumare & Medagli, 2015b)

Fig. 4/Sl. 4: *Ophrys sipontensis*

Fig. 5/Sl. 5: *Serapias orientalis* ***subsp.*** *apulica*

177. *Serapias bergonii* × *Serapias parviflora* (*Serapias ×barsellae* Lumare & Medagli) (Lumare & Medagli, 2015a)
178. *Serapias bergonii* × *Serapias politisi* (*Serapias ×marchiorii* Turco & Medagli) (Turco & Medagli, 2009, E)
179. *Serapias bergonii* × *Serapias vomeracea* subsp. *vomeracea* (N)
180. *Serapias cordigera* × *Serapias orientalis* subsp. *apulica* (*Serapias ×gennaioi* Turco & Medagli) (Turco & Medagli, 2012, L)
181. *Serapias cordigera* × *Serapias lingua* (*Serapias ×ambigua* Rouy) (E, L)
182. *Serapias cordigera* × *Serapias vomeracea* subsp. *vomeracea* (*Serapias ×kelleri* Camus) (Gennaio & Medagli, 2010)
183. *Serapias lingua* × *Serapias parviflora* (*Serapias ×todaroi* Tin.) (E, L, M)
184. *Serapias lingua* × *Serapias politisi* (*Serapias ×lupiense* Medagli, Bianco, D'Emerico & Ruggiero) (Medagli *et al.,* 1993, E)
185. *Serapias lingua* × *Serapias vomeracea* (*Serapias ×intermedia* Forestier) (E)
186. *Serapias orientalis* subsp. *apulica* × *Serapias vomeracea* (*Serapias ×garganica* H. Baumann & Künkele) (M, N)
187. *Serapias parviflora* × *Serapias politisi* (*Serapias ×demericoi* Medagli & Turco) (Medagli & Turco, 2010-2011, I)
188. *Serapias parviflora* × *Serapias vomeracea* (*Serapias ×ruggiero* Medagli & Turco) (F, Medagli & Turco, 2010-2011, M)

Come si può osservare l'elenco floristico è costituito da 102 entità ripartite in sedici generi. Tale considerevole numero, facendo riferimento a Peruzzi (2010) e agli elenchi pubblicati in Conti *et al.* (2005), GIROS (2009) e Pezzetta (2011), costituisce oltre il 49 % delle Orchidaceae presenti sul territorio nazionale e contribuisce a collocare la Puglia insieme a Abruzzo e Toscana tra le regioni d'Italia più ricche e importanti per il patrimonio orchidologico. I dati riportati nella Tabella 1 mostrano che la Puglia con il 4,34 % è al primo posto in scala nazionale per quanto riguarda l'incidenza percentuale delle Orchidaceae sulla flora regionale. Seguono Molise

Tab. 1: Incidenza delle Orchidacee sulla flora regionale
Tab. 1: Delež kukavičevk (Orchidaceae) v deželni flori

Regione	Totale Orchidacee presente	Totale taxa flora regionale[2]	% Orchidacee sulla flora regionale
Valle d'Aosta	41	2190	1,87
Piemonte	63	3630	1,73
Lombardia	64	3332	1,92
Trentino Alto Adige	67	3043	2,2
Veneto	74	3587	2,06
Friuli Venezia Giulia	72	3347	2,15
Liguria	82	3324	2,47
Emilia Romagna	76	2821	2,69
Toscana	96	3541	2,71
Marche	68	2713	2,5
Umbria	67	2396	2,8
Lazio	84	3302	2,54
Abruzzo	97	3409	2,84
Molise	80	2440	3,28
Campania	81	3102	2,61
Puglia	102	2352	4,34
Basilicata	86	2694	2,37
Calabria	88	2787	3,16
Sicilia	76	3106	2,45
Sardegna	63	2620	2,4

[2] Fonte /Vir: Peruzzi (2010)

(3,2 %), Calabria con il 3,17 %, Abruzzo con 2,84 % e Toscana con il 2,7 1%.

All'insieme delle varie specie e sottospecie si aggiungono 188 ibridi per un ammontare complessivo di 290 taxa. Tra essi, quattro nuovi per la Regione sono stati segnalati (R. Souche, *com. pers.*): *Ophrys bertolonii* × *Ophrys brutia*, *Ophrys cinnabarina* × *Ophrys holosericea* subsp. *gracilis*, *Ophrys holosericea* subsp. *paolina* × *Ophrys neglecta* e *Ophrys lojaconoi* × *Ophrys passionis* subsp. *passionis*. Nell'elenco floristico quattro ibridi segnalati da Griebl (2014) formati da *Ophrys holosericea* e un altro taxon sono stati riportati con l'indicazione *Ophrys holosericea* s. l. poiché, alla luce delle recenti revisioni tassonomiche, nella Regione la presenza di *Ophrys holosericea* subsp. *holosericea* è da escludersi. Nell'elenco non sono state considerate diverse segnalazioni ritenute molto dubbie ed entità segnalate in passato che non sono state riconfermate dalle ricerche attuali. Tra queste le seguenti specie segnalate da Fenaroli (1974): *Ophrys insectifera* L., *Ophrys lunulata* Parl., *Ophrys scolopax* Cav. subsp. *scolopax*, *Orchis militaris* L. (confermata da Biscotti (2002) ma esclusa in pubblicazioni successive) e *Serapias neglecta* De Notaris. Altre entità dubbie segnalate da Del Fuoco (2003) per il Gargano sono le seguenti:

- *Ophrys bilunulata* Risso, un'entità Mediterraneo-Occidentale, confermata da Hertel & Presser (2009) che ad avviso di Delforge (2005) in Italia è presente in Liguria, non è riportata tra le *Ophrys* garganiche da Rossini & Quitadamo (2014), mentre GIROS (2009) e Romolini & Souche (2012) non la riportano nell'elenco dei taxa presenti in Italia.
- *Ophrys araneola* subsp. *virescens* (Philippe ex Gren.) Kreutz, un'entità Mediterraneo-Occidentale riportata anche in GIROS (2009), non confermata in pubblicazioni successive e pertanrto da assegnare a entità simili.

Un altro taxon dubbio, non riportato nell'elenco è *Ophrys exaltata* subsp. *exaltata* Ten. che è stato segnalato da De Martino & Centurione (2001), Del Fuoco (2003) e GIROS (2009) ma che non è confermato da Delforge (2005) e Romolini & Souche (2012).

Nonis (2012) segnala in provincia di Lecce *Orchis elegans* Heuff. un taxon secondo Delforge (2005) tipico delle zone umide nord-balcaniche, che ad avviso dello scrivente potrebbe essere una varietà cromatica di *Anacamptis palustris* o è da attribuire a qualche ibrido.

Si può osservare dall'elenco floristico che il gruppo di *Ophrys holosericea* è rappresentato con dieci entità: *Ophrys cinnabarina*, *Ophrys candica*, *Ophrys holosericea* subsp. *apulica*, *Ophrys holosericea* subsp. *gracilis*, *Ophrys holosericea* subsp. *paolina*, *Ophrys holosericea* subsp. *parvimaculata*, *Ophrys lacaitae*, *Ophrys oxyrrhinchos* subsp. *celiensis*, *Ophrys oxyrrhinchos* subsp. *ingrassiae* e *Ophrys pinguis*. Secondo Del Prete (1982) il taxon ha subito uno smembramento in entità di dubbio valore tassonomico. Il processo dall'epoca in cui furono fatte tali considerazioni si è ulteriormente incrementato e, in accordo con l'autore si può sostenere che piccole differenze morfologiche sono state interpretate come caratteri distintivi per la descrizione di nuove entità.

Devey *et al.* (2009) hanno dimostrato che tra le popolazioni dello stesso territorio appartenenti al gruppo in considerazione, avviene un flusso genico. Ciò porta alla formazione di molti individui con caratteri intermedi che talvolta può rendere difficoltosa la classificazione. Di conseguenza solo altri studi e ricerche, che tengano conto non solo degli aspetti morfologici che nelle *Ophrys* sono variabilissimi, potranno portare chiarezza e confermare o no il rango tassonomico di tante specie considerate dubbie.

Identiche considerazioni possono essere applicate al gruppo di *Ophrys exaltata-sphegodes* che nella regione è rappresentato da undici taxa (Ophrys *brutia*, *Ophrys exaltata* subsp. *archipelagi*, *Ophrys gravinensis*, *Ophrys incubacea* subsp. *incubacea*, *Ophrys mateolana*, *Ophrys minipassionis*, *Ophrys murgiana*, *Ophrys passionis* subsp. *passionis*, *Ophrys sipontensis*, *Ophrys sphegodes* e *Ophrys tarquinia*) e più in generale, secondo Delforge (2005) è costituito da trenta taxa cui sono da aggiungere numerosi altri descritti successivamente. Ad avviso di Sedeek *et al.* (2014), dal punto di vista genetico *Ophrys sphegodes* e *Ophrys exaltata* sono indistinguibili. In alcuni casi le differenze morfologiche non sono corrisposte da isolamento riproduttivo oppure esemplari simili sono parzialmente isolati dal punto di vista riproduttivo. Queste considerazioni dimostrano che i concetti di specie biologica e di specie filogenetica nei casi esaminati non coincidono, che i soli caratteri morfologici non sono sempre utilizzabili con estrema chiarezza per classificare singoli individui e quindi probabilmente il numero delle "buone specie" effettivamente valide potrebbe ridursi.

Analoghe considerazioni si possono applicare anche al gruppo di *Ophrys fusca-iricolor* rappresentato in regione con nove taxa: *Ophrys calocaerina*, *Ophrys forestieri*, *Ophrys fusca* subsp. *fusca*, *Ophrys fusca* subsp. *funerea*, *Ophrys fusca* subsp. *lucana, Ophrys fusca* subsp. *lupercalis, Ophrys iricolor* subsp. *eleonorae, Ophrys iricolor* subsp. *lojaconoi* e *Ophrys lucifera*. Le segnalazioni di *Ophrys fusca* subsp. *fusca* sono da ritenere dubbie poiché secondo Delforge (2005), GIROS (2009) e Romolini & Souche (2012) in Italia la specie è assente e probabilmente è sostituita dalle subsp. *lupercalis* e *funerea*. Tuttavia, in attesa di chiare e future ricerche si è preferito mantenere la vecchia denominazione e riportare il taxon nell'elenco.

Altrettanto dubbie sono le segnalazioni dei gruppi di *Epipactis helleborine* e di *Epipactis leptochila* che secondo Rossini & Quitadamo (2014) sul Gargano sono presenti rispettivamente il primo con tre sottospecie e il secondo con due. La classificazione del genere *Epipactis* non è sempre facile poiché è caratterizzato da un›alta variabilità morfologica, da adattamenti in base alle caratteristiche ecologiche territoriali e da un'elevata ibridazione. Di conseguenza è ragionevole presumere che la presenza di entità simili in un ambito geografico abbastanza ristretto, possa essere ricondotta a un unico taxon.

Possono essere considerate dubbie anche le segnalazioni di alcuni ibridi cui concorrono:

- Entità appartenenti allo stesso gruppo. In questo caso gli individui di natura ibridogena potrebbero rappresentare delle forme intermedie tra i taxa da cui avrebbero origine.
- Entità considerate di discutibile rango tassonomico e quindi soggette a nuovi studi e ricerche.

Secondo Rossi *et al.* (1990) sotto il profilo puramente matematico il genere *Ophrys* può generare oltre 2000 entità d'ibridi e quindi non è da escludere che possano essere individuati entro breve tempo altri cui possono concorrere le entità di recente descrizione. Ad avviso di Schiestl (2005), Cozzolino & Scopece (2008) e Turco *et al.* (2012), i fenomeni d'ibridazione, molto frequenti all'interno delle *Ophrys* hanno un ruolo importante nella produzione di nuovi taxa, poiché gli ibridi, oltre ad acquisire caratteri morfologici propri, sono in grado di produrre nuovi feromoni capaci di attrarre insetti impollinatori diversi da quelli dei progenitori. Di conseguenza, la presenza di numerosi ibridi non è escluso che possa portare all'individuazione e descrizione di altre specie per la regione. Alla luce di tali considerazioni si può ritenere provvisorio il numero dei taxa compresi nell'elenco poiché suscettibile sia di possibili diminuzioni sia d'incrementi.

La presenza in un territorio di più specie appartenenti allo stesso gruppo porta a pensare che si siano verificati processi di speciazione simpatrica in cui una da una popolazione presente in un'area, a causa d'isolamento riproduttivo, si evolvono organismi capaci di assumere caratteri diversi. Un particolare processo simpatrico è la speciazione per ibridazione in cui dalla combinazione di più genomi delle specie parentali si originano nuovi organismi con caratteri propri. Spesso gli ibridi di prima generazione sono sterili. Con una certa frequenza essi si rincrociano con una specie parentale e producono individui fertili con propri caratteri morfologici e corredo genetico. Detto fenomeno chiamato introgressione gioca un ruolo importantissimo nella formazione di nuovi taxon tra le Orchidacee in generale e le *Ophrys* in particolare. Secondo Cozzolino & Widmer (2005) nelle popolazioni simpatriche, l'isolamento riproduttivo è causato da barriere post zigotiche dovute ad arrangiamenti cromosomici.

Nell'ambito in esame è stato dimostrato che hanno un›origine introgressiva le seguenti specie: *Ophrys oxyrrhinchos* subsp. *ingrassiae* (Turco *et al.*, 2011) e *Ophrys murgiana* (Medagli & Cillo, 2009). Ad avviso di Devey *et al.* (2008) hanno le stesse origini e caratteristiche anche *Ophrys lacaitae*, *Ophrys holosericea* subsp. *apulica* e *Ophrys garganica* (*Ophrys passionis*). Probabilmente anche le specie endemiche appartenenti al gruppo di *Epipactis helleborine* presenti sul Gargano, se confermate nel loro rango tassonomico, potrebbero avere la stessa origine.

La distribuzione delle Orchidacee suddivide la Puglia in quattro grosse macroaree:

- il Gargano con 85 tra specie e sottospecie cui si aggiungono 103 ibridi (Perilli, 2013, Perilli 2015; Griebl, 2014; Rossini & Quitadamo, 2014);
- le Murge (che in parte sono comprese nella Provincia di Taranto e in quella di Matera appartenente alla Basilicata) con 62 taxa e 23 ibridi (Medagli & Cillo, 2009; D'Alonzo, 2013; Siletti & Medagli, 2015);
- la Provincia di Taranto con oltre 40 specie e 10 ibridi (Terzi *et al.,* 2010; Campochiaro & Dura, 2011; Dura, 2002, 2004a, 2004b, 2010, 2011; Dura *et al.,* 2011);
- il Salento in cui sono segnalate rispettivamente 39 tra specie e sottospecie e 62 ibridi (Gennaio *et al.,* 2010; Medagli & Turco, 2010-11, 2011; Turco & Medagli, 2012; Turco *et al.,* 2012; Gennaio & Medagli, 2013, 2015; Gennaio, 2014; Lumare & Medagli 2015a, 2015b).

Fanno da contorno l'Appennino Dauno con 33 entità (Santoro, 2006, 2010; Wagensommer *et al.,* 2014), il Parco Naturale di Lama Balice con 17 di cui 15 specie e due ibridi (De Leo, 2015) e le isole Tremiti con 15 (Wiebalck, 1985; Rossini & Quitadamo, 2014).

Dall'elenco floristico e dal confronto sulla distribuzione dei vari taxa nelle altre regioni italiane (Pezzetta, 2011) emerge che i generi *Anacamptis* con 7 taxa, *Neotinea* con 4, *Ophrys* con 48 e *Serapias* con 9 raggiungono la maggior diversità.

Un'altra importante caratteristica è data dal fatto che nella regione, diverse entità raggiungono un limite di distribuzione geografica. Infatti:

raggiungono il limite settentrionale di distribuzione geografica *Anacamptis collina, Epipactis schubertiorum*, *Epipactis helleborine* subsp. *aspromontana* e *Serapias bergonii*;

raggiungono il limite occidentale di distribuzione geografica *Ophrys argolica* subsp. *biscutella*, *Ophrys candica* e *Serapias politisii*.

Altre entità molto più numerose raggiungono in Puglia il limite orientale di distribuzione geografica e questo è dovuto essenzialmente alla sua particolare posizione e al fatto che le contiguità territoriali con le regioni vicine hanno consentito scambi e migrazioni floristiche da varie direzioni.

L'analisi corologica e ipotesi sulle rotte migratorie

Dall'analisi corologica, com'è visibile nella Tabella 2, si può osservare che domina l'elemento mediterraneo con 37 taxa e nel suo ambito sono più numerose le entità stenomediterranee. Esso è seguito dagli elementi:

Tab. 2: Corotipi delle Orchidacee pugliesi
Tab. 2: Korotipi kukavičevk (Orchidaceae) v Apuliji

Elementi geografici	Numero taxa	%
	34	33,34
Endemico	31	
Subendemico	3	
Mediterraneo	**37**	**36,27**
Eurimediterraneo	12	
Stenomediterraneo	16	
Mediterraneo Orientale	6	
Mediterraneo Occidentale	3	
Eurasiatico	**14**	**13,73**
Eurasiatico s.s.	6	
Europeo-Caucasico	4	
Eurosiberiano	1	
Paleotemperato	3	
Nordico	**2**	**1,96**
Circumboreale	2	
Europeo	**10**	**9,80**
Europeo s.s.	1	
Centro Europeo	3	
Appennino-Balcanico	5	
Sud-Est Europeo	1	
Mediterraneo-Atlantico	**5**	**4,90**
Subatlantico	1	
Mediterraneo-Atlantico s.s.	3	
Totale	**102**	**100**

endemico con 34, eurasiatico con 14, europeo con 10, mediterraneo-atlantico con 5 e nordico con due taxa. Nella composizione della flora pugliese e quindi anche tra le Orchidaceae si riduce l'incidenza dei taxa appartenenti ai seguenti corotipi: Eurosiberiano, Artico-Alpino, Orofita-Sud-Europeo, Mediterraneo-Montano, Eurasiatico e Circumboreale.

Oltre il 33 % delle orchidacee regionali sono endemiche o subendemiche. Tutto il territorio pugliese e in particolar modo il Gargano per il genere *Ophrys* in ambito nazionale, rappresentano un importantissimo centro d'origine e di diversificazione floristica che contribuisce a rendere la regione, un'incredibile miniera che attrae studiosi di tutta Europa alla ricerca di novità (Gennaio *et al.,* 2010; Marchiori *et al.,* 2000; Griebl, 2014; Rossini & Quitadamo, 2014).

Tra le numerose entità endemiche alcune sono presenti anche in altre regioni peninsulari, mentre altre sono esclusive della Puglia. Sono da considerare endemismi esclusivi pugliesi: *Epipactis leptochila* subsp subsp. *umbrae*, *Ophrys gravinensis*, *Ophrys holosericea* subsp. *paolina*, *Ophrys mateolana*, *Ophrys mattinatatae*, *Ophrys murgiana, Ophrys oestrifera* subsp. *montis-gargani, Ophrys oxyrrhinchos* subsp. *celiensis, Ophrys oxyrrhinchos* subsp. *ingrassiae*, *Ophrys pseudomelena*, *Ophrys tardans* e *Serapias orientalis* subsp. *apulica*.

Sono da considerare endemismi esclusivi dell'Italia centro-meridionale: *Epipactis meridionalis*, *Ophrys cinnabarina*, *Ophrys fusca* subsp. *lucana*, *Ophrys holosericea* subsp. *apulica*, *Ophrys holosericea* subsp. *gracilis, Ophrys lucifera*, *Ophrys minipassionis, Ophrys peucetiae, Ophrys pinguis*, *Ophrys promontorii, Ophrys sipontensis* e *Ophrys tarquinia*.

Sono da considerare endemismi esclusivi dell'Italia meridionale: *Epipactis helleborine* subsp. *aspromontana*, *Epipactis helleborine* subsp. *schubertiorum*, *Ophrys holosericea* subsp. *parvimaculata*, *Ophrys iricolor* subsp. *eleonorae*, *Ophrys iricolor* subsp. *lojaconoi*, *Ophrys scolopax* subsp. *conradiae* e *Ophrys tarentina*.

In Puglia sono segnalate anche cinque specie appennino-balcaniche, sei mediterraneo-orientali, una sud-est europea, tre corotipi di particolare interesse fitogeografico che potrebbero rappresentare forme relittiche e attuali testimonianze di processi migratori avvenuti in ere geologiche passate tra la penisola italiana e balcanica.

In una pubblicazione sulle orchidacee della Croazia (Kranjčev, 2005) si segnala la presenza nella Dalmazia continentale e/o nelle sue isole le seguenti specie: *Ophrys brutia, Ophrys bertoloni* subsp. *bertoloniformis*, *Ophrys holosericea* subsp. *gracilis* e *Ophrys lupercalis*. Se le ricerche successive le confermeranno, il loro areale si estenderebbe e anziché essere considerate endemiche italiane rientrerebbero a pieno titolo nel corotipo appennino-balcanico che quindi crescerebbe di numero.

Un altro gruppo interessante è quello dei corotipi mediterraneo-atlantico e mediterraneo-occidentale che sono rappresentati in totale da 8 taxa e documentano possibili movimenti migratori avvenuti in direzione orientale. In effetti, secondo Pezzetta (*dati non pubbl.*) la Puglia è la regione adriatica in cui sono presenti in maggior quantità entità floristiche a baricentro occidentale (subatlantiche, ovest-europee, mediterraneo-occidentali, mediterraneo-atlantiche e mediterraneo-macaronesiche) che raggiunsero l'ambito in epoche passate percorrendo diverse rotte emigratorie: da sud attraverso Sicilia, Calabria e Basilicata e da nord-ovest scendendo lungo la penisola. Nel caso delle orchidacee la maggior presenza di entità orientali rispetto a quelle occidentali porta a propendere per uno spostamento del baricentro floristico verso est e a una maggiore influenza della componente di questo gradiente direzionale.

Al fine di formulare ipotesi più o meno attendibili riguardo eventuali rotte migratorie seguite, si è deciso di considerare il genere *Ophrys* che con oltre 90 taxa (Romolini & Souche, 2012) è il più rappresentativo delle orchidacee della flora italiana e con 48 anche di quella pugliese. Soliva *et al.* (2001) evidenziano che il genere *Ophrys* è abbastanza diffuso nel bacino del Mediterraneo e in base ad analisi filogenetiche ipotizzano che la sua diversità è il risultato di una recente radiazione. Tuttavia non risolvono il problema dell'ambito, ove potevano essere presenti le entità ancestrali né tantomeno chiariscono come si sarebbero diffuse. Di conseguenza ai nostri fini si continuano a utilizzare alcune vecchie ipotesi che consentono per il momento di chiarire il problema delle possibili rotte migratorie.

Secondo Nelson (1962) le ofridi sono di origine orientale e attraverso il Gargano sarebbero giunte in Italia subendo altri processi di speciazione e di migrazione lungo la penisola. Depongono a favore delle sue teorie:

- la presenza di 18 specie corrispondenti al 50 % delle *Ophrys* garganiche anche in Dalmazia e le sue isole;
- la presenza sul Gargano di *Ophrys holosericea* subsp. *apulica*, *Ophrys mattinatatae* e *Ophrys oestrifera* subsp. *montis-gargani* che possono essere considerati taxa vicarianti accomunati da origini, migrazioni e processi di speciazione allopatrica con le seguenti entità presenti nella Dalmazia continentale e le sue isole: *Ophrys pharia* P. Devillers & J. Devillers-Terschuren, *Ophrys rhodostephane* P. Devillers & J. Devillers-Terschuren e *Ophrys zinsmeisteri* A. Fuchs & Ziegenspeck.

La presenza in Dalmazia anche di *Orchis quadripunctata* potrebbe rappresentare un altro elemento che confermerebbe gli scambi floristici delle orchidacee lungo la rotta in oggetto.

Tuttavia l'assenza sulle isole Tremiti di molte *Ophrys* presenti sul Gargano potrebbe non confermare le ipotesi di Nelson. Questo gruppo d'isole nei processi migratori tra le due sponde dell'Adriatico poteva fungere da ponte intermedio e su di esse dovrebbero essere presenti le entità che ne avrebbero usufruito.

Altri fatti portano a pensare che la porta d'ingresso del genere *Ophrys* e di altre Orchidacee per l'Italia possa essere stato anziché il Gargano, il Salento, e cioè la porzione del territorio pugliese distante in linea d'aria solo 80 km dalle coste albanesi e che è delimitata a nord dalla linea che congiunge Taranto a Torre Canne e che confina con le subregioni dell'Anfiteatro tarantino, della Murgia dei trulli e della Cimosa litoranea (Bissanti, 1991). Secondo Marchiori *et al.* (1998) la comune vicinanza tra le coste garganiche e salentine con quelle della penisola balcanica ha consentito di fingere da ponte di transito per numerosi organismi animali e vegetali. Gargano e Salento dal punto di vista floristico sono caratterizzati da proprie entità endemiche, in alcuni casi presenti da una parte ma non nell'altra. Questo dimostra che tra i due ambiti nel passato non ci sono stati molti scambi floristici e che in ognuno di essi i singoli taxa hanno proprie origini, storie evolutive e processi migratori.

Nel corso di ere geologiche passate le coste greco-albanesi e quelle salentine a più riprese oltre a beneficiare di collegamenti terrestri diretti furono caratterizzate anche da una maggiore vicinanza a causa dei fenomeni di variazioni del livello marino (eustatismo glaciale) che favorirono i processi di dispersione degli organismi animali e vegetali in entrambe le direzioni. Secondo Francini Corti (1953, 1966, 1967) il collegamento terrestre che si creò nel Miocene medio tra quest'area pugliese e la parte meridionale delle terre dell'Egeo fu l'origine delle migrazioni floristiche di entità definite "paleoegeiche". Tuttavia poiché si suppone che le *Ophrys* ebbero origine 4,7 Ma e continuarono a differenziarsi sino a 0,4 Ma (Inda *et al.* 2012) l'ipotesi delle migrazioni mioceniche per tale genere è da escludersi. Durante il Pleistocene, secondo Ozenda (1994) il Salento e il settore meridionale della Grecia molto più vicine rispetto all'era attuale, funsero da isole di rifugio per la flora mediterranea, mentre il Gargano e il resto della Puglia erano dominati da formazioni vegetali a steppa e steppa alberata tipiche di ambiti continentali freddi. Di conseguenza è da presumere che il genere *Ophrys* e altri tipici dell'ambiente mediterraneo dovevano essere assenti nei territori dell'ex Jugoslavia posti a monte e a nord del Gargano, mentre solo nel Salento ove arrivarono dalla penisola egea, trovarono in tale periodo le condizioni ambientali idonee per la loro sopravvivenza. L'ipotesi di diffusione attraverso il ponte salentino-albanese muove dall'osservazione anche di altri fattori tra cui: la condivisione di specie, le affinità genetiche esistenti tra entità presenti sulle opposte sponde e l'analisi degli areali dei taxa che costituiscono i gruppi di *Ophrys argolica* e di *Ophrys bornmuelleri*.

Il Salento condivide con la Grecia-Albania 10 entità del genere *Ophrys*. Secondo Delforge (2005) il gruppo di *Ophrys argolica*, di origine egea, è costituito nel complesso da 13 specie distinte con 4 presenti in Italia (*Ophrys crabronifera, Ophrys pollinensis*, *Ophrys biscutella* e *Ophrys morisii*) e il resto nella Grecia continentale, nelle isole ionie ed egee. Esclusa *Ophrys biscutella* che è segnalata solo sull'isola di Korčula, il gruppo è completamente assente nel territorio continentale dell'ex Jugoslavia posta di fronte e a nord del Gargano stesso. Questa particolarità porta a pensare che l'emigrazione della specie ancestrale del gruppo non possa che essere arrivata da sud e di conseguenza la rotta percorsa per arrivare nella penisola italiana doveva essere stata quella che passa attraverso la Grecia, l'Albania e il Salento.

Un'altra prova a sostegno di tale tesi la fornisce il gruppo di *Ophrys bornmuelleri* che secondo Delforge (2005) è costituito da 17 specie che ora occupano grosso modo lo stesso territorio del gruppo di *Ophrys argolica* e sono assenti nei territori dell'ex Jugoslavia posti a nord del Gargano. Di tale gruppo fanno parte 5 specie presenti in Italia. Di esse una è presente nel Gargano (*Ophrys parvimaculata*), 2 nel Salento (*Ophrys candica* e ancora *Ophrys parvimaculata)* e le altre in Sicilia e Sardegna. Altre prove a sostegno di tale rotta migratoria sono fornite da:

- *Serapias politisii* presente a Corfù e nella Grecia continentale e assente nell'ex Jugoslavia (Delforge, 2005);
- le affinità genetiche riscontrate da Musacchio *et al.* (2006) tra le popolazioni salentine e albanesi di *Anacamptis palustris* che deporrebbero per l'esistenza di unico centro d'origine e da movimenti migratori avvenuti lungo il canale di Otranto;
- l'assenza nei territori dell'ex Jugoslavia di entità appartenenti al gruppo di *Serapias orientalis* (Greuter) H. Baumann & Künkele che in Italia è presente con due specie - *Serapias orientalis* subsp. *apulica* e *Serapias orientalis* subsp. *siciliensis* Bartolo & Pulv.

Si può ammettere che la diffusione delle ofridi dalla penisola balcanica a quella italiana sia avvenuta, senza usufruire di collegamenti terrestri diretti e potrebbe essere stata affidata all'azione del vento, come ipotizzato per i gruppi di *Ophrys oestrifera* da Van De Vijer *et al.* (2010) e *Ophrys dinarica* Kranjcev & P. Delforge da Buono & Gransinigh (2011). In generale i piccolissimi semi delle orchidacee sono facilmente trasportabili dal vento e quindi la diffusione anemocora può essere estesa anche ad altre entità della famiglia. Secondo altre ipotesi gli ambienti steppici e di steppa alberata che caratterizzavano il paesaggio di gran parte delle penisole balcanica e italiana durante le glaciazioni quaternarie consentiva la sopravvivenza di orchidacee del genere *Ophrys* che oggi vegetano sino a 1000 metri di quota e ciò non escluderebbe la loro presenza sul territorio posto di fronte al Gargano da cui sarebbero partite le ondate migratorie in direzione occidentale.

CONCLUSIONI

L'elenco floristico riportato dimostra l'importanza del patrimonio orchidologico della Puglia in cui si registra la maggior presenza di taxa di orchidacee tra tutte le regioni italiane ed evidenzia le particolarità fitogeografiche dell'ambito in esame. Molte entità prima segnalate e non riconfermate dimostrano che le ricerche condotte in quest'ambito geografico offrono il terreno di confronto tra i vari studiosi che utilizzano ciascuno un proprio metodo di classificazione e ricerca e ripropongono il tema della corretta definizione e caratterizzazione dei taxa.

La tendenza in atto è in generale orientata verso la descrizione di nuove entità sulla base di sottili caratteri morfologici che in genere portano alla riduzione dell'intervallo di variabilità dei caratteri stessi per ognuno di essi. Poiché in natura i fenomeni biologici seguono leggi continue, se si riduce l'intervallo di variabilità delle specie, ne consegue che la discriminazione dei caratteri in alcuni casi può diventare molto difficile se non impossibile. La descrizione di nuovi taxa basata solo sui caratteri morfologici non considera gli aspetti evolutivi, causa frequenti disaccordi tra gli studiosi e spesso non considera i polimorfismi esistenti in natura (specie politipiche, razze cromosomiche, ecc.). Secondo Vereecken *et al.* (2010) il concetto di specie morfologica non può essere applicato con sufficiente chiarezza alle *Ophrys* che sono caratterizzate da una grande morfologia floreale. Di conseguenza è da supporre che un approccio filogenetico che prevede l'uso di marcatori molecolari porterebbe da una parte a una valutazione più rigorosa dei criteri utilizzati per discriminare le specie e dall'altro, molto probabilmente a un loro ridimensionamento. Alla luce di queste considerazioni si ritengono necessarie una definizione di specie condivisa da tutti e revisioni tassonomiche che possano portare a classificazioni più sicure.

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KUKAVIČEVKE APULIJE (JUŽNA ITALIJA)

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POVZETEK

Apulija je dežela v južni Italiji, ki pokriva 19.540,9 km² površine. Lahko se pohvali z izjemno floristično dediščino, ki je bila ocenjena na 2.352 taksonov ob koncu leta 2010. Članek predstavlja floristični seznam vseh kukavičevk (Orchidaceae), vključno s hibridi. Prikazana je tudi korološka analiza. Avtorji poročajo o 102 vrstah in podvrstah, ki jim dodajajo še 188 hibridov. Korološka analiza poudarja razširjenost mediteranskih elementov in endemitov.

Ključne besede: Orchidaceae, regionalni seznam vrst, Apulija, južna Italija

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OCENE IN POROČILA

RECENSIONI E RELAZIONI

REVIEWS AND REPORTS

Egidio Trainito, Mauro Doneddu: NUDIBRANCHI DEL MEDITERRANEO. 2a edizione, riveduta e ampliata. Il Castello, 2014, 192 p.

Sea slugs are beautiful benthic molluscs mostly characterized by bright colouration and striking forms. During the course of evolution, their shell has been drastically reduced or has even disappeared. Of all the sea slugs, the nudibranchs are the most attractive to divers and underwater photographers because of their vivid colour patterns and a variety of forms. However, they are also interesting for some peculiar biological features, such as food specialization, bioactive compounds production, camouflage, mimicry, kleptoplasty and others.

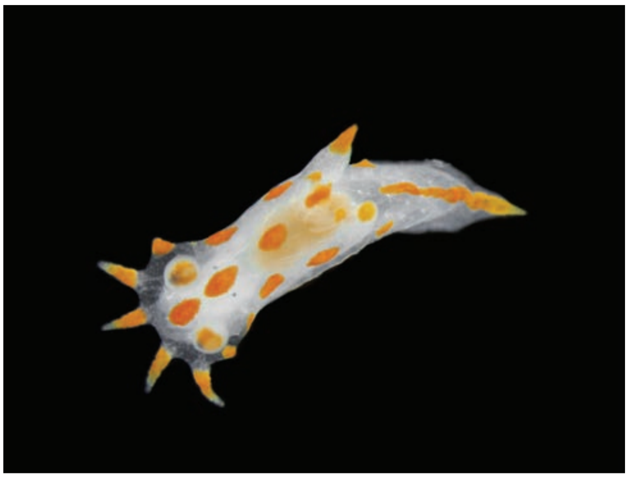
Egidio Trainito is a well-known Italian marine biologist, author of numerous books on the matter of biodiversity in marine ecosystems. His production of scientific writings consists of a series of illustrated guides in the Italian language, including *Conchiglie del Mediterraneo* (Seashells of the Mediterranean), *Nudibranchi del Mediterraneo* (Nudibranchs of the Mediterranean), and others in English such as *Dive the World: The Most Fascinating Diving Sites* and *Atlas of Mediterranean Flora and Fauna*. He also published a specialized book on opisthobranchs, entitled *Mediterranean Harlequins - A Field Guide to Mediterranean Sea Slugs* (2003).

The co-author, Mauro Doneddu is also intrigued by all kinds of biodiversity topics and has already published several monographs on Sardinian butterflies, Mediterranean molluscs and even a book on the wild orchids of Sardinia.

Despite the fact that nudibranchs and other sea slugs are a favourite subject of underwater photographers, there are, paradoxically, only a few books available on the respective Mediterranean species. In some of these works, sea slugs are presented with drawings, for instance, in Pruvot-Fol (1954): *Faune de France*: *Mollusques opisthobranches* or Barletta (1980): *Gasteropodi nudi. Guida per il riconoscimento*. In others, the writings are enriched by amazing illustrations, as is the case with the excellent monograph by Schmekel & Portmann (1982) entitled *Opisthobranchia des Mittelmeeres*. *Des limaces de Reve* (Bielecki *et al.*, 2011) and *Sea Slugs of the Algarve* (Calado & Silva, 2012) on the other hand, are monographs based on excellent photographs. Although there are no such publications to date on the Adriatic Sea, a book entitled *Il regno dei nudibranchi* (The Realm of Nudibranchs), dealing with the nudibranchs of the restricted area of the Riviera del Conero (area close to Ancona) was recently published by Federico Betti.

The book by Trainito and Doneddu on Mediterranean nudibranchs is an expanded and revised second edition. The main emphasis in it is given to photographs. In fact, the great majority of species are illustrated with excellent close-up photos taken in the natural environment. This volume is an improvement over the previous edition in that it brings more information about the Mediterranean species in term of their distinguishing characteristics, distribution and up-to-date records. The title is somehow misleading, though, as not only nudibranchs, but also other opisthobranch sea slug orders, such as Cephalaspida, Anaspida, Saccoglossa, Umbraculoida, Pleurobranchomorpha, Thecosomata, Acochlidacea, Gymnosomata and Rhodopida, are included in the book.

The monograph presents 363 species and over 650 photographs of Mediterranean Sea slugs made by 77 photographers. After short introductive chapters on nudibranchs and their revised taxonomy, the great bulk of the book is dedicated to a short description of the species and notes on biogeography completed with rich photographic material. On the one hand, it is still rather difficult for a reader to identify certain specimens from photographs or in hand, such as those of the genus *Doto* or to determine dorid species, and there are many taxonomic problems yet to solve, as well. On the other hand, the authors tried to collect all available data on



Polycera quadrilineata ***(Photo/Foto: B. Mavrič)***

Thordis filix ***(Photo/Foto: B. Mavrič)*** certain less known species, found only in one or few occasions, which give added value to the book. In such cases photographs are generally not available, so the authors provided authentic illustrations of such species derived from their first descriptions.

In the recent decades, a considerable number of opisthobranch species were discovered due to a growing scientific interest in and divers' fascination with all parts of the Mediterranean Sea. One of such results is also the present book, which synthesises the research findings on opisthobranchs obtained via diving excursions around the Mediterranean and through collection of available data in scientific literature. To my opinion, this publication should be considered as the ultimate guide for marine biologists, but will prove equally helpful to divers, snorkelers, naturalists, scientists and photographers. I am not at all surprised that it is already considered a bible on Mediterranean nudibranchs by certain malacologists.

Lovrenc Lipej

NAVODILA AVTORJEM

1. Revija ANNALES (*Anali za istrske in mediteranske študije* Series historia naturalis) objavlja **izvirne znanstvene** in **pregledne članke** z naravoslovnimi vsebinami, ki obravnavajo posebnosti različnih podpodročij sredozemskega naravoslovja: morska biologija in ekologija, ihtiologija, geologija s paleontologijo, krasoslovje, oljkarstvo, biodiverziteta Slovenije, varstvo narave, onesnaževanje in varstvo okolja, fizična geografija Istre in Mediterana idr. Vključujejo pa tudi **krajše** znanstvene prispevke o zaključenih raziskovanjih., ki se nanašajo na omenjeno področje.

2. Sprejemamo članke v angleškem, slovenskem in italijanskem jeziku. Avtorji morajo zagotoviti jezikovno neoporečnost besedil, uredništvo pa ima pravico članke dodatno jezikovno lektorirati.

3. Članki naj obsegajo do 48.000 znakov brez presledkov oz. 2 avtorski poli besedila. Članek je mogoče oddati na e-naslov annales@mbss.org (zaželjeno) ali na elektronskem nosilcu (CD) po pošti na naslov uredništva.

Avtor ob oddaji članka zagotavlja, da članek še ni bil objavljen in se obvezuje, da ga ne bo objavil drugje.

4. Naslovna stran članka naj vsebuje naslov članka, ime in priimek avtorja (avtorjev), ime in naslov inštitucije, kjer je (so) avtor(ji) zaposlen(i) oz. domači naslov in naslovom elektronske pošte (samo prvi oz. korespondenčni avtor).

5. Članek mora vsebovati **povzetek** in **izvleček.** Izvleček je krajši (cca. 10 vrstic) od povzetka (cca. 30 vrstic).

V *izvlečku* na kratko opišemo namen, metode dela in rezultate. Izvleček naj ne vsebuje komentarjev in priporočil.

Povzetek vsebuje opis namena in metod dela ter povzame analizo oziroma interpretacijo rezultatov. V povzetku ne sme biti ničesar, česar glavno besedilo ne vsebuje. V povzetku se avtor ne sklicuje na slike, tabele in reference, ki so v članku.

6. Avtorji naj pod izvleček članka pripišejo ustrezne **ključne besede** (največ 6). Zaželjeni so tudi angleški (ali slovenski) prevodi izvlečka, povzetka, ključnih besed, podnapisov k slikovnemu in tabelarnemu gradivu. V nasprotnem primeru bo za prevode poskrbelo uredništvo.

7. Glavni del besedila naj vključuje sledeča poglavja: Uvod, Material in metode, Rezultati, Razprava ali Rezultati in razprava, Zaključki (ali Sklepi), Zahvala (če avtor želi), Literatura. Dele besedila je možno oblikovati v podpoglavja (npr. Pregled dosedanjih objav v Uvodu, Opis območja raziskav v Material in metode). Podpisi k slikam so priloženi posebej za poglavjem Literatura.

8. Tabele avtor priravi posebej na ločenih straneh v programu Word, tako kot rokopis, jih zaporedno oštevilči in opremi z naslovom – kratkim opisom. V glavnem delu besedila se sklicuje na tabele tako, da jih na ustreznem mestu označi z npr. "(Tab. 1)".

9. Slikovno gradivo (grafi, zemljevidi, fotografije, table) avtor posreduje v ločenih datotekah (jpeg, tiff) z najmanj 300 dpi resolucije pri želeni velikosti. Največja velikost slikovnega gradiva je 17x20 cm. Vsa potrebna dovoljenja za objavo slikovnega gradiva (v skladu z Zakonom o avtorski in sorodnih pravicah) priskrbi avtor sam in jih predloži uredništvu pred objavo članka. Slike je potrebno tudi podnasloviti in zaporedno oštevilčiti (glej točko 7). V glavnem delu besedila se avtor sklicuje na slike tako, da jih na ustreznem mestu označi z npr. "(Sl. 1)".

10. Bibliografske opombe, s čimer mislimo na **citat** – torej sklicevanje na druge publikacije, sestavljajo naslednji podatki v oklepaju: *avtor* in *leto izida*; npr. (Novak, 2007). Če sta dva avtorja, se izpišeta oba (Novak & Kranjc, 2001), če so trije ali več pa se izpiše samo prvi, ki mu sledi okrajšava *et al.* (Novak *et al.*, 1999). Več citatov je med seboj ločenih s podpičjem in si sledijo kronološko - z naraščajočo letnico izdaje, npr. (Novak *et al.*, 1999; Adamič, 2001; Kranjc & Zupan, 2007). Osebno informacijo (ustno, pisno) izpišemo prav tako v oklepaju z navedbo kratice imena in priimka posredovalca informacije, za vejico pa dodamo "osebno sporočilo", npr. (J. Novak, *osebno sporočilo*).

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Primeri navajanje različnih tipov bibliografskih podatkov:

članki v revijah:

Klock, J.-H., A. Wieland, R. Seifert & W. Michaelis (2007): Extracellular polymeric substances (EPS) from cyanobacterial mats: characterisation and isolation method optimisation. Mar. Biol., 152, 1077-1085.

Knjige in druge neserijske publikacije (poročila, diplomska dela, doktorske disertacije):

Wheeler, A. (1969): The fishes of the British Isles and North-West Europe. McMillan, London, 613 p.

Poglavje v knjigi:
McEachran, J. D. & C. Capapé (1984): Myliobatidae. In: Whitehead, P. J. P., M. L. Bauchot, J.-C. Hureau, J. Nielsen & E. Tortonese (eds.): Fishes of the North-eastern Atlantic and the Mediterranean, Vol. 1. Unesco, Paris, pp. 205-209.

12. Drugo: latinski izrazi kot npr. *in vivo*, *in situ*, *e.g.*, *i.e.*, ter rodovna (*Myliobatis* sp.) in vrstna (*Myliobatis aquila*) imena se izpišejo v fontu italic. Kadarkoli je možno, se uporabljajo enote iz sistema SI (Système international d'unités).

13. Prvi odtis člankov uredništvo pošlje avtorjem v **korekturo.** Avtorji so dolžni popravljeno gradivo vrniti v enem tednu. Besedilo popravljamo s korekturnimi znamenji, ki jih najdemo na koncu Slovenskega pravopisa (2001), Ljubljana, ZRC SAZU, 24–25.

Širjenje obsega besedila ob korekturah ni dovoljeno. Druge korekture opravi uredništvo.

14. Za dodatna pojasnila v zvezi z objavo člankov je uredništvo na voljo.

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4. Ogni articolo deve essere corredato da: **titolo**, nome e cognome dell'autore (autori), denominazione ed indirizzo dell'ente di appartenenza o, in alternativa, l'indirizzo di casa, nonché l'indirizzo di posta elettronica (solo del primo autore o dell'autore di corrispondenza).

5. I contributi devono essere corredati da un **riassunto** e da una **sintesi**. Quest'ultima sarà più breve (cca. 10 righe) del riassunto (cca 30 righe).

Nella *sintesi* si descriveranno brevemente lo scopo, i metodi e i risultati delle ricerche. La sintesi non deve contenere commenti e segnalazioni.

Il *riassunto* riporterà in maniera sintetica lo scopo, i metodi delle ricerche e l'analisi ossia l'interpretazione dei risultati. Il riassunto non deve riferirsi alle tabelle, figure e alla bibliografia contenuta nell'articolo.

6. Gli autori sono tenuti ad indicare le **parole chiave** adeguate (massimo 6). Sono auspicabili anche le traduzioni in inglese (o sloveno) della sintesi, del riassunto, delle parole chiave, delle didascalie e delle tabelle. In caso contrario, vi provvederà la Redazione.

7. Il testo principale deve essere strutturato nei seguenti capitoli: Introduzione, Materiali e metodi, Risultati, Discussione o Risultati e discussione, Conclusioni, Ringraziamenti (se necessari), Bibliografia. Il testo può essere strutturato in sottocapitoli (ad es. sottocapitolo Rassegna delle pubblicazioni nell'Introduzione; sottocapitolo Descrizione dell'area di ricerca nel capitolo Materiali e metodi). Le didascalie devono essere presentate separatamente, a seguito del capitolo Bibliografia.

8. Le tabelle saranno preparate in forma elettronica come il manoscritto (formato Word) e allegate in fogli separati alla fine del testo. Gli autori sono pregati di contrassegnare ogni tabella con un numero e il titolo ossia una breve descrizione. Nel testo la tabella viene richiamata come segue: (Tab. 1).

9. Il materiale grafico (grafici, carte geografiche, fotografie, tavole) va preparato in formato elettronico (jpeg o tiff) e consegnato in file separati, con una definizione di 300 dpi alla grandezza desiderata, purché non ecceda i 17x20 cm. Prima della pubblicazione, l'autore provvederà a fornire alla Redazione tutte le autorizzazioni richieste per la riproduzione del materiale grafico (in virtù della Legge sui diritti d'autore). Tutto il materiale grafico deve essere accompagnato da didascalie (vedi punto 7) e numerato.. Nel testo i grafici vengono richiamati come segue: (ad es. Fig. 1).

10. I riferimenti bibliografici (citazioni) richiamano un'altra pubblicazione (articolo). La nota bibliografica, riportata nel testo, deve contenere i seguenti dati tra parentesi: *cognome dell'autore*, *anno di pubblicazione*, ad es. (Novak, 2007). Se gli autori sono due, verranno indicati entrambi (Novak & Kranjc, 2001), nel caso di tre o più autori verrà indicato soltanto il primo, seguito dall'abbreviazione *et al.* (Novak *et al.*, 1999). Vari riferimenti bibliografici in una stessa nota vanno divisi dal punto e virgola e segnalati in ordine cronologico, ad. es. (Novak *et al.*, 1999; Adamič, 2001; Kranjc & Zupan, 2007). La testimonianza (orale, scritta) verrà indicata tra parentesi con l'abbreviazione del nome e con il cognome di chi l'ha trasmessa, seguiti dalla virgola e la dicitura "informazione personale", ad es. (J. Novak, *informazione personale*).

11. La bibliografia completa va inserita in ordine alfabetico nel capitolo Bibliografia. L'autore indicherà esclusivamente i lavori e le edizioni citati nell'articolo. Se si citano più lavori dello stesso autore, verranno indicati prima in ordine cronologico i lavori in cui l'autore appare solo, poi quelli in cui l'autore compare assieme ad un secondo coautore, seguiti infine da quelli in cui egli compare tra più coautori. I nomi delle riviste in cui sono pubblicati i lavori citati saranno indicati nella forma abbreviata (abbreviazioni ufficialmente riconosciute). Gli articoli inediti si possono citare soltanto se sono in corso di pubblicazione, facendo loro seguire la dicitura "in corso di pubblicazione". Gli articoli, non ancora recensiti non possono essere citati.

Esempio di lavoro bibliografico:

Articoli in riviste:

Klock, J.-H., A. Wieland, R. Seifert & W. Michaelis (2007): Extracellular polymeric substances (EPS) from cyanobacterial mats: characterisation and isolation method optimisation. Mar. Biol., 152, 1077-1085.

Libri ed altre pubblicazioni non periodiche (relazioni, tesi di laurea, dissertazioni di dottorato):

Wheeler, A. (1969): The fishes of the British Isles and North-West Europe. McMillan, London, 613 p.

Capitoli di libro:

McEachran, J. D. & C. Capapé (1984): Myliobatidae. In: Whitehead, P. J. P., M. L. Bauchot, J.-C. Hureau, J. Nielsen & E. Tortonese (eds.): Fishes of the North-eastern Atlantic and the Mediterranean, Vol. 1. Unesco, Paris, pp. 205-209.

12. Altro: Le espressioni latine come ad es. *in vivo*, *in situ*, *e.g.*, *i.e.*, i nomi dei generi famiglie (*Myliobatis* sp.) e delle specie (*Myliobatis aquila*) si scrivono con il carattere italic. Quando possibile saranno utilizzate le unità del sistema SI (*Système international d'unités*).

13. Gli autori ricevono le **prime bozze** di stampa per la revisione. Le bozze corrette vanno quindi rispedite entro una settimana alla Redazione. In questa fase, i testi corretti con segni adeguati (indicazioni in merito si trovano alla fine della pubblicazione "Slovenski pravopis" (2001), Ljubljana, ZRC SAZU, 24-25, non possono essere più ampliati. La revisione delle bozze è svolta dalla Redazione.

14. La Redazione rimane a disposizione per eventuali chiarimenti.

LA REDAZIONE

INSTRUCTIONS TO AUTHORS

1. The journal ANNALES (*Annals for Istrian and Mediterranean Studies,* Series historia naturalis) publishes **original scientific** and **review articles** in the field of natural studies related to the specifics of various subfields of Mediterranean natural studies: marine biology and ecology, ichthyology, geology with paleontology, karst studies, olive growing, biodiversity of Slovenia, nature protection, pollution and environmental protection, physical geography of Istria and the Mediterranean, etc. It also publishes **short** scientific papers on completed research projects related to the above-mentioned sub-fields.

2. The articles submitted can be written in the English, Slovene or Italian language. The authors should ensure that their contributions meet acceptable standards of language, while the editorial board has the right to have them language edited.

3. The articles should be no longer than 48,000 characters (spaces excluded) or 32 typewritten double-spaced pages. They can be submitted via e-mail annales@mbss.org (preferably) or regular mail, with the electronic data carrier (CD) sent to the address of the editorial board.

Submission of the article implies that it reports original unpublished work and that it will not be published elsewhere.

4. The **title page** should include the title of the article, the name and surname of the author(s), their affiliation (institutional name and address) or home address, and e-mail address (of the first author or the corresponding author only).

5. The article should contain the **summary** and the **abstract,** with the former (c. 30 lines) being longer than the latter (c. 10 lines).

The *abstract* contains a brief description of the aim of the article, methods of work and results. It should contain no comments and recommendations.

The *summary* contains the description of the aim of the article and methods of work and a brief analysis or interpretation of results. It can contain only the information that appears in the text as well. It should contain no reference to figures, table and citations published in the main text.

6. Beneath the abstract, the author(s) should supply appropriate **keywords** (max 6) and, if possible, the English (or Slovene) translation of the abstract, summary, keywords, and captions to figures and tables. If unprovided, the translation will be provided by the editorial board.

7. The **main text** should include the following chapters: Introduction, Material and Methods, Results, Discussion or Results and Discussion, Conclusion, Acknowledgement (not obligatory), References. Individual parts of the text can form a sub-chapter (e.g. Survey of Previous Studies under Introduction; Description of Research Area under Material and Methods). Captions to figures should appear on a separate page beneath References.

8. Each **table** should be submitted on a separate page in Word programme (just like the main text). It should be numbered consecutively and supplied with the title – brief description. When referring to the tables in the main text, use the following style: (Tab. 1).

9. Illustrative matter (diagrams, maps, photographs, plates) should be submitted as separate files (in jpeg or tiff format) and saved at a minimum resolution of 300 dpi per size preferred, with the maximum possible publication size being 17x20 cm. Prior to publication, the author(s) should obtain all necessary authorizations (as stipulated by the Copyright and Related Rights Act) for the publication of the illustrative matter and submit them to the editorial board. All figures should be captioned and numbered consecutively (cf. Item 7). When referring to the figures in the main text, use the following style: (Fig. 1).

10. **Bibliographic notes** or **citations** – i.e. references to other articles or publications – should contain the following data: *author* and *year of publication,* e.g. (Novak, 2007). If there are two authors, include both surnames (Novak & Kranjc, 2001); if there are more than two authors, include the surname of the first author followed by a comma and the abbreviation *et al.* (Novak *et al.*, 1999). If there is more than one reference, separate them by a semicolon and list them in ascending chronological order, e.g. (Novak *et al.*, 1999; Adamič, 2001; Kranjc & Zupan, 2007). When citing information obtained through personal communication (oral, written), provide the initial letter of the name and full surname of the informant followed by a comma and the phrase *personal communication,* e.g. (J. Novak, *personal communication*).

11. The entire list of **bibliographic data** should be published under References in alphabetical order. The author(s) should list only the works cited in the article. If you are listing several works by the same author with some of them written in co-authorship, first list those written by the author him/herself, then those written in co-authorship with another author, and finally those written in co-authorship with more than one author, with the entries listed in chronological order. The names of journals in which the works cited were published should be abbreviated (cf. list of official journal abbreviations). Unpublished articles can be cited only if they have been approved for publication, which should be indicated by adding the phrase *in press* to the end of the relevant bibliography entry.

Some examples of how to cite different types of bibliographical data:

Articles published in serial publications:

Klock, J.-H., A. Wieland, R. Seifert & W. Michaelis (2007): Extracellular polymeric substances (EPS) from cyanobacterial mats: characterisation and isolation method optimisation. Mar. Biol., 152, 1077-1085.

Books and other non-serial publications (reports, diploma theses, doctoral dissertation):

Wheeler, A. (1969): The fishes of the British Isles and North-West Europe. McMillan, London, 613 p.

Chapters published in a book:

McEachran, J. D. & C. Capapé (1984): Myliobatidae. In: Whitehead, P. J. P., M. L. Bauchot, J.-C. Hureau, J. Nielsen & E. Tortonese (eds.): Fishes of the North-eastern Atlantic and the Mediterranean, Vol. 1. Unesco, Paris, pp. 205-209.

12. Miscellaneous: Latin phrases such as *in vivo*, *in situ*, *e.g.*, *i.e.*, and names of genera (*Myliobatis* sp.) and species (*Myliobatis aquila*) should be written in italics. Whenever possible, use the SI units (Système international d'unités).

13. The authors are sent the **first page proofs**. They should be returned to the editorial board within a week. When reading the proofs, the authors should use the correction signs listed at the end of the book Slovenski pravopis (2001), Ljubljana, ZRC SAZU, 24–25.

It is not allowed to lengthen the text during proof-reading. Second proof-reading is done by the editorial board.

14. For additional information regarding article publication contact the editorial board.

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SLIKA NA NASLOVNICI:
Kukavičnice so s svojimi barvitimi in nenavadnimi cvetovi že od nekdaj navdihovale številne fotografe. Na sliki je križanec med vrstama *Serapias vomeracea* in *Anacamptis laxiflora*, fotografiran na otoku Krku (Hrvaška). (Foto: M. Lipovšek)
Sl. 1: Purpurna močvirnica *Epipactis purpurata* domuje v vlažnih listnatih in mešanih gozdovih. V Sloveniji je redka in uvrščena na Rdeči seznam ogroženih vrst. (Foto: A. Pezzetta)
Sl. 2: Mačje uho na sliki, *Ophrys iricolor* subsp. *eleonorae*, ki je endemit južne Italije, je dobilo latinsko ime podvrste po kraljici Eleonori iz Arboree (XIV. stoletje), znani med drugim tudi po nekaterih prvih naravovarstvenih aktih, ki jih je uvedla, da bi zavarovala sredozemskega sokola. (Foto: A. Pezzetta)
Sl. 3: Mačje uho vrste *Ophrys sipontensis* je endemična vrsta, ki jo najdemo na Monte Garganu in delu Apulije. Je značilna sredozemska vrsta, ki se pojavlja v makiji in garigi. (Foto: A. Pezzetta)
Sl. 4: Za mačja ušesa (rod *Ophrys*) je značilno, da imajo cvetove podobne telesu čebel, os in drugih kožokrilcev. Samce poleg oblike cveta pritegnejo tudi vonjave, podobne samičjim, ki jih izločajo žleze cvetov mačjih ušes. Na sliki podvrsta čmrljelikega mačjega ušesa *Ophrys holosericea* subps. *parvimaculata*. (Foto: A. Pezzetta)
Sl. 5: Med florističnimi posebnostmi Apulije je tudi vrsta mačjega ušesa *Ophrys argolica* subsp. *biscutella,* ki je endemit južne Italije. Spoznamo ga po dveh svetlih pegah na medeni ustni, ki spominjata na očala, zato bi ga lahko poimenovali očalasto mačje uho. (Foto: A. Pezzetta)
Sl. 6: Ralovec podvrste *Serapias orientalis* subsp. *apulica* je endemit dežele Apulije. Nekateri mu priznavajo status samostojne vrste *Serapias apulica*. (Foto: A. Pezzetta)

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