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An updated checklist and biogeography of the Sardinian large branchiopods, with a focus on Spinicaudata (Crustacea, Branchiopoda)

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SUMMARY

The large branchiopod fauna of Sardinia is reviewed based both on literature and newly collected data. Based on the available evidence, 13 taxa are present on the island (8 Anostraca, 2 Notostraca, and 3 Spinicaudata). Among them, the finding of the spinicaudatan *Leptestheria dahalacensis* is new for Sardinia, while the spinicaudatans *Cyzicus bucheti* and *Eulimnadia* sp. were overlooked in the most recent synopses on the fauna of the island due to misidentifications. Conversely, *Cyzicus tetracerus* and *Limnadia lenticularis*, previously erroneously reported based on misidentifications, must be excluded from the fauna of Sardinia. The finding of *Eulimnadia* sp. is the first record in Europe and the northernmost record of the genus. The occurrence of *Leptestheria dahalacensis* in Sardinia is rather unexpected and probably due to its accidental introduction linked with rice cultures. At least four of the 13 Sardinian large branchiopod species are absent from the Italian mainland and Sicily, stressing the uniqueness of its fauna and its significant contribution to the Mediterranean inland water crustacean diversity.

INTRODUCTION

Due to their size, ancient history, distinctive ecology, and evolutionary conserved morphology, the large branchiopods are considered flagship taxa for temporary ponds and pools (Dumont and Negrea 2002; Brendonck et al. 2008), and an appealing study

group for evolutionary research (e.g., Gueriau et al. 2016). Unfortunately, their unsettled taxonomy and the lack of accurate information about their distribution preclude in-depth biogeographical analyses and the implementation of effective conservation practices. These shortcomings are particularly evident for the order Spinicaudata, plagued by *"the most confused taxonomy of any branchiopod group*" (Rogers 2020).

According to Mura (2006), the only representative of the Spinicaudata occurring in Sardinia is *Cyzicus tetracerus* (Krynicki, 1830), a widespread Palearctic species also reported from the neighbouring Maghrebian and European countries (e.g., Brtek and Thiéry 1995; Marrone et al. 2016; Alfonso 2017). However, contrasting information is reported in the available literature, so that even this single report is doubtful and in need of revision. Much taxonomic confusion and dubious identifications also beset the Anostraca and Notostraca in Sardinia, and several reports need re-examination. Consequently, we carried out a critical review of the Sardinian large branchiopod fauna, accompanied by new field sampling and the study of spinicaudatan specimens deposited in public and private natural history collections. The aim of the paper is to offer an updated checklist with accompanying bibliography and distributional notes for all large branchiopods known for Sardinia up to now, as well as a critical review of Sardinian Spinicaudata, the least known taxon in the study area.

MATERIALS AND METHODS

The study area includes Sardinia and the circum-Sardinian islands. Due to its geographic position, the climate of Sardinia is typically Mediterranean, with dry and hot summers and relatively rainy and mild winters. Mean annual rainfall ranges from 411 along the southern coast to more than 1215 mm in the mountainous regions. Mean annual temperature ranges from 12 °C to 18 °C (Canu et al. 2014).

A comprehensive review of the available literature dealing with all Sardinian large branchiopods was compiled, considering the incompleteness of the bibliography included in Mura (2006) and the systematic changes that took place in the last fifteen years. Due to the economic importance of the anostracan genus *Artemia*, the literature dealing with this genus is enormous, so that producing an exhaustive bibliographic review about Sardinian brine shrimps was considered unfeasible for the present work. However, attention was paid to review and mention all references that included relevant systematic and distributional data.

The private crustacean collections of Vezio Cottarelli, Fiorenza Gabriella Margaritora and Graziella Mura were carefully screened for the occurrence of Spinicaudata collected in Sardinia. Moreover, the catalogue of the Crustacean Collection of the Museum für Naturkunde (Berlin, Germany), where the occurrence of a spinicaudatan sample collected in Sardinia was reported by Daday (1915), was consulted through its web interface (https://www.museumfuernaturkunde.berlin/en/science/crustacea, accessed on 18/03/2021).

The crustacean fauna of 106 Sardinian temporary ponds and pools scattered throughout the island was investigated through field surveys carried out in the spring of 2008 (March), 2010 (March), 2018 (March), 2019 (June, September, and December), and 2021 (March and May), focusing on the localities where spinicaudatans were previously reported (Fig. 1). Crustacean samples were collected by means of a 125 µm mesh-sized towing net in the open waters and a 200 µm mesh-sized hand net in the benthic and littoral zones. Moreover, a 600 µm mesh-sized hand net was specifically used for collecting adult large branchiopods, which are able to actively avoid the denser nets and, especially when present in low densities, may be overlooked when using the conventional zooplankton sampling techniques. Collected samples were fixed *in situ* in 95% ethanol. During the summer months sediment samples were collected in selected localities

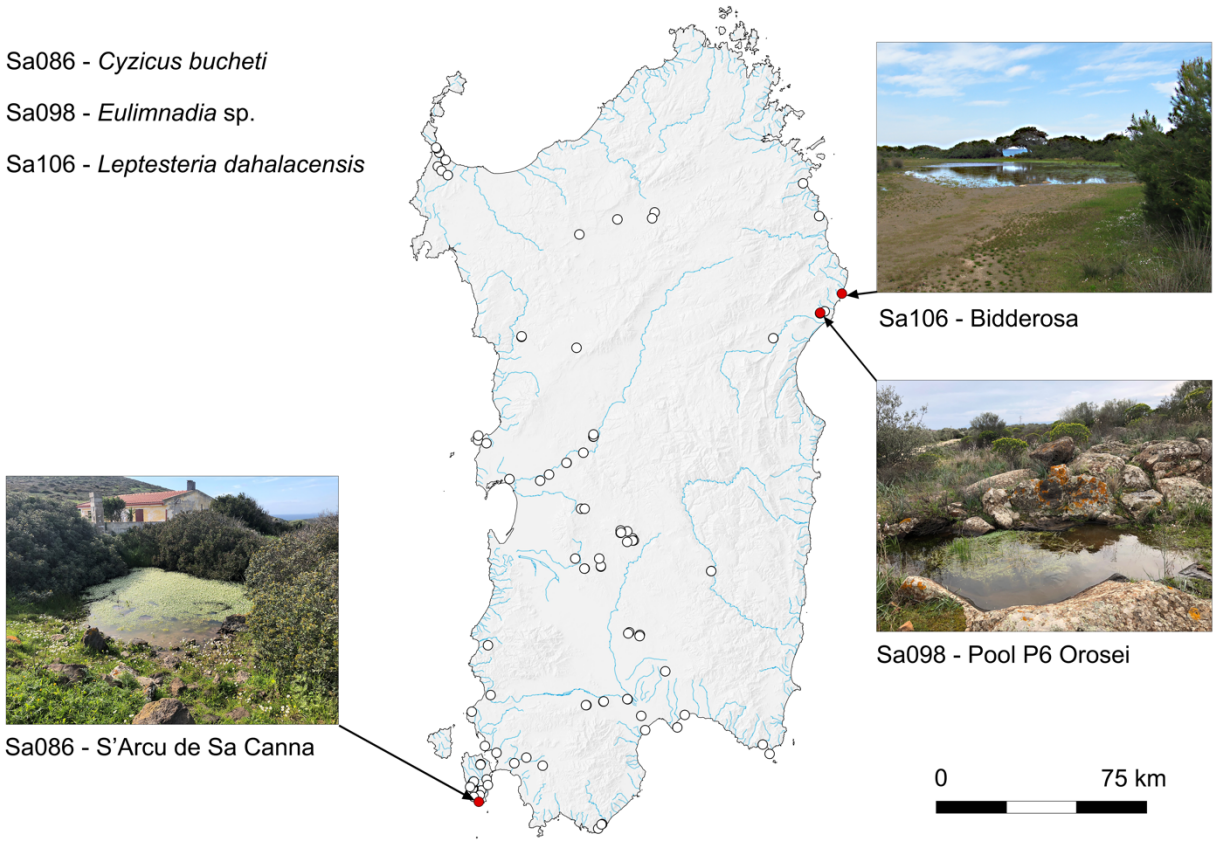

Figure 1. Map of the sampled water bodies (white dots) and of the occurrence localities of Spinicaudata (red dots). S'Arcu de Sa Canna (Sant'Antioco, CI), coord.: 38.966896 N; 8.416305 E; Bidderosa (Orosei, NU), coord.: 40.463614 N; 9.803591 E; Pool P6 (Orosei, NU), coord.: 40.407674 N; 9.719177 E (coordinates provided: WGS84 datum; longitude and latitude in decimal degrees).

from the deepest part of the dry water bodies; collected sediment was cultured in the laboratory following the "Sars' method" as described in Marrone et al. (2019a), implementing different temperatures and photoperiods. Collected and cultured large branchiopod specimens were identified according to Daday (1915, 1923, 1925), Straškraba (1966), Cottarelli and Mura (1983), Thiéry (1987), Alonso (1996), and Rabet et al. (2015).

RESULTS AND DISCUSSION

Literature review

Our critical literature review allowed us to make some amendments about the nomenclature and spelling of the taxa mentioned by Mura (2006) (Table 1). Moreover, four taxa reported in the literature were not mentioned for the fauna of the island by Mura (2006). These are the anostracans *Chirocephalus diaphanus* Prévost, 1803, reported from Sant'Antioco island by Cottarelli and Mura (1995), *Artemia persimilis* Piccinelli and Prosdocimi, 1968, reported from Santa Gilla saltworks by Piccinelli and Prosdocimi (1968), and *Branchipus blanchardi* Daday, 1908 and *Branchinecta* sp., both collected from Oristano rice fields by Benassi (1983).

The literature study also revealed references mentioning three spinicaudatan species in Sardinia: *Cyzicus bucheti* (Daday, 1915), *Cyzicus tetracerus* (Krynicki, 1830), and

an unidentified Limnadiidae. These occurrence data were not consistently reported in later papers and synopses.

Cyzicus bucheti was reported from Sardinia by Daday (1915) in the monograph with its original description (sub *Caenestheriella Bucheti*). Daday's report for Sardinia was based on a single female specimen stored in the "*collectione Musaei nat. hist. Berolinensis*" (Daday, 1915), and then reported as such by Brtek and Thiéry (1995), unfortunately without precise locality. The species was not mentioned by other authors who investigated the large branchiopod fauna of Sardinia (e.g., Stella et al. 1972; Stella and Margaritora 1976; Cottarelli and Mura 1983); therefore, *C. bucheti* was not included in the checklists of the Italian fauna compiled by Cottarelli et al. (1995) and Mura (2006).

Table 1. Checklist of the large branchiopods (Branchiopoda: Anostraca, Notostraca, and Spinicaudata) of Sardinia. See the text for a discussion on the scientific names used for each taxon in previous literature.

Taxa	Sources
Anostraca Sars, 1867	
Artemiidae Grochowski, 1895	
Artemia salina (Linnaeus, 1758) *Artemia* sp. "parthenogenetic strain"	Artom 1905, 1906; Daday 1910; Stefani 1960, 1961, 1964; Bowen & Sterling 1978; Cottarelli & Mura 1983; Mura 1986, 1987; Vanhaecke et al. 1987; Baratelli et al. 1988, 1990; Mura 1993; Brtek & Thiéry 1995; Cottarelli et al. 1995; Cottarelli & Mura 1995; Mura 1995a, 1995b; Triantaphyllidis et al. 1998; Mura 1999, 2001; Nascetti et al. 2003; Mura & Brecciaroli 2004; Baxevanis et al. 2006; Mura 2006; Muñoz et al. 2008; Maniatsi et al. 2011; Maccari et al. 2013; Mechaly et al. 2013; Sainz-Escudero et al. 2021.
Branchipodidae Milne-Edwards, 1840	
Branchipus schaefferi Fischer, 1834	Cottarelli & Mura 1973, 1983; Brtek & Thiéry 1995; Cottarelli et al. 1995; Mura 1999, 2006.
Chirocephalidae Daday, 1910	
Chirocephalus salinus Daday, 1913	Stella et al. 1972; Cottarelli & Mura 1973; Stella & Margaritora 1975-1976; Trentini & Marini 1978; Margraf & Maas 1982; Cottarelli & Mura 1983; Mura 1986, 1987; Brtek & Thiéry 1995; Cottarelli et al. 1995; Mura et al. 1997; Mura 1999; Ketmaier et al. 2003; Mura 2006; Reniers et al. 2013.
Chirocephalus diaphanus Prévost, 1803	Cottarelli et al. 1995; Cottarelli & Mura 1995; Mura 2001.
Tanymastigidae Brtek, 1972	
Tanymastix stagnalis (Linnaeus, 1758)	Artom 1927; Cottarelli et al. 1970; Stella et al. 1972; Cottarelli & Mura 1973; Stella & Margaritora 1975-1976; Cottarelli & Mura 1983; Brtek & Thiéry 1995; Cottarelli et al. 1995; Cottarelli & Mura 1995; Mura 1999, 2001, 2006; Ketmaier et al. 2005; Rodriguez-Flores et al. 2020.
Tanymastix stellae Cottarelli, 1967	Cottarelli 1967; Stella et al. 1967; Bianchi-Bullini et al. 1968; Stella et al. 1972; Cottarelli & Mura 1973, 1983; Mura 1986, 1999, 2006; Brtek & Thiéry 1995; Cottarelli et al. 1995.

Taxa	Sources
Thamnocephalidae Packard, 1883	
Phallocryptus spinosus (H. Milne Edwards, 1840)	Cottiglia & Tagliasacchi-Masala 1969; Tagliasacchi-Masala 1969; Cottarelli et al. 1970; Cottarelli & Mura 1973, 1983; Mura 1986, 1993; Brtek & Thiéry 1995; Cottarelli et al. 1995; Cottarelli & Mura 1995; Mura 1999, 2001, 2006; Ketmaier et al. 2008.
Notostraca Sars, 1867	
Triopsidae Keilhack, 1909	
Triops cancriformis (Bosc, 1801)	Ghigi 1921; Stella et al. 1967, 1972; Zaffagnini & Trentini 1980; Cottarelli & Mura 1983; Brtek & Thiéry 1995; Cottarelli et al. 1995; Mantovani et al. 2004; Mura 2006; Mantovani et al. 2008; Velonà et al. 2009; Buscaino et al. 2021.
Lepidurus cf. *couesii* Packard, 1875	Stella & Margaritora 1975-1976; Margraf & Maas 1982; Cottarelli & Mura 1983; Brtek & Thiéry 1995; Cottarelli et al. 1995; Mura 2006; Innocenti 2009; Bagella et al. 2010; Korn et al. 2013; Mathers et al. 2013; Boix et al. 2017.
Spinicaudata Linder, 1945	
Cyzicidae Stebbing, 1910	
Cyzicus bucheti (Daday, 1913)	Daday 1915; Brtek & Thiéry 1995; Cottarelli & Mura 1995; Cottarelli et al. 1995; Mura 2001.
Limnadiidae Burmeister, 1843	
Eulimnadia sp.	Stella et al. 1967, 1972; Cottarelli & Mura 1983; Brtek & Thiéry 1995; Cottarelli et al. 1995.
Leptestheriidae Stebbing, 1902	
Leptestheria dahalacensis (Rüppell, 1837)	Present work.

Natural history collections review

A sample labelled "*Estheria cycladoides* - Sardinien - Staudinger" (sample reference number: 3879 of the Crustaceans Collection) is stored in the "Museum für Naturkunde" (Berlin, Germany); this sample was collected by O. Staudinger and its identification was corrected to "*Canestheriella bucheti*" (sic!) in the catalogue itself. This specimen is likely the one mentioned for Sardinia by Daday (1915, pag. 139).

A vial containing *Cyzicus* specimens stored in ethanol and labelled "*Cyzicus grubei*, S'Arcu de Salanu, Sant'Antioco (CA)" was stored in the F.G. Margaritora collection (Fig. 2a). The locality reported on the label ("S'Arcu de Salanu") is to be considered a *lapsus calami*, since this toponym does not exist on Sant'Antioco island, and it is probably an erroneous hand transcription of the original label (Margaritora, *ex verbis*). This sample was provided to F.G. Margaritora by V. Cottarelli, and includes the specimens reported later *sub Cyzicus tetracerus* by Cottarelli and Mura (1995). Based on their morphology, the specimens can be tentatively identified as *C. bucheti*, although the differentiation from the congeneric *C. grubei* (Simon, 1886) is problematic when based on morphological characters alone (see below). Two *C. bucheti* specimens were deposited in the collection of the Zoology Section "La Specola," Natural History Museum, University of Florence (Italy), collection number MZUF 664.

No Sardinian spinicaudatans were stored in the crustacean collections of G. Mura and V. Cottarelli.

Field surveys and laboratory cultures

Despite the significant sampling effort carried out in Sardinian ponds and pools, no active stages of spinicaudatans were collected. Conversely, the laboratory culturing of collected sediment samples led to the hatching of spinicaudatans from two sites, coded "Sa098 – Pool P6 Orosei", and "Sa106 – Bidderosa" (Fig. 1). Moreover, thanks to the courtesy of N. Di Domenico (*ex verbis*) and M. Cobolli (*in litteris*), we were able to locate the site where the *Cyzicus* sample studied by Cottarelli and Mura (1995) was collected, which corresponds to our site "Sa086 – S'Arcu de Sa Canna".

Notwithstanding the claim that the Orosei rock pools cited by Cottarelli (1967) and Stella et al. (1972) had been destroyed (Cottarelli and Mura 1973, 1983; Mura 1999; Ketmaier et al. 2005), we were able to trace their exact location thanks to the 'field notes' of Stella and Margaritora kindly provided by F.G. Margaritora. In addition, also a comparison of pools and ponds in the area with available photographs taken by Stella and co-workers confirmed that these pools still exist and are in quite good condition (Fig. 1). The rock pool labelled "P6" in Stella et al. (1972) is here coded "Sa098 – Pool P6 Orosei". Several individuals of a limnadiid species hatched from the sediment from this site cultured at 26°C with a 12-h photoperiod. Due to the presence of a pedunculated frontal organ, of a ventral spiniform process on the telson, of sinuate cercopods slightly longer than telson ventral margin, and of a projecting mound bearing the caudal filament, they could be ascribed to the genus *Eulimnadia* as characterised by Rogers et al. (2012) and Rabet et al. (2015) (Fig. 2b). The species-level identification and the molecular characterization of this taxon is currently underway. One specimen was deposited in the collection of the Zoology Section "La Specola," Natural History Museum, University of Florence (Italy), collection number MZUF 663.

Numerous leptestheriids hatched from the sediment collected in a temporary pool at Bidderosa and cultured at 20°C with a 14-h photoperiod. They could be identified based on morphology as *Leptestheria dahalacensis*

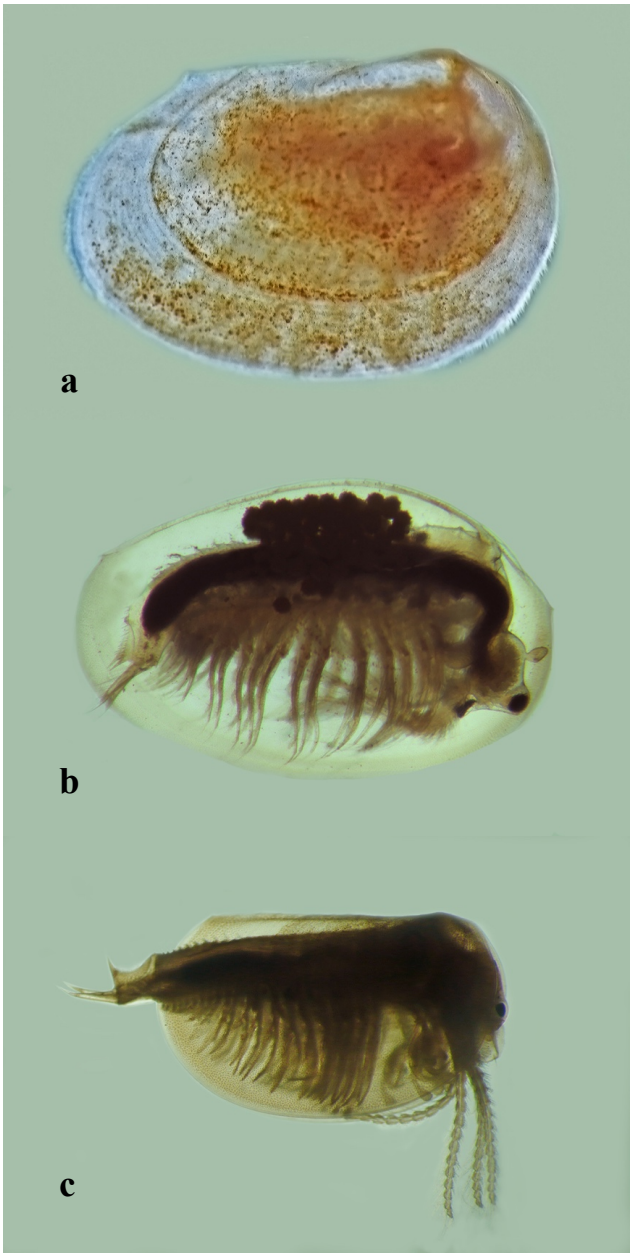

Figure 2. Spinicaudata found in Sardinia. (a) *Cyzicus bucheti*; (b) *Eulimnadia* sp.; (c) *Leptestheria dahalacensis*.

(Rüppel, 1837) (Fig. 2c). Four *L. dahalacensis* specimens were deposited in the collection of the Zoology Section "La Specola," Natural History Museum, University of Florence (Italy), collection number MZUF 662.

DISCUSSION

Anostraca

Overall, eight anostracan species belonging to five different families are reported with certainty from Sardinia (Table 1). Conversely, the occurrence of *Artemia persimilis*, *Branchipus blanchardi* and *Branchinecta* sp. (Piccinelli and Prosdocimi 1968; Benassi 1983) were based on single reports each, and no recent confirmation for the presence of these species is available (e.g., Triantaphyllidis et al. 1998; Mechaly et al. 2013). These last reports are probably due to misidentifications of *Artemia salina*, *Branchipus schaefferi* and *Phallocryptus spinosus*, which are known with certainty to occur in the localities mentioned by Piccinelli and Prosdocimi (1968) and Benassi (1983), respectively. *Artemia persimilis*, *Branchipus blanchardi* and *Branchinecta* sp. are thus excluded from the Sardinian fauna.

The parthenogenetic *Artemia* populations reported *sub* "*Artemia parthenogenetica* Barigozzi, 1974" by Mura (2006) are here reported as *Artemia* sp. "parthenogenetic strain" (Table 1). The taxonomy and nomenclature of *Artemia* parthenogenetic populations is still unsettled (e.g., Asem et al. 2020; Sainz-Escudero et al. 2021), and the binomen *Artemia parthenogenetica* itself is considered a *nomen dubium* by Rogers (2013).

Within the family Chirocephalidae, *Chirocephalus salinus* Daday, 1913 is widespread throughout the island (Mura 2006), whereas the congeneric *C. diaphanus* is to date only reported from a single temporary pond on Sant'Antioco island (Cottarelli and Mura 1995). Considering the unsettled taxonomy and nomenclature of the *diaphanus*-group within the genus *Chirocephalus*, and of the possible presence of cryptic species currently lumped under widely-used binomia (Reniers et al. 2013; Cottarelli et al. 2017; Marrone et al. 2019a), a morphological and molecular characterization of Sardinian *Chirocephalus* populations and their comparison with topotypical specimens for the available *Chirocephalus* specific epithets is desirable.

Two *Tanymastix* species are reported from Sardinia, the widespread *Tanymastix stagnalis* (Linnaeus, 1758) and the Sardinian endemic *Tanymastix stellae* Cottarelli, 1967. The records of the latter species from Corsica (Defaye et al. 1998) and Capraia (Bianchi-Bullini et al. 1968; but see Cottarelli and Mura 1973) are unsubstantiated and thus here ignored. *Tanymastix stellae* was considered extinct by Mura (1999) due to presumed habitat destruction (Cottarelli and Mura 1973, 1983; Ketmaier et al. 2005). However, the occurrence sites reported by Cottarelli (1967) and Stella et al. (1972) were rediscovered; they were holding thriving *Tanymastix* populations, which are currently under study.

Notostraca

Both notostracan genera *Triops* and *Lepidurus* are known from Sardinia (Table 1). The widespread Palearctic *Triops cancriformis* (Bosc, 1801) seems to be limited to rice fields and natural pools occurring along the coastal areas (e.g., Ghigi 1921; Stella et al. 1967, 1972; Mantovani et al. 2004; Buscaino et al. 2021). Conversely, *Lepidurus* cf. *couesii* inhabits temporary ponds located in the inner parts of the island (Stella and Margaritora 1975-1976; Mura 2006). This last taxon was reported as *Lepidurus apus* (Linnaeus, 1758) by Margraf and Maas (1982), Bagella et al. (2010), and Boix et al. (2017), and as *Lepidurus apus lubbocki* (Brauer, 1873) by Cottarelli et al. (1995) and Mura (2006); however, molecular evidence proved that the Sardinian *Lepidurus* populations belong to a different taxon, close to *L. couesii* Packard, 1875 (Mathers et al. 2013).

Spinicaudata

The occurrence of three spinicaudatan species belonging to three different families is here confirmed for the fauna of Sardinia: *Cyzicus bucheti* (Cyzicidae), *Eulimnadia* sp. (Limnadiidae) and *Leptestheria dahalacensis* (Leptestheriidae) (Table 1). Conversely, as there is no support for the actual occurrence of *Cyzicus tetracerus* and *Limnadia lenticularis* in Sardinia, these species must currently be excluded from Sardinian large branchiopod checklist.

In agreement with Daday (1915) and Brtek and Thiéry (1995), *Cyzicus bucheti* is confirmed to be present in Sardinia. The morphological re-analysis of the samples from Sant'Antioco island discovered in the F.G. Margaritora crustacean collection allowed us to confirm that the previous report of *C. tetracerus* for Sardinia by Cottarelli and Mura (1995) is due to a misidentification and should be corrected to *Cyzicus bucheti*. However, it is worth stressing that currently no unambiguous morphological characters are available to discriminate between *C. bucheti* and the closely-related *C. grubei* (e.g., Alonso 1996). Pending a review of the group including both morphological and molecular data, we conservatively decided to ascribe the Sardinian *Cyzicus* to *C. bucheti*, in agreement with the identification provided with the original description in Sardinia by Daday (1915). *Cyzicus bucheti* is often erroneously considered as a Moroccan endemic species (Rogers 2020), although the occurrence of the species in Sardinia was already reported by Daday (1915) in his original description of the taxon. In addition, the *Cyzicus* population from Menorca should possibly also be ascribed to *Cyzicus bucheti* (see: Pretus 1990; Alonso 1996).

The unidentified Limnadiidae that hatched from the sediment collected from "Sa098 – Pool P6 Orosei" could be ascribed to the genus *Eulimnadia* based on morphology (see above). This is the first record of the genus *Eulimnadia* from Europe, and its species-level identity is currently under study. Its local and regional distribution is likely underestimated due to its short life cycle and the requirement of relatively high temperatures for hatching (Rabet et al. 2015).

The occurrence of *Leptestheria dahalacensis* in Sardinia was rather unexpected since this species has a distribution range encompassing central and eastern Europe, whereas it is absent from the western Mediterranean countries (Brtek and Thiéry 1995), with the only possible exception of Sicily (Marrone and Mura 2006). The reported occurrence of the species in Menorca is due to a misidentification of the congeneric *L. mayeti* (see: Pretus 1990 and Alonso 1996). The occurrence of the species in Sardinia could be due to an anthropogenic introduction, possibly followed by further local dispersal, as also proposed for the Belgian (Brendonck et al. 1989) and Greek (Marrone et al. 2019b) populations of the species.

Biogeographical remarks

Considering the amendments and nomenclatural corrections mentioned above, the updated checklist of the large branchiopods of Sardinia includes 13 taxa (table 1), i.e., nearly 50% of the whole Italian large branchiopod fauna (Mura 2006; Alfonso 2017; present work). Interestingly, it includes several taxa which are not known from the Italian mainland and Sicily: the anostracans *Tanymastix stellae* and *Chirocephalus salinus*, and the spinicaudatans *Eulimnadia* sp. and *Cyzicus bucheti*. Moreover, the identity of the Sardinian populations of the notostracan *Lepidurus* cf. *couesii* is currently unclear, although Sardinian populations are possibly closely related to the *Lepidurus* species occurring in Apulia (Mathers et al. 2013; Alfonso 2017).

The spinicaudatan fauna of Sardinia is quite peculiar: *Cyzicus bucheti* is a typical west-Mediterranean species, *Eulimnadia* sp. is a taxon with southern affinities, whereas *Leptestheria dahalacensis* is widely distributed

in central and eastern Europe, and possibly a non-native species in Sardinia. Interestingly, none of these species are currently known to occur in Corsica (Culioli et al. 2006), southern mainland France (e.g., Defaye et al. 1998; Waterkeyn et al. 2009), mainland Spain (Sala et al. 2017), Sicily and Peninsular Italy (Marrone and Mura 2006; Mura 2006, except for *L. dahalacensis* occurring in northern Italian rice fields east of the Apennines and possibly introduced in Sicily). The occurrence of *Cyzicus bucheti* in Sardinia confirms the west-Mediterranean zoogeographical affinity of the island. A similar pattern is found in other inland water crustaceans, such as the diaptomid copepods *Hemidiaptomus roubaui* (Richard, 1888) and *Copidodiaptomus numidicus* (Gurney, 1909) (Marrone et al. 2017; Alfonso et al. 2021).

Based on currently available data, we can conclude that the Sardinian large branchiopod fauna is rather unique in the context of the Mediterranean area and deserve adequate monitoring and management actions. Moreover, further surveys aimed at better understanding and characterising it are desirable. In particular, the molecular characterization of Sardinian large branchiopods is needed, especially since the presence of cryptic or overlooked diversity within Branchiopoda is nowadays well known (e.g., Schwentner et al. 2020).

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