

Freshwater large branchiopods in Portugal: an update of their distribution

Margarida Machado¹, Luís Cancela da Fonseca^{3,4} and Margarida Cristo^{1,2,*}

¹ CCMAR - Centro de Ciências do Mar, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.

² FCT - Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.

³ MARE - Marine and Environmental Sciences Centre, Laboratório Marítimo da Guia, Av. N. Sra. do Cabo, 939, 750-374, Portugal.

⁴ CTA - Centro de Ciências e Tecnologias da Água, Universidade do Algarve Campus de Gambelas, 8005-139 Faro, Portugal.

* Corresponding author: mcristo@ualg.pt

Received: 31/10/16

Accepted: 12/05/17

ABSTRACT

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This study is based largely on 20 years of field and laboratory work, with surveys conducted by the authors and some other researchers. During this period several studies dealing with freshwater large branchiopods (FLB) were carried out, resulting in scientific publications and project reports. The distribution of FLB in Portugal was presented in 2 international scientific meetings, but apart from a first paper by Vianna-Fernandes in 1951 and an update done by ourselves in 1999 concerning the southwest Portugal, no other information has been published. Therefore, this work intends to bring up to date the known distribution of this faunal group in freshwater temporary systems. This is pertinent because of the recent revision of the taxon *Triops cancriformis* on the basis of genetic analyses. The Portuguese populations were assigned either to the Portuguese endemism *T. vicentinus*, or to *T. baeticus*, the more widely spread Iberian species, both belonging to the *T. mauritanicus* complex. Furthermore, a new species, *Tanymastigites lusitanica* was found and described in Portugal. More recently, a male free (or, at least, a strongly female-biased) metapopulation of *T. cancriformis* was discovered in a rice field in the central region of Portugal. Here we present an updated status of FLB species of Portuguese temporary lentic systems and their distribution, plotted on a UTM (10 × 10 km) grid. A total of 505 sites (temporary ponds or assemblages of closely located, not individual temporary pools) have been surveyed (455 by us). In 241 of these (47.7%) at least one species of FLB was found on at least one occasion. Of the 505 sites, only 87 are located north of the Tagus River and of these, at least one species was found in only 17 (19.5%). South of the Tagus River (Alentejo and Algarve), 53.6% of the sites were inhabited by at least one species. This list comprises 7 anostracans, 2 spinicaudatans and 4 notostracans, including 3 species endemic to the Iberian Peninsula and another 3 to Portugal.

Key words: Temporary ponds, anostracans, notostracans, spinicaudatans, endemic species, conservation status.

RESUMO

Grandes branquiópodes dulçaquícolas em Portugal: atualização da sua distribuição

O presente trabalho é, maioritariamente, o resultado de 20 anos de trabalho de campo e de laboratório, com campanhas realizadas pelos autores e por outros investigadores. Durante este período foram realizados vários estudos de que resultaram diversas publicações e relatórios de projectos. O conhecimento da distribuição dos grandes branquiópodes dulçaquícolas (GBD) em Portugal baseia-se num primeiro artigo de 1951 de Vianna-Fernandes e de uma actualização do conhecimento publicada por nós em 1999 e relativa ao sudoeste de Portugal. A sua actualização foi posteriormente apresentada em dois encontros científicos internacionais, mas nunca foi publicada. Assim, este trabalho pretende actualizar o conhecimento sobre a distribuição, em Portugal, deste grupo faunístico de sistemas lênticos temporários. Esta intenção é pertinente, visto que, na última década, o taxon *Triops cancriformis* foi revisto com base em análises genéticas. As populações portuguesas

foram atribuídas quer ao endemismo português *T. vicentinus*, quer a *T. baeticus*, a espécie com distribuição mais alargada na península ibérica, ambas anteriormente incluídas no complexo *T. mauritanicus*. Além destas, uma nova espécie, *Tany-mastigites lusitanica* foi descoberta e descrita em Portugal. Mais recentemente, foi encontrada num campo de cultivo de arroz na região central de Portugal, uma metapopulação de *T. cancriformis* aparentemente composta unicamente por fêmeas. Apresenta-se neste trabalho o estado actual do conhecimento sobre a distribuição dos GBD de sistemas lênticos temporários portugueses, em mapas de quadrícula UTM (10 × 10 km). Foram prospectados um total de 505 sítios (charcos temporários ou conjuntos de charcos com localização próxima, não individualizável), 455 pelos autores. Em 241 deles (47.7%) foi observada a presença de pelo menos uma espécie de GBD numa ocasião. Dos 505 sítios, apenas 87 estão localizados a Norte do rio Tejo, e nestes apenas em 17 (19.5%) foi detectada a presença de pelo menos 1 espécie de GBD. A sul do rio Tejo (Alentejo e Algarve), em 53.6% dos sítios foi encontrada pelo menos uma espécie de GBD. Esta lista inclui 13 espécies (7 *Anostraca*, 2 *Spinicaudata* e 4 *Notostraca*), entre as quais 3 endemismos ibéricos e outros 3 endemismos portugueses.

Palavras-chave: Charcos temporários, *Anostraca*, *Notostraca*, *Spinicaudata*, espécies endémicas, estatuto de conservação.

INTRODUCTION

Large branchiopods are a group of crustacean species (Branchiopoda: *Anostraca*, *Notostraca*, *Spinicaudata*, *Laevicaudata*, and *Cyclestherida*) that inhabit almost exclusively, temporary aquatic systems (Brendonck *et al.*, 2008). They play an important ecological role in lentic systems as detritivores, herbivores, carnivores and/or scavengers, contributing for example to the control of mosquitoes and to mitigating eutrophication by feeding on organic detritus and decaying organic matter. They are also important in the trophic chain as a food source for insects, amphibians and birds. They are considered a flagship group for freshwater temporary lentic systems (FTLS) and for temporary pond invertebrates (Brendonck *et al.*, 2008). Until a few decades ago, studies on these systems were scarce (Hildrew, 1985 and references therein; Boix *et al.*, 2012; Jeffries *et al.*, 2016), most probably due to the generally small size of FTLS, their ephemeral nature and the relative unpredictability of the duration and timing of their flooding periods. In Europe, the considerable increase of scientific interest in these environments over the last two decades (Boix *et al.*, 2016b and references therein) has been favored by the classification of Mediterranean Temporary Ponds as a priority freshwater habitat under Directive 92/43/CEE. As a consequence of the increasing scientific in-

terest in temporary ponds, namely in their functioning and biodiversity, many studies on freshwater large branchiopods (FLB) from several areas in the world have been published (e.g. Brendonck *et al.*, 2008 and references therein) and many new species have been recently discovered and described (e.g. Belk & Pereira, 1982; Thiéry, 1988; Brendonck *et al.*, 1992; Rogers, 2001; Belk & Rogers, 2002; Balaraman & Nayar, 2004; Olesen & Timms, 2005; Cottarelli *et al.*, 2007; Cohen, 2008; Alonso & Garcia-de-Lomas, 2009; Alonso & Ventura, 2013; Machado & Sala, 2013; Rogers *et al.*, 2013; Timms, 2013; Boix *et al.*, 2016a; Korn & Hundsdoerfer, 2016). In Portugal, the first published scientific paper dealing with FLB inhabitants of temporary ponds and pools (Vianna-Fernandes, 1951), reported the occurrence of 4 new species: the anostracans *Tanymastix lacunae* Daday, 1910 (= *T. stagnalis* (L., 1758)) and *Streptocephalus torvicornis* (Waga, 1842), the notostracan *Triops cancriformis mauritanicus* (Ghigi, 1921) and the spinicaudatan *Caenestheriella grubei* Daday, 1915 (= *Cyzicus grubei* (Simon, 1886)). With the exception of one specimen of *Apus cancriformis* Schäffer, 1756 (= *Triops cancriformis* (Lamarck, 1801)) registered in the catalogue of the invertebrate collection of the Zoological Museum of Coimbra University (Carvalho, 1944), no FLB species were known to occur in Portugal until the publication of this paper.

After a second paper by Vianna-Fernandes published in 1957 the Portuguese FLB fauna remained forgotten during decades, likely due to the minor attention given to their ephemeral habitats. As in other European countries, the classification of Mediterranean Temporary Ponds as a priority habitat has contributed to drawing attention of Portuguese researchers to the FTLs and their communities.

The work on the Portuguese FLB fauna was restarted by the present authors 45 years after the 1951 publication of Vianna-Fernandes. Since then, several studies were carried out, resulting in scientific papers and reports (Reis *et al.*, 1997; Machado *et al.*, 1999a; Machado *et al.*, 1999b; Cristo *et al.*, 2002; Grosso-Silva & Soares-Vieira, 2002; Cancela da Fonseca *et al.*, 2008; Presado *et al.*, 2010; Gascón *et al.*, 2012; Machado & Sala, 2013; Sousa, 2015). Some updates of its distribution have been presented in 2 international scientific meetings (Machado *et al.*, 2004;

Sala *et al.*, 2010). Nevertheless, updated statuses of FLB in Portugal have not yet been published.

Furthermore, in the last decade, the taxon *Triops cancriformis* have been revised on the basis of genetic analyses. In a first step, Korn *et al.* (2006) reinstated the subspecies *cancriformis* and *mauritanicus* to full species status and raised to subspecies rank two Northern African lineages within *T. mauritanicus*. Later, four more Iberian endemic lineages within *T. mauritanicus* were recognized and full species statuses were attributed to all six *T. mauritanicus* lineages (Korn *et al.*, 2010). The populations in Portugal were assigned either to the Portuguese endemism *Triops vicentinus*, or to *Triops baeticus*, the more widely distributed Iberian species. The only specimen of *Apus cancriformis* (= *Triops cancriformis*) included in the crustacean collection of the Zoological Museum of Coimbra University (Carvalho, 1944) proved to belong to *T. baeticus* (Machado, 2014). More recently, a

Table 1. Species of FLB in the mainland of Portugal: Total number of sites with FLB; number of sites where each species co-occurs with *n* other species (higher values in **bold**); percentage of sites with each species in relation to the total number of sites with FLB; number of sites with *x* number of species; mean species number per site. *Espécies de GBD em Portugal continental: número total de sítios com GBD; número de sítios com coocorrências de GBD (valores mais elevados a negrito); percentagem de sítios em que ocorre cada espécie em relação ao número total de sítios com GBD; número de sítios com x número de espécies; número médio de espécies por sítio.*

Species	No. of sites where the species co-occur with <i>n</i> other species						Sites Total no.	Sites % of Total with FLB
	<i>n</i> = 0	<i>n</i> = 1	<i>n</i> = 2	<i>n</i> = 3	<i>n</i> = 4	<i>n</i> = 5		
<i>C. diaphanus</i>	41	31	24	21	3	1	121	50.2
<i>T. stagnalis</i>	18	43	9	13	2	1	86	35.7
<i>B. cortesi</i>	12	38	9	11	3	0	73	30.3
<i>T. baeticus</i>	8	22	16	16	3	1	66	27.4
<i>C. grubei</i>	1	7	10	14	1	1	34	14.1
<i>T. vicentinus</i>	1	7	11	4	0	0	23	9.5
<i>Tanyastix</i> sp. 1	8	1	4	2	0	0	15	6.2
<i>B. schaefferi</i>	7	2	1	2	0	1	13	5.4
<i>S. torvicornis</i>	2	0	3	6	1	1	13	5.4
<i>T. lusitanica</i>	8	1	0	0	0	0	9	3.7
<i>M. maroccana</i>	0	3	1	1	1	0	6	2.5
<i>T. cancriformis</i>	1	0	1	0	0	0	2	0.8
<i>L. apus</i>	0	1	0	0	0	0	1	0.4
No. of species per site	X = 1	X = 2	X = 3	X = 4	X = 5	X = 6	Total	
No. sites with FLB	107	78	30	22	3	1	241	
Mean no. of species/site								1.9

male free (or, at least, a strongly female-biased) population of *T. cancriformis* was discovered in a rice field in the central region of Portugal (Machado *et al.*, 2016).

This study is the result of 20 years of survey efforts during which the environmental characterization of the FTLS was carried out on most occasions. This characterization, comprising estimation of the plant coverage and measurements of pond surface and depth, temperature, pH, oxygen concentration, percentage of dissolved oxygen, conductivity and turbidity of the water, has been mostly presented and discussed by Cancela da Fonseca *et al.* (2008). Nevertheless, the distributions of the FLB in Portugal have not been updated since the survey of large branchiopods in the southwest part of the country, published in 1999 (Machado *et al.*, 1999a), which was the start of the systematic inventory of these organisms. Since then, a huge amount of FLB new locations were recorded. Consequently, this study aims to present the updated FLB inventory and distribution in Portugal and to evaluate the current conservation status of the Portuguese FLB fauna.

MATERIAL AND METHODS

Data on presence/absence of Freshwater Large Branchiopods were mainly obtained from several surveys carried out from March 1996 to April 2016. Many of the FTLS were sampled during the course of several projects, both institutional and personal. Some were discovered based on personal information or through published work (e.g. Grosso-Silva & Soares-Vieira, 2002; Sousa, 2015). Others were detected from satellite images from Google Earth, sometimes complemented by the inspection of military charts with located ponds, or even by chance when in the field. The FTLS were visited at least once during the flooded period and, whenever possible, close to its beginning. The geographic coordinates of Portuguese freshwater temporary lentic systems with FLB species were registered using either a Magellan GPS ProMARK XTM or a Garmin-GPS72.

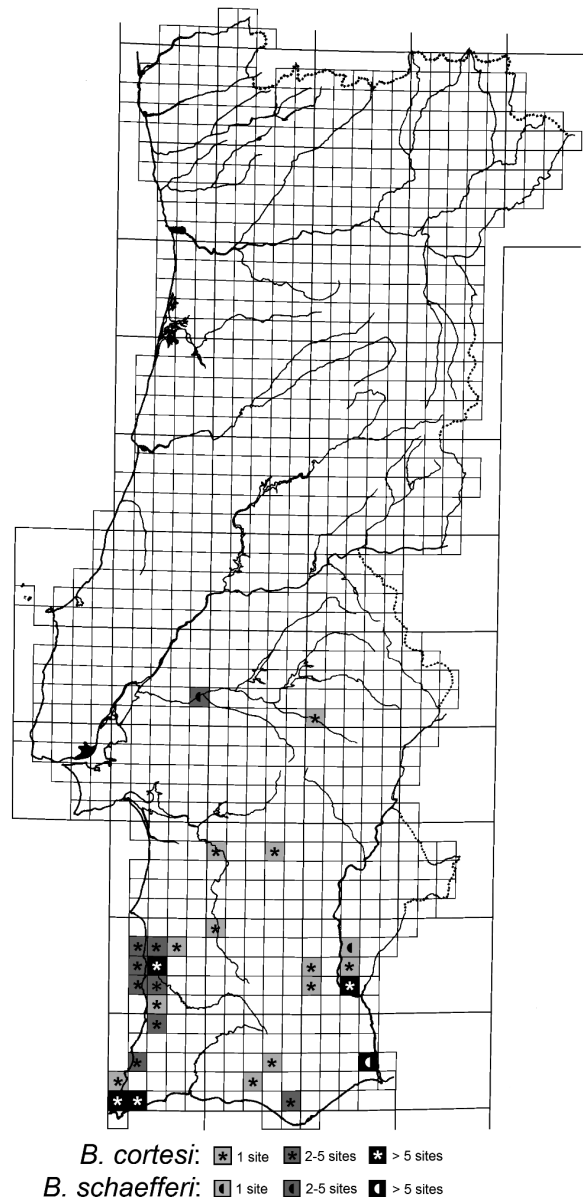


Figure 1. Distribution of *Branchipus cortesi* and *Branchipus schaefferi*, plotted on the map of Portugal on a UTM (10 × 10 km) grid base. *Distribuição de Branchipus cortesi e Branchipus schaefferi, em Portugal (quadricula UTM-10 × 10 km).*

Sampling of the FLB community was performed by trawling with a round hand net (30.5 cm mouth diameter, 1 mm² mesh size) in all possible microhabitats. FLB were identified by eye to genus level in the field. Nevertheless, from each FTLS and at least once, a small

sample of each species found was fixed in 4% formaldehyde or in 70-99.8° ethanol and taken to the lab for identification to species level. Although some FTLs were not sampled by us, all the collected FLB were studied by ourselves.

The identification was based on Cottarelli & Mura (1983), Thiéry (1987), Petkovski (1995), Alonso (1996), Brtek & Mura (2000), Korn *et al.* (2006; 2010) and Machado & Sala (2013). The nomenclature followed Brtek (2002), Weekers *et al.* (2002) and Machado & Sala (2013).

The collected data were complemented by the information published by Vianna-Fernandes (1951), Grosso-Silva & Soares-Vieira, 2002, Korn *et al.* (2006; 2010), Cancela da Fonseca *et al.* (2008) and Machado *et al.* (2016).

A map with a UTM grid (10 × 10 km) adapted from Franco & Afonso (1982) was georeferenced into the Portuguese coordinate system (Portuguese_National_Grid; Projection: Transverse_Mercator; Datum: D_Lisbon) using the software package ArcGIS 9.2 (ESRI, 2006). The geographic coordinates of all sites where at least one FLB species has been found were inserted in a Geographic Information System, as an ArcGIS database (Booth & Mitchell, 2001), and plotted on the map.

RESULTS

A total of 505 sites (temporary ponds or assemblages of closely located, not isolated temporary pools) were surveyed (455 by us) in mainland Portugal. In 241 of those (47.7%) at least one species of FLB was found on one occasion. Of the 505 sites, only 87 are located North of Tagus River and at least one species was found in 17 (19.5%) of these. South of the Tagus River (Alentejo and Algarve), 53.6% of the sites were inhabited by at least one FLB species.

A total of 13 FLB species were identified: 7 anostracans, 4 notostracans and 2 spinicaudatans. A maximum of six coexisting species were found in only one FTLs and the mean number of co-occurring species was 1.9 (Table 1). Data on the current occurrence of these species are detailed in Table 1. The inventory of Fresh-

water Large Branchiopods in Portugal mainland is:

- Class Branchiopoda Latreille, 1816
- Order Anostraca Sars, 1867
- Family Branchipodidae Milne-Edwards, 1840
- Genus *Branchipus* Schaeffer, 1766
- Branchipus cortesi* Alonso & Jaume, 1991
- Branchipus schaefferi* Fischer, 1834
- Family Chirocephalidae Daday, 1910
- Genus *Chirocephalus* Prévost, 1820 (in Jurine)
- Chirocephalus diaphanus* Prévost, 1820 (in Jurine)
- Family Streptocephalidae Daday, 1910
- Genus *Streptocephalus* Baird, 1852
- Streptocephalus torvicornis* (Waga, 1842)
- Family Tanymastigidae Brtek, 1972
- Genus *Tanymastigites* Brtek, 1972
- Tanymastigites lusitanica* Machado & Sala, 2013
- Genus *Tanymastix* Simon, 1886
- Tanymastix stagnalis* (Linnaeus, 1758)
- Tanymastix* sp.1
- Order Notostraca Sars, 1867
- Family Triopidae Keilhack, 1909
- Genus *Lepidurus* Leach, 1819
- Lepidurus apus* (Linnaeus, 1758)
- Genus *Triops* Schrank, 1803
- Triops cancriformis* (Lamarck, 1801)
- Triops baeticus* Korn, 2010
- Triops vicentinus* Korn, Machado, Cristo & Cancela da Fonseca, 2010
- Order Spinicaudata Linder, 1945
- Family Cyzicidae Stebbing, 1910
- Genus *Cyzicus* Audouin, 1837
- Cyzicus grubei* (Simon, 1886)
- Family Leptestheriidae Stebbing, 1902
- Genus *Maghrebestheria* Thiéry, 1988
- Maghrebestheria maroccana* Thiéry, 1988

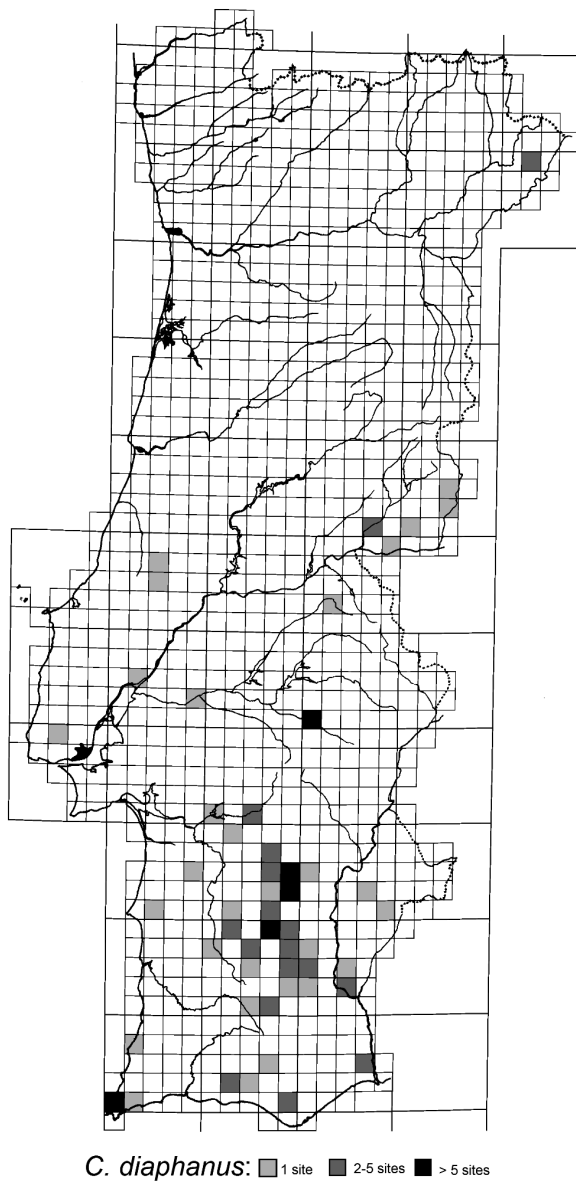


Figure 2. Distribution of *Chirocephalus diaphanus*, plotted on the map of Portugal on a UTM (10×10 km) grid base. *Distribuição de Chirocephalus diaphanus em Portugal (quadrícula UTM- 10×10 km).*

Figures 1 to 6 show the distribution of these 13 FLB species plotted on the map of Portugal on a UTM (10×10 km) grid base.

The most common species is *C. diaphanus*, followed by *T. stagnalis* and *B. cortesi*. The rarest species (< 10 sites) are *L. apus*, *T. cancriformis*, *M. maroccana*, and *T. lusitanica* (Table 1).

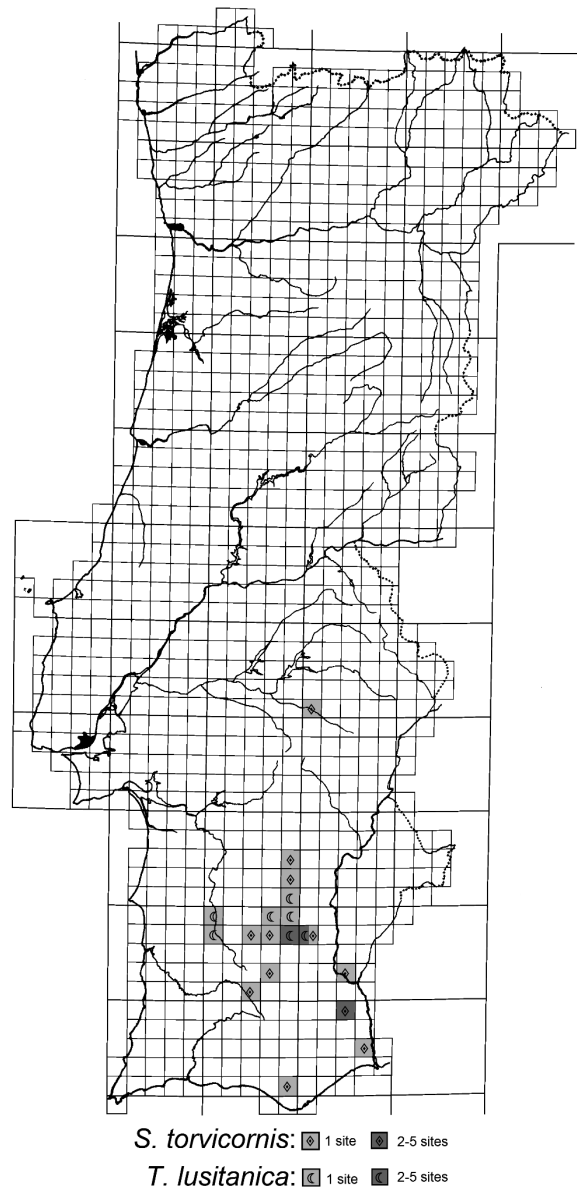
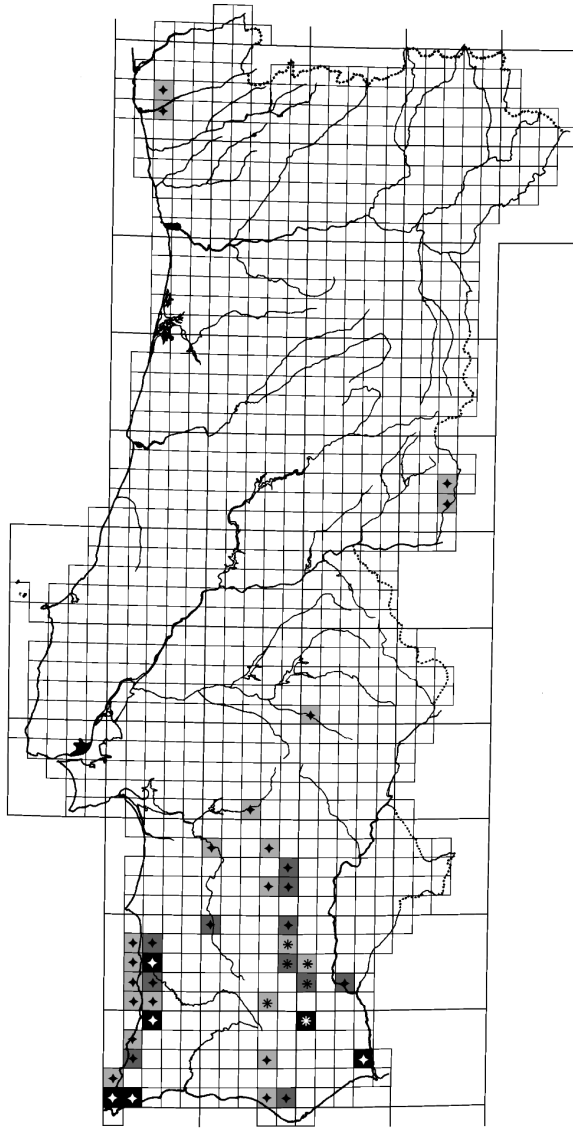


Figure 3. Distribution of *Streptocephalus torvicornis* and *Tanyastigites lusitanica* plotted on the map of Portugal on a UTM (10×10 km) grid base. *Distribuição de Streptocephalus torvicornis e Tanyastigites lusitanica em Portugal (quadrícula UTM- 10×10 km).*

Streptocephalus torvicornis shows a scattered distribution in the Alentejo and Algarve whereas *Tanyastigites lusitanica* was found in only 7 UTM grid squares located in a small central area of South Alentejo (Fig. 3).

Tanyastix sp.1 is a new species for science which is currently being described (M. Machado

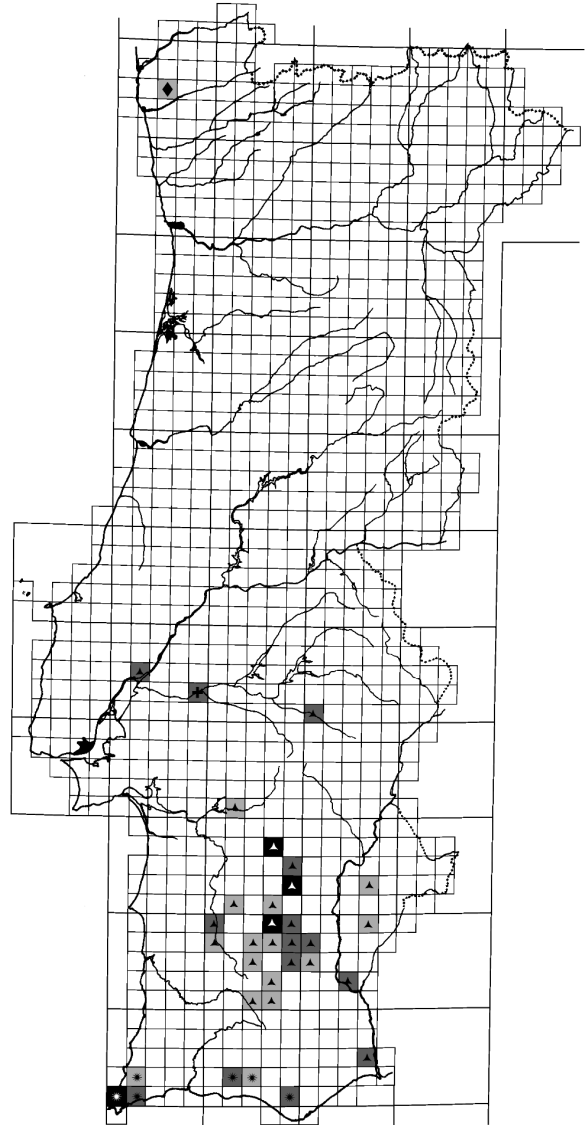


T. stagnalis: ◻+ 1 site ◻+ 2-5 sites ◻+ > 5 sites
Tanymastix sp.1: ◻+ 1 site ◻+ 2-5 sites ◻+ > 5 sites

Figure 4. Distribution of *Tanymastix stagnalis* and *Tanymastix* sp.1 plotted on the map of Portugal on a UTM (10 × 10 km) grid base. *Distribuição de Tanymastix stagnalis e Tanymastix sp.1 em Portugal (quadrícula UTM-10 × 10 km).*

and J. Sala, unpublished data.). It is roughly confined to a rectangular area of 1500 km², located in the central part of southern Alentejo and the northern Algarve (Fig. 4).

Triops baeticus is the species of this genus with the largest geographical distribution in Portugal, whereas *Triops vicentinus* shows a distribution limited to the west and central Algarve



L. apus: ◻△ 1 site ◻△ 2-5 sites ◻△ > 5 sites
T. cancriformis: ◻+ 2 sites ◻+ 1 site ◻+ 2-5 sites ◻+ > 5 sites
T. baeticus: ◻+ 1 site ◻+ 2-5 sites ◻+ > 5 sites
T. vicentinus: ◻+ 1 site ◻+ 2-5 sites ◻+ > 5 sites

Figure 5. Distribution of *Lepidurus apus*, *Triops cancriformis*, *Triops baeticus* and *Triops vicentinus* on the map of Portugal on a UTM (10 × 10 km) grid base. *Distribuição de Lepidurus apus, Triops cancriformis, Triops baeticus e Triops vicentinus em Portugal (quadrícula UTM-10 × 10 km).*

and *Triops cancriformis* is known only from 2 close sites in Portugal. *Lepidurus apus* is the rarest FLB species in Portugal, being known only from 1 FTLS, a small, shallow puddle corresponding to flooded tracks left by farm tractors in the middle of a cultivated field (Fig. 5).

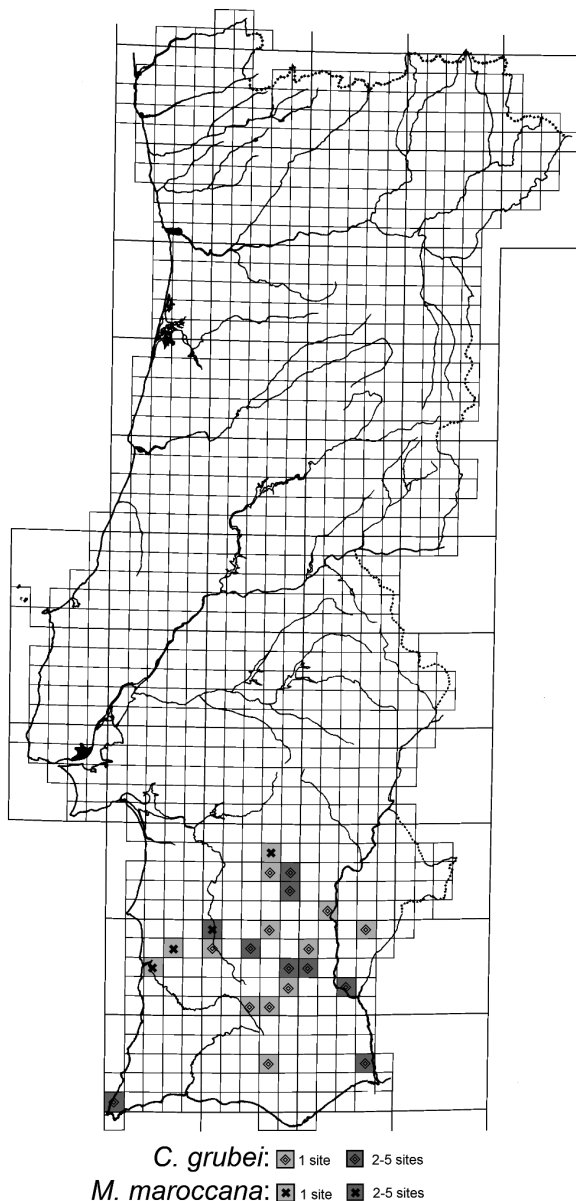


Figure 6. Distribution of *Cyzicus grubei* and *Maghrebestheria maroccana* plotted on the map of Portugal on a UTM (10×10 km) grid base. *Distribuição de Cyzicus grubei e Maghrebestheria maroccana em Portugal (quadrícula UTM- 10×10 km).*

The current known distribution of *Cyzicus grubei* in Portugal is limited to the southern part of the country (southern half of Alentejo and Algarve). *Maghrebestheria maroccana* has been found in only 6 sites in a very restricted Alentejo area (Fig. 6).

Table 1 highlights the difference in the highest number of sites where n other species co-occurred with each species. Most anostracan species were mainly found alone, with the exception of *T. stagnalis* and *B. cortesi*, which normally shared their biotope with 1 more FLB species, as did the notostracan *T. baeticus* and the spinicaudatan *M. maroccana*. The notostracan *T. vicentinus* was mostly found together with 2 more species and the anostracan *S. torvicornis*, as well as the spinicaudatan *C. grubei*, with 3 more species.

In Table 2 the FLB species richness found in Portugal, and also South of the Tagus River is compared with that reported from other European, Mediterranean and west Asian countries/regions from the Palaearctic.

DISCUSSION

During the first half of the last century the occurrence of only 4 freshwater large branchiopod taxa (2 Anostraca, 1 Spinicaudata, and 1 Notostraca) was reported from Portugal (Carvalho, 1944; Vianna-Fernandes, 1951). Their distribution was restricted to four sites: one north and very close to the Tagus River (Azambuja) and three (Torrão, Beja and Castro Verde) in the central and south Alentejo (cf. Machado *et al.*, 1999a). The eventual existence of a fifth species, *Cyzicus tetracerus* (Krynicky, 1830), although reported by Brtek & Thiéry (1995) who did not mention the source of the information, has not yet been confirmed in Portugal. Since we started our work on FLB in the SW Portugal, the number of species has increased from 4 to 7 (4 Anostraca, 2 Spinicaudata, and 1 Notostraca), with greater understanding of their known distribution in the country and in the Iberian Peninsula (Machado *et al.*, 1999a).

Nevertheless, the percentage of the surveyed area in the country was still very low (less than 5%). Nowadays the total surveyed area is approximately 70% of Portugal and we report here the existence of 13 species (7 Anostraca, 2 Spinicaudata, and 4 Notostraca), 6 more species than 18 years ago. In general we found the same species

in the same places (cf. Machado *et al.*, 1999a), but their distribution has greatly expanded due to the increased surveying (Figs. 1 to 6).

FLB occurrence and distribution

Anostracans

B. cortesi was mainly found in the southwest edge of the country where it was first discovered in Portugal (Machado *et al.*, 1999a). Previously it had been reported in 40 out of 129 Portuguese sites with at least one FLB species (Cancela da Fonseca *et al.*, 2008). This species is an Iberian endemism (Alonso, 1996). It often co-occurs with *T. stagnalis* (Cancela da Fonseca *et al.*, 2008). It prefers FTLs with low conductivity (Alonso, 1996; Cancela da Fonseca *et al.*, 2008).

B. schaefferi, has been reported from 5 more sites than the previously published data (Cancela da Fonseca *et al.*, 2008). In agreement with the latter study, we found this species in poorly-vegetated ponds. It is a Palearctic species (Alonso, 1996), widely distributed in the steppe and wood steppe zones of continental Europe and on the western Mediterranean islands of Sardinia, Sicily and Malta (Brtek & Thiéry, 1995). In Portugal as in southern Spain (Alonso, 1985; Boix *et al.*, 2007; Fahd *et al.*, 2009; Prunier & Saldaña, 2010; Prunier *et al.*, 2011) the species is relatively rare, with a scattered distribution. Nevertheless, it is frequent in the northern part of Spain (Alonso 1996), being the most common FLB species in Catalonia (Gascón *et al.*, 2012) and in Comunitat Valenciana (Sahuquillo, 2012).

C. diaphanus is a euryoecious (e.g. Mura, 1999; Cancela da Fonseca *et al.*, 2008) Palearctic species (Alonso, 1996), widely distributed in Europe (Brtek & Thiéry, 1995). In Portugal it is the most common anostracan, as it is also in several other southern European countries, such as France (Defaye *et al.*, 1998), Italy (Mura, 2001), and Spain (Alonso, 1996). In relation to the previous published data (Cancela da Fonseca *et al.*, 2008), the number of known sites increased from 65 to 121 and the known distribution is expanded to the northeast of Portugal.

T. lusitanica is only known from the very same sites that were reported when it was first described (Machado & Sala, 2013). It has always been found in small and very shallow puddles or rainpools on unpaved clayish or sandy roads. Typically, the water is very turbid, with low mineralization and poorly colonized by aquatic vegetation (Cancela da Fonseca *et al.*, 2008; Machado & Sala, 2013). This species, the only representative of the genus in Europe, is also endemic to Portugal (Machado & Sala, 2013).

T. stagnalis was previously reported from 1 Portuguese site located in the central part of Alentejo (Vianna-Fernandes, 1951), and is nowadays the only FLB species in Portugal with the known occurrence increasing in percentage in relation to the former published data (31.8% to 35.7%). Previously it was found in 41 sites (Cancela da Fonseca *et al.*, 2008). It is a Palearctic (Alonso, 1996) widespread (Brtek & Thiéry, 1995) species which has been considered as a cold stenothermal species (see Alonso, 1995; Mura, 1999, 2001). Nevertheless it often occurs in water temperatures of 25-30 °C, e.g. in Austria (Eder *et al.*, 1997) and in Portugal (Cancela da Fonseca *et al.*, 2008), suggesting that it is a eurythermal species. The data here presented expands its distribution to the southwest edge of Iberian Peninsula (Alonso, 1985; Machado *et al.*, 1999a).

Since the last published data (Cancela da Fonseca *et al.*, 2008) only one more site was added to the distribution of *Tanymastix* sp.1. To date it seems that this species is also endemic to Portugal. It prefers shallow pools with short hydroperiods and relatively low water conductivity (Cancela da Fonseca *et al.*, 2008).

The distribution of *S. torvicornis* has broadened in 2 sites relative to the previously published data (Gascón *et al.*, 2012). It prefers small and deep ponds with moderate to long hydroperiods and waters with relatively low conductivity (Cancela da Fonseca *et al.*, 2008) and seems to avoid large FTLs. It is a Eurasian species, found in the Mediterranean region, Asia Minor and Central Asia (Alonso, 1996), being one of the most common anostracans in some Mediterranean countries (Beladjal *et al.*, 2003), such as

Morocco (Thiéry, 1986) and Algeria (Samraoui, *et al.*, 2006). In Spain, Alonso (1996) have reported it as very frequent in the hydrographic

sedimentary basins from the South, namely in the low Guadalquivir river region. Nevertheless, this species seems to be rare nowadays (Boix *et*

Table 2. Total number of sites with FLB, total number of FLB species, maximum number of co-occurring species and mean species number per site (\bar{X}) with FLB found in several studied European, Mediterranean and west Asian countries/regions from Palearctic. – information not explicit and impossible to infer from references. Area information (km²), whenever absent from references, was found in the web. *Número total de sítios com GBD, número de espécies de GBD, número máximo de espécies que coocorrem e número médio de espécies por sítio (\bar{X}) com GBD encontrados em diversos países/regiões paleárticas europeias, mediterrânicas e do próximo oriente. – informação não explícita ou impossível de inferir dos dados das referências. A informação sobre a área (km²), sempre que não referida, foi retirada da “web”.*

Geographical Zone	Area	Sites with FLB		Species		Reference
		no.	no.	Max. no./site	\bar{X}	
Iberian Peninsula	582 000	—	22	6	—	Alonso (1996); Sala <i>et al.</i> (2016)
Portugal (mainland)	89 000	241	13	6	1.9	Present study
Portugal (South of Tagus River)	39 850	224	12	6	2.0	Present study
Spain (mainland)	493 000	63	15-16	5	1.5	Alonso (1985); Korn <i>et al.</i> (2006; 2010)
Spain (mainland)	493 000	—	19	6	—	Alonso (1996); Korn <i>et al.</i> (2006; 2010); Sala (pers. com.)
Girona Province	5600	75	6	3	1.2	Gascón <i>et al.</i> (2012)
Comunitat Valenciana	23255	56	9	6	1.3	Rueda <i>et al.</i> (2006); Zierold <i>et al.</i> (2007); Sahuquillo (2012); Forner i Valls & Brewster (2013); Soria <i>et al.</i> (2016)
Extremadura Province	41 643	25	7-8	4	1.7	Pérez-Bote <i>et al.</i> (2006; 2008); Korn <i>et al.</i> (2010)
Córdoba province	13 769	81	8	5	1.5	Prunier & Saldaña (2010); Prunier <i>et al.</i> (2011)
Murcia Region	11 313	17	3	3	1.2	Verdiell-Cubedo & Boix (2014)
Málaga Province	7276	20	4	3	1.4	Rippol Rodríguez <i>et al.</i> (2013)
Doñana Natural Area	1200	25	8	6	2.1	Boix <i>et al.</i> (2007); Fahd <i>et al.</i> (2009)
Menorca	702	20	4	3	1.7	Boix <i>et al.</i> (2010)
France	635 121	—	14	—	—	Defaye <i>et al.</i> (1998)
Italy	251 537	—	14	—	—	Cottarelli & Mura (1983); Mura (2001); Beladjal <i>et al.</i> (2003)
Former Yugoslavia	288 364	—	18	—	—	Petrov & Petrov (1997)
Banat Province (form. Yugosl.)	9300	68	10	7	2.6	Petrov & Cvetković (1997)
Austria (eastern)	42 000 approx.	—	15	—	—	Eder <i>et al.</i> (1997)
Belgium	30 528	—	7	—	—	Vanschoenwinkel <i>et al.</i> (2013)
Denmark	42 400	—	6-7	—	—	Damgaard & Olesen (1998)
Morocco (West)	445 050	101	16	10	—	Thiéry (1986; 1991)
Morocco North and West)	—	108	14	6	2.7	Van den Broeck <i>et al.</i> (2015)
Algeria	2 382 000	—	15	—	—	Beladjal <i>et al.</i> (1995); Samraoui <i>et al.</i> (2006)
Numidia (Algeria)	—	19	4	2	1.6	Samraoui & Dumont (2002)
Tunisia	163 610	—	11	—	—	Turki & Turki (2010); Korn <i>et al.</i> (2006)
Iran	1 648 000	15	9	4	1.6	Atashbar <i>et al.</i> (2014)

al., 2007; García de Lomas & García, 2008; Fahd *et al.*, 2009; Prunier & Saldaña, 2010; Prunier *et al.*, 2011) with the exception of Murcia Region where it is common in temporary freshwater ponds used by cattle (Verdiell-Cubedo & Boix, 2014). *Notostracans*

L. apus was first discovered by Grosso-Silva & Soares-Vieira (2002) in Correlhã, Ponte de Lima. Seven years later we have found it in the very same location (confirmed with Grosso-Silva, pers. com.), in very turbid and low oxygenated water (L. Cancela da Fonseca, M. Machado and M. Cristo, unpublished data). It is a Palearctic species (Alonso, 1996; Rogers, 2001), widely distributed in Europe (Brtek & Thiéry, 1995). In Spain it is also quite rare, occurring only in the north (Alonso, 1996).

T. cancriformis, has been recently discovered in rice fields of Vale do Sorraia, Ribatejo (Machado *et al.*, 2016). This finding represents a new species for the Portuguese FLB fauna, enlarging its geographical distribution to the Southwest edge of the Iberian Peninsula and Europe. It is a Palearctic species (Alonso, 1996), widely distributed in Europe (Brtek & Thiéry, 1995) and Asia, including India and Japan (Korn *et al.*, 2006).

T. baeticus, is a species recently described following a classification based on genetic sequences (Korn *et al.*, 2010). It is one of the new species assigned to the *Triops mauritanicus* lineage (Korn *et al.*, 2006) endemic to the Iberian Peninsula. In Portugal, as well as in Spain (Korn *et al.*, 2010; this study) it is the species of the *mauritanicus* complex with the widest distribution.

T. vicentinus, also one of the recently described Iberian species of the *mauritanicus* complex, is a Portuguese endemism. Besides the scarcity of known sites, it also has a very restricted distribution area in the west and central Algarve (Korn *et al.*, 2010; this study).

Spinicaudatans

C. grubei is an endemism of the Iberian Peninsula and Balearic Islands (Alonso, 1996). In

relation to the previous published data (Cancela da Fonseca *et al.*, 2008), the number of known sites increased from 22 to 34. Although it is one of the most euryoecious Portuguese species, it prefers relative deep ponds with long hydroperiods (Cancela da Fonseca *et al.*, 2008). In Spain it is also a relatively rare species (Alonso, 1985; Fahd *et al.*, 2009; Boix *et al.*, 2007, 2010), with the exception of Extremadura, where Pérez-Bote *et al.* (2006) found it in 36% of the ponds with FLB.

M. maroccana is known from just one more site than those previously reported (Cancela da Fonseca *et al.*, 2008; Gascón *et al.*, 2012). It occurs in the northwest of Morocco (Van den Broeck *et al.*, 2015), from where the species was described (Thiéry, 1988). It is also present in Spain (Alonso, 1996), where with the exception of the Doñana Natural Area (Boix *et al.*, 2007) it has been rarely found (Alonso 1996; Pérez-Bote *et al.*, 2006; Prunier & Saldaña, 2010; Prunier *et al.*, 2011; Gascón *et al.*, 2012; Sahuquillo, 2012). It is a sensitive species which is absent from large FTLS and prefers ponds rich in aquatic vegetation, with low turbidity waters and relatively low conductivity (Cancela da Fonseca *et al.*, 2008).

FLB faunal richness

Of the 13 FLB species known to occur in Portugal, all but *Lepidurus apus* are present in the area south of the Tagus River (Table 2, Fig. 5), whereas north of the Tagus River only 4 species were recorded: the anostracans *Chirocephalus diaphanus* and *Tanymastix stagnalis* and the notostracans *Lepidurus apus* and *Triops baeticus* (Figs. 2, 4 and 5), the last one found very close to the Tagus margins.

The apparent scarcity of the FLB fauna north of the Tagus River both in species richness and in occurrence, may be due to the fact that this part of the country has been much less surveyed (fewer sites, visited at most on two occasions) than the areas south of the Tagus River (many more sites sampled, some of them on a weekly basis). Hence an effort should be made to intensify surveys in that part of the country.

In the Iberian context, the Portuguese FLB fauna should be considered rich, since Portugal, occupying just 15.3% of that region, accounts for 59.1% of the species known to occur in the Iberian Peninsula (see Table 2).

The number of species recorded in Portugal is similar to or higher than reported for France, Italy, Belgium, Denmark, Tunisia and Iran, but lower than for Spain, the former Yugoslavia and Algeria (Table 2). Nevertheless, considering the species richness in relation to area, that reported for Portugal is only lower than those of Belgium and Denmark. Comparing the number of FLB species recorded south of the Tagus River region (STRr) to those of the other regions/provinces, it is only lower in relation to the eastern half of Austria and to North-Western Morocco (Table 2). However a completely different picture is apparent when considering the species richness in relation to the area, with that of STRr being only higher than that reported for Extremadura and Murcia (Spain).

Maximum number of co-occurring species and mean species number per site seem to be better indicators of species richness for comparison purposes and have been used in other studies (Boix *et al.*, 2007; 2010). The maximum number of co-occurring species both in Portugal (Tables 1 and 2) and in STRr (Table 2) is only lower than that reported for the Banat Province and also for Western Morocco 3 decades ago (Thiéry, 1991). Presently, the value for North-Western Morocco is the same as for Portugal (Van den Broeck *et al.*, 2015, table 1; Table 2). Regarding the mean species number per site, Portugal presents the highest value of all the countries under consideration and that found for STRr is only lower than those relative to Banat Province and to North-Western Morocco, being similar to that inferred for Doñana Natural Area.

Differences were found in species coexistence related to order: whereas notostracans and spinicaudatans were mostly found with other species, anostracans were mainly found alone. These differences should be related to the diverse lifespans exhibited by the different groups of FLB. In general, anostracan species have the shortest life cycle (Cancela da Fonseca *et al.*,

2008) and hence are able to survive in quite ephemeral temporary waters as well as in those with larger flooding period (Beladjal *et al.*, 2003). This is the case of all Portuguese freshwater anostracans (Cristo *et al.*, 2002; Cancela da Fonseca *et al.*, 2008), with the exception of the lonely short-lived *T. lusitanica* (Table 1; see also Machado & Sala, 2013) and of the long-lived *S. torvicornis* (Beladjal *et al.*, 2003). *S. torvicornis*, together with *C. grubei*, are the species with the longest lifespans recorded in Portugal (Cancela da Fonseca *et al.*, 2008; M. Machado, per. obs.), with the latter typically lasting until the habitat dries out (Cristo *et al.*, 2002). Species with extended life cycles are unable to survive in short-lasting temporary waters, needing deeper, longer-lasting ponds to complete their life cycles and, hence, to persist in the biotope (Beladjal *et al.*, 2003). Many of the FLB with short lifespans can also be present in long-lived FTLS. *T. baeticus*, *T. vicentinus* and *M. maroccana* have intermediate lifespans (Cristo *et al.*, 2002; Cancela da Fonseca *et al.*, 2008; present authors, per. obs.). The positive and strong association already reported to exist between *T. stagnalis* and *B. cortesi* (Cancela da Fonseca, 2008) explains why these short-lived species were usually found with one more species instead of being normally alone.

Another remarkable fact with FLB in Portugal is the total absence of co-occurrence of congeneric species which is not the case in other countries like Spain (Miracle *et al.*, 2008) and Morocco (Pérès, 1939; Thiéry, 1991).

FLB conservation status in Portugal

Portuguese FLB fauna has a high conservation value. Almost half the FLB currently known to occur in Portugal are endemic species: 3 to Portugal – *T. lusitanica*, *Tanyastix* sp.1 and *T. vicentinus*, and another 3 to the Iberian Peninsula – *B. cortesi*, *T. baeticus* and *C. grubei*. Nevertheless 7 species were only found in 15 FTLS or less, 4 of them being known from less than 10 sites (see results). Seven of the 13 species have a restricted (*C. grubei*) or very restricted distribution (the 3 Portuguese endemisms plus *L. apus*,

T. cancriformis and *M. maroccana*) (Figs. 1-6).

Portuguese FLB species are all exclusive inhabitants of temporary aquatic systems. However, the ephemeral nature, the small size and the limited visibility of these systems lead to a lack of recognition of their values and functions which results in them being readily destroyed or transformed all around Europe (Gauthier *et al.*, 2004) and other parts of the world, before the opportunity to fully study their communities and functioning (e.g. Belk, 1998).

A large number of studies have called attention to the increasing rate of disappearance of FTLs in general and Mediterranean Temporary Ponds in particular (e.g. Lumbreras *et al.*, 2016 and references therein) and to the causes of their destruction (e.g. Machado *et al.*, 1999a; Presado *et al.*, 2010; Boix *et al.*, 2012 and references therein; Lumbreras *et al.*, 2016). In the southwest region of Portugal, Ferreira & Beja (2013) reported the disappearance of 56% of 296 temporary ponds between 1991 and 2009, due mainly to agricultural activities. As stated in Belk (1998) “The overriding requirement for protecting invertebrates is habitat conservation”.

As their survival is strictly dependent on temporary pond preservation, the conservation status of the Portuguese FLB fauna is therefore of great concern because of the scarcity of these habitats and the threats to which they are currently exposed (e.g. Ferreira & Beja, 2013), even in the case of the most common species. In Portugal, as in many other countries, none of the FLB species is red listed. Hence it is urgent to intensify the warnings to the appropriate national or international entities, in order to ensure implementation of conservation laws concerning the Portuguese FLB fauna. As stated by Miracle *et al.* (2008), nowadays “large branchiopods are endangered invertebrates of greatest conservation need”.

ACKNOWLEDGEMENTS

We are very grateful to Ana Abreu, Ana Maria Vidal, António Luís, Bárbara Monteiro, Carlos Noivo, Carlos Pacheco, Cristina Cardoso, Débora Nascimento, Edgar Gomes, Eliana Galioto,

Fernando Pereira, Horácio Costa, Inês Cardoso, João Puga, João Reis, Jordi Sala, Jorge Roque, Luís Palma, Luis Sousa, M. José Caramujo, Manuel João Pinto, Mário Reis, Miguel Pais, Mirjam van de Vliet, Nelson Fonseca, Núria Salvador, Patrícia Presado, Pedro Beja, Pedro Portela, Pedro Rocha, Pedro Veiga, Rita Alcazar, Sérgio Ribeiro, Sofia Castel-Branco, Susana Coelho, Tiago Marques, Tiago Mourinho, and Udo Schwarzner, for providing information on site location and/or helping in the field work and/or sending FLB samples for identification.

This study received national funds through FCT - Foundation for Science and Technology, projects UID/Multi/04326/2013, UID/MAR/04292/2013 and was also partially funded by: LIFE + Nature Project “Conservação de charcos temporários na costa Sudoeste de Portugal”; “Estudo da Ecologia das Lagoas Temporárias do Sudoeste de Portugal”-Protocolo de colaboração CCMAR-PNSACV, ICN funding; “Identificação dos Elementos de Conservação (Fauna de Crustáceos Filópodes e Anfíbios) nos Charcos temporários do Parque Natural do Vale do Guadiana e Áreas limítrofes”-Protocolo de colaboração CCMAR-ICN, ICN funding; “Estudo dos Grandes Branquiópodes das Lagoas Temporárias na área de influência dos Parques Naturais do Tejo Internacional e da Serra de Aires e Candeeiros”-Collaboration with the PNTI and PNSAC chiefdoms; Consórcio BIO3 / Matos, Fonseca & Associados, Estudos e Projectos, Lda-“Plano de Conservação para os Charcos Temporários Mediterrânicos na região de implementação do EFMA”, EDIA funding. Additional thanks are due to Karim Erzini, who kindly reviewed the English of the manuscript. We also thank two anonymous reviewers for their helpful suggestions that improved the manuscript.

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