Updated checklist and distribution of large branchiopods (Branchiopoda: Anostraca, Notostraca, Spinicaudata) in Tunisia

FEDERICO MARRONE^{1,*}, MICHAEL KORN², FABIO STOCH³, LUIGI NASELLI-FLORES¹, SOUAD TURKI⁴

¹ Dept. STEBICEF, University of Palermo, via Archirafi, 18, I-90123 Palermo, Italy

² Limnological Institute, University of Konstanz, Mainaustr. 252, D-78464 Konstanz & DNA-Laboratory, Museum of Zoology, Senckenberg Natural History Collections Dresden, Königsbrücker Landstrasse 159, D-01109 Dresden, Germany

³ Dept. of Life, Health & Environmental Sciences, University of L'Aquila, Via Vetoio, I-67100 Coppito, L'Aquila, Italy

⁴ Institut National des Sciences et Technologies de la Mer, Rue du 02 mars 1934, 28, T-2025 Salammbô, Tunisia * e-mail corresponding author: federico.marrone@unipa.it

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SUMMARY

Temporary ponds are the most peculiar and representative water bodies in the arid and semi-arid regions of the world, where they often represent diversity hotspots that greatly contribute to the regional biodiversity. Being indissolubly linked to these ecosystems, the so-called "large branchiopods" are unanimously considered flagship taxa of these habitats. Nonetheless, updated and detailed information on large branchiopod faunas is still missing in many countries or regions. Based on an extensive bibliographical review and field samplings, we provide an updated and commented checklist of large branchiopods in Tunisia, one of the less investigated countries of the Maghreb as far as inland water crustaceans are concerned. We carried out a field survey from 2004 to 2012, thereby collecting 262 crustacean samples from a total of 177 temporary water bodies scattered throughout the country. Large branchiopod crustaceans were observed in 61% of the sampled sites, leading to the identification of fifteen species. Among these, the halophilic anostracan Branchinectella media is here reported for the first time for the country; conversely, four of the species reported in literature were not found during the present survey. Based on literature and novel data, the known large branchiopod fauna of Tunisia now includes 19 species, showing a noteworthy species richness when the limited extension of the country is considered. For each species, the regional distribution is described and an annotated list of references is provided. Under a conservation perspective, the particular importance of the temporary ponds occurring in the Medjerda river alluvial plain is further stressed. In this location,

several large branchiopod taxa with different ecological requirements converge and form unique and species-rich assemblages that should be preserved.

INTRODUCTION

Temporary ponds, i.e. those water bodies naturally experiencing alternate phases of flooding and drought according to seasonal climatic patterns, are widespread in the arid and semi-arid regions of the world. In the Mediterranean region, they are widely recognised for the noteworthy biological diversity they host, which contributes disproportionately to the regional biodiversity when their limited extension is considered (e.g. Williams 2006, Naselli-Flores and Barone 2012). Temporary ponds host several peculiar aquatic taxa that are exclusive to them, and which present a set of adaptations for their survival during the dry phases of the water bodies, and for their passive dispersal (e.g. Incagnone et al. 2015). Although protected in Europe and included as 'Priority Habitats' under the auspices of the 'Habitats Directive 92/43/EEC', the number of temporary ponds drastically decreased in the last decades due to climate change, the increased demand of land for agriculture and urban development, and the overexploitation of water resources (Stoch and Naselli-Flores 2014). Being indissolubly linked to these ecosystems, and exerting a remarkable appeal on the public, the so-called "large branchiopods" are unanimously considered "flagship taxa" of these ecosystems (e.g. Brendonck et al. 2008) and subjected to the same threats.

In the Maghreb, Moroccan and Algerian large branchiopod faunas are relatively well-known (see Thiéry 1987, van den Broeck et al. 2015, Samraoui et al. 2006, and references therein), and are characterised by a high diversity and a pronounced degree of endemism. Conversely, to date an updated and comprehensive synoptic inventory of Tunisian Anostraca, Notostraca and Spinicaudata has not been compiled, and only scattered records are available for several species. This prevents from getting an exact knowledge of their local distribution, and from detecting the threatened taxa, e.g. those with a restricted distribution.

In an attempt to fill this gap, we performed an exhaustive review of the available literature dealing with Tunisian large branchiopods, and we carried out an extensive sampling campaign in Tunisia from 2004 to 2012.

In this paper, the most updated information on the presence and distribution of large branchiopods in Tunisian temporary ponds is reported, whereas an analysis of the ecological factors affecting their distribution has been published in Stoch et al. (2016).

MATERIALS AND METHODS

THE STUDY AREA

Tunisia is situated on the Mediterranean coast of North Africa, between Algeria and Libya, encompassing an area of about 164,000 km². Despite its relatively small size, the country has a noteworthy geographical and climatic diversity, ranging from Mediterranean to desert climates and ecosystems. The northern part of the country with a Mediterranean climate is characterised by the Medjerda mountains, the valley of the Medjerda river, the plain of Tunis (i.e. the alluvial plain of the Medjerda river) and the Cap Bon Peninsula. The northern slope of the Medjerda Mountains is the most rain-laden region of the country: in the western part of this region average precipitation ranges from 800 to 1,500 mm y⁻¹; the average annual temperature is 17-18 °C. The Cap Bon Peninsula and the plain of Tunis are characterised by lower rainfalls (400-600 mm y⁻¹) and an annual average temperature of

17-19 °C. South of the valley of the Medjerda river begins the Tell, a plateau representing the easternmost part of the Atlas Mountains. The average annual temperature in the Tell is between 15 and 19 °C, and the rainfall ranges from 200 to 600 mm y⁻¹. The Sahel, a hot arid lowland steppe, spreads from east of the Tell to the Mediterranean coast; it is characterised by annual rainfall of approximately 200-400 mm, and an average annual temperature of 19-20 °C. Finally, south of a direct line connecting the oases of Gafsa and Gabés there is the desert, comprising desert steppe, the Great Eastern Erg (sand-dune desert), the *hamada* (rocky desert) and the *chotts* (salt-lake depressions). It is characterized by rainfall values ranging from 75 to 200 mm y⁻¹ and average temperatures of 19 to 22 °C.

Based on the climatic and physiographic features outlined above, Gauthier (1928a; 1933a) classified the country and neighbouring Algeria into three zones: (1) a "zone pluvieuse", i.e. a humid area with Mediterranean climate, and mean annual rainfall >500 mm; (2) a "zone substeppique", including the eastern Mediterranean part of the country, the Tell plateau, and the northern coast of the Sahel, with mean annual rainfall between 300 and 500 mm, and (3) a "zone steppique", i.e. arid, steppe and semi-desert area, with mean annual rainfall below 300 mm. The importance of the isohyets of 300 and 500 mm y⁻¹ in determining different crustacean assemblages in the Maghreb has been widely accepted and implemented, e.g. by Dumont et al. (1979), Thiéry (1991), Turki and El Abed (1999), Samraoui and Dumont (2002), and Stoch et al. (2016).

BIBLIOGRAPHICAL REVIEW AND NOMENCLATURE

A database of the large branchiopod crustaceans reported for Tunisia was compiled from an extensive literature search. The bibliographical services of the "Biodiversity Heritage Library" (http://www.biodiversitylibrary.org/) and of "The digital library of the Royal Botanic Garden (CSIC)" (http://bibdigital.rjb.csic.es/ing/presentacion.php) have been extensively used. Due to the economic importance of *Artemia*, literature about this genus is virtually enormous and mostly focused on aquaculture, so that producing an all-encompassing review on Tunisian brine shrimps was out of the scope of the present work. However, attention was paid to review all the references that include relevant systematics, as well as biogeographical and distributional data.

All obtained occurrence data were critically revised and, when possible, checked through dedicated sampling surveys. Large branchiopod nomenclature was based on Korn et al. (2006, 2010, 2013) and Korn and Hundsdoerfer (2006) for the Notostraca, and on Belk and Brtek (1995) and Rogers (2013) for the Anostraca, with the only exception of *Phallocryptus spinosus* (Milne-Edwards, 1840). In fact, the halophilic thamnocephalid originally described as *Branchipus spinosus* by Milne-Edwards (1840), transferred to the genus *Branchinecta* Verrill, 1869 by Simon (1886) and later to the genus *Branchinella* Sayce, 1903 by Daday (1910), was convincingly demonstrated to belong to the genus *Phallocryptus* Biraben, 1951 by Rogers (2003). However, Rogers (2003) did not apply the agreement in gender (ICZN Code, Art. 31.2, Fourth Edition in force since 1 January 2000), using the binomen *Phallocryptus spinosa*, i.e. maintaining the feminine Latin gender ending that was used when the species was transferred to the genus *Branchinecta* by Simon (1886). Such a binomen was erroneously adopted by subsequent workers (e.g. Ketmaier et al. 2008, Alonso and Ventura 2013, Rogers 2013, Atashbar et al. 2014). We herein correct this oversight; since the species is currently ascribed to the masculine genus *Phallocryptus*, its correct name is *Phallocryptus spinosus* (Milne-Edwards, 1840).

Due to the lack of a sound revision of the Palaearctic Spinicaudata, in the present work we refer to the collected species by use of the most commonly used binomia in the recent literature.

A complete list of the references pertaining to each species is reported in the "Results" section.

SAMPLING

Tunisian mainland and its main islands (Kerkennah and Djerba) were sampled from 2004 to 2012; the vast majority of the sites was sampled in winter and early spring, when the probability of finding the temporary ponds in their "ponding phase" is highest. In order to account for the seasonal successions and the possible inter-annual differences of the faunal assemblages, about 30% of the sampled sites were visited several times in different months and years. Each sampled site was univocally identified by an alphanumeric code, and its coordinates were registered in situ with a GPS device. Respective altitude values were obtained through the software Google Earth© (www.google.com/earth/).

Crustacean samples were collected by means of three different nets: a 125 μ m mesh-sized towing net was used in the open waters; a 200 μ m hand net was used for the benthic and littoral zones, and a further 600 μ m hand net was specifically used for catching adult large branchiopods, which are known to be able to actively avoid the denser nets and, especially if present in low densities, may be easily overlooked when using the conventional zooplankton sampling techniques. Attempts were made to sample every type of microhabitat present within each sampled site. Collected samples were fixed *in situ* in 95% ethanol in order to make them available for both morphological studies and molecular analyses.

When large branchiopod nauplii, juveniles and or immature specimens were observed, the collected samples were duplicated and in part kept alive in order to let them reach sexual maturity in laboratory cultures.

Branchiopods were sorted under a stereomicroscope, prepared according to Alonso (1996) and Dumont and Negrea (2002), and then identified according to Thiéry (1987, 1996), Alonso (1996) and Korn et al. (2006, 2010).

Collected samples are presently stored in FM's crustacean collection at the University of Palermo, Italy and in the Museum of Zoology, Senckenberg Natural History Collections Dresden, Germany.

RESULTS

Overall, 262 samples were collected from a total of 177 temporary ponds (Fig. 1, Table 1), leading to the retrieval of 15 large branchiopod species. These, added to the four large branchiopod species reported in the literature, but not found in the course of the present survey, lead to a total of 19 species for the country (Table 2). The occurrence of large branchiopods was registered in 107 out of the 177 sites studied, i.e. in 61 % of the sampled ponds. The occurrence sites for each of the recorded species are shown in Fig. 2 and Fig. 3. An annotated list of the Tunisian large branchiopod species is provided below.

Code	Location	Longitude (E)	Latitude (N)	Elevation (m a.s.l.)	Таха
F001	La Goulette	10.293178	36.815549	0	
F002	La Goulette	10.290118	36.813608	0	
F003	La Marsa	10.299812	36.886894	3	
F004	Gammarth	10.291864	36.894066	0	
F005	La Marsa	10.301739	36.888036	3	
F006	La Marsa	10.300410	36.882489	1	Cdia
F007	Djebel Oust	10.101859	36.587455	64	Cdia
F008	Zana	9.995808	37.031263	7	Cdia
F009	El Alia	9.999483	37.148530	30	Cdia
F011	Tinja	9.760281	37.165999	3	Bsch, Cdia, Lalu
F013	Tinja	9.760802	37.166355	3	Cdia
F014	Oued El Amor	8.755628	36.927287	3	Cdia, Lalu
F015	Sejnane	9.233482	37.056287	138	Cdia
F016	El Kraten - Kerkennah	11.257612	34.821888	0	Bsch
F017	El Kraten - Kerkennah	11.258134	34.823366	0	Pspi
F021	Al Hammah	9.548813	33.822781	70	
F022	Radouan	9.251739	33.744458	85	
F026	Ghidma	8.796029	33.426094	20	
F027	Chott El Maseg	8.633431	33.438654	18	
F029	Nouail	8.844055	33.505862	25	
F030	Bchelli	8.898202	33.624237	19	
F032	Hajeb El Ayoun	9.542656	35.415363	304	Bsch, Tper, Stor, Lmay, Tgra
F034	Kairouan	10.116522	35.753299	34	
F037	Raoued	10.231609	36.954036	0	Bsch, Cdia
F039	Gammarth	10.288483	36.903883	0	
F040	Raoued	10.227447	36.955043	0	
F041	Raoued	10.183117	36.944956	5	Cdia, Tsim
F042	Raoued	10.212327	36.955595	1	Bsch
F043	Sebkha El Ariana	10.269296	36.926615	0	
F044	Les Berges du Lac	10.272840	36.850707	0	
F045	Bou Hnach	10.105755	36.947523	5	Bsch, Cdia,Ctet, Tsim
F046	El Hisiane	10.154198	37.002173	1	Cdia, Ctet
F047	El Hisiane	10.158451	36.995056	0	Bsch, Stor, Cdia, Bfer, Ctet, Lmay Tsim
F048	Raoued	10.217618	36.966277	0	Cdia, Ctet, Tsim
F049	Sebkha Sedjoumi	10.163131	36.783188	3	
F050	La Goulette	10.293716	36.818229	0	Pspi

TABLE 1. List and characteristics of the sampled sites. See Table 2 for the acronyms.

Code	Location	Longitude (E)	Latitude (N)	Elevation (m a.s.l.)	Таха	
F051	La Goulette	10.297632	36.818394	0	Pspi	
F052	La Goulette	10.294604	36.816994	0	Pspi	
F054	Tinja	9.758928	37.169217	1	Cdia, Ctet, Lalu	
F057	Tinja	9.762371	37.165517	3		
F059	Protville	10.048230	36.976907	7		
F060	Fouchana	10.180207	36.711302	10		
F061	Fouchana	10.184006	36.716664	11		
F063	Ben Arous	10.264861	36.735240	9	Bsch, Cdia, Tsim	
F064	Sulayman	10.493709	36.690177	12		
F065	Sulayman	10.504716	36.690975	11	Cdia, Tsim	
F066	Sulayman	10.504991	36.690729	11	Bsch, Tsim	
F067	Sulayman	10.461404	36.699492	1		
F068	Sulayman	10.461033	36.700606	1		
F069	Dar Belouar	10.330632	36.045580	18	Bsch, Tper, Stor, Tsim	
F070	Metbassta	10.158927	35.821102	28		
F071	Garaa Metbassta	10.146367	35.796014	26	Bsch, Lmay	
F072	El Bathen	9.982058	35.765742	41	Bsch, Stor, Tper, Lmay, Tgra	
F073	Sidi Naji	10.058864	36.041103	105	Bsch, Tgra	
F074	Sidi Naji	10.058329	36.040990	105	Bsch	
F076	Rades	10.274467	36.791206	0		
F077	Rades	10.275925	36.786548	0		
F078	Rades	10.273724	36.781577	0	Bsch, Cdia	
F079	Rades	10.263266	36.769772	0	Pspi	
F080	Rades	10.263266	36.769772	0	Pspi	
F081	Rades	10.264426	36.769453	0		
F082	Sebkha El Ariana	10.267158	36.929054	0	Pspi	
F083	Sebkha El Ariana	10.252743	36.937382	0	Pspi	
F084	Raoued	10.220210	36.956236	1	Bsch, Cdia, Ctet, Tsim	
F085	Raoued	10.214045	36.970442	0		
F086	Raoued	10.184419	36.947855	4	Tsim	
F087	Bou Hnach	10.165290	36.978583	1	Cdia, Ctet, Tsim	
F088	Sulayman	10.492831	36.674855	13	Cdia, Ctet, Tsim	
F091	Charfine	10.695688	36.677916	242	Cdia	
F092	Errahma	10.727949	36.718888	99	Cdia	
F093	Oum Dhouil	10.790902	36.728837	91	Stor, Tcan	
F094	Oum Dhouil	10.790545	36.729347	90	Stor, Tsim	
F095	Oum Dhouil	10.790309	36.728819	91		

Code	Location	Longitude (E)	Latitude (N)	Elevation (m a.s.l.)	Таха	
F096	Manzil Tamim	10.894614	36.784799	15		
F097	Kelibia	11.095134	36.836063	3		
F099	El Haouaria	11.028908	37.023158	20	Cdia	
F101	El Haouaria	11.009341	37.043707	19	Bsch, Cdia, Tsim	
F102	Zouiet el Mgaiez	10.858409	36.924689	89		
F103	Enchaa	10.849900	36.900072	98	Cdia, Ctet	
F104	Manzil Hurr	10.931882	36.728885	8	Bsch, Stor, Cdia, Tsim	
F105	Sebkha Chibal	10.914250	36.654575	0	Pspi	
F106	Protville	10.078471	36.963133	6	Cdia, Ctet, Tsim	
F108	Parc de l'Ichkeul	9.707563	37.111015	0	Cdia	
F109	Sidi Othman	9.933141	36.962526	6	Cdia	
F110	Sidi Othman	9.930017	36.961469	7		
F111	Sidi Othman	9.937308	36.932214	9	Pspi	
F112	Sebkha el Jem	10.727158	35.166092	40	Pspi	
F113	Sebkha Sidi el Hani	10.417505	35.547783	32		
F114	Kerkennah	11.180175	34.740341	0	Pspi	
F115	Ouled Kacem - Kerkennah	11.168808	34.692374	0		
F118	Al Hushayshinah	10.228906	34.390504	3	Pspi	
F119	Al Hushayshinah	10.252606	34.386720	0	Bmed, Pspi	
F121	Sidi Makhlouf	10.417293	33.599618	2	Asal	
F122	Sidi Makhlouf	10.538237	33.598119	0		
F123	Sidi Makhlouf	10.542966	33.596193	0	Pspi	
F124	DJerba	10.748290	33.760047	1	Bsch	
F131	Chott El Guettar	8.935240	34.319596	217	Pspi	
F136	Jilmah	9.306290	35.303194	443	Bsch, Tper, Stor, Lmay, Tgra	
F139	Henchir el Haouareb	9.764359	35.562475	191	Bsch, Stor, Tgra	
F147	El Bathen	9.939861	35.748336	45	Bsch, Tper, Stor, Ctet, Lmay, Tsin	
F149	El Bathen	10.030332	35.699531	67	Bsch	
F153	Haffuz	9.716648	35.638288	307	Bsch, Stor	
F154	Dawwar Shaib	9.619487	35.722778	346		
F156	Bir Khalifah	9.199006	35.924475	870	Cdia	
F157	El Kef	8.855797	36.198843	514	Cdia	
F158	Jendouba	8.780255	36.473554	148	Cdia, Tsim	
F160	Firnanah	8.697033	36.641052	233	Cdia	
F164	Ain Draham	8.679090	36.743350	603		
F166	Ain Draham	8.705565	36.727138	597		
F168	Jaballah	8.745653	36.898059	17	Lalu	

Code	Location	Longitude (E)	Latitude (N)	Elevation (m a.s.l.)	Таха	
F169	Tabarka - Oued El Amor	8.755804	36.928252	3	Cdia, Lalu	
F170	Tabarka	8.755627	36.931136	4	Cdia, Lalu	
F171	Tabarka	8.756221	36.931245	4		
F180	Dawwar Mraf	9.373443	37.138851	87	Ctet, Lalu	
F184	Ghezala	9.580266	37.078381	20	Cdia	
F185	Mateur	9.623718	37.055436	9	Cdia	
F188	Chott El Djerid	8.476603	33.930777	14	Asal	
F190	Kalaat El Andalous	10.187319	37.089569	0		
F192	Kalaat El Andalous	10.135314	37.032921	0		
F193	El Hisiane	10.142575	37.018556	0		
F195	Oulad Cheikh Ayed	9.767422	36.652830	88		
F196	Oued Zarga	9.417019	36.696022	140		
F198	Bulla Regia	8.755233	36.557274	160	Cdia	
F199	Bou Hnach	10.138912	36.975729	2	Stor, Ctet, Tsim	
F200	Bou Hnach	10.140491	36.976138	1	Stor, Cdia, Ctet, Tsim	
F201	Sebkha Bou Jemel	11.162801	33.215326	0	Pspi	
F202	Chenini	10.237606	32.867673	542		
F203	Chenini	10.237606	32.867673	540		
F204	Chott el Djerid	8.733384	33.813802	21		
F205	Chott El Djerid	8.540688	33.902514	15	Asal	
F208	La Marsa	10.302282	36.883036	1		
F210	Parc de l'Ichkeul	9.673126	37.119058	20	Cdia, Ctet, Tcan	
F217	Sidi Bouzid	9.291635	35.074755	395	Bsch, Tper, Tgra	
F218	Henchir el Haouareb	9.764114	35.562278	189	Bsch, Tgra	
F219	Oued El Mekta	10.169650	35.599940	64	Bsch, Tper, Stor, Ctet, Lmay, Tgra	
F220	Enfidha	10.394997	36.104167	9	Bsch	
F221	Ouled Amer	10.318323	36.013054	13	Bsch, Stor, Tsim	
F222	Garaa Metbassta	10.147774	35.799165	32	Bsch, Stor, Lmay, Tgra	
F223	Tinja	9.759166	37.170549	0		
F224	Dawwar Mraf	9.375271	37.137493	88	Lalu	
F225	Enchaa	10.850495	36.902902	98	Cdia	
F226	Kairouan	10.114025	35.734434	35	Pspi	
F227	Kairouan	10.121209	35.763389	33		
F228	Raoued	10.208718	36.973155	0	Stor	
F229	Menzel Djemil	9.940044	37.214411	3	Cdia, Ctet, Tcan	
F230	Menzel Abderrahmane	9.834465	37.229895	15	Cdia, Lalu	
F231	Parc de l'Ichkeul	9.697821	37.110325	0		

Code	Location	Longitude (E)	Latitude (N)	Elevation (m a.s.l.)	Таха	
F232	Teskraia	9.520817	37.206117	17	Cdia, Ctet, Lalu	
F233	Dawwar Mraf	9.375803	37.138077	85	Cdia	
F234	Dawwar Mraf	9.338640	37.138865	87	Cdia, Ctet, Lalu	
F235	Kchatlia	8.846171	36.967102	10		
F238	Jendouba	8.776094	36.484553	145	Cdia	
F239	Sidi Hassoun	10.846156	36.840147	94	Cdia, Ctet, Tsim	
F240	Garaa El Khala	10.849002	36.847052	98	Cdia, Tsim	
F241	Kairouan	10.106397	35.706564	47	Bsch	
F242	Bou Hsan	9.984556	35.804985	44	Bsch, Tper, Tgra	
F243	Sidi Salem	9.551206	37.132686	20	Cdia	
F244	Teskraia	9.578804	37.192282	9	Cdia	
F245	Teskraia	9.554502	37.206334	10	Lalu	
F246	Dawwar Mraf	9.324672	37.150623	99	Lalu	
F247	Oued Bou Hsan	9.988914	35.813740	45	Bsch, Stor, Tgra	
F248	Garaa Machaar	9.199651	37.086655	153		
F249	Tabarka	8.761124	36.936295	1		
F250	Chott El Gharsa	8.069397	34.153165	-10	Apar	
F251	Dawwar Mraf	9.262662	37.122932	98	Cdia, Ctet, Lalu	
M027	Protville	10.076986	36.965331	7	Tsim	
M037	Dawwar Mraf	9.387517	37.137803	87	Cdia, Lalu	
M039	Dawwar Mraf	9.386086	37.137672	86		
M042	Bou Salem	8.928211	36.583283	130	Cdia, Tcan	
M043	Essebt	8.871919	36.547789	132	Tcan	
M044	Jendouba	8.778864	36.480525	146	Tsim	
M045	Jendouba	8.779967	36.472531	149	Tsim	
M048	Menzel Guemoudi	8.989708	34.632492	406	Bsch	
M053	Kerker	10.643233	35.484819	44	Bsch	
M062	Sidi el Hani	10.258450	35.680553	84	Stor	
M063	Sidi el Hani	10.254961	35.683981	77	Stor, Tsim	
M064	Znetti	10.221464	35.682031	46	Bsch, Stor, Lmay, Tgra, Tsim	

TABLE 2. List of large branchiopod species occurring in Tunisia. For each species, the number of sites at which the species was observed in the course of the current survey is reported. The acronyms refer to the codes used in Table 1.

Species and taxonomical hierarchy	Number of sites	Acronym
Anostraca		
Family Artemiidae		
Artemia franciscana Kellogg, 1906	0	-
Artemia salina (Linnaeus, 1758)	3	Asal
Artemia sp. (parthenogenetic strain)	1	Asp
Family Branchinectidae		
Branchinecta ferox (Milne-Edwards, 1840)	1	Bfer
Family Branchipodidae		
Branchipus schaefferi Fischer, 1834	36	Bsch
Family Chirocephalidae		
Subfamily Chirocephalinae		
Chirocephalus diaphanus Prévost, 1803	55	Cdia
Chirocephalus recticornis (Brauer, 1877)	0	-
Subfamily Branchinectellinae		
Branchinectella media (Schmankewitsch, 1873)	1	Bmed
Family Streptocephalidae		
Streptocephalus bimaris Gurney, 1909	0	-
Streptocephalus torvicornis (Waga, 1842)	21	Stor
Family Tanymastigidae		
Tanymastigites perrieri (Daday, 1910)	8	Tper
Family Thamnocephalidae		
Phallocryptus spinosus (Milne-Edwards, 1840)	18	Pspi
Notostraca		
Family Triopsidae		
Triops cancriformis (Bosc, 1801)	5	Tcan
Triops granarius (Lucas, 1864)	12	Tgra
Triops simplex Ghigi, 1921	28	Tsim
Lepidurus apus lubbocki Brauer, 1873	15	Lalu
Spinicaudata		
Family Cyzicidae		
Cyzicus tetracerus (Krynicki, 1830)	21	Ctet
Family Leptestheriidae		
Leptestheria mayeti (Simon, 1885)	9	Lmay
Family Limnadiidae		
Eulimnadia sp.	0	-

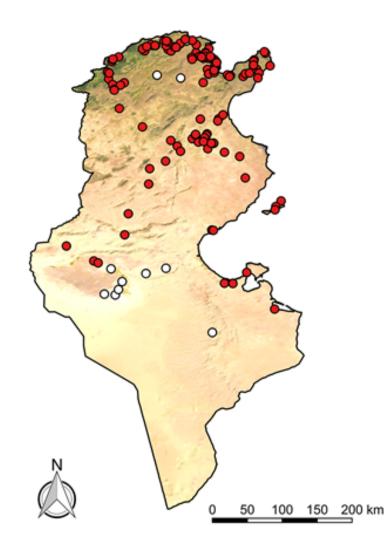


FIGURE 1. Location of the sampled sites. Red dots: sites where at least one large branchiopod species was collected; white dots: sites where no large branchiopods were collected.

Order ANOSTRACA

Family Artemiidae Grochowski, 1895

Artemia franciscana Kellogg, 1906

This alien anostracan species of Nearctic origin was reported for the country by Ben Naceur et al. (2010a). Unfortunately, no accurate description of the methods used for the identification of the species were reported by the authors, so that the presence of the species in Tunisia should be confirmed. *Artemia franciscana* is already known to occur in several circum-Mediterranean countries, where it proved to be able to replace the native *Artemia* strains (e.g., Amat et al. 2005, Muñoz et al. 2014), so that its possible presence in Tunisia does not appear unlikely.

Other references: Ben Naceur et al. (2012a, 2013a)

This species was not found during our survey.

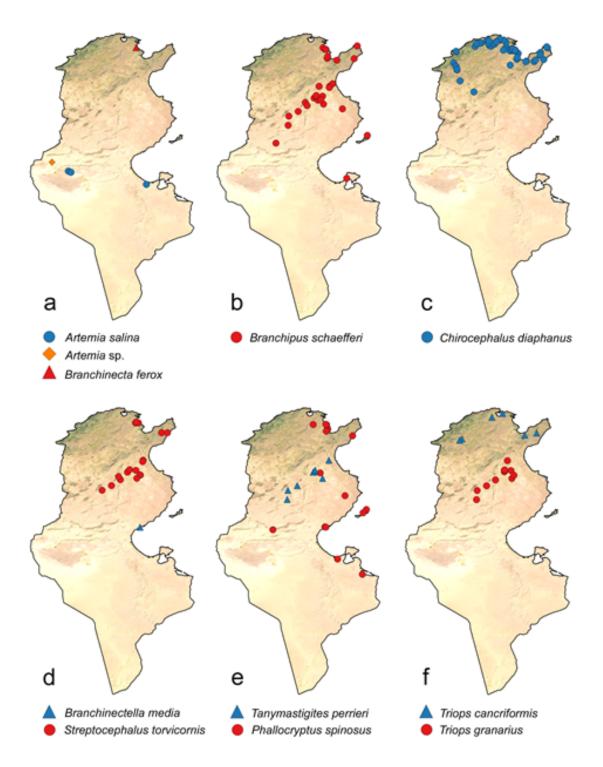


FIGURE 2. Distribution maps of selected Tunisian large branchiopod species based on present survey.

Artemia salina (Linnaeus, 1758)

The presence of the autochthonous Mediterranean gonochoric *Artemia* species was first reported for the country by Daday (1910). *A. salina* was sometimes reported in the literature under the junior synonym *Artemia tunisiana* Bowen and Sterling, 1978 (e.g.: Mura 1990, Barigozzi and Baratelli 1993, Triantaphyllidis et al. 1997a, 1997b). It is widespread in the

country, occurring wherever suitable habitats are available, sometimes co-occurring with *Phallocryptus spinosus* (Ben Naceur et al. 2009b).

In the light of the impressive amount of published papers dealing with *Artemia salina* in Tunisia, the list of references reported below is admittedly incomplete. Other references : Gurney (1909); Seurat (1922); Gauthier (1928a); Dumont et al. (1979); Van Ballaer et al. (1987); Vanhaecke et al. (1987); Triantaphyllidis et al. (1998); Toumi et al. (2005); Kaiser et al. (2006); Ben Naceur et al. (2008a, 2008b, 2009a, 2009b, 2010a, 2010b, 2011a, 2011b, 2012a, 2012b, 2012c, 2012d, 2013a, 2013b); Guermazi et al. (2008, 2009); Muñoz et al. (2008); Mahdhi et al. (2010, 2012); Ghomari et al. (2012); Ladhar et al. (2014).

During the present survey the species was collected at 3 sites (Table 1, Table 2; Fig. 2a).

Artemia sp. (parthenogenetic population)

The binomen *Artemia parthenogenetica* Bowen and Sterling, 1978 comprises a nonmonophyletic assemblage of parthenogenetic lineages with different levels of ploidy (e.g. Baxevanis et al. 2006, Muñoz et al. 2010, Maniatsi et al. 2011). In accordance with Baxevanis et al. (2006) and Rogers (2013) we herein refer to asexual forms of *Artemia* as populations instead of treating them as separate taxa. The occurrence of parthenogenetic populations of *Artemia* is known for the whole Maghreb, including Tunisia, where it was to date recorded from a single locality, i.e. the Sebkha Sedjoumi (Ghomari et al. 2012) (reported as "Sebkha Sijoumi" by the authors).

In the course of our samplings, a parthenogenetic population of *Artemia* was observed in a single pool within the Chott el Gharsa (Table 1, Table 2; Fig. 2a).

Family Branchinectidae Daday, 1910

Branchinecta ferox (Milne-Edwards, 1840)

This rare but widely distributed species was reported for the alluvial plain of the Medjerda river by Massal (1951). *B. ferox* is regionally extremely localised, and its Tunisian occurrence localities are situated about 1000 km east of the closest Algerian sites (i.e. the environs of Mécheria, Gauthier 1928a), and 600 km south-east of the Balearic occurrence sites of the species (Pretus 1990). It is known to occur in Spain (Alonso 1996), western Maghreb (Gauthier 1928a, Thiéry 1991, van den Broeck et al. 2015), in the Middle East, Turkey, Ukraine, and from the Balkans to the Pannonian lowland (cf. Daday 1910, Petkvoski 1991, Mura et al. 2005, and references therein). The observed "pincers-like" distribution closely resembles that of the anostracan *Streptocephalus torvicornis* (Waga, 1842) (Dumont et al. 1995) and of the calanoid copepod *Neolovenula alluaudi* (Guerne & Richard, 1890) (Alfonso and Belmonte 2013), thus suggesting a shared post-glacial northward overland dispersal through two separate routes (a western and a eastern one) for these temporary pond-dwelling crustaceans (see Incagnone et al. 2015).

During the present survey, the species was collected at a single site (Table 1, Table 2; Fig. 2a), which lies in the same area where the species was observed by Massal (1951).

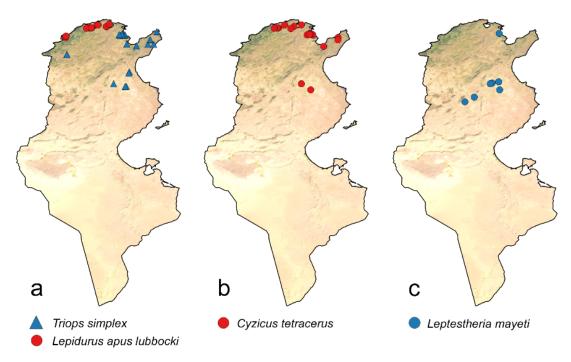


FIGURE 3. Distribution maps of selected Tunisian large branchiopod species based on present survey.

Family Branchipodidae Milne-Edwards, 1840

Branchipus schaefferi Fischer, 1834

The occurrence of *B. schaefferi* in Tunisia was first reported by Simon (1886). The species was later collected by Seurat (1922), Gauthier (1928a), Massal (1951) and Dumont et al. (1979). Cottarelli and Mura (1983) reported the occurrence of the closely related species *Branchipus pasai* Cottarelli, 1968 in "Northern Africa" without providing any precise occurrence locality. However, the latter species occurs in Lampedusa island, which is located on the Tunisian shelf, so that a possible occurrence of *B. pasai* in Tunisia should be checked. However, as stressed by Gandolfi et al. (2015), the taxonomy of some closely related *Branchipus* species (i.e. *B. schaefferi*, *B. pasai*, and *B. visnyai* Kertész, 1956) is to date quite confuse. Accordingly, pending a sound revision of the genus, in the present work we retain the use of the binomen *B. schaefferi* for all Tunisian populations.

Other reference: Turki and Turki 2010.

In the course of our survey, the species was collected at 36 sites (Table 1, Table 2; Fig. 2b).

Family Chirocephalidae Daday, 1910

Subfamily Chirocephalinae Daday, 1910

Chirocephalus diaphanus Prévost, 1803

The species was first reported for the country by Gurney (1909). All the localities of the species are limited to the northern part of the country, so that Gauthier (1928a) indicated *C. diaphanus* as one of the characteristic species of the "zone pluvieuse". In neighbouring Algeria, the

occurrence of the closely related *C. salinus* Daday, 1913 was reported by Samraoui et al. (2006). However, the taxonomy of the *Chirocephalus* species of the *diaphanus*-group is currently under revision, and it is possible that the taxa occurring in Maghreb belong in fact to several different species (Reniers et al. 2013).

Nourisson and Lenel (1968) reported the occurrence of a gynandromorph specimen of *C. diaphanus* collected near Ez-Zahra, northern Tunisia.

Other references: Daday (1910); Seurat (1922); Massal (1951); Turki and Turki (2010).

In the course of our survey, the species was collected at 55 sites (Table 1, Table 2; Fig. 2c), proving to be the commonest anostracan species in the country.

Chirocephalus recticornis (Brauer, 1877)

The species was described by Brauer (1877) as *Branchipus recticornis* based on some female specimens sent him by M. Fischer, and a single male obtained by Brauer himself upon culturing some mud collected *"in einem Tümpel in Tunis"* (in a pool in Tunis). No further records of this quite peculiar chirocephalid species have ever been published. Pesta (1921) reported on some specimens of the species stored in the natural history Museum of Vienna that likely represent the material studied by Brauer (1877).

Other references: Simon (1886), Daday (1910), Brtek (1966), Belk and Brtek (1995), Mura (2001), Rogers (2013).

This species has not been found in the course of the present survey.

Family Chirocephalidae Daday, 1901

Subfamily Branchinectellinae Brtek, 1966

Branchinectella media (Schmankevitsch, 1873)

Gauthier (1928a) reported a halophilic anostracan species which he referred to under the binomen *Branchinella media* Schmankevitsch, 1873 to have occurred in Sebkha Sedjoumi, a site located in northern Tunisia. Interestingly, in contrast to the currently used taxonomical arrangement (Belk and Brtek 1995, Rogers 2013), Gauthier considered *B. media* Schmankevitsch, 1873 (now: *Branchinectella media* (Schmankevitsch, 1873)) and *Branchinectella salina* Daday, 1908 to be two different species, which sometimes co-occur (cf. Gauthier 1928a, pages 362, 374, 375). Furthermore, he is not consistent with the genus he attributes the species to, e.g. at page 359 (Gauthier 1928a) he wrote "… et *Branchinecta* sp. (vraisemblablement *Branchinella media*)…". In fact, Gauthier considers *Branchinella media* as a synonym of *Branchipus spinosus* Milne-Edwards, 1840 (see note 2, page 90, in: Gauthier 1928b), so that we should actually consider Gauthier's records of '*Branchinella media*' as pertaining to *Phallocryptus spinosus* (Milne-Edwards, 1840). Consequently, no reliable record of *Branchinectella media* in Tunisia exists in the available literature. However, the nomenclature pertaining to this taxon distributed from the African deserts to the Arctic is likely to be revised.

In the course of our survey, the species was collected at a single site (Table 1, Table 2; Fig. 2d), which constitutes the first reliable record of the species for the country.

Family Streptocephalidae Daday, 1910

Streptocephalus bimaris Gurney, 1909

The species was described based on specimens once collected near Oued Tinja by Gurney (1909), and never collected again. Linder (1941) questioned that the female and male specimens described by Gurney (1909) did in fact belong to the same species, and suggested that the presumptive *S. bimaris* females could actually have been immature *Chirocephalus* specimens. Conversely, according to Belk and Brtek (1995), *S. bimaris* might be a senior synonym of the Ethiopian species *Streptocephalus chappuisi* Brehm, 1935.

Other references: Daday 1910, Hamer et al. 1994, Rogers 2013.

This species has not been found in the course of the present survey.

Streptocephalus torvicornis (Waga, 1842)

Gauthier (1928a) is the first author who collected the species in Tunisia, although it is possible that the *Streptocephalus* sp. collected near Tindja by Gurney (1909) was, in fact, *S. torvicornis*. The presumptive subspecies *S. torvicornis torvicornis* (Waga, 1842) and *S. torvicornis bucheti* Daday, 1910 appear to be indistinguishable and thus lack any taxonomical meaning (see: Daday 1910, Thiéry 1987, 1996, Dumont et al. 1995, Alonso 1996, Rogers et al. 2013), so that we refer to the taxon as *S. torvicornis*. Beladjal and Mertens (2003) provided data on the morphological variability of the resting eggs of a Tunisian population of the species.

Other references: Massal 1951, Dumont et al. 1991, 1995, Hamer et al. 1994, Turki and Turki 2010.

In the course of our survey, the species was collected at 21 sites (Table 1, Table 2; Fig. 2d).

Family Tanymastigidae Brtek, 1972

Tanymastigites perrieri (Daday, 1910)

Tanymastigites perrieri was first collected in the country by Gauthier (1928a). It is a taxon typical of steppe and desert regions, known for the whole Maghreb (Gauthier 1928a, Thiéry 1987, 1991).

Other references: Turki and Turki 2010 (as "*Tanymastigites* sp.), Machado and Sala 2013, Cohen 2013.

In the course of our survey, the species was collected at 8 sites (Table 1, Table 2; Fig. 2e).

Family Thamnocephalidae Packard, 1883

Phallocryptus spinosus (Milne-Edwards, 1840)

This halophilic anostracan species was collected in Sebkha Sedjoumi by Gurney (1909, as "*Branchinecta* sp."; the name of the salt lake in question is misspelled as "Sebkha Sedjouma" by this author) and Gauthier (1928a, as "*Branchinella media*", see also Gauthier, 1928b). Unfortunately, due to the instability inherent to the nomenclature of this species and of the other halophilic anostracan *Branchinectella media* (see also the comments above related to this

species), respective records apparently were overlooked, so that Ben Naceur et al. (2009b) reported the presence of the species in Tunisia as a first record for the country. Interestingly, Ben Naceur et al. (2009b) collected the species in Sebkha Sedjoumi as well, in good accordance with the findings of Gurney (1909) and Gauthier (1928a).

Other reference: Turki and Turki 2010.

In the course of our survey, the species was collected at 18 sites (Table 1, Table 2; Fig. 2e).

Order NOTOSTRACA

Family Triopsidae Keilhack, 1909

Triops cancriformis (Bosc, 1801)

Simon (1886) and Seurat (1922) are the first authors who reported the occurrence of '*Apus cancriformis*' in the country; however, it cannot be ascertained whether their records are to be ascribed to *T. cancriformis*, *T. simplex*, or to both species. Gauthier (1934) explicitly excluded the possibility that *T. cancriformis* s.s. was among his samples collected in Tunisia, however, a comparison of molecular and morphological characters suggests that the latter are in part misleading and may lead to erroneous determinations so that identifications should ideally be supported by investigations of the reproductive mode and/or molecular data (see Korn et al. 2006). The first reliable records of the species for Tunisia are thus the ones reported by Korn et al. (2006).

Other reference: Korn et al. (2010).

In the course of our survey, the species was collected at 5 sites (Table 1, Table 2; Fig. 2f).

Triops granarius (Lucas, 1864)

The species was first reported for Tunisia by Simon (1886) and Gauthier (1928a) under the binomen *Apus numidicus* Grube, 1865. *Triops numidicus* was synonymised with *Triops granarius* (Lucas, 1864) by Longhurst (1955). Later, Brtek (1997) listed *T. numidicus* as the valid species name. However, Korn and Hundsdoerfer (2006) showed that Longhurst's (1955) treatment was correct and that *Triops granarius* (Lucas, 1864) is indeed the valid species name for this notostracan under the ICZN rule of priority. Korn and Hundsdoerfer (2006) further emphasized the noteworthy cryptic diversity lumped under this binomen. Korn et al. (2013) even demonstrated that *T. granarius* is paraphyletic with respect to the remaining congeneric species, thus further highlighting the need for a revision of the taxon. Other references: Seurat (1922), Longhurst (1955), Turki and Turki (2010). In the course of our survey, the species was collected at 12 sites (Table 1, Table 2; Fig. 2f).

Triops simplex Ghigi, 1921

The species was described based on Libyan specimens, and Ghigi (1921) himself attributed to this species certain Tunisian notostracan samples stored in the Museum of Genova (Italy). The species was confirmed to be present in the country by Gauthier (1934) and Longhurst (1955) (using the trinomen *T. cancriformis simplex*) and by Korn et al. (2006; revising its name to '*T. mauritanicus simplex*'). Korn et al. (2010) reinstated it to full species status. The available information regarding the taxonomical identity of the *Triops* populations reported by Simon (1886), Seurat (1922), and Gauthier (1928a) does not allow for an exact determination.

Other references: Gurney (1923), Gauthier (1928a), Colosi (1923), Ghigi (1924), Longhurst (1958).

In the course of our survey, the species was collected at 28 sites (Table 1, Table 2; Fig. 3a).

Lepidurus apus lubbocki Brauer, 1873

This taxon, originally described as *L. lubbocki*, was first collected in Tunisia by Gurney (1909). Longhurst (1955) treated it as a subspecies of *L. apus*. Although recent molecular evidence suggests that this taxon probably deserves species status (e.g. Mantovani et al. 2009, Korn et al. 2013), further evidence is needed in order to clarify which of the populations presently attributed to this subspecies might actually refer to the former *L. lubbocki* described by Brauer (1873). The situation is complicated by the fact that *L. a. lubbocki* turned out to be paraphyletic with respect to the remaining species in the genus hitherto studied via molecular tools (see Figs. 1 and 2 in Korn et al. 2013). Furthermore, no specimens from the type locality have ever been investigated using modern methods, so that a possible taxonomical re-instatement should await a profound re-investigation of Circum-Mediterranean populations. Pending a formal revision of the Palaearctic species of the genus *Lepidurus*, we provisionally retain the trinomen proposed by Longhurst (1955).

Other references: Seurat (1922), Longhurst (1955), Turki and Turki (2010). In the course of our survey, the species was collected at 15 sites (Table 1, Table 2; Fig. 3a).

Order SPINICAUDATA

Family Cyzicidae Stebbing, 1910

Cyzicus tetracerus (Krynicki, 1830)

This spinicaudatan species was first reported for the country by Grube (1865) and Simon (1886) (as *Estheria cycladoides* Joly, 1841), and later confirmed by Gurney (1909), Daday (1915; as *Cyzicus cycladoides*), Gauthier 1928a, 1933b (as *E. cycladoides*). More recently, it was recorded by Dumont et al. (1979) and Turki and Turki (2010) who, following Šrámek-Hušek et al. (1962), treated *E. cycladoides* as a junior synonym of *Cyzicus tetracerus*, thus opting for the use of the latter name. Consequently, in the present survey we use the binomen *C. tetracerus* for the Tunisian populations of this species.

Other references: Seurat (1922), Straškraba (1966).

In the course of our survey, the species was collected at 21 sites (Table 1, Table 2; Fig. 3b).

Family Leptestheriidae Daday, 1923

Leptestheria mayeti Simon, 1885

The species was first recorded for the country by Gauthier (1928a) and recently reported by Turki and Turki (2010). No further records for the country are available. In Algeria, the presence of the closely related *Leptestheria cortieri* Daday, 1923 is reported (Samraoui et al. 2006), and the presence of *L. lybica* Colosi, 1920 was reported in Libya (Colosi 1920), although both might in fact be synonyms of *L. mayeti* (see Thiéry 1996, but see also Straškraba 1966).

Other references: Daday (1915), Thiéry (1987), Jaume (1989), Alonso (1996).

In the course of our survey, the species was collected at 9 sites (Table 1, Table 2; Fig. 3c).

Family Limnadiidae Burmeister, 1843

Eulimnadia sp.

Rabet et al. (2015) reported the occurrence of some juvenile limnadiid spinicaudatans in central Tunisia. Unfortunately, it was not possible to ascertain whether they belong to a new, yet undescribed species or if they might be ascribed to one of the *Eulimnadia* species known to date. The collection and characterization of further samples of this taxon would be desirable.

DISCUSSION

THE LARGE BRANCHIOPOD FAUNA OF TUNISIA

In the course of this survey, 14 out of the 18 formerly reported species could be confirmed for the fauna of the country. Furthermore, the halophilic anostracan *Branchinectella media* is herein reported for the first time for the country, leading to a total of 19 large branchiopod species (Table 2). No evidence for the presence of *Chirocephalus recticornis* and *Streptocephalus bimaris* was found, in spite of an extensive sampling effort spent in the localities where these species had been reported from. The species *Artemia franciscana* and *Eulimnadia* sp., both recently reported for the country (Ben Naceur et al. 2010a, Rabet et al. 2015) were not recovered as well. Conversely, *Branchinecta ferox* is here reported several decades after its last report for the country (Massal 1951).

The large branchiopod fauna of Tunisia is rich and diverse, and it is rather similar to that of the neighbouring North African countries Algeria and Libya. Conversely, the fauna of Morocco, in particular that found in the parts of the country that are situated west of the High Atlas mountains, is peculiar and well-characterised. This is mainly due to the high incidence of endemic and sub-endemic species such as *Linderiella africana* Thiéry, 1986, *Tanymastix affinis* Daday, 1910, *Tanymastigites brteki* Thiéry, 1986, *Cyzicus bucheti* (Daday, 1913), *Maghrebestheria maroccana* Thiéry, 1988, *Eocyzicus saharicus* Gauthier, 1937 and *Triops mauritanicus* Ghigi, 1921.

However, the high incidence of species in Tunisia that to date are only known from single occurrence localities, i.e. *B. ferox, B. media, C. recticornis, S. bimaris,* and *Eulimnadia* sp., stresses the possibility that we are still far from getting an exhaustive picture of the actual diversity of large branchiopod crustaceans in the country, which might in fact constitute a hotspot for large branchiopod diversity in the central Mediterranean area.

The composition and distribution of the fauna of the Sahel and of the Mediterranean part of the country are presently known quite accurately. However, further attempts should be made in order to check for a possible presence of *Tanymastix stagnalis* (Linnaeus, 1758) in the country, particularly in the governorate of Jendouba, which is geographically close to the *T. stagnalis* populations reported for Algerian Numidia, (e.g. Samraoui and Dumont 2002) and appears to provide suitable conditions for the species (F.M., own observations). Similarly, future studies should intensify sampling efforts in order to investigate the fauna of the Tell plateau and of the southern part of the country in more detail, since only a few water bodies could hitherto be investigated in these areas; this is in part due to the fact that temporary ponds are often difficult to identify in regions with low and irregular precipitation.

Regarding the southern part of the country, it would in particular be desirable to locate and study suitable habitats in the south-easternmost part of the country, in order to check for the possible presence of some steppe species reported for neighbouring Algeria and Libya, e.g. *Streptocephalus rubricaudatus* (Klunzinger, 1867) and *Tanymastigites* spp. (see Brtek 1972, Samraoui et al. 2006).

LARGE BRANCHIOPOD DISTRIBUTION WITHIN THE COUNTRY

Based on the available data, it is possible to differentiate four major ecological groups within the Tunisian large branchiopods (see Stoch et al. 2016 for a discussion). These show, in fact, quite a sharp latitudinal gradient, with some species being confined to the more arid areas of Tunisia that are located in the southern and central portion of the country (i.e., *Tanymastigites perrieri*, *Triops granarius* and *Leptestheria mayeti*), whereas others are strictly linked to the more humid areas of northern Tunisia (i.e., *Chirocephalus diaphanus* and *Lepidurus apus lubbocki*). A third group comprises the halophilic taxa linked to the heavily mineralised water of sebkhas and chotts, i.e., *Artemia salina*, *Phallocryptus spinosus*, and *Branchinectella media*, whose distribution is mostly driven by the occurrence of suitable saline habitats themselves. A fourth and last group comprises the more euryecious species, occurring throughout most of the country without a clear geographical pattern. Finally, the scarce data presently available for the Tunisian populations of *Branchinecta ferox*, *Chirocephalus recticornis*, *Streptocephalus bimaris* and *Eulimnadia* sp. prevent us from formulating any hypothesis on their ecological preferences and distribution.

Hitherto, the ranges of *Streptocephalus torvicornis* and *Leptestheria mayeti* in Tunisia were considered to be confined to the Sahel, whereas in the course of this survey we collected these species as far north as in the plain of the Medjerda river, and in the Cap Bonn Peninsula. The floodplain of the Medjerda river is in fact a hot-spot of large branchiopod diversity in the Mediterranean bioregion: an area of a few square kilometres between the northern shore of the Sebkha El Ariana and the mouth of the Medjerda river itself hosts 9 large branchiopod species, with large branchiopod multiple co-existence of up to 7 species at a single site. Such a high diversity is comparable to that of the Chaouia Plain in Morocco (Thiéry 1991, van den Broeck et al. 2015).

It is noteworthy that the Medjerda floodplain is the only region within Tunisia where it is possible to observe the co-existence of large branchiopod species typical of the steppe and of the temperate areas of the country, these two species assemblages being usually strictly allopatric. The high faunal diversity of the Medjerda floodplain, which has been observed also in other crustacean taxa (F. Marrone, unpubl. data) and amphibians (Sicilia et al. 2009), is likely due to the location of the area at the boundary of two climatic regions (i.e. the steppic and the Mediterranean ones, referring to the "zone substeppique" and "zone pluvieuse" of Gauthier 1928a), and to the existence of a local patchwork of temporary ponds with different characteristics. An attentive management of this area and the conservation of its network of natural and semi-natural water bodies would thus be desirable in order to grant the long-term conservation of this diversity hotspot of Tunisian large branchiopods. Strict control of land reclamation and urban development planning are the only effective approaches to preserve existing Tunisian wetlands and their biota. For this reason, we suggest to implement some environmental obligations in selected parts of Medjerda floodplain. In particular, preserving and encouraging traditional agricultural and pastoral practices could be an effective tool for the protection of large branchiopod habitats in the area.

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