

First record of the tadpole shrimp *Triops cancriformis* (Lamarck,1801) (Crustacea: Branchiopoda: Notostraca) in Portugal

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ABSTRACT

First record of the tadpole shrimp *Triops cancriformis* (Lamarck,1801) (Crustacea: Branchiopoda: Notostraca) in Portugal

Notostracan crustaceans identified as *Triops cancriformis* according to the presently accepted morphological criteria were recorded for the first time in Portugal in 2007. All previous records of *Triops* in Portugal belong to *mauritanicus* lineage species i.e. *T. vicentinus* or *T. baeticus*. A specimen purportedly belonging to *T. cancriformis* (Carvalho, 1944) has been re-identified by Machado in 2014 as *T. baeticus* after morphological examination. During 2007, hundreds of individuals of *T. cancriformis* were observed throughout the rice paddies on the northern margin of Sorraia River (Vale do Sorraia, Coruche). In the last 9 years, monthly checks during the rainy season have failed to record high population abundances and only a few specimens have been observed in the flooded tracks left by trucks and other heavy machinery on the elevated margins of the paddies. The low number of individuals observed in the latter years possibly results from changes of ecological conditions. Thus far, males of *T. cancriformis* have not been recorded, which may indicate that the observed population is either androdioecious or made of hermaphrodite or parthenogenetic populations. This situation contrasts with the other confirmed populations of this species in the Iberian Peninsula that are gonochoric. Parthenogenetic/hermaphrodite/androdioecious lineages, present in Northern and Central Europe, are considered to have derived from gonochoric Iberian populations in the Pleistocene which makes this finding all the more interesting in evolutionary terms. Has this population resulted from a recent recolonization from non-Iberian populations? Or has it derived directly from the assumed Iberian Pleistocene refuge? The potentially high dispersal abilities of *Triops* diapausing cysts and the possibility of hermaphrodite/parthenogenetic reproduction favour the 1st hypothesis of recolonization. Possible sources of individuals are (i) cysts attached to migratory birds arriving possibly from Southern France or Northern Italy and that are regularly seen feeding at these rice fields or ii) cysts unwittingly transported with the rice seeds used in the Vale do Sorraia. Both are in accordance with the assumption that nongonochoric reproductive mode confers a colonization advantage over gonochoric populations, which lack evidence of fast long distance dispersal ability. Future multilocus phylogenetic analysis is expected to clarify the origin of *T. cancriformis* found in the Sorraia's rice fields.

Key words: Large branchiopods, rice fields, temporary ponds, androdioecy, hermaphrodites, gonochoric.

RESUMO**Primeiro registo do grande branquiópode *Triops cancriformis* (Lamarck, 1801) (Crustacea: Branchiopoda: Notostraca) em Portugal**

Crustáceos identificados como *Triops cancriformis* (Crustacea, Notostraca) de acordo com os critérios morfológicos actualmente adoptados, foram registados pela primeira vez em Portugal em 2007. Todos os *Triops* anteriormente encontrados em Portugal pertencem a espécies da linhagem mauritanicus, ou seja *T. vicentinus* ou *T. baeticus*. A identificação de uma amostra existente no Museu de Coimbra e referida como *T. cancriformis* (Carvalho, 1944) foi emendada para *T. baeticus* em 2014, por M. Machado, após análise morfológica. Em 2007, foram observadas centenas de indivíduos de *T. cancriformis* nos arrozais da margem norte do rio Sorraia (Vale do Sorraia, Coruche). Prospecções mensais durante a época das chuvas nos últimos 9 anos, não voltaram a evidenciar abundâncias tão elevadas, tendo-se apenas observado alguns indivíduos em sulcos inundados deixados por camiões e máquinas agrícolas nas margens elevadas de alguns canteiros de arroz. Este reduzido número de indivíduos observado nos últimos anos resulta, possivelmente, de alterações nas condições ecológicas. Não se encontraram, até agora, indivíduos machos de *T. cancriformis*, o que pode indicar que a população observada é androdióica, ou hermafrodita, ou partenogenética. Esta situação contrasta com as outras populações confirmadas desta espécie na Península Ibérica, que são gonocóricas. Linhagens partenogenéticas/hermafroditas/androdióicas, presentes no Norte e Centro da Europa, são consideradas como tendo derivado de populações gonocóricas ibéricas no Pleistoceno, o que torna esta descoberta ainda mais interessante em termos evolutivos. Terá essa população resultado de uma recolonização recente com origem em populações não-ibéricas? Ou derivará directamente do assumido refúgio pleistocénico Ibérico? A capacidade potencialmente elevada de dispersão de cistos de *Triops* em diapausa e a possibilidade de reprodução hermafrodita/partenogenética favorecem a hipótese de recolonização recente a partir de (i) cistos transportados por aves migratórias possivelmente provenientes do sul de França ou do norte de Itália e que são regularmente vistas a alimentarem-se nestes campos de arroz ou de (ii) cistos inadvertidamente introduzidos via sementes de arroz utilizadas no Vale do Sorraia. Ambas as possibilidades estão em conformidade com o pressuposto de que o modo reprodutivo não gonocórico confere vantagens de dispersão e colonização relativamente a populações gonocóricas, que não evidenciam capacidade de dispersão rápida a longa distância. Espera-se que uma futura análise filogenética multilocus possa vir a esclarecer a origem de *T. cancriformis* nos arrozais do Sorraia.

Palavras-chave: Grandes branquiópodes, arrozais, charcos temporários, androdióica, hermafroditas, gonocóricas.

INTRODUCTION

Species of the tadpole shrimp *Triops* (Schrank, 1803) have a scattered distribution in all continents except Antarctica (Vanschoenwinkel *et al.*, 2012; Korn *et al.*, 2013). *Triops* morphology in the fossil record appears to be indistinguishable from that of modern forms by the Upper Triassic (Trusheim, 1938) and morphological similarities may extend to the Permian (Guthörl, 1934; Gand *et al.*, 1997; Korn *et al.*, 2013). The conservative form exhibited by modern *Triops* is typically accompanied by high morphological individual variability, and low number of known morphological characters of taxonomic significance. This rendered correct species identification nearly impossible until the revision by Longhurst in 1955 who retained only taxonomic entities that could be distinguished (i.e. diagnosable morphospecies). Molecular phylogenetic studies in

the last decades, allied with morphological re-investigation, have clarified various instances of cryptic diversification and led to taxonomic revisions worldwide (Korn *et al.*, 2013; Mathers *et al.*, 2013; Korn & Hundsdoerfer, 2016; Meusel & Schwentner, 2016). In the Mediterranean region, at least 2 *taxa* formerly attributed to *Triops granarius* (Lucas, 1864), and at least 7 *taxa* formerly attributed to *Triops cancriformis sensu lato* are known to occur (Korn *et al.*, 2006; 2010; Korn & Hundsdoerfer, 2006; 2016). Korn *et al.* (2006), using mitochondrial 16S and 12S rDNA sequences, investigated the phylogenetic relationships among the three subspecies of *Triops cancriformis* recognized at the time and identified two distinct lineages.

One lineage encompasses *T. c. cancriformis* populations from central and northern Europe and samples from northern Spain (Espolla temporary pond, Girona, 42°09'02"N, 02°45'60"E)

that had been hitherto classified as *T. cancriformis* of the *simplex* form (Alonso, 1985; 1996). The common lineage of the population of Espolla and the other European populations has been corroborated in a subsequent study by Zierold *et al.* (2007). These authors named the individuals from Espolla as *T. c. simplex* referring to the previous identification by Alonso (1985; 1996) and Boix *et al.* (2002), and observed that this population and other population from “El Puig” (inaccurate coordinates 39°34'24"N, 00°16'43"E, possibly 39°34'24"N, 00°16'43"W near El Puig de Santa Maria, Comunitat Valenciana, Spain) shared haplotype H1 with five *T. c. cancriformis* individuals from four different populations in Germany. The authors remarked that “the two morphologically described subspecies *T. c. cancriformis* and *T. c. simplex*, which show different reproductive modes, are not represented by distinct monophyletic lineages”. As noted by Korn *et al.* (2006) after morphological re-examination of specimens with comparison to molecular data, the former *T. c. simplex* cannot be reliably separated from *T. c. cancriformis*, using the morphological characters that had hitherto been used to distinguish among subspecies of *T. cancriformis*.

The second lineage of *Triops cancriformis sensu lato* comprises all populations of former *T. c. mauritanicus* and the northern African populations of *T. c. simplex* (Ghigi, 1921) (Korn *et al.*, 2006). This lineage contains the predominant species in the Iberian Peninsula, *T. mauritanicus*, which was originally established by Ghigi (1921), later considered a subspecies of *T. cancriformis* by Longhurst (1955), and re-instated to full species status by Korn *et al.* in 2006. The clarification of the gross phylogenetic relationships within the group resulted in the inclusion of the northern African populations of the former *T. cancriformis simplex* as a subspecies of *T. mauritanicus*. Later records have shown that *T. m. simplex* is also present in Spain. In 2007, a population of *Triops* from Ares del Maestre (40°25'19"N, 00°04'13"W, Comunitat Valenciana, Spain) was identified by Zierold *et al.* (2007) under the invalid name *T. cancriformis mauritanicus*, based on nucleotide sequencing in

two mtDNA genes. Korn *et al.* (2010) comparing the corresponding COI sequence retrieved from GenBank with those obtained from a representative subset of *T. mauritanicus* samples re-assigned that population to *T. m. simplex*. Interestingly, Korn *et al.* (2010) noted that *T. c. cancriformis* (first lineage) and *T. m. simplex* (second lineage) co-occur on a regional scale in northern Spain, highlighting the need to perform a phylogeographic study allied to a sound morphological re-investigation of northern Spanish populations.

Further analysis by Korn *et al.* in 2010 of main lineages in *Triops mauritanicus* Ghigi, 1921 in the south-western Iberian Peninsula indicated the presence of four more clades. As their data suggested that these clades represent distinct species, Korn *et al.* (2010) described the Iberian lineages as *T. baeticus* Korn, *T. emeritensis* Korn & Pérez-Bote, *T. gadensis* Korn & García-de-Lomas and *T. vicentinus* Korn, Machado, Cristo & Cancela da Fonseca, and reinstated *T. simplex* Ghigi, 1921 to full species status. *T. mauritanicus* Ghigi, 1921 is represented by populations in northern Africa alone (Korn *et al.*, 2006; 2010). Six species are therefore recognized as present in the Iberian Peninsula: five species from the *mauritanicus* lineage plus one species from the *cancriformis* lineage.

Contrasting with their conserved morphology, *Triops* exhibit a wide range of reproductive modes. The ancestral reproductive mode is apparently gonochorism (Pannell, 2002; Zierold *et al.*, 2007; Mathers *et al.*, 2013) with equal sex ratio populations and cross-fertilization necessary to produce viable eggs (Korn *et al.*, 2006). Certain populations lack males and individuals possess ovotestis and have the ability to breed in isolation (Sassaman, 1991; Zaffagnini & Trentini, 1980). In other populations, males and hermaphrodites co-occur in varying frequency, and there are varying levels of self-fertilisation and outcrossing; a system named androdioecy (Pannell, 2002). The inbreeding depression, reduction in effective recombination rates and reduction in effective population size resulting from the deleterious effects of self-fertilisation (Glemin *et al.*, 2006) may be off-set by the benefits of coloniza-

tion advantage and reproductive assurance in a metapopulation (Baker, 1955; see Mathers *et al.*, 2013). On the one hand, the often unpredictable environment of ephemeral ponds where branchiopod populations have strong inter-annual density fluctuations could drive the evolution of androdioecy by ascribing higher fitness to self-fertilising hermaphrodites relative to females (Weeks *et al.*, 2006). On the other hand, considering that *Triops* produce long-lived dormant cysts that survive during adverse periods and also provide the dispersal stage (Fryer, 1988; Longhurst, 1955), evolution towards self-fertile hermaphroditism could both increase the success of populations and its dispersal probabilities.

Triops Iberian populations are considered gonochoric while Central and Northern European populations where males are absent or present in a low proportion, are considered hermaphrodite and/or androdioecious (Zierold *et al.*, 2007; 2009; Korn *et al.*, 2006; Mathers *et al.*, 2013). Some controversy remains regarding the reproductive strategy in some Central Euro-

pean and Italian populations with suggestions of parthenogenesis or a mixture of gonochoric and hermaphroditic reproduction in a German population (Engelmann *et al.*, 1997), and meiotic parthenogenesis and selfing hermaphroditism in some Italian populations (Trentini & Scanabissi, 1979; Zaffagnini & Trentini, 1980). Studies in the last decade have shown that modern parthenogenetic/hermaphrodite/androdioecious lineages of *Triops cancriformis* present in Northern and Central Europe are considered to have derived from those located in glacial Pleistocene refuges after the last glacial maximum; i.e. possibly in southern Iberia, Sardinia, Sicily and areas of mainland Italy and Greece (Korn *et al.*, 2006; Zierold *et al.*, 2007).

Here we report the first record of the tadpole shrimp *Triops cancriformis* (Lamarck, 1801) in rice fields of southern Portugal. Contrasting to the other Iberian populations of *Triops* which are purportedly all gonochoric, males were absent from the sampled populations from the rice fields on the north margin of Sorraia River.

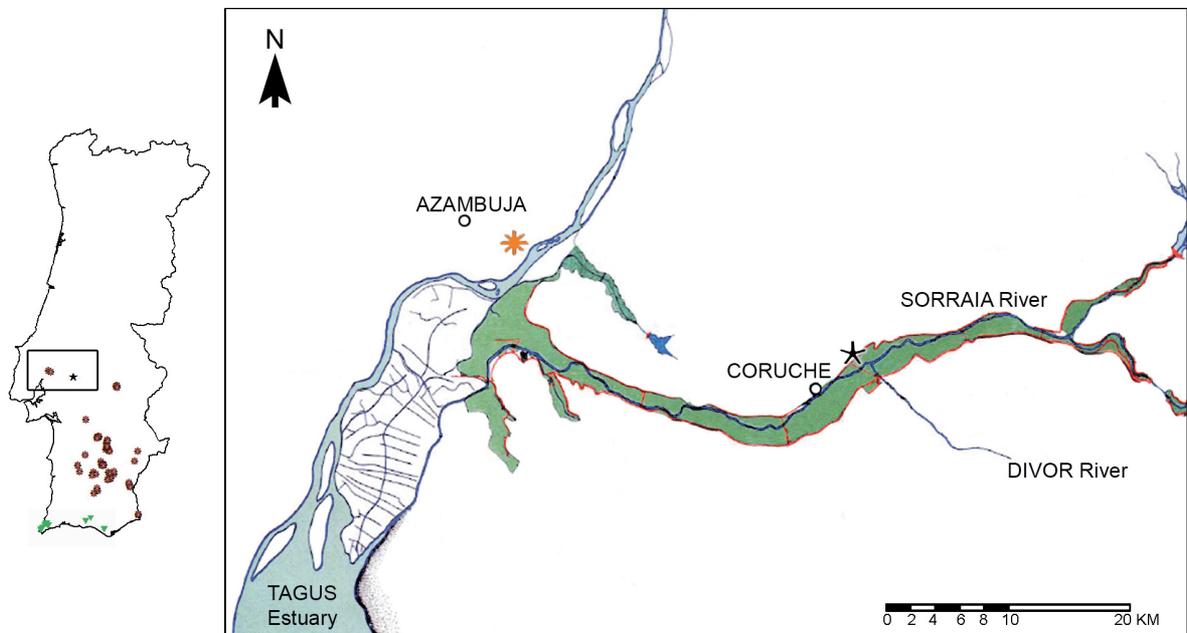


Figure 1. Geographical records for *T. baeticus* (asterisks), *T. cancriformis* (black star) and *T. vicentinus* (triangles) in Portugal and in Vale do Sorraia. Distribution of *T. baeticus* and *T. vicentinus* according to M. Machado, L. Cancela da Fonseca and M. Cristo (unpublished data). *Registos geográficos de T. baeticus* (asteriscos), *T. cancriformis* (estrela negra) e *T. vicentinus* (triângulos) em Portugal e no Vale do Sorraia. Distribuição de *T. baeticus* e *T. vicentinus* segundo M. Machado, L. Cancela da Fonseca e M. Cristo (dados não publicados).

The absence of males suggests a prevailing hermaphrodite or androdioecious sexual system in the populations and we present and discuss two hypotheses for this possible absence of gonochorism. The 1st hypothesis proposes colonization by a single or few selfing hermaphrodites or parthenogenetic individuals, and the 2nd hypothesis considers that *Triops cancriformis* from Vale do Sorraia derived directly from gonochoric populations from the Iberian Pleistocene refuge.

MATERIALS AND METHODS

Study site

The rice fields surveyed in this study are located in the Tagus river basin (38°58'42"N, 8°29'0"W), Portugal, in the alluvial plains of the Sorraia river (Fig. 1). These alluvial plains, dominated by rice fields and irrigation crops, are bordered by open oak forest. The paddies are filled with rainwater from October to March when soil is prepared for the cultivation of rice (*Oriza sativa*). During the period of rice cultivation, from April/May to September/October, the water level in the paddies is controlled through the irrigation system of locks, boxes and channels (Associação de Regantes e Beneficiários do Vale do Sorraia, 2012). The exotic plant *Azolla filiculoides*, the exotic red swamp crayfish *Procambarus clarkii* and mosquitofish *Gambusia holbrooki* were present in the majority of the inspected paddies. Avian fauna, which are recognized as long distance dispersal vectors (Viana *et al.*, 2016) and use rice fields as feeding areas (Elphick, 2010 and references therein), were assessed by walking transects from the outer edge of the fields in direction of the river, with the aid of binoculars. Field collected information was complemented with literature records for the area and information obtained from researchers involved in ringing-recovery programmes for aquatic birds (V. Encarnação and E.D. Galioto). Supplementary information on migratory birds was collected from BirdLife International (2015)

and literature. The avian species feeding in the area are: flamingos *Phoenicopterus roseus* (partially migrant), black-winged stilts *Himantopus himantopus* (migrant) spoonbills *Platalea leucorodia* (migrant), great white egrets *Egretta alba* (syn. *Ardea alba*, migrant), cattle egrets *Bubulcus ibis* (partially migrant), little egrets *Egretta garzetta* (migrant to nomadic), night heron *Nycticorax nycticorax*, (migrant), grey heron *Ardea cinerea* (partially migrant or sedentary), purple heron *Ardea purpurea* (migrant), white stork *Ciconia ciconia* (migrant or sedentary), the northern lapwing *Vanellus vanellus* (migrant) and mallard *Anas platyrhynchos* (sedentary or nomadic).

Sampling and Identification

The rice fields were inspected for the presence of large branchiopods every month from October to May (2007-2016), or during the period when they were filled with rainwater. Flooded expanses within the rice paddies were prospected with dip-nets (0.250 mm pore mesh size) or hand held sieves (1 mm pore mesh size). Large branchiopods were collected in May 2012, in February and April 2014, and March, 2016. After collection, specimens were sorted, anaesthetized with carbonated water and preserved in 70% ethanol for laboratory identification. The morphology of adult *Triops* was studied according to Korn *et al.* (2006) and Longhurst (1955) and specimens were identified following the criteria adopted by Korn *et al.* (2006). The recorded biological data were: (i) telson length ratio calculated as the ratio of furcal spine length to the distance between furcal spine tip and the anterior-lateral edge of the telson (see figure 1 in Korn *et al.*, 2006); carapace length (measured at carina level); (ii) number and type of carina spines; (iii) number of apodous abdominal segments, and (iv) sex. Morphological females present a brood pouch at each side of the body that results from a modification of the endopodite and the exopodite of each branch of the 11th pair of thoracopods.

RESULTS

Morphological observation of the sampled 40 specimens revealed that 28 were juveniles and 12 were adults (Table 1). It was possible to recognize the presence of a pair of brood pouches in all individuals, which proved them to be morphological females. Males have not been recorded, which may indicate that the observed population is either androdioecious or made of hermaphrodite or parthenogenetic individuals.

The 12 adults exhibited small furcal spines as shown by a lower telson length ratio (0.21-0.29; mean value: 0.25) than that of the *mauritanicus* lineage (≥ 0.38) in the Iberian Peninsula (Table 1), with the exception of *T. simplex* (see Introduction). This morphological characteristic together with the sex ratio of the sample allowed us to unambiguously identify the specimens as *Triops cancriformis*. The specimens were compared with *T. baeticus* belonging to the *mauritanicus* lineage of *Triops*, and previously collected in

Table 1. Morphological characteristics of *Triops* (Vale do Sorraia) and diagnostic characteristics of *T. cancriformis* and of the endemic Iberian species of *T. mauritanicus* lineage as defined by Korn *et al.* (2006) and Longhurst (1955). Carina spines are counted from the distal spine; carapace length is measured at carina level in both adults (Ad) and juveniles (Juv); telson length ratio characterizes the length of furcal spines (see Methods). All observed specimens, including juveniles, were females (F); sex was determined based on morphology. States of the most important character for species identification are presented in bold. *Características morfológicas de Triops (Vale do Sorraia) e características diagnosticantes de T. cancriformis e das espécies Ibéricas endêmicas da linhagem T. mauritanicus, tal como definidas por Korn et al. (2006) e Longhurst (1955). Os espinhos da carena são contados a partir do espinho distal; o comprimento da carapaça é medido ao nível da carena em adultos (Ad) e juvenis (Juv); o rácio do comprimento do telson caracteriza o comprimento dos espinhos da furca (ver Métodos). Todos os espécimens observados, incluindo os juvenis, eram fêmeas (F); o sexo foi determinado com base na morfologia. Os estados do carácter mais relevante para a identificação das espécies estão em negrito.*

Characteristics exhibited by Sorraia individuals (Ind.)										
Ind. (n = 40)	Sex	Carina spines		Telson length ratio		Number of apodous segments in females			Carapace Length (mm)	
		Type	N.er	Median	Range	Mean ± SD	Range	Mean ± SD		95% confidence intervals
12 Adults	F	minute	0-13	4	0.21-0.29	0.25 ± 0.023	4.5 - 6.0	4.96 ± 0.547	4.653 - 5.272	16.6-38 (12 Ad) 4 - 9 (28 Juv)
<i>Triops cancriformis</i>										
Korn <i>et al.</i> , 2006	Mostly small		0-30, often 0-4		Haplotype group means: 0.21-0.27		–	–		4.0 - 5.6 (a) 4.0 - 6.8 (b)
Longhurst, 1955a	Small		0-10, often 2-3		size of furcal spines: small		4 - 6	–	–	
<i>Triops mauritanicus</i>										
Endemic Iberian Species Korn <i>et al.</i> , 2006, 2010	Gradient sized, largest next to distal spine		Numerous, often > 50		Haplotype group means: \geq 0.38-0.44 (c)		–	–		4.7 - 6.2 (c)
Longhurst, 1955	Strong, largest sub-equal to distal spine		–		Strong furcal Spines		5-7	–	–	

(a, b, c) Values inferred from Figure 5-A and 5-B in Korn *et al.* (2006) considering (a) only nongonochoric populations, (b) including the gonochoric population from Espolla, and (c) Iberian populations of the *mauritanicus* complex.

Azambuja, approximately 40 km northwest of the rice fields sampled in this study (Fig. 2). It is also interesting to note the minute carina spines

anterior to the distal spine observed in the specimens identified as *T. cancriformis* relative to the larger spines observed in *T. baeticus*.

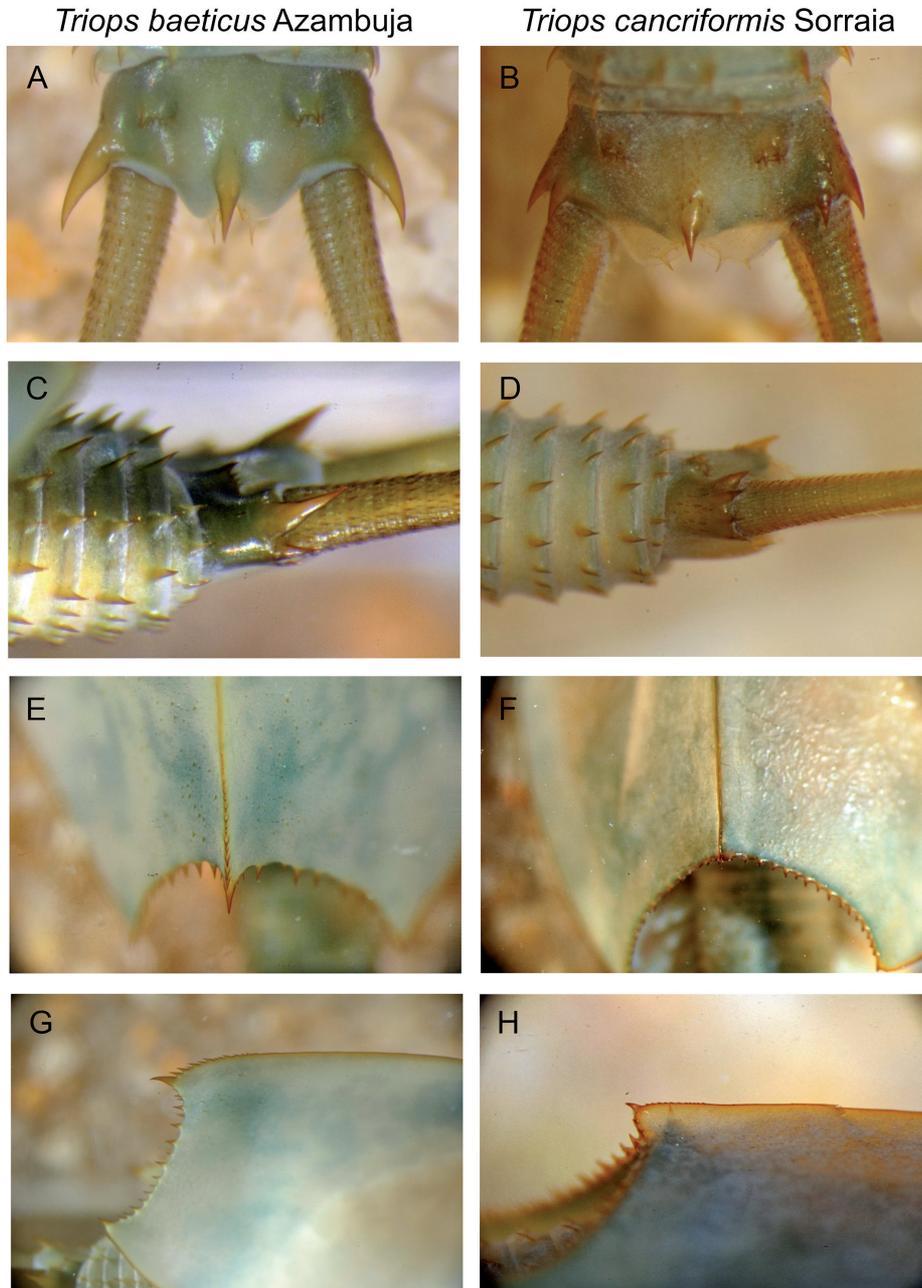


Figure 2. Morphological characteristics of *T. cancriformis* from Vale do Sorraia compared with those of *T. baeticus* from Azambuja: Posterior part of the body, dorsal view (A, B); posterior part of the body, lateral view (C, D); posterior part of carapace carina, dorsal view (E, F); posterior part of carapace carina, lateral view (G, H). *Comparação das características morfológicas de T. cancriformis do Vale do Sorraia com as de T. baeticus da Azambuja: parte posterior do corpo, visão dorsal (A, B); parte posterior do corpo, visão lateral (C, D); parte posterior da carena da carapaça, visão dorsal (E, F); parte posterior da carena da carapaça, visão lateral (G, H).*

DISCUSSION

The *Triops* population inhabiting the rice fields of Vale do Sorraia could be unambiguously ascribed to the species *Triops cancriformis* both by morphological analysis and by the observed sex ratio that suggests a nongonochoric reproductive type. The absence of males in a sample of 40 individuals strongly suggests that these either reproduce by selfing hermaphroditism or automictic parthenogenesis, or by androdioecy (Zierold *et al.*, 2009; Mathers *et al.*, 2013). These are the most widespread reproductive modes in *T. cancriformis*, which has only one known gonochoric population i.e. that of the Espolla temporary pond in the eastern Iberian Peninsula (Korn *et al.*, 2006; Boix *et al.*, 2002). Scanabissi *et al.* (2005) reported the occurrence of a population with equal sex ratio in Corbières, southern France referenced to Nourisson & Thiery (1988). This is probably a mistake because Nourisson & Thiery do not make such claim in their article, and as noted by Korn *et al.* (2006), “Thiéry (1988) only reported one population of *Triops* (*T. c. cancriformis*) in that region (Opul temporary pond), from which Knoepffler (1978) reported the complete absence of males in a sample of 300 specimens”. Zierold *et al.* (2007) noted that the population from Espolla (and other from El Puig, Spain) shared haplotype H1 with five *T. c. cancriformis* individuals from four different populations in Germany, and concluded that the populations that “show different reproductive modes, are not represented by distinct monophyletic lineages”.

The Sorraia population also presents morphological similarity to *T. simplex*. In fact, morphological characters distinguishing *T. cancriformis* from *T. simplex* are not known (Korn *et al.*, 2006) and in the past, populations of *T. cancriformis* have been attributed to the former *T. c. simplex*, considered a sub-species of *T. cancriformis* at the time (Alonso, 1985; 1996; Boix *et al.*, 2002; Zierold *et al.*, 2007). Only recently the classification based on genetic sequences has been established for these species by Korn *et al.* (2006; 2010). *T. simplex* is present in Eastern Spain with a population in Ares del Maestre

(Comunitat Valenciana), a location well within the distribution area of *T. cancriformis* in the Iberian Peninsula, and that may explain why some authors disagree on the taxonomic identity of *Triops* populations in that region (see Korn *et al.*, 2006; 2010 and Zierold *et al.*, 2007). Nevertheless, all reported populations of *T. simplex* are gonochoric (Longhurst, 1955; Thiéry, 1996; Korn *et al.*, 2006; Hassan, 2015) which enables the assignment of the Sorraia population to the species *Triops cancriformis* (where non-gonochoric populations are known) without performing a genetic study.

The presence of this hermaphroditic/parthenogenic or androdioecious population of *T. cancriformis* in the Iberian Peninsula contrasts with the gonochoric nature of that from Espolla and makes its finding all the more interesting in evolutionary terms. The gonochoric reproductive mode is considered to be the ancestral condition (Zierold *et al.*, 2007 and references therein). Korn *et al.* (2006) hypothesized that a nongonochoric lineage has originated from a gonochoric ancestor located in Spain that would form parthenogenetic/hermaphroditic and androdioecious populations. This nongonochoric lineage would further diverge while spreading eastwards during postglacial periods in the Pleistocene, namely to mainland Italy and Israel (see Korn *et al.*, 2006 and references therein). These two areas should have not lost *Triops* populations during the Ice Ages, and like the Iberian Peninsula could have offered a Pleistocene refuge to ancient lineages. The parthenogenetic/hermaphrodite/androdioecious lineages present nowadays in Northern and Central Europe are considered to have derived from those glacial Pleistocene refuges after the last Ice Age (Korn *et al.*, 2006; Zierold *et al.*, 2007).

Consequently, the question arises concerning the origin of the population recently found in Sorraia rice fields. Has it derived directly from the assumed Iberian Pleistocene refuge? Or has it resulted from a recent colonization from non-Iberian populations? To discuss the first hypothesis, we have to consider that the only confirmed *T. cancriformis* population in Iberia (from Espolla, Spain) is gonochoric. Recent-

ly, other population of *Triops* was recorded by Sahuquillo (2012) at Rebasador (39°49'31"N, 00°39'51"W, Comunitat Valenciana, eastern Spain), which is located 387 km SE from Espolla and 83km SE to Ares del Maestre. This population is probably gonochoric since 7 males were observed in a sample of 12 specimens. The morphology of the individuals was consistent with both *T. cancriformis* and *T. simplex* (see Table 4.2 in Sahuquillo, 2012) and the unambiguous identification of the Rebasador population requires a genetic study. If this population belongs indeed to *T. cancriformis*, it should have derived directly from the gonochoric ancestor located in Spain, as hypothesized by Korn *et al.* (2006). Considering that the population from El Puig (Comunitat Valenciana, Eastern Spain, which is ca. 58 Km from Rebasador) has the same haplotype than that of Espolla (Zierold *et al.* 2007), it would be relevant to genetically analyse the three populations plus the population from Sorraia to clarify how they are related. Unhappily the reproductive mode of the El Puig population is not mentioned by Zierold *et al.* (2007). If this population and that from Sorraia are both nongonochoric and share the same haplotype with the population from Espolla, they may both derive from the primitive nongonochoric lineage, as hypothesized by Korn *et al.* (2006) (see above). If only the population from Sorraia is nongonochoric and share the haplotype with the population from Espolla, then the population from Sorraia may be the only known population derived from the primitive nongonochoric populations from the Iberian Pleistocene refuge. As there is no evidence of long-distance dispersal for gonochoric populations (Korn *et al.*, 2006), the probability that the Portuguese population has derived directly from gonochoric populations from the Iberian Pleistocene refuge is quite low.

The potentially high dispersal abilities of *Triops* spp. diapausing cysts and the reproductive mode inferred for the Portuguese population seems to favour the 2st hypothesis of recent colonization from non-Iberian populations, as a single selfing hermaphrodite or parthenogenetic individual can theoretically originate a new population while a minimum of two cysts

originating individuals of different sex would be required to start a population (Korn *et al.*, 2006; Zierold *et al.*, 2007). The hermaphrodite or parthenogenetic population would also have a greater reproductive potential (i.e. all individuals lay eggs) relative to gonochoric populations composed of roughly 50% males (Zaffagnini & Trentini, 1980). The founding cyst(s) could result from long-distance passive dispersal through external transport by migratory birds. Rice fields are highly frequented by migrant waterbirds, functioning normally as feeding areas (Elphick, 2010 and references therein). In the Sorraia rice pads several bird species could be observed including flamingos, black-winged stilts, spoonbills (V. Encarnaç o, pers. obs.), great white egrets and northern lapwings. Except for the spoonbill, these species use or visit the rice fields from Camargue (France, Tourenq *et al.*, 2001; 2003). National ringing-recovery programs for aquatic birds have shown that *E. alba* comes from the central Europe (mainly Hungary) while the other species arrive from Camargue and/or from the mainland of Italy (V. Encarnaç o, pers. com.). At Camargue, the occurrence of maleless populations of *T. cancriformis* is known from 1951 (Zaffagnini & Trentini, 1980; Pont & Vaquer, 1986). In mainland of Italy, where no gonochoric populations of this species have been found (Korn *et al.*, 2006; Mantovani *et al.*, 2008), *T. cancriformis* is known to occur in a considerable number of rice fields (Zaffagnini & Trentini, 1980; Golfieri & Bonato, 2014). For some of these populations, males have been reported as absent, while for the major part of populations the sex system is unknown (Golfieri & Bonato, 2014). Gill *et al.* (2005) has confirmed the ability of an aquatic bird species to perform non-stop flights as long as 11 000 km. Flamingos, black-winged stilts and spoonbills, coming from the South of France or from the Italian rice fields to those at Vale do Sorraia, are able to cover the distance through one nonstop flight (V. Encarnaç o, pers. com.). Hence they can act as vectors for the colonization of those Portuguese rice fields by a nongonochoric population of *T. cancriformis* from Central Europe, when transporting cysts attached to feathers or to their legs (Viana *et al.*, 2016).

The other possible vector for this colonization is the rice seed that is used for production in the Vale do Sorraia. Seeds are routinely imported from Italy, which is the main rice producer in Europe, by rice growers associations and companies e.g. Lusosem that imports seeds from Almo Company (Novara, Italia) (Lusosem, 2010; A. S. Almeida, pers. com.). Some dry *Triops* cysts can eventually be unwittingly transported with those seeds as it is known to occur with other crustaceans inhabiting temporary freshwater habitats (McKenzie & Moroni, 1986) and with the main introduced weeds in rice fields (Vasconcelos *et al.*, 1999).

Other possibility for the origin of the Portuguese population would be a modification of the second hypothesis i.e. it derives from the *T. cancriformis* populations inhabiting rice fields of northern and eastern Spain, which had themselves originated from Central and Northern European populations. Indeed, *Triops* has been recorded in Spanish rice fields since the early 20th century (Arévalo, 1915; Font de Mora, 1923; Bolivar, 1926; all cited in Boix, 2002). Later, this taxon was reported from several other rice fields from northeastern to eastern Spain (Forés *et al.*, 1986; Boix, 2002). Some of those populations should be nongonochoric as stated by Longhurst (1955). Populations from eastern Spain (Comunitat Valenciana) previously reported as *T. cancriformis simplex* by Alonso (1985) may belong to *T. cancriformis* as suggested by the results of Zierold *et al.* (2007). These authors identified 2 haplotypes of *T. cancriformis* from Ullal de Baldovi, which is completely surrounded by Albufera de València rice fields. Those haplotypes are closer to some haplotypes from Central and northern Europe than to the supposedly primitive haplotype from Espolla. This strongly suggests that the *T. cancriformis* population from Albufera de València rice fields has originated from Central and northern Europe populations through long distance dispersal recent events. Cysts from the Spanish populations inhabiting rice fields could have been transported by aquatic birds and then disperse and colonize the Sorraia rice fields. Internal transport by birds is also a possibility. The resistant stages of some

invertebrate are viable after 26 h retention in the gut of mallards (Green & Figuerola, 2005). Considering a distance of approximately 750 km between Albufera de València and Sorraia rice fields where mallards have been observed (Albufera de València, 2009) and that mallards can fly up to 110 km/h (Wikipedia, 2016), these birds may disperse *Triops* resistant eggs.

The origin of the population of *T. cancriformis* inhabiting the rice fields of Sorraia can only be clarified by multilocus phylogenetic analysis of *Triops* specimens from rice field populations from Camargue, Italy and the Iberian Peninsula. An exhaustive genetic study of the *Triops* populations known hitherto in the northern and eastern Spain and previously identified as *T. c. simplex*, integrating information on the sexual systems of the populations, is imperative to fully understand the distribution patterns of both *T. cancriformis* and of the several other *Triops* species that are known to coexist in the Iberian Peninsula.

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REFERENCES

- ALBUFERA DE VALÈNCIA. 2009. Avifauna. Available at: <http://www.albufera.com/parque/book/export/html/6261> (Accessed 1.10.2016; in Spanish).
- ALONSO, M. 1985. A survey of the Spanish euphyllopoda. *Miscellània Zoològica*, 9: 179–208.
- ALONSO, M. 1996. *Crustacea, Branchiopoda*. In: *Fauna Ibérica*, vol. 7. (M. A. Ramos *et al.*, Ed.).

- Museo Nacional de Ciencias Naturales, CSIC, Madrid. DOI:10.1163/193724097X00189
- ASSOCIAÇÃO DE REGANTES E BENEFICIÁRIOS DO VALE DO SORRAIA. 2012. Agricultural map. Available at: http://www.arbvs.pt/carta_agricola (Accessed 22.10.2016; in Portuguese).
- BAKER, H.G. 1955. Self-compatibility and establishment after 'long-distance' dispersal. *Evolution*, 9: 347–349. DOI:10.2307/2405656
- BIRDLIFE INTERNATIONAL. 2015. Species. Available at: <http://www.birdlife.org/datazone/species> (Accessed at 22.10.2016).
- BOIX, D. 2002. Aportació al coneixement de la distribució d'anostracis i notostracis (Crustacea: Branchiopoda) als Països Catalans. *Butlletí de la Institució Catalana d'Història Natural*, 70: 55–71.
- BOIX, D., J. SALA & R. MORENO-AMICH. 2002. Population dynamics of *Triops cancriformis* (Crustacea: Branchiopoda: Notostraca) of the Espolla temporary pond in the northeastern Iberian peninsula. *Hydrobiologia*, 486: 175–183. DOI:10.1023/A:1021350802144
- CARVALHO, R.N. 1944. Catálogo da coleção de invertebrados de Portugal existentes no Museu Zoológico da Universidade de Coimbra. Crustacea. *Memórias e Estudos do Museu Zoológico da Universidade de Coimbra*, 160: 1–15.
- ELPHICK, C. 2010. Why Study Birds in Rice Fields?. *Waterbirds* 33 (Special Publication 1): 1–7. DOI:10.1675/063.033.s101
- ENGELMANN, M., T. HAHN & G. HOHEISEL 1997. Ultrastructural characterization of the gonads of *Triops cancriformis* (Crustacea, Notostraca) from populations containing both females and males: no evidence for hermaphroditic reproduction. *Zoomorphologie*, 117: 175–180. DOI:10.1007/s004350050042
- FORÉS, E., M. MENÉNDEZ & F.A. COMÍN. 1986. Contribución al conocimiento de crustáceos y rotíferos del Delta del Ebro. *Miscellània Zoològica*, 10: 105–111.
- FRYER, G. 1988. Studies on the functional morphology and biology of the Notostraca (Crustacea: Branchiopoda). *Philosophical Transactions of the Royal Society of London, Series B*, 321: 27–124. DOI:10.1098/rstb.1988.0091
- GAND, G., J. GARRIC & J. LAPEYRIE. 1997. Biocénoses à triopsidés (Crustacea, Branchiopoda) du Permien du bassin de Lodève (France). *Geobios*, 30: 673–700. DOI:10.1016/S0016-6995(97)80157-X
- GHIGI, A. 1921. Ricerche sui Notostraci di Cirenaica e di altri paesi del Mediterraneo. *Atti della Società italiana di scienze naturali e del Museo civico di storia naturale di Milano*, 60: 161–188.
- GILL, R.E., T. PIERSMA, G. HUFFORD, R. SERVRANCKX & A. RIEGEN. 2005. Crossing the ultimate ecological barrier: evidence for an 11000-km-long nonstop flight from Alaska to New Zealand and eastern Australia by bar-tailed godwits. *The Condor*, 107:1–20. DOI:10.1650/7613
- GLEMIN, S., E. BAZIN & D. CHARLESWORTH. 2006. Impact of mating systems on patterns of sequence polymorphism in flowering plants. *Proceedings of the Royal Society B Biological Sciences*, 273: 3011–3019. DOI:10.1098/rspb.2006.3657
- GOLFIERI, B. & L. BONATO. 2014. Recent distribution of *Triops cancriformis* in northern Italy (Crustacea: Notostraca). *Bollettino del Museo Civico di Storia Naturale di Verona*, 38: 127–131.
- GREEN, A.J., & J. FIGUEROLA. 2005. Recent advances in the study of long-distance dispersal of aquatic invertebrates via birds. *Diversity and Distributions*, 11: 149–156. DOI:10.1111/j.1366-9516.2005.00147.x
- GUTHÖRL, P. 1934. Die Arthropoden aus dem Carbon und Perm des Saar-Nahe-Pfalz-Gebietes. *Im Vertrieb bei der Preußischen Geologischen Landesanstalt*, 44(4): 1–279.
- HASSAN, M.M.S.M. 2015. The large branchiopod, *Triops cancriformis simplex* (Gihl, 1921) (Crustacea: Notostraca) in temporary rain pools, recorded for the first time in Taif-KSA. *The Journal of Basic and Applied Zoology*, 69: 1–9. DOI:10.1016/j.jobaz.2015.04.006
- KORN, M. & A.K. HUNDSDOERFER. 2006. Evidence for cryptic species in the tadpole shrimp *Triops granarius* (Lucas, 1864) (Crustacea: Notostraca). *Zootaxa*, 1257: 57–68.
- KORN, M., F. MARRONE, J.L. PÉREZ-BOTE, M. MACHADO, M. CRISTO, L. CANCELADA FONSECA & A.K. HUNDSDOERFER. 2006. Sister species within the *Triops cancriformis* lineage (Crustacea, Notostraca). *Zoological Scripta*, 35(4): 301–322. DOI:10.1111/j.1463-6409.2006.00230.x
- KORN, M., A.J. GREEN, M. MACHADO, J. GARCÍA-DE-LOMAS, M. CRISTO, L. CANCELADA FONSECA, D.A. FRISCH, J.L. PÉREZ-BOTE & A.K. HUNDSDOERFER. 2010. Phylogeny, molecular ecology and taxonomy of southern Ibe-

- rian lineages of *Triops mauritanicus* (Crustacea: Notostraca). *Organisms Diversity and Evolution*, 10(5): 409–440. DOI:10.1007/s13127-010-0026-y
- KORN, M., N. RABET, H.V. GHATE, F. MARRONE, A.K. HUNDSDOERFER. 2013. Molecular phylogeny of the Notostraca. *Molecular Phylogenetics and Evolution*, 69(3): 1159–1171. DOI: 10.1016/j.ympev.2013.08.006
- KORN, M. & A.K. HUNDSDOERFER. 2016. Molecular phylogeny, morphology and taxonomy of Moroccan *Triops granarius* (Lucas, 1864) (Crustacea: Notostraca), with the description of two new species. *Zootaxa*, 4178(3): 328–346. DOI:10.11646/zootaxa.4178.3.2
- LONGHURST, A.R. 1955. A review of the Notostraca. *Bulletin of the British Museum (Natural History)*, 3, 1–57. DOI:10.5962/bhl.part.4119
- LUSOSEM. 2010. Rice. Available at: <http://www.luso-sem.pt/arroz.html/A=14> (Accessed 22.10.2016; in Portuguese).
- MACHADO, M. 2014. Amendment to the register of the specimen n° 212 in Carvalho, R. N., 1944. Catálogo da coleção de invertebrados de Portugal existentes no Museu Zoológico da Universidade de Coimbra. *Crustacea. Memórias e Estudos do Museu Zoológico da Universidade de Coimbra*, 160: 1–15.
- MANTOVANI, B., M. CESARI, A. LUCHETTI & F. SCANABISSI. 2008. Mitochondrial and nuclear DNA variability in the living fossil *Triops cancriformis* (Bosc, 1801) (Crustacea, Branchiopoda, Notostraca). *Heredity*, 100: 496–505. DOI:10.1038/hdy.2008.3
- MATHERS, T.C., R.L. HAMMOND, R.A. JENNER, T. ZIEROLD, B. HÄNFLING & A. GÓMEZ. 2013. High lability of sexual system over 250 million years of evolution in morphologically conservative tadpole shrimps. *BMC Evolutionary Biology*, 13: 30. DOI:10.1186/1471-2148-13-30
- McKENZIE, K.G. & A. MORONI. 1986. Man as an agent of crustacean passive dispersal via useful plants – exemplified by Ostracoda ospiti esteri of the Italian ricefields ecosystem – and implications arising therefrom. *Journal of Crustacean Biology*, 6(2): 181–198. DOI:10.2307/1547979
- MEUSEL, F. & M. SCHWENTNER. 2016. Molecular and morphological delimitation of Australian *Triops* species (Crustacea: Branchiopoda: Notostraca) – large diversity and little morphological differentiation. *Organisms, Diversity & Evolution*, DOI:10.1007/s13127-016-0306-2. DOI:10.1007/s13127-016-0306-2.
- NOURISSON, M. & A. THIERY. 1988. Introduction à la systématique des organismes des eaux continentales françaises. 9. Crustacés Branchiopodes (Anostracés, Notostracés, Conchostracés). *Bulletin mensuel de la Société Linnéenne de Lyon*, 57: 75–95 and 104–135. DOI:10.3406/linly.1988.10826
- PANNELL, J.R. 2002. The evolution and maintenance of androdioecy. *Annual Review of Ecology and Systematics*, 33: 397–425. DOI:10.1146/annurev.ecolsys.33.010802.150419
- PONT, D. & A. VAQUER. 1986. Influence du phyllo-pode *Triops cancriformis* (Bosc.) sur la biocénose des rizières de Camargue. *Acta Oecologica*, 7: 75–88.
- SAHUQUILLO, M. 2012. *Distribution and diversity of crustacean communities in Mediterranean ponds (Eastern Spain): relationship with climatic and limnological factors*. Ph.D. Thesis. University of Valencia, Spain.
- SASSAMAN, C. 1991. Sex ratio variation in female-biased populations of Notostracans. *Hydrobiologia*, 212: 169–179. DOI:10.1007/978-94-011-3366-1_19
- SCANABISSI, F., E. EDER & M. CESARI. 2005. Male occurrence in Austrian populations of *Triops cancriformis* (Branchiopoda, Notostraca) and ultrastructural observations of the male gonad. *Invertebrate Biology*, 124: 57–65. DOI:10.1111/j.1744-7410.2005.1241-07.x
- THIÉRY, A. 1996. Large Branchiopods (Crustacea: Anostraca, Notostraca, Spinicaudata, Laevicaudata) from temporary inland waters of the Arabian Peninsula. *Fauna of Saudi Arabia*, 15: 37–98.
- TOURENQ, C., R.E. BENNETTS, H. KOWALSKI, E. VIALET, J.-L. LUCCHESI, Y. KAYSER & P. ISENMANN. 2001. Are ricefields a good alternative to natural marshes for waterbird communities in the Camargue, southern France? *Biological Conservation*, 100(3): 335–343. DOI:10.1016/S0006-3207(01)00037-4
- TOURENQ, C., N. SADOUL, N. BECKA, F. MESLÉARD & J.-L. MARTIN. 2003. Effects of cropping practices on the use of rice fields by waterbirds in the Camargue, France. *Agriculture, Ecosystems and Environment*, 95: 543–549. DOI:10.1016/S0167-8809(02)00203-7
- TRENTINI, M. & F. SCANABISSI. 1979. Ultrastructural observations on the oogenesis of *Triops can-*

- cancriformis* (Crustacea, Notostraca). *Cell and Tissue Research*, 194: 71–77. DOI:10.1007/BF00209234
- TRUSHEIM, F. 1938. Triopsiden (Crust. Phyll.) aus dem Keuper Frankens. *Palaeontologische Zeitschrift*, 19: 198–216.
- VANSCHOENWINKEL, B., T. PINCEEL, M.P.M. VANHOVE, C. DENIS, M. JOCQUE, B.V. TIMMS, B.V., BRENDONCK L. 2012. Toward a Global Phylogeny of the “Living Fossil” Crustacean Order of the Notostraca. *PLoS ONE* 7: e34998. DOI:10.1371/journal.pone.0034998
- VASCONCELOS, T., M. TAVARES & N. GASPAR. 1999. Aquatic plants in the rice fields of the Tagus Valley, Portugal. *Hydrobiologia*, 415: 59–65. DOI:10.1023/A:1003873315570
- VIANA, D.S., L. SANTAMARÍA & J. FIGUEROLA. 2016. Migratory Birds as Global Dispersal Vectors. *Trends in Ecology & Evolution*, 31(10): 763–775. DOI:10.1016/j.tree.2016.07.005
- WEEKS, S.C., T.F. SANDERSON, S.K. REED, M. ZOFKOVA, B. KNOTT, U. BALARAMAN, G. PEREIRA, D.M. SENYO & W.R. HOEH. 2006. Ancient androdioecy in the freshwater crustacean *Eulimnadia*. *Proceedings of the Royal Society B: Biological Sciences*, 273: 725–734. DOI: 10.1098/rspb.2005.3370
- WIKIPEDIA. 2016. Lavanco. Available at <https://gl.wikipedia.org/wiki/Lavanco> (Accessed 1.10.2016).
- ZAFFAGNINI, F. & M. TRENTINI. 1980. The distribution and reproduction of *Triops cancriformis* (Bosc) in Europe (Crustacea Notostraca). *Monitore Zoologico Italiano (N S)*, 14: 1–8. DOI:10.1080/00269786.1980.10736342
- ZIEROLD, T., B. HANFLING, & A. GÓMEZ. 2007. Recent evolution of alternative reproductive modes in the ‘living fossil’ *Triops cancriformis*. *BMC Evolutionary Biology*, 7: 161. DOI:10.1186/1471-2148-7-161
- ZIEROLD, T., J. MONTERO-PAU, B. HÄNFLING & A. GÓMEZ. 2009. Sex ratio, reproductive mode and genetic diversity in *Triops cancriformis*. *Freshwater Biology*, 54: 1392–1405. DOI:10.1111/j.1365-2427.2009.02191.x

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