

Contribution to the knowledge of the freshwater Ostracoda fauna in continental Portugal, with an updated checklist of Recent and Quaternary species

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ABSTRACT

Ostracods have a ubiquitous distribution, a high diversity of life history strategies and an extensive fossil record. These characteristics make the group a useful study tool in multiple disciplines. In Portugal, very few studies on recent freshwater ostracods have been published. This is particularly important given the need for an accurate database of both autoecology and geographical distribution of the extant species for a correct use of these crustaceans as environmental proxies. Six temporary ponds were periodically sampled during the wet season 2005/2006, in the natural park "Parque Natural do Sudoeste Alentejano e Costa Vicentina" (PNSACV) north of Vila Nova de Milfontes, SW Portugal. The relationship between ostracod assemblages and habitat features was analysed. In parallel, to assess relative ostracod species richness and diversity, faunistic results are also presented based on a regional (Alentejo) survey carried out during the same season. The semi-permanent nature of the study area in PNSACV may contribute to the observed relative high ostracod richness. Yet other environmental factors, such as pH and pond hydroperiod variation, may restrict the local ostracod species distribution and its abundance. The most abundant and/or common ostracod species were eurytopic forms: *Cypridopsis vidua*, *Cypris bispinosa* and *Herpetocypris chevreuxi*. A total of 14 species was identified, of which three named and one most probably undescribed candonid species new to Portugal: *Bradleycypris obliqua*, *C. bispinosa*, *Paralimnocythere psammophila* and *Fabaeformiscandona* sp. ex gr. *fabaeformis*. In the regional survey nine species were identified. *Heterocypris* sp., *Ilyocypris* aff. *lacustris* and *Tonnacypris lutaria* were recorded in the regional survey but not present in PNSACV, with the latter two also new in Portugal. To favour further faunistic and ecological research on ostracods in Portugal, a distributional checklist of Recent and Quaternary freshwater inland Ostracoda known from Portuguese mainland is given.

Key words: autoecology, distribution check-list, hydroperiod variation, temporary ponds

1. INTRODUCTION

The Ostracoda are small bivalved crustaceans abundant in nearly all non-marine and marine habitats, but also found in semi-terrestrial environments (Cohen & Morin 1990; Holmes & Chivas 2002). The taxon exhibits a high taxonomical diversity and an extensive fossil record, product of an easily fossilizable calcitic bivalve carapace (Moore 1961). Moreover, an impressive diversity of life history strategies and reproductive modes has been reported, in particular within the non-marine ostracods (Chaplin *et al.* 1994; Butlin *et al.* 1998). Their narrow environmental preferences (e.g., Carbonel *et al.* 1988) define them as potential bioindicators for monitoring of recent freshwater conditions (e.g., Roca *et al.* 2000; Külköylüoğlu 2004) and excellent proxies of past environmental changes (Carbonel *et al.* 1988). Given these unique characteristics, the group provides an excellent study tool in multidisciplinary disciplines as (palaeo)ecology or evolutionary biology.

Although the study of ostracods was initiated in the 18th century (Oertli 1982), regionally only the Holarctic fauna of freshwater ostracods is considered reasonably well documented (Meisch 2000; Martens *et al.* 2008).

Still, Portugal is an exception. The present study aims at extending the knowledge on Portuguese freshwater ostracod fauna.

The earliest known evidence of freshwater ostracods in mainland Portugal is that of Petkovski (1963; 1964) with reference to two species from mainland Portugal, unfortunately not providing any geographical details. Subsequently, Paulo (1969) recorded three freshwater species from the Douro region, North Portugal, one of which (*Herpetocypris mateusorum*) new to science. Much of the knowledge on the recent freshwater ostracod fauna in mainland Portugal results from the work carried out by Paulo & Moutinho (1983). These records are based on surveys carried out within Portugal (continent and islands), and extending from fresh and brackish waters to marine habitats. The article gives a list of 23 freshwater species with geographical and some ecological details. More recently González-Mozo *et al.* (1996) in a taxonomic revision of the genus *Herpetocypris* provided new records for four species of this genus in two regions of Portugal. In the same year, Baltanás *et al.* (1996) released a faunistic list of recent freshwater ostracods occurring in the Iberian Peninsula, including continental Portugal. Yet, all records but one

within the list result from the previously published data, little extending our knowledge of the Portuguese fauna. The latest published papers including new records of Recent fresh- and brackish-water ostracod species for Portugal were that of Cearreta *et al.* (2003), Cabral *et al.* (2006), Hilbich *et al.* (2008) and Loureiro *et al.* (2009).

Horne (2004) in the Fauna Europaea database recapitulated all records from continental Portugal and provided a species list of 27 inland freshwater ostracod species although the record of *Dolerocypris sinensis* (Sars 1903) is most probably erroneous (C. Meisch, pers. comm.). The five species of the families Leptocytheridae and Loxoconchidae plus *Cyprideis torosa* (Jones 1859), classified as brackish and/or marine forms, were not considered in the present work. More information on these species may be found in recent studies on fossil and recent material in mainland Portugal (e.g., Hindson & Andrade 1999; Hindson *et al.* 1999; Cabral *et al.* 2006; Loureiro *et al.* 2009). The record of 26 species for mainland Portugal is modest if compared to that of mainland Spain with 80 recent inland freshwater species (Horne 2004), or with those of other European countries of similar geographical area as Austria (81 species) or Hungary (86 species) (Meisch 2000). Continual work in mainland Portugal is, therefore, required for a real picture of the freshwater ostracod fauna, especially in view of the work known from Spain. Such a study seems even more justified by the fact that, among the species known to occur in continental Portugal, four have not (yet) been recorded in Spain.

Continental Portugal, given its geographical position and geophysical features, is characterised by dry summers and moderate rainy winters with natural large permanent water bodies virtually absent (Ribeiro & Lautensach 1988). Therefore, temporary pools play an essential role as repositories of a diverse and valuable genetic heritage, with Temporary Mediterranean Ponds classified as a priority habitat by the European Directive 92/43/EEC (Habitats Directive, Annex I, Council Directive 92/43/EEC). The freshwater ostracods are one of the most characteristic and abundant group of meiobenthic invertebrates in lentic shallow waters (Giere 2009). In this work we present data collected in six temporary ponds monitored over one hydroperiod in the natural park "Parque Natural do Sudoeste Alentejano e Costa Vicentina" (PNSACV), Baixo Alentejo, Portugal. The sampling area was chosen because of its relatively limited human occupation, easy field accessibility and presence of large depressed zones favourable to keep frequent shallow water bodies.

Possible relationships between habitat features and ostracod assemblage dynamics were analysed. To assess the relative ostracod species richness of the study area, located in a protected area, a parallel survey was carried out in temporary ponds in the Alentejo region (Alto and Baixo Alentejo). The pooled results and species list

produced were compared to previously published data for continental Portugal.

2. MATERIAL AND METHODS

2.1. Regional settings

2.1.1. Natural park PNSACV

The PNSACV is located on the SW coastline of Portugal. The park is 110 km long with an area of approximately 57,000 ha, where several natural habitats may be found (e.g., cliffs, beaches, small islands, fossil reefs, small canyons, the River Mira estuary, dunes, salt marshes, temporary ponds). The detailed geology of the zone may be checked in the Carta Geológica de Portugal (1:200,000), Folha 7, Serviços Geológicos de Portugal (Oliveira 1984). It is essentially represented by sands, sandstones and gravels from the Plio-Pleistocene and less frequently from the Holocene and Recent; to the interior and in the south, the area contains a wide variety of Palaeozoic rocks. In the northern part of the park (near V. N. Milfontes), in depression areas of the dunes of Holocene age, some surface water bodies can be found during the rainy season. A larger one (ca 500 × 120m), stands out for its size and semi-permanent nature, almost permanent, which is probably caused by the existence of accumulated mud at the bottom preventing infiltration of the rain waters. The catchment area is broadly delimited in figure 1. The region is under a Mediterranean climate, with an average annual precipitation between 570 mm in inland and 720 mm close to the coast, where Atlantic influence is recorded (www.meteo.pt).

2.1.2. Regional survey

The Alentejo region (Alto and Baixo Alentejo) (Fig. 2) has a diverse geology (Carta Geológica de Portugal, 1:500,000, Serviços Geológicos de Portugal, Oliveira *et al.* 1992), mostly represented by Palaeozoic metamorphic and igneous rocks. Regarding the ponds sampled in the Alentejo region, local geology has little importance, since all are of temporary nature, located along roads, in small roadside depressions. The climate of the region is also Mediterranean, but with precipitation decreasing towards the inner area.

2.2. Sampling

2.2.1. Natural park PNSACV

The area of the study sites is approximately 1 km² and it is located at altitudes of ca 50–58 m a.s.l. (Tab. 1). Since the sampled area is located inside a natural park, it is under reduced human activity, serving as pasture grounds. Sampling was carried out on three occasions (22 December 2005, 5 January 2006 and 27 April 2006), surveying a total of six temporary ponds (Fig. 1). Despite being part of a single basin area, all ponds were isolated at the sampling times except ponds Font 2, 3 and 4 which were connected in April (coded for this date as Font 2-4).

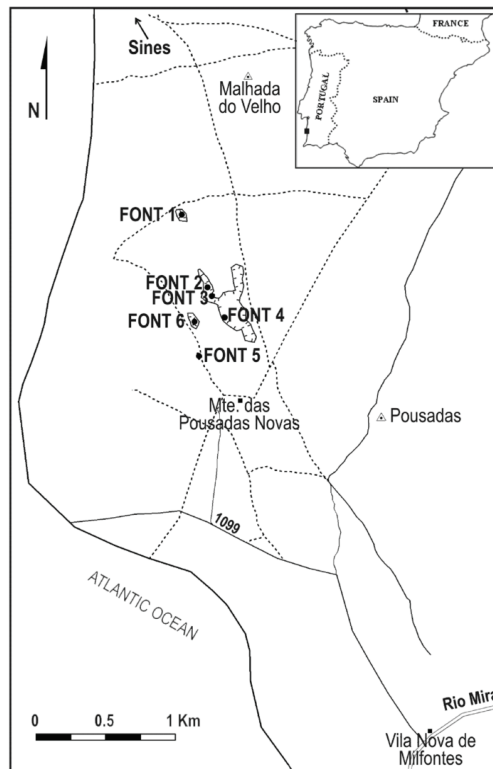


Fig. 1. Location map of the study sites with indication of the six ponds sampled in the natural park PNSACV.

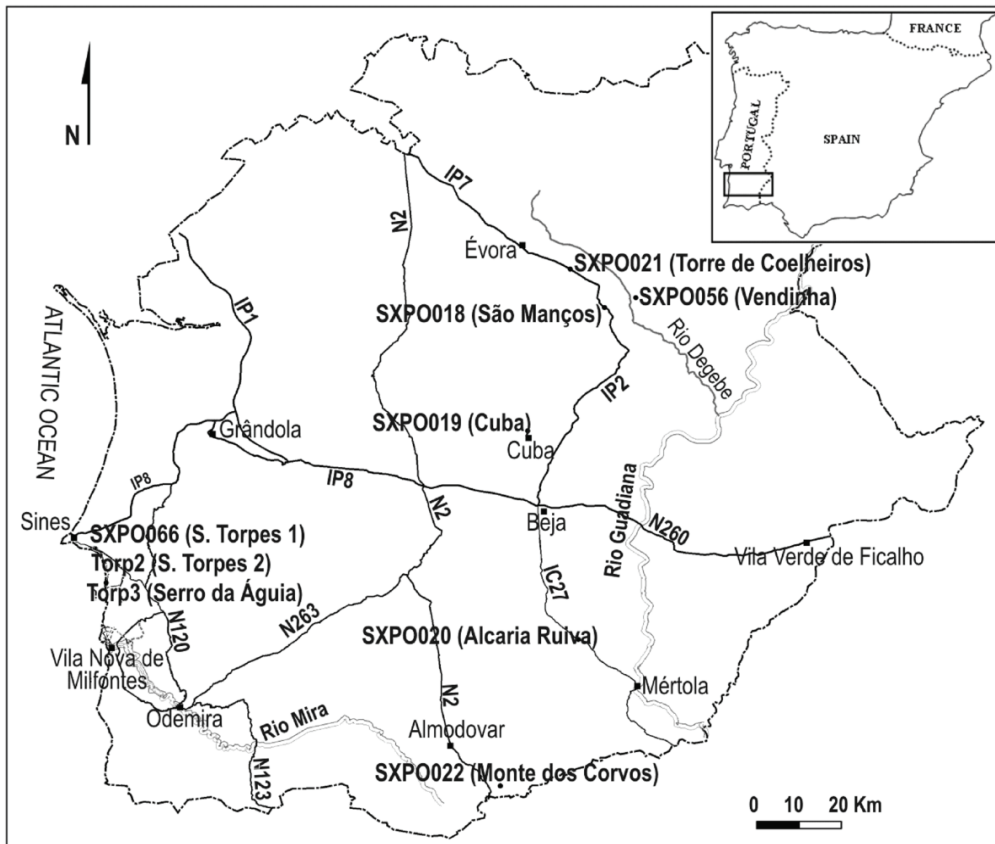


Fig. 2. Location map of the nine ponds sampled in the Alentejo regional survey.

Tab. 1. Pond names, codes, values for the environmental variables in chronological order (December-January-April), coordinates and altitudes for the six ponds sampled in the natural park PNSACV.

Pond Code		Font 1 1	Font 2 2	Font 3 3	Font 4 4	Font 5 5	Font 6 6
Water temperature (°C)	Temp	14.5-26.3	13.9-13.5-18.9	16.4-18.9	10.0-9.2-18.9	9.6-22.7	20.8
Salinity (‰)	Sal	0.6-0.1	0.3-0.3-0.2	0.5-0.2	1.3-0.9-0.2	0.7-0.1	0.4
pH	pH	6.0-7.0	6.3-6.3-6.5	6.5-6.5	5.0-5.0-6.5	5.0-6.3	6.4
Conductivity ($\mu\text{S cm}^{-1}$)	Cond	1589-689	1095-1071-885	1445-885	2799-1955-885	1198-777	1213
Dissolved Oxygen (%)	O ₂	95.1-2.0	66.8-28.1-20.1	44.4-20.1	11.0-14.7-20.1	69.0-2.2	9.4
Latitude		N37°45'26"	N37°45'09"	N37°45'07"	N37°45'02"	N37°44'53"	N37°45'01"
Longitude		W8°47'52"	W8°47'46"	W8°47'45"	W8°47'42"	W8°47'48"	W8°47'49"
Altitude (m a.s.l.)		58	56	56	55	50	55

Tab. 2. Pond names, field codes, closest localities, coordinates and altitudes for the nine ponds sampled in the Alentejo regional survey, plus information on ostracod species recorded in each pond. ♂ - includes records of males in species reproducing primarily by parthenogenesis.

Pond name	São Manços	Cuba	Alcaria Ruiva	N18 2 nd	Monte dos Corvos	N256 2 nd	S. Torpes 1	S. Torpes 2	Serro da Águia
Code	SXPO018	SXPO019	SXPO020	SXPO021	SXPO022	SXPO056	SXPO066	Torp2	Torp3
Closest locality	São Mancos	Cuba	Alcaria Ruiva	Torre de Coelheiros	Monte dos Corvos	Vendinha	Sines	Sines	Sines
Latitude	N38°26'37"	N38°10'58"	N37°44'29"	N38°31'31"	N37°25'56"	N38°27'34"	N37°56'05"	N37°56'00"	N37°51'42"
Longitude	W7°44'01"	W7°53'50"	W7°47'20"	W7°48'23"	W7°57'19"	W7°40'02"	W8°49'07"	W8°48'58"	W8°47'36"
Altitude m a.s.l.	187	186	163	235	336	199	10	7	17
<i>Candona</i> sp. ex gr. <i>neglecta</i>			X						
<i>Cypridopsis hartwigi</i>				X	X				
<i>Cypridopsis vidua</i>			X						
<i>Eucypris virens</i>	X	X	X♂	X	X♂	X	X	X	X
<i>Heterocypris incongruens</i>	X	X		X	X	X	X	X	X
<i>Heterocypris</i> sp.			X						
<i>Ilyocypris gibba</i>								X	X
<i>Ilyocypris</i> aff. <i>lacustris</i>		X		X			X		
<i>Paralimnocythere psammophila</i>			X						
<i>Tonnacypris lutaria</i>	X			X					

Ponds were shallow (depth <1.5 m) and rainwater fed. Water presence in the area was first confirmed in December 2005. Pond hydroperiod was variable throughout the sampling period conditioning sampling regularity: Ponds Font 2 and Font 4 were filled in December 2005; the remaining ponds were filled in January except for pond Font 6, still empty at this date. The last pond was sampled in the last sampling date only (27 April 2006). The bottom of the ponds was (partially) covered by grassy vegetation dominated mostly by the common reed *Phragmites* sp. (not observed in site Font 1 in January).

Ostracods were sampled semi-quantitatively by sweeping the bottom (superficial sediment and adjacent water column) of the pond using a rectangular hand net (25 × 15 cm; mesh size: 0.12 mm) at two to six sampling points, depending on pond area. The samples were pooled and immediately preserved in 30% ethanol. Ostracod abundances were standardized for all ponds and expressed as number of individuals per sampling unit (*ca* 1 m² of sampled surface area; coded as ind. s.u.⁻¹). At each sampling occasion, water temperature, salinity, pH, conductivity and dissolved oxygen were measured in situ using electronic probes.

2.2.2. Regional survey

The regional survey was carried out from December 2005 until April 2006. Samples were taken during single visits in nine ponds (site names, locations and coordinates are given in table 2). Ponds sampled were located within the region Alentejo (Alto and Baixo Alentejo) but outside the protected area of the natural park PNSACV, in distinctly human impacted areas (Fig. 2). All ponds were temporary, small and shallow water bodies (max. size 50 × 25 m; depth <1.5 m). Ostracods were collected with a rectangular hand net (25 × 15 cm; mesh size: 0.12 mm) and immediately fixed in 30% ethanol.

2.3. Laboratory work

Samples were washed under tap water through a 0.25 mm mesh and preserved in 70% ethanol. Ostracods were sorted using a stereomicroscope (40× magnification), counted and identified to the species level (when possible) under a stereomicroscope (100× magnification) or light transmitted microscope (up to 400× magnification) using the keys of Meisch (2000). When ostra-

Tab. 3. Categorized densities of ostracod taxa recorded in the six ponds of the natural park PNSACV per sampling occasion (Dec – 22.12.2005, Jan – 5.1.2006, Apr – 27.4.2006). Ponds are listed and coded in table 1; Scale: 1: 1 ind. s.u.⁻¹, 2: 2-10 ind. s.u.⁻¹, 3: 11-100 ind. s.u.⁻¹, 4: 101-1000 ind. s.u.⁻¹, 5: >1000 ind. s.u.⁻¹.

Ponds	Dec		Jan					Apr			
	2	4	1	2	3	4	5	1	2-4	5	6
<i>Bradleycypris obliqua</i> (Brady, 1868)								5	4		5
<i>Candona</i> sp. ex gr. <i>neglecta</i>		3									
<i>Cyprina ophthalmica</i> (Jurine, 1820)		4			3	4	3		4		
Cyprididae indet. juv.	4	3	3	4	4	1	5	3	5	4	5
<i>Cypridopsis hartwigi</i> (G.W. Müller, 1900)									1	2	3
<i>Cypridopsis vidua</i> (O.F. Müller, 1776)				4				3	5	5	5
<i>Cypris bispinosa</i> Lucas, 1849				4	2	1	4	4	4	5	4
<i>Eucypris virens</i> (Jurine, 1820)				3				3			
<i>Fabaeformiscandona</i> sp. n. ex gr. <i>fabaeformis</i>		3							2		
<i>Herpetocypris chevreauxi</i> (Sars, 1896)				4	3			2	4	5	4
<i>Heterocypris incongruens</i> (Ramdohr, 1808)								2			
<i>Ilyocypris gibba</i> (Ramdohr, 1808)								3			
<i>Paralimnocythere psammophila</i> (Flössner, 1965)				4	4			3	4	3	3
<i>Pseudocandona marchica</i> (Hartwig, 1899)								3			
<i>Sarsocypridopsis aculeata</i> (Costa, 1847)				2	2				2		
Ostracoda indet. juv.		2		3				3	2	3	3

cod densities in a given sample exceeded 1000 ind. s.u.⁻¹, the identifications were limited to the animals found in a randomly selected fraction of the total sample (usually ½). Ostracod valves were illustrated using Scanning Electron Microscopy (JEOL-JSM5200LV).

2.4. Statistical analysis

Principal component analysis (PCA) was applied to summarise the most important environmental gradients in our sites in order to compare these with observed ostracod assemblage structures. Detrended correspondence analysis (DCA) was applied to assess the range of the data set used and linear ordination models were chosen (length of gradient SD <2); variables were centred and divided by standard deviation (Lepš & Šmilauer 2003). From the five variables (Tab. 1), water temperature was removed in the final PCA eliminating strong correlations (highly correlated with salinity and pH; Spearman's correlation = -0.80 and 0.83, respectively, $p < 0.05$). Conductivity values were log transformed prior to ordination. Relationships among ostracod site assemblages were examined using a non-metric Multi-Dimensional Scaling (MDS) method based on species relative abundances (categorized densities as explained in Tab. 3) and Bray-Curtis similarity coefficient. Ordination analyses were performed with CanocoWin 4.5 (Ter Braak & Šmilauer 2002); normality assumptions and Spearman's correlation tests were analysed with the statistical program Rwin 2.8.1 (R Development Core Team 2008); MDS procedure was run on PRIMER ver. 6.1.10 software (Clarke & Gorley 2006).

3. RESULTS

3.1. Natural park PNSACV

The ranges of the physical and chemical water parameters are reported in table 1. Water conductivity and salinity variation were minimal/absent between Decem-

ber and January in Font 2. In Font 4 the variation of these parameters was more evident. An overall decrease in water conductivity and salinity was observed in the five ponds sampled between January and April, whilst the temperature increased in this time period (Tab. 1). In the weeks prior to the April sampling, high precipitation was recorded, which might have contributed to the temporal variation of the recorded variables. Seasonal decrease of dissolved oxygen was intense, in particular in ponds Font 1, 2 and 5, most probably caused by the variation in primary productivity over the season (Tab. 1). However, in Font 4 this pattern was not observed. Slightly acidic pH values prevailed in all ponds in the study area (pH range: 5.0-7.0). Macrophyte coverage increased between samplings, influencing primary production and affecting seasonal pH variation, as well as dissolved oxygen percentage of saturation.

A total of 14 ostracod species (Tab. 3) were identified of which two were assigned to the species group only, namely *Candona* sp. ex gr. *neglecta* and *Fabaeformiscandona* sp. ex gr. *fabaeformis*, the latter probably representing a new species (see comments in 4.1.). Of these, three species were a new record in mainland Portugal: *Bradleycypris obliqua*, *Cypris bispinosa* and *Paralimnocythere psammophila*. Species are illustrated in Appendix Plates A1-A3. The collected material includes also very young juvenile specimens which could not be identified to the species level and were assigned to the family level (Cyprididae). Damaged and/or incomplete specimens were left as indeterminate (Tab. 3).

On average 4.6 species were found per sample, with overall ostracod species richness increasing over the season. The sites with the highest ostracod richness were Font 1 and Font 2-4 in April with nine species identified (Tab. 3). In Font 1, only cypridid juveniles were present in January. An overall increase in ostracod density over the season was also observed, even under

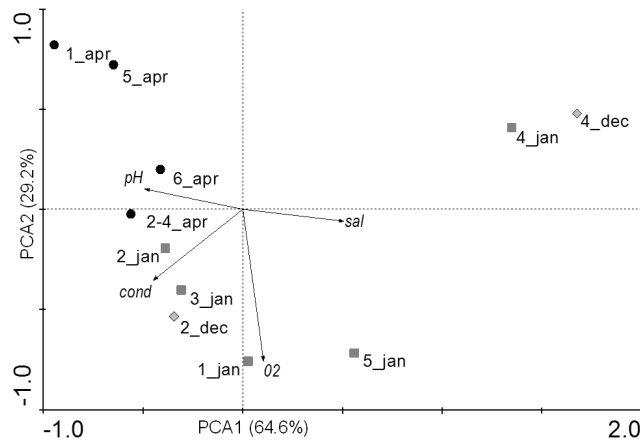


Fig. 3. PCA ordination diagram based on four environmental variables measured at 11 occasions in the six study sites in the natural park PNSACV. Environmental variables and ponds are listed and coded in table 1. Sampling visit is indicated after the pond code (dec – December; jan – January; apr – April).

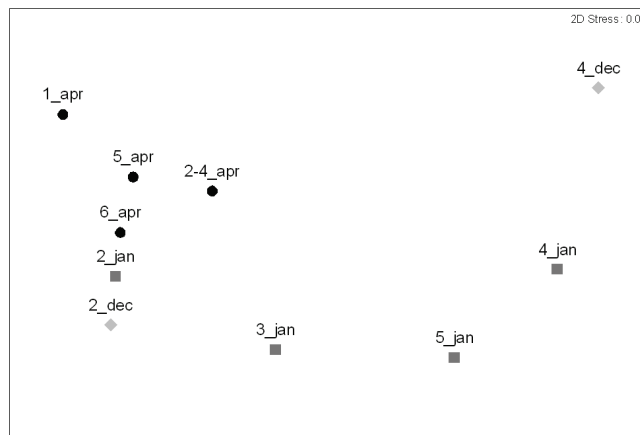


Fig. 4. Multi-dimensional scaling ordination scatter plot of the sample ostracod assemblages based on Bray-Curtis similarities on categorized species densities as indicated in table 3. Sample and pond codes as in figure 3 and table 1.

pond expansion after high precipitation in April. Species emergence and succession was variable among ponds. *Eucypris virens* was one of the first ostracod species present after inundation along with *Cyprina opthalmica*, *Cypris bispinosa*, *Fabaeformiscandona* sp. n. ex gr. *fabaeformis*, *H. chevreuxi* and *P. psammophila*. *Cypridopsis vidua* and *S. aculeata* were observed in January. *Bradleycypris obliqua*, *I. gibba*, *H. incongruens* and *P. marchica* were recorded in April only along with *C. hartwigi*. The most abundant (density) species in this study was *Cypridopsis vidua*. The most common species (present in all six ponds) were *C. bispinosa* and *Herpetocypris chevreuxi*. *Heterocypris incongruens*, *Ilyocypris gibba* and *Pseudocandona marchica* were recorded in one sample only each.

The first two axes extracted by PCA accounted for a cumulative percentage of 93.7% of explained environmental variance. The occurrence of only eleven samples restricted any further ordination methods. The first axis (PCA 1: 64.6%) is mainly correlated to pH and salinity

while PCA 2 (29.2%) is negatively correlated to dissolved O₂ (Fig. 3). The samples with positive scores in the first axis are samples collected from the most acidic waters and with highest salinity. Samples collected from pond Font 4 in December and January and Font 1, Font 5 and Font 6 in April, with positive scores regarding axis 2, are the samples with the lowest oxygen and highest temperature.

The ordination of the samples by the MDS technique based on faunistic similarity (Fig. 4) exhibited high similarity with that of the PCA ordination on the basis of abiotic factors (Fig. 3). Samples taken in December and January from Font 4 and in January from Font 5, when the lowest pH and highest salinity was recorded, were dominated mainly by *C. opthalmica* (Tab. 3). On the other hand, the cluster of the remaining samples (Fig. 4), with negative scores in the first PCA axis (Fig. 3), were samples mostly dominated by *H. chevreuxi*, *P. psammophila* and/or *C. bispinosa* (Tab. 3).

3.2. Regional survey

From the survey carried out in Alentejo region, 10 ostracod species were recorded (three identifications questionable, see point 4.1.) belonging to four families (Tab. 2). Of these, two species were new records for mainland Portugal (*Tonnacypris lutaria* and *P. psammophila*) while *Heterocypris* sp., *Ilyocypris* aff. *lacustris* and *T. lutaria* were not recorded in the natural park PNSACV.

Species richness was on average 3.3 per pond (range: 2-5). The most common species was *Eucypris virens* followed by *H. incongruens* (occurring in nine and seven ponds, respectively). The least frequent species was *P. psammophila*, present in one pond only.

4. DISCUSSION

4.1. Species richness: new records and taxonomic remarks

Specimens assigned to the genus *Candona* (Appendix A1, figure a) may belong to *C. neglecta*, however the only male found among collected juveniles and females was damaged, not allowing an unambiguous identification to the species level. Regarding *Heterocypris* sp. (Appendix A2, figure f), the specimens retrieved were <1.1 mm in length, belonging to the *exigua* class size of the genus (Petkovski *et al.* 2000). Specimens resemble *H. exigua* Gauthier & Brehm (1928) the typical representative of this class. However, as the material consists only of females and juveniles (no males found), their specific assignment is ambiguous, and the record was left in open nomenclature as *Heterocypris* sp. Both *Candona* sp. ex gr. *neglecta* and *Heterocypris* sp. were not included in the annotated distributional checklist of Recent and Quaternary freshwater inland Ostracoda known from Portuguese mainland (Tab. 4).

Fabaeformiscandona sp. n. ex gr. *fabaeformis* (Appendix A1, figures b, c) most probably represents a yet non described species. Our specimens resemble those of *F. fabaeformis* (Fischer 1851) with differences mainly in the shape of the female genital organ and of the M-process of the hemipenis, which is distally distinctively bifurcated. The latter character is shared with *Eucandona svetozari* (Petkovski & Karanovic 2004) from specimens collected in Macedonia and with those from Spain originally assigned by Mezquita & Sanz-Brau (1997) to *F. fabaeformis*. However, specimens collected in the present study differ from *E. svetozari* in valve shape. A detailed description of these specimens and a discussion on their taxonomic relationship will be published elsewhere. It is worth to mention that all former Portuguese records of *F. fabaeformis*, both recent and fossil, are given with some reserve (see Paulo & Moutinho 1983; Cabral *et al.* 2005a; Minati *et al.* 2008). Therefore, these records may represent another species,

while *F. fabaeformis* does not occur in Portugal or even in the whole Iberian Peninsula.

Until recently, only five species were known from the modern biotopes of the region Baixo Alentejo (Tab. 4; Paulo & Moutinho 1983; Cearreta *et al.* 2003). In the region Alto Alentejo, on the other hand, there were no known published studies (Tab. 4). With the addition of the data from the present study a total of 18 recent freshwater ostracod species has now been recorded in the regions Alto and Baixo Alentejo. The present study also resulted in the discovery of six ostracod species new to recent continental Portuguese fauna (*B. obliqua*, *C. bispinosa*, *I. aff. lacustris*, *P. psammophila*, *T. lutaria* and the already mentioned *F. sp. n. ex gr. fabaeformis*) increasing a total number of the recent freshwater ostracod species recorded in mainland Portugal to 32 (Tab. 4).

The occurrence of *B. obliqua*, *C. bispinosa*, *P. psammophila* and *T. lutaria* in continental Portugal was not surprising given their presence in mainland Spain. However, if *C. bispinosa* (previously recorded in 1997 in Mata dos Medos, Costa da Caparica, ca 10 km from Lisbon; collection M.C. Cabral and R. Guerreiro, unpubl.) and *T. lutaria* are rather well known species, *P. psammophila* has only been sporadically reported in Europe. Although the species records in northern Europe derive mostly from lakes (Meisch 2000), its ecological preferences appear broader, as implied from its presence in temporary ponds (Smith & Horne 2004; Martins *et al.* 2009) but also in a karstic lake (Mezquita *et al.* 1996) and in springs (Mezquita *et al.* 1999). The presence of bisexual populations of *B. obliqua* is of great interest given that male records for this species were reported from few sites in North Africa (Gauthier 1928; Klie 1943), one site in Croatia (Petkovski 1964) and one site in Central Spain (Roca *et al.* 2000) only. The species, despite the rather broad-based habitat incidence, has only few scattered records in Europe (though being known to occur in the archipelagos of Madeira and Azores) (Meisch 2000). A detailed description of the males of *B. obliqua* will be published elsewhere.

Along with the new records for continental Portugal, the finding of few other species is worth noting. The specimens of *C. vidua* (Appendix A3, figure a) recovered in the present sampling had brownish carapaces and as such may be assigned to *C. parva* Müller, 1900 which is considered by Meisch (2000) an intraspecific form of *C. vidua*. It is also worth noting the presence of *C. vidua* in Font 1 where oxygen level is very low. This may indicate that the species tolerance to low oxygenated waters may be wider than reported (see Meisch 2000). *Ilyocypris lacustris* is a rather rare European species with a few known published records in lakes only (Meisch 2000). Our specimens slightly differ in the carapace shape from those originally illustrated by Kaufmann (1900) and as such are denoted here with some reservation as *I. aff. lacustris* (Appendix A1, figure g).

Tab. 4. Faunal checklist (in alphabetical order) of Recent (R) and fossil Quaternary (Holocene – H and Pleistocene – P) freshwater Ostracoda species recorded in mainland Portugal. References ordered in chronological order : 1. Petkovski 1963; 2. Petkovski 1964; 3. Paulo 1969; 4. Paulo & Moutinho 1983; 5. Antunes *et al.* 1996; 6. Baltanás *et al.* 1996; 7. Gonzáles-Mozo *et al.* 1996; 8. Cearreta *et al.* 2003; 9. Cabral *et al.* 2004; 10. Cabral *et al.* 2005a; 11. Cabral *et al.* 2005b; 12. Hilbich *et al.* 2008; 13. Minatti *et al.* 2008. Species recorded in the present study are marked as NP (natural park) or/and RS (regional survey); species new to Portugal are in bold. ♂ – includes records of males in species reproducing primarily by parthenogenesis; ^a – as *Candona cf. neglecta*; ^b – dubious identification; ^c – refers to records originally ascribed by Petkovski (1963) to *C. brincki* (see synonymy in Petkovski *et al.* 1993); ^d – reported as *Cypris cf. pubera*; ^e – reported as *Darwinula cf. stevensoni*; ^f – reported as belonging to the genus *Candona*; ^g – reported as *Fabaeformiscandona cf. fabaeformis*; ^h – refers to records originally ascribed to bisexual populations of *H. reptans* (see discussion in Gonzáles-Mozo *et al.* 1996 and Meisch 2000); ⁱ – reported as belonging to the genus *Erpetocypris*; ^j – reported as *Erpetocypris cf. reptans*; ^k – reported as *Ilyocypris* gr. *gibba*; ^l – reported as *Eucypris cf. ornata*; ^m – reported as belonging to the genus *Cypridopsis*; ⁿ – includes records of *P. villosa crassipes* Masi, 1905; ^o – reported as *Candona parallela*.

	Age	Minho	T. Montes e Alto Douro	Douro Litoral	Beira Litoral	Beira Alta	Beira Baixa	Ribatejo	Estremadura	Alto Alentejo	Baixo Alentejo	Algarve	?
<i>Bradleycypris obliqua</i> (Brady, 1868)	R										NP [♂]		
<i>Candona angulata</i> Müller, 1900	P											10	
<i>Candona lindneri</i> Petkovski, 1969	R			4									
<i>Candona neglecta</i> Sars, 1887	R,P			4							4,RS ^a	5 ^a ,10,13	
<i>Candona sanociensis</i> Sywula, 1971	H											9	
<i>Cyclocypris laevis</i> (Müller, 1776)	H											9	
<i>Cypria exsculpta</i> (Fischer, 1855)	R			4 ^b									
<i>Cypria ophthalmica</i> (Jurine, 1820)	R	4		4	4			4			NP	4	
<i>Cypridopsis hartwigi</i> Müller, 1900	R							4		RS	NP,RS		
<i>Cypridopsis lusatica</i> Schäfer, 1943	R												1 ^c
<i>Cypridopsis vidua</i> (Müller, 1776)	R,H,P		4		4			4			4,8,NP,RS	10	
<i>Cypris bispinosa</i> Lucas, 1849	R										NP		
<i>Cypris falki</i> Janz, 1997	P											10	
<i>Cypris pubera</i> (Müller, 1776)	P											5 ^d	
<i>Darwinula stevensoni</i> (Brady & Robertson, 1870)	R,H,P			4							4,8 ^e	10,12,13	
<i>Eucypris virens</i> (Jurine, 1820)	R	4								RS	NP,RS		
<i>Fabaeformiscandona fabaeformis</i> (Fischer, 1851)	R,P			4 ^{b,f}								10 ^g , 13 ^g	
<i>Fabaeformiscandona</i> sp. n. ex gr. <i>fabaeformis</i>	R										NP		
<i>Herpetocypris brevicaudata</i> Kaufmann, 1900	R			4 ^h	7			4 ^h					2 [♂]
<i>Herpetocypris chevreuxi</i> (Sars, 1896)	R			4 ⁱ ,7				4 ⁱ	4 ⁱ		NP		
<i>Herpetocypris intermedia</i> Kaufmann, 1900	R				7								
<i>Herpetocypris mateusorum</i> Paulo, 1969	R	4 ⁱ		3 ⁱ ,4 ⁱ	4 ⁱ	7		4 ⁱ	4 ⁱ				
<i>Herpetocypris reptans</i> (Baird, 1835)	R,P			4 ⁱ								5 ^j	
<i>Heterocypris incongruens</i> (Ramdohr, 1808)	R,P	4		3 [♂] ,4 [♂]				4	4	RS	NP,RS	10	
<i>Heterocypris salina</i> (Brady, 1868)	R	4		4					4			4	
<i>Ilyocypris bradyi</i> Sars, 1890	H,P										8	10,13	
<i>Ilyocypris gibba</i> (Ramdohr, 1808)	R,H,P										4,8,NP,RS	4,5 ^k ,10,13	
<i>Ilyocypris aff. lacustris</i> Kaufmann, 1900	R										RS		
<i>Ilyodromus viridulus</i> (Brady, 1886)	R				6								
<i>Koencypris cf. ornata</i> (Müller, 1776)	H										8 ^l		
<i>Limnocythere inopinata</i> (Baird, 1843)	H,P										8	10	
<i>Paralimnocythere compressa</i> (Brady & Norman, 1889)	P											10	
<i>Paralimnocythere messanae</i> Martens, 1992	P											10	
<i>Paralimnocythere psammophila</i> (Flössner, 1965)	R										NP,RS		
<i>Penthesilenula brasiliensis</i> (Pinto & Kotzian, 1961)	P											10,13	
<i>Plesiocypridopsis newtoni</i> (Brady & Robertson, 1870)	R	4 ^l		4 ^m	4 ^m		4 ^m	4 ^m			4 ^m	4 ^m	
<i>Potamocypris unicaudata</i> Schäfer, 1943	R					4							
<i>Potamocypris villosa</i> (Jurine, 1820)	R			3,4 ⁿ	4				4				
<i>Pseudocandona albicans</i> (Brady, 1864)	R			4 ^o									
<i>Pseudocandona marchica</i> (Hartwig, 1899)	R			4 ^f							NP		
<i>Pseudocandona</i> sp. ex gr. <i>zschokkei</i> (Wolf, 1920)	P											10	
<i>Sarscypridopsis aculeata</i> (Costa, 1847)	R				4 ^m						NP	4	
<i>Sclerocypris cf. bicornis</i> (Müller, 1900)	P											10,11	
<i>Strandesia vavrai</i> (G.W. Müller, 1898)	R			4	4								
<i>Tonnacypris lutaria</i> (Koch, 1838)	R									RS			
<i>Vestalenula cylindrica</i> (Straub, 1952)	P											10,13	
<i>Vestalenula paglioli</i> (Pinto & Kotzian, 1961)	P											10,13	
<i>Zonocypris cf. costata</i> (Návra, 1837)	P											9,10,11	

4.2. Environmental factors affecting ostracod distribution in the natural park PNSACV

Freshwater ostracod species are composed of generalist species (cosmopolitans with wide environmental tolerances), specialist species with (in general) limited geographical distributions, and species which fall in between both extremes (Meisch 2000; Klkylođlu 2004). Consequently, studies of seasonal freshwater ostracod assemblages and their ecological predictors have since long been under study. The multivariate analysis (PCA) applied to summarise the most important environmental gradients in the studied sites within PNSACV showed that over 60% of the environmental variation was explained by the axis 1 and that this axis was highly correlated to pH and salinity (Fig. 3). Salinity and temperature have been suggested as primary factors constraining ostracod ecology and distribution (Neale 1964). The influence of pH on ostracod species distribution is related with problems in carapace calcification during the post-embryonic development, when calcium uptake in acid waters is difficult (Griffiths & Holmes 2000). It has been recognized that at a pH <5 ostracod species are in general absent (Boomer *et al.* 2006). Although in the samples collected in PNSACV salinity was within freshwater limits, with relatively low variation, pH was slightly acidic to neutral. These factors together with temperature (highly correlated with salinity and pH), dissolved oxygen and organic matter content (among others) may have constrained the ostracod species presence and temporal succession. This containment is patent in the MDS analysis, confirming the main separation observed in the PCA: Samples with highest salinity and the lowest pH are characterised by the dominance of the eurytopic species *C. ophthalmica*. The general commonness and dominance of widely tolerant species (e.g., *C. vidua*, *E. virens*, *H. incongruens*) in addition to pH euryplastic species further support this idea.

Temporary water bodies usually have a large surface area to volume ratio, and for this reason they show hydrological characteristics very susceptible to weather fluctuation (Brnmark & Hansson 1998). In Mediterranean temporary habitats hydrological conditions may function as a primer conductor in species distribution (Quintana *et al.* 1998). Therefore, despite the small area studied, species distribution in time and space varied among ponds as a probable effect of pond hydroperiod differences. Hydroperiod also influences habitat longevity and consequently community structure and complexity. Studies on temporary ponds suggest that species richness typically increases as the length of the flooded period in ponds increases (e.g., Neckles *et al.* 1990; Eyre *et al.* 1992). In this way, the observation that species richness per pond increased over the season was expected in the present study, as a combination of the presence of pure ephemeral inhabitants (e.g., *H. incongruens*) to species typical of more "mature" habitats

(e.g., *H. chevreuxi*) and of the succession of early form species (e.g., *E. virens*) to summer forms (e.g., *I. gibba*). As a consequence, it is also understandable the observed seasonal increase in total ostracod density. This seasonal trend of increase was in great part caused by the emergence and population growth of *C. vidua* in April.

The nature of the "species reservoir" present in the sediment as diapausing eggs (egg bank) should also be considered when analysing the habitat characteristics. The study area was affected by severe droughts in 2004 and 2005, with precipitation records at about 40% of the expected average annual precipitation for this region. In fact 2005 was the driest year recorded in continental Portugal since 1931, with the extreme drought recovering to a normal situation in March 2006 (www.meteo.pt). Yet, the presence of *C. vidua*, *I. gibba*, *P. psammophila* and *Pseudocandona marchica* which may be present in temporary water bodies but prefer permanent habitats (Meisch 2000) indicates that the study area may have a temporal to semi-permanent character. The presence of deep muddy sediment layers in the central zone of study area (the large depression inclosing ponds Font 2, 3 and 4), may permit water retention for long periods of time in this sector. Last, although salinity variation was reduced during the sampling period here studied, it is expected that highly variable water chemical conditions may occur, depending on annual hydroperiod conditions. The incidence of *C. bispinosa*, *H. chevreuxi* and *Sarscypridopsis aculeata*, with preference for brackish waters although inhabiting also pure freshwaters (Meisch 2000), may indicate such conditions. Moreover, the co-occurrence of *H. chevreuxi* and *S. aculeata*, as observed in January and April during this study, may be perceived as an indication of increasing salinity (Meisch 2000) which may have been restricted by high precipitation in early April. Considering the close distance to the sea, potential influence of sea water by sea spray or through the aquifer system should also be considered.

4.3. Species richness: natural park PNSACV vs regional survey

The surveyed ponds outside the natural park were ephemeral, rainwater fed, subjected to highly fluctuating conditions and located in rather altered human areas (road side ditches or agriculture grounds). Unlike the study area, which as a product of its unique geology may hold water/humidity for rather longer periods of time, the nine ponds in the regional survey are subjected to annual drying events, being more sensitive to annual hydrological conditions. Consequently as a combination of higher human activity and environmental unpredictability, the presence of lower ostracod richness was expected in the regional surveyed ponds (10 species comparing to 14 in the natural park PNSACV, with 6 species in common). Moreover, the presence of rather typical inhabitants of ephemeral waters and, in particu-

lar, cosmopolitan species such as *E. virens* and *H. incongruens* was expected.

The unique settings of the study area in the natural park PNSACV define these habitats as exceptional, allowing the coexistence of both cosmopolitan and less tolerant species, and temporary to permanent water bodies' inhabitants. It should be noted though that one of the ponds with highest ostracod richness was Font 1 which exhibits the most short-lived characteristics. Font 1 is mostly dominated by sandy substrate of reduced water retention, with maximum depth below 0.5 m. Therefore, despite the importance of the semi-permanent nature of the study site, other factors may contribute to the local ostracod richness of the study site.

4.4. Known fossil Quaternary freshwater ostracod fauna in Portugal

Most of the knowledge on the non-marine ostracod fauna from the Quaternary in continental Portugal derives from an outcrop assigned to the ?Pleistocene, located at Boliqueime, Algarve region (Cabral *et al.* 2005a). The corresponding deposits are interpreted as reflecting a fluvial- or alluvial-palustrine setting, with shallow, permanent waters of variable energy, developed under a warm climate, seasonally contrasting as regards to humidity. Up to now nineteen species have been recorded from this outcrop, from which five are common to the fauna studied in the Natural Park PNSACV and regional survey (Tab. 4): *C. vidua*, *H. incongruens*, *I. gibba*, *C. neglecta* and *F. cf. fabaeformis*. All of these species are tolerant to several environmental factors, usually inhabiting small ponds of permanent or temporary waters, having a wide distribution in Europe.

The presence of one species, *Paralimnocythere compressa* (Brady & Norman, 1999), though unrepresented in the Recent fauna, must also be mentioned. *P. compressa* is morphologically very similar to *P. psammophila*, with the characteristics of the valves from both species almost impossible to distinguish. It is possible that the fossil population, mainly composed of juveniles (most difficult to identify), actually belongs to *P. psammophila*, widening the record of this species in Portugal to the Pleistocene.

5. CONCLUSIONS

In our study area, ostracod species distribution seems to have been conditioned by environmental settings with pH but also salinity as main variables and dissolved oxygen as a secondary determinant. The potential of mainland Portugal and of non-permanent water bodies as important reservoirs of ostracod fauna richness was patent, despite the small size of the studied area. Still, other characteristics of the study area, not only pond hydroperiod, should be considered when explaining the relative high ostracod richness encountered.

Temporary habitats may hold significant biodiversity serving as repositories of a diverse and valuable genetic heritage. These environments may provide habitat for rare and endangered species (Collinson *et al.* 1995 and references therein). The loss of Mediterranean temporary ponds as a result of human activities will conduct to the loss of unique ecological sanctuaries and vast biodiversity within the Iberian Peninsula, much of which remain poorly known (or even unreported). To an extent, the lack of correct management of temporary ponds reflects the lack of information about their ecology (Collinson *et al.* 1995).

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A P P E N D I X

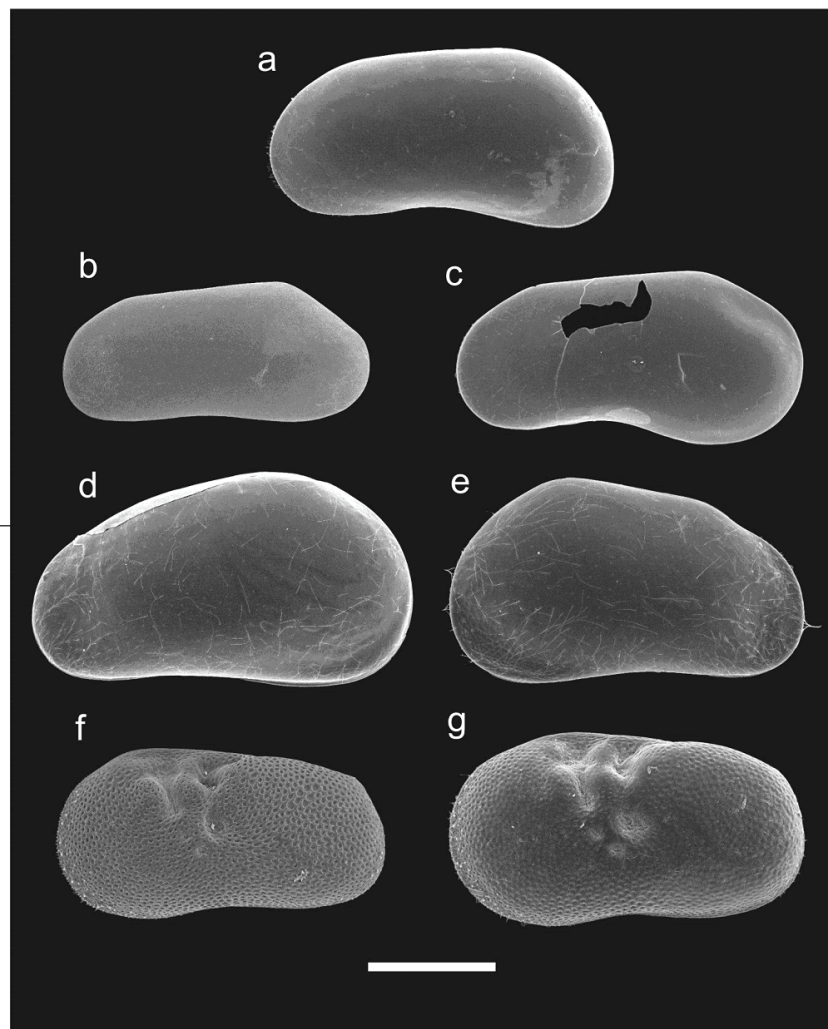


Plate A1. SEM pictures of the ostracod species recorded in the region Alentejo. **a.** *Candona* sp. ex gr. *neglecta*: female, lateral view, left valve (scale bar = 382 μ m; sample: SXPO020); **b.** *Fabaeformiscandona* sp. n. ex gr. *fabaeformis*: **b.** subadult female, lateral view, left valve (scale bar = 287 μ m; sample: Font 2-4 Apr); **c.** male, lateral view, left valve (scale bar = 382 μ m; sample: Font 2-4 Apr); **d.** *Pseudocandona marchica*: **d.** male, lateral view, left valve (scale bar = 383 μ m; sample: Font 1 Apr), **e.** female, lateral view, right valve (scale bar = 378 μ m; sample: Font 1 Apr); **f.** *Ilyocypris gibba*: female, lateral view, left valve (scale bar = 385 μ m; sample: Torp2); **g.** *Ilyocypris* aff. *lacustris*: female, lateral view, left valve (scale bar = 389 μ m; sample: SXPO019).

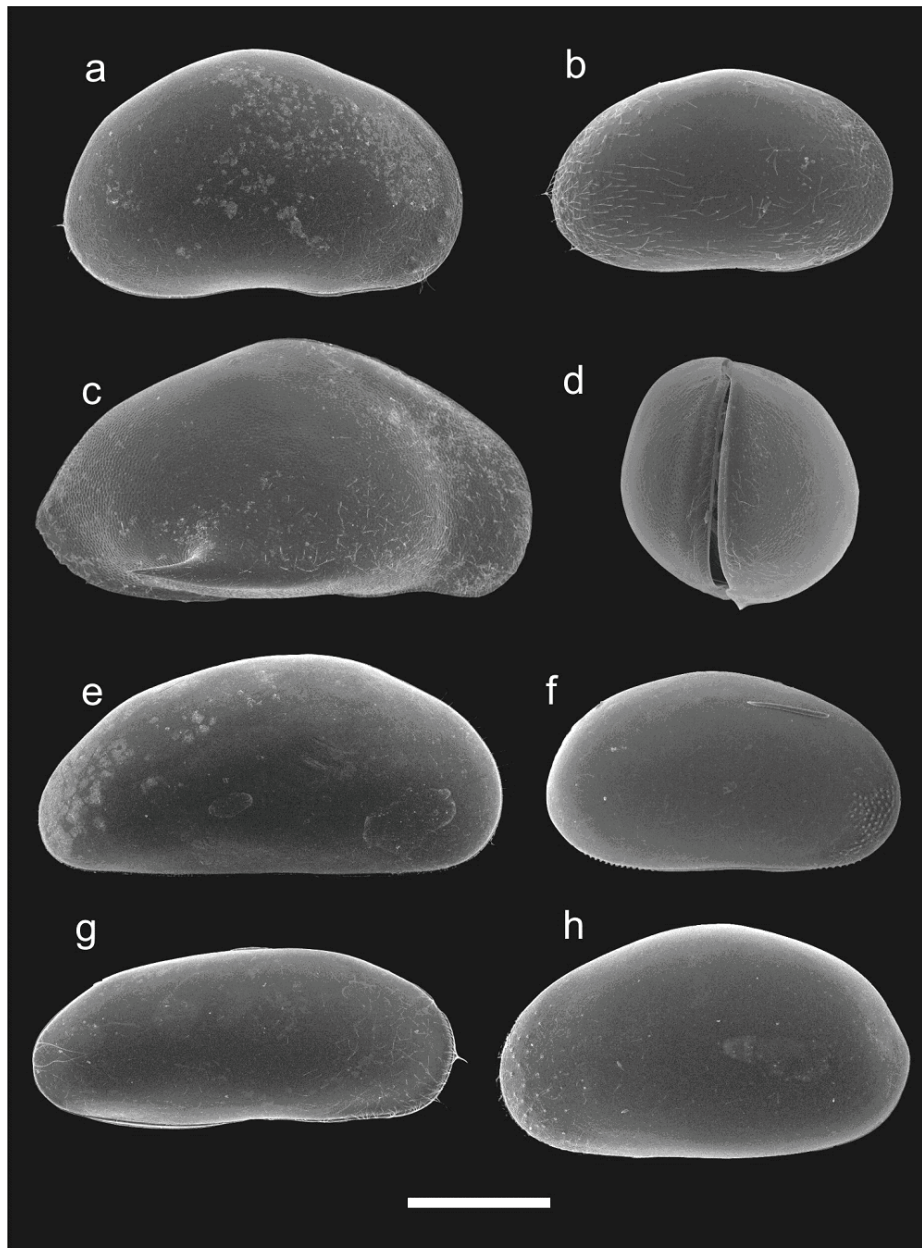


Plate A2. SEM pictures of the ostracod species recorded in the region Alentejo (continued). **a.** *Eucypris virens*: female, lateral view, right valve (scale bar = 778 μm ; sample: Font 2 Jan); **b, d.** *Bradleycypris obliqua*: **b.** female, lateral view, right valve, **d.** female, carapace frontal view (scale bar = 516 μm ; sample: Font 2-4 Apr); **c.** *Cypris bispinosa*: female, lateral view, right valve (scale bar = 809 μm ; sample: Font 2-4 Apr); **e.** *Tonnacypris lutaria*: female, lateral view, right valve (scale bar = 706 μm ; SXP0021); **f.** *Heterocypris* sp.: female, lateral view, right valve (scale bar = 376 μm ; sample: SXPO020); **g.** *Herpetocypris chevreuxi*: female, lateral view, right valve (scale bar = 776 μm ; sample: Font 2-4 Apr); **h.** *Heterocypris incongruens*: female, lateral view, left valve (scale bar = 517 μm ; sample: Torp 2).

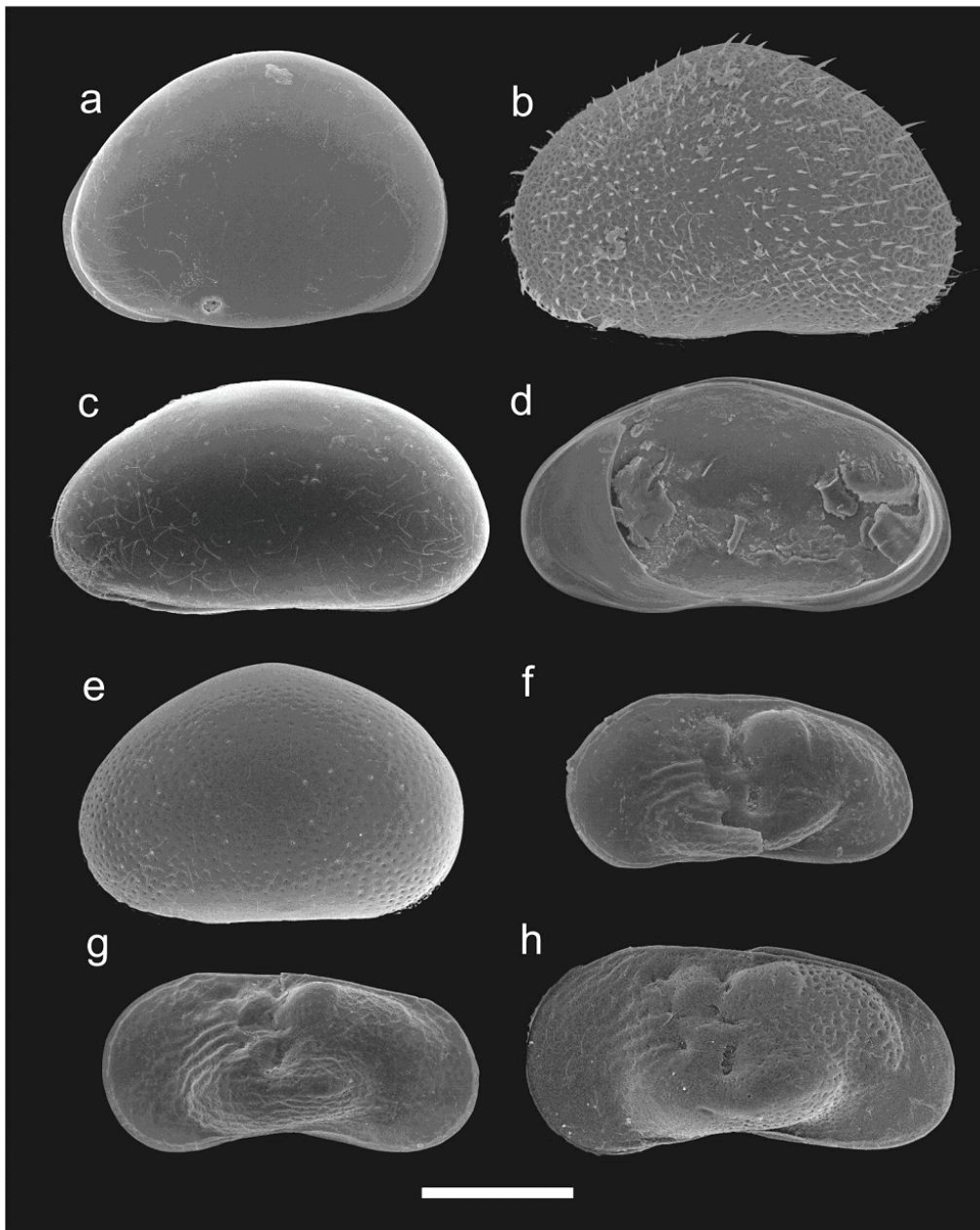


Plate A3. SEM pictures of the ostracod species recorded in the region Alentejo (continued). **a.** *Cypria ophthalmica*: female, lateral view, left valve (scale bar = 259 μm ; sample: Font 3 Jan); **b.** *Sarscypridopsis aculeata*: female, lateral view, left valve (scale bar = 259 μm ; sample: Font 2 Jan); **c-d.** *Cypridopsis hartwigi* (scale bar = 252 μm ; sample: Font 2-4 Apr): **c.** female, lateral view, left valve, **d.** female, internal view, right valve; **e.** *Cypridopsis vidua*: female, lateral view, left valve (scale bar = 196 μm ; sample: Font 2-4 Apr); **f-h.** *Paralimnocythere psammophila*: **f.** juvenile carapace, left side (scale bar = 208 μm ; sample: Font 2 Dec), **g.** male carapace, left side (scale bar = 208 μm ; sample: Font 2-4 Apr), **h.** female, lateral view, left valve (scale bar = 252 μm ; sample: Font 2 Jan).

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