Thematic set:
Cyprideis torosa

JM-TMS
Journal of Micropalaeontology

https://doi.org/10.1144/jmpaleo2016-029 | Vol. 36 | 2017 | pp. 80–93

The Neogene Mediterranean origin of *Cyprideis torosa* (Jones, 1850)

Elsa Gliozzi[1]*, Julio Rodriguez-Lazaro[2] & Radovan Pipik[3]

[1] Dipartimento di Scienze, Università degli Studi Roma Tre, Largo S. Leonardo Murialdo, 1 – 00146 Rome, Italy
[2] Department Estratigrafía y Paleontología, Facultad de Ciencia y Tecnología UPV/EHU, Apartado 644, 48080 Bilbao, Spain
[3] Earth Science Institute, Slovak Academy of Sciences, Dumbierska 1-SK-97411, Banska Bystrica, Slovak Republic
* Correspondence: elsa.gliozzi@uniroma3.it

Abstract: Although *Cyprideis torosa* is one of the most studied ostracods, its ecophenotypic variability has always impeded a clear definition of its carapace morphology. As a consequence, it is often difficult to identify this species in fossil material and very little is known about its phylogenetic origin. In this paper, we attempt to answer two main questions: when and from what species did *C. torosa* originate? To reach this goal we first analysed living and Recent populations of *C. torosa* collected from different salinity environments to define its variability. We found that the valve outline, the size of rounded normal sieve pores and the percentage width of the anterior inner lamella seem to vary independently of salinity. Secondly, to look for possible ancestors we analysed several Neogene species and found that *Cyprideis* sp. from the mid-Serravallian of Spain could possibly be the common ancestor of the Late Miocene Mediterranean and Lake Pannon lineages and that *C. torosa* is strictly linked to the Mediterranean stock, particularly to the phyletic lineage *C. ruggierii*–*C. crotonensis*. In this framework *Cyprideis* gr. *torosa* originated in the late Tortonian and *C. torosa* s.s. is thought to have been differentiated from *C. crotonensis* at the beginning of the Calabrian.

Keywords: *Cyprideis*; Mediterranean; Paratethys; evolution; comparative morphology

Supplementary material: biometric tables and the list of the examined material is available at https://dx.doi.org/10.6084/m9.figshare.c.3636170.v1

Received 27 July 2016; **revised** 8 September 2016; **accepted** 13 September 2016

The genus *Cyprideis* Jones, 1857 was established, as a possible subgenus of *Candona* Baird, on Recent specimens of *Candona torosa* Jones, 1850 on the basis of its

> oblong carapace, marginal edges thickened, presence of selvage, left valve hinge with anterior and posterior sockets and central small teeth (the opposite in the right valve), surface of the valve punctated, upper antennae not plumous as well as the low pair (Jones 1857, p. 20).

Whatley *et al.* (1998) and Tibert *et al.* (2003) amended the diagnosis based on the carapace, indicating as diagnostic characters the quadripartite entomodont hinge, frontal V-shaped scar, strong sexual dimorphism with inflated female posterior and sieve pores of type C (of Puri 1974). Owing to the strong ecophenotypic plasticity, size and ornamentation are not considered as valid diagnostic characters (Wouters 2002), although the position of strong spines (particularly when they are present also in juveniles) has been considered a valid criterion for the specific identification of *Cyprideis* species by Ligios & Gliozzi (2012) and Gross *et al.* (2014).

The genus *Cyprideis* is supposed to have originated from the Cretaceous genus *Fossocytheridea* Swain & Brown (Tibert *et al.* 2003) and, so far, its oldest known species is the Late Oligocene–Early Miocene *Cyprideis traisensis* Malz & Triebel from the Mainz Basin (Germany) (Malz & Triebel 1970). The fossil record depicts clear different speciation events in the New World (Caribbean area – Van den Bold 1976; Western Amazonia – Gross *et al.* 2014 with references therein) and Eurasia (Decima 1964; Krstić 1968*a*,*b*; Ligios & Gliozzi 2012). In the Caribbean area *Cyprideis* is known since the Early Miocene, with *Cyprideis* aff. *C. ovata* (Mincher), and another 14 species. In Western Amazonia (Brazil), a *Cyprideis* species flock comprised of at least 30 species radiated from the late Serravallian to early Tortonian time (Whatley *et al.* 1998; Gross *et al.* 2014). In Eurasia, the early evolution of the genus *Cyprideis* is documented by very few records: one species from the Early Miocene (late Burdigalian, i.e. Late Ottnangian/Early Karpatian) of Croatia (Hajek Tadesse *et al.* 2009), two (or three) species from the mid-Late Serravallian (Sarmatian) of Bulgaria (Stancheva 1963), and one species from the mid-Serravallian of Spain (Rodriguez-Lazaro, unpublished data). Conversely, the genus *Cyprideis* underwent very important adaptive radiations during the Late Miocene and Pliocene in isolated brackish basins of Mediterranean and Paratethys areas. At least 18 species of *Cyprideis* are known in the Palaeomediterranean marginal-marine and Italian brackish athalassic domains (Sissingh 1972; van Harten 1975*a*, 1980*a*, *b*; Ligios & Gliozzi 2012; Gliozzi, unpublished data), 16 species and subspecies in the Anatolian Peninsula (Bassiouni 1979), 29 species in the Pannonian Basin (Reuss 1850; Méhes 1908; Pokorný 1952; Kollmann 1960; Krstić 1968*a*, *b*), and 3 species in the eastern Paratethys (Pobedina *et al.* 1956; Mandelstam *et al.* 1962).

At present, *Cyprideis* is represented by 23 species, geographically limited to continents which probably represent natural barriers for species dispersal. *Cyprideis beaconensis* (Leroy), *C. beaveni* Tressler & Smith, *C. bensoni* Sandberg, *C. castus* Benson, *C. edentata* Klie, *C. mexicana* Sandberg, *C. multidentata* Hartmann, *C. ovata* (Mincher), *C. pacifica* Hartmann, *C. salebrosa* van den Bold, *C. saetosa* Hartmann and *C. similis* (Brady) live in the Americas, including the Caribbean region, while *C. remanei* Klie, *C. limbocostata* Hartmann and *C. nigeriensis* Omatsola are recorded in Africa (van Harten 1990; Wouters 2002, 2003). The Lake Tanganyika species flock is at present made of six endemic species of *Cyprideis* and five phyletic, morphologically highly variable, descending genera whose radiation occurred *c.* 15 Ma (*Archeocyprideis*, *Kavalacythereis*,

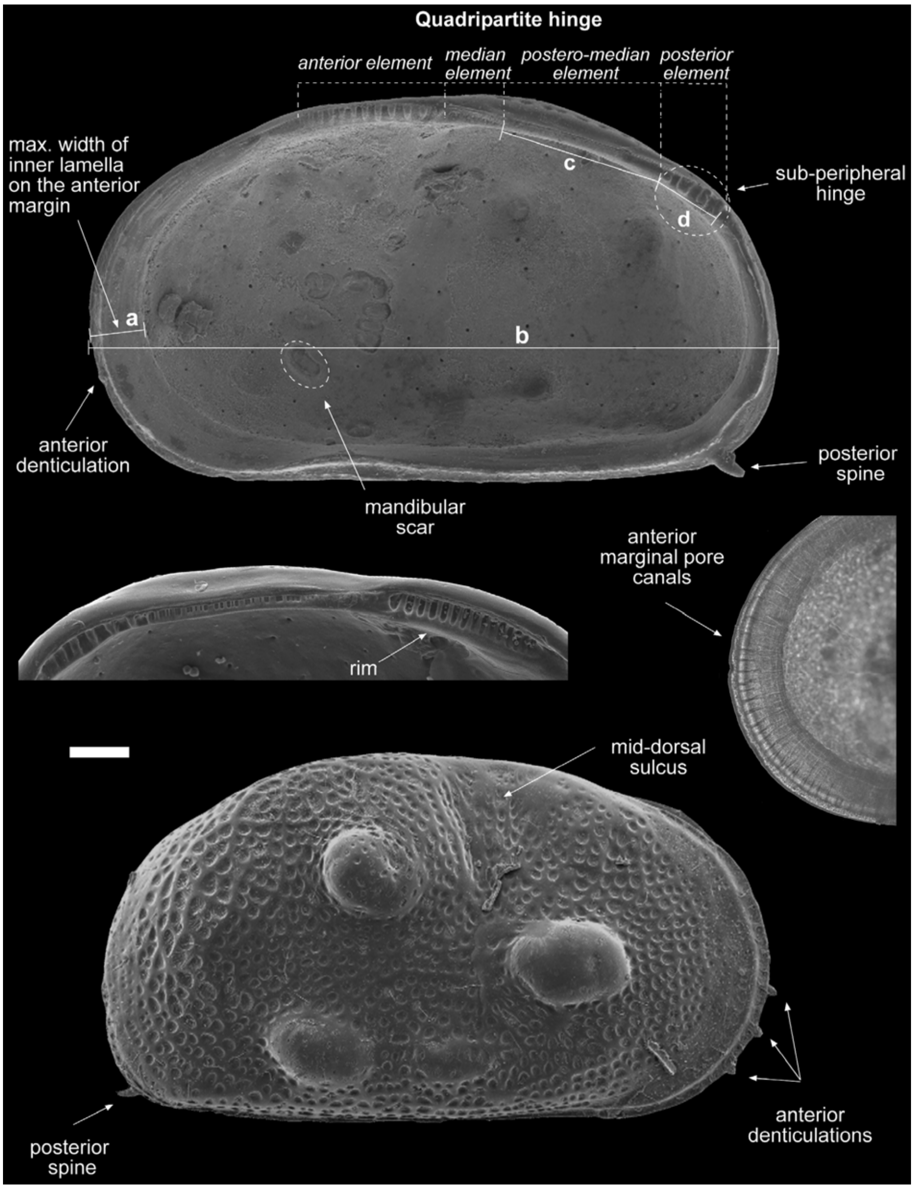


Fig. 1. Detailed morphological characters of *Cyprideis torosa* and biometric ratios analysed in this paper: a/b × 100 = percentage width of the anterior marginal area on the total length of the valve; c/c + d × 100 = percentage length of the postero-median element of the hinge on the total posterior denticulated area. The hinge is defined as peripheral when the teeth overpass the dorsal border, sub-peripheral when they are near the dorsal border, internal when they are well below the dorsal border. Scale bar 0.1 mm. All specimens are from Lake Trasimeno, sample PAN 8.

Mesocyprideis, *Romecytheridea*, *Tanganyikacythere* – Wouters & Martens 2007; Schön & Martens 2012). Furthermore, two endemic species (*C. stenopora* Triebel and *C. pedashenkoi* (Daday)) have been recovered, respectively, from the Galapagos Islands (Sandberg 1964) and the Lake Issyk-Kul (Kyrgyzstan, Central Asia). *Cyprideis torosa* (Jones) displays the largest geographical distribution among all *Cyprideis*, namely in coastal oligo-miohaline (brackish) waters of Europe, Asia and Africa (Meisch 2000; Wouters 2002, 2016).

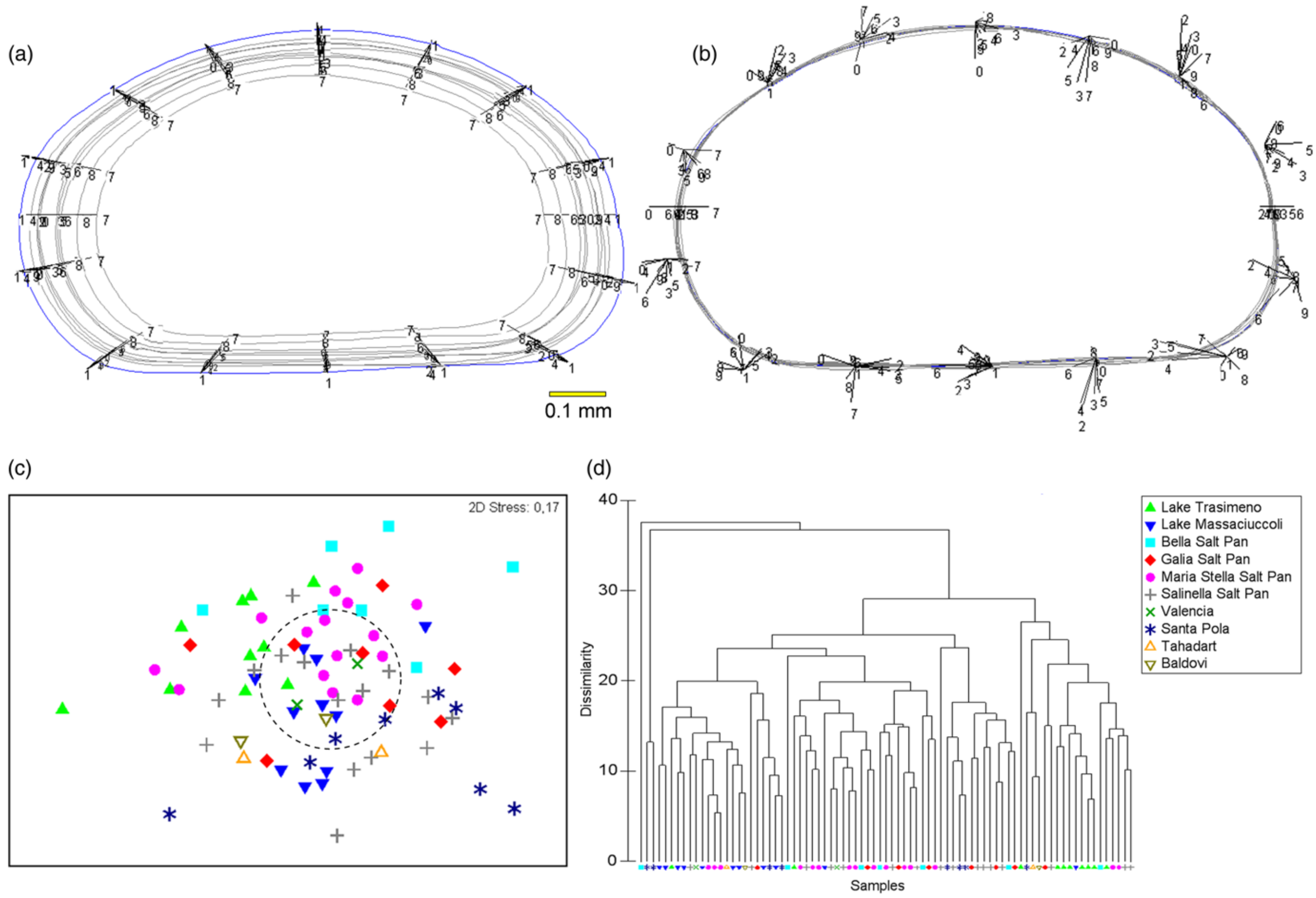
According to van Harten (1990) the wide dispersal area of some living species (*C. salebrosa*, *C. beaconensis* and, in particular, *C. torosa*) is mainly due to passive dispersal by birds, and that author envisages in their shallow-water preferences and brood-care the main characters that enhance their potential to survive temporary hostile conditions during transport. Van Harten (1990) suggests that the extraordinary potential of endemic speciation of the genus *Cyprideis*, particularly in the brackish Lake Pannon, could be due to adaptations to deep environments that, on one side, would provide new ecological niches and, on the other, could have prevented the geographical dispersal of the species. Such a hypothesis seems to be at least plausible since the Recent Tanganyika species flock, according to Wouters & Martens (1994, 1999), could be due to bathymetric segregation. Moreover, this hypothesis is also in agreement with the occurrence of three endemic *Cyprideis* species in the brackish and rather deep Late Miocene Baccinello lacustrine basin (Tuscany, central Italy) (Ligios *et al.* 2008, 2012; Ligios & Gliozzi 2012).

At present, *C. torosa* seems the only living ostracod species of the genus in the Palaearctic region that inhabits freshwater, brackish or even hyperhaline waters where salinity can reach up to 200‰ (Aladin & Potts 1996; Gamenick *et al.* 1996; for details of *C. torosa* geographical distribution see the article by Wouters (2016)). Although it is one of the most studied ostracod species due to its great phenotypic plasticity and value as a (palaeo)ecological and geochemical proxy for reconstruction of past marginal-marine, freshwater and hyperhaline environments, its origin is still unknown. According to Decima (1964), *C. torosa* was derived from *Cyprideis tuberculata* Méhes during the Messinian, while van Harten (1990) suggests its Pliocene origin from the Mediterranean *C. agrigentina* Decima.

This paper attempts to shed light on the time of appearance and the evolutionary history of this species through the re-definition of its specific diagnostic characters and comparison with similar species from the Neogene of the Mediterranean and Paratethys.

Cyprideis torosa: shell description

The taxonomic history of *Cyprideis torosa* is somewhat confused. First, Sandberg (1964) and, most recently, Kempf (2016) gave a



(e)

	Trasimeno	Massaciuccoli	Bella	Galia	Maria Stella	Salinella	Valencia	Santa Pola	Tahadart	Baldovi
Trasimeno	0	0.428	0.482	0.484	0.331	0.428	0.283	0.678	0.561	0.365
Massaciuccoli	0.428	0	0.678	0.494	0,283	0.388	-0.101	0.457	0.086	-0.053
Bella	0.482	0.678	0	0.070	0.218	0.326	-0.117	0.494	0.649	0.386
Galia	0.484	0.494	0.070	0	0.212	-0.010	-0.282	0.199	0.345	0.168
Maria Stella	0.331	0,283	0.218	0.212	0	0.155	-0.182	0.533	0.436	0.332
Salinella	0.428	0.388	0.326	-0.010	0.155	0	-0.218	0.238	0.345	0.173
Valencia	0.283	-0.101	-0.117	-0.282	-0.182	-0.218	0	0.030	0.000	-0.500
Santa Pola	0.678	0.457	0.494	0.199	0.533	0.238	0.030	0	0.073	0.112
Tahadart	0.561	0.086	0.649	0.345	0.436	0.345	0.000	0.073	0	-0.500
Baldovi	0.365	-0.053	0.386	0.168	0.332	0.173	-0.500	0.112	-0.500	0

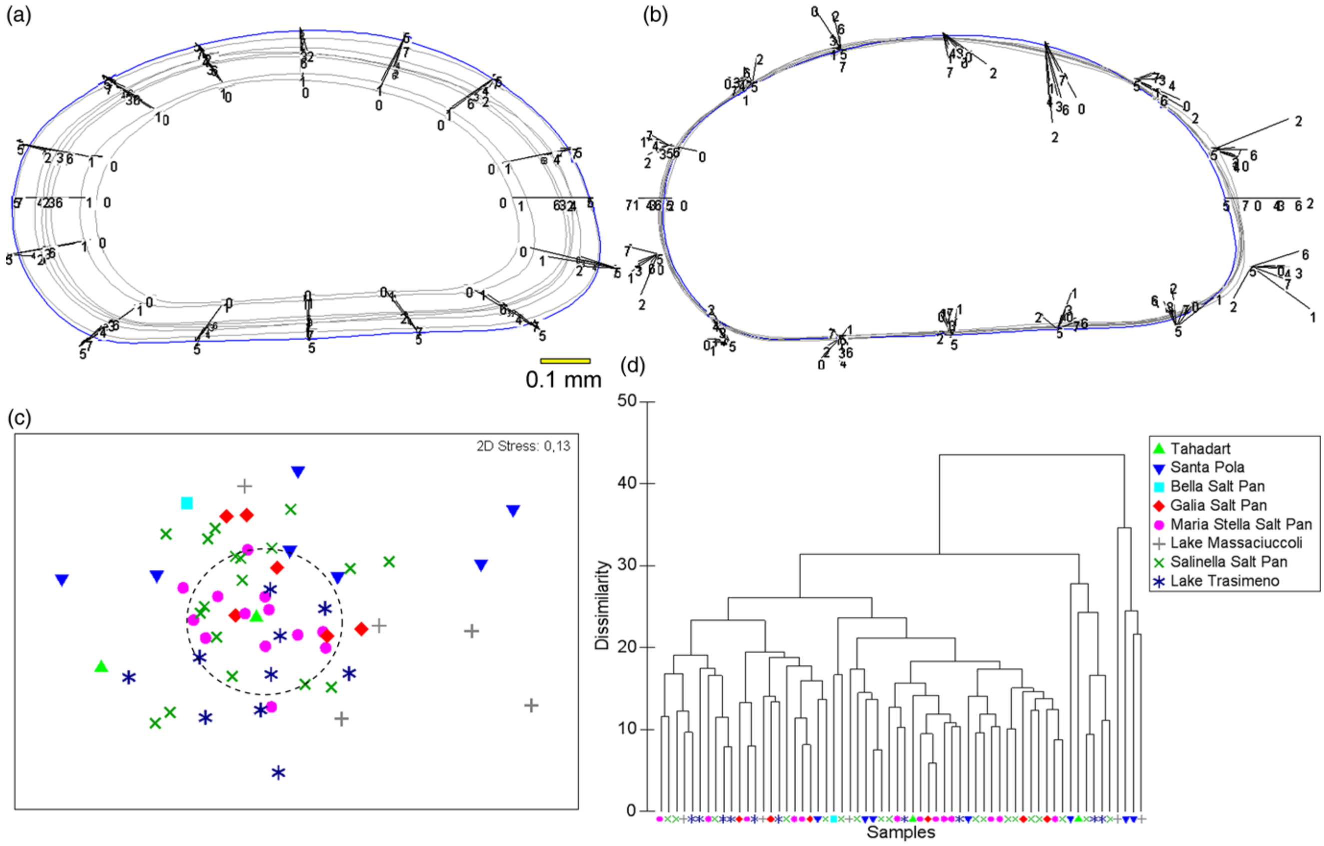
Fig. 2. Results of the geometric-morphometric and multivariate analyses on the female right valve of different populations of living and recent *Cyprideis torosa*. (**a**, **b**) Superimposition of the mean outlines of *C. torosa* from Lake Trasimeno (0), Lake Massaciuccoli (1, blue in online version), Bella salt pan (2), Galia salt pan (3), Maria Stella salt pan (4), Salinella salt pan (5), Valencia (6), Santa Pola (7), Tahadart (8) and Baldovi (9) in (a) 'not normalized for area' and (b) 'normalized for area' modes (MORPHOMATICA outputs have been realized with 6 iterations; in (a) the delta vector scale is 6, in (b) the delta vector scale is 4). Scale bar 0.1 mm. (**c**) nMDS plot in 'normalized for area' mode. (**d**) Dendrogram of the cluster analysis (Euclidean distance measure and the unweighted pair group method using arithmetic average – UPGMA) in 'normalized for area' mode. (**e**) Results of the analysis of similarities (ANOSIM) pairwise tests in 'normalized for area' mode. Significance level for all the tests $P < 0.1\%$.

thorough historical overview of the nomenclature and taxonomy of the genus and species that have been definitively accepted since Sars (1925) but still continue to be confused. A thorough synonymy of *Cyprideis torosa* until 1962 is reported by Sandberg (1964). It is worth noting that *Cyprideis pedashenkoi* Daday, considered by Sandberg (1964) after Klie (1937) and Lindroth (1953) to be a synonym of *C. torosa*, and indicated by Kempf (2016) as a possible subspecies of *C. torosa*, has been revised recently by Schornikov (2015) and definitely separated as an endemic species of Lake Issyk-Kul (Kyrgyzstan) on the basis of the morphology of the soft parts.

But, which are the shell characters peculiar to *Cyprideis torosa* that can be used to distinguish it from other *Cyprideis* species? This is the basic question that must be answered before any debate on its origin and time of speciation.

Wagner (1957), Sandberg (1964) and Decima (1964) gave detailed descriptions of the shell of *Cyprideis torosa*, but these concern general features that could be referable also to other fossil *Cyprideis* species, as *Cyprideis agrigentina* Decima, *Cyprideis ruggierii* Decima and *Cyprideis crotonensis* Decima (Ligios & Gliozzi 2012) are all very similar to *C. torosa*. Herein we will describe some particular features of the *C. torosa* shell, derived from living or sub-fossil specimens sampled from different salinity environments, in order to include the complete ecophenotypical variability of the species.

The studied samples were collected from two low oligohaline athalassic lakes in central Italy (Lake Massaciuccoli in Tuscany and Lake Trasimeno in Umbria, with salinities, respectively, of 0.5 and 0.9‰), three marginal-marine environments in Spain (Valencia, Baldovi and Santa Pola with low mesohaline salinities) and in Morocco (Tahadart, low mesohaline salinity) and four hyperhaline salt pans at Trapani (Sicily) (Maria Stella, Galia, Salinella and Bella, with calculated salinities respectively of 44, 61, 70 and 80‰) (the brackish-water classification follows the Venice System of Remane & Schlieper 1958).



(e)

	Tahadart	Santa Pola	Bella	Galia	Maria Stella	Massaciuccoli	Salinella	Trasimeno
Tahadart	0	-0.006	0	0.250	0.232	0.182	0.109	0.188
Santa Pola	-0.006	0	-0.075	0.077	0.476	0.104	0.300	0.486
Bella	0	-0.075	0	0.378	0.740	0.320	0.170	0.773
Galia	0.250	0.077	0.378	0	0.124	0.249	-0.027	0.245
Maria Stella	0.232	0.476	0.740	0.124	0	0.656	-0.002	0.171
Massaciuccoli	0.182	0.104	0.320	0.249	0.656	0	0.521	0.465
Salinella	0.109	0.300	0.170	-0.027	-0.002	0.521	0	0.112
Trasimeno	0.188	0.486	0.773	0.245	0.171	0.465	0.112	0

Fig. 3. Results of the geometric-morphometric and multivariate analyses on the male right valve of different populations of living and recent *Cyprideis torosa*. (**a, b**) Superimposition of the mean outlines of *C. torosa* from Santa Pola (0), Tahadart (1), Bella salt pan (2), Galia salt pan (3), Maria Stella salt pan(4), Lake Massaciuccoli (5, blue in online version), Salinella salt pan (6), Lake Trasimeno (7) in (a) 'not normalized for area' (b) and 'normalized for area' modes (MORPHOMATICA outputs have been realized with 6 iterations; in (a) the delta vector scale is 6, in (b) the delta vector scale is 1). Scale bar 0.1 mm; (**c**) nMDS plot in 'normalized for area' mode; (**d**) Dendrogram of the cluster analysis (Euclidean distance measure and the unweighted pair group method using arithmetic average - UPGMA) in 'normalized for area' mode; (**e**) Results of the analysis of similarities (ANOSIM) pairwise tests in 'normalized for area' mode. Significance level for all the tests $P < 0.1\%$.

On this bulk of individuals we carried out several detailed observations, morphometric measurements and analyses, such as the lateral valve outline and size (Supplementary material, table 1), the percentage presence of anterior denticulation on the anterior margin and of postero-ventral spine(s) in the postero-ventral margin, the diameter of rounded normal sieve-pores (Supplementary

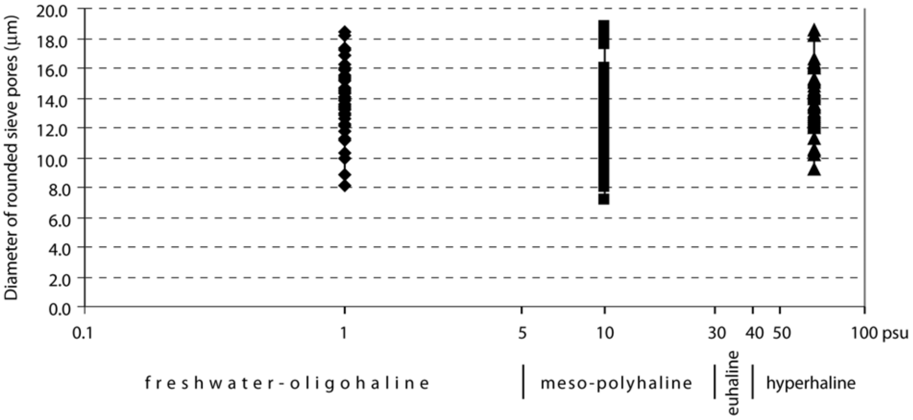


Fig. 4. Variability of the diameter of rounded normal sieve pores in living or Recent *C. torosa* specimens collected in environments with different salinities. For further information, see Supplementary material, table 2.

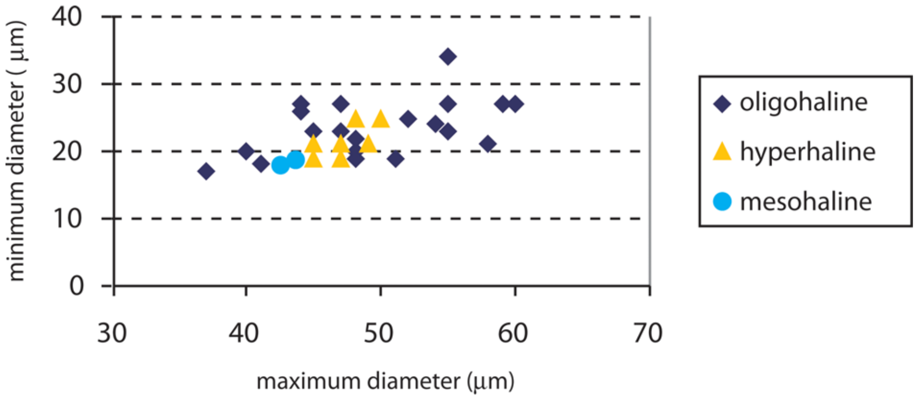


Fig. 5. Scatter diagram of maximum and minimum diameter of the mandibular scar in *C. torosa* from oligohaline, mesohaline and hyperhaline studied waterbodies.

material, table 2), the number of marginal pore canals, the shape and size of the mandibular scar (Supplementary material, table 3), the position of the hinge relative to the dorsal margin, the shape of teeth on the anterior and posterior elements of the left valve (LV) hinge, the number of teeth on the anterior and posterior elements of the LV hinge; the percentage of the denticulated postero-median element length of the LV hinge compared with the total posterior elements, the width of inner lamella on the anterior margin compared with the total length of the valve, using a stereomicroscope in transmitted light and a scanning electron microscope (Fig. 1). The geometric-morphometric analyses of the outline of the right female and male valves were performed using the software TPSdig (Rohlf 2009) to digitize the images, the B-splines algorithm to approximate the outlines (Baltanás & Danielopol 2011), the software MORPHOMATICA v. 1.6.01 (Linhart *et al.* 2007) to compare them and the software PRIMER (Clarke & Gorley 2006) to elaborate statistically the results of the Euclidean distance matrix obtained by MORPHOMATICA.

Table 1. *Comparison of some selected valve characters in living (or Recent) specimens of C. torosa from environments with different salinities*

	Athalassic oligohaline	Marginal-marine brackish	Hyperhaline salt pans
	Lake Trasimeno, Lake Massaciuccoli	Valencia, Tadhart, Baldovi, Santa Pola	Maria Stella, Galia, Salinella, Bella salt pans
	(Living and Recent)	(Living and Recent)	(Recent)
Maximum width (µm) of anterior inner lamella (a in Fig. 1)	50 – 90	41 – 75	47 – 72
% max width of anterior inner lamella/max length of the valve (a/b in Fig. 1)	6.9	5.8	6.4
Shape of the mandibular scar (Fig. 1)	elliptical	elliptical	elliptical
% presence of posterior spine on the right valve (Fig. 1)	94.1 (number of spines: 1)	63 (number of spines: 1)	90.9 (number of spines: 1)
% presence of denticles on the anterior margin of the RV (Fig. 1)	82.4 (number of denticles: 1 – 3)	52 (number of denticles: 1 – 3)	81.8 (number of denticles: 1 – 4)
Number of teeth on the anterior element of the hinge (Fig. 1)	11 – 15	13 – 14	13 – 15
Number of teeth on the posterior element of the hinge (Fig. 1)	5 – 6	5 – 7	5 – 6
% postero-median element of the hinge length/total posterior (c/c+d in Fig. 1)	59 – 75	58 – 64	64 – 78
Position of the hinge in comparison with the dorsal margin (Fig. 1)	peripheral to sub-peripheral	peripheral to sub-peripheral	peripheral

As in any other population of animals, superimposed non-normalized mean outlines of *C. torosa* adult populations differ in size, the smallest being about 77% of the size of the biggest individuals (Figs 2a and 3a). Superimposed normalized mean outlines show that a small disparity in outline is expressed in both sexes at the anterior margin, anterodorsal margin in the area of the anterior hinge element, posteroventral margin and posterior part of the ventral margin (Figs 2b and 3b). In addition, males display higher variability at the dorsal and posterior margin in comparison to the females and this variability is most pronounced between the males from Bella, Galia and Maria Stella salt pans.

Most individuals from all the sampled habitats occur in the centre of the normalized for area n-MDS plot (Figs 2c and 3c). Some specimens fall out of this cloud (showing a greater morphometric distance). They were collected in the oligohaline Lake Trasimeno, the mesohaline Santa Pola and the hyperhaline Bella and Salinella samples in the case of females, and from the oligohaline Massaciuccoli marsh and the mesohaline Santa Pola locality in the case of males. This observation matches with the results of the cluster analysis which clearly separates three females from Bella and Santa Pola and four males from Massaciuccoli and Santa Pola (Figs 2d and 3d). ANOSIM Pairwise test (Figs 2e and 3e) implies low morphometric differences between the measured individuals (Global R = 0.308 for females and 0.222 for males). The highest differences are between males from the oligohaline Massaciuccoli marsh v. the hyperhaline Maria Stella salt pan and the oligohaline Lake Trasimeno and the hyperhaline Bella salt pan (respectively R = 0.66 and 0.77) and females from the mesohaline Santa Pola locality v. the hyperhaline Salinella and oligohaline Trasimeno ones (respectively R = 0.53 and 0.68).

In conclusion, the males display larger morphometric variability in outline than females (see also Grossi *et al.* 2016), but the morphometrical space in which this variability is expressed differs for the sexes from the same locality. Furthermore, Figures 2 (female right valve (RV)) and 3 (male RV), show that the valve outline is independent of the environmental salinity and only the shell size is somewhat affected by it, in that the oligohaline forms are slightly larger than the others and the hyperhaline valves are the smallest (see Boomer *et al.* (2016) for more details).

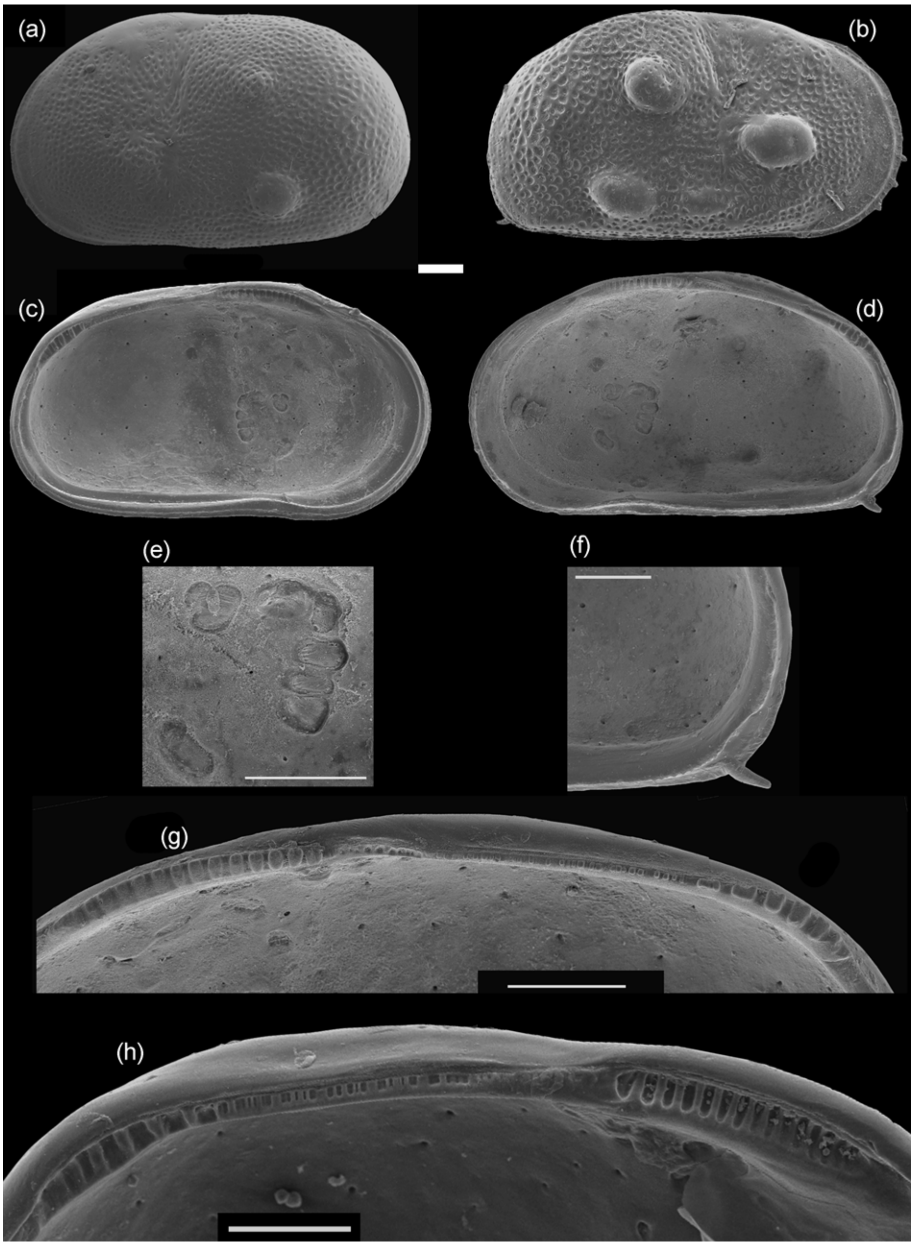


Fig. 6. Morphology of the female valves of living *C. torosa*. (**a**) left valve in external view (Lake Trasimeno, sample PAN 15); (**b**) right valve in external view (Lake Trasimeno, sample PAN 7); (**c**) left valve in inner view (Lake Trasimeno, sample PAN 9); (**d**) right valve in inner view (Lake Trasimeno, sample PAN 9); (**e**) detail of the muscle scars in a right valve (Lake Trasimeno, sample PAN 9); (**f**) detail of the postero-ventral angle of a right valve in inner view (Lake Trasimeno, sample PAN 9); (**g**) detail of the hinge of a right valve (Salinella salt pan, Trapani); (**h**) detail of the hinge of a left valve (Lake Massaciuccoli, sample LM 2). Scale bars 0.1 mm.

Also, the diameters of the normal rounded sieve pores (Fig. 4) and the elliptical shape and size of the mandibular scar are not influenced by salinity (Fig. 5).

In general, from Table 1 it is possible to observe that none of the considered characters are environmentally cued (at least for what concerns salinity) but not all of them can be considered valid characters to describe the shell of *C. torosa*. For example, the occurrence of posterior spine(s) and anterior denticulations in Figure 1 in the right valve is rather variable (as already observed by Ligios & Gliozzi 2012) as well as the maximum width of the anterior inner margin/max. length of the valve ratio. Thus, hereinafter we give a complete description of the carapace and valves of *C. torosa*, merging those from Wagner (1957), Sandberg (1964) and Decima (1964) with our own observations.

Shape

Female (Fig. 6)

The carapace is massive, subcuneiform in dorsal view, weakly to strongly inflated posteriorly due to the presence of the brood pouch, and with a more or less evident dorso-median sulcus, anterior end slightly pointed, posterior end broadly rounded. The left valve is

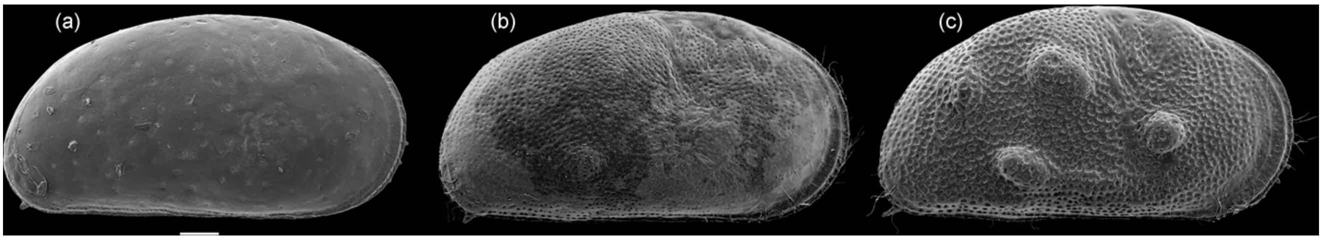


Fig. 7. Ornamentation in *C. torosa* is an ecophenotypical character: (**a**) smooth (Maria Stella salt pan); (**b**) pitted (Lake Trasimeno, sample PAN 8); (**c**) reticulate (Lake Trasimeno, sample PAN 9). Generally the reticulated morph is coupled with the presence of nodosity. Scale bar 0.1 mm. All male right valves.

Table 2. *Comparison of some selected valve characters in selected species of Cyprideis from Palaeomediterranean and Paratethys*

	C. pannonica Skalica	*C. pannonica* Sankt Margarethen	*C. sublittoralis* Hodonin	*C. macrostigma*	*C. tuberculata*	*C. seminulum*	*C. alberti*	*Cyprideis* sp. (Spain)	*C. ruggierii*	*C. agrigentina*	*C. crotonensis*	*C. torosa*
Shell thickness	thin/thick	thin/thick	thin/thick	thick	thick	thin/thick	thin/thick	thick	thick	thick	thick	thin
Posterior carapace in dorsal view	scarcely inflated to inflated	scarcely inflated to strongly inflated	inflated	inflated to strongly inflated	inflated to strongly inflated	scarcely inflated to inflated	flat	scarcely inflated	inflated	inflated	scarcely inflated	scarcely inflated to inflated
Mid dorsal sulcus (Fig. 1)	remarkable, deep and large	remarkable, deep and large	negligible to remarkable	shallow to deep, large	deep and large	shallow, negligible	negligible to weak	remarkable	weak to pronounced	negligible	negligible to weak	remarkable
Number of anterior marginal pore canals (Fig. 1)	34 – 43	33 – 44	34 – 47	27 – 37	36 – 46	30 – 37	25 – 33	36	37 – 45	34 – 42	33 – 46	30 – 40
Average diameter of rounded normal pore-canals (μm)	2.8	4.6	3.7	4	3.7	3.2	3.4	9.1	14.8	10.6	12.8	13.3
% max width of anterior inner margin/max length of the valve (a/b in Fig. 1)	7.1	6.6	7.3	7.2	7.5	8.0	7.6	6.2	8.7	8.3	7.1	6.4
Shape of the mandibular scar (Fig. 1)	elliptical	elliptical to large subelliptical	elliptical to elliptical large	elliptical	elliptical	elliptical narrow to elliptical	elliptical	sub-elliptical to elongated crescent	elliptical	elliptical	elliptical	elliptical
% presence of posterior spine on the right valve	80 (number of spines: 1; PV list always present)	100 (number of spines: 1; PV list always present)	80 (number of spines: 1; PV list occasionally present)	86 (number of spines: 1–3; PV list can occur)	70 (number of spines: 1; PV list can occur	0	0	70 (number of spines: 1)	76 (number of spines: 1)	83 (number of spines: 1–2)	57 (number of spines: 1–2)	83 (number of spines: 1)
% presence of anterior denticles on the right valve	81 (number of rudimentary denticles: 2 – 6)	100 (number of tiny denticles: 5 – 7)	100 (number of denticles: 4 – 8	96 (number of denticles: 3 – 6)	100 (number of tiny denticles: 5 – 7)	100 (number of denticles: 6 – 9)	100 (number of tiny denticles: 9 – 14)	64 (number of denticles: 2 – 6)	0	12 (number of denticles: 1 – 3)	65 (number of denticles: 1 – 5)	72 (number of denticles: 1 – 4)
% presence of posterior spine on the left valve	0	0	0	0	0	0	0	0	0	0	0	0
% presence of anterior denticles on the left valve	0	40 (number of denticles: 2 – 5)	80 (number of denticles: 1–8, frequently rudimentary)	93 (number of denticles: 1 – 5; occasionally rudimentary)	82 (number of tiny denticles: 2 – 5)	88 (number of denticles: 4 – 8)	85 (number of tiny denticles: 2 – 12)	0	0	4 (number of denticles: 2)	48 (number of denticles: 1 – 4)	0
Number of teeth on the anterior plate of the hinge	0 – 13	10 – 16	7 – 13 (occasionally 0)	0 – 11	0 (occasionally up to 7)	11 – 14 (occasionally 5)	10 – 13	10 – 16	14 – 15	14 – 16	16	11 – 15
Shape of the teeth on the anterior plate of the hinge	square to rectangular, sometimes	rectangular to square, some of them subtle	rectangular, square and rounded, tiny;	smooth, if present subtle	smooth, if present subtle	rectangular, triangular and square,	rectangular	squared	rectangular narrow	rectangular narrow	rectangular narrow	ovoidal or rectangular narrow

	tiny or smooth		occasionally smooth	rectangular to square		frequently divided						
Rim below the anterior element of the left valve hinge (Fig. 1)	faint	faint	not present	negligible to faint	faint	median	strong	strong	strong	strong	strong	negligible to faint
Number of teeth on the posterior plate of the hinge	0 – 7	5 – 7 (occasionally 2)	4 – 7 (occasionally 0)	0 – 5	0, 4 – 5	4 – 6	4 – 6	5 – 8	5 – 6	6 – 7	5 – 6 (the last one frequently V-shaped)	5 – 7
% length of the postero-median element of the hinge/total posterior (c/c+d in Fig. 1)	66.1	62.6	61.5	75.3	69.4	59.2	63.7	54.5	65.7	63.7	64 – 72	58 – 78
Hinge thickness	thin	thin/thick	thin to thin/thick	thin/thick	thin	thin, thin/thick	thin/thick	thick	thick	thick	thick	thin
Position of the hinge in comparison with the dorsal margin	sub-peripheral	sub-peripheral	sub-peripheral	sub-peripheral	sub-peripheral	sub-peripheral	sub-peripheral	peripheral to sub-peripheral	sub-peripheral to internal	sub-peripheral to internal	sub-peripheral to internal	peripheral to sub-peripheral

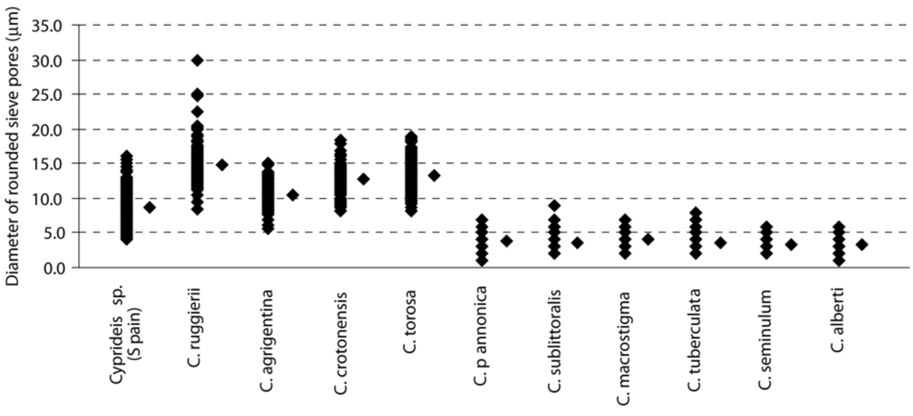


Fig. 8. Variability of the diameter of rounded normal sieve pores in several species of *Cyprideis*.

larger than the right one. In lateral view the valves are subovate. The right valve displays an arched dorsal margin with sub-central maximum height, broadly rounded anterior margin with a posterior cardinal angle obtuse and rounded, posterior margin steeply sloping, postero-ventral margin rounded, and ventral margin straight. Left valve similar, with a flat dorsal margin slightly sloping down posteriorly, posterior margin rounded. In both valves the dorso-median sulcus can vary from weakly to considerably depressed.

Male (see Fig. 7)

The carapace is longer than the female, being lanceolate in dorsal view, with sides nearly parallel and both ends slightly pointed. In lateral view the valves are elongated and sub-elliptical. The right valve shows broadly rounded anterior margin, gently arched dorsal margin, posterior cardinal angle marked by a gradual change in the slope, posterior margin flatly sloping, postero-ventral margin acutely rounded, and ventral margin straight. The left valve displays a flat dorsal margin slightly sloping down posteriorly, ventral margin straight to slightly concave below the central muscle scar field and postero-ventral margin more broadly rounded. The dorso-median sulcus is generally weak and shallow.

Ornamentation

Wagner (1957), Decima (1964) and particularly Sandberg (1964) describe very accurately the surface ornamentation – the ecophenotypic characters of *C. torosa* that depend mainly on salinity (Fassbinder 1912; Triebel 1941; Wagner 1957; Van Morkhoven 1962; Carbonel 1988; van Harten 1996; Keyser & Aladin 2004; Keyser 2005; Frenzel *et al.* 2012) but can be induced by physiological and genetic changes as well (Kilenyi 1972; van Harten 1975*b*, 2000; Bodergat 1983, 1985). It is just as important to note that the species displays ornamentation that ranges from smooth to punctate to faintly reticulated and noded (Fig. 7). Below the threshold of 8–9 psu valves can be noded, and up to 8 nodes, although differently developed, occur at the exact locations indicated by Sandberg (1964). The anterior margin of the right valve is without or with one to five small, sharp, widely spaced denticles. The postero-ventral margin of the right valve is without a spine or carries one, rarely two, rather strong spines directed postero-ventrally. Left valves are without anterior denticulation and posterior spines.

Internal characters (Fig. 1)

The inner lamella is rather narrow, being widest on the anterior margin where its width varies from 5.8 to 6.9% of the total length of the valve; it is concave outwards at the postero-ventral margin of the right valve. A vestibule is absent. Approximately 30 – 40 straight (very rarely bi- or trifurcated) marginal pore canals are found on the anterior margin, while those on the ventral margin are short and straight; they are characteristically thickened in the middle. The normal pore canals are all sieve-type, rounded, elongated or irregular in outline. The percentage of different sieve pore outlines is known as an ecophenotypic character, strictly linked to salinity (Rosenfeld & Vesper 1977; Pint *et al.* 2012). Conversely, the dimensions of the rounded sieve pores seem to be unaffected by salinity. The adductor muscle scars are arranged in a vertical row of four scars. The antennal scar is V-shaped. Behind it, there is an evident sub-circular fulcral point. The mandibular scar is elliptical in shape. The hinge is quadripartite. On the right valve it is composed of an anterior denticulate element with 11 – 15 trapezoidal sometimes bipartite teeth, a short median element with small sockets, a rather long posteromedian denticulate element and a posterior tooth plate with 5 – 7 more or less quadrangular and sometimes bipartite teeth. The elements of the right valve are opposite to those of the left valve. Generally a faint rim is developed below the anterior element in the left valve.

***Cyprideis torosa*: origin**

Although *C. torosa* is one of the most studied ostracod species, the time of its appearance and its ancestors are still unclear. The first author who attempted to depict its evolutionary history was Decima (1964) who suggested, in agreement with Pokorný (1952), that *C. torosa* could be strictly linked to the *Cyprideis heterostigma* (Reuss) group from the Late Miocene Lake Pannon, in particular to *Cyprideis sublittoralis* Pokorny. In more detail, Decima (1964) supposed that a common ancestor (similar to *Cyprideis ruggierii* Decima) gave rise to two lineages, one including *Cyprideis pannonica* (Mehés), *C. agrigentina* Decima, *C. crotonensis* Decima, *C. alberti* Kollmann, and *C. seminulum* (Reuss), the other with *Cyprideis tuberculata* (Mehés), *C.* gr. *heterostigma* and *C. macrostigma* Kollmann. Within this latter lineage, *C. torosa* originated in the post-evaporitic Messinian (three valves referred by him to *Cyprideis torosa* subsp. indet. from Eraclea Minoa succession in Sicily) (note that Ligios & Gliozzi (2012) referred those valves to the Messinian species *C. agrigentina* Decima).

In her revision of *Cyprideis* from the Congeria Beds (Lake Pannon), Krstić (1968*a*, *b*) included in the 'group *torosa*' several species, among which were *C. seminulum*, *C. pannonica* and *C. macrostigma*, clearly stating that some of those species are intermediate between *C. seminulum* and *C. torosa*, particularly *C. pannonica*. According to Krstić (1968*b*) none of the species described by Decima (1964) from the Neogene of Italy were present in Lake Pannon.

In their revision of the Neogene *Cyprideis* of Italy, Ligios & Gliozzi (2012) included in the '*C. torosa* group' the species

C. ruggierii, *C. agrigentina* and *C. crotonensis* on the basis of the strong morphological resemblance of the valve outline. According to Decima (1964) and Ligios & Gliozzi (2012) the latter three species are phylogenetically related and van Harten (1990) suggested that *C. torosa* could represent the Pliocene ‘daughter’ of the post-evaporitic Messinian *C. agrigentina*, although subsequently (van Harten (2000), followed by Wouters (2002)), *C. torosa* was indicated to have originated in the Late Miocene.

To unravel the complex history of the origin of *C. torosa* we analysed in detail the morphology of *C. pannonica*, *C. alberti*, *C. seminulum*, *C. sublittoralis*, *C. macrostigma*, *C. tuberculata*, *C. ruggierii*, *C. agrigentina*, and *C. crotonensis*, comparing them with *C. torosa*. In particular we have analysed: (a) *C. torosa* living and Recent specimens from Italy, Spain and Morocco used to define the morphological characters of the species (see details above); (b) *C. ruggierii*, *C. agrigentina* and *C. crotonensis* specimens from Decima’s original collection and Gliozzi’s collection and database, also following the original descriptions of Decima (1964) and the revision of Ligios & Gliozzi (2012); (c) *C. pannonica* from pre-Lake Pannon deposits (Middle Miocene), *C. alberti*, *C. seminulum*, *C. sublittoralis*, *C. tuberculata* and *C. macrostigma,* all from Lake Pannon and from Pipik’s collection and database, also following the original descriptions of Reuss (1850), Méhes (1908), Pokorný (1952) and Kollmann (1960); (d) *Cyprideis* sp. specimens from the Ebro Basin (Spain), from Rodriguez-Lazaro’s collection; (e) *Cyprideis* sp. specimens from Croatia (=*C. sublittoralis* in Hajek Tadesse *et al.* 2009), through SEM pictures kindly made available by V. Hajek-Tadesse. For further information, see Supplementary material, Table 4.

Table 2 summarizes some morphological characters of the examined species. It is rather evident that some of those characters, such as the number of teeth in the hinge, the anterior denticulations and the number of marginal pore canals, display a great intraspecific variability, thus do not seem to be clearly species-specific, while others, such as the mandibular scar shape, width of the inner lamella on the anterior margin, presence of posterior spine on the left valve, are rather similar in all the species and typical of the genus. Finally, very few of them could be useful differentiating characters: the diameter of the normal sieve pores (Fig. 8), which clearly separates the *Cyprideis* from Lake Pannon from those of the Palaeomediterranean and Mediterranean species, and the valve dimensions, *C. macrostigma*, *C. crotonensis* and *C. torosa* being among the largest species (Table 3).

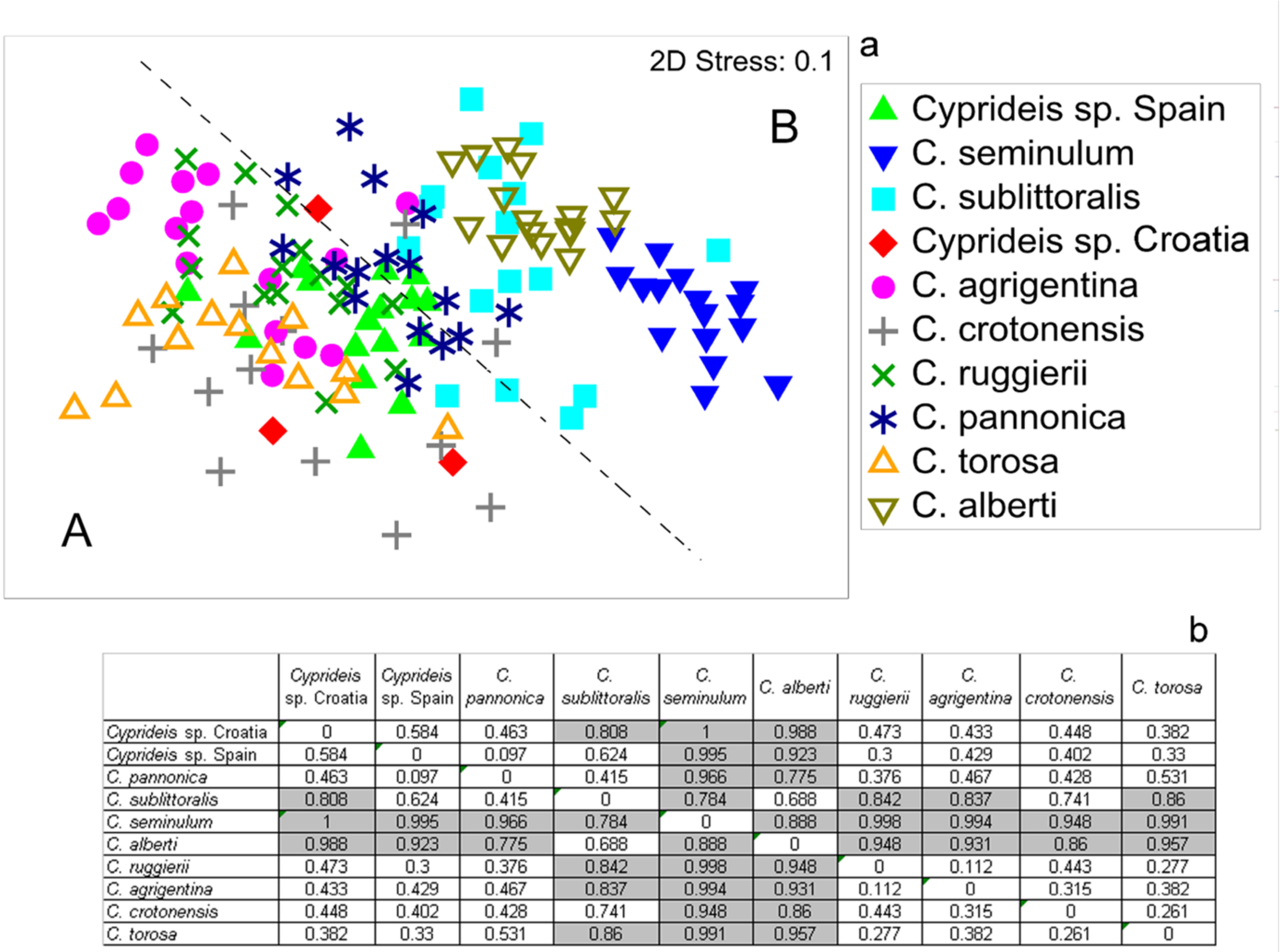
Since most of the taxonomic papers dealing with *Cyprideis* (Pokorný 1952; Kollmann 1960; Krstić 1968*a*, *b*; Bassiouni 1979; Gross *et al.* 2008, 2014; Ligios & Gliozzi 2012) base the specific distinction within the genus mainly on the form of the valves (i.e. their shape and size according to Danielopol *et al.* 2011), we have applied the geometric-morphometric analysis to the female right valves of *Cyprideis* sp. (Spain), *Cyprideis* sp. (Croatia), *C. pannonica*, *C. alberti*, *C. seminulum*, *C. sublittoralis*, *C. ruggierii*, *C. agrigentina*, and *C. crotonensis*, comparing them with *C. torosa* and we have processed the obtained results through ANOSIM pairwise tests and nMDS analysis.

According to ANOSIM analysis (Fig. 9a), the Lake Pannon *C. seminulum* is statistically dissimilar from all the examined species and *C. alberti* is dissimilar from all except *C. sublittoralis*. Conversely, Lake Pannon *C. pannonica* displays similarities with all the remaining species which, in their turn, are not statistically separated from each other. Similarly, the nMDS plot (Fig. 9b) shows a clear separation of *C. seminulum* and *C. alberti* from the other species. If we ideally trace an oblique line which divides the plot of Figure 9a into low left (A) and high right (B) triangular areas it is possible to observe that the Lake Pannon species are roughly located in B, while the Palaeomediterranean species are mainly in A. *Cyprideis* sp. from Spain and *C. pannonica* dots are mainly

Table 3. *Comparison of length and height of the right female valve in selected species of Cyprideis from Palaeomediterranean and Paratethys*

	Cyprideis seminulum	*Cyprideis alberti*	*Cyprideis sublittoralis*	*Cyprideis macrostigma*	*Cyprideis tuberculata*	*Cyprideis pannonica* SM	*Cyprideis pannonica* SKA	*Cyprideis* sp. (Spain)	*Cyprideis ruggierii*	*Cyprideis agrigentina*	*Cyprideis crotonensis*	*Cyprideis torosa*
Length (mm)												
n	30	29	29	20	30	30	29	4	51	23	19	128
SD	0.020	0.015	0.053	0.023	0.024	0.036	0.036	0.065	0.045	0.034	0.046	0.063
max	0.91	0.73	1.17	0.99	0.96	1.01	0.91	0.78	1.06	0.99	1.02	1.10
min	0.83	0.67	0.97	0.90	0.86	0.86	0.75	0.66	0.84	0.87	0.82	0.77
mean	0.88	0.70	1.03	0.95	0.90	0.95	0.81	0.73	0.92	0.93	0.97	0.97
Height (mm)												
n	30	29	29	20	30	30	29	4	51	23	19	128
SD	0.016	0.075	0.032	0.017	0.015	0.020	0.024	0.029	0.030	0.026	0.028	0.062
max	0.49	0.40	0.64	0.56	0.52	0.56	0.52	0.44	0.63	0.59	0.57	0.63
min	0.44	0.36	0.52	0.49	0.46	0.48	0.41	0.37	0.47	0.49	0.46	0.43
mean	0.46	0.38	0.56	0.53	0.50	0.53	0.45	0.41	0.52	0.54	0.55	0.55

n, number of specimens; SD, standard deviation.



	Cyprideis sp. Croatia	*Cyprideis* sp. Spain	*C. pannonica*	*C. sublittoralis*	*C. seminulum*	*C. alberti*	*C. ruggierii*	*C. agrigentina*	*C. crotonensis*	*C. torosa*
Cyprideis sp. Croatia	0	0.584	0.463	0.808	1	0.988	0.473	0.433	0.448	0.382
Cyprideis sp. Spain	0.584	0	0.097	0.624	0.995	0.923	0.3	0.429	0.402	0.33
C. pannonica	0.463	0.097	0	0.415	0.966	0.775	0.376	0.467	0.428	0.531
C. sublittoralis	0.808	0.624	0.415	0	0.784	0.688	0.842	0.837	0.741	0.86
C. seminulum	1	0.995	0.966	0.784	0	0.888	0.998	0.994	0.948	0.991
C. alberti	0.988	0.923	0.775	0.688	0.888	0	0.948	0.931	0.86	0.957
C. ruggierii	0.473	0.3	0.376	0.842	0.998	0.948	0	0.112	0.443	0.277
C. agrigentina	0.433	0.429	0.467	0.837	0.994	0.931	0.112	0	0.315	0.382
C. crotonensis	0.448	0.402	0.428	0.741	0.948	0.86	0.443	0.315	0	0.261
C. torosa	0.382	0.33	0.531	0.86	0.991	0.957	0.277	0.382	0.261	0

Fig. 9. Results of the geometric-morphometric and multivariate analyses on the female right valve of different species of *Cyprideis*: (**a**) results of the analysis of similarities (ANOSIM) pairwise tests in 'normalized for area' mode; (**b**) nMDS plot in 'normalized for area' mode. For explanations, see text.

distributed at the boundary between areas A and B. The position of *Cyprideis* sp. from Croatia is unclear, possibly due to the scarce material (only three valves).

The ANOSIM pairwise tests and the nMDS analysis seem to support the clear separation between the Palaeomediterranean and Lake Pannon species proposed by Krstić (1968*b*). Furthermore, the distribution of *Cyprideis* sp. from Spain (mid-Serravallian) and *C. pannonica* (latest Serravallian) at the boundary between areas A and B suggest that the former could represent the ancestor of both the Palaeomediterranean and Lake Pannon lineages and that the latter could be at the origin of the adaptive radiation of *Cyprideis* in the Paratethyan realm. This hypothesis is not inconsistent with present knowledge about the palaeogeography of both areas during the Middle and Late Miocene. According to the most recent palinspastic reconstructions of the Neogene of the Tethys area (Popov *et al.* 2004, 2006; Neubauer *et al.* 2015*a*, *b*), during Middle Miocene up to mid-Serravallian time the western and eastern sector of the basin were connected to the north, through the south Alpine corridor, whereas during the late Serravallian the corridor closed, giving rise to the spectacular adaptive radiation of endemic species in Lake Pannon.

In this framework, *C. torosa* would be linked to an exclusively Palaeomediterranean phyletic lineage, starting from *Cyprideis* sp. from Spain through *C. ruggierii* and *C. crotonensis*, contradicting both the hypotheses proposed by Decima (1964) and Krstić (1968*a*, *b*). The record of *C. torosa* in the Sarmatian of Bulgaria (Stancheva 1963 as *C. littoralis*) should be discarded or checked carefully.

The time of the first occurrence of *C. torosa* is still a matter of debate. There are several records of *C. torosa* or *Cyprideis* ex gr. *torosa* during the Neogene (Italy – e.g. Ruggieri 1958; Bossio *et al.* 1993, 1994, 1997; Sarti & Testa 1994; Paratethys – e.g. Agalarova *et al.* 1961; Bodina 1961; Mandelstam *et al.* 1962; Karmishina 1975; Stoica *et al.* 2013; van Baak *et al.* 2016). The Italian specimens were checked by Ligios & Gliozzi (2012) and proved to be referable to *C. agrigentina*. Unfortunately, we were unable to check the eastern Paratethyan material.

If our hypothesis that *C. torosa* is derived from *C. crotonensis* is correct, we must take into account that the morphometric differences between *C. torosa* and *C. crotonensis* are subtle (Table 2) (*C. crotonensis* shell is thicker, the dorso-median sulcus is less marked, the outline is slightly more convex in the middle ventral margin, sometimes there are anterior denticles on the left valve, the rounded normal pore canals are slightly smaller on average, the rim below the anterior element of the left valve hinge is thicker, the anterior inner margin is proportionally wider). Thus, the distinction between the two species requires well-preserved and abundant specimens and a detailed analysis. At present, such data are available only for some Pleistocene populations of central Italy: the Gelasian specimens from Dunarobba (Tiberino Basin) (Gliozzi unpublished material) and Case Strinati (Rieti Basin) (Barberi *et al.* 1995; Gliozzi & Mazzini 1998), and the Calabrian recoveries from S. Faustino (Tiberino Basin) (Ambrosetti *et al.* 1995) and Pietrafitta (Umbria) (Gliozzi unpublished material). Although the normalized outlines of those species are completely superimposed (Fig. 10), as shown in Figure 11, Dunarobba and Case Strinati specimens display thick shells, a faint dorso-median sulcus, a conspicuous rim below the anterior element of the hinge, a relatively broad inner lamella on the anterior margin (respectively 7 and 6.9%), and small rounded

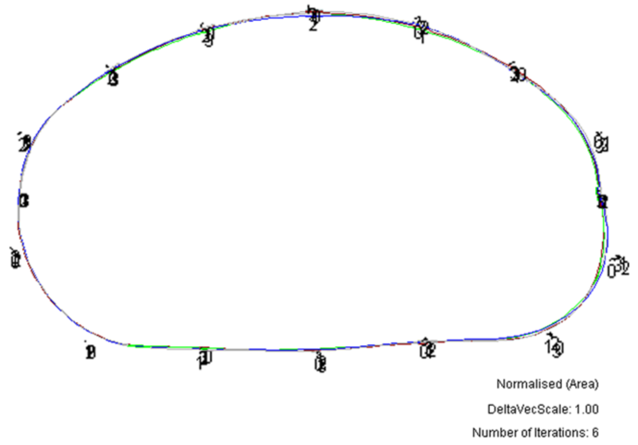


Fig. 10. Superimposition of the mean outlines of the female right valve of Pleistocene *Cyprideis* from sample DUN 3 from Dunarobba (red, contour 0), Case Strinati (grey, contour 1), Pietrafitta (blue, contour 2) and S. Faustino (green, contour 3) from MORPHOMATICA in 'normalized for area' modes. See online version for colour.

sieve pores (on average respectively 12.4 and 11.9 µm), thus they could be referable to *C. crotonensis*. Conversely, S. Faustino and Pietrafitta specimens show thin shells, a marked dorsal sulcus, a faint rim, a narrower inner lamella on the anterior margin (6.4% for S. Faustino specimens), and larger rounded sieve pores (on average, respectively 14.8 and 15.8 µm), more similar to *C. torosa*. The present analysis, limited to some Italian populations, suggests that *C. torosa* could have first occurred in the Early Pleistocene (Calabrian) in the Mediterranean area.

In summary, our research is still in need of further studies on more fossil specimens from Eurasia. At the moment, with the available data, we propose a Palaeomediterranean origin of *C. torosa* and we suggest two possible hypotheses. (1) If we accept the specific distinction of *C. ruggierii*, *C. agrigentina*, *C. crotonensis* and *C. torosa* (according to Decima (1964) and Ligios & Gliozzi (2012)), we propose a gradual evolution along this phyletic lineage and suggest a younger, Early Pleistocene origin for *C. torosa,* with the first occurrence at the beginning of the Calabrian. (2) If we consider that the subtle morphological differences of the shells of those four species are insufficient to separate them into valid species but, at least, they mirror only chronosubspecies or minor variations of different populations along time, we can trace back the origin of *Cyprideis* gr. *torosa* or *C. torosa* to the Miocene (late Tortonian).

Further analyses are needed on more Plio-Pleistocene fossil *Cyprideis* to accept or discard one or the other of the hypotheses.

Conclusions

The detailed morphometric analyses carried out on different *Cyprideis torosa* populations collected from several waterbodies with different salinities (oligohaline, mesohaline, hyperhaline) showed that, apart from the well-known salinity-cued polymorphism of some morphological characters, such as size, ornamentation and normal sieve pore shape (see among others Frenzel *et al.* 2012 with references; Pint *et al.* 2012 with references; Boomer *et al.* 2016), it is possible to define some other characters that seem genetically fixed and not salinity-dependent. Among these, and one of the most important, is the valve outline, particularly in females, but also the size of the rounded normal sieve pores and the proportion of the width of the anterior inner lamella, which displays a 'normal' intraspecific variability. The recovery of these characters could be useful for recognizing the species when fossil and the soft parts are not available.

The valve outline, in particular, analysed using the geometric-morphometric approach and the multivariate analysis of the results, proved to be a very useful character for recognizing the similarities among species and for confirming that the typical '*torosa*' group outline is recognizable among Palaeomediterranean Neogene species, while some Late Miocene Paratethyan taxa show statistically significant dissimilarities. The results of our analyses have shown that *Cyprideis* sp. from the mid-Serravallian of Spain (which appeared before the separation of the Palaeomediterranean

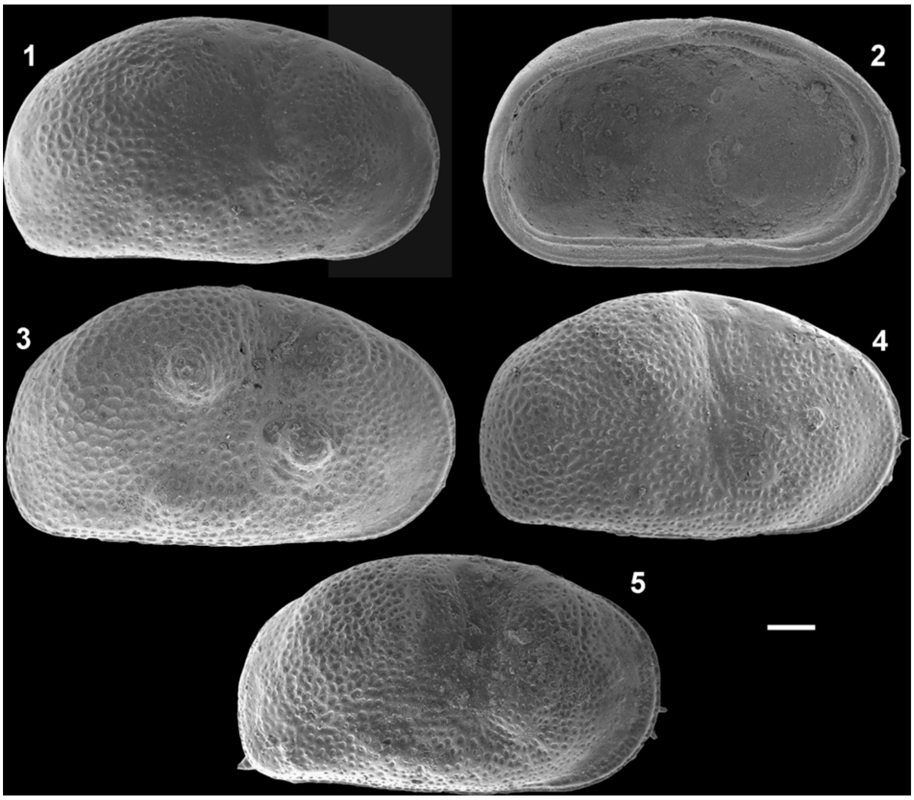


Fig. 11. SEM pictures of the female valves of *Cyprideis* recovered from the Early Pleistocene of central Italy. 1. *C. crotonensis*, right valve lateral external view – Dunarobba (sample DUN3). 2. *C. crotonensis*, left valve internal view – Dunarobba (sample DUN3). 3. *C. crotonensis*, right valve lateral external view – Case Strinati (sample CS8). 4. *C. torosa*, right valve lateral external view – S. Faustino (sample 7C). 5. *C. torosa*, right valve lateral external view – Pietrafitta (sample below the lignite layer). Scale bar 0.1 mm.

and Paratethys domains) and *C. pannonica* from the latest Serravallian of pre-Lake Pannon (which appeared just after the separation), display rather similar outlines, although the species can be differentiated by the different diameters of the rounded sieve pores, the development of the rim below the anterior element of the left valve hinge and the different proportions of the two posterior elements of the left valve hinge. Furthermore, while Palaeomediterranean Late Miocene and Pliocene species, such as *C. ruggierii*, *C. agrigentina* and *C. crotonensis*, preserved a '*torosa*' outline, Late Miocene Lake Pannon taxa, such as *C. sublittoralis* and, particularly, *C. alberti* and *C. seminulum*, show different outlines.

As a conclusion, we hypothesize that *Cyprideis* sp. from the mid-Serravallian of Spain could represent the ancestor of the two stocks and it split subsequently into *C. ruggierii* in the Palaeomediterranean domain and *C. pannonica* in the Paratethys domain. A huge amount of data will be necessary to define with certainty the time of appearance of *C. torosa*, since literature reports this species from a very wide geographical area and stratigraphic range. Ligios & Gliozzi (2012) have already studied several Italian fossil recoveries and concluded that all the Late Miocene (late Messinian) '*C. torosa*' were misidentified and must be ascribed to *C. agrigentina*. In this paper, we have analysed some published and unpublished records of *Cyprideis* from Italian Plio-Pleistocene localities and concluded that, possibly, *C. torosa* appeared in the second part of the Early Pleistocene (Calabrian), being derived from *C. crotonensis*. The separation between these two species is often a difficult task, since, due to the variability of the fossil assemblages, they seem to grade one into the other and only the extreme morphotypes show diagnostic differences. So, it seems that the appearance of *C. torosa* in the Early Pleistocene (Calabrian) could just represent a 'starting' hypothesis and further analyses are needed on more Plio-Pleistocene fossil assemblages. On the other hand, it is possible also to consider that the *C. ruggierii*–*C. torosa* phyletic lineage is represented only by chronosubspecies. In this case, the appearance of *C. torosa* could date back to the Late Miocene (late Tortonian).

Acknowledgements and Funding

The authors wish to thank Valentina Hajek-Tadesse for making available her *Cyprideis* specimens from Croatia. Driss Nachite and Pere Anadon are thanked for the loan of specimens from Morocco and Spain. We are grateful to Patrick DeDekker, David Horne and Alan Lord for their critical revision and valuable suggestions that helped us to improve this paper. This research was supported by the Department of Science (Roma Tre) research funding (EG) and by the research project VEGA 2/0056/15 (RP). JR-L acknowledges Spanish Ministry project MICINN (Ref. CGL2011–23438).

Scientific editing by Alan Lord

References

Agalarova, D.A., Kadyrova, Z.K. & Kulieva, S.A. 1961. *Ostrakody Pliotsenovykh i postpliotsenovykh otlozheniy Azerbaydzhana.* [*Ostracoda from Pliocene and Post-Pliocene deposits of Azerbaijan*]. Baku: 1–420 [in Russian].

Aladin, N.V. & Potts, W.T.W. 1996. The osmoregulatory capacity of the Ostracoda. *Journal of Comparative Physiology, B*, **166**, 215–222.

Ambrosetti, P., Basilici, G. *et al.* 1995. Il Pleistocene inferiore nel ramo Sud-Occidentale del Bacino Tiberino (Umbria): aspetti litostratigrafici e biostratigrafici. *Il Quaternario*, **8**, 19–36.

Baltanás, A. & Danielopol, D.L. 2011. Geometric morphometrics and its use in ostracod research: a short guide. *Joannea Geologie und Paläontologie*, **11**, 235–272.

Barberi, R., Cavinato, G.P., Gliozzi, E. & Mazzini, I. 1995. Late Pliocene–Early Pleistocene paleoenvironmental evolution of the Rieti Basin (Central Apennines). *Il Quaternario*, **8**, 515–534.

Bassiouni, M.A. 1979. Brackische und marine Ostrakoden (Cytherideinae, Hemicytherinae, Trachyleberidinae) aus dem Oligozän und Neogen der Türkei (Känozoikum und Braunkohlen der Türkei. 22. *Geologisches Jahrbuch, B*, **31**, 3–195.

Bodergat, A.-M. 1983. Les ostracodes, témoins de leurs environnement: approche chimique et écologie en milieu lagunaire et océanique. *Documents des laboratoires de Géologie de Lyon*, **88**, 1–246.

Bodergat, A.-M. 1985. Composition chimique des carapaces d'Ostracodes, Paramètres du milieu de vie. *In:* Oertli, H.J. (ed.) *Atlas des ostracodes de France (Paléozoique-Actuel)*. Bulletin du Centres des Recherches Exploration-Production Elf-Aquitaine, Mémoire, **9**, 379–386.

Bodina, L.E. 1961. Ostrakody Tretichnykh otlozheniy zaysanskoy i iliyskoy depressii [Ostracoda from tertiary deposits of the Zaysan and Iliy depressions]. *Trudy Vsesoyuznogo Neftyanogo Nauchno-Issledovatelskogo Geologo-Razvedochnogo Instituta (VNIGRI)*, **n.s. 170**, 43–139.

Boomer, I., Frenzel, P. & Feike, M. 2016. Salinity-driven size variability in *Cyprideis torosa* (Ostracoda, Crustacea). *Journal of Micropaleontology*, first published online July 4, 2016, http://doi.org/10.1144/jmpaleo2015-043

Bossio, A., Costantini, A. *et al.* 1993. Rassegna delle conoscenze sulla stratigrafia del Neoautoctono Toscano. *Memorie della Società Geologica Italiana*, **49**, 17–98.

Bossio, A., Cerri, R., Mazzei, R., Salvatorini, G. & Sandrelli, F. 1994. The neoautochthonous succession of the Spicchiaiola-Pignano area, east of Volterra (Southern Tuscany, Italy). *Memorie della Società Geologica Italiana*, **48**, 425–430.

Bossio, A., Foresi, L.M., Mazzanti, R., Mazzei, R. & Salvatorini, G. 1997. Note micro Paleontologiche sulla successione miocenica del Torrente Morra e su quella pliocenica del bacino dei fiumi Tora e Fine (Province di Livorno e Pisa). *Atti della Società Toscana di Scienze Naturali Memore, A*, **104**, 85–134.

Carbonel, P. 1988. Ostracoda and the transition between fresh and saline waters. *In:* De Deckker, P., Colin, J.-P. & Peypouquet, J.-P. (eds) *Ostracoda in the Earth Sciences*. Elsevier, Amsterdam, 157–173.

Clarke, K.R. & Gorley, R.N. 2006. *Primer ver. 6: computer program*. Primer-E Ltd, Plymouth.

Danielopol, D.L., Gross, M., Harzhauser, M., Minati, K. & Piller, W.E. 2011. How and why to achieve greater objectivity in taxonomy, exemplified by a fossil ostracod (*Amplocypris abscissa*) from the Miocene Lake Pannon. *Joannea Geologie und Paläontologie*, **11**, 273–326.

Decima, A. 1964. Ostracodi del genere *Cyprideis* Jones del Neogene e del Quaternario italiani. *Palaeontographia Italica*, **57**, 81–133.

Fassbinder, K. 1912. Beiträge zur Kenntnis der Süßwasserostracoden. *Zoologische Jahrbücher Abteilung für Anatomie und Ontonogenie der Tiere*, **32**, 533–576.

Frenzel, P., Schulze, I. & Pint, A. 2012. Noding of *Cyprideis torosa* valves (Ostracoda) – a proxy for salinity? New data from field observations and a long-term microcosm experiment. *International Review of Hydrobiology*, **97**, 314–329.

Gamenick, I., Jahn, A., Vopel, K. & Giere, O. 1996. Hypoxia and sulphide as structuring factors in a macrozoobenthic community on the Baltic Sea shore: colonization studies and tolerance experiments. *Marine Ecology – Progress Series*, **144**, 73–85.

Gliozzi, E. & Mazzini, I. 1998. Palaeoenvironmental analysis of Early Pleistocene brackish marshes in the Rieti and Tiberino intrapenninic basins (Latium and Umbria, Italy) using ostracods. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **140**, 325–333.

Gross, M., Minati, K., Danielopol, D.L. & Piller, W.E. 2008. Environmental changes and diversification of *Cyprideis* in the Late Miocene of the Styrian Basin (Lake Pannon, Austria). *Senckenbergiana lethaea*, **88**, 161–181.

Gross, M., Ramos, M.I.F. & Piller, W.E. 2014. On the Miocene *Cyprideis* species flock (Ostracoda; Crustacea) of Western Amazonia (Solimões Formation): Refining taxonomy on species level. *Zootaxa*, **3899**, 1–69.

Grossi, F., Da Prato, S. & Gliozzi, E. 2016. Is the occurrence of sigmoidal ventral border in *Cyprideis torosa* (Jones, 1850) valves linked to salinity? A morphometrical analysis approach. *Journal of Micropaleontology*, first published online December 22, 2016, http://doi.org/10.1144/jmpaleo2016-018.

Hajek Tadesse, V., Belak, M., Sremac, J., Vrsaljko, D. & Vacha, L. 2009. Early Miocene ostracods of the Sadovi section (Mt Požeška Gora, Croatia). *Geologica Carpathica*, **60**, 251–262.

Jones, T.R. 1850. Description of the Entomostraca of the Pleistocene beds of Newbury, Copford, Clacton, and Grays. *Annals and Magazine of Natural History, London, series 2*, **6**, 25–28.

Jones, T.R. 1857. A monograph of the Tertiary Entomostraca of England. *Monographs of the Palaeontological Society of London*, **9**, 1–68.

Karmishina, G.I. 1975. Ostrakodi Pliozena iga europeiskoi vasti SSSR i ik stratigrafueskoe znavenie. *Ministertuo Vscego srednego spezialnogo obrazovania RSFSR, Saratov*, 1–53.

Kempf, E.K. 2016. Historical development of nomenclature and taxonomy of *Cyprideis torosa* (Ostracoda). *Journal of Micropalaeontology,* first published online May 24, 2016, http://doi.org/10.1144/jmpaleo2016-011

Keyser, D. 2005. Histological peculiarities of the noding process in *Cyprideis torosa* (Jones) (Crustacea, Ostracoda). *Hydrobiologia*, **538**, 95–106.

Keyser, D. & Aladin, N.V. 2004. Noding in *Cyprideis torosa* and its causes. *Studia Quaternaria*, **21**, 19–24.

Kilenyi, T.I. 1972. Transient and bilance generic polymorphism as an explanation of variable noding in the ostracode *Cyprideis torosa*. *Micropaleontology*, **18**, 47–63.

Klie, W. 1937. Ostracoden und Harpaticoden aus brackigen Gewässer an der bulgarisches Küste des Schwarzen Meeres. *Mitteilungen aus den Königlichen Naturwissechaftlichen Instituten in Sophia, Bulgaria*, **10**, 1–42.

Kollmann, K. 1960. Cytherideinae und Schulerideinae n. subfam. (Ostracoda) aus dem Neogen des östlichen, Österreich. *Mitteilung der Geologischen Gesellschaft in Wien*, **51**, 89–195.

Krstić, N. 1968*a*. Ostracodes des couches congeriennes: 1. *Cyprideis* I. *Bulletin du Muséum d'Histoire Naturelle de Belgrade, A*, **23**, 107–151.

Krstić, N. 1968*b*. Ostracodes des couches congeriennes: 3. *Cyprideis* II. *Bulletin du Muséum d'Histoire Naturelle de Belgrade, A*, **23**, 153–183.

Ligios, S. & Gliozzi, E. 2012. The genus *Cyprideis* Jones, 1857 (Crustacea, Ostracoda) in the Neogene of Italy: a geometric morphometric approach. *Revue de Micropaleontologie*, **55**, 171–207.

Ligios, S., Benvenuti, M., Gliozzi, E., Papini, M. & Rook, L. 2008. Late Miocene palaeoenvironmental evolution of the Baccinello–Cinigiano Basin (Tuscany, central Italy) and new autoecological data on rare fossil fresh- to brackish-water ostracods. *Palaeogeography, Palaeoecology, Palaeoclimatology*, **264**, 277–287.

Ligios, S., Anadón, P. *et al.* 2012. Ostracoda and Mollusca biodiversity and hydrochemical features of Late Miocene brackish basins of Italy. *Geobios*, **45**, 351–367.

Lindroth, S. 1953. Taxonomic and zoogeographical studies of the ostracod fauna in the inland waters of East Africa. *Zoologiska Bidrag från Uppsala*, **30**, 1–156.

Linhart, J., Brauneis, W. & Neubauer, W. 2007. *Morphomatica, version 1.6.0*, http://palstrat.uni-graz.at/methodsinostracodology/methodsinostracodology.htm

Malz, H. & Triebel, E. 1970. Ostracoden aus dem Sannois und jüngeren Schichten des Mainzer Beckens. 2: *Hemicyprideis* n. g. *Senkenbergiana Lethaea*, **51**, 1–47.

Mandelstam, M.I., Markova, L.P., Rosyjeva, T.R. & Stapanaitys, N.E. 1962. *Ostrakodi pliotsenovykh i postpliotsenovykh otlozheniy Turkmenistana* [*Ostracoda from Pliocene and post-Pliocene deposits of Turkmenistan*]. Academy Nauk SSR, Turkmenistan, Ashkhabad, 1–288 [in Russian].

Méhes, G. 1908. Beiträge zur kenntnis der Pliozänen ostrakoden Ungarns. II Die Darwinulidaeen und Cytherideaidaeen der unterpannonischen Stufe. *Supplement Földtani Közlöny*, **38**, 601–635.

Meisch, C. 2000. *Freshwater Ostracoda of western and central Europe. Süsswasserfauna von Mitteleuropa 8/3*. Spektrum, Gustav Fischer, Heidelberg, 1–522.

Neubauer, T.A., Harzhauser, M., Kroh, A., Georgopoulou, E. & Mandic, O. 2015*a*. A gastropod based biogeographic scheme for the European Neogene freshwater systems. *Earth Science Reviews*, **143**, 98–116.

Neubauer, T.A., Harzhauser, M., Georgopoulou, E., Kroh, A. & Mandic, O. 2015*b*. Tectonics, climate, and the rise and demise of continental aquatic species richness hotspots. *Proceedings of the National Academy of Sciences*, http://doi.org/10.1073/pnas.1503992112

Pint, A., Frenzel, P., Fuhrmann, R., Scharf, B. & Wennrich, V. 2012. Distribution of *Cyprideis torosa* (Ostracoda) in Quaternary Athalassic Sediments in Germany and its Application for Palaeoecological Reconstructions. *International Review of Hydrobiology*, **97**, 330–355.

Pobedina, V.M., Voroshilova, A.G., Rybina, O.I. & Kuznetsova, Z.V. 1956. *Spravochnik po mikrofaune Sredne – I verkhnemiotsenovykh otlozeniy Azerbaydzhana* [*Handbook on the microfauna of Middle and Upper Miocene deposits of Azerbaijan*]. Academy Nauk SSR, Azerbaijan, Baku, 1–192 [in Russian].

Pokorný, V. 1952. Skořepatci t. zv. 'basálmiho horizontu subglobosových vrestev' (Pliocen) v Hodoníně. *Sbornik Ustředniho Ústavu Geologickeho, oddil Paleontologicky*, **19**, 229–396.

Popov, S.V., Rögl, F., Rozanov, A.Y., Steininger, F.F., Shcherba, I.G. & Kovac, M. 2004. Lithological-paleogeographic maps of Paratethys. 10 Maps. Late Eocene to Pliocene. *Courier Forschungsinstitut Senckenberg*, **250**, 1–46.

Popov, S.V., Shcherba, I.G., Ilyina, L.B., Nevesskaya, L.A., Paramonova, N.P., Khondkarian, S.O. & Magyar, I. 2006. Late Miocene to Pliocene palaeogeography of the Paratethys and its relation to the Mediterranean. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **238**, 91–106, http://doi.org/10.1016/j.palaeo.2006.03.020

Puri, H.S. 1974. Normal pores and the phylogeny of Ostracoda. *Geoscience and Man*, **6**, 137–151.

Remane, A. & Schlieper, C. 1958. Die Biologie des Brackwassers. *Die Binnengewässer*, **22**, 1–348.

Reuss, A.E. 1850. Die fossilen Entomostraceen des österreichschen Tertiärbeckens. *Abhandunglen Haidingers naturwissenschaft*, **3**, 41–92.

Rohlf, F.J. 2009. *tpsDig, digitize landmarks and outlines, version 2.14*. Department of Ecology and Evolution, State University of New York at Stony Brook, New York, http://life.bio.sunysb.edu/morph/

Rosenfeld, A. & Vesper, B. 1977. The variability of the sieve-pores in recent and fossil species of *Cyprideis torosa* (Jones, 1850) as an indicator for salinity and paleosalinity. *In:* Löffler, H. & Danielopol, D. (eds) *Aspects of Ecology and Zoogeography of Recent and Fossil Ostracoda*. Junk Publishers, The Hague, 55–67.

Ruggieri, G. 1958. Gli esotici neogenici della colata gravitativa della Val Marecchia (Appennino Romagnolo). *Atti dell'Accademia di Scienza Letterature Arti Palermo*, **4**, 49–217.

Sandberg, P.A. 1964. The Ostracod Genus *Cyprideis* in the Americas. *Stockholm Contributions in Geology*, **12**, 1–178.

Sars, G.O. 1925. *An Account of the Crustacea of Norway with Short Descriptions and Figures of All the Species*. Bergen Museum, Bergen, **9** Ostracoda (parts 5–12), 73–208.

Sarti, G. & Testa, G. 1994. Studio geologico-stratigrafico della successione tortoniana superiore-pliocenica del margine occidentale del bacino di Volterra (Pisa). *Memorie Descrittive della Carta Geologica d'Italia*, **49**, 189–228.

Schön, I. & Martens, K. 2012. Molecular analyses of ostracod species flocks from Lake Baikal and Lake Tanganyika. *Hydrobiologia*, **682**, 91–110.

Schornikov, E.I. 2015. The taxonomic position of the *Cyprideis* species (Ostracoda, Cytheroidea, Cytherideidae) that occurs in Lake Issyk-Kul, N.E. Kyrgystan. *In:* Gliozzi, E., Pugliese, N. & Pipik, R. (eds) *Advances in Ostracodological Research – Selected Papers from the 17th International Symposium on Ostracoda*. Micropaleontology, **61**, 37–48.

Sissingh, W. 1972. Late Cenozoic Ostracoda of the South Aegean Island Arc. *Utrecht Micropaleontological Bulletins*, **6**, 1–187.

Stancheva, M. 1963. Ostrakodna fauna ot Neogena v Severozapadna Bulgaria: 2. Sarmatski Ostrakodi [Ostracoda from the Neogene of Northwestern Bulgaria: 2. Sarmatian Ostracoda]. *Trudove Vurjhu Geologiyata na Bulgariya, Seriya Paleontologiya*, **5**, 5–73 [in Bulgarian].

Stoica, M., Lazăr, I., Krijgsman, W., Vasiliev, I., Jipa, D. & Floroiu, A. 2013. Paleoenvironmental evolution of the East Carpathian foredeep during the late Miocene–early Pliocene (Dacian Basin; Romania). *Global and Planetary Change*, **103**, 135–148.

Tibert, N.E., Colin, J.-P., Leckie, M.R. & Babinot, J.-F. 2003. Revision of the ostracode genus *Fossocytheridea* Swain and Brown, 1964: Mesozoic ancestral root for the modern eurytopic *Cyprideis* Jones. *Micropaleontology*, **49**, 205–230.

Triebel, E. 1941. Zur Morphologie und Ökologie der fossilen Ostrakoden. Mit Beschreibung einiger neuer Gattungen und Arten. *Senkenbergiana*, **23**, 294–400.

van Baak, C.G.C., Stoica, M., Grothe, A., Aliyeva, E. & Krijgsman, W. 2016. Mediterranean–Paratethys connectivity during the Messinian salinity crisis: The Pontian of Azerbaijan. *Global and Planetary Change,* http://doi.org/10.1016/j.gloplacha.2016.04.005

Van den Bold, W.A. 1976. Distribution of species of the tribe Cyprideidini (Ostracoda, Cytherideidae) in the Neogene of the Caribbean. *Micropaleontology*, **22**, 1–43.

van Harten, D. 1975*a*. *Cyprideis baetica* n. sp. (Ostracoda, Cytheridae) from the Miocene of southeastern Spain. *Scripta Geologica*, **32**, 1–17.

van Harten, D. 1975*b*. Size and environmental salinity in the modern euryhaline ostracod *Cyprideis torosa* (Jones, 1850), a biometrical study. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **17**, 35–48.

van Harten, D. 1980*a*. On *Cyprideis undosa* Van Harten, sp. nov. *A Stereo Atlas of Ostracod Shells*, **7**, 81–88.

van Harten, D. 1980*b*. On *Cyprideis exuberans* Van Harten, sp. nov. *A Stereo Atlas of Ostracod Shells*, **7**, 89–100.

van Harten, D. 1990. The Neogene evolutionary radiation in *Cyprideis* Jones (Ostracoda: Cytheracea) in the Mediterranean Area and the Paratethys. *Courier Forschungsinstitut Senckenberg*, **123**, 191–198.

van Harten, D. 1996. *Cyprideis torosa* (Ostracoda) revisited. Of salinity, nodes and shell size. *In:* Keen, C. (ed.) *Proceedings of the Second European Ostracodologists' Meeting*. British Micropalaeontological Society, London, 191–194.

van Harten, D. 2000. Variable noding in *Cyprideis torosa* (Ostracoda, Crustacea): an overview, experimental results and a model from Catastrophe Theory. *In:* Horne, D.J. & Martens, K. (eds) *Evolutionary Biology and Ecology of Ostracoda*. Hydrobiologia, **419**, 131–139.

Van Morkhoven, F.P.C.M. 1962. *Post-Paleozoic Ostracoda, 1*. Elsevier, Amsterdam, 1–204.

Wagner, C.W. 1957. *Sur les ostracodes du Quaternaire Récent des Pays-Bas et leur utilisation dans l'étude géologique des dépôts Holocènes*. Mouton & Co., Gravenhage, 1–259.

Whatley, R.C., Munoz-Torres, F. & Van Harten, D. 1998. The ostracoda of an isolated Neogene saline lake in western Amazon basin. *In:* Crasquin-Soleau, S., Braccini, E. & Lethiers, F. (eds) *What about Ostracoda!* Bulletin des Centres de Recherches Elf Exploration-Production, Mémoire, **20**, 231–245.

Wouters, K. 2002. On the distribution of *Cyprideis torosa* (Jones) (Crustacea, Ostracoda) in Africa, with the discussion of a new record from the Seychelles. *Bulletin de l'Institut royal des sciences naturelles de Belgique, Biologie*, **72**, 131–140.

Wouters, K. 2003. New records of the genus *Cyprideis* (Crustacea, Ostracoda) from South Africa. *Bulletin de l'Institut royal des sciences naturelles de Belgique, Biologie*, **73**, 161–165.

Wouters, K. 2016. On the modern distribution of the euryhaline species *Cyprideis torosa* (Jones, 1850) (Crustacea, Ostracoda). *Journal of Micropaleontology*, first published online May 3, 2016, http://doi.org/10.1144/jmpaleo2015-021

Wouters, K. & Martens, K. 1994. Contribution to the knowledge of the *Cyprideis* species flock (Crustacea: Ostracoda) of Lake Tanganyka, with the description of three new species. *Bulletin de l'Institut royal des sciences naturelles de Belgique, Biologie*, **64**, 111–128.

Wouters, K. & Martens, K. 1999. Four new species of the *Cyprideis* species flock (Crustacea: Ostracoda) of Lake Tanganyika. *Bulletin de l'Institut royal des sciences naturelles de Belgique, Biologie*, **69**, 67–82.

Wouters, K. & Martens, K. 2007. Three new species of the *Cyprideis* species flock (Crustacea, Ostracoda) of Lake Tanganyika (East Africa). *Bulletin de l'Institut royal des sciences naturelles de Belgique, Biologie*, **77**, 147–160.