Cyprideis torosa: a model organism for the Ostracoda?

Patrick De Deckker1* & Alan Lord2*
1 Research school of Earth Sciences, The Australian National University, Canberra ACT 2601, Australia
2 Senckenberg Forschungsinstutit, Senckenberganlage 25, D-60325 Frankfurt-am-Main, Germany
*Correspondence: patrick.dedeckker@anu.edu.au; alan.lord@senckenberg.de

In 1990 Danielopol et al. described the ostracod genus Cytherissa as ‘the Drosophila of paleolimnology’ in the sense of a model organism for their purposes at that time. In the intervening years Drosophila is no longer seen by biologists as the perfect test model and, for example, the nematode worm Caenorhabditis elegans is now viewed as preferable because ‘the fly is much more complex than the worm and the anatomy of the nervous system has not reached the level of completeness achieved for the worm’ (Brenner 2003, p. 278). For some years attention has focused on Cyprideis torosa (Jones, 1850), especially since the pioneering work of Rosenfeld & Vesper (1977) on sieve-pore variability in this species in relation to salinity, because torosa is a particularly widely distributed euryhaline living and fossil ostracod species. Cyprideis torosa is not only biogeographically widespread but occurs in a salinity range from freshwater to hypersaline, tolerant of a wide range of temperature, oxygen and substrate conditions, and also has a large, well-calciﬁed and easily preserved carapace. The species first occurs in sedimentary formations of early Pleistocene age but may be older. Therefore, it has the potential to be an ostracod model organism. This set of thematic papers is designed to summarize our current knowledge of one of the most important living ostracod species, its distribution, ecology, morphological response to environmental pressures, and molecular characterization, together with our understanding of its origins and value for palaeoenvironmental interpretation. The ultimate aim is to deﬁne potentially rewarding research targets using C. torosa as a model organism.

Layout

The papers herein were ﬁrst published electronically (see data on individual papers) reﬂecting the order in which manuscripts were submitted, reviewed and accepted. Thus, later authors had the beneﬁt of being able to read and cite papers already published Online First. Since manuscripts were handled editorially piecemeal there are inconsistencies evident that we hope the reader will forgive, especially in relation to citation of salinity (see below). In the printed part, we have rearranged the papers into what we consider to be a more logical sequence.

Salinity

Salinity is cited in a variety of ways in the literature and herein: ‘‰’ ‘psu’ ‘ppm’ and ‘S’. Salinity is a ratio and requires no units; however, ARL has respected author preferences in the submitted manuscripts especially when salinity has been quoted from literature or other older sources. It is also very important to know and record how salinity was measured, especially since several references relate to work performed several decades ago. For instance, salinity could be established by weighing the total residue left after evaporating water, and thus salinity was listed as the amount of Total Dissolved Solids [=TDS], but we now know that organic compounds will occur among the post-evaporation residue, and this does not directly relate to water salinity. Measuring electrical conductivity has been for many decades the preferred method for calculating salinity, but this is based on waters having a NaCl dominance, and tables have become available to relate water salinity to electrical conductivity. Nevertheless, some saline waters may be dominated by different ions, such as sodium carbonate, calcium sulphate etc., and therefore salinity calculated by correlation with electrical conductivity has to be carefully assessed. Nevertheless, it is clearly established that torosa and, by similarity, other Cyprideis species – all being of marine ancestry – are found in the alkalinity-depleted chemical pathway of waters as deﬁned by Eugster & Jones (1979) which are dominated by Na and ≤Cl ions.

The reason for establishing the salinity of the water in which the ostracod lived is rather important when assessing osmoregulation processes, and when linking salinity changes to climatic and environmental changes. The latter two parameters are also of direct relevance when studying fossil ostracod material.

The papers

As will become clear, for obvious reasons, this set of papers is dedicated to our late colleague Amnon Rosenfeld (1944 – 2014); see Keyser & Honigstein (2016).

Frogley & Whittaker (2016) discuss the original description of C. torosa from Pleistocene deposits on the Thames Estuary (UK), reﬁgure the original illustrations and the scanning electron micrographs of lectotypic material of Kilenyi & Whittaker (1974) and brieﬂy outline the career of the describer of the species Thomas Rupert Jones (1819–1911). Kempf (2016) complements the historical dimension with a review of the nomenclature and taxonomy of C. torosa since its description.

Wouters (2016) brings up to date his earlier surveys of the biogeographical distribution of living C. torosa (Wouters 2002, 2003) in Europe, Africa and Asia and raises the question of the presence, or not, of the species in Australia. Schön, Halse & Martens (2017) use molecular phylogenetic methods to investigate the occurrence of torosa in Australia, concluding that the species is not present and that two other different species are probably present on the continent, thereby demonstrating that torosa is not a cosmopolitan species.

From its well-calciﬁed valves and common occurrence torosa is an attractive subject for geochemical study (trace-elements and stable-isotopes) and Holmes & De Deckker (2016) review data from specimens from in vitro cultures and a range of natural environments. This paper clearly identiﬁes that there are still several unknowns about ostracod shell composition and its relationship to ambient conditions, and therefore calls for additional investigations. Thus, in an application of trace-element chemistry Wansard, De Deckker & Julià (2016) combined with the pioneer work of Heip (1976a, b), who measured ambient water temperature for torosa...
Three papers investigate the relationships between carapace characteristics and salinity. Frenzel, Ewald & Pint (2016) describe experiments with cultured *torosa* material to broadly confirm the relationship between sieve-pore shape and salinity recognized by Rosenfeld & Vesper (1977) in the oligohaline to mesohaline range; however, the stability of the host water body seems to be a factor. Boomer, Frenzel & Feike (2016) analyse the relationship between size (as length, length/height) and salinity and show that while there is a relationship it is not a simple quantitative one, but a number of questions are raised about a complex relationship in need of further research. Grossi, Da Prato & Gliozzi (2016) apply morphometrics to understand the relationship between salinity and the valve outline of living and Recent (sub-fossil) *torosa* from Italy, concluding that two morphotypes can be recognized: forms with a straight ventral margin from oligohaline waters and forms with a more sigmoidal ventral margin profile apparently related to higher salinity waters. Carapace characteristics of living and Recent *torosa* are analysed by Gliozzi, Rodriguez-Lazaro & Pipik (2016) in order to clearly define the species for comparison with fossil *Cyprideis* forms. By this means *Cyprideis* gr. *torosa* is recognized in the Palaeo-Mediterranean late Tortonian (late Miocene) and *C. torosa sensu stricto* appears to have evolved in the early Calabrian (early Pleistocene).

Cabrál, Fatale, Lopes, Freitas & Andrade (2016) provide a very full account of living and Holocene *torosa* from mainland Portugal, the living material analysed as adults and juveniles from lagoons and estuaries, with associated ostracod species documented. Pint & Frenzel (2016) focus on taxa occurring with *torosa* as important guides to salinity variations in the host milieu, given that *torosa* is usually monospecific only in hypersaline waters. Scharf, Herzog & Pint (2016) review the occurrence of *torosa* and associated taxa in German coastal waters and especially from natural and anthropogenic saline waters in central Germany.

 Appropriately the final paper returns to the type area of *Cyprideis torosa*, the Thames Estuary. Horne, Bernardout & Whittaker (2016) examine the occurrence of *torosa* and associated ostracods from (marine isotope stage) MIS 9 and MIS 11 interglacial deposits of the early Thames–Medway river system by comparison with the study of ostracod biofacies in the modern Thames Estuary of Kilenyi (1969).

Quo vadis *Cyprideis torosa*?

Upon reading the articles presented in this volume, it is clear that *Cyprideis torosa* needs to be further investigated so as to render this species a better biological, geological and environmental indicator. Below, we suggest several topics which we consider to be worth pursuing so as to better understand this species.

Noding

The formation of nodes in *torosa* has now been finally resolved and interpreted by Keyser & Aladin (2004) and Keyser (2005). The latter paper demonstrates that the changes in ornamentation and the cellular layers of the epidermis occur in areas of the valves and underlying epidermis that are linked. The same author (Keyser 2005) postulated that noding is caused by the inability of the ostracod to regulate its internal osmotic pressure during moulting in low salinity waters (<<6). Keyser (2005) concluded that this must therefore be considered as a phenotypic rather than genetic response. This is in contrast to what had been previously postulated (see discussion in Keyser 2005, pp. 100–101). Keyser (2005, p. 101) clearly defined that ’nodes occur only outside the isthmus of the shell when the outer and inner epidermal cell layer are connected’. When raising the ambient osmolarity in the body fluid during moulting, pressure disrupts the old cuticle on the edge of the calcified zone. Further, Keyser & Aladin (2004), through analysis of micro-cryoscopical measurements of the internal osmoregulation, found that at salinities below 6.2, the osmolarity of the hemolymph in *torosa* results from a hyperosmotic condition, whereas above that value, the conditions are isosmotic. The upper salinity value for the waters in which ostracods were analysed by Keyser & Aladin (2004) was 44. There is nevertheless a need to investigate the occurrence of nodation at a much higher salinity (up to 96) in which Schonikov (1973) found noded *torosa* in the Aral Sea. Is isosmotic condition still prevalent in water salinities as high as three times that of seawater?

Despite the fact that Keyser & Aladin (2004) indicated that *torosa* has problems with osmoregulation, the presence of nodes on valves still be assumed to infer low salinities (<6) if the nodation at the Aral Sea site of Schonikov (1973) is to be explained. The processes involved in the formation of nodes in *torosa* are elegantly presented with clear and ample illustrations in Keyser (2005) and Keyser & Aladin (2004). Finally, we wish to query the statement made by Keyser (2005, p. 106) when he discusses noding that the moulting process is interlinked due to ’low amounts of calcium ions within the animal, reducing sharply the flexibility of desmosomes and muscles’. We suggest that additional investigation should concentrate on the composition of the hemolymph fluid, so as to determine if the alkalinity of this fluid (via HCO3-) may, in fact, be the controlling factor (see further discussion below).

Long-term ecological observations

Vesper (1972a, b) carried out a very detailed analysis of the morphology and ecology of *torosa* (along the River Schlei and areas in the Schleswig-Holstein region, northern Germany) which was almost coincident with the study made by Heip (1976a, b; Herman & Heip 1982; Heip in Herman et al. 1983), who continuously sampled *torosa* in a brackish-water site (Dievengat, northern Belgium), which also occurs in the cold, nearctic region of Europe. There is, therefore, a need to carry out a similar long-term study of *torosa* under the influence of a Mediterranean climate under which temperature regimes, alkalinity, pCO2 and water chemistry composition would vary differently from the Belgian and German sites. For example, Heip (1976a), who identified that *torosa* is a detritivore that feeds principally on the vast bacterial biomass, pointed out that at higher temperatures such a biomass would be enhanced. This needs to be investigated in areas such as the Camargue in the Rhône Delta and the salinas of the Santa Pola region in northwestern Spain where *torosa* commonly abounds in saline lakes.

Heip (1976a) identified that temperature is the most important factor influencing the life cycles of *torosa*, for which ostracod larval development lasted 129–152 days over 3 years, with no adult lasting into the second year. Would a similar life strategy occur under the warmer Mediterranean climate?

It is unfortunate that neither Vesper nor Heip measured alkalinity of the waters during their long investigations of the life cycles of *torosa*, and this needs to be examined in the future so as to better understand ostracod shell composition. Alkalinity, combined with ionic analysis of the ambient waters will lead to identification of the calcite saturation nature of the waters in which ostracods moult and grow. Some of these parameters may be important factors controlling ostracod valve calcification and also perhaps hemolymph composition that in turn is now known to affect the nodosity...
of torosa. We may have to wait until analytical techniques are improved to enable us to measure alkalinity in such small samples, but perhaps the use of tiny pH electrodes would already suffice to establish alkalinity levels in the hemolymph.

**Productivity**

Concerning the productivity studies of torosa in Dievengat by Heip (1976a, b) and Herman et al. (1983), figures are staggering. Two methods used to establish production of torosa return values of 9.7 and 9.2 g of dry weight per m² per year, with even a total biomass value once found by Heip (1976b) reaching 48.9 g dry weight per m² per year; an amazing phenomenon which clearly identifies that torosa is on top of the food chain (Heip 1976a, b). This author also showed that the number of individuals found in his four-year sampling varied between 20,000 and 40,000 individuals per m² with, in one instance, numbers reaching 1.8 million specimens per m² (at that time adult specimens amounted to c. 15% of the population). It is interesting to note, therefore, that the level of calcium and bicarbonate of the water in which torosa live in large numbers need to be constantly high. Geochemical analysis of ostracod valves, such as δ13C for comparison against dissolved inorganic carbon (DIC) composition, should also guide us to establish past productivity levels at fossil sites.

**Temperature requirements**

Heip (1976a) also identified that moulting to adulthood can occur at Dievengat only once the ambient water temperature is above 15°C. Hence, the following question can be asked: is the temperature requirement the same for other locations, or remained the same during glacial/colder periods? Wansard et al. (2016) already considered this issue for the glacial period in Lake Banyoles in northern Spain where torosa was found to thrive.

**Genetic investigations and passive transport**

The genetic differentiation of torosa needs to be further documented. Already, Syyvula et al. (1995) have found two distinct genetic populations, one along the coasts of England and The Netherlands, and the other along the southern coast of the Baltic Sea. Such a surprising find challenges the concept that a continuous exchange of gene pool exists, via passive transport [see Sandberg & Plusquellec (1974) for a thorough review of dispersal processes, although they did not cite transport by boats and fishing equipment between different water bodies], so a north–south transit from the Neartic down to at least Lake Turkana in Kenya where torosa occurs (see Syyvula et al. 1995) would help identify the likely presence of several gene pools that may be linked to bird pathways and the presence of past environmental conditions, such as glacial erosion, and climatic events. Van Harten (1996) already invoked this when considering the transient and balanced genetic polymorphism and environmentally-cued capability of forming nodes on torosa originally postulated by Kilenyi (1972), Schön et al. (2017) used a different approach to Syyvula et al. (1995) (namely DNA extraction for several Australian taxa) who carried out an electrophoretic survey of allozyme variation.

It is clear that further genetic studies are essential to resolving questions of gene pools, of the number of cryptic species present and relationships between Cyprideis torosa and other congeners in Europe and surrounding regions, as well as for the numerous Cyprideis species now reported from the Americas (see Sandberg 1964; Sandberg & Plusquellec 1974). This may also help to determine the evolutionary origin of torosa itself.

**Brood care**

Finally, brood care is an important characteristic of the life strategy of torosa. Already, Sandberg (1964) has discussed this phenomenon which had been recognized as far back as 1866 by G.O. Sars (see Sandberg’s 1964 review, p. 53). Sandberg & Plusquellec (1974) showed this to offer a distinct advantage for dispersal that can also help the ostracod tolerate environmental stresses (such as salinity change, anoxic condition, as well as temporary desiccation conditions). Also, can we establish the diet of the instars remaining inside the carapace of the adult mother? What is the chemical composition of the instar valves, in particular with respect to δ13C? Surprisingly, neither Heip (1976a, b) nor Vesper (1972a) discuss the brood care phenomenon. More research is required concerning brood care in Cyprideis, which as a genus is never found in ephemeral waters; it clearly inhabits permanent waters.

**Shell chemistry**

Already, Meyer et al. (2016) have compared morphological and geochemical variations in two species of Cyprideis (C. salebrosa and C. americana) in the neotropics of the Americas and showed changes in calcite saturation between two seasons in Shell Creek of Florida (with only two analyses, unfortunately). Such a change could be of importance to explain the δ13C and δ18O composition of ostracod shells. What these authors did not investigate is exactly where Cyprideis valves are calcified. We already know that torosa, for example, is part of the meiofauna (Heip 1976a, b; Herman et al. 1983) and that the chemical composition of pore fluids may have a different composition with respect to δ13C compared to the supernatant water. The important work of Decrouy et al. (2011) at Lake Geneva/Leman in Switzerland indicates that pore fluids may have a different composition from the supernatant water and consequently ostracod valve composition with respect to δ13C relates to pore water isotopic composition for forms dwelling interstitially. This phenomenon may explain why Marco-Barba et al. (2012), who analysed the isotopic composition of torosa from several water bodies near Valencia in Spain, showed no correlation with the ambient waters that they analysed. Perhaps this may explain also why those authors mentioned above found no correlation between the Mg/Ca of ostracod valves and water temperature, in contrast with the in vitro experiments made by De Deckker et al. (1999) on the Australian species C. australis (see Schön et al. (2017) for the taxonomic discussion of this species). An important question remains: was the Mg/Ca of the pore waters of the Valencia lakes the same as those of the measured lake waters? Nevertheless, Marco-Barba et al. (2012) reported that the δ13C of torosa in the Valencia lakes is c. 2% lower than expected from the δ13C of the dissolved inorganic carbon of the lake water, from which these authors conclude that calcification must take place intrafamally.

**And finally**

Concerning the estimation of the alkalinity of the waters in which torosa thrives, it may be possible to investigate the B/Ca and the boron isotopic composition of ostracod valves, such as has been carried out for foraminifera (Rae et al. 2011) and corals (Pelejero et al. 2005) as a proxy for water alkalinity. The increase in atmospheric CO2 since the beginning of the industrial revolution may already affect the distribution of torosa in some water bodies. This may explain, for example, why Pint et al. (2012) failed to find live specimens of torosa in inland waters in Germany (up to 300 km from the coast) whereas in such areas ample fossil torosa material was found in Holocene and interglacial deposits.

Obviously, for environmental monitoring, additional physico-chemical parameters need to be acquired to render torosa an