The larval stages of *Triops cancriformis* (Branchiopoda, Notostraca) - SEM-studies on the ultrastructural development of the early larva.

Abstract:
Comprising both the well known anostracan Artemia and the predatory cladoceran Leptodora, the Branchiopoda is a very heterogeneous group. Traditionally the Anostraca, Notostraca, Spinicaudata, Copepoda and Copepoda are named the “larger” Branchiopoda. Among these, the Notostraca is not the most extensively studied group. The specimens used in this study are hatched from commercially available cysts. In our study, we give a description of the larval series of *Triops cancriformis* at the ultrastructural level. We have identified the larval stages 1-5 and a further 10 different juvenile / subadult stages. In stage I larva, the carapace can be recognized as a pair of dorso-lateral “humps” posterior to the large dorsal organ. The maxillules appear in stage II; at stage III the larva is probably capable of feeding. The tips of the long setae on the proximal endites on the antenna have also become bifid at this stage. The stage V larva has a reduced mandibular palp and several other advanced features.

The main focus of the study is on the description of the development of some important characters of the larva (antennae, the labrum, trunk limbs). As our primary tool, we are using SEM but other techniques such as histology for light microscopy and whole mounts have proved useful. Our aim is to discuss the data obtained in a general “large-branchiopod” ontogeny context. We hope that the described characters will prove to be useful in the ongoing phylogenetic analyses of the Branchiopoda.

Materials and methods:
Dried cysts obtained from “Triops CC” (Erich Eder from Vienna) were hatched in destilled water. The larvae were fixed in a 2.5% glutaraldehyde solution, dehydrated in a graded alcohol series, critical point dried in a JEOL JFC-2300HR coater. Observations were made in a JEOL JSM-840 or JES JSM-6335F scanning electron microscope with digital cameras.

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First SEM-micrographs of the stage I larva. 1a: Postero-lateral view. The developing carapace can be seen as distinct dorso-lateral “humps”. This suggests a hatched origin of the adult univalved shell in the Notostraca. 1b: Dorsal view. The abdomen is rounded and the 2nd antennae are proportionally large. The dorso-lateral “humps” (arrows) are easily overlooked in this view (same specimen as 1a). 1c: Ventral view. The small labrum and the non-developed mandibles clearly indicate that this stage is not feeding (different specimen than 1a1b). Abbr: A1=first antenna, A2=second antenna, Do=dorsal organ, Mdp=mandibular palp. All scale bars are 100 µm.

The stage II larva. 2a: Lateral view. The stage II larva is easily recognisable by the presence of the conical furcal rami, the larger carapace and the more elongate shape of the larva. 2b: Ventral view. 6-7 rows of primordial trunk limbs can be seen and the abdomen is conical in shape (different specimen than 2a). 2c: The mouth region of the larva. The labrum almost covers the mandibles and 1st maxillae are already present (same specimen as 2b). Abbr: Fr=furcal rami, La=labrum, M1=1st maxilla. All scale bars are 100 µm.

The stage III larva. 3a: Ventral view. 10-12 rows of primordial trunk limbs are now present and the abdomen is almost cylindrical as in the adult. 3b: The tip of the large seta on the proximal endite of the 2nd antenna is now bifid and the labrum covers the grinding surface of the mandible completely. 3c: The mandible still retains a palp and the anterior trunk limbs have developed enough to form a rudimentary food groove. Abbr: Tl=trunk limbs. Scale bar in 3a is 100 µm. In 3b and 3c scale bars are 10 µm.

Stage IV and V larvae. 4: The stage IV larva in ventral view. Though still using larval propulsion (i.e. swimming primarily with the 2nd antennae) the larva has many adult features. The carapace is of almost adult shape, the endites are well developed (but still mostly cylindrical) and the food groove is apparent and usable. 5: The stage V larva in ventral view. The mandibular palp is now beginning to atrophy as well as the 2nd antennae. The abdomen now has a distinctive adult appearance and the furcal rami are very prominent. Scale bars are 100 µm.

Three sub-adult larvae in different developmental stages. 6: Ventral view of a subadult larva. The mandibular palp is now rudimentary and the labrum is a flat “lid” on the mandibles. Although very adult-like in overall appearance, this specimen still has its 2nd antennae. Some endites of the trunk limbs are still cylindrical rather than flattened. 7: In this specimen, the ventral view reveals a pair of 2nd antennae further reduced in size. The folding of the carapace around the trunk is a typical shrinkage artefact in specimens of this size. 8: This specimen is the oldest one observed in SEM so far and only fails the criterium of adulthood by not being sexually mature. All scale bars are 1 mm.