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An exceptional event during the moulting process of an eutardigrade: two bucco-pharyngeal apparatuses present

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Summary

A specimen of *Diphascon* (*Diphascon*) *procerum* with two bucco-pharyngeal apparatuses (one of which should have been expelled during moulting process) is described, and that anomalous condition is discussed.

It is well known that tardigrade moult follows precise stages in which well defined events occur (Marcus, 1929; Baumann, 1961, 1966; Ramazzotti 1962; Kristensen, 1976; Grohé, 1976; Walz, 1982). All tardigrades undergo the following stages in order to moult:

1) Expulsion of the sclerified structures of the bucco-pharyngeal apparatus (cuticle of the oral cavity, buccal tube, stylets and their supports, pharyngeal tube if present, cuticular covering of the pharyngeal lumen with placoids and of the oesophagus).
   The musculature associated to the stylets is kept.
2) The mouth closes and for some time, from one to three days, the animals cannot feed; this stage is defined as "*simplex*". The musculature associated with the stylets is surrounded by the salivary glands which, after the expulsion of the sclerified structures of the bucco-pharyngeal apparatus, move forward
3) The salivary glands produce the new cuticle of the oral cavity, the new buccal tube, stylets and their supports, and pharyngeal tube if present; the pharyngeal epithelium produces its cuticular covering (and the placoids), as does the oesophageal epithelium. The related stylet muscles, which have been retained, attach to the new stylets. Also during the "*simplex*" stage, the new cuticle covering the whole body and the new claws are produced.
4) The animal abandons the old cuticle and the moulting process is therefore completed.

Those four stages are the same in all tardigrades and, until now, no variations or aberrations have ever been reported. Surprisingly, we found an exceptional specimen of eutardigrade (*Diphascon (Diphascon) procerum* Pilato, Sabella & Lisi, 2014) with two complete bucco-pharyngeal apparatuses (Fig. 1).

The studied specimen, mounted in polyvinyl-lactophenol, is deposited in the Binda & Pilato collection (Department of Biological, Geological and Environmental Sciences, University of Catania) (slide No. 3172).

The newly formed apparatus was complete in all its various parts starting from the mouth cavity (Fig. 1A, B); also the apophyses for the insertion of the stylet muscles (Fig. 1B), as well as the stylet furcae, the entire bucco-pharyngeal tube (Fig. 1B) and the placoids are clearly visible; however, the pharyngeal bulb is not connected to the oesophagus. The apparatus which should have been expelled, is placed caudally to the first, and also shows all its parts. However, it is not connected to, and is far away from, the mouth (Fig. 1A), but its pharyngeal bulb is connected to the oesophagus (Fig.1C).

Since that specimen does not show a double cuticle, and has not produced new claws (Fig. 1B, D), one can conclude that it was not moulting. Instead, this animal had completed a moult without expelling the old buccal apparatus, while the new one was regularly formed and the old cuticle was regularly abandoned.

This animal, when the slide was mounted, was active, therefore it had survived without apparent damage in the moult and was coping, at least temporarily, with the anomaly of not having expelled the old bucco-pharyngeal apparatus.

It must be stressed that the new bucco-pharyngeal apparatus is not connected to the oesophagus; therefore, we find it very improbable that the specimen would have been able to survive and restore a normal condition.

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techniques (e.g. Cesari, *et al.,* 2013). This incongruence between morphology and molecular data comes out when mapping the phylogeny of taxa to distinguish population groups (Cesari, *et al.,* 2013); currently, without understanding what has been affected by the genetic changes. This emphasises that morphological observation and molecular techniques are both tools used in the identification of taxa and as such should be used in an integrated approach, giving neither technique priority over the other.

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