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Comments. Discussions

Interpreting early developmental stages of pycnogonids
Интерпретируя ранние стадии развития пикногонид

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Determining the stage at which a pycnogonid hatches can be challenging, and interpreting images made of that stage may contribute to the challenge. A recent publication on these pages (Fornshell, 2014) provides an informative example. The second sentence of the Results states that: "A true protonymphon larva was not found in archived material of either of the species studied here" (p. 327, col. 1). However, the legend for both Fig. 1A (p. 328) and 3A (p. 330) states: "molting of the protonymphon into the second instar". The resulting confusion then seems to have led to an opinion (p. 334) that the "sequence of larval development" is misinterpreted in Ferrari et al. (2011) and Fornshell and Ferrari (2012), and that "a true protonymphon larva does occur in this genus". These contradictions provide a useful platform to discuss the evidence that should be used in interpreting the early stages of development of *Ammothea*, as well as the advantages and disadvantages of line drawings, photographs and scanning electron micrographs for depicting these stages.

Here, the interpretation of the outer covering shown on the SEMs of Fig. 1A and Fig. 3A is a problem. The surface of this outer covering in both figures is smooth and uncomplicated; there is no indication of an exoskeletal covering of any limb. Therefore the covering is not an exuvia and the animal is not molting. The presence of the bud pair of limb IV cannot be determined on the emerging animal in Fig. 1A and Fig. 3A because the posterior part of the animal is concealed by the outer covering. Thus it is impossible to know if the emerging animal is a protonymphon (without the bud pair of limb IV) or a second instar (with the bud pair of limb IV). However, it seems more reasonable to interpret this outer covering as having contained the embryo that is in the process of being released. Furthermore, Fig. 3B is a recently molted specimen whose segmented limb IV is not yet extended. This stage is a third instar, not a second instar.

Because the stage of development cannot be determined by direct observation from the presence of the bud pair of limb IV in the SEMs, two other issues are raised (Fornshell, 2014). Appressed appendages are said to occur only on the protonymphon. However, limbs of a protonymphon are not shown for comparison, so no judgment can be made about this statement. Furthermore, appendages of later instars appear pressed closely to the center of the body (e.g. Fig. 3D). The second point raised is that six of seven species of *Ammothea*, whose early development is known, hatch as a protonymphon. However, the number of species of *Ammothea* is not given, nor are relationships among these species mentioned. Furthermore, it is not clear that the genus *Ammothea* has been diagnosed by set of synapomorphies. So this second point also fails careful scrutiny.

Relying on SEMs to interpret a developmental stage can be problematical. Once a specimen has been prepared for SEM, in effect fossilized, only a very limited number of further observations of that specimen can be made. In contrast, a specimen that has been photographed or from which a line drawing has been prepared can be further manipulated to provide information on external and internal characters of that specimen. So for example, the specimen of *Ammothea glacialis* (Hodgson, 1909) photographed for Ferrari et al. (2011: Fig. 1B) was further manipulated to determine that the outer covering did not bear projections of the covering of any limb, so that covering enclosed the embryo. Furthermore the emerging specimen possessed the bud pair of limb IV, so the specimen hatched as a second instar. Such manipulations cannot be extended to a specimen that has been prepared for SEM. Thus the advantage of SEM to resolved detailed structure must be balanced against this loss of further manipulation of the specimen.

The issue that six of seven species of *Ammothea* whose early development is known hatch as a protonymphon needs further consideration because the statement "a true protonymphon larva does occur in this genus" (Fornshell, 2014: 334) is technically correct. Species of *Ammothea* that hatch as a protonymphon have been reported. However, the important question is do all species of *Ammothea* hatch as a protonymphon? The answer is that not all species of *Ammothea* hatch as a protonymphon. It seems quite likely that the early sequence of development within *Ammothea* has evolved to include some species that hatch as a protonymphon and other species that hatch as a second instar with the bud pair of limb IV (e.g. Ferrari et al., 2011). Furthermore, there is the possibility that the early sequence of development may vary within a species of *Ammothea*. Specifically, if a male bears on his oviger a linear arrangement of clutches of offspring from several different females, as has been reported for *A. hilgendorfi* (see Barreto, Avise, 2008), does the location of the clutch or the age of clutch effect the stage that the developing pycnogonid is released?

In conclusion, when attempting to interpret the stage of development of a pycnogonid emerging from an outer covering, the following should be considered:

1. Is the surface of outer covering smooth and uncomplicated? Then it is the outer covering containing the embryo.
2. Does the outer surface bear projections that cover the limbs? Then that covering is an exuvia.
3. Does the inner stage bear only transformed limbs I–III? Then that stage is a protonymphon.
4. Does the inner stage bear the bud pair of limb IV? Then that stage is a second instar.

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