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External morphology of the two cypridiform ascothoracid-larva instars of *Dendrogaster*: The evolutionary significance of the two-step metamorphosis and comparison of lattice organs between larvae and adult males (Crustacea, Thecostraca, Ascothoracida)

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Abstract

We describe the external morphology of the two cypridiform larval instars (first and second ascothoracid-larvae, or "a-cyprids") of the ascothoracidan genus *Dendrogaster*. Ascothoracid-larvae of five species were studied with light and scanning electron microscopy, including both ascothoracid-larval instars in *Dendrogaster orientalis* Wagin. The first and second instars of the ascothoracid-larvae differ in almost all external features. The carapace of instar 1 has a smooth surface and lacks pores, setae, and lattice organs, while instar 2 has all these structures. The antennules of the first instar have only a rudimentary armament, the labrum does not encircle the maxillae, thoracopods 2–3 are not armed with a plumose coxal seta, and the abdomen is four-segmented (versus five-segmented in instar 2). Thus, the first ascothoracid-larva of *Dendrogaster* represents a transitional, generally brooded stage between the naupliar stages and the dispersive and fully functional second ascothoracid-larva that accomplishes settlement. The presence of two instars of ascothoracid-larvae (a-cyprids) in members of the order Dendrogastrida differs from the single cypridiform instar found in the Cirripedia (cyprid) and Facetotecta (y-cyprid), and we discuss the evolutionary significance of these ontogenies. We found lattice organs in both the second ascothoracid-larvae and in adult males of *Dendrogaster*. We could not observe both ascothoracid-larvae and males in any single species, but our data suggests that the lattice organs change significantly at the molt between these two instars. The lattice organs of second ascothoracid-larvae have no distinct keel and are situated in wide, shallow pits, whereas they have the ground pattern "crest-in-a-trough" morphology in adult males of two additional species examined for comparison. The positions of the terminal pores of lattice organs 1 and 2 also seem to change during maturation. These findings show that comparative data on lattice organ morphology for phylogenetic purposes must derive from strictly homologous instars, viz., the second

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ascothoracid-larva (a-cyprid) of the Ascothoracida, the y-cyprid of the Facetotecta, and the cyprid of Cirripedia. The ascothoracid-larvae of *Dendrogaster* and those of the family Ascothoracidae have four pairs of lattice organs, which suggests that this genus and family form a monophylum, to the exclusion of *Ulophysema*, which then brings into question the monophyly of the Dendrogastridae. *Ulophysema* is currently placed in the Dendrogastridae, but its second ascothoracid-larva has lattice organs of different and more plesiomorphic number and morphology. We briefly review lattice organ morphology across the Thecostraca. These organs are normally considered structures of the cypridiform larva and their presence in adult (males) Ascothoracida is unique in the Thecostraca. The continued morphological modification of these sensory structures in males compared to ascothoracid-larvae may suggest that they originated in adult thecostracans, but have come to be functional in the cypridiform larvae as well.

Keywords: Ascothoracida; Cyprid larvae; Larval instars; Morphology; SEM; Lattice organs; Cuticular microstructures; Phylogenetic relationships

1. Introduction

Recent studies have revealed the importance of larval morphology in reconstructing the phylogeny of the Thecostraca, and the cypridiform larva becomes especially important in understanding how thecostracans first became sessile and diversified into the impressive range of morphologies and modes of life seen in extant forms (Grygier 1983b, 1987d; Itô and Grygier 1990; Jensen et al. 1994a; Kolbasov et al. 1999; Høeg and Kolbasov 2002; Rybakov et al. 2003; Høeg and Møller 2006; Kolbasov and Høeg 2007).

Within the Thecostraca, the subclass Ascothoracida has adopted a parasitic way of life with echinoderms and anthozoans as their hosts. The approximately 100 described species are classified in two orders, Laurida and Dendrogastrida (Grygier 1987b). In the ground pattern of the Thecostraca larval development comprises six instars of pelagic nauplii and a terminal cypridiform larva adapted for attachment (Grygier 1987d; Høeg et al. 2004). Some Ascothoracida retain this scheme and may even have planktotrophic nauplii, but the majority of the species either release lecithotrophic nauplii or brood their offspring inside the mantle cavity until their release as ascothoracid-larvae (Itô and Grygier 1990). The cypridiform larvae of the Thecostraca share a number of putative synapomorphic similarities such as a large head shield (carapace) with five pairs of lattice organs, a pair of prehensile antennules, six pairs of specialized, biramous and natatory thoracopods, and a pair of frontal filaments associated with the compound eyes (Grygier 1987d; Høeg et al. 2004). In the Ascothoracida, the cypridiform stage is traditionally called the "ascothoracid-larva" but was renamed "a-cyprid" by Høeg et al. (2004) to emphasize the homology with the facetotectan y-cyprid (see Itô 1985) and the true cirripede cyprid (see Glenner 1998). Here we use "ascothoracid-larva" whenever we mean "a-cyprid" and employ "ascothoracidan" as an adjective of the taxon. We use the term "head shield" for the "carapace" of the ascothoracid-larva to emphasize its development as an enlargement of the naupliar head shield (Walossek et al. 1996).

Unlike all other Thecostraca, the species of the order Dendrogastrida (families Dendrogastridae, Ascothoracidae and Ctenosculidae) possess two consecutive cypridiform instars, called first and second ascothoracid-larvae. The first is normally retained within the mantle cavity with only the second being eventually released into the plankton to locate a new host or mate (Brattström 1948; Wagin 1976; Grygier and Fratt 1984; Grygier 1987c; Grygier and Høeg 2005), but one species has been inferred to also have a free first ascothoracid-larva (Grygier 1991a). Species of the Laurida have, as far as is known, only one ascothoracid-larva, called "Tessmann's larva" in the Lauridae. No one has cultured the larvae through both ascothoracid-larval instars, so the time of development remains unknown.

Sexes are separate in the Ascothoracida, except for the hermaphroditic Petrarcidae (Grygier 1987a, b), and dwarf males, little or just moderately modified compared to the ascothoracid-larvae, are known from most taxa with modified females. Ascothoracidans whose adult females resemble ascothoracid-larvae are probably close to the ground pattern, but in many species the adult females show hypertrophy of the head shield (or mantle) and reduction of segmentation in the antennules, thoracopods, thorax, and abdomen.

The chemosensory lattice organs are highly modified setae and an autapomorphy of the class Thecostraca (Jensen et al. 1994a, b; Høeg et al. 1998; Kolbasov et al. 1999; Høeg and Kolbasov 2002; Rybakov et al. 2003). In the ground pattern, found in the Facetotecta and some Ascothoracida, they occur with five pairs on the head shield of the cypridiform larva, with each individual organ having the form of a crest-like structure (the modified seta) traversing a shallow depression and equipped with a posteriorly situated terminal pore. Within the Ascothoracida there are numerous variations both in the number of lattice