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External morphology of cypris larvae of two species of *Trypetesa* Norman, 1903 (Crustacea: Thecostraca: Cirripedia: Acrothoracica: Trypetesidae)

Наружная морфология циприсовидных личинок двух видов *Trypetesa* Norman, 1903 (Crustacea: Thecostraca: Cirripedia: Acrothoracica: Trypetesidae)

G.A. Kolbasov[1] & J.T. Høeg[2]
Г.А. Колбасов[1], Й.Т. Хёг[2]

[1] Department of Invertebrate Zoology, the White Sea Biological Station, Biological Faculty, Moscow State University, Moscow 119899 Russia.
[2] Institute of Zoology, University of Copenhagen, Universitetsparken 15, DK-2100, Copenhagen, Denmark.
[1] Кафедра зоологии беспозвоночных, Беломорская Биологическая Станция, Биологический факультет МГУ, Москва 119899 Россия.

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КЛЮЧЕВЫЕ СЛОВА: Cirripedia, Acrothoracica, циприсовидная личинка, морфология, родственные связи, СЭМ.

ABSTRACT: The external morphology of the cypris larvae of two closely related acrothoracican species *Trypetesa lampas* (Hancock, 1849) and *T. lateralis* Tomlinson, 1953 has been investigated with a scanning electron microscopy. Special attention was given to the structure of lattice organs, antennules, thoracopods and telson. The trypetesid cyprid larvae have lattice organs with pore fields, fourth antennular segment with subterminal and terminal setae and slightly cleft telson. These characters bring together both trypetesid and lithoglyptid cypris larvae. Four-segmented antennules with the attachment organ on the third segment, subterminal and terminal setae on the fourth segment and lattice organs with pore fields are synapomorphies for all cirripede cypris larvae. These characters support a monophyletic taxon Cirripedia comprising the Acrothoracica, Thoracica and Rhizocephala.

РЕЗЮМЕ: Наружная морфология циприсиовидных личинок двух родственных видов *Trypetesa lampas* (Hancock, 1849) и *T. lateralis* Tomlinson, 1953 была исследована с помощью сканирующего электронного микроскопа. Специальное внимание уделялось таким структурам как решетчатые органы, антеннулы, торакоподы и тельсон. Циприсовидные личинки трипетесид имеют решетчатые органы с пористым полем, четвертый антеннулярный сегмент с субтерминальными и терминальными щетинками и слегка расщепленный тельсон. Эти признаки сближают циприсовидных личинок семейств Lithoglyptidae и Trypeteidae. Такие признаки, как четырехсегментные антеннулы с прикрепительным органом на третьем сегменте, субтерминальными и терминальными щетинками на четвертом и решетчатые органы с пористым полем — синапоморфии для всех циприсовидных личинок усоногих ракообразных. Они говорят о монофилии таксона Cirripedia, состоящего из надотрядов Acrothoracica, Thoracica и Rhizocephala.

Introduction

The Acrothoracica represent the most plesiomorphic cirripede taxon [Anderson, 1994; Glenner et al., 1995; Kolbasov et al., 1999]. The larval characters, especially those of the cypris larvae, are urgent for resolving of the relationships within the Thecostraca, because their adult stages are highly modified by symbiotic or sessile way of life [Grygier, 1987a; Jensen et al., 1994; Kolbasov et al., 1999]. Jensen et al. [1994] used SEM for the study of the lattice organs of both the Ascothoracida and Cirripedia. They found some putative synapomorphies between the Ascotoracida and Acrothoracica and putative synapomorphies that group all three cirripede superorders into a monophyletic taxon. Spears et al. [1994], using molecular data, supposed that the Acrothoracica should be suggested as close relatives of the Ascothoracida and argued the monophyly of the Cirripedia. Recently Kolbasov et al. [1999] investigated with SEM almost all external cyprid characters in two acrothoracican species of the genus *Lithoglyptes* Aurivillius, 1892. In contrast to Spears et al. [1994], they showed that all three cirripede taxa compose a monophyletic taxon, although the Acrothoracica exhibit a row of common characters with the Ascothoracida.

All previous works on the morphology of the cypris larvae of *Trypetesa* [Tomlinson, 1955; Turquier, 1967, 1970, 1971] based on light microscopy. Most of them described the changes occurred during a metamorphosis from cyprid to adult female or male.

Here we study such structures as lattice organs, antennules, thoracopods and telson of the *Trypetesa* cyprid and compare them with the analogous structures of cyprids of the lithoglyptid acrothoracicans [Kolbasov et al., 1999].

Material and methods

Although most acrothoracican species brood their larvae inside the mantle sac until the cypris instar [Tomlinson, 1969] the species of the *Trypetesa* have free-swimming larval instars.

The cyprids of *T. lampas* were reared in the Kristineberg Marine Research Station (59°15.79′N, 11°27.79′E, Sweden) from the culture of adult barnacles, burrowed the buccinid gastropod shells, occupied by hermit-crab *Pagurus bernhardus* (Linnaeus, 1758).

Two just-settled cyprids (future dwarf males) of *T. lateralis* were found in Tomlinson's material (California, Monterey Bay, Monterey Country, Point Pinos, in gastropod shell of *Tegula* Born, 1778, occupied by hermit-crabs) deposited in the Zoological Institute RAS (St.-Petersburg).

All material was preserved in 70% alcohol. We examined five cypris larvae of *T. lampas* and two cypris larvae of *T. lateralis* with SEM. All larvae were post-fixed with 2% OsO_4 for 2 hrs, dehydrated in acetone, and critical point dried in CO_2. Dried larvae of were sputter-coated with platinum-palladium or gold and examined at 15 kv accelerating voltage (with a JEOL JSM-840 SEM in Copenhagen and a HITACHI S405A SEM in Moscow).

Results

General appearance

The cypris headshield or carapace has spindle-shaped form (Fig. 1A), with rounded anterior and truncated posterior ends and curved dorsal margin. They measure ca. 450–500 μm in length. Their shape is rather similar to the shape of *Weltneria* cypris larvae [Jensen et al., 1994; own data], the height ratio of the carapace is ca. 2:1, whereas the same ratio for the cypris larvae of the genus *Lithoglyptes* is 3:1 [Kolbasov et al., 1999]. The surface of the carapace is smooth, with a few small pores. We did not find conspicuous furrows at the anterior end, as in the cyprids of *Lithoglyptes* [Kolbasov, et al. 1999].

The rounded, conspicuous frontolateral pores are similar to those in *Lithoglyptes* and are situated near the anteroventral margin of the carapace (Fig. 1B). The pores are elongated (3 by 6 μm) and surrounded by a simple cuticular ridge (1.36 μm high).

Lattice organs

The lattice organs of the cyprids of *T. lampas* were studied in detail with SEM [Jensen et al., 1994] and TEM [Høeg et al., 1998]. The shape and location of all five pairs of lattice organs (two anterior and three posterior pairs) are similar in both species of *Trypetesa*. As in all acrothoracican cypris larvae, the second pair of lattice organs has an anterior terminal pore, whereas other pairs bear posterior terminal pores (Fig. 1C-G). All lattice organs are in the elongate and slightly curved depressions. A distinct medial keel was observed in the lattice organs of *T. lateralis*, whereas thoseof *T. lampas* lack such a conspicuous keel (Fig. 1C, D). All surface of the lattice organs of *T. lateralis* are densely covered by small pores (Fig. 1G). It seems that the lattice organs of *T. lampas* have a smooth surface (Fig. 1D, F), although Jensen et al. [1994] described a medial keel sometimes covered with very small pores.

Cypris body

The cypris body is completely covered by carapace (Fig. 1A). The anterior half of the mantle cavity is occupied by antennules (Fig. 2A, B), compound eyes and frontal filaments [Kolbasov et al., 1999]. The thorax with six natatory thoracopods, the rudimentary abdomen and the telson with furca are situated in the posterior half of the mantle cavity (Fig. 2A).

Antennules

Four-segmented antennules (Fig. 2B–D) exhibit almost the same morphology as in the cypris larvae of *Lithoglyptes* [Kolbasov et al., 1999]. The big first segment is smooth and lack any denticles and setae. The cylindrical second segment is ca. 104 μm long (Fig. 2B), with a postaxial seta 2 (psII), number 13 in the classification of Nott & Foster [1969], inserted ventrodistally (Fig. 2C). The hoof-shaped third segment (Fig. 2C) measures ca. 24 by 14 μm, with conspicuous ventral attachment disc, covered with a dense carpet of cuticular villi. Maybe due to poor fixation, we found only a postaxial sensillum (pas) at the postaxial margin of the segment (Fig. 2C). The cylindrical fourth segment (Fig. 2C, D) measures ca. 7 by 4 μm and inserts laterally on the third segment. It is armed with three subterminal and five terminal setae (Fig. 2D). It differs from the fourth segment of *Lithoglyptes* species in having three, instead of four, subterminal setae. The subterminal setae have an equal length (ca. 5 μm), with terminal pores. The terminal setae are of different sizes. Two short and tiny setae surround a biggest terminal seta, that is probably homologous to the aesthetasc of other cirripede cypris larvae. The fifth seta is long and narrow.

Oral pyramid

An undifferentiated oral pyramid or buccal cone is situated on the ventral side just anterior to the thorax

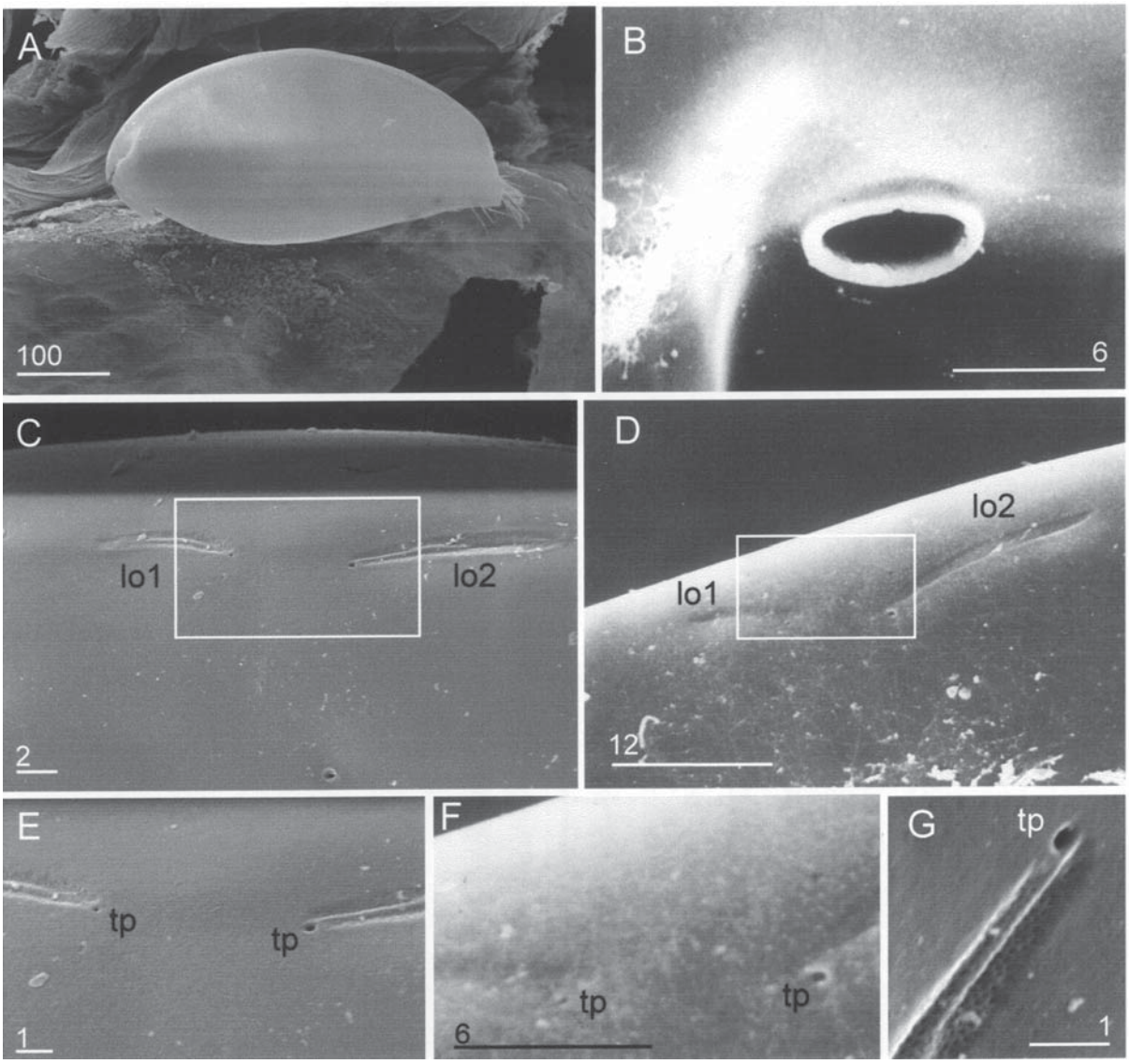

Fig. 1. A — just settled cypris larvae of dwarf male, general view, B — frontolateral pore, C, D — lattice organs, anterior pairs (first pair left), E — parts of lattice organs from rectangle area in C, F — parts of lattice organs from rectangle area in D, G — magnificated part of lattice organ of second pair in C. (A,C,E,G — *T. lateralis*, B,D,F — *T. lampas*). Abbreviations: lo1 — lattice organ of first pair; lo2 — lattice organ of second pair; tp — terminal pore of lattice organ. Scale bars in μm.

Рис. 1. A — недавно осевшая циприсовидная личинка карликового самца, общий вид, B — фронтолатеральная пора, C, D — решетчатые органы, передние пары (первая пара слева), E — части решетчатых органов из прямоугольной области на рис. C, F — части решетчатых органов из прямоугольной области на рис. D, G — увеличенная часть решетчатого органа второй пары с рис. C. (A,C,E,G — *T. lateralis*, B,D,F — *T. lampas*). Обозначения: lo1 — решетчатый орган первой пары; lo2 — решетчатый орган второй пары; tp — терминальная пора решетчатого органа. Масштаб в мкм.

(Fig. 2F). It represents a small protrusion with the rudiments of the mouth parts.

Thorax and thoracopods

The thorax forms the posterior third of the body (ca. 154 μm long). It consists of six segments each bearing a pair of natatory, biramous thoracopods (Fig. 2A, E, F). Grygier [1983, 1987a] and Grygier & Ohtsuka [1995] indicate that both, the Thecostraca and the Maxillopoda, in general have a 5-7-4 tagmosis scheme. Kolbasov et al. [1999] supposed that the seventh thoracal segment may be one of the four rudimental segments described for the abdominal area in *Lithoglyptes* cypris larvae.

We could not observe in details all six pairs of thoracopods which also were described for the cypris larva of *Trypetesa nassarioides* Turquier, 1966 by Turquier [1967] with a light microscopy. They resemble those, described from the cypris larvae of *Lithoglyptes* [Kolbasov et al., 1999]. A two-segmented protopod (coxa and basis) carries a two-segmented exopod and a three-segmented endopod (Fig. 2E, F). Their setation is similar to that

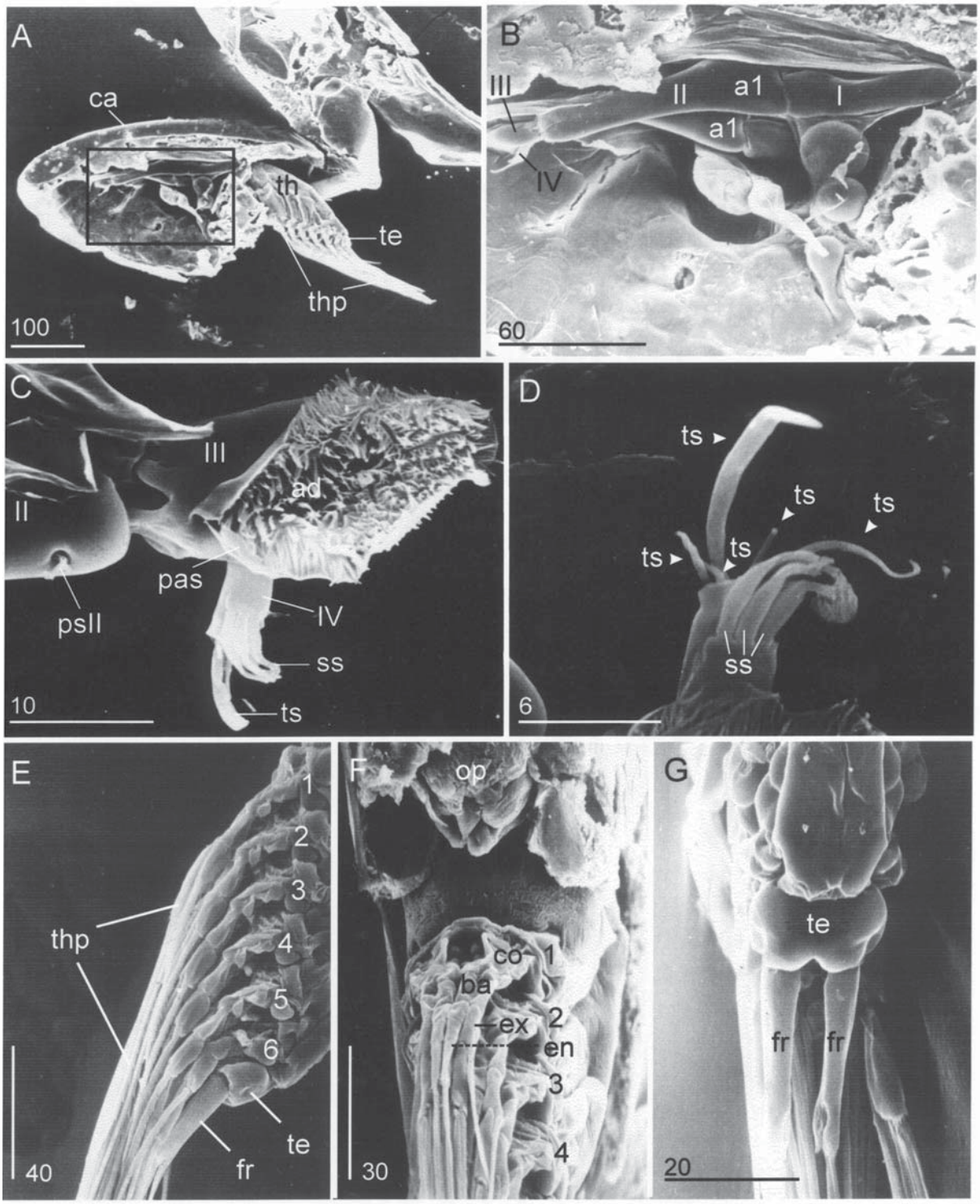

Fig. 2. *T. lampas*: A — cypris larva, lateral view of left side (carapace removed), B — anterior part of cypris body from rectangle area in A, C — antennule, distal part, D — fourth antennular segment, E — posterior half of cypris body, lateral view (thorax, thoracopods, telson and furcal rami), F — oral pyramid and anterior part of thorax, ventral view, G — posterior part of thorax with telson and furcal rami, dorsal view. Abbreviations: a1 — antennules (I — IV numbers of antennular segments); ad — attachment disc; ba — basis; ca — carapace; co — coxa; en — endopod; ex — exopod; fr — furcal rami; op — oral pyramid; pas — postaxial sensillum; psII — postaxial seta 2; ss — subterminal setae; te — telson; th — thorax; thp — thoracopods (1–6 numbers of thoracopods); ts — terminal setae. Scale bars in μm.

described from the cypris larvae of *Lithoglyptes* and *T. nassarioides*. A single stout seta is inserted laterodistally at the first exopod segment, its denticles are shorter than in the analogous one in *Lithoglyptes*. This type of seta is characteristic for all previously investigated cyprid larvae, but the degree of armament varies between different acrothoracican species.

Telson and furcal rami

The telson measures ca. 20 by 11 μm and has a conspicuous notch at the posterior margin (Fig. 2G). This notch is much deeper in the telson of the cypris larvae of *Lithoglyptes* [Kolbasov et al, 1999] and is distinctly cleft. Two long and narrow furcal rami are inserted at the posterior margin of the telson (Fig. 2G). They consists, as in the cypris larvae of *Lithoglyptes*, of a single segment with a tuft of long, distal setae.

Discussion

Lattice organs

The first pair of lattice organs of the Acrothoracica retains in all investigated species a plesiomorphic posterior position of the terminal pore. The acrothoracican lattice organs have a 'keel in a trough' morphology by Jensen et al. [1994]. These characters are plesiomorphic for Thecostraca and are also described for Ascothoracida [Jensen et al., 1994] and Facetotecta [own data]. Whereas other species of Cirripedia, belonging to the superorders Thoracica and Rhizocephala, have the lattice organs of the 'pore field type' [Jensen et al., 1994], i.e., an oval or elongate area perforated by numerous small pores.

Jensen et al. [1994] indicated that the lattice organs of Acrothoracica lack true pores. Kolbasov et al. [1999] wrote that the elongate depression of the lattice organs of *Lithoglyptes* 'has a lattice bottom due to minute perforations in the epicuticle, but TEM investigation of similar organs in the acrothoracican *T. lampas* revealed that they lack pores in the underlying procuticle [Høeg et al., 1998]'.

The lattice organs of *T. lateralis* and also lattice organs of other acrothoracican species [own data] possess distinct and dense minute pores, having a similar morphology to analogous ones in the thoracican barnacles. These facts stimulate us to reexamine our data on the lattice organs ultrastructure in Acrothoracica. We reinvestigate the TEM ultrastructure of the lattice organs of Acrothoracica of the study of Høeg et al. [1998] and established that they also penetrate the procuticle, whereas the lattice organs of Ascothoracida and Facetotecta lack any pores [Jensen et al., 1994; own data].

It means that the field of pores of the lattice organs is a synapomorphy for Cirripedia and distinguish them from other taxa of Thecostraca (Ascothoracida and Facetotecta).

Antennules

The four-segmented antennules with big first and second segments, special attachment disc on the hoof-shaped third segment and small fourth segment inserted laterally on the third segment are synapomorphies for all Cirripedia [Kolbasov et al., 1999]. There may be some variations on the number and form of setae of the fourth segment [Clare & Nott, 1994; Høeg & Rybakov, 1996; Kolbasov et al. 1999; Moyse et al., 1995; Walker, 1985].

A complete discussion on the structure of the fourth segment and the character of its setation in the cirripede taxa was made in Kolbasov et al. [1999] paper. Therefore here we concentrate only on some interesting details found in *Trypetesa* cypris larva.

Only three, instead of four in *Lithoglyptes*, subterminal setae were observed in *Trypetesa* cypris larva. Four terminal setae are suggested as a ground pattern for cirripede cypris larvae. Three subterminal setae of *Trypetesa* should be considered as an apomorphic character for this genus. The cypris larvae of *Trypetesa* and *Lithoglyptes* lack a distinct aesthetasc formed by a terminal seta in Thoracica and Rhizocephala. Anyway we can not consider an absence of aesthetasc as an synapomorphy of the Acrothoracica. Our data on the cypris larva of *Weltneria spinosa* Berndt, 1907 revealed the presence of a distinct aesthetasc in this species.

Telson and furcal rami

Following to Grygier [1987b], Kolbasov et al. [1999] and Walossek et al. [1996] one-segmented furcal rami are present in all cirripede cypris larvae. The basal segment of the two-segmented furcal rami, described in some thoracican cypris larvae [Glenner & Høeg, 1995; Walker & Lee, 1976], is a derivative of the telson cleavage. Probably Turquier [1967] made a similar mistake, when he described two-segmented furcal rami for *T. nassarioides*.

We observe a different degree of the telson cleavage in the cypris larvae of the acrothoracican species. The telson of *T. lampas* has only a small median notch, whereas telsons of *Lithoglyptes* species [Kolbasov et al., 1999], a new species of the genus *Kochlorine* Noll, 1892 and *W. spinosa* possess a deep medial cleft [own data].

Рис. 2. *T. lampas*: A — циприсовидная личинка, вид сбоку, левая сторона (карапакс снят), B — передняя часть тела циприсовидной личинки из прямоугольной области на рис. A, C — антеннула, дистальная часть, D — четвертый антеннулярный сегмент, E — задняя половина тела циприсовидной личинки, вид сбоку (торакс, торакоподы, тельсон и фуркальные ветви), F — ротовая пирамида и передняя часть торакса, вентральная сторона, G — задняя часть торакса с тельсоном и фуркальными ветвями, дорсальная сторона. Обозначения: a1 — антеннулы (I — IV номера антеннулярных сегментов); ad — прикрепительный диск; ba — басис; ca — карапакс; co — кокса; en — эндоподит; ex — экзоподит; fr — фуркальные ветви; op — ротовая пирамида; pas — постаксиальная сенсилла; psII — постаксиальная щетинка 2; ss — субтерминальные щетинки; te — тельсон; th — торакс; thp — торакоподы (1–6 номера торакоподов); ts — терминальные щетинки. Масштаб в мкм.

The cypris larvae of Facetotecta [Grygier, 1987b, Itô & Ohtsuka, 1984; own data] possess an entire telson. It means that this morphology of the thecostracan telson should be considered as plesiomorphic. This character of the telson is more plesiomorphic in *T. lampas* in comparison with other studied acrothoracican barnacles, although the genus *Trypetesa* is regarded traditionally as the most advanced within the Acrothoracica [Tomlinson, 1969].

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