



A clearly identifiable postlarva in the life cycle of a new species of *Pliciloricus* (Loricifera) from the deep sea of the Angola Basin*

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Abstract

A newly discovered species of *Pliciloricus* from the deep sea of the Angola Basin (Namibia, Atlantic) seems to have two types of postlarvae in its life cycle. Type I is a simplified but clearly identifiable postlarval stage, the other type II consists only of a thin layer of cuticle as the remnant of a postlarva. Both types contain adults that have moulted from them. The simplified type I postlarva has a fully developed lorica with an ornamentation identical to that of the adult, but other body regions are reduced with only a few structures left. The discovery of this clearly identifiable postlarva is important, because it supports the conclusion that *Pliciloricus*-species originally moults from postlarval stages as well as other taxa of Loricifera. Furthermore, it could be concluded that the simple cuticle layer surrounding most adults found during their metamorphosis is the remnant of a postlarval stage. The life cycle of the new species seems to include two phases. After to the bisexual is a unisexual phase, represented by a simplified parthenogenetic adult stage which lacks most parts of the adult morphology. The adults of *P. diva* sp. n. differ from other species in having among others (1) a mouth cone with four strong cuticular bars plus eight primary oral ridges; (2) leaf-like clavoscalids which are very broad basally and narrow distally, and have more than 22 transverse cross walls; (3) a strongly sclerotized double-organ consisting of four rami; (4) large spinoscalids of second row shorter than clavoscalids, (5) short type B spinoscalids of fourth row with claw-tips and with a double row of five teeth as well as distally with a double row of minute denticles; (6) an anterior margin of the lorica with bicuspid protrusions and specific crescent-shaped ornamentations; (7) a midventral plica with five bar-like transversal strengthened ridges. Distinguishing features of the Higgins-larva are (1) short clavoscalids with broad second segments; (2) a collar with seven flosculi located in small pits; posterolateral setae being short but strong and pod-like. The study also revealed new information about the double-organ of the adult and the buccal structures of the Higgins-larva.

Key words: deep-sea meiofauna, Angola Basin, DIVA 1

Introduction

During the DIVA 1 and –2 expeditions (Latitudinal Gradients of Deep-Sea BioDiversity in the Atlantic Ocean) the meiofauna of the Angola Basin were studied. The aim of the DIVA expeditions was to enhance the knowledge of the benthic fauna inhabiting sediments in Atlantic deep-sea basins from pole to pole (Martínez & Schminke 2005). The 160 multicore samples obtained during the DIVA 1 expedition contained 280 Loricifera. One successful multicore tube contains on average 2–3 specimens of Loricifera in strong contrast to the dominant taxa, Nematoda (2000 specimens) and Copepoda, Harpacticoida (500 specimens). Loricifera make up no more than 0.2% of the total meiofauna in all samples analysed to date. More than 95% of the specimens obtained so far have been larval instars and many of them contain eggs. These have most likely

been produced by parthenogenetic simplified adults (Gad 2005a). Many new species of Pliciloricidae have been discovered in the deep sea of the Angola Basin indicating that they are widely distributed (Gad 2001, 2002, 2005a). Every fourth specimen turned out to be a representative of *Pliciloricus* most of which were larval instars (Gad 2005a).

Loricifera are an exclusive taxon of marine meiofauna. The presence of Loricifera seems often linked to low nutrient sediments (Gad 2004a, 2005a). Loricifera have been found worldwide from the shallow subtidal zone down to the deep sea (7 to 8260 m depth, see Kristensen & Shirayama 1988; Todaro & Kristensen 1998; Kristensen 1983, 2003; Kristensen & Gad 2004; Gad 2004a, 2005b; Heiner & Kristensen 2005; Heiner 2008). The adults have a bilaterally symmetrical trunk and a size of around 180–320 µm. Their body is composed of five regions: (1) a mouth cone, (2) an introvert, (3) a neck, (4) a thorax, and (5) an abdominal region called lorica. Higgins-larvae measure 100–800 µm in length and have bodies composed of similar regions as adults. They mainly differ in having a collar region as closing apparatus for when the introvert is withdrawn into the body, the cuticle of the lorica is thinner, and the caudal end is equipped with strong setae and ventrally locomotory appendages called toes.

The genus *Pliciloricus* includes 11 described species, which exhibit a broad variety of features (Higgins & Kristensen 1986; Kristensen & Shirayama 1988; Heiner & Kristensen 2005; Gad 2005a, c). Adults of *Pliciloricus* characteristically have a large mouth cone, a double-organ in the second row of spinoscalids, eight single trichoscalids arranged in alternation with seven double trichoscalids on the neck region, a clearly defined lorica with about 22 double ridges, and a broad transformed midventral plica (Higgins & Kristensen 1986; Gad 2005a, c). The Higgins-larvae have an indistinct neck region, a well developed collar as an intermediate region between head and flexible thorax, an abdomen with a less developed lorica and about 20 deep longitudinal folds, two pairs of short anterior setae, and a caudal end with three pairs of posterior setae, and spinose or tubular toes (Higgins & Kristensen 1986; Gad 2005a, c).

Since the description of *Nanaloricus mysticus* and its life cycle (Kristensen 1983, 1991a), many new details about the life cycles of Loricifera have been discovered (Kristensen & Brooke 2002; Kristensen 2002, 2003; Gad 2002, 2004a, 2005a, b, c, d, e; Heiner & Neuhaus 2007; Heiner 2008). The life cycles of Loricifera are known to include bisexual adults, a series of larval instars called Higgins-larvae and a juvenile or postlarval stage, and all life history stages and instars are separated by a moult (Kristensen & Brooke 2002). Because of the paucity of observations of live specimens of Pliciloricidae, the exact number of larval instars remains uncertain. Their number has been deduced from the size classes found. Based on this assumption probably up to seven larval instars occur in the life cycles of *Pliciloricus*-species. This assumption may not be wholly satisfactory but it is currently the best estimate for comparison of life cycles and morphological transformations of life history stages (Gad 2005a, c). According to this assumption the last instar larva, which contains adults moulting from a postlarva, is likely to typically be the seventh instar in species of *Pliciloricus*. Opinions vary on how the number of larva should be named, for example Heiner (2008) preferred the term penultimate instar larva in the description of a new *Rugiloricus*-species, which contained new observations of the life cycle especially the reduced postlarvae.

The discovery of several new *Pliciloricus*-species in the deep sea of the Angola Basin (Gad, 2005a) and on the plateaus of seamounts (Gad 2004a; Heiner & Kristensen 2005) has added important new information. First, in addition to mature adult males and females there is in some life cycles a simplified adult, which seems to reproduce parthenogenetically (Gad 2005a, c). Second, there is the tendency in the last (seventh instar) Higgins-larva of some *Pliciloricus*-species to reduced more and more some of its features, e.g. some toes (Gad 2005c). This observations let to the hypothesis that a thin layer of cuticle which surrounds adults after metamorphosis could be the remnant of the postlarval stage. As long as this was unknown *Pliciloricus*-species were assumed to directly develop from larvae into adults (Kristensen & Brooke 2002). To date there is no clear indication for the occurrence of a postlarva in the life cycles of *Pliciloricus*-species. This is contrast with some *Rugiloricus*-species, which are representatives of the other genus of Pliciloricidae (Higgins & Kristensen 1986; Kristensen & Brooke 2002; Gad 2004a), for which a simplified postlarva with an unfinished adult morphology is known.

Material and Methods

This publication is a result of the DIVA 1 and –2 (Latitudinal Gradients of Deep-Sea BioDiversity in the Atlantic Ocean) expedition. The main material was sampled during DIVA 1 no. 48/1 of R/V "Meteor" to the Angola Basin (Southeast Atlantic) in 2000. Sampling of the meiofauna at the two stations (346 in the north and 331 in the south) was carried out with a multicorer by Dr. Elke Willen (at that time associate of AG Zoosystematik und Morphologie, C. v. O. University of Oldenburg) and Dr. Kai Horst George (DZMB, Deutsches Zentrum für Marine Biodiversitätsforschung, Forschungsinstitut Senckenberg, Wilhelmshaven). Additional material was found in samples taken during the DIVA 2 Expedition (Cape Town - Mindelo) with R/V "Meteor" (M63/2) in spring 2005. Sampling was also carried out with a multicorer at 5649 m depth at station 50 MUC 6 (Angola Basin). Sampling and sorting of the meiofauna on board was carried out by the staff of the DZMB (Deutsches Zentrum für Marine Biodiversitätsforschung, Forschungsinstitut Senckenberg, Wilhelmshaven).

The upper five centimetres of all multicorer samples together with the remaining supernatant water, which was filtered through a 40 µm mesh, were fixed and preserved with 4% buffered formalin. The meiofauna was extracted using the differential flotation method with the colloidal silica gel Levasil® 200 (40 %, viscosity 20, density 1,29) and centrifuging the sample for five minutes at 4,000 rpm (adapted from McIntyre & Warwick 1984; Higgins & Thiel 1988). The loriferans were sorted with the aid of an Irwin loop under a stereomicroscope (LEICA MZ8). Before and during sorting they were placed in a 70% ethanol medium, later transferred to glycerol and mounted in glycerin-paraffin-beewax preparations, sealed with Glyceel (adapted from Higgins & Thiel 1988; Brown 1997).

Microscopic investigation was carried out with LEICA interference-microscopes (DMLB with UCA condenser, IC prism and additional magnification x1.5 and x2). Photographs were taken with a DMLB microscope and a computerised digital camera (ColourView system). Illustrations were made with the DMLB microscope and with the aid of a drawing tube (mirror technique and macro-apparatus FS25PE). The terminology used in text and figures is adapted from Higgins and Kristensen (1986) and Gad (2005a, c). Species are differentiated on morphological basis.

Morphological terms used in text and figures

* marks structures of postlarva; same structures found in bisexual adults unmarked.

Ad	bisexual adult
af	anal field
ap	anal plate
apl	anterior plate of prepharyngeal armature
apo	apodeme
ba	basis of spinoscalids and of double-organ
bar	cuticular bar
bc	buccal channel
bg ₁	anterior cuticular bridge
bg ₂	posterior cuticular bridge
bp	basal plate
bp ₁₋₂ a	type A basal plates of first to second row
bp ₃ a	type A basal plates of third row with single type A trichoscalid
bp ₁₋₂ b	type B basal plates of first to second row
bp ₃ b	type B basal plates of third row with double type B trichoscalid
br	brain
bra ₁	anterior double bracelet
bra ₂	posterior single bracelet

bu	buccal tube
bug	buccal gland
cgl	caudal gland
clo	cloaca
co	collar
cr ₁	first row of (clavo)scalids
cs	clavoscalid
ct	claw-tip
cw	cross wall
d	dorsal
den	denticle
do	(primary) double-organ
ds ₁₋₄	first to fourth dorsal spines
ec	end cone
ed	anterior edge of lorica
eg	egg
em	embryo
fl ₁	papillate flosculus located on surface
fl ₃	papillate flosculus located in pit
fs ₁₋₂	first to second fringe of spinules
gu	midgut
ho	hook
ia	prepharyngeal armature
in	introvert
Lar I–VII	first to seventh instar Higgins-larva
lo	lorica
lop	longitudinal protrusion
lr	longitudinal fold of larval lorica
lr ₁	longitudinal (primary) ridge of adult lorica
ls ₁	anterolateral seta
ls ₂	anteroventral seta
lw	longitudinal weal
mc	mouth cone
mcr	mouth cone retractor
mcs ₁₋₃	first to third section of mouth cone
mo	mouth opening
ms	midventral oral seta
nk	neck
ngl	neck gland
oc	oocyte
oe	oesophagus
olp	oral loop
or	(primary) oral ridge of adult
os	oral stylet
ost	oral stria of Higgins-larva
ot	oral tooth
ov	ovary
p ₁₋₅	placoids of first to fifth row

pb	pharyngeal bulb
pc	pharyngeal crown
pl	plica
Pla	postlarva
plm	midventral transformed plica
psc	protoscalid
pt	pore of tubular part of toe
pro	protrusion
re	retractor
ra ₁	inner ramus of double-organ
ra ₂	outer ramus of double-organ
ro	round structure
ru	ruff
sAd	simplified (parthenogenetic) adult
sc	scalid
se ₁	posterodorsal seta
se ₂	posterolateral seta
se ₃	posteroterminal seta
sg ₁₋₄	first to fourth segment
sk	stalk of mouth cone
spr	row of spinules
spz	spermatozoa
sr ₂₋₉	second to ninth row of (spino)scalids
sr ₄₋₆ a	type A (spino)scalids of fourth to sixth row
sr ₄₋₆ b	type B (spino)scalids of fourth to sixth row
ss	spinoscalid
ssm	midventral transformed spinoscalid
st	end spine of toe
tb	toe base
te	testis
th	thorax
thp ₃	third row of adult thoracic plates
thr ₁₋₆	first to sixth row of larval thoracic plates
tm	transversal muscle
to	toe
tr	trichoscalid
tr ₁	lower (primary) ramus of type B trichoscalids
tr ₂	upper (secondary) ramus of type B trichoscalids
tr a	type A single trichoscalid
tr b	type B double trichoscalid
tri	transversal ridge
tu	tubular part of toe
tv	transversal constriction of lorica
v	ventral
wa	wart

Taxonomy

Loricifera Kristensen, 1983

Pliciloricidae Higgins & Kristensen, 1986

Pliciloricus Higgins & Kristensen, 1986

Type species. *Pliciloricus enigmaticus* Higgins & Kristensen, 1986

Pliciloricus diva sp. n.

Type material: includes eight specimens, the holotype and paratypes from the DIVA 1 expedition have been deposited in the type collection (UNIOL) now in the charge of the DZMB. The additional material from the DIVA 2 expedition are lodged at the central Senckenberg Institute and Museum Frankfurt (SMF).

Holotype: adult male, with good fixation of external structures, DIVA-1, station 331/1 MUC 8 (mounted on slide UNIOL-2003.0029).

Paratype I: fully extended adult male, with good fixation of internal structures, DIVA-2, station 50 MUC 6 (slide SMF 16898).

Paratype II: fully extended Higgins-larva (of 4th or 5th instar), DIVA-1, station 346/3 MUC 11 (slide UNIOL-2003.030).

Paratype III: mature male with withdrawn introvert surrounded by simplified postlarva (type I) and enclosed into the exuvium of a seventh instar Higgins-larva, DIVA-1, station 331/1 MUC 8 (slide UNIOL-2003.034).

Paratype IV: mature female with withdrawn introvert surrounded by simplified postlarva (type I) both enclosed into exuvium of seventh instar Higgins-larva, DIVA-1, station 346/7 MUC 1 (slide UNIOL-2003.035).

Paratype V: young female with withdrawn introvert surrounded by reduced postlarva (type II) both enclosed into exuvium of seventh instar Higgins-larva, DIVA-2, station 50 MUC 6 (slide SMF 16899).

Paratype VI: simplified parthenogenetic female in immature stage surrounded by cuticles of reduced postlarva (type II) and of seventh instar Higgins-larva, DIVA-2, station 50 MUC 6 (slide SMF 16900).

Paratype VII: simplified parthenogenetic female in late stage of maturity enclosed together with egg, embryo and first instar larva inside exuvium of seventh instar Higgins-larva, DIVA-2, station 50 MUC 6 (slide SMF 16901).

Paratype VIII: exuvium of seventh instar Higgins-larva; DIVA-1, station 346/5 MUC 11 (mounted on slide UNIOL-2003.038 respectively).

Type locality: Holotypic male originate from the deep sea of the Angola Basin (Atlantic) near the coast of Namibia (Africa), DIVA 1, station 331 (two hauls).

Station data: 19° 07,0' S / 003° 52,0' E; 5423 m depth, July 17, 2000, DIVA 1, station 346 (eight hauls).

Type habitat: oligotrophic to eutrophic environment; sediment type white to light beige; with mud contents in the surface layer reaching 90 - 99% and decreasing towards 12 cm sediment depth; total carbon contents between 8 and 8,7%; with high amounts of globularian foraminiferans; sediments well oxygenated down to a depth of 20 cm; 94–171 mV measured in surface sediments; bottom temperature 2.48°C; salinity 34.8‰ (Kröncke & Türkay 2003).

Additional material from DIVA 2 expedition also from the deep sea of the Angola Basin. Station data: 9° 56,0' S / 0° 54,1' E; 5648 m depth, March 11, 2005. other environmental data not yet available.

Etymology: From the Latin *diva* (goddess or famous lady), but in this case the species name refers to the acronym DIVA of the scientific expedition "Latitudinal Gradients of Deep-Sea BioDIVERSity in the Atlantic Ocean" during which the new species was discovered.

Measurements: Morphometric data of holotypic and paratypic males listed in Table 1 and of paratypic Higgins-larva listed in Table 2.

TABLE 1. Morphometric data of males of *P. diva* sp. n.

Adult males:	No.	Holotype	Paratype I
Length of:			
Body		302 µm	220 µm
Mouth cone		56 µm	46 µm
Introvert		62 µm	32 µm
Lorica		160 µm	95 µm
Anal cone		66 µm	45 µm
Primary double-organ		110 µm	85 µm
Secondary double-organ		22 µm	16 µm
lower trichoscalid ramus		115 µm	82 µm
upper trichoscalid ramus		108 µm	77 µm
Clavoscalids of first row (cr ₁)	8	136 µm	120 µm
Spinoscalids of second row (sr ₂)	9	112 µm	100 µm
Spinoscalids of third row (sr ₃)	15	105 µm	96 µm
A-spinoscalids of fourth row (sr _{4a})	15	68 µm	59 µm
B-spinoscalids of fourth row (sr _{4b})	15	115 µm	111 µm
Spinoscalids of fifth row (sr ₅)	30	126 µm	115 µm
Spinoscalids of sixth row (sr ₆)	30	120 µm	113 µm
Spinoscalids of seventh row (sr ₇)	30	115 µm	109 µm
Spinoscalids of eighth row (sr ₈)	30	110 µm	101 µm
Spinoscalids of ninth row (sr ₉)	30	12 µm	10 µm
Diameter of:			
Lorica		115 µm	90 µm

TABLE 2. Morphometric data of Higgins-larva of *P. diva* sp. n.

Higgins-larva:	No.	Paratype II
Length of:		
Body		162 µm
Mouth cone		28 µm
Midventral setae		4 µm
Introvert		19 µm
Lorica		70 µm
Toes		35 µm
Spine-tip of toes		10 µm
Anterolateral seta		14 µm
Anteroventral seta		14 µm
Posterodorsal seta		25 µm
Posterolateral seta		18 µm
Posteroterminal seta		10 µm
Clavoscalids of first row (cr ₁)	8	35 µm
Spinoscalids of second row (sr ₂)	10	35 µm
Spinoscalids of third row (sr ₃)	15	22 µm
Spinoscalids of fourth row (sr ₄)	14	18 µm
Spinoscalids of fifth upper subrow (sr ₅)	7	10 µm
Spinoscalids of fifth lower subrow (sr ₅)	8	3.5 µm
A-spinoscalids of fifth row (sr ₆)	7	2 µm
B-spinoscalids of fifth row (sr ₆)	8	2 µm
Diameter of:		
Lorica		60 µm

Description of adult male

External morphology (Figs. 1, 2A, B, 4A, D, E)

Body of holotypic male divided into stalked mouth cone, introvert, neck, thorax, and loricate abdomen. Thorax not fully extended in mounted specimen (Fig. 4A), all other body regions totally extended; total body length 302 μm , as measured from tip of mouth cone to caudal end; maximal width 115 μm , at level of anterior loric. Loric ventrally flattened with pronounced bilateral symmetry of trunk.

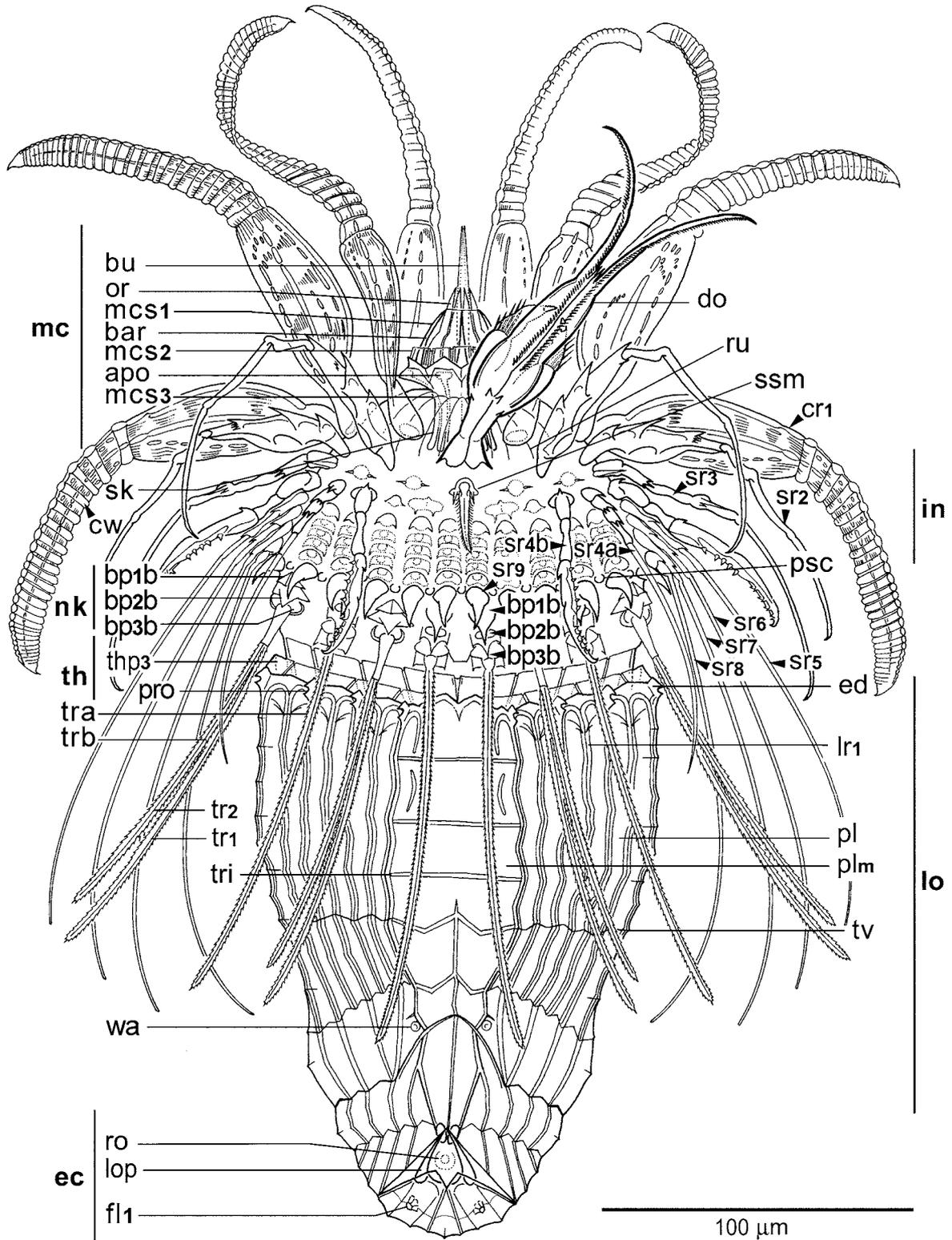


FIGURE 1. *Pliciloricus diva* sp. n., holotype, adult male, ventral view.

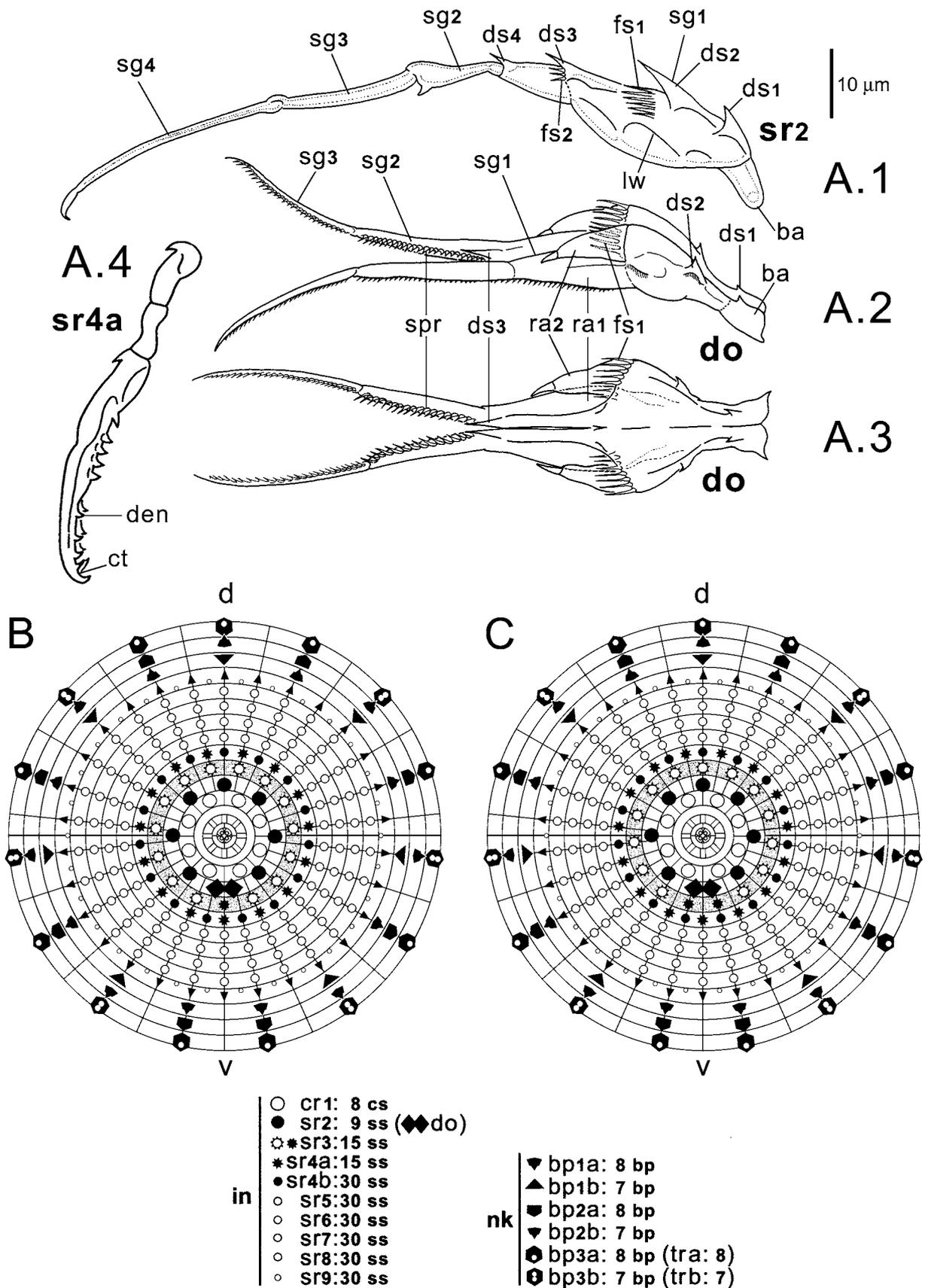


FIGURE 2. A *Pliciloricus diva* sp. n., details of scalids: double-organ compared with untransformed spinoscalids of second row of scalids; A.1, free spinoscalid, lateral view; A.2, double-organ, lateral view; A.3, double-organ, dorsal view; A.4, type B spinoscalid of fourth row; B, diagram of scalid arrangement on the head (= introvert and neck) of *Pliciloricus diva* sp. n., compared with the one of C, *Pliciloricus corvus* Gad, 2004.

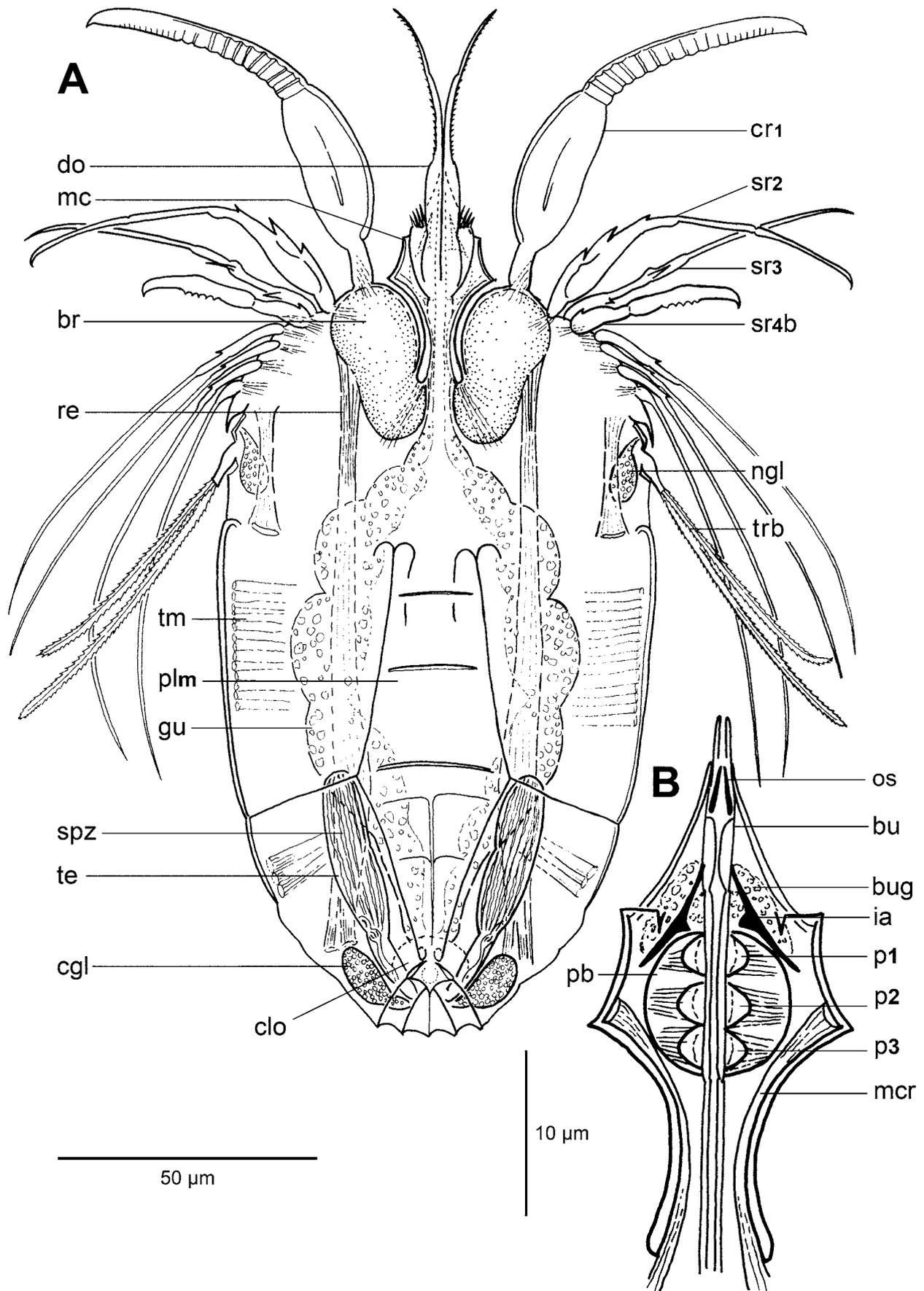


FIGURE 3. *Pliciloricus diva* sp. n., paratype I, adult male; A, half schematic illustration of anatomy with details in different optic layers; B, internal structures of the mouth cone, both ventral view.

Mouth cone (mc, Fig. 1) large, reaching nearly 20 % of body length; divided into three sections. First or distal most section (mcs₁) conical and pointed; mouth opening located terminally. Buccal tube (bu) being sclerotized part of buccal channel, short, narrow (3 µm), smooth, and weakly sclerotized; extended telescopically from the mouth (not in paratypic male which is drawn in Fig. 3B); distally with four minute oral stylets (os, for internal structures see also description of paratypic male below and Fig. 3B). Cuticle of first section strengthened by eight elevated, and strong longitudinal primary oral ridges (or), secondary oral ridges not observed (Fig.1). Second section (mcs₂) surrounding first section frill-like; bearing six anterior, strong longitudinal cuticular bars (bar); the posterior area divided into 12 plates folded transversally together. Mouth cone reaching broadest width at beginning of third section, marked by eight triangular formations. Third section (mcs₃) narrowing posteriorly and forming a narrow stalk (sk); stalk basally surrounded by circular fibres of the ruff (ru). Fibres extending as part of cuticle from the base of the stalk down to first row of clavoscalids.

Introvert (in, Figs. 1, 4A, 2B) spherical, densely covered with nine transversal rows of closely standing scalids.

First row (cr₁, Figs. 1, 4A) with eight strongly sclerotized and anteriorly oriented clavoscalids. All clavoscalids long (reaching over 50% of total body length), unsegmented and consisting of narrow, stalk-like bases and broad, flattened elements. Elements with reinforced dorsal margins and with pronounced median axis; subdivided into distal and proximal parts; ending in small ventral tips. Distal part longer and narrower than basal one; with more than 22 strong but narrow transverse cross walls (cw) with distally diminishing intervals between them. Intervals flat with fine longitudinal striae on lateral surfaces; alternation of protruded cross walls and flat intervals giving margins of distal parts an undulated appearance. Proximal parts visibly enlarged, lateral surfaces together with pronounced median axis with specific pattern of dots and fine striae. Second row (sr₂, Figs. 1, 2A.1, 4B) with nine strong spinoscalids being more sclerotized than others, oriented posteriorly, distinctly shorter than clavoscalids, and arranged in alternation to them. Pair of midventral-most spinoscalids fused to (primary) double-organ (Figs. 2A.2, A.3, 4A); seven free spinoscalids (Fig. 2A.1) strong, leg-like and composed of four segments. First segment (sg₁) large, angular, and broad; both lateral surfaces with three longitudinal arched weals (lw); segment also with four dorsal thorns (ds₁–ds₄) and two dorsolateral fringes of small spinules (fs₁, fs₂). Second segment (sg₂) short with swollen joint which bears a ventral tooth; next segment filiform connected by swollen hinge-joint to second segment; last segment (sg₄) as long as first one, ending in distinct sickle-like tip.

Double-organ (do, Figs. 2A.2, A.3, 4A) strongly sclerotized, rigid and bifurcated. A comparison in Figs. 2A.1–A.3 with an untransformed spinoscalid of the second row best demonstrates its transformation. Double-organ divided into two branches being subdivided into an inner and an outer ramus. Both branches arising from a common stem-like base (ba), which is movably connected with the introvert. Bases of double-organ with two small dorsal thorns (ds₁) having their equivalents in first dorsal thorn of free spinoscalids. Inner or ventral rami (ra₁) long and divided into three segments with many similarities and with free spinoscalids. First segments of ventral rami with second (ds₂) dorsal thorn having its equivalents in first dorsal thorn of free spinoscalids. Basal part of first segment (sg₁) enlarged, dorsally with shield-like duplication, longitudinally fused, and reaching 30% of length of double-organ. Two comb-like dorsal fringes of spinules (fs₁) located distally. Distal part of first segment free, not fused, tapers distally and ends in two inner thorns (ds₃). Next two segments filiform, free, and equipped in a saw-like manner with inner row of stiff minute spinules (spr). Third segment (sg₃) of inner ramus being a long movable spine. Both outer rami (ra₂) shorter, basally swollen and fused longitudinally with inner rami, ending in a pronounced tooth-like tip.

Third row (sr₃, Fig. 1) with 15 small leg-like, filiform spinoscalids consisting of four segments, 14 of them with following structure: First segment short and large, with double bases, flanked by two spines and divided by constriction in the middle, ending with fringe of minute spinules. Second segment short, narrow, with two swellings, and distinct hinge-joint flanked by two spines. Third segment short; fourth segment long, bristle-

like, and distally sickle-shaped. Fifteenth spinoscalid of this row in midventral position beneath double-organ transformed (ssm), distinctly shorter (12 μm) and consisting of a base and a short, flattened element with rows of fine, hairy setules along both lateral margins (in this position two spinoscalids are sometimes modified to a secondary double organ e.g. in *P. corvus* and *P. shukeri*).

All following scalid rows consist of at most 30 spinoscalids and are inserted on small arched basal plates. Fourth row armed with two different types of 15 spinoscalids in alternating arrangement.

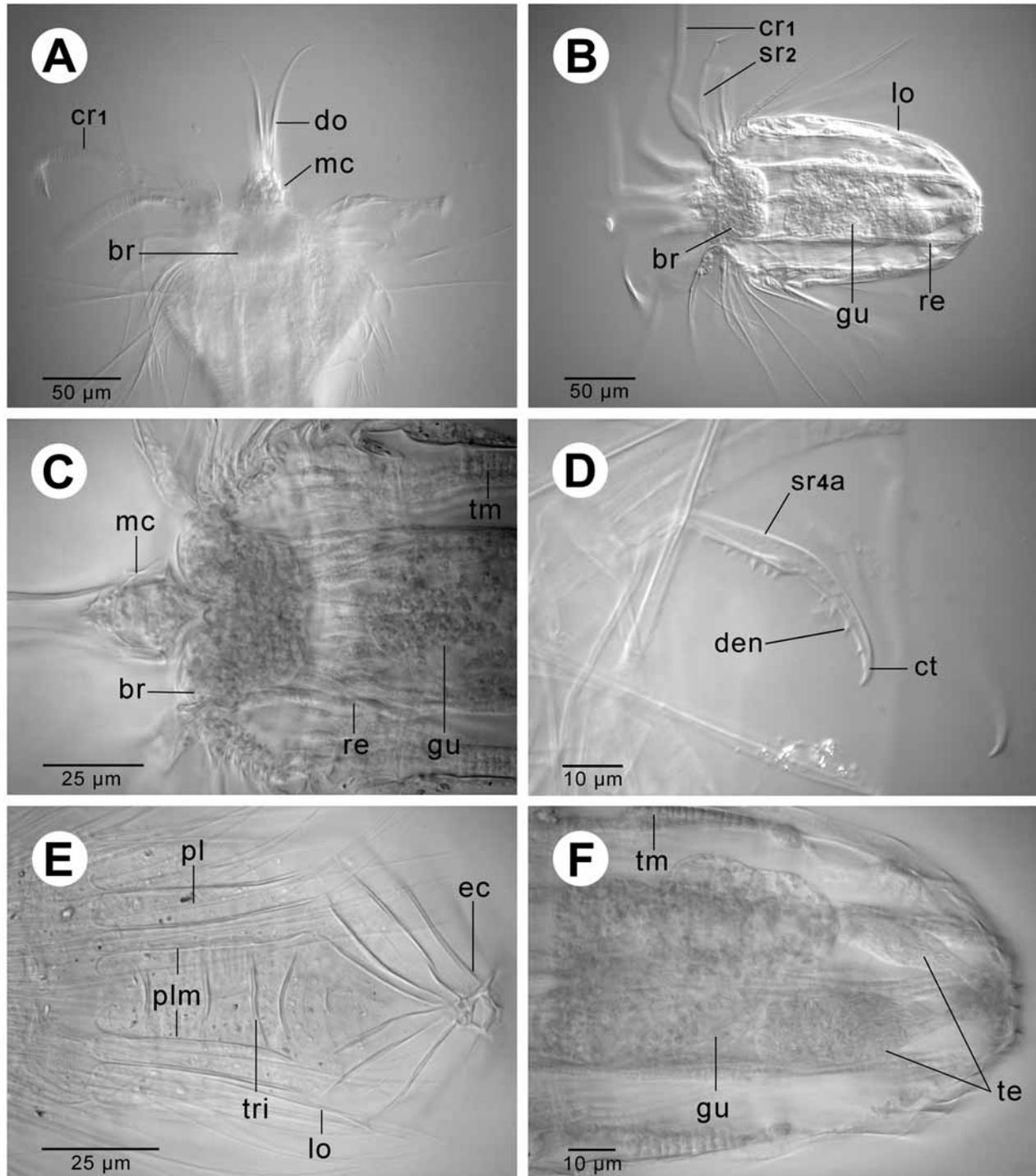


FIGURE 4. *Pliciloricus diva* sp. n., interference contrast light micrographs, holotypic male: A, anterior body region; paratype male: B, habitus; C, mouth cone and introvert; D, type B scalid of fourth row; E, lorica and posterior end; F, internal view on testis.

Type A spinoscalids (sr_4a , Fig. 1) long, filiform and divided into four segments. First segment short with separate base and a dorsal tooth, being divided by constriction in the middle, and ending with fringe of minute spinules. Second and third segments short, whilst fourth segment long and bristle-like.

Type B spinoscalids (sr_4b ; Figs. 1, 2A.4, 4D) short, strongly sclerotized, consisting of three segments. First segment more swollen than in type A scalids; base separated and also with small tooth. Second segment short and narrow with transversal constriction in its middle. Third segment enlarged, subdivided into three parts; first part round in cross section, and dorsally with small tooth; second part slightly narrower, laterally flattened, ventrally equipped with double row of five small teeth (Figs. 2A.4, 4D); third part jaw-like, ventrally with double row of small denticles (den), ending in claw-tip (ct).

Fifth row (sr_5 , Fig. 1) with 30 uniform spinoscalids, seta-like, consisting of three filiform segments, first two segments short, ending in dorsal thorns, third segment long.

Sixth row (sr_6 , Fig. 1) with 30 uniform spinoscalids, similar to spinoscalids of fifth row.

Seventh row (sr_7 , Fig. 1) with 30 uniform spinoscalids, similar to spinoscalids of fifth row.

Eighth row (sr_8 , Fig. 1) with 30 uniform spinoscalids, of more hair-like structure, consisting of two segments; first one short and bulbous; second one long and thin.

Ninth row (sr_9) equipped with 30 small and short teeth-like scalids.

Additional row of small protoscalids (psc, of an additional row ?) between eighth and ninth row (Fig. 1); covered by, and alternating with, spinoscalids of eighth row.

Neck (nk, Fig. 1) with eight single type A trichoscalids (tr a) and seven double type B trichoscalids (tr b) in alternating arrangement and covered with three transverse rows of accessory basal plates.

(Fig. 1) long, thin, rigid, flat, and with very fine serrated lateral margins; a third hardly visible serrated margin perpendicular to the median axis. Central channel usually extending inside, not visible. All trichoscalids end in simple pointed tips. Type A trichoscalids long and single, type B trichoscalids of two rami arising from a long, smooth common shaft; upper (secondary) ramus (tr_2) of type B trichoscalids slightly shorter than lower (primary) one (tr_1), but identical in structure and width. Midventrally there are two single type A trichoscalids standing close together; middorsally there is one type B double trichoscalid.

Accessory basal plates (Fig. 1) standing close together in rows. Well-defined basal plates of the first row represent two types; eight large, leaf-like, and pointed protrusions representing type A basal plates of the first row (bp_1a) alternating with seven smaller and triangular type B basal plates which have median keels. Type A basal plates of second row (bp_2a) are angular, tricuspid, and with oval depression. They alternate with larger triangular type B basal plates, carrying a large tooth. Single type A trichoscalids arise with short shafts from large basal plates (bp_3a), subdivided into a round upper and rectangular lower part. Double type B trichoscalids insert via a long shaft in round depressions of less defined angular type B basal plates.

In summary the number and arrangement of scalids (cs = clavoscalids, ss = spinoscalids, tr = trichoscalids) and basal plates (= bp) belonging to the head can be expressed in the following scalid formula: $8cs, 9ss(1do)+14ss/1ss, 15a+15b ss, 30ss, 30ss, 30ss, 30ss, 30ss$ (in) / $8a+7b bp / 8a+7b bp / 8a+7b bp, 8a$ (single)+ $7b$ (double) tr (nk)

See also diagram in Fig. 2B as additional demonstration of the scalid arrangement.

Thorax (th, Fig. 1) movable, retracted, and with thin cuticle subdivided into three transverse rows of moderately developed thoracic plates.

Lorica (lo, Figs. 1, 4E) well-developed, consisting of thick cuticle covering abdomen and being divided longitudinally into 22 secondary plicae (pl). Each plica composed of two primary ones (giving the impression that the lorica consists of 44); each primary plica is framed by a strongly developed longitudinal primary ridge (lr_1), adjacent plicae therefore separated by double ridges. Without additional single or secondary ridges. Midventral transformed plica (plm, Figs. 1, 4E) broad, being the result of total longitudinal fusion of four primary plicae, ending posteriorly in two small roundings. Anteriorly there are two short longitudinal bars. Lorica transversally subdivided by number of indistinct transversal ridges (tri); five of them are stronger and clearly visible in the midventral plica which posteriorly also has an incomplete longitudinal ridge along the

midventral axis. Anterior edge (ed) of lorica paved with crescent-shaped ornamentation and 22 dicuspid protrusions (pro). Lorica divided halfway into two halves by a transverse constriction (tv). Posterior lorica flexible, consisting of transverse row of plates separated by zigzag border; last part of lorica separated as end cone. Midventral plicae posteriorly flanked by a pair of ventral warts (wa).

End cone (ec, Figs. 1, 4E) consisting of two transverse rows of plates; with round structure (ro) flanked by two short longitudinal protrusions (lop). Caudally anal field composed of many small anal plates surrounding terminal anus. Anal field with a pair of flosculi (fl₁) composed of papillae. Holotypic male with two saccate testes compactly filled with flagelliform spermatozoa.

Anatomy (Figs. 3A, B, 4B, C, F)

Body of paratypic male (Fig. 4B) divided into same body regions as described in holotype and identical with that in all general external features, apart from being slightly smaller (see Table 1).

Mouth cone (Fig. 3B) fully extended and stalked; accompanied by close-fitting double organ.

Buccal structures (Fig. 3B, 4C) identical as in holotype. Internally, buccal channel extends straight through mouth cone. Pharyngeal bulb small and round, located in anterior part of mouth cone making up the second section. Buccal channel (bu) in front of pharyngeal bulb supported longitudinally by three small symmetrical bracelets (ia). Cells of buccal glands (bug) surround pharyngeal bulb and occupy space anterior of bracelets. Inner walls of pharyngeal bulb paved with three transversal rows of small placoids (p₁₋₃) each row containing three placoids. Oesophagus narrow, and continues through the stalk. Apodemes of mouth cone retractors connect anteriorly with inner wall of second section. Eight mouth cone retractors (mcr) run through the stalk and extend through the brain. Brain (br, Figs. 4B, C) visible as large and dense mass of cells filling out the anterior part of introvert.

Main musculature (Figs. 3A, 4C) consists of large layers of transverse muscle (tm) being most distinct in anterior and posterior region of lorica. Pair of large main retractors of introvert (re) run dorsally down from brain to caudal end. Smaller neighbouring retractors of introvert connected posteriorly with ruff fibres. Longitudinal muscles located mainly inside anterior half of trunk where they run down from last row of thoracic plates to mid transversal constriction of lorica. Loose grid of characteristic transverse muscles bundles in thoracic region. Scalid rows on introvert with own band of ring muscle. Each scalid has its separate diagonal muscle for separate movement.

Digestive system (Figs. 3A, 4C, F) beyond oesophagus continuing as voluminous midgut (gu) with large absorptive cells, followed by narrow hindgut, and round cloaca cavity (clo) which opens dorsocaudally.

Thorax contains series of eight neck glands (ngl), which open into basal plates above double trichoscalids via pores. Two oval caudal glands (cgl) present. Large pair of saccate testes (te, Fig. 4F) marking lateral sides of lorica and being densely filled with fibroid spermatozoa (spz) like in holotype. Protonephridia indistinct. Ducts of both testes end in cloaca (clo).

Description of female

Two mature females with retracted body (Fig. 8E) were found still enclosed in the cuticle of seventh instar larvae and both surrounded by cuticles of postlarvae. One female has moulted from a type I, and the other from a type II postlarva (see below). Adult females seem to be morphologically identical with males but differ internally by having paired ovaries of which only one is enlarged and filled with large oocytes and/or one large egg.

Description of simplified parthenogenetic female (Fig. 5A, B; 6A–F)

Simplified, not free-living adult stage represent unisexual phase of life cycle, with sack-like trunk, lacking buccal structures and introvert.

Body (Figs. 5A, B, 6A, C, D) retracted, with thin, weakly developed cuticle, consisting of indistinct thorax and abdomen, both united to simple sack-like trunk, containing ovaries. Introvert as anterior part of head lacking, only neck region present.

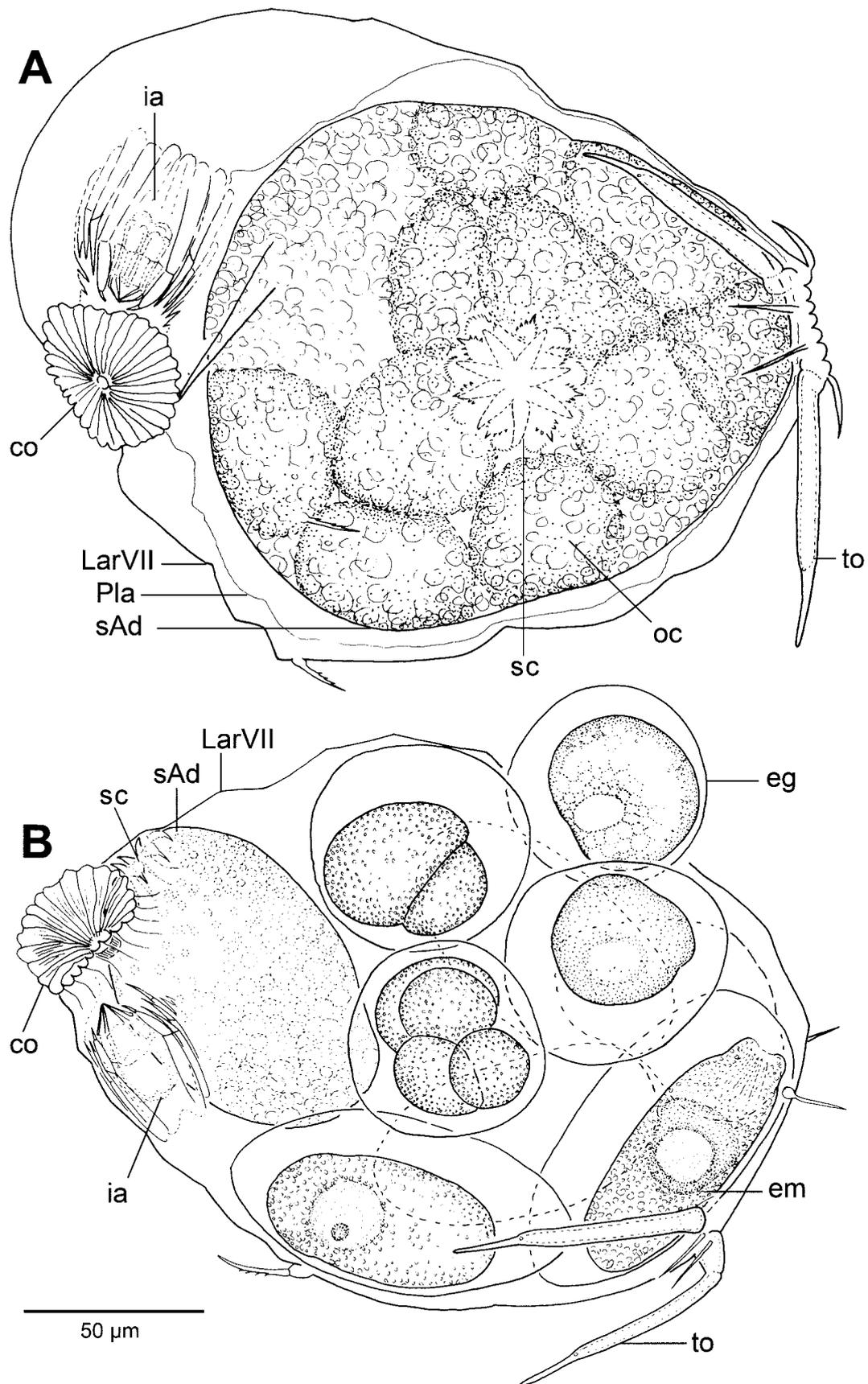


FIGURE 5. *Pliciloricus diva* sp. n., A, exuvium of seventh larval instar with retracted introvert containing reduced postlarve (type II) and simplified parthenogenetic adult (female) being in immature phase, both developing ovaries containing many oocytes; B, simplified parthenogenetic adult (female) in phase of disintegration, which is still enclosed together with released eggs and embryos inside exuvium of seventh instar Higgins-larva (cuticle of type II postlarve disintegrated), drawings in ventrolateral view.

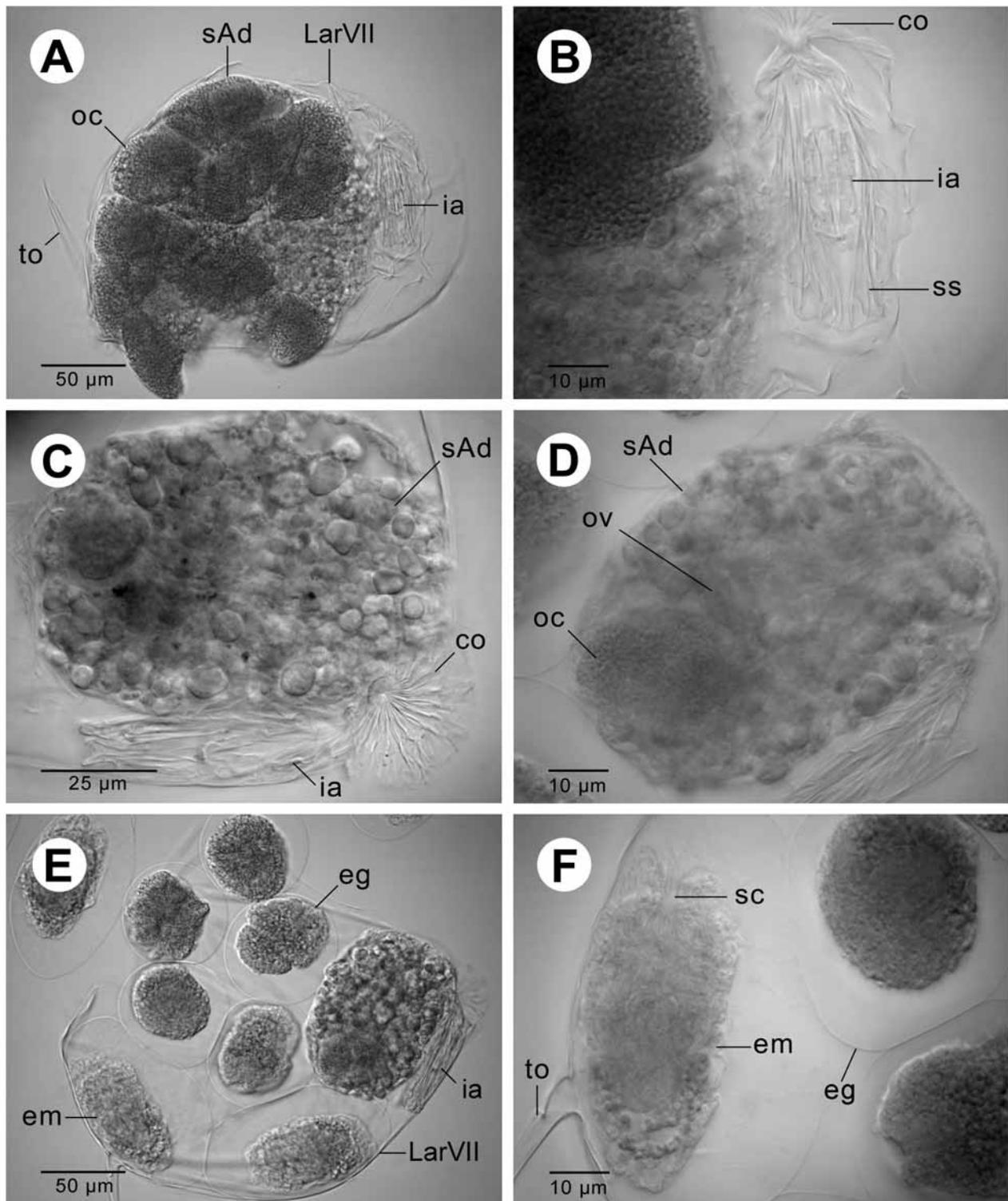


FIGURE 6. *Pliciloricus diva* sp. n., interference contrast light micrographs. A, simplified parthenogenetic adult (female) in immature phase (same specimen as in Fig. 5A); B, cuticle structures of surrounding seventh larval instar, closed collar, retracted scalids, and prepharyngeal armature; C, simplified parthenogenetic adult (female) in late phase of maturity (same specimen as in Fig. 5B) with cuticle structures of surrounding seventh larval instar; D, simplified parthenogenetic adult as unisexual active stage; E, same specimen which is still enclosed together with released eggs and embryos inside exuvium of Higgins-larva; F, focus on eggs and embryo.

Neck with eight large hook-like scalids (sc) arranged tightly in one circle at anterior end. Additional two rows of indistinct tiny spiny scalids it was not clearly visible how many scalids were present per row .

Simplified adult female (sAd, Figs. 5A, 6A, B) surrounded by thin cuticle of reduced postlarva (type II) and additionally enclosed in retracted exuvium of seventh instar Higgins-larva (LarVII). The latter showing characteristic features like structure of toes and trunk setae, but having introvert retracted and collar closed (Figs. 5A, B, 6A–C, E). One simplified female (paratype VI) found in immature phase (Figs. 5A, 6A, B, size 226 μm), which was starting to form twelve oocytes (oc) inside developing ovaries (ov). Other simplified female (paratype VII) being in phase of disintegration (Figs. 5B, 6C–F), body shrunk (“rest body”, size 90 μm) and tissue extensively disintegrated, even so the cuticle of type II postlarva. Exuvium of surrounding seventh instar Higgins-larva (LarVII, Figs. 5B, 6E) acts as shelter for nine released eggs (eg, diameter 40 μm) of which some have already developed to embryos (em, length 60 μm) or first instar larvae.

Description of Postlarvae (Figs. 7B–C, 8A–E)

Two types of postlarvae were found enveloped by exuvia of seventh instar Higgins-larvae. These postlarvae are non free-living instars and contain adults, which have moulted from them.

Type I postlarva (Figs. 7B–C) has a simplified adult morphology, the anterior region being simpler than the posterior one:

Body length 175 μm ; maximal body width 94 μm ; exuvium with features of later adult morphology. Anterior half of trunk with thin cuticle ($> 1 \mu\text{m}$, see arrow in Figs. 7B and 8C) representing introvert and thoracic region.

Introvert densely covered with minute protoscalids (psc), which could be identical in number and arrangement with later scalids of the adult introvert (too small to allow distinction of rows in LM). There is no mouth cone nor any internal buccal structures.

Neck and thoracic region not clearly defined, with wrinkled cuticle and many longitudinal and transverse folds, posteriorly with a circle of eight hooks (ho) which have their equivalents in the eight type A basal plates (bp_{1a}) on the neck of adults (Fig. 1). Posterior half of trunk representing armoured abdominal region with fully developed lorica.

Lorica (lo) with thick cuticle (3 μm , see arrow in Figs. 7B and 8C), easily visible in LM, and almost identical in structure with lorica of adults but less prominent: distinct anterior margin with cuticle reinforcement consisting of protrusion and crescent-shaped ornamentation, lorica composed of 44 primary plicae surrounded by well-developed double ridges, modified midventral plicae with five transversal ridges. End cone (ec, Fig. 7B) distinct and ventrally with a round structure.

Type II postlarva (Figs. 5A, 8E) consisting only of uniform thin cuticle without any structures surrounding adults during metamorphosis as type I postlarva.

Description of Higgins-larva (Figs. 8F, 9A, B)

Body (Figs. 8F, 9A, B) bottle-like, divided into conical mouth cone, introvert, neck, collar, thorax, and slightly loricate abdomen.

All body regions fully extended; length 167 μm (from tip of mouth cone to caudal end); maximal body width 63 μm (in middle of lorica).

Mouth cone (mc; Figs. 9A, B, 10A.1, A.2) divided into three sections. First section (mcs₁) narrow, cylindrical, and telescopically retractable into slightly broader second section. Cuticle with six massive basal longitudinal cuticular bars (bar). Terminal end has mouth opening, which is surrounded by external armature (Fig. 10A.2). Second (mcs₂) and third (mcs₃) sections of mouth cone broad and separated by transverse constriction; or second section slightly conical and anteriorly with six oral loops (olp); cuticle of both sections sculptured by six fine longitudinal oral striae (ost). Single midventral oral seta (ms) situated at base of mouth cone (Fig. 10A.2).

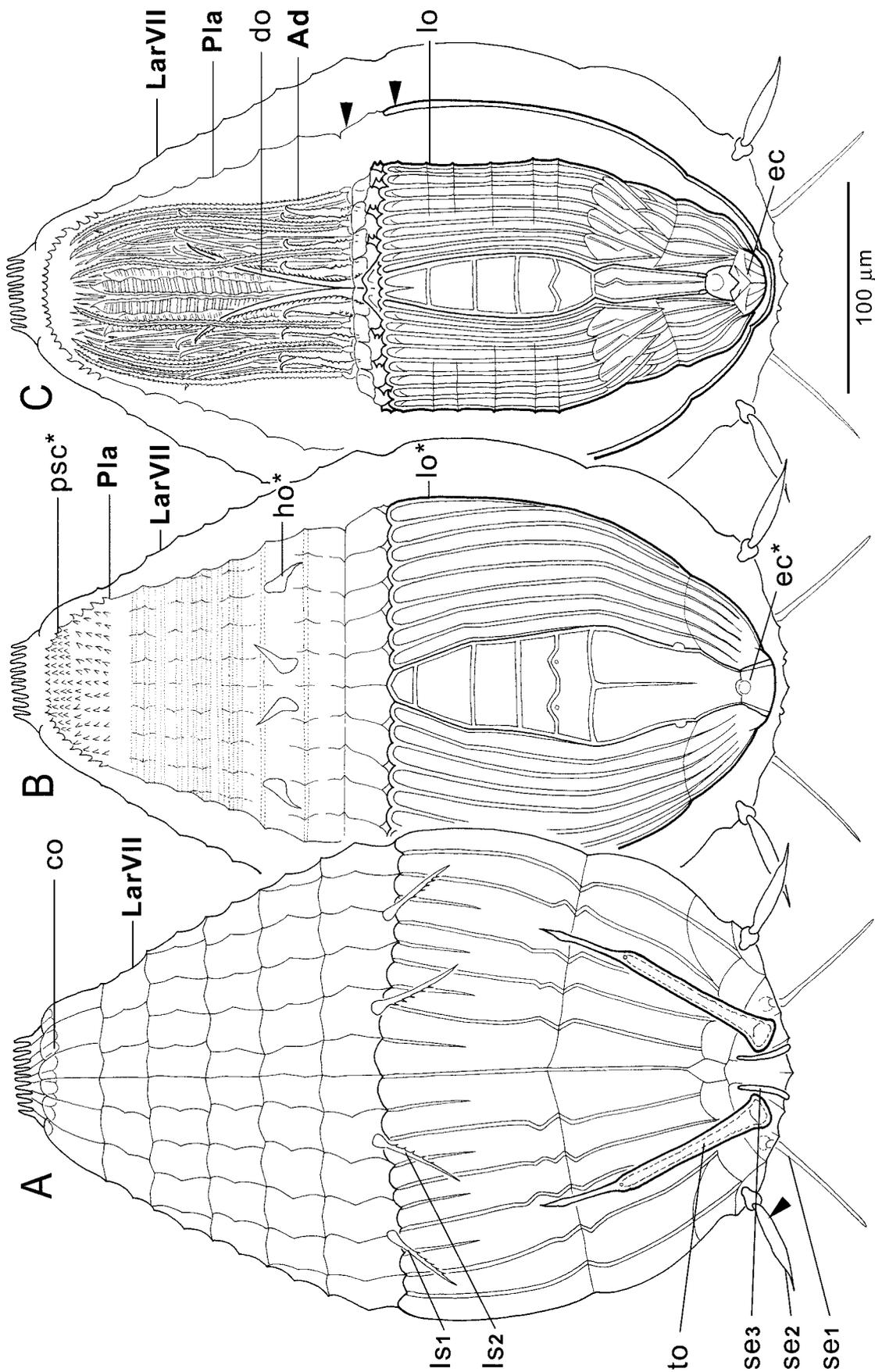


FIGURE 7. *Pliciloricus diva* sp. n., paratype III, a seventh instar Higgins-larvae containing an adult male having moulted from a type I simplified postlarva (same specimen as in Fig. 4A–D), all stages in ventral view. A, external view of exuvium of seventh instar Higgins-larva with retracted head and with the collar closed (withdrawn scalds and buccal armatures not drawn, see Fig. 8A, arrow marks characteristic anterolateral seta, compare with Fig. 9); B, view of enclosed simplified postlarva with adult-like lorica; C, view of enclosed fully developed mature adult with retracted body, ready to leave both exuvia (arrows point to different thickness of cuticle of simplified postlarva: anterior part comprising head and thorax with thin cuticle, posterior part consisting of lorica with thick cuticle, compare with Fig. 8C, ventral view).

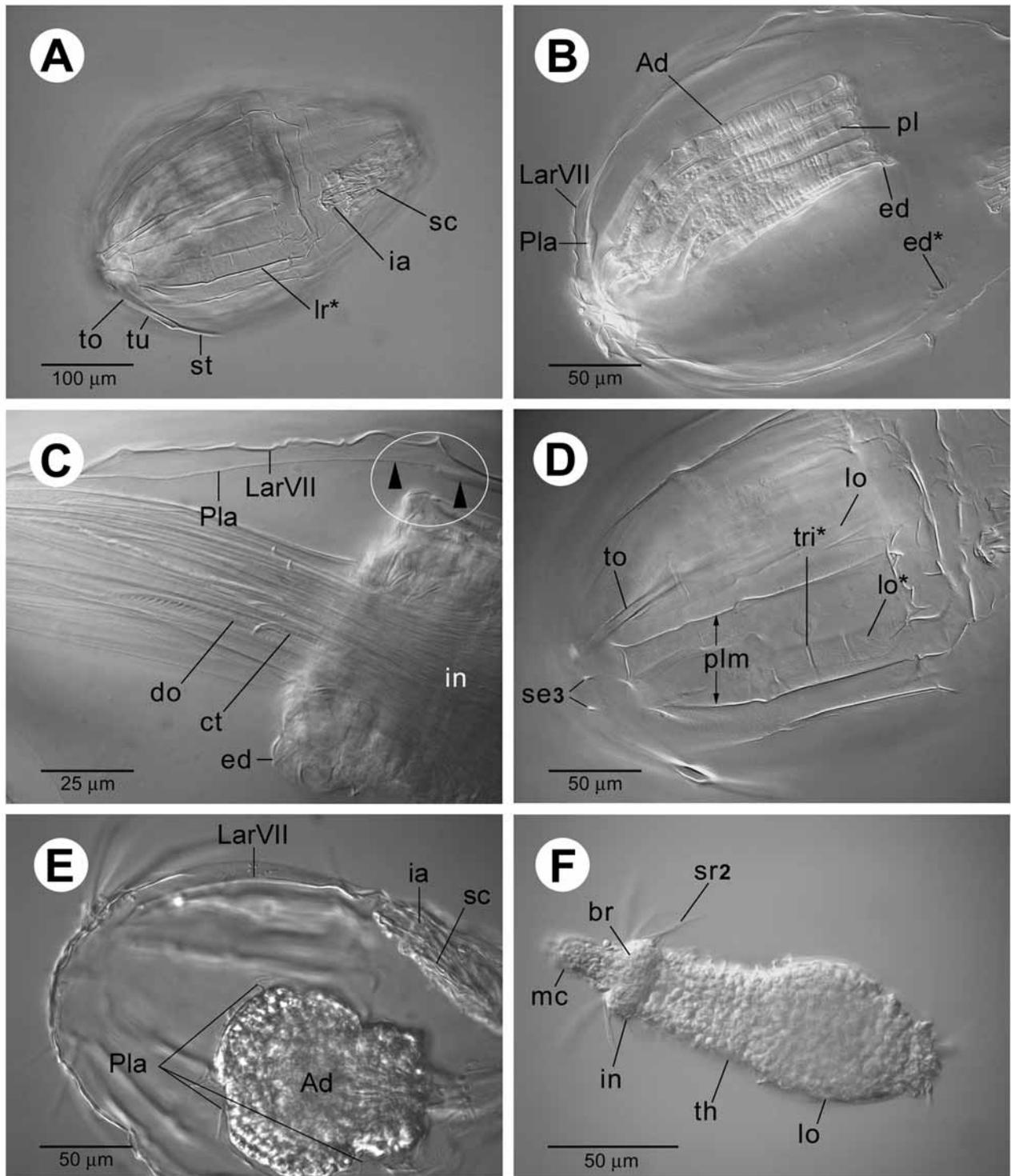


FIGURE 8. *Pliciloricus diva* sp. n., interference contrast light micrographs. A, exuvium of seventh larval instar with retracted introvert containing type I postlarva and adult (same specimens as drawn in Fig. 7A–C); B, focus on enclosed adult; C, anterior region with focus on both stages enclosed into larval exuvium (arrows point to different thickness of cuticle of simplified type I postlarva); D, ornamentation of midventral plica of postlarval lorica shining through larval cuticle; E, young adult female enclosed in reduced type II postlarva and exuvium of retracted seventh larval instar; F, habitus of paratypic Higgins-larva.

Internally there is a buccal channel (bc) with complex adjunctive structures (Fig. 10A.1). Pharyngeal bulb and prepharyngeal armature well recognizable in the paratypic Higgins-larva due to good fixation. Mouth

opening (mo) located terminally on the first section, which can be seen extended from the second section. Mouth opening externally surrounded by six oral teeth (ot) in front of longitudinal cuticular bars. Ring of six long and broad oral stylets (os) inside the mouth and partly pushed out from it. Basal stylets followed by broad and cylindrical prepharyngeal armature. Cuticle of narrow buccal channel weakly sclerotized and smooth, not forming a buccal tube, but surrounded by hexaradially symmetrical prepharyngeal armature over its entire length.

Prepharyngeal armature (ia, Fig. 10A.1) consisting of six longitudinal bracelets arranged in two stories, an anterior and a posterior one. Anteriorly, bracelets (bra_1) stand in pairs, their bases bifurcate and are connected by six upper transversal bridges (bg_1), and their other ends are connected with bases of oral stylets. Bracelet pairs covered anteriorly by six hyaline valve-like armature plates (apl) forming an outer cylinder. Posteriorly, bracelets continue as single straight elements (bra_2) connected with each other also by transverse bridges (bg_2). Behind bridges, single bracelets converge towards buccal channel, their posterior ends being fused with pharyngeal crown.

Pharyngeal bulb (pb, Fig. 10A.1) large, rounded at both ends, and slightly cylindrical. Anterior part of bulb marked by a sclerotized ring-like structure called pharyngeal crown (pc). Crown with three small apodemes (apo). Muscles of bulb in longitudinal section arranged in five layers of three radial muscles. Lumen walls of pharynx bulb sclerotized by five rows of placoids each row containing three of them (p_1-p_5). Each placoid subdivided by constriction into two halves, from both of which extends a small apodeme (apo). Pharyngeal bulb followed by short oesophagus (oe). Buccal channel continues through oesophagus and round mass of brain (br) that fills the space in the larval head (Fig. 8F).

Introvert (in, Fig. 9A, B) as anterior region of head, spherical, densely covered with four scald rows (Fig. 8F).

First row (cr_1 , Figs. 9A, B, 10B) with eight long and strong clavoscalids, being laterally flattened and divided into three segments; first segment with narrow base and as long as second segment; second segment broad and laterally flattened; third segment being small and spinose; ventral-most pair of clavoscalids slightly shorter and thinner.

Second row (sr_2 , Figs. 9A, B, 10B) with ten strong spinoscalids of nearly same length as clavoscalids and divided into two segments; first segment arising with broad base from introvert, tapering distally, lateral surfaces with longitudinal weal; second segment spine-like, as long as first one, with fine ventral serration consisting of three minute denticles near the tip.

Third row (sr_3 , Figs. 9A, B, 10B) with 15 strong spinoscalids of same structure as those of second row, but slightly shorter.

Fourth row (sr_4 , Figs. 9A, B, 10B) with 14 strong spinoscalids of same structure as those of second row, but clearly shorter.

Neck (nk, 9A, B, 10B) as posterior region of head not distinct from introvert, and carrying fifth and sixth row of scale-like scalds.

Fifth row (Figs. 9A, B, 10B) with 15 scalds of two different types arranged in two alternating subcircles. Upper subcircle with seven large type B scalds (sr_5b), consisting of two segments; first segment conical, with distal dorsal tooth; second segment rigid and spinous. Lower subcircle with eight type A scalds (sr_5a); consisting of protruding double leaf-like elements, each with reinforced inner margins. Dorsally, type A double elements shorter, more triangular, protruded, and hook-like.

Sixth row (Figs. 9A, B, 10B)) also contains 15 scalds of two different types arranged in two subcircles. Seven minute double spines (sr_6b) alternate with eight small single rectangular plates (sr_6a), which posteriorly are tricuspid. In summary the number and arrangement of scalds (cs = clavoscalids, sc = scale-like scalds, ss = spinoscalids) belonging to the head can be expressed in the following scald formula: $8cs, 10ss, 15ss, 14ss$ (in) / $7b+8a$ sc, $7b+8a$ sc (nk)

See also diagram in Fig. 10B as additional demonstration of the scald arrangement.

Collar (co, Figs. 9A, B) intermediate between neck and thorax; well defined and distinct anteriorly and posteriorly. Cuticle of collar with many longitudinal folds and subdivided by transversal constriction; with seven papillate flosculi located along constriction in large distinct pentagonal pits (2–3 µm deep) called therefore pit flosculi (fl₃).

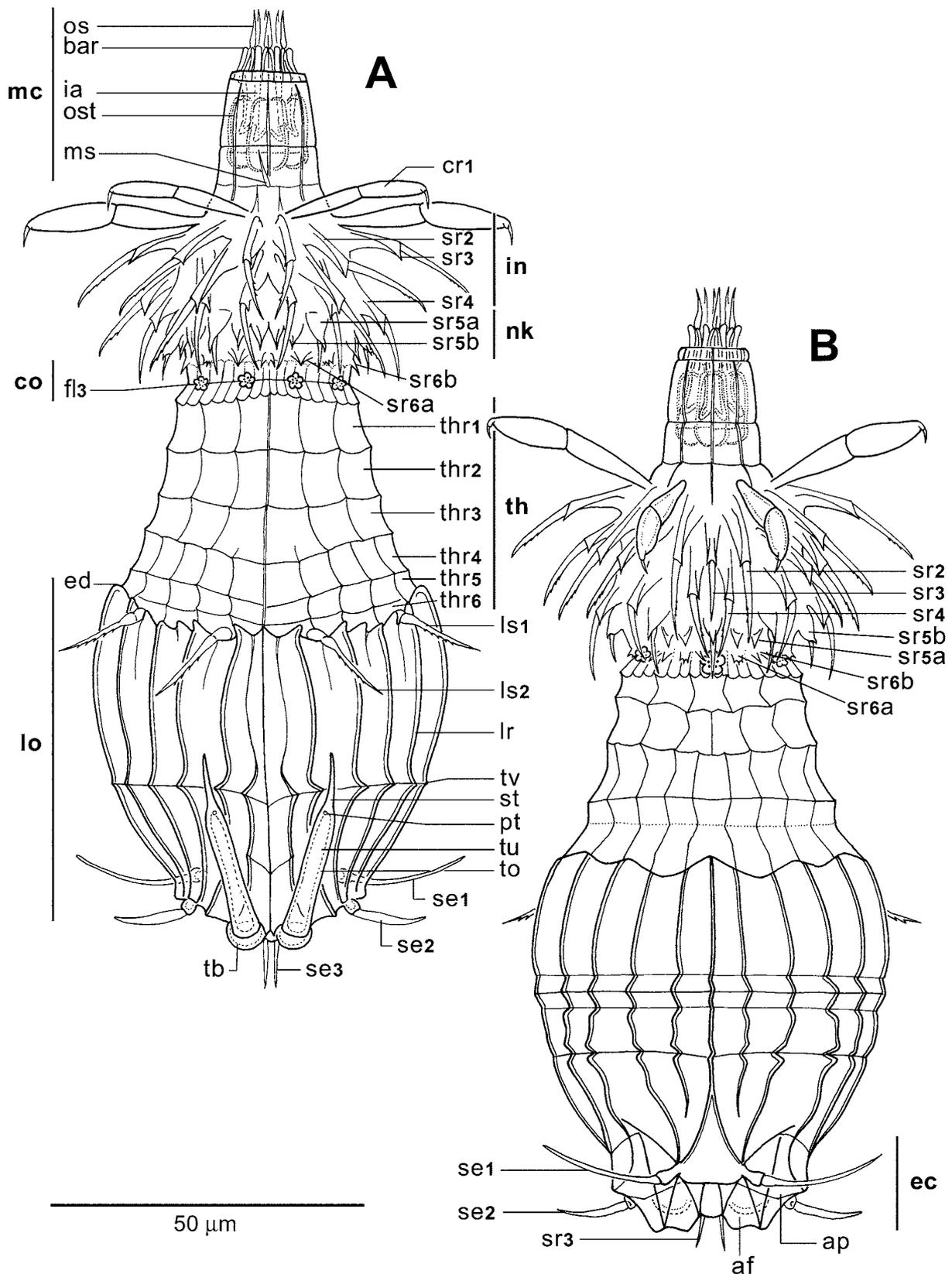


FIGURE 9. *Pliciloricus diva* sp. n., Higgins-larva, paratype II, habitus. A, ventral view; B, dorsal view.

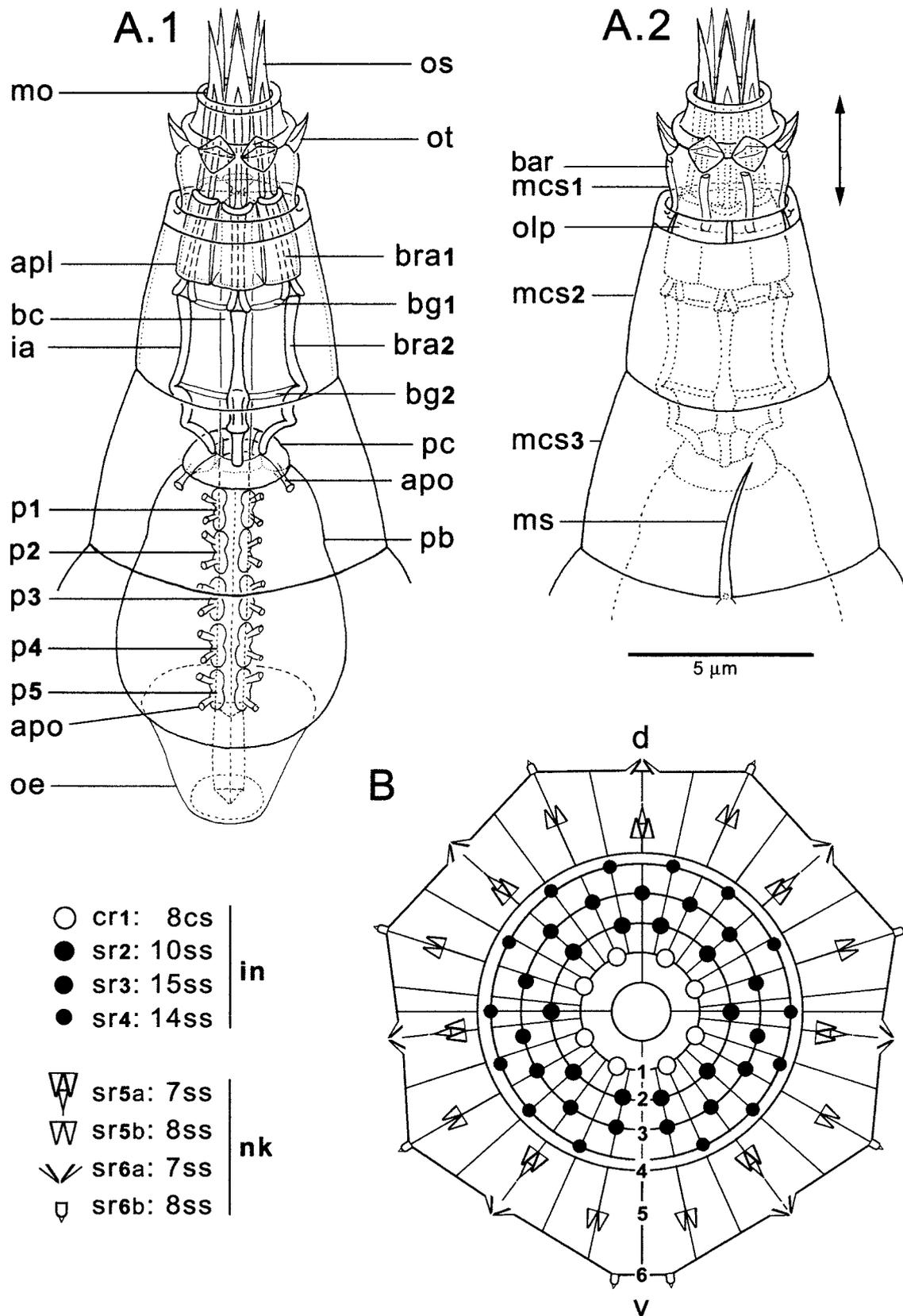


FIGURE 10. *Pliciloricus diva* sp. n., buccal channel of paratype Higgins-larva with buccal structures like prepharyngeal armature and pharyngeal bulb, ventral view. A.1, internal features; A.2, external features; B, diagram of scalid arrangement on the head (introvert and neck) of paratype Higgins-larva.

Thorax (th, Figs. 9A, B) consisting of thin and flexible cuticle divided into six ventral and five dorsal transversal rows of thoracic plates (thr_{1-6}), highest number of 19 thoracic plates in fifth row, all plates can transversely be folded once. Transition zone between thorax and loricate abdomen with two pairs of short setae; anterolateral setae (Is_1) and anteroventral setae (Is_2) have the same structure: filiform, arising from small swollen bases and with fine serrated inner margins.

Loricate abdomen (lo, Figs. 9A, B) urn-shaped, moderately armoured, with 22 primary longitudinal folds (lr) separating 22 plicae; ventrally secondary longitudinal folds present between plicae, not extending very far posteriorly. Lorica divided ventrally into two parts by a transverse constriction (tv) halfway. Toes (to, Fig. 9A) long (55% of lorica length), rigid, and extending pair-wise ventrally from caudal end; articulating with ball-and-socket-joints on moderately developed basal plates. Toes with enlarged bases (tb), followed by hollow tubes (tu) (60% of length of toes) and ending in solid end spines (st); tubes opening with a pore (pt) at the beginning of the end spine. Two pairs of large caudal glands with long ducts to tubular parts of toes occupy posterior most region of abdomen. Other caudal appendages include three pairs of posterior setae. Posterodorsal setae (se_1), modestly long, filiform, located dorsally at end of lorica; posterolateral setae (se_2) short, sickle-shaped and visibly swollen pod-like; posteroterminal setae (se_3) short, robust, spinous, standing close together between toes.

End cone (ec, Figs. 9A, B) distinct, consisting of two rows of anal plates (ap), forming terminal anal field (af); anal cone with anus situated terminally on anal field.

Observations concerning life history stages and life cycle of *P. diva* sp. n.

The following observations on life history stages and instars of *P. diva* sp. n. add new information to the understanding of similar life cycles of *Pliciloricus*-species also found in the Angola Basin. The hypothetical life cycle of *P. diva* include a series of instars of Higgins-larvae, two types of postlarvae (type I simplified, type II reduced), and next to bisexually males and females a simplified stage reproducing parthenogenetically (Fig. 11A). The life cycle, as figured out so far, probably has two phases (Fig. 11B PhI, PhII): (1) a bisexual phase with adults of both sexes finishing their metamorphosis enclosed in one (simplified) or other (reduced) type of postlarva and (Fig. 11B PhI); (2) a parthenogenetic phase with a simplified adult only (Fig. 11B PhII). The discovered bisexual adults shows no external sexual dimorphism (Fig. 11B PhI a, b). The males have a pair of testes, while the females have one matured ovary, which produces large single eggs, whereas the other ovary is not fully developed. From the egg, the first instar Higgins-larva hatches (Fig. 11B PhI c). The sixth or seventh larval instars are larger and have a greater volume than adults. The seventh or last instar Higgins-larvae (Fig. 11B PhI e) are without any sign of reduction: for example toes, setae, and scalids are of normal length. When this seventh instar Higgins-larvae still contains adults in metamorphosis the introvert is, in all observed cases, withdrawn. Then the collar closes the thorax and shows a spoked-wheel-like structure. The seventh instar Higgins-larva has two possibilities of further development: it can develop into a mature male or female or into a parthenogenetic simplified female. The bisexual adults can probably moult from either of the two different types of postlarvae (Fig. 11B PhI g, h), which both shows clear signs of reduction and are not free-living stages. The type II postlarva (Fig. 11B PhI h) is strongly reduced to not more than a thin cuticle (as found in most other known *Pliciloricus*-species, see section "discussion" below). The type I postlarva (Fig. 11B PhI g) has a simplified anterior body region but shows a well developed lorica with the same ornamentation as seen later in the adults. The parthenogenetic adult is probably also a non free-living stage because it has a simplified body with numerous reductions (Fig. 11B PhII i). The trunk is a simple sack containing the mature ovaries that produces the many large eggs stored inside. There is no introvert but the neck region is preserved bearing eight large hooks and a few additional tiny spines. The parthenogenetic simplified adult, moulting from a type II reduced postlarva, disintegrates more or less completely after all eggs have been formed and released from the mature ovary.

FIGURE 11. A, overview of life history stages of *Pliciloricus diva* sp. n., Ad = adult, Lar I – Lar VII = first to seventh instar Higgins-larva, Pla = type I and type II postlarva; B, schematic and hypothetical life cycle of *Pliciloricus diva* sp. n., PhI = bisexual phase, PhII = parthenogenetic (unisexual) phase; **a**, mature male, **b**, mature female, **c**, first instar Higgins-larva, **d**, sixth instar Higgins-larva, **e**, seventh instar Higgins-larva with retracted head and closed collar, **f**, type I simplified postlarva moulted from seventh instar Higgins-larva, male or female moulting either (?) from **g** type I simplified or from **h** type II reduced postlarvae, being during metamorphoses still enclosed in exuvium of seventh instar Higgins-larva, **i**, simplified parthenogenetic adult (female) seems to moult generally (?) from type II reduced postlarva, large eggs are produced parthenogenetically in the developing ovaries.

Discussion

Taxonomic comments and new observed structures

The holotypic and one paratypic male are the only adult specimen of the new species with an extended body found free in the sediment. Three other fully developed adults (one male and two females) all of which look like the specimen in Figs. 7c, 8e were found with a withdrawn head enclosed in a simplified postlarva, which in turn was enclosed in the seventh instar Higgins-larva. Contraction of the trunk depends on the medium in which the holotypic male specimen was stored. First the holotypic male was placed in distilled water in which the body especially the introvert was extended and covered by a loose cover slip for the descriptive drawing. Later when the same specimen was transferred to the glycerol and prepared to the glycerin-paraffin-slides in which it is stored now, the body was contracted again. Internally two strong retractor muscles extend from the caudal end of the lorica to the pharyngeal bulb in the introvert. Many circular muscles are clearly visible and the gut is empty and collapsed.

The genus *Pliciloricus* to date includes eleven described species. They are distinguished by features of either male or female, as only rarely are both sexes known (Higgins & Kristensen 1986; Kristensen & Shirayama 1988; Gad 2005c, e; Heiner & Kristensen 2005). These species exhibit a broad variety of features. Seven of the eleven species, including the type species *P. enigmaticus* and also *P. diva* sp. n., share many features indicative of a very close relationship. A differential diagnosis in form of a comparison of the distinguishing features of the adults is given in Table 3.

The adult of *Pliciloricus diva* sp. n. is characterized as a new species by the following features: (1) mouth cone with short and narrow buccal tube, and with four strong cuticular longitudinal bars in addition to the eight primary oral ridges in the first section; (2) leaf-like enlarged basal part of clavoscalids much broader than distal part and with a pattern of longitudinal striae, bars, and dots on both lateral surfaces, narrow distal part of clavoscalids with more than 22 protruding and narrow standing transverse cross walls; (3) a pair of ventral scalids in the second row being transformed into a strongly sclerotized double-organ consisting of four rami; (4) large leg-like, segmented and free spinoscalids of second row shorter than clavoscalids, first segment of spinoscalids angular and with four dorsal thorns and two fringes of minute spinules; (5) last segment of type B scalids in the fourth row basally with double row of five teeth and distally with double row of minute denticles and terminal claw-tip; (6) anterior lorica margin with small bicuspid protrusions and specific crescent-shaped ornamentation; (7) midventral plica with five bar-like transverse strengthened ridges, two short longitudinal bars posteriorly, one median longitudinal ridge and two small roundings posteriorly; (8) posterior part of lorica flexible, with two transversal rows of plates, (9) round or rosette structure on the end cone flanked by two short longitudinal protrusions.

TABLE 3. Comparison of adult characters of *P. diva* sp. n. with the most similar *Pliciloricus*-species.

Adult characters:	<i>P. diva</i> sp. n.	<i>P. enigmaticus</i> Higgins & Kristensen, 1986	<i>P. pedicularis</i> Gad, 2003	<i>P. corvus</i> Gad, 2003	<i>P. senicirrus</i> Gad, 2003	<i>P. hadalis</i> Kristensen & Shirayama, 1988
Mouth cone	8 oral ridges, 4 oral bars	8 oral ridges, 4 oral bars	8 oral ridges	8 oral ridges, 4 oral bars	8 oral ridges	8 oral ridges
Clavoscalids	unsegmented, leaf-like, flattened, with 22 cw	unsegmented, leaf-like, flattened, with 13 cw	unsegmented, leaf-like, flattened, with 26 cw	unsegmented, leaf-like, flattened, with 15 cw	unsegmented, leaf-like, flattened, with 32 striae	segmented (?), spinous, flattened, with 3 cw
Second row scalids	4 subdorsal thorns, 2 fringes of spinules	no subdorsal thorns, 2 fringes of spinules	2 subdorsal thorns, 3 fringes of spinules	no subdorsal thorn, 4 fringes of spinules	1 subdorsal thorn, fringes of spinules not observed	1 subdorsal thorn, fringes of spinules not observed
Second row with Double-organ (do₁)	<i>P. enigmaticus</i> -type	<i>P. enigmaticus</i> -type	<i>P. enigmaticus</i> -type	derived from <i>P. enigmaticus</i> -type	different type (?)	different type (?)
Third row with Double-organ (do₂)	(1 transformed scalid)	not observed	2 transformed scalids	2 transformed scalids	not observed	not observed
Type-b scalids of fourth row	claw-tipped, rows of subventral denticles	claw-tipped, rows of subventral denticles	claw-tipped, rows of subventral denticles	hook -tipped, smooth	claw-tipped, rows of subventral denticles	hook -tipped, smooth
Ninth row scalids	tricuspid	tricuspid	simple tooth-like	simple tooth-like	leaf-like	simple tooth-like
Lorica margin	crenate-shape ornament with protrusions	crenate-shape ornament	crenate-shape ornament with small plates above	crenate-shape ornament with protrusions	crenate-shape ornament with small plates above	crenate-shape ornament with small plates above
Midventral plicae	5 transversal ridges	6 transversal ridges	4 transversal ridges	6 transversal ridges	5 transversal ridges	not observed
Full transversal rid-ges dividing lorica	less distinct (number?)	well distinct, 7 ridges	absent	well distinct, 11 ridges	absent	absent
Sensory wards	2 subventral	4 subventral	2 (2) subventral	4 subventral	2 subventral	not observed
End cone	well distinct, with round structure and longitudinal protrusions	well distinct, with round structure	less distinct, with round structure	well distinct, with round structure and longitudinal protrusions	less distinct, with round structure	absent
Additional unique features	lorica and end cone with two rows of plates	clavoscalids with asymmetrical tips	trichoscalids with cross walls	transformed double-organ	type-b scalids of fourth row subventrally winged	type-b scalids of fourth row basally swollen
Postlarva	enlarged basal element of clavoscalids laterally with dots, striae, bars partly reduced	tips of second row scalids with subventral denticles reduced	midventral plicae with additional longitudinal ridges reduced	additional sensory spots on the lorica reduced	end cone with 20 papillate flosculi reduced	just 2 rows of basal plates on interthorax, with special structure not found

The (primary) double-organ of *P. diva* sp. n. is most similar to that of *P. enigmaticus*. Both inner or ventral rami seem to represent former free spinoscalid, because they still show features found in the untransformed free spinoscalids e.g. fringes of spines, dorsal thorns, and rows of spinules. The outer or dorsal rami of the double-organ as present in *P. diva* sp. n. seems to have no equivalents in the free spinoscalids of the second row. The same is true for the double-organ of *P. corvus* which, however, belongs to a different type of double-organ (Gad 2005c). A possible explanation for the presence of the outer rami may be additional spinoscalids in the second row as found in *Pliciloricus shukeri* Heiner & Kristensen, 2005 from the Faroe Bank (Heiner & Kristensen 2005) and the Angola Basin (Gad unpubl.). Adult Pliciloricidae generally have a maximum of nine spinoscalids in the second row (Higgins & Kristensen 1986) but the adults from the Angola Basin seem to have four or six additional spinoscalids and a double-organ lacking outer rami. The additional spinoscalids differ from the nine large, leg-like spinoscalids generally found in the second row (Higgins & Kristensen 1986). They are shorter than the other free spinoscalids of the second row but not as filiform as the spinoscalids of the third row. This indicates that the number of nine spinoscalids found on the second row of scalids of published *Pliciloricus*- and *Rugiloricus*-species (Higgins & Kristensen 1986; Kristensen & Shirayama 1988; Gad 2005a, c) does not seem to be the highest possible number of scalids in this row. Perhaps there are more basic Pliciloricidae in which the second row, if fully developed, may consist of 15 spinoscalids as a maximum number, as in the case in the third row of spinoscalids of known Pliciloricidae (Higgins & Kristensen 1986; Kristensen & Shirayama 1988; Gad 2005a, c; Heiner & Kristensen 2005). Such additional free spinoscalids in the second row could be an explanation for the outer branch of the double-organs of *P. diva* sp. n. and *P. corvus*. Two of these additional spinoscalids could have become part of the double-organ, so that it would consist of four transformed spinoscalids, each ramus representing a former free spinoscalid.

Another assumed transformation is observed in the third row of scalids where a small spinoscalid is located in the midventral position (ssm, Fig. 1). In *P. corvus* a secondary double-organ consisting of two transformed hairy spinoscalids is present in this location (Gad 2005c). The scalid arrangement on the head as depicted in Figs. 2B, C demonstrates that the number and arrangement of scalids and basal plates are nearly identical in *P. diva* sp. n. and *P. corvus*. They agree in having a third row with 15 spinoscalids but the position of these scalids is reversed so that there is a difference of half a position between *P. diva* sp. n. (Fig. 2B) and *P. corvus* (Fig. 2C). As a result *P. diva* sp. n. has one scalid on the midventral axis, whereas *P. corvus* has two scalids on both sides of this axis.

A simplified adult as a parthenogenetic stage is, apart from *P. diva* sp. n., known from *P. pedicularis* and *P. corvus* (Gad 2005a, c). These simplified adults differ mainly in the structure of hooks, scalids and pads on the persisting neck region (Gad 2005a, c) see also Table 3.

A differential diagnosis for the Higgins-larvae is given in form of a comparison of the distinguishing features in Table 4. The Higgins-larva of the new species is separated from other species by the following features: (1) short clavoscalids with broad second segment; (2) collar with seven large pit-flosculi (see below); (3) posterolateral setae short but massive and pod-like; (4) toes moderately long, length relation of tubular part and end spine 60 : 40.

The scalid arrangement of the Higgins-larva of *P. diva* sp. n. can be expressed in the following scalid formula: 8cs, 10ss, 15ss, 14ss (in) / 7b+8a sc, 7b+8a sc (nk), which is identical for *P. pedicularis*, and *P. corvus* (Gad 2005a, c). The Higgins-larvae of *P. senicirrus* have only eight instead of 14 scalids in the fourth row. This is expressed in the formula: 8cs, 10ss, 15ss, 8ss (in) / 7b+8a sc, 7b+8a sc (nk) (Gad 2005c, e). Except for the Higgins-larva of *P. pedicularis* which has clavoscalids divided into four segments instead of three and which is equipped with an additional dorsal row of small spinules, the scalid structure is very uniform in *P. gracilis*, *P. corvus*, and *P. senicirrus*.

TABLE 4. Comparison of larval characters of *P. diva* sp. n. with the most similar *Pliciloricus*-species.

Larval characters:	<i>P. diva</i> sp. n.	<i>P. enigmaticus</i> Higgins & Kristensen, 1986	<i>P. pedicularis</i> Gad, 2003	<i>P. corvus</i> Gad, 2003	<i>P. senicirrus</i> Gad, 2003	<i>P. hadalis</i> Kristensen & Shirayama, 1988
Mouth cone	8 bars, 8 teeth, 8 stylets, and midventral setae	8 bars, 8 teeth, 8 stylets, and midventral setae	8 bars, 8 teeth, 8 stylets, and midventral setae	8 bars, 8 teeth, 8 stylets, and midventral setae	8 bars, 8 teeth, 8 stylets, and midventral setae	8 bars, 8 stylets
Clavoscalids	3 segments, smooth first and second segment 50:50	3 segments, smooth first and second segment 50:50	4 segments, subdorsal row of spinules first and second segment 55:45	3 segments, smooth first and second segment 50:50	3 segments, smooth first and second segment 65:35	3 segments, smooth first and second segment 60:40
Collar	7 pit flosculi	7 papillate flosculi	flosculi not observed	7 papillae	flosculi not observed	flosculi not observed
Anterior setae	very short with row of 5-6 short spinules	very short with row of 5-6 short spinules	very short with row of 5-6 short spinules	very short with row of 5-6 short spinules	long with row of 12-14 long spinules	short branched without spinules
Toes	not longer than lorica, tubular part long, end spine short	not longer than lorica, tubular part long, end spine short, with serrated margin	longer than lorica, tubular part very long, end spine short	not longer than lorica, tubular part long, end spine short	not longer than lorica, tubular part and end spine equal in length	longer than lorica, tubular part short, end spine very long
Posterior setae	all three pairs short, posterolateral setae pod-like posteroterminal setae shortest	all three pairs short, posterolateral setae filiform posteroterminal setae shortest	all three pairs short, posterolateral setae filiform with pedicels posteroterminal setae shortest	all three pairs short, posterolateral setae filiform posteroterminal setae shortest	all three pairs very long posteroterminal setae longest	all three pairs short, posterolateral setae filiform posteroterminal setae shortest

The external and internal buccal armatures of the Higgins-larvae of all four species compared (*P. diva* sp. n., *P. gracilis*, *P. pedicularis*, and *P. corvus*) are very similar. Only excellently fixed material, as in the case of *P. diva* sp. n., allows investigation of all details of their complex structures with LM. The only remarkable difference is that the oral stylets of *P. diva* sp. n. are longest compared with the Higgins-larvae of the other species and that the placoids of the pharyngeal bulb are clearly paired and equipped with apodemes. Newly observed structures in *P. diva* sp. n. are the oral loops (olp) at the anterior end of the second section of the mouth cone. The small double apodemes of the placoids and these oral loops are difficult to see in the other investigated larvae so that it remains unclear whether they are lacking or not clearly visible.

On the collar of the Higgins-larvae of some *Pliciloricus*-species seven flosculi composed of papillae surrounding a large pore (or just large pores, or just large papillae) have been discovered; e.g. in *P. gracilis*, *P. senicirrus*, and *P. corvus* (Higgins & Kristensen 1986; Gad 2005a, c). The Higgins-larvae of *P. diva* sp. n. also have seven flosculi on their collar, but these flosculi represent a new type. They have a structure known from papillate flosculi (Kristensen 1991b; Gad 2005a, c), but they are of bigger size and located in pentamerous pits formed by the body cuticle. To distinguish this kind of flosculi from papillate flosculi (fl₁) found in the most Loricifera (Higgins & Kristensen 1986; Kristensen 1991b; Gad 2005a, c) and collar flosculi (fl₂) known so far exclusively from *P. corvus* (Gad 2005c) they are named here, pit-flosculi (fl₃).

Life cycles and the occurrence of postlarvae in *Pliciloricus*-species

It is an advantage for studying loriciferans that one stage or instar of the life cycle contains the following one or ones. As an example, the exuvium of the seventh larval instar may contain the exuvium of the postlarva and the adult that has moulted from it. This is the case in *P. diva* sp. n. (Figs. 7A–C, 8A–E). Furthermore, loriciferans are so transparent that often all features of the enclosed stage/s can clearly be seen with the microscope. These advantages help enormously in identifying the different life stages, in assigning them to the different species, and in making plausible reconstructions of the life cycles without observing live Loricifera. Key characters to determine whether the extended Higgins-larva (Figs. 9A, B) and the retracted larva containing postlarva and adult belong to the same species are the specific structure and length relation of the loricate abdomen, the posterior and anterior setae (see arrow in Fig. 7A), and the toes. Key characters to determine whether the free adult and the retracted ones enclosed in the seventh instar larva belong to the same species are the specific structure of lorica, end cone, trichoscalids, (primary) double-organ, and tips of the type B spinoscalids of the fourth spinoscalid row (Fig. 2A.4).

Kristensen and Brooke (2002) concluded that in some species of Pliciloricidae a postlarva is absent. Especially species of the genus *Pliciloricus* were said to develop from Higgins-larvae directly to adults. Observations made on *P. corvus* and *P. senicirrus* from the Great Meteor Seamount (Gad 2004a, 2005c) and on *P. pedicularis* from the Angola Basin (Gad 2005a) indicate that postlarvae are always present, although often only in the form of a simple exuvium surrounding adults after metamorphosis. This is also the case in known species of the genus *Rugiloricus*. A delicate cuticle layer surrounds the adults of *Rugiloricus carolinensis* Higgins & Kristensen, 1986 (Gad, unpubl.) and can also be seen in the drawings accompanying the description of *Rugiloricus ornatus* Higgins & Kristensen, 1986 (Higgins & Kristensen 1986).

The study of *P. diva* sp. n. has clearly shown that there is a postlarva in its life cycle, even though this is in a simplified or reduced form. This observation is regarded as evidence that all simple cuticular layers surrounding adults in other *Pliciloricus*-species are remnants of the postlarval stage. Such cuticular layers have been observed in *P. enigmaticus*, *P. pedicularis*, *P. senicirrus*, *P. corvus* and *P. leocaudatus* (see Higgins & Kristensen 1989; Gad 2005a, c; Heiner & Kristensen 2005). In this regard *P. diva* sp. n. is pivotal for the understanding of the process of reduction of the postlarval stage. Doubts remains whether there are any Loricifera at all which develop directly from larvae to adults by totally skipping-out the postlarval stage.

What could be a not fully-developed postlarva was first mentioned (Higgins & Kristensen 1986) for *Rugiloricus cauliculus* Higgins & Kristensen, 1986. Many more of these particular life history stages have in the meantime been found in samples from the plateau of the Great Meteor Seamount (Gad 2004a) or from

sediment of the Faroe Bank (Heiner & Kristensen 2005; Heiner 2008). There is no doubt that these stages are postlarvae and characteristic for species being similar to *R. cauliculus* (see Gad 2004a). These postlarvae represent a special type because they are not just simplified by showing an “unfinished adult morphology” (Higgins & Kristensen 1986). Although the scalids of the introvert are present as protoscalids, the neck region is more developed and characterized than others by eight large hooks. These hooks are believed to have their equivalents in the eight type A basal plates of the first row found on the neck of adults moulting from these postlarvae (Gad 2004a, 2005a). In this type of postlarva buccal structures are nearly totally lacking. The postlarval trunk is armoured with a thick cuticle without forming a lorica as observed in the postlarva of *P. diva* sp. n. Instead the cuticle is divided into transverse plate rows. Considering these morphological features (Kristensen & Brooke 2002; Kristensen 2003; Gad 2004a) it can be concluded that this *Rugiloricus*-postlarva is a life history stage which does not feed or move freely in the sediment. Additional features like the extra thick cuticle, the eight large hooks probably responsible for anchoring and the fact that all postlarvae found so far contain mature adults having moulted from them, indicate that this type of postlarva may be a dormant stage (Gad 2004a).

In contrast to the *Rugiloricus*-postlarva, the postlarva of *P. diva* sp. n. has an abdomen already armoured with the species-specific lorica, which is identical with the one of the adults, but the anterior region is almost free of structures. Only very tiny protoscalids indicate that other body regions like the introvert are present as protoformations. These protoscalids are even smaller than those of the *Rugiloricus*-postlarvae mentioned above and hardly recognizable. Postlarvae of *Pliciloricus*-species have so far never been found free in the sediment as have several *Rugiloricus*-postlarvae from the plateau of Great Meteor Seamount (Gad 2004a). That is why the type I simplified postlarva of *P. diva* sp. n. is assumed to be a non free-living instar, which is generally enclosed in the exuvium of the seventh instar Higgins-larva. The only Loricifera with a free-living and feeding postlarvae known so far are found in species of Nanaloricidae, in which the postlarva is a juvenile stage between the Higgins-larva and the adult (Kristensen 1991a, 2003; Kristensen & Gad 2004; Gad 2004b; Kristensen et. al. 2007; Heiner & Neuhaus 2008).

To sum up, it can be concluded that a basic life cycle in Loricifera includes a series of Higgins-larvae, a postlarva and bisexual adults (Kristensen & Brooke 2002; Kristensen 2003; Gad 2005a, b; Heiner 2008). All other life cycles which include other life history stages, seem to be derived from this basic life cycle which is similar to that of Priapulida, where larval, postlarval and adult stages have been known already for a long time (van de Land 1970; Kirsteuer 1976; Adrianov & Malakhov 1996). According to present knowledge Priapulida are the most likely the sister group of Loricifera (Lemburg 1999).

The observed tendency in the life cycle of many *Pliciloricus*-species (Gad 2005c) including the type II postlarva of *P. diva* sp. n. to reduce the postlarva may be a sign of fast metamorphosis. The opposite would be cases in which the postlarva is transformed into a dormant stage to postpone metamorphosis, as in the type I postlarva of *P. diva* sp. n. However, it remains unclear whether two types of postlarva really belong to the life cycle of a single species, as seems to be the case in *P. diva* sp. n. Perhaps the life cycle of this species is less derived than that of the other known *Pliciloricus*-species which have already reduced the postlarva and even started to partly reduce the last or seventh larval instar (Gad 2005c). Another possibility is that the type I postlarva as found in *P. diva* sp. n. is also a part of the life cycles of other species but has not been found so far. One could also speculate that the two types of postlarvae have evolved in response to changes of environmental conditions: optimal conditions result in faster development with a type II postlarva, suboptimal conditions result in dormancy with a type I postlarva.

It seems that in Pliciloricidae the postlarva is no longer used for postembryonic growth, as in Nanaloricidae and in the closely related Priapulida (Lemburg 1999), because the seventh instars of Higgins-larvae are sometimes much larger than the adults (Gad 2005b). They seem therefore, to be free to evolve along two directions: either reduction or new function. The new function could be to serve as a dormant stage.

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