

# ON *AFROCYPRIS BARNARDI* G. O. SARS, 1924 (OSTRACODA), A SECOND GIANT OSTRACODE WITH ADDITIONAL APPENDAGES

BY

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## ABSTRACT

*Afrocypris barnardi* G. O. Sars, 1924, a giant temporary pool ostracode from South Africa, is here redescribed, based on new material. It is only the second time that this species has been found. The present morphological analyses show that the copulatory appendages of both sexes have unusual characteristics. In the female, at least three paired appendage-like structures (R-appendages) are associated with the Female Reproductive Organ (FRO). This is the second time that such structures are described from non-marine ostracodes, *Liocypris grandis* (G. O. Sars, 1924) having six pairs of such structures. The male copulatory organs have a Penile Attachment uniting the proximal sides of both hemipenes, and in *Afrocypris barnardi* an asymmetrical, finger-like structure is associated with this PA.

The discovery of female R-appendages in a second species strengthens the interpretation of these structures as (part of) true appendages, and reinforces the hypothesis that the FRO is homologous to more than one ancestral somite. However, it remains unclear how many body segments are involved in the origin of the FRO, and three, five, or even six somites are all possible. In addition, these R-appendages can be ancestral, atavistic, or even apomorphic. The position of the two taxa with such appendages in a phylogeny of the Cyprididae as a whole will constitute a test to discriminate between these three possibilities.

## ZUSAMMENFASSUNG

Basierend auf neuen Funden wird *Afrocypris barnardi* G. O. Sars, 1924, eine Groß-Ostracodenart aus ephemeren Kleingewässern Südafrikas, hier wiederbeschrieben. Die Funde stellen erst den zweiten Nachweis dieser Art dar. Die neue morphologische Untersuchung zeigt, daß die Kopulationsorgane beider Geschlechter ungewöhnliche Merkmale aufweisen. Dem externen weiblichen Kopulationsapparat (FRO) sind mindestens drei paarige, extremitätenartige Anhänge (R-Appendages)

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zugeordnet. Erst zum zweiten Mal werden solche Strukturen für eine Süßwassertostracodenart nachgewiesen: *Liocypris grandis* (G. O. Sars, 1924) besitzt sechs solcher Anhänge.

Das Kopulationsorgan des Männchens besitzt einen Anhang (PA), welcher die proximalen Bereiche der beiden Hemipenes verbindet. Auf diesem Anhang inseriert bei *Afrocypris barnardi* zudem eine asymmetrische fingerförmige Struktur.

Die Entdeckung von zusätzlichen extremitätenartigen Anhängen des weiblichen Kopulationsapparates in einer zweiten Ostracodenart verstärkt ihre Interpretation als (Teile von) echte(n) Extremitäten und unterstützt die Hypothese, daß der weibliche Kopulationsapparat mehr als einem ursprünglichen Segment homolog ist. Allerdings bleibt unklar, wieviele Körpersegmente in die Bildung des Kopulationsapparates involviert sind; drei, fünf oder sogar sechs Segmente wären denkbar. Zudem können diese zusätzlichen Anhänge sowohl ancestrale, als auch atavistische oder apomorphe Merkmale darstellen. Erst die Positionierung der beiden Taxa mit solchen Anhängen in einer Phylogenie der Cyprididae wird eine Entscheidung ermöglichen.

## INTRODUCTION

Ostracodes are unusually conservative with regard to the number and form of their appendages. Whereas valve size, structure, and shape can vary widely amongst even closely related groups, most (all?) podocopid ostracodes have 7 pairs of appendages (A1, A2, Md, Mx1, and three pairs of thoracopods), a pair of copulatory organs, and a pair of caudal structures, previously named furcae, now referred to as caudal rami. The giant ostracode, *Liocypris grandis* (G. O. Sars, 1924), described by Sars (1924) from South Africa, was redescribed by Martens (2003). Surprisingly, five pairs of additional (putative) appendages associated with the Female Reproductive Organ (FRO) were found. Because of their apparent association with reproductive functions (as they are absent in males, as well as in juvenile females: they all appear during the last moult), these structures were named R1 to R5, from anterior to posterior. Apart from the most caudally inserted R5, which resembles an Anlage of a thoracopod, the structures have a morphology unlike anything thus far described from podocopid ostracodes. This discovery provoked the erection of a new subfamily for this unusual species and genus, the Liocypridinae Martens, 2003. Moreover, Matzke-Karasz & Martens (2005) showed that all of these additional structures indeed are directly connected to the FRO, and this indicates that reproductive organs in ostracodes, or at least in Cyprididae, may be homologous to several post-cephalic somites. Tsukagoshi & Parker (2000) had previously postulated that both male and female copulatory organs would have been derived from a single somite, albeit a different one in both sexes. The discovery of the additional appendages (Martens, 2003) and the demonstrated homology with the FRO (Matzke-Karasz & Martens, 2005) provided interesting counter-evidence to this single-somite homology for both FRO and hemipenis. However, as the structures R1-R5 were thus far found in a single species only, the basis to reject the said hypothesis remained slim.

Here, we describe and analyse similar (though not identical) additional structures in a second giant ostracode genus from South African temporary pools, *Afrocypris barnardi* G. O. Sars, 1924. Because of the presence of these structures (putatively homologized to R0, R1, and R5), the monospecific genus *Afrocypris* is also transferred to the Liocypridinae, although a number of structural differences with *Liocypris* are discussed. While at the same time redescribing the full morphology of both sexes, a basal (asymmetrical) structure at the fusion of both hemipenes in males was noted. A potential connection, either in homology or in function, of male and female additional structures is unclear at present.

Abbreviations used in text and figures. A1, antennula; A2, antenna; Cp, carapace; CR, caudal ramus; dls, dorsolateral shield of hemipenis; H, height of valves; L, length of valves; LV, left valve; Md, mandibula; ms, medial shield of hemipenis; Mxl, maxillula; R, Rome Organ; R0, R1, and R5, additional appendages in adult females; RV, right valve; T1, first thoracopod; T2, second thoracopod; T3, third thoracopod; vls, ventrolateral shield of hemipenis. The chaetotaxy of the limbs follows the model proposed by Broodbakker & Danielopol (1982), revised for the A2 by Martens (1987). The higher taxonomy of the Ostracoda follows the synopsis by Horne et al. (2002).

#### MATERIAL AND METHODS

Investigations were carried out on five females and four males of *Afrocypris barnardi*, preserved in 70% ethanol. The material is deposited in the State Museum Windhoek, Coll. Nr. SMN 51306, collected March 1988 by B. Curtis in Makuri, Bushman Land. Light microscopy was carried out with a Leica MZ Apo microscope; for SEM, we used a BalTec CPD 030 Critical Point Drying System and a Leitz AMR 1200, a LEO 1430VP, and a Philips XL30 scanning electron microscope. From one valve, a series of pictures has been photographed with a Canon 350 D camera and a macroscope MZM 1 (ASKANIA Mikroskop Technik Rathenow), which subsequently has been mounted electronically to yield a deep-focus picture with the software AUTOMONTAGE (Synoptics Group, U.K.).

#### RESULTS: TAXONOMIC DESCRIPTIONS

Class OSTRACODA Latreille, 1806  
Subclass PODOCOPA G. W. Müller, 1894  
Order PODOCOPIDA G. O. Sars, 1866  
Suborder CYPRIDOCOPINA Baird, 1845  
Superfamily CYPRIDOIDEA Baird, 1845  
Family CYPRIDIDAE Baird, 1845

### **Liocypridinae** Martens, 2003

Diagnosis [modified after Martens, 2003]. — Giant Cyprididae ( $L \geq 5$  mm), with elongated or arched, laterally compressed carapaces, wide anterior calcified inner lamella. Non-reproductive appendages typical of the family. A1 without flagellated claws. Müller-organ present between A1 and A2. A2 in males with both claws G3 and G1 short and claw-like. Mx1 with smooth Zahnborsten on third endite, terminal segment of palp sub-quadrate or rectangular. Md with beta-seta stout and hirsute, gamma-seta stout and hirsute in the terminal half. T1-palp in females long and stout, three terminal setae relatively short; 'c' seta absent; male prehensile palps asymmetrical, right palp larger than left palp. T2 a walking leg, with penultimate segment divided. T3 a slender cleaning leg, apically with a pincer. Caudal rami symmetrical, slender, attachment without Triebel's loop. Adult females with a series of additional, paired appendages associated with the genital operculum. Hemipenis large, lateral shield consisting of 2-3 lobes.

Remarks. — The subfamily was erected by Martens (2003) to include the type genus, *Liocypris*. Especially the presence of the additional appendages associated with the female reproductive organ in the type species (and thus far single species, *Liocypris grandis*) lead to the exclusion of this genus and species from any existing subfamily in the Cyprididae. The monospecific genus *Afrocypris* is now added to the Liocypridinae, as also females of the species, *A. barnardi*, appear to have some additional appendages associated with the female reproductive organ.

### **Afrocypris** G. O. Sars, 1924

Type species. — *Afrocypris barnardi* G. O. Sars, 1924 (by original designation and monotypy).

Diagnosis. — Giant species and genus, with elongated valves; RV with selvage anteriorly widely inwardly displaced, posteriorly submarginal. LV without selvage but with inner list, ventral margin slightly sinuous. A1 with third segment unusually elongated, c. 3 times as long as wide. Mx1 with second palp-segment subquadrate, 3<sup>rd</sup> endite with 2 smooth Zahnborsten and some further claw-like setae, respiratory plate with 5 reflexed setae. T1 in female with unusually long endopodite, distally with 3 short setae and in some cases with an additional, lateral seta. T2 elongated, penultimate segment divided, seta d1 longer than d2. T3 with a distal pincer. Attachment of caudal ramus with additional, lateral branch, the latter connecting to the female copulatory organ. Female copulatory organs associated with a number of additional appendages, in attempted homology to those of *Liocypris* named R0, R1, and R5. Hemipenis stout, with large shield of the peniferum and with well-developed sclerified inner structures; at fusion of both hemipenes, an

additional, basal structure carrying an asymmetrical, pointed finger is present. Prehensile palps asymmetrical, both segments of right prehensile palp larger than in left palp, this right palp also with two small setae at joint between both segments.

***Afrocypris barnardi* G. O. Sars, 1924**

(figs. 1-7, 9)

Type locality. — Okualuthi and Tamansu in Ovamboland.

Type material. — Natural History Museum, Oslo; South African Museum, Cape Town.

Other material used. — SMN 51306, c. 15 males, females, and juveniles, collected March 1988 by Barbara Curtis in Makuri, Bushman Land.

Diagnosis. — As for the genus.

Redescription of female. — Carapace elongate in lateral view, with broadly rounded anterior and bluntly rounded posterior margin. Dorsal margin straight over about 2/3 of its length, slightly sloping towards the caudal side. Carapace in dorsal view narrow, greatest width approximately situated in the middle, no anterior or posterior overlap, but RV extending beyond LV anteriorly. LV overlapping RV ventrally. External valve surface smooth. Pore canals numerous and branched, about twice as long along the anterior margin than along the posterior margin.

RV (fig. 1B) with anterior selvage inwardly displaced over a considerable distance (c. 1/3 of the distance between valve margin and inner margin). Posterior selvage distinct from valve margin, but submarginal. No inner or outer lists. Muscle scars (fig. 1G) a paw print, Md scars elongate, central muscle scars in 3 rows, from posterodorsal to anteroventral: 2-3-2, relatively widely spaced. Anterior margin widely rounded, with a long and straight antero-dorsal edge, leading to a clear antero-dorsal angle. From here, the dorsal margin leads straight but obliquely to a much lower postero-dorsal angle. The posterior margin forms a much narrower curve than the anterior margin. Ventral margin almost straight.

LV (fig. 1A) without selvage but with inner list, strongly developed along ventral margin, and along the ventral parts of anterior and posterior margins. Ventral margin slightly sinuous. Valve outline as in RV.

A1 (fig. 2A) 7-segmented, first segment with one small dorsal and two longer ventral setae. Second segment with small, medio-ventral Rome Organ, and a very small dorso-apical seta. Third segment unusually elongated ( $L = c. 3 \times \text{width}$ ), with one longer dorso-apical and one shorter ventro-apical seta. Fourth segment about 1.5 times as long as wide, ventro-apically with one very short and one short seta, dorso-apically with 2 long natatory setae. Fifth segment subquadrate, with 4 long natatory setae. Sixth segment with 3 long natatory setae, and one smaller (dorso-apical) seta. Terminal segment small, with two long natatory setae, one long aesthetasc Ya and one seta of intermediate length (almost as long as aesthetasc).

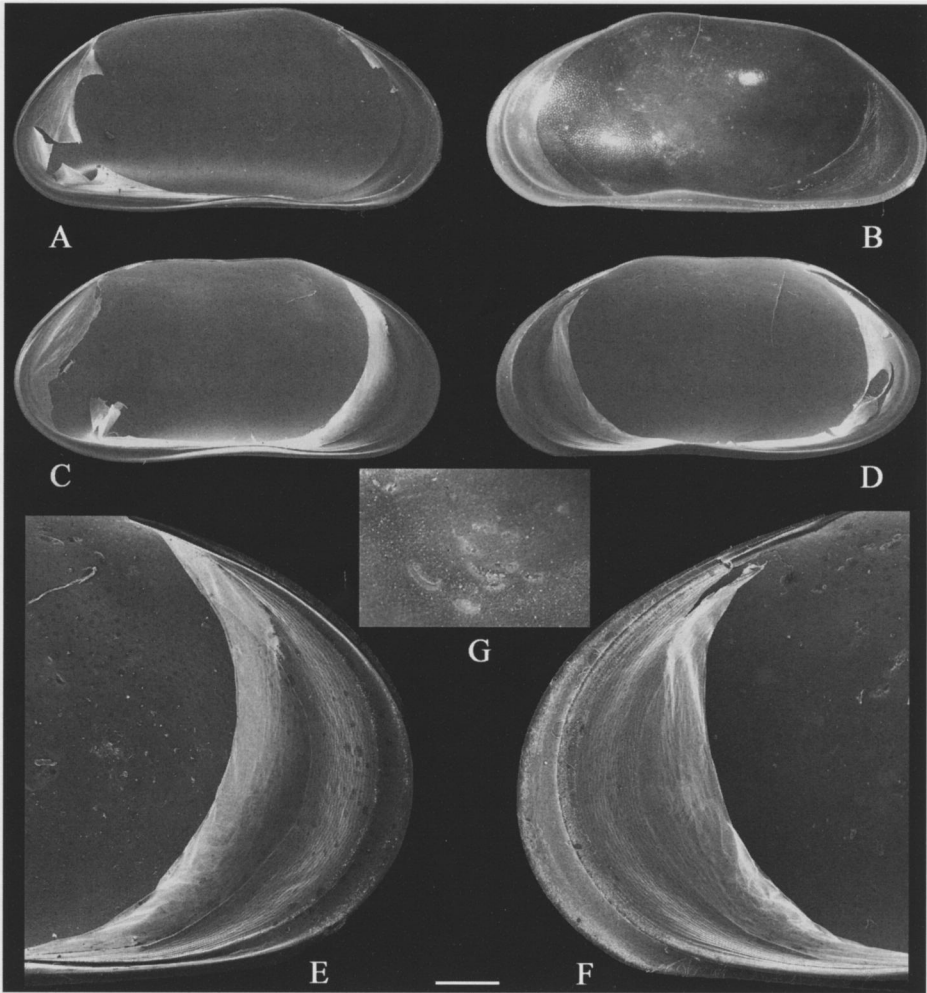


Fig. 1. *Afrocypris barnardi* G. O. Sars, 1924: valves, internal view. A, C-F, SEM images; B, G, light microscopic images. A, female LV; B, female RV; C, male LV; D, male RV; E, detail of C; F, detail of D; G, detail of B. Scale = 1000  $\mu\text{m}$  for A-D; 444  $\mu\text{m}$  for G; 370  $\mu\text{m}$  for E, F.

A2 (fig. 3A, C, D) with aesthetasc Y very small, c. 1/6 of length of ventral side of first endopodal segment, natatory setae long, reaching beyond the middle of the end claws and with 4 t-setae. Chaetotaxy of last two segments as typical of female Cyprididae, i.e., three z-setae, claws G1 and GM the longest, G3 only slightly shorter, Gm about 2/3 of length of GM, and G2 short, less than half the length of G1. Aesthetasc y3 slightly shorter than accompanying seta, seta 'g' shorter than claw Gm.

Md-palp (fig. 2G, K) on first segment with seta s1 broader than seta s2, alpha-seta shorter than half the length of s2, 4th seta smooth, and longer than the s-setae;

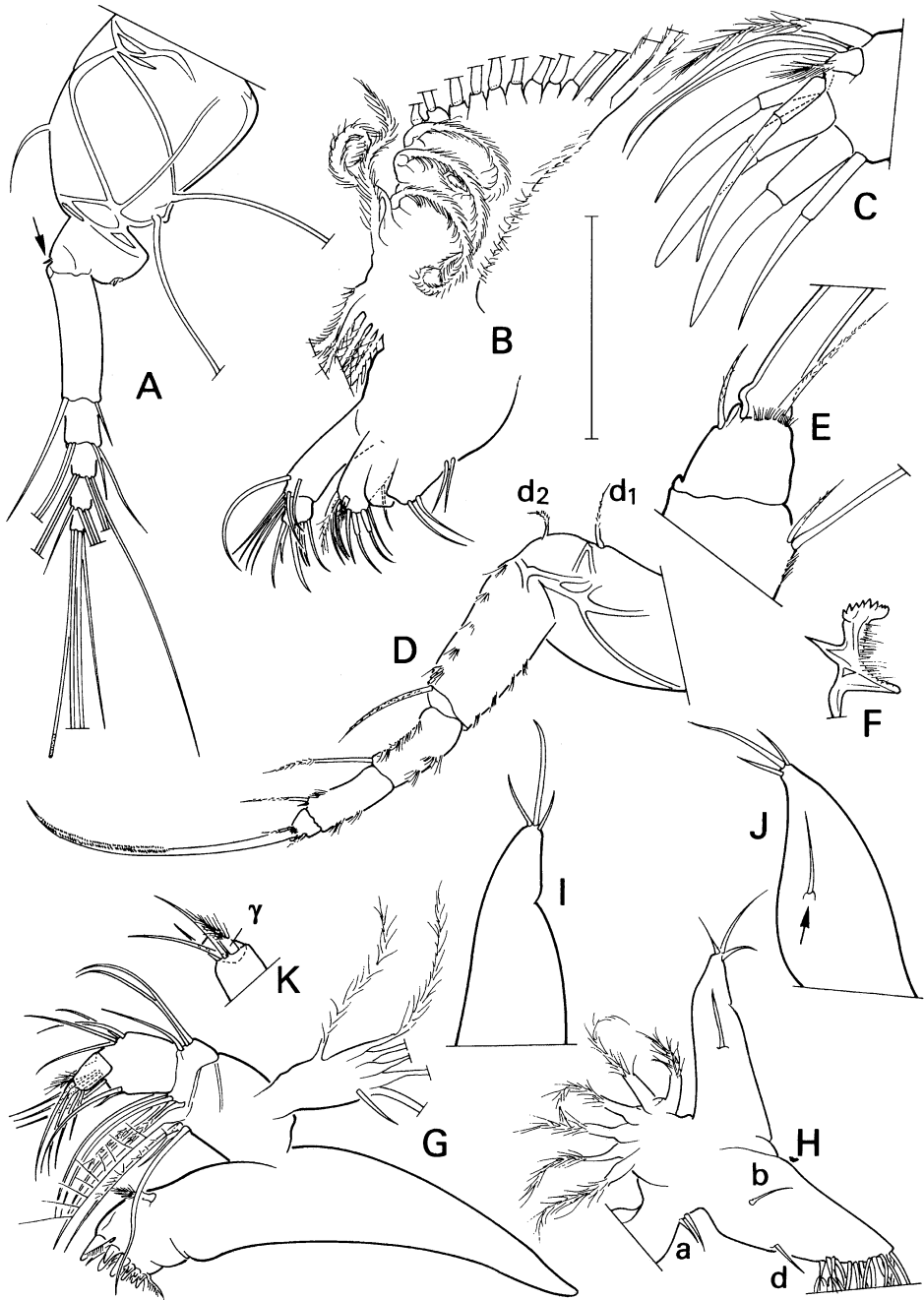


Fig. 2. *Afrocypris barnardi* G. O. Sars, 1924, female: A, antennula; B, maxillula; C, detail of 3<sup>rd</sup> endite of maxillula; D, T2; E, distal segment of T2; F, rake-like organ; G, mandibula; H, T1; I, palp of left T1; J, palp of right T1; K, detail of penultimate segment of mandibular palp. Scale = 250  $\mu\text{m}$  for J, K; 167  $\mu\text{m}$  for A, B, D, F, G, H, I; 42  $\mu\text{m}$  for C, E.

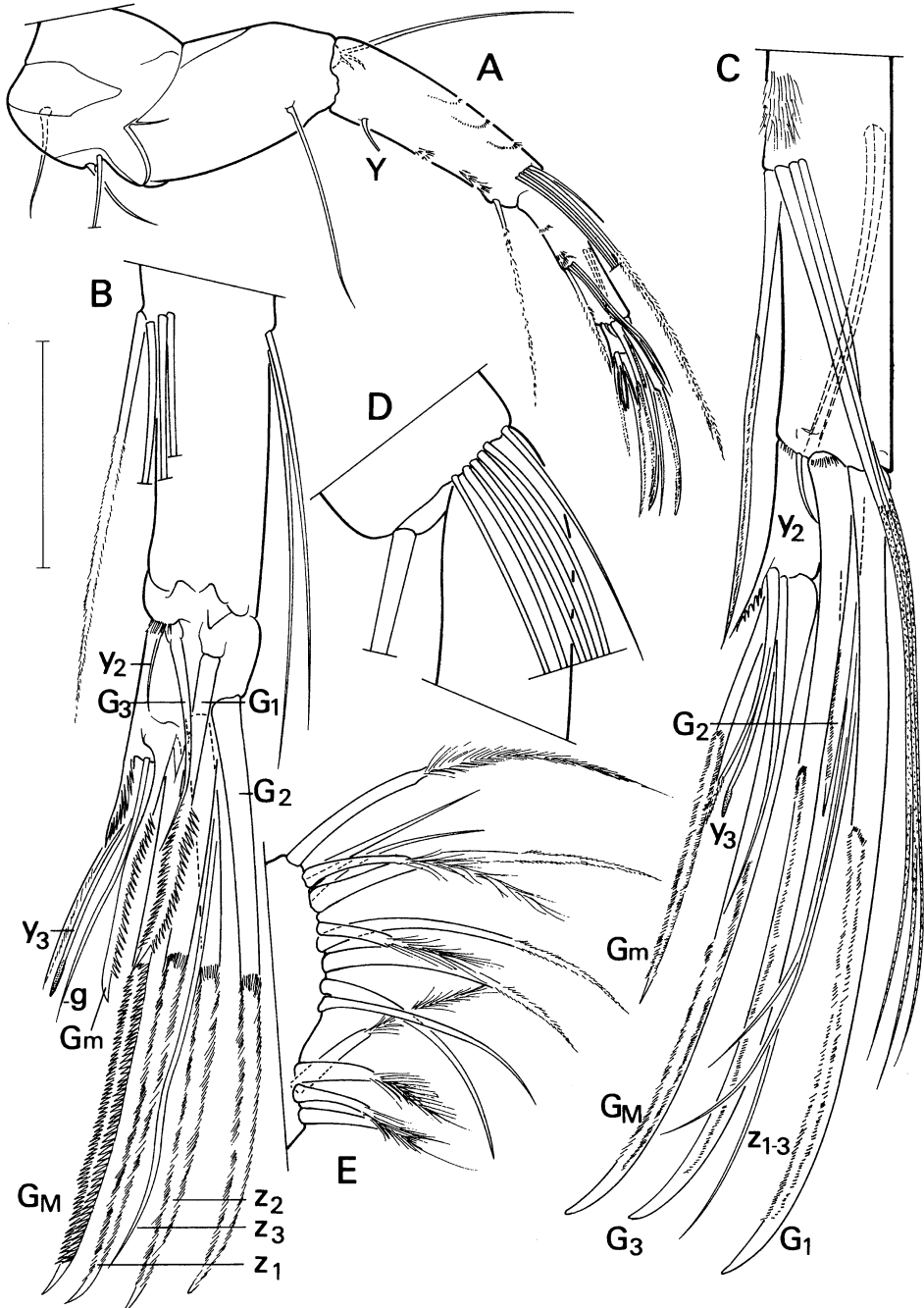


Fig. 3. *Afrocypris barnardi* G. O. Sars, 1924: A, female antenna; B, male antenna, detail of chaetotaxy of distal two segments; C, female antenna, detail of chaetotaxy of distal two segments; D, female antenna, detail of attachment of natatory setae; E, female T1, detail of basipodite. Scale = 167  $\mu\text{m}$  for A; 42  $\mu\text{m}$  for B-E.



respiratory plate with 5 apical and one lateral respiratory rays of unequal length plus an additional subapical seta. Second segment with 3 dorsal setae, two longer, subequal, one shorter; ventral side with a cluster of 5 setae: 3 long and smooth, one shorter and hirsute, beta-seta relatively narrow, hirsute. Third segment dorsally with a group of 4 smooth, subapical setae, two longer, two shorter, medially with one stout and hirsute gamma-seta, ventrally of which are three slim, but longer setae, ventrally with 2 smooth, subapical setae, one long and one shorter (less than 1/4 of the longer one). Terminal segment about twice as long as basal width, tapering towards the distal side, apically with 3 long and 4 shorter setae, all smooth.

Rake-like organ present (fig. 2F).

Mxl (fig. 2B, C) with palp two-segmented, first segment elongated, with 5 dorsal-apical and 2 subapical setae; second segment subquadrate, apically with 6 setae, 3 long and claw-like, three short. Third endite ventrally with one medio-lateral seta, dorsally with one hirsute subapical seta; apically with 8 smooth setae, two of which large and stout Zahnborsten, also smooth. First and second endite without special features. Respiratory plate with 5 proximal setae.

Basipodite of T1 (fig. 2H) with 2 short and smooth unequal a-setae, 1 short and smooth b-seta and one short and smooth d-seta; apically with 10 apical and 4 subapical setae of varying length and appearance. Exopodite (branchial plate) consisting of 6 hirsute rays.

T1 (fig. 2I, J) with large and elongated palp, distally with three relatively short setae, central one the longest, lateral ones less than half the length of the central one, right palp with additional lateral seta; exopodite with 5 rays, basipodite as in the male.

T2 (fig. 2D, E) a walking limb, with seta d1 about twice the length of d2 and penultimate segment divided, all segments elongate.

T3 (fig. 4B, C) a cleaning limb, distally with a pincer-organ, consisting of fourth segment fused with distal part of third segment.

Caudal rami (fig. 4E) slender and symmetrical, with two slender claws and 2 short setae. Attachment (fig. 4D) slender and without loops. At about one-third of main branch, an additional, ventrally pointing lateral branch entering the R5.

Female reproductive organs large and symmetrical, medially with some additional appendages associated with these organs. These are called R-appendages, in attempted homology with those described for *Liocypris grandis* (see Discussion).

R0 (figs. 4F, 5E, F) consists of a paired group of 6-7 small, tube-like structures, some of which are bifurcated.

Appendage R1 (figs. 4F, 5C, D) is a worm-like, tubular structure, not well-sclerotized, distally bluntly pointed.

R5 (fig. 5A, B) strongly resembles a (thoracic, see below) limb Anlage as illustrated for other Cypridae (cf. Smith & Martens, 2000). It forms a direct

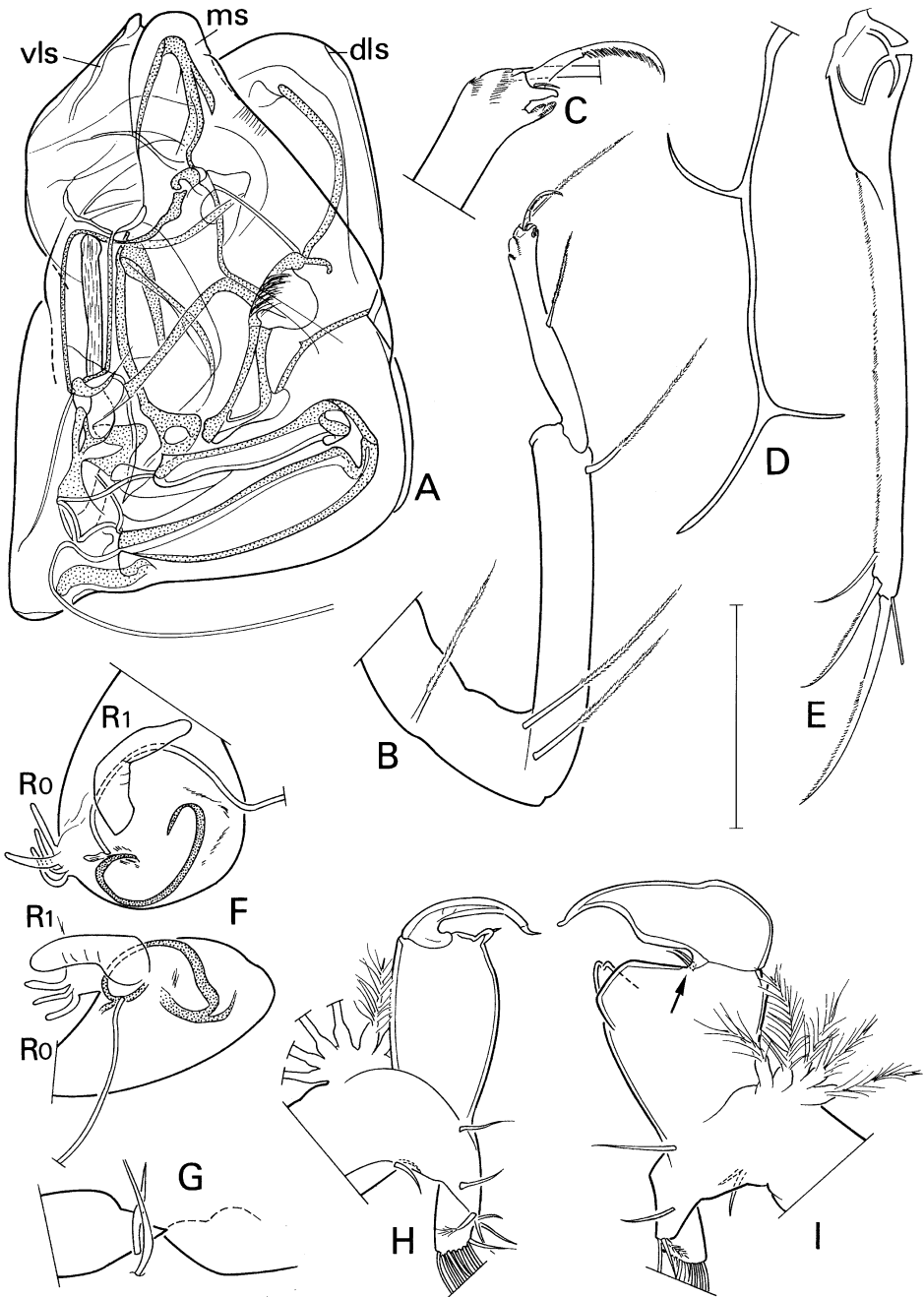


Fig. 4. *Afrocypris barnardi* G. O. Sars, 1924: A, male hemipenis; B, female T3; C, female T3, detail of pincer; D, female, attachment of caudal ramus; E, female caudal ramus; F, detail of female reproductive organ (FRO), showing R0 and R1; G, male right T1, detail with atypically inserted setae; H, male left T1; I, male right T1 (arrow indicating atypically inserted setae figured in G). Scale = 278  $\mu$ m for A, F; 167  $\mu$ m for D, E, H, I; 139  $\mu$ m for B; 70  $\mu$ m for C; 27  $\mu$ m for G.

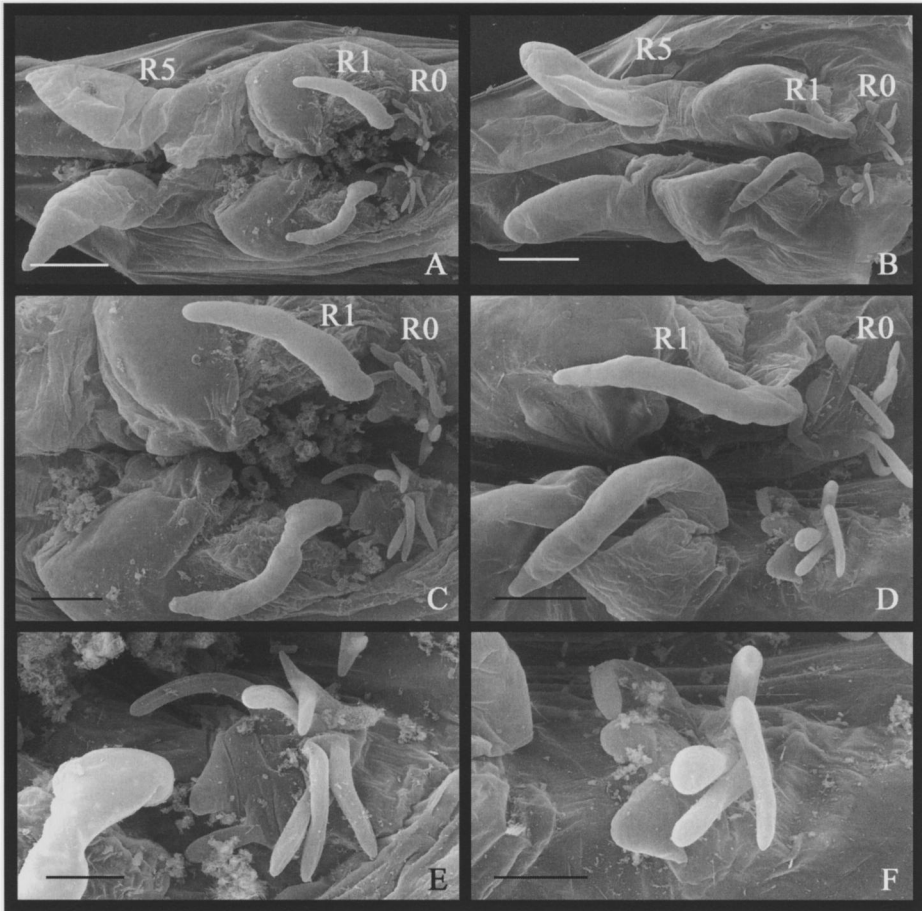


Fig. 5. SEM images of *Afrocypris barnardi* G. O. Sars, 1924, two female specimens (one on left side, one on right side): A, B, overview of FRO with R0, R1, and R5; C, D, detail of R0 and R1; E, F, detail of R0. Scales: 200  $\mu\text{m}$  for A, B; 100  $\mu\text{m}$  for C, D; 50  $\mu\text{m}$  for E, F.

continuation of the caudal corner of the FRO. The structure points in a caudal direction, but does not reach the attachment of the caudal rami.

Abdomen and thorax of adult females dorsally and ventrally without remnant traces of segmentation.

Redescription of male. — Valves generally shorter than in the female. Dorsal margin almost parallel to ventral margin; anterior and posterior margins less produced than in the female (fig. 1C, D).

A1, Md, Mx1, T2, T3, and caudal rami as in the female, attachment of caudal ramus without additional ventral lateral branch.

A2 (fig. 3B). Sexual dimorphism of distal chaetotaxy pronounced. On penultimate segment: z3 a long seta, z2 and z1 long, slender claws; G2 a long, stout claw,

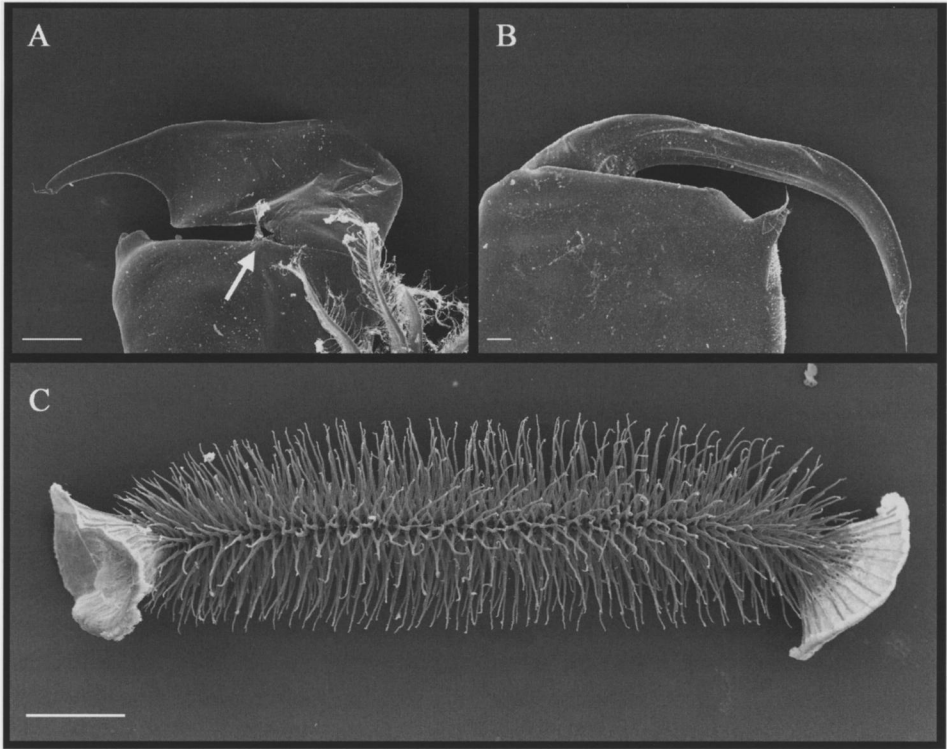


Fig. 6. SEM images of *Afrocypris barnardi* G. O. Sars, 1924, males: A, distal part of right T1 (arrow indicates atypically inserted setae); B, distal part of left T1; C, chitinous skeleton of Zenker Organ. Scales: 100  $\mu\text{m}$  for C; 20  $\mu\text{m}$  for A; 10  $\mu\text{m}$  for B.

G1 and G3 short claws, the latter almost a seta. On terminal segment: Gm short, about half of GM, the latter distally serrated; aesthetasc y3 and accompanying seta about as long as claw Gm, seta 'g' slightly longer.

T1 Endopodites 2-segmented, prehensile palps. Right palp (figs. 4I, G, 6A) the largest, first segment distally dilated, ventro-apically with a cone-like protuberance, apically with two small setae near insertion of distal segment, distal segment resembling a birds-head, with long, pointed ventral tip carrying elongated sensory organ. Left palp (figs. 4H, 6B) with first segment with rounded lateral sides, ventro-apically with one protuberance carrying two minute sensory organs; second segment narrow and rounded, distally tapering and apically with one sensory organ.

Zenker organs (fig. 6C) large and well-developed, with indeterminate number of spinous whorls.

Hemipenis (fig. 4A) large, lateral shield with 2 large and prominent lobes, with pronounced three-dimensional structure; medial shield simple, bluntly pointed, reaching the tips of the lobes of the lateral shield. Inner anatomy with elongate

labyrinth, post-labyrinthal inner spermiduct simple, without extra coils and surrounded by sclerotized structures. Bursa copulatrix with stout, elongated rod.

Underneath the relaxed hemipenes, a so far unknown unpaired, asymmetrical extension present. This Penile Attachment (PA, fig. 7A-E) is globular, c. 700  $\mu\text{m}$  long and 350  $\mu\text{m}$  wide. Left side of the PA without connection to the main body, but at mid-length showing an outwardly pointing finger-like structure. This 'finger' is c. 400  $\mu\text{m}$  long, 100  $\mu\text{m}$  thick, partly supported by a chitinous framework, not articulated, and distally tapering. Tip narrowly rounded.

Length of spermatozoa: c. 4 mm.

Measurements. — Following Sars (1924): length of adult female up to 5.30 mm.

New measurements (in mm): lengths, female RV: 5.3-5.4 (5.34; n = 3); female LV: 5.2-5.4 (5.32; n = 4); male RV: 5.0-5.4 (5.09; n = 6); male LV: 4.9-5.1 (5.01; n = 4); heights, female RV: 2.4-2.6 (2.47; n = 3); female LV: 2.4-2.6 (2.50; n = 4); male RV: 2.3-2.5 (2.38; n = 5); male LV: 2.3-2.4 (2.30; n = 4).

Remarks. — Upon the discovery of the additional structures in males and females of *A. barnardi*, the material of *Liocypris grandis* used by Martens (2003) and Matzke-Karasz & Martens (2005) was re-examined:

1. The tube-like clusters, here named R0, in the female of *A. barnardi* also occur in females of *Liocypris*, which is even visible in the SEM illustrations in Martens (2003). However, as they are much smaller, they were at that stage not recognized as belonging to the morphology of the animal itself. It is here confirmed that R0 occur in both *Liocypris grandis* and in *Afrocypris barnardi*.

2. The additional asymmetrical basal structure on the male hemipenes, or penile attachment PA, is also present in *Liocypris grandis*. However, only a small bulge is developed, where *Afrocypris barnardi* is showing the finger-like structure (fig. 8A, B).

3. The attachment of the caudal ramus has an additional dorsal branch in the female, entering the R5 (longer than drawn in Martens, 2003); this branch is absent in the male.

## DISCUSSION

### The female reproductive organ

As indicated in the introduction, the discovery of the additional appendages R1-R5 in *Liocypris grandis* constituted counter-evidence for the hypothesis that the FRO was homologous to a single (thoracic) somite (Tsukagoshi & Parker, 2000), but the fact that these additional structures were known from a single species only weakened the power of the falsification. The discovery of similar structures in a

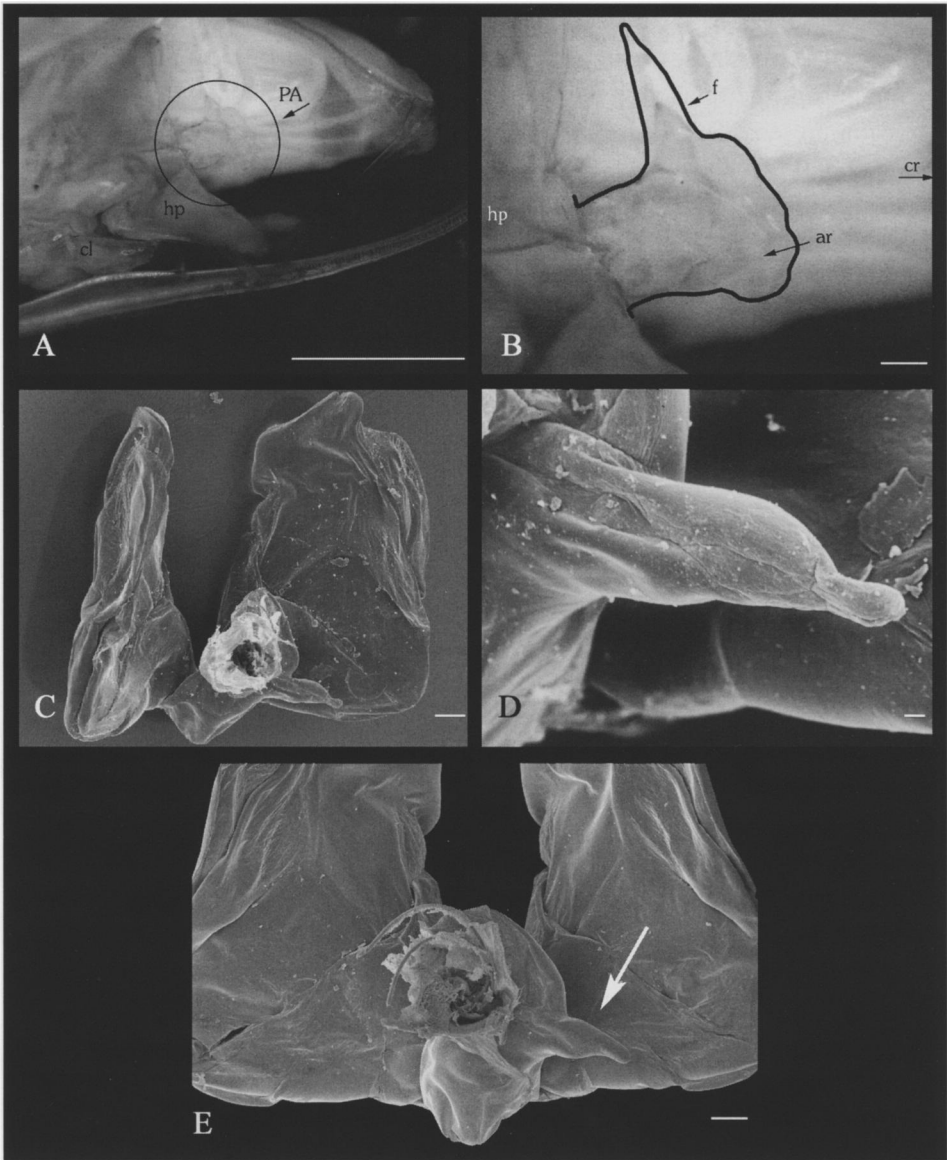


Fig. 7. *Afrocypris barnardi* G. O. Sars, 1924, males, all hemipenes with penile attachment (PA): A, light microscopic view of left body side in right valve; B, detail of A, showing finger-like structure of the PA; C, interior view of the hemipenes with PA; D, detail of C with finger-like structure; E, hemipenes and PA of another specimen than that in C. Abbreviations: ar, attachment region; cl, 'clasping organ' of T1; cr, caudal ramus; f, finger-like structure; hp, hemipenes. Scales: 2000  $\mu\text{m}$  for A; 100  $\mu\text{m}$  for B, C, E; 10  $\mu\text{m}$  for D.

second species strengthens the arguments against the said hypothesis on the one hand, but the addition of the R0, the dissimilarity of the R1 between the two taxa,



Fig. 8. SEM images of *Liocypris grandis* (G. O. Sars, 1924), males: A, hemipenes with penile attachment PA; B, detail of A, showing an asymmetrical bulge located where *Afrocypris barnardi* G. O. Sars, 1924 developed a finger-like structure. Scales: 100  $\mu\text{m}$  for A, B.

and the absence of the R2-R4 in *Afrocypris barnardi*, on the other hand, further confuse the situation.

The discovery of the additional appendages in *Afrocypris barnardi* appears to strengthen the interpretation of such structures as (part of) true appendages, although no final conclusions can as yet be drawn. If one continues to consider the said structures as being homologous to appendages, then the structures can represent the equivalent to 3, 5, or 6 appendages (and related somites). Let us first consider the situation in *Liocypris*. If R0-R5 all represent separate appendages, then 6 body segments have been involved in the origin of the FRO. However, the cluster of tube-like structures in the R0 cannot readily be equated with known

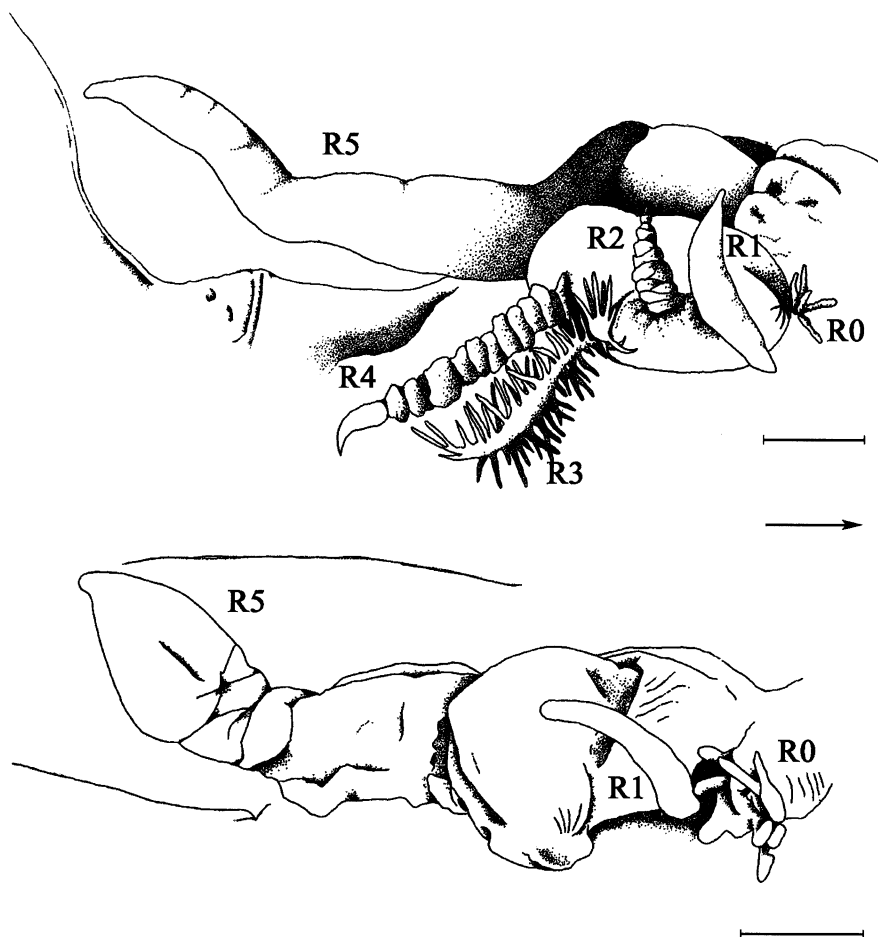


Fig. 9. Drawings of additional appendages in *Liocypris grandis* (G. O. Sars, 1924) (above) and *Afrocypris barnardi* G. O. Sars, 1924 (below). The *L. grandis* FRO is here corrected for the R0, which were missing in Martens (2003). Scales: 200  $\mu$ m.

structures, so at present it appears more prudent not to consider these minute structure as representing an appendage. If R1-R5 represent 5 appendages, then 5 somites are involved. If R1+R2 and R3+R4 represent 2 appendages, then 3 somites are involved.

In *Afrocypris*, only R0, R1, and R5 are present (following our present intuitive homologies based on position); R2-R4 are absent. Continuing to ignore R0, the situation in *Afrocypris* could be fitted into either the 3- or the 5-somite scenarios (fig. 10). The discovery of the additional appendages in *Afrocypris*, therefore, does not allow a distinction between either hypothesis.



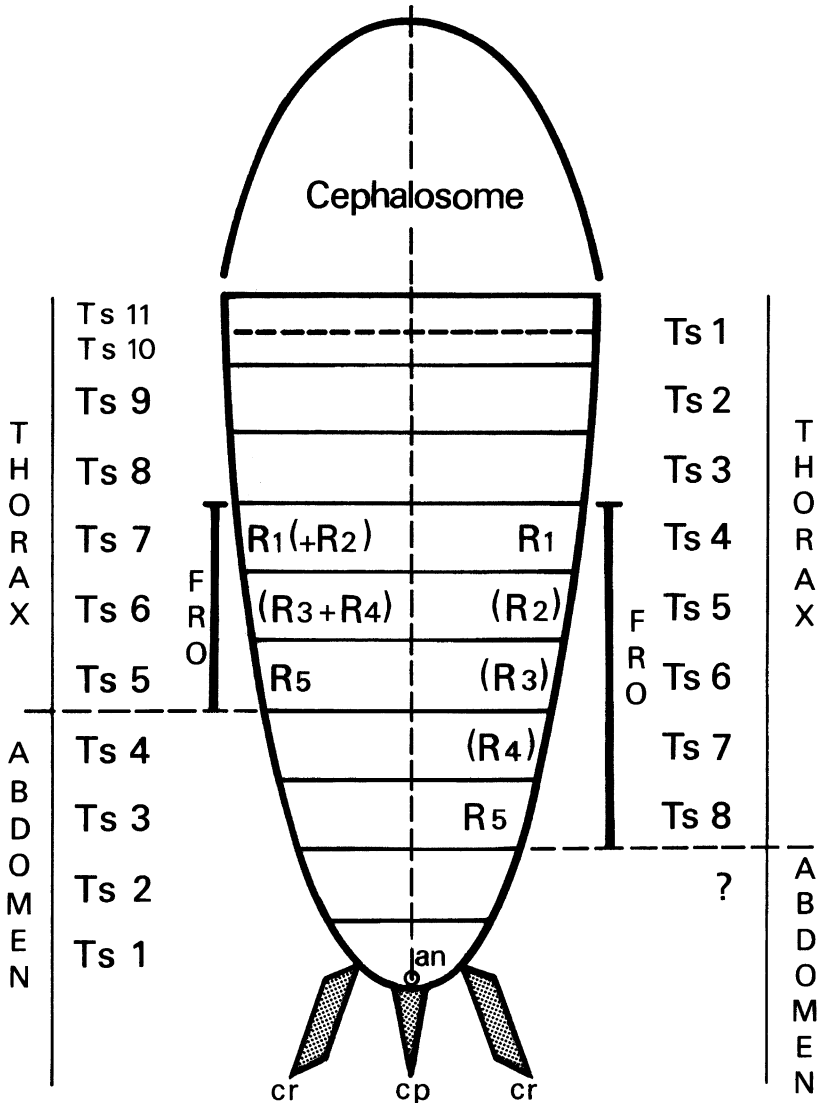


Fig. 10. Schematic dorsal view of a podocopine ostracode, further developed from Tsukagoshi & Parker (2000) and Matzke-Karasz & Martens (2005). Somite numbering and division of the body into thorax and abdomen according to Tsukagoshi & Parker (2000) on the left, according to Matzke-Karasz & Martens (2005) on the right.

The left bar 'FRO' indicates the '3-segment-scenario' in which the appendages R1+R2 and R3+R4 represent two appendages in all. The right bar 'FRO' indicates the '5-segment-scenario' in which each of the appendages R1-R5 represents one somite. While all appendages R1 to R5 are present in *Liocypris grandis* (G. O. Sars, 1924), the appendages R2 to R4 (here in parentheses) are missing in *Afrocypris barnardi* G. O. Sars, 1924. Note: the appendages R0 are ignored in this scheme, because they cannot be readily equated with known structures. Abbreviations: an, anus; cp, caudal process; cr, caudal ramus; FRO, female reproductive organ; Ts, trunk somite.

### The male reproductive organ

In male cypridoidean ostracodes, the hemipenes are normally attached directly to the main body by their most proximal, joined bases. Here, the long sperm ducts lead symmetrically from the Zenker Organs of both body sides to the point of attachment of the hemipenes, and no additional structure is interposed. In *Afrocypris* as well as in *Liocypris* males, the distal symmetry of the spermiducts within the main body, as well as the symmetry of their transition point are broken up by the asymmetrical PA.

Since the FRO in *Afrocypris barnardi* is associated with at least three additional, paired appendages, it appears likely that the PA plays a role during copulation by, e.g., interconnecting with the female copulatory appendages. Since all investigations were carried out on fixed collection specimens, copulations could not be monitored and only hypotheses may be presented here.

It has been shown for different cypridoidean species, that in case of ventral to dorsal mating position, the male copulatory organ is turned around 180 degrees before copulation (Smith et al., in press). In case the male copulatory organ in *Afrocypris barnardi* is turned prior to mating, this movement is likely to also lift the PA out of its idle position and to turn it around until its finger-like extrusion appears in a central position. If so, this 'finger' might then interact with any or all of the R-appendages of the female.

The asymmetrical PA is attached to the main body on the right side only (fig. 7 C-D). Therefore, spermiducts are both entering the PA through the right side, meaning that distal portions of the spermiducts within the main body, leading from the Zenker Organs to the hemipenes, are no longer mirror images of each other.

Evolutionarily, the PA might be seen either as new morphological feature, developed, e.g., to optimize contact with the female during copulation by interaction with the female additional appendages, or as a remnant of (a) former segmental appendage(s), with or without function.

### Implications for podocopid phylogeny

The five additional structures associated to the FRO in *Liocypris grandis* were interpreted as being homologous to five somites, the FRO thus representing at least five somites (Matzke-Karasz & Martens, 2005). This interpretation implies an evolutionarily more ancestral state of segmental reduction in this species. Since the females in *Afrocypris grandis* also have at least three pairs of additional appendages on their FRO, also here an evolutionary older anatomical status in both sexes of this species appears likely. The fact that the 'finger' of the PA bears a

(simple) chitinous framework resembling those of 'real' appendages, supports the hypothesis of an ancestral segmental situation.

The presence of additional appendages related to the FRO in the two species thus far considered raises questions about the phylogenetic relevance of such structures. As outlined by Martens (2003) and confirmed by Matzke-Karasz & Martens (2005), the R0-R5 can either be ancestral, or they can be atavistic (ancestral characters that disappeared in the course of evolution, re-appearing after a certain time in isolated cases), or they can be fully apomorphic features.

In the first case, an ancestral nature of the R0-R5 appendages, the logical conclusion would be that *Liocypris* is the most ancestral taxon in the Cyprididae (and maybe even in the Podocopa), and that *Afrocypris* is a more derived taxon within the same lineage. The phylogeny of the Cyprididae remains unresolved at present, but attempts to clarify the phylogeny of the Podocopa (cf. Horne et al., 2005) do not place Cypridoidea as an ancestral group within the Podocopa. More ancestral taxa such as Cytheroidea and Puncioidea do not seem to show these structures. Therefore, the present hypothesis seems unlikely.

If the R-appendages are atavistic, they can appear at any given position within phylogeny, and *Liocypris* and *Afrocypris* do not necessarily belong to ancestral lineages, while even their position within the same lineage could be doubted.

Finally, if the R-appendages are newly formed, apomorphic structures, one could assume that the two taxa would belong to (a) more recently derived lineage(s).

The discovery of the R-appendages in *Afrocypris* does not allow immediate discrimination between the three scenarios. However, molecular phylogenies of the Cyprididae will provide further clues as to which scenario should be preferred, now that R-appendages have been found in at least two different taxa.

If *Afrocypris* and *Liocypris* will not cluster together in the tree, the possibility that the R-appendages are independent, atavistic structures will gain ground. If both taxa cluster together in a basal position, the ancestral nature of the characters will be corroborated. If the two taxa cluster in a more derived lineage, the structures might be seen as being apomorphic.

## CONCLUSIONS

A second giant temporary pool species from South Africa has been shown to have a set of additional R-appendages associated with the FRO. In addition, re-description of *Afrocypris barnardi* and of *Liocypris grandis*, has revealed additional unusual morphologies in the male copulatory appendages.

The presence of additional R-structures in a second species, after *Liocypris grandis*, strengthens the hypothesis that the FRO originated out of more than a

single (thoracic) somite. However, it is at present still not possible to unequivocally confirm whether this FRO is homologous to 3, 5, or even 6 body segments. In addition, it also remains uncertain if such structures are ancestral, atavistic, or apomorphic. This being said, their presence in two genera (and species) will allow to discriminate between these three possibilities by assessing the position of these taxa within a phylogeny of the Cyprididae as a whole.

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