A redescription of *Eucyclops serrulatus* (Fischer, 1851) (Crustacea: Copepoda: Cyclopoida) and some related taxa, with a phylogeny of the *E. serrulatus*-group

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Eucyclops serrulatus (Fischer, 1851), the type species of the genus *Eucyclops*, is redescribed from specimens found in the St. Petersburg area, Russia (type locality) and compared with specimens from Siberia, western Europe and North Africa. A neotype is selected. Cultures were set up, and interpopulation hybridization as well as hybridization with related species was attempted. The classical description of external body morphology was combined with pore signature mapping and with DNA nuclear small subunit (18S) ribosomal gene sequence analysis. Comparisons with *E. dumonti* Alekseev, 2000, *E. hadjebensis* Kiefer, *E. speratus* Lilljeborg, *E. turcomanus* Lindberg, and *E. pectinifer* (Cragin, 1883) were carried out. A phylogenetic tree based on molecular information shows that *E. serrulatus* and *E. speratus* should be regarded as separate species. *E. dumonti* also deserves species status, but not *E. hadjebensis*. A cladistic tree based on the pore pattern of the cephalosome agrees well with a tree based on the sequence of the 18S rDNA gene. Cephalosome (and probably metasome) pore patterns seem useful to elucidate relationships within genera, while urosomal pore patterns better reflect the relationship between genera. *E. serrulatus* occurs in three morphological forms over most of its range; one of these (C) might be a rare ('recessive') morphotype, while forms A and B differ in microhabitat choice, but hybridize when living together. The same polymorphism also occurs in an American species (*E. prionophorus*), and therefore two hypotheses regarding its origin are advanced: either forms A and B evolved during the glacial episode (Pleistocene origin), separately on both sides of the Atlantic, or the polymorphism was already present in the ancestor of the *serrulatus*-group, and was later lost in some but not in all species (Pliocene origin.)

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Introduction

The taxonomy of the large cyclopoid genus *Eucyclops*, burdened with faulty species descriptions, is complex and chaotic. To a certain extent this is because the original description of the type species of the genus, *Eucyclops serrulatus* (Fischer, 1851), was incomplete. This taxon was discovered in the mid-19th century in a pond at Petershoff, the summer resort of the Tsar close to St. Petersburg, Russia (Fischer 1851). An earlier description, *Cyclops agilis* Koch, 1838, sometimes equated with *E. serrulatus*, was published in the third volume of Koch's study on the fauna from Germany. *Cyclops agilis* is only one of a series of copepods described by Koch, very few of which are still valid because both his descriptions and figures were extremely sketchy. The single illustration of *C. agilis*, collected from around Regensburg, reproduced in Fig. 4: 1, shows a cyclopoid that cannot be assigned to a particular genus. It could belong to *Eucyclops*, but also to *Metacyclops*, *Acanthocyclops*, *Tropocyclops*, *Diacyclops*, or even *Thermocyclops*. Only Sars (1918) picked up the name *agilis*, based on the 'agile swimming habits' of the species, although this attribute holds for numerous other cyclopoids. Generally, European authors except Gurney (1933) did not follow him, but most American authors did. Thus, via the link Koch-Sars-Gurney a *serrulatus*-like species that occurs over most of Canada and the USA is still widely known under

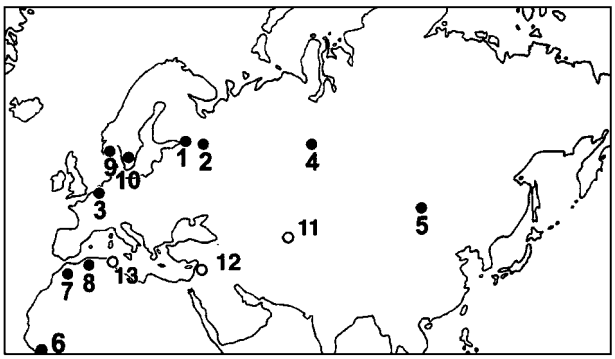
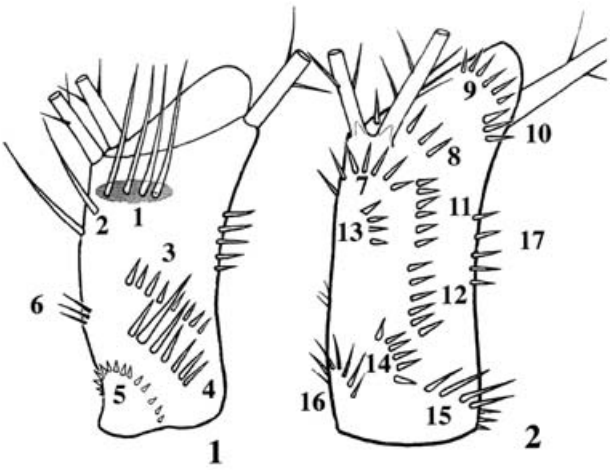


Fig. 1 Collecting sites of specimens of the *Eucyclops serrulatus*-group (filled circles), except *E. turcomanus* (open circles); details in text.



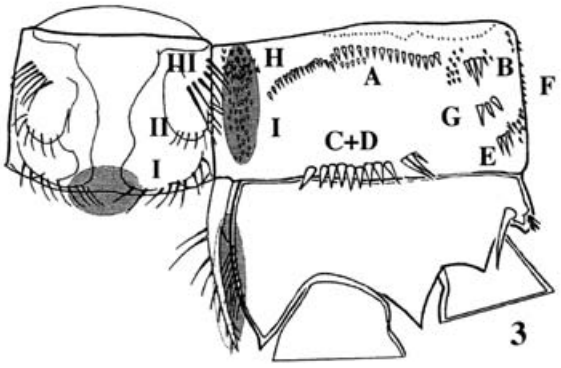


Fig. 2 Numbering system for micromorphological patterns in *Eucyclops*: 1 — basipodite of A2, posterior; 2 — A2 basipodite, anterior; 3 — intercoxal sclerite and coxa of P4.

the name of an animal of German origin. On the other hand, the name and description given by Cragin (1883) to this animal (see below) have remained unrecognized.

For well over a century, *E. serrulatus* has been reported from water-bodies around the world (Dussart & Defaye 1985). Its cosmopolitan nature remained unchallenged until some taxa that superficially look like *E. serrulatus* were separated from it (Dussart 1986; Reid 1995; Ishida 1997, 1998). Some of these may indeed be valid species, others mere forms of *E. serrulatus*. Progress in the taxonomy of this species-group is impossible without a detailed re-description of *E. serrulatus*, the type species, supported by information on inter- and infra-population variability. One of us (J. P.) started working on *E. serrulatus* in the late 1970s and became impressed with the strong morphological variability of the species (hereafter referred as forms A, B and C (morphological types)), even within the population from the type locality at St. Petersburg. Therefore, the present study uses a number of methods, in which results obtained by one method are used to verify those obtained by other methods, in hopes of reaching a consensus and of finding new criteria of species delimitation.

Materials and methods

Eucyclops serrulatus samples from the *terra*-type locality (sites 1, 2), western Europe (site 3), Siberia (site 4), and northern Africa (site 7) were used for the various types of research outlined below (Fig. 1). Material used to study other species will be specified under the heading of each species.

Type locality

For hybridization experiments, pore signature mapping and molecular genetics, we used animals collected in May–June 1999 in Tavricheski Park, St. Petersburg, and in the main accumulation pond of the Petershoff palace fountain (another candidate for the status of type water-body). For comparative morphological research, pore signature mapping and molecular genetics, we also collected animals in the Orlov Pond in Petershoff, in a roadside ditch on the way between Petershoff and St. Petersburg, in the small River Sablinka, 50 km from St. Petersburg in the direction of Moscow, and in the small River Ravan, 100 km from St. Petersburg in the direction of Moscow (Fig. 2, site 2). These samples yielded *E. serrulatus*, but also *E. speratus* Lill. and *E. macruroides*, and were used in DNA studies and for comparative morphology.

Western Europe (Ghent, Belgium: 51°05′N, 3°45′E). For hybridization, pore signature mapping and molecular genetics we collected material in the pond of the botanical garden of Ghent University in June 1999, as well as animals from roadside ditches in the vicinity of Ghent in July 1999.

Siberia (Tumen, Western Asian Russia: 57°N, 67°E). A pond in the park of the Tumen Agriculture Academy, May 1999, sampled to collect material for hybridization experiments, pore mapping and molecular genetics. For comparative morphological research we also used a population collected in temporary ponds, near roadside ditches and marshes close to Tumen in April–May 1999.

Northern Africa. Material collected in the Atlas Mountains (sites 7 and 8) since July 1971 includes a taxon hereinafter referred to as *E. serrulatus hadjebensis* (Kiefer). In the foothills and lowlands of Morocco and Algeria, a *serrulatus*-like animal that corresponds to forms B and C *sensu* this paper was encountered, in an area that extended as far south as the central Saharan Ahaggar mountains. This animal has a manuscript name (without taxonomic relevance), *Eucyclops asymmetricus* Dumont & Pensaert, because its caudal rami often have an asymmetric serra (Fig. 15: 11).

In all *Eucyclops* species examined, topotypical specimens were used. For a comparison with other species of *serrulatus*-group we included samples from Northern Africa (*E. hadjebensis*, site 8), Norway (site 9) and a small river in the vicinity of Lund, Sweden (type locality of *E. speratus* Lilljeborg, site 10), Mali (site 6, for *Afrocyclops gibsoni*), Afghanistan (site 11 type locality of *E. turcomanus*, Lindberg), Jordan and Algeria (sites 12, 13, extra localities for *E. turcomanus*), and Pennsylvania, eastern USA, for *E. pectinifer* Cragin (site not indicated in Fig. 1).

The material was collected from the littoral zone. For hybridization, animals collected in the field or animals collected from type locality and reared under controlled conditions were used. These experiments were conducted in the Zoological Institute of the Russian Academy of Sciences. For DNA and pore signature analyses, animals preserved in 80% ethanol were used. For morphological studies, specimens were preserved in 80% ethanol or 4% formalin.

External morphology

Abbreviations used in the text, tables and figures: A1, antennula; A2, antenna; P1–P4, swimming legs; P5–P6, rudimentary legs; Enp, endopodite; Exp, Exopodite; II — anterolateral (lateral) caudal seta; III — posterolateral (outermost) caudal seta; IV — outer terminal (terminal median external) caudal seta; V — inner terminal (terminal median internal) caudal seta; VI — terminal accessory (innermost) caudal seta; VII — dorsal seta.

Animals were placed in glycerol, dissected under a stereomicroscope, and drawings were made at 1000× under a Leitz Medilux compound microscope equipped with a camera lucida. Drawings were scanned, corrected and scaled using Adobe Photoshop (version 5.0). For micropatterns on both sides of the antennal basipodite we applied the numerical scheme illustrated in Fig. 2 based on accumulation in one picture of most micropatterns of the appendages known in cyclopids (after Huys & Boxshall 1991). For mapping rows of spinules and setules on the coxa and intercoxal sclerite of P4 we mainly followed Einsle (1985).

Pore pattern mapping

The integumental perforation pattern was determined on adult females, according to Fleminger (1973), Baribwegure & Dumont (1999, 2001), and Alekseev & Naumova (2005).

Animals were heated for 3 h in KOH 10%, rinsed in distilled water and 70% alcohol, and stained in 1% chlorazol black in 70% alcohol. Tungsten needles were used to dissect animals under a Wild M3 dissecting microscope. Perforations were observed and mapped at 1000× using an Olympus Medilux-12 microscope equipped with a camera lucida. The mapping of the integumental pore signature included the rostrum, cephalosome, metasome, urosome and furca. A full mapping required about 10 specimens. Thus, the body parts figured are not from a single specimen and should not be used to evaluate individual variation.

To compare perforations patterns between species, all positions were numbered. In some positions near to the lateral curvature of the cephalosome, difficulties were encountered and assignments were tentative. In cases where pore migration was suspected, and when positions had either a single or a double pore, the position in question was always coded as 2 (with double pore). The number and position of pores on some metasome segments of some species (e.g. *E. macruroides*) appeared erratic. Because of the uncertainty involved, only the dorsum of the cephalosome was used, rather than its rostral zone and flanks, for comparative taxonomic and phylogenetic study (Fig. 3). Thus, a maximum of 42 pore positions was specified, tabulated (0, absence of pores, 1 pore, 2 pores, exceptionally 3 pores), and subjected to phylogenetic analysis using the neighbor joining (NJ) and maximum parsimony (MP) algorithms in PAUP* 4.0b8.

Bootstrap analyses were performed to assess the stability of each branch-point in the tree. The NJ analysis used 1000 replicates to assess the reliability of individual branches in the phylogenetic tree. The MP analysis used 1000 replicates to assess the reliability of individual branches in the phylogenetic tree obtained by a heuristic search with simple addition, tree bisection reconnection (TBR) branch swapping, multiple trees retained, no steepest descent, rearrangements limited to 100 000 and accelerated transformation. Trees were displayed with TreeView 1.6.6 (Page 1996).

Hybridization experiments

Possible hybridization among forms and local populations across two subsequent generations was assessed by reciprocal crosses under laboratory conditions.

Males and females, collected in the field or reared under controlled conditions, were separated at the copepodid V stage in 50 mL transparent plastic containers. After maturing we put females and males in every container. For each combination of forms or populations we did at least two experiments with opposite sex representation. Experiments took place at room temperature (18–22 °C) and under standard feeding conditions. To each container we added weekly several drops of a laboratory culture of *Scenedesmus* sp. mixed with recently collected zooplankton killed by heating to 50–60 °C. The presence of offspring was checked at 2–3 day intervals.

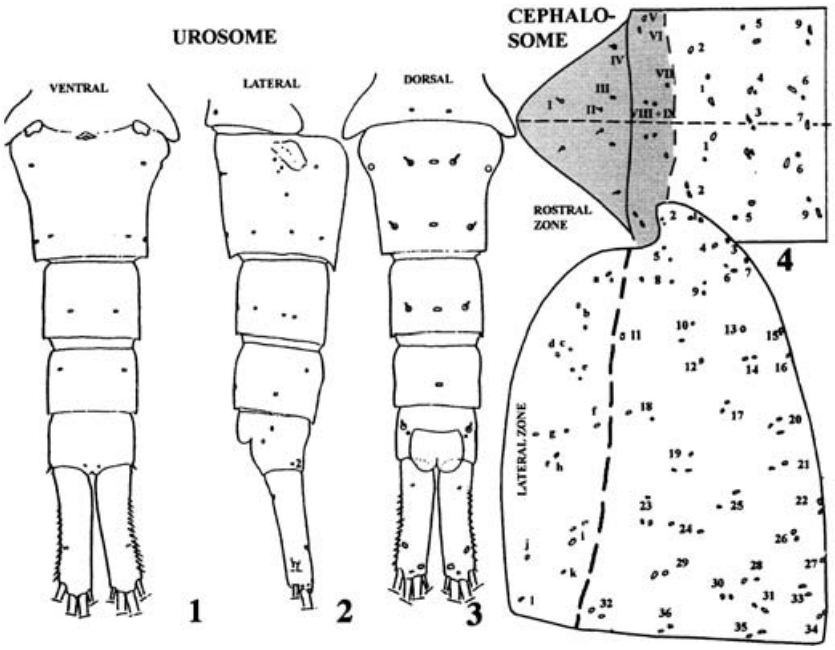


Fig. 3 Integumental pore mapping in *Eucyclops*: 1–3 — urosomal segments; 4 — cephalosome, dorsal and lateral.

Molecular genetic analysis

Preparation of nucleic acids, PCR amplification, and sequencing reactions. About 200 eggs from several females were isolated under aseptic conditions, and washed with 70% ethanol solution to remove accompanying microorganisms. Total DNA was prepared according to the protocol of the Puregene™ DNA isolation kit type D-5000 A (Gentra Systems, Inc., BIOzym, Landgraaf, The Netherlands). 18S rDNA was amplified using the polymerase chain reaction (PCR) with Qiagen DNA polymerase (Westburg, Leusden, The Netherlands). Eukaryote-specific primers complementary to the 5′-terminus (5′-TACCTGGTTGATCCTGCCAG-3′) and 3′-terminus (5′-TGATCCTTCCGCAGGTTCACCT-3′) were used to amplify the entire 18S rDNA gene (except for the 5′- and 3′-primer sites). Cycling conditions were 95 °C for 1 min, 55 °C for 1.5 min, and 72 °C for 2 min, for 35 cycles. PCR products were used for direct sequencing employing BigDye™ technology and the protocol of the ABI Prism BigDye terminator cycle sequencing ready reaction kit; they were subsequently analysed on an ABI Prism 377 DNA sequencer (PE Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). The 18S rDNA sequences were aligned using ClustalW (Thompson *et al.* 1994) and optimized using Genedoc (Nicholas *et al.* 1997) with published 18S rDNA sequences based on the conservation of both primary sequence data and secondary structure features (http://www.psb.ugent.be/rRNA/ssu/index.html/).

Alignment and phylogenetic analysis

The DNA sequences of the complete 18S gene of all taxa were aligned with ClustalW 1.64b (default settings) to create an initial dataset. The alignment of the 18S gene was manually optimized using Genedoc with published 18S rDNA sequences based on the conservation of both primary sequence data and inferred secondary structure features (Nelles *et al.* 1984) (http://wwwrrna. uia.ac.be/ssu/index.html). First, the likelihood ratio test (LRT) and Akaike information criteria (AIC) in ModelTest 3.06 (Posada & Crandall 1998) were used to determine the appropriate substitution model of DNA evolution that best fitted the dataset. *Macrocyclops albidus* and *Ectocyclops polyspinosus* were used as outgroup. The dataset was analysed with NJ, MP and the maximum likelihood (ML) algorithms in PAUP* 4.0b10 to resolve the phylogenetic relationships. Bootstrap analyses were performed to assess the stability of each branch point in the tree and were considered as an index of support for a particular clade, and not as a statement about probability in a statistical sense (Hillis & Bull 1993). For each dataset the appropriate DNA evolution model with corresponding nucleotide frequencies, substitution rates and types, and Ti/Tv ratios was determined by ModelTest 3.06, and used for NJ, MP and ML algorithms in PAUP*. NJ analysis was performed with PAUP* by application of the selected ML substitution model to the NJ algorithm. The nonparametric bootstrap analysis used 1000 replicates to assess the reliability of individual branches in the phylogenetic tree (Felsenstein 1985).

Equally weighted MP analyses were performed with PAUP*. Heuristic search settings were: stepwise taxon addition, TBR branch swapping, multiple trees retained, no steepest descent, rearrangements limited to 10 000 000, and accelerated transformation. The nonparametric bootstrap analysis used 1000

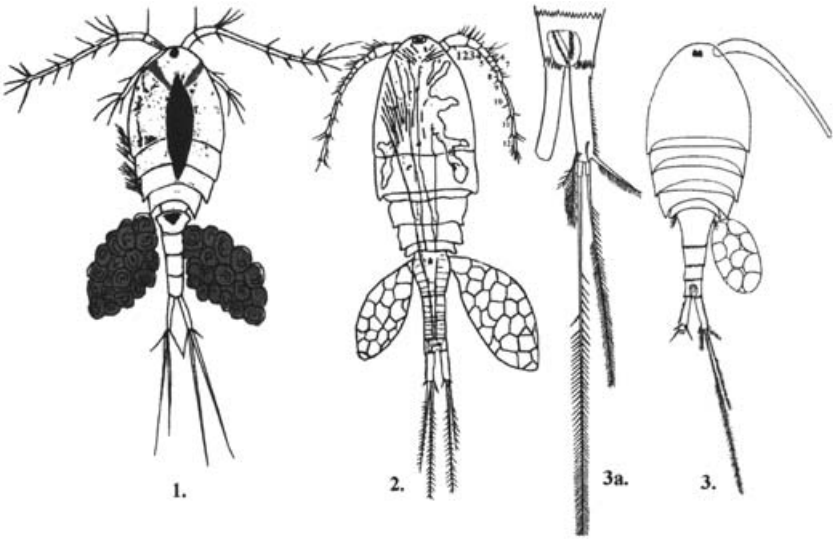


Fig. 4 Original illustrations of 1 — *Cyclops agilis*; 2 — *Cyclops serrulatus*; 3 — *Cyclops pectinifer*, body, 3a — caudal rami. 1, after Koch (1838); 2, after Fischer (1851); 3, 3a, after Cragin (1883).

replicates to assess the reliability of individual branches in the phylogenetic tree obtained by heuristic search with stepwise sequence addition (Felsenstein 1985). The consistency index (CI) (Klug & Farris 1969), retention index (RI) and rescaled consistency index (RC) (Farris 1989) were computed to estimate the amount of phylogenetic signal available for parsimony analysis. For ML analysis, the substitution model of DNA evolution with corresponding parameters that best fitted the data was determined by the LRT and AIC using ModelTest 3.06. Heuristic search settings were stepwise taxon addition, TBR branch swapping, MulTrees option in effect, no steepest descent, and rearrangements limited to 1 000 000. The non-parametric bootstrap analysis with 100 replicates was used to assess the reliability of individual branches in the phylogenetic tree obtained by heuristic search with stepwise sequence addition (Felsenstein 1985). Pairwise sequence divergence data between taxa were computed. Absolute distance values and distances based on a ML distance matrix (PAUP*), with appropriate parameters for the correct DNA evolution model (ModelTest) were calculated for the dataset. Trees were displayed with TreeView 1.6.6.

Results

***1. Eucyclops serrulatus* (Fischer, 1851)**

Synonymy: see Dussart & Defaye, 1985.

Eucyclops asymmetricus Dumont & Pensaert in Dumont (1979) (*nomen nudum*).

Probable new synonyms: *Eucyclops speratus* Dumont & Decraemer, 1977; *Eucyclops speratus ifniensis* Dumont & Decraemer, 1977.

Material examined. Neotype: dissected female neotype, catalogue number 55031, dissected male (accession number 55032), 8 September 1983, from pond in Petershoff, possibly S. Fischer's type locality (Fischer 1851). The material was deposited in the Federal Collection N 96-03-16, Zoological Institute of the Russian Academy of Sciences, St. Petersburg.

Type locality: Petershoff, 18 km W of St. Petersburg, Russia (60°00′N, 30°55′E).

Justification of neotype: Sebastian Fischer was a medical doctor, a graduate of Munich University, who moved to St. Petersburg around 1843 to work at the court of the Tsar. He was probably invited by Dr Brandt, another copepodologist and employee of the Russian court (Fischer 1848). His two major copepod papers, published in 1851 and 1853, were based on work carried out in his spare time. Although he was affiliated with the Imperial Academy of Sciences, there is no trace of collections by Fischer at the Zoological Institute of the Academy of Sciences in St. Petersburg. We also explored the zooplankton collection of the Zoological Museum of Moscow State University and no samples labelled with Fischer's name or the Petershoff area were found. After about a decade, Fischer returned to Germany and continued to publish for a while, but again, no evidence of any of his material surviving has come to light. Consequently, we consider it lost. The description of *Cyclops serrulatus*, published in 1851, is not complete, but the accompanying figures (Fig. 4) unequivocally show a cyclopoid with a 12-segmented antennule and a serrated furca of medium length. The setulation of the terminal setae of the furca corresponds to 'type A' of the present paper.

Description

Female neotype: corresponding to type A, as defined hereafter. Body colour rusty brown, rarely dark brown or greyish. Full length without caudal seta 1117 μm, with seta 1690 μm. Cephalosome as long as wide, maximum width close to posterior margin (Fig. 5: 1). Last segment of prosome with group of

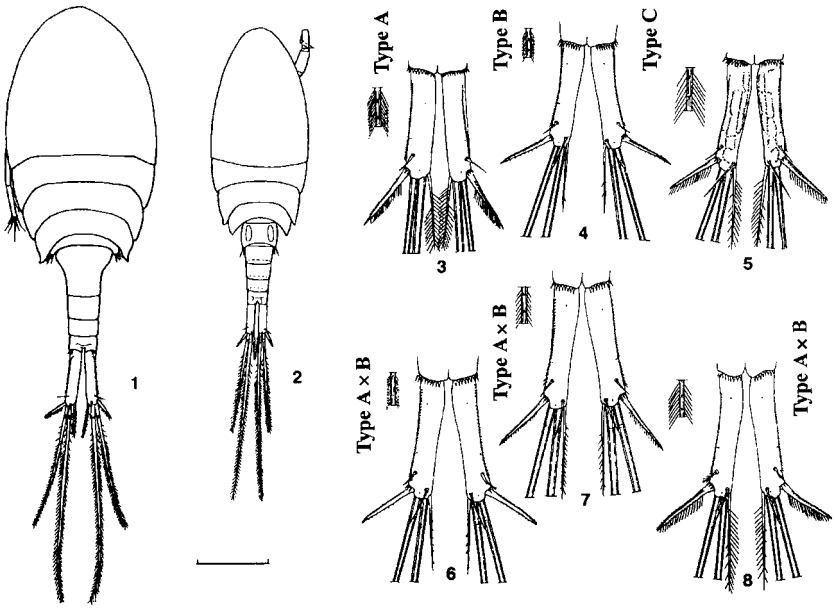


Fig. 5 *Eucyclops serrulatus* (Fischer, 1851) from the type locality. 1 — female, dorsal view; 2 — male, dorsal view; 3–8 — female caudal rami in forms A, B, C, and hybrids. Scale bar: 1,2 = 75 μm, 3–8 = 40 μm.

short lateral setules. Genital double somite as long as wide, with seminal receptacle as in Fig. 9: C. Caudal rami 5 times longer than wide, with longitudinal row of spinules along most of outer edge of each ramus. Six setae at distal part of ramus. Length proportions of four terminal setae, beginning from II to VI 1/4.8/7.5/1.31. Dorsal seta about half the length of seta VI, covered with long setules on both sides; II a spiny seta with dense setules on both sides, but longer on inner margin, about 3 times as long as dorsal seta yet distinctly shorter than seta VI. Antennule 12-segmented, reaching caudal edge of first free metasomal somite, with finely denticulated membrane along three distalmost segments (Fig. 9: 1; 10: 1). Setation of antennular segments, beginning from first, 8/4/2/6/4/2/2/3/2/2/3/8. First segment with curved row of spinules at its base; outermost spinules the longest. Antenna with 1-segmented basipodite, 3-segmented endopodite. Exopodite represented by a long, barbed seta. Posterior face of basipodite A2: apical group N1 with 4–6 long setules and group N2 with variable number of the same setules (2–4) subdistally along inner margin (Fig. 3: 3); with three diagonal and parallel rows of spinules (N3–5) and two groups of marginal spinules (N17) and (N15) (Fig. 10: 3). Anterior face of basipodite A2: 3–5 strong spinules subdistally (N8), long row of 15–17 relatively small spinules medially (N11 + N12); N13 and N14 represented by two isolated groups of spinules (Fig. 10: 2). Labrum (Fig. 10: 6) with 10–12 large teeth in central part and with two groups of small teeth on lateral outgrowths. Gnathobase of mandible (Fig. 10: 5) with six teeth, rudiment of endopodial segment with two long setae covered with long setules and one short naked seta. Maxillula (Fig. 10: 4) with six strong teeth and two strong setae; palp with seven setae, surface without ornamentation. Maxilla (Fig. 10: 7) 4-segmented, praecoxa with two strong median setae; syncoxopodite with two endites; proximal endite with one, distal endite with two setae; basal endite with two strong spines and one small seta near site of fusion of rudimentary endopodite. The latter 2-segmented; first segment with strong spine and slender seta; second segment with three elements in all. Maxilliped (Fig. 10: 8) 4-segmented, syncoxopodite with two strong setae medially and one small seta distally. Basipodite with two setae of different lengths and ornamented with strong spinules close to proximal setae and with two sets of spinules medially and subdistally along outer magin. First and second endopodal segments with two elements each. Swimming legs 1–4 (Figs 7 and 8) with 3-segmented rami, spine (roman numerals) and seta (arabic numerals) formula as follows:

	Coxa	Basis	Exopodite	Endopodite
Leg 1	0–1	1-I	I-1; I-1; III-5	0–1; 0–2; 1,I,4
Leg 2	0-I	1-0	I-1; I-1; IV-5	0–1; 0–2; I,4
Leg 3	0-I	1-0	I-1; I-1; IV-5	0–1; 0–2; 1,I,4
Leg 4	0-I	1-0	I-1; I-1; III-5	0–1; 0–2; 1,II,2

Distal segment of P4Enp elongated, 2.6 times as long as wide, with two strong apical spines; inner spine 1.32 times as long as outer spine. All setae on all segments of P1–P4 uniformly seta-like, not constricted, flanked with series of long setules at both edges. Setules at least twice as long as distance between them (Fig. 7: 8). Inner edge of basis of P1–4 with group of long setules. Coxa of P1–4 (Fig. 2: 3) with strong spine, dense hair-setae on inner side and large gap among short hair-setae on inner side. Caudal side of coxa with tiny spinules on inner

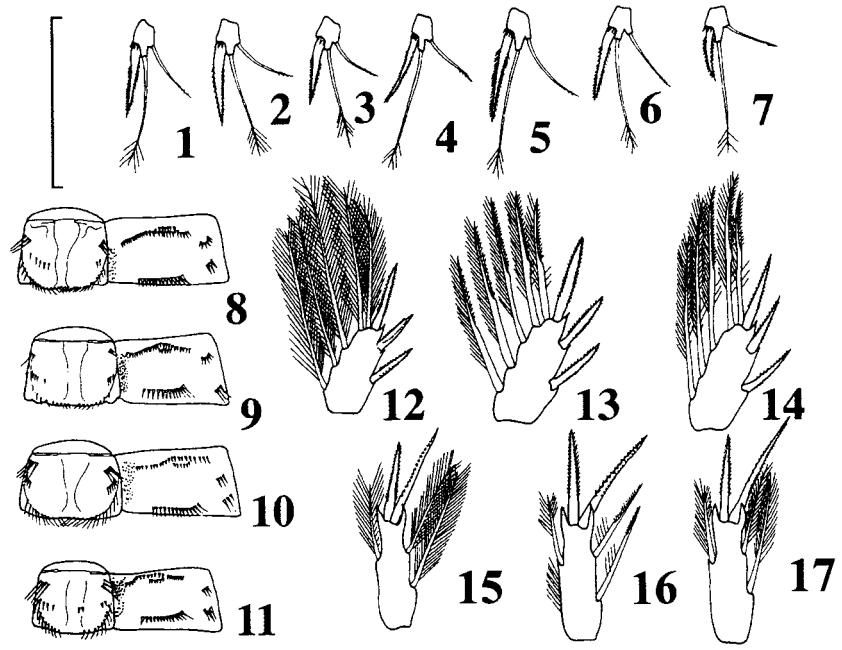


Fig. 6 *Eucyclops serrulatus* (Fischer, 1851), female, type locality. Variation in leg structure in forms A, B and hybrids A × B: P5 form A (1, 5), form B (2, 4) and hybrids A × B (3, 6, 7); intercoxal sclerite and coxa, form A (10), form B (9) and hybrids A × B (8, 11); distal segment exopodite P4 form A (12), form B (14) and hybrids A × B (13); distal segment endopodite P4 form A (15), form B (16) and hybrids A × B (17). Scale bar = 50 μm.

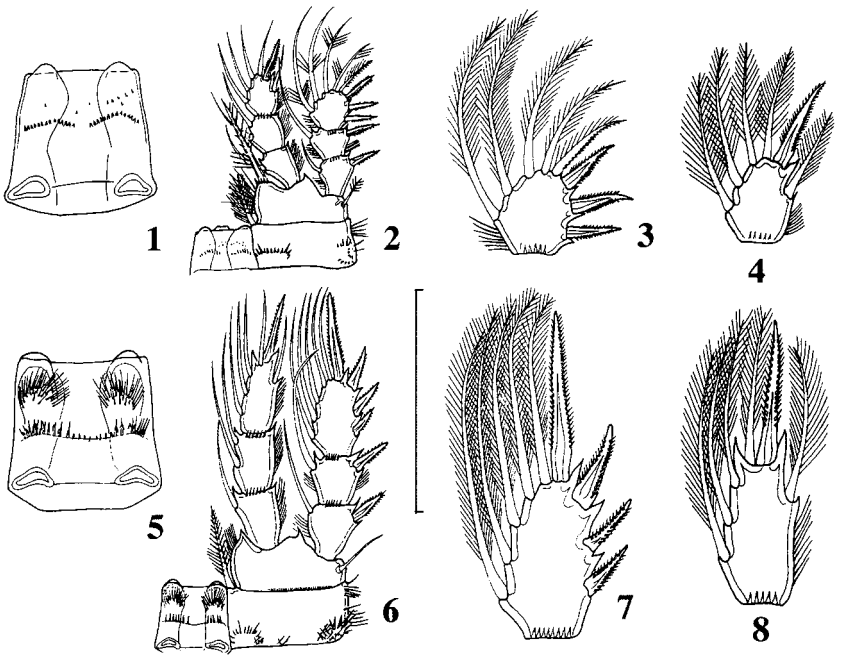


Fig. 7 *Eucyclops serrulatus* (Fischer, 1851), female form A from the type locality: 1 — intercoxal sclerite P1; 2 — P1, posterior; 3 — distal segment of P1exopodite; 4 — distal segment of P1 endopodite; 5 — intercoxal sclerite of P2; 6 — P2, posterior; 7 — distal segment of P2 exopodite; 8 — distal segment of P2 endopodite. Scale bar: 1, 3–5, 7–8 = 50 μm; 2, 6 = 100 μm.

side, not organized in groups, and groups of spinules and setules representing formula A – B – C + D – E – H (Fig. 2: 3). Intercoxal sclerite of P1 (Fig. 7: 1) with two protuberances, a row of small denticles around midway and two groups of finest spinules on body of protuberances, not extending beyond edge. Intercoxal sclerites P2–3 also with protuberances on free edge and with groups of setules as shown in Figs 7: 5 and 8: 1. Intercoxal sclerite P4 (Fig. 8: 8) with protuberances wide but not extending beyond edge of sclerite; two groups of incurved setules along edge, two groups of setules and spinules on body of sclerite. Rudimentary P5 (Fig. 9: 3) 1-segmented, with knife-like inner spine and two setae; outer seta sequal in length to spine, middle seta about 1.3–1.5 times as long as spine. Egg sacs with 35–40 eggs each.

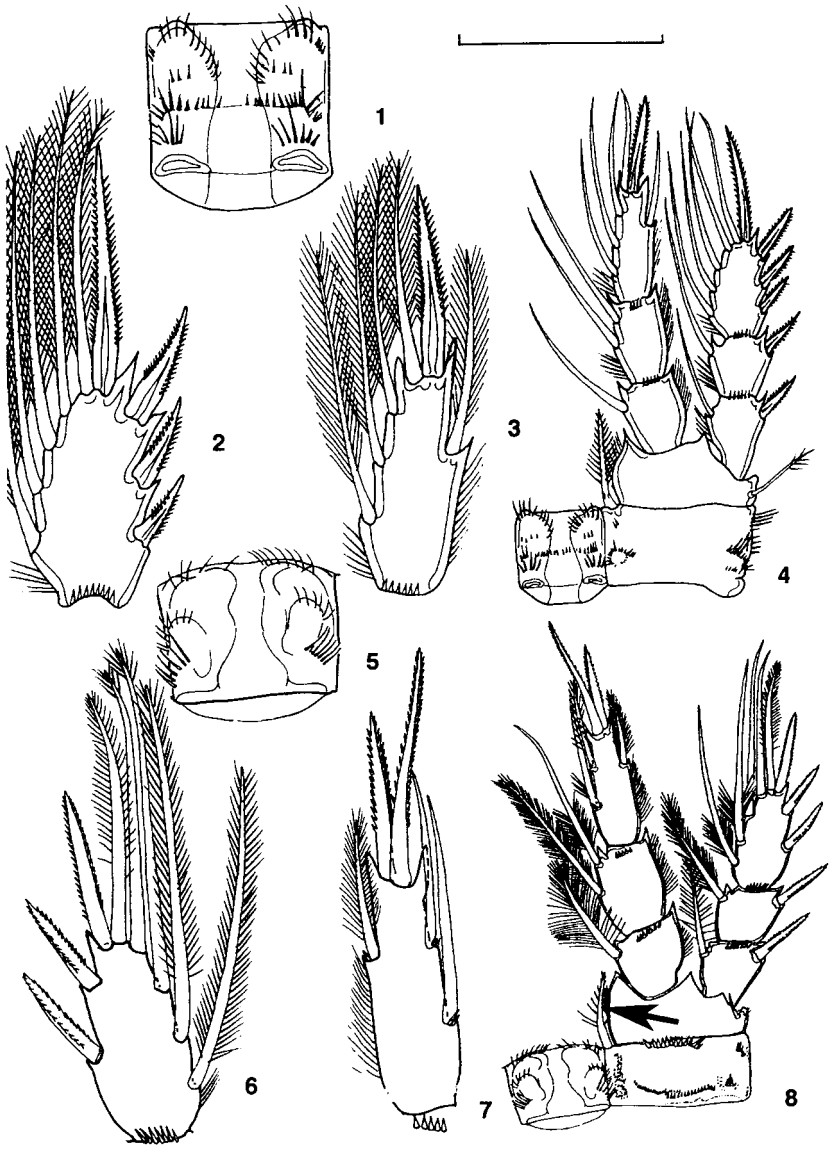


Fig. 8 *Eucyclops serrulatus* (Fischer, 1853), female form A from the type locality: 1 — intercoxal sclerite of P3; 2 — distal segment of P3 exopodite; 3 — distal segment of P3 endopodite; 4 — P2, posterior; 5 — intercoxal sclerite of P4; 6 — distal segment of P4 exopodite; 7 — distal segment of P4 endopodite; 8 — P4, posterior. Scale bar: 1–3, 5–7 = 40 μm; 4, 8 = 80 μm.

Male: body length 810 μm, with caudal setae 1230 μm. Cephalosome (Fig. 5: 2) 1.3 times as long as wide, with maximal width close to its posterior end. Last segment of prosome smooth, last urosomal segment with row of denticles on caudal side. Caudal rami (Fig. 11: 7) 4.3 times longer than wide, without lateral spinules. Slender innermost terminal seta about twice the length of spine-like outermost seta. Lateral seta shifted to dorsal side, with several spinules at base. Dorsal seta near insertion of innermost seta, about 0.8 times as long as outermost seta. Antennule (Fig. 9: 2) 14-segmented with six setae and three aesthetascs on first segment. Segments 2, 3, 4, 6 and 10 also with aesthetascs. Antennal basipodite (not shown) basically as in female, with 4–6 long setules posteriorly (N1, N2); anteriorly with three rows of strong spinules and additional row of spinules subdistally. Morphology of mouth parts and P1 basically as in female. Coxopodites and intercoxal sclerites P1–P3 as shown in Fig. 11: 1–3. Inner edge of basis P4 (Fig. 11: 4) with short setules, coxopodite P4 with strong spine, bearing 8–10 stiff inner hair-setae and 2–3 hair-setae at top and one at foot of spine. Coxopodite P4 (Fig. 11: 4) with narrow row of small spinules on inner side and several groups of spinules A – B – C + D – F posterior. Intercoxal sclerites P4 (Fig. 11: 4) with small protuberances, strong hair-setae on free edge, and three groups of setules on both sides. Distal segment of endopodite of P4 3 times longer than wide, with inner spine as long as segment and 1.4 times as long as outer spine. P5 (Fig. 11: 5) with inner spine slightly shorter than in female, outer seta as long as spine, middle seta 1.4 times

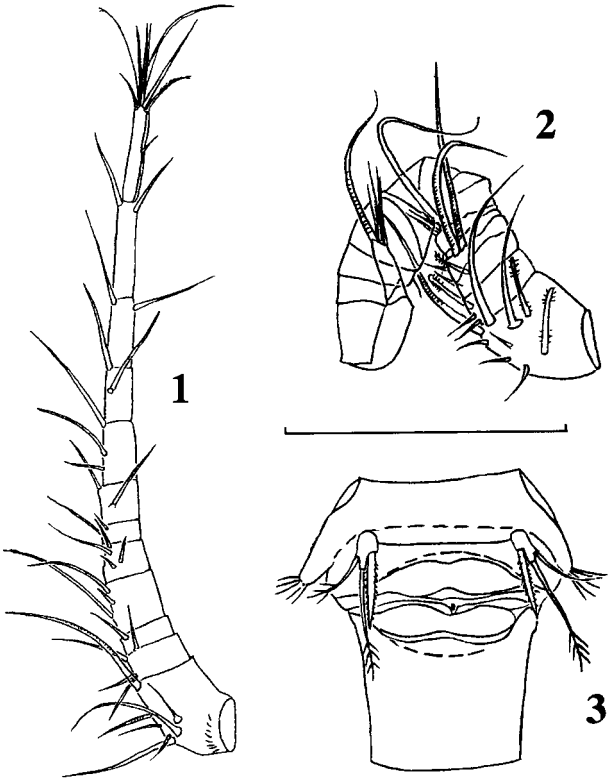


Fig. 9 *Eucyclops serrulatus* (Fischer, 1853), form A from type locality: 1 — A1, female; 2 — A1, male; 3 — genital double somite, female. Scale bar: 1 = 175 μm; 2, 3 = 150 μm.

as long as spine. P6 (Fig. 11: 6) with inner spine and two setae with length proportions, beginning from outer seta, 1/0.75/1.1.

Intrapopulation variation: there was appreciable variation between the topotypical specimens and other populations, mainly in the setation of the swimming legs and caudal rami. We identified three forms, of which specimens were deposited under accession number 55033 in the St. Petersburg collection mentioned earlier. A weak asymmetry (less than 5% totally) in body proportions and micropatterns was found in the topotypical population.

Form A (with long setules): plumose setae of the endopodite and exopodite segments of P1–P4 (Fig. 6: 12,17) and caudal setae with dense long setules (Fig. 5: 3). Intercoxal sclerite P4 (Fig. 6: 10) with dense long setules, sometimes as long as half of sclerite width. Innermost apical spine of P4Enp3 1.5–1.6 times as long as outermost apical spine and about 1.4 times as long as supporting segment. Outer seta relatively long, reaching middle of outermost apical spine. P4Exp3 (Fig. 6: 12) with relatively short distal spine, about 0.7 times as long as supporting segment and about half as long as distalmost lateral seta. P5 with relatively slender spine as long as outer seta (Fig. 6: 5). Caudal rami 5.0–5.7 times longer than wide, somewhat divergent; posterolateral spine-like seta with a row of spinules along outer margin and with long setules on inner edge; terminal accessory seta with long setules on both sides, about 1.3–1.5 times long than posterolateral seta (Fig. 5: 3). Inner and outer terminal setae with dense and long setules. At some distance from setal base, all individual setules are much longer than the distance between them. For variation of microcharacters of antennal basipodite and coxa of P4 in form A, see Tables 1, 2.

Table 1 Variation of microcharacters on antennal basipodite in *Eucyclops*. Hair-setae in roman, denticles in arabic numerals; numbers of characters correspond to Fig. 2.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
E. serrulatus form A St. Petersburg, Russia	VI–VIII	I–II	8–9	7–8	12–16	1–4	4–5	0	5–8	0	5–6	6–8	0	5–8	4–5	0
E. serrulatus form B St. Petersburg	V–IX	III–IV	7	9	13	4–5	3–4	0	5–6	0	5–6	7	2	3–4	4–5	0
E.serrulatus form A Norway	IV	I–II	6–10	8–9	15–18	0	4	0	6–7	0	5–6	6–7	4	3–6	6–7	0
E. hadjebensis Atlas Mts, Morocco	VI	I	7	9	12	0	5	0	2	2	7	6	0	0	8	0
E. speratus Lund, Sweden	0	0	0	7	12	0	5–8	0	3	7	3–5	4–5	?	2–5	4–6	5
E. dumonti Central Mongolia	0	0	0	7	15	0	4–7	0	6–8	0	8	8–9	4–5	5–8	6	0
E. turcomanus Herat Prov., Afganistan	VIII	III	5	6	18	3	4	5	4	0	5	6	2	7	5	0
E. pectinifer Philadelphia, USA	VII	5	6	7	18	0	7	0	4	1	5	8 + I	1	8	2–6	5
E. macruroides Lund, Sweden	0	0	3–11	7–8	9–12	0	0	0	7	0	0	0	0	0	6	0

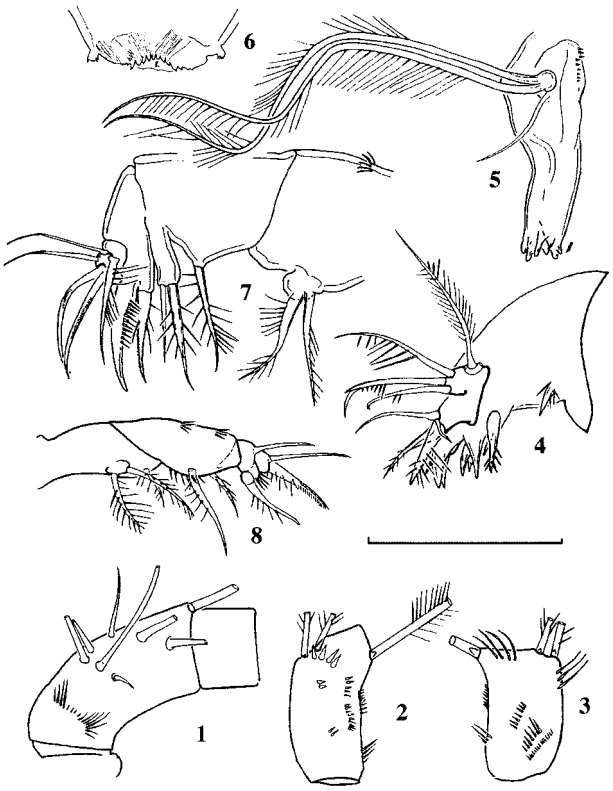


Fig. 10 *Eucyclops serrulatus* (Fischer, 1853), female form A from type locality: 1 — first segment of A1, 2 — basipodite of A2, anterior; 3 — basipodite A2, posterior; 4 — maxillule; 5 — mandible; 6 — labrum, 7 — maxilla; 8 — maxilliped. Scale bar: 1 = 4, 7, 8–50 μm; 5, 6 = 40 μm.

Form B (with short setules): typified by a reduction of the length of the setules of exopodal (Fig. 6: 14) and endopodal (Fig. 6: 16) setae of at least P4 and of the setae of the caudal rami (Fig. 5: 4). Distal half of setae of P4Enp3 and most setae of P4Exp3 (Fig. 6: 14,16) stylet-shaped, narrowed and with much shorter setules than on proximal part. Intercoxal sclerite of P4 with short marginal setules of about 1/3 or less the membrane width (Fig. 6: 9). Innermost distal spine of P4Enp3 (Fig. 6: 16). 1.25–1.44 (1.35 ± 0.02) times longer than outermost distal spine and 0.95–1.2 (1.04 ± 0.022) times longer than supporting segment. Outer seta of this segment relatively short, reaching distal part of outer distal process of segment and 0.5 times as long as outer apical element. P4Exp3 (Fig. 6: 14) with relatively long apical spine about 0.9 times as long as segment itself and about 0.7 times as long as nearest apical seta. Caudal rami (Fig. 5: 4) 4.0–5.1 (4.6 ± 0.1) times longer than wide; outermost spiny-form seta smooth; innermost seta hairless, 0.95–1.09 (1.04 ± 0.02) times as long as outermost seta. Inner spine of P5 (Fig. 6: 2) usually slightly longer than outer seta. For microcharacters of antennal basipodite as well of P4 coxa in form B, see Tables 1, 2.

Form C (pitted form): similar to form A but provided with a pitted cephalosome, urosome, caudal rami and first antennae (Fig. 5: 5). In the Petershoff ponds, form C was rare during spring (15%) but became more frequent in autumn (40%). Forms A and B were most abundant in spring (about 40% each); hybrids between them became dominant in summer and autumn.

Interpopulation variation in morphology: forms A, B, and C were also found in Ghent, but only forms A and B were present in the Tumen population. Most North African populations of *E. serrulatus*, including *E. serrulatus hadjebensis*, belong to or are close to form B. Forms A and B did not show variation in antennal basipodite microcharacters and these are consequently used here to define *E. serrulatus* (Tables 1, 2). *Eucyclops speratus ifniensis* Dumont & Decraemer, from the high mountain lake Ifni in the Morocco Atlas Mountains, is really a representative of form C of *E. serrulatus*. In North African populations, such phenotypes were rare.

Pore signature analysis: specimens of *Eucyclops serrulatus* from the type locality, from Ghent and from Tumen were compared.

Table 2 Micro-characters on coxa and intercoxal sclerite of P4 in *Eucyclops*. Coding corresponds to Fig. 2.

Species	A	B	C + D	E	F	G	H	J	I	II	III	Hair–setae on basipodite
E. serrulatus A StPetsb	Y	4–5	12	2–4	N	N	Y	Y	LH	SH	LH	Y
E. serrulatus B StPetsb	Y	5	12–14	3	N	N	Y	Y	SH	SH	LH	Y
E. serrulatus A Norway	Y	5	14	3–4	N	N	Y	Y	SH	SH	LH	Y
E. serrulatus A Ghent	Y	4	13	2	N	N	Y	Y	H	H	H	Y
E. s. hadjebensis	Y	5	10–11	2	N	N	Y	Y	LH	LH	LH	Y
E. speratus	Y	5	27	8	N	N	Y	Y	LH	H	LH	Y
E. dumonti	Y	2–3	10–12	2	N	N	Y	Y	SH	SH	SH	N
E. macruroides	Y		20	6					H	H	H	Y
E. turcomanus	Y	4	10	4	N	N	Y	Y	LH	SH	LH	Y
E. pectinifer	Y	3	10	5	N	N	Y	Y	LH	SH	LH	Y

Y, present; N, absent; ?, character not seen; H, hair-setae; LH, long hair-setae; SH, short hair-setae.

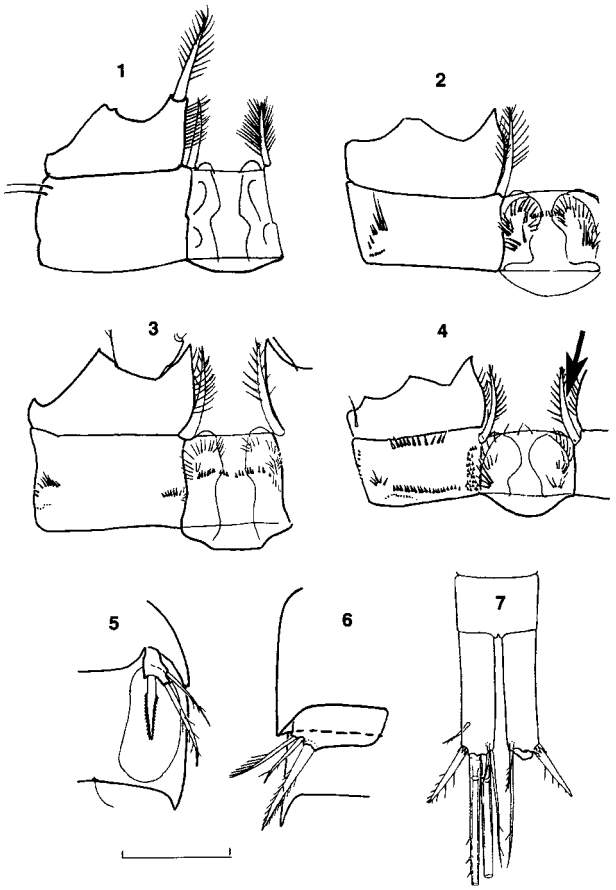


Fig. 11 *Eucyclops serrulatus* (Fischer, 1853), male form A from type locality: 1–4 — coxa with intercoxal sclerite of P1–4; 5 — P 5; 6 — P6; 7 — caudal rami, ventral. Scale bar: 1–6 = 50 μm; 1 = 100 μm.

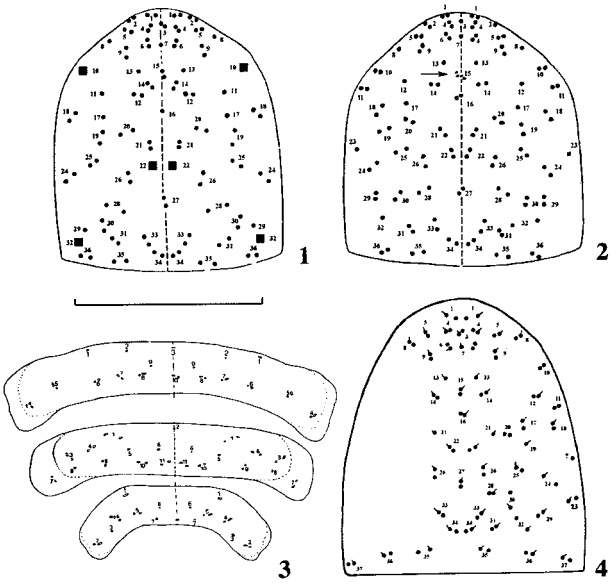


Fig. 12 *Eucyclops serrulatus* (Fischer, 1853), female, pore signatures: 1 — dorsal part of cephalosome, form B from the type locality; 2 — dorsal part of cephalosome, form A from Ghent, Belgium; 3-1-3 free somites of metasome, form A from the type locality; 4 — dorsal part of cephalosome, form A from the type locality. Scale bar = 250 μm.

The pore signature of the type population from Petershoff is described and remarks on the differences with other populations are given. In general, all pores were distributed symmetrically to the medio-sagittal axis of the body. The pore signature of *E. serrulatus* is described in detail, including zones not necessarily documented in other species. The rostrum has eight pores in four positions (I–IV), symmetrically distributed, and ten, again in four groups, posterior to the rostrum on the convex dorsum of the cephalosome (V–IX) (Fig. 3: 4). Cephalosome (Fig. 12): number of perforations 141–165; 131–155 dorsally, 21–22 laterally. The population from Belgium (155 pores) had more pores than that from Russia (131–136). Within the same population, variation in the number of pores affected positions 10, 11, 12, 16, 22, 24, 29, 30 and 32. This variation may involve positions completely missing (10, 22, 32), or doubletons being transformed to singletons or vice versa. In some cases, pores appear to form functional groups and can migrate from one position to another (e.g. exchange of pores was seen between groups 11 and 12). In one specimen from Ghent, position 15, otherwise fixed, was fragmented into four small pores. This appears to be a malformation. All other positions (1–9, 13–15, 21, 26, 28–31, 33–37) were constant in the three populations. Pore pairs 33–34 together form a U-shape, close to the mid-distal margin, which is characteristic of the *serrulatus*-group (see below for more examples). Metasome 1 (second pedigerous segment) (Fig. 12: 3a) has 25 perforations; in some Russian specimens (St. Petersburg and Tumen) it had 29 pores. Metasome 2 (third pedigerous segment) (Fig. 12: 3b): all three populations had 33 pores arranged in a similar pattern. Metasome 3 (fourth pediger) (Fig. 12: 3c): Belgian specimens had 28 pores, those from Russia, 21. Fifth pediger (Fig. 3: 3): two pores. Urosome (Fig. 3: 1,2,3): three populations with a constant number of 46 pores (20 dorsal, 17 ventral and 9 lateral) on urosome and furca, as follows: eight dorsal, six ventral and two lateral on the genital double-somite; three dorsal, three ventral and two lateral on second somite. Third somite with five pores: one dorsal, two lateral and two ventral. Anal somite with six pores: four dorsal and two ventral. Caudal rami (Fig. 3: 3): each ramus with four pores (two dorsal, two ventral), a pore close to the distal margin of the anal somite and a posterior one close to the insertion site of the dorsal seta (Fig. 3: 3). There is a ventrolateral pore close to the insertion site of the lateral setae, and one at the distal margin of the caual rami, as described by Fiers *et al.* (1996).

Table 3 Hybridization of forms A, B, and C from Ghent in intrapopulation trials.

Combinations	Offspring in first generation?	Hybrids in offspring	Offspring in second generation?
Type A × Type B	+	A–B	+
Type A × Type C	+	A	+
Type B × Type C	+	B	+
Type A × Type A	+	A	+
Type B × Type B	+	B	+
Type C × Type C	+	C	+

Table 4 Hybridization of *Eucyclops serrulatus* from Tumen and St. Petersburg (StPetsb).

Combinations	Sex ratio	Offspring in first generation	Offspring in second generation
Tumen males x StPetsb females	3 : 6	+	+
Tumen females x StPetsb males	6 : 3	+	+
Tumen males x Tumen females	3 : 6	+	+
StPetsb males x StPetsb females	6 : 3	+	+

Hybridization experiments

Intrapopulation hybridization: for these experiments forms A, B and C from Ghent were used (Table 3). A preliminary survey on *E. serrulatus* from the type locality in 1983 had revealed much intrapopulation variability. Based on differences in caudal seta construction, caudal rami proportion and presence of spine patterns on the body surface, morphotypes A, B, and C were identified (Figs 5, 6, Tables 1, 2). In 1999, these forms were found to co-occur in the same water-bodies, in ponds in Tavricheski Garden, St. Petersburg, in the botanical garden of the University of Ghent and in the park of the Agriculture Academy of Tumen (A and B only). No reproductive isolation was found among any of them. Hybridization of forms A and B produced animals with intermediate characters in caudal proportions and setal armament. Such intermediate forms were also found in samples collected from the field (Figs 5–8). Form C seems to be a recessive form, appearing only by combining parents with the same genotype. It was rare in natural populations at all three sites. In Petershoff its abundance increased in the autumn.

Table 5 Hybridization of *Eucyclops serrulatus* from St. Petersburg (StPetsb), Tumen and Ghent.

Combinations	Sex ratio	Offspring in first generation	Offspring in second generation
StPetsb males x Ghent Females	3 : 6	+	+
StPetsb females x Ghent males	6 : 3	+	+
StPetsb male x StPetsb females	6 : 3	+	+
Ghent males x Ghent females	6 : 3	+	+
Tumen females x Ghent males	6 : 3	+	+

Interpopulation hybridization: we hybridized 'distant' populations in two steps. In May 1999 we hybridized populations from Tumen and St. Petersburg using form A from Tumen and forms A and B from St. Petersburg. The next set of experiments was done in Ghent. We hybridized animals from St. Petersburg (forms A–C) with animals from Ghent (Ge). We also hybridized animals from Tumen (form A) with animals from Ghent (forms A–C) (Table 5). These experiments revealed no genetic isolation among these three widely disjunct populations.

Interspecies hybridization: we tested the hybridization of three species: *E. serrulatus* from the type locality (all three forms) with *E. speratus* (St. Petersburg area, Tavricheski Park) and with *E. macruroides* (St. Petersburg area, Tavricheski Park); *E. macruroides* was also crossed with *E. speratus*. No hybrids between *E. serrulatus*, *E. speratus* and *E. macruroides* were obtained (Table 6).

Table 6 Hybridization of three forms of *Eucyclops serrulatus* with *E. speratus* and *E. macruroides* (all from St. Petersburg, May–June 1999).

Combinations	*E. speratus* males	*E. speratus* females	*E. macruroides* males	*E. macruroides* females
Females *E. serrulatus* Type A	NO		NO	
Females *E. serrulatus* Type B	NO		NO	
Females *E. serrulatus* Type C	NO		NO	
Males *E. serrulatus* Type A		NO		NO
Males *E. serrulatus* Type B		NO		NO
Males *E. serrulatus* Type C		NO		NO
Females *E. macruroides*	NO		YES	
Females *E. speratus*	YES		NO	

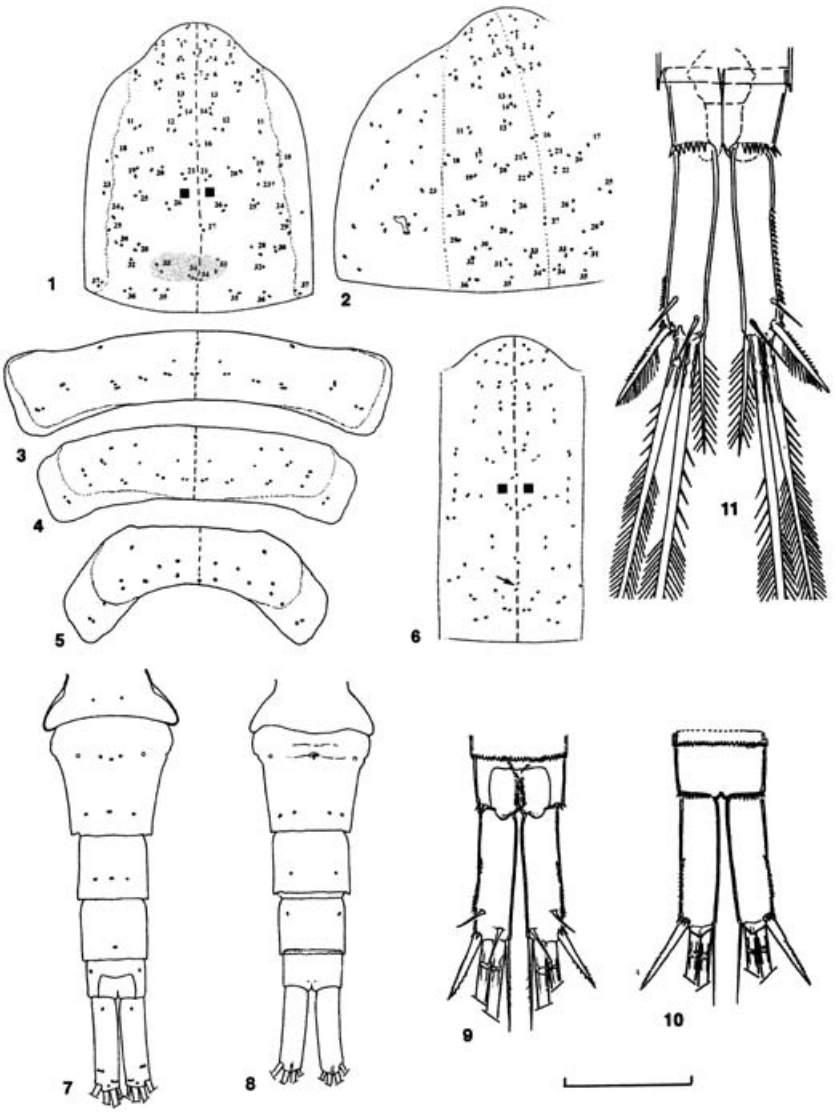


Fig. 13 *Eucyclops serrulatus hadjebensis* Kiefer, 1926 **comb. nov**., female from type locality, Source Vittel at El Hajeb, Middle Atlas Mountains, Morocco (1–10) and *Eucyclops 'asymmetricus'* = hybrid *E. serrulatus* forma A × *Eucyclops serrulatus hadjebensis* (11): 1, 2, 6 — cephalosome pore signature in different specimens; 3–5 — metasomite 2–4 pore signature; 7, 8 — urosomite, dorsal and ventral view, respectively; 9 — caudal rami, dorsal; 10 — caudal rami, ventral (another specimen); 11 — caudal rami, dorsal. Scale bar: 1–8 = 100 μm; 9, 10–75 μm; 9, 11 = 60 μm.

***2. Eucyclops serrulatus hadjebensis* Kiefer, 1926 comb. nov.**

Material examined: holotype on slides N 00834, 00835 and 00836 of Kiefer's collection in Karlsruhe, Germany, from a well in Oued Guigou, Morocco (leg. Dollfus). Specimens from the presumed type locality, Source Vittel near El Hajeb (loc. 8), Middle Atlas, Morocco, July 1971 (leg H. Dumont & W. Decraemer).

Reported from El Hajeb in the Middle Atlas (Kiefer 1926) and later found in an artesian pond at Guelma, Algeria (Roy & Gauthier 1927). Found in great numbers in a cold-water mineral spring ('Source Vittel') close to the type locality (Dumont & Decraemer 1977). Kiefer (1954) indicated the strong variability in *serrulatus*-like animals in Morocco.

Description: with short furcal rami (L/W = 3.5); longitudinal row of small spinules along outer margin of caudal rami located on distal half reduced to between half and one third the length of a ramus. Outermost seta with spinules on both sides, innermost seta with short stiff setules (Fig. 13: 9). Antennule of 12 segments with hyaline, finely denticulated membrane along three distalmost segments. Mouthparts (not shown) as in *E. serrulatus*. Basipodite of antenna with six hair-setae in position N1 and one setule in position N2 posteriorly, most other characters on both sides as in *E. serrulatus* (Table 1). P4: spine of coxopodite with a gap in the setules lining its outer margin, intercoxal sclerite with long setules along outer margin, other characters of P4 coxopodite as in *E. serrulatus* (Table 2). A feature stressed as diagnostic by Kiefer was that the setae on the distal segment of P4 exopodite are knife-shaped, with the constricted margin set with spinules, while the inner rim has 'normal' setules. This, in fact applies to P3 and P2 as well; only P1 has more or

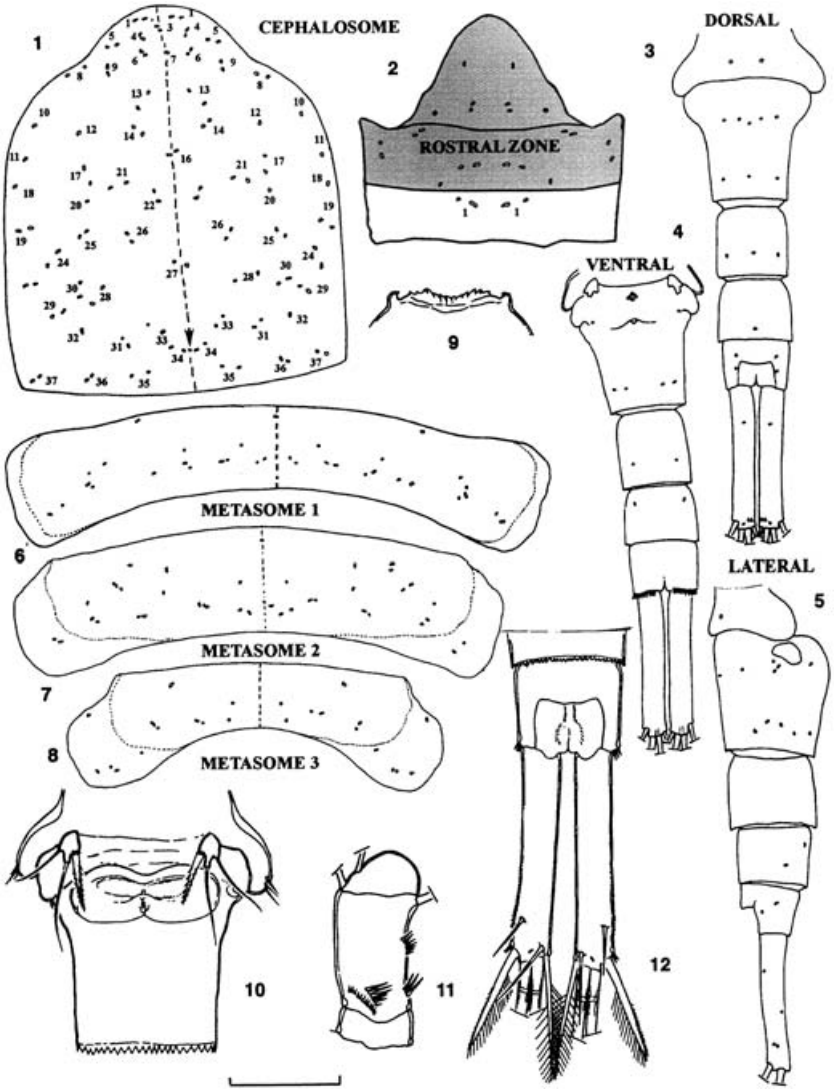


Fig. 14 *Eucyclops speratus* (Lilljeborg, 1901), female from the type locality, Lund area, Sweden: 1 — pore signature on cephalosome; 2 — rostral zone; 3–5 — urosome; 6–8 — metasome; 9 — labrum; 10 — genital double somite, ventral; 11 — A2 basipodite, posterior; 12 — caudal rami, dorsal. Scale bar: 1, 3–8, 10 = 100 μm; 2, 12 = 75 μm, 9, 11 = 50 μm.

less 'normal' cylindrical setae (but see Discussion for the significance of this character).

The pore pattern of the cephalosome and pedigers is shown in Fig. 13. The similarity with *E. serrulatus* is striking, with the typical U-shaped positions created by pore positions 33–34 (Fig. 13: 6) present. Some variability was noted, with positions 21 and 22 occasionally absent and, in one case, a couple of extra pores present in the opening to the 'U'. Pore pattern on urosome (Fig. 13: 7–8) indistinguishable from that of *E. serrulatus*.

3. Eucyclops speratus (Lilljeborg, 1901)

Synonymy: see Dussart & Defaye 1985.

Material examined: a number of females (accession number 55036 in collection N 96-03-16 of the Zoological Institute, Russian Academy of Sciences, St. Petersburg) from a small river in the vicinity of Lund, Sweden (Lilljeborg's type locality). From these, a female neotype was selected earlier by V. Alekseev and deposited under number 55034 in the collection of the Zoological Institute, Russian Academy of Sciences.

Description: full body length without setae 1090 μm; with caudal setae, 1490 μm.

Cephalosome as long as wide. Last segment of prosome with group of long seta-like spinules in lateral margin. Genital double somite (Fig. 14: 10) as long as wide, seminal receptacle typical for *Eucyclops*. Caudal rami (Fig. 14: 12) about 6.5 times longer than wide, with longitudinal row of small spinules along outer margin dorsally in distal half (but with significant variation in this row length in type group, range: 0.25–0.75 of caudal rami length). Length proportion of four terminal setae, beginning from III to VI 1/5/8/1.5. Dorsal seta about half as long as VI seta, and II seta about half as long as III seta. Antennule 12-segmented, reaching posterior border of first

thoracic somite, with a finely serrated hyaline membrane along last three segments (Fig. 14: 6). Setation of antennular segments, beginning with first, 8/4/2/6/4/2/2/3/2/2/3/7. Antennal basipodite without group of hair-setae distally on posterior face, with three parallel rows of spinules placed diagonally in central part and with two groups of spinules and setules laterally (Fig. 14: 11). On posterior face, four strong spinules on distal outgrowth, several spinules near base of long seta, with two rows of 10–12 spinules medially and two groups of two spinules (Table 1). Labrum with 6–8 teeth on distal edge, similar to that of *E. serrulatus*. Gnathobase of mandible with six teeth, rudiment of endopodal segment with two long setae and short naked seta. Maxillule with six strong teeth and two setae, strong seta in praecoxal arthrite; palp with seven setae, and row of spinules. Maxilla 4-segmented, praecoxa with two strong setae; coxa with medial strong spine and small seta and strong spine distally; basal endite with two strong spines and small setae close to endopodite, bearing two long spines, one plumose seta and two bare elements. Maxilliped 4-segmented, praecoxa and coxa with two strong medial setae and small setae distally; basis with two setae of different length and group of strong spinules near insertion of setae; first segment of endopodite with strong spine and group of setules around segments of endopodite, bearing one strong spine and two hairless setae. P1Exp3 and P4 with four spines and five setae; P2–3 with four spines and five setae. P1–3Enp3 with one spine and five setae. P4Enp3 elongated, 2.6 times as long as wide, with two strong distal spines, inner spine 1.4–1.6 times as long as outer spine. Inner edge of basis of P1–P4 with long setules. Coxa of P1–P4 with spine, bearing dense setules on both sides. Coxa P4 with row of long spines (25–27) along distal border and several other groups of spinules and setules posteriorly (Table 2). Intercoxal sclerite of P1 with protuberances, row of small spinules in middle part and without setules distally. Intercoxal sclerites of P2–P3 with protuberances and with groups of hair-setae distally. Intercoxal sclerite P4 without protuberances, with long and dense setules distally, and with two other groups of long setules on posterior face (Table 1). P5 1-segmented, with knife-like inner spine and two setae, outer seta shorter or slightly longer than spine, middle seta about 1.8 times as long as spine (Fig. 14: 10). Egg sacs with about 20 eggs each.

Male: not studied.

Pore pattern shown on Fig. 14: 1–8. Urosomal pore signature as in *E. serrulatus*.

4. Eucyclops turcomanus Lindberg, 1959

Synonymy: Eucylops abdelkader Dumont & Pensaert in Dumont (1979) (*nomen nudum*).

Material examined: ten females from Lindberg's original material and type locality, the spring Tagao Boraq at Qal'eh Nou, Herat Province, 990 m, Afghanistan, 24 October 1957. From these, a lectotype was selected and mounted on a slide in glycerol sealed with Canadian balsam, deposited in the Institute of Natural Sciences, Brussels (accession number of lectotype 30252). Eighteen females from Lake Oubeira, Algeria, 24 March 1977, and 12 females from a pond ('birket'), 165 km S. of Amman, Jordan (30°35′N, 35°50′E), 16 April 1978.

Described by Lindberg (1959) from Afghanistan, and found here again in 1960 (Lindberg 1960). The Mediterranean findings are the first non-mesasiatic records of what appears to be a species with an Irano-Turanian geographical range.

Brief description and pore patterns

Female: colour and habitus as in *E. serrulatus*. Antennule 12-segmented, with hyaline membrane fringing the last three segments. Mouthparts and genital segment basically as in *E. serrulatus*. Armament of basipodite of antenna as in Fig. 15: 3–4. P1: intercoxal sclerite with well-developed protuberances; some spinules at about half of sclerite width. Spine on inner distal corner of basipodite particularly strong, extending to halfway third segment of endopodite. P2 also with protruding tubercles on free margin of intercoxal sclerite, and semicircle of setules. Spines on exopodite robust. P3 with small protruding tubercles on intercoxal sclerite and two rows of stiff setae. Four of the five internal setae of the exopodite stylet-like. P4: apex of intercoxal sclerite fringed with long setules, divided into three groups. A series of 4–5 long and fine basal spines, and three rows of juxtaposed short spinules at about half length. Apical segment of exopodite with apical stylet-like seta. P4Enp3 and P5 as in *E. serrulatus*. Caudal rami about 5 times as long as wide, with complete longitudinal row of spinules. II spiny seta with row of spinules along outer margin, and three or four spinules along inner margin. Seta VI naked, slightly longer than outermost seta. IV–V setae peculiar, naked for about 3/5 of their length, and apical 2/5 set with numerous short spines.

Pore pattern (Fig. 15: 1): rostral and postrostral pores as in *E. serrulatus*. Axial pores 15 and 27 missing; positions 10, 11 and 21 also missing. Positions 18 and 23 with three, not two or a single pore. Posterior U (positions 33 and 34), and posterior doublets 35–37 typical for the *serrulatus*-group. First and third metasomal segments largely as in *E. serrulatus*, but second with several extra pairs of pores, especially in posterior-median sector. Urosomal pore pattern as in *E. serrulatus*.

Male: not studied.

5. Eucyclops dumonti Alekseev, 2000

Material examined: 26 females from the type locality, lake Bur-Nuur, *c.* 100 km N. of Ulan Bator, Central Mongolia. Type series

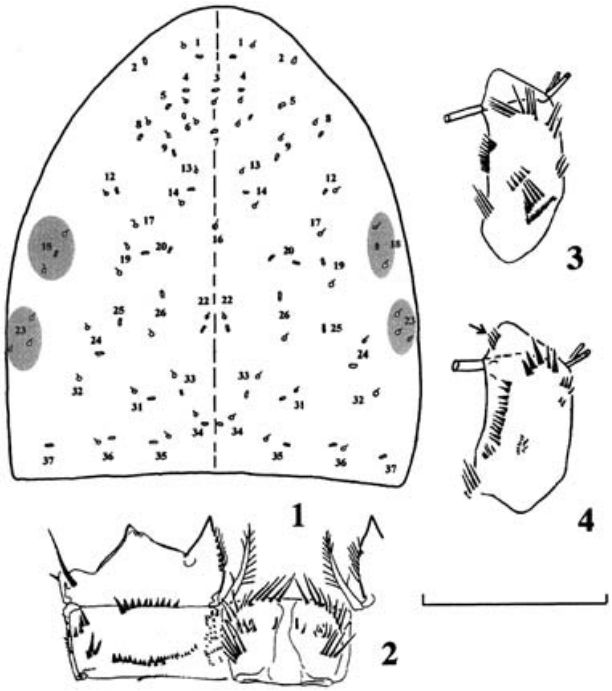


Fig. 15 *Eucyclops turcomanus* Lindberg, 1952, female from type locality, spring Tagao Boraq at Qal'eh Nou, Herat Province, Afghanistan. 1 — cephalosome pore signature; 2 — coxa with intercoxal sclerite of P4, posterior; 3, 4 — basipodite of A2 in anterior and posterior view, respectively. Arrow indicates spinule pattern missing in *E. serrulatus*. Scale bar: 1 = 100 μm; 2 = 75 μm, 3, 4 = 50 μm.

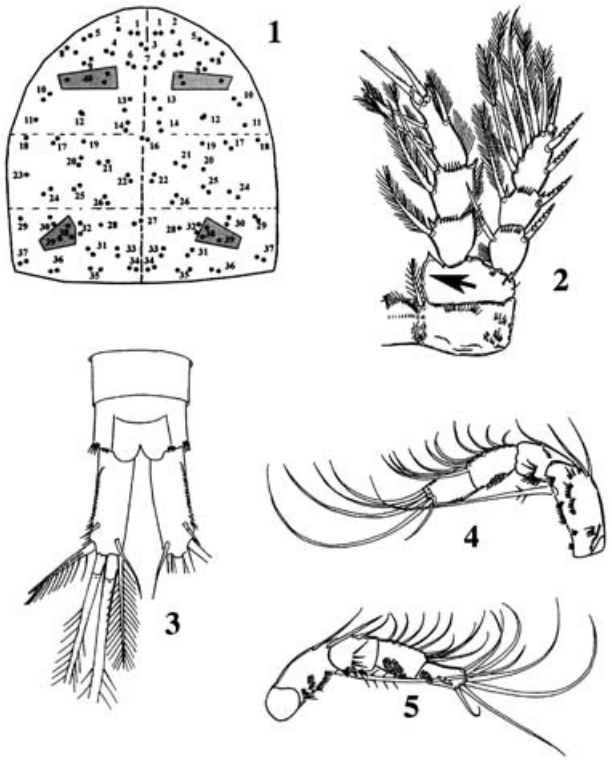


Fig. 16 *Eucyclops dumonti* Alekseev, 2000, female from type locality, Lake Bur-Nuur, Central Mongolia: 1 — cephalosome pore signature; 2 — P4; 3 — caudal rami dorsal; 4 — A2, anterior; 5 — A2, posterior. Scale bar: 1 = 100 μm; 2–3 = 75 μm; 4–5 = 60 μm.

in the Zoological Institute of the Russian Academy of Sciences, St. Petersburg (holotype accession number N 55021).

Brief description and differential diagnosis: for a full description, see the original paper (Alekseev 2000). Female length (without setae *c*. 930 μm) and habitus much like that of *E. serrulatus*. Caudal rami short, about 2.9 times al long as wide, similar to *E. hadjebensis*, but with complete longitudinal row of spinules (Fig. 16: 3). Shape and ornamentation of caudal setae similar to *E. serrulatus* type A. Mouthparts as in *E. serrulatus*. Basipodite of antenna on posterior face without long setules subdistally as in *E. serrulatus* (Fig. 16: 4–5). Highly diagnostic armament of leg base of P4, which is setulose in *E. serrulatus* and all *serrulatus*-like taxa studied here, but naked in *E. dumonti* (Fig. 16: 2, arrow). Coxal spine of P4 fully setulated along its outer margin in *E. dumonti* (Fig. 16: 2), while there is a gap in the setulation in *E. serrulatus*.

For a full description of the male, which is structurally similar to the female, see Alekseev (2000).

Pore patterns: the pore pattern of the cephalosome (Fig. 16: 1) reveals most of the typical pores found also in *E. serrulatus*, including the mid-posterior U formed by positions 33 and 34. However, some extra positions occur, both caudally (38 and 39) and frontally (40). The metasomal pattern (not shown) is similar to that in *E. serrulatus*, and the urosomal pattern conforms to that of the genus. For information on the 18S rDNA gene, and the position of *E. dumonti* in the phylogeny of the *E. serrulatus*-group, see below.

***6. Eucyclops pectinifer* (Cragin, 1883)**

Material examined: 56 individuals (17 females, 10 males and 29 copepodites) from the littoral of Gees Lake near Philadelphia, Pennsylvania, USA, in the same general area (New England) as Cragin's type locality (a pond on the campus of Harvard University) were studied. Female neotype bears accession number 55037 and is deposited in the Federal Collection of the Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia.

Justification of a neotype: Francis W. Cragin, a native of Kansas, spent the year 1880–1 at the Lawrence Scientific School of Harvard College and was invited to the laboratory of marine biology of Alexander Agassiz at Newport, RI. Although mainly

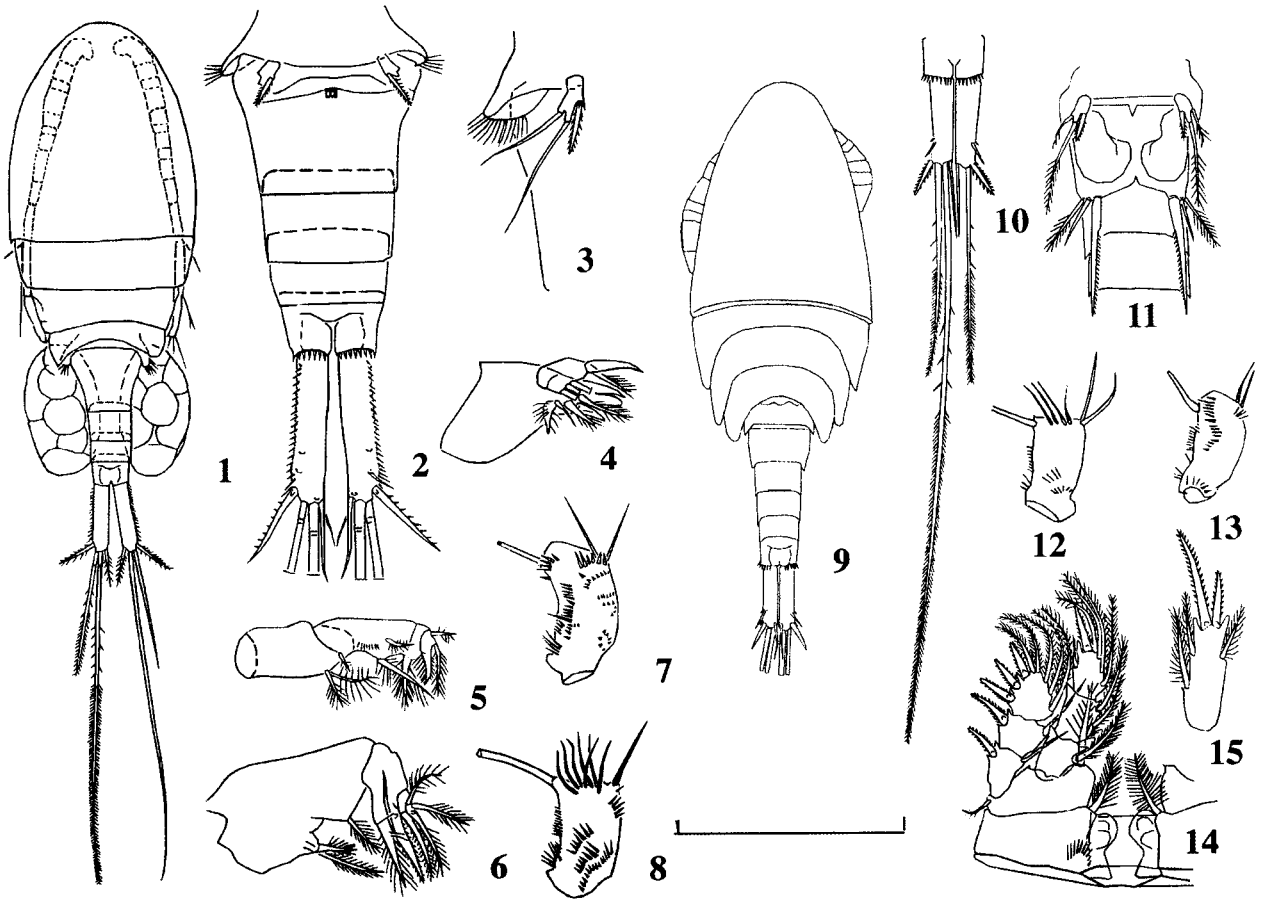


Fig. 17 *Eucyclops pectinifer* (Cragin, 1883) neotype from Gees Lake near Philadelphia, PA, USA. 1–8 female, 9–15 male: 1 — body, dorsal; 2 — urosomites and caudal rami, ventral; 3 — P5; 4 — maxillule; 5 — maxilliped; 6 — maxilla; 7, 8 — A2 basipodite, anterior and posterior, respectively; 9 — body, dorsal; 10 — caudal rami, dorsal; 11 — urosomites with P5–P6; 12, 13 — basipodite of A2, posterior and anterior, respectively; 14 — P1; 15 — distal segment of P4 endopodite. Scale bar: 1, 9 = 400 μm; 2, 10 = 200 μm, 14 = 150 μm; 14 = 75 μm; 3–6, 12, 13, 15 = 70 μm.

a herpetologist, he collected plankton at Cambridge, MA, and published a paper (Cragin 1883) about the copepods found therein. He later returned to Kansas, became an administrator, and never published on zooplankton again (Willard 1938). He seems not to have conserved his early 1880s collections. Nothing could be found in Harvard Museum, and nothing in the Smithsonian Institution either (*fide* Frank Ferrari); we are therefore compelled to accept that all material, including types, has been lost.

Description

Female: body dark brown, sometimes black; length without caudal setae 1030 μm; with caudal setae, 1610 μm. Cephalosome (Fig. 17: 1) 1.2 times as long as wide, maximum width at last third. Last segment of prosome with lateral group of stiff setules. Genital double somite as long as wide; seminal receptacle as shown in Fig. 17. Caudal rami (Fig. 17: 2) 5 times longer than wide, with longitudinal row of spinules along most of outer edge of each ramus. Lateral seta about half dorsal seta and about 0.3 times the outermost seta. Length proportions of four terminal setae, beginning from III to VI 1/3.2/8.7/1.1. Antennule 12-segmented, with fine denticulated or almost smooth hyaline membrane along three distalmost segments. Setation of antennular segments beginning from first: 8/4/2/6/4/2/2/3/2/3/3/8. First segment with curved row of spinules at its base. Anterior surface of antennal basipodite (Fig. 17: 7) with group of spinules near insertion of single seta; six long spinules near insertion of two setae along inner margin; longitudinal group with one long setule in distal part (arrow); several groups of tiny spinules. Posterior face of antennal basipodite (Fig. 17: 8) with seven long setules subdistally and group of short setules along inner margin distally; with three diagonal and parallel rows of spinules (N3–5) and one group of marginal spinules (N17). Labrum (not shown) as in *E. serrulatus*.

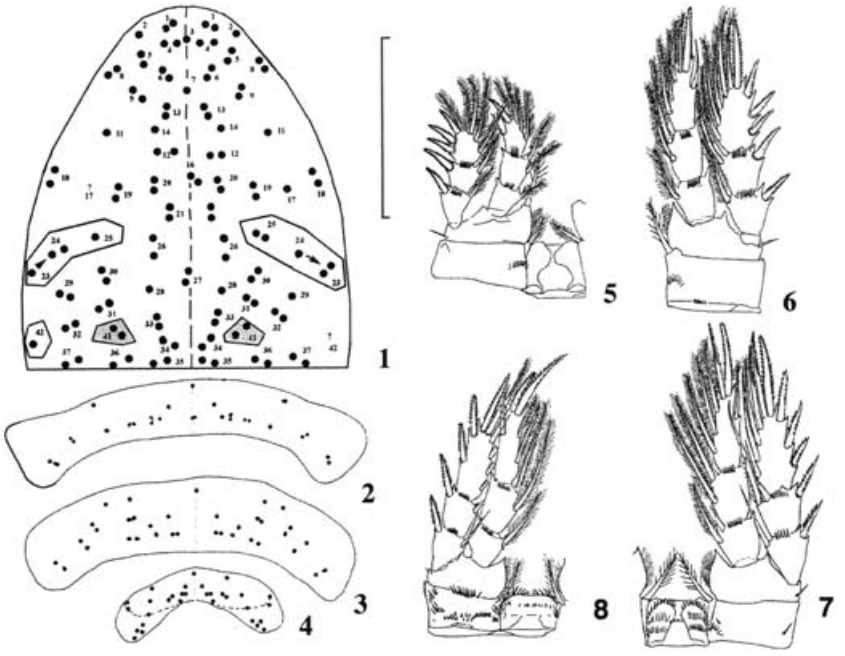


Fig. 18 *Eucyclops pectinifer* (Cragin, 1883) female, neotype from Gees Lake: 1 — cephalosome pore signature; 2–4 — metasomal pore signatures; 5–8 = P1 – P4. Scale bar: 1–4 = 150 μm; 5–8 = 75 μm.

Gnathobase of mandible (Fig. 17) with six large teeth, basis of mandible with long row of long setules around rudiment of endopodital segment with two long and one small seta distally. Maxillule (Fig. 17: 4) biramous with seven strong teeth and two small and one strong seta at praecoxal arthrite; palp with seven setae, surface without ornamentation. Maxilla (Fig. 17: 6): praecoxa with two strong median setae. Maxilliped (Fig. 17: 5) 4-segmented, syncoxopodite with two endites; proximal endite with one, distal endite with two setae; basal endite with two strong spines, one small seta and group of strong spinules near site of fusion of rudimentary endopodite. The latter 2-segmented; first segment with two strong spines and slender seta; second segment with three elements in all. Swimming legs 1–4 with 3-segmented rami (Fig. 18: 5–8), spine (roman numerals) and seta (arabic numerals) formula as follows:

	Coxa	Basis	Exopodite	Endopodite
Leg 1	0–1	1-I	I-1; I-1; III-5	0–1; 0–2; 1,I,4
Leg 2	0-I	1-0	I-1; I-1; IV-5	0–1; 0–2; 1,I,4
Leg 3	0-I	1-0	I-1; I-1; IV-5	0–1; 0–2; 1,I,4
Leg 4	0-I	1-0	I-1; I-1; III-5	0–1; 0–2; 1,II,2

Distal segment of P4Enp elongated, 3.2 times as long as wide, with two strong apical spines; inner spine 1.32 times as long as outer spine. Inner margin of basis P1–4 with long setules. Coxa of P1–4 with strong spine, dense hair-setae on inner side and large gap among short hair-setae on inner side. Caudal side of coxa with tiny spinules on inner side, not organized in groups, and groups of spinules and setules representing formula A – B – C + D – E – H (Fig. 18: 8). Intercoxal sclerite of P1 (Fig. 18: 5) with two protuberances, a row of small denticles around midway and two groups of finest spinules on body of protuberances, not extending beyond edge. Intercoxal sclerites of P2–P3 also with high protuberances on free edge and groups of setules as shown in Fig. 18: 6,7. Intercoxal sclerite of P4 (Fig. 18: 8) without protuberances with row of stiff incurved setae near outer edge and group of spinules at mid-length; two groups of incurved setae along edge, two groups of setules and spinules on body of sclerite. Coxa of P4 with strong spine bearing stiff setae on inner margin and only 1–2 setae on outer margin. Setae of P4Exp3 and apical seta of P4Enp3 knife-shaped, as in type B of *E. serrulatus*. All other limb setae cylindrical. P5 (Fig. 17: 3) 1-segmented with short inner spine and two setae; outer seta subequal in length to spine, middle seta about 2.5 times as long as spine. Egg sacs (Fig. 17: 1) divergent, with 20–25 eggs each.

Male: body length 716 μm; with caudal setae, 1266 μm. Cephalosome (Fig. 17: 9) 1.2 times as long as wide, with maximal width close to its posterior end. Last segment of prosome without lateral setae. Caudal rami (Fig. 17: 10) 4.3 times longer than wide, seta III about half of seta VI; V seta 2.8 times longer than seta IV. Antennule 14-segmented; antennal basipodite (Fig. 17: 12–13) as in female, with four long setules posterior (N1, N2) and two diagonal rows of 4–5 spines each; anterior with fewer shorter and slender spines than in female. Morphology of mouth parts (not shown) and P1 (Fig. 17: 14) as in female. Coxopodites and intercoxal sclerites P1 as shown in Fig. 17: 14. Intercoxal sclerites P4

(not shown) and spinules on coxal segment basically as in female. Distal segment of endopodite of P4 3.1 times as long as wide, with inner spine 1.8 times as long as outer spine (Fig. 17: 15). P5 (Fig. 17: 11) with inner spine shorter than in female, outer seta 1.15 as long as spine, middle seta about 3 times as long as spine. P6 (Fig. 17: 11) with strong inner spine reaching fourth pediger and two setae with length proportions, beginning from outer seta, 1/0.87/1.8.

Differential diagnosis: Cragin's picture of *E. pectinifer* (Fig. 4: 3) shows an unmistakable *Eucyclops* of the *serrulatus* group, with strong serra and setulation of the terminal furcal setae of type A. He found the female of his species easy to separate from *E. serrulatus* by the length of the furcal setae: the outer seta is less than half as long as the inner one. Additional distinctive characters occur on the caudal side of the antennal basipodite: the lateral group of hair-setae is more than 3 times shorter than the top group, and the first (top) diagonal line consists of long spines similar in length to the spines of the second line. In *E. serrulatus* and most other species studied, the longest spines of the antennal basipodite are in the second row. The frontal side of the antennal basipodite has a longitudinal group with at least one long hair-like spine in its distal part. Morphometric differences between *E. pectinifer* and *E. serrulatus* from Petershoff, type A are given in Table 7.

Pore pattern (Fig. 18: 1–4): rostral and postrostral pores as in *E. serrulatus*. Cephalosome with axial pore position 15 lacking. Position 10 apparently absent (but this also happens at times in *E. serrulatus*); asymmetries (no pore, or single pore on one half of the body, double pore on the other) in positions 17, 23, 24, 25, 29, 42. Specific and supplementary positions 41 and 42 present (asymmetric). Typical posterior U of *serrulatus* group present. In one specimen, an axial pair of pores inside the opening of the U. Positions 23–24–25 again seem to form a functional unit, with pore capable of migrating between positions (identity of position 23 is slightly uncertain). Metasome 1 with position 2 absent, metasome 2 with position 8 absent. Metasome 3 with an extra pair of lateral pores and positions 6 and 7 double (single in *serrulatus*). Urosomal pore pattern as in *E. serrulatus*.

Male: not studied.

7. *Eucyclops macruroides* (Lilljeborg, 1901)

Material examined: 12 males, 23 females, collected in a small river in the vicinity of Lund, Sweden (*terra typica*), in collection of V. Alekseev.

Eucycops macruroides does not belong to the *serrulatus* group; it has a row of robust denticles along the terminal segment of the 12-segmented antennule, and extremely long caudal rami. Numerous other anatomical microcharacters also set it well aside (see Table 2). It is here included to serve as an in-genus outgroup in the phylogenetic evaluation of the pore signature.

Female pore pattern (Figs 19, 20): rostral and postrostral positions as in *E. serrulatus*, and therefore likely to be diagnostic at the genus level only. Cephalosome with reduced pore complement. Positions 8, 10, 11, 15, 19 (?), 22, 23 (?), 31, 32 and 34 missing. The last couple of pores particularly significant, because they are part of the posterior U-shape, which is only partly expressed in this species. Some positions are single, against double in the *serrulatus*-group (4, 5 and 30), but here as well as in other species, left-right asymmetries occur (e.g. position 2, which could be either a singleton or a doubleton). The metasomal pattern is puzzling, because many small, asymmetric pores were found. It appears that metasome segments 1 and 2 have at least two extra pairs of lateral pores, while metasomal segment 3 has extra pores in the basal zone of the mid-dorsum. Apparently, this species shows a tendency for a

Table 7 Morphometric comparison of *Eucyclops pectinifer* with *E. serrulatus*.

Measurements and indices	***E. pectinifer***			***E. serrulatus* StPetsb area**		
	Range	Mean ± SD	*n*	Range	Mean ± SD	*n*
Length (μm)	1000–1080	1028 ± 0.015	12	970–1130	1043 ± 0.018	12
L/W gen. som.	1.00–1.02	1.01 ± 0.011	12	0.842–1.09	0.942 ± 0.034	12
L/W c.r.	4.85–5.35	5.15 ± 0.031	24	3.86–6.00	4.79 ± 0.061	28
Si/So c.r.	1.00–1.14	1.14 ± 0.015	24	0.95–1.53	1.486 ± 0.16	27
Si/L c.r.	0.56–0.59	0.583 ± 0.16	24	0.513–0.956	0.690 ± 0.126	28
Smi/Smo c.r.	2.00–2.63	2.15 ± 0.082	19	1.267–1.568	1.482 ± 0.085	22
L/W endP4	3.12–3.21	3.18 ± 0.234	24	2.13–3.00	2.54 ± 0.247	28
SPi/SPe endP4	1.68–1.85	1.79 ± 0.115	24	1.214–1.583	1.317 ± 0.105	28
SPi/L endP4	0.91–1.21	1.1 ± 0.051	24	0.882–1.125	1.04 ± 0.069	28
AS1/AS3	1.23–1.54	1.41 ± 0.061	22	1.267–1.54	1.427 ± 0.082	12

SD, standard deviation; *n*, number; StPetsb, St. Petersburg; L/W, length to width ratio; gen. som., genital somite; c.r., caudal rami; Si/Se, ratio of innermost setae to outermost seta; Si/L c.r., ratio of innermost setae to caudal ramus. Smi/Sme, ratio of inner medial setae to outer medial seta; endP4, terminal endopodite segment of P4; SPi/SPe, internal to outer spine ratio; SPi/L, ratio of internal spine to length of distal endopodite segment of P4; AS1/AS3, ratio of first to third segment of antennula.

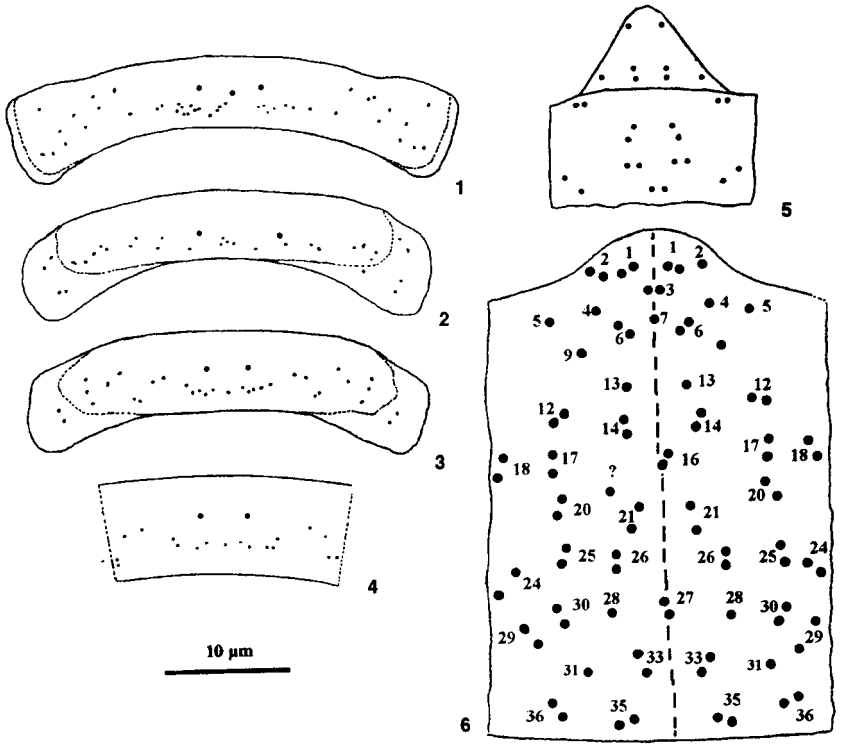


Fig. 19 *Eucyclops macruroides* (Lill.), female from Lund, Sweden: 1–4 — metasomal pore signatures; 5 — rostral signature; 6 — cephalosome pore signature, central part. Scale bar: 1–4 = 100 µm; 5–6 = 85 µm.

fragmentation of pores into porules. Urosomal pore pattern as in *E. serrulatus*.

Male: not studied.

***8. Afrocyclops gibsoni* (Brady, 1904)**

Material examined: two females, littoral of Lake Faguibine, Internal delta of Niger River, Mali, 1 March 1976 (for more information about the locality, see Dumont *et al*. 1981). The type locality is Natal, South Africa, but the species is distributed Africa-wide.

Brief description: habitus, size and gross morphology much like a *Eucyclops*, but caudal rami strongly elongated (up to 10 times as long as wide) and completely devoid of longitudinal row of spinules (Fig. 20: 6–7). Pore pattern: see Fig. 20: 1–5. Even though *Afrocyclops* is generically distinct from *Eucyclops*, it proved possible to homologize many pore positions. On the cephalosome, even more positions are vacant than in *E. macruroides*: 4, 8, 12, 13, 15, 16, 17, 23, 26, 27, 28, 30, and 34. The number of axial positions is reduced to two, and the posterior U is absent. Positions 2, 20, 21 and 22 were singletons, position 18 was asymmetric, and position 19 had three pores (pore exchange between 19 and 20?). It should be noted that 21 and 22 could have been interpreted as a single position; that both are represented by a singleton was thus an arbitrary decision. Free metasome segment 1 (Fig. 20: 2) with only six pores on either side of the longitudinal axis is also relatively deficient in pores, but metasome segment 2 has a surplus of pores (Fig. 20: 3–4), which seem to vary quite a bit. Metasome segment 3 has seven pores on each side of the midline, of which one is a triplet (Fig. 20: 5). Urosome pore pattern distinct from that of *Eucyclops* (Fig. 20: 2–5): genital segment dorsally with a triplet of pores about halfway, and a row of four posterior pores. Second segment dorsally as in *Eucylops*, but third also with three pores, and anal segment with six dorsal pores (against four in *Eucyclops*).

Phylogenetic relationships

Cephalosome pore pattern-based phylogeny: the coding system defining the state of the pore-characters is shown in Table 8. A consensus tree including eight taxa, with *E. macruroides* and *A. gibsoni* as outgroups, is shown in Fig. 21: it reveals all taxa in the *serrulatus*-group to be closely related. *E. serrulatus* and *E. hadjebensis* cluster together, and form a clade distinct from that containing *E. speratus*, *E. dumonti* and *E. pectinifer*. *Eucylops dumonti* and *E. speratus* come out as close relatives, a position also supported by morphology. *E. turcomanus* is the most distant member of this group although with low bootstrap support. *Eucyclops macruroides* and *Afrocyclops gibsoni* have high bootstrap support and belong to a clade that could be called a 'non-*serrulatus* group'.

Table 8 Coding system for pore positions on dorsum of cephalosome (0 = position vacant, 1 = 1 pore, 2 = doublet; 3 = triplet).

	1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2
serrulatus Petersburg type B	2 2 2 2 2 2 1 2 2 2 2 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 0 0 0 0 0
hadjebensis	2 2 2 2 2 2 1 2 2 0 2 2 2 2 0 2 2 2 2 2 2 2 1 2 2 2 2 2 2 2 2 1 2 2 2 2 2 2 0 0 0 0 0
speratus	2 0 2 2 2 2 1 2 2 1 1 1 2 2 0 2 2 2 1 2 1 2 2 0 2 2 2 2 2 2 2 2 1 2 2 2 2 2 0 0 0 0 0
dumonti	2 1 2 2 2 2 1 2 2 2 1 1 2 2 0 2 2 2 1 1 2 2 2 1 2 2 2 2 1 2 2 2 2 2 2 2 2 2 2 1 3 0 0
turcomanus	2 1 2 2 1 2 1 2 2 2 0 0 2 1 2 0 1 1 3 2 2 0 2 3 2 1 1 0 1 0 0 2 1 2 2 2 2 2 0 0 0 0 0
macruroides	2 2 2 1 1 2 1 0 1 0 0 2 1 2 0 2 2 2 2 0 2 2 0 0 2 2 2 2 2 1 2 2 1 0 2 0 2 2 2 0 0 0 0 0
pectinifer	2 1 1 2 2 2 1 2 2 0 1 2 2 1 0 2 1 2 2 2 2 2 0 1 2 2 2 2 1 2 2 2 2 2 2 2 2 0 1 0 0 0 2 1
Afrocyclops gibsoni	2 1 2 0 2 2 1 0 2 2 2 2 0 0 2 0 0 0 2 3 1 1 1 0 2 2 0 0 0 1 0 1 1 2 0 2 2 2 2 0 0 0 0 0

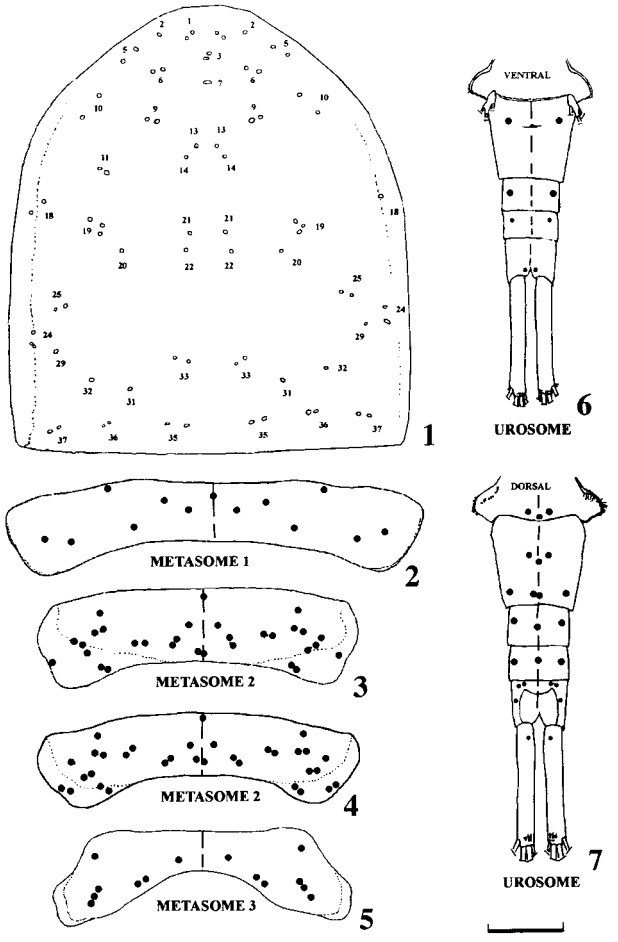


Fig. 20 *Afrocyclops gibsoni*, female from Mali, pore signature: 1 — cephalosome; 2–5 — metasomites 2–5; 6, 7 — urosomites in ventral and dorsal view. Scale bar 1–7 = 75 μm.

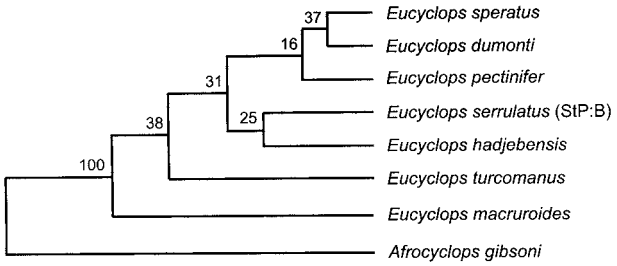


Fig. 21 Maximum parsimony (MP) tree based on pore signature data. The MP analysis used 1000 replicates to assess the reliability of individual branches in the phylogenetic tree; bootstrap support expressed as a percentage.

18S rDNA-based phylogeny: we obtained 18S rDNA gene sequences for three populations of *E. serrulatus* and for four related congeners (not exactly the same taxa as in the pore-pattern study). The gene is 1808–9 base pairs long, and its G + C content varies between 49.14% (*E. speratus*) and 50.61% (*M. albidus*) (Table 9). There is no sequence variation among the five *E. serrulatus* populations studied. Unfortunately, no representative from the North African Atlas Mountains could be included. Phylogenetic analysis, using the methods described above, resulted in the tree shown in Fig. 22. The tree topology, genetic distances and absolute nucleotide differences between taxa show that populations of *E. serrulatus* from Petershoff and Ghent and their hybrids cluster closely, including forms A and B, with *E. speratus* closely related but definitely a distinct taxon. A North American *E. serrulatus* sequence taken from the data bank stands well away from this '*serrulatus* s. str. group'. The sequence was deposited by Dr T. Spears (Tallahassee), who told us that its origin was a culture obtained from a commercial supplier in the north-east USA. It is definitely not *serrulatus*, and its origin suggests it is *E. pectinifer*. A final clade, grouping *E. dumonti* and *E. macruroides*, is somewhat surprising in that *E. dumonti* is morphologically closer to the *serrulatus*-group than to *E. macruroides*, but this clade may represent an artifact that would disappear if more species were included in the tree.

Discussion

By any standard and method, considerable intra- and inter-population variability was encountered in *E. serrulatus*. The type population contained three forms (A, B and C) that differ in setae morphology of the caudal rami and the swimming legs (Table 1), but all easily mated and had fertile offspring across

Table 9 Gene length, GC content and accession number of the ribosomal 18S gene and geographical origin of the species used in this study.

Species	18S (bp)	GC percentage	EMBL acc.	Geographical origin	Collector
Eucyclops serrulatus FA	1809	49.25	AJ746330	St. Petersburg, Russia	V. Alekseev
Eucyclops serrulatus FA × FB	1809	49.25	AJ746331	St. Petersburg, Russia	V. Alekseev
Eucyclops serrulatus FA	1809	49.25	AJ746332	Tumen, Russia	V. Alekseev
Eucyclops serrulatus FA	1809	49.25	AJ746328	Ghent, Belgium	V. Alekseev
Eucyclops 'serrulatus'	1809	49.25	L81940	north-east USA	T. Spears
Eucyclops speratus	1809	49.14	AJ746333	St. Petersburg, Russia	V. Alekseev
Eucyclops dumonti	1808	49.28	AJ746335	Central Mongolia	V. Alekseev
Eucyclops macruroides	1809	49.25	AJ746329	St. Petersburg, Russia	V. Alekseev
Ectocyclops polyspinosus	1808	49.56	AJ746336	Montreal, Canada	V. Alekseev
Macrocyclops albidus	1808	50.61	AJ746334	Ghent, Belgium	V. Alekseev

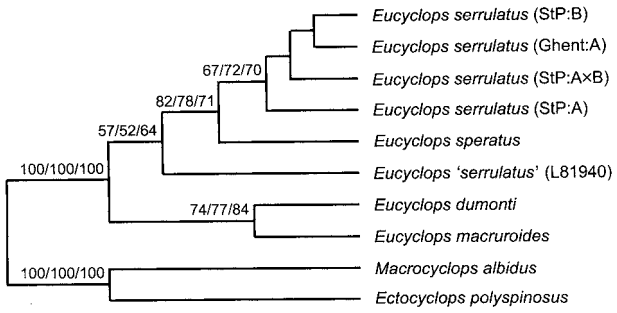


Fig. 22 Phylogeny based on 18S ribosomal DNA sequences; consensus tree for neighbor joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) methods. The NJ analysis used the optimality criterion distance, a bootstrap method with NJ search, ties broken randomly and distance measure set to maximum likelihood using the data as determined by ModelTest. The MP analysis generated three most parsimonious trees (MPTs) of 98 steps (CI = 0.9388, RI = 0.8333, RC = 0.7823). Bootstrap method with heuristic search, stepwise taxon addition, TBR branch swapping, MULTREES option, no steepest descent, rearrangements limited to 10 000 000, and accelerated transformation. The ML analysis with GTR + G + I, gamma correction, R = (0.9413, 1.6963, 0.7570, 0.5504, 3.3251), Pinv = 0.7499, and gamma shape parameter = 0.8368 generated a tree with a log likelihood of –3109.09943. The numbers along the branches indicate NJ (first number), MP (second number) and ML (third number) bootstrap support, and are expressed as a percentage.

two generations. Hybrids were morphologically identifiable and also occurred in the natural population of the type locality. Cross-breeding was equally easy between specimens of groups A, B and C obtained from Siberia (Tumen) and western Europe (Ghent). A single substitution in the 18S rDNA gene between forms A and B can therefore not have much taxonomic weight. Forms attributable to forms A–C were also found in North Africa by Kiefer (1954) and by ourselves, and at least two groups (probably A and B) were reported from Britain by Gurney (1933). *Eucyclops hadjebensis* Kiefer was found to be so close in all microcharacters examined (unfortunately no specimens were available for cross-breeding or for genetic analysis), that we reduce it to a subspecies of *serrulatus*, geographically isolated in springs in the Atlas mountains of North Africa. The comparative rarity of form C over the entire range of *E. serrulatus* suggests that a recessive gene controls the pit-pattern. Pit-patterned phenotypes are also known in other cyclopids, like *Acanthocyclops signifer* Mazepova from Lake Baikal and in *Eucyclops speratus infniensis* Dumont & Decraemer, 1977 from Morocco. The latter was singled out as a subspecies based on evidence of its extended furcal rami, but it is here considered to represent form C of *E. serrulatus*. Similarly, analysis of the pore signature revealed only small differences between forms A and B (Fig. 12: 1–2, Tables 1, 2), and these differences (pore positions expressed as either singletons or doubletons, or present on one side of the integument but not on the other) were equally common within and between groups. They are therefore considered as individual variations. Other minor interpopulation differences include spine group 13 of the antennal basipodite of form B in the type population, but this was also present in form A from localities in Scandinavia (Table 1). Also, a difference in the length of the setules of the coxal membrane of P4 in forms A and B exists (setules long, short, absent) (Fig. 6: 9–10), but such differences cannot be more than individual variation (Tables 2, 3). *Eucyclops serrulatus* (including *E. hadjebensis*) can, however, be unequivocally diagnosed by the following morphological characters: caudal side of basipodite of A2 with two groups of long setules, one subdistally, one on the distal inner margin, frontal side of basipodite A2 without spinule group 9, Coxa of P4 with row of 12–14 stong denticles subdistally (groups C + D), coxal spine with a gap in the strong setules on outer margin (Fig. 8: 8, arrow, and Fig. 11: 4, arrow), and basis of P4 with long dense setules on inner surface.

To separate *E. speratus* from *E. serrulatus*, the length-of-furca character is not very useful, as broad overlap occurs. However, in *E. speratus* there are no setules on the caudal side of the antennal basipodite, no gap in the setules on the outer margin of the coxal spine of P4, but a high number of spinules in groups C + D (25–28) and in E (8) on the coxa of P4.

Eucyclops dumonti lacks setules on the inner surface of the basipodite of P4 (Fig. 16: 2, arrow). Additionally, it has only 15–18 spines in groups C + D. Apart from that, it shares more characters with *E. speratus* than with *E. serrulatus* (except for the large difference in length/width ratio of the caudal rami): no hair-setae on posterior face basipodite of A2, anterior face of basipodite A2 with a group of spines at position 9, both sides of coxal spine of P4 homogeneously setulose. *Eucyclops turcomanus* is close to *E. serrulatus* but a spine group 9 is present on anterior face of the basipodite of A2, and the coxa of P4 has a reduced number (< 12) of spines in groups A + D. *Eucyclops pectinifer* is also close to *E. serrulatus* (see differences in size, Table 8) but the posterior surface of the basipodite of A2 has only one group of setules subdistally (1), while group 2 is represented by strong spinules (Fig. 17, arrow). In the medial row (12), the anterior surface shows one or two long spines (arrow), and the coxa of P4 has about 10 long setae instead of denticles in groups A + D. All this suggests a closely related group of species, confirmed by the phylogenetic tree based on cephalosomal pore patterns (Fig. 21). As might be expected, *E. serrulatus* and *E. hadjebensis* cluster together, as do *E. speratus* and *E. dumonti*; *E. turcomanus* appears to be the most distant member of what could be called the '*serrulatus*-group'.

Morphological characters uniting this group include a 12-segmented A1 with a finely serrated membrane along the three distalmost segments, caudal rami of variable length but always at least partly with longitudinal row of setules on the outer margins; antennal basipodite posteriori at least with one group of long setules subdistally (groups 1, 2). The base of the cephalosome has a medial U-shaped figure formed by the eight pores of positions 33 and 34, and an identical pore-configuration of the metasomal and urosomal segments.

Eucyclops macruroides differs by a coarsely spinulated antennal apex, an extremely long furca practically devoid of spinules on its outer margins, and absence of the dorsal U-shaped pore figure of the base of the cephalosome. The pore configuration on the metasomal segments might also be diagnostic, but needs more study. The 18 S rDNA-based tree reveals the identity of the different morphotypes of *E. serrulatus* and their hybrids, confirms *E. speratus* as a separate taxon, and also strongly suggests that the *E. serrulatus* from North America taken from the DNA database is different from *E. serrulatus* known in Europe and Asia. It either represents *E. pectinifer* or another related North American taxon. Somewhat puzzling is the clustering of *E. dumonti* with *E. macruroides*, but this may be an artefact that might disappear with more extensive taxon sampling. At the genus level, *Afrocyclops* and *Eucyclops* can be separated by the morphological characters currently considered standard for these genera, but also by the pore pattern on the cephalosome, metasome and urosome. This opens new possibilities for study, the hypothesis to be tested being that closely related cyclopoid species can be identified by specific pore configurations on the cephalosome, while species groups additionally have differences in the metasome pore configurations, and related genera differ by the number and position of their urosomal pores.

A question that needs to be addressed is that of the origin of the two main forms of *E. serrulatus*, and how these forms persist in shallow lakes with no apparent possibility to avoid mixing. *Eucyclops serrulatus* is well adapted to inhabit the littoral and near-bottom zone of lakes. In Lake Sevan, for example, one of us (V. A.) collected specimens from depths of 5–6 m to 120 m. But even in such a large lake, the near-shore area is at risk of drying up in summer, which is the time for reproduction of this cyclopoid. Lakes under a strong selecting factor like a summer drought in their littoral could support the coexistence of two forms if one were adapted to deep seasonal changes in the littoral and the other to stable limnetic conditions. Form A indeed seems adapted to survive in biotopes at risk of drying up; it is found in temporary water bodies, while form B occurs mainly in permanent waters and springs.

This speculation should be checked experimentally against a similar adaptation reported in the near bottom-thermophilic *Macrocyclops albidus* (Alekseev 1985, 1990). Here, a population collected in the near-shore area of Lake Uchenoe showed alternative photoperiodic responses in producing diapausing stages. While one part of the population produced a maximum of diapausing stages under a long-day photoperiod, another part did so under short-day conditions. Hybrids showed intermediate photoperiodic responses. A diapause during summer (long day) would be adaptive in temporarily drying-up environments like shallow bays or near-shore pools, an environment relatively shielded from fish predation. Alternatively, the population living in deep water can breed longer and at the best time, but is exposed to a higher risk of fish predation.

It is possible that a comparable difference in habitat choice, linked to a different cue for initiating dormancy, perpetuates the coexistence of the two forms of *E. serrulatus*. But what is the origin of these two forms? One possibility is that they are of glacial age. In the Old World, the existence of two main Pleistocene refuge areas, one in the west (Iberia-North Africa), one in the east (the Balkans and West Asia as far as the Ponto-Caspian depression) is well known (De Lattin 1967). Here, plants and animals retreated during glacial advances, and evolved in isolation. Each time the glaciers retreated, they recolonized the vacated territories, at various speeds, hybridizing where the east and west re-invading fronts met (Hewitt 2000). *Eucyclops serulatus* is a rapid invader, and may quickly have extended north and west, with the ecological differences between the forms preventing the formation of a typical cline or hybrid zone, and slowing down introgression.

In North America, two coalescing ice-sheets, the Laurentide and the Cordilleran, divided the continent into two blocs: Beringia in the north-west, and an area below the Great Lakes

in the south (Pielou 1991). The possibility of independent evolution in disjunct refugia therefore also exists in the New World. To the best of our knowledge, *E. pectinifer* does not show polymorphism, but yet another member of the *serrulatus*-group, *E. prionophorus* (Kiefer) does. Although no fresh specimens were available for DNA and pore-pattern analysis, we found that variation similar to that in *E. serrulatus* occurs in the armament of caudal seta and distal segment of P4. *Eucyclops prionophorus* females have only one group of setules at the top of the caudal side of basipodite A2, and a short A1 that does not reach the first free somite, while males have a much longer spine on P6 than *E. serrulatus*.

Upon checking the types of *E. prionophorus* (Kiefer collection, Karlsruhe Museum of Natural History), we found that slides NN1506–1508 from New Haven (north-east USA) can be attributed to form A, while specimens from a southern population (Paraguay) belong to forms A, B and hybrids between them (slides NN 3103–3106). One possibility, therefore, is that form A originated close to, or north-west of, the glaciers; this hypothesis implies that the polymorphism embodied in the existence of forms A and B arose at least twice during the Pleistocene. An alternative hypothesis is that these two forms were already present in the common ancestor of *E. serrulatus* and *E. prionophorus*, and were retained in some, but not all, of its descendents. This would place the origin of the polymorphism back in the Pliocene at least. Testing which of these hypotheses is nearer to the truth will need additional molecular and morphological analysis.

A final word needs to be said about the geographical range of *E. serrulatus sensu lato*. Clearly, it extends from North Africa, the Mediterranean basin, continental Europe and Russia to most of Siberia and perhaps Central Asia. In North (and possibly) South America, it is, however, replaced by related species. In South-east Asia we know of *serrulatus*-like specimens close to type A of the type population (Northern Thailand and Southern China, Guangdong Province). On the Mongolian plateau, it seems to be replaced by *E. dumonti*, and related but possibly distinct species occur in Japan (Ishida 1997, 1998) and perhaps elsewhere in the Far East and in Lake Baikal (Alekseev 1989). In Australia, *serrulatus*-like animals occur, but preliminary cross-breeding experiments by one of us (J. P.) established a genetic incompatibility between specimens from South Australia and from Ghent. In Africa south of the Sahara, finally, countless records have claimed to establish the presence of *E. serrulatus*, but all of these are based on identifications using obsolete species definitions, and are therefore up for revision.

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