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## A new species within the *Eurytemora affinis* complex (Copepoda: Calanoida) from the Atlantic Coast of USA, with observations on eight morphologically different European populations

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### Abstract

*Eurytemora carolleae* sp. nov. (Crustacea: Copepoda: Calaniformes) is described from the Chesapeake Bay, USA. The new species belongs to the Atlantic clade of the *Eurytemora affinis* complex outlined by previously published molecular work but poorly characterized morphologically. To discriminate *E. carolleae* we compare specimens from the Atlantic USA clade with specimens from the type population of *E. affinis* (Poppe, 1880) from the Elbe River Estuary (Germany), as well as with eight other European coastal populations. Several important morphological characters clearly separate the North American *E. cf. affinis* from the European clade that include both sexes: a large outside orientated dent on the mandible, and clearly observable seta segmentation in the caudal rami and swimming legs. Unlike *E. affinis*, the newly described species possesses wing-like outgrowths on the genital double-somite and a very small spine near the distal seta insertion point in P5 in females. In males, the specific characters include naked dorsal and ventral sides of the caudal rami, and a cylindrical shape of exopod on the left P5, in contrast to a triangular shape of the segment in *E. affinis*. The new species was also found in Canada (St. Lawrence Estuary) and as an invasive species in the Baltic Sea. *Eurytemora carolleae* is possibly widely distributed along the North American Atlantic coast, as well as in inland waters from Great Lakes to Mexico.

**Key words:** Copepod diversity, taxonomy, sibling species, North Atlantic, estuary

### Introduction

The estuarine copepod, *Eurytemora affinis* (Poppe, 1880) was suspected for a long time of being a species with a Holarctic distribution, having been recorded in cold and temperate latitudes of North America, Europe, and Asia (Dussart & Defaye 2002; Lee & Frost 2002). In Europe and North American Atlantic areas, this species is known from coastal brackish water environments, but also from large continental lakes like Ladoga Lake in Europe or Lake Erie in North America (Rylov 1922; Croskery 1978).

In the Baltic Sea *E. affinis* is a dominant copepod, both in littoral and pelagic ecosystems (Telesh & Hercloss 2004). In Asia, it is known from the Caspian Sea and from fresh water lakes in Japan (Lee 2000; Dussart & Defaye 2002). Due to its worldwide distribution and the key role in food-webs, *E. affinis* has been recently suggested as a model species in estuarine ecosystems (Souissi *et al.* 2010)

In contrast to morphology-based taxonomy, recent molecular-genetic and cross-hybridization studies demonstrated that *E. affinis* represents, in fact, a complex of sibling species with highly similar morphologies but isolated within the past million years (Knowlton 1993; Lee 1999, 2000; Lee & Frost 2002). Laboratory cross-hybridization studies between two divergent clades of *E. affinis* from Europe and North America indicated that hybrids in the second generation were sterile (Souissi S., pers. com). *Eurytemora cf. affinis* from Chesapeake Bay, USA was recently found in the Gulf of Finland in the Baltic Sea (Alekseev *et al.* 2009). Lee & Frost (2002) performed some morphometric analyses of a limited number of characters in the major clades of *E. cf. affinis*, including the American North Atlantic, Asian and European clades. Their previous study discovered that all the major clades of *E. affinis* are morphologically divergent from Europe.

The results of our analyses allow us to support the opinion of Lee (2000) on the possible validity of the Atlantic clade of *E. cf. affinis* from the USA as a new species. The present study is the first description of a cryptic species within this *E. affinis* complex.

## Material and methods

The type material for this new species was selected from a sample collected on 16 April 2008 by Dr. D. Kimmel (East Carolina University, USA) in the Chesapeake Bay, Atlantic coastline of USA. We also used material from the St. Lawrence Estuary, Canada donated by Dr. G. Winkler (ISMER, University of Québec at Rimouski, Canada). For comparison we used specimens from The Elbe, Germany (*terra typica* for *Eurytemora affinis* (Poppe, 1880) collected in 2006 twice per season by Dr. Winkler and Dr. Boillens and preserved in the Marine Station of Wimereux, University of Lille 1, France (Prof. S. Souissi collection). Individuals from eight other European populations of *E. affinis* were also collected by the authors in 2008 and 2009 (Table 1 and Fig. 1).

TABLE 1. *Eurytemora* sampling sites in Europe and North America.

Country, area	Locality/№ on the map	Date
USA, Atlantic coast	Chesapeake Bay/1	April 2008
Canada, Quebec	R. St. Lawrence estuary/2	July 2008
Germany, Hamburg	R. Elbe estuary/3	March 2006
Spain, Seville City	R. Guadalquivir/11	January 2009
France, Atlantic coast	R. Gironde estuary/6	April 2006
France, Atlantic coast	R. Loire estuary/5	June 2009
France, Atlantic coast	R. Seine estuary/4	July 2009
Belgium, North Sea coast	R. Schelde estuary/7	April 2006
Latvia Baltic Sea coast	Riga Bay/8	June 2008
Finland Baltic Sea, Helsinki	City Fish Port /9	August 2009
Russia, Baltic Sea, Gulf of Finland	R. Luga estuary/10	August 2008

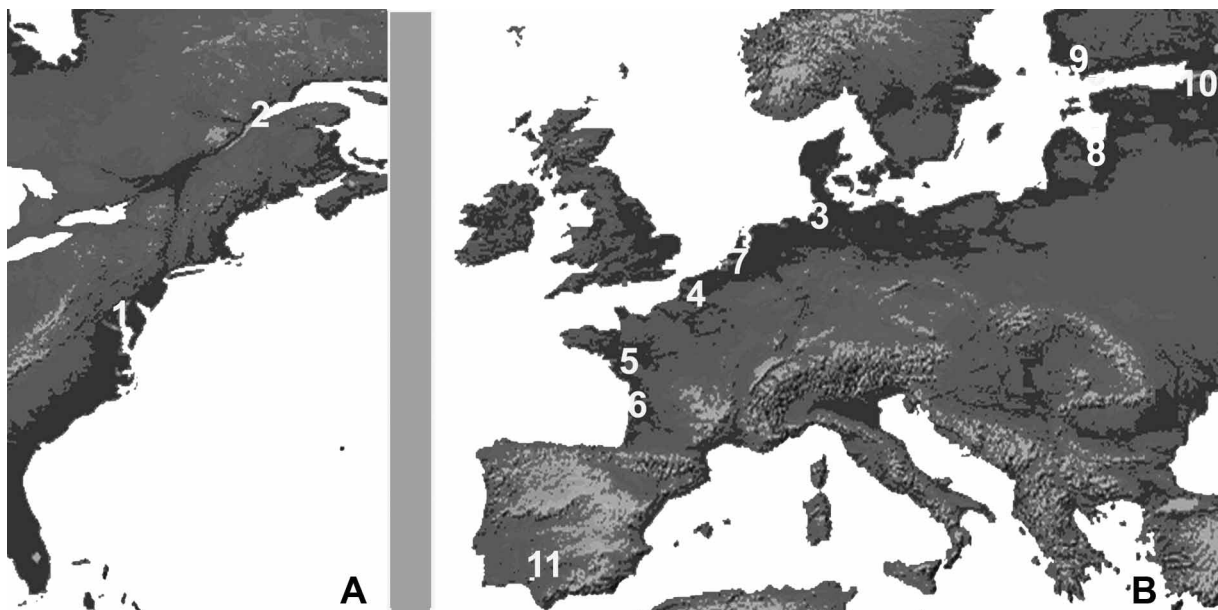


FIGURE 1. Sampling map of *Eurytemora carollaeae* sp. nov. in North America (A) and *Eurytemora affinis* (Poppe, 1880) in Europe (B).

The samples were sorted in the laboratory under a dissection microscope (Olympus, SZX2), and about 60 adults from each population were selected for analyzes. Before dissection, copepod adults were photographed with a digital camera and measured with an ocular micrometer (5 µm resolution). After dissection, the individuals were placed on slides in pure glycerol, covered with a cover slip and ringed with Canadian balsam. The slides were then observed at maximum resolution up to 1000 x (Plan objective 100 x, oil immersion) under a compound microscope (Zeiss IMAGER) equipped with Nomarski system for differential interference contrast microscopy and a drawing tube. Initial pencil drawings were converted to India ink and placed on A4 size tracing paper. The tables were scanned at a resolution of 600 dpi, numbered and reorganized with a computerized graphics program (Adobe Photoshop 7).

To delineate the species, all morphological characters were checked in both sexes, including not only secondary sexual dimorphic characters typically used in copepod taxonomy, but also mouth appendages, micro characters of the fifth legs, seta structure and body shape.

To observe variation between *E. carolleae* **sp. nov.** and *E. affinis* (Poppe, 1880), as well as among 8 local populations of *E. affinis* in Europe, we measured for both sexes caudal rami length and width; in the swimming legs 1 and 4 the distal exopod segment and distal spine lengths were also measured.

In females, we measured additionally leg 5 exopod spine lengths, and for the genital double-somite we determined the length and width in the anterior (W1) and posterior (W2) sides of the somite (Fig 2). For males, length and maximal width of the first segment of leg 5 exopod were measured. Statistical analyses were performed in Statistica-7 program.

The type slides for *E. carolleae* were placed in the type collection of the Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia (ZIN RAN ) under reference numbers 55052–55054. Slides with dissected individuals from 8 European populations of *E. affinis*, including the type population from the Elbe River, are stored in the Copepod division of the Russian Federal Collection N 96–03–16.

For terminology we mainly followed Huys & Boxshall (1991). Abbreviations used are: END, endopod; EXP, exopod; BAS, basipod; P1–P4, swimming legs 1–4; P5, leg 5.

## Systematics

### Class Copepoda H. Milne Edwards, 1840

### Order Calanoida Sars, 1903

### Superfamily Diaptomoidea Baird, 1850

### Family Temoridae Giesbrecht, 1893

### Genus *Eurytemora* Giesbrecht, 1881

#### *Eurytemora carolleae* **sp. nov.**

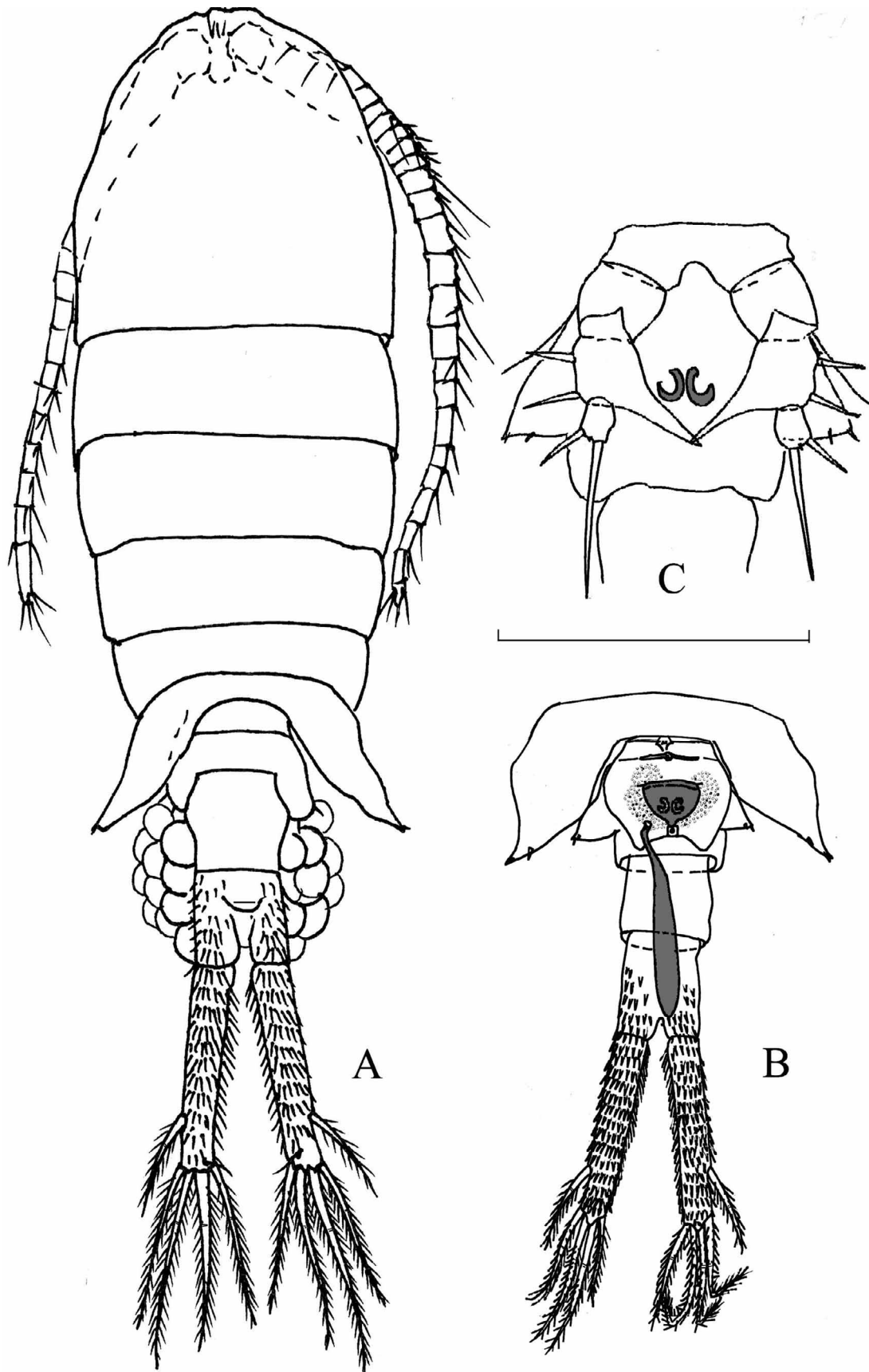
(Figs 2–7, 8A, 9A–D)

**Type material.** Holotype, ZIN RN 55052, a female dissected on 1 slide; from the Chesapeake Bay, USA (39°23.81'N, 76°03.32'W), collected by Dr Kimmel, 16 April 2008.

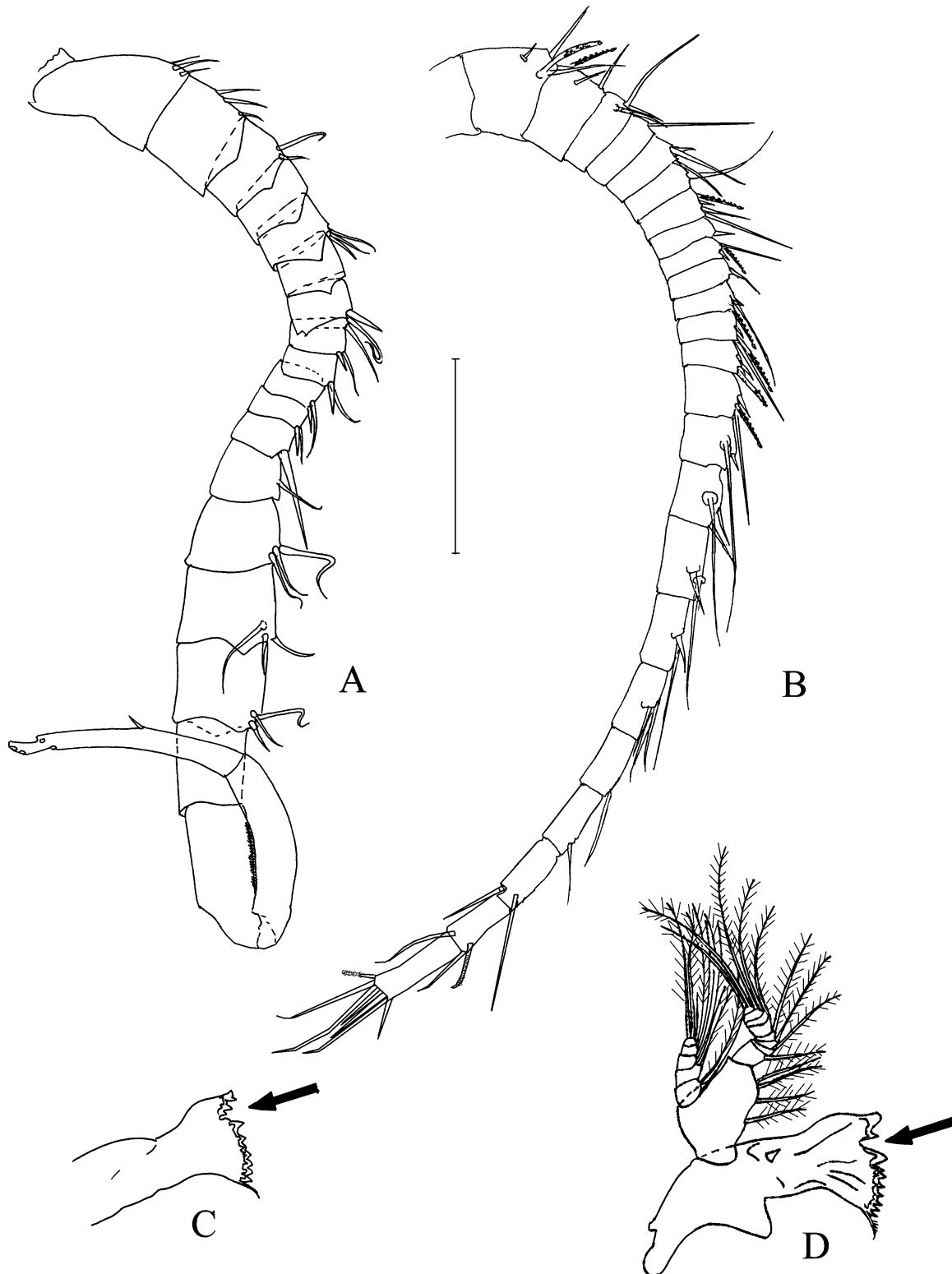
Allotype, ZIN RN 55053, a male dissected on 1 slide; from the Chesapeake Bay, USA (39°23.81'N, 76°03.32'W).

Other paratypes, ZIN RN 55054/1–10, 15 females and 15 males from the Chesapeake Bay, USA (39°23.81'N, 76°03.32'W), dissected on 10 slides (3 individuals per 1 slide)

**Description.** Female (Figs. 2–5). Body transparent, genital double-somite yellowish brown. Length measurements: Full body length without caudal setae 1160 µm, with caudal setae 1655 µm.; cephalosome 418 µm and 4 free thoracic segments 1/2/3/4 = 90/70/70/40 µm. Urosome 460 µm, genital double-somite 130 µm, 3 following somites 1/2/3 = 80/60/60 µm and caudal rami 130 µm.



**FIGURE 2.** *Eurytemora carolleae* sp. nov., female, ZIN 55050 (holotype): A, habitus, dorsal view; B, urosome, ventral view; C, genital double-somite with P5, ventral view. Scale bar: A, B, 300  $\mu$ m; C, 150  $\mu$ m.



**FIGURE 3.** *Eurytemora carolleae* sp. nov. A, male antennula; B, female left antennula; C, male gnathobasis of mandible; D, female mandible with palp. Arrows indicate separate processor on gnathobasis. Scale bar: A, B, 125µm; C, D, 62.5 µm.

Cephalothorax (Fig. 2A) as long as wide, with maximum width close to middle, frontal part of cephalothorax oval.

Last thoracic somite with 1–2 small spines on wing-like outgrowths of lateral margin.

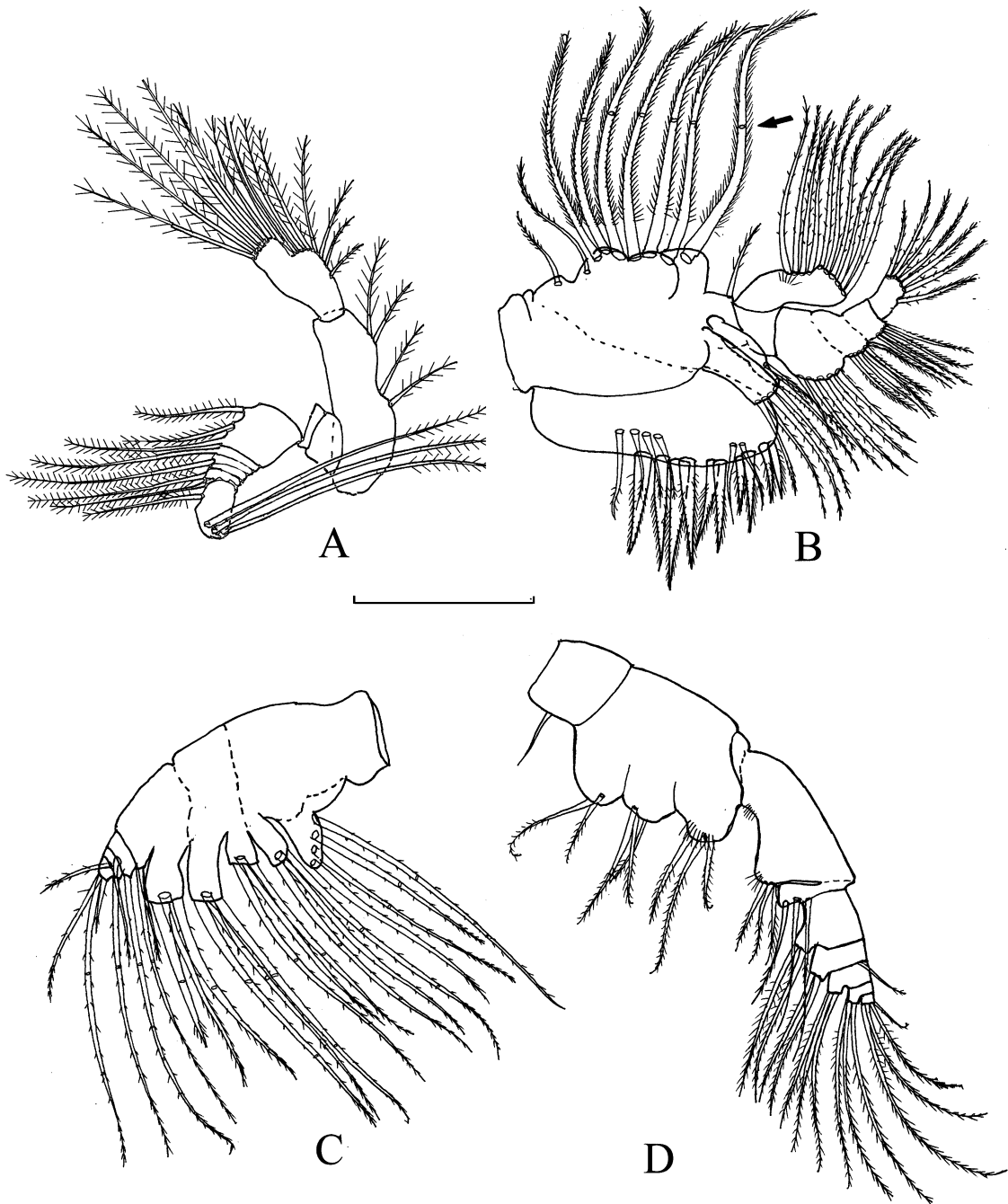
Genital double-somite 1.5 times as wide as long, due to wing-like outgrowths in anterior part of the somite, with two relatively long spines on both sides, with seminal receptacle as shown in Figs. 2B–C.

Caudal rami (Fig. 2B) divergent, 6.2 times as long as wide, with long and strong hair-setae on both sides, as well as on last abdominal somite.

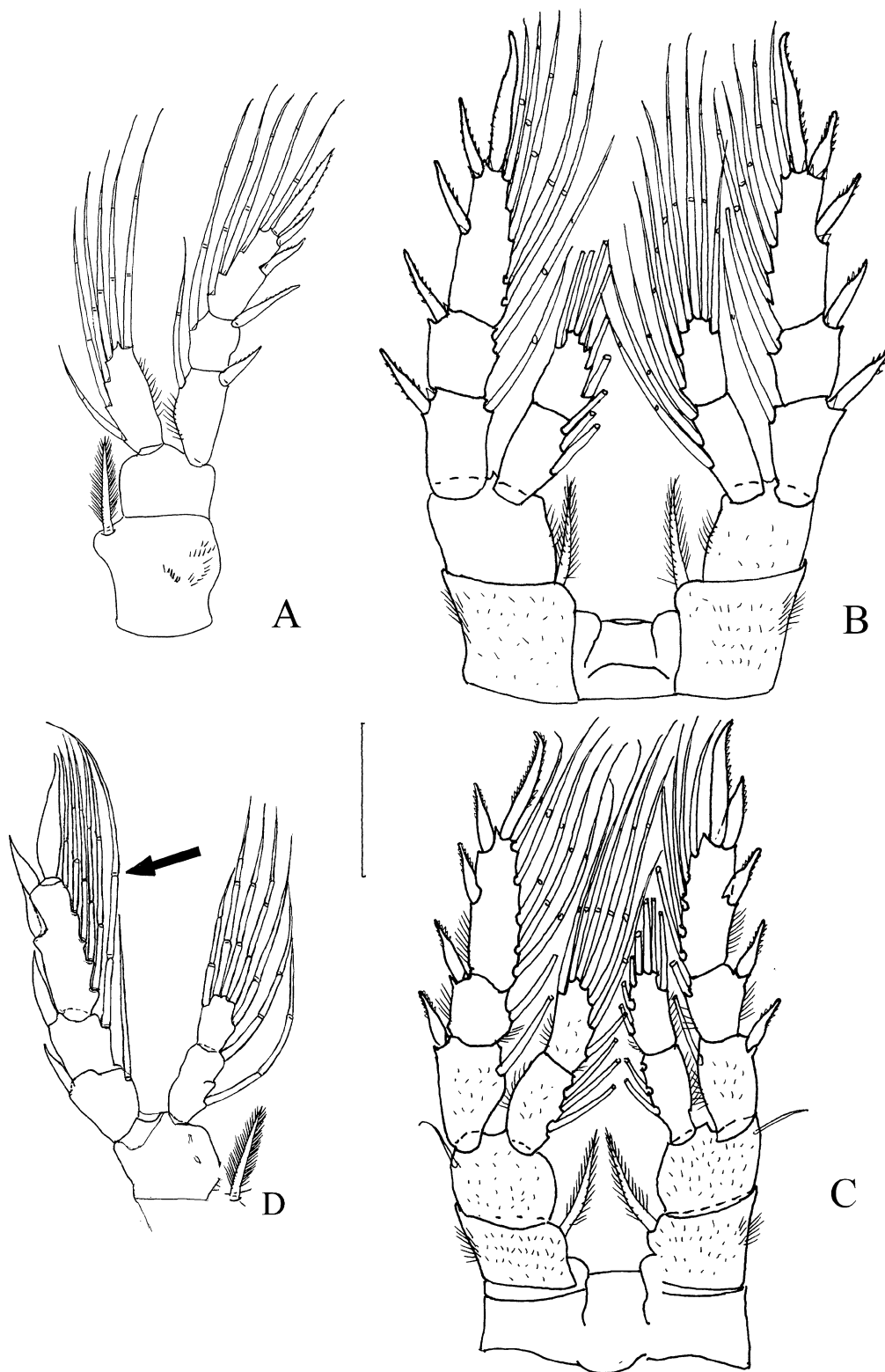
Length proportions of terminal setae, beginning from outermost caudal seta: 1/1.3/1.15/1. Length proportions of dorsal and lateral setae to outermost seta 0.25 and 0.6 times respectively. All caudal setae with clearly observable segment-like divisions.

Antennules (Fig. 3A) of 25 segments, reaching end of third free thoracic somite, setation of segments beginning from first segment: 0/4/4/3/1/3/1/4/1/3/1/3/3/2/3/2/3/2/2/3/1/1/2/3/7. Most segments with 2 and more setae provided with 1 aesthetasc, last (distal) segment with 6 setae and 1 aesthetasc.

Antenna (Fig. 4A) biramous, composed of 2-segmented protopod, 2-segmented endopod and 6-segmented exopod. First exopod segment with 3 setae, second segment with 2 setae, 3–5 segments with 1 seta each, 6 (distal) segment with 4 setae. First endopodal segment with 2 setae, second with 9 setae laterally and 7 setae at distal end.



**FIGURE 4.** *Eurytemora carolleae* sp. nov., female, ZIN 55050 (holotype): A, antenna; B, maxillula, ventral view; C, maxilla; D, maxilliped. Scale bar: 50  $\mu$ m. Arrow indicating seta segmentation.



**FIGURE 5.** *Eurytemora carolleae* sp. nov., female, ZIN 55050 (holotype): A, left swimming leg 1, anterior view; B, swimming legs 2, anterior view; C, swimming legs 3, anterior view; D, swimming leg 4, anterior view. Scale bar: 100  $\mu$ m. Arrow indicating seta segmentation.

Mandible (Fig. 3D) composed of coxa with gnathobasis, one of its tooth (outermost) significantly larger than other and separated from them. Coxa in middle with biramous mandibular palp, basis with 4 setae, 8-segmented exopod and 5-segmented endopod. Distal segment of exopod with two sub-equal setae, other segments bearing single long seta each. Distal segment of endopod with 2 long setae and 1 short seta, other segment with 2 long setae each.



Maxillula (Fig. 4B) biramous and composed of precoxa with medial arthrite bearing 10 strong claw-like spines, 6 relatively long setae; coxa with elongated endite bearing 5 long and one short setae and outer outgrowth with 7 very strong sub-equal in length setae and 2 thin setae. Basis composed of basal endite, 1-segmented endopod with 10 long subequal setae and 4-segmented exopod bearing 5–5–4–7 (distally) long setae.

Maxilla (Fig. 4C) uniramous, composed of precoxa with 2 endites; distal endite bearing 3 long setae, first segment with 3 setae, coxa with two endites bearing 3 long setae each and 5-segmented endopod including basal endite with 3 setae and 4 short segments bearing 2 and 3 long distal setae.

Maxilliped (Fig. 4D) uniramous and 9-segmented, composed of short precoxa with 1 seta and long coxa with 3 hill-shaped endites bearing 2–3–3 setae; basis more wide in distal part with 3 long setae and with group of long hairs; endite with 3 long setae followed with 5-segmented endopod armed with 2–3–4–3–4 (distally) setae.

Swimming legs P1–4 (Figs. 5A–D) consist of coxa and basis bearing 3-segmented exopod and 1 (P1) or 2-segmented endopod (P2–4). Coxae connected with smooth coxal membrane. First and second exopodal segments in each leg with 1 spine outside and 1 seta inside. In P1 endopod with 6 setae, in P2–P3 first segment with 3, distal segment with 6 setae, in P4 first segment with 2, distal segment with 5 setae. Formula for spine (Arabic) and seta (Roman) for distal exopodal segments in P1–4 as follows: 3V–3V–2V–3V. All setae in swimming legs with clear seen segment-like divisions (Fig. 5D, indicated with arrow). Distal spines in exopod P4 very strong in middle part, sub-equal or slightly shorter than nearest setae and shorter than distal segment length (Fig. 5D). Lateral edge of coxa in P1–2 with groups of long hair-setae. Both sides of P1–P4 covered with very short hair-setae.

Rudimentary P5 (Fig. 2C) uniramous and 4-segmented, narrow coxal plate bearing 1-segmented basis with strong inner outgrowth and 2 spines, distal spine slightly longer than first one. One-segmented exopod with long apical seta and lateral spine about half of apical seta. Tiny spine inserted between these two appendages about 10% or less of short spine length and shorter than distal spine width in insertion place.

Egg sac with up to 60 eggs.

Male. Body length 1720  $\mu\text{m}$ , with caudal setae 1780  $\mu\text{m}$ , in live and freshly conserved specimens blue-grey in colour or colorless.

Cephalothorax as long as wide, with maximum width close to caudal end, anterior part of cephalothorax round shaped.

Last thoracic somite without wings and spine on lateral margin (Fig. 6A)

Abdomen 5-segmented.

Caudal rami: 9 times as long as wide without setules or hair-like seta on dorsal and ventral side but with long hair-seta on inner part of cauda. Terminal setae ratio beginning from outermost caudal seta: 1/1.15/1.25/1. Lateral seta about 0.8 times longer than outermost seta, dorsal seta very short, located near innermost seta insertion place (Fig. 6A).

Right Antennule (Fig. 3B) 21-segmented, 4 and 6 segments without setae, 7–12 segments with strong spines, strongest spine (twice as long as any other spine) at segment 12; 19–20 segments with denticulate plates at inner edges, distal segment with several sensitive papilla. Other segments with regular setae as in Fig. 2. Left antennule 25 segmented armed as in female (see Fig. 2).

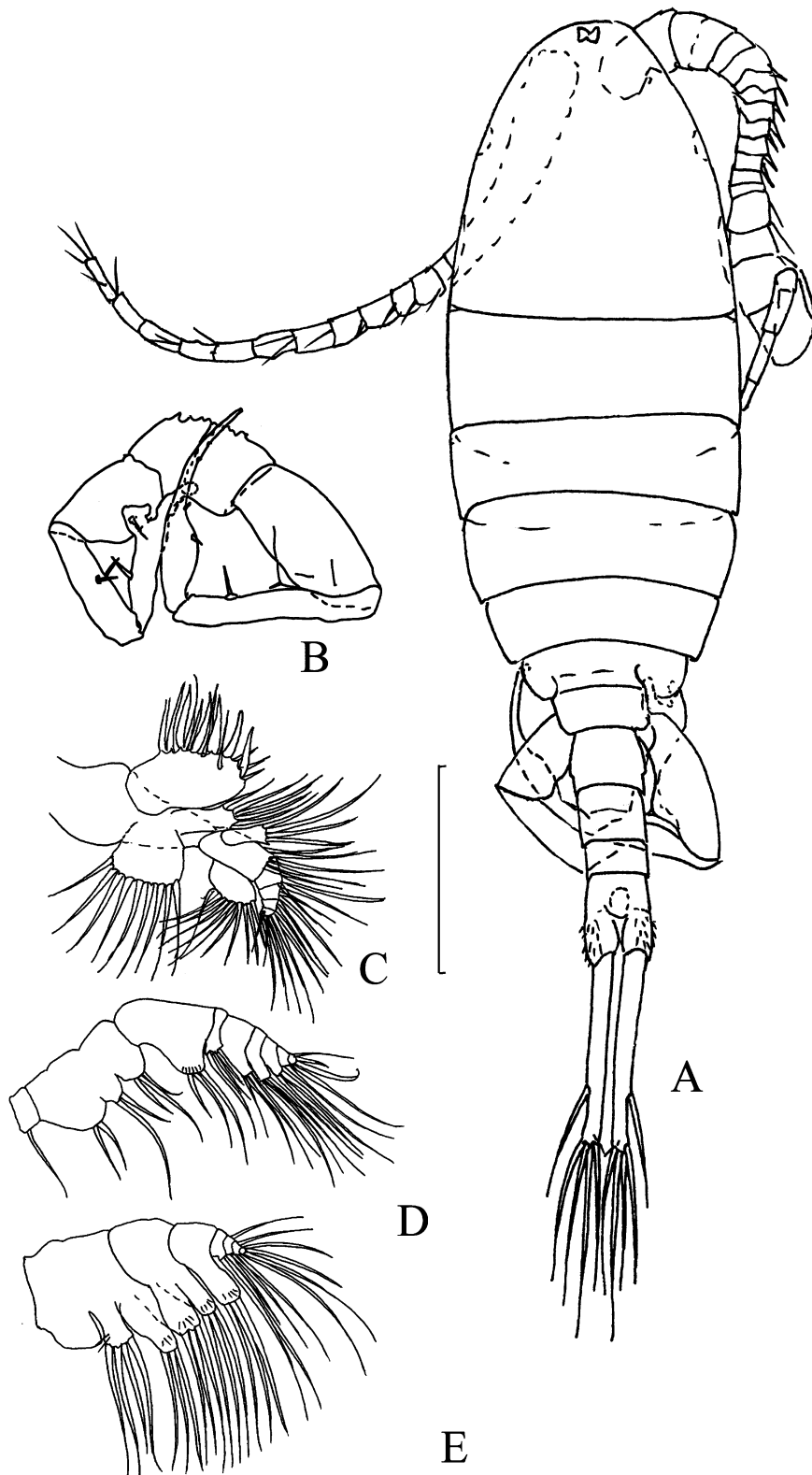
Antenna biramous (not shown) with 2-segmented protopod, 2-segmented endopod and 6-segmented exopod. Setation as in female (see Fig. 4A).

Mandible of same construction as in female, gnathobasis with outermost tooth-like processor significantly larger than other and separated from them with a gap indicated in picture Fig. 3C with arrow.

Maxillula (Fig. 6C) biramous, basically as in female, with precoxa bearing 8 strong claw-like spines, 6 relatively long setae. Coxa with elongated endite bearing 6 long setae and outer outgrowth with 9 very strong sub-equal in length setae. Basis composed of basal endite, 1-segmented endopod with 10 long subequal setae and 4-segmented exopod bearing 5–5–4–7 (distally) long setae.

Maxilla (Fig. 6E) similar to female: precoxa with 2 endites; distal endite with 3 long setae, first segment with 3 setae, coxa also with two endites bearing 3 long setae each and 5-segmented endopod including basal endite with 3 setae and 4 short segments bearing 2 setae; distal segment with 4 setae.

Maxilliped (Fig. 6D) 9-segmented, composed of short precoxa with 1 seta and long coxa with 3 hill-shaped endites bearing 2–2–3 setae; basis more wide in distal part with 3 long setae and with group of long hair-setae near setae insertion place. Endopod 5-segmented with 2–2–4–3–4 (distally) setae.



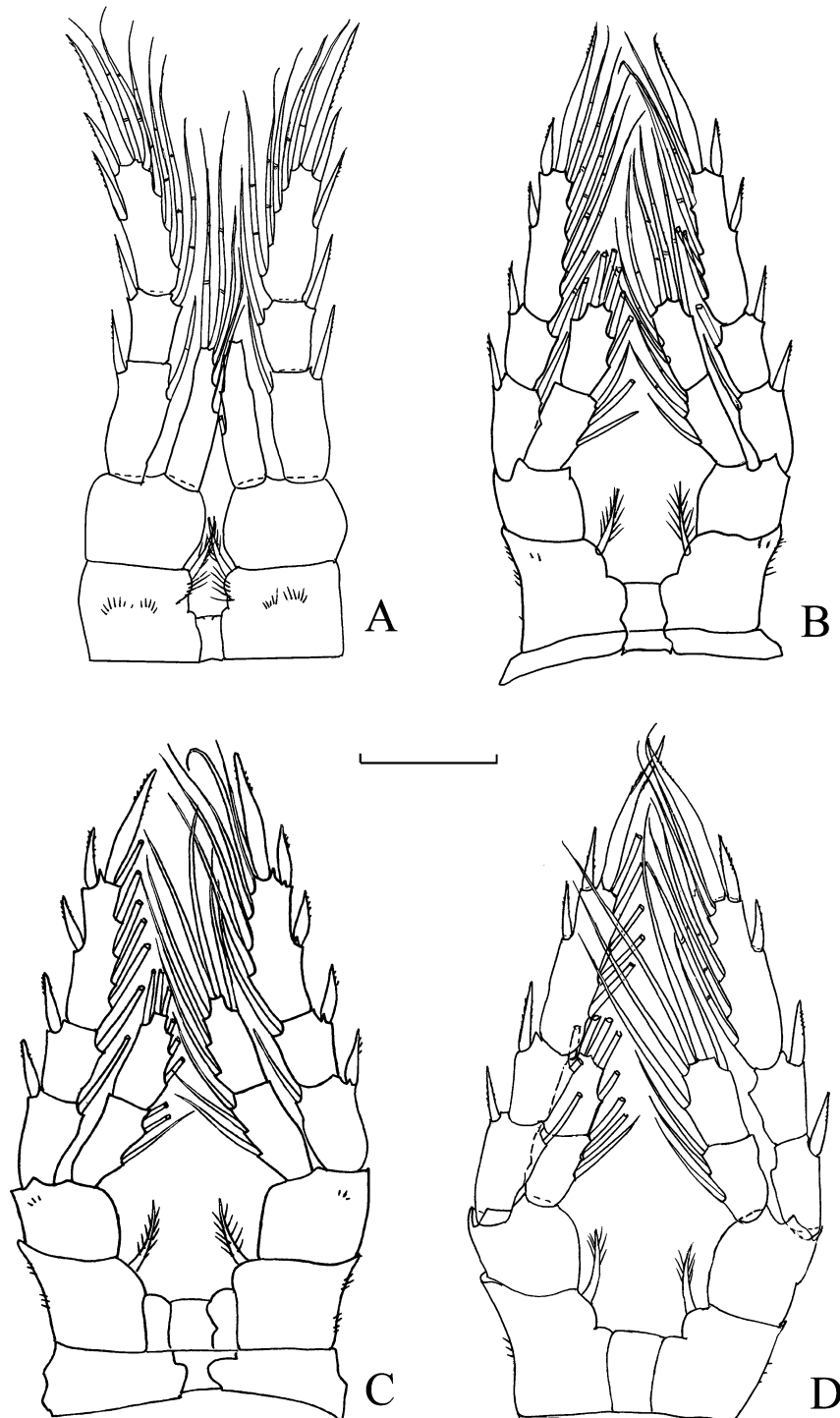
**FIGURE 6.** *Eurytemora carolleae* sp. nov., male, ZIN 55051 (paratype): A, habitus, dorsal view; B, leg 5, anterior view; C, maxillula, anterior view; D, maxilliped, anterior view; E, maxilla, anterior view. Scale bar: A, 325  $\mu$ m; B, 250  $\mu$ m; C–E, 125  $\mu$ m.

Swimming legs P1–4 (Figs. 7A–D) constructed basically like in female. Formula for spine (Arabic) and seta (Roman) for distal exopod segments in P1–4 as follows: 3V–3V–2V–3V. All setae in swimming legs with clear seen segment-like divisions. Distal spines in exopod P1–4 not so strong in middle part as in female, slightly shorter

than nearest setae and longer than distal segment length (Fig. 7D). Lateral edge of coxa in P1–4 with groups of long hair-setae. Both sides of P1–P4 covered with very short hair-setae.

Rudimentary legs P5 (Fig. 6B): right leg with basipodal segment cylindrical in shape and with small hill on inner side pointed with long spine, distal bent segment with several sensitive pores and two short spines in middle. Left leg basipod also cylindrical about 1.45 times as long as wide, next exopodal segment with two long spine in middle part, distal segment with strong long spine in middle and hook in end similar in construction to *E. affinis*.

**Etymology.** The new species is named in honor of Dr. Carol Lee, Wisconsin University, Madison, USA, in recognition of her pioneering work on cryptic species within the *E. affinis* complex.



**FIGURE 7.** *Eurytemora carolleae* sp. nov., male, ZIN 55051 (paratype): A, swimming leg 1, anterior view; B, swimming leg 2, anterior view; C, swimming leg 3, anterior view; D, swimming leg 4, anterior view. Scale bar: 100  $\mu$ m.

**Remarks.** The new species was discriminated from *E. affinis* (Poppe, 1880) that inhabits European coastal brackish and fresh water habitats. *Eurytemora carolleeae* sp. nov. differs from *E. affinis* by a combination of characters that includes in both sexes: clearly seen segment-like divisions in setae on P1–P4 and caudal rami; mandible with large outside orientated tooth separated with a gap from other teeth (Figs. 3A, 9).

Females *E. carolleeae* are equipped with wing-like outgrowths of the genital double-somite that significantly increases the proportion between anterior and posterior parts of the somite (Fig. 9C; Table 2). This species has P5 with a very small (tiny) spine in the second exopodal segment placed between two distal spines. The length of this tiny spine is less than the width of the nearest spines, or about 10 % of the short distal spine length (Table 2).

**TABLE 2.** Selected morphometric indexes in females of *E. carolleeae* sp. nov. and *E. affinis* (Poppe, 1880) from their type localities. Mean + standard deviation (Min–Max). In bold – significant difference with  $p < 0.05$ . \*Only in one female among 56 examined, possibly an aberrant specimen.

Species/Indexes	Caudal rami, L/W	Genital somite, W2/W1	Leg 5, tiny spine/spine1	Leg 4, Long spine/Distal Segment
<i>E. carolleeae</i>	6,06+0,531 (5–7,4)	1,54+0,103 (1,35–1,67)	0,11+0,029 (0,06– 0,2*)	0,85+0,068 (0,70– 0,99)
<i>E. affinis</i>	6,08+0,638 (5,2– 7,8)	1,24+0,075 (1–1,39*)	0,25+0,053 (0,18– 0,36)	0,94+0,066 (0,8 –1,07)

**TABLE 3.** Selected morphometric indexes for *E. carolleeae* sp. nov. and *E. affinis* (Poppe, 1880) in males from the type localities. Mean + standard deviation (Min–Max).

Species/Indexes	Caudal rami, L/W	Leg 5 Basipod left, L/W	Leg 4, Long spine/Distal Segment	Leg 1, Long spine/Distal Segment
<i>E. carolleeae</i>	9,56+0,936 (8,06–11,07)	1,43+0,13 (1,27–1,6)	1,06+0,060 (0,95–1,14)	1,1+0,117(1–1,39)
<i>E. affinis</i>	7,77+1,037 (6–10,45)	0,96+0,049 (0,91–1,08)	1,14+0,052 (1,03–1,25)	1,21+0,081 (1,06–1,38)

**TABLE 4.** Selected morphometric indexes in female and male *E. carolleeae* sp. nov. (USA, Chesapeake Bay) and *E. affinis* (Poppe, 1880) (from several European localities). Populations from the type localities are indicated in bold.

Species	AREA	Female		Male	
		Caudal rami, L/W	Leg 5, tiny spine/spine1	Caudal rami, L/W	P5 basipod left, L/W
<i>E. carolleeae</i>	CHESAPEAKE BAY	6,06	0.095	9.56	1.43
<i>E. affinis</i>	RIGA BAY	7.59	0.292	10.86	0.98
<i>E. affinis</i>	SCHELDE	7.03	0.31	8.01	0.92
<i>E. affinis</i>	SEINE	7.61	0.241	8.77	0.94
<i>E. affinis</i>	LOIRE	6.26	0.196	7.93	1.1
<i>E. affinis</i>	ELBE	6,08	0.25	7.77	0.96
<i>E. affinis</i>	GUADALQUIVIR	–	–	12.91	1
<i>E. affinis</i>	GIRONDE	7.87	0.239	7.04	0.98

In males of *E. carolleeae* the caudal rami are naked on both dorsal and ventral sides, left rudimentary P5 with exopod of cylindrical shape, and length/width proportion not less than 1.25 times (Table 3, Fig. 9).

In *E. affinis* only a small proportion of adults in some local populations (see below) have setae on swimming legs with unclear segment-like divisions. They have mandible with more or less equal teeth; the outside tooth is not separated from neighboring teeth by a gap.

Female *E. affinis*, in contrast to the newly described species, has a genital somite with only small upper outgrowths (if present) which do not look like wings; the ratio between anterior and posterior parts in the genital somite is significantly less than 1.5 (see Table 2). In P5 the tiny spine is much longer than width of the nearest distal spines and usually is more than 15 % of the short distal spine length (Table 4).

Male *E. affinis* always has caudal rami with at least several spines on the dorsal surface. In P5 the left exopodal segment has a triangular shape outgrowth that decreases length/width proportion in this segment up to 1 or even less (see Table 3).

### Variability among European populations of *E. affinis* (Poppe, 1880)

*Eurytemora affinis* is known for significant morphological variation among individuals from local populations (Dussart 1967). Variability in significant characters in *E. affinis* was checked here and compared among eight distant Atlantic and Baltic populations, inhabiting river estuaries. Hereafter we present brief comments on morphological variations found among eight European populations of *E. affinis*.

**Gironde River Estuary, France.** About 15% of the population had setae with segment-like divisions in P1–P4. Other signs of *E. affinis* (P5 construction, shape of teeth in mandible) both in male and female were close to the type population from the Elbe River (see Table 2, 4).

**Guadalquivir River in Seville, Spain.** With exception of the Basque Country (Albaina et al. 2009), this is the only river in Spain where *E. affinis* has been found. In January (date of sampling) we found only adult males and copepodid 5 females; there were no adult females. Males had typical triangular outgrowths on the left basipod of P5, caudal rami were long and narrow ( $L/W=12,9+1,3$ ), but always with at least a few tiny spines on the dorsal surface of rami.

**Helsinki Fishing Port, Finland.** In some males and females we found setae with clearly observable segment-like divisions. Caudal rami of males were quite long, about 11 times as long as wide, but always with tiny hairs and dents on the dorsal surface. Other characters were the same as in the type population of *E. affinis* from the Elbe River (see Table 2, 4).

**Loire River Estuary, France.** More than 50% of the *E. affinis* females examined for swimming legs had setae with segment-like divisions. We also found several females with segment-like division in caudal rami setae similar to *E. carolleae*. One female had wing-like processes on the genital double-somite similar to American females from the Chesapeake Bay. The tiny spine in P5 in *E. affinis* females in the Loire was less than in the type population ( $0,196 + 0,035$ ). This index on average was significantly larger than in *E. carolleae* sp. nov. Only in one female from the Loire it was similar to the North-American species. At least some females of *Eurytemora* in The Loire are morphologically close to the North-American species. In several seaports in the Baltic we found an invasion of *E. carolleae*, and therefore we can suspect a similar penetration of the same species in the Loire estuary also. This assumption should be tested with molecular-genetic tools.

**Luga River Estuary, Gulf of Finland, Baltic Sea, Russia** (Fig. 8). Here individuals of both taxa were found living together (Alekseev et al 2009). *Eurytemora carolleae* females can be easily separated from *E. affinis* by lower length/width proportions in the genital double-somite and in the caudal rami, and by the frontal part of the cephalothorax being oval in *E. carolleae* and triangular in *E. affinis* (see Fig. 8). In males, *E. affinis* length/width proportion in the basipod of left P5 was about 1, whereas in *E. carolleae* it was never less than 1.4.

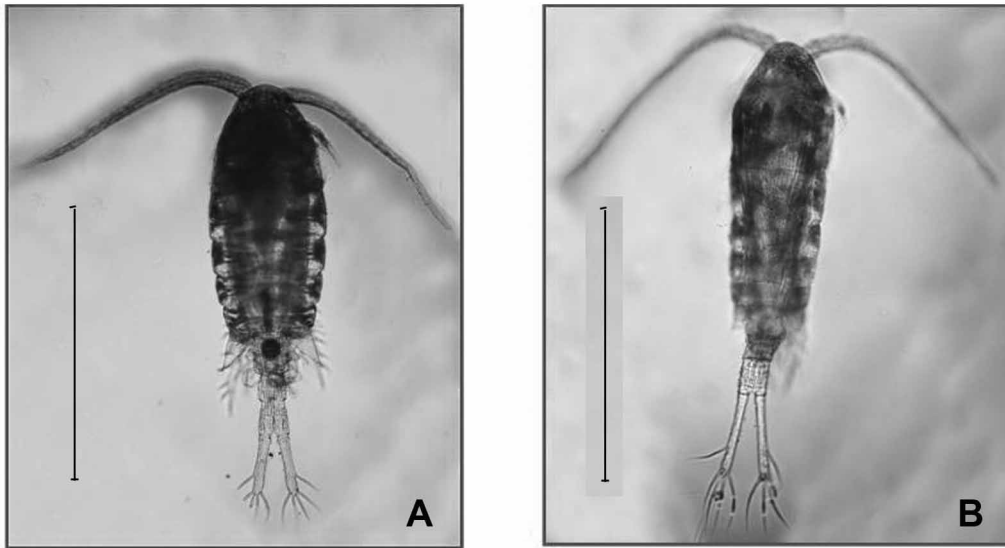
**Riga Bay, Baltic Sea, Latvia.** Females of *E. affinis* at this site have the tiny spine on the distal exopodite segment P5 that is about 30% of the nearest small spine 1 length (longest ratio among 8 populations studied). In males, caudal rami have groups of strong denticles on the dorsal surface.

**Seine River Estuary, France.** About 35 % of females exhibited swimming legs that had setae with slightly seen segmentation but no segments were found in caudal rami setae as in *E. carolleae*. Genital double-somite ratio ( $W2/W1$ ) in the Seine population ( $1.23 + 0.089$ ) was also significantly less than in *E. carolleae*. In *E. affinis* females in the Seine Estuary the tiny spine of leg 5 was significantly longer ( $0.24 + 0.025$ ) than in *E. carolleae* (see Tables 2, 4). All *E. affinis* males in the Seine Estuary had caudal rami with spines on the dorsal surface and a large outgrowth on the left basipod of P5.

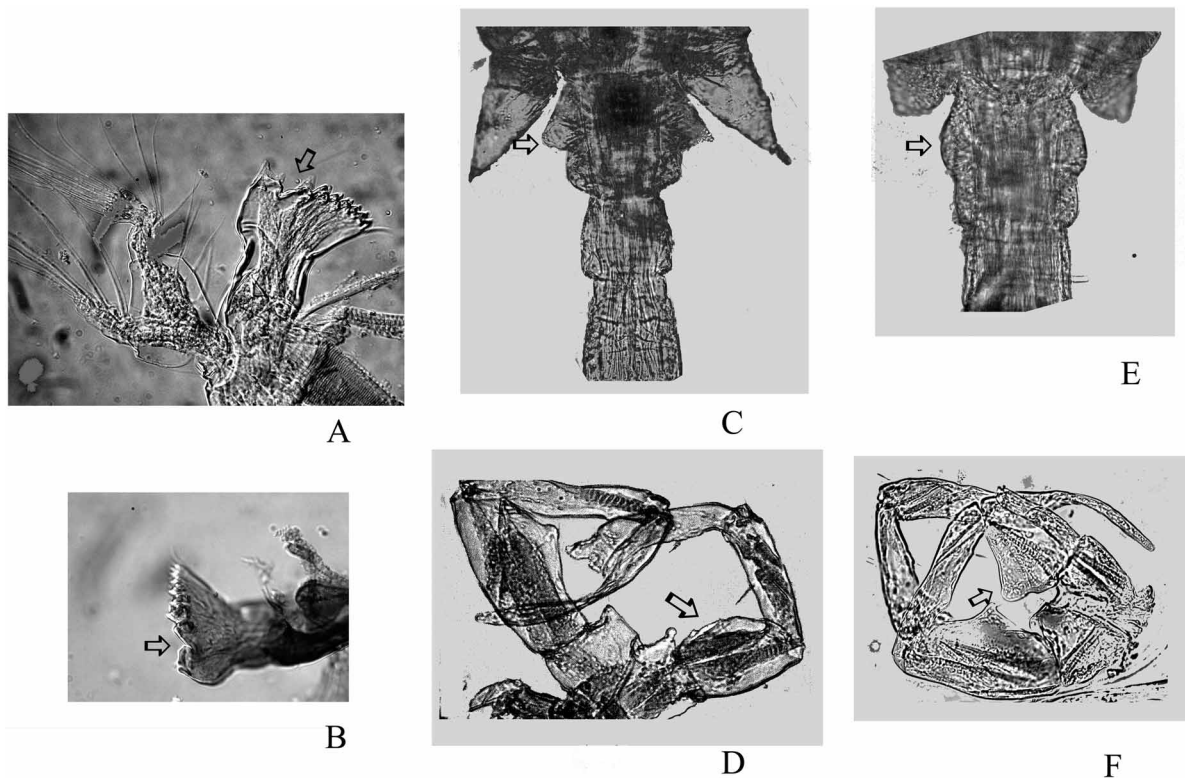
**Schelde River Estuary, Belgium.** The tiny spine on P5 of *E. affinis* females in the Schelde had the maximum length among all populations studied (Table 4). In male P5 the left basipod L/W ratio also had a minimal value (see Table 4). This *E. affinis* population seems like more morphologically divergent from *E. carolleae* than other European populations.

This overview lets us conclude that characters used here to discriminate *E. carolleae* are sufficient to distinguish all geographically distant populations of *E. affinis* from the Northern part of the Gulf of Finland in the Baltic

to the South of Spain (see Fig. 1). Only a low number of individuals from some populations of *E. affinis* showed a small overlap in certain, but not all, characters with *E. carolleae*. In some cases (for example population in the Ust-Luga), it was caused by the *E. carolleae* invasion that was confirmed by molecular-genetic tools (Alekseev *et al.* 2009).



**FIGURE 8.** *Eurytemora carolleae* sp. nov. (A) and *E. affinis* (Poppe, 1880) (B) from the Luga Bay, The Finish Gulf, Baltic Sea. (Photo: Mrs Natalia Sukhikh)



**FIGURE 9.** *Eurytemora carolleae* sp. nov. (A–D) and *E. affinis* (Poppe, 1880) (E–F): A, female mandible, arrow indicating a gap; B, male mandible, arrow indicating a gap; C, female genital somite with wing-like outgrowth; D, male leg 5 with arrow indicating left basipod; E, female genital somite without wing-like outgrowth; F, male P5, arrow indicating left basipod. (Photo: Mrs Natalia Sukhikh)

## Discussion

Lee and Frost (2002) combined the molecular genetic results of Lee (2000) with brief morphometric analysis of *Eurytemora cf. affinis* collected from 43 sites around the Holarctic. They had already proposed that the European populations are quite distinct from the North Atlantic clade and also from all the other clades of *E. affinis* (Poppe, 1880). Though they declared that there are morphological distances among the studied *E. affinis* populations, in fact they did not suggest any clear morphological differences between the North Atlantic and European clades. In their conclusion, they revealed morphological stasis in the *E. affinis* group and concluded that due to the long-term reproductive isolation and molecular evolution, the four major Asian, European, North Atlantic and Pacific clades became a complex sibling species “where speciation was accompanied by lack of morphological differentiation.” Lee & Frost (2002) used for their analysis 3–5 individuals from each site. For morphometry, they measured a limited number of structures. Female characters included genital double-somite proportion, and ratio of the 22nd and 24th segments of the first antenna. Male characters were limited to some ratios in P5 and, as in the female, the ratio of the 22nd and 24th segments of the first antenna. These characters were selected by the authors because they are typically used for copepod taxonomy. We argue that in a sibling species complex this approach might not be an effective tool to distinguish closely related species.

That is why in our study we examined a larger amount of material. In the case of morphological variability up to 60 specimens from the type populations were measured. Additionally, before selecting morphometric indexes, we examined about 25 morphological structures in *E. affinis* from the type locality in the Elbe estuary and in specimens from the North Atlantic clade from the Chesapeake Bay. Then all the selected morphological differences were checked (in the case of morphometric indexes with appropriate statistic methods) in 8 other distant populations in these two species. This analysis let us come to some extent to the opposite conclusion than Lee & Frost (2002) on the possibility of separating and describing a new species in this complex based on external morphology.

Dodson *et al.* (2010) used measurements of 26 characters from 125 female specimens to determine patterns of morphological variation in *Eurytemora* species within the ancestral range in Alaska. The goal of their study was to analyze morphological variation and divergence within this genus, focusing on *Eurytemora* species that inhabit North America. As a result they excluded from consideration morphological characters of *E. affinis* from Europe and were not able to separate the North Atlantic clade from the native *E. affinis*.

One of the possible additional explanations why previous researchers did not find morphological differences between these two taxa could be the lack of material from the type population from the Elbe River in their analysis. Without a re-description or at least detailed analysis of the type population of a species described in the 19th century, to our mind, it is impossible to separate a new species in a sibling species complex.

On the level of molecular-genetic distances among clades studied by Lee (2000), it is also clear to us that so-called Asian and Pacific clades, which are not of course taxonomically valid names if based on genetic differentiation alone, very soon will appear as new species or at least subspecies. A preliminary morphological survey (Aleksseev, unpublished) indicates that they can be discriminated from *E. affinis* and *E. carolleae* **sp. nov.** These taxa, when described, will define the *E. affinis* complex more clearly based on morphological differentiation. This will be especially important if more new species of this complex cross geographical borders via human mediated vector.

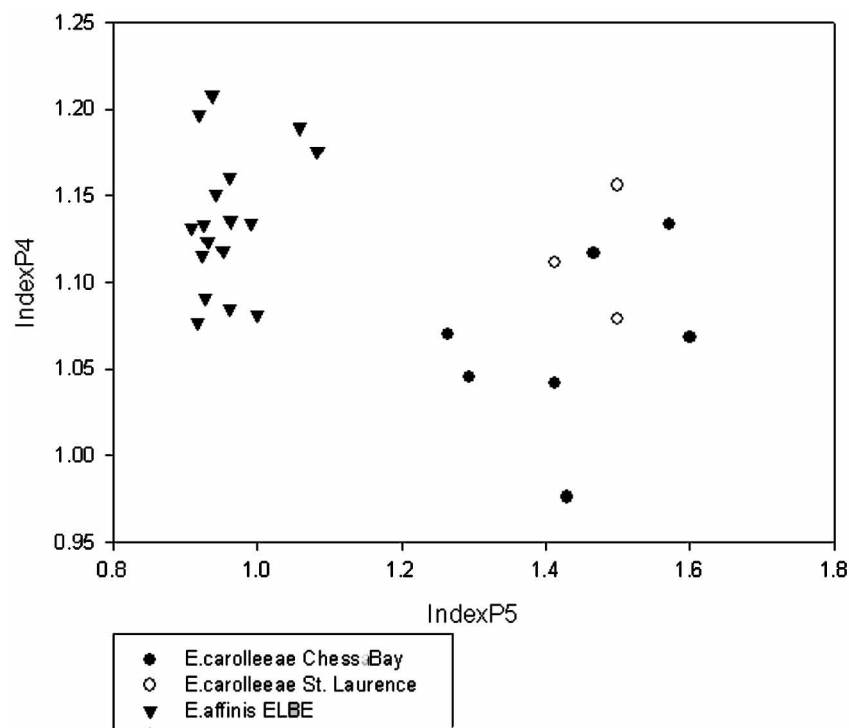
Among the European populations studied here practically no significant variation was observed in the P5 characters both in males and females. In female *E. affinis* from the most studied European sites, the tiny spine in distal exopod segment P5 was about 20% and sometimes about 30% of the nearest small spine in length. In males, *E. affinis* length/width proportion in basipod left P5 was about 1, due to an asymmetrical outgrowth on the inner side of the leg they possess (see Table 4). These characters of P5 construction are recommended as the most valuable ones for morphological separation between these two taxa because, due to the recent invasions, they can be found together, both in Europe and in North America (Busch & Brenning 1992; Aleksseev *et al.* 2009).

Compared to the other 16 congeners, *E. carolleae* differs by a combination of characters that in females include: genital double-somite with lateral “wings” of different sizes, symmetrical P5 in the first exopod segment with smooth inner outgrowth, oriented under 45 degrees to the segment axes and with two sub-equal spines; coxae of legs 1–4 with long setae at inner side; more or less symmetrical abdomen; caudal rami and last abdominal segment covered with dense spines.

Males in *E. carolleae* differ from other congeners by: a long seta at inner side coxa in legs 1–4; 4-segmented right leg in P5; caudal rami naked on both dorsal and ventral surfaces; last abdominal segment covered with rather

strong dents; 8–12 segments of the first antenna armed with spines, the spine at 12th segment at least two times as long as other spines; in P5 left basis more or less cylindrical in shape, provided with two small round shape outgrowth, the first segment of left exopod without very long spine distally and the second (distal) segment with 2 lobes at the end.

Beside the Atlantic coast of USA *E. carolleae* was also found in samples collected in Canada (The St. Lawrence Estuary, Dr. G. Winkler donation). Specimens from these two populations are morphologically close, but clearly different from *E. affinis* from the Elbe River population (Fig. 10). Analysis of published drawings and digital pictures of *E. cf. affinis* lets us conclude that there is at least one record of *E. carolleae* in freshwater environments in Mexico from a reservoir in a desert area (Suárez-Morales *et al.* 2008).



**FIGURE 10.** Morphological indexes in *Eurytemora affinis* (Poppe, 1880) from the type locality and *E. carolleae* **sp.nov.** from the Chesapeake Bay, USA and St. Lawrence Bay, Canada. Males: IndexP4, distal spine/segment length ration in EXP P4; IndexP5, L/W ratio in BAS P5 left. For more explanation see text.

Specimens of the Atlantic clade of *E. affinis* from the Chesapeake Bay (= *E. carolleae*) were recently found in the Gulf of Finland, Baltic Sea (Aleksiev *et al.* 2009).

*Eurytemora carolleae* shows a tendency to advance further inland in continental waters (Lee & Peterson 2003; Suárez-Morales *et al.* 2008). In the new invasive area in Europe its distributive range will possibly be significantly modified in the near future. On the other hand, all previous records of *E. affinis* in North America should now be checked again to avoid mixing the newly described *Eurytemora carolleae* with *E. affinis* that recently invaded from Europe (Busch & Brenning 1992; Debiase & Taylor 1993; Suárez-Morales & Reid 1994, 1998; Suárez-Morales *et al.* 2008).

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## References

- Albaina, A., Villate, F. & Uriarte, I. (2009) Zooplankton communities in two contrasting Basque estuaries (1999–2001): reporting changes associated with ecosystem health. *Journal of Plankton Research*, 31, 739–752.
- Alekseev, V.R., Abramson, N.I. & Sukhikh, N.M. (2009) Introduction of Sibling Species to the Ecosystem of the Baltic Sea. *Doklady Biological Sciences*, 429, 1–4.
- Busch, A. & Brenning, U. (1992) Studies on the status of *Eurytemora affinis* (Poppe, 1880) (Copepoda, Calanoida). *Crustaceana*, 62, 13–38.
- Croskery, P. (1978) The freshwater co-occurrence of *Eurytemora affinis* (Copepoda: Calanoida) and *Manayunkia speciosa* (Annelida: Polychaeta): possible relicts of a marine incursion. *Hydrobiologia*, 59, 237–241.
- Debiase, A.E. & Taylor, B.E. (1993) New occurrences of *Eurytemora affinis* and *Epischura fluviatilis*, freshwater calanoid copepod species of the family Temoridae, in South Carolina. *The American Midland Naturalist*, 130, 386–392.
- Dodson, S.I., Skelly, D.A. & Lee, C.E. (2010) Out of Alaska: Morphological evolution and diversity within the genus *Eurytemora* from its ancestral range (Crustacea, Copepoda). In: L. Naselli-Flores & G. Rossetti (Eds.) *Fifty years after the “Homage to Santa Rosalia”: Old and new paradigms on biodiversity in aquatic ecosystems*. *Hydrobiologia*, 653, 131–148.
- Dussart, B. (1967) *Les Copépodes des eaux continentales d'Europe occidentale. Tome I: Calanoïdes et Harpacticoides*. N. Boubée et Cie, Paris, 500 pp.
- Dussart, B. & Defaye, D. (2002) *World directory of Crustacea Copepoda of inland waters. I – Calaniformes*. Backhuys Publishers, Leiden, 276 pp.
- Huys, R. & Boxshall, G.A. (1991) *Copepod evolution*. The Ray Society, London, 468 pp.
- Knowlton, N. (1993) Sibling species in the sea. *Annual Review Evolution and Systematics*, 24, 189–216.
- Lee, C.E. (1999) Rapid and repeated invasions of fresh water by the copepod *Eurytemora affinis*. *Evolution*, 53, 1423–1434.
- Lee, C.E. (2000) Global phylogeography of a cryptic copepod species complex and reproductive isolation between genetically proximate “populations”. *Evolution*, 54, 2014–2027.
- Lee, C.E. & Frost, B.W. (2002) Morphological stasis in the *Eurytemora affinis* species complex (Copepoda: Temoridae). *Hydrobiologia*, 480, 111–128.
- Lee, C.E. & Petersen, C.H. (2003) Effects of environmental acclimatization on adult salinity tolerance in the freshwater invading copepod *Eurytemora affinis*. *Physiological and Biochemical Zoology*, 76, 296–301.
- Poppe, S.A. (1880) Über eine neue Art calaniden Gattung *Temora* Baird. *Naturwasser Verhart Bremen*, 7, 55–60.
- Rylov, V.M. (1922) On new species Copepoda–Calanoida. *Proceedings of Sain–Petersburg Society of Naturalists*, 52, 67–78. [in Russian]
- Souissi, A., Souissi, S., Devreker, D. & Hwang, J.S. (2010) Occurrence of intersexuality in a laboratory culture of the copepod *Eurytemora affinis* from the Seine estuary (France). *Marine Biology*, 157, 851–861.
- Suárez-Morales, E. & Reid, J.W. (1994) Adiciones a la fauna plánctica de aguas continentales de Quintana Roo, México. *Avicent Chetumal*, 9, 29–33.
- Suárez-Morales, E. & Reid, J.W. (1998) An updated list of the free living freshwater copepods (Crustacea) of Mexico. *The Southwestern Naturalist*, 43, 256–265.
- Suárez-Morales, E., Reid, J.W. & Gasca, R. (2000) Free-living marine and freshwater Copepoda (Crustacea) from Mexico. In: J. Llorente, E. Gonzalez & N. Papavero (Eds.), *Biodiversidad, taxonomía y biogeografía de Artrópodos de México. Hacia una síntesis de su conocimiento*, 2, CONABIO & UNAM, Mexico, pp. 171–190.
- Suárez-Morales, E., Rodríguez-Almaraz, G., Gutiérrez-Aguippe, M.A. & Walsh, E. (2008) The coastal-estuarine copepod, *Eurytemora affinis*, (POPPE) (CALANOIDA, TEMORIDAE), from arid inland areas of Mexico: an expected occurrence? *Crustaceana*, 81, 679–694.
- Telesh, I. & Heerkloss, R. (2004) *Atlas of estuarine zooplankton of the southern and eastern Baltic sea. Part II: Crustacea*. Verlag Dr. Kovac, Hamburg, 118 pp.