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Redescription of *Acanthocyclops vernalis* (Fischer, 1853) and *Acanthocyclops robustus* (Sars, 1863) from neotypes, with special reference to their distinction from *Acanthocyclops americanus* (Marsh, 1892) and its invasion of Eurasia

Victor R. Alekseev[1,*], Maria R. Miracle[2,†], Maria Sahuquillo[2,3] and Eduardo Vicente[2]

[1] Zoological Institute of the Russian Academy of Sciences, Universitetskaya Embankment 1, St Petersburg 199034, Russia.
[2] Department of Microbiology and Ecology, ICBiBE, University of Valencia, 46100 Burjassot, Valencia, Spain.
[3] Subdirecció General del Medi Natural, Generalitat Valenciana, València, Spain.
[†] Deceased

[*] Corresponding author: alekseev@zin.ru

ABSTRACT

Redescription of *Acanthocyclops vernalis* (Fischer, 1853) and *Acanthocyclops robustus* (Sars, 1863) from neotypes, with special reference to their distinction from *Acanthocyclops americanus* (Marsh, 1892) and its invasion of Eurasia

This paper provides a redescription of *Acanthocyclops vernalis* and *A. robustus* neotypes collected in their type localities in Russia (Orlov pond, Peterhof) and Norway (a stream outcoming from a lake in Oslo) with a special attention to their morphological differentiation from *A. americanus*. Due to overmixing of these species throughout most of the last century and fast arrival and spreading of the aggressive planktonic predator *A. americanus*, all previous indications of *A. robustus* should be checked and confirmed. More than 200 localities in Valencia region (Spain) and more than 30 localities in Armenia, Belgium, Egypt, Finland, France, Kazakhstan, Norway, Russia, Sweden and Tunisia were checked for the presence of *A. americanus*. In all these countries the invader was found in large numbers as dominant species often substituting other native *Acanthocyclops* species in limnetic communities. A brief history and possible explanation of the successful invasion of *A. americanus* in Eurasia is discussed.

Key words: *Acanthocyclops robustus*; *Acanthocyclops vernalis*; *Acanthocyclops* redescriptions; cyclopoid taxonomy; *Acanthocyclops americanus*; biological invasion, distribution in Eurasia

RESUMEN

Redescripción de los neotipos de* Acanthocyclops vernalis *(Fischer, 1853) y* Acanthocyclops robustus *(Sars, 1863), con especial atención a sus diferencias morfológicas con* Acanthocyclops americanus *(Marsh, 1892) y su invasión en Eurasia

Este trabajo proporciona una nueva descripción de los neotipos de A. vernalis *y* A. robustus *recopilados en sus localidades tipo en Rusia (estanque de Orlov, Peterhof) y Noruega (una corriente que sale de un lago en Oslo), con especial atención a su diferenciación morfológica respecto a* A. americanus. *Debido a la mezcla de estas especies prácticamente durante todo el siglo pasado y la rápida llegada del agresivo depredador planctónico* A. americanus, *todas las indicaciones anteriores de presencia de* A. robustus *deben verificarse y confirmarse. Se estudia la presencia de* A. americanus *en más de 200 localidades en la región de Valencia (España) y más de 30 ubicaciones en Armenia, Bélgica, Egipto, Finlandia, Francia, Kazajstán, Noruega, Rusia, Suecia y Túnez. En todos estos países, la especie invasora se encontró en grandes cantidades y como forma dominante, a menudo sustituyendo a las especies nativas de* Acanthocyclops *en las comunidades limnéticas. También se presenta una breve historia y posible explicación de la exitosa invasión de* A. americanus *en Eurasia.*

Palabras clave: Acanthocyclops robustus*;* Acanthocyclops vernalis*; redescripción de* Acanthocyclops*; taxonomía de ciclópidos;* Acanthocyclops americanus*; invasión biológica, distribución en Eurasia*

INTRODUCTION

Acanthocycops americanus (Marsh, 1892) a planktonic cyclopid first described from the Great Lakes in Northern America was found soon after by Lowndes (1926, 1928a) in Great Britain and later on in several other countries of the Old World (Dussart, 1967; Monchenko, 1961; Alekseev & Kosova, 1976). It became the first documented invasive copepod species transported from the Americas to Europe. In the last century it was also found in limnetic communities throughout Eurasia. After Kiefer's (1976) mistaken designation of *A. robustus* (G. O. Sars, 1863) as an older synonym of *A. americanus*, a lot of data indicating their differentiation has been accumulated (Alekseev *et al.*, 2002, Lewis *et al.*, 2004, Alekseev & Monchenko, 2011, Miracle *et al.*, 2013). Clearly visible ecological differences among planktonic *A. americanus* and littoral-benthic *A. robustus* in combination with limitation of classical morphological features used in taxonomy of this cyclopids caused it to become a challenge for several generations of European and American copepodologists.

To solve these taxonomical and ecological problems, representative populations from type localities of the respective taxa were analyzed morphologically and genetically using mitochondrial COI and 12S rRNA markers (Miracle *et al.*, 2013). Molecular-genetic analysis revealed that *A. robustus*, *A. americanus* and *A. vernalis* are well-separated species. All studied European populations of *A. americanus* had small genetic distances to native American reference population obtained from *terra typica*. Consequently, all genetically studied *A. americanus* populations in Eurasia are recognized as a recent biological invasion as it was proposed by Lowndes (1926) (Miracle *et al.*, 2013).

Soon after, a paper appeared on this *robustus*-group, trying to reincarnate wrongly described *A. einslei* and *A. trajani* (Anufriieva *et al.*, 2014). The authors of the paper totally ignored molecular-genetic evidence on the similarity/equivalence of *A. einsli* to *A. robustus* and *A. trajani* to *A. americanus*. They concentrate only on morphological differences quite common in different cyclopid population which is not enough nowadays when molecular-genetic tools have clearly corroborated that the morphologically different populations belong to the same species. Even excellent morphologists in XXI century cannot ignore results of molecular-genetic species validation that was confirmed for this *Acanthocyclops robustus - vernalis* complex recently (Błędzki & Rybak, 2016). A good example could be found in *Cyclops abyssorum* group: many local populations of this group were described as separate species but in direct hybridization experiment of Einsle (1996) easily crossed with each other.

A. americanus dominates in many separated and even isolated waterbodies in Europe except in Arctic areas in Scandinavia and Russia. This species became a main summer planktonic invertebrate predator in large Ukrainian (Dnieper River) and Russian (Don River and Volga River) reservoirs. *A. robustus* should not be a dominating planktonic species due to preference to benthic (littoral) biotopes. All these water-reservoirs appeared during the 50-60s of the last century after creation of cascade of hydropower electric stations. In the Rivers Volga, Dnieper and Dnestr no species of this genus was found in limnetic plankton. *A. americanus* was found in the Caspian Sea, Lake Sevan (Caucasus region), Lake Aral area, Lake Chany and northern reservoirs built at the Yenisey River (Western and Central Siberia). Recently, this species was found in North Africa, the Nile River delta and in Tunisian fresh waters (Victor Alekseev personal data, not published).

At the end of the XIX century, three species of the *Acanthocyclops robustus* group were described variously as: *Acanthocyclops vernalis* Fisher (1853) from the Saint Petersburg area (Russia); *Acanthocyclops robustus* Sars (1892) from the Oslo area (Norway); and *Acanthocyclops americanus* by Marsh (1892) from Wisconsin (USA).

A. americanus was redescribed on the basis of a neotype obtained from *terra typica* (Miracle *et al.*, 2013). At the same time two other closely related sister-species, *A. vernalis* and *A. robustus*, have not yet been redescribed following modern standards. That can create confusion

between them and *A. americanus* or some other similar species of *Acanthocyclops*.

This paper provides a redescription of *A. vernalis* and *A. robustus* from the type localities with a special attention to differentiation from *A. americanus*. In addition, we provide new ecological features on *A. americanus* biology and current distribution based, first, on extensive European samplings and, second, on intensive and detailed samplings over a single Mediterranean area, Valencia region, as a case study. Both based on collection from authors and using the morphological differentiation detailed in the first part of the paper. Also, a possible brief history of biological invasion of *A. americanus* in Eurasia is discussed.
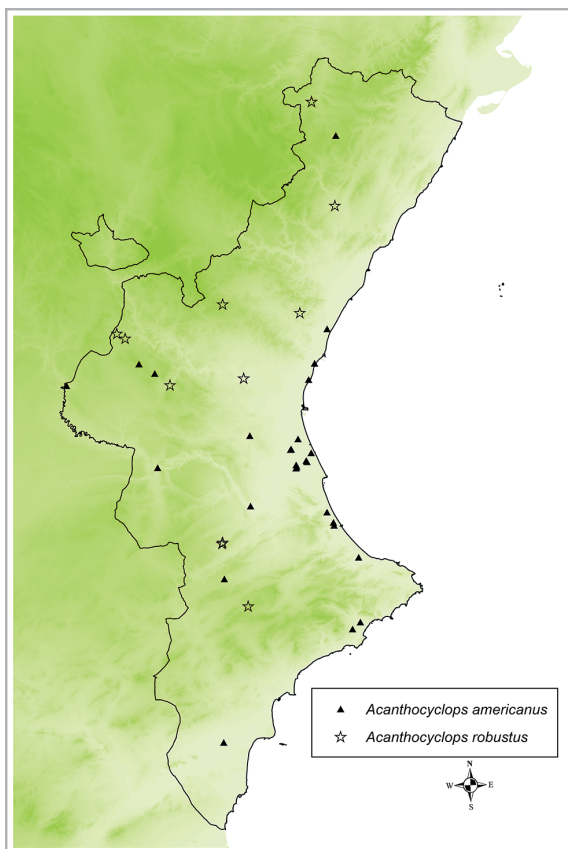

Figure 1. Sampling sites of *Acanthocyclops* species in Valencia region with indication of found species. *Sitios de muestreo de las especies de* Acanthocyclops *en la región de Valencia con indicación de las especies encontradas.*

MATERIALS AND METHODS

1.- Morphological description of *A. americanus*

Morphological description of *A. americanus* had been done on material from *terra typica*, obtained from Prof. Stainly Dodson, Wisconsin USA (Miracle *et al.*, 2013). Hereafter we have used neotype specimens of two morphologically close species which consist of: 10 ♂ and 20 ♀ of *A. vernalis* selected from a sample taken in April 1998 in Orlov pond, Peterhof, St-Petersburg, the type locality for this species as it was described by S. Fischer (1853); 8 ♀ and 2 ♂ of *A. robustus* selected from a sample collected in a stream connected with Lake Maridalsvann, Oslo, Norway. A classical taxonomical method of external morphology examination has been used. Adult females and males before dissecting were photographed from dorsal side for a common view of animal with a 12-megapixel digital photo camera equipping Zeiss ImagerA1 microscope. Then specimens were dissected in a drop of glycerol on a microscope glass covered with a cover glass supported by droplets of plasticine on opposite corners to prevent damage of taxonomically important parts. Drawings were made at 400-1000x magnification with microscope equipped with Nomarski optics and a camera *lucida*, converted to ink, then digitized with scanner (resolution 1200 dpi) and used for mounting in drawings sheet. Each picture had a scale bar added. All slides obtained and used for study were catalogued and placed in the Federal Collection of Zoological Institute of RAS #96-03-16 (Russia)

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Abbreviations used in description and drawings

A1 – antennula; A2 – antenna; CR – caudal rami; CPT – cephalosome; GDS – genital double somite; Enp – endopodite; Exp – exopodite; P1-4 – swimming legs 1-4; P5-6 – rudimental legs; TH1-4 – free thoracic somite

2.- Growth experiments

Experiments and field observation were conducted in the Volga River delta laboratory. In a tempo-

rary waterbody placed in Astrakhan State Nature Reserve regularly filled with Volga River water during Spring flooding, we filtered on a daily basis 100 liters with a 10 liter can via an Apstein zooplankton net (mesh size 70 μm) to observe the reactivation of copepod dormant stages present in sediment and population dynamics in several *Acanthocyclops* species coexisting together in the same pool. Species identification was done for females, males and diapausing copepodite stages 4-5 to the last stage. Special taxonomic keys were used for identification (Alekseev, 2000).

For growth experiments, 5-7 female of *Acanthocyclops americanus* and *A. vernalis* with egg sacs were picked up from the pool and incubated in lab until nauplii hatching out. Females were immediately killed and identified to subspecies level. Offspring was used in two types of experiments.

1. In lab conditions 30-50 nauplii of two *Acanthocyclops* species (*A. americanus* and *A. vernalis* as a control) were grown separately (one individual per experimental vial) and measured daily under microscope with ocular micrometer (resolution 7 μm). Each nauplius was placed in 20 ml vial and at copepodite stages they were transferred to 100 ml vials. Water change and organism measurements were done daily at the same time for each animal. Food for nauplii (protists and algae) and for copepodites and adult (crustaceans freshly killed by heating) was replenished after measurements once a day, temperature in lab was checked every 8 hours. Development time for each stage was first converted to 20 ºC and then estimated as an average % from female development time. Metabolic rate and productivity were calculated after Edmondson & Winberg (1972) and also converted to 20 ºC following Crog's evacuation.

2. At least 30 individuals of both species (control groups) were placed in glass tubes covered on both sides with a double mesh membrane and reared directly in pool under natural conditions. Organisms were checked, counted and measured several times until maturation and became reference groups for laboratory grown ones.

Female fecundity was evaluated in about one hundred females randomly collected during the time of the species presence in zooplankton of the temporary waterbody. Embryonic development time was estimated in lab under controlled temperature. Observation for egg hatching time was carried out with a frequency of about one hour.

3.- Distributions: sites and sampling methods

3. 1.- Sampling methods and sites in Valencia region, Spain

First references to the presence of *Acanthocyclops* in Valencia came in the 70s with the study of Albufera lagoon (Blanco, 1976) although unfortunately no specimens are available. Under the supervision of one of us (Dr. Miracle) several studies focused specifically on zooplankton in this coastal lagoon surrounding marshlands and other neighbouring coastal wetlands began in 1982 and have continued, until today (Oltra, 1993; Oltra & Miracle, 1984; Alfonso & Miracle, 1990; Alfonso, 1996; Miracle & Sahuquillo 2002, Moss *et al.*, 2003). The crustacean communities in inland ponds were studied during 2006 and 2007 in extensive surveys to cover the different eco-regions of the Comunitat Valenciana (Sahuquillo & Miracle, 2013). Overall, we sampled 140 ponds (86 rain-fed ponds, 24 stream- or surface water–fed ponds and 30 spring- or groundwater-fed ponds) (Fig. 1). In all cases, to obtain the fullest possible representation of the planktonic and littoral microcrustacean communities in each site, we took semi-quantitative net samples from different mesohabitats (open waters of the central area, the vegetated littoral and very shallow shores). Littoral and plant-associated microinvertebrates were sampled by sweeping a 90 μm hand-net through the vegetated areas and the shallow shores. From open waters, we took samples with 45 μm towing nets. All the material collected was fixed in 4 % formaldehyde and, additionally, some samples were preserved in alcohol for further molecular analyses, when needed.

3.2.- Sampling methods and sites outside Spain

Sites outside Spain where *A. americanus* was found and collected by one of us (V. A.), included 12 countries (Fig. 2). In deep lakes and water reservoirs, for sampling a tow net with open hole
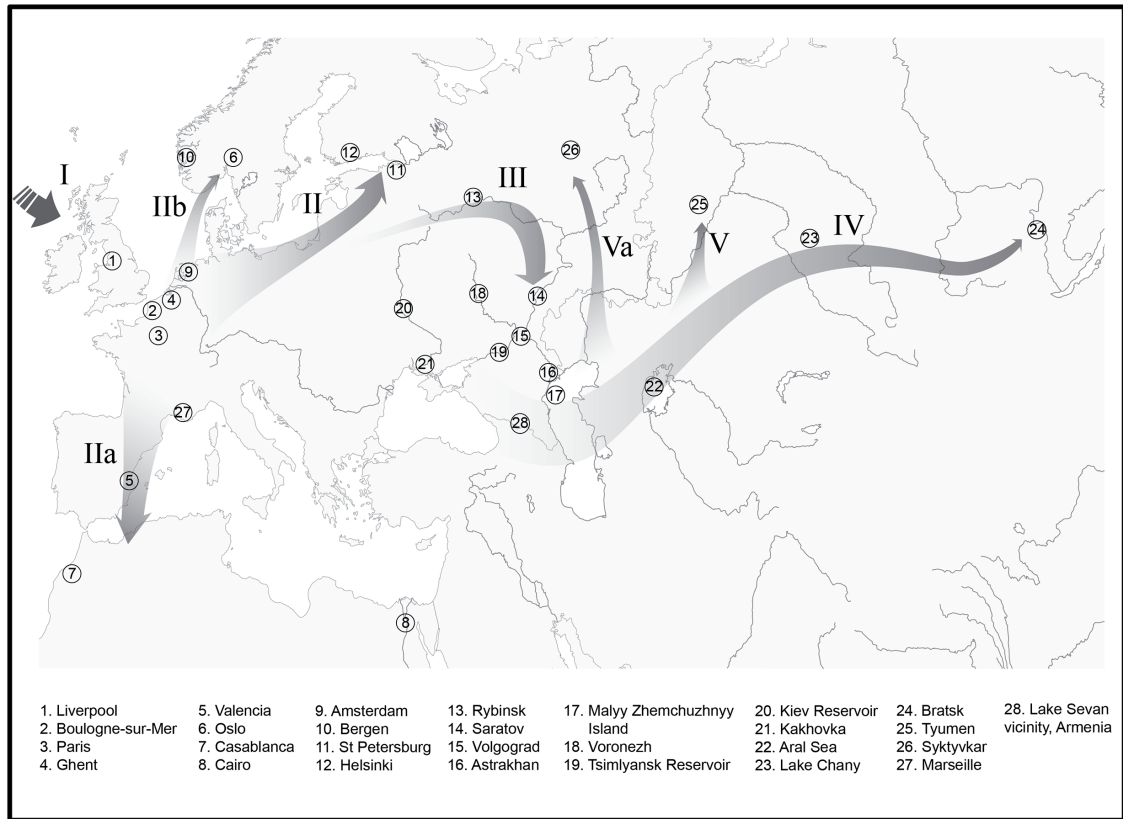
Figure 2. Sampling sites of *Acanthocyclops americanus* found in Eurasia. Numbers indicate the name of the places (see additional information in the Materials and Methods chapter). Arrows indicate the major seasonal aquatic bird migrations. *Lugares de muestreo de* Acanthocyclops americanus *en Eurasia. Los números indican el nombre de los lugares (ver información adicional en el capítulo de Material y Métodos). Las flechas indican las principales rutas estacionales de migración de las aves acuáticas.*

25 cm diameter and mesh size 100 μm was moved from bottom to surface in the central or the deepest parts of lake. In other shallow places, including 3 type localities in Norway, Russia and USA, 50-100 l water with 10 l can via a handle net (mesh size 100 μm) were filtered. Two types of samples were usually collected: formalin conserved for morphological studies and strong alcohol conserved for molecular-genetic and/or morphological studies.

Armenia: 1 shallow lake-let near Lake Sevan, the Caucasus Mountains; Belgium: 3 sites in Ghent and its vicinity; Egypt: 2 sites in Chair vicinity; Finland: 3 sites in Helsinki vicinity; France: 3 sites in Paris, 2 sites in Wimeraux, 3 sites in Camarg, Marseille vicinity; Germany: 3 sites in Plön vicinity; Kazakhstan: 1 site in Aral vicinity; Norway: 2 sites in Oslo vicinity; Russia: more than 20 sites all over the country from St Petersburg in the West to Irkutsk in the East and from Salekhard in the North to Caspian Sea in the South; Sweden: 2 sites in Stockholm vicinity; Tunisia: 2 sites in Hammamet and Tunis; Ukraine: 3 sites in Western, Southern and Eastern parts of the country.

RESULTS AND DISCUSSION

In many localities these three species were found together and in the same place. In large lakes and water reservoirs they maintained different niches as American invasive species is a typical planktonic form while *A. vernalis* and *A. robustus* inhabit littoral areas. Sometimes, as in shallow water bodies like temporary pools in the Volga
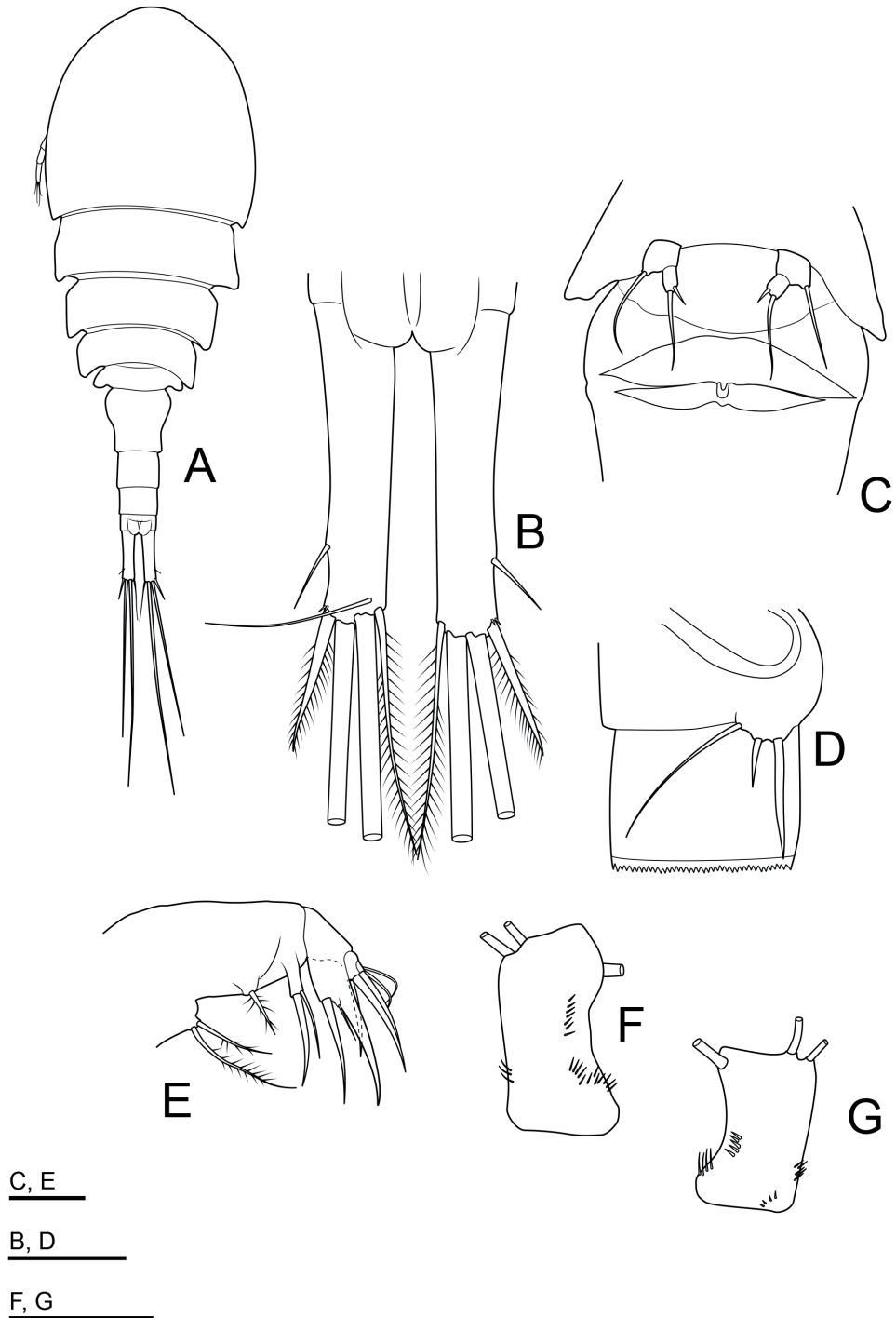
Figure 3. *Acanthocyclops robustus*, holotype of female (A, B, C, E, F, G) and paratype of male (D). Acanthocyclops robustus, *holotipo de hembra (A, B, C, E, F, G) y paratipo de macho (D).*
A - habitus, dorsal; B - caudal rami, dorsal; C - genital segment, ventral; D - P6 ♂; E - maxilla; F - antennal basipodite, frontal; G - antennal basipodite, caudal.
Scale bars: A - 220μm; B, C, D - 50μm; E - 20μm; F, G - 40μm.
Original, V.A.

River delta, they can be found in a sample collected by a handle net among waterplants. Actually, that became one of the reasons why F. Kiefer recognized *A. americanus* as a junior synonym of *A. robustus* after collecting a reference sample in the type waterbody near Oslo (Alekseev *et al.*, 2002). The species being very close to each other show also high variability in classical morphological features.

To avoid the mixing up of these closely related species, hereafter we present descriptions for the three species of the *robustus*-group.

Morphological peculiarities in the *americanus-vernalis-robustus* group
Redescription of *Acanthocyclops robustus* (G.O. Sars, 1863)
(Fig. 3, Fig. 4)

Cyclops robustus Sars, 1863
Acanthocyclops (Acanthocyclops) robustus, Dussart, 1969
Acanthocyclops (s. str.) *vernalis* f. *robusta*, Monchenko, 1974
Acanthocyclops vernalis robustus, Rylov, 1948
Acanthocyclops einslei: Mirabdullayev and Defaye, 2004; Vezhnovets, 2005; Dussart and Defaye, 2006; Rybak and Błędzki, 2010; Blaha, 2010
Acanthocyclops robustus: Kiefer, 1978; Einsle, 1993, 1996; Alekseev, 1995; Mirabdullayev and Defaye, 2004; Dussart and Defaye, 2006; Rybak and Błędzki, 2010, Miracle *et al.*, 2013.

Justification of neotype

Victor Alekseev explored Zoological collections in several locations connected with Sars' work. In Oslo, Lund and Bergen no samples or slides that could be attributed to *A. robustus* were found. Our conclusion is that type material of Sars related to this species does not exist. Lake Maridalsvann is a large waterbody to the north of Oslo practically in the same state. Victor Alekseev visited it in June 2010 and sampled zooplankton in the lake and littoral zone plankton and meiobenthos in the lake and related with its waterbodies: bogs, small rivers coming into the lake. Regarding the plankton in the lake, only *Acanthocyclops americanus*

was found in large densities. In littoral areas few members of other *Acanthocyclops* species which could be attributed to *A. robustus* were collected. Finally, in an unnamed stream coming into the lake, only one group of individuals was collected which could be identified as *A. robustus* males, females or copepodite later stages, dark in color, and they clearly different from planktonic form of *Acanthocyclops* from the lake. We used these individuals both for molecular-genetic and morphological studies. The neotype and type group of *A. robustus* were selected from this sample and are described hereafter.

Material examined. The neotype female and paratype male dissected and mounted in glycerol surrounded with Canadian balsam on slides N56736a and N56736b and deposited into the type collection of Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia.

Female description
(see Fig. 3, Fig. 4)

Body robust, slightly flattened dorso-ventrally, dark-brown in color. Full body length without caudal setae 1300 µm. Cephalosome as long as wide, with maximum width close to its middle. Lateral angles 5th thoracic segment with winged outgrowths, directed laterally. Genital double-somite about as long as wide, round-shaped, receptaculum seminis as in figure 3. Anal segment with row of small dense spinules, proctodeum with single row of setules on both sides. Caudal rami parallel without hairs on inner or outer margins, 5 times as long as wide. Length proportions of distal setae, beginning from outer terminal seta: 1.0/6.7/9.2/1.7. Inner terminal seta shorter than ramus length. Dorsal seta about 1.2 times as long as outer seta and lateral seta slightly longer than ramus width.

Antennule of 17 segments, not reaching distal margin of cephalothorax. Setation of antennulary segments (aesthetascs in Roman numbers) beginning from first: 8/4/2/6/3+I/1/2/ 1/1/0/1+I/2/0/1/ 2/2+I/7+I. Antenna composed of 1-segmented basipodite bearing exopodite represented by a long seta and the two short setae on opposite side, and a 3-segmented endopodite. Basipodite A2 ornamentation as in figures 3 F and G. Three
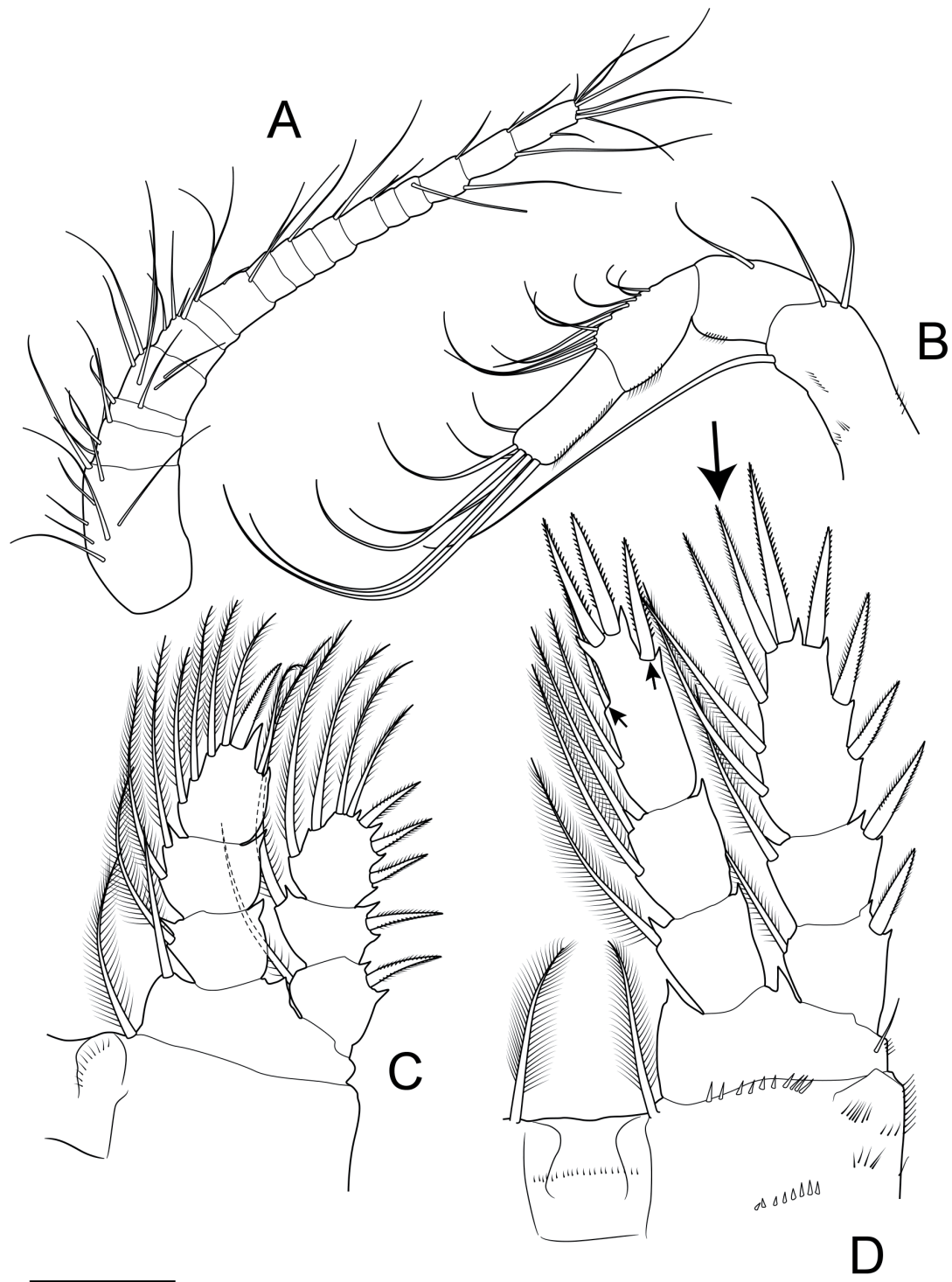
Figure 4. *Acanthocyclops robustus*, female holotype (A-D). Acanthocyclops robustus, *holotipo de hembra (A-D).*
A - antennule; B - antenna; C - P1; D - P4.
Scale bars: A - 200μm; B - 50μm; C, D - 100μm.
Original, V.A.

following segments (endopodite) bearing subsequently: one, nine and seven setae.

Gnathobase of mandible with six strong teeth, rudiment of endopodal segment with 2 long and 1 short seta 1. Maxillula with 3 strong and 3 small teeth, 2 strong setae; maxillulary palp with 7 setae, different in length. Maxilla of 5 segments, praecoxa with 2 strong setae in its middle part; coxa with strong seta in middle and endite bearing 2 claw-like setae; basal endite with 2 very strong claw-like spines, both with row of spinules and small seta near place of fusion of rudimentary endopod. Endopodite 1 with 3 clawlike setae and endopodite 2 bearing distally 2 long setae. Maxilliped of 4 segments, praecoxa + coxa with 2 strong setae in middle part and small setae at their distal end; basis with 2 setae of different length and 3 groups of strong spinules near insertion of setae; first segment of endopodite with strong spine and rudimentary endopod, bearing strong spine and 2 hairless setae.

P1–4 with three-segmented rami. Distal segments of exopodite of legs 1/2/3/4 with 3/4/4/4 spines, respectively. Distal segments of endopodite of legs 1–2 with 1 spine and 5 setae. P4 Enp3 about 2 times as long as wide and inner apical spine 1.13 times longer than outer spine. Lateral outer seta of this segment transformed in strong spine inserted not at same distance from end of segment as inner lateral seta (see Fig. 4D, small arrows). Setae of P4 Exp3 with very short hairs in distal part and long hairs in proximal part (see Fig. 4D, large arrow). P1-3 intercoxal plates with hills and without hairs on its free edge. P4 intercoxal plate with small hills and a row of short denticules in middle part. P4 coxopodite with two rows of spinules near distal and proximal margins and two groups of longer and thinner spinules near lateral margin. Rudimentary P5 two-segmented, basipodite with long outer seta. Distal segment with long apical seta and short inner spine; inner spine slightly more than half of segment length and exceeds segment width.

Male description
(see Fig. 3D and Fig. 7C)

Body length without caudal setae 925 μm. Cephalosome 1.2 times as long as wide, with maximal width close to end of its length. Caudal rami 3.8 times as long as wide, slender inner seta about twice length of outer spine-like seta. Lateral seta placed without spinules in its base. Dorsal seta placed near inner seta insertion, about as long as outer seta. Antennule 12-segmented. Setation of antennulary segments (aesthetascs in Roman numbers) beginning from first: 7(III)/3/1/6(I)/1/1/ 1/4(I)/0/1/0/7. Antennary basopodite ornamentation as in female. Morphology of mouth appendages and Leg 1–3 basically as in female. Distal segment of P4 Enp about 2,5 times as long as wide, with inner apical spine shorter than segment and 1.15 times as long as outer apical spine. Insertion of lateral seta/spine in this segment as in female. Inner edge of basis of P4 with short hair-setae, coxa of P4 with strong spine. Caudal side of P4 coxa with same groups of spinules as in female but fewer in number. Intercoxal sclerite of P4 without hills and hair-setae on its free edge, but with short row of small spinules in middle. Rudimentary P5 two-segmented, with setae and spine of similar proportion to female. Rudimentary P6 with very short middle seta and very strong inner spine almost reaching length of outer seta (relative lengths 1.0/0.6/0.95) clearly separating it from other species of this group.

Redescription of *Acanthocyclops vernalis* (Fischer, 1853)
(Fig. 5)

Cyclops vernalis Fischer, 1853
Cyclops parcus Herrick, 1882
Cyclops robustus (part.) Lilljeborg, 1901
Cyclops lucidulus G.O. Sars, 1863
Cyclops (Acanthocyclops) vernali Pesta, 1928; Kiefer, 1929; Yeatman, 1944
Acanthocyclops (Acanthocyclops) vernalis Dussart, 1969
Acanthocyclops (s. str.) *vernalis vernalis* Monchenko, 1974
Acanthocyclops (s. str.) *vernalis* f. *robusta* Monchenko, 1974
Acanthocyclops vernalis: Rylov, 1948; (part.) Margalef, 1953; Kiefer, 1978; Einsle, 1993; (part.) Einsle, 1996; Alekseev, 1998; Vezhnovets, 2005; Dussart and Defaye, 2006; Rybak and Błędzki, 2010

Justification of neotype

Cyclops vernalis was described in the middle of XIX century by Fischer from a sample collected in Orlov pond, Peterhof town, St. Peterburg vicinity, Russia. Victor Alekseev checked collections of two Russian Zoological museums that existed at that time: collection of Zoological museum of Imperial Academy of Science in St. Petersburg and collection of Zoological museum of Moscow State University, Moscow. No samples attributed to Fischer were found nor any other materials related to copepods except description on paper. The type Orlov pond at Peterhof still exists and contains all five species described by Fischer including *A. vernalis*. This information let us conclude that type materials of Fischer except description on paper were lost and they do not exist now. Fortunately, the type waterbody, a small Orlov pond continued to exist in the same place all this time and no other *Acanthocyclops* species except *A. vernalis* were found
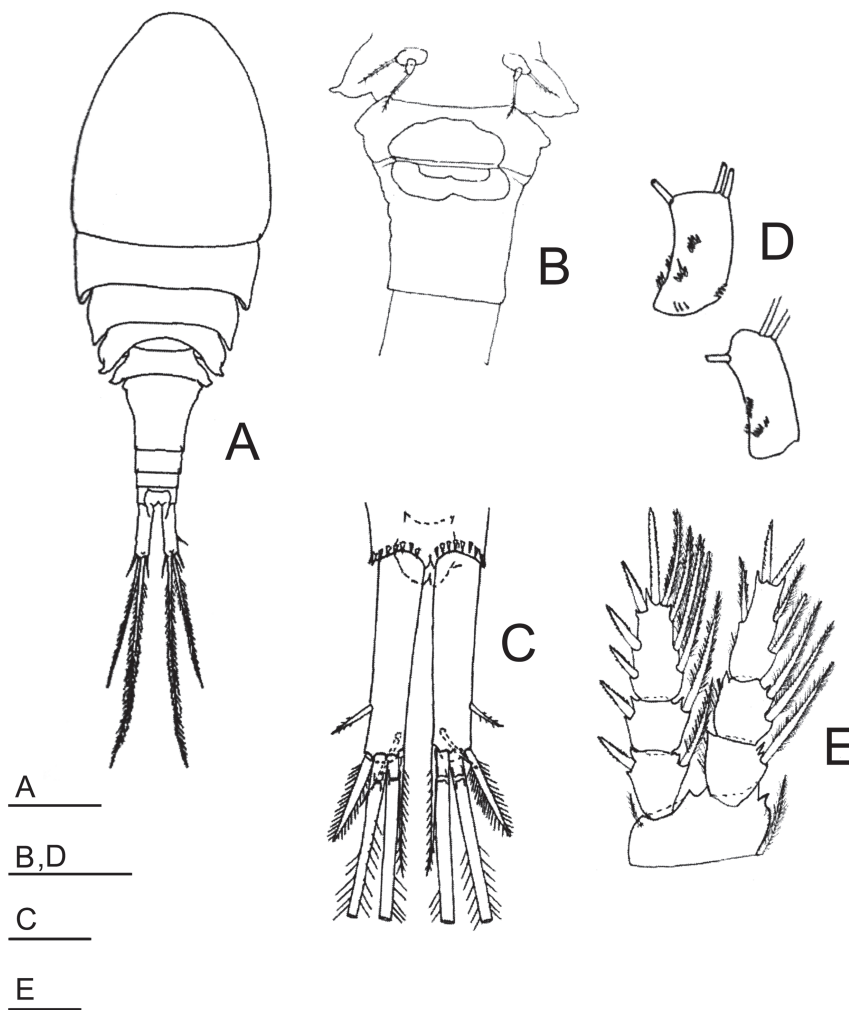

Figure 5. *Acanthocyclops vernalis*, holotype of female. Acanthocyclops vernalis*, holotipo de hembra.*
A - habitus, dorsal; B - genital segment, ventral; C - caudal rami, ventral; D - antennal basipodite, frontal; D1 - antennal basipodite, caudal; E - P4.
Scale bars: A - 200µm; B - 100µm; C - 50µm; D, D1 - 25µm; E - 50µm.
Original, V.A.

after its observation in April 1998 and all following visits up to now, approximately one-two times a year. That grants us the splendid possibility to establish the neotype of *A. vernalis* with a high probability from the same population that Fischer worked on.

Material examined. The neotype female and paratype male dissected and mounted in glycerol surrounded with Canadian balsam on slides N56736 and N56737 and deposited into the type collection of Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia. The type series also includes two females and one male preserved in pure glycerol.

Female description
(see Fig. 5)

Body transparent, colorless. Full body length without caudal seta 1375 µm. Genital double-somite approximately as long as wide, with angular anterior part and sharpened lobes, receptaculum seminis with an anterior depression. Caudal rami parallel or slightly divergent, without hairs on inner margin, 5.5 times as long as wide. Inner seta 1.15 times longer than outer seta. Dorsal seta shorter than outer seta. Lateral setae not reaching ramus length. Length proportions of distal setae, beginning from outer terminal seta: 1/8/12/1.3.

Antennule 17-segmented almost reaching distal margin of cephalosome. Setation of antennulary segments (aesthetascs in Roman numbers) beginning from first: 8/4/2/6/3+I/1/2/1/1/0/1+I/2/0/1/2/2+I/7+I. Shortest setae of distal segment less than length of distal segment. Antenna composed of 1-segmented basipodite bearing exopodite represented by a long seta and the two short setae on opposite side, and a 3-segmented endopodite. Basipodite A2 ornamentation as in figure 5. Three following segments (endopodite) bearing subsequently: one, nine and seven setae.

Gnathobase of mandible with six teeth, rudiment of endopodial segment with 2 long setae and 1 short seta. Maxillula with three strong and three small teeth, two strong setae; maxillar palp with seven setae, different in length. Maxilla of five segments, praecoxa with two strong setae in its middle part; coxa with strong seta in the

middle and an endite bearing two claw-like setae; basal endite with two very strong claw-like spines, both with a row of spinules and small setae near the place of fusion of the rudimentary endopod. Endopodite 1 with three clawlike setae and endopodite 2 bearing distally two long setae. Maxilliped of four segments, praecoxa + coxa with two strong setae in the middle part and small setae at their distal end; basis with two setae of different length and three groups of strong spinules near the insertion of the setae; first segment of endopod with strong spine and rudimentary endopod, bearing a strong spine and two hairless setae.

Swimming legs 1–4 with three-segmented rami. Distal segments of endopodite of Legs 1–2 with one spine and five setae. Distal segment of endopod Legs 3–4 elongated, with two strong spines at its end. In P4 Enp3 2.9 times as long as wide and outer apical spine slightly longer than inner one (1.2 times). Insertion of the lateral outer seta not at the same level from the end of the segment as the second lateral inner seta. Outer lateral seta of this segment not transformed in spine as it was in previous species, all setae homogeneously pinnated. Intercoxal plate of P4 with small hills and row of denticules in middle. P4 coxopodite with row of spinules along proximal margin, two groups of spinules at distal margin and groups of spinules near lateral margin. Rudimentary P5 two-segmented, basal segment with long outer seta. Distal segment with long apical seta and short inner spine less than half of segment length with few spinules at the base.

Male description
(see Fig. 5G)

Body length without furcal seta 915 µm. Cephalosome 1.3 times as long as wide, with maximal width close to end of its length. Caudal rami 4.3 times as long as wide, slender inner seta more than twice length of outer spine-like seta. Lateral seta placed without spinules in its base. Dorsal seta placed near inner seta insertion, about 1.2 times longer than outer seta. Antennule 12-segmented. Setation of antennular segments (aesthetascs in Roman numbers) beginning from first: 7(III)/3/1/6(I)/1/1/1/4(I)/0/1/0/7. Antennal baso-
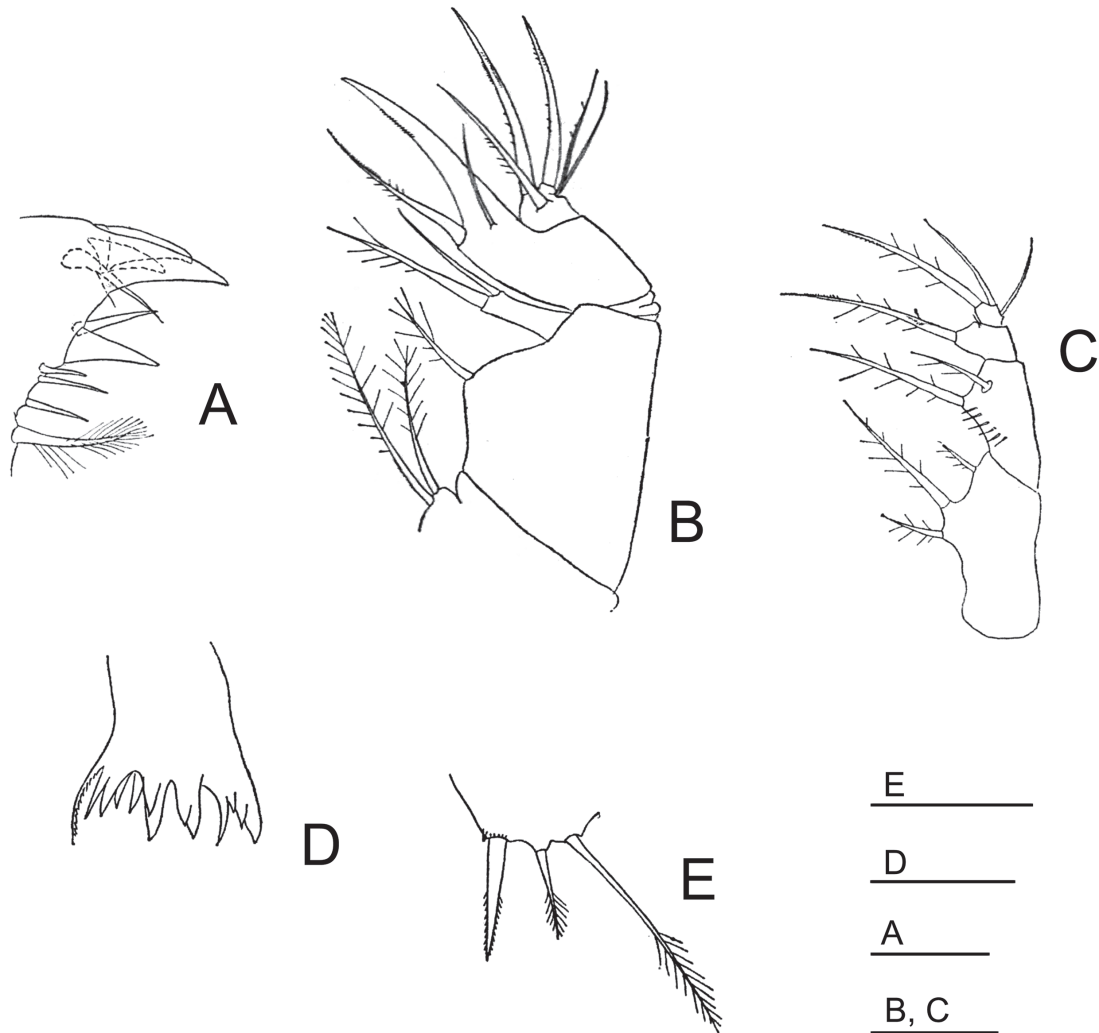
Figure 6. *Acanthocyclops vernalis*, female from the type seria (A-D), male (E). Acanthocyclops vernalis*, hembra del tipo seria (A-D), macho (E).*
A - gnathobase of maxilla; B - maxilla; C - maxiliped; D - mandibula, distal part; E - male, rudimentary leg 6.
Scale bars: A, D, E - 30μm; B, C - 50μm.
(After Monchenko, 1973 with changes)

podite ornamentation as in female. Morphology of mouth appendages and Leg 1–3 basically as in female. Distal segment of endopod Leg 4 about three times as long as wide, with inner apical spine about as long as the segment and 1.25 times as long as outer apical spine. Insertion of lateral seta in this segment as in female. Intercoxal sclerite Leg 4 without hills and hair-setae on its free edge, but with short row of small spinules in middle. Rudimentary Leg 5 two-segmented, with setae

and spine of similar proportion to female. Rudimentary P6 with strong inner spine, middle seta shorter than spine and a very long outer seta (relative lengths 1.0/0.6/1.4).

Detailed redescription of *A. americanus* neotype from the type locality was done in our previous study (Miracle *et al.*, 2013). Hereafter we decided to briefly remind the most important characters to show as the greatest possible difference in morphology among these three species.

Acanthocyclops americanus (Marsh, 1892)
(Fig. 6)

Cyclops viridis americanus Marsh, 1892
Cyclops americanus Marsh, 1920
Acanthocyclops americanus f. *spinosa* Monchenko, 1961: Vezhnovets, 2005
Acanthocyclops robustus f. *limnetica* Petkovski, 1975
Acanthocyclops robustus: (part.) Kiefer 1976; Fryer, 1985; Dodson, 1994; Lescher-Moutoué, 1996; Einsle, 1996; Dussart and Defaye, 2006; Rybak and Błędzki, 2010
Acanthocyclops trajani Mirabdullayev and Defaye, 2002; Dussart and Defaye, 2006; Rybak and Błędzki, 2010; Blaha, 2010
Acanthocyclops (Acanthocyclops) americanus: Dussart,1969
Acanthocyclops americanus: Rylov, 1948; Alekseev, 1995; Vezhnovets, 2005; Alekseev *et al.*, 2002

Female. Body colorless, length 1100-1600 µm. Egg sacs with large number of transparent small size eggs. Cephalosome as narrow ellipsoid with maximal width close to middle. Fifth segment without long wings laterally. Genital double-somite round-shaped in lateral sides, 1.1-1.3 times as long as wide, with seminal receptacle with wide transparent zone in its frontal part (see Fig. 7). Caudal rami parallel or divergent, 4.5-6.5 times as long as wide. Innermost seta sub-equal to caudal rami length. Antenna composed of 1-segmented basipodite bearing exopodite represented by a long seta and the two short setae on opposite side, and a 3-segmented endopodite. Basipodite A2 ornamentation as in figure 6D,E. Mouth appendages
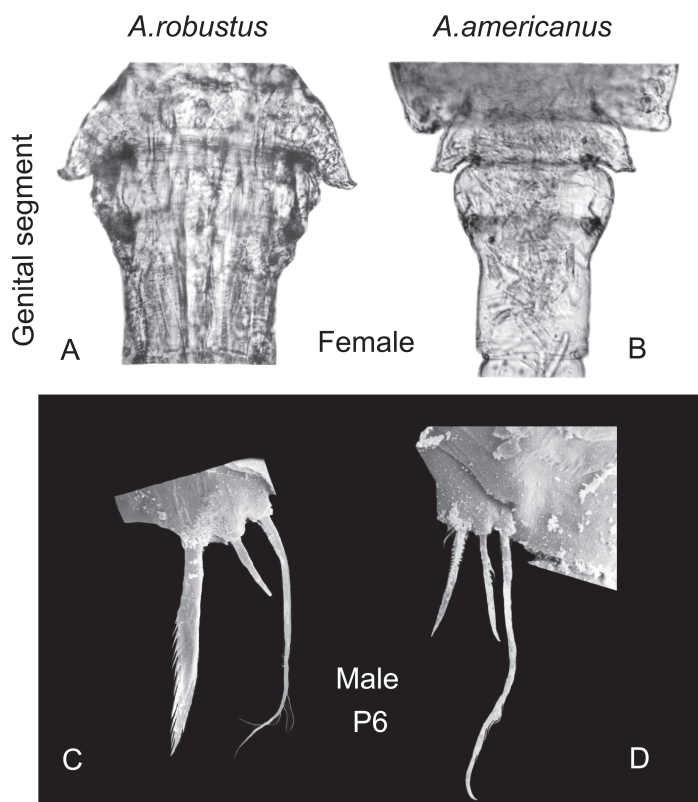

Figure 7. Comparative illustration showing the clear differences between female (A, B) and male (C, D) of *Acanthocyclops robustus* (A, C) and *Acanthocyclops americanus* (B, D). *Ilustración comparativa que muestra las claras diferencias entre la hembra (A, B) y el macho (C, D) de* Acanthocyclops robustus *(A, C) y* Acanthocyclops americanus *(B, D).*

as in other *Acanthocyclops* species.

P4 Enp3 2.6 times as long as wide; inner apical spine 1.1 times as long as outer spine and longer than segment itself. Insertion of the lateral outer seta approximately at the same level from the end of the segment as the second lateral inner seta. Intercoxal sclerite Leg 4 with small hills and row of small denticules in middle part. Coxa Leg 4 with four groups of denticles typical for several species of *robustus*-group. Distal segment exopod Leg 4 with distal seta longer than nearest distal spine. Rudimentary Leg 5 two-segmented, basal segment with long outer seta. Distal segment with long apical seta and short inner spine less than half of segment length.

Male. Body length without caudal seta 900-1100 μm. Cephalosome 1.3 times as long as wide, with maximal width close to end of its length. Caudal rami 4-5 times as long as wide, slender inner seta more than twice length of outermost spine-like seta. Lateral seta placed without spinules in its base. Dorsal seta placed near innermost seta insertion, about 1.2 times longer than outer seta. Antennule 12-segmented.

Antennal basopodite ornamentation as in female. Distal segment of endopod Leg 4 about 2.5 times as long as wide, with inner apical spine about as long as the segment and 1.25 times as long as outer apical spine. Insertion of lateral seta/spine in this segment as in female. Rudimentary Leg 5 as in female. Rudimentary Leg 6 with inner weak spine, middle seta slightly shorter than spine and a very long outer seta 2 times longer than inner spine. (Fig. 7D).

Comments on *robustus*-group differentiation

Several morphological differences among the specimens from the type localities were found to assign the specimens unambiguously to the mentioned species of *Acanthocyclops* (Table 1).

Male differences in P6 construction among these species is also very clear and stable as observed in many populations inhabiting Eurasia (Alekseev & Monchenko, 2011). In *A. americanus* male inner spine is weak and similar in size to middle seta when outer seta is very long and twice of inner spine length (Fig. 7D). In *A. robus-*

Table 1. The most important morphological features in female that separate invasive *A. americanus* from native sister species in Eurasia. *Características morfológicas más importantes en hembras para separar la especie invasora* A. americanus *de las especies nativas de Eurasia.*

Characters/species	A. americanus	A. vernalis	A. robustus
Body shape and color	Elongated, colorless	Robust, yellowish	Flattened, dark brown
Cephalosome L/Wratio	>1	1	1 or <1
Caudal rami shape and L/W ratio	Divergent	Slightly divergent	Parallel
CR innermost/ outer seta ratio	>2		1.7
GDS shape and L/W ratio	round laterally >1	Angular laterally =1	round laterally =1
P4 End3 distal seta inserted places	At the same distances from end	At different distances from end	At different distances from end
P4 End3 L/W ratio	More than 3	2.5 - 3	Less than 2.5
P4 End3 inner distal spine/L	More than 1	About 1	Less than 1
P4 Exp3 distal seta / distal spine	Seta>spine	Subequal	Seta<spine

tus this spine is strong and twice of middle seta length and subequal to outer length (Fig. 7C). In *A. vernalis* these appendages are of intermediate size and length (see Fig. 5). As mentioned above for females differences in P4 distal segment construction are similar to males and can be used for species separation purposes.

Microcharacters of the antennary basipodite that are so good taxonomic characters for species discrimination in some genus of cyclopoids such as *Eucyclops* or *Mesocyclops*, in our opinion, are too variable at population level in the genus *Acanthocyclops* to be used for species separation, at least in the *robustus* group (Lewis *et al.*, 2004; Alekseev, 2015).

The critical point that lets us separate Eurasian species from the invasive American species became molecular genetic assessment conducted by Maria Rosa Miracle (Miracle *et al.*, 2013). This study also improved species synonymy. *Acanthocyclops americanus* from Wisconsin (USA) and *A. trajani* described from Etàng de Noes (France) were morphologically and genetically alike as the same happening to *A. robustus* and *A. einslei* Mirabdullayev & Defay, 2004 (Alekseev & Monchenko, 2011; Miracle *et al.*, 2013).

Key for species identification in *robustus*-group

Females

1. P4 End3 inner distal seta and lateral seta inserted at the different levels from the segment end .... *A. robustu*s (G.O. Sars, 1863) [Native species within the Palearctic Region in Spain in rivers and springs (Maria Miracle, unpublished)]
- P4 End3 inner distal seta and lateral seta inserted at the same level from the segment end 2
2. P4 End3 apical inner spine shorter than apical outer spine .... *A. vernalis* (Fischer, 1853) [Native species within the Palearctic Region but missing in Spain (probably)]
- P4 End3 apical inner spine equal or slightly longer than apical outer spine .... *A. americanus* (Marsh, 1892)

Males

1. Rudimental P6 with weak inner spine; outer seta twice of its length .... *A. americanus* (Marsh, 1892)
- This spine is strong; outer seta shorter ....…. 2
2. Inner spine in P6 about as long as outer seta and twice of middle seta .... *A. robustus* (G. O. Sars, 1863)
- Inner spine significantly shorter .... *A. vernalis* (Fischer, 1853)

Distribution of *A. americanus* and *A. robustus* at Valencia Region, eastern Spain

Long time uncertainty of identification within the *robustus*-group caused by biological invasion of *A. americanus* in Eurasia resulted in wrong determination of species in most European countries (except Russia and Ukraine) and an absence of relevant information on these two species interaction as well as real areal occupied by *A. americanus* in XX century. Only recently, after molecular-genetic confirmation, regarding the invasive origin of *A. americanus* in Europe, this situation begins to turn better but still needs improving (Alekseev & Monchenko, 2011; Miracle *et al.*, 2013).

The morphological characters selected by us here in separating *A. americanus* from the native Eurasian species *A. robustus* were hereafter used to reveal modern distribution of *A. americanus* and *A. robustus* as well as the coexistence of these species in Valencia area, Spain. Thus, this becomes the first detailed study so far on the problem in Europe.

A database of the presence of these two *Acanthocyclops* species occurring in the Valencia region (eastern Iberian Peninsula) was compiled from several extensive sampling surveys, carried out by the authors under direction of Dr. Miracle through several different research projects from 2000 to 2017. In total we collected data for more than 200 sampled sites. Sampled sites included: coastal lagoons (with low marine influence) and their surrounding temporary marshes along the Valencia coast; small ponds fed by rain (temporary ponds) or springs (permanent ponds) and sites associated with fluvial courses and major rivers.

Table 2. Characteristics of wetlands where *Acanthocyclops* species have been found in Valencia region from a total of more than 200 sites studied. *Características de los humedales en los que se encontraron especies de* Acanthocyclops *en la Comunitat Valenciana de un total de más de 200 lugares estudiados.*

Type of sites	A. americanus (Number of sites, %)	A. robustus (Number of sites, %)	Conductivity (µS/cm)
Coastal lagoons and marshes	8	0	1800 - 3000
River ponds	5	4	538 - 1330
Permanent ponds	12	0	300 - 3050
Temporary ponds	2	10	93 - 585
TOTAL	27 (66%)	14 (34%)	

Acanthocyclops species were found in 41 sites (Table 2). They were never found together in the same aquatic habitat and none of these species has been found in brackish waters. The distribution of both species highlights some of their differences in ecology. *A. americanus* was the most frequent species found in the current survey. In accordance with the data available in the studied area, *A. robustus* has not been found in any coastal lagoon, neither in permanent ponds, where was possibly substituted by the other species. It is important to note that the most permanent ponds sampled were also located in coastal areas. Ponds associated with rivers harbors both species approximately in equal proportions. The greatest part of sites with *A. robustus* are small temporary ponds isolated from water courses. From our sampling experience, these two species have been seen to occupy different habitats: *A. robustus* showing littoral preferences and *A. americanus* being more pelagic. Spatially (see Fig. 1) most sites with *A. robustus* are located in inland areas. This distribution possibly suggests that in the more connected coastal areas with greater anthropogenic influence, the invasive *A. americanus* species has displaced the native *A. robustus* species, while the latter is only preserved in small isolated ponds and in rivers in the most unpopulated inland area.

Other countries

In most sites outside Spain these two species were also found separately with two exceptions that became important for our study as we explain in the history of biological invasion of *A. americanus* and reasons why it was so successful.

The first exception – the type locality of *A. robustus*. In the type for *A. robustus* lake in Oslo (Norway) *A. americanus* was dominant in plankton when *A. robustus* was found in small numbers in a small shallow stream out-coming from the lake (Alekseev & Monchenko, 2011). This sampling, done at the end of June 2010, confirms our previous inference that in the 60s Kiefer also sampled *A. robustus* in the same lake and getting them together came to the mistaken conclusion on their identity (Alekseev *et al.*, 2002). As a result he down-graded *A. americanus* as a synonym of a younger *A. robustus*. That mistake in effect blocked studies on the *A. americanus* invasion in Palearctic for more than 50 years. Hereafter we try to restore the main elements of the history of biological invasion of this species in Eurasia.

The second exception – seasonally temporary water bodies in the River Volga delta. The spring filling temporary water bodies in the Volga delta are highly productive and short time (1-3 months) existing habitat where species competition is low due to extremal environmental time limitation. This lets many species to co-exist effectively producing dormant stages highly resistant to desiccation and in the same site up to 200 copepod species including all three from the robustus group can be found living together (Alekseev, 1980). These special conditions are very suitable to study and compare life-cycle

parameters in these morphologically close sister-species.

A history of biological invasion of *A. americanus*

According to literature data in native habitat (the Americas) a planktonic cyclopid *Acanthocyclops* morphologically close to *A. vernalis* and *A. robustus* was described from the Great Lakes in North America (Marsh, 1892). Soon after, it was found in U. K. (Lowndes, 1928) possibly after oversea transport with ship ballast waters (Alekseev *et al.*, 2002). In native habitat it is also known as a planktonic form, often dominating in eutrophic water bodies in Canada and Mexico (V. A. personal data).

In Spain and in France it was found in the middle of the last century (Dussart, 1967). As *A. robustus* f. *pelagica* it was registered in Hungary (Petkovski, 1975). Also it was presented in Kiefer's sample collected at south Norway (Oslo) (see Fig. 2) where it co-existed with *A. robustus*. This co-existence of *A. americanus*, dominating the plankton of the lake, and *A. robustus*, in low densities inhabiting small rivers connected with the lake, was confirmed by V. A. sampling at the same site in 2010.

It was a dominant species in Belgian creeks and in ponds of the Central City Park in Ghent, whereas *A. robustus* was only found in one location, a roadside ditch (Alekseev *et al.*, 2003).

V. Rylov a very accurate taxonomist did not report any *A. americanus* in Russia before 1941 but included it in his key for cyclopids of USSR as a potential invader (Rylov, 1948). So we can suppose that *A. americanus* was not in Russia at that time as well as in European continental countries it was only found in the middle of last century.

Consequences after creation of large limnetic habitats (cascades of hydro-electric power stations with water reservoirs) on the Volga, Don, Dnepr and Dnestr Rivers

Acanthocyclops americanus was found for the first time in Russia in Rybynsk water reservoir (on the River Volga, started on 1941, and finished in 1947) in 1957 (Prof. A. Monakov personal com-munication to V.A.), then in Ukraine in Kievskoye reservoir (on the River Dnepr) and in Dnestr reser-voir (Monchenko 1961, 1974). After creating a cascade for the hydro-power stations on the River Volga (1950-1960s) this species was found first in Saratov reservoir, then in Volgagrad reservoir (Vijushkova, 1962). In both reservoirs *A. ameri-canus* was a dominative cyclopid in summer zooplankton (Vijushkova, 1962). In 1974 this species was also found in high densities in tempo-rary waterbodies in the River Volga Delta (Alek-seev & Kosova, 1975). Few years later *A. ameri-canus* was the only planktonic crustacean reported in the Caspian Sea (sea lagoons and rain pools on the island Zhemchuzhniy) (Chuykov, 1986). It was also present in Gulf of Finland (the Baltic Sea near St. Petersburg), 1981; in Armenia, near Lake Sevan (Caucasus), 1990; in Kazakstan near Lake Aral; and in lake Chanu (1985), in Western Siberia (a lake near the Tola River, Tumen, 1987); in Central Siberia, Bratsk water reservoir (the River Enisey), 1988. In 2015 it was found in a tempo-rary rain pool near Syktyvkar (the northern Ural Mountains), and the same year in Hanko island near Helsinki (Finland). In 1996 and in 2003 it was found in Egypt (Nile river near Cairo). Recently it was also indicated in the Lena River delta (Abramova *et al.*, 2017). Most of the lines connecting the sampling sites where *A. ameri-canus* was found are similar to flyways of aquatic birds seasonal migrations.

Biology of *Acanthocyclops americanus*, an invasive species in the Volga versus *verna-lis-robustus* native species

Life cycle parameters that include growth and productivity rates, egg size and fecundity in *A. americanus* was compared to the same rates in *A. vernalis* obtained at the same temperature and food conditions (Alekseev, 1980). The invasive species had shorter maturation time (about 12 % faster at 20 ºC), higher metabolic level as well as feeding and productivity rates. If we compare time from egg laying until dormant stage (Copepodid 4), *A. americanus* show times shorter than those for *A. vernalis*. All these biological features along with higher fecundity are thought to be responsible for this invasive species to

increase its competitive edge and substitute similar sized sister species in their niches.

Acanthocyclops americanus seasonality in the River Volga delta

This species was found in highest density not in permanent water bodies (rivers, lakes etc.) but in temporary ones (Alekseev & Kosova, 1976). In spring river flooding pools that filled with water every year and are used by local and migrating fish for breeding and short time feeding, all these three species coexist together in more or less equal proportions (Alekseev, 1980). They appear at the very first stage of the spring water flooding.

There was some regularity in the appearance of these species at 4th copepodid stages immerged in wet or just covered with thin films of water soil after 9-11 month dormancy in dry conditions.

A. vernalis appears first, then in one week comes *A. robustus* and finally when water temperature reaches 18-20 ºC *A. americanus* are found among reactivated formerly dormant copepods. In few days copepodites become adults (males in 1-3 days earlier) and immediately start reproducing. Before fish hatching the first generation of tiny nauplii appear and very soon they substitute adults consumed by young fish. Being numerous and small they can escape complete elimination by countless fish. Before the drying up of the water bodies, when fish are gone, populations of these species are ready for dormancy at copepodite 4th stage thus they finish their life cycle having only one or two generations in a season. Even in these ephemeral water bodies we can observe differences in ecological demands between the invasive (*Acanthocyclops americanus*) and native (*A. vernalis* and *A. robustus*) species (Alekseev, 1980).

Appearance times for these species in the temporary water bodies were related to water temperature. At the very beginning of spring, when river water just arrived in this pool and temperature was about 14 ºC, *A. vernalis* copepodite stages were found emerging from the soil and in few days became adults and started breeding. At 16 ºC, copepodite *A. robustus* appeared and turned to maturation and breeding. Soon after, at 18-20 ºC, *A. americanus* and *A. americanus*

spinosus had appeared. *A. americanus* seems to be more sensitive to temperature at the beginning of the life cycle and that may become a limiting factor in distribution of the species in the Arctic zone. Climate warming, on the other hand, must be a favorable factor to overcome this obstacle and to increase areal distribution northwards.

Small egg size and significantly higher female fecundity in invasive species could give them competitive advantages but only in high trophic conditions. If food is limited, especially in naupliar stages of development, species with large egg size will survive better. So the native species are deemed to be more effective in the early spring time and late fall season whereas invasive species should be more competitive in early summer time when small sized prey such as protist and rotifers are numerous.

Other studies on this species showed other advantages such as omnivorous diet (Sarma *et al.*, 2019), rapid population growth rates, higher fecundity, effective predation on other cyclopid species and use of aquatic birds for dispersal in a similar way to Cladocerans (Ślusarczyk *et al.*, 2019). Also the naupliar stages outcompete herbivorous cladocerans and thus possibly are dominant in zooplankton communities (Enríquez-García *et al.*, 2013). We think that the fast and wider distribution of *A. americanus* became possible due to all mentioned above biological features.

CONCLUSIONS

1. The putative invasive species *Acanthocyclops americanus* can now be easily separated from the two native similarly sized species *A. vernalis* and *A. robustus*, both using morphological characters and molecular techniques. The most important taxonomical differences in females are related with P4 structure, in males with P6 armament.

2. Study on copepod diversity carried out in about 200 sites in Valencia region (Spain), revealed the presence of *Acanthocyclops* species in 41 sites: in 66 % of the sites the genus is represented by the putative invasive species *A. americanus* and only in 34 % sites *A. robustus* was the species found. These two species were never found together at the same place.

3.   Analysis of literature and our own sampling data on distribution of *A. americanus* in Eurasia lets us assemble a possible story of its invasion begun in early XX century from U.K.; today, the species is distributed throughout the Palearctic Region.

4.   Experimental study of life cycle in *A. americanus* and the two native species showed a competitive advantage of the invasive planktonic species in limnetic communities in high trophic conditions.

5.   Eutrophication, creation of large limnetic habitats (water reservoirs) and climate warming were favorable factors for the great success of its invasive and colonizing abilities to dominate in such a huge territory within a century.

6.   There is a high risk that *A. americanus* in the near future may expand its area to the Arctic zone due to climate warming. As it was observed in many cases in shallow water bodies, *A. americanus* became the dominant planktonic species. Arctic water bodies are mainly small and shallow with limited number of invertebrate species. *A. americanus* as a very effective predator may dramatically change these vulnerable ecosystems. The last finding of *A. americanus* in the Arctic zone (Comi Republic and North Urals) recently sampled by E. Fefilova (personal communication) confirmed these preliminary conclusions.

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