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Morphological differentiation of some populations of the genus *Cyclops* (Copepoda: Cyclopoida) from Bohemia (Czech Republic)

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Abstract. Groups of spines forming the coxal ornamentation of the fourth pair of swimming legs and some other morphological details are used to distinguish species of the genus *Cyclops* O. F. Müller, 1776, from several habitats in the Czech Republic. The identity of populations based on these characters agrees with that derived from allozyme analyses. Besides the easily distinguished species *Cyclops vicinus* Uljanin, 1857 and *C. insignis* Claus, 1857, three other species were found: *C. furcifer* Claus, 1857 (in temporary waters) and probably *C. strenuus* Fischer, 1851 and *C. abyssorum* G. O. Sars, 1863 (in permanent waters).

Morphology, cryptic species, coxal ornamentation, species identification, habitats, *Cyclops*, Czech Republic, Palaearctic region

INTRODUCTION

Although the detailed taxonomic differentiation of the common species of cladocerans in this country has long tradition (e.g., Kurz 1874) and was greatly enhanced by Hrbáček in his early publications (1959a, b), common cyclopoid copepods were believed to be a well-known group of simply delimited species with good differentiating morphological characters (Šrámek-Hušek 1938, 1953). Even the most common limnetic genus *Cyclops* O. F. Müller, 1776, was supposed to be represented by just two limnetic species, *C. strenuus* Fischer, 1851, and *C. vicinus* Uljanin, 1857, and two others inhabiting rather small water bodies (*C. insignis* Claus, 1857, and *C. furcifer* Claus, 1857). The fifth species listed in the Bohemian faunistic records was originally described by Šrámek-Hušek (1937) as *C. bohemicus* Šrámek-Hušek, 1937, from the lake Černé Jezero in the Šumava Mountains and later assigned to the complex of *C. abyssorum* Sars, 1863. Now it is supposed to be extinct at the original type locality.

On the other hand, the parts of Europe rich in numerous and deep lakes (both northern Europe and the subalpine countries) are known to have a higher number of closely related and morphologically very similar limnetic species of the genus *Cyclops* (e. g., *C. scutifer* Sars, 1863, *C. abyssorum*, *C. lacustris* Sars, 1863, *C. kolensis* Lilljeborg, 1901, *C. bohater* Kozminski, 1933). Their species identity and mutual relationships were quite complicated and unclear. Kozminski (1936) introduced a detailed and elaborate morphometric analysis using about 30 statistically evaluated characters to distinguish closely related species. Even using this approach the specific identity of some populations remained uncertain (Einsle 1975). Their differentiation was enabled by two new techniques

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This contribution is dedicated to Doc. RNDr. Jaroslav Hrbáček, DrSc., upon the occasion of the 80th anniversary of his birthday (12th May 1921).

introduced only a quarter of century ago. Beermann (1977) discovered the partial elimination of chromatine from the chromosomes of early embryonic cells of a cleaving egg. The timing of this process into the exact phase of the egg cleveage, specific for each of the very closely related species, allowed Einsle (e.g., 1996a, b) to identify and describe some of the so far unrecognized species. He also used another technique, i.e. the electrophoretic separation of allozyme (for copepods used earlier by Boileau and Hebert 1988) to distinguish such very similar species. Combining these two approaches with the analysis of previously neglected details of morphological structures (Einsle 1985), he was better able to delimite the existing species and to describe some new species (*C. heberti* Einsle, 1996, *C. singularis* Einsle, 1996, and *C. stagnalis* Einsle, 1996).

The aim of this work is to describe some detailed morphologic characters of several Bohemian populations of the genus *Cyclops* which we have collected and to compare their species identity with the recentmost descriptions.

MATERIAL AND METHODS

Plankton samples were collected with a coarse plankton net (0.38 mm mesh size) either by vertical hauls from deep reservoirs and ponds or by pouring water gathered by 1-litre wide-mouth bottle from shallow pools through the net. If possible, adult females were individually selected immediately from the fresh live material and then preserved in 4% formalin; otherwise the whole sample was preserved in the same way. Preserved animals were dissected in a drop of glycerin on a microscopic glass. The 4th pair of swimming legs and its posterior face ornamentation were examined. The ornamentation of the coxal segment of the basipodite of this pair of legs consists of up to seven groups (Fig. 1, A to F) of little spines or setulae. They are arranged and placed in characteristic positions (Einsle, 1996b) on the coxal segment. Moreover, the praecoxal plate connecting the coxae (intercoxal coupler) may be either nake or covered with setulae and either simply even at the distal margin or humped on each side near the inner lateral seta of the coxa (Fig. 1, H – the letter symbols follow Einsle, 1996b). The humps may either be short and not exceeding the margin of the plate or they may project significantly

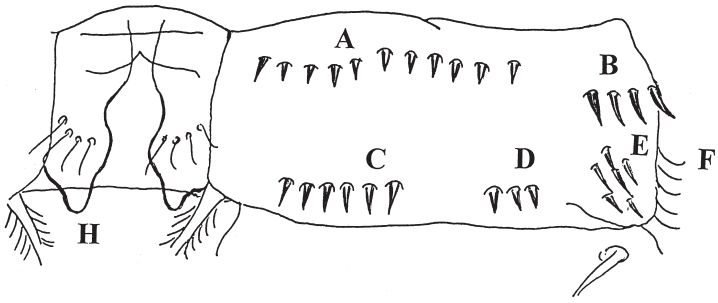

Fig. 1. Ornamentation pattern of the coxal segment of the 4th pair of swimming legs in *Cyclops* O. F. Müller: general scheme. The letters follow conventional labelling by Einsle (1985). A to F: groups of spines or setulae; H: the humps of the intercoxal coupler.

beyond the coupler margin. Other characters which we have recorded were the length of antennules as compared with the length of the cephalothoracic body region, the shape of the genital segment of females, the position of the lateral spine at the inner side of the distal segment of the 5th legs, and the length of apical spines of the last segment of endopodite of the 4th pair of swimming legs.

We collected and examined material from various types of habitats ranging from deep reservoirs (Slapy Reservoir on the Vltava River in Central Bohemia, Římov Reservoir on the Malše River in South Bohemia) to ephemeral puddles filled with rain water. Small permanent waters were represented by carp ponds and also by permanent pools in the innundation areas of the Labe (Elbe) River nr. Velký Osek (north of Kolín, Central Bohemia) and of the Upper Lužnice River (south of Suchdol nad Lužnicí). Besides these materials collected for the purpose of this work, we also examined some of our older collections or samples kindly supplied by colleagues.

RESULTS

The specimens of cyclopoid copepods of the genus *Cyclops* we have examined can be distinguished into five morphologically different types. The first of these types, which can be easily recognized, is represented by *C. insignis*, the species differring from all the others within the genus *Cyclops* by its 14-segmented antennules when adult. Adults of all the other species of this genus have 17-segmented antennules, with a characteristic group of four short segments, which are just as long as they are wide, in the middle of the antennule (segments 8 through 11).

One of the other four types we collected is undoubtedly a common species *C. vicinus*. The typical characters of *C. vicinus* are the "wings", i.e. widely extended lateral lobes of the 4th thoracomer, together with another constant character of this species, the spine formula 2.3.3.3. The last character may occur in some of the other *Cyclops* species which otherwise have the spine formula 3.4.3.3. Therefore we describe some details of *C. vicinus* morphology in comparison to the details of the remaining three types.

The ornamentation of the coxal segment of the basipodite of the 4th pair of swimming legs and the ornamentation of the intercoxal plate of this pair of legs (the coupler)

Cyclops vicinus. (Ornamentation pattern ABCDEF, Fig. 2). Group A consists of 20–35 mostly small spines in a few rows. Some spines may be longer than the others. Group B: 4–7 short thick spines. Group C: 7–12 long thick spines of equal length. Group D: 1 or 2 short thick spines. Group E: numerous thin spines covering each other. Group F: short fine setae along the distal half of the outer coxal edge. Intercoxal coupler: naked, with a pair of little humps.

Type I (Ornamentation pattern ACD, Fig. 2). Group A consists of 18–25 spines, the half of them close to the coupler being finer, longer and set into slightly bent arch, the external half of them being shorter, especially those in the middle of the row. Group C: 3–5 thicker and longer spines, slightly bent outward from the intercoxal coupler. Group D: 1–3 short and thick spines. The humps on the intercoxal coupler do not extend beyond the coupler margin, the coupler bears one row of fine long setae in the middle of its length. Groups B, E, F: missing.

Type II (Ornamentation pattern ACDE, Fig. 2). Group A: 15–23 thick spines shorter than in type I, often placed on the cuticular lobe. Group C: 6–8 thick spines, those in the middle being longer than the external ones. Group D: 2–5 thick long spines. Group E: large number (up to more than 10) of short or long thin spines partially covering each other. The intercoxal coupler with a pair of humps extending well beyond its distal edge; the humps and the area between them are covered with a row of very long fine setae. Groups B, F: missing.

Type III (Ornamentation pattern ABC, Fig. 2). Group A: a row of 25–30 short minute spines, with the internal half of the row often in two parallel curved lines. Group B: from 3 up to 7 or 10 long weak spines. Group C: long row of 14 to 22 short and strong spines of equal length. Intercoxal coupler: without any extending hump, covered with numerous fine long setae in its distal part. Groups D, E, F: missing.

Position of the lateral spine of the 2nd segment of the 5th leg

Cyclops vicinus: inserted at the middle of the segment, not longer than the segment itself.

Type I : at the middle of the segment, as long as the segment.

Type II: at the middle of a very long and slender segment, shorter than the segment.

Type III: the spine shifted to the distal end of a shorter and wider segment.

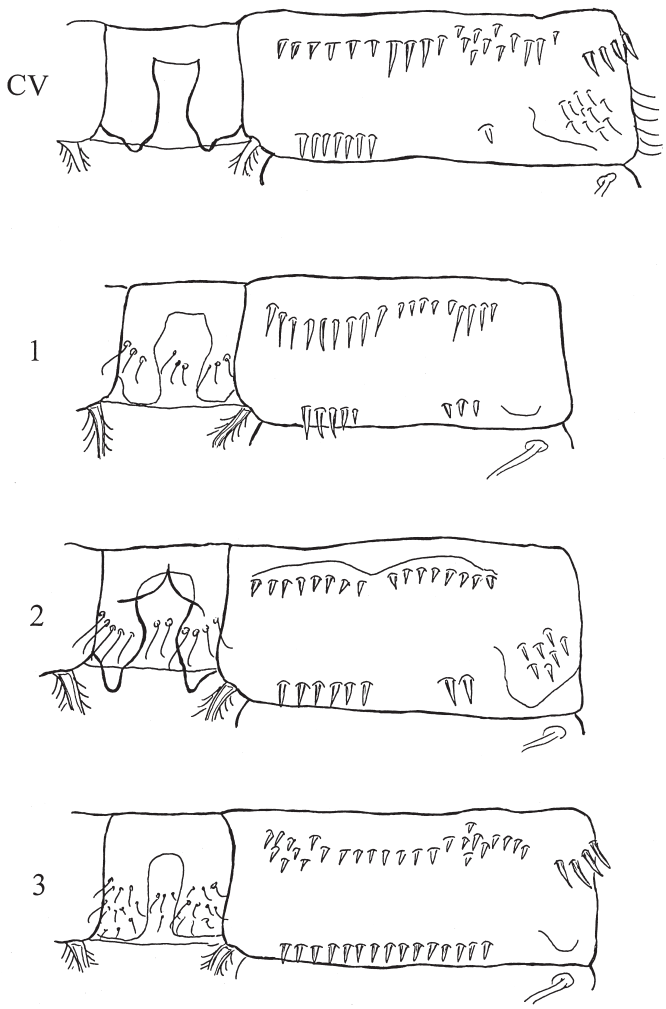

Fig. 2. Ornamentation pattern of the coxal segment of the 4th pair of swimming legs found in the examined populations: CV = *Cyclops vicinus* Uljanin, 1857, 1 to 3 = Types I to III.

The length of the outer apical spine of the 4th endopodite

Cyclops vicinus: one third of the length of the inner apical spine.

Type I: 1/3 to 1/2 of the length of the inner apical spine.

Type II: 1/2 to 2/3 of the length of the inner apical spine.

Type III: 1/3 of the length of the inner apical spine.

Shape of the genital segment of female

Cyclops vicinus, Type I, Type II: Broadest at anterior part and gradually narrowing at posterior part.

Type III: Anterior part wide, round-shaped, then abruptly narrowing into a cylindrical caudal part.

Habitats and localities

Cyclops insignis. Spring species in small permanent waters. Two permanent backwaters, Upper Lužnice River nr. Dvory nad Lužnicí, many samples from 1992–1994, J. Hrbáček legit; the same backwaters, 8.3. 1995, 21.3. 1995, Holý (1996); extensive flat shoal overgrown with willow and alder shrub, Starý Vrbenský Pond nr. České Budějovice, 28.3. 1996, 11.4. 1996, 26.3. 1997, 9.4. 1997, M. Lavická legit.

Cyclops vicinus. The most frequent species of the genus *Cyclops* in Bohemian ponds, reservoirs and permanent backwaters. Almost permanent inhabitant of limnetic region with maximum density in spring when its copepodid larvae emerge from the cocoons diapausing on the bottom. Examples of localities: Slapy Reservoir, Římov Reservoir (Brandl 1994); ponds nr. Blatná (Kořínek et al. 1987); ponds Starý Vrbenský, Nový Vrbenský, Starý Haklovský, Nový Haklovský nr. České Budějovice, year-round collections 1992, 1993, Z. Brandl legit; permanent backwater, Upper Lužnice River nr. Lesní Chalupy, 27. 4. 1999, M. Lavická legit; permanent backwater, Labe (Elbe) River nr. Velký Osek, 22. 4. 1999, M. Lavická legit.

Populations of the Type I. Permanent backwater, Upper Lužnice River nr. Lesní Chalupy, 27. 4. 1999; permanent backwater, Labe (Elbe) River nr. Velký Osek, 22. 4. 1999; Starý Vrbenský Pond nr. České Budějovice, 5. 5. 1999; Římov Reservoir, 2. 6. 1999, all M. Lavická gblegit.

Populations of the Type II. Permanent backwater, Upper Lužnice River nr. Lesní Chalupy, 27. 4. 1999; Římov Reservoir, 2. 6. 1999, all M. Lavická legit; Slapy Reservoir, material cultivated from the female collected on 6. 3. 1999 by J. Hrbáček.

Populations of the Type III. Ephemeral pool filled only by rain water, with maximum depth of 0.07 m, grassland and shrubs at the abandoned millitary training area, NW edge of the town České Budějovice, 27. 5. 1999, M. Lavická legit.

The identity of Types I to III

When the ornamentation patterns and the other details described above are compared with the most recent descriptions of such characters given by Einsle (1996a, 1996b) we can state that:

Type I fits best to the description of these characters of either *C. strenuus* or *C. stagnalis*

Type II is closest to the description of the lowland populations ("*C. divulsus"* group) of *C. abyssorum* or to the description of *C. singularis*

Type III is less ambiguous being closely similar to the description of *C. furcifer* with uniform spine formula 3.4.3.3 (the rarer of the two possible formulas of this species).

DISCUSSION

All five *Cyclops* species mentioned in the previous paragraph may occur in Bohemian water bodies. All these species are very similar in their morphology and differ in very small and unstable details. Einsle (1996b) uses the point of insertion of the lateral spine on the 2nd article of the 5th legs

(at the middle or nearer to the basis of the article). This character is very uneasy to quantify. Another character separating *C. strenuus* from *C. stagnalis* is the length of the antennules. According to Einsle (1996b), they reach just the end of the cephalothorax in *C. stagnalis* but up to the middle of the 3rd thoracomer in *C. strenuus*. This character was found to be very variable at least in our populations by Lavická (2000). Similarly, the width of the 4th thoracomer is used to differentiate *C. strenuus* from *C. abyssorum* (e. g. Einsle 1975), the maximum width of this segment being in the middle of its length in *C. strenuus* but in the slightly extended corners of the posterior part in *C. abyssorum*. However, evaluation of this character is not always easy especially in preserved material.

Thus the morphological characters do not offer a reliable way to identify a given population up to the species level. Another differentiation can be made using the stage of the egg cleavage in which redundant heterochromatin is eliminated. This "chromatin diminution" takes place in a definite cleavage stage specific for individual species which might differ also in the amount of such eliminated material (from small particles up to huge amount of heterochromatin, Beermann, 1966, Einsle, 1996b). The timing of diminution into the 4th or the 5th cleavage stage also separates the species in the two couples (*C. strenuus* + *C. stagnalis* and *C. abyssorum* + *C. singularis*) each of which has the same coxal ornamentation in both species of a couple. The procedure to find out this stage is relatively simple but hundreds to thousands individuals have to be examined until the eggs are found in the relevant stage of their embryonic development. Therefore we lack this information for any of the examined populations having the ornamentation of Types I, II or III.

However, the relevant five species differ from each other in their habitats. Three of them, *C. furcifer*, *C. stagnalis* and *C. singularis* are known to dwell in the ephemeral habitats of temporary ponds and pools which often completely dry off. On the other hand, both *C. strenuus* and *C. abyssorum* (including its lowland group of populations known as the "*C. divulsus*-group") are inhabitants of permanent waters ranging from small permanent ponds to large and deep lakes, although *C. strenuus* can be found in temporary pools, too. At least the populations of the Types I and II we examined came from permanent waters which included even two large reservoirs. The reservoir populations should therefore represent *C. strenuus* rather than *C. stagnalis* (Type I, Římov Reservoir) and *C. abyssorum* (Type II, Slapy Reservoir and Římov Reservoir) rather than *C. singularis*.

The question of species identity of the populations which belong to the type having the same coxal ornamentation pattern was elucidated by parallel allozyme examination of the same populations by Lavická (2000). She applied the electrophoretic separation of enzymes arginine phosphokinase (APK) and glutamateoxaloacetate transferase (GOT) made on the cellulose-acetate plates to all the populations of Type I, II and III we collected. With two loci of the GOT and three of APK enzymes she was able to distinguish three genetic types within the examined material. (The fourth one was represented by populations of *Cyclops vicinus*.)

The most important result is that each of these three genetic types corresponded to one of the above described types of coxal ornamentation patterns. Thus, the populations having the same type of coxal ornamentation represent also one genetically uniform type – and therefore they belong to one species. When we take into account the properties of habitats of the Types I and II, they are most probably identical with *C. strenuus* and *C. abyssorum* (lowland *"C. divulsus*-group" of populations), respectively. The third Type III may represent an isolated local population of *C. furcifer* with stabilized spine formula 3.4.3.3, which otherwise is the less common of the two possible formulas of this species (Einsle 1996b).

The occurrence of other species of the genus *Cyclops*, e.g. *C. stagnalis*, *C. singularis* or even *C. heberti* Einsle, 1996, and *C. bohater* Kozminski, 1933, is still possible in the Czech Republic. A population of the mountain subalpine type of *C. abyssorum* ("*C. praealpinus*-group") lived in the

past in the Šumava Mountains' lake "Černé jezero" ("*C. bohemicus"* of Šrámek-Hušek, 1937). In more recent years it was found in another of the Šumava Mountains' lakes, the "Prášilské jezero" (e.g. Fott et al. 1994). Its relation to the lowland populations of *C. abyssorum* should be re-examined. Numerous populations of *C. abyssorum* inhabit mountain lakes in neighbouring Austria and Slovakia. Further reasearch of the genus *Cyclops* and its distribution in this country is still needed and should be preferably made using all three possible approaches: detail examination of morphology, enzyme electrophoresis and the analysis of chromatine diminution.

A c k n o w l e d g e m e n t s

We are honoured to be invited to participate in this special issue dedicated to Doc. Jaroslav Hrbáček on the occassion of his 80th birthday. The first author is especially happy to do so with a paper on the detail morphology used to identify species – the approach which J. Hrbáček always promoted and taught his students. The study was supported by the grant F0149/1999(G4) of the Ministry of Education, Youth, and Sports. J. Hrbáček kindly supplied some samples of copepods. C. M. Steer greatly improved the English of this paper.

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