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A new Central European species of the *Daphnia curvirostris* complex, *Daphnia hrbaceki* sp. nov. (Cladocera, Anomopoda, Daphniidae)

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Abstract

Although systematics of the cladoceran genus *Daphnia* (Cladocera: Daphniidae) has been intensively investigated for decades using both morphological and genetic approaches, new lineages are being discovered on all continents, including in well-studied regions. Among Holarctic daphnids, *Daphnia curvirostris* Eylmann, 1887 held an interesting position, sharing some morphological characters of both the *D. pulex* and *D. longispina* groups. Recently, additional species of the *D. curvirostris* complex have been discovered in the Eastern Palaearctic. Here, we describe a new species in this complex from Central Europe, *D. hrbaceki* **sp. nov**. It was discovered in small, newly created fishless pools in the Czech Republic, and an additional sample of apparently the same taxon was collected in 1951 in Slovakia. *D. hrbaceki* is the closest yet known relative of *D. curvirostris*, but remains genetically divergent from all members of the complex (based on the sequences of three mitochondrial genes: 12S, COI, and ND2). In general, adult females of this species are morphologically very similar to *D. curvirostris*. Unlike the latter species, *D. hrbaceki* may develop a specific hump-shaped dorsal outline of the carapace, presumably an inducible defence against invertebrate predators. Juveniles of the new species occasionally form neckteeth, which may also be retained in adult individuals. The species also shows substantial variation in the size of spines in the middle pecten of the postabdominal claw, similarly as in the Japanese member of the species complex, *D. tanakai* Ishida, Kotov & Taylor, 2006. This variable character of spine size in the postabdominal middle pecten (a transition from the *pulex* to the *longispina* group character), as well as a bent and heavily setulated terminal seta on the male 2nd endopodite (considered as the *pulex* group character), are typical for the new species. *D. hrbaceki* also differs from *D. curvirostris* as well as other members of the complex in the ephippial surface ultrastructure. Our study demonstrates the utility of such ultrastructural characters in *Daphnia* taxonomical studies.

Key words: taxonomy, new species, inducible defences, ephippia ultrastructure

Introduction

Water fleas of the genus *Daphnia* (Anomopoda: Daphniidae) are an important group in the zooplankton of inland water bodies, particularly in temperate zones. Their position in pelagic food webs, linking primary producers in phytoplankton and planktivorous consumers, especially fish, makes daphnids some of the keystone taxa in lake ecosystems. In addition, several *Daphnia* species have become model organisms in a number of research fields, including evolutionary biology or applied sciences such as ecotoxicology (Peters & de Bernardi 1987; Benzie 2005). In comparison with other cladoceran taxa, the genus *Daphnia* can be considered extremely well-known (Forró *et al.* 2008), and is among the most intensively studied aquatic invertebrates. However, there are still substantial gaps in knowledge of the diversity and systematics of this ecologically important model taxon. As in other cladoceran groups, undescribed lineages are being discovered in all biogeographic regions (see, e.g., Adamowicz *et al.* 2009), and many apparently widespread taxa turn out to be cryptic species complexes if studied in detail (Forró *et al.* 2008).

Until recently, *Daphnia curvirostris* Eylmann, 1887 belonged to a group of rather unusual *Daphnia* species which turned out to belong to the same genetic lineage in different biogeographic zones, despite its very broad distribution including the Palaearctic, Africa and North America (Benzie 2005). However, two new closely related species from the *D. curvirostris* complex were recently described from the eastern Palearctic: *Daphnia tanakai*

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Ishida, Kotov & Taylor, 2006 from Japan and *Daphnia sinevi* Kotov, Ishida & Taylor, 2006 from East Russia. Additional genetic evidence (Kotov *et al.* 2006) indicates that the diversity within this species complex in the eastern Palaearctic is even higher; apparently, this region may have been a diversification centre of the complex.

The *D. curvirostris* complex has several interesting morphological features. Despite belonging phylogenetically to the *D. longispina* group (Adamowicz *et al.* 2009) that mostly consists of pelagic taxa from larger water bodies, members of the *D. curvirostris* complex usually inhabit smaller water bodies, and share some ecological as well as morphological characteristics with the *D. pulex* group. Among the *D. longispina* group, the *D. curvirostris* complex is unique in having an enlarged middle pecten of spines on the postabdominal claw (i.e., a pecten of the *pulex* type, which has been used as the main differentiating character between the *longispina* and *pulex* groups; see, e.g., Glagolev 1995). Interestingly, it has been shown for *D. tanakai* that this feature, believed to be very stable in higher taxonomic groups, can be variable within a single species, and even within a single population (Ishida *et al.* 2006). However, *D. tanakai* remains so far the only taxon within the *D. curvirostris* species complex for which such variation has been documented. Another morphological character that has received recent attention was the ability to form neckteeth, an antipredator morphological structure, in *D. sinevi*, another newly described Far East taxon of the complex (Kotov *et al.* 2006). By documenting for the first time that such feature also exists in *curvirostris*-like taxa (Kotov *et al.* 2006), this discovery provided additional evidence that neckteeth apparently originated several times independently in *Daphnia* (Colbourne *et al.* 1997).

In Europe, from which *D. curvirostris* was originally described (Eylmann 1887), this taxon seemed to be very homogeneous. However, the *Daphnia* fauna of the Western Palaearctic regions is far from fully explored, as documented by recent discoveries of a number of cryptic lineages within the genus in this biogeographic region (Petrusek 2003; Petrusek *et al.* 2008; Adamowicz *et al.* 2009; Petrusek *et al.* 2009). In this paper, we describe a new species from the *D. curvirostris* complex, *Daphnia hrbaceki* **sp. nov.**, collected from small pools in Central Europe (Czech Republic and Slovakia). A single sample of *Daphnia* of unusual morphology was collected in Slovakia in 1951 (from a pool at the village Rimavská Baňa); however, no additional material of this taxon was available until recently, when similar individuals were found in a newly recreated small fishless pool in the Czech protected landscape area Kokořínsko. The finding of this apparently rare species, which shares several characteristics with the above-mentioned Eastern Palaearctic taxa, demonstrates that pond and pool habitats may harbour substantial cryptic diversity even in seemingly well-explored regions.

Material and methods

Sampling. Zooplankton samples were collected by plankton nets (mesh sizes 100–200 µm). Localities in the protected landscape area Kokořínsko (Central Bohemia, Czech Republic) were visited three times per year (spring, summer, autumn) in five consecutive years from 2005 to 2009. All samples were preserved either with 96% ethanol or by the addition of formalin to a resulting formaldehyde concentration of approx. 4%. The sample from Rimavská Baňa (southern Slovakia) was collected in 1951 during a student field course and preserved with formalin.

Morphological analyses. We used samples of several related or morphologically superficially similar taxa for comparison with the putative new species (Table 1): *Daphnia curvirostris*, *D. tanakai*, *D. sinevi*, *Daphnia longispina* (O. F. Müller, 1776), *Daphnia minnehaha* Herrick, 1884, *Daphnia pulex* Leydig, 1860, and *Daphnia* sp. (morphotype FLO9), a North American taxon labeled in several publications as *D. arenata* which nevertheless must be considered a *nomen nudum* (see below for a discussion of its nomenclature).

Material used for mounting in permanent slides was transferred to ethanol and stained with lignin pink and chlorazol black E dyes for 24 hours. After staining, specimens were dehydrated with 2-2-dimethoxypropane for 10–15 minutes, then transferred into xylene and mounted in Canada balsam (Kořínek 1999). To see details of the exoskeleton, some specimens were heated for 30 minutes in 10% potassium hydroxide or lactic acid, and washed in distilled water before mounting.

For morphological analyses, we used optical as well as scanning electron microscopy (SEM). Photographs were taken by a Nikon DXM1200F digital camera attached to a Nikon Eclipse E400 optical microscope. Every object under the microscope was photographed several times with different depths of focus. Resulting pictures were consequently merged into one completely sharp picture (Extended Depth of Field).

TABLE 1. Material examined in this study. Abbreviations of country names: BG—Bulgaria, CA—Canada, CH—Switzerland, CZ—Czech Republic, DE—Germany, IL—Israel, JP—Japan, PL—Poland, RU—Russia, SK—Slovakia, UG—Uganda, US—United States of America. Abbreviations of personal names*:* AGK—A. G. Kirdyasheva, AP—A. Petrusek, AYS—A. Y. Sinev, DV—D. Vondrák, EK—E. Kočárek, MČ—M. Černý, FK—F. Kubíček, HK—H. Kling, HL—H. Loffler, JH—J. Hrbáček, KO—K. Okamoto, OA—O. Albertová, PDNH—P. D. N. Hebert, PJJ—P. J. Juračka, VK—V. Kořínek. Samples used for genetic analyses are marked with asterisks. Precision of geographic coordinates depends on availability of data or size of the locality.

Locality	Geographical coordinates	Sampling date	Collected by:
***Daphnia hrbaceki* sp. nov.**			
CZ: Kokořínsko, pool #17 in Český příkop (type locality)*	N 50°28’54” E 14°41’10’	12 July 2006	PJJ
CZ: Kokořínsko, pool #18 in Žďárský důl	N 50°29’11” E 14°41’24’	10 November 2006	PJJ
SK: Rimavská Baňa, shallow pool	N 48° E 19°	27 April 1951	OA
Daphnia curvirostris			
CZ: Libický luh near Velký Osek, fluvial pools	N 50°06’ E 15°10’	April 2007	VK
CZ: Přerov, fluvial pools (including Karasí pool)	N 50°10’ E 14°48’	13 samples between 1964 and 2007	VK
CZ: Kokořínsko, Tupadly, experimental pools	N 50°26’16” E 14°28’20”	23 October 2007	DV
CZ: Kadov, fishpond Paseka	N 49°25’25” E 13°47’50”	22 August 1991	VK
CZ: Tchořovice, fishpond Radov	N 49°25’28” E 13°49’13”	22 June 1985	VK
CZ: Slatina, large temporary marsh on a meadow	N 49°23’48” E 13°44’55”	28 April 2008	VK
CZ: Kateřina, nature reserve Soos, pool	N 50°09’ E 12°24’	19 April 1959	JH
CZ: Lednice, pools in the Dyje River alluvial plain	N 48°48’ E 16°50’	15 samples between 1948 and 2007	VK + other collectors
CZ: Havraníky, shallow pool	N 48°48’54” E 16°00’16”	7 June 2001	VK
CZ: Mutěnice, forest pool	N 48°54’ E 17°04’	23 April 1969	FK
CZ: Kunovice, forest fluvial pool	N 49°02’ E 17°30’	31 March 2007	MČ
CZ: Moravičany, temporary fluvial pools	N 49°45’21” E 16°58’40”	2 April 2007	MČ
SK: Vinné, Vinianské Lake	N 48°49’06” E 21°59’12”	22 May 1964	JH
PL: Wipsowo, small pool in a peat bog east of village	N 53°54’ E 20°49’	21 August 1958	JH
BG: Chelopechene, shallow puddle at fish farm	N 42°44’ E 23°27’	14 October 1987	VK
IL: Netanya, temporary pool Dora	N 32°17’25” E 34°50’45”	20 January 2004	AP
UG: Ruwenzori Range, Bujuku Lake	N 0°22’36” E 29°53’35”	September 1967	HL

continued next page

TABLE 1. (continued)

Locality	Geographical coordinates	Sampling date	Collected by:
RU: Borok, temporary puddles	N 58°03’ E 38°13’	11 June 2004	AGK
Daphnia minnehaha			
CA: Ontario, Experimental lake area: Lake #81 (Patalas,1971)	N 49°38’49” W 94°04’27”	24 September 1971	HK
CA: vicinity of lake #81, small pool	as above	29 August 1971	VK
***Daphnia* morphotype FLO9**			
US: Oregon, Florence, coastal pond #9	N 44°, W 124°	16 May 1989 15 April 1993	PDNH MČ
US: Oregon, Florence, Sutton Lake	N 44°03’40” W 124°05’21”	16 April 1993	MČ
Daphnia tanakai			
JP: Honshu, Tateyama Mountains, Lake Mikuriga-ike	N 36°34’54” E 137°35’49”	25 September 1978	KO
Daphnia sinevi			
RU: Nakhodka, pond in Avangard	N 42°48’ E 132°53’	25 September 2004	AYS
Daphnia longispina			
CZ: Mirovice, abandoned clay pit	N 49°31’01” E 14°03’23”	21 September 1986	VK
DE: Ismaning, Ismaninger Fischteiche, large fishpond	N 48°13’00” E 11°46’08”	22 September 2004	AP
CH: Valais, shallow pond above Great St. Bernard pass	N 45°52’16” E 07°10’12”	6 September 2005	AP
Daphnia pulex			
CZ: Chlístovice, pond	N 49°53'06" E 15°13'30"	1 October 1995	VK
Daphnia obtusa			
CZ: Kokořínsko, Medonosy, small shallow pool *	N 50°30’06” E 14°29’07”	9 March 2010	PJJ

Specimens preserved in 96% ethanol or formalin solution were used for SEM analyses. To clean the surface of foreign particles, specimens were treated with hot 10% potassium hydroxide for 5 to 10 minutes. Remnants of alkali were washed out in distilled water. Specimens were then dehydrated in a graded acetone series and then dried either by critical point drying (using the dryer BAL-TEC CPD 030) or with organic volatile matter hexamethyldisalazane (Laforsch & Tollrian 2000). Dehydrated specimens or body parts were gold-coated for 5 minutes in argon plasma at 10^{-1} millibar vacuum in the BAL-TEC Sputter Coater SCD 050. Gold-coated objects were observed in the JEOL JSM-6380 LV scanning electron microscope at 15 kV. Background surrounding the object was replaced in the micrographs by solid black.

Genetic analyses. To characterise the morphologically unusual *Daphnia* population from the Czech Republic, we amplified three mitochondrial genes commonly used in *Daphnia* diversity studies. Genes for the small ribosomal subunit (12S rRNA) and for the cytochrome c oxidase subunit I (COI) have been traditionally used in studies on *Daphnia* phylogeny (e.g., Schwenk *et al.* 2000; Colbourne *et al.* 2006; Petrusek *et al.* 2009), and are available for the vast majority of *Daphnia* species so far genetically analysed (see Adamowicz *et al.* 2009). Sequences of these genes deposited in the public database (GenBank accession numbers HM625747 for 12S and HM625748 for COI) are therefore useful for any future studies analysing new or rare species in a wider context. The third chosen marker, the rapidly evolving gene for NADH dehydrogenase subunit 2 (ND2), has recently been used to characterise Eastern Palaearctic members of the *D. curvirostris* complex and their phylogenetic relationships (Ishida *et al.* 2006; Kotov *et al.* 2006), and it remains the only mitochondrial marker available for those taxa. We therefore used it to reconstruct the phylogenetic position of the Czech taxon within the *D. curvirostris* complex, as well as in the wider phylogenetic context. In particular, we included in the phylogenetic analysis the specimen representing *Daphnia obtusa* Kurz, 1874, a species common in the studied area and co-occurring with the studied taxon at its type locality.

Nucleic acid isolation, amplification and sequencing followed previously published protocols. DNA was extracted from single *Daphnia* individuals preserved in ethanol by proteinase K digestion (Schwenk *et al.* 1998). Fragments of 12S rDNA and COI genes were amplified using standard protocols as in Schwenk *et al.* (2000). For ND2, we followed the protocol provided in Ishida *et al.* (2006), using the primer combination MetF2 and TrpR. PCR products were purified and sequenced on ABI 3730XL capillary sequencers by a third party (Macrogen, Seoul, Korea). Resulting sequences (deposited in GenBank under accession numbers HM625747-HM625750) were aligned with sequences of other relevant *Daphnia* species (retrieved from GenBank) using the ClustalW algorithm (Thompson *et al.* 1994) in MEGA version 4 (Tamura *et al.* 2007). The alignments were checked by eye and corrected according to the translated amino-acid alignment, and sequence divergences (Kimura 2-parameter model) were calculated by the same software.

Phylogenetic relationships among species within the *Daphnia curvirostris* complex, including selected taxa from other species complexes of the *D. longispina* group and three members of the *D. pulex* group as an outgroup, were subsequently assessed using a part of the ND2 gene, which was available for all relevant taxa (alignment length 932 bp). We used jModeltest (Posada 2008) to select the best model of nucleotide substitution, and assessed the phylogeny using the Bayesian inference (BI) in MrBayes version 3.1.2 (Ronquist & Huelsenbeck 2003), and Maximum Likelihood (ML) and Maximum Parsimony (MP) analyses in PAUP* 4.0b10 (Swofford 2002). In BI, two parallel runs of four Monte Carlo Markov chains were run for 3 million generations, trees were sampled every 100 generations, and the first 20% of sampled trees were discarded as a burn-in phase. In PAUP, heuristic searches were conducted with tree bisection-reconnection branch swapping and 10 random sequence taxon additions; branch support was evaluated by nonparametric bootstrapping with 100 (ML) and 1000 (MP) pseudoreplicates.

Abbreviations. CL—Chlístovice, Czech Republic; ELA—Ontario, Canada; FL—Florence, Oregon, USA; H—Havraníky, Czech Republic; I—Ismaning, Germany; K—Kokořínsko (type locality), Czech Republic; KP—Karasí pool, Czech Republic; LL—Libický luh, Czech Republic; LM—Lake Micuriga, Japan; RB—Rimavská Baňa, Slovakia; GSB—Great St. Bernard pass, Switzerland**.**

Results

Taxonomy

Order Anomopoda Sars, 1865

Family Daphniidae Straus, 1820

Genus *Daphnia* Müller, 1785

Daphnia hrbaceki sp. nov.

(Figs 1–8)

Etymology. The new species is dedicated to the eminent Czech hydrobiologist Jaroslav Hrbáček (1921–2010), who initiated complex ecological studies of *Daphnia* populations in the former Czechoslovakia. The name in the Czech language also reflects the hunched body shape of some individuals.

Type locality. A small fishless, recently (2004) excavated pool in the valley Český příkop (protected landscape area Kokořínsko, Czech Republic); N 50°28'54", E 14°41'10", alt. 289 m above sea level. The pool is 7 m long and 3 m wide with maximal depth ca. 2 m, situated in a deep, shaded valley with a cold microclimate. The type series was collected on 5 November 2007 by P. J. Juračka.

Holotype. Adult parthenogenetic female (total body length 1.7 mm) mounted in Canada balsam and stained with a mixture of lignin pink and chlorazol black E; Natural History Museum, London (NHM 2010.39).

Allotype. Adult male (body length without shell spine 1.0 mm) mounted and stained as above (NHM 2010.40).

Paratypes. Males and females (45 specimens), preserved in 96% ethanol and a small amount of glycerol (NHM 2010.53-62). Additional specimens from the type series are deposited in the collection of the National Museum, Prague (P6E3005).

Ephippial female (total body length 1.5 mm) stained and mounted as above (NHM 2010.41).

Dissected parthenogenetic female treated with hot 10% potassium hydroxide and mounted as above (NHM 2010.42).

Females and males (13 specimens) stained and mounted as above (NHM 2010.43-52).

Diagnosis. *Parthenogenetic female* with median keel on head shield, some populations with induced necktooth on its posterior margin. Similar neckteeth may be present in juveniles and males. Antennule completely reduced, median mound strongly vaulted with reticulated apex. Ocellus pigmented. Shallow cervical depression. Shell spine short or absent. Gnathobase of second thoracic limb extended distally into angular projection. Postabdominal claw with second (middle) pecten of spinules or teeth of variable size and shape: either spinules slightly longer than those in proximal pecten, or large teeth longer than width of claw.

Ephippium saddle-shaped, dorsal ridge smooth (without spinules), only reticulated; posterior carapace margin included into ephippium. Ephippial surface ultrastructure with many minute pits surrounded by fine lamellae.

Male with medium-sized rostrum hardly covering antennular socket. Antennule short, two to three times longer than wide. One of the three terminal setae on 2nd endopodite bent and heavily setulated. Pre-anal margin of postabdomen weakly depressed, anal margin convex.

Size. Total body length (without shell spine): parthenogenetic female 1.0–1.7 mm; ephippial female 1.2–2.0 mm; male 0.9–1.2 mm.

Description. Parthenogenetic female. *Head*: high, strongly vaulted apical part with median keel increasing in width dorsally. Keel extremely developed in some individuals; forming hump-shaped structure (Figs. 1C, E; 2B, F). Neckteeth rarely present in adult females (Figs. 1E; 2B, E). Dorsal margin with shallow cervical depression (Fig. 1B). Frontal contour of head concave above rostrum. Rostrum not prominent, its tip bent ventrally in some specimens. Tip of rostrum obtusely rounded and split into two lobes by suture or line between head shield and ventral side of head in lateral aspect (Fig. 4C, E). Mid-antennular mound well developed, markedly reticulated on apex. Optic vesicle contiguous with frontal part of head. Ocellus pigmented. Fornix rounded at base of second antenna.

Antennule: not protruding, its body reduced, seen as lateral areole on median mound with 9 sensory setae; single lateral seta anterior to areole (Fig. 4C, E).

Antenna: Setal formula of natatory setae: 0-0-1-3/1-1-3. Presumably sensorial setae and spinules: two setae on concertina-like basal joint, one apical spine-like on its outer side, one seta on inner side between both branches, one apical spinule on dorsal margin of second segment 4-segmented branch. Dark rings at base of distal part of swimming setae may be present in some individuals or populations. Surface of all segments covered with transversal groups of small teeth.

First maxilla: carrying three robust, curved and heavily setulated setae and one short stump-like distal seta.

Carapace: approximately sub-ovoid, length of posterior spine variable, forming up to 15% of body length (without shell spine) or completely reduced. Spinules on ventral margin cover 1/3 to 2/3 of its length, spinulation on dorsal margin developed only in posterior 1/4 of margin or only near posterior spine. Spinulation of dorsal margin completely missing in some individuals. Fringe of sub-marginal setae absent.

Thoracic limbs: agree with the re-description of *Daphnia curvirostris* in Ishida *et al.* (2006) with the exception of 2nd limb gnathobase, which extends in front of longest clearing seta into noticeable rectangular corner or small lobe (Fig. 5E, F).

Postabdomen: elongated, tapering distally, pre-anal face even, covered with scattered groups of fine spinules, anal margin slightly convex, fringed with up to 15 strong teeth that increase in length distally. Distal portion of postabdominal setae slightly shorter than proximal one. Abdominal processes gradually diminishing distally, first twice as long as second, third reduced to 1/3 up to 1/2 of second one in specimens preserved in formalin. Terminal claw long, with three groups (pectens) of teeth and spinules. Proximal one of 13–19 minute spinules, middle pecten variable in size: either 8–9 large teeth markedly longer than width of claw or 11–13 spinules that only slightly exceed in length those of other two pectens. Distal row of about 60 fine spinules, not reaching tip of claw. Differences in size and length of claw spinules were observed among samples collected in different times of season, and between individuals from the wild and those cultured in laboratory (Fig. 3A–D).

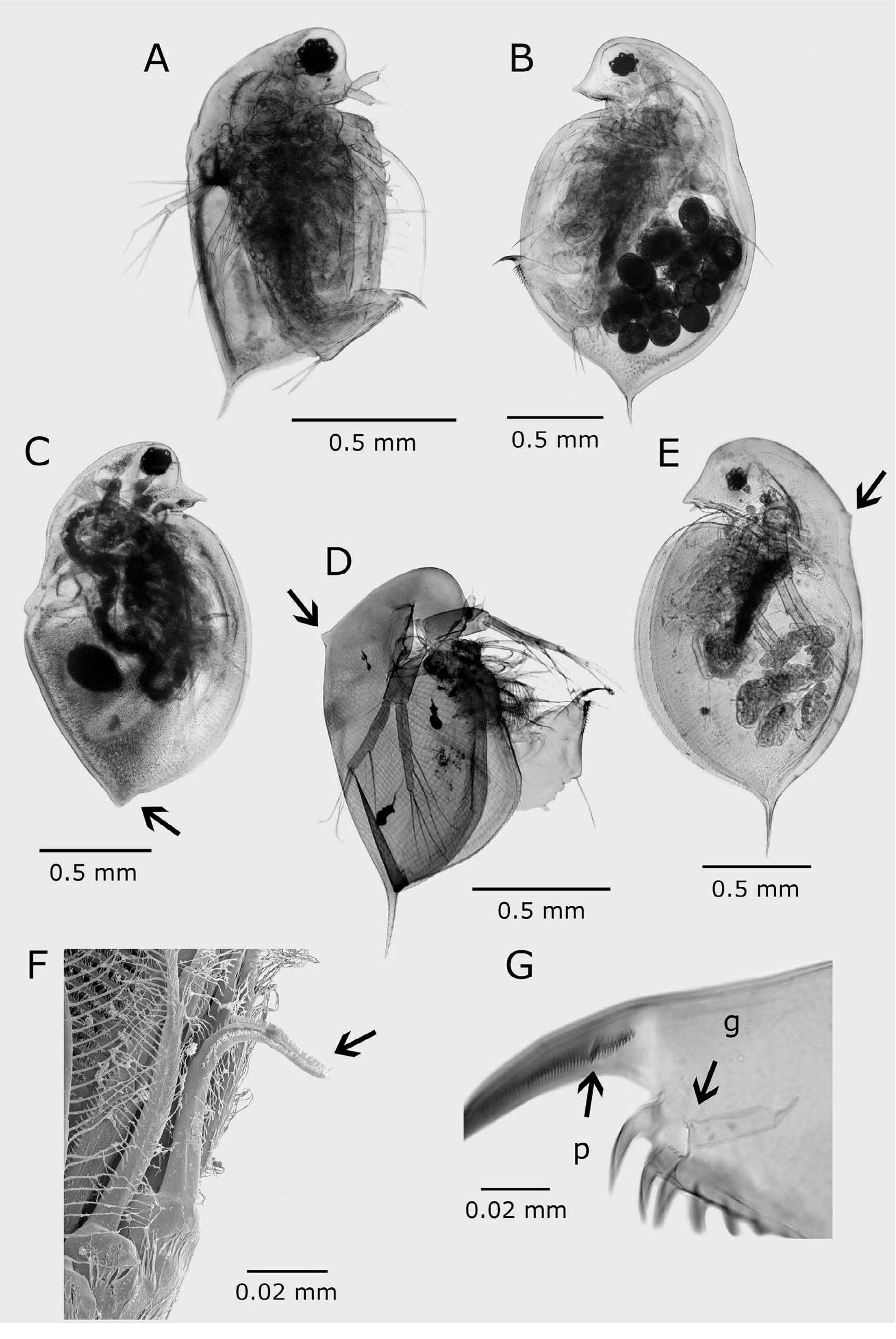

FIGURE 1. *Daphnia hrbaceki*. A. Adult male (K). B. Adult parthenogenetic female (K). C. Adult ephippial female (K). D. Adult male (RB) with necktooth indicated by arrow. E. Adult parthenogenetic female (RB) with morphology presumably induced by invertebrate predators; arrow indicates a hump-shaped dorsal outline of the carapace. F. Adult male (RB), hook-like apical seta (2nd limb) indicated by arrow. G. Adult male (RB), postabdomen (contrast increased at gonopore area); arrows indicate gonopore (g) and middle pecten on postabdominal claw (p).

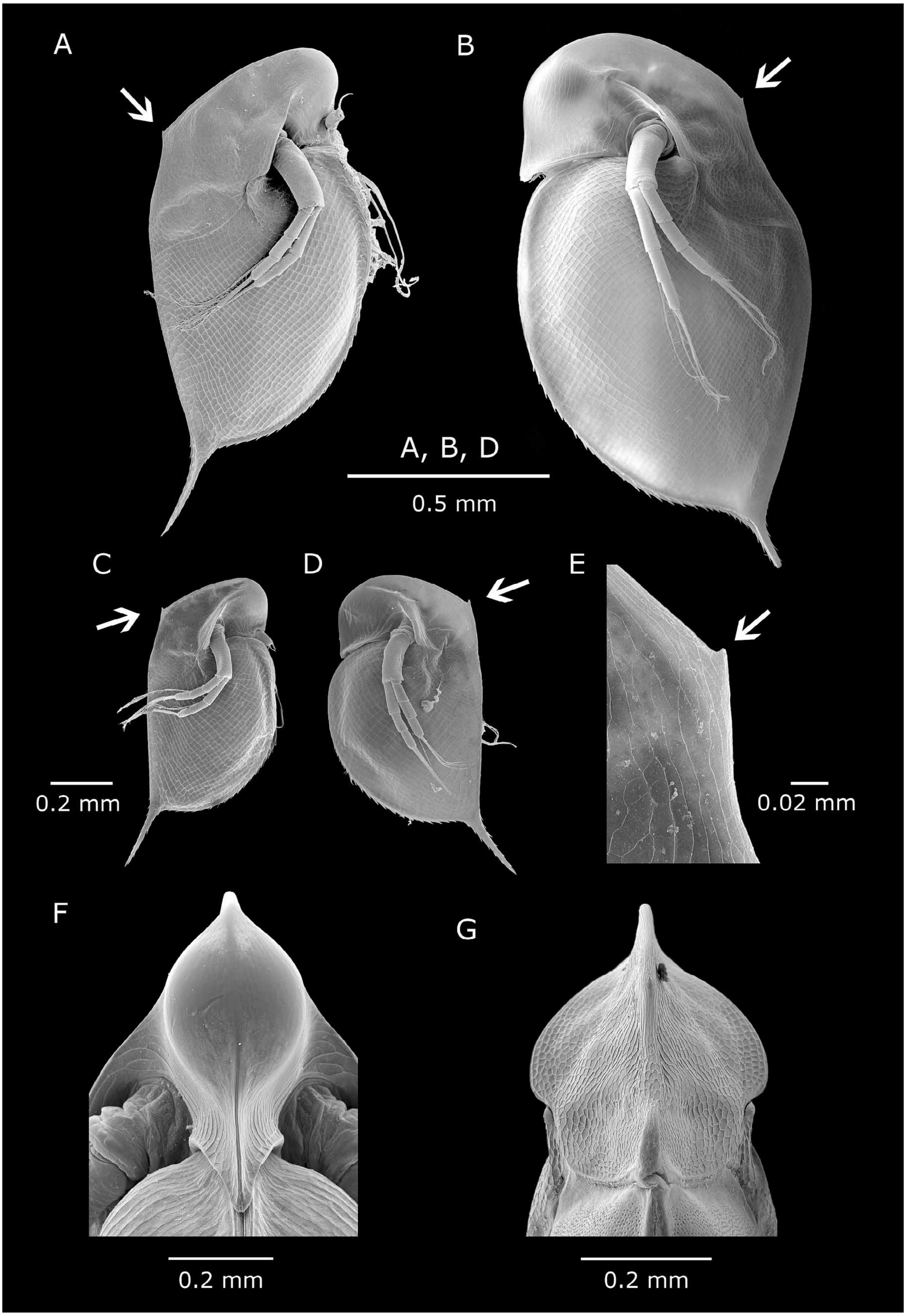

FIGURE 2. *Daphnia hrbaceki*. Arrows indicate neckteeth. A. Adult male (RB); B. Adult parthenogenetic female (K); C. Juvenile male (RB); D. Juvenile female (RB); E. Subadult female (RB), detail of necktooth; F. Head of adult parthenogenetic female (K) in antero-ventral aspect. G. Head of adult ephippial female (K) in dorsal aspect.

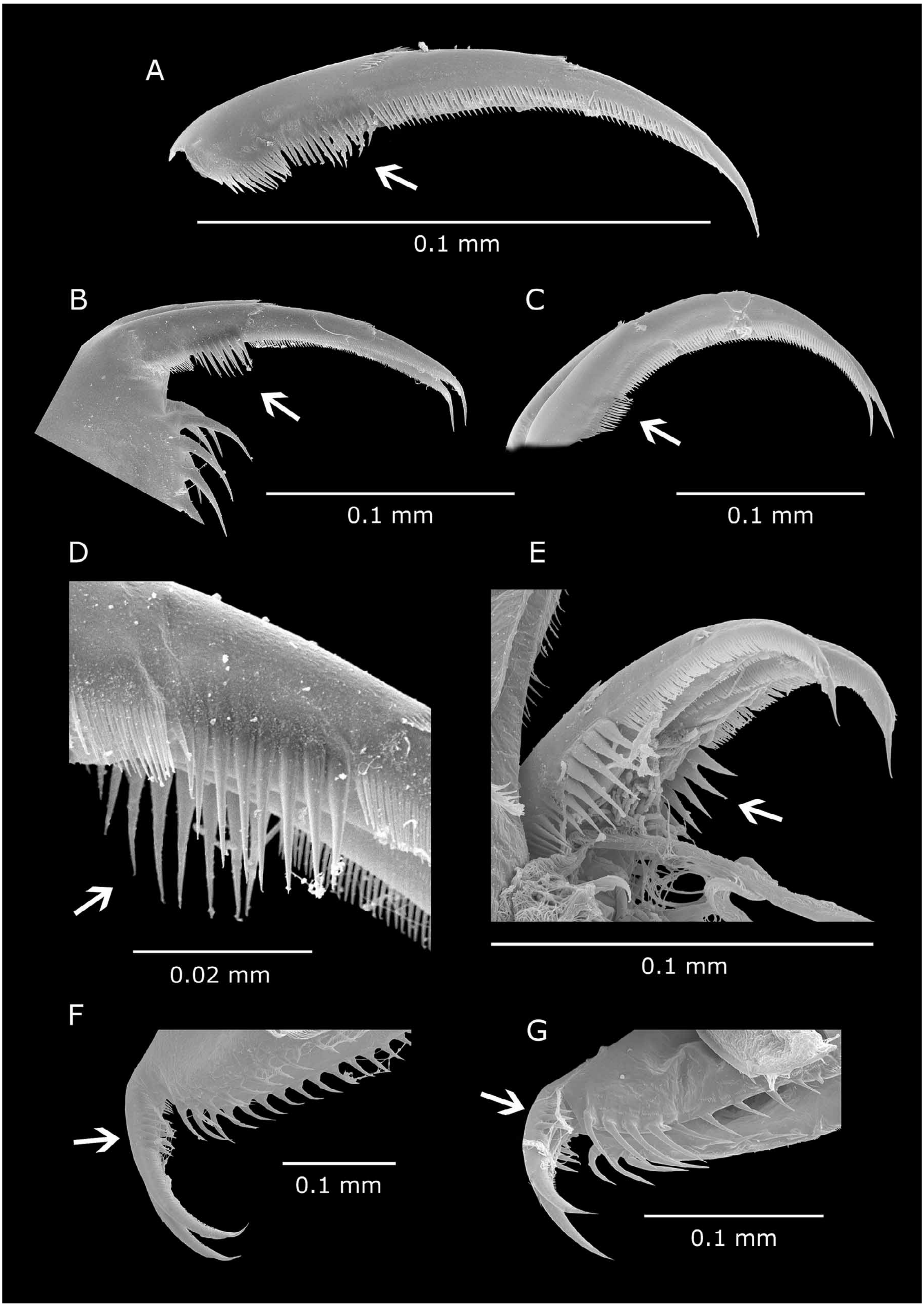

FIGURE 3. Comparison of postabdominal claws. Arrows indicate second (middle) pecten of spinules or teeth of postabdominal claw. A. *Daphnia hrbaceki*, adult female (K). B. *D. hrbaceki*, adult female from laboratory culture (K). C. *D. hrbaceki*, adult female (RB). D. *D. hrbaceki*, adult female from laboratory culture (K); detail of middle pecten. E. *D. hrbaceki*, adult male from laboratory culture (K). F. *Daphnia* sp. (morphotype FLO9), adult female (FL). G. *D. minnehaha*, adult female (ELA).

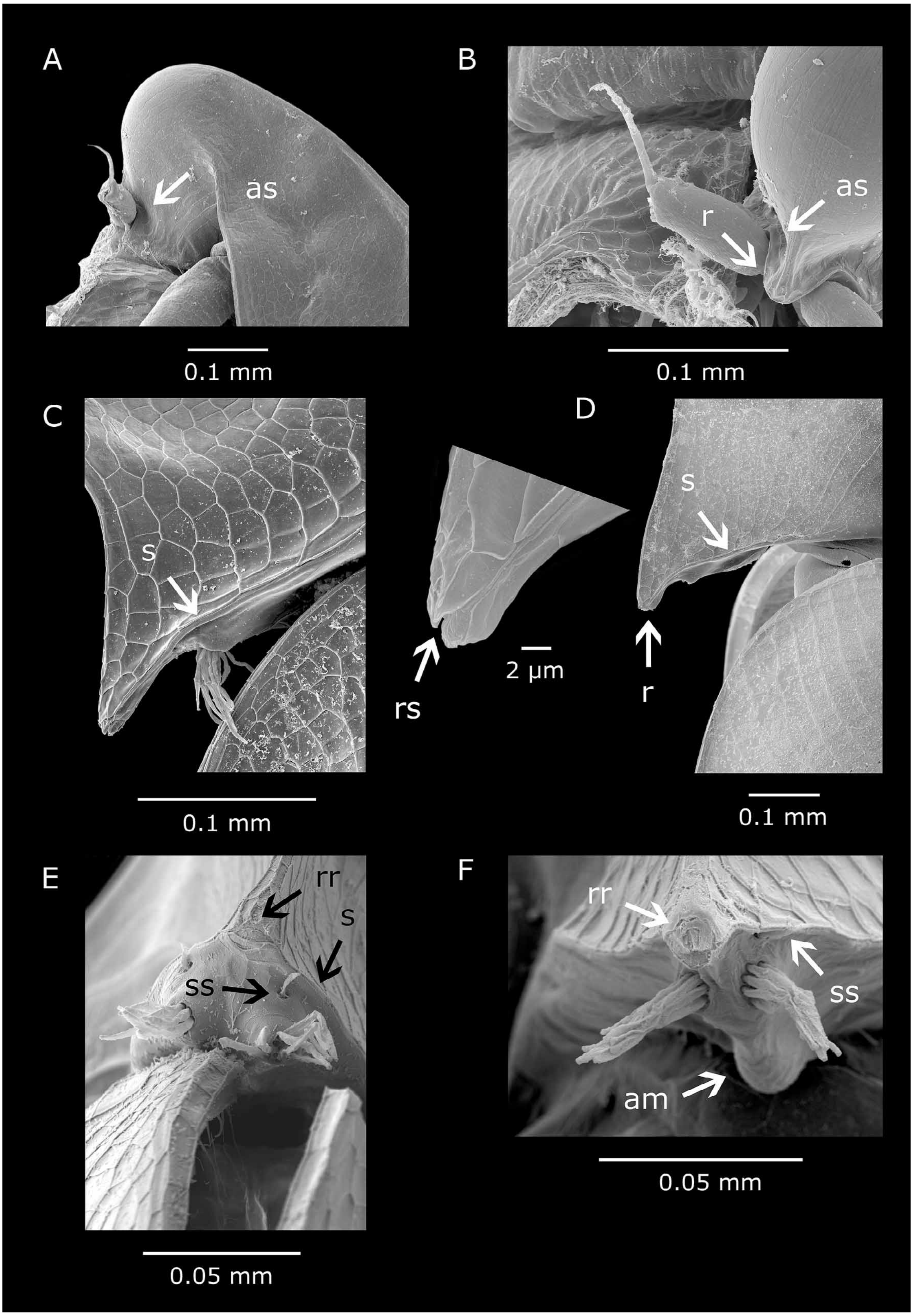

FIGURE 4. *Daphnia hrbaceki* and *Daphnia curvirostris*. Arrows indicate male antennular socket (as), suture between head shield and ventral side of head (s), single lateral seta anterior to areole (ss), rostrum (r), reticulation on the tip of rostrum (rr), apparent split of the rostrum (rs) and antennular mound (am). A, B. *D. hrbaceki*, head of adult male (RB) C. *D. hrbaceki,* adult female (K); rostrum and antennule, lateral aspect. D. *D. curvirostris*, adult female (H); rostrum and antennule, lateral aspect; detail of the rostrum tip in lateral aspect shown on the left. E. *D. hrbaceki*, adult female (K); rostrum, postero-frontal aspect. F. *D. curvirostris,* adult female (KP); rostrum, frontal aspect.

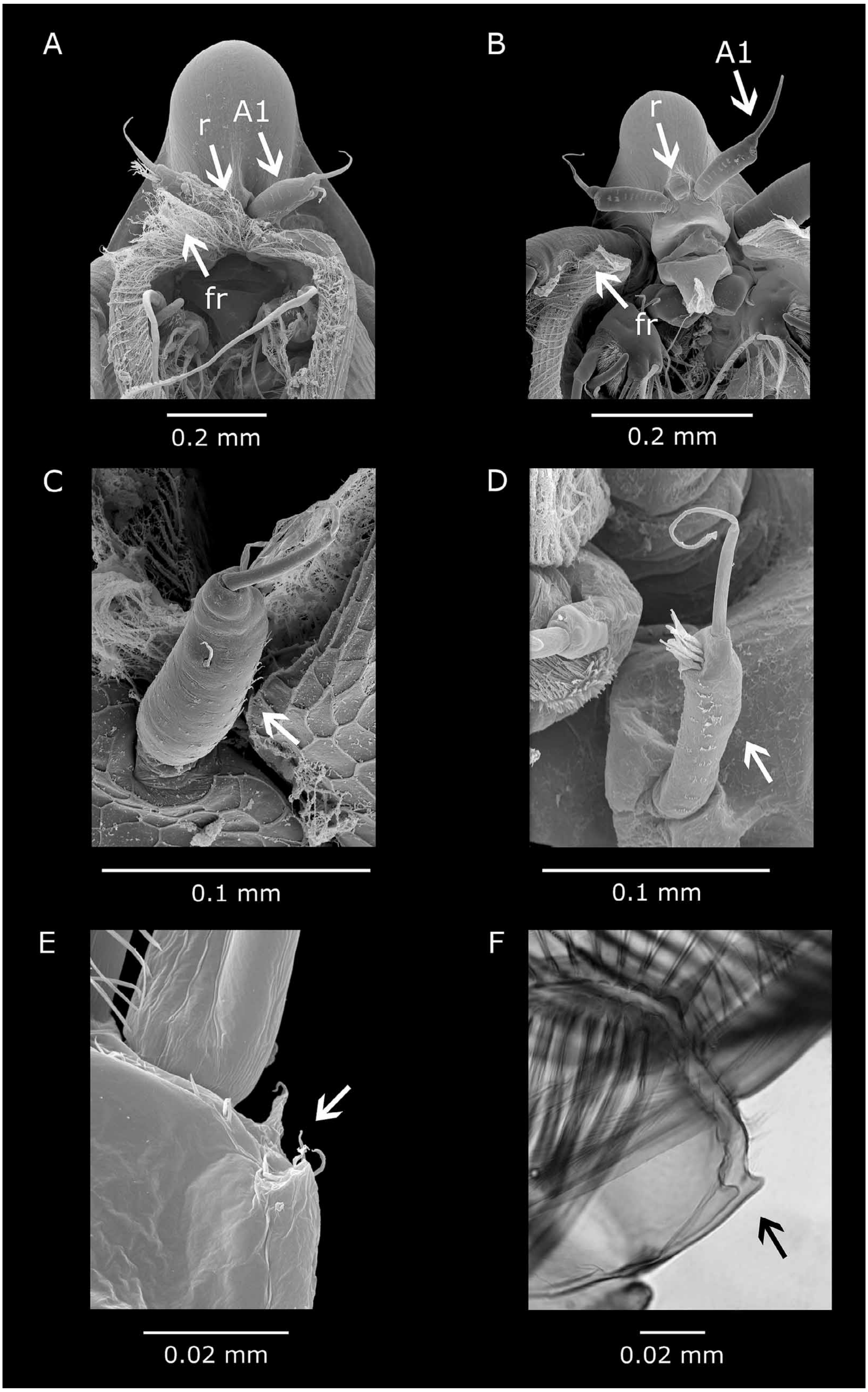

FIGURE 5. *Daphnia hrbaceki* and *Daphnia curvirostris*. A. *D. hrbaceki*, adult male (RB); antennules (A1) and rostrum (r), arrow (fr) indicates valves fringed with row of long, sub-marginal feathered setae. B. *D. curvirostris*, adult male, arrows as in Fig. 5 A (LL). C. *D. hrbaceki*, adult male (K); antennule (indicated by arrow). D. *D. curvirostris,* adult male (KP); antennule (indicated by arrow). E, F. *D. hrbaceki*, adult females (RB); 2nd thoracic limb, gnathobase, arrows indicate gnathobase extending distally into angular projection.

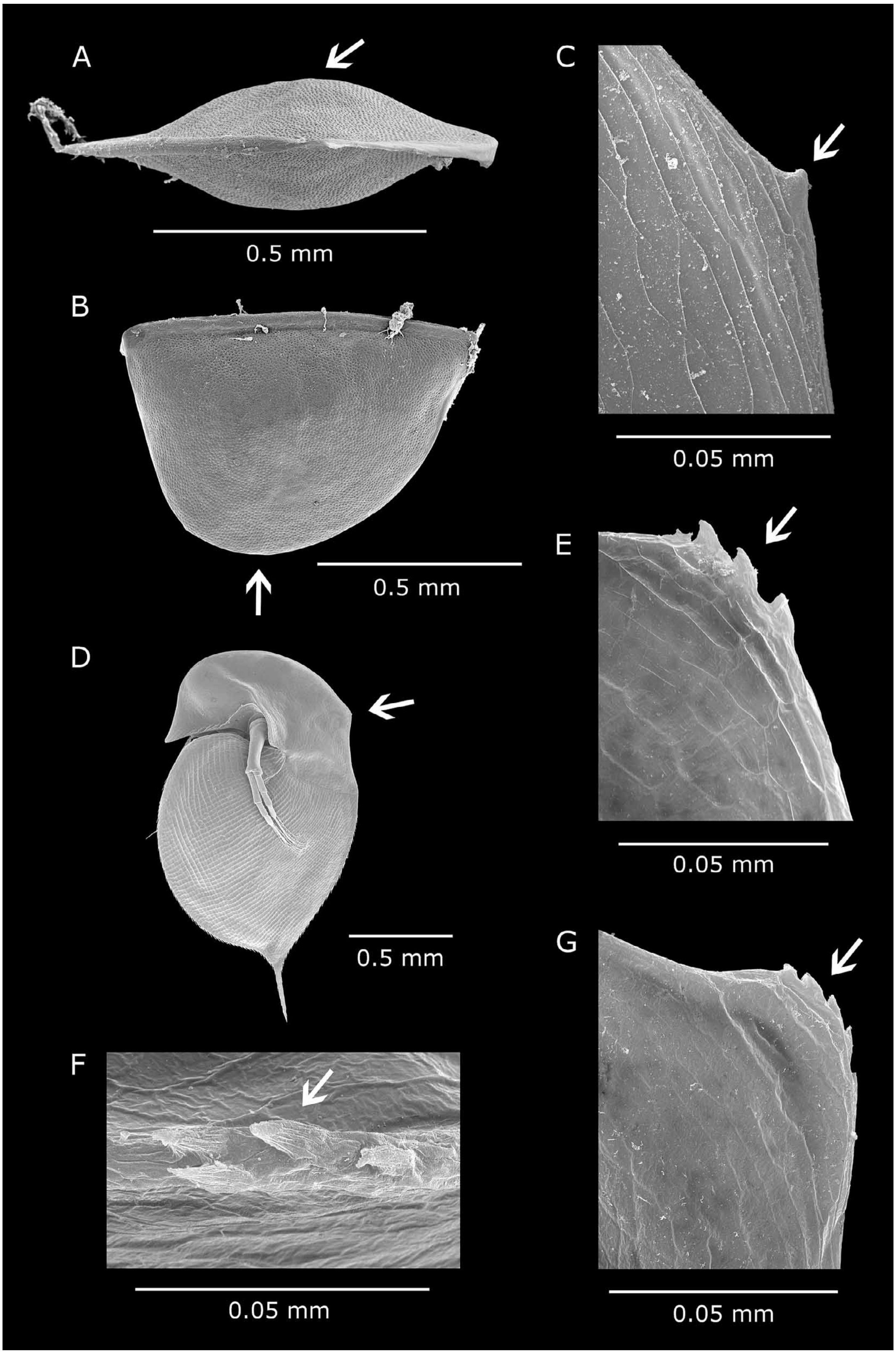

FIGURE 6. Ephippium and neckteeth. A. *Daphnia hrbaceki*, dorsal aspect of free ephippium (K); arrow indicates evenly shaped convex outline lacking any concavity between the two egg chambers. B. *D. hrbaceki*, lateral aspect of free ephippium (K); arrow indicates position of maximal width of the ephippium. C. *D. hrbaceki*, adult female (RB); detail of necktooth (indicated by arrow). D. *D. minnehaha*, adult female (ELA) with neckteeth (indicated by arrow). E. *D. minnehaha*, adult female (ELA); detail of neckteeth (indicated by arrow). F. *D. minnehaha*, juvenile female (ELA); dorsal aspect, detail of neckteeth (indicated by arrow). G. *Daphnia* sp. (morphotype FLO9) (FL); detail of neckteeth (indicated by arrow).

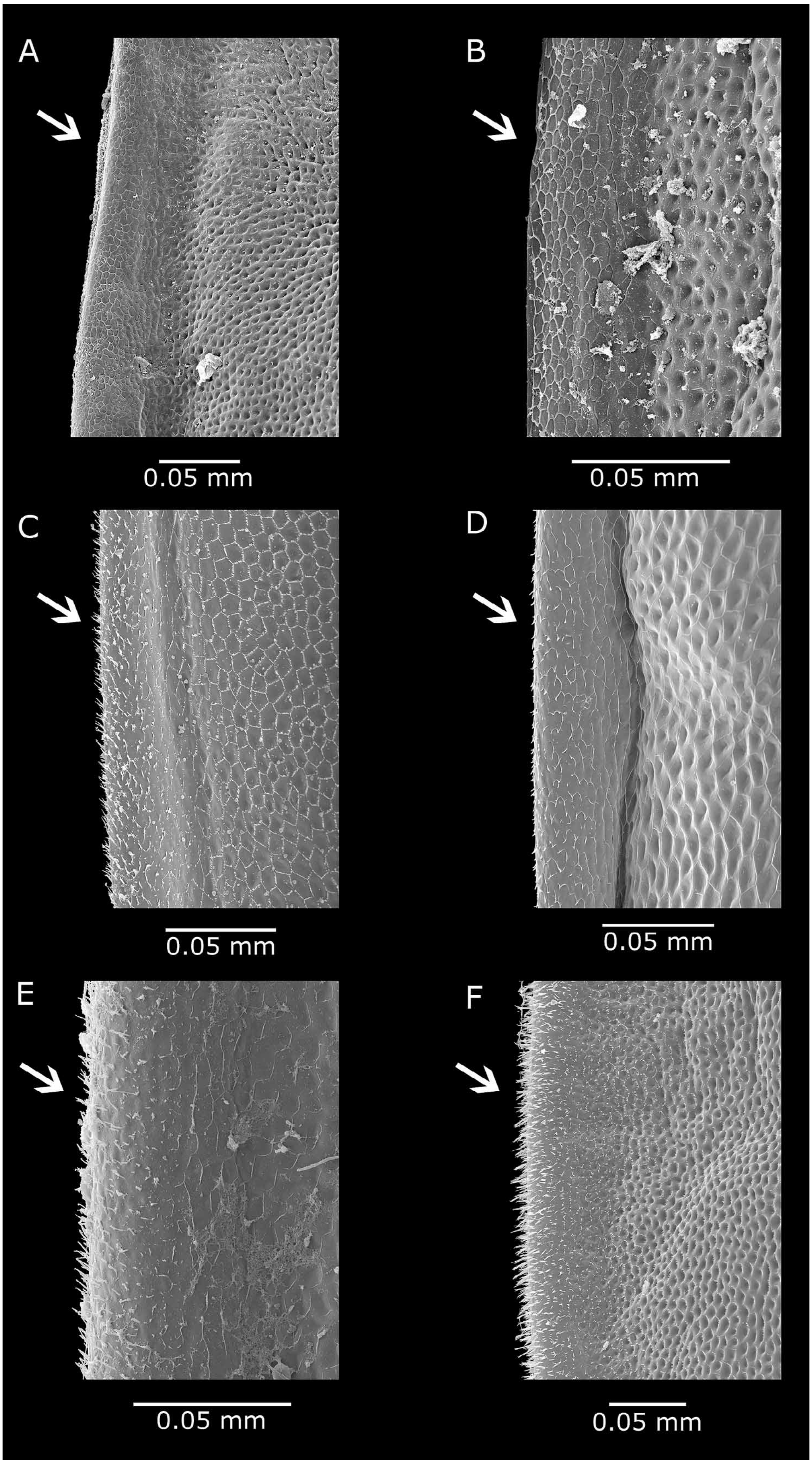

FIGURE 7. Comparison of ultrastructures of ephippial dorsal ridges with various development of spinulation or reticulation (indicated by arrows). A, B. *Daphnia hrbaceki* (K). C. *D. curvirostris* (H). D. *D. tanakai* (LM). E. *Daphnia* sp. (morphotype FLO9) (FL). F. *D. minnehaha* (ELA).

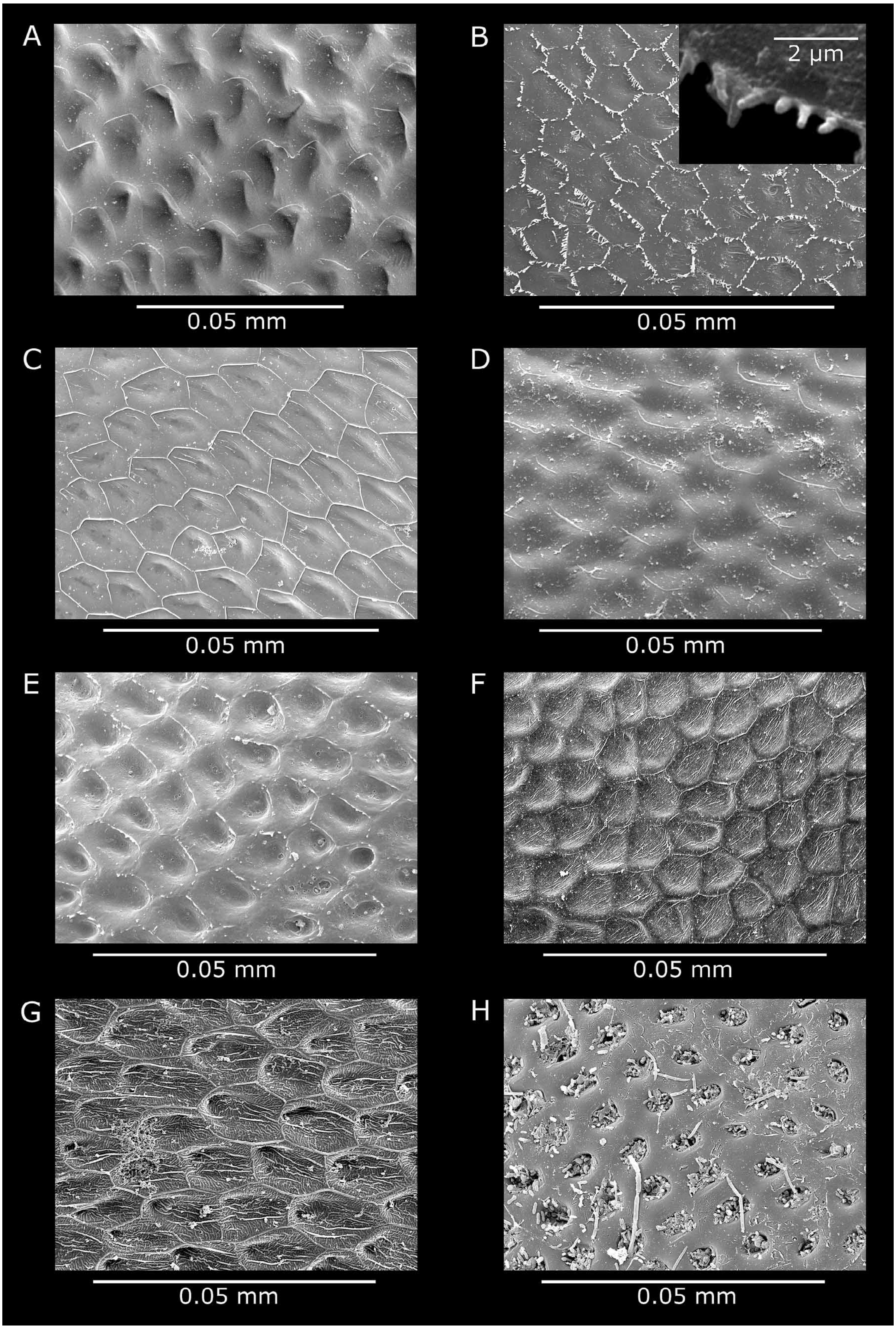

FIGURE 8. Comparison of ephippial surface ultrastructures. A. *Daphnia hrbaceki* (K). B. *D. curvirostris* (LL); detail shown in inset. C. *D. tanakai* (LM). D. *Daphnia* sp. (morphotype FLO9) (FL). E. *D. minnehaha* (ELA). F. *D. pulex* (CL). G. *D. longispina* from high-altitude temporary pool (GSB). H. *D. longispina* from lowland fishpond (I).

Ephippial female. Dorso-posterior part of head shield swollen, forming bulge over dorsal suture between carapace and head shield (Figs. 1C, 2G). Ephippial surface covered with sclerotized pneumatic cells reaching up to postero-dorsal angle of shell without any gap. Two resting eggs perpendicular to dorsal margin, egg chambers well separated from each other. Free post-molting ephippium (Fig. 6A, B) asymmetrically saddle-shaped, with maximal width between centre and proximal third of its length. Dorsal ridge without any spinescence, only reticulated (Fig. 7A, B). Postero-dorsal corner includes part of vaulted posterior margin and remnants of short shell spine, which is lost in older, freely floating ephippia. Surface ultrastructure with many minute pits surrounded by fine lamellae (Fig. 8A).

Male. *Head*: rounded in frontal part around optic vesicle, apical contour only feebly convex, gradually descending dorsally to level of attachment of posterior antennal muscle or to necktooth (if present) (Figs. 1A, 2A, 4A). Compound eye large, filling half of frontal portion of head shield, ocellus pigmented. Obtuse rostrum short, covering only antennular socket. Antennular (ventral) part of head extends ventrally forming posterior wall of antennular sockets (Fig. 4A, B).

Antennule: in adult males directed towards compound eye, its segment short, two to three times longer than wide, reaching hardly to pigmented part of compound eye. Flagellum inserted on conical butt elevated over shallow socket for sensory papillae. Dorsal seta inserted distally at about four fifths of antennular length (Figs. 4B, 5C).

Antenna: surface sculpture of all segments weaker than in female.

Carapace: ventral aspect: wide anterior gap between valves fringed with row of long, sub-marginal feathered setae. Setae most densely spaced along anterior fold of valves, gradually shortened to mid carapace margin (Fig. 5A). No gap or sub-marginal setae at distal part of ventral margin, only small marginal spines and groups of sub-marginal setules present. Dorsal margin feebly convex.

Thoracic limbs conform with the description of *Daphnia curvirostris* male in Ishida *et al.* (2006). Hook-like seta of 2nd limb is shown in Fig. 1F.

Postabdomen: all abdominal processes reduced, proximal one very small, others mostly missing. Pre-anal part with shallow depression, anal margin convex, fringed with up to 12 lateral spines (Fig. 1D). Gonopores open ventrally of last three largest marginal spines (Fig. 1G). Distal part of postabdominal setae slightly shorter than their proximal part. Middle pecten on terminal claw with either 6–7 spines or 10–12 spinules (Fig. 3E).

Differential diagnosis. The new species has to be differentiated from several other taxa present in the region of its occurrence: *Daphnia curvirostris*, members of the *Daphnia pulex* group, and *Daphnia longispina* (O. F. Müller, 1776), as well as related taxa in Asia and two taxa showing some similarities in North America. The main differential characters are listed in Tab. 2. Among locally occurring species, females in the *D. pulex* group are clearly distinguished by well developed antennules protruding from the antennular mound which contrast with the reduced, non-protruding antennule of *Daphnia hrbaceki*. *Daphnia longispina* has a flat, reduced inter-antennular mound, but parthenogenetic females in some of the populations are difficult to distinguish from those of *D. hrbaceki* that do not have enlarged middle pecten of the postabdominal claw. The *Daphnia longispina* ephippium is also widest in the anterior third of its length, its dorsal ridge covered with spinules and a shell spine always part of the free ephippium compared with the smooth dorsal ridge of the *Daphnia hrbaceki* ephippium whose greatest width is about mid-length. The apical stiff seta on the male second endopodite of *Daphnia longispina* is S-shaped, armed with a row of robust teeth or thorns; that of *D. hrbaceki* is hook-like (Fig. 1F), its distal part fringed on both margins with dense rows of spinules. The *Daphnia curvirostris* ephippium is asymmetrically saddle-shaped and widest at the proximal third of its length, with the dorsal ridge covered densely with minute spinules (Fig. 8B). Ephippial surface covered with small pits framed with rows of small, blunt spinules (Fig. 8B). *D. curvirostris* males have a longer basal segment of the antennule reaching nearly to the anterior margin of the pigmented part of the compound eye (Fig. 5D), whereas this reaches only the posterior contour of the eye in *D. hrbaceki* (Fig. 5C).

Two other related species have been described from eastern Asia (Japan and the Russian Far East): *Daphnia tanakai* and *Daphnia sinevi*. The ephippium of *Daphnia tanakai* does not include the carapace posterior margin; the postero-dorsal corner of the ephippium is obtusely rounded. The ephippial dorsal ridge is covered with sparsely distributed fine spinules (Fig. 7D). A wide gap is present between ephippial surfaces (covered with large sclerotized cells) and the posterior margin of carapace, separated by ecdysial suture. Ephippial surfaces are covered with shallow dimples and a pattern of hexagonal fine lamellae (Fig. 8C). Males have a reduced rostrum and a long antennule. *Daphnia sinevi* has a robust inter-antennular mound with slightly protruding tips of antennules. The

TABLE 2. Main differential morphological characters among *Daphnia hrbaceki*, *Daphnia curvirostris*, *Daphnia tanakai*, *Daphnia sinevi*, *Daphnia longispina* (populations from small, fishless habitats), *Daphnia minnehaha* and *Daphnia* sp. (morphotype FLO9, denoted as *D. arenata* in some studies).

Character	*D. hrbaceki* **sp. nov**.	*D. curvirostris*	*D. tanakai*	*D. sinevi*	*D. longispina*	*D. pulex*	*D. minnehaha*	morphotype FLO9
Female								
head								
documented ability to produce neckteeth (Fig. 6)	yes	yes (rarely)	no	yes	yes	yes	yes	yes
antennule body (Fig. 4)	reduced	reduced	reduced	reduced	reduced	prominent	slightly prominent	slightly prominent
carapace								
sub-marginal row of long setae	absent	absent	absent	absent	absent	absent	absent	present
thoracic limbs								
2nd gnathobase-posterior margin (Fig. 5)	extended, keel-like	rounded	rounded	rounded	rounded	rounded	rounded	rounded
postabdomen - terminal claw								
middle pecten (Fig. 3)	large teeth or fine spinules	large teeth	large teeth of fine spinules	large teeth	fine setules	large teeth	large teeth	large teeth
ephippium								
postero-dorsal portion of carapace incorporated in ephippium	yes	yes	not	yes	yes	yes	yes	yes
dorsal spinescence (Fig. 7)	absent	present	present	present	present	present	present	present
surface ultrastructure (Fig. 8)	minute pits surrounded by fine lamellae	minute pits surrounded by rows of blunt spinules	shallow dimples with remnants of longitudinal striation	not studied	variable	dimples surrounded with well developed lamellae	shallow pits surrounded by toothed lamellae	shallow dimples with remnants of surrounding lamellae
Male								
2nd limb, terminal seta on last endite	bent	bent	bent, but variable	strongly bent	not bent	bent	bent	robust. slightly bent
distal portion	setulated	setulated	setulated	setulated	with strong teeth	setulated	setulated	setulated
postabdomen								
dorsal margin, anal region	even	even	slightly convex	slightly convex	even	shallow depression	deep depression	deep depression
1st postabdominal processes	reduced	reduced	reduced	reduced	reduced	long, reaching over insertion of postabdominal setae	short, reaching up to insertion of postabdominal setae	short, reaching up to insertion of postabdominal setae

ephippium is saddle-shaped, widest at the posterior third of its length, its dorsal ridge with fine spinules. The postero-dorsal corner of the ephippium is horn-shaped, not rounded. Male has long, slender antennule.

The body shape of some individuals of *D. hrbaceki* may superficially resemble *Daphnia minnehaha* (Fig. 6E) and *Daphnia* sp. (morphotype FLO9, denoted as *D. arenata* in some studies) occurring on the North American continent. Both species have antennular tips partly protruding from the base of the head shield, and individuals of the FLO9 morphotype carry a row of sub-marginal plumose setae similar to those in the *D. obtusa* complex or in the subgenus *Ctenodaphnia* (this characteristic of the American taxon was omitted in Hebert 1995); such a row of plumose setae is not observed in *Daphnia hrbaceki* and *D. curvirostris*. Dorsal ridges of ephippia of both American species are covered with spinules (Fig. 7E, F) in contrast to the smooth reticulated dorsal ridge of *D. hrbaceki*. Males of American species have a deep depression in the pre-anal part of the postabdomen contrasting with the even or slightly convex anal region in *D. hrbaceki*.

Other material examined. *Daphnia hrbaceki*: Czech Republic, Kokořínsko, small pool (N 50°29'11", E 14°41'24"), 13 July 2006, P. J. Juračka legit. *Daphnia* cf. *hrbaceki*: Slovakia, Rimavská Baňa, (48.5° N, 19.9° E) fluvial pool, 27 April 1951, O. Albertová legit. The first author sampled most pools in the vicinity of Rimavská Baňa village recently (three times in 2006–7) but without success. In the original sample from the mid 20th century, no other *Daphnia* species was present.

Distribution. So far, *Daphnia hrbaceki* has only been found in two isolated pools in Central Bohemia and at another locality in south-eastern Slovakia (for the Slovak sample, no DNA data is available). Apart from the type locality, the species was found in a similar pool created in 1999, located about 500 m away. Cladoceran fauna of the region where *D. hrbaceki* was discovered had been studied for at least one century. The species is thus certainly very rare and it is difficult to judge the area of its distribution. However, other populations may have escaped detection (being confused with *D. curvirostris* or other species) if individuals did not exhibit the characteristic hump-shaped body profile.

Ecology. The species was sampled in the summer zooplankton and survived up to the beginning of winter. It was outcompeted in spring by co-occurring *Daphnia obtusa*. Both species coexisted in summer. Summer water conditions: conductivity fluctuated within the range 39–768 $\mu S.cm^{-1}$; pH 5.7–7.8; temperature up to 17.4 °C; dissolved oxygen 1.8–10.4 $mg.l^{-1}$. The species was successfully cultivated in the laboratory on a diet of green algae (mostly *Scenedesmus*).

Genetic analyses. All analysed mitochondrial genes of the analysed Czech *Daphnia* clearly showed a considerable divergence from all other so-far genetically characterised species in the genus: the genetically most similar species, *Daphnia curvirostris*, diverged by 13% at 12S, 23% at COI, and 41% at ND2 (all Kimura 2-parameter distances); other analyzed species, including all other known members of the *D. curvirostris* complex, diverged substantially more (over 46.8% at ND2; Fig. 9). The divergence of the syntopically occurring *D. obtusa* (belonging to the *D. pulex* group) from *D. hrbaceki* exceeded 63% at ND2. No variation in sequences of any of the three mitochondrial genes was observed in several analysed individuals of *D. hrbaceki*.

The GTR+I+G model of nucleotide substitution consistently performed best among the different approaches to model selection, based on the 932 bp long alignment of ND2 sequences. All applied methods of phylogenetic reconstructions supported the sister relationship between the new species and *D. curvirostris* despite their relatively high divergence. The support for monophyly of the *D. curvirostris* complex was weaker but the whole complex was unambiguously assigned as a sister taxon of the *D. longispina* complex (Fig. 9).

Taxonomic and nomenclatural comments. *Daphnia hrbaceki* could be characterized both morphologically and genetically. Its morphological peculiarities have been known for more than fifty years, but difficult to evaluate as there was only a single sample from Slovakia available. The recent discovery of populations in Central Bohemia allowed DNA analyses and a comparison of both morphology and genetics with recently described East Asian taxa. The morphological diagnosis of the species and its membership within the *D. curvirostris* complex were thus substantiated.

Comparison with other taxa described over century ago from Japan (*Daphnia whitmani* Ishikawa, 1895 and *Daphnia morsei* Ishikawa, 1895) is difficult as the original drawings are inadequate and the descriptions do not mention some important characters. For instance, the ephippium of *D. whitmani* is traced as not reaching to the posterior margin of carapace in Fig. 4 in Ishikawa (1895), but clearly incorporating it in Fig. 4b in the same work. In general, *D. whitmani* seems to be similar to the recently described *Daphnia sinevi*. The male of *D. morsei* has a remarkably deep depression of the pre-anal or anal part of the postabdomen. A genetically clearly divergent

Daphnia population found recently in Japan may have belonged to this taxon (Kotov *et al.* 2006). Recent genetic analysis (Kotov & Taylor 2010) nevertheless suggested that the above-mentioned taxa described by Ishikawa likely belong to the *D. pulex* group and are therefore unrelated to the *D. curvirostris* complex.

Both American species mentioned in the differential diagnosis are in great need of re-description. Hebert (1995) documented some of their morphology on his CD-ROM on North American *Daphnia* fauna. While *Daphnia minnehaha* was described by Herrick (1884) according to the rules applied in the time of publication and the use of this name is not in doubt, the description of *Daphnia arenata* is lacking some of the attributes required by the International Code of Zoological Nomenclature. No types were designated, the description contained neither a short diagnosis nor differential diagnosis, and the text of the description itself was substituted by a set of microphotographs illustrating selected morphological characters. Coastal pond #9 at Florence (Oregon) was designated the type locality. The name *Daphnia arenata* has already been used in other regular publications (e.g., Colbourne *et al.* 1997; Benzie 2005; Mergeay *et al.* 2008). This situation clearly suggests that the name has to be considered a *nomen nudum*. The problem with the nomenclature of several North American taxa first named in Hebert (1995) is discussed in details in Benzie (2005). Therefore we prefer to label our comparative material as *Daphnia* sp. (morphotype FLO9).

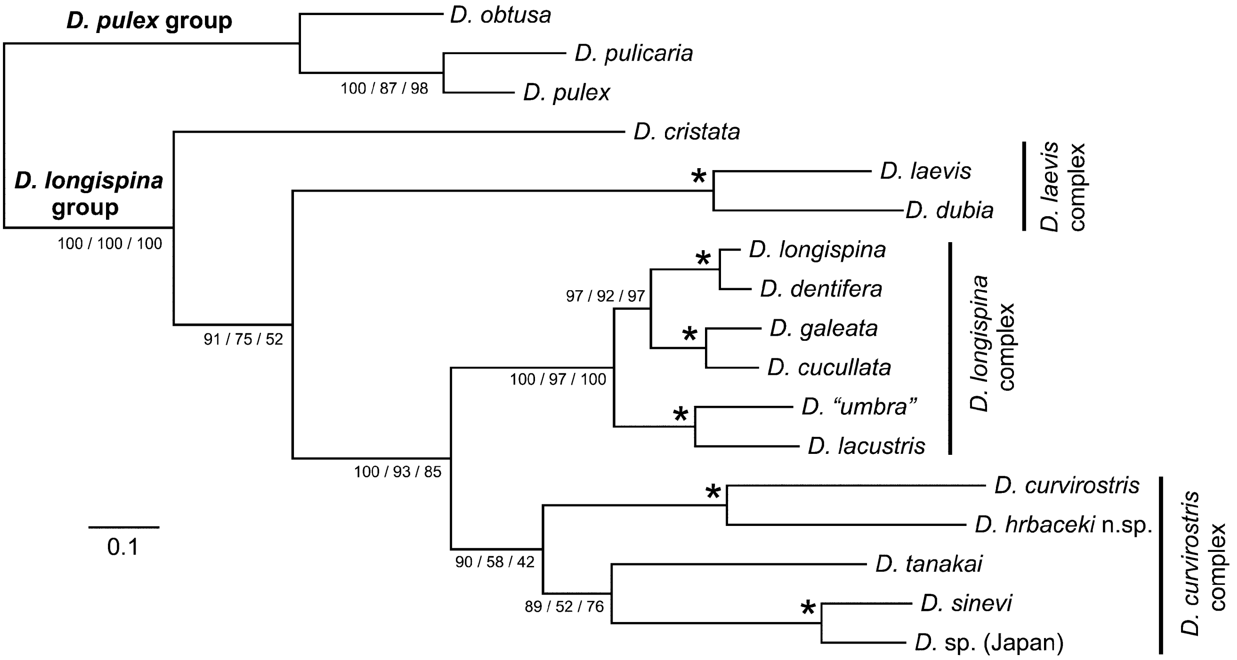

FIGURE 9. Relationship among species of the *Daphnia curvirostris* complex and its position relative to other species complexes (represented by selected taxa) of the *D. longispina* group (nomenclature of the *D. longispina* complex follows Petrusek *et al.* 2008). Three members of the *D. pulex* group, including *D. obtusa* coexisting with *D. hrbaceki*, were used as outgroups. The tree was constructed by the Bayesian inference of phylogeny from a partial sequence of the mitochondrial ND2 gene. Node support is provided for Bayesian inference, Maximum Likelihood and Maximum Parsimony analyses, asterisks indicate sister species with support at least 99% in all three analyses. Vertical bars delineate species complexes, scale indicates 10% divergence.

Discussion

Daphnia hrbaceki is the closest relative of *D. curvirostris* identified to date, although the level of genetic divergence between these two species is substantial. The new species shares several characteristics with other recently described species of the *D. curvirostris* complex. It is the second *Daphnia* species of the *curvirostris* complex after *D. tanakai* that shows substantial variation in the size of the middle postabdominal pecten even within the same population. This confirms that this character may not be as stable as previously thought, and populations differing solely in such a feature should be carefully compared by other means (see also Ishida *et al.* 2006 and Kotov *et al.* 2006 for discussion).

Our study has some implications for the use of certain morphological characters in *Daphnia* taxonomy. In particular, it demonstrates the usefulness of structures on the ephippial surface; the ephippial ultrastructure is a character reliably differentiating *D. hrbaceki* from morphologically similar *D. curvirostris*. On the other hand, we could not support the use of the differential character for the species in the *D. curvirostris* complex introduced in Kotov *et al.* (2006): the lateral bilobate aspect of the rostral part of the head. The detailed analysis of our SEM pictures (Fig. 4C–F) shows that ventral (antennular) part of the head is separated by a more or less noticeable suture present in all *Daphnia* species. The more or less swollen or vaulted tip of the antennular plate is variably expressed in living individuals and may be influenced with formalin or ethanol preservatives.

Known populations of *D. hrbaceki* are characterised by the presence of antipredator morphological structures. Juveniles commonly formed neckteeth (Fig. 2C, D), previously documented within the *D. curvirostris* complex only in the recently described *D. sinevi* (Kotov *et al.* 2006). However, neckteeth seem to be occasionally observed in *D. curvirostris* as well. Careful inspection of the comparative material originating from Czech pools with *Chaoborus* larvae revealed that a small necktooth in the first and occasionally in the second juvenile instars is commonly present but missing in older instars and adults. Interestingly, it might be retained also in adult males, as seen in some specimens collected in Central Bohemia (D. Vondrák, unpubl. data). The presence of this morphological feature in the *D. curvirostris* complex therefore deserves further attention. Small fishless pools, the habitat of the above-mentioned species as well as of *D. hrbaceki*, are often characterised by strong invertebrate predation (Arnott & Vanni 1993). Larvae of *Chaoborus* phantom-midges, which are commonly observed in the type locality of *D. hrbaceki*, are among the most important predatory invertebrates in such habitats (e.g., Kvam & Kleiven 1995; Young & Riessen 2005). Neckteeth, formed especially in juvenile individuals of various *Daphnia* species (Colbourne *et al.* 1997; Kotov *et al.* 2006), have long been known to efficiently increase resistance to this predator (Havel & Dodson 1984; Repka *et al.* 1995). Additionally, Laforsch *et al.* (2004) recently showed that the defensive mechanism accompanying neckteeth formation is much more complex, and involves substantial strengthening of the whole carapace.

D. hrbaceki is able to retain the neckteeth after achieving maturity (Figs. 1D, E; 2A, B, E), a feature rarely observed in other *Daphnia* species. Such *D. hrbaceki* adults usually exhibit morphotypes with a hump-shaped dorsal body outline, a prominent feature that first suggested that the studied population is unique. Among other congeneric species, the North American *D. minnehaha* (which also tends to form hump-shaped morphs in the presence of predators) may retain neckteeth after maturity, usually in conditions of low food concentration and high *Chaoborus* predation pressure. With a better food supply, adults of this species may tend to lose neckteeth (Riessen & Young 2005). *Daphnia hrbaceki* seems to show a similar reaction to food conditions and predator density, as suggested by changes of the prevailing morphotypes in the type locality over time. A year after the habitat was created, under high transparency (Secchi depth over 1 m) and apparently low food densities, hump-shaped adults with neckteeth were frequent in the population (around 80% of all adult individuals). Two to three years later, the nutrient content of the pool seems to have increased: transparencies dropped to 20 cm, chlorophyll-a concentration reached 50 $\mu g.l^{-1}$ in summer, and the pool surface started to be overgrown by macrophytes. Correspondingly, hump-shaped *Daphnia* forms were very rare in the population, and adults with neckteeth were not observed in three consecutive seasons (2007–9). As adults of *D. hrbaceki* not showing antipredator defence structures are hardly distinguishable from *D. curvirostris*, it is not surprising that this species would have escaped attention even if it was common in the Central European landscape.

Apparent morphological similarity is the most common reason why cryptic species are overlooked in nature (Pfenninger & Schwenk 2007). It is therefore possible that *D. hrbaceki* lives also in other regions but has not been recorded in the recent decades. However, genetic analyses of different European populations of *D. curvirostris* suggest that cryptic species within this complex are rare. Černý and Hebert (1999) screened 17 Czech and Slovak populations using allozyme analysis. All analysed populations belonged apparently to a single species despite substantial intraspecific variation. Similar results were obtained by Michels *et al.* (2003) from an analysis of ten Belgian populations; in that case, allozyme analysis was verified by sequencing of a mitochondrial gene. Screening of COI variation of selected *D. curvirostris* individuals from various habitats across the Western Palaearctic, from Spain to Israel, also did not reveal any cryptic lineage (A. Petrusek, unpublished data).

D. hrbaceki therefore seems to be relatively rare species in Europe. Possibly, its centre of distribution is not in Central Europe from which we describe it but elsewhere, and it was introduced to the region from some distant source. Several non-indigenous cladoceran species, including *Daphnia*, have widely dispersed across continents thanks to human activities (e.g., Havel & Medley 2006; Mergeay *et al.* 2006), and at least one *Daphnia* species, *D. ambigua* Scourfield, 1947, was actually described from its invaded range. First recognized as a distinct species in

Europe, it is a North American invader spreading only in the recent decades (Dumont 1974; Žofková *et al.* 2002). An unusual genetic lineage with *D. similis*-like morphology but genetically clearly divergent, discovered in a temporary pool in Munich, Germany (Petrusek 2003; Adamowicz *et al.* 2009), might also be a case of a long-range introduction within the Palaearctic region.

The failure to recognize *D. hrbaceki* earlier, despite its potential to form conspicuous morphotypes, may also have an ecological explanation. This species seems to be a relatively weak competitor, at least in comparison with *D. obtusa* inhabiting similar habitats in the landscape surrounding the type locality. The latter species coexists with *D. hrbaceki* in both its presently known Czech localities, and outcompetes it in the spring and early summer. The type locality, artificially re-created at a site which used to be a wetland with a tiny ephemeral pool, offered an opportunity for colonization by a species that might not be successful in later stages of succession. It is not unlikely that *D. hrbaceki* will be completely replaced by *D. obtusa* in the future. A similar case was documented in Belgium, where a population of the *Daphnia atkinsoni* complex, previously not recorded in that country, colonized a newly created pool. Originally reaching high densities, it was largely replaced by *D. magna* which appeared in the pool later (Louette & De Meester 2004). It is possible that *D. hrbaceki* is favoured in young habitats in the beginning of the zooplankton assemblage process, especially in the studied region where zooplankton is apparently not dispersal-limited (P. Juračka, unpublished data). However, we cannot rule out that this species used to live at the site in the past, and the present population was founded from the resting egg bank.

Daphnia hrbaceki was discovered in newly created pools, which were dug in the Kokořínsko landscape protected area for conservation purposes of rare species of aquatic macrophytes, molluscs and amphibians. Conservation of those well-known vulnerable flagship taxa may have large impact on other organisms as well (Walpole & Leader-Williams 2002). Newly created pools have an important role as refuges from predators found in permanent waters, particularly fish (Wellborn *et al.* 1996), as biocorridors and habitats for a wide range of aquatic taxa (Santamaría 2002), and may offer opportunities for species that are usually outcompeted by other dominant species later during succession (Zedler 2003). Our discoveries of a new *Daphnia* species in Central European pools and other cryptic lineages of the genus found in such habitats in the Western Palaearctic (e.g., Adamowicz *et al.* 2009; Petrusek *et al.* 2009) stress the importance of small and temporary waters for preserving aquatic biodiversity.

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References

Adamowicz, S.J., Petrusek, A., Colbourne, J.K., Hebert, P.D.N. & Witt, J.D.S. (2009) The scale of divergence: a phylogenetic appraisal of intercontinental allopatric speciation in a passively dispersed freshwater zooplankton genus. *Molecular Phylogenetics and Evolution*, 50, 423–436.

Arnott, S.E. & Vanni, M.J. (1993) Zooplankton assemblages in fishless bog lakes: Influence of biotic and abiotic factors. *Ecology*, 74, 2361–2380.

Benzie, J.A.H. (2005) *The genus* Daphnia *(including* Daphniopsis*) (Anomopoda: Daphniidae)*. In Dumont, H. J. F. (ed.), *Guides to the Identification of the Microinvertebrates of the Continental Waters of the World*, 21. Kenobi Productions, Ghent & Backhuys Publishers, Leiden, 376 pp.

Colbourne, J.K., Hebert, P.D.N. & Taylor, A.D. (1997) Evolutionary origins of phenotypic plasticity in *Daphnia*. *In:*

Givnish, T.J. & Sytsma, K.J. (Eds.) *Molecular Evolution and Adaptive Radiation*. Cambridge University Press, pp. 163–188.

Colbourne, J.K., Wilson, C.C. & Hebert, P.D.N. (2006) The systematics of Australian *Daphnia* and *Daphniopsis* (Crustacea: Cladocera): a shared phylogenetic history transformed by habitat-specific rates of evolution. *Biological Journal of the Linnean Society*, 89, 469–488.

Černý, M. & Hebert, P.D.N. (1999) Intercontinental allozyme differentiation among four holarctic *Daphnia* species. *Limnology and Oceanography*, 44, 1381–1387.

Dumont, H.J. (1974) *Daphnia ambigua* Scourfield, 1947 (Cladocera: Daphniidae) on the European continent. *Biologisch Jaarboek - Dodonaea*, 42, 112–116.

Eylmann, E. (1887) Beitrag zur Systematik der europäischen Daphniden. *Berichte der Naturforschenden Gesellschaft zu Freiburg I. B.*, 2, 61–148.

Forró, L., Korovchinski, N.M., Kotov, A.A. & Petrusek, A. (2008) Global diversity of cladocerans (Cladocera; Crustacea) in freshwater. *Hydrobiologia*, 595, 177–184.

Glagolev, S.M. (1995) Genus *Daphnia*. *In:* Alexeev, V.R. (Ed.) *Key to freshwater invertebrates of Russia and adjacent lands, 2. Crustaceans*. Zoological Institute, Russian Academy of Sciences, St. Petersburg, pp. 48–58.

Havel, J.E. & Dodson, S.I. (1984) *Chaoborus* predation on typical and spined morphs of *Daphnia pulex*: Behavioral observations. *Limnology and Oceanography*, 29, 487–494.

Havel, J.E. & Medley, K.A. (2006) Biological invasions across spatial scales: intercontinental, regional, and local dispersal of cladoceran zooplankton. *Biological Invasions*, 8, 459–473.

Hebert, P.D.N. (1995) The *Daphnia* of North America: an illustrated fauna. CD-ROM. University of Guelph, Guelph

Herrick, C.L. (1884) A final report on the Crustacea of Minnesota, included in the orders Cladocera and Copepoda, together with a synopsis of the described species in North America, and keys to the known species of the more important genera. 12th annual report. Geological and natural history survey of Minnesota: Johnson, Smith & Harrison, Minneapolis, 191 pp.

Ishida, S., Kotov, A.A. & Taylor, D.J. (2006) A new divergent lineage of *Daphnia* (Cladocera: Anomopoda) and its morphological and genetical differentiation from *Daphnia curvirostris* Eylmann, 1887. *Zoological Journal of the Linnean Society*, 146, 385–405.

Ishikawa, C. (1895) Phyllopod Crustacea of Japan. *Daphnia whitmani*. *Zoological Magazine of Tokyo*, 7, 147–154.

Kořínek, V. (1999*) A guide to limnetic species of Cladocera of African inland waters (Crustacea, Branchiopoda).* Occasional Publication 1, International Association of Theoretical and Applied Limnology, Geneva, 57 pp.

Kotov, A.A. & Taylor, D.J. (2010): A new African lineage of the *Daphnia obtusa* group (Cladocera: Daphniidae) disrupts continental vicariance patterns. *Journal of Plankton Research*, 32, 937–949.

Kotov, A.A., Ishida, S. & Taylor, D.J. (2006) A new species in the *Daphnia curvirostris* (Crustacea: Cladocera) complex from the eastern Palearctic with molecular phylogenetic evidence for the independent origin of neckteeth. *Journal of Plankton Research*, 28, 1067–1079.

Kvam, O.V. & Kleiven, O.T. (1995) Diel horizontal migration and swarm formation in *Daphnia* in response to *Chaoborus*. *Hydrobiologia*, 307, 177–184.

Laforsch, C., Ngwa, W., Grill, W. & Tollrian, R. (2004) An acoustic microscopy technique reveals hidden morphological defenses in *Daphnia*. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 15911–15914.

Laforsch, C. & Tollrian, R. (2000) A new preparation technique of daphnids for Scanning Electron Microscopy using hexamethyldisilazane. *Archiv für Hydrobiologie*, 149, 587–596.

Louette, G. & De Meester, L. (2004) Rapid colonization of a newly created habitat by cladocerans and the initial build-up of a *Daphnia*-dominated community. *Hydrobiologia*, 513, 245–249.

Mergeay, J., Verschuren, D. & De Meester, L. (2006) Invasion of an asexual American water flea clone throughout Africa and rapid displacement of a native sibling species. *Proceedings of the Royal Society B-Biological Sciences*, 273, 2839–2844.

Mergeay, J., Aguilera, X., Declerck, S., Petrusek, A., Huyse, T. & De Meester, L. (2008) The genetic legacy of polyploid Bolivian *Daphnia*: the tropical Andes as a source for the North and South American *D. pulicaria* complex. *Molecular Ecology*, 17, 1789–1800.

Michels, E., Audenaert, E., Ortells, R. & De Meester, L. (2003) Population genetic structure of three pond-inhabiting *Daphnia* species on a regional scale (Flanders, Belgium). *Freshwater Biology*, 48, 1825–1839.

Peters, R.H. & de Bernardi, R. (Eds.) (1987) *Daphnia*. *Memorie dell'Instituto Italiano di Idrobiologia*, 45, 1–502.

Petrusek, A. (2003) The populations of the *Daphnia similis* species complex in Germany after 110 years—a new case of species introduction? *Senckenbergiana biologica*, 82, 11–14.

Petrusek, A., Hobæk, A., Nilssen, J.P., Skage, M., Černý, M., Brede, N. & Schwenk, K. (2008) A taxonomic reappraisal of the European *Daphnia longispina* complex (Crustacea, Cladocera, Anomopoda). *Zoologica Scripta*, 37, 507–519.

Petrusek, A., Tollrian, R., Schwenk, K., Haas, A. & Laforsch, C. (2009) A ”crown of thorns” is an inducible defense that protects *Daphnia* against an ancient predator. *Proceedings of the National Academy of Sciences of the United States* *of America*, 106, 2248–2252.

Pfenninger, M. & Schwenk, K. (2007) Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. *BMC Evolutionary Biology*, 7, 121.

Posada, D. (2008) jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution*, 25, 1253–1256.

Repka, S., Walls, M. & Ketola, M. (1995) Neck spine protects *Daphnia pulex* from predation by *Chaoborus*, but individuals with longer tail spine are at a greater risk. *Journal of Plankton Research*, 17, 393–403.

Riessen, H.P. & Young, J.D. (2005) *Daphnia* defense strategies in fishless lakes and ponds: one size does not fit all. *Journal of Plankton Research*, 27, 531–544.

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.

Santamaría, L. (2002) Why are most aquatic plants widely distributed? Dispersal, clonal growth and small-scale heterogeneity in a stressful environment. *Acta Oecologica*, 23, 137–154.

Schwenk, K., Sand, A.B. & Brehm, M. (1998) Genetic markers, genealogies and biogeographic patterns in the cladocera. *Aquatic Ecology*, 32, 37–51.

Schwenk, K., Posada, D. & Hebert, P.D.N. (2000) Molecular systematics of European *Hyalodaphnia*: the role of contemporary hybridization in ancient species. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 267, 1833–1842.

Swofford, D.L. (2002) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)*. *Version 4*. [Computer software.] Sinauer Associates, Sunderland.

Tamura, K., Dudley, J.N.M. & Kumar, S. (2007) MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, 24, 1596–1599.

Thompson, J.D., Higgins, D.G. & Gibson, T.J. (1994) Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22, 4673–4680.

Walpole, M.J. & Leader-Williams, N. (2002) Tourism and flagship species in conservation. *Biodiversity and Conservation*, 11, 543–547.

Wellborn, G.A., Skelly, D.K. & Werner, E.E. (1996) Mechanisms creating community structure across a freshwater hydroperiod gradient. *Annual Review of Ecology and Systematics*, 27, 337–363.

Young, J.D. & Riessen, H.P. (2005) The interaction of *Chaoborus* size and vertical distribution determines predation effects on *Daphnia*. *Freshwater Biology*, 50, 993–1006.

Zedler, P.H. (2003) Vernal pools and the concept of 'isolated wetlands'. *Wetlands*, 23, 597–607.

Žofková, M., Kořínek, V. & Černý, M. (2002) Two recent immigrants into Czech aquatic habitats: *Daphnia ambigua* and *D. parvula* (Crustacea: Cladocera). *Acta Societatis zoologicae Bohemicae*, 66, 221–230.