

A new African lineage of the *Daphnia obtusa* group (Cladocera: Daphniidae) disrupts continental vicariance patterns

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The *Daphnia* (*Daphnia*) *obtusa* group (Cladocera: Daphniidae) is commonly studied, but there is little known of the affinities of African or Asian representatives. We found a new endemic *obtusa*-like taxon of *Daphnia* to be common in lakes, pools and puddles of the Bale Mountains of Ethiopia (3857–4100 m.a.s.l.). We provide a phylogenetic analysis using DNA sequences from two mitochondrial genes and a detailed morphological description of this species as *Daphnia izpodvala* sp. nov. Among the characters that separate *D. izpodvala* sp. nov. from other members of this group are a large ocular dome, bordered by a distinct pre-ocular and a deep post-ocular depression. The closest relative according to our genetic evidence is North American *Daphnia pileata*. However, several *obtusa*-like species remain unexamined. We also found two new lineages in Japan that appear to have no known close relatives. These Japanese species are likely to have been described in the nineteenth century and later falsely synonymized with *D. obtusa* or other species from Europe. Our results bolster the accumulating evidence for cladoceran regionalism but indicate that strict continental vicariance may not explain the biogeography of the *D. obtusa* complex.

KEYWORDS: Cladocera; systematics; new species; COI; 12S; zoogeography

INTRODUCTION

Standing freshwaters remain among the most threatened and sensitive ecosystems to global change. However, there are still many invertebrate groups of freshwater ecosystems that have a poorly understood biodiversity. Current estimates for the diversity of the Cladocera (Crustacea: Branchiopoda), for example, are probably too low by 2- to 4-fold (Adamowicz and Purvis, 2005; Forró *et al.*, 2008). A major contributor to the species problem in Cladocera has been false cosmopolitanism (Frey, 1987; Forró *et al.*, 2008). Often species have been assigned to European taxa without detailed

morphological analysis. Transcontinental introductions have further compounded the issue, and in some cases, it is difficult to determine whether specimens are introduced or native (Taylor and Hebert, 1993; Mergeay *et al.*, 2005a).

Africa is among the most poorly studied regions, with even the best-known cladoceran genus, *Daphnia* O. F. Müller, 1785 (Anomopoda: Daphniidae), requiring extensive revision. Most of the detailed descriptions of *Daphnia* from this continent were made many years ago and limited to South Africa (Sars, 1895, 1916; Wagler, 1936). For East Africa, only a few inadequate

descriptions exist (Weltner, 1898; von Daday, 1910b; Brehm, 1912). Jenkin (Jenkin, 1934) made adequate descriptions and realistic drawings of several Kenyan cladocerans, but recorded only one species of *Daphnia*, *D. barbata* Weltner, 1898. Four species of *Daphnia* were recorded in Sudan (Dumont and Verheye, 1984; Dumont *et al.*, 1984). Mergeay *et al.* (Mergeay *et al.*, 2005b) established the presence of 11 species of *Daphnia* in Kenya, with genetic confirmation. Only two species of *Daphnia* (*Ctenodaphnia*) are known from the Ethiopian Rift Valley lakes, *D. (C.) barbata* Weltner, 1898 and *D. (C.) magna* Straus, 1820 (Elizabeth *et al.*, 1992; Green, 1995). Dumont and Verheye (Dumont and Verheye, 1984) recorded *D. (C.) lumholtzi* Sars, 1885 for Ethiopian Nile Basin and Elizabeth *et al.* (1992) detected *D. (C.) magna* in Lake Hayq, but the species then disappeared. von Daday (von Daday, 1910a, b) reported *D. psittacea* Baird, 1850 from east Africa, but this species could have been confused with *D. magna*. Dejenie *et al.* (Dejenie *et al.*, 2008) reported four species of *Daphnia*, *D. (C.) barbata*, *D. (C.) carinata*, *D. (C.) magna* and *D. (D.) pulex*, from the highland reservoirs of Tigray, Northern Ethiopia. Their “*D. carinata*” is in fact a cryptic species of the *Daphnia similis* complex (J. Mergeay, personal communication). The only three species of the subgenus *Daphnia* (*Daphnia*) reported from Ethiopia are: *D. longispina* (O. F. Müller, 1776) (Brunelli and Cannici, 1940; Wodajo and Belay, 1984; Dumont, 1994), “American” *D. cf. pulex*, introduced in the mid-1920s (Mergeay *et al.*, 2005a, 2006), and *D. obtusa* (Löffler, 1978). The status of other *pulex*-like populations (Dejenie *et al.*, 2008) needs further verification.

Daphnia obtusa has been reported from every continent save Antarctica (Benzie, 2005). The species has apparently been recently introduced across continents into Australia (Benzie and Hodges, 1996). Schwartz *et al.* (Schwartz *et al.*, 1985) found that *D. obtusa*-like species were common in North America. Penton *et al.* (Penton *et al.*, 2004) demonstrated that two lineages were common in North America. Adamowicz *et al.* (Adamowicz *et al.*, 2009) reported that the “*D. obtusa*” complex is composed of 12 species that form monophyletic groups according to continental affiliations (the nine northern hemisphere species also form a monophyletic group). Still, there are no detailed morphological or genetic analyses of “*D. obtusa*” from Africa or east Asia. The species has been reported from high altitude lakes in West and South Africa (Löffler, 1968; Green and Kling, 1988; Green, 1995).

Interestingly, there is a paleolimnological record of “*Daphnia obtusa*” from Ethiopia extending back to the Late Pleistocene, suggesting that the species is not a recent invader (Löffler, 1978). Moreover, the species appears

highly pigmented, which is uncharacteristic of *D. obtusa* from Europe. Still, Europe and Africa are connected by bird flyways, which are potential dispersal agents of cladocerans, so the question of the affinities of the African *D. obtusa* remains open. *Daphnia obtusa*-like species have been reported from Japan as well (Ishikawa, 1895a, b, 1896). *Daphnia mitsukuri* Ishikawa, 1896 is clearly part of the *D. obtusa* complex, as it possesses setae on the ventral margin of its valves (Kotov *et al.*, 2006), whereas *Daphnia morsei* Ishikawa, 1895 and *Daphnia whitmani* Ishikawa, 1896 have been regarded as a junior synonyms of *D. obtusa* (Richard, 1896; Uéno, 1927), *D. pulex* (Tanaka, 1997) or *Daphnia curvirostris* (Richard, 1896).

Here, we investigate about 50 samples of *Daphnia* from Ethiopia and more than 100 samples from Japan and assess the phylogenetic affinities and taxonomy of “*D. obtusa*”-like specimens. We find that east African specimens are indeed different from *D. obtusa* and are likely an endemic whose closest affinity is to a New World member of the *D. obtusa* complex. Japanese species that resemble *D. whitmani* and *D. morsei* also appear as a unique species, not closely related to *D. obtusa*, *D. pulex* or *D. curvirostris*.

METHOD

Molecular phylogenetics

Specimens of *D. obtusa*-like animals were isolated from Ethiopian lakes, South American ponds and Japan. DNA extraction from single ethanol-preserved animals was achieved using the DNA Quickextract extraction kit from Epicentre. Fifty microlitres of PCRs contained 5 μ L of extracted DNA template, 10 \times PCR buffer [50 mM KCl, 1.5 mg MgCl₂, 10 mM Tris-HCl, pH 8.3, 0.01% (w/v) gelatin], 2 mM of each dNTP, 1 μ M of each primer and 1 U of *Taq* DNA polymerase. We used primers from Taylor *et al.* (Taylor *et al.*, 1996) for the mitochondrial 12S rRNA gene fragment and Folmer *et al.* (Folmer *et al.*, 1994) for the mitochondrial COI fragment. The PCR temperature profiles were: 40 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 2 min, and final extension at 72°C for 5 min. DNA was sequenced by Sanger methods at the University of Washington High Throughput Genomics Facility. Sequencher 4.8 (Gene Codes Corp.) was used to assemble, edit and verify open-reading frames.

Bioinformatics

Sequences related to *D. obtusa sensu lato* were obtained from GenBank using the same matching pairs for COI

and 12S rRNA genes as Adamowicz *et al.* (Adamowicz *et al.*, 2009) and aligned with the new sequences from the present study (Table I). As there were no indels, the alignment of COI was trivial. MAFFT (Katoh *et al.*, 2008) was used to align the 12S rRNA gene sequences with the Q-INS-i algorithm that considers secondary structure. A concatenated alignment of the COI and 12S rRNA gene sequences was made. Kimura's two-parameter distance was calculated in MEGA 4.0 (Tamura *et al.*, 2007). We carried out maximum likelihood (ML) analyses with RAxML 7.0.4 (Stamatakis *et al.*, 2008) using the GTR substitution matrix, a gamma parameter for among-site rate variation and an invariable sites parameter. For bootstrapping, RAxML estimated the number of required pseudoreplicates using the Pearson average of 100 random splits. A partition file was input for the COI sequences to obtain specific model parameters for each codon position. For

Bayesian analysis, we used Mr Bayes 3.1.2 (Ronquist and Huelsenbeck, 2003) and set the parameter to match the RAxML analysis (NST = 6; rates = INVGAMMA, with a protein-coding sites partitioned according to codon position and gene). Two independent and simultaneous Markov chain Monte Carlo analyses of 15 heated and 1 cold chains were run for 6 million generations with sampling from the chain every 100 generations. Default prior settings were used with the exception of the "ratepr" parameter, which was set to "variable" so that partitions could evolve at different rates. Analysis proceeded until proxies for chain convergence were achieved (average standard deviation of split frequencies <0.01). We discarded the initial 50% of trees as a "burn-in". A 50% majority-rule consensus tree with posterior probability (PP) values for each node was constructed from the remaining Bayesian trees. Branch support was the proportion of trees that

Table I: GenBank accessions, studies, species used and locations of current study specimens used for phylogenetic analyses

Species	COI gene accession no.	12S rRNA gene accession no.	References
<i>Daphnia "arenata"</i> Hebert, 1995	FJ427493	FJ427424	Colbourne and Hebert (1996)
<i>Daphnia "melanica"</i> (name introduced in Hebert, 1995 but without formal description)	FJ427495	FJ427428	Colbourne and Hebert (1996), Adamowicz <i>et al.</i> (2009)
<i>Daphnia "middendorffiana"</i> (not identical with <i>Daphnia middendorffiana</i> Fischer, 1851)	FJ427496	FJ427429	Colbourne and Hebert (1996), Adamowicz <i>et al.</i> (2009)
<i>Daphnia ambigua</i> SA	AF523692	FJ427423	Hebert <i>et al.</i> , (2003), Adamowicz <i>et al.</i> (2004)
<i>Daphnia ambigua</i> Scourfield, 1947	AF523687	AF523716	Hebert <i>et al.</i> (2003)
<i>Daphnia catawba</i> Coker, 1926	AY380454	FJ427425	Penton <i>et al.</i> (2004), Colbourne and Hebert (1996)
<i>Daphnia</i> cf. <i>parvula</i> sp2-SA	AY323126	FJ427442	Adamowicz <i>et al.</i> (2004)
<i>Daphnia cheraphila</i> Hebert and Finston, 1996	FJ427494	FJ427426	Adamowicz <i>et al.</i> (2009)
<i>Daphnia izpodvala</i> sp. nov.	GU595182– GU595189	GU595178– GU595181	Four different water bodies in Bale Mountains, see sections "Type locality" and "Other material studied"
<i>Daphnia</i> JPN sp. 1	GU595190– GU595191	GU595177	This study; pond near Hibara Park, Tokoname, Japan (34.857815N, 136.888565E); Coll. Seiji Ishida
<i>Daphnia</i> JPN sp. 2	GU595192– GU595193	GU595176	This study; pond near Hibara Park, Tokoname, Japan (34.857815N, 136.888565E); Coll. Seiji Ishida
<i>Daphnia minnehaha</i> Herrick, 1884	FJ427497	FJ427430	Adamowicz <i>et al.</i> (2009)
<i>Daphnia neo-obtusa</i> Hebert, 1995	AY380450	FJ427431	Penton <i>et al.</i> (2004); Colbourne and Hebert, (1996)
<i>Daphnia obtusa</i> s.s. Kurz, 1874 (CZ)	FJ427498	FJ427432	Adamowicz <i>et al.</i> (2009)
<i>Daphnia oregonensis</i> Korínek and Hebert, 1996	FJ427503	FJ427440	Adamowicz <i>et al.</i> (2009)
<i>Daphnia parvula</i> s.s. Fordyce, 1901	FJ427504	FJ427441	Colbourne and Hebert (1996), Adamowicz <i>et al.</i> (2009)
<i>Daphnia peruviana</i> Harding, 1955	AY323070	FJ427443	Adamowicz <i>et al.</i> (2004)
<i>Daphnia pileata</i> Hebert and Finston, 1996	AY380453	FJ427444	Penton <i>et al.</i> (2004), Adamowicz <i>et al.</i> (2009)
<i>Daphnia prolata</i> Hebert and Finston, 1996	AY380452	FJ427445	Penton <i>et al.</i> (2004), Adamowicz <i>et al.</i> (2009)
<i>Daphnia pulex</i> s.s. Leydig, 1860	EU152320	FJ427446	Mergeay <i>et al.</i> (2008)
<i>Daphnia pulicaria</i> s.s. Forbes, 1893	FJ427505	FJ427448	Colbourne and Hebert (1996)
<i>Daphnia tenebrosa</i> Sars, 1898	FJ427506	FJ427450	Colbourne and Hebert (1996)
<i>Daphnia villosa</i> Korínek and Hebert, 1996	FJ427507	FJ427451	Adamowicz <i>et al.</i> (2009)
<i>obtusa</i> group: unnamed species sp2-EUR	FJ427499	FJ427433	Adamowicz <i>et al.</i> (2009)
<i>obtusa</i> group: unnamed species sp3-EUR	FJ427500	FJ427434	Adamowicz <i>et al.</i> (2009)
<i>obtusa</i> group: unnamed species sp4-NA	FJ427501	FJ427435	Colbourne and Hebert (1996); Adamowicz <i>et al.</i> (2009)
<i>obtusa</i> group: unnamed species sp5-NA	FJ427502	FJ427436	Adamowicz <i>et al.</i> (2009)
<i>obtusa</i> group: unnamed species sp6-SA	AY323049	FJ427437	Adamowicz <i>et al.</i> (2004)
<i>obtusa</i> group: unnamed species sp7-SA	AY323059	FJ427438	Adamowicz <i>et al.</i> (2004)
<i>obtusa</i> group: unnamed species sp8-SA	AY323065	FJ427439	Adamowicz <i>et al.</i> (2004)

CZ, Czech Republic; EUR, Europe; JPN, Japan; NA, North America; SA, South America.

contained a clade, representing the PP of clade existence, given the data and the model of evolution. Trees were rooted with *Daphnia ambigua*, a species for which there is independent evidence of outgroup status to *pulex*-type species.

Morphology

For morphological study, animals were picked from the sample, placed on slides (in a drop of a glycerol–formaldehyde mixture) and studied under an optical microscope *in toto*. Then, five adult and five juvenile females were dissected for analysis of appendages. We applied a system of setae enumeration initially proposed for chydorids (Kotov, 2000) and recently applied to *Daphnia* (Ishida *et al.*, 2006; Kotov *et al.*, 2006).

RESULTS

Molecular phylogenetics

The phylogenetic analysis revealed that the Ethiopian specimens lacked close relatives among the known members of the *D. obtusa* complex (Fig. 1). The closest relative of the Ethiopian *Daphnia* was the North American *Daphnia pileata*, with an average K80 distance of 17.9% for COI. The grouping of *D. pileata* with *D. izpodvala* sp. nov. from Africa disrupted the monophyly of the North American and the Palearctic clades of *D. obtusa*-like species. We note that there was little bootstrap and PP support for much of the among group associations. The Japanese specimens that resemble *D. whitmani* and *D. morsei* were clearly in the

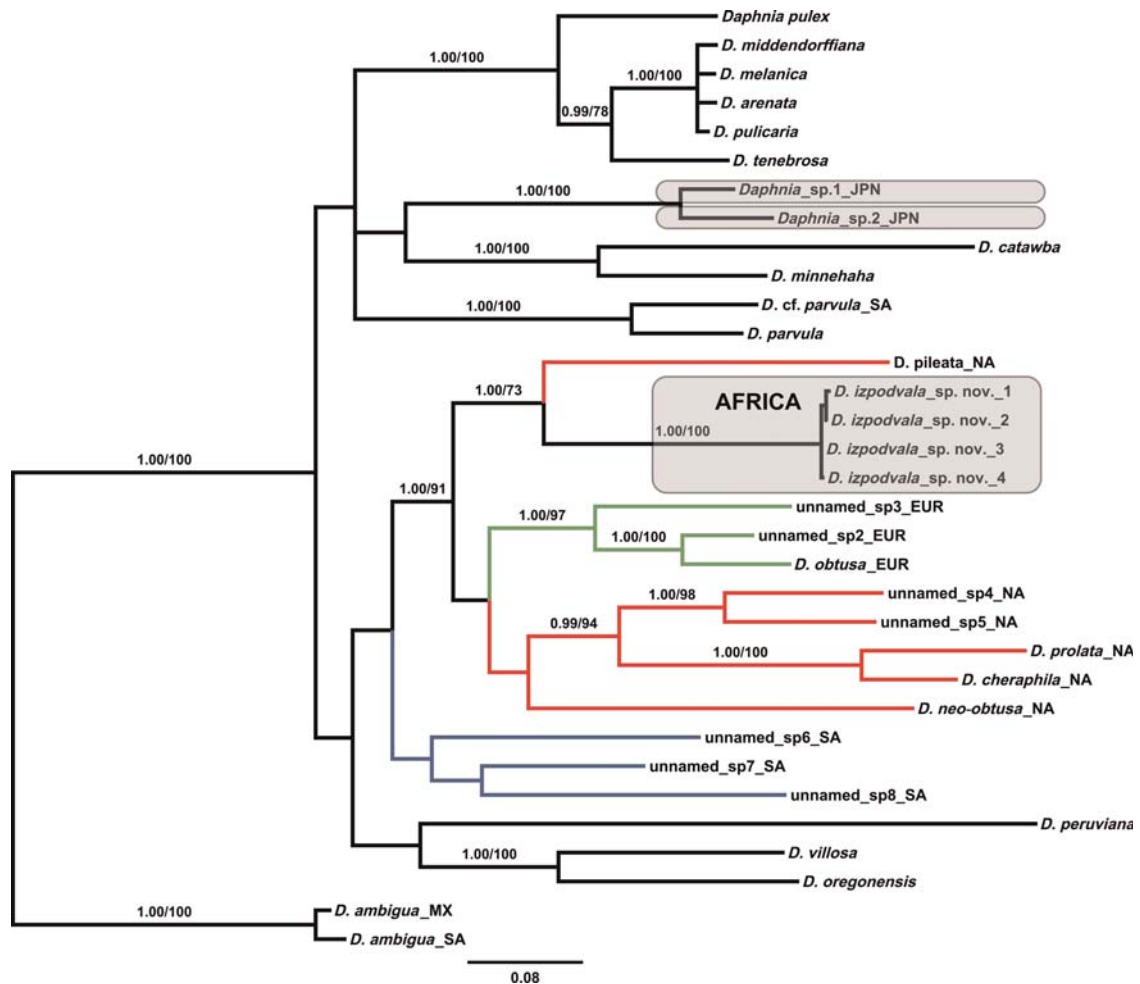


Fig. 1. Phylogeny of the *D. pulex* group *sensu lato* based on a concatenated alignment of DNA sequences from the COI and 12S rRNA genes. Support values are shown above branches with greater than 0.95 Bayesian posterior probability and 70% non-parametric bootstrap values. New lineages described in the present paper are shown in grey boxes. Note that some taxa contain multiple identical sequences. The sequence source information is provided in Table I. A colour version of this figure is available online. Colored branches represent continental affiliations of taxa in the *Daphnia obtusa* group: North America (red), South America (blue), and Europe (green).

pulex group *sensu lato*, but were more closely related to the *Daphnia catawba*/*Daphnia minnehaha* clade than to the *pulex* complex in the strict sense. The K80 distance of Japanese species to *D. pulex*, *D. obtusa* and *D. minnehaha* ranged from 22% to 25%. The distance between the Japanese species was 9.1%. The mean distance for COI of *D. izpodvala* sp. nov. (two specimens from each of four water bodies) was 0.4% (with a maximum value of 0.7%).

Taxonomy

Daphnia izpodvala sp. nov.

Daphnia obtusa Kurz in Löffler 1978: 1133.

Type locality. A small unnamed lake 1 (06°51.272'N, 39°53.574'E, 4070 m.a.s.l.) near Mountain of Konte, Sanetti tableland, Bale Mountains, Ethiopia.

Holotype. Adult parthenogenetic female in 90% alcohol, MGU MI 93. Label of the holotype: “*Daphnia izpodvala* sp. nov., adult parthenogenetic female from a small lake 1 (06°51.272'N; 39°53.574'E, *h* = 4070 m.a.s.l.) near Mountain of Konte, Bale Mountains, Ethiopia col. in 10.ii.2008 by O.A. Krylovich, A.N. Babenko, B.F. Khassanov and A.B. Savinetsky, HOLOTYPE”.

Paratypes (all from *type locality*). Forty parthenogenetic females in 90% alcohol, MGU MI 94. Forty parthenogenetic females in 90% alcohol, AAK 2009-020. Forty parthenogenetic females in 90% alcohol, USNM 1124006.

Other material studied (excluded from type series). Two hundred females from a lake (06°51.297'N, 039°52.851'E, 4100 m.a.s.l.) near Mountain of Konte, collected on 14 February 2007; 100 females from a roadside lake (06°47.078'N, 039°48.257'E, 3857 m.a.s.l.), collected on 15 February 2007; 300 females from a small lake 2 near Mountain of Konte, collected on 10 February 2008; 50 females from Crane Lake (06°53.596'N, 39°54.498'E, 3970 m.a.s.l.), near Mountain of Konte, collected on 10 February 2008.

All samples were from Sanetti tableland, Bale Mountains; samples of 2007 were collected by O. Krylovich, B. Khassanov and A.B. Savinetsky, and samples of 2008 were collected by O. Krylovich, A. Babenko, B. Khassanov and A. Savinetsky.

Etymology. The species epithet is derived from the Russian phrase for “from the basement”, a homage to the Paleocology group of the Russian Academy of Sciences who collected the original specimens (see above).

Short diagnosis. Parthenogenetic female. Body with dark brown carapace pigmentation, subovoid and lacking caudal spine. Rostrum short, ventral margin of head with a distinct pre-ocular depression, eye capsule

occupies whole anterior-most head portion; a distinct post-ocular depression behind it. Head shield with a crest on its posterior 1/4. Spinules present only on posterior half of dorsal margin, no spinules on ventral margin, a continuous row of setae on inner side of valve ventral margin, with bases located relatively far from the margin, a series of especially long setae in its middle. First abdominal segment with a very long (longer than post-abdominal claw) process; second segment with a moderate process, bent posterior; third segment with a very low, mound-like process; fourth segment lacking of a process, with straight dorsal margin. Pre-anal angle and post-anal angle on post-abdominal dorsal margin not expressed. On outer side of post-abdominal claw, first (proximal) pecten consisting of 6–8 thin teeth; second (medial) pecten consisting of 8–11 large teeth (as long as claw diameter at base); the third pecten consisting of numerous setules. Body of antenna I well developed, tips of aesthetascs projected remarkably beyond tip of rostrum, antennular sensory seta arise from base of mound of the antenna I and reaching tip of rostrum. On limb I with setae 1 and 2, long bearing short setules distally, seta 3 short, seta 4 even shorter than 3, two ejector hooks of different length. Limb II with seta 1 on its distal endite almost as long as each of two other setae on this endite, armed with fine setules distally; gnathobase with four anterior setae and numerous (15–16) posterior setae. Limb III with seta 2 of exopodite bearing short setules; inner-distal portion of limb III with seta 1 long, seta 2 long, endite 2 with a large anterior seta (3) and two posterior setae (c–d); endite 1 with a rudimentary anterior seta (4) and four posterior (e–h) setae. Gnathobase III–IV with numerous posterior soft setae, each with a chitinous insertion within basal portion of distal segment. Limb V with exopodite supplied with two small, thin distal setae and a large lateral seta.

Ephippial female, male unknown.

Size up to 1.95 mm.

Description. Adult parthenogenetic female. Body with dark brown carapace pigmentation, subovoid in lateral view, maximum height in posterior half, dorsal margin of valves obviously elevated above head, regularly convex (Fig. 2A), a minute depression between head and rest of body (Fig. 2B). Postero-dorsal angle lacking of a caudal spine, as a rounded triangle, ventral margin regularly convex.

Head with a relatively short rostrum (Fig. 2B, C, E and F), its tip not bent, in lateral view, the tip subdividing into two lobes by a “line” of pre-rostral fold; posterior margin of head with a prominence proximally to antenna I; ventral margin of head with a distinct pre-ocular depression. Eye capsule occupies whole

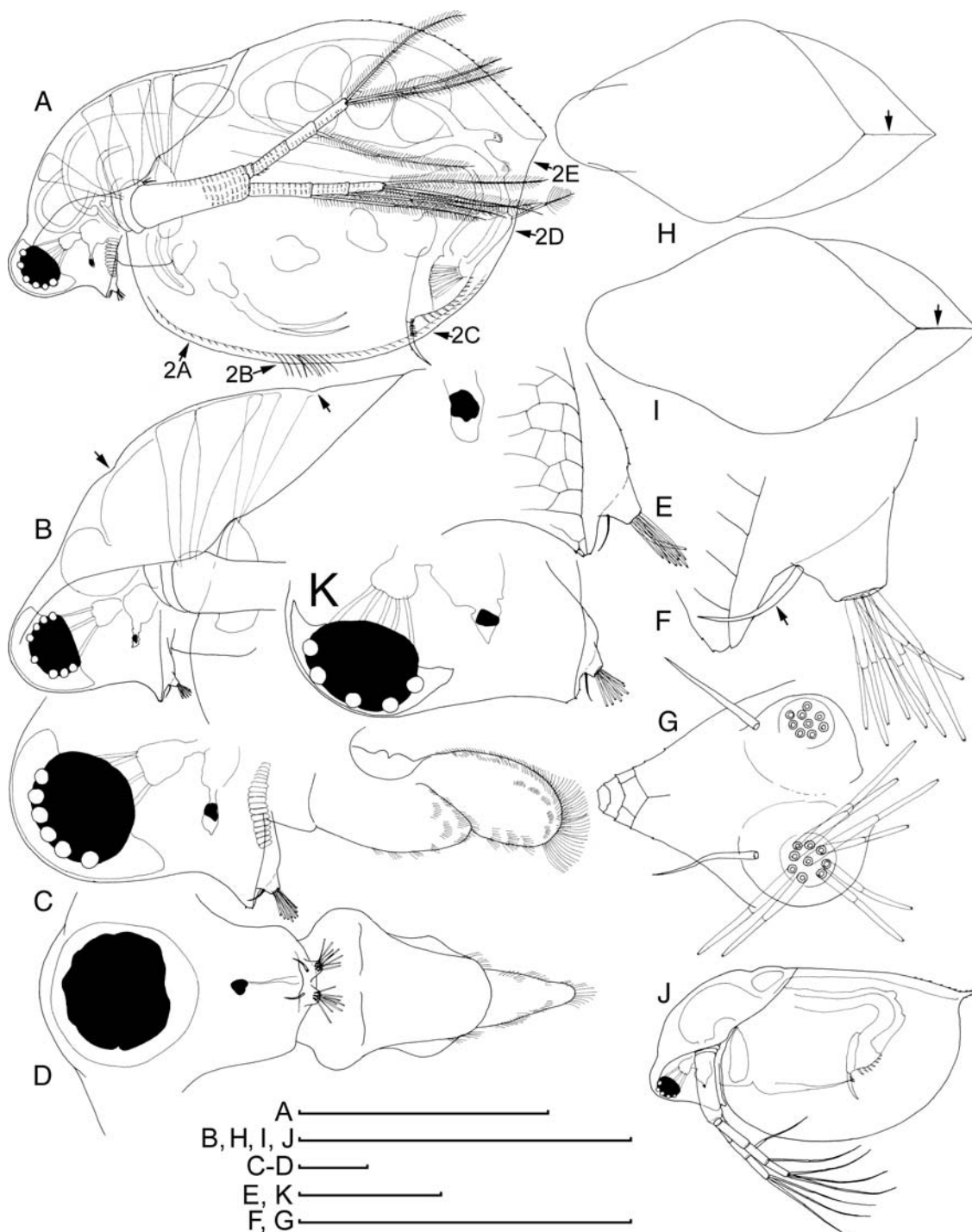


Fig. 2. *Daphnia (Daphnia) izpodvala* sp. nov., parthenogenetic female from a small unnamed lake 1 near Mountain of Konte, Sanetti tableland, Bale Mountains, Ethiopia. (A) Large adult, lateral view. (B and C) Head, lateral view. (D) Head, ventral view. (E and F) Rostrum and antenna I, lateral view. (G) The same, posterior view. (H and I) Head shield. (J) Juvenile. (K) Its head. Scale bars: A, B, H, I, J, 1 mm and C–G, K, 0.1 mm.

anterior-most head portion; a distinct post-ocular depression behind it; two very shallow depressions bordered region of antennal muscles attachment anteriorly and posteriorly (Fig. 2B, arrows). Compound eye large;

ocellus small and located far from base of antenna I (Fig. 2C–E). Head shield widest in middle, with rounded fornices, with a crest on its posterior 1/4 (Fig. 2H and I, arrow). Labrum with a wide, fleshy

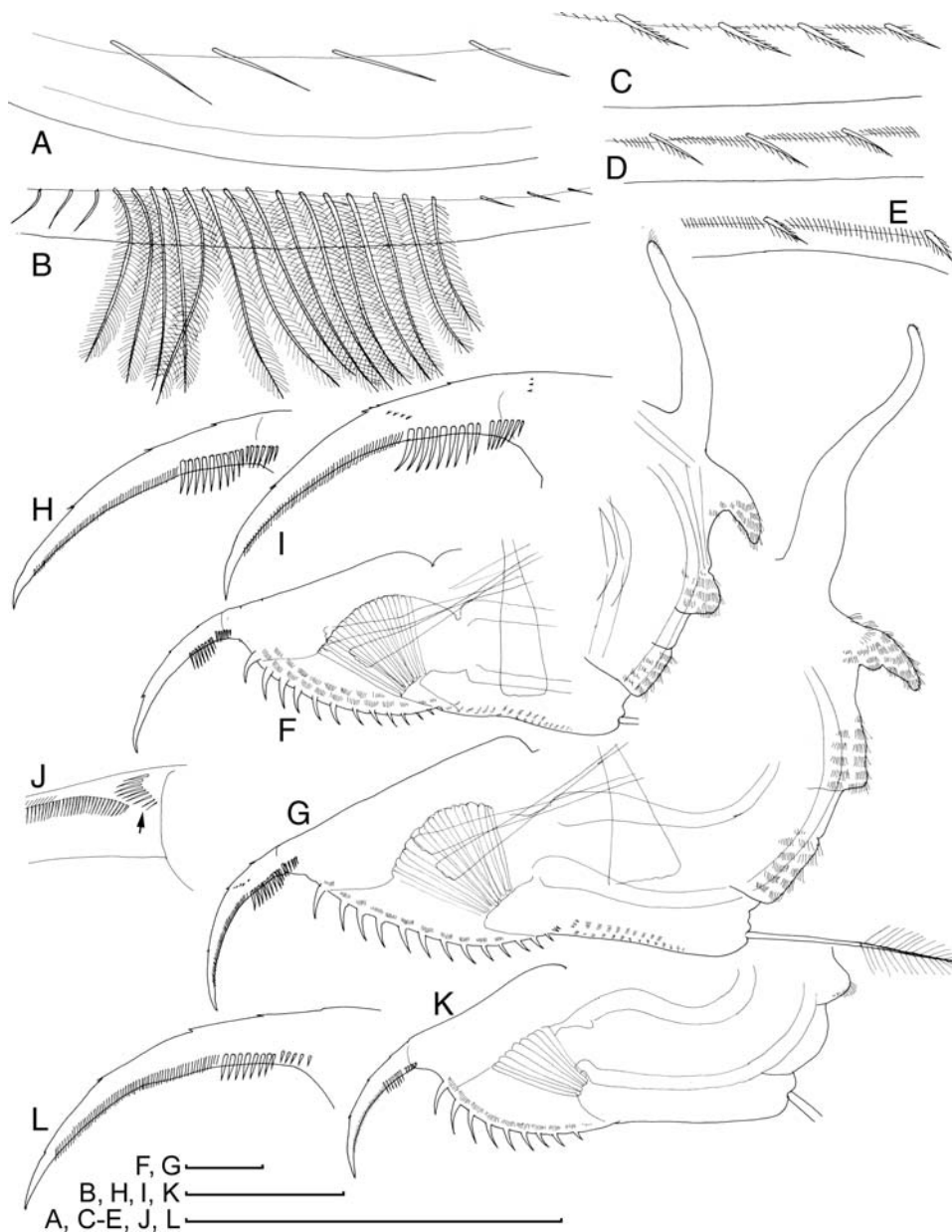


Fig. 3. *Daphnia* (*Daphnia*) *izpodvala* sp. nov., parthenogenetic female from a small unnamed lake 1 near Mountain of Konte, Sanetti tableland, Bale Mountains, Ethiopia. (A) Anterior portion of valve, inner view. (B) Central portion of ventral margin. (C and D) Postero-ventral margin. (E) Posteriormost portion of valve, closest to postero-dorsal angle. (F and G) Abdomen and post-abdomen of adult. (H and I) Post-abdominal claw, outer view. (J) Basal portion of post-abdominal claw, inner view. (K and L) Post-abdomen and post-abdominal claw of juvenile. Scale bars: 0.1 mm.

main body and a large, setulated distal labral plate, compressed laterally (Fig. 2C and D).

Carapace subovoid, spinules present only on posterior half of dorsal margin, no spinules on ventral margin (Fig. 2A). A continuous row of setae on inner side of valve ventral margin, with bases located relatively far from the margin; these setae sparse, relatively short in anterior half of ventral margin (Fig. 3A), then a series of

about 14–16 long setae in middle (Fig. 3B), then setae again short and sparse, with minute setules between them (Fig. 3C–E). In Fig. 2, we marked regions of ventral margin, represented in Fig. 3A–E by corresponding letters.

Abdomen relatively short, consisting of four segments, the first (basal most) abdominal segment with a very long (longer than post-abdominal claw) process;

the second segment with a moderate process, bent posterior; the third segment with a very low, mound-like process; on all processes, there are transverse rows of minute setules; the fourth segment lacking of a process, with straight dorsal margin, also with setules (Fig. 3F and G).

Post-abdomen elongated, tapering distally, with ventral margin straight, lacking of setules (Fig. 3F and G). Pre-anal margin of moderate length (longer than post-anal portion), slightly concave, with series of minute setules. Pre-anal angle and post-anal angle not expressed. Numerous paired spines on post-anal and anal portion, their size increasing distally. Post-abdominal seta approximately as long as pre-anal margin, its distal segment somewhat shorter than basal one. Post-abdominal claw regularly bent, with a pointed tip (Fig. 3H and I). On outer side, three successive pectens along the dorsal margin: the first (proximal) pecten consisting of 6–8 thin teeth; the second (medial) pecten consisting of 8–11 large teeth (as long as claw diameter and base); the third pecten consisting of numerous setules, approximately three to four times shorter than those in the second pecten, not reaching the tip of claw. Rows of denticles at the ventral margin of the claw. On inner side of claw, a series of thin, long spinules basally (Fig. 3J, arrow).

Body of antenna I well developed, with nine aesthetascs of different length terminally (Fig. 2F and G), their tips projected beyond tip of rostrum, antennular sensory seta (Fig. 2E, arrow) fine, arise from base of mound of the antenna I and reaching tip of rostrum. Antenna II with coxal part possessing two short sensory setae of different length (Fig. 4A, arrow). Basal segment elongated, a well-developed (remarkably longer than the basal segment of exopod) distal sensory seta on its posterior face (Fig. 4B, arrow), minute distal spine at its anterior face (Fig. 4C, arrow). Antennal branches longer than basal segment, all with series of minute denticles (Fig. 2A). Spines on apical segments rudimentary (Fig. 4D), spine on the second segment of exopod small (its length less than half of diameter of third segment) and thin (Fig. 4B). Antennal formula: setae 0-0-1-3/1-1-3. Each swimming seta with basal and distal segments bilaterally setulated, a chitinous insertion within distal segment near joint with basal segment (Fig. 4D, arrow).

Limb I with ovoid epipodite (Fig. 4E); accessory seta absent; outer distal lobe (Fig. 4F: ODL), with a long seta unilaterally armed distally with short setules, and a short, thin seta; inner distal lobe (Fig. 4F: IDL), or endite 4, with a single, long anterior seta 1, bearing short setules distally. Endite 3 with a long anterior seta 2 and two posterior setae (a–b). Endite 2 with a short

and thin anterior seta 3 and two posterior setae (c–d). Endite 1 with a short anterior seta 4 and four posterior setae (e–h). Two ejector hooks of different length.

Limb II with a small, subovoid epipodite (Fig. 4G); distal portion as a large lobe bearing a large, soft, distal seta and a large, soft, lateral seta. Four endites bearing five setae, among them, a stiff, anterior seta (Fig. 4H: 1) almost as long as each of two other setae on this endite, armed with fine setules distally. Gnathobase with two rows of setae: four anterior setae (Fig. 4I: 1–4) and numerous (15–16) posterior setae of gnathobasic “filter plate” (a–p).

Limb III with a large pre-epipodite, subglobular epipodite and a flat exopodite bearing four distal setae (Fig. 4J: 1–4), among them seta 2 with short setules (Fig. 4K) and two lateral (Fig. 4J: 5–6) setae. Inner-distal portion of limb with four endites: endite 4 with a single, long anterior seta (Fig. 4L: 1) and a posterior (a) seta; endite 3 with a single anterior seta (2) and a single posterior (b) seta; endite 2 with a large anterior seta (3) with a small sensillum near its base, and two posterior setae (c–d); endite 1 with a rudimentary anterior seta (4) and four posterior (e–h) setae. The rest of limb inner-distal portion as a singular large lobe, modified gnathobase, bearing numerous posterior soft setae, each with chitinous insertion within basal portion of distal segment (Fig. 4M), a single, relatively long anterior seta (Fig. 4L: 1) in its distal corner and two short anterior setae (2 and 3) in middle of filter comb.

Limb IV with a large, setulated pre-epipodite, large, ovoid epipodite and wide, flat exopodite, with protruding and setulating inner-distal angle, and bearing four distal and two lateral setae (Fig. 4N: 1–6). Inner-distal portion of this limb with completely fused endites, distally with two setae of unclear homology (Fig. 4O), the most part of limb inner margin is a gnathobase filter plate consisting of numerous posterior setae.

Limb V with a setulated pre-epipodite, large, subovoid epipodite, triangular exopodite supplied with two small, thin distal setae and a large lateral seta (Fig. 4P). Inner limb portion as an ovoid flat lobe, with setulated inner margin and a single, large seta.

Juvenile female. Body with almost straight dorsal margin and a short caudal needle, head with shorter rostrum and rudimentary pre-ocular and post-ocular depressions (Fig. 2J and K). Post-abdomen as in adult (Fig. 3K), post-abdominal claw with spinules in first and second pecten less numerous, shorter, thicker (Fig. 3L) than in adult.

Ephippial female, male unknown.

Size. Holotype 1.95 mm, parthenogenetic females 0.65–1.95 mm.

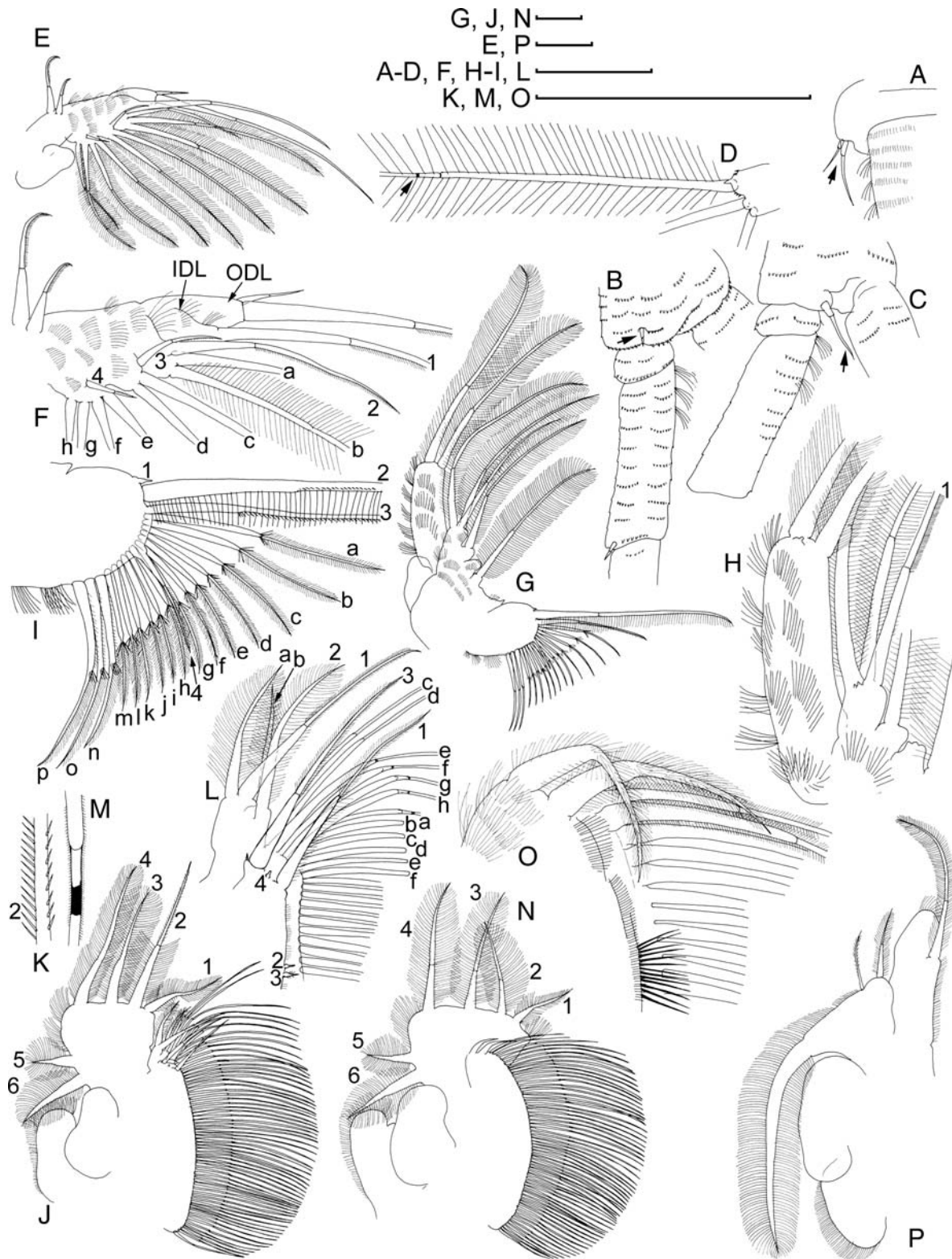


Fig. 4. *Daphnia* (*Daphnia*) *izpodvala* sp. nov., head and thoracic appendages of adult parthenogenetic female from a small unnamed lake 1 near Mountain of Konte, Sanetti tableland, Bale Mountains, Ethiopia. (A) Sensory setae on coxal portion of antenna II. (B and C) Distal portion of basal segment and proximal portion of exopod, anterior and posterior view. (D) Distal segment of exopod and apical swimming seta. (E and F) Limb I. (G, H and I) Limb II, its distal portion and gnathobase. (J, K, L and M) Limb III, seta 2 of its exopod, inner-distal portion and filtering seta of gnathobase. (N and O) Limb IV and its inner-distal portion. (P) Limb V. Scale bars: 0.1 mm.

Differential diagnosis. Among numerous taxa of the *pulex*-group, there are only two species with a large ocular dome, bordering by a distinct pre-ocular and a deep post-ocular depression, *D. izpodvala* sp. nov. and *D. marcahuasensis* (Valdivia Villar and Burger, 1989). The latter was earlier erroneously placed to the genus *Daphniopsis*, which is apparently a junior synonym of *Ctenodaphnia* (Glagolev, 1986), but *D. marcahuasensis* lacks the diagnostic characters of *Ctenodaphnia* and resembles *obtusa*-like species of the *pulex*-group. *Daphnia izpodvala* sp. nov. differs from the former (Valdivia Villar and Burger, 1989) in having: (i) more numerous and closely located teeth in the medial pecten of post-abdominal claw; (ii) longer process on second abdominal segment; and (iii) smaller antenna I, body of which does not project beyond the tip of rostrum.

Distribution. *Daphnia izpodvala* sp. nov. is very common in the standing waters of the Bale Mountains, Ethiopia, 3857–4100 m.a.s.l.

DISCUSSION

Our results support the existence of three separate African and Asian lineages of *Daphnia* that have been synonymized with *D. obtusa*, none of which bear a close relationship to *D. obtusa* s. str. based on mitochondrial DNA. We describe one of these, from Ethiopia, as a new species, *D. izpodvala* sp. nov. There are clearly continental species clusters within the *obtusa* clade (i.e. species with ventral margin seta), but our phylogenetic analyses indicate that the position of the African *D. izpodvala* sp. nov. breaks up the Holarctic clade and the North American clade. Thus, the patterns of evolution are inconsistent with strict continental vicariance. The position of *D. izpodvala* sp. nov. with *D. pileata* is well supported, but could also be the result of a systematic bias. A test of homogeneity of substitution patterns (Tamura *et al.*, 2007) for COI reveals that *D. pileata* has significantly different patterns of substitution from three of five North American members of the complex, but lacks significantly different patterns of substitution from its phylogenetic sister group, *D. izpodvala*. Still, both the rDNA gene and the protein-coding gene, which are expected to have different biases, revealed the same association of *D. izpodvala* sp. nov. with *D. pileata* (though with weaker support). More genetic data will be needed to rule out a systematic bias attracting *D. pileata* to *D. izpodvala* sp. nov. The results do highlight the complexity of biogeographic inference with older radiations of the cladocerans (Frey, 1987; Kotov, 2007) when there are likely several extinct (Korovchinsky, 2006) or

non-sampled lineages and tree shapes appear sensitive to systematic biases.

Despite a dispersal connection by avian flyways, we can find no evidence for affinities between the European *D. obtusa* complex and Ethiopian *D. izpodvala* sp. nov. Glagolev (Glagolev, 1986) and Alonso (Alonso, 1996) redescribed “*D. obtusa* s. str.” from Europe in detail, but then it was concluded that there are four distinct *obtusa*-like lineages in Europe (Petrušek *et al.*, 2005; Adamowicz *et al.*, 2009). According to photos of Petrušek *et al.* (Petrušek *et al.*, 2005), all of these species lack the large ocular dome and have a remarkably longer rostrum than *D. izpodvala* sp. nov. Other *obtusa*-like taxa are present in Africa, but their morphology (Green and Kling, 1988; Seaman *et al.*, 1999; Kořínek, 2002) is of the “European” type as well. Green (Green, 1995) reported on the altitudinal distribution of *D. obtusa* in tropical Africa, but unfortunately we lack the names and exact locations for the high mountain lakes where he reports this species. Recently, Benzie (Benzie, 2005) synonymized the South African *D. propinqua* Sars 1895 and *D. tenuispina* Sars 1916 with *D. obtusa*. But Sars never described or illustrated setules on the ventral margin of valves, and Kořínek (Kořínek, 2002) regarded *Daphnia propinqua* as a valid taxon. So, the status of South African *obtusa*-like populations must be re-evaluated.

We note that the separation of the Bale Mountain endemic lineage took place before the Pleistocene aridization, an important event in African zooplankton biogeography (Dumont, 1980). Löffler (Löffler, 1978) proposed that “*D. obtusa*” penetrated the Ethiopian Mountains from Europe after their Pleistocene glaciation. But he apparently misidentified the currently described *D. izpodvala* sp. nov. as “heavily pigmented” *D. obtusa*. The independent origin of cuticular pigmentation associated with high latitude/altitude species in *Daphnia* is well known (Colbourne *et al.*, 1997). The South American *Daphnia peruwiana* from the high Andes, for example, is also pigmented, as is a newly discovered Andean endemic from the subgenus *Ctenodaphnia* (Kotov *et al.*, unpublished results). Here, we describe yet another independent origin of cuticular pigmentation in high mountain *Daphnia* and the first case from the *D. obtusa* group. South American species in the *D. obtusa* complex are genetically distant from *D. izpodvala* sp. nov. The rare Andean species, *D. marcahuasensis*, does share the ocular dome character, pigmentation, shell spine truncation and ventral margin setation with *D. izpodvala* sp. nov. Whether this character sharing is indicative of shared ancestry or convergence in montane habitats is presently unknown and molecular information for *D. marcahuasensis* is missing.

Populations from the Bale Mountains belong to an endemic species, apparently absent in the Palaearctic. In contrast to Löffler's opinion, we propose that *D. izpodvala* sp. nov. colonized the young post-glacial Bale mountain lakes from an African refugium as with other Bale Mountain endemics (Umer *et al.*, 2007). Waterfowl are very common on the lakes and pools of the Bale Mountains (A.B. Savinetsky, personal observation) and have a good chance of carrying ephippia of *Daphnia* species from nearby lakes (i.e. from the closest Rift Valley). Yet, *D. izpodvala* sp. nov. has not been replaced by the "aggressive" American clone, as in some Kenyan and Ethiopian lakes (Mergeay *et al.*, 2005a, 2006), perhaps indicating an altitudinal fitness advantage for *D. izpodvala* sp. nov. High mountain localities are a recent focus of cladoceran taxonomy because they often appear to contain endemics (Smirnov *et al.*, 2006). Several species of the cladocerans endemic to the high mountains of Africa and South America remain undescribed (Adamowicz *et al.*, 2004, 2009).

The affinities of the Japanese *Daphnia mitsukuri* Ishikawa, 1896 are also unknown. Two other Japanese species that are *obtusum*-like (and have been synonymized with *D. obtusum*), but lack ventral margin setation, are *Daphnia whitmani* Ishikawa, 1895 and *Daphnia morsei* Ishikawa, 1895. We found two new lineages in Japan whose females cannot be distinguished from the original descriptions of these species. Ishikawa stated that the main difference between these species was the anal denticle number (9 for *whitmani* and about 13 for *morsei*), the width of the carapace of females—we find these differences in the present study but the character states for males are missing. We have deferred assigning our new lineages from Japan to these lowland Japanese species until male character states, such as the deep post-abdominal embayment of male *D. morsei* is assessed. Our results do clearly show the existence of two unknown Japanese lineages in the *pulex* group *sensu lato*. Japanese lineages have recently been found in several other cladoceran genera (Kotov *et al.*, 2006; Ishida *et al.*, 2006; Kotov *et al.*, 2009; Xu *et al.*, 2009).

Despite the long historical study of the *pulex* s. lat. group, a global revision is urgently needed.

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REFERENCES

- Adamowicz, S. J. and Purvis, A. (2005) How many branchiopod crustacean species are there? Quantifying the components of underestimation. *Global Ecol. Biogeogr.*, **14**, 455–468.
- Adamowicz, S. J., Hebert, P. D. N. and Marinone, M. C. (2004) Species diversity and endemism in the *Daphnia* of Argentina: a genetic investigation. *Zool. J. Linn. Soc.*, **140**, 171–205.
- Adamowicz, S., Petrussek, A., Colbourne, J. *et al.* (2009) The scale of divergence: a phylogenetic appraisal of intercontinental allopatric speciation in a passively dispersed freshwater zooplankton genus. *Mol. Phylogenet. Evol.*, **50**, 423–436.
- Alonso, M. (1996) *Crustacea, Branchiopoda. Fauna Iberica*, Vol. 7 Consejo Superior de Investigaciones Científicas, Madrid.
- Benzie, J. A. H. (2005) *The Genus Daphnia (Including Daphniopsis) (Anomopoda: Daphniidae). Guides to the Identification of the Microinvertebrates of the Continental Waters of the World*, Vol. 21 Kenobi Productions, Ghent and Backhuys Publishers, Leiden.
- Benzie, J. A. H. and Hodges, A. M. A. (1996) *Daphnia obtusum* Kurz, 1874 emend Scourfield, 1942 from Australia. *Hydrobiologia*, **333**, 195–199.
- Brehm, V. (1912) Die Cladoceren. Die Cladoceren. *Wissenschaftliche Ergebnisse der Deutschen Zentral-Africa-Expedition 1907–1908, unter Führung Adolf Friedrichs, Herzog zu Mecklenburg*, **3**, 167–174.
- Brunelli, G. and Cannici, E. G. (1940) Le caratteristiche biologiche del Lago Tana. *Missione di Studio al Lago Tana. Centre Studi per l'Africa Orientale Italiana*, **4**, 71–115.
- Colbourne, J. K. and Hebert, P. D. N. (1996) The systematics of North American *Daphnia* (Crustacea: Anomopoda): a molecular phylogenetic approach. *Phil. Trans. Roy. Soc. Lond. B*, **351**, 349–360.
- Colbourne, J. K., Hebert, P. D. N. and Taylor, D. J. (1997) Evolutionary origins of phenotypic diversity in *Daphnia*. In Givnish, T. J. and Sytsma, K. J. (eds), *Molecular Evolution and Adaptive Radiation*. University Press, Cambridge, pp. 163–189.
- Dejenie, T., Asmelash, T., De Meester, L. *et al.* (2008) Limnological and ecological characteristics of tropical highland reservoirs in Tigray, Northern Ethiopia. *Hydrobiologia*, **610**, 193–209.
- Dumont, H. J. (1980) Zooplankton and science of biogeography: the example of Africa. In Kerfoot, W. C. (ed), *Evolution and Ecology of Zooplankton Communities*. University Press, Hanover, NH, pp. 685–696.
- Dumont, H. J. (1994) On the diversity of the Cladocera in the tropics. *Hydrobiologia*, **272**, 27–38.
- Dumont, H. J. and Verheye, H. M. (1984) The nature and origin of the crustacean zooplankton of Sahelian Africa, with a note on the *Limnomedusa*. *Hydrobiologia*, **113**, 313–325.

- Dumont, H. J., Pensaert, J. and El Moghraby, A. I. (1984) Cladocera from the Sudan: Red Sea Hills, Jebel Marra and valley of the main Nile. *Hydrobiologia*, **110**, 163–169.
- Elizabeth, K., Getachew, T., Taylor, W. D. *et al.* (1992) Eutrophication of Lake Hayq in the Ethiopian Highlands. *J. Plankton Res.*, **14**, 1473–1482.
- Folmer, O., Black, M., Hoeh, W. *et al.* (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.*, **3**, 294–299.
- Forró, L., Korovchinsky, N. M., Kotov, A. A. *et al.* (2008) Global diversity of cladocerans (Cladocera; Crustacea) in freshwater. *Hydrobiologia*, **595**, 177–184.
- Frey, D. G. (1987) The taxonomy and biogeography of the Cladocera. *Hydrobiologia*, **145**, 5–17.
- Glagolev, S. M. (1986) Morphology, systematics and geographic distribution of the cladoceran genus *Daphnia* from Eurasia. PhD Thesis. IEMEZh of AN SSSR, Moscow (In Russian)
- Green, J. (1995) Altitudinal distribution of tropical planktonic Cladocera. *Hydrobiologia*, **307**, 75–84.
- Green, J. and Kling, G. W. (1988) The genus *Daphnia* in Cameroon, West Africa. *Hydrobiologia*, **160**, 257–261.
- Hebert, P. D. N., Witt, J. D. S. and Adamowicz, S. J. (2003) Phylogeographical patterning in *Daphnia ambigua*: Regional divergence and intercontinental cohesion. *Limn. Ocean.*, **48**, 261–268.
- Ishida, S., Kotov, A. A. and Taylor, D. J. (2006) A new divergent lineage of *Daphnia* (Cladocera: Anomopoda) and its morphological and genetical differentiation from *Daphnia curvirostris* Eylmann, 1887. *Zool. J. Linn. Soc.*, **146**, 385–405.
- Ishikawa, C. (1895a) Phyllopod Crustacea of Japan. *Daphnia Morsei*. *Zool. Mag.*, **7**, 137–142.
- Ishikawa, C. (1895b) Phyllopod Crustacea of Japan. *Daphnia Whitmani*. *Zool. Mag.*, **7**, 147–154.
- Ishikawa, C. (1896) Phyllopod Crustacea of Japan. (VIII) *Daphnia Mitsukuri*, n. sp. *Zool. Mag.*, **8**, 55–57.
- Jenkin, P. M. (1934) Report on the Persy Sladen Expedition to some Rift Valley Lakes in Kenya in 1929. VI. Cladocera from the Rift Valley Lakes in Kenya. *Ann. Mag. Nat. Hist.*, **10th Ser.**, **13**, 137–160, 281–308.
- Katoh, K., Asimenos, G. and Toh, H. (2009) Multiple alignment of DNA sequences with MAFFT. *Methods Mol. Biol.*, **537**, 39–64.
- Kořínek, V. (2002) Cladocera. In Fernando, C. H. (ed.), *A Guide to Tropical Freshwater Zooplankton*. Backhuys Publishers, Leiden, pp. 69–122.
- Korovchinsky, N. M. (2006) The Cladocera (Crustacea: Branchiopoda) as a relict group. *Zool. J. Linn. Soc.*, **147**, 109–124.
- Kotov, A. A. (2000) Redescription and assignment of the chydorid *Indialona ganapati* Petkovski, 1966 (Branchiopoda: Anomopoda: Aloninae) to *Indialonini*, new tribus. *Hydrobiologia*, **439**, 161–178.
- Kotov, A. A. (2007) Jurassic Cladocera (Crustacea, Branchiopoda) with a description of an extinct Mesozoic order. *J. Nat. Hist.*, **41**, 13–37.
- Kotov, A. A., Ishida, S. and 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. *J. Plankton Res.*, **28**, 1067–1079.
- Kotov, A. A., Ishida, S. and Taylor, D. J. (2009) Revision of the genus *Bosmina* Baird, 1845 (Cladocera: Bosminidae), based on evidence from male morphological characters and molecular phylogenies. *Zool. J. Linn. Soc.*, **156**, 1–56.
- Löffler, H. (1968) Die Crustaceenfauna der Binnengewässer Ostafrikanischer Hochberge. *Hochgebirgsforschung*, **1**, 107–170.
- Löffler, H. (1978) Limnological and paleolimnological data on the Bale Mountain Lakes (Ethiopia). *Verh. Internat. Verein. Theor. Angew. Limnol.*, **20**, 1131–1138.
- Mergeay, J., Verschuren, D. and De Meester, L. (2005a) Cryptic invasion and dispersal of an American *Daphnia* in East Africa. *Limnol. Oceanogr.*, **50**, 1278–1283.
- Mergeay, J., Verschuren, D. and De Meester, L. (2005b) *Daphnia* species diversity in Kenya, and a key to the identification of their ephippia. *Hydrobiologia*, **542**, 261–274.
- Mergeay, J., Verschuren, D. and De Meester, L. (2006) Invasion of an asexual American water flea clone throughout Africa and rapid displacement of a native sibling species. *Proc. Roy. Soc., B-Biol. Sci.*, **273**, 2839–2844.
- Mergeay, J., Aguilera, X., Declerck, S. *et al.* (2008) The genetic legacy of polyploid Bolivian *Daphnia*: the tropical Andes as a source for the North and South American *D. pulicaria* complex. *Mol. Ecol.*, **17**, 1634–1635.
- Penton, E. H., Hebert, P. D. N. and Crease, T. J. (2004) Mitochondrial DNA variation in North American populations of *Daphnia obtusa*: continentalism or cryptic endemism? *Mol. Ecol.*, **13**, 97–107.
- Petrusek, A., Bastiansen, F. and Schwenk, K. (2005) European *Daphnia* Species (EDS)—taxonomic and genetic keys. [Build 2005-09-07 beta]. CD-ROM, distributed by the authors. J.W. Goethe-University, Frankfurt am Main, Germany and Charles University, Prague, Czechia.
- Richard, J. (1896) Révision des Cladocères. Deuxième Partie. Anomopoda. Famille III.—Daphnidae. *Ann. Sci. Nat. Zool.*, **8th Ser.**, **2**, 187–363.
- Ronquist, F. and Huelsenbeck, J. P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.
- Sars, G. O. (1895) On some South-African Entomostraca raised from dried mud. *Skr. VidenskSelsk. Christiania, Mat.-naturv. Kl.*, **8**, 1–56.
- Sars, G. O. (1916) The fresh-water Entomostraca of the Cape Province (Union of South Africa). Part 1: Cladocera. *Ann. South Afr. Mus.*, **15**, 303–351.
- Schwartz, S. S., Innes, D. J. and Hebert, P. D. N. (1985) Morphological separation of *Daphnia pulex* and *D. obtusa* in North America. *Limnol. Oceanogr.*, **30**, 189–197.
- Seaman, M. T., Kok, D. J. and Watson, M. (1999) Cladocera. In Day, J. A., Stewart, B. A., Moor, I. J., Louw, A. E. *et al.* (eds), *Guides to the Freshwater Invertebrates of Southern African. Crustacea I*. WRC Report No. TT 121/00, pp. 1–126.
- Smirnov, N. N., Kotov, A. A. and Coronel, J. (2006) Partial revision of the *aduncus*-like species of *Pleuroxus* Baird, 1843 (Chydoridae, Cladocera) from the southern hemisphere with comments of subgeneric differentiation within the genus. *J. Nat. Hist.*, **40**, 1617–1639.
- Stamatakis, A., Hoover, P. and Rougemont, J. (2008) A rapid bootstrap algorithm for the RAxML web servers. *Syst. Biol.*, **57**, 758–771.
- Tamura, K., Dudley, J., Nei, M. *et al.* (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol. Biol. Evol.*, **24**, 1596–1599.
- Tanaka, S. (1997) Notes on the Cladocera of Japan—4. Family Daphniidae Straus, 1820—2. Genus *Daphnia*, subgenus *Daphnia*. *Mem. Fac. Educ. Toyama Univ., B-Nat. Sci.*, **49**, 55–66.
- Taylor, D. J. and Hebert, P. D. N. (1993) Cryptic intercontinental hybridization in *Daphnia* (Crustacea): the ghost of introductions past. *Proc. Roy. Soc. London, B-Biol. Sci.*, **254**, 163–168.

- Taylor, D. J., Hebert, P. D. N. and Colbourne, J. K. (1996) Phylogenetics and evolution of the *Daphnia longispina* group (Crustacea) based on 12S rDNA sequence and allozyme variation. *Mol. Phylogenet. Evol.*, **5**, 495–510.
- Uéno, M. (1927) The freshwater Branchiopoda of Japan I. *Mem. Coll. Sci., Kyoto Imp. Univ., Ser. B.*, **2**, 259–311.
- Umer, M., Lamb, H. F., Bonnefille, R. *et al.* (2007) Late Pleistocene and Holocene vegetation history of the Bale Mountains, Ethiopia. *Quaternary Sci. Rev.*, **26**, 2229–2246.
- Valdivia Villar, R. S. and Burger, L. O. (1989) Descripción de *Daphniopsis marahuasensis* sp. nov. (Cladocera: Daphniidae) del Perú, con la inclusión de una clave de identificación de las especies del Género. *Amazoniana*, **10**, 439–452.
- von Daday, E. (1910a) Collections recueillies par M. le Baron M. de Rothschild dans l'Afrique Orientale (Abyssinie et Ethiopie). Entomostraces d'eau douce. *Bull. Soc. Zool. France*, **16**, 253–265.
- von Daday, E. (1910b) Untersuchungen über die Süßwasser-Mikrofauna Deutsch-Ost-Afrikas. *Zoologica*, **59**, 1–316.
- Wagler, E. (1936) Die Systematik und geographische Verbreitung des Genus *Daphnia* O. F. Mueller mit besonderer Berücksichtigung des sudafrikanischen Arten. *Arch. Hydrobiol.*, **30**, 505–556.
- Weltner, W. (1898) Die Cladocren Ost-Afrikas. *Deutsch-Ost-Africa*, **4**, 1–14.
- Wodajo, K. and Belay, A. (1984) Species composition and seasonal abundance of zooplankton in two Ethiopian Rift Valley lakes—Lakes Abiata and Langano. *Hydrobiologia*, **113**, 129–136.
- Xu, S., Kotov, A. A., Hebert, P. D. N. *et al.* (2009) The non-cosmopolitanism paradigm of freshwater zooplankton: insights from the global phylogeography of the predatory cladoceran *Polyphemus pediculus* (Crustacea, Onychopoda). *Mol. Ecol.*, **18**, 5161–5179.