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A new divergent lineage of *Daphnia* (Cladocera: Anomopoda) and its morphological and genetical differentiation from *Daphnia curvirostris* Eylmann, 1887

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The systematic biology of the subgenus *Daphnia s.s*. remains confused. Prior attempts at resolution used chiefly postabdominal claw morphology, chromosome numbers and rRNA gene sequences as characters for higher-level relations. Still, several taxa, such as *Daphnia curvirostris* Eylmann, 1878, have unclear affiliations. We addressed the position of *D. curvirostris* in this genus by estimating phylogenetic trees from a rapidly evolving protein coding gene (ND2), conducting broad geographical comparisons and carrying out detailed morphological comparisons. The Japanese '*curvirostris*' was found to be a new divergent lineage in the subgenus *Daphnia*, and to possess distinctive morphological characteristics from *D. curvirostris*. We described this new species as ***Daphnia tanakai* sp. nov.**, and redescribed *D. curvirostris*. The polymorphic postabdominal claw morphology and the distinctive chromosome number of *D. tanakai* sp. nov. provided evidence for rapid evolution of these traits. Our new morphological, chromosomal and genetic assessment of *Daphnia* weakened the argument for division of the subgenus *Daphnia* (*Daphnia*) O. F. Müller, 1785 *sensu* Johnson, 1952, into two further subgenera.

ADDITIONAL KEYSWORDS: chromosome – Daphnidae – mitochondrial DNA – morphology – phylogeny – postabdominal claw – subgenera – taxonomy.

INTRODUCTION

The taxonomy of the subgenus *Daphnia* remains notoriously confused. Phenotypic plasticity (especially of head and carapace shape), hybridization, cryptic intercontinental introductions and poor taxonomic descriptions have hindered the understanding of species boundaries (Taylor & Hebert, 1993, 1994; Schwenk, Posada & Hebert, 2000). Most of divergent *Daphnia* species groups (as assessed by DNA sequence comparisons) have been recognized over 100 years ago (Brooks, 1957; Colbourne & Hebert, 1996). Nevertheless, in some groups of water fleas (e.g. family Bosminidae), the sampling of new habitats combined with detailed genetic and morphological analysis yielded the discovery of new subgenera (Taylor, Ishikane & Haney, 2002). As detailed among-continent comparisons that assess both genetic and morphological variation in water fleas are still rare, more discoveries are certainly expected (Colbourne *et al*., 1998; Černý & Hebert, 1999).

Morphological systematists traditionally separated the subgenus *Daphnia* (*Daphnia*) O. F. Müller, 1785 *sensu* Johnson 1952, into the *longispina* and *pulex* groups (see Brooks, 1957). Many authors considered the postabdominal claw morphology or chromosome number to have undergone conserved evolution (Brooks, 1957; Beaton & Hebert, 1994; Colbourne, Hebert & Taylor, 1997), and formed the groups largely on this evidence. The paired postabdominal claws are posterior to the anus, contain three pectens and are believed to function as cleaners for the filtering limbs (Fryer, 1991). The proximal and medial pectens of the *Daphnia pulex* group have fewer, but more robust teeth than the pectens of the *Daphnia longispina*

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group. The chromosome numbers largely agree with the designation of two major groups based on claws, as the *pulex* group contains $2n = 24$ chromosomes, and the *longispina* group contains the ancestral daphniid chromosome number of $2n = 20$. However, several taxa are either morphologically intermediate or chimeric between the *pulex* and *longispina* groups (Johnson, 1952). As a result, some authors divided the *pulex* and *longispina* groups based on different characters from the claw. Alonso (1996), for example, proposed that the size of the anterior seta on the distalmost endite in the female limb II, the size of the flagellum on antenna 1 in the male, the size of the anterior setae on endites 2 and 3 in the male limb I, and the curvature of the anterior seta on the distalmost endite in the male limb II, were more important characters than the claw. Thus according to Alonso (1996), *Daphnia parvula* Fordyce, 1901, with robust teeth in the medial pecten, should be a member of the *longispina* group, while molecular phylogeny has shown that *D. parvula* is a member of the *pulex* group (Colbourne & Hebert, 1996; Schwenk *et al*., 2000).

Molecular phylogenetic studies, based on conserved genes (12S rRNA, COI, 28SrRNA), largely agreed with the claw-chromosome clades but failed to resolve some deeper clade relations and position of some 'orphan' species (Lehman *et al*., 1995; Colbourne & Hebert, 1996; Taylor, Hebert & Colbourne, 1996; Schwenk *et al*., 2000; Omilian & Taylor, 2001). The positions of *Daphnia curvirostris* Eylmann, 1878, the *Daphnia laevis* species complex and the *Daphnia longiremis* species complex, for example, remain unresolved in molecular phylogeny. The uncertainty is due to weak clade support, weak sampling or a molecular evolutionary bias in the data. *Daphnia laevis* and *D. longiremis* should belong to the *longispina* group because they possess both the *longispina* type of claw and the ancestral chromosome count of $2n = 20$. *Daphnia curvirostris* is more enigmatic. European and North American *D. curvirostris* have the *pulex* type of claw but the ancestral chromosome number of $2n = 20$ (Trentini, 1980; Beaton & Hebert, 1994). The initial molecular study of *D. curvirostris* used 288 base pairs (bp) of 12S rRNA and grouped *D. curvirostris* in the *D. longispina* group (Lehman *et al*., 1995). Nevertheless, the support values were modest (51–70%), and only one species of the *Daphnia longispina* group was included in the analysis (*Daphnia galeata*), increasing the risk that the *D. curvirostris*/*D. galeata* clade resulted from a long-branch attraction artefact. Indeed, later studies examined the same 12S rRNA gene with more species, and the support for *D. curvirostris* being in the *D. longispina* clade decreased to <50% (Colbourne & Hebert, 1996; Schwenk *et al*., 2000). Finally, Tanaka & Tominaga (1986) described *D. curvirostris* from Japan where specimens had a unique chromosome number ($2n = 22$) and possessed either the *pulex* type of claw or the *longispina* type of claw. Therefore, morphology, chromosome number and DNA sequence evidence presently fail to provide strong evidence for the evolutionary affiliations of *D. curvirostris*.

We aimed to address the *D. curvirostris* species problem by estimating phylogenetic trees from a more rapidly evolving gene (mitochondrial ND2), conducting broad geographical comparisons and carrying out detailed morphological comparisons. We specifically addressed the phylogenetic position of *D. curvirostris*, and the agreement of the ND2 tree with the evolutionary groups predicted from claw morphology and chromosome number. The results necessitated a revision of *Daphnia curvirostris* and the description of a genetically divergent species of *Daphnia*.

MATERIAL AND METHODS

Sampling

We compared geographically distant populations of *D. curvirostris* from Japan ($N = 2$), Europe ($N = 1$) and North America ($N = 1$) with 12 species of the *longispina* group ($N = 13$), three species of the *pulex* group ($N = 4$) and one species of the subgenus *Ctenodaphnia* ($N = 1$) (Table 1). Japanese *curvirostris* from two populations were identified according to Tanaka & Tominaga (1986). Species from the other populations were identified according to Brooks (1957), Flössner (2000) and Taylor *et al*. (1996).

DNA extraction and sequencing

Total genomic DNA was extracted using QuickExtract (Epicentre). Samples were homogenized in 30–50 μL of the QuickExtract solution, incubated at 65 °C for 2 h and 95 °C for 20 min, and stored at −20 °C. We developed specific primers for the mitochondrial ND2 gene (~ 1000 bp) of the *longispina* and *pulex* groups by comparing flanking tRNA genes of *D. pulex* from North America (Crease, 1999; GenBank accession number NC000844), *D. galeata* from Japan (S. Ishida & D. J. Taylor, unpubl. data) and *Eubosmina coregoni* from North America (S. Ishida, M. Faustova & D. J. Taylor, unpubl. data): MetF1 (5′-TAA AGC TAG TGG GTT CAT GCC CC-3′) at *D. pulex* mtDNA genome position 150–172, MetF2 (5′-TGG GTT CAT GCC CCA TTT ATA G-3′) at *D. pulex* mtDNA genome position 159–180, MetF3 (5′-GTT CAT GCC CCA TTT ATA GGT TA-3′) at *D. pulex* mtDNA genome position 162–186, CysR (5′-AGT TGA AAA GAG TCA ACG TCG CA-3′) at *D. pulex* mtDNA genome position 1424–1402 and TrpR (5′-GAA GGT TTT TAG TTT AGT TAA CTT AAA ATT CT-3′) at *D. pulex* mtDNA genome position 1217–1186.

Table 1. *Daphnia* species subjected to DNA sequencing

Taxon	Sampling loality	GenBank accession number
***D. tanakai* sp. nov.**	Midori-ga-ike (Midori), Toyama, Japan	DQ132616, DQ132617
***D. tanakai* sp. nov.**	Kagami-Ike (Kagami), Gifu, Japan	DQ132618
D. curvirostris Eylmann, 1887	Pilgrim Hotsprings, Alaska, USA	DQ132619
D. curvirostris Eylmann, 1887	Somotor, Slovakia	DQ132620
D. galeata Sars, 1864	Galeairy Lake, Ontario, Canada	DQ132605
D. cucullata Sars, 1862	Somotor, Slovakia	DQ132606
D. thorata Forbes, 1893	Flathead Lake, Montana, USA	DQ132607
D. rosea Sars, 1862	Vysne Furkotske, Slovakia	DQ132608
'D. umbra'	Pond near Richards Bay, NWT, Canada	DQ132609
D. longispina O. F. Mueller, 1785	Pond, South of Muono, Lapin laani, Finland	DQ132610
D. longiremis Sars, 1862	Melville Peninsula, NWT, Canada	DQ132611
D. hyalina Leydig, 1860	Buttermere, England, UK	DQ132612
D. cristata Sars, 1862	Puruvesi, Ita-Suomen laani, Finland	DQ132613
D. laevis Birge, 1879	Cape Cod, Massachusetts, USA	DQ132614
D. dubia Herrick, 1883 emend. Herrick, 1885	Pond, South of Cornerbrook, Newfoundland, Canada	DQ132615
D. ambigua Scourfield, 1947	Copeland Lake, Colorado, USA	DQ132621
D. pulex Leydig, 1860	Nome, Alaska, USA	DQ132626
D. pulex Leydig, 1860	Horni Luznice, Czech	DQ132622
D. pulicaria Forbes, 1893	Crooked Lake, Indiana, USA	DQ132623
D. dentifera Forbes, 1893	Crane Lake, Indiana, USA	DQ132624
D. dentifera Forbes, 1893	Oh-zuka-Ike, Toyama, Japan	DQ132625
D. magna Straus, 1820	Clone from WARD'S Natural Science, USA	DQ132627

The primer combination of MetF1 and CysR was used for amplification of *D. magna*, MetF2 and TrpR were used for amplification of *D. longispina* and Japanese *curvirostris*, and MetF3 and TrpR were used for amplification of the other species. Each 50 μL PCR reaction consisted of 5 μL extracted DNA, 10× PCR buffer [50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl pH 8.3, 0.01% (w/v) gelatin], 2 mM each dNTPs, 1 μM each primer and 1 U *Taq* DNA polymerase. PCR thermal cycling parameters were 40 cycles of 94 °C for 45 s, 48 °C for 45 s and 72 °C for 1.5 min with RoboCycler (Stratagene), and 45 cycles of 94 °C for 30 s, 48 °C for 30 s and 72 °C for 1.5 min with Peltier Thermal Cycler (MJ Research). PCR products were gel-purified using the Amicon kit for DNA extraction, cycle-sequenced with ABI BigDye Terminators and sequenced in both directions using a capillary-based DNA sequencer (ABI3100). Sequences were assembled, edited with SEQUENCHER 4.2 (Gene Codes Corporation) and aligned manually with SeAl 2.0 (Rambaut, 1996). The alignment length was 965 bp nucleotides and 321 translated amino acids. There was an insertion at nucleotide alignment position 247–249 in *D. curvirostris* from the Midori-ga-ike (Japan) population and the European population, a deletion at nucleotide alignment position 910–912 of *D. longiremis*, and an insertion at nucleotide alignment position 913–915 of *D. longiremis* and *D. longispina*. All indels, which involved three nucleotides (a codon), failed to disrupt the open reading frame.

Phylogenetic analyses

Nucleotide alignments were subjected to minimum evolution (ME), maximum parsimony (MP) and maximum likelihood (ML) in PAUP v4.0b10 (Swofford, 2002), and Bayesian inference (BI) as implemented in MrBayes v.3.0b3 (Huelsenbeck, Rannala & Masly, 2000; Huelsenbeck & Ronquist, 2001). The best-fit GTR + I + G model was selected by hierarchical likelihood ratio tests of the program MODELTEST 3.0 (Posada & Crandall, 1998). ME analysis used the distance matrices of the best-fit GTR + I + G model, and the support was estimated by 1000 bootstrap replicates with tree bisection-reconnection (TBR) branch swapping. MP analysis used a heuristic search with TBR branch swapping, and the support was estimated by 1000 bootstrap replicates with TBR branch swapping. ML analysis was based on the best-fit GTR + I + G model performed by a heuristic search with TBR branch swapping, and the support was estimated by 1000 bootstrap replicates with no branch swapping. BI analysis sampled 10 000 trees from Markov chain Monte Carlo (MCMC) sampling based on the GTR

model with the partition of three codon characters. We removed the first 300 trees found to eliminate variance prior to convergence on the Markov chain, and exposed the remaining trees to 50% majority rule consensus tree analysis in PAUP. Tests of statistical significance of the difference in tree topologies were carried out in PAUP* using the SH test with RELL bootstrapping (1000 replications) (Shimodaira & Hasegawa, 1999).

Amino acid alignments were subjected to MP in PAUP and BI in MrBayes. MP analysis used a heuristic search with TBR branch swapping and was supported by 1000 bootstrap replicates with TBR branch swapping. BI analysis sampled 10 000 trees from MCMC sampling based on the mtREV priors for amino acid sequences. We removed the first 300 trees found to account for variance due to convergence on the Markov chain, and exposed the remaining trees to 50% majority rule consensus tree analysis in PAUP.

To construct trees, a species of the subgenus *Ctenodaphnia* (*Daphnia magna*) was used as the outgroup because previous studies of phylogeny have provided substantial evidence that the subgenus *Ctenodaphnia* is not included in the monophyletic subgenus *Daphnia sensu* Johnson, 1952.

Morphological comparison

No existing paper has compared both DNA sequence and detailed morphological variation in daphniids. Moreover, many recent papers conflict with the International Code of Zoological Nomenclature (ICZN, 2000), leading to problems with the separation of subgenera and the identification of studied species. Our aim was to give as detailed as possible a comparison of all accessible *curvirostris*-like populations from Europe, Asia and North America, with special attention to postabdomens and appendages. Animals were picked from samples preserved in formalin or alcohol, placed on slides (in a drop of a glycerol–formaldehyde mixture) and studied under an optical microscope *in toto*. Then, at least five adult and two juvenile females, and at least two adult males (if present), from each population were dissected for analysis of appendages.

There are two rows of setae on the inner portion of the limbs of all anomopods, named in different styles by different authors. Here, we used the terminology of Alonso (1996) who called these rows of setae 'anterior' and 'posterior'. In this article we apply to *Daphnia* a system of enumeration of setae, earlier suggested for chydorids (Kotov, 2000) and macrothricids (Kotov & Hollwedel, 2004). Anterior setae of (i) inner-distal limb portions and (ii) distal armature of gnathobases are numbered here from distalmost to basalmost elements; posterior setae of the filter plate of gnathobases are lettered, also from distalmost to basalmost elements.

RESULTS

Molecular phylogenetics

The 965 bp nucleotide and 321 translated amino acid (aa) alignments were compared for 16 species sampled from 21 populations. The 965 bp nucleotide sequences had 672 bp variable sites and 629 bp parsimony informative sites. The 321 translated aa sequences had 206 aa variable sites and 183 aa parsimony informative sites. Two best trees of 2816 steps were found from MP searches for nucleotide sequences. One best tree of 920 steps was found from MP searches for amino acid sequences. One best ML tree for nucleotide sequences was found that had a likelihood score of $-\ln L = 11440.4361$. All trees from the nucleotide and amino acid sequences were concordant to the ME bootstrap consensus tree from the nucleotide sequences (Fig. 1).

The taxa with 20 chromosomes and the *longispina* type of claw failed to form a monophyletic group. The *longiremis-cristata* and *laevis-dubia* complexes were found to be basal lineages of the *longispina* group. There was strong support for *D. curvirostris* belonging to the *longispina* group by bootstrap support values (Fig. 1) and an SH test ($-\ln L_{\text{Ml not in longispina}} = 11459.6326$, $P < 0.05$). In contrast, the monophyly of all '*curvirostris*' was unresolved in trees (Fig. 1) and an SH test ($-\ln L_{\text{Ml not monophyly}} = 11441.9384$, $P = 0.505$).

Japanese '*curvirostris*' formed a monophyletic clade that was distantly related to other *curvirostris* in North America and Europe. Four specimens from Midori-ga-ike (the population of the *longispina* type claw) had the same sequence (Midori 1 in Fig. 1), but one had a unique sequence (Midori 2 in Fig. 1). The uncorrected *p*-distance between the two genotypes was 0.1%. All five specimens from Kagami-ike (the population of the *pulex* type claw) had the same sequence (Kagami in Fig. 1). The average *p*-distance between these two populations was 1.5%, while the average *p*-distance between Japanese '*curvirostris*' and other *curvirostris* in North America and Europe was 35%.

The chromosome number of the *longispina* group appeared to change twice on the tree (Fig. 2). The change of chromosome number from $2n = 20$ to $2n = 24$ occurred in the lineage leading to the *Daphnia pulex* group, whereas the change of chromosome number from $2n = 20$ to $2n = 22$ occurred in the lineage leading to the Japanese '*curvirostris*'.

The tree also indicated that the ancestral claw with pronounced pectens has been regained in *Daphnia curvirostris*. The variable claw states between the ancestral and the *longispina* types were shared between closely related populations of the Japanese '*Daphnia curvirostris*' (1.5% divergence).

Our results show that Japanese '*D. curvirostris*' is a very divergent lineage (35%) from European

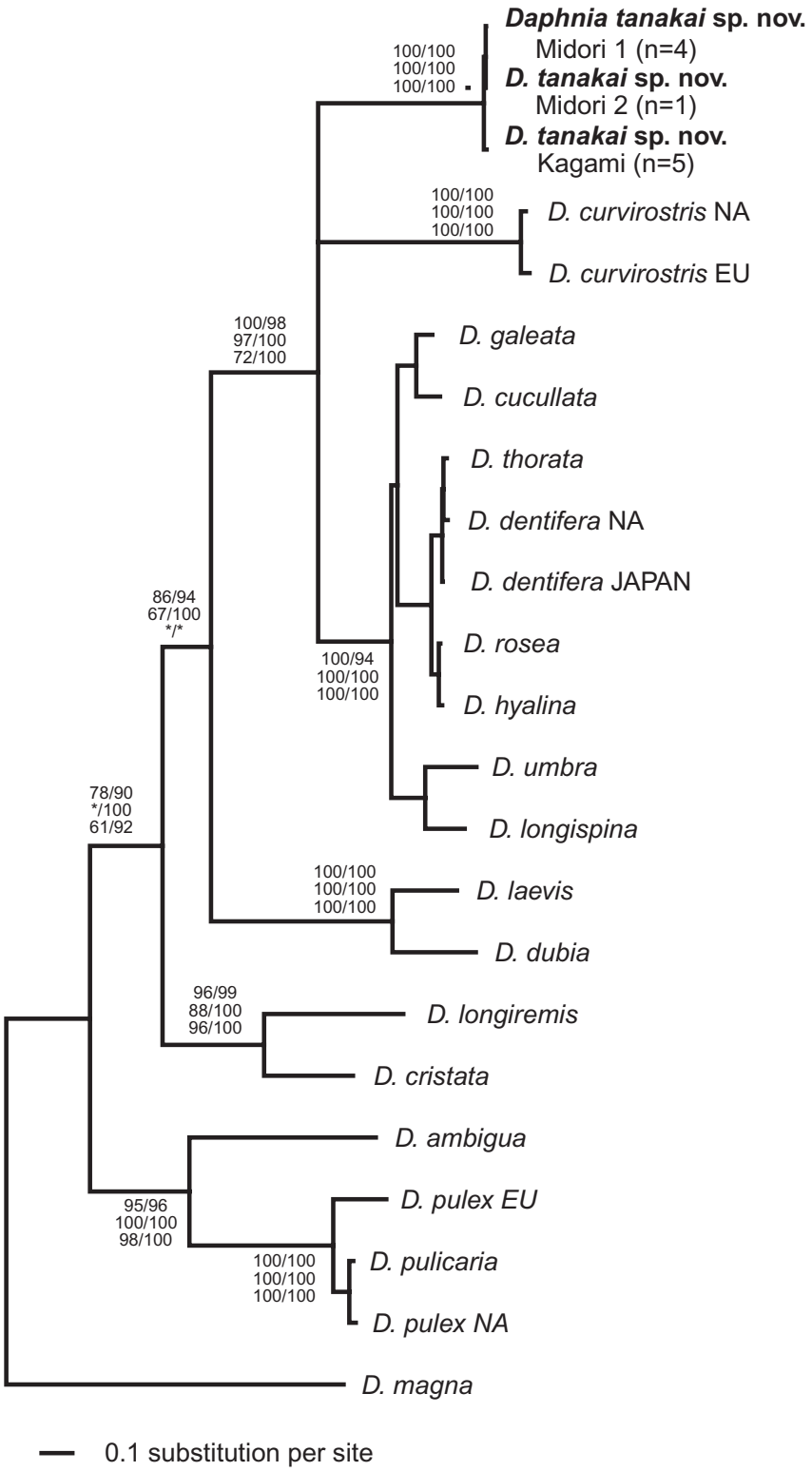

Figure 1. ME bootstrap consensus tree of *Daphnia* ND2 sequences. The numbers on each branch show support values of the branch. Upper numbers indicate ME, and ML bootstrap support values for nucleotide sequences. Middle numbers indicate MP bootstrap support values and Bayesian clade credibility values for nucleotide sequences. Lower numbers indicate MP bootstrap support values and Bayesian clade credibility values for amino acid sequences. Asterisks indicate no support values.

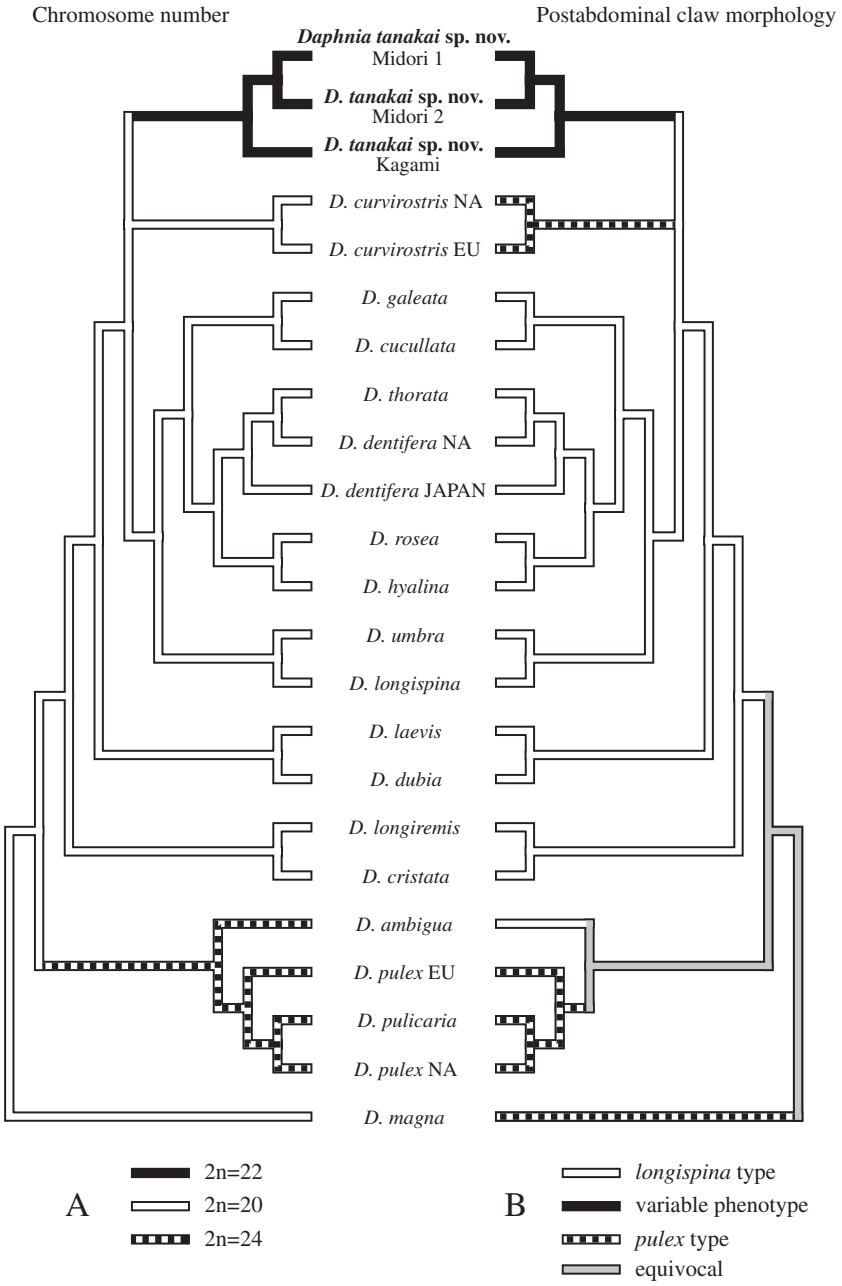

Figure 2. Mapping the characters of chromosome number and postabdominal claw morphology onto the *Daphnia* ND2 consensus tree (Fig. 1). A, the left cladogram shows the evolution of chromosome number. Black line denotes $2n = 22$, white line denotes $2n = 20$ and dot line denotes $2n = 24$. B, the right cladogram shows the evolution of postabdominal claw morphology. Black line denotes variable phenotype between the *longispina*-claw and *pulex*-claw types, white line denotes the *longispina*-claw type, dot line denotes the *pulex*-claw type and grey line denotes equivocal.

or North American *Daphnia curvirostris s.s*. The Japanese lineage is potentially one of the oldest lineages in the subgenus *Daphnia* and shows more divergence from *D. curvirostris* than is found in the proposed 'subgenera' *Daphnia* and *Hyalodaphnia* (i.e. the *pulex* group, see Fig. 1). In addition to genetic divergence, the Japanese lineage possesses a unique chromosome number and many diagnostic morphological characters that separate it from *D. curvirostris*. We therefore described this lineage as a new species, *Daphnia tanakai* sp. nov.

TAXONOMY

(1) DAPHNIA CURVIROSTRIS EYLMANN, 1887 EMEND JOHNSON, 1952

Daphnia curvirostris Eylmann, 1887: 17–19; Richard, 1896: 264–267, plate 23, figs 7 and 15,16,17; Johnson, 1952: 448–450, figs 4(b), 6(a)–(d) and 9(b); Hrbáček, 1959: 124–125, figs 4(B) and 5(A); Šrámek-Hušek, Straškraba & Brtek, 1962: 213–214, fig. 75(A)–(F); Flössner, 1972: 129–130, fig. 57(A)–(C); Margaritora & Ferrara, 1974: 9–12, figs 4, 5, 6, 7, 8, 9, 10, 11; Margaritora, Stella & Mastrantuono, 1977: 161; Negrea, 1983: 117–120, figs 44, 45; Hollwedel & Poltz, 1985: 57–59, fig. 5; Margaritora, 1985: 130–133, fig. 54(A)–(G); Flössner, 1986: 8–10, figs 2, 3; Glagolev, 1986: 56–58, fig. 1(A)–(D); Hebert & Loaring, 1986: plate 2, figs 7, 8, 9, plate 3; Glagolev, 1995: 55–56, plate 47, figs 7, 8, 9, 10, 11, 12; Alonso, 1996: 155–157, fig. 69; Flössner, 2000: 158–160, fig. 59(A)–(J).

Not *Daphnia curvirostris* Eylmann in Tanaka & Tominaga, 1986: 35–42, figs 2–7; Tanaka, 1997: 57–58, fig. 3.

? *Daphnia whitmani* Ishikawa, 1895: 147–153, plate 22, figs 1–5.

Type locality

"Hannover: Graben mit viel pudrescirenden Substanzen in der Landdrosfei Stade", Lower Saxony, Germany.

Type material

Apparently lost.

Material examined

Germany: A small man-made pool on Juist Island, coll. 06.vii.1995 by W. Hollwedel. Romania: Lac Popina, Ascunsa, coll. 11.v.1966 by S. Negrea. Georgia: Puddle near River Bzyb′, region of Pistunda, Abkhazia, coll. 31.x.1980 by M. B. Berkinblit. Ukraine: A fish pond, L'vov Area, unknown collector. Lake Chernoe, near Petrushki, Kiev Area, coll. 15.viii.1985 by A. Shkrabaluk. Pools near River Desna, town of Chernigov, coll. 01.viii.1987 by M. Rodionov. Un-named lake near Dnestr, Zaporozhje, coll. 30.viii.1987 by D. Sil'chenko. Lakes Pervij Liman and Vtoroj Liman, Slaviansk, Donetsk Area, coll vii.1985 by O. Y. Lisatchev. A pond in town of Lugansk and a tributary of the Lugan′ River, Lugansk Area, coll. 05.ix.1987 by E. Belostotskaya. Russia: Several water bodies in delta of the Volga River, Astrakhan Area, coll. in 1977–82 by unknown collector. A pool in delta of Volga, near Krit, Volgograd Area, coll. V. Smirnov. A pond in UAZ factory, Ulyanovsk, coll. 08.vii.1985 by D. Sedekhmenov. Ponds near River Kama, Nabereznije Chelni, Tatarstan Autonomous Republic, coll. 26.vii.1985 by A. V. Gladushevsky. Two puddles in town of Cheremshan, Tatarstan Autonomous Republic, coll. 15.viii.1985 by R. Aleeva. Lake Chirtovo, Tver Area, coll. 02.vii.1982 by E. Mnatsakanova. A pool in Bitsa forestpark, town of Moscow, coll. 27.ix.1982 by A. V. Matveev. Lake Glubokoe, Moscow Area, coll. vii–ix.2004 by A. A. Kotov. Two pools in town of St Petersburg, coll. 02.vi.1985 by A. V. Makrushin. A puddle near shore of White Sea, near Kem′, Karelian Autonomous Republic, coll. viii.1985 by A. S. Kondrashev. Several rockpools in islands of Keretskij and Kem'ludskij archipelagos in White Sea, coll. in 2002–04 by S. M. Glagolev. A small sandy lake near Puiko, Yamal Peninsula, coll. 04.viii.1908 by B. M. Zhitkov. Ponds near Irkutsk and in this town, coll. in 1983–85 by A. Y. Nikitina. Pools near Lake Baikal, coll. 19–20.viii.1982 by S. M. Glagolev. USA: Pilgrim Hotsprings, Seward Peninsula, Alaska, coll. 12.xiii.2003 by D. J. Taylor.

Short emended diagnosis

Female. Body subovoid, caudal spine well developed. Rostrum long, with bent tip. Spinules cover no more than 1/2 of ventral margin of carapace and no more than 1/4 of its dorsal margin. First abdominal process long, bent anteriorly, second process bent distally, third process massive. Postabdominal claw long, the proximal pecten consisting of 8–10 stout, thin teeth, the second pecten consisting of 10–14 large teeth. Antenna I with completely reduced body, a short antennular sensory seta arising immediately from head surface. Limb I with a long anterior seta 1, long seta 2, very short seta 3 and short seta 4. Limb II with long anterior seta 1.

Ephippium with axes of eggs perpendicular to its dorsal margin, postero-dorsal portion of valves with caudal spine incorporated into ephippium.

Adult male. Head with well developed rostrum. Abdomen without processes on three distal segments, basalmost segment with a small process. Postabdomen shape and armature in general as in female, gonopore opens subdistally, without a genital papilla. Antenna I relatively short, antennular sensory seta thin and short; flagellum on top of a conical, postaesthetasc process, its distal segment with a hooked tip. Inner distal lobe (IDL) of limb I with a bent copulatory hook, and two setae of different size; endite 3 with four setae, anterior setae 3 and 4 large that these in female. On distalmost endite of limb II, anterior seta 1 hook-like, setulated distally, setules on basal portion of distal segment relatively robust.

Size. Females up to 2.9 mm, males 0.8–1.2 mm.

Redescription

Adult parthenogenetic female. Body subovoid in lateral view, maximum height in the middle (Fig. 3A). Dorsal margin of valves slightly elevated above head, regularly convex, a shallow depression between head and rest of body. Postero-dorsal angle with a well developed caudal spine (Fig. 3B), ventral margin convex. Head with a long rostrum, in lateral view, its tip noticeably bent, and subdividing into two lobes by a 'line' of prerostral fold; posterior margin of head slightly convex; ventral margin of head with a depression expressed in different extension in different specimens from a single population (Fig. 3C–E). No crest or large helmet on head, compound eye large, ocellus small and located far from base of antenna I. Labrum with a short, fleshy main body and a large, setulated distal labral plate (Fig. 3C).

Carapace subovoid, the spinules usually cover no more than 1/2 of its ventral margin and no more than 1/4 of its dorsal margin (less frequently up to 1/3). In postero-ventral portion of valve, on inner face of valve, a row of setules, organized in short series (Fig. 3F); at posterior portion of valve, each series terminating in a setulated spine (Fig. 3G).

Abdomen relatively short, consisting of four segments. The first (basalmost) abdominal process especially long, slightly bent anteriorly, the second (middle) process well developed, characteristically bent distally; the third (distalmost) process globose; the fourth segment lacking a process (Fig. 3H). Postabdomen elongated, tapering distally, with ventral margin almost straight and lacking setules. Preanal margin long, almost straight, with series of minute setules. Preanal and postanal angle not expressed. Paired spines on postanal and anal portion, their size continuously increasing distally. Postabdominal seta approximately as long as preanal margin, its distal segment shorter than basal one. Postabdominal claw long, regularly bent, with a pointed tip (Fig. 3I, J). On outer side, three successive pectens along the dorsal margin: the first (proximal) pecten consisting of 8–10 stout, thin teeth; the second (medial) pecten consisting of 10–14 large teeth; the third pecten consisting of numerous fine setules, not reaching the tip of claw. Fine denticles at middle of ventral margin, and at distal end of medial pecten.

Antenna I with completely reduced body, nine aesthetascs (of different length) and a fine, short antennular sensory seta arising immediately from head surface (Fig. 3K, L). Antenna II with coxal part possessing two short sensory setae of different length (Fig. 4A). Basal segment elongated, with a small distal sensory seta at posterior face (inner apical seta in Kořínek & Villalobos, 2003) (Fig. 4B, arrow). Antennal branches elongated, 4-segmented exopod slightly shorter than 3-segmented endopod, all with numerous series of denticles, especially long, delicate setules on distal segment of endopod (Fig. 4C). Antennal formula: setae 0-0-1-3/1-1-3. Each swimming seta with basal and distal segments bilaterally setulated, a weakly pigmented insertion within distal segment near joint with basal segment (Fig. 4D, arrow). Spines on apical segments rudimentary. Spine on the second segment of exopod rudimentary.

Maxilla II as a lobe with three fully setulated setae (Fig. 4E).

Limb I with ovoid epipodite; 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 very 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, globular epipodite; 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. 4G: 1) almost as long as each of two other setae on this endite, armed with fine setules distally (Fig. 4H). Gnathobase with two clear rows of setae: four anterior setae (Fig. 4I: 1–4) and 10–11 posterior setae of gnathobasic 'filter plate' (a–j).

Limb III with a large pre-epipodite, subglobular epipodite and a flat exopodite bearing four distal and two lateral setae (Fig. 4J). Inner-distal portion of limb with four endites: endite 4 with a single anterior seta (Fig. 4K: 1) and a posterior (a) seta; endite 3 with a single anterior seta (2) and a single posterior (b) seta; endite 2 with a rudimentary anterior seta (3) and two posterior setae (c, d); endite 1 with a large anterior seta (4) and four posterior (e–h) setae. Small sensillae near setae 3 and 4. The rest of limb inner portion as a singular large lobe, bearing numerous (45–54 in large adults from Glubokoe Lake) posterior soft setae (Fig. 4L) and a single, relatively long anterior seta (Fig. 4K: 1) in its distal corner. This limb part probably represents a modified gnathobase III.

Limb IV with a large, setulated pre-epipodite, ovoid epipodite and wide, flat exopodite, with protruding and setulating inner-distal angle, and bearing four distal and two lateral setae (Fig. 4M). Inner-distal portion of this limb with completely fused endites, distally with 2 setae of unclear homology (Fig. 4N), the most part of the limb inner margin is a gnathobase filter plate consisting of numerous (38–48 in adults from Glubokoe Lake) posterior setae.

Limb V with a setulated pre-epipodite, subovoid epipodite, triangular exopodite bearing two small distal

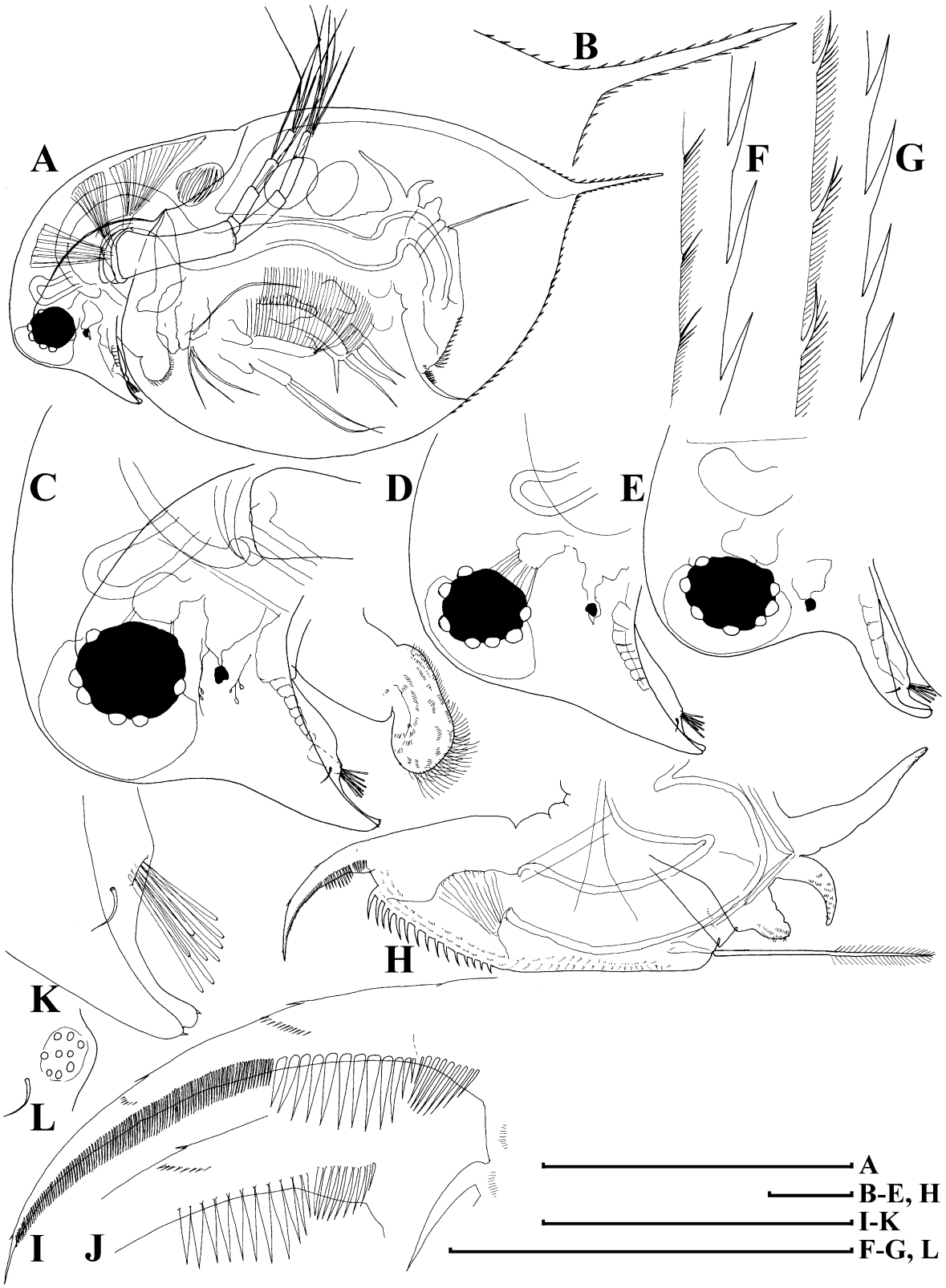

Figure 3. *Daphnia curvirostris*, large parthenogenetic female from Lake Glubokoe, Moscow area, European Russia, collected on August 9, 2004 by AAK. A, lateral view. B, caudal spine. C–E, head. F, G, armature of postero-ventral and posterior region of valve. H, postabdomen. I, J, postabdominal claw. K, L, antenna I in lateral and posterior view.

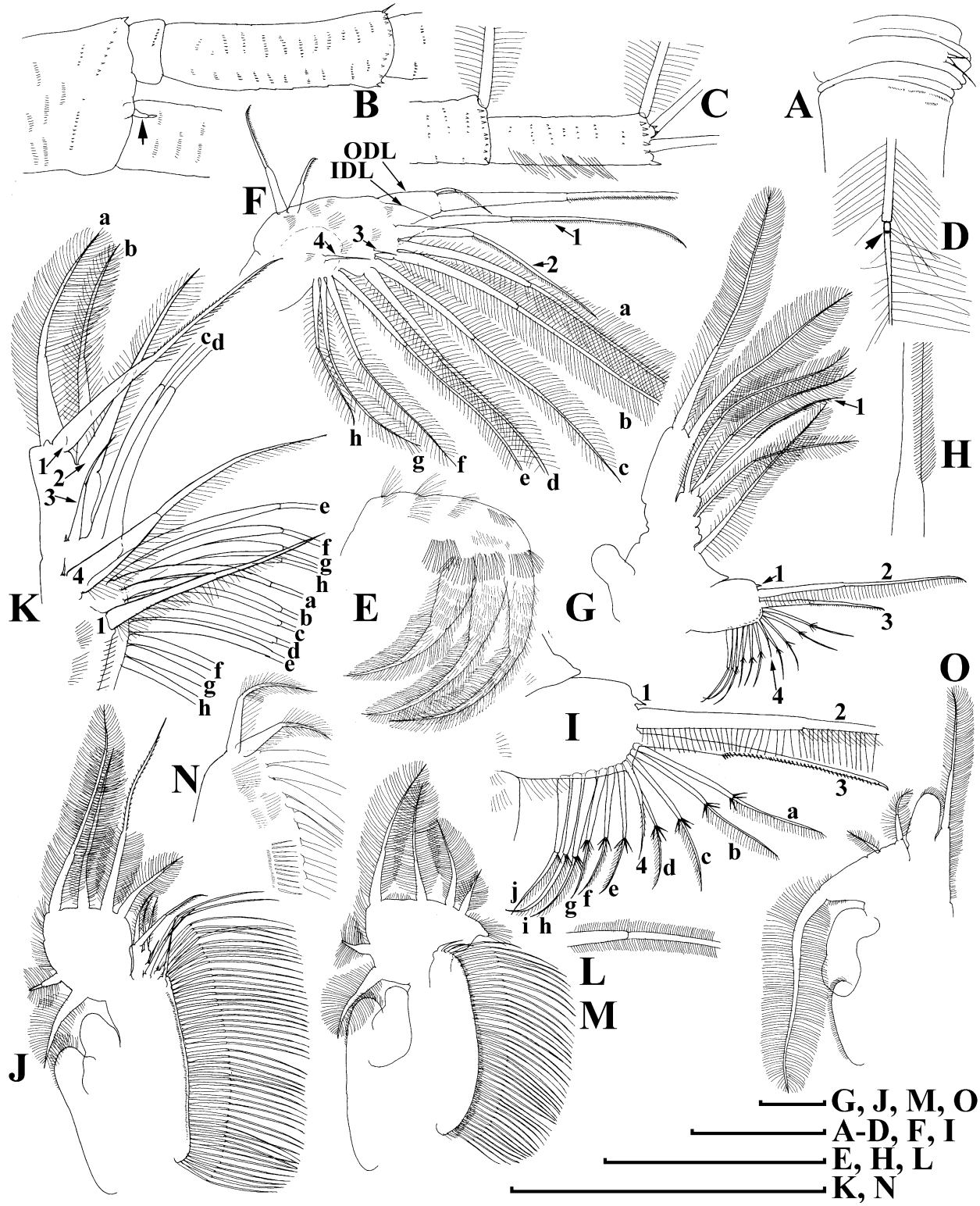

Figure 4. *Daphnia curvirostris*, appendages of parthenogenetic female from Lake Glubokoe, European Russia. A, coxal part of antenna II. B, distal portion of basal segment and basal portion of branches. C, distal portion of endopod. D, swimming seta. E, maxilla I. F, limb I: ODL indicates outer distal lobe; IDL indicates inner distal lobe. G–I, limb II, second seta on its inner-distal end, and gnathobase II. J–L, limb III, its inner-distal portion and filtering seta of gnathobase. M, N, limb IV and its inner-distal portion. O, limb V.

setae and a large lateral seta (Fig. 4O). Inner limb portion as an ovoid flat lobe, with setulated inner margin and a single, large seta.

Ephippial female. In contrast to parthenogenetic female, dorsal margin of valves almost straight (Fig. 5A), dorsal wall of carapace additionally chitinized, forming a dorsal plate, covered with fine spinules (Fig. 5B). Ephippium with two resting eggs, axes of which perpendicular to its dorsal margin, egg chambers well separated from each other (Fig. 5C), most part of ephippium additionally darkly pigmented and covered with sculpturing of polygonal cells, postero-dorsal portion of valves with caudal spine incorporated into ephippium.

Adult male. Body subovoid, dorsal margin of valves almost straight, not elevated above head, shallow depression between head and valves, postero-dorsal angle distinct, with a short caudal spine (Fig. 5D). Head with a well developed rostrum, region of antenna I joint with a distinct depression (Fig. 5E, arrow). Anteriormost extremity completely occupied with optic vesicle, a shallow supra-occular depression posteriorly to it. Eye large, ocellus small, but relatively larger than that in female.

Valve with antero-ventral angle distinctly prominent posteriorly, whole ventral margin with long, numerous setae submarginally on inner face of valve (Fig. 5F, G). Postero-ventral portion of valve with marginal denticles, short setae located submarginally on inner face of valve, rows of fine setules between these setae (Fig. 5H).

Abdomen without processes on three distal segments, basalmost segment with a small process (Fig. 5I, J, arrow). Postabdomen shape and armature in general as in female, but preanal margin shorter and preanal angle expressed. Paired teeth small. Gonopore opens subdistally, without a genital papilla. On outer surface of postabdominal claws, a basal pecten of fine setules, second pecten of 6–7 teeth increasing in size distally, third pecten consisting of fine, numerous setules.

Antenna I relatively short for a *Daphnia* male, slightly and regularly curved, with series of fine setules (Fig. 5E); antennular sensory seta thin, reaching distal end of antenna I body (Fig. 5K, arrow); aesthetascs of different length, largest aesthetasc longer than antenna I maximum diameter. Male seta (flagellum) on top of a conical, distal (postaesthetasc) process. This seta long, bisegmented, its distal segment with a hooked tip. Antenna II thin, with groups of short setules on endopod distal segment (Fig. 5L).

Limb I: ODL large (Fig. 5M), bearing a rudimentary seta and a very large seta (Fig. 5N) supplied with minute setules distally (Fig. 5O); IDL with a bent copulatory hook, and two setae of different size (Fig. 5M: 1 and 1′); in contrast to female, endite 3 with four setae (additional seta of unclear homology marked as 2′), seta 2 shorter than that in female, setae 2 and 1 larger than those in female.

Limb II: distalmost endite with a modified, hook-like anterior seta 1, setulated distally, setules on basal portion of distal segment relatively robust (Fig. 5P: 1).

Size. Length of adult females from Glubokoe Lake 1.35–1.75 mm (without caudal spine), males 0.97–1.07 mm; range of female size for all studied Palaearctic populations 1.07–2.88 mm (Glagolev, 1986); females 0.7–2.9 mm, males 0.8–1.2 mm according to Flössner (2000).

Taxonomic comments

Although Eylmann's (1887) description was relatively detailed, subsequent authors confused his *D. curvirostris* with *D. pulex*. Johnson (1952) was the first investigator to demonstrate unequivocally that *D. curvirostris* is a distinct species, and reported a set of its diagnostic traits. Flössner (2000) listed *D. curvirostris* var. *insulana* Richard, 1896 and *D. longispina* var. *simulans* Sars, 1903 as junior synonyms of *D. curvirostris*, but to our mind, both these taxa were described too superficially for any conclusions about conspecifity.

Distribution

Daphnia curvirostris is chiefly a Palaearctic species from temporary water bodies and is common in many European countries, i.e. Spain (Alonso, 1996), Italy including Sardinia (Margaritora, 1985), Czech Republic (Šrámek-Hušek *et al*., 1962), Romania (Negrea, 1983), Germany, Sweden, southern Norway (Flössner, 2000), British Isles and Corfu (Greece) (Johnson, 1952). The species is found in some Mediterranean Asian countries, i.e. Turkey (Margaritora *et al*., 1977) and Israel (Flössner, 2000). *Daphnia curvirostris* is distributed in Russia from the St Petersburg area to the Far East, preferring the southern half of the country (Glagolev, 1995). It is also reported from Mongolia (Flössner, 1986). In North America, *D. curvirostris* is very rare and recorded from only a few ponds in Tuktoyaktuk and Old Crow in north-western Canada (Hebert & Loaring, 1986). Duffy *et al*. (2000) provided genetic evidence that *D. curvirostris* had invaded Onondaga Lake in upstate New York. Here, we report that *D. curvirostris* is also present in north-western Alaska in a thermally disturbed, high conductivity pond (26.7 mS/cm) at the Pilgrim Hotsprings (65°5′13″N, 164°55′20″W). Records from African mountains (Harding, 1957; Mergeay, Verschuren & De Meester, 2005) exist but these designations need confirmation with direct morphological or genetic comparisons with *D. curvirostris*.

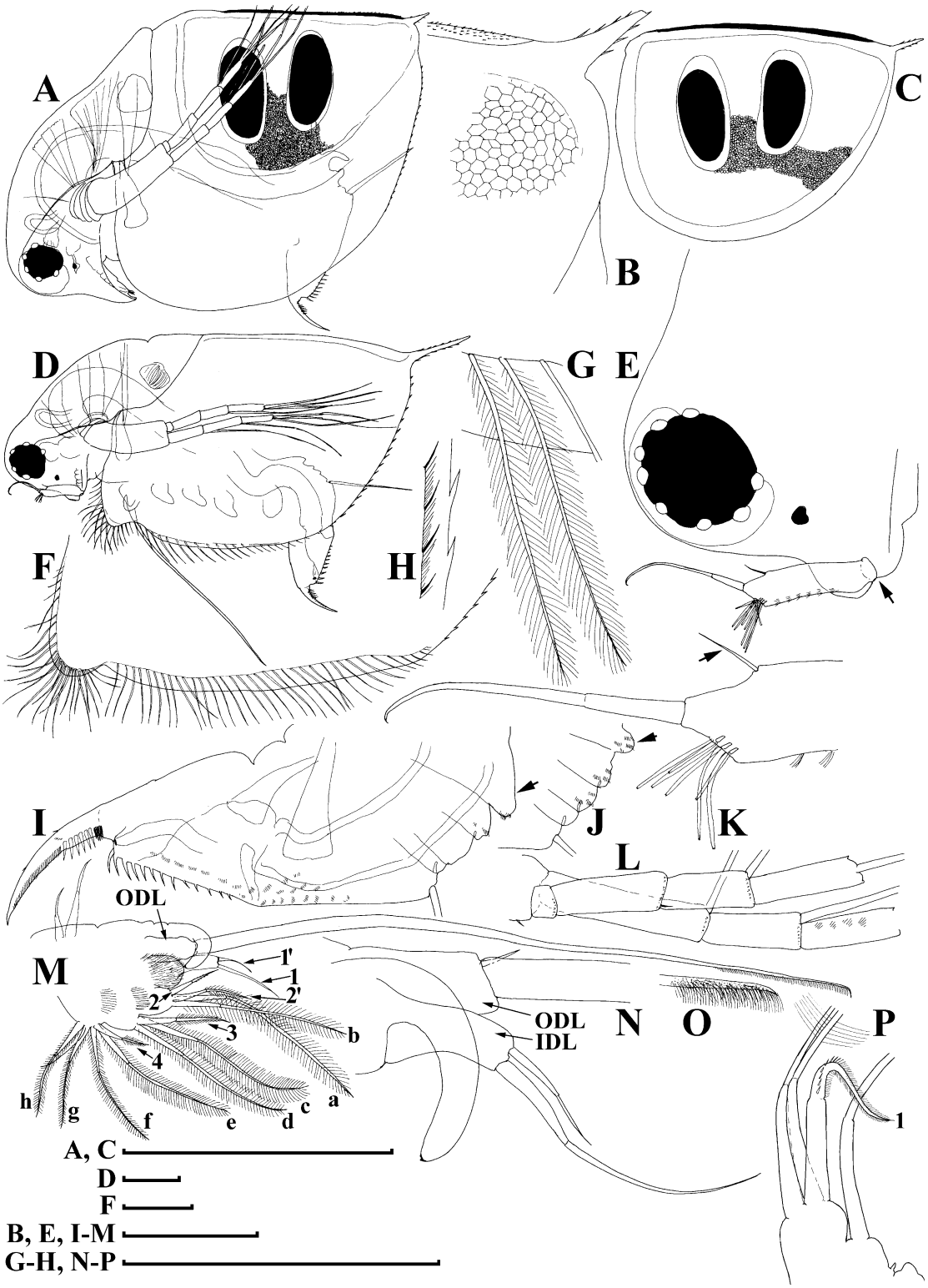

Figure 5. *Daphnia curvirostris* from Lake Glubokoe, Moscow area, European Russia, collected on September 9, 2004 by N. N. Smirnov. A, B, ephippial female and its postero-dorsal region. C, fresh ephippium. D, adult male. E, male head. F, G, armature of ventral margin of valve. H, armature of posterior portion of valve. I, J, postabdomen and abdomen. K, antenna I. L, antenna II. M, N, limb I and its distal portion. O, armature of distal portion of largest seta of outer distal lobe. P, inner-distal portion of limb II.

(2) **DAPHNIA TANAKAI SP. NOV.**

Daphnia ambigua Scourfield in Uèno & Tanaka, 1960: 296, figs 1, 2.

Daphnia curvirostris Eylmann in Tanaka & Tominaga, 1986: 35–42, figs 2–7; Tanaka, 1997: 57–58, fig. 3; Tanaka, 1998: 30, fig. 1(A), (B).

Not *Daphnia whitmanni* Ishikawa, 1895: 147–153, plate 22, figs 1–5.

Etymology

This species is dedicated to Dr S. Tanaka, renowned Japanese cladocerologist, who found this species and supplied us with a part of material.

Type locality

Lake Midori-ga-ike, Hida Mountain Range, Honshu Island, Japan. This is a medium-sized (maximum length 157 m), shallow (maximum depth 1.65 m) mountain lake located at 2430 m above sea level (36°34′39″N, 137°36′1″E). The type series was collected on 30 viii.2004 by S. Tanaka.

Holotype

One female (1.5 mm in body length), deposited at the National Science Museum, Tokyo, Japan, catalogue number NSMT-Cr 16117.

Allotype

One male, deposited at the National Science Museum, Tokyo, Japan, catalogue number NSMT-Cr 16118.

Paratypes

Thirty females and males, deposited at the National Science Museum, Tokyo, Japan, catalogue number NSMT-Cr 16119; 30 females and males, deposited at the Zoological Museum of the Moscow State University, Moscow, catalogue number MGU Ml 34; about 30 females and males in the personal collection of AAK, catalogue number AAK 2004–056.

Other material examined

Japan, Honshu Island, Hida Mountain Range: Lake Midori-ga-ike, coll. 09.ix.1979 by S. Tanaka. Lake Kagami-ike, coll. 01.ix.2004 by S. Tanaka.

Short diagnosis

Female. Body subovoid, caudal spine completely absent or short. Rostrum relatively short. Spinules on ventral and dorsal margin normally completely reduced, or present only in region of postero-dorsal angle. First abdominal process relatively long, slightly bent, second process short, the third small and rounded. Postabdominal claw long, size of teeth in basal and, especially, medial pecten varies significantly between populations from *longispina* to *pulex* type. Antenna I with almost completely reduced body. Limb I anterior setae 3 and 4 larger than similar setae in *D. curvirostris*. Limb II with anterior seta 1 about 3/4 length of posterior seta.

Ephippium with axes of eggs perpendicular to its dorsal margin, postero-dorsal portion of valves not incorporated into ephippium.

Adult male. Head with reduced rostrum. Abdomen with a process on second (from distal end) segment. Postabdomen with convex ventral margin, gonopore opens subdistally, without a genital papilla. Antenna I short, antennular sensory seta short and thin, flagellum with slightly curved, hooked tip. Limb I with stiff setae 2–3 times larger than in *D. curvirostris*. Limb II: on inner-distal portion, the anterior seta 1 slightly bent.

Size. Females up to 1.79 mm, males 0.95–1.13 mm.

Description

Adult parthenogenetic female. Body subovoid in lateral view, maximum height in middle (Fig. 6A). Dorsal margin of valves slightly elevated above head, slightly convex, a shallow depression between head and rest of body. Postero-dorsal angle well expressed, but caudal spine completely absent or very short. Head with rostrum well developed, but significantly shorter than that in *D. curvirostris*, in lateral view, its tip projected posteriorly, not subdividing into two lobes; posterior margin of head convex. Compound eye large, ocellus small and located far from base of antenna I.

Carapace subovoid, in large females spinules on ventral and dorsal margin normally completely reduced, but if caudal spine expressed, these spinules present on it and in region of postero-dorsal angle. In postero-ventral portion of valve, on inner face of valve, a row of setules, organized in short series (Fig. 6C); at posterior portion of valve, each series terminating in a setulated spine (Fig. 6D), finer than that in *D. curvirostris*.

Abdomen relatively short, consisting of four segments. The first (basalmost) abdominal process relatively long, but shorter than that in *D. curvirostris*, slightly bent anteriorly, the second (middle) process short, the third (distalmost) small and rounded; the fourth segment lacking a process (Fig. 6E). Postabdomen with preanal margin long, almost straight, covered with series of minute setules. Preanal and postanal angle not expressed. Paired spines on postanal and anal portion, their size continuously

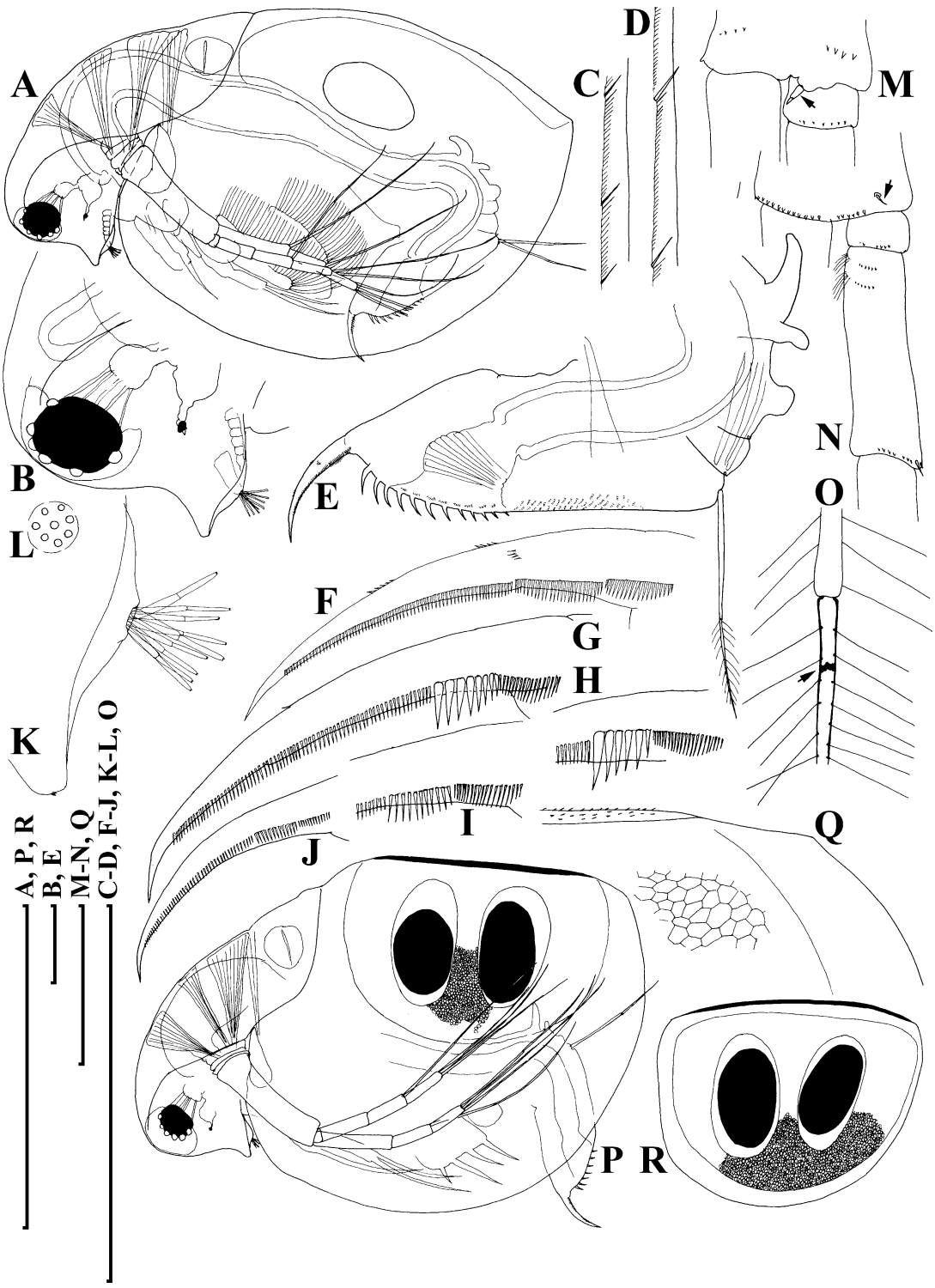

Figure 6. ***Daphnia tanakai*** **sp. nov.** from Lake Midori-ga-ike, collected on August 30, 2004 by S. Tanaka (A–F, K–O) and Lake Kagami-ike, collected on September 01, 2004 by S. Tanaka (G–J, P–R); both lakes are in Hida Mountain Range, Honshu Island, Japan. A, parthenogenetic female, lateral view. B, head of parthenogenetic female. C, D, armature of postero-ventral and posterior region of valve. E, postabdomen. F–I, postabdominal claws of adults. J, postabdominal claw of juvenile. K, L, antenna I in lateral and distal view. M, N, distal portion of basal segment in posterior and anterior view. O, swimming seta. P, Q, ephippial female and postero-dorsal portion of its carapace. R, ephippium.

increasing distally. Postabdominal seta approximately as long as preanal margin, its distal segment shorter than basal one. Postabdominal claw long, regularly bent, with a pointed tip. On outer side, three successive pectens along the dorsal margin, but size of teeth in basal and, especially, medial pecten varies significantly between populations, and even within a single population, from *longispina* type to *pulex* type. All females from Lake Midori-ga-ike have postabdominal claws of *longispina* type (Fig. 6F), in contrast, majority of females from Lake Kagami-ike had *pulex* type claws (Fig. 6G, H), while small part has intermediate claws (Fig. 6I); finally, some juveniles were of *longispina* type (Fig. 6J). All females have fine rows of denticles at middle of ventral margin, and at distal end of medial pecten.

Antenna I with almost completely reduced body, nine aesthetascs of different size arising immediately from head surface, antennular sensory seta not found (Fig. 6K, L). Antenna II in general as in previous species. A small sensory seta posteriorly at distal margin of basal segment (Fig. 6M, arrow), a small distal seta was found at its anterior face (Fig. 6N, arrow). A weakly pigmented insertion within distal segment of swimming seta (Fig. 6O, arrow) located further from joint with basal segment compared with *D. curvirostris*. Spine on the second segment of exopod also rudimentary.

Limb I (Fig. 7A, B) with outer distal lobe had the second (smaller) seta larger, anterior seta 2 asymmetrically armed (Fig. 7C). Anterior setae 3–4 (Fig. 7D) larger than similar setae in *D. curvirostris*, each accompanied by a minute sensillum. Limb II (Fig. 7E) with anterior seta 1 (Fig. 7F) shorter than that in *D. curvirostris*, and armed with shorter setules (Fig. 7G). Gnathobase II with shorter setules on distal segment of seta 2, and small denticles on seta 3, 10 posterior setae of gnathobasic 'filter plate' (Fig. 7H: a–j). Limb III (Fig. 7I) very similar to that of *D. curvirostris*, but in its inner portion (Fig. 7J) posterior seta 1 armed in different way, seta 3 rudimentary, seta 4 relatively short; gnathobasic seta 1 with naked basal segment and short setules distally, a small sensillum near it, 44–49 posterior soft setae in filter plate III. Limb IV as in previous species, 36–41 posterior setae in filter plate IV (Fig. 7K, L). Limb V as in previous species, but distalmost seta of exopodite armed distally with short setules (Fig. 7M, N).

Ephippial female. Dorsal margin of valves almost straight. Dorsal wall of carapace was additionally chitinized, formed a dorsal plate, had fine spinules (Fig. 6P, Q). Ephippium with two resting eggs, axes of which perpendicular to its dorsal margin (Fig. 6R), postero-dorsal portion of valves not incorporated into ephippium.

Adult male. Body low, subquadrangular, dorsal margin of valves straight, not elevated above head, shallow depression between head and valves (Fig. 8A), postero-dorsal angle distinct, with a distinct caudal spine protruding postero-dorsally, minute spinules on the spine and dorsal margin of carapace (Fig. 8B). Head with reduced rostrum (Fig. 8C).

Valve with antero-ventral angle slightly prominent ventrally, whole ventral margin with long, numerous setae submarginally on inner face of valve (Fig. 8D), rows of fine setules at posterior margin of valve on its inner face, organized in series (Fig. 8E).

Abdomen with a process on second (from distal end) segment (Fig. 8F, arrow). Postabdomen with convex ventral margin, preanal angle smoothed, paired teeth small. Gonopore opens subdistally, without a genital papilla. Only males with postabdominal claws of *longispina* and intermediate types were found (Fig. 8G).

Antenna I short for a *Daphnia* male, almost straight, antennular sensory seta short and thin, not reaching base of male seta (flagellum) (Fig. 8C, H), which is as long as body of antenna I, with slightly curved distal portion, supplied with minute setules and a spinule at its tip (Fig. 8I).

Limb I with a wider copulatory hook (Fig. 8J, K), both setae of IDL and anterior setae 2–3 significantly larger than those in *D. curvirostris*. On inner-distal portion of limb II, the anterior seta 1 asymmetrically setulated distally, slightly (Fig. 8L) moderately (Figs 8M, O) or significantly (Fig. 8N) bent.

Size. Juvenile and adult females from Lake Midori-ga-ike 0.64–1.79 mm, ephippial females 1.35–1.75 mm, ephippium 0.70–0.78 mm, adult males 0.95–1.13 mm according to our measurements; adult parthenogenetic females from the same lake 1.64 ± 0.11 mm according to Tanaka & Tominaga, 1986).

Differential diagnosis

Daphnia tanakai sp. nov. and *D. curvirostris* Eylmann, 1887 are superficially similar in morphology, but *D. tanakai* sp. nov. is unique in the following characteristics: (1) short rostrum, not subdivided into two lobes by fornix line in lateral view; (2) no denticles on posterior portion of valves; (3) all postabdominal processes shorter; (4) size of teeth in two basal pectens on postabdominal claw varies significantly from *longispina* to *pulex* type; (5) on limb I, anterior seta 3 normally developed; (6) anterior seta 1 on distalmost endite of limb II short; (7) on limb III, seta 3 rudimentary. In addition, the male of *D. tanakai* sp. nov. has: (8) a reduced rostrum; (9) a postabdomen with inflated ventral margin; (10) an abdomen with a process on second segment (from basal side); (11) a sensory seta on antenna I very short, and not reaching bases of male seta (flagellum); (12) on limb I, large anterior

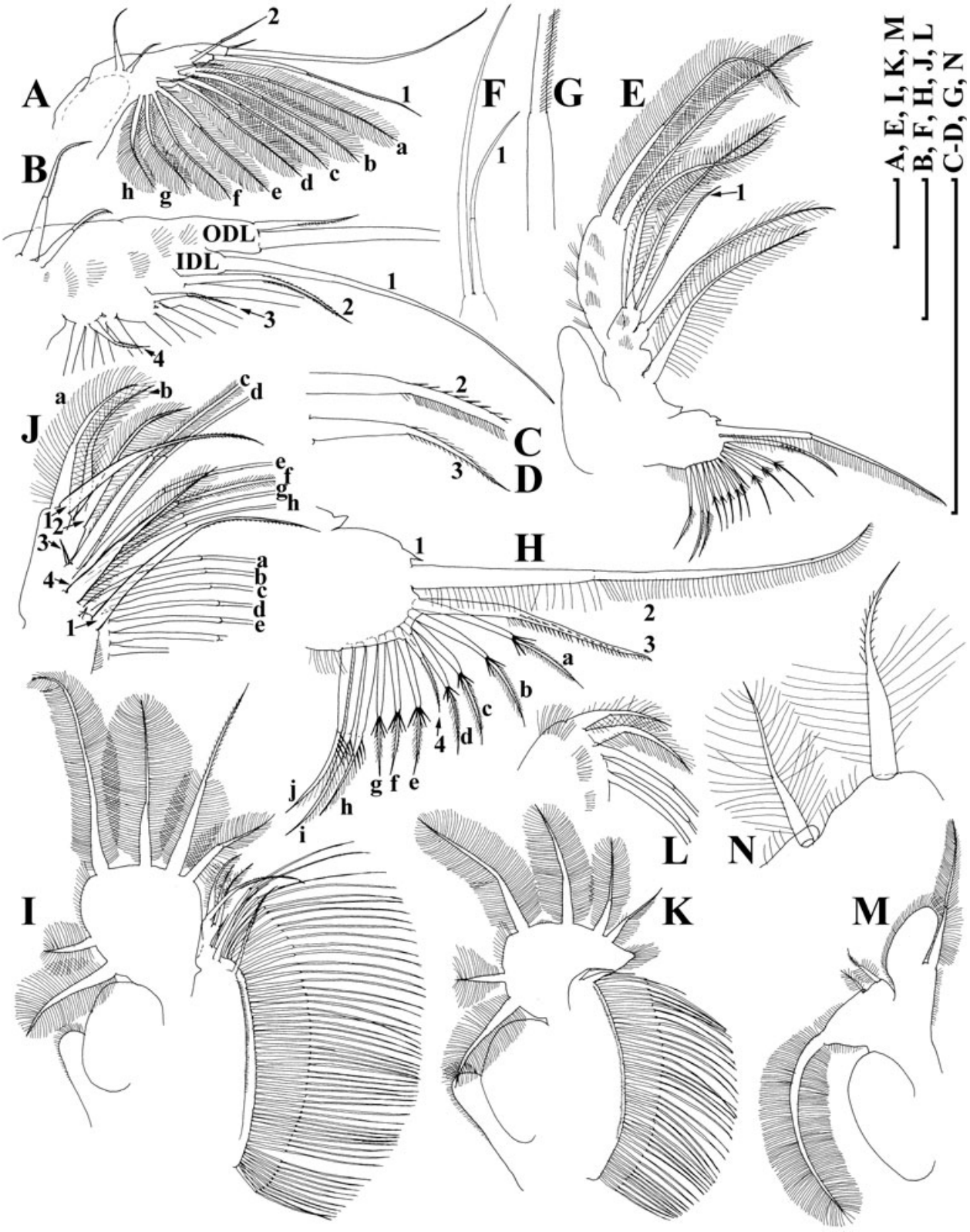

Figure 7. ***Daphnia tanakai*** **sp. nov.**, thoracic limbs of parthenogenetic female from Lake Midori-ga-ike, Japan. A, B, limb I. C, D, anterior seta on its endite 3 and 2. E, limb II. F, G, stiff seta on its inner-distal end. H, gnathobase II. I, J, limb III and its inner-distal portion. K, L, limb IV and its inner-distal portion. M, N, limb V and distal portion of its exopodite.

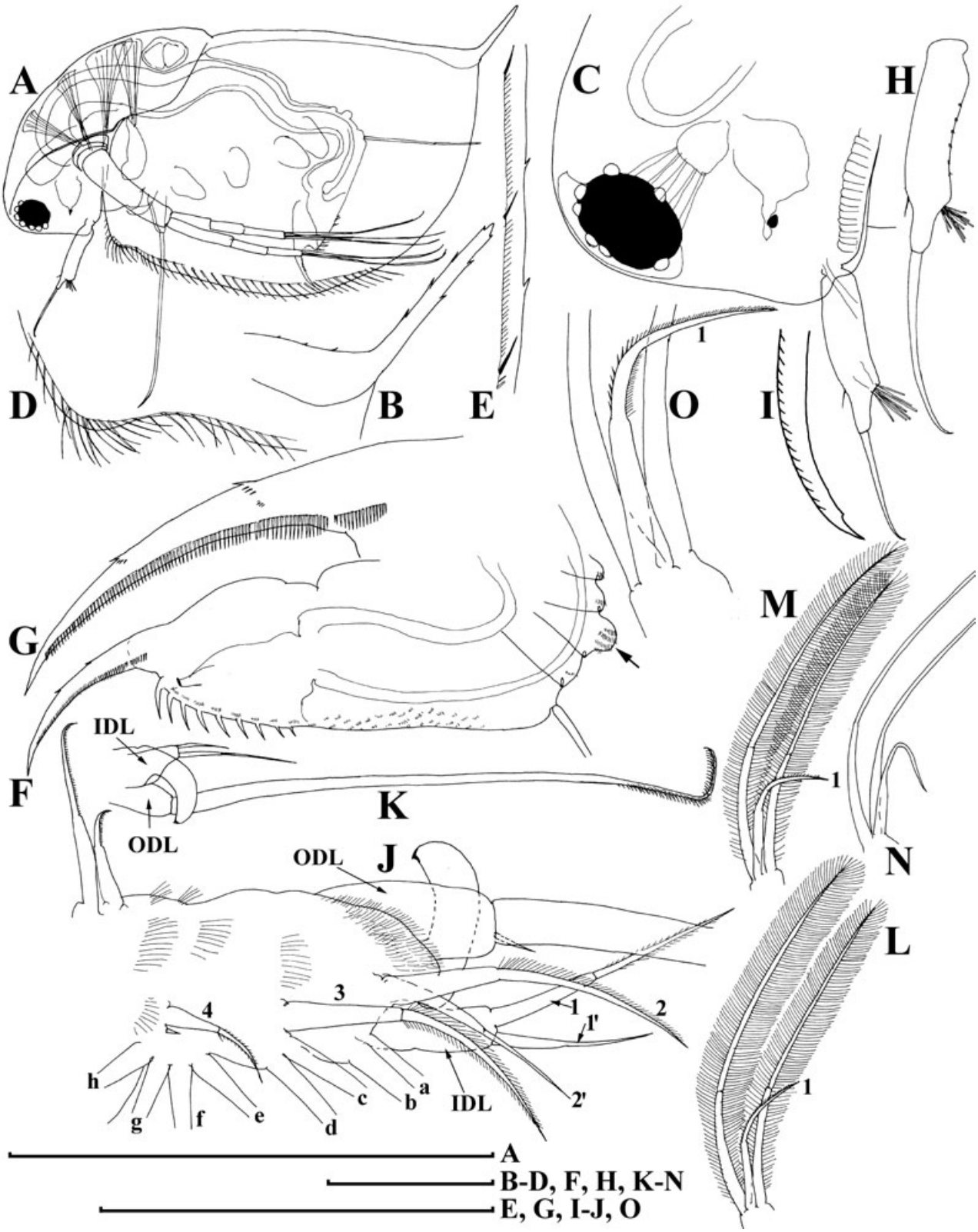

Figure 8. ***Daphnia tanakai*** **sp. nov.**, male from Lake Midori-ga-ike, Japan. A, lateral view. B, caudal spine. C, head. D, E, armature of antero-ventral and posterior portion of valve. F, G, postabdomen and postabdominal claw. H, male antenna I. I, tip of male seta ('flagellum') on antenna I. J, K, limb I and its distal portion. L–O, distal-most endite of limb II.

setae 2–3; (13) anterior seta 1 on distal-most endite of limb II only slightly curved not hook-like.

Uèno & Tanaka (1960) assigned *D. tanakai* sp. nov. specimens to *Daphnia ambigua* Scourfield, 1947, but unlike *D. tanakai* sp. nov., the female of *D. ambigua* has aesthetascs reaching the tip of the rostrum, well developed abdominal processes, two abdominal processes in the male, and a male flagellum possessing a spoon-like widening at the tip.

Daphnia dentifera Forbes, 1893 is a morphologically similar species to *D. tanakai* sp. nov. However, unlike *D. tanakai* sp. nov., *D. dentifera* adults in the late stage moult have a dark insertion within the distal segment of the swimming setae (see Benzie, 2005). Also, *D. dentifera* males have a reduced flagellum on antenna I that is subequal to the aesthetascs (see Brooks, 1957; Benzie, 2005).

Daphnia pulex Leydig, 1860 can be distinguished from *D. tanakai* sp. nov. by antenna I in the female. *Daphnia pulex* possesses a distinct tubular extension from the anntennular mound.

Daphnia parvula Fordyce, 1901 is another morphologically similar species to *D. tanakai* sp. nov. However, unlike *D. tanakai* sp. nov., *D. parvula* males have a reduced flagellum on antenna I that is only slightly longer than the aesthetascs (see Brooks, 1957; Alonso, 1996). Also, *D. parvula* lacks the ocellus pigmentation that is present in *D. tanakai* sp. nov. Finally, Penton & Crease (2004) presented robust phylogenetic evidence that *D. parvula* is a member of *pulex* clade, whereas we have shown here that *D. tanakai* sp. nov. is a member of the *D. longispina* clade.

Taxonomic comments

This taxon was first determined as *D. ambigua* and then as *D. curvirostris* (Uèno & Tanaka, 1960; Tanaka & Tominaga, 1986). We found that the specimens examined here represent a separate species, differing from *D. ambigua* and *D. curvirostris* morphologically (see Differential diagnosis), chromosomally (Tanaka & Tominaga, 1986; Beaton & Hebert, 1994) and genetically (see Fig. 2).

Daphnia whitmani Ishikawa, 1895 was described from the vicinities of Tokyo. According to Ishikawa's realistic pictures (Ishikawa, 1895: plate 21), this animal seems to be a species similar to *D. curvirostris* but has a shorter rostrum. In contrast to *D. tanakai* sp. nov. (also found in Japan), the female of *D. whitmani* has long postabdominal processes, its male has a well developed rostrum, long flagellum and sensory seta on antenna 1, and relatively short stiff setae on endites 2 and 3 of the limb I, like *D. curvirostris*. Perhaps, *D. whitmani* is an ecological morph of *D. curvirostris* with a shorter rostrum. No other *curvirostris*-like species have been described from Asia.

Distribution

At present, *D. tanakai* sp. nov. is known only from several fishless pools and ponds in the Hida Mountain Range (2070–2550 m above sea level), Honshu Island, Japan.

DISCUSSION

The validity and relations of the subgenera of *Daphnia* have been controversial throughout the history of daphniid systematic biology. For example, species of the genus *Daphniopsis* Sars, 1903, group with the subgenus *Daphnia* (*Ctenodaphnia*) Dybowski & Grochowski, 1895 in phylogenetic analyses (Colbourne & Hebert, 1996; Omilian & Taylor, 2001; Hebert *et al*., 2002). Also, the subgenus name *Daphnia* (*Hyalodaphnia*) Schödler, 1866 has been misapplied in recent studies (e.g. Colbourne & Hebert, 1996; Schwenk *et al*., 2000; Penton & Crease, 2004). The recent applications of the 'subgenus *Hyalodaphnia*' contain the type species of the genus *Daphnia*, namely *D. longispina* O. F. Müller, 1785. However, the type species of a genus is, at the same time, a type of nominotypical subgenus (Article 44.1 of the ICZN, 2000), so *D. longispina* must belong to the subgenus *Daphnia s.s*., not to any other subgenus (see Johnson, 1952; Brooks, 1957; Flössner, 1972, 2000). Importantly, Schödler (1866), although aware of the existence *D. longispina*, established his genus *Hyalodaphnia* without including *D. longispina* as a member. His *Hyalodaphnia* lacked an ocellus, a feature that is prominent in *D. longispina*. If the *pulex* group does warrant a higher taxonomic ranking, then a new name must be suggested because the subgenus *Daphnia* is reserved for the group containing *D. longispina*.

Nevertheless, if the gene tree presented in Figure 1 is correct, then we find little objective basis upon which to erect additional subgenera to *Daphnia sensu* Johnson, 1952. For example, our tree provides the first strong evidence that the claw character can rapidly evolve. Some species such as *D. parvula* show claw pecten length variation, but not of the magnitude seen in *D. tanakai* sp. nov. (i.e. from *D. pulex* type to *D. longispina* type). *Daphnia tanakai* sp. nov. possessed a variable claw morphology and the habitat correlation described by Tanaka & Tominaga (1986), in which the pronounced pectens are found in shallow pools and the reduced pectens are found in deeper ponds; this is supported in our study. Interestingly, this habitat–claw morphology association is present throughout the subgenus *Daphnia*, with ephemeral shallow pond dwellers possessing pronounced claw pectens (*D. curvirostris*, *D. tanakai* sp. nov. and many *D. pulex* group species), and deeper pond and lake species possessing reduced claw pectens (most of the *D. longispina* group plus *D. ambigua*, *D. parvula* and

D. retrocurva) (Brooks, 1957; Colbourne *et al*., 1997). The exceptions to the pattern are the putative recent colonists of large lakes, *Daphnia pulicaria* and *Daphnia catawba*, which have pronounced pectens. The prominence of the pecten varies in other daphniid genera, such as *Moina*, *Ceriodaphnia* and *Simocephalus*, but less is known of the phylogeny of these groups (Goulden, 1968; Flössner, 2000; Orlova-Bienkowskaya, 2001). Clearly, *D. tanakai* sp. nov. would be a very informative group for studying the functional morphology and evolution of the postabdominal claw.

Other morphological characters suggested for discrimination of the *pulex* and *longispina* groups (Alonso, 1996) are also diminished by our results. In *D. tanakai* sp. nov., the curvature of the anterior seta on the second limb of the male is variable, rendering this character polymorphic. Other potential sources of new morphological characters, i.e. female and male thoracic limbs, have not been well studied at present.

Our results also suggest that chromosome evolution has been less conserved than proposed for daphniids. Although we did not independently count the chromosomes of *D. tanakai* sp. nov. from Tanaka & Tominaga (1986), it is unlikely that the $2n = 22$ conclusion is a counting error. Counting errors from chromosome squashes usually result when some chromosomes are overlapping in the preparations, yielding a reduced count of the actual number, but $2n = 22$ is an increased count over *D. curvirostris*, the *D. longispina* group and the ancestral condition of $2n = 20$. The chromosomal justification for subgenera is diminished by this new character state in *Daphnia*. The *pulex* group cannot be justified as a subgenus solely on its possession of a derived chromosome number without elevating *D. tanakai* sp. nov. to a new subgenus.

Finally, the genetic divergences of clades have been used to justify subgenera (Colbourne & Hebert, 1996). Here again we find little justification for elevating the *pulex* group to a new subgenus. We did find that the *pulex* group was basal to the other *Daphnia* taxa that we sampled, but there are also at least two other very divergent clades in the *longispina* group (the *D. laevis* clade and the *D. longiremis* clade). Also, the most divergent *pulex* group species were sampled in our study, and the within clade divergence of the *pulex* subgenus is less than the divergence found within at least three of the *longispina* group lineages. The ND2 tree based on divergence is more of a grade than two discrete subgeneric clades.

We have identified and described a new divergent species of *Daphnia* from Japan and presented the first gene tree for the genus *Daphnia* based on the ND2 gene. The tree is largely consistent with estimates based on other genes (12S rDNA, COI, 28S rDNA), but the ND2 gene has more robust support for the clades examined. The ND2 tree reveals that *D. tanakai* sp. nov. represents one of the most genetically divergent lineages in the subgenus *Daphnia*. Although the ND2 gene seems to possess clock-like properties (based on branch length evenness), further genetic analyses are needed to confirm that the divergence of *D. tanakai* sp. nov. is not a gene-specific rate acceleration. Japan may represent an important area for cladoceran diversity because it probably lacked permafrost during the Pleistocene and, unlike much of Beringia, represented a refugium for temperate species. Other endemic Japanese cladoceran species have recently been described (e.g. Kotov & Tanaka, 2004) and there are likely several more undescribed Japanese species.

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