

Phylogeny of Iranian species of the genus *Daphnia* O. F. Müller, 1785 (Crustacea: Branchiopoda: Anomopoda) based on morphological characters

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Daphnia is a wide spread member of Cladocera living in different aquatic environments ranging from hypersaline swamps to freshwater lakes, lagoons, streams and rivers. To improve our knowledge on the diversity of the genus *Daphnia* and promote its biogeographical information in Iran, an investigation on the morphology and identification of the Iranian species of the genus was carried out during the spring of 2013 and 2014. The Southern Caspian Sea Basin (SCSB) and Urmia Lake Basin (ULB) *Daphnia* fauna has been studied based on historical literature records and new collections. Zooplanktons were sampled from 29 randomly chosen localities across ca. 2500 km in the SCSB and ULB basins including both permanent habitats (lakes and reservoirs), and small temporary water bodies (ponds and lagoons), both freshwater and saline. Cladistic analysis of Iranian species of genus *Daphnia*, based on 64 morphological characters obtained from the literature, confirmed the traditionally basic division of the genus into two subgenera, *Daphnia* and *Ctenodaphnia*. This split was supported by enough number of synapomorphies. The relationships between all species within both subgenera were determined clearly. A regional identification key to ten Iranian species of the genus *Daphnia* was provided.

Key words: Cladocera, *Daphnia*, Cladistic Phylogeny, Morphology, Key, Iran, SEM

INTRODUCTION

The cosmopolitan genus *Daphnia* O. F. Müller, 1785 (Class: Branchiopoda, Order: Anomopoda, Family: Daphniidae), includes more than 200 known species of freshwater zooplanktons found around the world (Kotov et al. 2013). These filter feeder organisms are ecologically well-known and inhabit most types of freshwater habitats; in a range of water bodies, from small temporary pools to very large lakes (Ebert, 2005). *Daphnia* are one of the important taxa that used as a model organism in aquatic ecology, molecular and evolutionary biology (Jaromir & Petrusek, 2011).

Two out of three *Daphnia* subgenera (*Ctenodaphnia* and *Daphnia*) are known to occur in Iran. Three species of the subgenus *Ctenodaphnia* known from the Palearctic region, including *D. magna* Straus, 1820; *D. atkinsoni* Baird, 1859; *D. similis* Claus, 1876 were listed from Iran (Löffler 1961). Also, the members of the subgenus *Daphnia* including *D. longispina*, O.F.M. 1776; *D. pulex* Leydig, 1860; *D. obtusa* Kurz, 1874 exist in Iran (Löffler 1961). Recently some already reported species such as *D. similis*, *D. magna*, *D. pulex*, and *D. longispina* (Aghaei moghadam & Aslan Parviz, 2003), *D. longispina*

(Sabkara, & Makaremi, 2003), *D. pulex* and *D. similis* (Haghparsat & Bastami, 2011 and Haghparsat, et al. 2011), *D. pulex* (Haghparsat, et al. 2012), *D. pulex* and *D. longispina* (Salavatian et al, 2012) rediscovered from different geographical regions of Caspian basin in Iran. Recently, a revision study on *D. similis*-group in old world reported *D. sinensis* Gu, Xu, Li, Dumont et Han, 2013 from Khuzestan province of Iran (Popova et al. 2016); therefore, it is possible that most of old reports of *D. similis* in Iran misidentified with *D. sinensis*. In addition to above mentioned species, *D. cucullata* G.O. Sars, 1862, was reported from Haraz River (Jafari et al., 2011). With considering *D. mediterranea* Alonso, 1985, *D. galeata* Sars, 1864 and *D. curvirostris* Eylmann, 1887 as new records from northwest of Iran (Mohammadyari et al., 2014), the number of species used in dataset raised up to ten.

The German entomologist Willi Hennig, it was mentioned that only shared derived characters could probably provide us information regarding phylogeny. Taxa that share many derived characters are grouped more closely together than those that do not (Hennig & Davis, 1999). Essentially, the cladistic or phylogenetic systematics methods depend on recognizing plesiomorphous and apomorphous states of a character for the given group. The most reliable approach to assess the relationship between two taxa on the basis of synapomorphies is using an outgroup (Benzie, 1986). In the present study, cladistic analyses of morphological characters scored from the literature is used to infer phylogenetic relationships among the Iranian species within the genus *Daphnia* and to considered whether the characters have been traditionally used to identify the species work properly to separate them within the genus. In addition, to investigate and analyze all published records and new findings data pertaining to genus *Daphnia* in Iran to date, in order to produce regional identification key.

MATERIAL AND METHODS

Study area and sampling

The area including the Urmia Lake (ULB) and southern Caspian Sea basins (SCSB) from the northwest to the northeast of the country ca. 2500 km in length was sampled in March–June, 2013 and 2014, using a 100-µm mesh net through vertical and horizontal hauls. The sampling area include 11 provinces (West Azerbaijan, Kurdistan, East Azerbaijan, Zanjan, Qazvin, Ardabil, Gilan, Mazandaran, Golestan, North Khorasan, and Razavi Khorasan) (Fig. 1). The collected samples were transferred to ethanol (96%) and kept at -20°C. Also, zooplankton collections (collected in 2007 and deposited in 70% ethanol) taken from the ULB were used in this study (See table 3). Zooplanktons were collected from 29 randomly chosen localities including both permanent habitats (lakes and reservoirs), and small temporary water bodies (ponds and lagoons), both freshwater and saline. In addition to sampled species, *Daphnia* records from literatures for the country were considered in this study.

Morphological studies

For examination of the specimens, the whole body and dissected adult individuals putted in glycerine were observed under a light microscope and a stereomicroscope. As there is no identification key for Iranian *Daphnia* spp., the morphological identifications were made based on Benzie (2005), Petrusek et al. digital key (2005) and the original species descriptions presented by Sars (1864), Eylmann (1887), Alonso (1985), Müller (1776), Kurz (1874), Straus (1820), Baird (1859) and Claus (1876). Populations of *Daphnia* species examined in this study are listed in Table 3. Some specimens were used for scanning electron microscopy (SEM). For SEM analysis, specimens preserved in 96% ethanol were used. To clean the debris, specimens were treated with hot 10% potassium hydroxide for 5-10 minutes. Remnants of alkali were washed out in distilled water. The standard dehydration procedure using a graded acetone-alcohol series followed, and finally the specimens were dried using hexamethyldisilazane for 45-60 minutes (Laforsch & Tollrian 2000). The dehydrated specimens were mounted on stubs and were gold-palladium coated for 2 minutes in

argon plasma in the mini Sputter Coater SC 7620. Then, coated specimens were observed using a scanning electron microscope (LEO-1450VP, Germany) at 20 KV with 2.5 nm resolving power at maximum voltage.

Cladistic analyses

Ten species extracted from the literatures and the present study were included in the analyses. A hypothetical outgroup were used as outgroup in the analyses. The dataset of morphological characters for all species was made using DEscription Language of TAXonomy DELTA 1.02 (2088) program (Dallwitz et. al, 1993). The selection of characters was mainly determined by the material available in the literatures and was modified whenever was needed. Character states for each species were settled from published descriptions and drawings and previous morphological works (Benzie, 1986 & 2005; Gießler et al., 1999). Moreover, the credibility of the characters responsible for any clade achieved in the analyses was discussed. The definitions of the character states and in what way every single taxon has been scored are given in Tables 1 and 2.

The nexus file provided by DELTA was used in PAUP Version 4.0b10 to construct the best tree. Parsimony analysis (command: heuristic search, random addition sequence, 1000 replications, tree-bisection-reconnection = TBR swapping), with all characters unordered, resulted in two final unrooted trees. Gaps are treated as "missing" and accelerated transformation (ACCTRAN) was used to character-state optimization. Branches collapsed (creating polytomies) if maximum branch length is zero. Optimal trees are saved from each replicate, even if they are not optimal. The consensus tree is displayed in Figure 14 and the corresponding apomorphy list of characters is appeared on each clade in the tree. In addition, for the need of simplified and standard identification, a dichotomous regional identification key has been generated using a database on species morphological diagnostic features in DELTA 1.02 (2088) program, containing all morphological studied species from Iran.

TABLE 1. Definitions of the 64 character states used in the cladistic analysis.

Character List		Character State				
		0	1	2	3	4
1	Head shape	narrow	Broad			
2	Head shape lateral view	evenly rounded	irregular shaped			
3	Ventral margin of head	convex	Straight	slightly concave	concave	
4	Basal margin of head	convex	Straight	moderately concave	concave	
5	Helmet	No	Variable	Yes		
6	Eye size	small	Medium	large		
7	Position of eye	close to frontal margin of head	quite away			
8	Ocellus	Un-pigmented	pigmented			
9	Ocellus	small	medium	large		
10	Head delineated from body by deep indentation	Yes	No			
11	Head enlargement	Whole	Spine	Laminar Crest		
12	Expansion of dorsal ridge on the head	without expansion	with few or no small spines	strongly and densely spined		
13	Lateral head grooves	Absent	Present			
14	Dorsal carina: invagination of cephalic shield	Absent	Very weak	Strong		
15	Dorsal carina anterior expansion	Absent	Equal or less than posterior	Greater than posterior	than	
16	Dorsocephalic suture, angle to long axis of body	>90°	90°	<90°		
17	Dorsocephalic deepening of pigmentation	Absent	Present			
18	Length of antenna	< 1/2 valve length	ca. 1/2 valve length	> 1/2 valve length	as long as valve length	
19	Valve margin	Rounded	Almost quadrate			
20	Valve reticulation	Irregular anastomising with cross connections	Regular subrectangular			
21	Mid-ventral carapace row of long setae	Present as long row	Present as short row	Absent		
22	Ventral carapace margin spinulation	Absent	Small	Medium	Large	
23	Ventral carapace margin spine number	Absent	Very few	Few	Many	
24	Ventral carapace margin spine distribution	absent	ca. 1/3 of shell margins	ca 1/2 of shell margins	> 1/2 of shell	
25	Dorsal carapace margin spine distribution	Absent	Near tail (or caudal outgrowth)	Posterior half	All	
26	Lateral compression	Normal	Great			
27	Fornices	rounded or blunt	Pointed			
28	Secondary fornices on the carapace valves	absent	present			
29	Rostrum ridge development	Weak	Medium	Strong		
30	Rostral ridge orientation	Vertical	Lateral			
31	Rostral ridge spinulate	No	Yes			
32	Rostrum spinulate	No	At tip	At anterior margin	Laterally	

Continue of table 1

Character List		Character State				
		0	1	2	3	4
33	Rostral curvature	Concave	Variable (Concave - convex)	Convex		
34	Rostrum length	short	Moderate	Long		
35	Rostrum tip	Pointed	Blunt	truncate		
36	Antennules	prominent	well developed	reduced to sensory setae only		
37	Endings of antennules and tip of rostrum	null	Short	long		
38	Endings of aesthetes and tip of rostrum	< tip	reach tip	>tip	arise at tip of rostrum	
39	Male antennule	Long, mobile	Short, fixed as female			
40	Male antennular seta tip	Tapering	Spatulate	Swollen	Absent	
41	Male antennular seta mid-point	Not thickened	Thickened			
42	Male postabdomen dorsal margin	Flat	Slightly sinuate	Sinuate distal to anal spines	Deeply sinuate distal to anal spines	Sinuate proximal to anal spines
43	Male anal spines	Continuous row from base of pa claw	Separated from base of pa claw			
44	Postabdomen shape	Broad, truncate	Tapering, non-truncate			
45	Postabdominal size	Relatively long	Relatively short			
46	Postabdomen dorsal margin	Sinuate	Flat	Slightly sinuate	Notched	
47	Postabdominal claw combs	1 only larger	Even	1 + 2 larger (1-2)	1+2 (1>2) larger	1 + 2 larger (1<2)
48	Anal spines continue as lateral row	No	Yes			
49	Ventro-distal end of postabdomen	With rows of spinules	without rows of spinules			
50	Number of developed abdominal processes	2	3	4		
51	First abdominal process	Slightly hairy	Not hairy	Hairy		
52	Relation of abdominal processes 1: 2: 3: 4	1>2>3>4	1>2=3>4	1>2>3=4		
53	Gap between postabdominal claw and row of anal teeth	No gap	Small gap			
54	Number of anal spines	5–10	10–15	15–20	5–15	10–20
55	Medial pecten on the postabdominal claw	not prominent	prominent			
56	Postabdominal claw curvature	Slight	Obvious			
57	Length of tail spine	Absent	very short	Short	moderate	long
58	Basal structure of tail spine	thickend and strong	slender thin and fragile			
59	Position of tail spine	in	dorsally from body length axis			
60	Ephippial Shape	ephippium elongated	Ephippium saddle-shaped with more or less straight dorsal margin			
61	Ephippium type	Egg chambers not differentiated: approximately mid-line broadest	Egg chambers not differentiated: anteriorly broadest	Egg chambers differentiated: mid-line broadest		
62	Ephippium anterodorsal angle	Not truncate	Truncate			
63	Ephippial eggs	eggs arranged diagonally or parallelly to its dorsal margin	eggs arranged perpendicularly to the dorsal margin			
64	Ephippium dorsal spinules	No	very small	small	well developed	

TABLE 2. Character states (as defined in Table 1) and data sources for each of the species considered in the analyses.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Outgroup	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>D. magna</i>	1	0	2	1	0	2	0	1	1	1	0	2	1	2	1	2	0	2	0	1	1	1	3	3	3	0	1	1	2	1	0	1
<i>D. similis</i>	1	0	1	2	1	2	0	1	1	1	0	1	0	2	1	2	0	2	0	1	1	1	3	2	3	0	1	1	0	0	0	0
<i>D. atkinsoni</i>	1	1	1	3	0	2	0	1	0	1	0	1	0	2	2	2	0	2	0	1	1	2	3	2	3	0	1	1	0	0	0	0
<i>D. mediterranea</i>	1	0	1	1	0	0	0	1	0	1	0	2	0	2	1	2	0	1	0	1	1	1	3	2	3	0	1	1	0	0	0	0
<i>D. pulex</i>	1	0	3	2	0	2	0	1	0	1	0	0	0	0	0	0	0	2	0	1	2	1	2	2	2	0	0	0	0	0	0	0
<i>D. obtusa</i>	1	0	3	2	0	2	0	1	1	1	0	0	0	0	0	0	0	3	0	1	1	1	2	2	1	0	0	0	2	0	0	0
<i>D. curvirostris</i>	1	0	3	1	0	2	0	1	2	1	0	0	0	0	0	0	0	3	0	1	2	1	2	1	1	0	0	0	0	0	0	0
<i>D. galeata</i>	0	0	2	1	1	1	0	1	0	1	0	0	0	0	0	0	0	3	0	1	2	1	3	2	1	0	0	0	0	0	0	1
<i>D. longispina</i>	0	0	2	1	1	2	0	1	0	1	0	0	0	0	0	0	0	2	0	1	2	2	3	2	1	0	0	0	1	0	0	0
<i>D. cucullata</i>	0	1	1	1	2	0	0	0	0	1	0	0	0	0	0	0	0	2	0	1	2	1	2	2	1	0	0	0	0	0	0	0
species	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64
Outgroup	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>D. magna</i>	0	0	0	0	1	2	0	0	0	3	0	1	1	0	2	0	1	2	0	2	0	2	0	1	2	0	1	0	2	0	0	3
<i>D. similis</i>	0	0	0	1	1	1	0	0	0	1	0	1	1	1	3	0	1	1	0	0	0	1	0	1	4	0	1	0	2	0	0	2
<i>D. atkinsoni</i>	1	0	1	0	2	2	0	0	0	3	0	1	1	2	4	0	1	1	0	1	1	3	1	1	1	0	1	0	2	0	0	3
<i>D. mediterranea</i>	0	0	1	0	2	2	0	0	0	2	1	1	1	1	4	0	0	0	0	2	1	2	1	1	2	0	0	0	2	0	0	3
<i>D. pulex</i>	0	0	0	1	1	1	0	0	0	0	0	1	1	1	4	0	1	1	1	0	1	4	1	1	2	0	0	1	1	0	1	1
<i>D. obtusa</i>	0	0	0	1	1	2	0	0	0	0	0	1	1	1	4	0	1	1	0	0	1	1	1	1	1	1	0	1	1	1	0	1
<i>D. curvirostris</i>	0	0	0	2	1	0	0	2	0	0	0	1	1	1	4	0	1	2	0	0	1	1	1	1	1	1	0	1	1	0	1	0
<i>D. galeata</i>	1	0	1	2	1	1	0	0	0	0	0	1	1	1	1	0	1	1	1	0	1	1	0	1	3	0	0	1	1	0	1	0
<i>D. longispina</i>	1	1	0	2	1	0	0	0	0	0	0	1	1	1	4	0	1	1	1	0	1	4	0	1	4	0	1	1	1	0	1	3
<i>D. cucullata</i>	1	0	1	2	1	3	0	0	0	0	0	1	1	1	4	0	1	1	1	0	1	0	0	1	3	1	1	1	1	0	1	0

RESULTS

Eight *Daphnia* species were identified in the collected samples based on morphological approaches. Totally, Four *Ctenodaphnia* (*D. magna*, *D. mediterranea*, *D. sinensis*, and *D. atkinsoni*), two species of the *D. pulex* group (*D. pulex* and *D. obtusa*), and four species of *D. longispina* group (*D. curvirostris*, *D. galeata*, *D. cucullata* and *D. longispina*) were used for analysis in this study.

Taxonomy

Order **Anomopoda** Sars, 1865

Family **Daphniidae** Straus, 1820

Genus ***Daphnia*** O. F. Müller, 1785

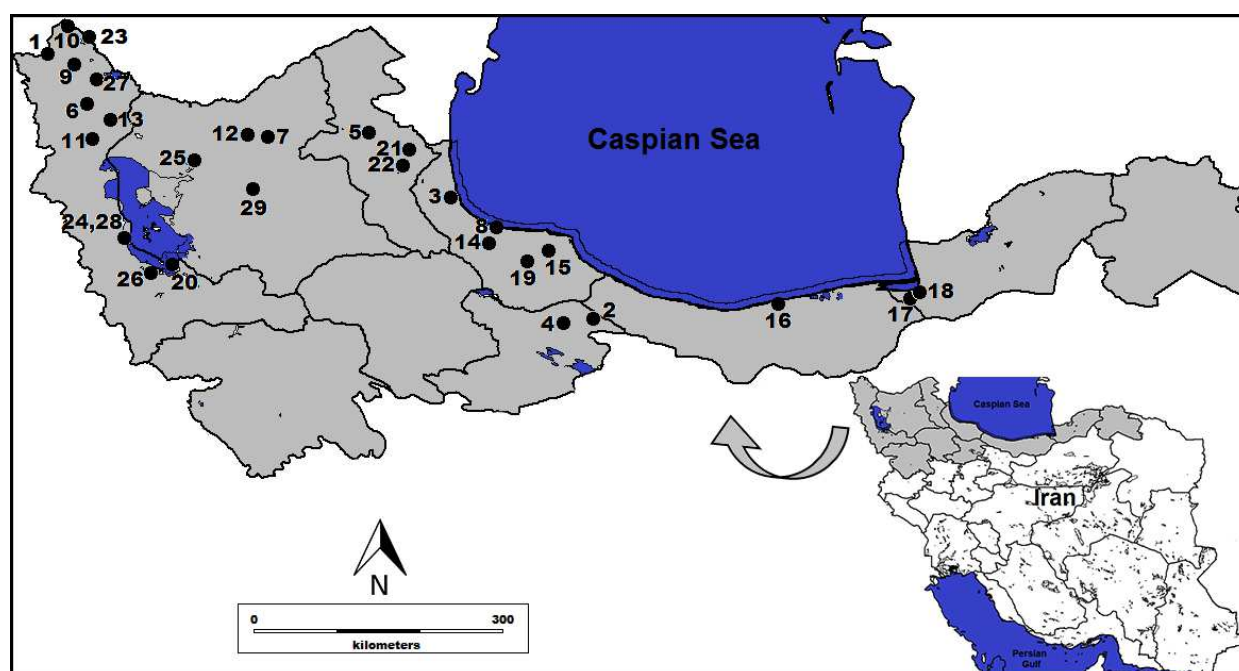


FIGURE.1. Distribution of *Daphnia* sample localities in the study area. The numbers in the map are related to the numbers (map code) in table 3.

Subgenus *Daphnia* O.F. Müller, 1785

Daphnia (Daphnia) obtusa (Kurz, 1874 emend. Scourfield, 1942)

Fig 2 (A-I)

Diagnosis

Female

Prominent medial pecten of post-abdominal claw (the *pulex* type), is the main differentiating character between the *longispina* (*D. galeata* and *D. longispina*) and *pulex* (*D. obtusa* and *D. curvirostris*) groups (figs 3H, 4E, 2G, 5I). Within group, *D. obtusa* is distinguished from *D. pulex* by having a submarginal row of long plumose setae in the mid-ventral carapace margin with small setae throughout (fig. 2G). Spinules on the ventral margin of the carapace are also smaller and widely spaced (figs 6C,G,I). Antennules well developed (figs 2A,C-E). Antennular mound is pronounced (figs 2A,C-E, 3D-E) and high. Rostrum ridge development is strong (figs 2A,C-E). Ventral carapace margin spine number is fewer than *D. galeata* and *D. longispina* (figs 3A,C,G, 4A,C, 2A,C,I). Tail-spine is short to medium (figs 2A-C). Spine size of comb 2 >> comb 1 >> comb 3 in postabdominal claw (fig. 2G). Fornix rounded (fig. 2F). With nuchal organ on the posterior margin of the head (fig. 2H). For detailed taxonomical characters of the species see Kurz (1874 emend. Scourfield, 1942) and Benzie (2005: 251, f. 998-1005, (♀)).

No male was observed in studied populations. For taxonomical characters of male *D. obtusa* see Kurz (1874 emend. Scourfield 1942) and Benzie (2005: 252, f. 1006-1009, (♂)).

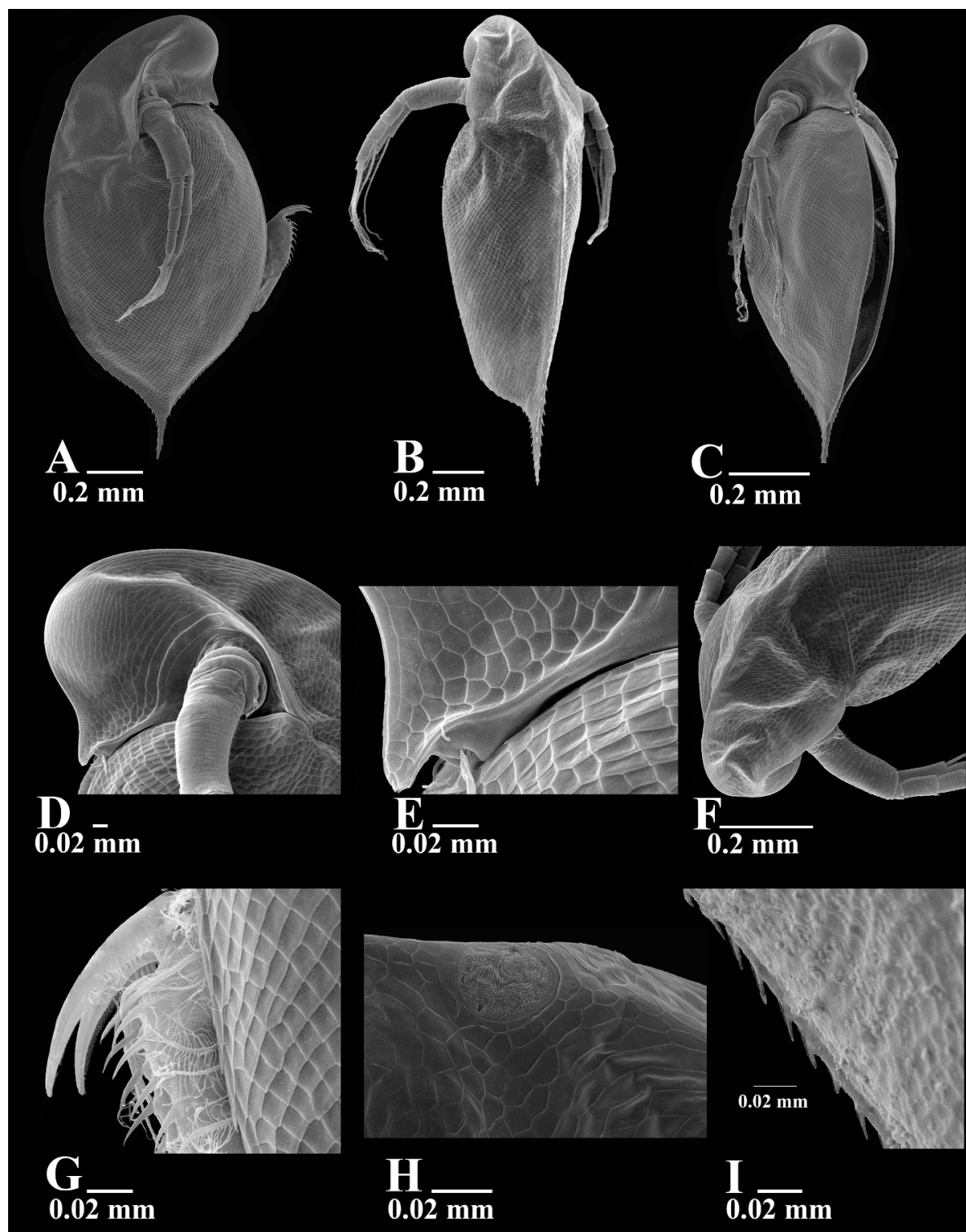


FIGURE 2. Female *Daphnia obtusa*. **A.** Lateral view (carapace broad oval); **B.** Dorsal view; **C.** Ventral view; **D.** Head rounded; **E.** Details of head surface, rostrum small pointed tip, antennule small; **F.** Fornix (rounded); **G.** Anal teeth strong, Postabdominal claw, Carapace surface ultra-structure; **H.** Nuchal organ; **I.** Detail of spinules on ventral margin on posterior half of carapace.

***Daphnia (Daphnia) pulex* (Leydig, 1860)**

East Azerbaijan (Löffler, 1961). No new specimens were examined in this study.

Daphnia (Daphnia) galeata* (Sars, 1864)*Fig 3 (A-I)****Diagnosis****Female**

This species can be distinguished from *D. longispina* by having high, pronounced antennular mound (Figs 3D,E) that is moderate or low in the latter. Medial pecten on the postabdominal claw (fig. 3H) is not prominent. Rostrum ridge development (Figs 3D,E) is weak. Rostrum is spinulated at tip (fig. 3E). Rostrum with blunt tip (figs 3A, 3D, 3E). Spine size of three combs in postabdominal claw are equal (fig. 3H). With several spinules on ventral margin on posterior half of carapace (figs 3G, 4C). Dorsal margin of tail spine with spinules (figs 3A,B). Tail-spine in *D. galeata* (figs 3A-C) and *D. longispina* (fig 4A) longer than of that in *D. obtusa* and *D. curvirostris*. Fornix rounded (fig. 3F). Carapace ultrastructure surface with regular rows of rectangular schema (fig. 3I). For detailed taxonomical characters of the species see Sars (1864) and Benzie (2005: 172 & 173, f. 611-617, (♀)). No male was observed in studied populations. For taxonomical characters of male *D. galeata* see Benzie, (2005: 174, f. 618-621, (♂)).

TABLE 3. List of *Daphnia* species, and their sampling sites. Water body (lake / pool / ditch / puddle / river / dam / lagoon), duration (permanent / temporary / ephemeral) and GPS are given.

Map code	Taxon	Collection Site (Main City)	Province	Waterbody	Latitude (Acc. 3m)	Longitude (Acc. 3m)
	Subgenus <i>Daphnia</i> :					
1	<i>D. longispina</i>	Yarim Qaya (Bazargan)	West Azerbaijan	Ephemeral lagoon	N39°24.451'	E044°25.725'
2	<i>D. longispina</i>	Evan (Moallem Kelayeh)	Qazvin	Permanent Lake	N36°28.931'	E050°26.706'
3	<i>D. longispina</i>	Seragah (Talesh)	Gilan	Permanent Dam	N 37°49.615'	E048°52.664'
4	<i>D. longispina</i>	Zereshk	Qazvin	Temporal Lagoon	N36°25.95'	E050°06.86'
5	<i>D. galeata</i>	Sabalan (Ardabil)	Ardabil	Permanent Dam	N38°32.057'	E047°58.553'
6	<i>D. galeata</i>	Agh chai (Chaipareh)	West Azerbaijan	Permanent Dam	N38°50.897'	E044°52.080'
7	<i>D. galeata</i>	Sattar Khan (Ahar)	East Azerbaijan	Permanent Dam	N38°29.231'	E046°51.685'
1	<i>D. curvirostris</i>	Yarim Qaya (Bazargan)	West Azerbaijan	Ephemeral lagoon	N39°24.451'	E044°25.725'
8	<i>D. curvirostris</i>	Bashman (Bandar Anzali)	Gilan	Ephemeral Lagoon	N37°29.638'	E049°22.532'
9	<i>D. curvirostris</i>	Khalaj (Maku)	West Azerbaijan	Permanent Wetland	N 39°17.172'	E044°43.470'
10	<i>D. curvirostris</i>	Beiri (Bazargan)	West Azerbaijan	Permanent Wetland	N39°42.711'	E044°39.127'
11	<i>D. curvirostris</i>	Qorogh (Khoy)	West Azerbaijan	Ephemeral Lagoon	N 38°27.866'	E044°55.525'
12	<i>D. curvirostris</i>	Varzaghan	East Azerbaijan	Ephemeral Lagoon	N38°30.688'	E046°37.918'
13	<i>D. curvirostris</i>	Bilavar (Khoy)	West Azerbaijan	Ephemeral Lagoon	N38°40.337'	E045°07.745'
14	<i>D. curvirostris</i>	Kasma (Somea Sara)	Gilan	Permanent River (Ditch)	N37°18.92'	E049°18.20'
15	<i>D. curvirostris</i>	Lahijan	Gilan	Temporary Lagoon	N37°13.972'	E049°57.193'
16	<i>D. curvirostris</i>	Ezbaran (Feridoon Kenar)	Mazandaran	Permanent Dam	N36°38.630'	E052°29.119'
17	<i>D. obtusa</i>	Ston Abad (Bandar Gaz)	Golestan	Permanent Lagoon	N36°42.17'	E053°56.55'
18	<i>D. obtusa</i>	Kord kuy	Golestan	Ephemeral Lagoon	N36°46.81'	E054°02.82'
	Subgenus <i>Ctenodaphnia</i> :					
19	<i>D. similis</i>	Shahrestan (Siahkal)	Gilan	Permanent Lagoon	N37°07.21'	E049°43.15'
20	<i>D. mediterranea</i>	Tappeh Rash (Miandoab)	West Azerbaijan	-	N 37° 05.167'	E 45° 47.867'
21	<i>D. atkinsoni</i>	Nojeh Deh (Ardabil)	Ardabil	Ephemeral Lagoon	N 38°21.095'	E048°24.947'
21	<i>D. magna</i>	Nojeh Deh (Ardabil)	Ardabil	Ephemeral Lagoon	N 38°21.095'	E048°24.947'
3	<i>D. magna</i>	Seragah (Talesh)	Gilan	Permanent Dam	N 37°49.615'	E048°52.664'
7	<i>D. magna</i>	Sattar Khan (Ahar)	East Azerbaijan	Permanent Dam	N38°29.231'	E046°51.685'
10	<i>D. magna</i>	Beiri (Bazargan)	West Azerbaijan	Permanent Wetland	N39°42.711'	E044°39.127'
22	<i>D. magna</i>	Kammi Abad	Ardabil	Permanent Dam	N 38°10.383'	E048°21.090'
23	<i>D. magna</i>	Qara Qoyunlu (Maku)	West Azerbaijan	-	N 39° 35.283'	E 44° 52.983'
24	<i>D. magna</i>	Dolama (Urmia)	West Azerbaijan	Temporary Wetland	N37°22.631'	E045°16.114'
25	<i>D. magna</i>	Qom tappeh	East Azerbaijan	-	N 38° 13.633'	E 46° 02.633'
26	<i>D. magna</i>	Dorgeh Sangi (Naghadeh)	West Azerbaijan	Permanent Lake	N36°59.324'	E045°34.350'
27	<i>D. magna</i>	Marganlar (Poldasht)	West Azerbaijan	Permanent Dam	N 39°07.298'	E044°57.995'
28	<i>D. magna</i>	Rashakan (Urmia)	West Azerbaijan	-	N 37° 22.550'	E 45° 16.450'
29	<i>D. magna</i>	Quri Gol (Bostan Abad)	East Azerbaijan	Permanent Lake	N 37°55.019'	E046°41.764'

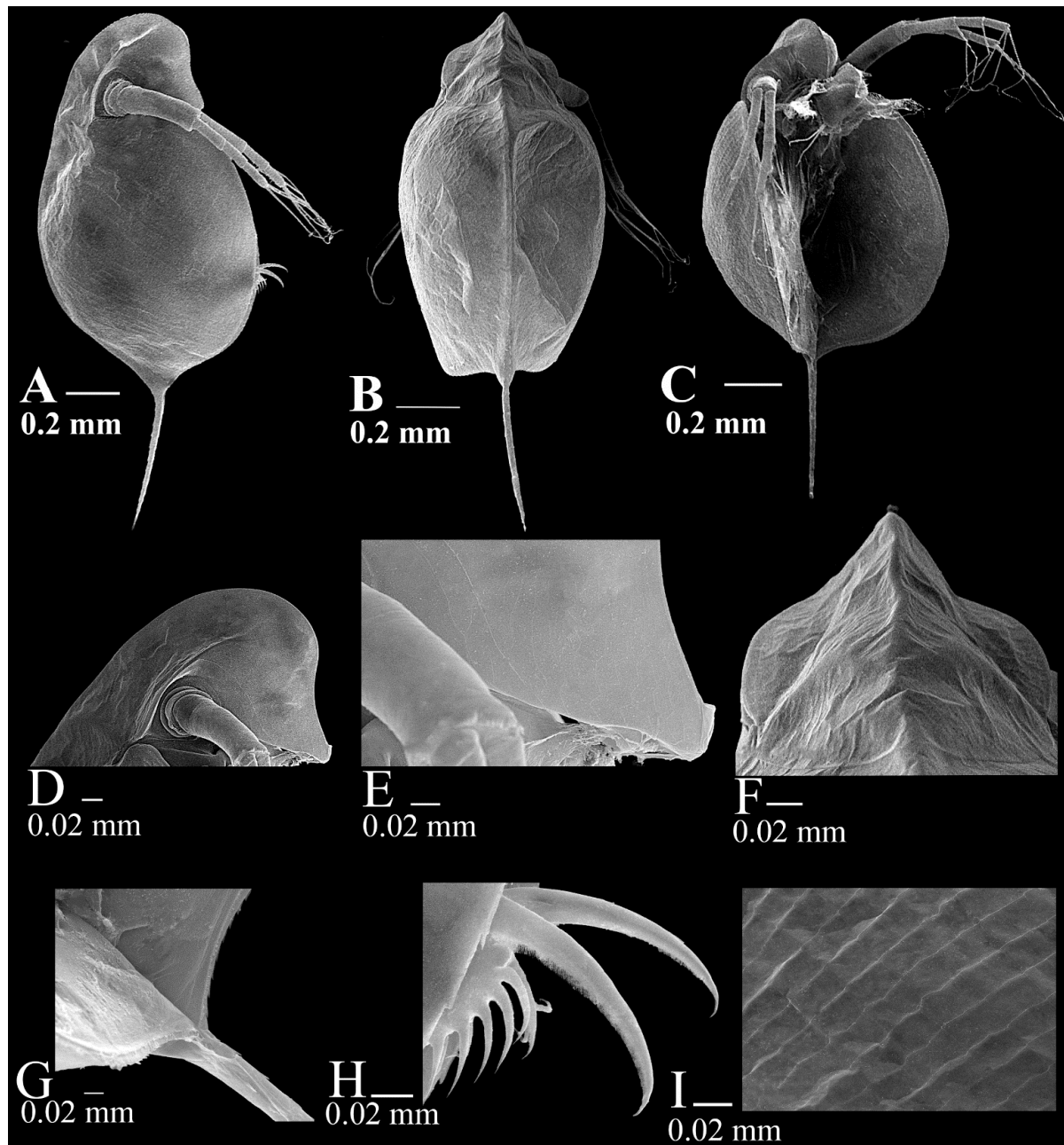


FIGURE 3. Adult parthenogenetic female *Daphnia galeata*. **A.** Lateral view (Carapace oval); **B.** Dorsal view; **C.** Ventral view; **D.** Head rounded; **E.** Rostrum short, blunt and antennule small; **F.** Fornix rounded; **G.** Detail of spinules on ventral margin on posterior half of carapace and tail spine; **H.** Postabdominal claw; **I.** Carapace surface ultra-structures.

***Daphnia (Daphnia) cucullata* (G.O. Sars, 1862)**

Mazandaran, Haraz River (Jafari et al., 2011). No new specimens were examined in this study.

***Daphnia (Daphnia) longispina* (O. F. Müller, 1776)**

Fig 4 (A-F)

Diagnosis

Female

This small to medium body size (fig. 4A) species can be distinguished from other species by having medium spines in ventral carapace margin (figs 4A,C) while in *D. galeata*, *D. curvirostris* and *D. obtusa* are small (figs 3A,C,G, 5A,F-G, 2A,C,I). *D. longispina* is also differentiated by posterior half widely spaced spine distribution in ventral margin of carapace (figs 4A,C). Carapace elongated and oval in lateral view and body laterally compressed (fig. 4A). Antennular mound is moderate or low (fig. 4D). Rostrum ridge development is medium. Medial pecten on the postabdominal claw is not prominent (fig. 4E). Spine size of comb 1 = comb 2 = comb 3 (fig. 4E). Tail-spine is medium to long (fig. 4A). Fornix rounded (fig. 4B). Carapace ultrastructure surface with regular rows of quadrangular schema (fig. 4F). For original description and detailed taxonomical characters of the species see O. F. Müller (1776) and Benzie (2005: 203 & 207, f. 761-767, (♀)).

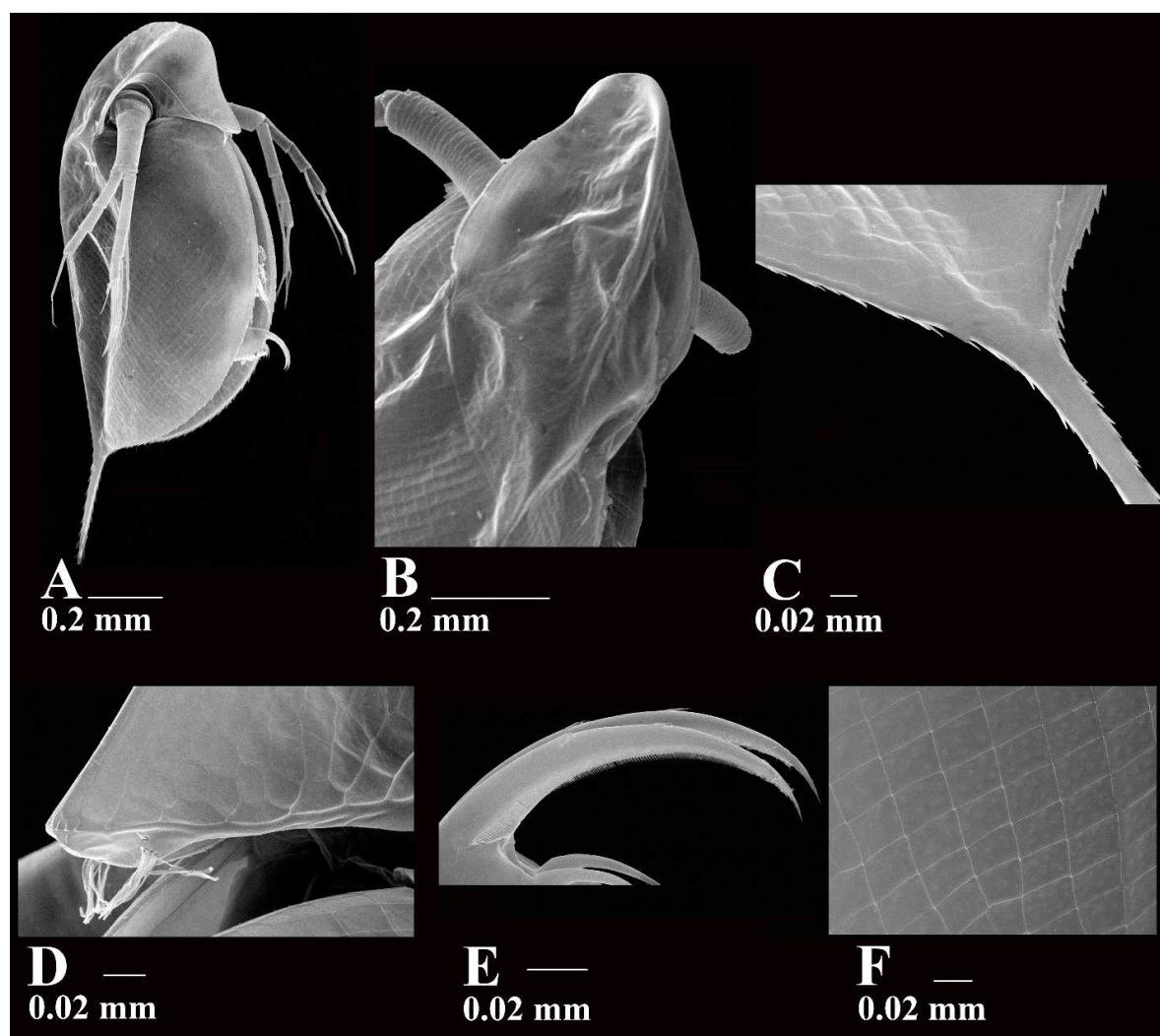


FIGURE 4. Parthenogenetic female *Daphnia longispina*. **A.** Lateral view (carapace elongate oval, head rounded, relatively long tail spine); **B.** Dorsal view of head, fornix rounded; **C.** Detail of separated spinules on posterior third of ventral margin of carapace and condensed on dorsal margin; **D.** Detail of head, rostrum long, pointed, antennule very small; **E.** Postabdominal claw external view **F.** Carapace surface ultra-structure.

Daphnia (Daphnia) curvirostris* Eylmann, 1887 emend. Johnson, 1952*Fig 5 (A-I) and Fig 6 (A-H)****Diagnosis****Female**

Members of the *D. curvirostris* complex share some morphological characteristics with the *D. pulex* group. Medial pecten on the postabdominal claw in *D. curvirostris* is prominent (fig. 5I). Ventral margin of carapace with few spines (figs 5F-G). Small spinules located over posterior third of ventral margin and posterior dorsal margin near tail spine, widely separated (figs 5F-G). Tail-spine short to medium (figs 5A-B). Rostrum ridge development weak (figs 5D,E). Antennular mound low (figs 5D-E). Prominent medial pecten, comb 2 large $\gg$ comb 1 $\geq$ comb 3 (fig. 5I). Fornix rounded (fig. 5C). Ultra-structure surface of carapace with rows of quadrangular schema near ventral margin (fig. 3G) and punctuated toward dorsal margins (fig. 5H). For original description and detailed taxonomical characters of the species see Eylmann, (1887) and Benzie, (2005: 140 & 141, f. 461-466, (♀)).

Male

Males are distinguished from other members of subgenus by having swollen antennular seta tip while in *D. obtusa*, *D. galeata*, and *D. longispina* has tapering form (figs 6A,C,E-G). Antennular sensory seta small and despite all studied species that inserted distally, inserted laterally (figs 6A,C,E-G). Comb 2 large = comb 1 $\geq$ comb 3 in post-abdominal claw. Fornix rounded (figs 6B,D). Spines on posterior third of ventral margin, and dorsal margin near tail spine (fig. 6H). For original description and detailed taxonomical characters of the species see Eylmann (1887) and Benzie (2005: 140 & 142, f. 467-470, (♂)).

Subgenus *Ctenodaphnia* Dybowski & Grochowski, 1895***Daphnia (Ctenodaphnia) sinensis* Gu, Xu, Li, Dumont et Han, 2013****Fig 7 (A-I)****Diagnosis****Female**

With less prominent antennules comparing with other Iranian members of subgenus *Ctenodaphnia* (figs 7A,C,D,F). Ventral carapace margin spine is small (figs 7A,C,G,H). With strong relatively long tail spine pointing backwards (figs 7A,C). Rostrum tip is pointed (figs 7A,C,D,F). Postabdomen dorsal margin is flat. Spine size of three combs on post-abdominal claw is comb 1 = comb 2 > comb 3 (fig. 7I). With closely packed spinules on ventral margin of carapace posterior to mid-line; dorsal margin of carapace with spinules (figs 7G,H). Dorsal carina well developed (figs 7B,E). For detailed description see Gu, Xu, Li, Dumont et Han, 2013.

No male was observed in the studied populations. For detailed description of the male see Gu, Xu, Li, Dumont et Han, 2013.

***Daphnia (Ctenodaphnia) similoides* (Hudec, 1991)**

Golestan Province, Pond NE of town Gorgan, Collector J. Mergeay, 2008 (Korinek, 2010). No new specimens were examined in this study.

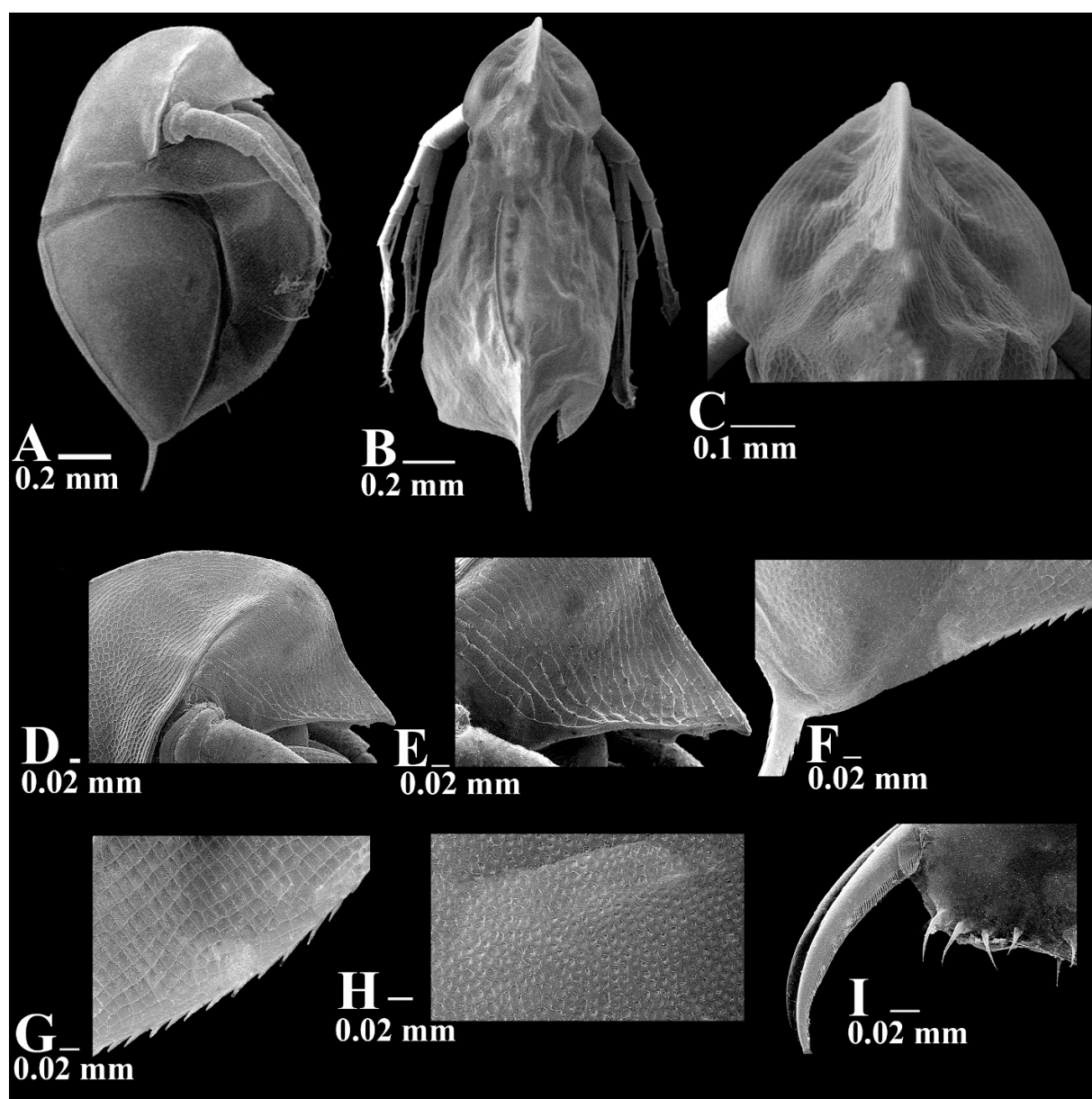


FIGURE 5. Adult parthenogenetic female *Daphnia curvirostris*. **A.** Lateral view (carapace oval); **B.** Dorsal view; **C.** Fornix rounded; **D.** Head broadly rounded; **E.** Rostrum (short, pointed tip), and reduced antennule; **F.** Detail of widely separated spinules on posterior third of ventral margin of carapace, Short tail spine; **G.** Detail of spinules on ventral margin on Anterior half of carapace; **H.** Carapace surface ultra-structures; **I.** Anal teeth strong, Postabdominal claw.

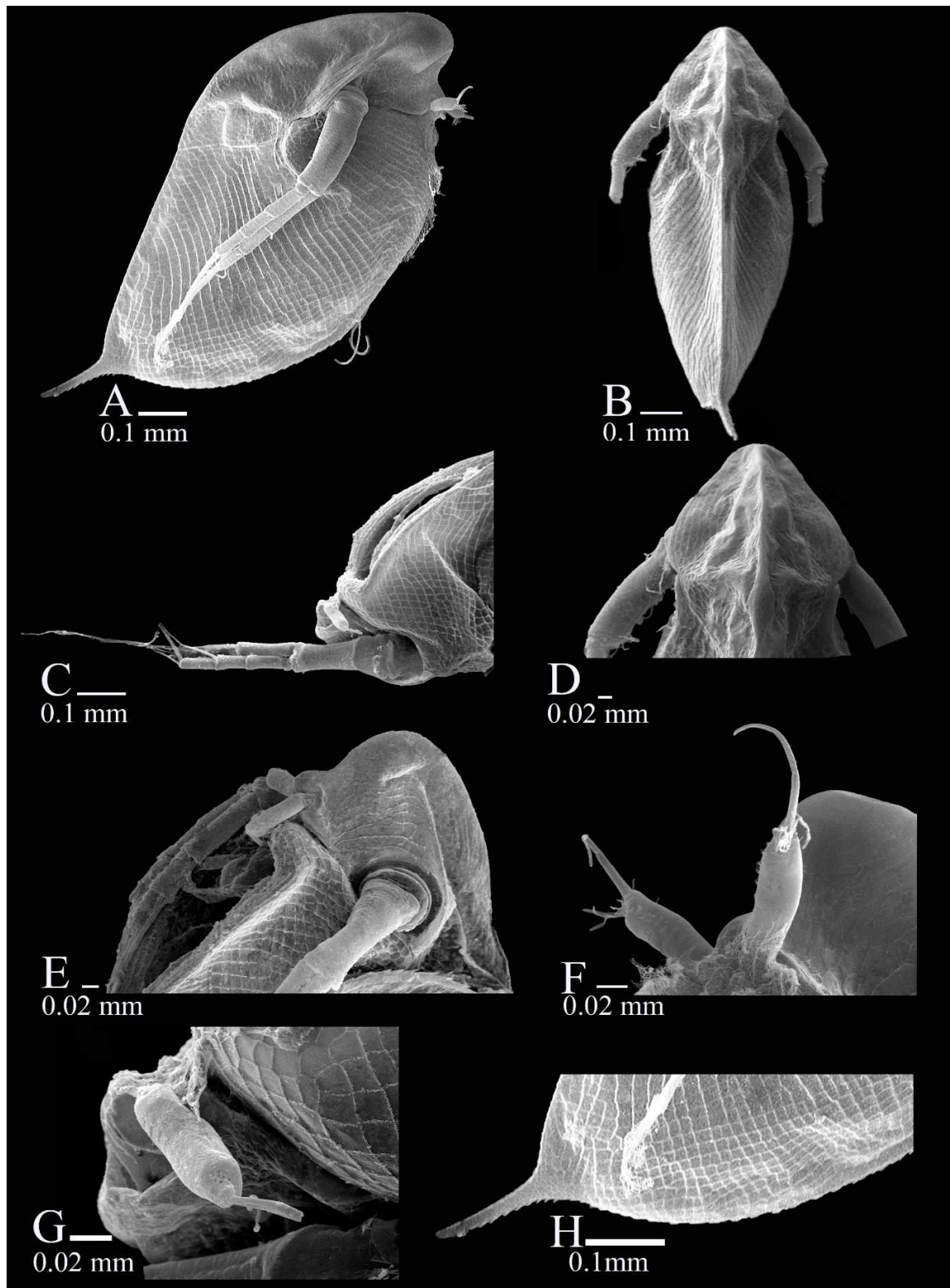


FIGURE 6. Adult male *Daphnia curvirostris*. **A.** Lateral view; **B.** Dorsal view; **C.** Lateral view with antenna **D.** Fornix rounded; **E., F. & G.** Antennule long, flagellum five times aesthete length, longer than antennule; **H.** Detail of widely separated spinules on posterior third of ventral margin of carapace, Short tail spine.

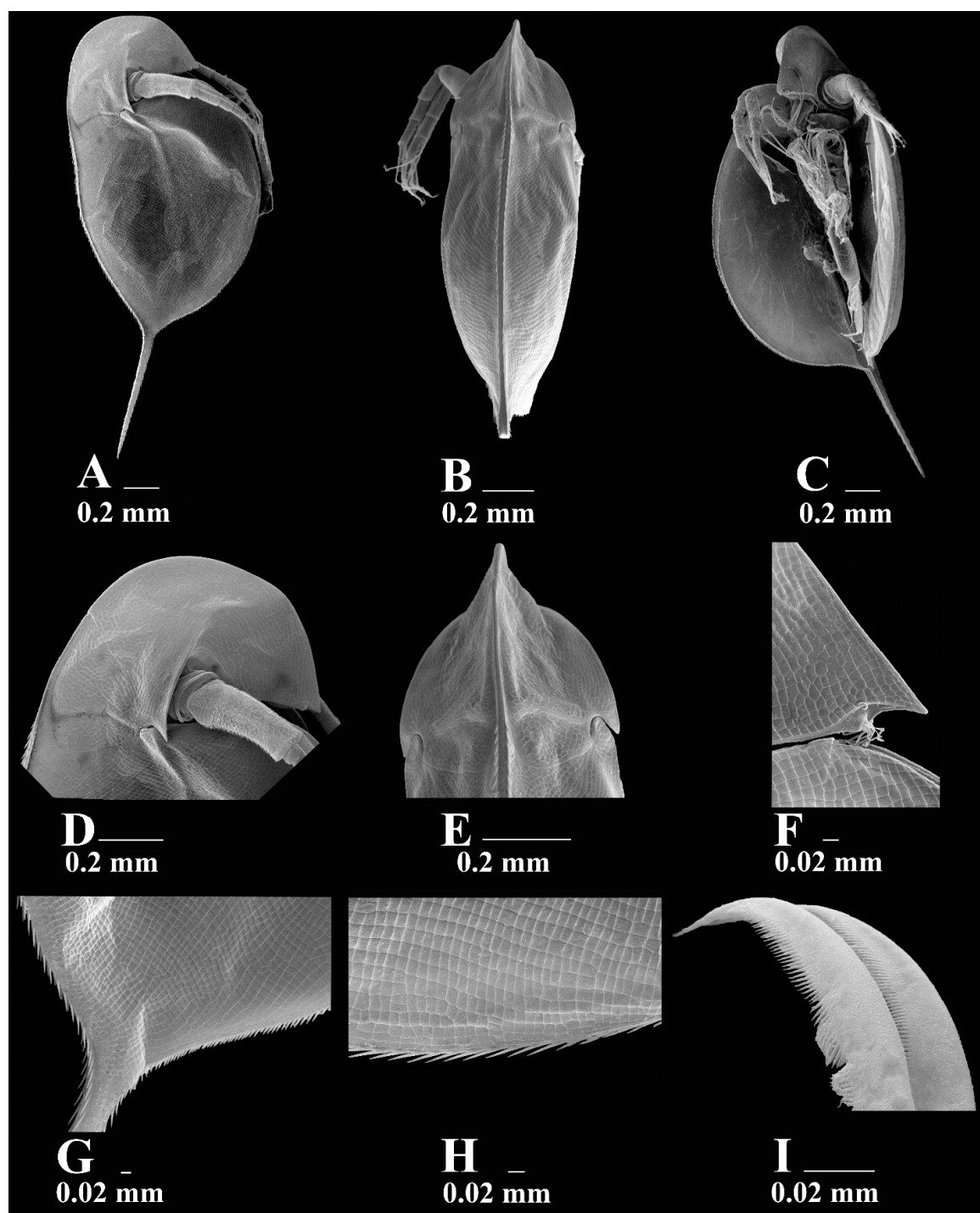


Figure 7. Adult parthenogenetic female *Daphnia sinensis*. **A.** Lateral view (carapace broad oval); **B.** Dorsal view; **C.** Ventral view; **D.** Head rounded; **E.** Fornix developed and sharply angled; **F.** Rostrum short and pointed, Antennule large; **G.** Detail of closely packed spinules on posterior of ventral margin of carapace; **H.** Carapace surface ultra-structure, detail of spinules on ventral margin on Anterior half of carapace; **I.** Postabdominal claw.

Daphnia (Ctenodaphnia) magna* (Straus, 1820)*Fig 8 (A-I) and Fig 9 (A-I)****Diagnosis****Female**

This species can be easily distinguished from all other members of subgenus *Ctenodaphnia* by its heavy and very large size of body (figs 8A,B), indented dorsal margin of carapace and deeply incised dorsal margin of the post-abdomen. Dorsal shield penetrates cephalic shield as narrow ridge half way up the head (figs 8A,C). Ventral carapace margin spine is small and located anterior to mid-line (figs 8A,B,H). Rostrum ridge development is strong and laterally oriented (fig 8F). Rostrum spinulate and pointed at tip (figs 8A,B,D-F). Dorsal margin of postabdomen is deeply sinuate. Spine size of three combs on post-abdominal claw is comb 1 = comb 2 > comb 3. Tail spine short (figs 8A,B). Ephippium D-shaped, black egg chambers on grey to white background oriented at an angle to spinose dorsal margin: with long spinose anterior and short posterior processes; surface ultrastructure with granulated projections (figs 8I,J,K). Carapace ultrastructure surface with rows of quadrangular schema (fig. 8G).

Male

The male *D. magna* is distinguished from other species by having sub-rectangular and large body size (fig. 9A), strongly sinuate post-abdomen dorsal margin close to terminal claw. Fornices is strong and make lateral rib (figs 9A,B,E). Antennules long (figs 9A-C,E-G). Medial pecten of post-abdominal claw with large spines (comb 2 > comb 1 > comb 3) (fig 9I). Ventral carapace margin with small spines (fig. 9D). Carapace ultrastructure surface with relatively regular rows of quadrangular schema (fig. 9D,H).

Daphnia (Ctenodaphnia) atkinsoni* (Baird, 1859)*Fig 10 (A-L) and Fig 11 (A-L)****Diagnosis****Female**

Antennules prominent (figs 10A,C,D,F,L). Cephalic extension of carapace expanded laterally into two lobes (figs 10A,B,D,E). Ventral carapace margin spine is medium (figs 10A,C,I,K). Rostrum tip is blunt (figs 10A,C,D,F,L). Dorsal margin of postabdomen is slightly sinuate. Anterior expansion of dorsal carina greater than the posterior one (figs 10A-F). Spine size of three combs on post-abdominal claw is comb 2 > comb 1 > comb 3 (figs 10J). Dorsal carina well developed, extends half way up the dorsal margin of head expanding anteriorly to form a lobe (figs 10A-E). Ephippium elongate oval, black egg chambers parallel to dorsal margin, anterior and posterior processes and spines on dorsal margin well-developed (fig. 10K). Ultrastructure of short regular spines on dorsal extension of the carapace into the head shield with parallel lines on theirs (fig. 10G). Carapace surface ultrastructure with irregular rows of quadrangular schema (fig. 10H). For detailed description of the species see Baird (1859) and Benzie (2005: 88, f. 84-92, (♀)).

Male

Fornices are sharp and broad in dorsal view with dorsal ridge with variably expanded lobes on head (Figs. 11B, D, E). With the distal lobe of the endopodite of the first limb with two setae, one of which is four times longer than the other. Medial pecten of post-abdominal claw with large spines in proximal combs (comb 1 > comb 2 > comb 3) (Fig. 9I). Anal spines few or absent. Antennule subequal to length of head, flagellum long, sensory seta inserted distally (Figs. 11A, C, D, F, H, K). Head ultrastructure surface with pentagons and hexagons schema (Fig. 11G). With small spines on ventral carapace margin (Fig. 11I). Carapace ultrastructure surface with rows of irregular quadrangular schema (Figs. 11I, K). Inner ventral margin of carapace with group of long setae centrally (Fig. 11J). For detailed description of male *D. atkinsoni* see Baird, (1859) and Benzie, (2005: 90, f. 93-97, (♂)).

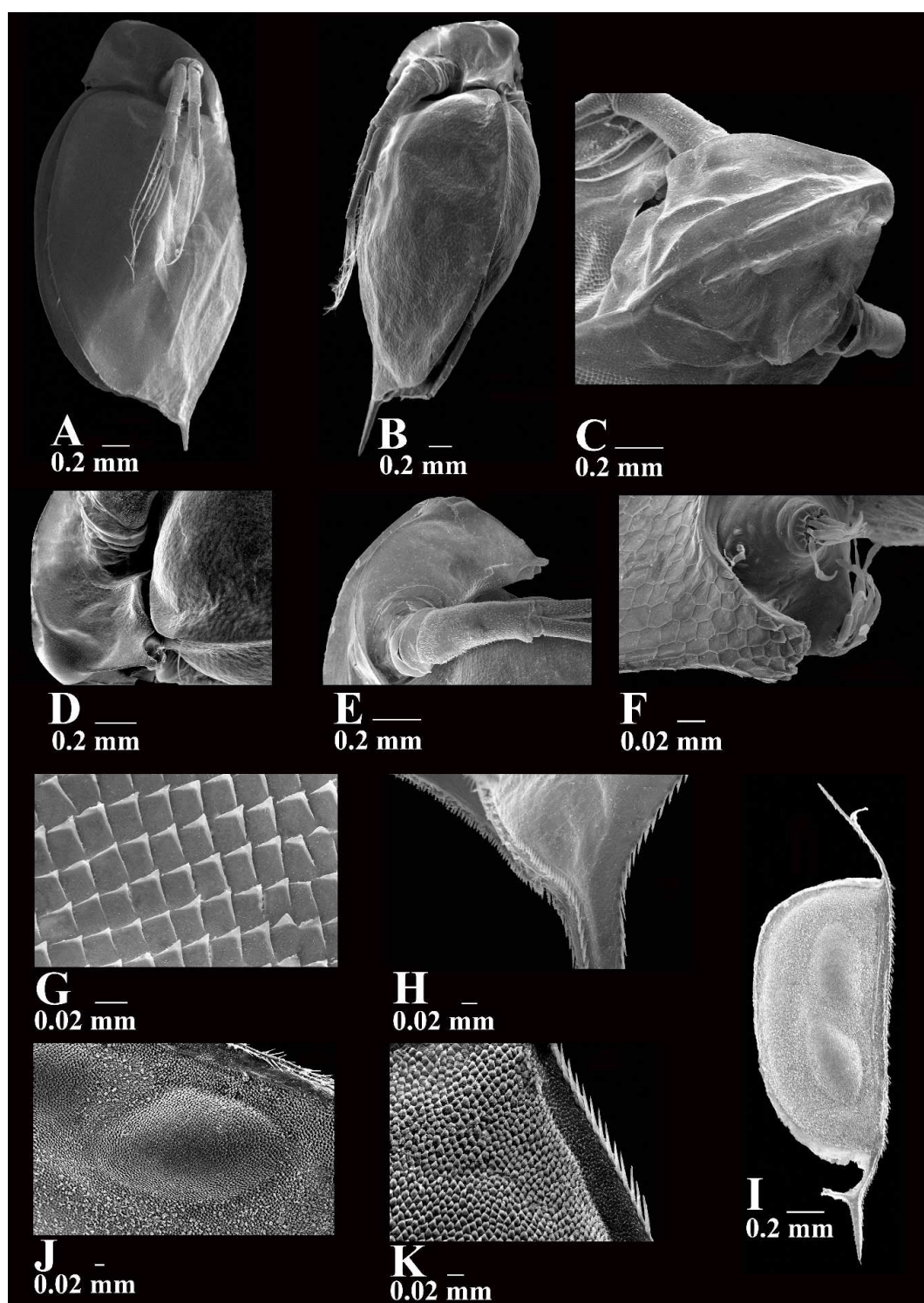


FIGURE 8. Adult ephippial female *Daphnia magna*. **A.** Lateral view (carapace broad oval, Tail spine short); **B.** Ventral view; **C.** Head broad in dorsal view, Fornix (strong, angled), secondary fornix strong; **D.** Antero-ventral view of head; **E.** Lateral view of head; **F.** Rostrum (short, pointed), antennule large; **G.** Carapace surface ultra-structure; **H.** Detail of small spines on posterior two-third of ventral margin of carapace and all dorsal margin; **I., J.** Ephippium; **K.** Ephippium surface ultra-structure.

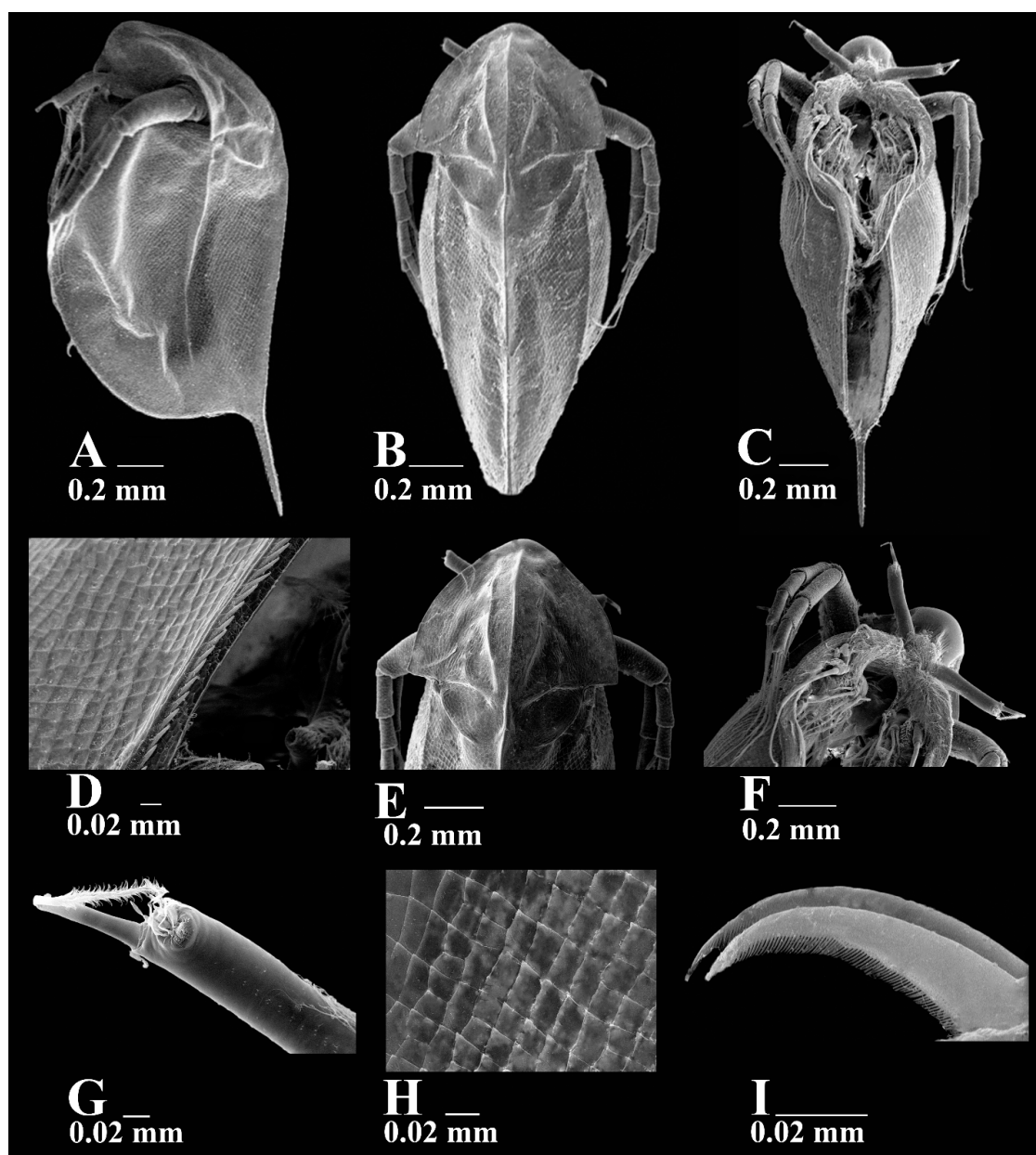


FIGURE 9. Adult male *Daphnia magna*. **A.** Lateral view; **B.** Dorsal view; **C.** Ventral view; **D.** Detail of spinules on ventral margin of carapace; **E.** Strong Fornices; **F.** Ventral aspect of head; **G.** Long antennule and flagellum; **H.** Carapace surface **I.** Postabdominal claw

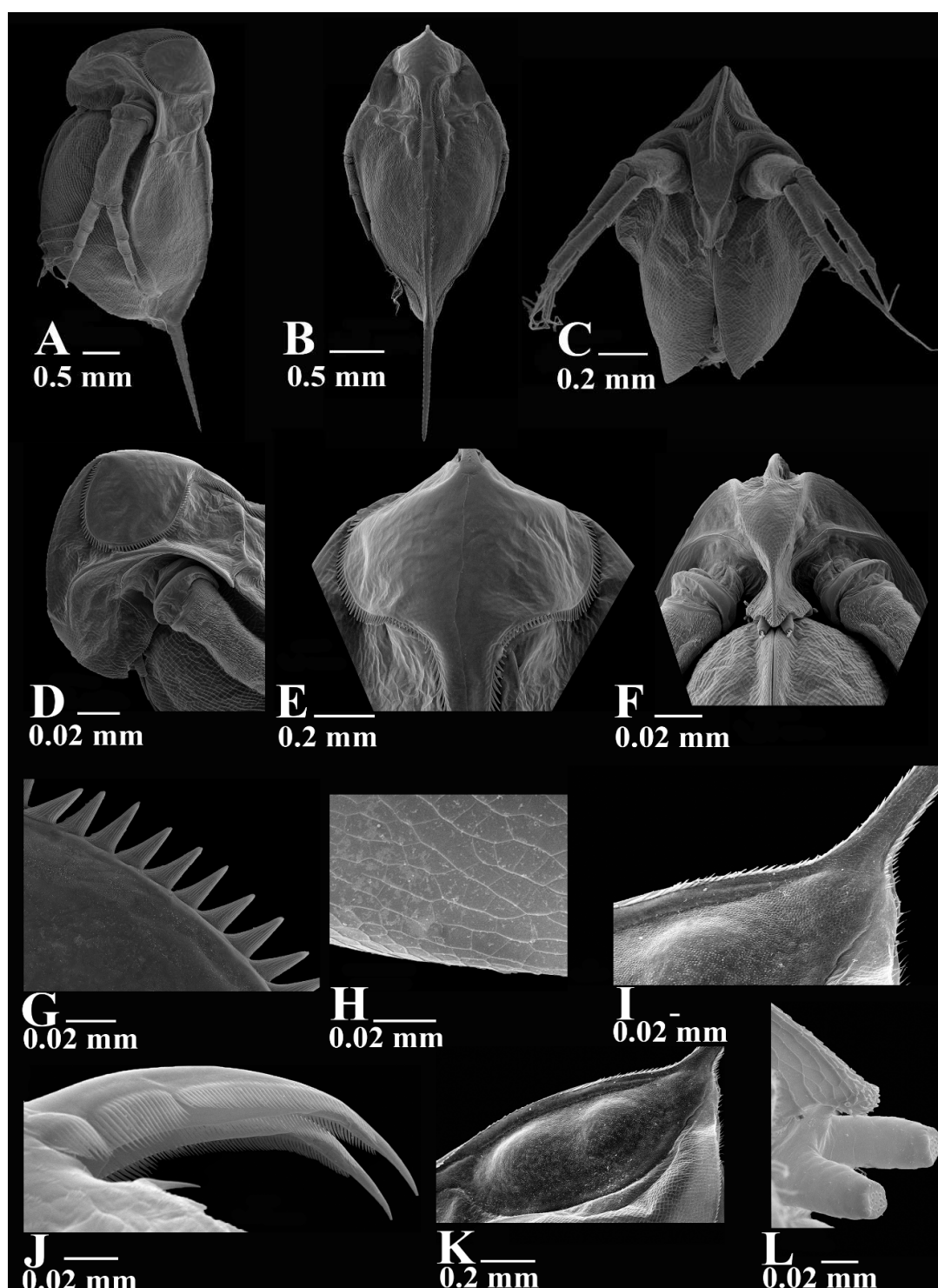


FIGURE 10. Adult ephippial female *Daphnia atkinsoni*. **A.** Lateral view (carapace oval); **B.** Dorsal view; **C.** Ventral view; **D.** Head very broad, Fornix sharply angled; **E.** Dorsal view of head shield; **F.** Ventral aspect of head; **G.** Detail of spinules on head shield; **H.** Carapace surface ultra-structure; **I.** Detail of spines on posterior ventral margin of carapace; **J.** Postabdominal claw; **K.** Ephippium, egg chambers parallel to dorsal margin; **L.** Rostrum short with rounded tip, antennule large.

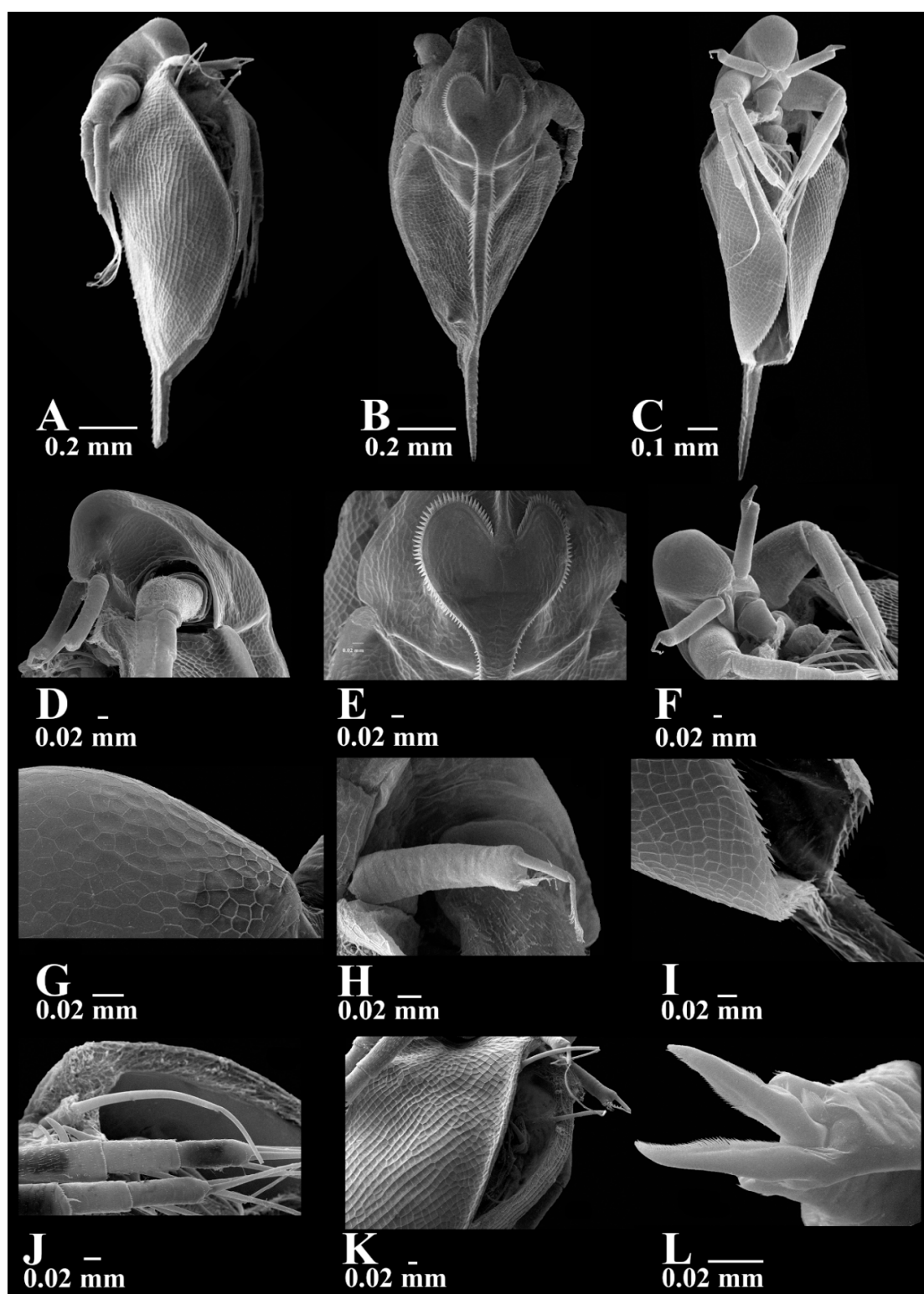


FIGURE 11. Male *Daphnia atkinsoni*. **A.** Lateral view; **B.** Dorsal view; **C.** Ventral view with antennules; **D.** lateral aspect of head; **E.** Details of head shield, Sharp and broad Fornices; **F.** Ventral aspect of head; **G.** Head surface; **H.** Antennule, flagellum long; **I:** Spines on posterior ventral margin of carapace; **J.** Inner ventral margin with group of long setae centrally; **K.** Carapace surface; **L.** postabdominal claw.

Daphnia (Ctenodaphnia) mediterranea* (Alonso, 1985)*Fig 12 (A-I) & Fig 13 (A-I)****Diagnosis****Female**

Antennules in all Iranian members of subgenus *Ctenodaphnia* (*D. magna*, *D. atkinsoni* and *D. mediterranea*) is prominent with exception of *D. sinensis* that is less prominent (figs 8A,B,D-F, 10A,C,D,F,I, 7A,C,D,F). With distinct supraocular depression. Ventral carapace margin spine is small (figs 12A,D,E). Rostrum tip is blunt. Post-abdominal dorsal margin is flat or straight (fig. 12A). Ventro-distal end of postabdomen with rows of spinules. Spine size of three combs on post-abdominal claw not exceptionally developed, comb 2 > comb 1 > comb 3 (figs 12F, 10J). Ephippium elliptic, dorsal spines well-developed, two egg chambers parallel with dorsal margin (figs 12B,C,H). Carapace ultrastructure surface with rows of quadrangular schema (fig. 12G). Ephippium ultrastructure surface with punctuated schema (figs 12H,I). For detailed taxonomical characters of the species see Alonso (1985) and Benzie (2005: 221 & 222, f. 834-841, (♀)).

Male

Anal spines separated from base of postabdominal claw (figs 13A,H). Antennules is longer than head (figs 13A,B,E,F). Post-abdominal claw with larger spines in proximal combs (comb 1 > comb 2 > comb 3) (figs 13H). Tail spine with stout base (fig. 13A). Distal lobe of the endopodite of the first limb with two setae, one of which is eight times longer than the other. Head surface with relatively parallel lines in lateral view (figs 13C,D). Small spines on ventral carapace margin (fig. 13G). Carapace ultrastructure surface with rows of quadrangular schema (fig. 13I). For detailed taxonomical characters of the species see Alonso (1985) and Benzie (2005: 221 & 222, f. 842-845, (♂)).

Our cladogram (Figure 14) contains ten cladistic nodes. Unrooted tree rooted using hypothetical outgroup method. From 64 total unordered characters, all characters had equal weight, 10 characters were constant, 12 variable characters were parsimony-uninformative and the number of parsimony-informative characters was 42. The cladistic tree demonstrated that two main clades occur within the genus *Daphnia* that corresponded to the two subgenera, *Daphnia* and *Ctenodaphnia*. Consistency, Retention, Rescaled consistency and Homoplasy indices of the parsimony tree are provided in table 4.

Sets of apomorphies including head shape (1), ventral margin of head (3), ocellus (9), expansion of dorsal ridge on the head (12), dorsal carina: invagination of cephalic shield (14), dorsal carina anterior expansion (15), dorsocephalic suture, angle to long axis of body (16), mid-ventral carapace row of long setae (21), dorsal carapace margin spine distribution (25), fornices (27), secondary fornices on the carapace valves (28), endings of aesthetes and tip of rostrum (38), male postabdomen dorsal margin (42), ephippium type (61) and ephippium dorsal spinules (64) are shared by the *Ctenodaphnia*, while characters ventral margin of head (3), mid-ventral carapace row of long setae (21), dorsal carapace margin spine distribution (25), antennules (36), gap between postabdominal claw and row of anal teeth (53), ephippial shape (60), ephippium type (61) and ephippial eggs (63) are common to subgenus *Daphnia*.

Within the subgenus *Ctenodaphnia*, *D. sinensis* is clearly differentiated by four derived characters from other members of the subgenus involving moderately concave basal margin of head (4), variable existence of helmet (5), well developed antennules (36) and 1+2 larger (1>2) postabdominal claw combs (47). Three other members of the subgenus *Ctenodaphnia*, *D. mediterranea*-*D. atkinsoni* clade, together with *D. magna* establish a well-defined clade differentiated from *D. sinensis* by expansion of dorsal ridge on the head (12), endings of aesthetes and tip of rostrum (38), male postabdomen dorsal margin (42), relation of abdominal processes 0: 1: 2: 3 (52), number of anal spines (54) and length of tail spine (57). *D. magna* is separated from *D. mediterranea*-*D. atkinsoni* clade by its slightly

concave ventral margin of head (3), presence of lateral head grooves (13), ventral carapace margin spine distribution $> 1/2$ of shell (24), strong rostrum ridge development (29), lateral rostral ridge orientation (30), rostrum spinulate at tip (32), spinulate postabdomen dorsal margin (46), postabdominal claw combs 1 + 2 larger (1-2) (47) and four abdominal processes (50) (fig. 14 and Table 2). *D. sinensis* is defined by moderately concave basal margin of head (4), variable helmet (5), well developed antennules (36) and combs 1+2 larger ($1>2$) (47). *D. mediterranea* is specified by several characters comprise small eye size (6), length of antenna ca. $1/2$ valve length (18), male postabdomen dorsal margin sinuate distal to anal spines (42), male anal spines separated from base of postabdominal claw (43), ventro-distal end of postabdomen with rows of spinules (49), two developed abdominal processes (50) and position of tail spine inside of body length axis (59). *D. atkinsoni* is indicated by irregular shape of head in lateral view (2), concave basal margin of head (4), expansion of dorsal ridge on the head with few or no small spines (12), anterior expansion of dorsal carina greater than posterior (15), medium spines on ventral carapace margin (22), concave or convex rostral curvature (33), slightly sinuate postabdomen dorsal margin (46), relation of abdominal processes $1>2=3>4$ (52), 5-15 anal spines (54) and very short tail spine (57).

The members of subgenus *Daphnia* constitute two main clades, the first one so-called genetically *D. longispina*-group comprises *D. longispina* with *D. cucullata*-*D. galeata* clades and the second is composed of *D. curvirostris* clade with *D. pulex*-*D. obtusa* clade that make *D. pulex* group (fig. 14). The first clade become different from the second by several characters including availability of helmet (5), rostral curvature (33), hairs on first abdominal process (51) and position of tail spine (59). The second clade is distinguished by having specific head shape (1), shape of ventral margin of head (3), length of antenna (18), ventral carapace margin spine number (23), medial pecten on the postabdominal claw (55) and length of tail spine (57). Within the *D. longispina*-group, *D. cucullata*-*D. galeata* clade separated from *D. longispina* clade based on four characters including eye size (6), shape of rostrum tip (35), aesthetes arise at tip of rostrum (38) and length of tail spine (57). *D. longispina* was defined by medium spines on ventral carapace margin (22), medium rostrum ridge development (29), medium rostrum (34), 10-20 anal spines (54) and well-developed ephippium dorsal spinules (64). *D. cucullata* is distinguished by irregular head shape in lateral view (2), straight ventral margin of head (3), having helmet (5), unpigmented ocellus (8), few spine on ventral carapace margin (23), aesthetes arise at tip of rostrum (38), 5-10 anal spines (54) and slender, thin and fragile tail spine (58). *D. galeata* is determined by medium eye (6), antenna as long as valve length (18), rostrum spinulate at tip (32), even combs (47) and position of tail spine on the body is dorsally from length axis (59).

Within the *D. pulex* group, *D. pulex*-*D. obtusa* clade separated from *D. curvirostris* clade based on the shape of basal margin of head (4), antennule development (36), endings of aesthetes and tip of rostrum (38) and ephippium dorsal spinules (64). *D. curvirostris* is defined by large ocellus (9), ventral carapace margin spine distribution ca. $1/3$ of shell margins (24), swollen male antennular seta tip (40), four developed abdominal processes (50) and slender thin and fragile tail spine (58). *D. obtusa* is differentiated from *D. pulex* by medium ocellus (9), mid-ventral carapace row of long setae present as short row (21), strong rostrum ridge development (29), endings of aesthetes exceed tip of rostrum (38) and position of tail spine on the body is dorsally from length axis (59). Finally, *D. pulex* is determined by length of antenna $> 1/2$ valve length (18), dorsal carapace margin spine distribution in posterior half (25), first abdominal process not hairy (51), 10-20 anal spines (54) and short tail spine (57).

TABLE 4. Some important scores are given for final tree.

Tree Length	CI Consistency index	RI Retention index	RC Rescaled consistency index	HI Homoplasy index	F value	F-ratio
136	0.6912	0.6038	0.4173	0.3088	142	0.2545

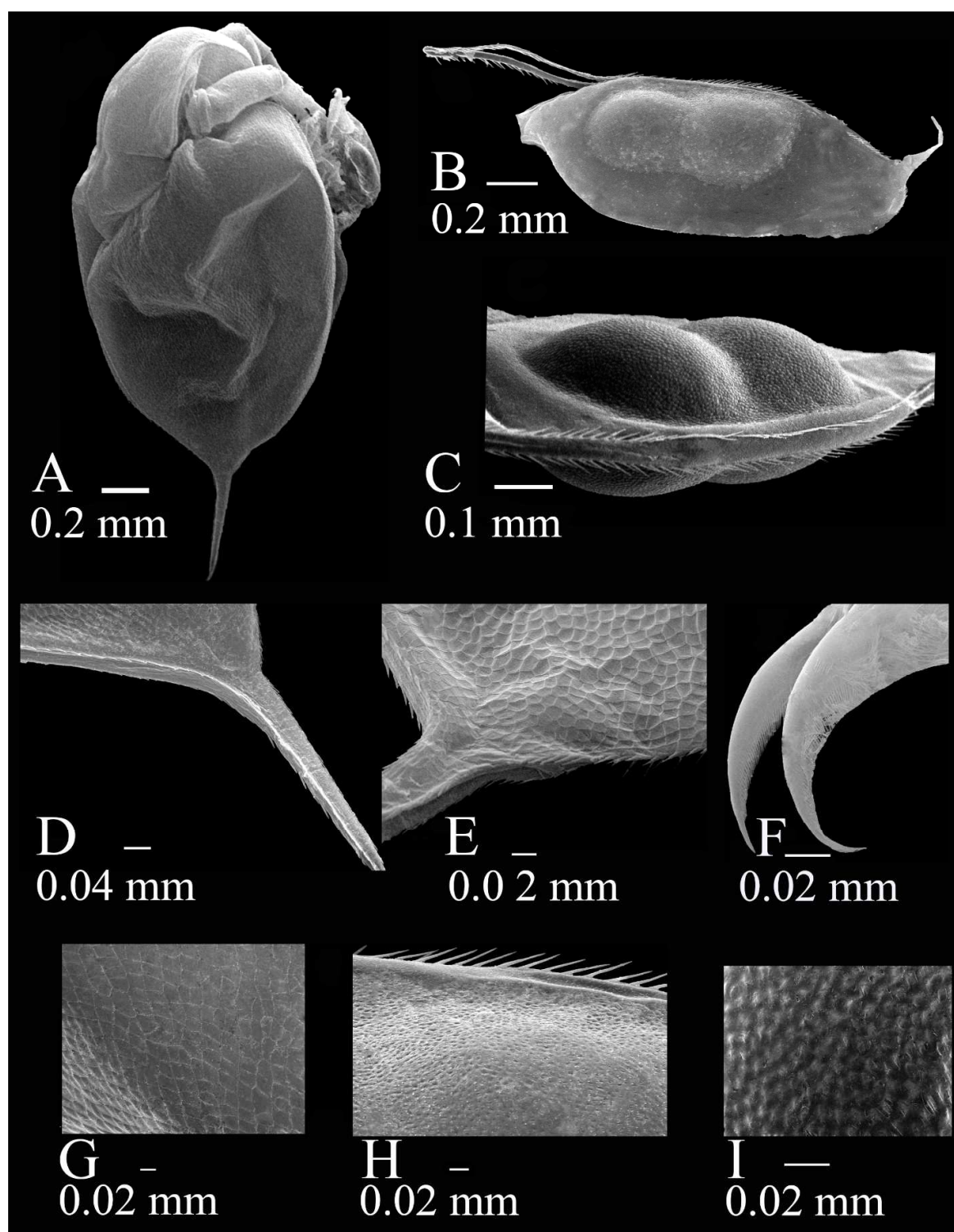


FIGURE 12. Adult ehippial female *Daphnia mediterranea*. **A**. Lateral view (body more oval, Head rounded); **B**. Lateral aspect of ehippium (elliptic, two egg chambers parallel with dorsal margin); **C**. Dorsal aspect of ehippium and carapace (densely covered in spines); **D**. Tail spine stout; **E**. Small spines on ventral margin of carapace posterior to mid-point; **F**. Postabdominal claw; **G**. Carapace surface; **H**. Ehippium surface; **I**. Ehippium surface ultrastructure.

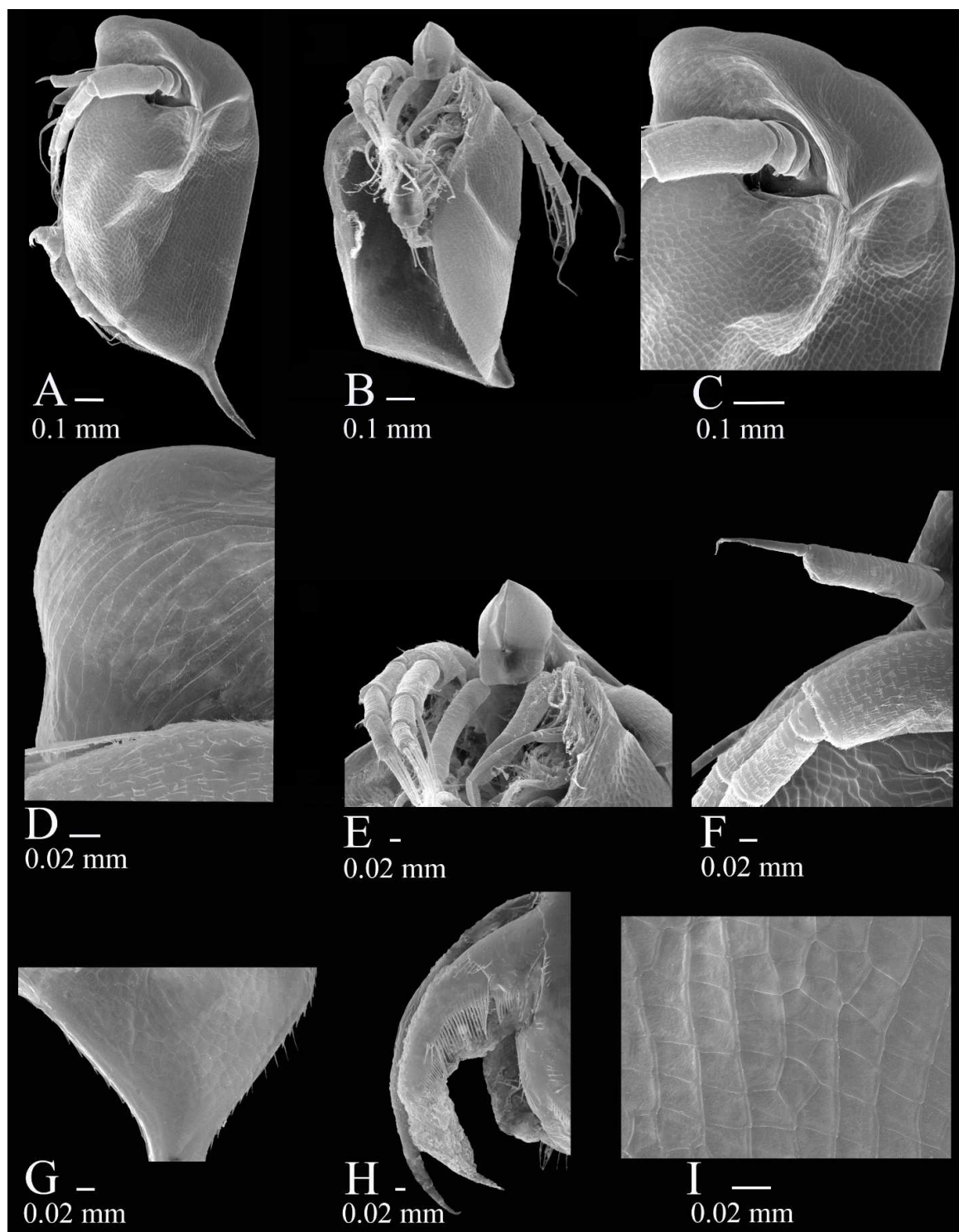


FIGURE 13. Adult male *Daphnia mediterranea*. **A.** Lateral view (tail spine with stout base, Post-abdomen dorsal margin straight except for distal bulge); **B.** Ventral view; **C.** lateral aspect of head; **D.** Head surface; **E.** Ventral aspect of head; **F.** Antennule very long, flagellum less than half length of antennule; **G.** Detail of spinules on ventral margin on posterior half of carapace; **H.** postabdominal claw; **I.** Carapace surface.

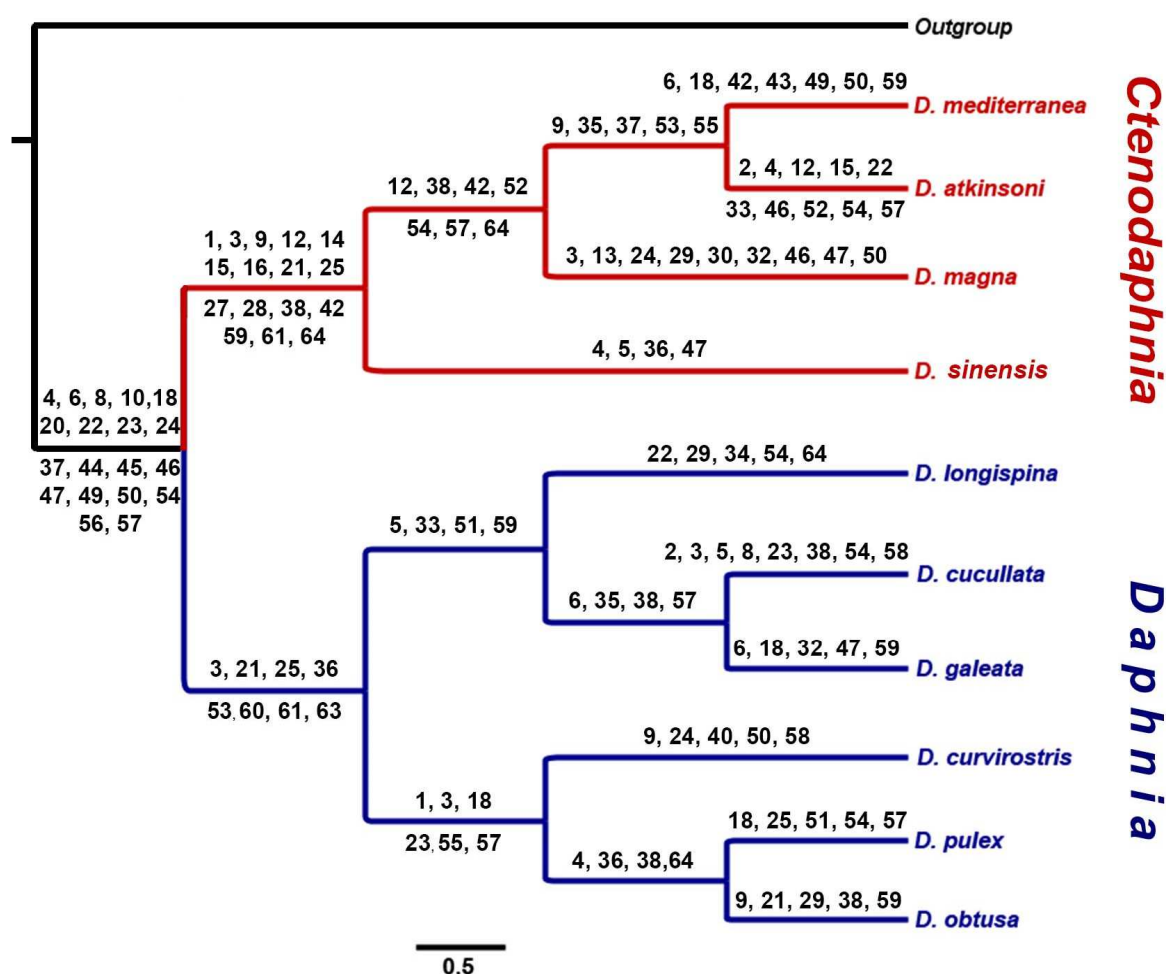


FIGURE 14. A parsimony tree illustrating the cladistic relationships of 10 *Daphnia* species. Hypothetical outgroup was used in the analyses. Characters defining a branch are noted upon each branch, and are identified by their code number. Details of the codes are given in Table 1.

The readers should be noticed about the below produced identification key that other *Daphnia* species may be present in Iran, and that some of the species included in the table belong to species complexes.

Identification key for Iranian species of the genus *Daphnia* investigated in this study:

1. Fornices rounded or blunt; dorsal ridge of the carapace not extends into the head shield; secondary fornices on the carapace valves absent 2
- Fornices pointed; dorsal ridge of the carapace extends into the head shield; secondary fornices on the carapace valves present 5
- 2(1). Medial pecten on the post-abdominal claw prominent 3
- Medial pecten on the post-abdominal claw not prominent 4
- 3(2). Antennules well developed; submarginal row of plumose setae on ventral carapace margin present *D. obtusa* Kurz 1874 emend. Scourfield 1942
- Antennules reduced to sensory setae only; submarginal row of plumose setae on ventral carapace margin absent *D. curvirostris* Eylmann 1887 emend. Johnson 1952

4(2).	Antennular mound high	<i>D. galeata</i> Sars 1864
	Antennular mound moderate or low	<i>D. longispina</i> Müller 1776
5(1).	Dorsal margin of carapace indented	<i>D. magna</i> Straus 1820
	Dorsal margin of carapace not indented	6
6(5).	Antennules prominent	7
	Antennules not prominent	<i>D. sinensis</i> Gu, Xu, Li, Dumont et Han, 2013
7(6).	Cephalic prolongation of carapace expanded laterally into two lobes.....	
		<i>D. atkinsoni</i> Baird 1859
	Cephalic prolongation of carapace not expanded	<i>D. mediterranea</i> Alonso 1985

CONCLUSION

A basic split of the genus into two subgenera, *Daphnia* and *Ctenodaphnia*, was demonstrated in the cladistic analysis. Overall, the subgenus *Ctenodaphnia* clade is strongly founded on 16 synapomorphies and the subgenus *Daphnia* is also powerfully defined as a clade by 8 shared characters. The *Ctenodaphnia* were strongly determined by a unique ephippium, the possession of a dorsal carina, and the invagination of the cephalic shield by the dorsal one, expansion of dorsal ridge on the head, angle to long axis of body, mid-ventral carapace row of long setae, dorsal carapace margin spine distribution, fornicies, secondary fornicies on the carapace valves and male postabdomen dorsal margin that confirmed the previously revealed importance of these characters in specifying of *Ctenodaphnia* (Benzie 1986 & 2005). Specific features of trunk limb morphology of *D. atkinsoni* (Glagolev & Alonso 1990) have been proposed to show the closeness of a *D. mediterranea*, that our findings verified this affinity by several characters (fig. 14).

Also, the members of subgenus *Daphnia* are common with ventral margin of head, mid-ventral carapace row of long setae, dorsal carapace margin spine distribution, length of antennules, gap between postabdominal claw and row of anal teeth, ephippial shape, ephippium type and ephippial eggs. All species in subgenus *Daphnia* were subjected to the nominate groups except for *D. curvirostris*. Within both subgenera this analyses exhibited much internal structure and the almost all of clades were powerfully defined by several synapomorphies (fig. 14).

Despite of the fact that classification of genus *Daphnia* like other taxa was established on morphological methods, the usefulness of these approaches was criticized by many works in the past especially after application of molecular methods. The lack of informative characters and in some cases shared characters between all groups (Benzie 1986), independently evolving of some characters for several times in the groups (Colbourne & Hebert 1996 & Colbourne et al. 1997), existence of cryptic species (Hebert, 1977; Petrusek et al., 2009; Crease et al. 2012), the issue of hybrids (Hobæk et al. 2004), the morphological plasticity in some groups (Wesenburg-Lund 1908, 1926; Hutchison 1967; Zaret 1980; Havel 1986; Pijanowska 1990) due to environment changes (Brooks 1957; Hebert 1978; Lampert & Wolf 1986; Mort 1989; Manca & Tognota 1993; Pijanowska 1992), making it difficult to assess the relationships among species or the true phylogenetic importance of the characters using morphological methods. Furthermore, assessing the phylogenetic relationships of *Daphnia* species in a regional level was successful but the deficiency of these approaches was revealed for whole genus in the worldwide study due to lack of characters (Benzie 1986). However, the molecular conformance of monophyletic status of the *Ctenodaphnia* (Lehman et al. 1995) and chromosomal grouping of all *Daphnia* species to nominate subgenera support the traditional morphology based groupings (Colbourne et al. 1997, Benzie 2005).

The present cladistic analysis of the genus *Daphnia* have clarified that several distinct groups can be identified within the genus, including the two subgenera, the *D. longispina* and *D. pulex* groups. These results with congruent with recent global molecular phylogeny of the genus (Adamowicz et al. 2009). Thus, the characters which could be scored from the literature for all taxa were sufficient to establish the existence and phylogenetical relationships of at least species found in the country. As well, this

study highlighted the need for meticulous morphological work rather than refuting of the morphological approach. In addition, morphological differences are usually found between the major groupings identified by molecular methods. Therefore, the collation of morphological and molecular methods would help to clarify ambiguous taxonomical issues of the genus.

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