



A contribution to the knowledge of the genus *Ceratophysella* Börner, 1932 (Collembola, Hypogastruridae) of Iran

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ABSTRACT. *Ceratophysella* Börner, 1932 is the second largest genus of the family Hypogastruridae and contains 146 species from all over the world, with nine species reported from Iran. Specimens were extracted from soil and leaf litter samples using a modified Berlese funnel. The DNA sequences of the mitochondrial COI gene were obtained from the whole body of *Ceratophysella* specimens. A Maximum Likelihood tree is also generated based on the sequences of COI gene representing the overall phylogenetic affinities of the known *Ceratophysella* species. In the present study, *C. communis* (Folsom, 1897) and *C. impedita* Skarzynski, 2002 are recorded for the first time from Iran. *Ceratophysella, communis* is re-described, and its diagnostic characters are illustrated. An identification key to *Ceratophysella* species of Iran is given.

Keywords: COI sequences, DNA barcoding, new records, redescription, taxonomy

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INTRODUCTION

Ceratophysella was erected by Börner (1932) as a genus for the species of *Hypogastrura* with an eversible integumental sac between the third and fourth antennal segments. Like the genus *Xenylla*, the genus *Ceratophysella*, with 146 species, is the second largest genus in the family, after *Hypogastrura* Bourlet, 1839 (Bellinger et al., 1996–2024). The genus includes several species-groups in which species are closely related and in some cases, it is difficult to differentiate them. For example, in *denticulata*-group, *C. stercoraria* (Stach, 1963), *C. gibbosa* (Bagnall, 1940), *C. caucasica* Martynowa, 1971 *sensu* Babenko et al., 1994, and *C. denticulata* (Bagnall, 1941) are too identical. So, the taxonomic position of this group is unclear and requires more studies (Skarżyński, 2000). DNA barcoding methods have provided researchers with ways to delimit such groups. Also, this technique is used frequently by researchers to describe new

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species of the class Collembola. Nakamori (2013) described a new species of *Ceratophysella* from Japan based on both morphological and molecular data. Skarżyński et al. (2018) examined the species complex *Xenylla maritima* using both morphological and molecular characters and reviewed the taxonomic status of nine forms in the *maritima* species group. Six were recognized as distinct species, and three as synonyms. Skarżyński et al. (2021) described a new species of *Ceratophysella* based on morphological data and DNA barcodes. Yoosefi Lafooraki et al. (2023) described three new species of *Isotomurus* from Iran and examined colour pattern credit as a tool for species identification using COI and 28SrRNA markers. Paśnik and Smolis (2024) tested taxonomic characters used in the classification of Neanurinae, and the results said that these characters are shared among members of different tribes.

In order to contribute to the knowledge of *Ceratophysella* genus in Iran, a taxonomical study based on both morphological and molecular data was made in Iran. For this objective, in the present paper, we provide the redescription of *C. communis* based on the Iranian material and phylogenetic tree for the genus. In the original description of the species, the dorsal body chaetotaxy, antennae chaetotaxy, mouthparts, and claws morphology are not described in detail, and the species is not included in the key of the genus presented in Thibaud et al. (2004). In this paper, we describe more characters for the species and present an identification key for all reported species of the genus from Iran.

MATERIAL AND METHODS

Morphological study. Specimens were collected from Iran, Mazandaran, Guilan, and Isfahan provinces. They were extracted using a modified Berlese funnel, placed in 96% ethanol, and stored at -20°C . For morphological analysis, specimens were cleared in Nesbitt's solution or 10% KOH and mounted in Hoyer's medium on microscope slides. They were studied using a Nikon® ECLIPSE E600 microscope. Materials are deposited in the collection of the Entomology lab in the Department of Plant Protection, Faculty of Crop Sciences, Sari University of Agricultural Sciences and Natural Resources (SANRU), Iran and in the Museo Nacional de Ciencias Naturales at Madrid, Spain (MNCN). *Abbreviations:* Abd. – abdominal segment; Ant. – antennal segment; PAO – post antennal organ; Th. – thoracic segment.

DNA barcoding. DNA was extracted from priori identified specimens of *Ceratophysella* using SinaPure DNA Kit (SinaClon, Tehran, Iran) following the manufacturer's standard protocols. For each species, we used a single specimen and DNA was extracted from the whole body. The COI region was amplified using LCO1490 and HCO2198 primers (Folmer et al., 1994). PCR products were checked on a 1% Agarose gel. Successful products were purified and sequenced by Sanger sequencing of Microsynth (Balgach, Switzerland). Sequences were blasted in GenBank, checked for possible errors, and then preliminarily aligned using MEGA 6.0 (Tamura et al., 2013). Alignments were checked and corrected manually. Intra- and interspecific divergences of COI were calculated based on K2P (Kimura, 1980) and uncorrected p-distances using MEGA 6.0. (Tamura et al., 2013). All sequences are available at NCBI GenBank. The jModeltest 0.1.1 was applied to select the best model of nucleotide substitution with Akaike Information Criterion (AIC) (Posada, 2008). The selected model was GTR+I+G. Maximum likelihood (ML) analysis. It was performed with raxmlGUI 3.1 using newly provided sequences, which were obtained from GenBank (Silvestro & Michalak, 2012).

RESULTS

Taxonomic hierarchy

Phylum Arthropoda von Siebold, 1848

Class Collembola Lubbock, 1871

Order Poduromorpha Börner, 1913

Family Hypogastruridae Börner, 1906

Genus *Ceratophysella* Börner, 1932

Type species: *Ceratophysella armata* (Nicolet, 1842)

Ceratophysella communis (Folsom, 1897)

Material examined. 8 / ♂♀, Iran, Isfahan Province, Isfahan, Nazhvan Forest Park, 32°38'14" N, 51°37'3" E, 1577 m, 16-XII-2021, Maryam Salimi leg.

Redescription (based on the specimens from Iran). Body length up to 1.8 mm. Colour dark greyish brown to black. Cuticular granulation fairly fine and even on body; 20–24 cuticular granules between p1 chaetae on Abd. V. Differentiation of dorsal chaetae into meso- and macrochaetae distinct (Fig. 1). Body chaetae long (ratio p1 mesochaetae and p2 macrochaetae on Th. II/inner edge of claws III = 0.6–0.7 and 1.6–1.8, respectively), fairly thin, tapering and pointed, macrochaetae distinctly longer, thicker and finely serrated (central macrochaetae blunt); arrangement of chaetae on head typical for the genus, spine-like chaetae absent; Thoracic terga II–III with m6 chaetae present and m3 chaetae absent; Abd. IV with p2 chaetae developed as macrochaetae, p1 as mesochaetae (dorsal chaetotaxy of type A), p3 chaetae present; p1 < p2 on Th. II to Abd. IV; Abd. I–III with 4 + 4, Abd. IV with 3 + 3, and Abd. V with two mesochaetae between median macrochaetae p2; a'2 chaeta on Abd. I–III present; body S chaetae relatively long (ratio S p4 and m7 on Th. II/inner edge of claws III = 1.2–1.4 and 1.0–1.2, respectively; ratio a1 mesochaeta and p1 macrochaeta/S chaeta on Abd. V = 0.5–0.6 and 1.3–1.4, respectively), thin and smooth; S chaeta on Th. II, III p4 and m7, Abd I–IV p5 and Abd V p3; S microchaeta (ms) on Th. II present; subcoxae I, II, III with 1, 3, 3 chaetae, respectively.

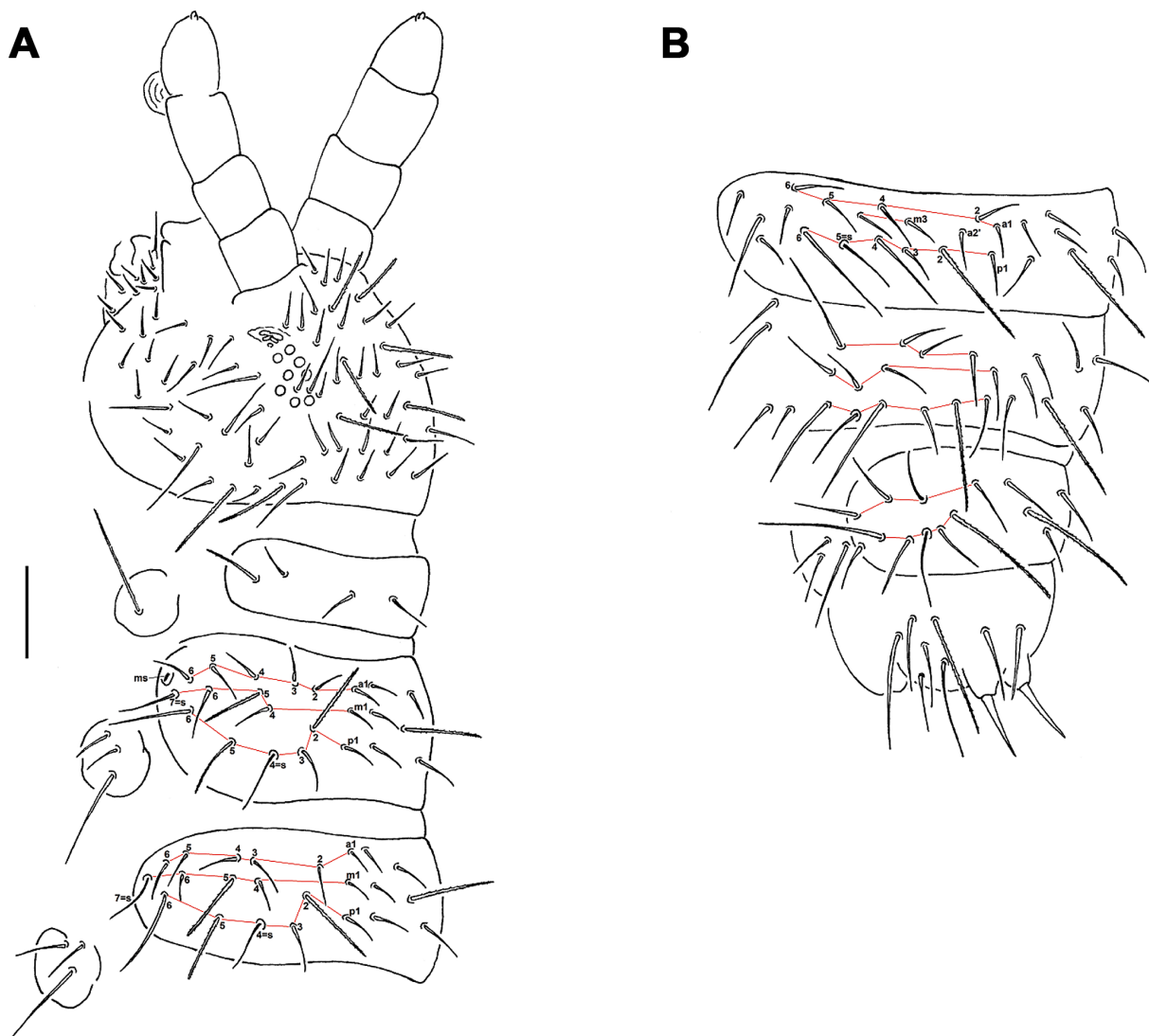


Figure 1. *Ceratophysella communis* (Folsom, 1897) from Iran. Habitus. Scale bar = 0.1 mm

Antennal segment IV with bilobed apical vesicle, subapical organite (or), microsensillum (ms), seven (2 lateral, 5 dorsal) cylindrical S chaetae that are extremely curved and fairly thick and about 10 short, apically oblique chaetae in ventral field. Ant. III organ normal with the five S chaetae. Eversible sac between antennal segments III and IV present. Antennal segment I with seven chaetae. Ocelli: 8 + 8. PAO about twice width of anterior ocellus, with four tubercles, two anterior distinctly larger than the posterior vesicles, and one lateral accessory tubercle. Labrum with 5, 5, 4 chaetae; 4 prelabrals present. Labial palp with 6 proximal chaetae. Maxillary palp simple with one sublobal hair. Tibiotarsi I, II, III with 19, 19, 18 chaetae, respectively; one longer pointed tenent hair on each leg. Claw with mid-inner tooth. Empodial appendage with broad basal lamella, reaching inner tooth or slightly beyond; ratio empodial appendage/inner edge of claws = 0.5–0.6. Ventral tube with 4 + 4 chaetae. Furca well developed. Ratio dens + mucro/inner edge of claw III = 2.7–2.9, ratio dens/mucro = 1.8–2.1. Dens with uniform fine granules and seven thin dorsal chaetae. Mucro wide at tip, boat-like, with large outer lamella. Retinaculum with 4 + 4 teeth. Anal spines long and curved, clearly longer than claws, on high papillae (ratio anal spines: papilla = 2.0–2.2; ratio anal spines/inner edge of claw III = 1.2–1.4).

Distribution. Widely distributed in East Asia, it has also been recorded in Canada, Japan, South America and Australia (Thibaud et al., 2004). This species has been recorded for the first time from Iran.

GenBank number. OR924466.

Ceratophysella impedita Skarzynski, 2002

Material examined. 1 ♀, Iran. Guilan Province, Fuman, Masouleh, 37°9'41" N, 49°0'18" E, 825 m, 06-III-2018, Elham Yoosefi Lafooraki leg; 1 ♂, 3 juvenils, Guilan Province, Rudbar, Oskolk, pine forest, 37°0'45" N, 49°34'58" E, 128 m, 04-III-2018, Elham Yoosefi Lafooraki leg.

Diagnosis. Tegumentary granulation quite strong, 8–12 granules between the p1 chaetae on Abd. V. Claw with internal teeth and without lateral teeth. Dens with 7 chaetae. Chaetotaxy of type B (Abd. IV with p1 chaeta longer than p2 chaeta). Chaeta a2' on Abd. I–III absent. The chaetae a4 and p3 on Abd. IV present.

Distribution. So far, it is only known in Poland (Thibaud et al., 2004). This species has been recorded for the first time from Iran.

GenBank number. OR924464.

Ceratophysella stercoraria Stach, 1963

Material examined. 6 ♀♀, Iran. Isfahan Province, Isfahan, Nazhvan Forest Park, 32°38'14" N, 51°37'3" E, 1577 m, 16-XII-2021, Maryam Salimi leg.; 2 ♀♀, Mazandaran Province, Sari, Doangeh, Bula (Boola) forest, 36°03'43.46" N, 53°14'01.77" E, 1423–1674 m, 19-IV-2021, Elham Yoosefi Lafooraki leg.

Diagnosis. Between the p1 chaetae on Abd. V, an area of very coarse granuli in a semicircular arrangement. Claw with internal tooth and two lateral teeth. Dens with 7 chaetae. Chaetotaxy of type A (Abd. IV with p1 chaeta shorter than p2 chaeta). Th. II and III with m6 chaeta, without m2 + m3. Abd. I–III and V with a2' chaetae.

Distribution. Eastern Europe, Central Asia (Thibaud et al., 2004). Already recorded in Iran (Shayanmehr et al., 2020).

GenBank number. OR924465.

DNA barcoding. The maximum likelihood (ML) tree based on mitochondrial COI sequences is provided in Fig. 2. Species of *Ceratophysella* are successfully discriminated using COI sequences. Sequences for *C. impedita* and *C. stercoraria* have been submitted to GenBank for the first time. Pairwise K2P distances between individuals were calculated (Table 1). The mean genetic divergence among the eight *Ceratophysella* species included in the analysis was 26.9% (ranging from 19.7% to 33.7%), and their mean intraspecific variation was 1.7% (ranging from 0.3% to 2.7%).

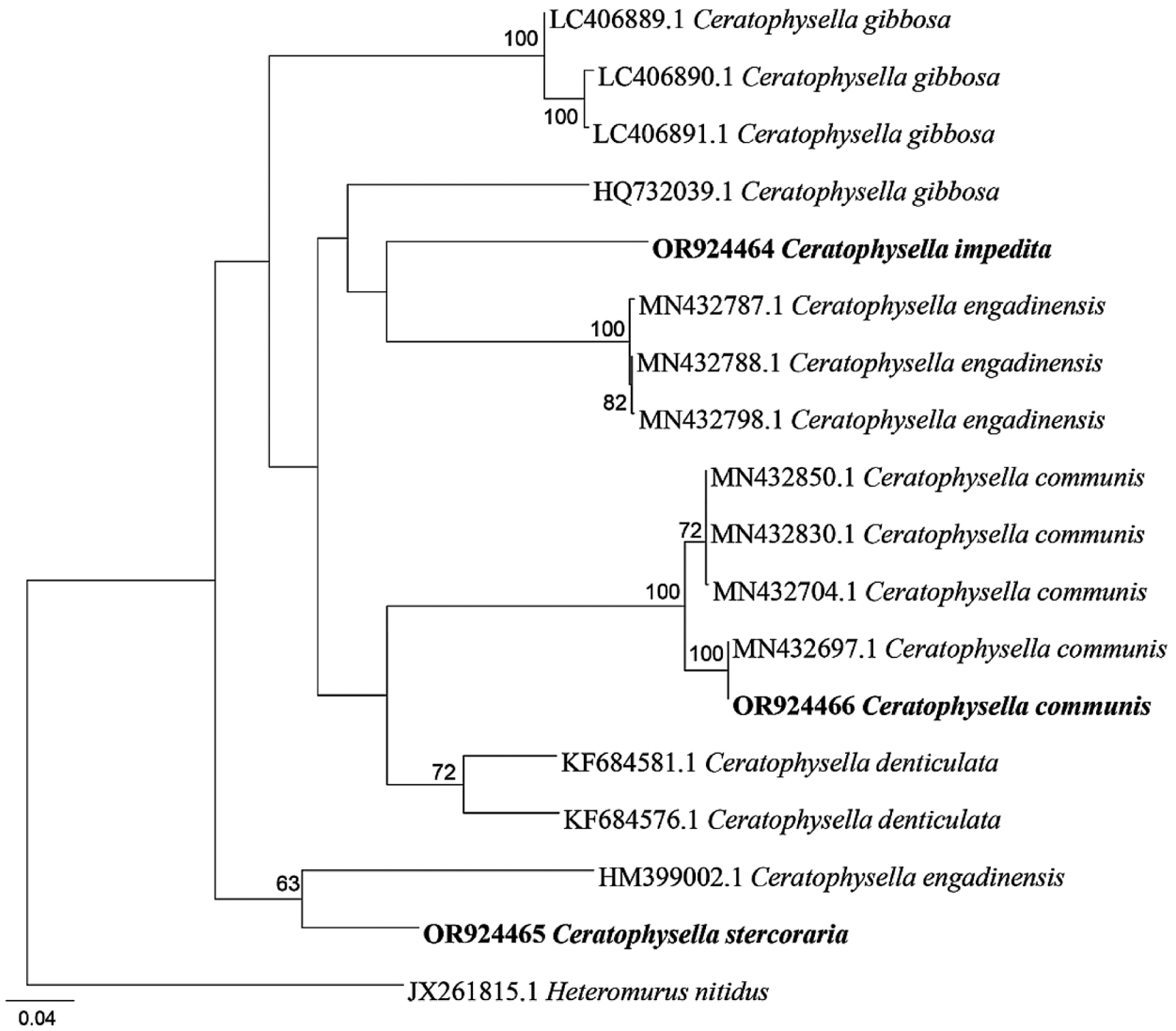


Figure 2. Maximum Likelihood consensus tree generated from the COI gene dataset with the GTR+I+G model. Bootstrap values of more than 50% are given for appropriate clades; newly obtained sequences are in bold letters, and others are from GenBank (NCBI).

Table 1. Intraspecific and interspecific genetic divergences among *Ceratophysella* species based on K2P distance percentages for COI.

SPECIES	Intraspecific divergence	<i>C. communis</i>	<i>C. impedita</i>	<i>C. stercoraria</i>	<i>C. denticulata</i>	<i>C. engadinensis</i>	<i>C. gibbosa</i>	<i>C. skarzynskii</i>	<i>C. liguladorsi</i>
<i>C. communis</i>	2.7	--							
<i>C. impedita</i>	--	29.2	--						
<i>C. stercoraria</i>	--	26.6	32.3	--					
<i>C. denticulata</i>	1.2	23.5	24.5	27	--				
<i>C. engadinensis</i>	--	27.9	33.7	21.5	27.7	--			
<i>C. gibbosa</i>	--	28.5	26.6	26.7	24.6	27.6	--		
<i>C. skarzynskii</i>	0.3	19.7	26.8	27.9	25.2	29.8	25.0	--	
<i>C. liguladorsi</i>	2.6	29.3	28.0	27.1	26.1	27.6	24.7	29.8	--

Key to Iranian species of *Ceratoophysella* Börner, 1932

- 1 Abd. IV chaeta p1 longer than p2 (type B chaetotaxy) (Fig. 3A). 2
 – Abd. IV chaeta p1 shorter than p2 (type A chaetotaxy) (Figs 1B, 3D, 3E). 5
- 2 Body granulation rather fine: more than 16 granules between p1 chaetae on Abd. V.
 *C. armata* (Nicolet, 1842)
- Body granulation rather coarse, especially on Abd. V-VI: 8-12 granules between p1 chaetae on Abd. V (Fig. 3A). 3
- 3 Head with spine-like chaetae (Fig. 3B). Abd. V with a strongly granulated hump between p1 chaetae (Fig. 3C). *C. tuberculata* Cassagnau, 1959
- Head without spine-like chaetae. Abd. V without such hump. 4
- 4 Microchaetae p3 on Abd. IV absent; Claws with two lateral teeth. *C. borealis* Martynova, 1977
- Microchaetae p3 on Abd. IV present (Fig. 3A); Claws without lateral teeth. ... *C. impedita* Skarżyński, 2002
- 5 Abd. IV with 2+2 medial microchaetae. Abd. V with a strongly granulated, wart-like hump between the p1 chaetae (Fig. 3D). *C. gibbosa* (Bagnall, 1940)
- Abd. IV with 3+3 medial microchaetae. Abd. V without such a wart-like hump (Fig. 3E). 6

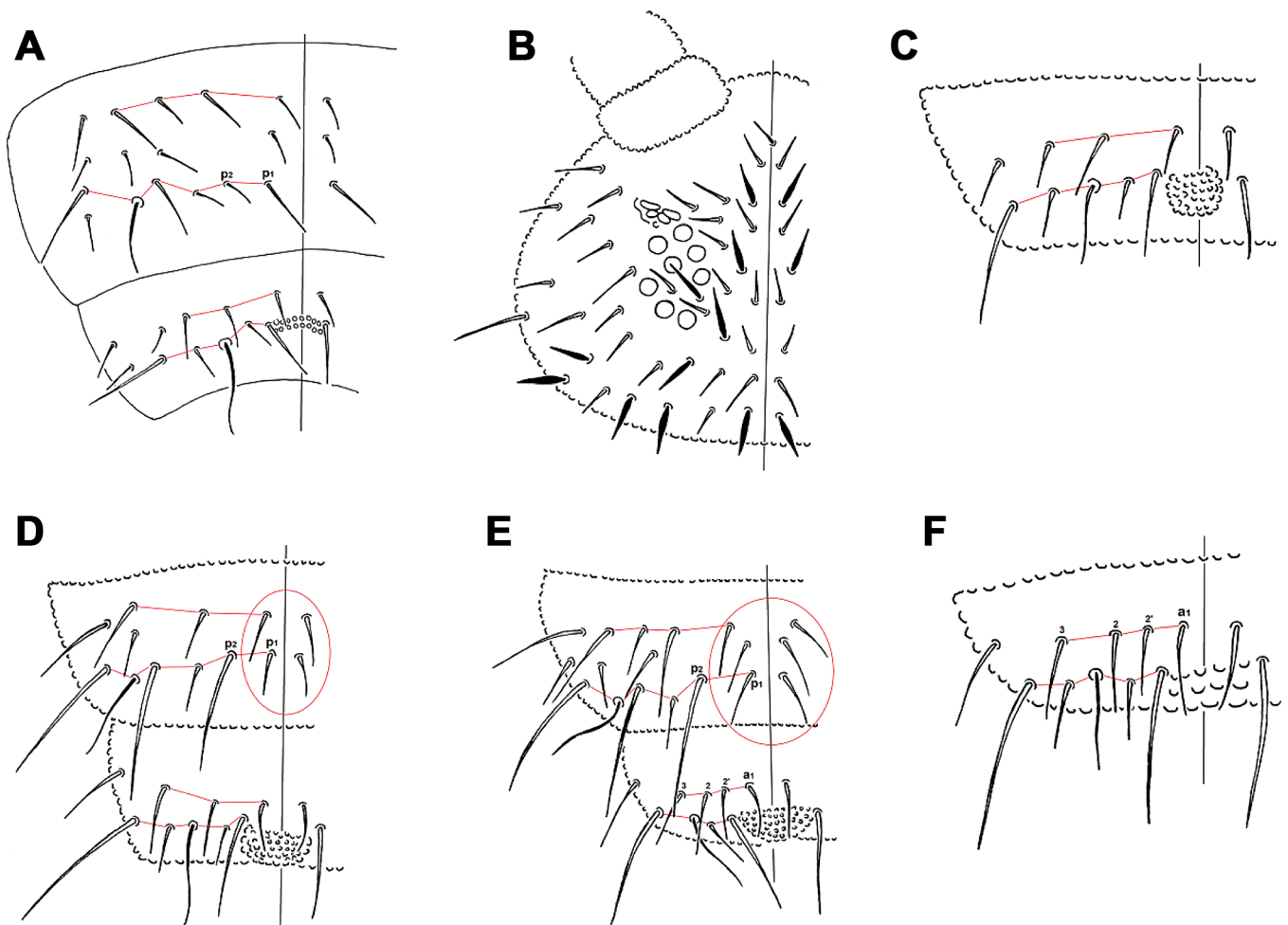


Figure 3. A. Chaetotaxy type B (Abd. IV chaeta p1 longer than p2); B: Head with spine-like chaeta; C. Abd. V with a strongly granulated hump between p1 chaetae; D. Abd. IV with 2+2 medial microchaetae; E. Abd. IV with 3+3 medial microchaetae; F. Abd. V with 4+4 a-chaetae between the bases of p5 macrochaetae (chaeta a2' present).

- 6 Abd. V with a semicircular hump between the p1 chaetae (Fig. 3E). *C. stercoraria* (Stach, 1963)
- Abd. V without such semicircular hump. 7
- 7 Abd. V with 4+4 a-chaetae between the bases of p5 macrochaetae (chaeta a2' present) (Fig. 3F).
..... *C. denticulata* (Bagnall, 1941)
- Abd. V with 3+3 a-chaetae between the bases of p5 macrochaetae (chaeta a2' absent) (Fig. 1B). 8
- 8 Ant. IV with simple apical bulb (Fig. 4A). 9
- Ant. IV with bilobed apical bulb (Fig. 4B). 10
- 9 Dens with 6 posterior thin chaetae (Fig. 4C). *C. succinea* (Gisin, 1949)
- Dens with 7 posterior chaetae, the 2 internal thickened (Fig. 4D). *C. engadinensis* (Gisin, 1949)
- 10 Dens with 7 posterior thin chaetae (Fig. 4E). Ant. IV with about 10 short, apically oblique sensory chaetae in ventral field (Fig. 4B). *C. communis* (Folsom, 1997)
- Dens with 7 posterior chaetae, the 4 internal thickened (Fig. 4F). Ant. IV with 20–25 short, slightly recurved sensory chaetae in ventral field. *C. alani* (Babenko, 1994)

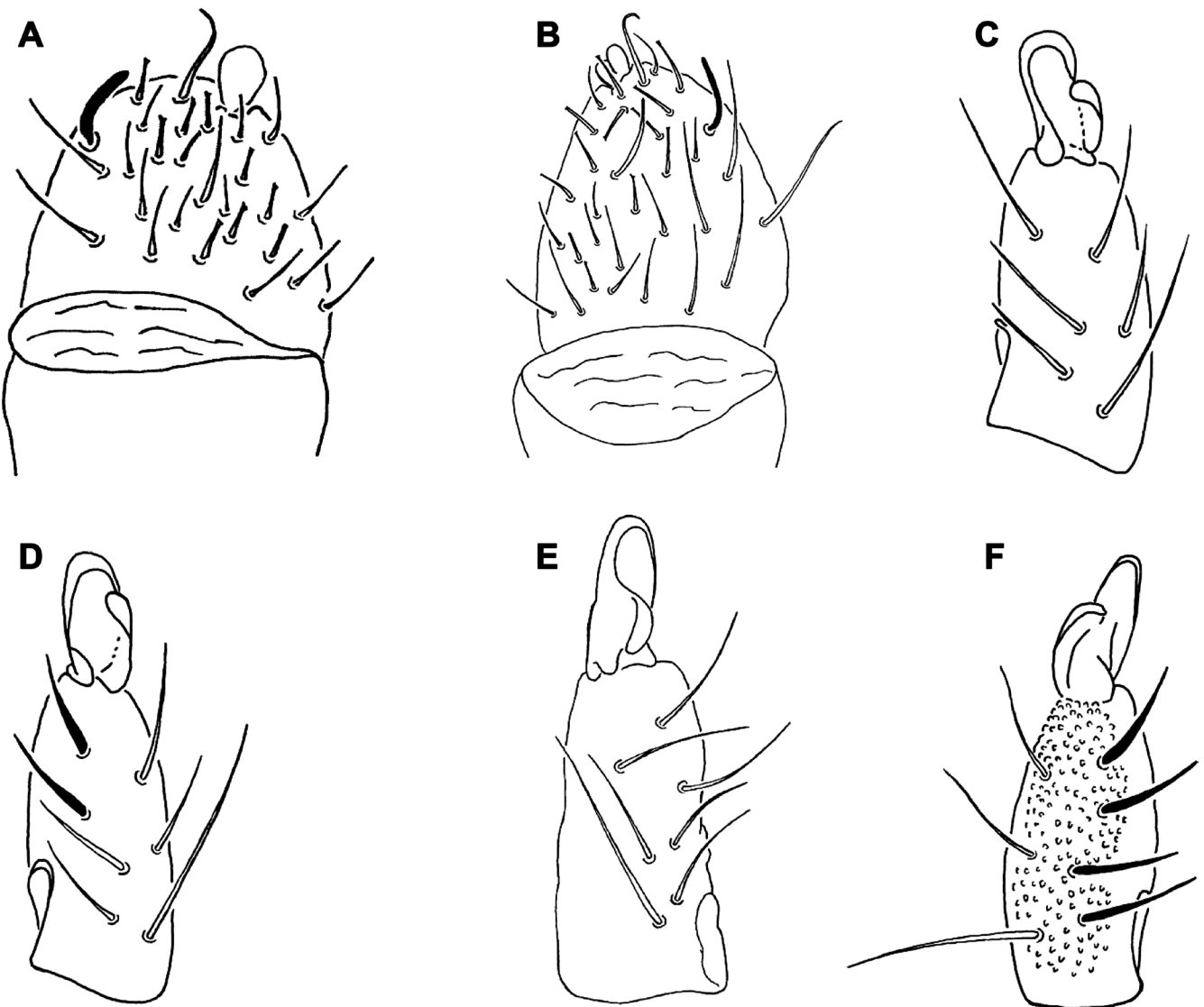


Figure 4. **A.** Ant. IV with simple apical bulb; **B.** Ant. IV with bilobed apical bulb and about 10 short, apically oblique sensory chaetae in ventral field; **C.** Dens with 6 posterior thin chaetae; **D.** Dens with 7 posterior chaetae, the 2 internal thickened; **E.** Dens with 7 posterior thin chaetae; **F.** Dens with 7 posterior chaetae, the 4 internal thickened.

DISCUSSION

The results of this study added two species of the family Hypogastruridae, including *Ceratophysella impedita* and *C. communis* to the Iranian Collembola fauna. As Thibaud et al. (2004) mentioned, *C. impedita* inhabits in litter of coniferous woods (in Poland). In this work, it is also found in a pine forest in the North of Iran. *C. communis* was already known from Asia, Australia, South America, Canada, and Japan. So, it is a sub-cosmopolitan species. Among all *Ceratophysella* species reported from Iran, six species, including *C. armata*, *C. borealis*, *C. denticulata*, *C. engadinensis*, *C. gibbosa*, and *C. succinea* are cosmopolitan or sub-cosmopolitan, and three species including *C. alani*, *C. stercoraria*, and *C. tuberculata* inhabit in Palaearctic region. Contrary to other genera in Poduromorpha, no endemic species were identified for the genus *Ceratophysella* in Iran. This can be due to a lack of enough studies on this genus in Iran. Most species reported from Iran are found in the North of the country, often from Hyrcanian forests, which possess both humid subtropical climate (*cfa*) and hot-summer Mediterranean climate (*csa*) according to Köppen climate classification (Beck et al., 2018). It is recommended that *Ceratophysella* fauna be investigated in other habitats of Iran, such as caves, mountains, deserts, etc., in different climates. On the other hand, difficulties in identifying *Ceratophysella* species, such as insufficient descriptions, lack of identification keys for some species, identical species in species-groups, etc., can slow down their study. Recently, DNA-based techniques have been successfully used for the molecular distinction of species of Collembola groups (Winkler et al., 2020; Valle et al., 2021; Jing et al., 2023; Yoosefi Lafooraki et al., 2023; Jia et al., 2024).

Analysis of morphological features is generally used in the taxonomy of the genus *Ceratophysella*. Recently, DNA barcoding methods have also been used, although on a small scale. Combined use of the morphological and molecular data not only helps to accurately identify collembolen species but also gives us a better view of the relationships between them. DNA barcoding data are available for only a few of the *Ceratophysella* species. This study is the first attempt to study DNA sequences of the genus *Ceratophysella* in Iran.

AUTHOR'S CONTRIBUTION

The authors confirm their contribution to the paper as follows: M. Salami: performed the project as an MSc thesis; M. Shayanmehr: encouraged M.S to investigate, supervised the project and the finding of this work; E. Yoosefi Lafooraki: advised the project and took the lead in writing the manuscript, M. Mohamadi Sharif, M.: advised the project and took the lead in writing the manuscript; J. Arbea: advised the project and took the lead in writing the manuscript. All authors read and approved the final version of the manuscript.

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AVAILABILITY OF DATA AND MATERIAL

The specimens listed in this study are deposited in the collection of the Entomology lab in the Department of Plant Protection, Faculty of Crop Sciences, Sari University of Agricultural Sciences and Natural Resources (SANRU), Iran and in the Museo Nacional de Ciencias Naturales at Madrid, Spain (MNCN), and are available from the curator, upon request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study only included arthropod material, and all required ethical guidelines for the treatment and use of animals were strictly adhered to in accordance with international, national, and institutional regulations. No human participants were involved in any studies conducted by the authors for this article.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

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دستاورد‌های نوین در مطالعه جنس *Ceratophysella* Börner, 1932 (Collembola: Hypogastruridae) در ایران

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چکیده: جنس *Ceratophysella* Börner, 1932، دومین آرایه بزرگ در خانواده Hypogastruridae و شامل ۱۴۶ گونه از سراسر جهان می‌باشد که ۹ گونه از ایران گزارش شده است. نمونه‌ها با استفاده از کیف برلیز اصلاح شده از نمونه خاک و بستر برگ استخراج شدند. توالی ژن COI میتوکندری از کل بدن نمونه‌های *Ceratophysella* به دست آمد. تبارنمای مشتق از تحلیل حداکثر احتمال بر اساس توالی‌های ژن COI نشان‌دهنده قرابت‌های فیلوژنتیک گونه‌های شناخته شده *Ceratophysella*، تولید شد. در پژوهش حاضر گونه‌های *C. communis* (Folsom, 1897) و *C. impedita* Skarzynski, 2002 برای اولین بار از ایران ثبت شدند. گونه *Ceratophysella communis*، مجدداً بازتوصیف و خصوصیات شناسایی آن ترسیم شد. کلید شناسایی گونه *Ceratophysella* ایران ارائه شد.

واژگان کلیدی: بارکدینگ دی.ان.آ.، بازتوصیف، توالی سیتوکروم اکسیداز ۱، تاکسونومی، گزارش جدید