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A new fairy shrimp *Phallocryptus tserensodnomi* (Branchiopoda: Anostraca) from Mongolia

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Abstract

A new species of *Phallocryptus* Biraben 1951 (Branchiopoda, Anostraca) from Mongolia is described. *Phallocryptus tserensodnomi* **sp. nov.** is close to *P. spinosa* (Milne-Edwards 1840), but both morphological and molecular analyses (Cytochrome Oxidase I, COI) indicate that they represent separate species. Most relevant differential features of the new species include: (1) frontal appendage provided with small ventral conical outgrowths; (2) second antennamere evenly curved, sickle-shaped; (3) distal fleshy process on labrum evenly curved forwards and tapering; (4) short stout acute spine-like projections present at each side of basal portion of gonopods; (5) female second antennae shorter and wider than in *P. spinosa*, tapering. Based on morphological comparisons the new species appears to be a Mongolian endemic, although some genotypes of presumed *P. spinosa* from Africa are similar to the new species, suggesting *P. tserensodnomi* might have a wider distribution.

Key words: Anostraca, Thamnocephalidae, *Phallocryptus tserensodnomi*, *Phallocryptus spinosa*, new species, Mongolia

Introduction

Mongolia harbours a vast array of lakes and wetlands scattered over a huge undisturbed territory. Ten limnological expeditions conducted all over the country during the last

eight years in the framework of the project “Biodiversity of Crustacea Entomostraca in the Palaearctic” in collaboration with the Water Research Center of the National University of Mongolia, have lead to the accumulation of an important collection of aquatic crustaceans, including large Branchiopoda. The Mongolian species of this group have been dealt with previously by several authors (Sars 1901; Brtek *et al.* 1984; Naganawa *et al.* 2001, 2002; Naganawa & Zagas 2002, 2003), but as Rogers (2005) pointed out, new records and the discovery of new species should be expected after extending the surveys to unexplored areas. Until now, there were nine fairy shrimp taxa reported from the country, two of them recently described as new species (Rogers 2005; Alonso 2008), whereas the ninth is a not yet described member of the genus *Phallocryptus* collected in saline lakes (Alonso 2010).

The genus *Phallocryptus* is one of the few members of the anostracan family Thamnocephalidae currently embracing three different species (Rogers 2003). They are halobionts living in arid and semiarid areas. Amongst them, *P. spinosa* is the only species showing a wide distribution, being present in western Eurasia and Africa, whereas the other two species are limited to the American continent, *P. wrighti* being widespread in Argentina while *P. sublettei* is a relict species from Texas, USA (Rogers 2003). In this study we describe a new species of *Phallocryptus* from Mongolia as *P. tserensodnomi* **sp. nov.** This taxon was initially reported by Alonso (2010) as *Phallocryptus* sp. and is closely related to *P. spinosa* (Milne-Edwards 1840). Both morphological and molecular analyses indicate nevertheless that they represent different species that probably originated by allopatric speciation.

Material and Methods

Samples were collected in the fordable areas of the lakes using a plankton net of 500 µm mesh size and fixed in 4% formaldehyde. Some specimens were fixed separately in 100% ethanol for genetic analyses. Specimens were treated with hot lactic acid and gently stained with chlorazol black to enable the observation of the finest integumentary structures. Dissections were made under a stereomicroscope, and drawings made with a camera lucida attached to an Olympus® BH-2 compound microscope equipped with phase contrast optics. Scanning electron microscopy used a FEI INSPECT (5350 NE Dawson Creek Drive Hillsboro, Oregon 97124, USA) Scanning Electron Microscope (ESEM) of the Museo Nacional Ciencias Naturales (Madrid, Spain). The ESEM

microscope in low vacuum mode admits hydrated samples to be studied in their original state with the large field detector (LFD), since it is close to the sample in order to avoid electron losses, the samples were observed with the Back Scattering Electron Detector (BSED). The SEM resolution at low-vacuum was at 4.0nm and 30kV (BSED). Salinity was measured *in situ* with a Krüss HR10 hand-held refractometer.

Genetic analyses. DNA was extracted from one or two individuals by proteinase K digestion. A portion of ca. 1–3 mm of each specimen was transferred to UV light-sterilized 200- μ L microcentrifuge tubes in 100 μ L proteinase K-buffer (Schwenk *et al.* 1998). Samples were incubated for 3 h at 56°C, followed by 10 min at 99°C, and 2-min centrifugation, and subsequently stored at -20 °C.

Fragments of a 658-nt section of the cytochrome c oxidase gene subunit 1 (COI) using primers LCOI490 (5'-GGTCAACAAATCATAAAGATATTGG- 3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAATCA-3') (Folmer *et al.* 1994) were amplified bidirectionally following the standards of DNA barcode of life (Ivanova *et al.* 2009). Previous *Phallocryptus* genetic studies used both mitochondrial COI and 16S rRNA genes to characterize the phylogeny of the group, and yielded nearly identical results. Therefore we used only one of the two genes to characterize our populations and compare them with previously published sequences. The total reaction volume (25 μ L) consisted of 1 \times PCR buffer (Silverstar, Eurogentec), 1.5 mm MgCl₂, 200 μ m of each dNTP, 0.2 μ m of each primer, 1 μ L of template DNA, 1–2 U Taq polymerase and UV light-sterilized mQ-H₂O. PCR amplifications involved a denaturing step of 5 min at 95°C, five cycles of 60 s at 95°C, 90 s at 45°C and 45 s at 72°C, followed by 30 cycles of 45 s at 95°C, 45 s at 50°C and 45 s at 72°C and a final elongation of 7' at 72°C. PCR products were purified and sequenced on ABI 3730XL capillary sequencers by a third party (Macrogen, Seoul, Korea). Resulting sequences (deposited in GenBank under accession numbers KF040444 to KF040446) were aligned with sequences of other relevant *Phallocryptus* species (retrieved from GenBank; Ketmaier *et al.* 2008) using the ClustalW algorithm (Thompson *et al.* 1994) in MEGA version 5 (Tamura *et al.* 2011). Among the four clades described by Ketmaier *et al.* (2008), we only included the sequences from clades I and IV since their three sequences of COI belonging to clades II and III seem not to belong to anostracans (Ketmaier *et al.* 2013). The alignments were checked by eye and corrected according to the translated amino-acid alignment, and sequence divergences (Kimura 2-parameter model) were calculated with the same software.

Phylogenetic relationships within the *Phallocryptus spinosa* complex were subsequently assessed using a part of the COI gene and using *Streptocephalus dorotheae* as outgroup. We used Modeltest (Posada 2008) to select the best model of nucleotide substitution, and assessed the phylogeny using the Bayesian inference (BI) in MrBayes version 3.1.2 (Ronquist & Huelsenbeck 2003), and Maximum Likelihood (ML) and Maximum Parsimony (MP) analyses in PAUP* 4.0b10 (Swofford 2002). In BI, two parallel runs of four Monte Carlo Markov chains were run for 3 million generations, trees were sampled every 100 generations, and the first 25% of sampled trees were discarded as a burn-in phase. In PAUP, heuristic searches were conducted with tree bisection-reconnection branch swapping and 10 random sequence taxon additions; branch support was evaluated by nonparametric bootstrapping with 100 (ML) and 1000 (MP) pseudoreplicates.

Molecular clock estimates are not very reliable for anostracans due to the lack of adequate fossil data. However, in order to provide a rough approximation of the divergence times between both species we used two crustacean clock calibrations of Knowlton & Weigt (1998) (1.4% sequence divergence per million years) and Wares (2001) (4.9% sequence divergence per million years). Both calibrations are based on COI data. Corrected average pairwise genetic sequence (using the best fit model for the COI dataset, GTR+G model) between haplotypes was used in all divergence time estimates.

Abbreviations. Collections: MNCN = Non-insect Invertebrates Collection of Museo Nacional Ciencias Naturales, Madrid, Spain. MA = Miguel Alonso. GM = Graziella Mura. TA = Theodore Abatzopoulos. JB = Ján Brtek.

Mongolian terms: “nuur” = lake, “toirom” = lagoon, “aimag” = province.

Results

Taxonomy

Class Branchiopoda Latreille, 1817

Order Anostraca Sars, 1867

Family Thamnocephalidae Packard, 1883

Subfamily Branchinellinae Daday 1910

Genus *Phallocryptus* Biraben 1951

***Phallocryptus spinosa* (Milne-Edwards, 1840)**

(Fig. 1)

Material examined. Laguna de Gallocanta (Gallocanta, Zaragoza, Spain), 40°58'06" N, 1°29'52"W, June, 2008, MA Collection; Laguna de Fuente de Piedra (Fuente de Piedra, Málaga, Spain), 37°06'34"N, 4°46'12" W, April, 2010, MA Collection; Adeg (Iran), 37°43'48.7" N, 45°14'54", May, 2011, MA Collection; Khaselou (Iran), 37°49'32"N, 45°50'7.4"E, GM Collection; Greece, TA Collection. Abe Istada lake (Ghazni, Afghanistan), 32°28'60" N y 67°55'0" E, JB Collection.

Short description. The species has been fully described by Cottarelli and Mura (1983), Alonso (1996) and Rogers (2003), among others. Here we focus only on the characters to be used in the differential diagnosis of the new species.

Male. Second antenna capable of extending backwards reaching to fourth thoracopod. Distal antennamere 1.5 times as long as proximal counterpart, proximal half curved (Fig. 1E). Frontal appendages smooth (Fig. 1A, B). Labrum with elongate stout digitiform fleshy process curved forwards ("L" shaped) on tip (Fig. 1C, D). Second genital segment, basal portion of gonopods and everted part of gonopods as figured (Fig. 1F–H). First four abdominal segments with distinctive midventral integumentary bulge on proximal margin (Alonso 1996: fig. 26I) covering set of subjacent small papillae. All abdominal segments with distal margin bearing medial pair of ventrally directed spine-like projections, those of first segment large, whereas those on other segments progressively smaller towards posterior (see Alonso 1996: fig. 26H); latter character subject to variability since Iranian and Greek populations, apart from that described by Rogers (2003), with midventral spine-like projections present only on first, second and sometimes third abdominal segments.

Female. Second antenna lamellar, four times longer than wide, counterparts fused medially at base and with sharp recurved apex.

Size. Medium-sized to large fairy shrimp. Total body length (including setae of cercopods) of larger specimens in Gallocanta Lake up to 40 mm.

Distribution and Ecology. Widespread in the dry areas of the Palearctic, such as the Circum-Mediterranean area, the eastern European steppes, the Middle East and some areas of Afghanistan and Kazakhstan to the east. It has been reported from Africa south to Botswana (several references in Belk & Brtek (1995), Rogers (2003)). It lives in temporary or permanent shallow lakes. Water quality ranges from mesosaline to

hypersaline (according to Hammer 1986) and with variable turbidity (Alonso 1990). In Gallocanta and Fuente de Piedra lakes, it co-occurs with halophyle species such as the cladocerans *Daphnia mediterranea* Alonso, 1985, *Moina salina* Daday, 1888 and the copepods *Arctodiaptomus (Rh.) salinus* Daday, 1885 and *Cletocamptus retrogressus* Schmankevitch, 1875 (Alonso 1998).

***Phallocryptus tserensodnomi* sp. nov.**

(Figs 2–5)

Etymology. Species named after J. Tserensodnom in recognition of his contributions to the knowledge of the Mongolian lakes, particularly the production of the first catalogue including more than 3,000 lakes.

Type locality. Shorvog nuur, code 342AR, Arkhangai aimag, Mongolia (47°39'0.54"N; 102°22'11.0" E).

Type material. Holotype. Undissected mature male preserved in 4% formaldehyde vial (MNCN accession number: 20.02/17242), coll. M. Alonso, September 2009.

Allotype. Undissected mature female preserved in 4% formaldehyde vial (MNCN 20.02/17243), coll. M. Alonso, September 2009.

Paratypes. 6 males and 10 females preserved in 4% formaldehyde vial (MNCN 20.02/17244), coll. M. Alonso, September 2009.

Comparative material examined. All in MA collection. Uizen nuur, code: 322SU, Sukhbaatar aimag, Mongolia (45°21'37.5''N; 113°18'16.2''E), September 2006; Mendbayar nuur, code 1874 DOD, Dornod aimag, Mongolia (47°51'51.7''N; 117°57'58.6''), September 2006; Khalkh Gol bagiin nuur 2, code 1875 DOD, Dornod aimag, Mongolia (47°49'22.2''N; 117°54'40.0''E), September 2006; Shiliin nuur, code 142TU, Tuv aimag, Mongolia (47°01'18.8''N; 106°07'31.7''E), September 2009; Khar nuuriin toirom, code 11TU, Tuv aimag, Mongolia (48,21271°N; 104,90981°E), August 2012. Information and pictures of sampling sites can be easily accessed on the web (at http://www.geodata.es/mongolian_lakes/).

Diagnosis. Male. Frontal appendages short, provided with small conical outgrowths. Distal antennameres long and narrow, evenly curved, sickle-shaped, reaching to IV–V thoracopods IV–V. Distal fleshy process on labrum evenly curved

forwards. Short stout spine-like projections present at each side of basal portion of gonopods. Distal margin of abdominal segments each provided with pair of large ventrolateral spine-like projections. Abdominal somites each with pair of medial spine-like projections on posterior margin; projections on first segment large, remainder smaller and progressively reduced towards posterior.

Female. Second antennae lamellar, broad, three times longer than wide, with broad pointed apex. Brood pouch fusiform, not protruding laterally from genital segments. Eggs sub-spherical with surface sculptured with shallow angular elongated depressed facets limited by blunt ridges.

Description. Male. Body unpigmented. *Head* (Fig. 2A–C) with rounded anterolateral angle barely covering eyestalk. Nuchal organ apparently absent. Eyes spherical with diameter longer than corresponding eyestalk.

First antennae (Fig. 2A, D) filiform, three times longer than eye diameter and shorter than proximal antennamere of second antenna. Distal end with three subdistal setae similar in length, each twice as long as broad. Longitudinal comb of eleven short aesthetascs disposed distally on lateral margin of antenna as figured.

Second antennae (Fig. 2A–C) long, capable of extending backwards to thoracopod IV or V. Proximal antennamere soft and corrugated, with patches of microtuberculate integument and sensillae distributed on dorsal and mediiodistal surface as figured. Distal antennamere narrow, twice length of proximal counterpart, evenly curved backwards, sickle-shaped and tapering into blunt apex. Frontal appendage short, half length of proximal antennamere, provided with small ventral conical outgrowths (Fig. 2E, F).

Labrum (Fig. 2B, H, I) subtrapezoidal, lacking distal protuberances; terminal fleshy process elongate and evenly curved forwards, tapering distally. Triangular setulose pad placed midway on ventral surface.

Phyllopodia with gross structure typical for genus (Fig. 3A–G). First thoracopod (Fig. 3A) attaining two-thirds size of fifth thoracopod (Fig. 3E); eleventh thoracopod (Fig. 3F) 0.6 times as large as fifth thoracopod; rest of thoracopods subsimilar in size. Praepodite (PE) oval, entire, with small notch placed midway of outer margin. Epipodite (EP) oval with smooth margin. Exopodite (EX) broad, “D”-shaped with plumose marginal setae. Endopodite (EN) broad; inner margin straight with marginal setae simple and sclerotized; length of setae variable both among individuals and between thoracopods of same specimen (even between left and right counterparts of same pair). First endite of thoracopods I–X with two submarginal spine-like setae on

anterior surface, distal one with tiny spine-like seta at base (Fig. 3D); counterparts on endite of eleventh thoracopod reduced. Second endite with two proximal setae submarginally on anterior surface, one of them reduced. Third and fourth endites of all limbs (Fig. 3G; I–XI) each with two submarginal spine-like setae on anterior surface, one of which reduced, and with three and two long plumose setae on posterior surface, respectively. Fifth endite with one submarginal spine-like seta on anterior surface, and two long plumose setae on posterior surface.

Genital segments (Fig. 4A, B, D) only slightly expanded and partially fused. Ventral surface of first segment smooth. Second genital segment (Fig. 4D) with short stout acute spine-like projection similar to those on abdominal segments placed posterolaterally at each side on ventral surface. Basal portion of gonopods short and rigid, microtuberculate, with inflated, rounded medial surface covered with densely set short chitinized denticles. Eversible part of gonopods consisting of unique short boom-like process provided with longitudinal row of denticles proximally on medial margin plus cluster of shorter denticles subdistally at each side (Fig. 4D, E).

Abdominal segments (Fig. 4A, B) bearing warty outgrowths provided with sensillae distributed as follows: posterodorsal pair on the first, third and fifth segments; posterolateral pair on the second and fourth segments; and two posterodorsal and one posterolateral on the sixth segment. In addition, all segments with pair of ventrolateral curved spine-like projections at each side close to posterior margin, sometimes appearing duplicate in most posterior segments (Fig. 4C). First segment with pair of large mid-ventral straight spine-like projections close to posterior margin; other segments with similar but reduced projections, although sixth frequently unarmed.

Cercopods five times longer than broad and three times longer than telson. Terminal setae implanted as in Fig. 4F.

Female. *First antennae* approximately twice as long as eye diameter and twice shorter than second antennae (Fig 5A).

Second antennae (Fig. 5A) soft, lamellar, three times longer than wide, with broad pointed apex. Patches of microtuberculate integument sparsely set on surface of antenna as figured. Counterparts fused medially at base, and forming obtuse angle in frontal view.

Genital segments (Fig. 5B, C) completely fused. Brood pouch fusiform, not protruding laterally, tapering posteriorly; apex reaching midway of second abdominal segment. Gonopore as illustrated in Fig. 5D, produced postero-ventrally.

Abdominal segments unarmed, lacking spine-like processes (Fig. 5B).

Eggs. Numerous, more than 500 per brood pouch. Diameter 250 μm . Sub-spherical, with surface sculptured with shallow angular elongated depressed facets limited by blunt ridges (Fig. 5E).

Size. Total length of holotype (including cercopods setae) 24.58 mm (head plus thorax 10.80 mm, abdomen 13.78 mm). Length of allotype 25.58 mm. Largest specimen recorded (female) 33.65 mm.

Differential diagnosis. Among members of the genus *Phallocryptus*, *P. spinosa* is closest to the new species. Nevertheless, *P. tserensodnomi* **sp. nov.** can be easily distinguished based on the following features: (1) frontal appendage is provided with small ventral conical outgrowths, unlike *P. spinosa*, which displays smooth frontal appendages; (2) second antennamere is evenly curved, sickle-shaped, more similar to that of *P. wrighii* and differing from the condition in *P. spinosa*, where the main curvature of segment is attained at its proximal half; (3) the distal fleshy process on the labrum evenly curved forwards and taper distally versus process “L” shaped in *P. spinosa*; (4) short stout spine-like projections are present on each side of basal portion of gonopods (versus projections absent in *P. spinosa*); (5) the first four abdominal segments have a distinctive midventral bulge on the integument on the anterior margin in *P. spinosa* (versus bulges absent in the new species); (6) the female second antennae are shorter and wider than in *P. spinosa*, and without the acute recurved antenna tip characteristic of this species. Genetic differences between both taxa are described in the following section.

Genetic analyses. The COI mitochondrial gene of *P. tserensodnomi* **sp. nov.** clearly showed a considerable divergence from all other so-far genetically characterised species/populations of the genus. *Phallocryptus tserensodnomi* **sp. nov.** is divergent from the genetically most similar species, *P. spinosa*, by 20% from the group IV; 19.2% from the Fuente de Piedra population; and by 10.9% from the group I (all Kimura 2-parameter distances) (Fig. 6). Group I was actually closer to *P. tserensodnomi* **sp. nov.** than to the group IV (19.2 %) or to the Fuente de Piedra population (18.2 %). Among the two populations analysed in this study (the Iberian sites of Gallocanta and Fuente de Piedra), the Gallocanta population was very similar to haplotypes belonging to the clade IV of Ketmaier *et al.* (2008), with 0.6 % divergence, while the population of Fuente de Piedra was more differentiated (6.7 %).

No variation in sequences of COI was observed in the two analysed individuals of *P. tserensodnomi* **sp. nov.** The GTR+G model of nucleotide substitution consistently performed best among the different approaches to model selection, based on the 508 bp alignment of COI sequences. All applied methods of phylogenetic reconstruction supported the sister relationship between the new species and *P. spinosa* despite their relatively high divergence. In addition, it suggests that the group I as described by Ketmaier *et al.* (2008) has a monophyletic origin with *P. tserensodnomi* **sp. nov.** Tentative molecular clock divergence time suggests that the separation between *P. tserensodnomi* and *P. spinosa* occurred between 4 and 15 Mya.

Distribution and ecology. So far *P. tserensodnomi* **sp. nov.** is a Mongolian endemic. It has been found in six lakes on the eastern half of the country, in the steppes and dry steppes (annual rainfall less than 350 mm) of the provinces of Arkhangay, Tuv, Sukhbaatar and Dornod, at altitudes from 588 to 1,346 m a.s.l. It is remarkable that the species has not been recorded in the western half of the country despite extensive sampling and presence of many suitable habitats, where its most common associated species are found. The lakes that it inhabits are shallow, temporary or permanent, but the latter freeze totally in the extremely cold Mongolian winter and therefore behave also as temporary. Waters are athalassic mesosaline, with salinities ranging between 10.8 and 25 g/L. Dominant ions are carbonate and/or chloride, and sodium. Water turbidity is high due to presence of suspended clay particles that are generally gray-coloured. No submerged macrophytes were recorded in any of these lakes. The accompanying crustacean community was composed of characteristic halophile species in Mongolia (Alonso 2010): the fairy shrimps *Artemia* cf. *sinica* Cai, 1989, *Galaziella mongoliana* (Uéno, 1940), *Branchinecta orientalis* Sars, 1901, and *Branchinectella media* Schmankewitsch, 1873; the cladocerans *Daphnia triquetra* Sars, 1903, *D. carinata* King, 1853, *Moina salina* Daday, 1888, *M. brachiata* (Jurine, 1820) and *Macrothrix* gr. *hirsuticonis* Norman & Brady, 1867; the copepods *Metadiaptomus asiaticus* (Ul'yanin, 1875) and *Thermocyclops kawamurai* Kikuchi, 1940; and some unidentified ostracods. It is remarkable that *P. tserensodnomi* **sp. nov.** normally co-occurs with some of the aforementioned fairy shrimps, in one case with three of them.

Discussion

The discovery of *P. tserensodnomi* **sp. nov.** brings to four the number of species of *Phallocryptus* currently known. It is very close to *P. spinosa* with which it shares gross morphological characteristics and habitat preferences. The main differences between them are related to a decrease of the number of structures in *P. spinosa* namely smooth male frontal appendages, shorter male second antennamere and lack of stout spine-like projections on the second genital segment on each side of gonopods. In other branchiopods (Chydoridae), these kinds of differences have been interpreted as an evolutionary trend (Adamowicz & Sacherová 2006), which suggests that *P. tserensodnomi* **sp. nov.** is evolutionarily closer to the common ancestor than *P. spinosa*. This is also in agreement with the shorter branch lengths corresponding to *P. tserensodnomi* in our phylogenetic tree (Fig. 6). There are no previous reports of any *Phallocryptus* either in Mongolia or in eastern Eurasia. Therefore, it must be considered new for the eastern Palaearctic.

The phylogenetic reconstruction supported the sister relationship between the new species and *P. spinosa* despite their relatively high divergence. In addition it suggests that the clade I of Ketmaier *et al.* (2008) (embracing populations from Botswana, Morocco and Algeria) has a monophyletic origin with *P. tserensodnomi* **sp. nov.**, suggesting that they may actually belong to the same species complex. Tentative molecular clock divergence estimates suggest that the separation between *P. tserensodnomi* and members of clade I of Ketmaier *et al.* (2008) occurred between 2 and 8 Mya. Alternatively *P. spinosa* could be a paraphyletic taxon. Comparison of published morphological descriptions of the Botswana (Brendonck & Riddoch 1997) and Morocco (Thiéry 1987) populations of this species with *P. tserensodnomi* are not conclusive taxonomically. The population from Botswana shows the second antennamere evenly curved, sickle-shaped and with short stout spine-like projections at each side of the basal portion of gonopods (see Brendonck & Riddoch 1997: figs 6a, c), features diagnostic of *P. tserensodnomi*. However, the drawings of Thiéry (1987) are not clear enough for precise taxonomic identification that they do not show the midventral bulge on the anterior margin of the first four abdominal segments, which are also absent in *P. tserensodnomi* (Thiéry 1987: fig. 92). Although further detailed morphological comparisons between populations of group I and *P. tserensodnomi* **sp. nov.** are required, it seems plausible that the three populations of the clade I of Ketmaier *et al.* (2008) are closer to *P. tserensodnomi*. They represent possibly an example of a pair of species that has arisen from a common ancestor by allopatric speciation, most

probably as a result of the rise of the western Himalayas and Altay mountain ranges, which formed during the Miocene, combined with a climatic change towards drier conditions that took place at the end of that epoch.

Regarding the two populations of *P. spinosa* from the Iberian Peninsula analysed, that from Gallocanta was included in their clade IV by Ketmaier et al. (2008), while that of Fuente de Piedra arose as sister to this clade, suggesting the existence of a cryptic lineage or species. Tentative molecular clock divergence times suggest that the Fuente de Piedra population separated from the main clade between 1 and 4.5 Mya, which is consistent with present knowledge of the age of some inland water bodies of the Iberian Peninsula (Alonso 1998).

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Figure Captions

FIGURE 1. *Phallocryptus spinosa* (Milne-Edwards, 1840). from Laguna de Gallocanta (Zaragoza, Spain). Male: A, head, lateral view; B, frontal appendages; C–D, labrum, lateral and ventral views; E, second antennamere; F, second genital segment, ventral view, with eversible portion of gonopods folded inside; G, basal portion of left gonopod; H, everted gonopod. Female: I, head, dorsal view.

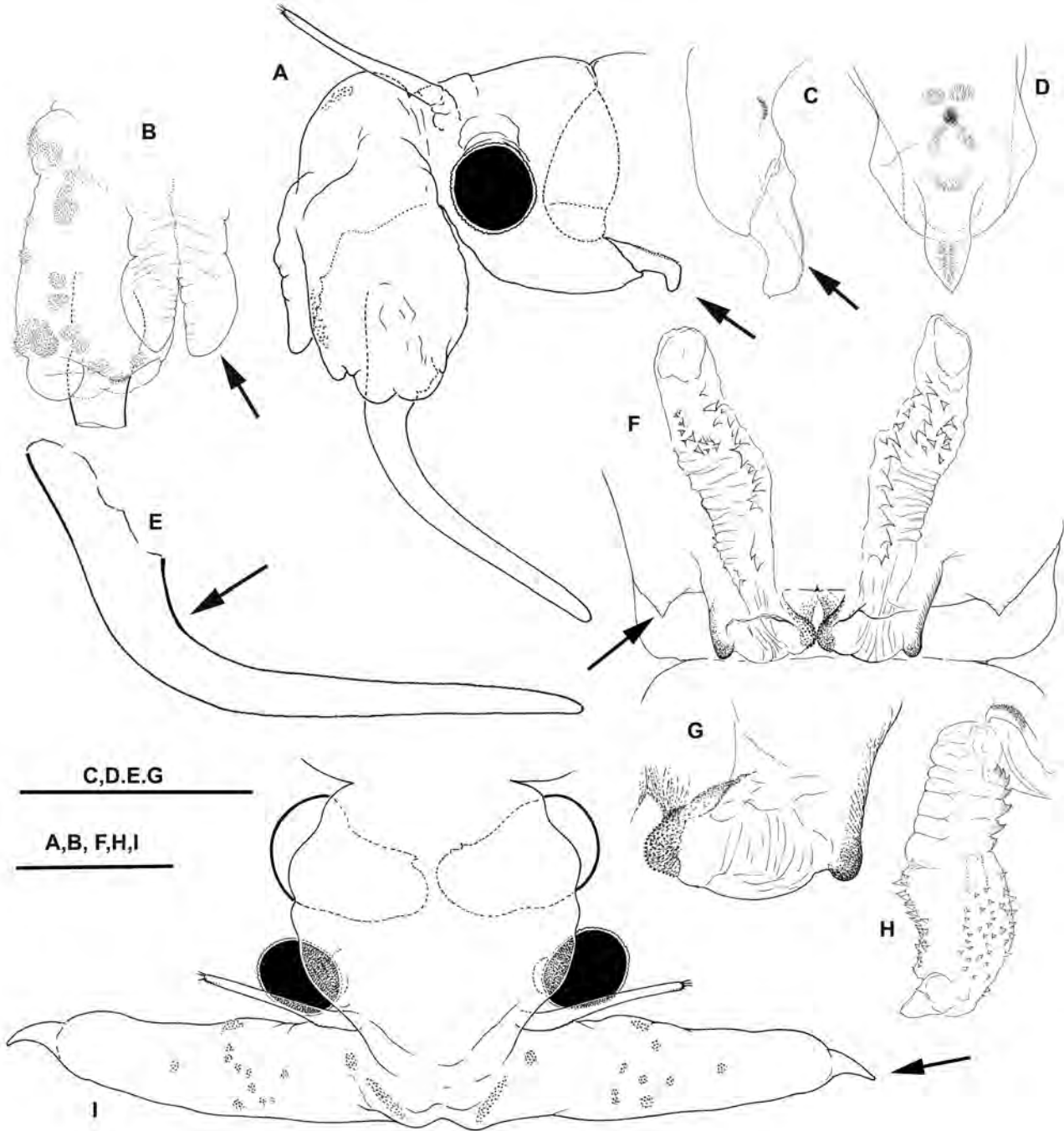
FIGURE 2. *Phallocryptus tserensodnomi* **sp. nov.** Male from Shorvog nuur, Arkhangai, Mongolia. A, head, dorsal view. B, head, lateral view. C, head, ventral view. D, tip of first antenna. E–F, frontal appendages, lateral view. G, second antennamere. H–I, labrum, lateral and ventral views.

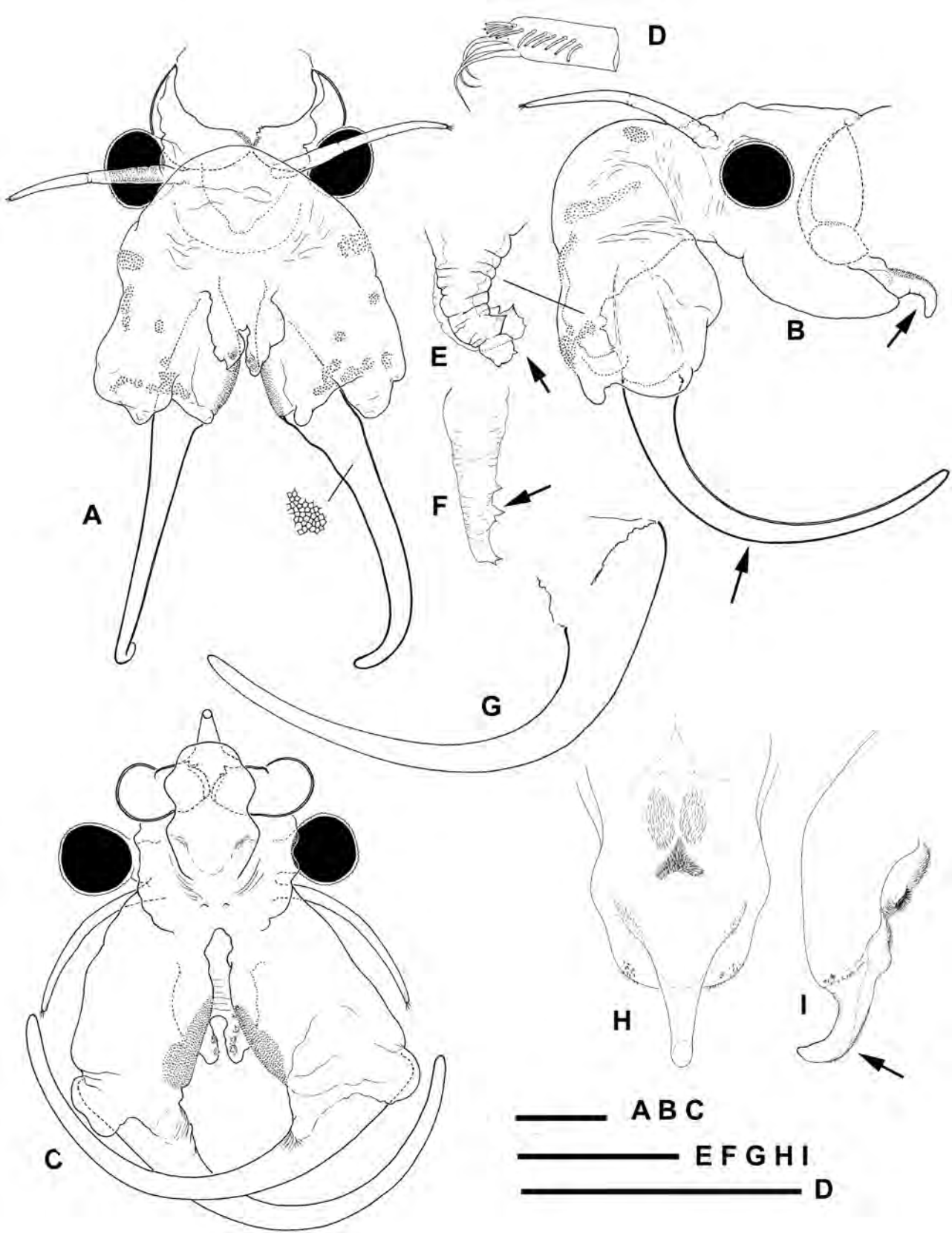
FIGURE 3. *Phallocryptus tserensodnomi* **sp. nov.** Male from Shorvog nuur, Arkhangai, Mongolia. A, first thoracopod. B, detail of plumose setae and corresponding basal scales on the exopodite. C, detail of distal simple setae on endopodite of eleventh thoracopod. D, distal submarginal spine-like setae on first endite. E, fifth thoracic limb. F, eleventh thoracic limb. G, arrangement of setae on endites of thoracic limbs I–XI.

FIGURE 4. *Phallocryptus tserensodnomi* **sp. nov.** Male from Shorvog nuur, Arkhangai, Mongolia. A–B, genital and abdominal segments, ventral and lateral views. C, posterior abdominal segments and telson, ventral view. D, second genital segment, ventral view; eversible portion of gonopods folded inside. E, everted gonopod. F, distal portion of cercopods.

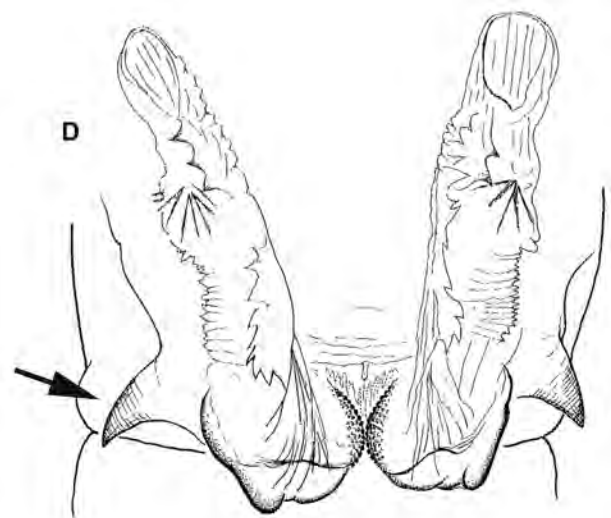
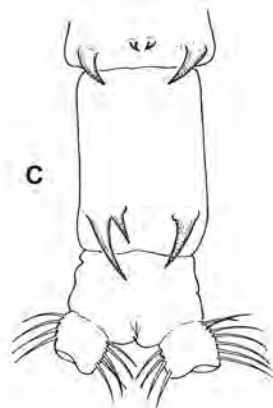
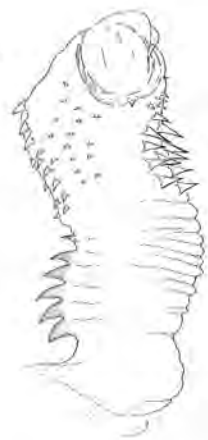
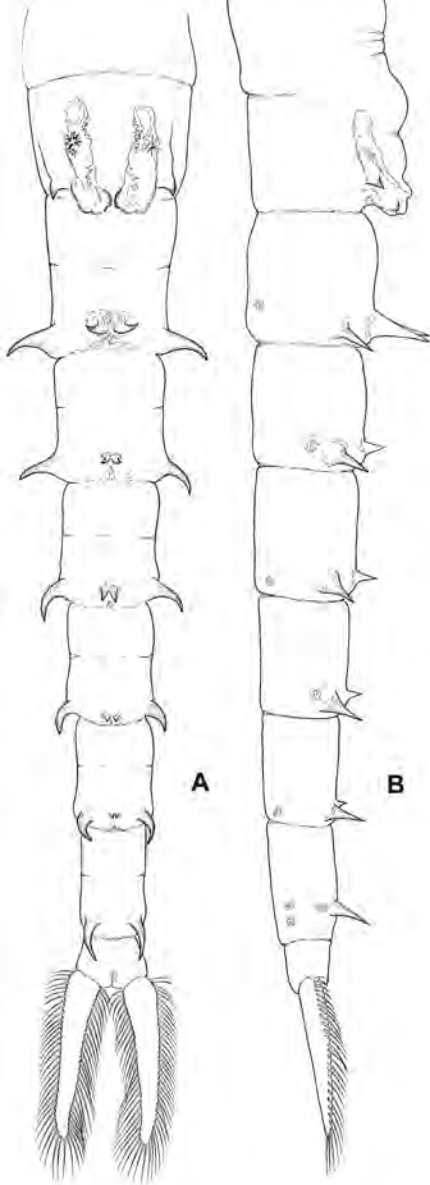
FIGURE 5. *Phallocryptus tserensodnomi* **sp. nov.** Female from Shorvog nuur, Arkhangai, Mongolia. A, head, dorsal view. B, genital and abdominal segments, lateral view. C, brood pouch, ventral view. D, gonopore, lateral view. E, egg.

FIGURE 6. Relationships among populations of *Phallocryptus tserensodnomi* **sp. nov.** and *P. spinosa* including those analysed in this study (in bold) and those previously published by Ketmaier *et al.* (2008). Letters are the haplotype name described in Table 1 of Ketmaier *et al.* (2008) and sampling site shown in brackets. The tree was constructed by the Maximum Parsimony of phylogeny from a partial sequence of the mitochondrial COI gene. Node support is provided for Maximum Parsimony, Maximum Likelihood and Bayesian inference analysis. Vertical bars delineate cluster names analysed by Ketmaier *et al.* (2008), scale indicates 10% divergence. Only nodes with a statistical support $\geq 50\%$ are labeled. Numbers in bold at each branch division are the lower and upper dates obtained from the two molecular clock calculations (see methods for details).







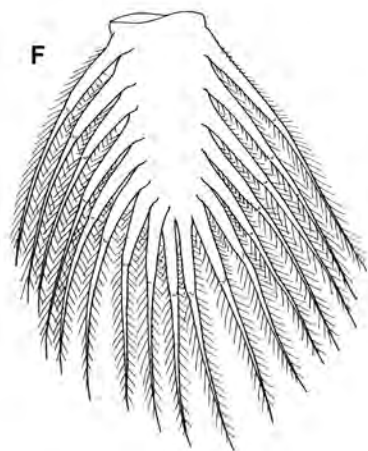


E

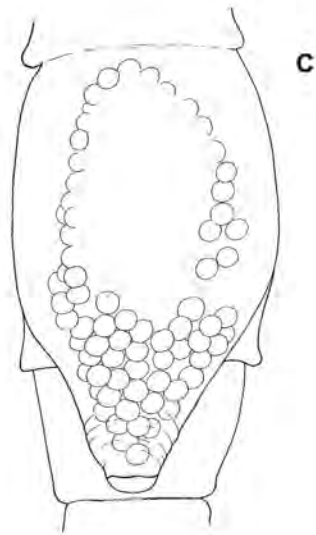
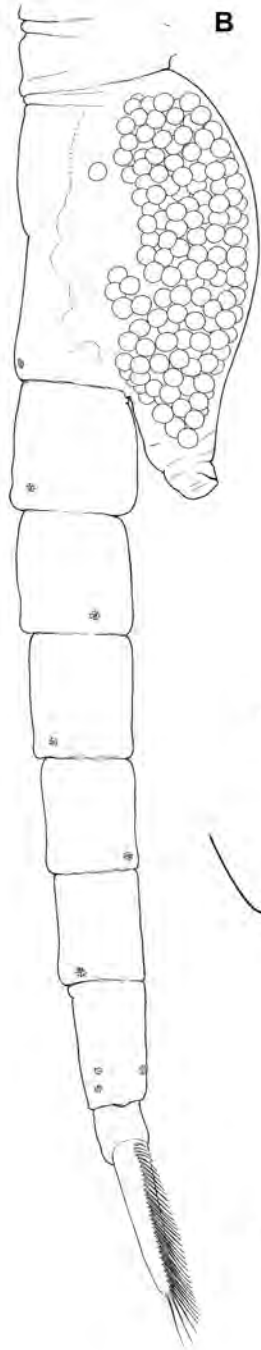
— A,B

— C

— D,E,F



F



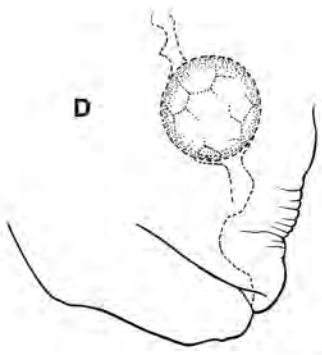
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— B,C

— A

— D



D

A

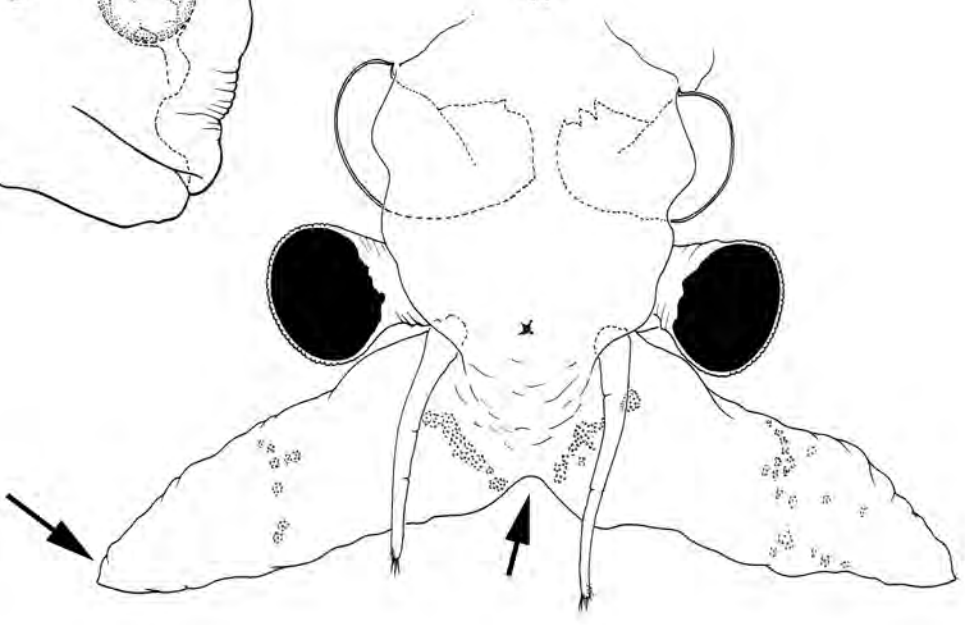


Figure 6.

