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A new species of *Litarachna* Walter, 1925 (Acari: Hydrachnidia: Pontarachnidae) from Corozal Bay (Belize), described based upon morphology and DNA barcodes

Lucia Montes-Ortiz^a, Tom Goldschmidt^b, Lourdes Vásquez-Yeomans^a, Manuel Elías-Gutiérrez^a

^a El Colegio de la Frontera Sur, Avenida Centenario km 5.5, Chetumal 77014, Quintana Roo, México.

^b Zoologische Staatssammlung München, Münchhausenstraße 21, D-81247 München, Germany.

Original research

ABSTRACT

A new planktonic species of the marine water mites of the family Pontarachnidae Koenike, 1910 is described from Corozal Bay, an estuarine system in Belize. The morphological description includes Scanning Electronic Microscope (SEM) images and is augmented by an analysis of DNA cytochrome c oxidase I (COI) sequences, the DNA barcode, used for the first time for a species description in this group.

Keywords Integrative taxonomy; *Litarachna belicensis* n. sp.; plankton; estuary; Chetumal Bay

Zoobank <http://zoobank.org/BFB2B682-ED8C-4272-954D-78EDD079A444>

Introduction

The Pontarachnidae Koenike, composed of two genera, *Pontarachna* Philippi, 1840 and *Litarachna* Walter, 1925, is the only water mite family with species occurring in marine habitats. Overall, 23 species of the genus *Litarachna* have been described (Chatterjee *et al.* 2019) mainly from the intertidal zone of tropical and temperate oceans including the Pacific, Atlantic, and Indian Ocean (Smit 2002; Pešić *et al.* 2012; Pešić *et al.* 2019; Chatterjee *et al.* 2019). Some species occur in estuarine systems as well as in freshwater, e.g. *Litarachna brasiliensis* Smit, 2007, while others live in shallow coastal marine waters, and some even extend to depths up to 70 m (Pešić *et al.* 2014), and one is a planktonic species (*Litarachna kamui* Uchida, 1935). Only four species are recorded from the tropical west Atlantic Ocean, with three occurring in the Caribbean Sea: *L. caribica*, *L. degiustii* and *L. lopezae* (Pešić *et al.* 2008; Pešić *et al.* 2012; Chatterjee *et al.* 2019). In general, the biology and ecology of this group of water mites is mostly unexplored, and little is known about distributional patterns and endemism, particularly in the marine provinces of the American continent (Chatterjee *et al.* 2019).

Here we describe a new species of *Litarachna* from Corozal Bay, an estuarine system in Belize, a region within the Yucatan Peninsula biogeographic zone. This species represents the second reported planktonic pontarachnid (*Litarachna*) mite. It is the first record for Belize, and we present the first DNA barcodes in a description for a member of this family.

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Corresponding author
Lucia Montes-Ortiz:
lumontes@ecosur.edu.mx

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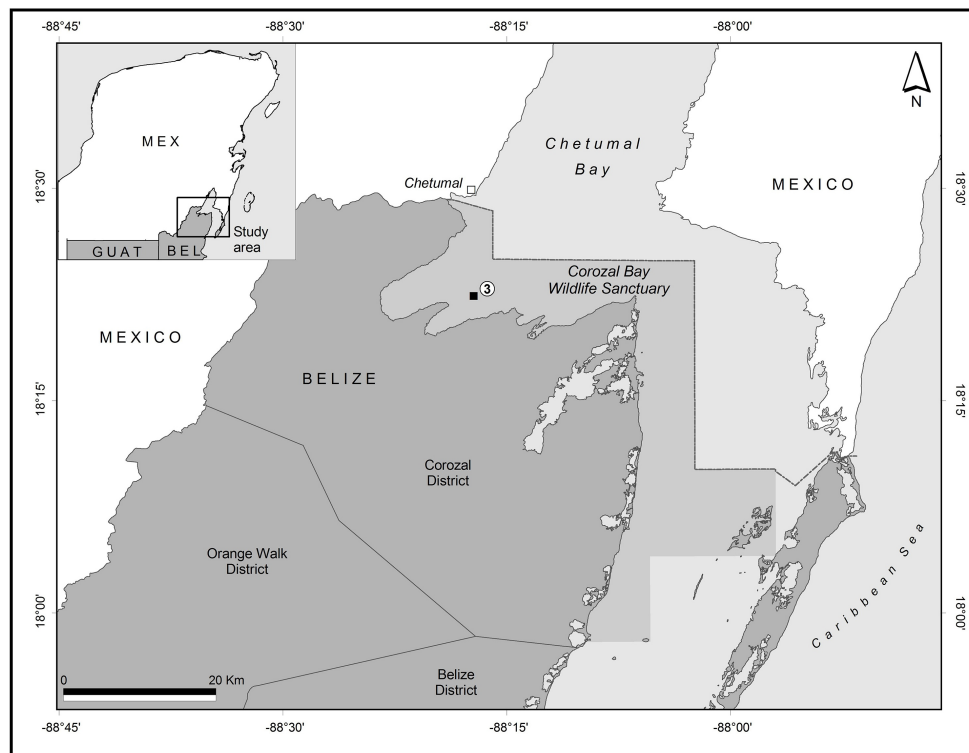


Figure 1 Sampling site of *Litarachna belicensis* n. sp. in Belize Bay.

Material and methods

The specimens were collected in open water from Corozal Bay, a binational embayment shared by Mexico and Belize, forming part of Chetumal Bay (Figure 1). A standard plankton net of 0.5 m mouth diameter and a mesh size of 0.333 mm was used to perform a vertical tow during a night sampling survey. The associated data in the collection site are presented in Table 1. Samples were sieved, washed and fixed using 96% ethanol and stored at -18 °C for seven days (Elías-Gutiérrez *et al.* 2018).

The mites were sorted under a stereomicroscope and one male, and one female were photographed using a Zeiss Discovery stereo microscope with an Eos Rebel T3i camera. SEM images were obtained from a Jeol JSM-SM-6010LA at the Chetumal unit of El Colegio de la Frontera Sur. An additional five individuals were used for molecular analyses using a non-destructive DNA extraction method (Porco *et al.* 2010). After the process, we recovered three specimens which were dissected and mounted in glycerin jelly. Whole specimens and the dissected parts were examined, measured and drawn under a compound microscope Olympus BX51 with a camera lucida, and the results were compared with prior documentation of this family of water mites (Cook 1996; Smit 2002; Smit 2007; Smit and Alberti 2010; Pešić *et al.* 2014; Moto and Abé 2014; Chatterjee *et al.* 2019). All specimens and preparations

Table 1 Sampling data.

| Site | Depth (m) | Salinity (ppt) | DO% | pH | Temp (°C) | Time in | Time out |
|---------|-----------|----------------|------|------|-----------|----------|----------|
| Point 3 | 2.8 | 20.7 | 6.71 | 8.02 | 26.1 | 20:46 pm | 20:57 pm |

were deposited in the Reference Collection of Zooplankton at El Colegio de la Frontera Sur (ECOSUR, Chetumal, Mexico).

Molecular analysis. DNA extraction was carried out using a standard glass fiber method (Ivanova *et al.* 2006) modified following Porco *et al.* (2010) for voucher recovery. Specimens were recovered after the lysis step from the glass fiber filter plates or the 96 well original plates and preserved in Koenike fluid.

After the DNA extraction, the PCR mixtures contained a final volume of 12.5 μ L, including 2 μ L of Hyclone ultra-pure water, 6.25 μ L of 10% trehalose (previously prepared: 5 g D-(+)-trehalose dehydrate, in 50 ml of total volume of molecular grade ddH₂O), 1.25 μ L of 10X PCR buffer, 0.625 μ L of MgCl₂ (50 mM), 0.0625 μ L of dNTP (10 mM), 0.125 μ L of each primer (10 μ M), 0.06 μ L of Platinum Taq DNA polymerase and 2 μ L of DNA template. Extracts were amplified with the Zooplankton primers (ZplankF1_t1 and ZplankR1_t1, see Prosser *et al.* 2013 for details). The reactions were cycled at 94 °C for 1 min, followed by five cycles of 94 °C for 40 sec, 45 °C for 40 sec and 72 °C for 1 min, followed by 35 cycles of 94 °C for 40 sec, 51 °C for 40 sec and 72 °C for 1 min, with a final extension of 72 °C for 5 minutes. PCR products were visualized on a 2% agarose gels (E-Gel 96 Invitrogen), and positive PCR products were selected for sequencing bidirectionally at Eurofins Scientific.

Sequences were edited using Codon Code v. 3.0.1 and uploaded to the Barcode of Life database (BOLD: available at www.boldsystems.org) and are in the public dataset DS-LITBEL. The sequences were included in a maximum likelihood (ML) tree generated with 10,000 replicates using MEGA version X (Kumar *et al.* 2018); two sequences from *L. communis* were mined from GeneBank database (<https://www.ncbi.nlm.nih.gov/genbank/>) and a group of ten sequences of *Atractides* genus were mined from BOLD to root the tree (Table 2); this genus was selected since they belong to the same Hygrobatoida superfamily as the pontarachnid mites and include high quality sequences.

All measurements are given in μ m. Terminology and abbreviations in the description of the new species follow Moto and Abé (2014) and Gerecke *et al.* (2016) except for the new abbreviations for the wheel acetabula.

Abbreviations pertaining to morphology of the body: Cx-I-IV = first to fourth coxae, Cxgl-2 = coxoglandularia 2, Cxgl-4 = coxoglandularia 4, L = length, Vgl = ventroglandularia, Vst = ventral setae, W = width, W-1 = wheel acetabula antero-lateral pair, W-2 = wheel acetabula antero-medial pair, W-3 = wheel acetabula posterior pair.

Table 2 Sequences used in the molecular study.

| Species | Locality | Deposition place | ID | Barcode Index Number (BIN) |
|-------------------------------------|---|---|-----------|----------------------------|
| <i>Litarachna communis</i> | Marine litoral Punta Spano, Corsica (France) | Naturalis Biodiversity Center, Leiden (Netherlands) | NLACA998 | ADD6045 |
| | | | NLACA1000 | |
| <i>Litarachna belicensis</i> n. sp. | Corozal bay (Belize) | El Colegio de la Frontera Sur, Unidad Chetumal (México) | YUCWM193 | AEB8019 |
| | | | YUCWM192 | |
| | | | CFWIA621 | ACE5393 |
| <i>Atractides</i> sp. | Los Angeles, E. Fork San Gabriel (United States of America) | Stroud Water Research Center (United States of America) | CFWIB562 | |
| | | | CFWIB563 | |
| | | | CFWIB564 | |
| | | | CFWIB565 | |
| | | | BACWM152 | ACX7786 |
| <i>Atractides</i> sp. | Bacalar, Quintana Roo (México) | El Colegio de la Frontera Sur, unidad Chetumal (México) | BACWM263 | |
| | | | BACWM044 | |
| | | | BACWM125 | |

Abbreviations pertaining to appendages (palp, leg): I- to IV-Leg-1-6 = first to sixth segments of leg I to IV, P-1 to P-5 = palp segments 1 to 5.

Abbreviations pertaining to molecular information: BIN = Barcode Index Number, COI = cytochrome oxidase subunit I.

Results

Family Pontarachnidae Koenike, 1910

Genus *Litarachna* Walter, 1925

Litarachna belicensis n. sp.

Zoobank: A09F5614-209C-4446-A6F6-5BE541E56D97

(Figures 2–6)

Type series — Holotype: female, Corozal Bay, Belize (18°37′27.7″ N 88°28′8.44″ W), 3–5 m depth, substrate mainly calcareous rocks and sand, also sea grass and some spots of fuzzy finger algae (*Batophora oerstedii*), collection from a planktonic sample from 2.8 m depth, 7 May 2019; dissected and slide mounted in glycerin jelly. Paratypes: one male, same collecting data and processing as holotype.

Diagnosis — Suture between Cx-II and Cx-III incomplete, suture between Cx-III and Cx-IV complete (Figure 6A and D). Postero-medial apodemes twice length of postero-lateral apodemes in both female and male. Ventral projection in P-2 (Figure 3B and 5B), in male very long perigenital setae surrounding the genital field, U-shaped (Figure 5A).

Description — Female (n=1): Idiosoma L/W 476/357. Anterior coxal group separated medially. Suture lines between Cx-II and Cx-III incomplete. Suture lines between Cx-I and Cx-II, and complete between Cx-III and Cx-IV. Posterior margin of Cx-IV with two pairs of long apodemes, extending beyond the genital field, postero-lateral apodemes half as long as postero-medial apodemes (Figures 2, 3, 6A–C)

Genital field L/W 78/47. Pregenital and postgenital sclerite fused, forming a ring around genital opening. Cxgl-2 and associated seta (*sensu* Cook 1974) lie between genital field and the fourth coxae. Posterior to the genital field, a lateral pair of Vgl, and three pairs of wheel-like acetabula (*sensu* Cook 1996), W-1 with nine radiating spokes, W-2 with eight and seven radiating spokes and W-3 with 10 radiating spokes (Figures 3 and 4). A pair of platelets bearing two pores latero-anteriorly to the excretory pore. Excretory pore sclerotized in subterminal position.

Palp (Figure 3C) total L 194, dorsal L (% of total L): P-1 27 (14%), P-2 33 (17%), P-3 40 (20%), P-4 71 (36%), P-5 25 (13%), P-2 bearing a ventral projection (Figure 3B). L of I-Leg-3-6: 34, 51, 60, 85; II-Leg-3-6: 40, 51, 88, 91; III-Leg-3-6: 28, 52, 95, 85 and IV-Leg-3-6: 57, 95, 119, 104, Leg III and Leg IV each with one swimming seta (Figure 3).

One ovigerous female from the material contained two eggs with a L/W 170/110 (Figure 8).

Male (n=1): Idiosoma L/W 400/348. First coxal plates fused as in the female, suture lines between Cx-II and Cx-III medially incomplete, suture lines complete between Cx-I and Cx-II, as well as between Cx-III and Cx-IV. Postero-medial apodemes twice as long as postero-lateral apodemes, reaching to posterior end of genital field (Figure 6D). Between posterior and lateral apodemes Cxgl-4.

Genital field L/W 35/28, genital sclerites forming a complete ring with four pairs of setae (Figure 5C), many long perigenital setae (>95) free in the integument surrounding the genital field in a U-shaped form with less dense cover of setae anteriorly and markedly increasing density towards posterior part (Figure 5B); one pair of tiny, wheel-like acetabula at center of perigenital setae, close to the genital opening (Figure 5C). Genital opening flanked by centrally extended lamellae, posterior to the genital field two pores and two pairs of wheel-like acetabula

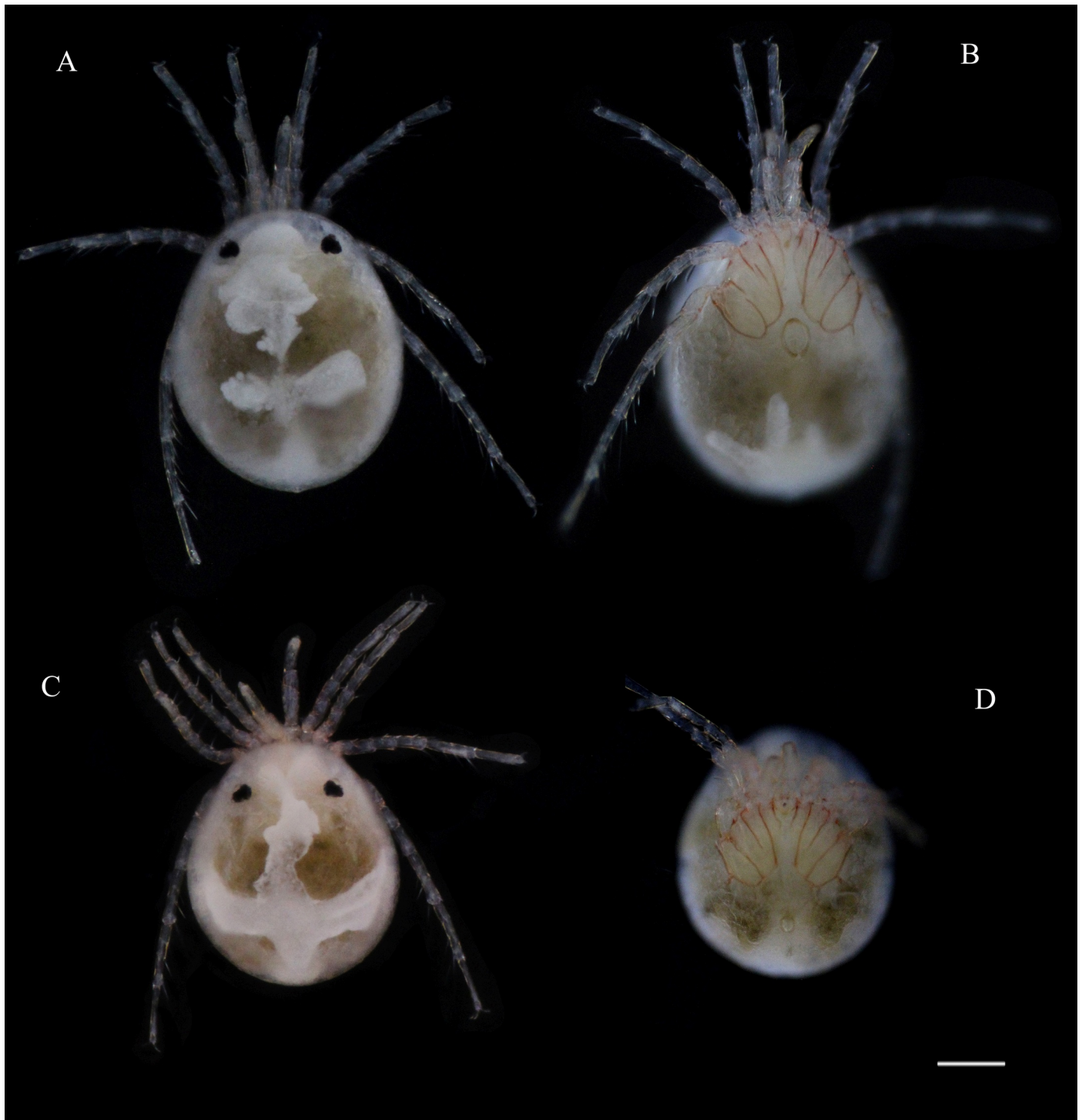


Figure 2 *Litarachna belicensis* n. sp. Female: A - Dorsal view; B - ventral view. Male: C - Dorsal view; D - Ventral view. Scalebar = 100 μ m.

(*sensu* Cook 1996), W-1, W-3 with nine radiating spokes, W-2 with six and four radiating spokes (Figure 5).

Palp (Figure 5A - B) total L 186, dorsal L (% of total L): P-1 28 (15%), P-2 38 (21%), P-3 40 (22%), P-4 57 (31%), P-5 22 (11%), P-2 bearing a ventral projection (Figure 5B). L

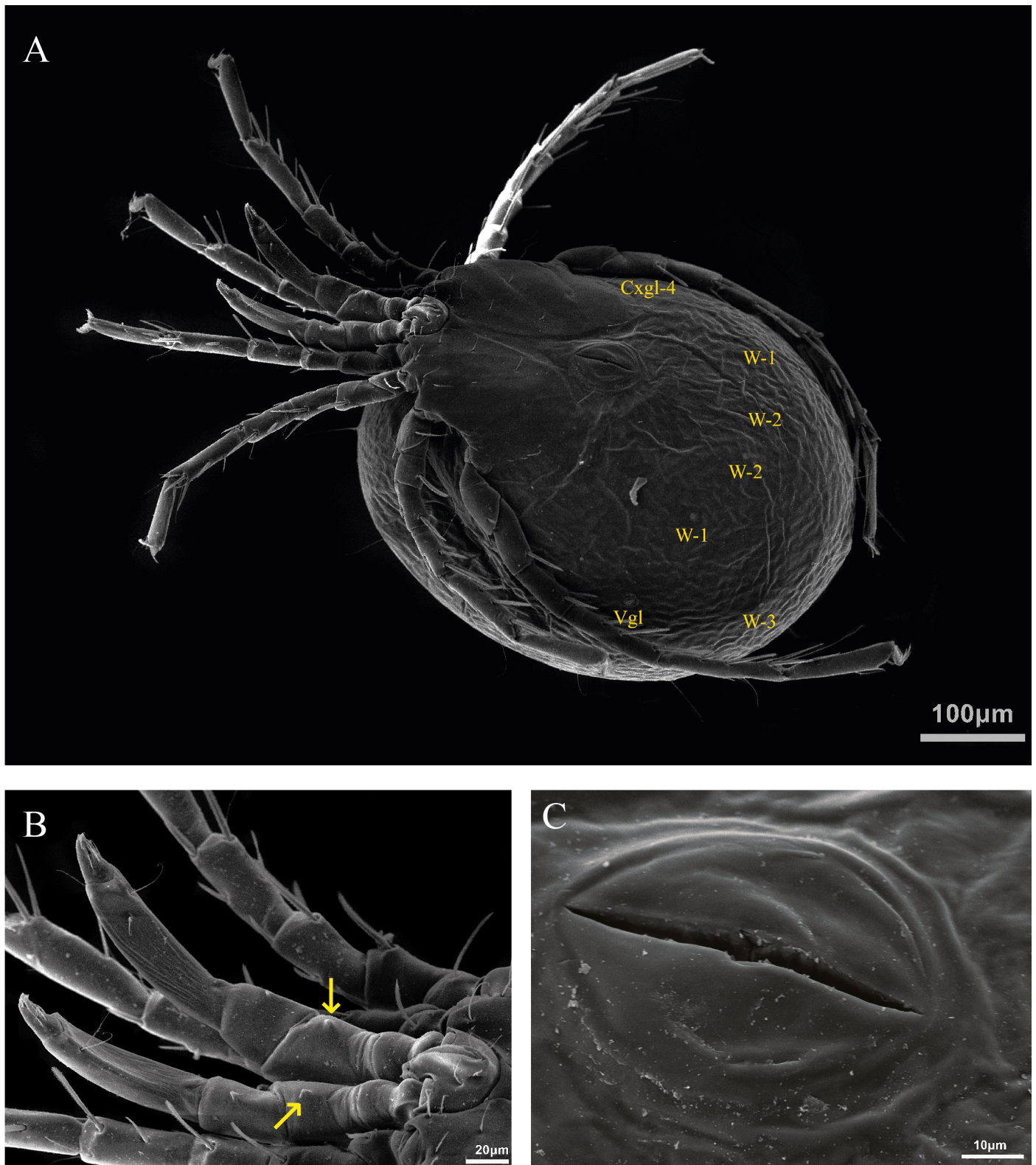


Figure 3 SEM micrographs of *Litarachna belicensis* n. sp. Female: A - Habitus (W-1 wheel acetabula, W-2, W-3); B - palps (arrows point at ventral processes on P-2); C - genital field.

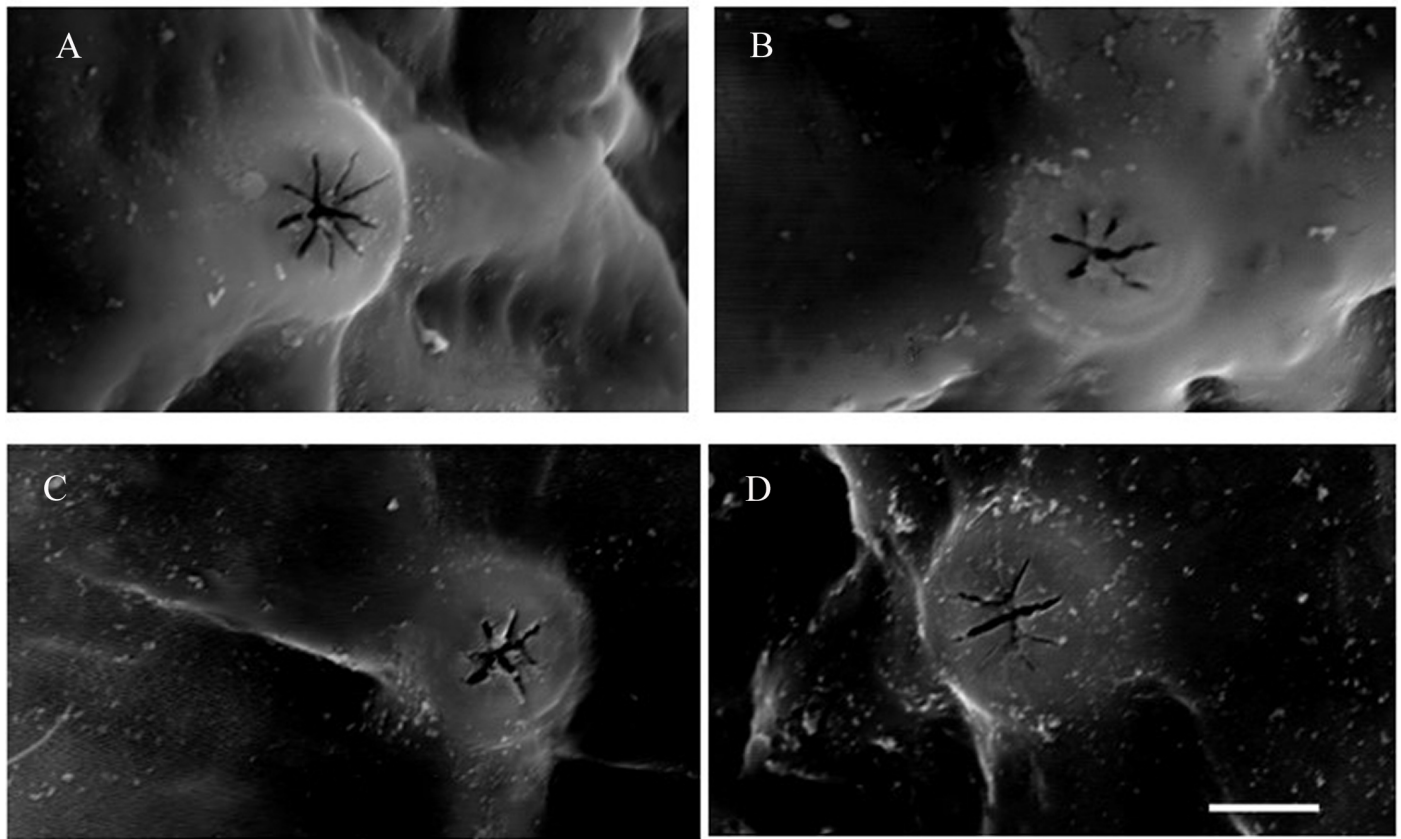


Figure 4 SEM micrographs of wheel acetabula in female of *Litarachna belicensis* n. sp. B, C – wheel acetabula 1; A, D – wheel acetabula 2. Scalebar = 5 μ m

of I-Leg-3-6: 39, 50, 64, 54; II-Leg-3-6: 39, 50, 64, 54; III-Leg-3-6: 39, 53, 92, 89 and IV-Leg-3-6: 46, 89, 100, 100.

DNA sequence (CO1) — Two sequences were obtained and resulted in the following consensus sequence:

```

CTCTATTTTG CTTTAGGAAG ATGATCAGGC ATAATGGGAA CAAGACTTAG 50
AACTTTAATT CGATTAGAAT TAGGTCAACC AGGAGCACTA ATTGGCAATG 100
AACAAATCTA TAACGTTATC GTAACAGCTC ATGCATTTAT TATAATTTTT 150
TTCATAGTCA TACCCATAAT AATTGGAGGT TTTGGAAATT GATTAGTTCC 200
GCTAATAATC AGAGCCCCCG ATATAGCCTT TCCCCGTATA AATAACATAA 250
GATTCTGACT TTTACCCCA GCCCTTATCC TTCTTTCAAC AAGATCCATA 300
AGATCAATAG GAGCTGGTAC AGGCTGAACA GTTTACCCTC CCCTCTCAAG 350
AAATTTGGCT CACTCAGGAC CATCCGTTGA CTTAACAATC TTCTCTCTCC 400
ATTTAGCTGG TATTTTCATCC ATCCTTGGGG CCATCAACTT TATAGCAACA 450
ATTATAAATA TAAAACCTAC CCATATAAAA ATGGAACAAG TACCCCTATT 500
TGTATGATCA ATTTTCATCA CAACCATTCT CCTCCTTCTT TCACTTCCAG 550
TCTTAGCAGG AGCCATTACT ATGCTTTTAA CTGACCGAAA CTTCAACACT 600
TCATTCTTTG ATCCAGCCGG TGGAGGTGAT CCAATTTTAT ACCAACATTT 650
    
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Etymology — This species is named for the country where it was collected.

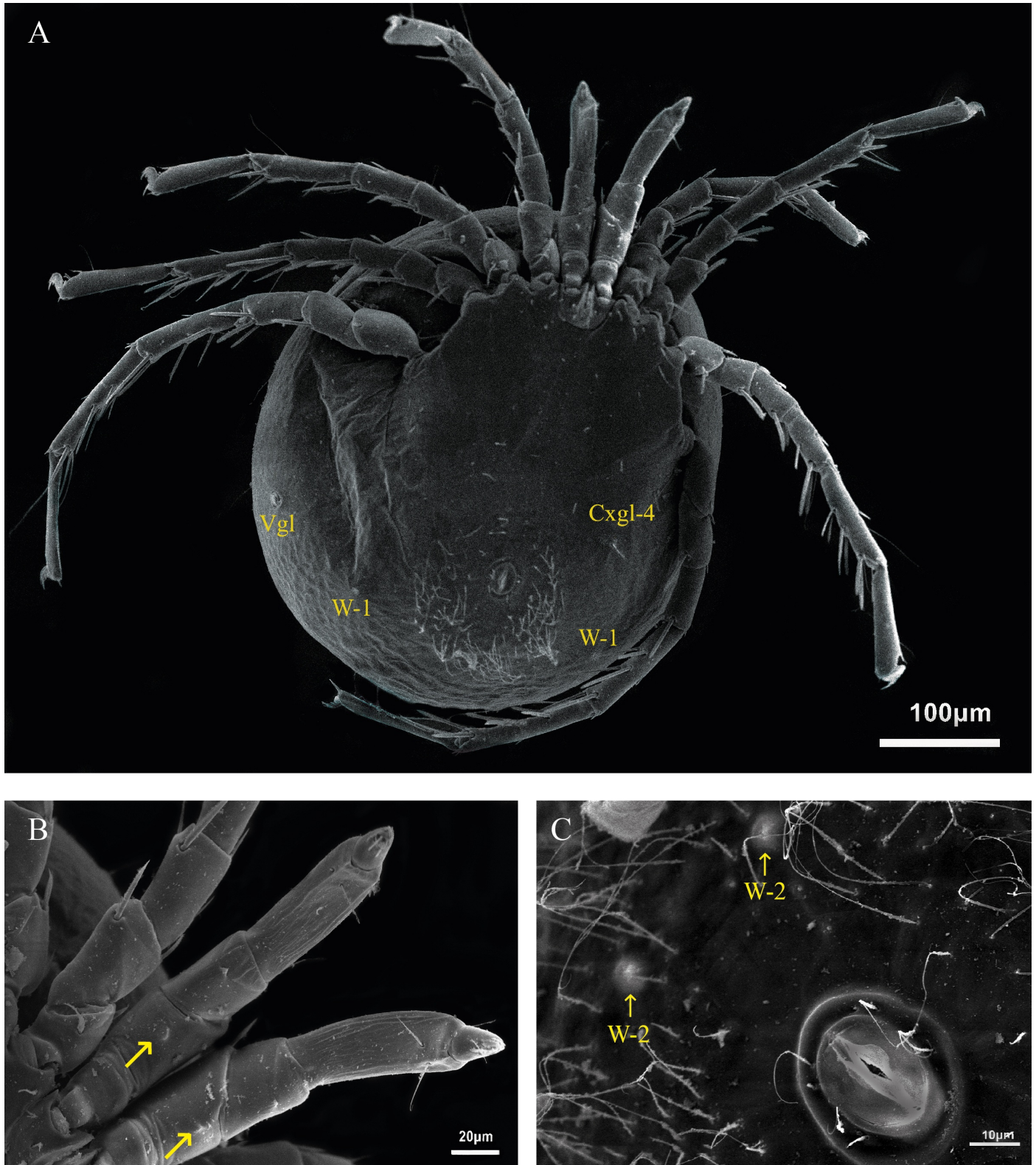


Figure 5 SEM micrographs of *Litarachna belicensis* n. sp. Male: A - Habitus; B - palps (ventral view, arrows point at ventral processes on P-2); C - genital field and area posterior to genital field, including medial pair of wheel-like acetabula (W-2) (note that the image is rotated, the upper left corner oriented to posterior).

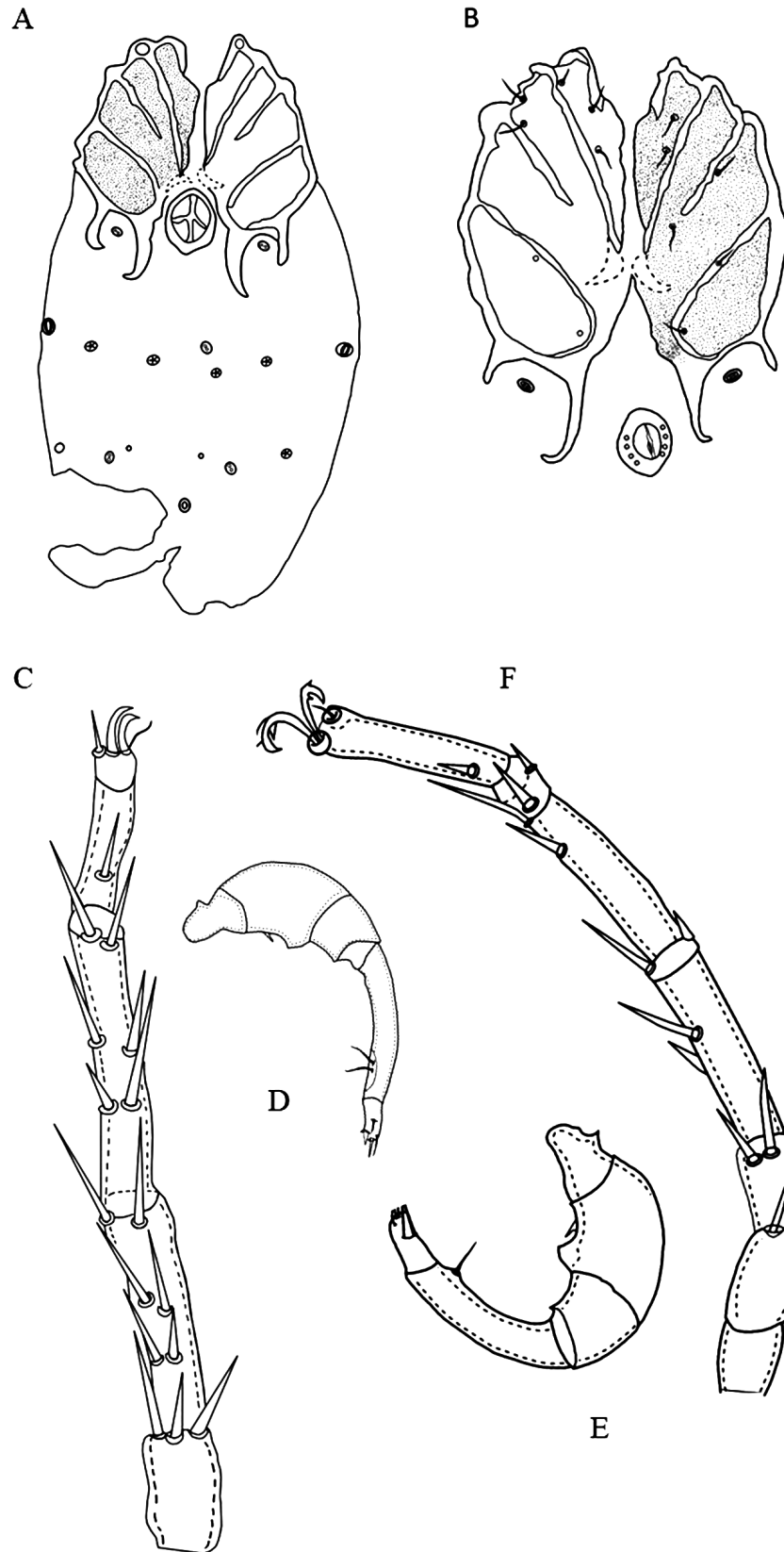


Figure 6 *Litarachna belicensis* n. sp. Female holotype A – idiosoma; C – IV leg; D – palp. Male paratype B – idiosoma; F – IV leg; E – palp.

Discussion

The new species *Litarachna belicensis* **n. sp.** is quite similar to *L. communis* Walter, 1925, originally described in France and with distribution in Croatia, Italy, Montenegro, Turkey (Mediterranean Sea) and Russia (Black Sea), in the complete suture lines between Cx-I and -II as well as Cx-III and -IV and the idiosoma length (*L. belicensis* 476 – *L. communis* 465); however, the new species differs from *L. communis* in the presence of a ventral projection on P-2 and in the absence of two spiniform setae on P-2. Furthermore, the two species differ in the length and width of the apodemes: in *L. belicensis* postero-medial hook shaped apodemes are wider and twice as long as postero-lateral apodemes, while in *L. communis* both postero-medial and lateral apodemes are the same length and wide. *Litarachna belicensis* **n. sp.** is similar to *L. degiustii* Cook, 1958 from Bahamas and Netherland Antilles (Caribbean Sea) and *L. caribica* Pešić *et al.* 2008 from Panama (Pacific Ocean) and Netherland Antilles (Caribbean Sea), in the shape and size of postero-medial apodemes. The new species differs from these two in the absence of a ventral setal tubercle on P-4, also in the size of postero-lateral apodemes (strongly reduced in the latter two species) and in the state of the suture lines from the coxal plates (both in *L. caribica* and in *L. degiustii* suture lines between Cx-II and Cx-III and between Cx-III and Cx-IV are incomplete).

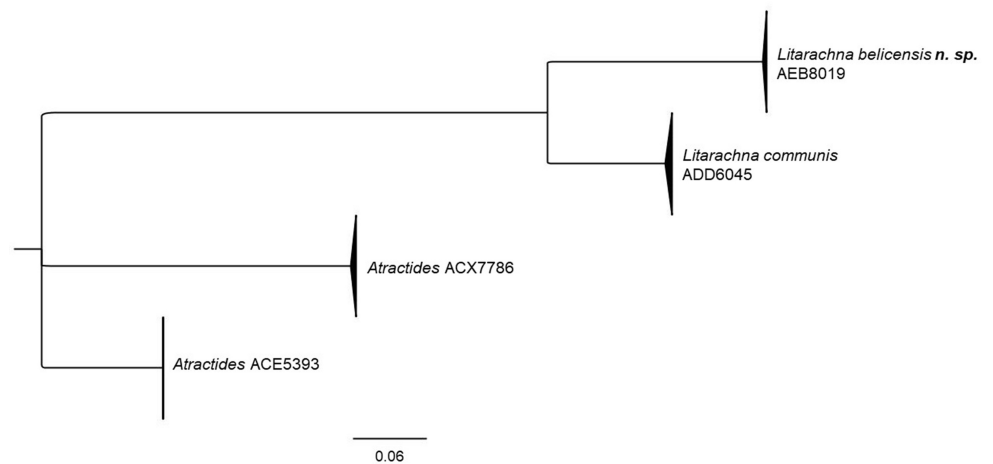


Figure 7 Maximum Likelihood tree, based on COI sequences.

The ML tree generated from COI DNA barcode sequences (Figure 7) reveals that *L. belicensis* **n. sp.** is a highly divergent lineage from *L. communis*, 23% different by K2P. This result agrees with other studies which report similar genetic distances for congeners in the group (Martin *et al.* 2010; Pešić and Smit 2020). The BOLD grouping algorithm assigns the two species to different Barcode Index Number, BINs: *L. communis* ADD6045 and *L. belicensis* **n. sp.** AEB8019 (Ratnasingham and Hebert 2013). These data suggest that *L. belicensis* **n. sp.** has a long independent history associated with an ancient separation from the European water mite *L. communis*, the same could probably be expected for other pontarachnid species in distant marine provinces.

This study is the first to apply an integrative morphological and genetic approach to species delimitation in this family. Our results, in addition to the biogeographic segregation of the new species, confirm the separate identity of *L. belicensis* as a new species. The integration of molecular data in this study and the future growth of DNA-sequence databases with high quality sequences and complete metadata should contribute to the elucidation of the



Figure 8 Ovigerous female recovered after DNA extraction, arrows point the eggs. Scalebar = 100 μm .

taxonomic placement of pontarachnid mites, especially the position of *Litarachna* within the Pontarachnidae as well as of Pontarachnidae within Hydrachnidia. Additional morphological and genetic studies of this enigmatic group will further help to understand their special biology and ecology and to clarify the distribution patterns and degrees of endemism.

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