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A new species of *Trhypochthoniellus* (Acari: Oribatida: Trhypochthoniidae) from Cuatro Ciénegas, Coahuila, Mexico, and a key to the world species

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Abstract

Trhypochthoniellus comprises nine species, being mostly associated to aquatic vegetation; *T. chilensis* Ermilov & Weigmann as the only known species from North and South America. This genus is recorded for the first time in Mexico. Herein we present the description, illustrations and SEM photographs of *Trhypochthoniellus churincensis* sp. nov., which was found in association to lignocellulolytic microfungal taxa in the oligotrophic freshwater oasis of Cuatro Ciénegas Basin, Coahuila, México. *T. churincensis* sp. nov. is morphologically similar to *T. longisetus* (Berlese) and *T. brevisetus* Kuriki; however, it differs from both taxa by the presence of nine genital setae (11 to 13 setae), setae *h3* subequal to other posterior notogastral setae; subcapitular setae *h* very short, and notogastral *c1* larger. Additionally, we discuss information on food preferences and reproductive mode obtained through the observation of one cultured population maintained under laboratory conditions. A key to the species of *Trhypochthoniellus* is provided.

Key words: Arid environment, oribatid diet, oligotrophic aquatic system, taxonomy

Introduction

Oribatid mites are a diverse group, representing a key element of the edaphic mesofauna. They are primarily terrestrial, with less than 1% of all known species truly aquatic (i.e., ca. 87 - 90 species from 10 genera), with reproduction and all stages of their life cycle living in freshwater. The oribatid genera containing (but not exclusively) aquatic members are *Mucronothrus*, *Trhypochthoniellus*, *Aquanothrus*, *Chudalupia*, *Tegeocranellus*, *Hydrozetes*, *Limnozetella*, *Limnozetes*, *Heterozetes*, *Zetomimus* (from 7 families). Even though aquatic oribatids have low species richness, these organisms may occur abundantly in a number of freshwater habitats, such as lentic (pools, lakes, water-filled microhabitats) or flowing waters (springs, rivers, streams). They do not swim and live mainly on aquatic plants, or in stream or lake sediments (Behan-Pelletier & Eamer 2007; Schatz & Behan-Pelletier 2008). The proportion of sexual reproduction in oribatid fauna is considerably lower in aquatic systems than in soil or litter (Behan-Pelletier & Eamer 2007) and many species, especially those in early-derivative taxa, are parthenogenetic (thelytokous) (Norton & Palmer 1991; Norton *et al.* 1993; Maraun *et al.* 2003). Maraun *et al.* (2019) found that the number of parthenogenetic oribatid mite species dominate in freshwater systems, peat bogs, acidic forest and glaciers foreland. In freshwater systems, genera such as *Limnozetes*, *Hydrozetes* and several taxa within Desmonomata as *Trhypochthoniellus longisetosus* and *Platynothrus peltifer* are dominant, and all with parthenogenetic species (Maraun *et al.* 2019).

Trhypochthoniellus (Crotonioidea: Trhypochthoniidae) was proposed by Willmann (1928) with *Trhypochthonius* (*Trhypochthoniellus*) *setosus* Willmann, 1928 as type species, recently Weigmann (1997, 1999) regarded as “forma” of *T. longisetus* (Berlese 1904), which has a cosmopolitan distribution. Currently, the genus comprises nine species and three subspecies, most of them associated to aquatic vegetation (Subías 2004). Six are known for the Oriental region: three were described from Japan (*T. ashoroensis* Fujikawa, 2000; *T. brevisetus* Kuriki, 2005; *T. taisetsuensis* Kuriki, 2005); one for Bali (*T. ramosus* Hammer, 1982), one for China (*T. qianensis* Hu & Jin, 2010) and one cited from Japan and Philippines (*T. porticus*); only one is reported for South America (Chile, *T. chilensis* Ermilov & Wiegmann, 2015), and the most recent described from South Africa *T. malaconothroiformis* Ermilov, Hugo-Coetzee & Theron, 2017.

Food specialization remains a key issue for understanding of animal species diversity but surprisingly, knowledge on feeding biology of many invertebrates remains poor and in some cases the available information is inconclusive. Investigations on the feeding biology of oribatid mites, including gut content analysis and analyses of enzyme activities indicate that most oribatids ingest a wide range of materials (Luxton 1972; Behan-Pelletier & Hill 1978; Siepel & de Ruiter-Dijkman 1993). Furthermore, results of food choice experiments suggest that oribatids feed preferentially on certain fungal species (Mitchell & Parkinson 1976; Kaneko *et al.* 1995; Kaneko 1988), preferring dark pigmented over hyaline fungi (Maraun *et al.* 1998). Investigations describing mite’s diet and trophic interactions have been gained from stable isotope techniques (using stable isotopes ¹⁵N and ¹³C) providing information on the dietary preferences, and to understand the trophic ecology of oribatid mites (Schneider *et al.* 2004; Maraun *et al.* 2011). Lehmitz and Maraun (2016) investigation on the heterogeneity within and between *Sphagnum* microhabitats, found that *Trhypochthoniellus longisetus* (a freshwater oribatid) was low in the food web and being a primary or secondary decomposer.

Invertebrate fauna of freshwater springs in the Cuatro Ciénegas Basin (CCB), Coahuila state, Mexico, has been relatively well documented (Dinger *et al.* 2005; Álvarez & Ojeda 2019; Ojeda & Gasca-Pineda 2019). These freshwater systems are known for their rich microbial diversity (Souza *et al.* 2006, 2012; Desnues *et al.* 2008), which includes microfungi. Velez *et al.* (2016) analyzed microfungal diversity and ecological patterns in three contrasting freshwater systems of the CCB (Churince, Becerra and Pozas Rojas). Their results evidenced the relevance of microfungal diversity, since this group of microorganisms represents an important indicator of trophic complexity and biotic interactions among microbial communities. Remarkably, during this study the authors discovered a large population of an aquatic oribatid mite associated to the lignocellulolytic microfungal taxa, this is the species here described.

Besides the complete description, illustrations and photographs that are presented; a key to all the species of *Trhypochthoniellus* is given. Baseline information about food preference and reproduction mode shown by *T. churincensis* sp. nov. kept in laboratory cultures is also given.

Material and Methods

Study site

The Cuatro Ciénegas Basin (CCB) is a small valley (about 1,500 km²) in central Coahuila, Mexico, formed by the mountain ranges of the Sierra Madre Oriental. Although it is in one of the driest areas of the Chihuahuan Desert (less than 200 mm of rainfall per year), it is estimated to contain more than 200 springs and other associated aquatic habitats with many endemic organisms and modern stromatolites. Different types of aquatic habitats occur in the basin: pozas (small springfed pools), lagunas (larger spring-fed lakes), playa lakes (large lakes fed by surface runoff, but

without outlets), ciénegas (shallow swamps), natural small rivers and human-made canals and ponds (Dinger *et al.* 2005).

Sampling

Specimens of *T. churincensis* sp. nov. used in this study were obtained from several wood pine block submerged at the Laguna Intermedia of the Churince system in 2014-2015 (Cuatro Ciénegas Basin, Coahuila, Mexico; details see Velez *et al.* 2018). Laboratory culture was started in November 2016 with 30 specimens for each block (total 5). Mites were cultured on a plaster of Paris/charcoal substrate (9:1) in plastic Petri dishes, at 23°C and watered with fresh tap-water to keep substrate submerged and air humidity at about 90%. About 50 specimens of the cultures were checked to take the 10 adult females that make up the type series. Additional specimens were collected from two other locations in the CCB (Poza La Becerra and Pozas Rojas); using hand nets and washing the vegetation that grows near the edge of the ponds, and preserved in ethilic alcohol 75% for transport and processing at the laboratory.

Mites were cleared in lactic acid and mounted in Hoyer's medium on temporary cavity slides for observation. Measurements are in micrometers (μm), and illustrations were made using a Nikon Optiphot-2 phase contrast microscope, with an adapted drawing tube. The body length was measured in lateral view, from tip of the rostrum to posterior edge of ventral plate. Notogastral width refers to the maximum width in dorsal aspect. Formulas for leg setation are given in parentheses according to the sequence trochanter-femur-genu-tibia-tarsus (femulus included). Formulas for leg solenidia are given in square brackets according to the sequence genu-tibia-tarsus. The general terminology we used follows Norton & Behan-Pelletier (2009). Images were obtained with an AxioCam MRC5 camera using a Carl Zeiss AxioZoom V16 microscope and SEM (Scanning Electron Microscope). Specimens were collected under the scientific collector's license issued to V. Souza (FAUT-0230), emitted by the Mexican environmental authority (SEMARNAT). Specimens were deposited at the following collections, National Acarological Collection (CNAC) of the Institute of Biology, UNAM, México City, and Oribatid collection, Laboratory of Ecology and Systematic of Microarthropods (LESM), Facultad de Ciencias, UNAM, México City.

Results

Trhypochthoniellus churincensis Ojeda sp. nov.

(Figs. 1–5)

Diagnosis

Body surface densely porose, notogaster covered by a well developed polygonal pattern. Rostral, lamellar and interlamellar setae setiform, smooth. Bothridial setae and bothidia absent. Fourteen pairs of setiform, thin, smooth notogastral setae. Setae *p3* absent and *fl* represented by alveoli. All setae setiform, smooth and ending in a very fine tip. Distance between insertions of setae *c1–c1* (67) subequal as *d1–d1* (69). Ventral setae setiform, smooth and fine. 9 (rarely 10) pairs of genital setae. Tridactylous. Leg trochanter IV with two dorsal strong triangular tubercles, genua IV with one seta.

Description

Measurements. Body length: 556 (holotype, female), 460–588 (9 paratypes, all females); notogaster width: 275 (holotype), 224–320 (9 paratypes).

Integument. Body color light brownish. Body surface cover by a densely fine pores. Interbothridial region and dorso-lateral parts of prodorsum and hysterosoma covered by a well developed net-like polygonal pattern (Figs. 1A–D).

Prodorsum. Rostrum rounded with 3–5 projections between rostral setae (*ro*) (Fig. 2A). Rostral (*ro*, 40), lamellar (*le*, 48), interlamellar (*in*, 99) and exobothridial (*ex1*, 12) setae, setiform, smooth and ending in a very fine tip; relative length, *ro*: *le*: *in* = 1: 1.2; 2.5. Lamellar setae (48) slightly longer than *ro* (40), with a well develop transverse ridge connecting insertions of *le*. Distance between *le*–*le* (27) subequal to *ro*–*ro* (33). Interlamellar seta (*in*) very long (99), extending far beyond insertion of dorsal seta *d*₂. Bothridial setae absents in all specimens. Exobothridial setae *ex1* (12) thin, setiform, smooth. Exobothridial setae *ex2* vestigial, represented by alveoli. Integument of interbothridial region and dorso-lateral parts covered with a well developed polygonal net-like pattern.

Notogaster. (Figs. 2A, B). Anterior margin straight with a transparent band. Posterior margin rounded, slightly V-shape. Surface of notogaster covered by a polygonal net-like pattern. Fourteen pairs of setiform, thin, smooth, notogastral setae ending in a very fine tip; *f1* represented only by alveoli, inserted anteriorly and far (38) from *h1*. Setae *p3* absent in all specimens. Opisthonotal gland opening (*gla*) distinct. Seta *c1* inserted near anterior margin (29), and passing *d1* insertion; *c2* short and nearest to anterior border (11); *cp* farthest (37). Setae *c1* and *c3*, and *d* medium size (53), setae in rows *h* and *p* longest (65). Distance between insertions of setae *c1*–*c1* subequal to *d1*–*d1* (67–69). Setae *h2* widely separated from each other (244), being closer to *h3* in dorsal view (62). Lyrifissures *ia* aligned obliquely, directing postero-lateral, situated just behind insertion of setae *c3* (Fig. 2A); *ip* aligned transversely in front of setae *h2*. Lyrifissures *im*, *ih* and *ips* openings distinct. Opisthonotal gland (*gla*) situated lateroabdominally and the opening found behind seta *e2*.

Gnathosoma. Subcapitulum diarthric, slightly longer than wider (73–82 × 69–73). Subcapitular setae setiform, smooth, *a* slightly longer than *m*, and *h* shortest, hardly visible because of punctuations on surface. Three pairs of adoral setae developed, similar in length (10–12), *or1* wide, dilated medio-distally, truncated, *or2* and *or3* setiform, smooth (Fig. 3A). Pedipalps (45–49) with setation 0-1-1-1-9 (+ω). Solenidion on tarsi thick, blunt-ended, eupathidium inserted on tubercle (Fig. 3B). Chelicerae (77–82) with two setae; *cha* (10) short, thick, barbed medio-distally, *chb* (15) setiform, smooth bending forward (Fig. 3C).

Epimeral region. Epimeral setal formula (from 1 to 4): 3-1-3-2. All setae thin and smooth; *1a*, *1c*, *2a*, *3a* and *4a* (10–12) shorter than others (18–20) (Fig. 4A).

Anogenital region. Nine pairs of genital setae thin and smooth (two specimens show 10 symmetric pairs of genital setae), smooth, *g1*–*g3* inserted closer to each other than *g4*–*g6*. Two pairs of adanal (*ad1*, 20; *ad2*, 18) and one pair of anal (*an*, 8) setae smooth, thin. Lyrifissures *ian* and *iad* distinct rounded (Fig. 4B).

Legs. (Figs. 5A–D). Tridactylous, median claw as long as lateral ones, claws smooth. Relative length of legs: IV > III > II > I; tarsus III and IV longer than tarsus I and II. Trochanter IV with two well develop dorsal triangular tubercles. Formulas of leg setation and solenidia: I (1-6-3-4-12) [1-1-3], II (1-5-3-3-11) [1-1-2], III (2-2-2-2-10) [1-1-0], IV (1-2-1-2-11) [0-1-0]. Setae of three types: setiform, smooth; spiniform, thick, smooth; and thickened or slightly dilated, barbed (as depicted in Fig. 5). Solenidion ω₂ on tarsus short. Solenidia on tibiae I slightly same length than coupled seta *d*. Solenidia on tibiae III subequal to coupled seta *d*; solenidia on tibiae IV shorter. Solenidia of genua I–III slightly shorter than coupled setae *d*. Solenidia on tibiae II longer than coupled seta *d*.

Material examined

Holotype (female) and 9 paratypes: Laguna Intermedia of the Churince system, Cuatro Ciénegas Basin, Coahuila, Mexico. 26°4.998' N, 102°14.923' W, 817 m asl, 18-XI-2014, P. Velez col.

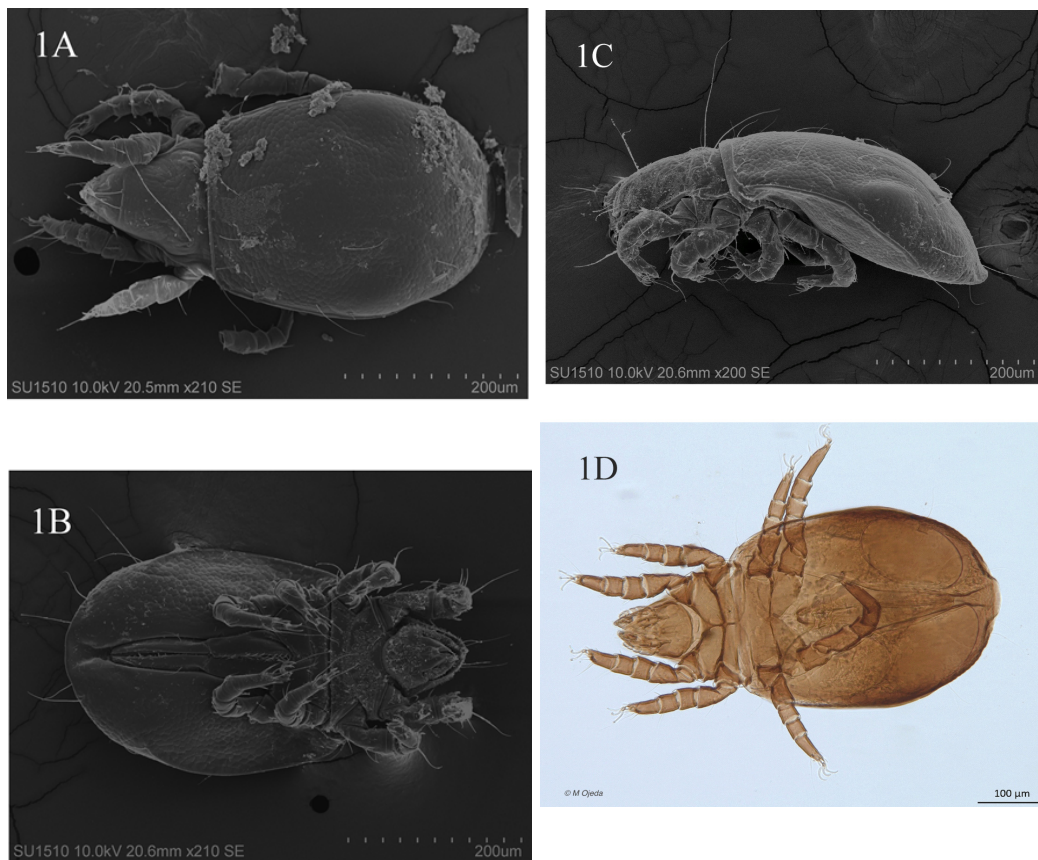


FIGURE 1. *Trhypochthoniellus churincensis* sp. nov., SEM images. A) Dorsal view, B) Ventral view, C) Lateral view, D) microphotograph, ventral view (showing color of specimens).

Other material examined

Poza La Bacteria, Road 30 Cuatro Ciénegas-Torreón, Coahuila, 26°50.405' N, 102°08.036' W, 771 m asl, 6-X-2016. M. Ojeda and A. Carlos col. Pozas Rojas, 26°52.273' N, 102°01.236' W, 714 m asl, 5-X-2016. M. Ojeda and A. Carlos col.

Type deposition

Holotype and nine paratypes (all females). Holotype and one paratype are deposited in CNAC; catalog numbers CNAC011469-011470. Eight paratypes are deposited in the Oribatid Collection of LESM; catalog numbers LESM1560-1568. Voucher material are deposited in the LESM.

Etymology

The specific name “*churincensis*” refers to the specimen’s site of collection, Churince, Cuatro Ciénegas Basin, Coahuila state, México.

Remarks

The new species shares with *Trhypochthoniellus longisetus* (Berlese 1904) forma *setosa* after Weigmann (1997, 1999), and *T. brevisetus* Kuriki (2005) the lack of bothridium and bothridial setae; body of medium size; absence of setae *p3*. It differs with *T. longisetus* forma *setosa* by 1) number of genital setae (10 vs 11–13), 2) number of notogastral setae (13 vs 14), 3) relative length *ro: le*; in (

1: 2; 3.5), 4) length of *le*, *ro*, *c2* and *h3* setae, and 5) absence of a slightly protruding expansion in the humeral portion. *T. churincensis* sp. nov. is distinguishable from *T. brevisetus* Kuriki (2005) by 1) number of genital setae (10 vs 11–13); 2) the position of the vestigial seta *fl* (*fl* close to *h1*), 4) queliceral setae *cha* shorter and apart from *chb* (*cha* minute and close to *chb*), 5) seta *h* on subcapitulum very short, minute (seta *h* large), and 6) Solenidion *w2* on tarsus II absent.

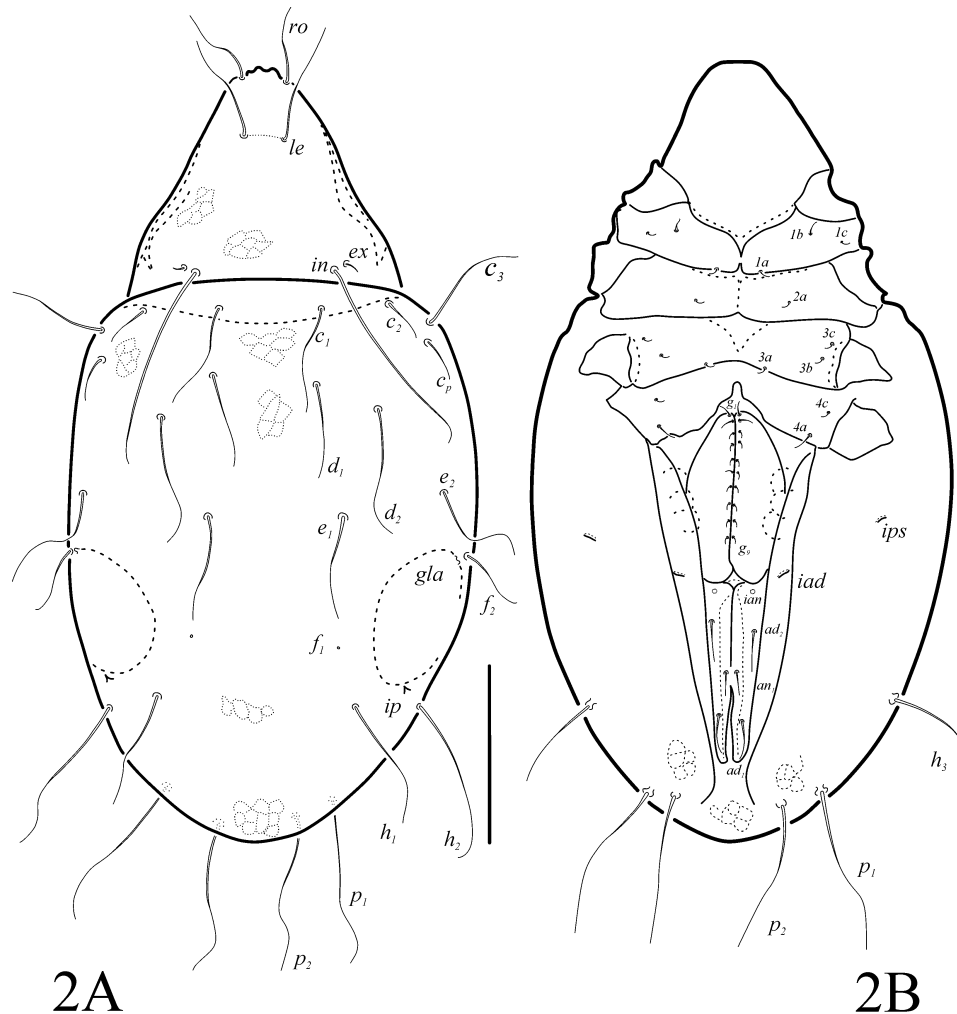


FIGURE 2. *Trhypochthoniellus churincensis* sp. nov., Adult female. A) Dorsal view, B) Ventral view (gnathosoma and legs not illustrated) Scale bar 100 µm.

Deichsel (2004), Weigmann (1997,1999) and Weigmann and Deichsel (2007) mentioned that oribatid freshwater aquatic taxa, e.g. *Trhypochthoniellus*, or *Hydrozetes* are highly variable in their morphology, and that in some cases the separation in different species is not appropriate, since such variation should be taken into account before describing a new species. However, the new species here described do not show broad differences, in the morphological characters that define the group. Observations on additional material from other two sites (La Becerra and Pozas Rojas) did not show broad biometric differences with those obtained from the Churince, and specimens show consistency in the absence of botridium and bothridial seta as well as number of genital setae. The molecular sequences obtained by Velez et al. (2018) indicated that *Trhypochthoniellus churincensis* sp. nov., represent a novel genetic lineage within the Crotonioidea, close to *Trhypochthonius cladonicolus*

(Willmann 1919). Topology of the ML tree was equivalent to Klimov et al. (2018), and the value supporting the branch containing CCB mites within *Trhypochthoniellus* was high (90%). Remarkably, mites were grouped within a single phylogenetic cluster, diverging from known taxa.

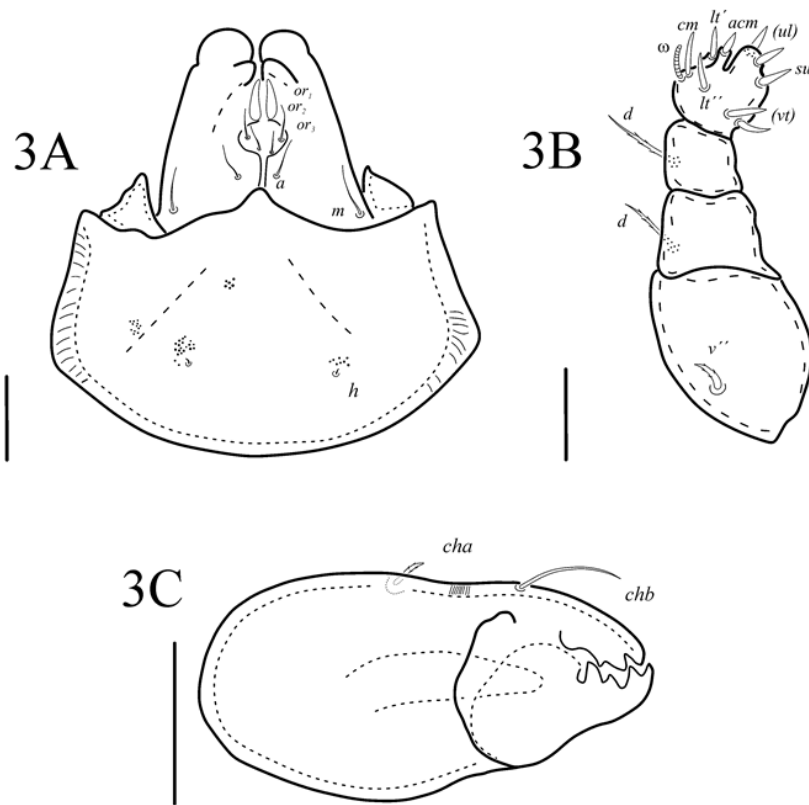


FIGURE 3. *Trhypochthoniellus churincensis* sp. nov., A) Subcapitulum, ventral view, B) Palp left paraxial view, C) Chelicera. Scale bars 20 µm.

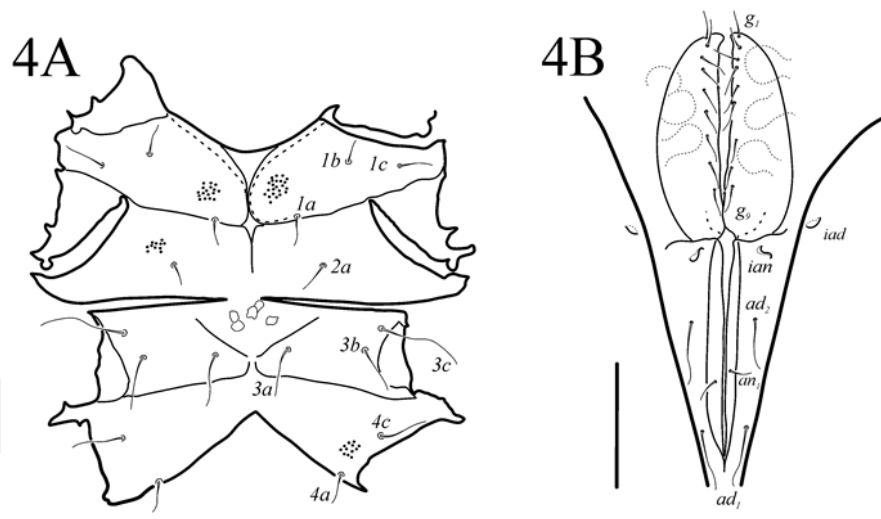


FIGURE 4. *Trhypochthoniellus churincensis* sp. nov., A) Epimeral region, B) Anogenital region. Scale bars 100 µm.

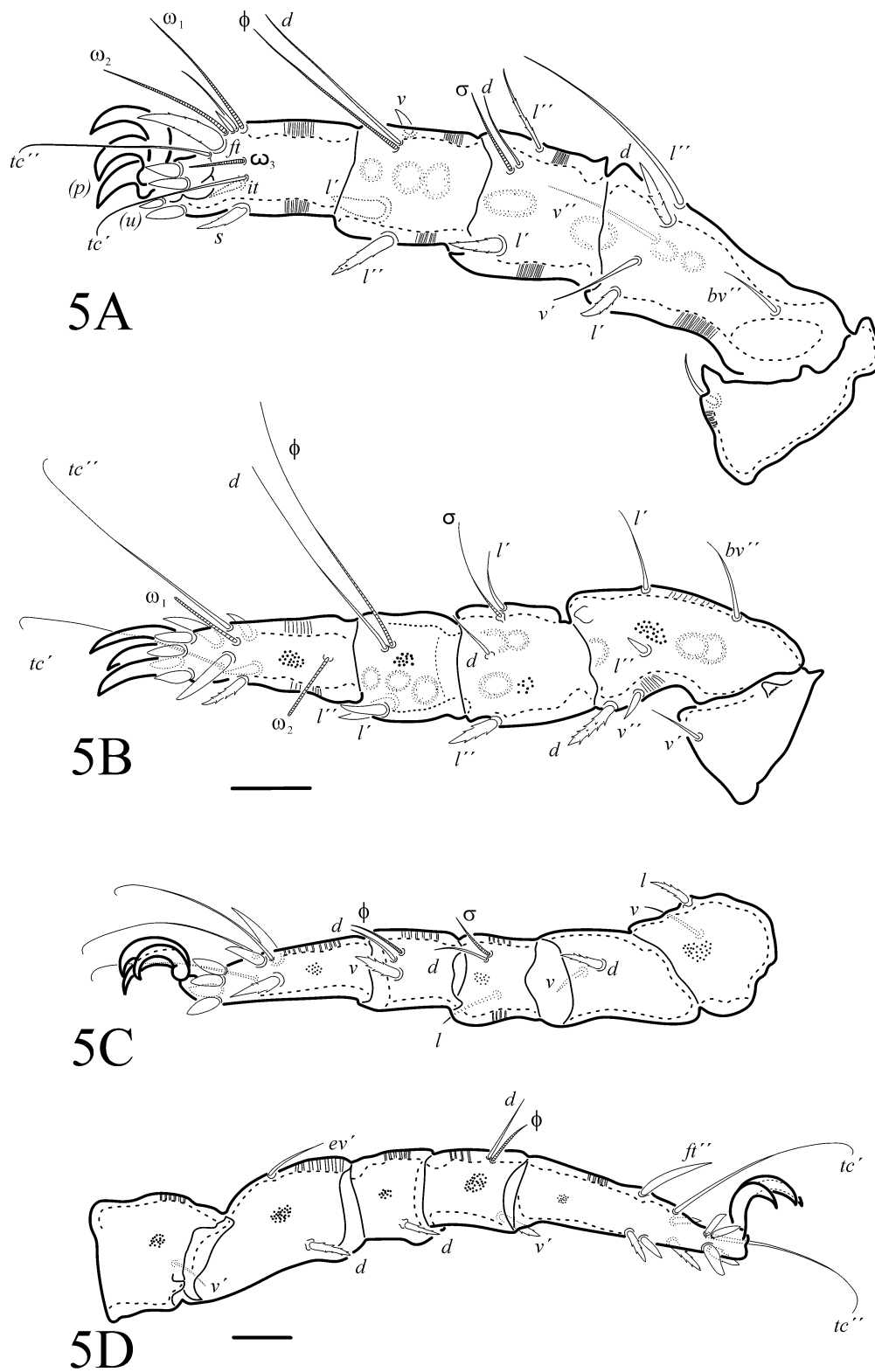


FIGURE 5. *Trhypochthoniellus churincensis* sp. nov., Adult. Legs. A) Leg I, B) Leg II, C) Leg III, D) Leg IV. Scale bars 20 μ m.

Observations on feeding biology

Food choice experiments suggest that oribatid mites preferentially feed on fungal species (Mitchell & Parkinson 1976, Kaneko et al. 1995, Maraun et al. 1998). Soil animals tend to be food generalists due to their close spatial association (Scheu & Setälä 2012).

In summary, mites belonging to *T. churincensis* sp. nov., have the ability to feed on the usually avoided and unsuitable *Aspergillus niger* (Velez et al. 2018), and seem to be mostly opportunistic feeders ‘choosy generalists’ (Schneider & Maraun 2005), but with preferences, possibly for more nutrient-rich or less toxic food, as observed for *Archeogozetes longisetosus* (Brückner et al. 2018). Food preference imprinting may be not beneficial for a highly opportunistic/generalist oribatid mite species like *T. churincensis* sp. nov., since such species need to switch food resources quite regularly to obtain exploitable nutrients in an environment with scarce and patchy distributed resources as happens in its habitat at Cuatro Ciénegas, Coahuila. Further investigations on trophic ecology using stable isotopes and feeding preferences in populations of *T. churincensis* sp. nov. will be of interest, to establish the specific role this species is playing in this particular ecosystem.

Parthenogenesis in *T. churincensis* sp. nov.

During the study we noted a complete absence of males suggesting a parthenogenetic reproductive strategy. We hypothesized that this strategy could also explain the absence of noticeable morphological variation between mites specimens collected from different sites in the valley (CCB), as La Becerra and Pozas Rojas sites, as they present a constant morphological characters that are defining this new species. Adoption of thelytokous (a type of parthenogenesis in which females are produced from unfertilized eggs) parthenogenesis has been widely reported in oribatid mites (Norton & Palmer 1991; Norton et al. 1993; Maraun et al. 2003, 2019).

We observed that *T. churincensis* sp. nov. reproduced differently on the three fungal species tested by Velez et al. (2018), evident in the growth time of the different stages of development and the number of immature individuals in each of the treatments use. This fact, combined with its unspecialized feeding habits, may have allowed this species to endure through the extreme and fluctuating temperature and water chemistry conditions in the ponds and lagoons of the CCB. Further investigations into reproductive modes and feeding preferences using stable isotope techniques in populations of *Trhypochthoniellus* species will be of interest, considering that most families of Crotonioidea are composed entirely of parthenogenetic species and knowledge about diet and trophic ecology of oribatid mite species mites is scarce.

Key to species of *Trhypochthoniellus*

1. Bothridial setae (sensilli) and bothridium absent 2
- Bothridial setae (sensilli) and bothridium present 6
2. 4 pairs of genital setae, 2 pairs of anal setae, 15 pairs of notogastral setae and without a bridge connecting the setae *T. ramosus* Hammer, 1982
- More than 4 pairs of genital setae, 1 pair of anal setae, 13–4 pairs of notogastral setae. 3
3. 7 pairs of genital setae finely barbulated, body surface densely porose, an irregular transverse ridge between rostral setae absent *T. malaconothroiformis* Ermilov, Hugo-Coetzee & Theron, 2017
- More than 9 pairs of genital setae smooth and fine, body surface with a polygonal pattern, an irregular transverse ridge between rostral setae present 4
4. 9 pairs of genital setae smooth, with a very fine tip (exceptionally 10), 14 pairs of notogastral setae *T. churincensis* sp. nov.
- More than 9 pairs of genital setae smooth, 13–14 pairs of notogastral setae. 5
5. 11 pairs of genital setae smooth (exceptionally 12 or 13), 13 pairs of notogastral setae, *ps3* absent. Seta *cha* long and apart from *chb* *T. longisetus* (Berlese 1904)

- 11 pairs of genital setae smooth (exceptionally 12 or 13), 14 pairs of notogastral setae, ps3 present. Seta *cha* minute, close to *chb* *T. brevisetus* Kuriki, 2005
- 6. 15 pairs of notogastral setae, setae *p3* present; 6 pairs of genital setae and tarsus monodactylous
..... *T. chilensis* Ermilov & Weigmann, 2015
- 14 pairs of notogastral setae, setae *p3* absent; 6 to 11 pairs of genital setae and tarsus tridactylous. 7
- 7. 8 to 9 pairs of barbulated genital setae, notogastral setae *c3* largest of row *c* ... *T. porticus* Fujikawa, 2000
- 6 to 11 pairs of smooth genital setae, notogastral setae *c3* different size of row *c* 8
- 8. 9 pairs of smooth genital setae, notogastral setae *c3* shortest of row *c*, body surface punctuated
..... *T. qianensis* Hu & Jin, 2010
- 6 to 11 pairs of genital setae, body surface different 9
- 9. 10 to 11 pairs of genital setae, sensillum with a short pedicellum, body size 540 x 323 µm
..... *T. ashoroensis* Fujikawa, 2000µm
- 6–8 pairs of smooth genital setae, sensillum with a medium size pedicellum, body size 427 µm
..... *T. taisetsuensis* Kuriki, 2005

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