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In memory of Gary Bauchan: Utilizing an integrated taxonomy approach for the description of a new species of *Gamasellodes* (Mesostigmata: Ascidae)

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Abstract

Gamasellodes garybauchani sp. nov. Rueda-Ramírez & Santos is described based on specimens collected at the Beltsville Agricultural Research Center (BARC) (Maryland, USA). Twenty-one specimens were used for the description, of which five were subjected to low temperature scanning electron microscopy (LTSEM), followed by DNA extraction for genetic analysis and subsequent slide mounting. The remaining 16 specimens were subjected to only DNA extraction for genetic analysis and subsequent slide mounting. Morphological characters of the new species observed both in the images obtained in LTSEM and in the observation of the mounted specimens are detailed. Molecular information available on the Barcode of Life Datasystem (BOLD) is presented. An update to the recent key to the *Gamasellodes* species is provided.

Key words: Ascidae, LTSEM, DNA barcodes, morphology

Introduction

Numerous soil predatory mite species belonging to the cohort Gamasina have been morphologically identified as potential biocontrol candidates of soil pests (Carrillo *et al.* 2015). However, an integrative approach can now also be adopted, in which morphological and molecular techniques are combined, resulting in identified voucher specimens and reliable molecular barcodes, openly accessible to the broad scientific community.

Ascidae is a diverse mite family with nearly 380 described species assigned to 17 genera (Moraes *et al.* 2016; Santos *et al.* 2021). *Gamasellodes* is commonly found in edaphic habitats or associated with insects. Twenty-eight species are presently known in this genus, most from soil and litter (Santos *et al.* 2021). Species of this genus have worldwide distribution described mainly from the Americas (Moraes *et al.* 2016; Santos *et al.* 2021).

A new *Gamasellodes* species was found in a recent study conducted at the USDA ARS Farming Systems Project (FSP), Beltsville Agricultural Research Center (BARC), Maryland, USA (Rueda-Ramírez *et al.* 2022, this special issue). The objective of this paper is to describe this new species providing morphological information, DNA barcodes and an updated dichotomous key to separate the world species of *Gamasellodes*.

Material and Methods

Mite collection. Soil samples were collected at the FSP, BARC, Maryland, USA (Latitude: 39.028026–39.029562; Longitude: -76.899278 – -76.894251) from conventional till, no till and organic farming systems, on April 30th, 2019. Each sample was collected with a core sampler (5 cm diameter and 5 cm depth). Soil was removed from the cores, gently mixed and then poured into a sieve 6 cm high and 10 cm in diameter. Sieves were incubated with or without soil amendments and the mites extracted as described by Rueda-Ramírez et al. (2022, this special issue).

LTSEM examination. Females and males were removed from ethanol 95%, arranged on double sided carbon tape mounted on standard brass plates, six to eight specimens per plate and frozen in liquid nitrogen. The brass plate containing the frozen sample was transferred to the Quorum PP2000 cryo transfer system (Quorum Technologies, East Sussex, UK) attached to an S-4700 field emission scanning electron microscope (Hitachi High Technologies America, Inc., Dallas, TX USA). The specimens were freeze etched inside the cryotransfer system to remove any surface contamination (condensed water vapor) by raising the temperature of the stage to -90 °C for 10–15 minutes. Following etching, the temperature inside the chamber was lowered below -130 °C, and the specimens were coated with a 10 nm layer of platinum using a magnetron sputter head equipped with a platinum target. The specimens were transferred to a pre-cooled (-130 °C) cryostage in the SEM for observation. An accelerating voltage of 5 kV was used to view the specimens. Images were captured using a 4pi Analysis System (Durham, NC USA).

DNA barcoding. Twenty-one specimens (including five specimens used for LTSEM imaging) were sent for molecular analysis at the Canadian Centre for DNA Barcoding (CCDB). The barcode region of cytochrome c oxidase I (COI) was sequenced with a cocktail (1:1 ratio) of LepF1/LepR1 (Hebert *et al.* 2003) and LCO1490/HCO2198 (Folmer *et al.* 1994) primers using standard protocols modified to allow voucher recovery following DNA extraction (Ivanova *et al.* 2007; Ivanova and Grainger 2007a, b; Porco *et al.* 2010). The DNA extracts were archived in -80 °C freezers at the Centre for Biodiversity Genomics (CBG; biodiversitygenomics.net), and the vouchered specimens were retained in 96-well microplates with 95% ethanol for subsequent morphological preparations. The sequences were assembled from forward and reverse chromatograms using CodonCode Aligner v. 4.2.7 and uploaded to BOLD. They were inspected for potential contamination or misidentification by examining their placement in a Neighbor-Joining tree and by querying each record against BOLD's complete reference library using the BOLD Identification Engine.

COI sequence similarities between the new species here described and other Ascidæ were visualized by first aligning representative DNA barcode sequences for each publicly accessible species (Table 1) using Muscle in MEGA6 (Tamura *et al.* 2013). Pairwise p-distances (with partial deletion) were estimated from the resulting alignment and visualized with a Neighbour-Joining tree, including *Rhodacarellus silesiacus* as an outgroup.

Mite identification and description. Morphological identification was performed based on the examination of 12 mites (measured only 6 females and 2 males) from which DNA had been extracted, and which had been mounted in Hoyer's medium on microscope slides. Illustrations of taxonomically important structures of the new species were made from photos taken with a digital camera AxioCam MRc5 connected to a Axiophot Zeiss microscope; photos and illustrations were then processed with a digital tablet, using the Adobe Illustrator® program (version 21.0.0, 2017). Measurements were taken with ZEN 2012 software (version 1.1.1.0). Measurements of each structure are given in micrometres, with the average measurement for the specimens examined followed (in parentheses) by the respective ranges, for variable structures.

Shield length refers to maximum distance between anterior and posterior margins, whereas shield width refers to maximum width, except for the sternal shield, for which width was taken at

level of coxae II. Length of epigynal shield included the anterior hyaline region. Leg lengths refer to the distance between the base of the coxa to the tip of the tarsus (not including pretarsus). Nomenclature of idiosomal setae is based on Lindquist & Evans (1965); leg chaetotaxy is based on Evans (1963, 1969) and pore-like structures on Athias-Henriot (1975). The number of teeth of each cheliceral digit does not include the apical 'hook'.

Type depositories of the new species are indicated in the 'Type specimens' section. Additionally, all voucher specimens (type specimens and other examined material) of the described *Gamasellodes* species were deposited in the United States National Mite Collection (USNM), USDA-ARS, Beltsville, Agricultural Research Centre, Beltsville, Maryland, USA.

TABLE 1. Ascidae species included in the Neighbor-Joining analysis with their corresponding voucher collection and GenBank accession numbers.

Species	Museum ID	BOLD Sequence ID	GenBank Accession Number
<i>Asca bicornis</i> (Canestrini & Fanzago, 1877)	BIOUG30422-B04	MSSCA514-16	MN348931
<i>Asca brachychaeta</i> Hurlbutt, 1963	BIOUG08018-A10	RBINA5574-13	MF916342
<i>Asca garmani</i> Hurlbutt, 1963)	USNMENT 01021857	PASMB152-19	OK072904
<i>Antennoseius</i> (<i>Antennoseius</i>) <i>avius</i> Karg, 1977	BIOUG06322-B05	SSPAA8424-13	KM830590
<i>Antennoseius</i> (<i>Vitzthumia</i>) <i>janus</i> Lindquist & Walter, 1989	MYMCA-05-67	MYMCA447-11	JX834536
<i>Arctoseius bilinear</i> Nasr, 1986	BIOUG01184-34	MYMCE699-12	JX834216
<i>Arctoseius brevicheles</i> Karg, 1969	DPMIT-36-66	CHACC161-10	JX837921
<i>Arctoseius cetratus</i> (Sellnick, 1940)	BIOUG05655-H09	SMTPB14723-13	KP979118
<i>Arctoseius confusus</i> Lindquist, 1961	08DPMIT-0498	CHACA498-09	JX833842
<i>Arctoseius kolymensis</i> Makarova & Petrova, 1992	DMPIT-30-27	CHACB786-10	HQ941484
<i>Arctoseius semiscissus</i> (Berlese, 1892)	BIOUG31745-C04	AGAKA234-17	MG410386
<i>Arctoseius taeniolatus</i> Athias-Henriot, 1961	BIOUG05655-H09	SMTPB14723-13	KP979118
<i>Gamasellodes</i> sp. JCS05	BIOUG04397-F06	SSBAD4022-12	KM837926
<i>Gamasellodes</i> sp. JCS06	BIOUG09080-D03	SSWEE011-13	KM828900
<i>Gamasellodes</i> sp. JCS07	BIOUG24569-B05	SWRON088-15	MG416480
<i>Neojordensia levis</i> (Oudemans & Voigts, 1904)	USNMENT 01021860	PASMB155-19	OK072955
<i>Leioseius minusculus</i> (Berlese, 1905)	BIOUG30428-D09	MSSCA1113-16	MN353977
<i>Iphidonopsis sculptus</i> Gwiazdowicz, 2004	BIOUG06184-D08	SSJAD1619-13	KM830507
<i>Iphidozercon altaicus</i> Gwiazdowicz & Marchenko, 2012	BIOUG05863-E10	SSPAA2270-13	KM838026
<i>Iphidozercon californicus</i> Chant, 1963	BIOUG16010-D06	SMTPI1028-14	MG411522
<i>Iphidozercon gibbus</i> (Berlese, 1903)	BIOUG01649-E04	JSJUN2325-12	KP979197
<i>Zerconopsis decemremiger</i> Evans & Hyatt, 1960	BIOUG11369-B01	CNKJB740-14	KR069970

Results

Gamasellodes Athias-Henriot, 1961

Gamasellodes Athias-Henriot, 1961: 480.

Gamasellodes.—Lindquist & Evans 1965: 42; Halliday et al. 1998: 21; Walter 2003: 2; Gwiazdowicz 2007: 67; Moraes et al. 2016: 22; Castro et al. 2020: 2.

Type species: *Gamasellodes vulgator* Athias-Henriot, 1961 by original designation.

Gamasellodes garybauchani sp. nov. Rueda-Ramírez & Santos
(Figures 1–23)

Diagnosis

Fixed cheliceral digit with five teeth. Anterior region of epistome with three-pointed, smooth projections of similar lengths (each bifurcate or not). Podonotal shield lightly ornate, with 16 pairs of setae. Opisthonotal shield lightly ornate, with 15 pairs of setae. Unsclerotised cuticle laterad podonotal and opisthonotal shields respectively with six and five pairs of setae. Sternal shield lightly ornate laterally; with three pairs of setae (*st1*–*st3*). Genital shield almost smooth, with round posterior margin. Ventrianal shield ellipsoidal, with three pairs of setae in addition to circumanal setae. Unsclerotised opisthogastric cuticle with six pairs of setae. Peritreme extending anteriorly almost to level of *z1*. Femur II and tibiae III and IV with respectively 11, 8, and 10 setae.

Description

Adult female (Figures 1–15, *n*=6)

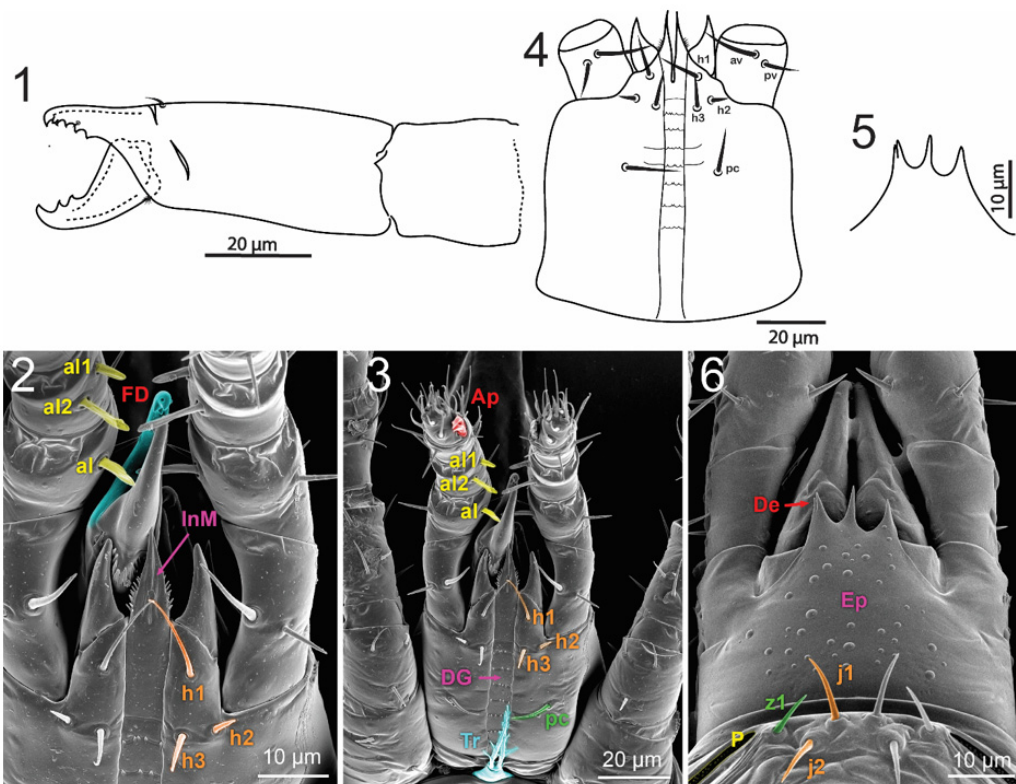
Gnathosoma. Chelicera (Figure 1, 2) with fringed coronet-like arthrodial process; basal segment of the chelicera 23 (19–26) long; section of second segment of the chelicera behind dorsal lyrifissure 46 (44–48); fixed chelicera digit 22 (21–24), with five teeth, the distal tooth offset and, therefore, not very evident in the mounted specimens, but evident in the LTSEM images; with a pore-like structure in the tip (Figure 2; FD colored blue); with spine-like *pilus dentilis*; dorsal seta thick and setiform, dorsal and antiaxial lyrifissures distinct; movable cheliceral digit 24 (24–25) long, with two teeth (Figure 1, 2). Number of setae on palp trochanter–tarsus: 2-5-6-14-15, aciculate and smooth, except *al* of femur and *al1* and *al2* of genu, stout and spatulated (Figure 2, 3); palptrochanter with outer seta (*pv*) 10 (9–11) shorter than inner seta (*av*) 19 (18–20) (Figure 2–4); apotele 2-tined, with one of the tines about 1.5 times as long as the other (Figure 2–4). Anterior region of the epistome tripartite, each prong each of them rarely bifurcate (Figure 5, 6). Deutosternal groove with eight transverse rows, the most distal smooth and others with 3–6 denticles each, delimited by subparallel lateral lines slightly converging posteriorly (Figure 3, 4). Internal malae totally separated from each other, each triangular, scarcely fringed in basal half, extending to the level of corniculus tip (Figure 3, 4). Corniculus horn-shaped, well separated from each other, subparallel to each other, about twice as long as its basal width; 18 (18–20) long and 9 (8–10) wide (Figure 3, 4). Hypostomal setae aciculate and smooth, *h3* about in longitudinal line with *h1* and mesad and slightly posteriad of *h2*, which is shorter (Figure 3, 4). Measurements of subcapitular setae: *h1* 14 (13–15), *h2* 10 (8–12), *h3* 12 (11–14), *pc* 11 (10–13); all setae aciculate and smooth.

Idiosoma. Length 323 (296–348), width 175 (159–191).

Measurements of setae: *j1* 11 (10–12), *j2* 12 (11–12), *j3* 12 (11–14), *j4* 12 (11–13), *j5* 13 (12–14), *j6* 12 (12–14), *z1* 8 (7–9), *z2* 13 (12–14), *z3* 12 (12–13), *z4* 14 (13–15), *z5* 13 (12–13), *z6* 11 (11–12), *s1* 10 (10–10), *s2* 9 (8–10), *s3* 14 (13–14), *s4* 14 (12–15), *s5* 14 (13–14), *s6* 13 (11–15), *r2* 10 (10–11), *r3* 11 (11–12), *r4* 10 (9–12), *r5* 11 (9–12), *J1* 11 (10–12), *J2* 12 (11–12), *J3* 10 (10–11), *J4* 13 (12–13), *J5* 7 (7–7), *Z1* 13 (13–14), *Z2* 13 (13–13), *Z3* 14 (13–15), *Z4* 15 (14–16), *Z5* 26 (25–27), *S1* 12 (11–12), *S2* 13 (12–14), *S3* 13 (13–14), *S4* 15 (14–15), *S5* 13 (12–14), *R1* 9 (8–9), *R2* 10 (10–10), *R3* 10 (10–11), *R4* 9 (8–10), *R5* 10 (10–11), *UR* 8 (7–9); all setae aciculate, smooth, except *Z5*, slightly stouter, distally barbed.

Dorsal idiosoma (Figures 7–8). Podonotal shield with irregular colliculate ornamentation, less evident between both lines of *j* setae; with delineated marginal strip posteriad *s2* (Figures 7–8); 162 (154–166) long and 149 (135–165) wide; with 16 pairs of setae (*j1*–*j6*, *z1*–*z6*, *s3*–*s6*), four pairs of distinguishable lyrifissures (*id1*, *id2*, *id5*, *id6*) and three pairs of pores (*gd1*, *gd2*, *gd5*). Unsclerotised

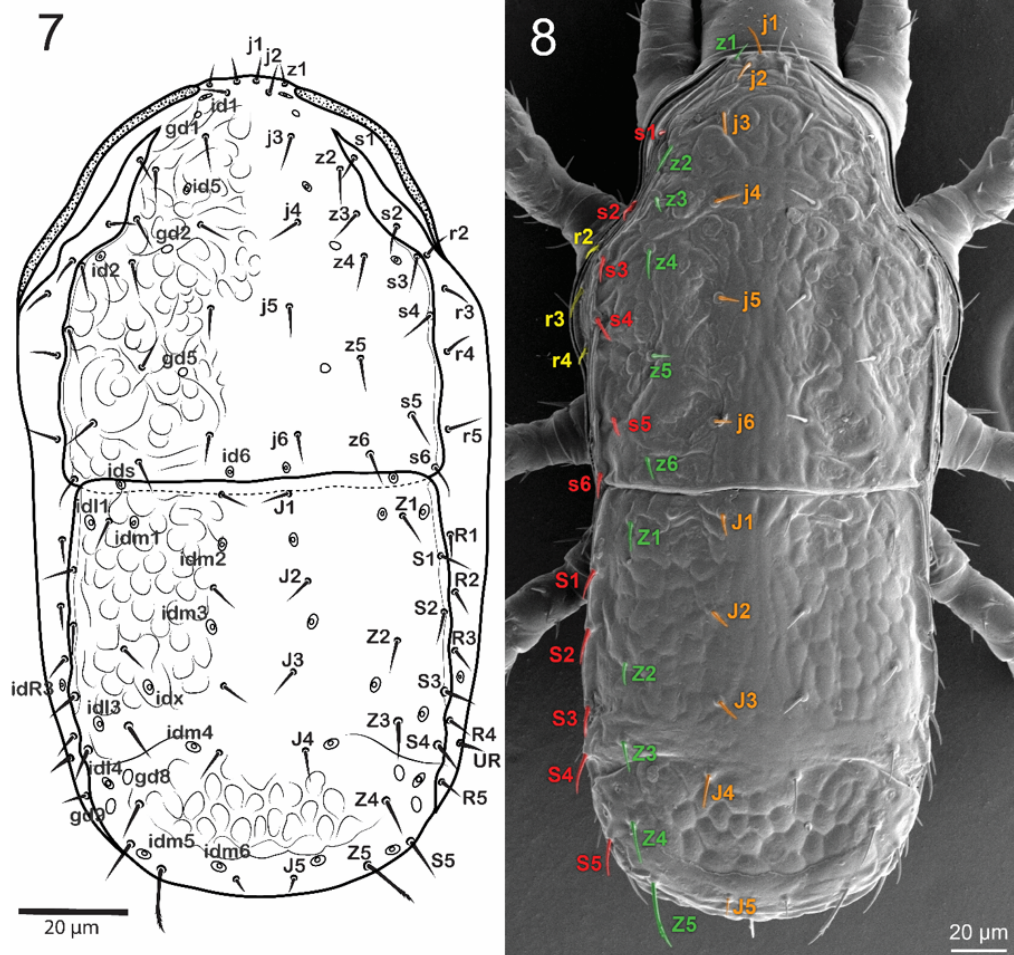
cuticle along lateral margins of podonotal shield with six pairs of setae (*s1*, *s2*, *r2–r5*); *r1* and *r6* absent. Opisthonotal shield with irregular colliculate ornamentation, less evident between lines of *J1–J4*; with a pair of curved lines behind *Z3* and *S4* extending from the shield margin to a point between *J4* and *Z3*; with a delineated marginal strip from anterior corner to *S3*; 156 (145–161) long and 146 (135–150) wide; with 15 pairs of setae (*J1–J5*, *Z1–Z5*, *S1–S5*), eleven pairs of distinguishable lyrifissures (*ids*, *idm1–idm6*, *idl1*, *idl3*, *idl4*, *idx*) and two pairs of distinguishable pores (*gd8*, *gd9*). Unsclerotized cuticle along lateral margins of opisthonotal shield with five pairs of setae (*R1–R5*); lyrifissure *idR3* distinct. On both shields, setae shorter than distance to respective closest neighboring setae.



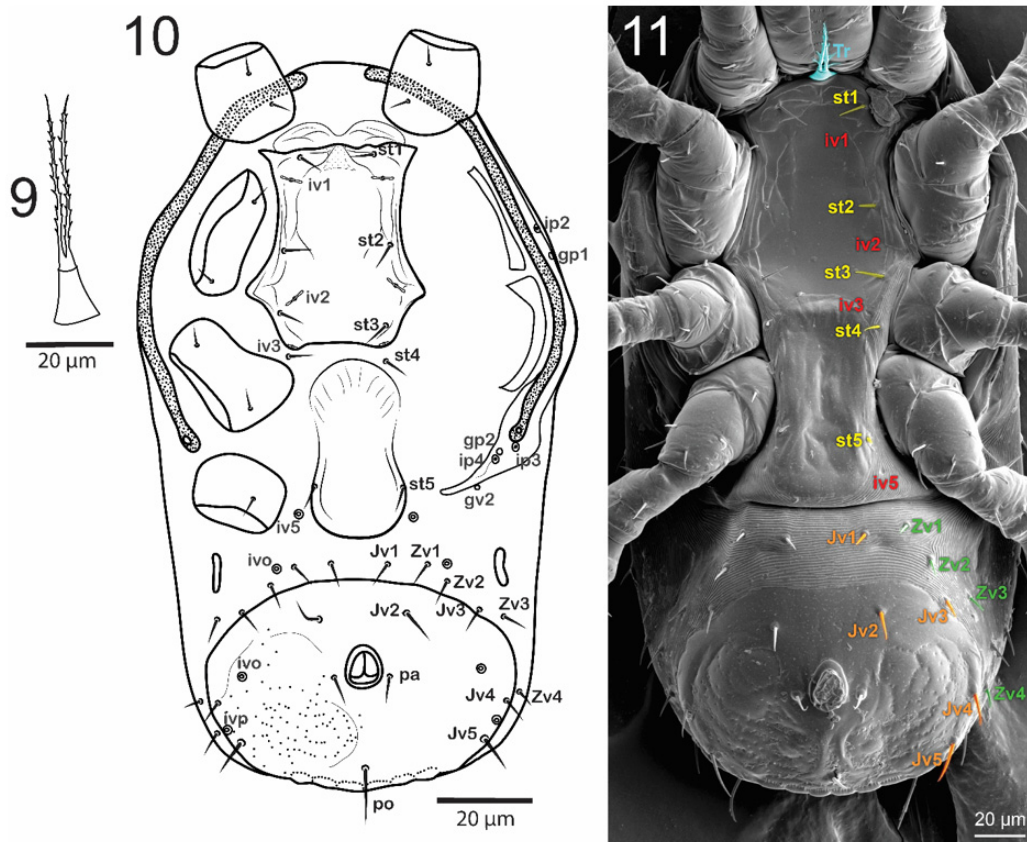
FIGURES 1–6. *Gamasellodes garybauchani* Rueda-Ramírez & Santos sp. nov., adult female. **1.** Drawing of antiaxial view of chelicera; **2.** LTSEM image of anterior part of ventral gnathosoma; **3.** LTSEM image of ventral view of gnathosoma; **4.** Drawing of ventral view of gnathosoma; **5.** Drawing of epistome; **6.** LTSEM image of dorsal view of gnathosoma. Ep = epistome, De = denticles, Ap = apotele, DG = deutosternal groove, Tr = tritosternum, FD = fixed digit, InM = internal malae, P = peritreme.

Ventral Idiosoma (Figures 9–11). Base of tritosternum 12 (12–13) long and 10 (9–10) wide proximally (Figure 9); laciniae 41 (40–44) long, separated for about 90 (82–95)% of their total length, pilose. Pre-sternal area weakly sclerotised, as two striate lobes (Figures 10; only visible in the mounted specimens). Sternal shield mostly smooth, with scant faint striae along lateral margins and with a variably shaped punctate central area next to anterior margin (only visible in the mounted specimens); with three pairs of setae (*st1–st3*), and three pairs of lyrifissures (*iv1–iv3*, sternal lyrifissure *iv1* posteriomersed to *st1*, *iv2* at the level of posterior margin of coxa II, and *iv3* marginal, at posterolateral protrusions); 86 (83–89) long and 54 (51–56) wide. Seta *st4* on unsclerotised cuticle. Section of endopodal plate between legs I–III fused with sternal shield and section between coxae

III–IV indistinct. Epigynial shield smooth, with round posterior margin, bearing *st5*; 83 (81–85) long and 39 (37–42) wide; distance *st5*–*st5* 35 (33–41). Lyrifissure *iv5* on unsclerotised cuticle, posterolaterad *st5*. Unsclerotised opisthogastric cuticle with six pairs of setae (*Jv1* and *Jv3*, *Zv1*–*Zv4*; *Zv3* of the left side on the ventrianal shield margin in one of the specimens) and a pair of elongate, longitudinal metapodal plates; with a pair of lyrifissures (*ivo*). Ventrianal shield transversally oval, smooth anteriorly and with coarse punctation and irregular folds of the cuticle posteriad anterior margin of anal opening; 92 (83–97) long and 133 (121–140) wide at level of *Jv4*; with three pairs of setae (*Jv2*, *Jv4* and *Jv5*) in addition to circumanals; anal opening small, about 15 (14–15) long; cribrum formed by a single transverse row of denticles that coincides approximately with the anterior margin of six transversely aligned narrow ellipsoidal structures, 60 (57–63) long. Exopodal plate (Figure 10) distinguishable as a pair of discrete, elongate and aligned fragments next to coxae II and III, and apparently a fragment fused with the peritrematic plate next to coxa IV. Pore *gv2* on unsclerotized cuticle. Measurements of setae: *st1* 13 (12–13), *st2* 13 (13–15), *st3* 13 (12–14), *st4* 11 (10–12), *st5* 11 (10–12), *Jv1* 12 (10–13), *Jv2* 16 (16–17), *Jv3* 10 (8–11), *Jv4* 13 (11–14), *Jv5* 19 (17–19), *Zv1* 10 (10–11), *Zv2* 10 (9–11), *Zv3* 7 (7–8), *Zv4* 10 (9–11), para-anal 15 (14–15), post-anal 26 (25–28); all setae aciculate and smooth.



FIGURES 7–8. *Gamasellodes garybauchani* Rueda-Ramírez & Santos sp. nov., adult female. 7. Drawing of dorsal idiosoma; 8. LTSEM image of dorsal idiosoma.



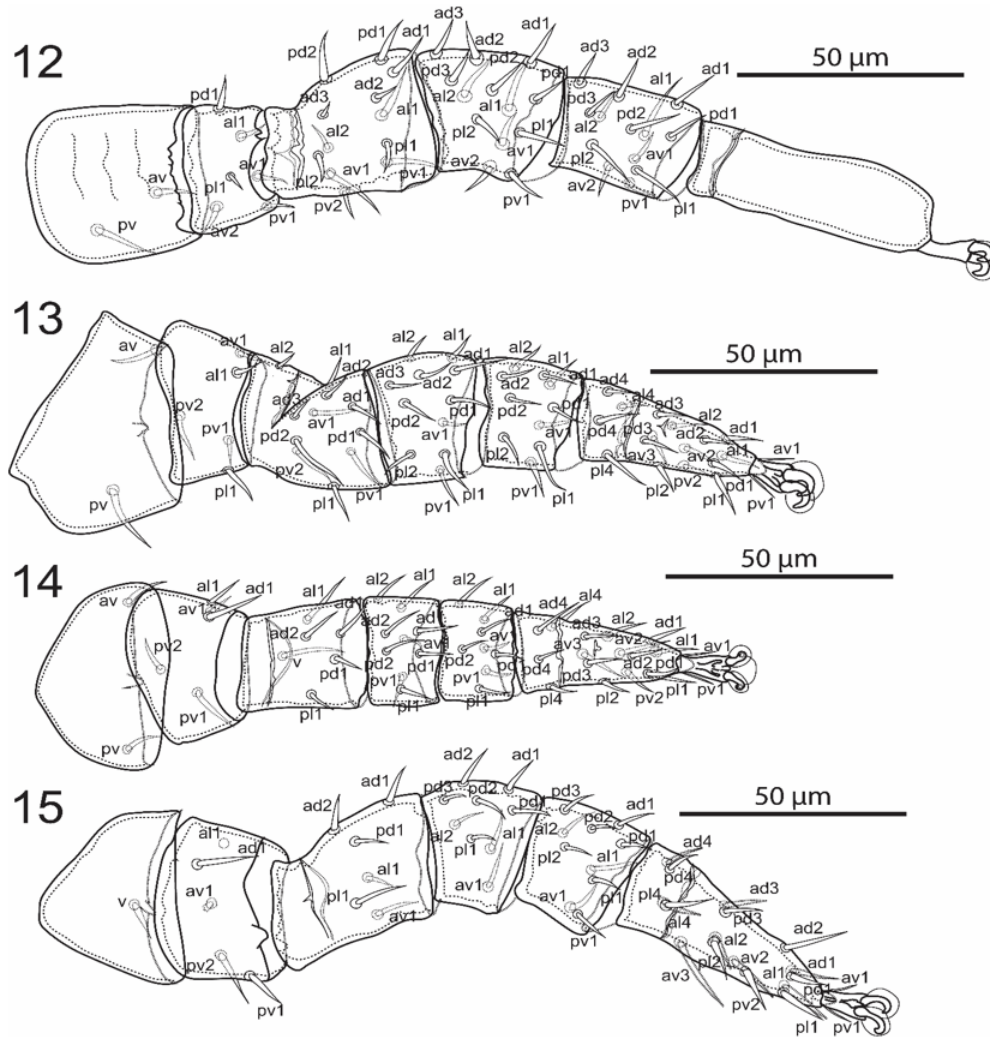
FIGURES 9–11. *Gamasellodes garybauchani* Rueda-Ramírez & Santos sp. nov., adult female. **9.** Drawing of Tritosternum; **10.** Drawing of ventral idiosoma; **11.** LTSEM image of ventral idiosoma. Tr = tritosternum.

Peritreme and peritrematic plate (Figures 10). Peritreme extending anteriorly almost to level of $z1$. Peritrematic plate fused anteriorly with dorsal shield at level of $s1$ and posteriorly with the posteriormost fragment of the exopodal plate next to coxa IV; on each side, with one lyrifissure ($ip2$) and one pore ($gp1$) between coxae II and III (both dorsad peritreme) and with two lyrifissures ($ip3$ and $ip4$) and one pore ($gp2$) in poststigmatic region of the plate.

Legs (Figures 12–15). Lengths of legs I–IV: 220 (210–228), 173 (157–185), 152 (142–159) and 190 (175–204), respectively. Setation (legs I–IV): coxae: I: 0, 0/1, 0/1, 0; II: 0, 0/1, 0/1, 0; III: 0, 0/1, 0/1, 0; IV: 0, 0/1, 0; trochanters: I: 1, 0/2, 1/1, 1; II: 1, 0/1, 0/2, 1; III: 1, 1/1, 0/2, 0; IV: 1, 1/1, 0/2, 0; femora: I: 2, 3/1, 2/2, 2; II: 2, 3/1, 2/2, 1; III: 1, 2/1, 1/0, 1; IV: 1, 2/1, 1/0, 1; genua: I: 2, 3/2, 3/1, 2; II: 2, 3/1, 2/1, 2; III: 2, 2/1, 2/1, 1; IV: 2, 2/1, 3/0, 1; tibia: I: 2, 3/2, 3/1, 2; II: 2, 2/1, 2/1, 2; III: 2, 1/1, 2/1, 1; IV: 2, 1/1, 3/1, 2; tarsi: I: not counted; II: 18; III: 18; IV: 18. All legs with pretarsi containing a pair of strongly sclerotized claws; median section of pulvillus of legs I–IV rounded.

Gnathosoma. Chelicera (16–18), with fringed coronet-like arthrodial process, as in adult female; basal segment of the chelicera 22–23; section of second segment of the chelicera behind dorsal lyrifissure 34–35; fixed chelicera digit 15, with three teeth and two basal protrusions being a small protrusion followed by a big one, with a pore-like structure in the tip; dorsal and antiaxial lyrifissures distinct, dorsal seta and *pilus dentilis* indistinct; movable cheliceral digit 17 long, with one tooth and with spermatodactyl, consisting of a grooved elongate process, distally curved and tapered, 22–23 long (Figure 16, 17). Number of palpal setae as in adult female; all aciculate and

smooth, except *al* of femur and *al1* and *al2* of genu, stout and spatulated; palptrochanter with outer seta (*pv*) 7–8 shorter than inner seta (*av*) 13–15; apotele 2-tined. Anterior region of the epistome tripartite, each prong rarely bifurcate, as in adult female (Figure 19). Internal malae totally separated from each other, each triangular, fringed proximoventrally, extending to the level of corniculus tip (Figure 17, 18). Deutosternal groove, corniculi (13–15 long and 6–7 wide) and subcapitular setae similar to adult female (Figure 18). Measurements of subcapitular setae: *h1* 11–12, *h2* 6–7, *h3* 9–10, *pc* 10; all setae aciculate and smooth.



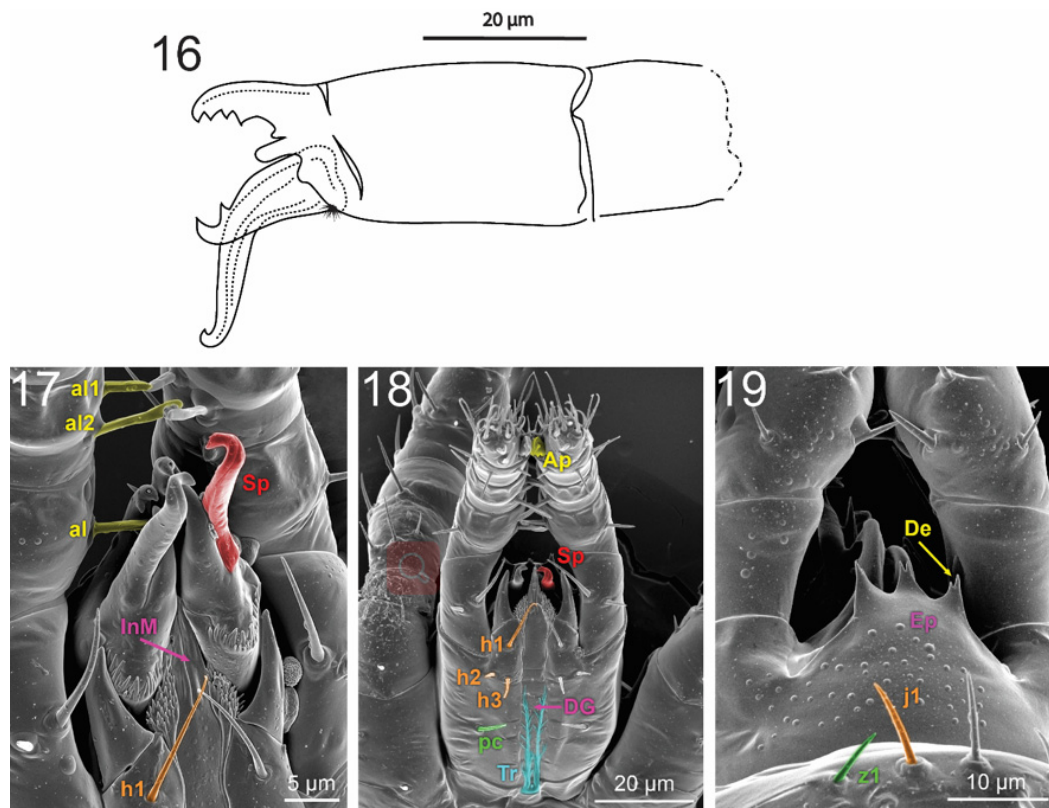
FIGURES 12–15. *Gamasellodes garybauchani* Rueda-Ramírez & Santos sp. nov., adult female. **12.** Leg I, coxa-tibia, posterolateral view (setae on tarsus I not illustrated); **13.** Leg II, posterolateral view; **14.** Leg III, dorsal view; **15.** Leg IV, posterolateral view.

Adult male (Figures 16–23, *n*=2)

Idiosoma. Length 245–251, width 139–140.

Dorsal idiosoma (Figures 20–21). Podonotal with irregular colliculate ornamentation, less evident between both lines of *j* setae; with delineated marginal strip posteriad *s2*, as in female (Figures 20, 21); 135–137 long and 118–119 wide; with 16 pairs of setae (*j1–j6*, *z1–z6*, *s3–s6*; *s6* from one side of one of the specimens on unsclerotized cuticle, Figure 21), five pairs of

distinguishable lyrifissures (*id1*, *id2*, *id4*, *id5*, *id6*) and three pairs of pores (*gd1*, *gd2*, *gd5*). Unsclerotised cuticle along lateral margins of podonotal shield with six pairs of setae (*s1*, *s2*, *r2*–*r5*); *r1* and *r6* absent. Opisthonotal shield with ornamentation similar to adult female including delineated marginal strip; 109–113 long and 113–116 wide; with setae, lyrifissures and pores similar to adult female. Unsclerotised cuticle along lateral margins also similar to adult female but in R-series three setae (*R1*–*3*). On both shields, setae shorter than distance to respective closest neighboring setae.



FIGURES 16–19: *Gamaselloses garybauchani* Rueda-Ramírez & Santos sp. nov., adult male. **16.** Drawing of paraxial view of chelicera; **17.** LTSEM image of anterior part of ventral gnathosoma; **18.** LTSEM image of ventral view of gnathosoma; **19.** LTSEM image of dorsal view of gnathosoma. Sp = spermatodactyl, InM = internal malae, Ap = apotele, DG = deutosternal groove, Tr = tritosternum, Ep = epistome, De = denticles.

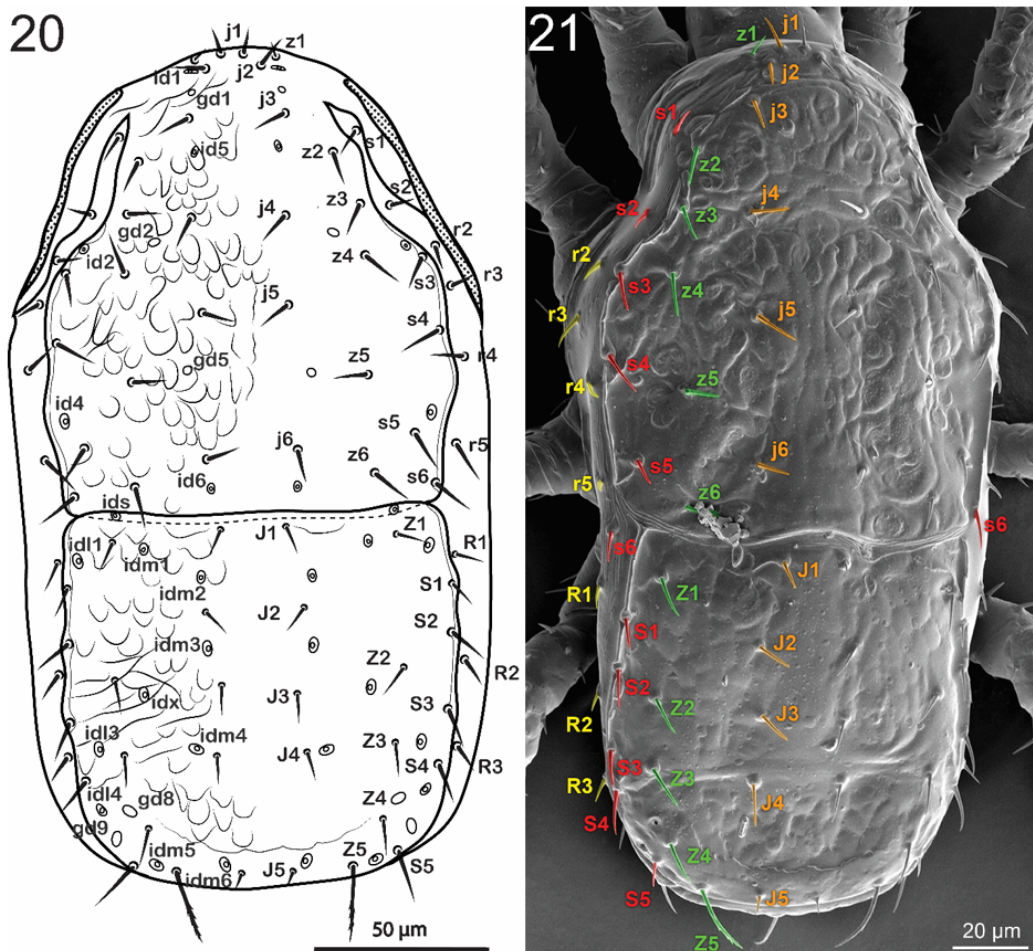
Measurements of setae: *j1* 8–9, *j2* 10–11, *j3* 11–12, *j4* 10–11, *j5* 10, *j6* 10, *z1* 6–7, *z2* 10–11, *z3* 10, *z4* 11–13, *z5* 10, *z6* 8–10, *s1* 8, *s2* 8–9, *s3* 10–12, *s4* 12–13, *s5* 11, *s6* 11, *r2* 8–9, *r3* 10, *r4* 9, *r5* 8, *J1* 9, *J2* 9–10, *J3* 9–10, *J4* 10–11, *J5* 4, *Z1* 11–12, *Z2* 11, *Z3* 11–13, *Z4* 10–12, *Z5* 21–23, *S1* 9–10, *S2* 9–11, *S3* 11–12, *S4* 11–13, *S5* 10, *R1* 8, *R2* 8, *R3* 8; all setae aciculate, except *Z5*, stouter, distally barbed.

Ventral Idiosoma (Figures 22–23). Base of tritosternum 8–9 long and 8 wide proximally; laciniae 29–31 long, separated for about 87–89% of their total length, pilose. Pre-sternal area similar to adult female. Sternogenital shield mostly smooth, with scant faint striae along lateral margins; 115–120 long and 44–47 wide; with five pairs of setae (*st1*–*st5*) and three pairs of lyrifissures (*iv1*–*iv3*, sternal lyrifissures *iv1* posteriolaterad to *st1*, *iv2* anteriolaterad to *st3*, and *iv3* anteriorad to *st4*). Anterior section of endopodal plate fused with sternogenital shield; fragment of endopodal plate between coxae III–IV indistinguishable. Lyrifissure *iv5* on unsclerotized cuticle, posterolaterad *st5*; *st5*–*st5* 21.

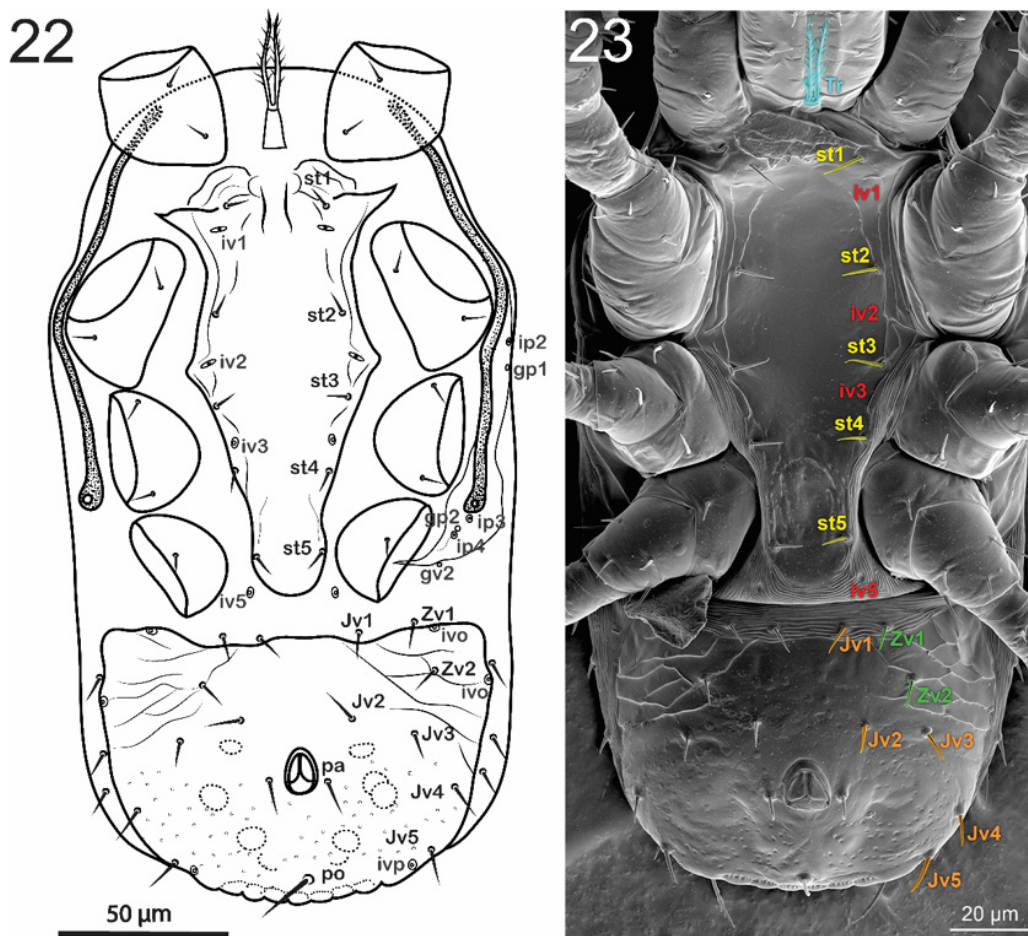
Unscerotised opisthogastric cuticle without distinguishable lyrifissures; with two pairs of setae (*Jv1* and *Zv1*). Ventrianal shield hemispheric, with anterolateral corners reticulate, region between *Jv1*, *Jv2* and *Zv2* smooth, and posterior half punctate; 81–82 long and 114–120 wide at level of *Zv2*; with five pairs of setae (*Jv2*–*Jv5*, *Zv2*), in addition to circumanals; with three pairs of lyrifissures (*ivo*, *ivp*); anal opening small, 12–14 long, located about centrally in the shield; cribrum formed by a transverse row of denticles, 45 long, that coincides approximately with the anterior margin of six transversely aligned narrow ellipsoidal structures. Metapodal plates incorporated to ventrianal shield. Pore *gv2* on unsclerotised cuticle. Measurements of setae: *st1* 10, *st2* 8–9, *st3* 8, *st4* 7, *st5* 7–8, *Jv1* 8–9, *Jv2* 11, *Jv3* 7, *Jv4* 8, *Jv5* 12, *Zv1* 6–7, *Zv2* 7, para-anal 9–11, post-anal 19–21; all setae aciculate and smooth.

Peritreme and peritrematic plate (Figures 22). Peritreme extending anteriorly to level between setae *z1* and *s1*. Peritrematic plate fused anteriorly with dorsal shield at level between *z1* and *s1* and apparently posteriorly with the posteriormost fragment of the exopodal plate next to coxa IV; lyrifissures and pores as in adult female.

Legs. Lengths of legs I–IV: 185–189, 152–156, 129–131 and 161–165, respectively. Setation, similar in number and form to adult female, no dimorphism evident.



FIGURES 20–21. *Gamasellodes garybauchani* Rueda-Ramírez & Santos sp. nov., adult male. **20.** Drawing of dorsal idiosoma; **21.** LTSEM image of dorsal idiosoma.



FIGURES 22–23. *Gamasellodes garybauchani* Rueda-Ramírez & Santos sp. nov., adult male. 22. Drawing of ventral idiosoma; 23. LTSEM image of ventral idiosoma. Tr = tritosternum.

Immatures. Not found.

Remarks. The *Gamasellodes* species with only three pairs of setae on ventrianal shield in addition to circumanals are *G. claudiae* Walter, 2003 (Walter 2003), *G. hildae* Jordaan, 1988 and *G. lentiformis* Tseng, 1989. *Gamasellodes claudiae* differs from the new species described here mainly by having a short peritreme (extending anteriorly to level of posterior margin of coxa II according to our observations of the holotype), the scanty ornamentation of the podonotal and opisthonotal shields, and the female with smaller and narrower ventrianal shield (88–100 x 89–105 wide), and male with 13–14 setae in the ventrianal shield. *Gamasellodes hildae* differs from *G. garybauchani* sp. nov. mainly by having a short peritreme (extending anteriorly to level of posterior margin of coxa II) and seta z1 absent. *Gamasellodes lentiformis* differs from the new species mainly by having two pairs of *R* setae on the unsclerotised cuticle along lateral margins of opisthonotal shield, setae Z5 aciculate and unsclerotised cuticle along lateral margins of ventrianal shield without setae.

Type specimens. A female holotype (USNMENT01021807 [PASMB102–19]) and five females paratypes (USNMENT01021811 [PASMB106–19], USNMENT01021812 [PASMB107–19], USNMENT01021891 [PASMB186–19]) and two males paratypes (USNMENT01021878 [PASMB173–19], USNMENT01021815 [PASMB110–19]) from soil of the Beltsville Agricultural

Research Center (BARC), Maryland, USA (all with barcode compliant sequences and five of them also with LTSEM images), deposited at the United States National Mite Collection (USNM), USDA-ARS, Beltsville, Agricultural Research Centre, Beltsville, Maryland, USA.

All specimen and sequence data are available on BOLD in the dataset DS-GGSN (doi: 10.5883/DS-GGSN) and on GenBank (Table 2).

TABLE 2. Summary of collection, specimen, and DNA barcode data for the 19 specimens of *Gamasellodes garybauchani* sp. nov. collected from the ARS Farming Systems Project at the Beltsville Agricultural Research Center (BARC) in Maryland, USA.

Specimen ID	Sequence Length	Identification Method	Voucher Status †	GenBank Accession
USNMENT 01021759	658	Morphological	Vouchered at USNM	OK073021
USNMENT 01021762 ⁺	658	Morphological	Vouchered at USNM	OK072935
USNMENT 01021763 ⁺	658	BIN Match	DNA Vouchered at CBG	OK073012
USNMENT 01021764 ⁺	658	Morphological	Vouchered at USNM	OK072893
USNMENT 01021766 ⁺	658	BIN Match	DNA Vouchered at CBG	OK072944
USNMENT 01021767 ⁺	658	Morphological	Vouchered at USNM	OK072983
USNMENT 01021806	658	BIN Match	DNA Vouchered at CBG	OK072895
USNMENT 01021807**	658	Morphological	Vouchered at USNM	OK073005
USNMENT 01021808	658	BIN Match	DNA Vouchered at CBG	OK072962
USNMENT 01021811*	658	Morphological	Vouchered at USNM	OK072930
USNMENT 01021812*	650	Morphological	Vouchered at USNM	OK073026
USNMENT 01021815*	658	Morphological	Vouchered at USNM	OK072903
USNMENT 01021850*	658	Morphological	Vouchered at USNM	OK073062
USNMENT 01021876	648	BIN Match	DNA Vouchered at CBG	OK072908
USNMENT 01021877	658	BIN Match	DNA Vouchered at CBG	OK072949
USNMENT 01021878*	658	Morphological	Vouchered at USNM	OK073044
USNMENT 01021884*	658	Morphological	Vouchered at USNM	OK072968
USNMENT 01021891*	658	Morphological	Vouchered at USNM	OK073009
USNMENT 01021893	658	Morphological	Vouchered at USNM	OK072967

**Holotype *Paratype

† Indicates whether a physical specimen voucher exists, or if the records is represented by a DNA voucher only. All physical specimens are also linked to a DNA voucher archived at the Centre for Biodiversity Genomics (CBG).

⁺Specimens with LTSEM images before DNA extraction.

Etymology. The specific name is given after Gary Bauchan. This species is in honor of Dr. Gary R. Bauchan for his support and dedication to the study of mites, particularly to the importance of mites in soil ecology through his spectacular LTSEM images.

DNA barcodes. DNA barcodes were recovered from 21 specimens including the holotype, five female paratypes, and two male paratypes. Five specimens were lost during molecular analysis but were assigned to this species by their membership in the same BIN (BOLD:ADZ7472, dx.doi.org/10.5883/BOLD:ADZ7472). The sequences ranged in length from 648–658 bp and possessed an average intraspecific p-distance of 0.40% (range: 0–1.40%). Comparison of these sequences to the BOLD reference library and to GenBank indicate that these are the first public sequence records for this species. The closest available match is a *Gamasellodes* species tentatively identified as JCS07 with 14.2% divergence (p-distance), and the Neighbour-Joining analysis demonstrates that the

nearest neighbour of most *Gamasellodes* are congenics (Figure 24). In fact, most Ascid genera with barcode coverage for two or more species form monophyletic clades in the tree, but some cases of non-monophyly persist. The low intraspecific divergences within *G. garybauchani* sp. nov., and large intraspecific divergences among *Gamasellodes* species suggest that DNA barcodes effectively delineate *Gamasellodes* species. Additional sampling will refine knowledge of interspecific divergences and improve evolutionary trees. However, additional markers may be needed to resolve deeper branches.

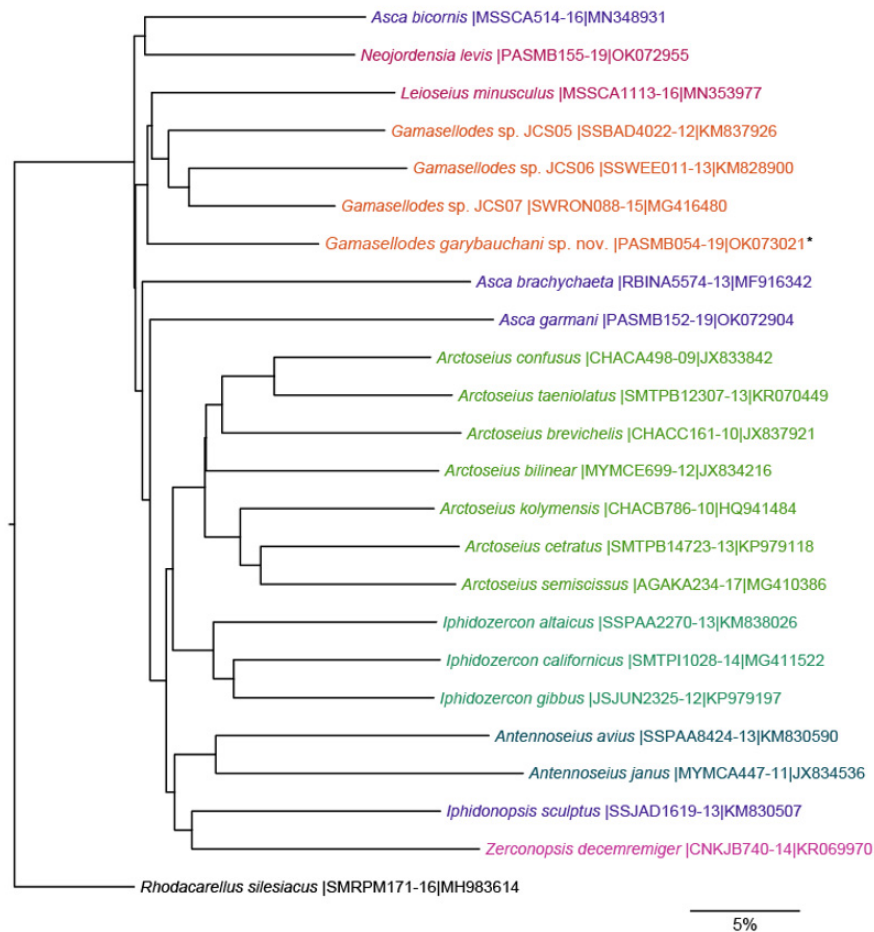


FIGURE 24. COI Neighbour-Joining tree for representatives of publicly available Ascid species including *Gamasellodes garybauchani* Rueda-Ramírez & Santos sp. nov. (indicated by an asterisk) and a Rhodacaridae (*Rhodacarellus silesiacus*) as an outgroup. Branch labels are colorized by genus and include each specimen's ProcessID (BOLD) and Accession number (GenBank).

A complement to the key to *Gamasellodes* species recently published by Castro et al. (2020) and modified by Mesa et al. (2021).

- [Couplets 1–3 unaltered]
- 4. Ventrianal shield with three pairs of setae in addition to circumanal setae5
 - Ventrianal shield with four pairs of setae in addition to circumanal setae.....8

5. Unsclerotised cuticle along lateral margins of opisthonotal shield with two pairs of R setae; unsclerotised cuticle of the opisthogaster with three pairs of setae (*Jv1*, *Zv1* and *Zv2*) *G. lentiformis* Tseng, 1989; Taiwan
 - Unsclerotised cuticle along lateral margins of opisthonotal shield with 6–7 pairs of *R/UR* setae; unsclerotised cuticle of the opisthogaster with 5–6 pairs of setae (*Jv1*, *Jv3* and *Zv1–Zv3*; *Zv4* present or absent) 6
 6. Anterior region of epistome with three distally denticulate projections; seta *z1* present. 6a
 - Anterior region of epistome with three pointed and smooth projections; seta *z1* absent *G. hildae* Jordaan, 1988; South Africa
 - 6a. Peritreme short, extending anteriorly slightly posteriad the level of *r3* *G. claudiae* Walter, 2003; USA
 - Peritreme extending anteriorly almost reaching the level of *z1* *G. garybauchani* Rueda-Ramírez & Santos sp. nov.; USA
- [Couplets 7–27 unaltered]

Discussion

In the present study *Gamasellodes garybauchani* sp. nov. Rueda-Ramírez & Santos is described utilizing an integrated taxonomy approach, using light and LTSEM microscopy as well as DNA barcoding. This species was one of the three most abundant mites extracted following the incubation with organic amendments (please refer to Rueda-Ramírez *et al.* (2022, this special issue) of this same special issue), in conjunction with a marked increase in bacteriovorous nematodes (Rueda-Ramírez *et al.* 2022, this special issue). Interestingly, *G. garybauchani* was not discovered in the pre-incubation freshly sampled material, suggesting the beneficial impact of bacterivorous nematodes as prey on the population growth of this predator. This is supported by previous studies where *G. vermivorax* was found to develop and oviposit when fed several species of free-living nematodes (Walter 1987; Walter *et al.* 1987; Walter & Ikonen 1989).

Features such as ornamentation and the pore-like structure at the tip of the cheliceral digits were better preserved and more pronounced in the LTSEM images. However, lyrifissures and structures under the sclerotized cuticle including the punctate region in the anterior medial region of the sternal shield needed to be observed with slides using light microscopy. Similarly, previous studies have demonstrated the advantages of observing such features in LTSEM images (Baker 1995; Oldfield *et al.* 1972; Otto 1999; Witalinski 1980; Witalinski & Borsuk 2002).

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