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## First record of the mite genus *Denheynaxoides* Smiley (Acari: Bdelloidea: Cunaxidae) for North America and a redescription of *D. americanus* Rocha *et al.*

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### Abstract

Here we present the first records of *Denheynaxoides* from North America: *D. americanus*, collected from coniferous litter, soil, and moss in Nova Scotia, Canada. These new records significantly expand the known distribution of the species. *Denheynaxoides americanus* is redescribed to include Canadian specimens and to rectify discrepancies in the species' description identified during examination of the holotype. The deutonymph of *D. americanus* is also described. Sequences, representing a 658 bp region of the mitochondrial cytochrome *c* oxidase subunit I (COI), were obtained from representative specimens collected in Canada. Comparison of these sequences with those from the Barcode of Life database (BOLD) suggests that a second species of *Denheynaxoides* may occur in British Columbia, Canada. A revised key to world species of *Denheynaxoides* is provided.

**Key words:** Canada, Cunaxidae, Brazil, *Denheynaxoides*, Nova Scotia

### Introduction

The predatory family Cunaxidae (Acari: Bdelloidea) is cosmopolitan, occurring in most terrestrial habitats and from a variety of substrates including soil, litter, moss, lichen and plants. In North America, knowledge of the species and genera that occur is scattered (Den Heyer 2011; Skvarla *et al.* 2014), but some efforts to document and describe cunaxids, particularly in the eastern United States have recently been undertaken (e.g., Skvarla *et al.* 2011; Skvarla and Dowling 2012).

The genus *Denheynaxoides* was erected by Smiley (1992) for specimens with smooth prodorsal sensilla (*at* and *pt*), setae *f*<sub>2</sub> and *h*<sub>2</sub> present, and trichobothria on tibia IV absent. Initially, the genus was included as a separate subfamily in Cunaxidae by Smiley (1992); however, Den Heyer and Castro (2009) instead included *Denheynaxoides* in the tribe Cunaxoidini within the subfamily Cunaxoidinae, as they considered the characters given by Smiley (1992) to be “only generic characteristics”. Sergeyenko (2011) later included *Denheynaxoides* in its own tribe within Cunaxoidinae, Denheynaxoidini, based on a suite of derived characters. This placement is supported by molecular data (Schwarzfeld and Nowell, unpub. data) and followed here.

Three species are presently included in the genus *Denheynaxoides*: *D. brevirostris* (Canestrini 1885), described from trees in Italy and recorded from moss on *Quercus pubescens* (Fagaceae) in Ukraine (Sergeyenko 2011) and the bark of various tree species in China (Lin 2001); *D. martini* Smiley 1992, described from an unknown host from New Zealand; and *D. americanus* Rocha *et al.* 2016, described from lichens and moss on the bark of *Ilex paraguariensis* (Aquifoliaceae) in Brazil.

In August 2017, specimens of *D. americanus* were collected from coniferous litter, soil, and moss in Nova Scotia, Canada. These specimens significantly expand the known distribution of the species and represent the first from the genus collected from edaphic habitats. Here we redescribe *D. americanus* to include Canadian specimens and molecular data, and to rectify discrepancies in the species' description identified during examination of the holotype. The deutonymph of *D. americanus* is also described.

## Material and methods

### *Sample collection*

Mites were extracted from coniferous litter, soil, and moss collected at Five Bridge Lakes Wilderness Area, Halifax Regional Municipality, Nova Scotia, Canada using Berlese funnels and stored in 95% ethanol at -4°C prior to sorting. Cunaxid mites were subsequently removed and stored in 95% ethanol at -80°C for later molecular and/or morphological analysis. Despite a notable attempt by M. Rocha (pers. comm.) to collect fresh specimens from the type locality of *D. americanus* in Brazil for inclusion in molecular analyses, none were obtained.

### *DNA extraction, amplification and sequencing*

Genomic DNA from 10 whole specimens was extracted using a DNeasy Blood & Tissue Kit (QIAGEN, Inc., Santa Clara, California, USA) following the manufacturer's protocol with some modification; (1) mite exoskeletons were recovered following the lysis step to permit morphological analysis; (2) following the second wash step, DNA was eluted with 100µL ddH<sub>2</sub>O, pH = 8.75 (Ricca Chemical Company, Maryland, USA), incubated for 5 minutes at room temperature (~20°C), and centrifuged for 1 minute at ≥6000 xg (8000 rpm). To increase DNA yield, this step was repeated once for a total of two elution steps. Eluted DNA was subsequently concentrated in a Vacufuge plus (Eppendorf, Hamburg, Germany) set to V-AQ (vacuum - aqueous) at 60°C for approximately 1 hour. DNA was resuspended in 20µL Buffer AE (Qiagen) and stored at -20°C.

The 5'-end of the COI region was amplified using the primer pair LCO1490 and HCO2198 (Folmer *et al.* 1994) in 25µL reaction volumes containing 12.5µL 2X Multiplex PCR Master Mix (QIAGEN, Inc.), 0.5µL (10 µM) each primer, 8µL of ddH<sub>2</sub>O and 4µL of template DNA. PCR amplification was performed in a Mastercycler Pro Thermal Cycler (Eppendorf), using the following protocol: initial denaturation cycle at 95°C for 15 min, followed by 40 cycles of 94°C for 30 sec, 45°C for 90 sec, 72°C for 90 sec, and a final extension at 72°C for 10 min. Products were visualized on a 1% agarose gel stained with ethidium bromide (Fisher Reagents, New Jersey, USA) to confirm the presence and size of amplification products and the absence of contamination.

PCR products were purified using Exo-Sap IT (Applied Biosystems, California, USA), following the manufacturer's protocol. Sequencing reactions were performed in 7.5µL reaction volumes with 0.75µL BigDye (Applied Biosystems), 1.5µL 5X BigDye Buffer (Applied Biosystems), 4.75µL ddH<sub>2</sub>O, 0.5µL primer and 2.5µL purified PCR product in a Mastercycler Pro Thermal Cycler with the following protocol: incubation at 96°C for 3 min, followed by 36 cycles of 95°C for 30 sec, 45°C for 15 sec, 60°C for 2 min. Sequencing was performed at the Ottawa Research and Development Centre Sequencing Facility, Agriculture and Agri-Food Canada (Ottawa, Ontario, Canada). Samples were precipitated with ethanol and re-suspended in Hi-Di formamide (LifeTechnologies, California, USA) and sequenced on an ABI 3500xl Genetic Analyzer (Applied Biosystems). Sequences were assembled, edited and aligned in Geneious (v. 11.0.3) and submitted to GenBank (MK462176-MK462185). These were compared with publicly available COI sequences

in the Barcode of Life Database (BOLD; [www.boldsystems.org](http://www.boldsystems.org)) and genetic distances were calculated in Mega X (v. 10.0.5) using the Jukes-Cantor model.

#### *Morphological analysis*

Specimens, both DNA extracted and non-extracted, were slide mounted in polyvinyl acetate (PVA; BioQuip, California, USA) on glass slides, cured for 3–5 days in an oven (40°C), and studied using a Leica DM6 B compound microscope equipped with differential interference contrast (DIC). Prior studies indicate that morphometrics do not differ significantly between DNA-extracted specimens and those that have not undergone extraction (Knee *et al.* 2012). All Canadian specimens are deposited in the Canadian National Collection of Insects, Arachnids and Nematodes (CNC) in Ottawa, Ontario, Canada. The holotype of *D. americanus* was borrowed from the University of São Paulo (ESALQ/USP) for comparison.

Illustrations were created using the techniques described in Fisher and Dowling (2010) with Corel Draw X7 and a Cintiq 13HD Tablet. Digital images were taken with a 5 mpx camera (DMC 4500) mounted on a Leica DM6 B microscope and stacked using the PMax option in Zerene Stacker V1.04. All illustrations and images are based on Canadian specimens.

Setal nomenclature follows that of Grandjean (1939, 1946) for hysterosomal setae and Fisher *et al.* (2011) for proterosomal setae, as in Skvarla *et al.* (2014). The following abbreviations are used: attenuate solenidion (*asl*); blunt rod-like solenidion (*bsl*); terminal solenidion (*tsl*); famulus (*fam*) (= peg organ) macroseta (*ms*); simple tactile seta (*sts*); peg-like epicoxal seta (*peg*). For leg chaetotaxy, counts are listed by leg segment, with the count for legs I–IV separated by “—”. For legs III and IV, the setal counts for basifemur and telofemur are separated by “/”, in that order.

All measurements are in micrometres and were taken as follows: idiosomal length and width were measured at the longest and widest parts, from the base of the hypognathum to the posterior end of the idiosoma, and anterior to legs III; leg length was measured along the dorsal surface of each leg, from the base of the trochanter to the apex of the tarsus (excluding the pretarsus); setal lengths were measured from the alveolus to the apex of the seta (note that for *pt*, *mps*, *f<sub>p</sub>*, *h<sub>1</sub>*, and long palpal setae, setae are hardly discernible at the apex, making measurements variable); distances between setae were measured from the centres of the alveoli; the length and width of the prodorsal shield were measured at the longest and widest parts; the length and width of the subcapitulum were measured at the longest and widest parts (note, however, that measurements of the width of the subcapitulum are highly variable, depending on the pressure applied during the slide-mounting process); the length of the palp was measured in a straight line from the base of the trochanter to the tip of the claw; the length of the chelicera was measured in a straight line from the base of the basal segment to the tip of the claw; the length of the moveable cheliceral digit was measured in a straight line from the articulation with the middle article to the apex.

#### **Molecular results**

A 658 bp sequence of COI was obtained for all 10 specimens of *Denheyrnaxoides* selected for molecular work. Overall, less than 1% sequence divergence was identified between them. In an unpublished molecular phylogeny for Cunaxidae based on COI (Schwarzfeld & Nowell, unpub. data), two sequences from specimens collected in British Columbia, Canada were found to be sister to those from Nova Scotia, with 88% similarity (Barcode Index Number (BIN): ACI5628; Sample ID's: BIOUG19442-H11 and BIOUG07137-G05). This suggests the presence of a second species of *Denheyrnaxoides* in western Canada. Unfortunately, physical vouchers for these specimens were

destroyed during tissue lysis (M. Young, pers. comm.), but a photograph of BIOUG19442-H11 was examined and supports the hypothesis (available on BOLD).

## Systematics

**Order Trombiformes Reuter, 1909**

**Suborder Prostigmata Kramer, 1877**

**Infraorder Eupodina Krantz, 1978**

**Superfamily Bdelloidea Dugès, 1834**

**Family Cunaxidae Thor, 1902**

**Subfamily Cunaxoidinae Den Heyer, 1979**

**Tribe Denheyernaxoidini Smiley, 1992 sensu Sergeyenko, 2011**

**Genus *Denheyernaxoides* Smiley, 1992**

***Denheyernaxoides americanus* Rocha et al. 2016**

## Diagnosis

Adult females of *Denheyernaxoides americanus* are most similar to those of *D. brevisrostris* (Canestrini 1885) in that they possess 4–5 *sts* on femora I–II, a prodorsal shield is present, the gnathosoma is indented only slightly posteroventrally, and dorsal cuticular striations between setae  $c_1$ – $e_1$  are transverse. Conversely, adult females of *D. martini* Smiley, 1992 possess 2–2 *sts* on femora I–II, a prodorsal shield is absent, the gnathosoma is deeply notched posteroventrally, and dorsal cuticular striations between setae  $c_1$ – $e_1$  are longitudinal.

Adult females of *D. americanus* differ from those of *D. brevisrostris* in having one long seta (vs two) on the palp femurogenou, four setae (vs five) on genu I, and three setae (vs four) on genu II and III in both Chinese (Lin 2001) and Ukrainian (Sergeyenko 2011) specimens of *D. brevisrostris*. Those of *D. americanus* further differ from the Ukrainian specimens in having five setae (vs six) on the palp femurogenou and two pairs of pregenital setae (vs three).

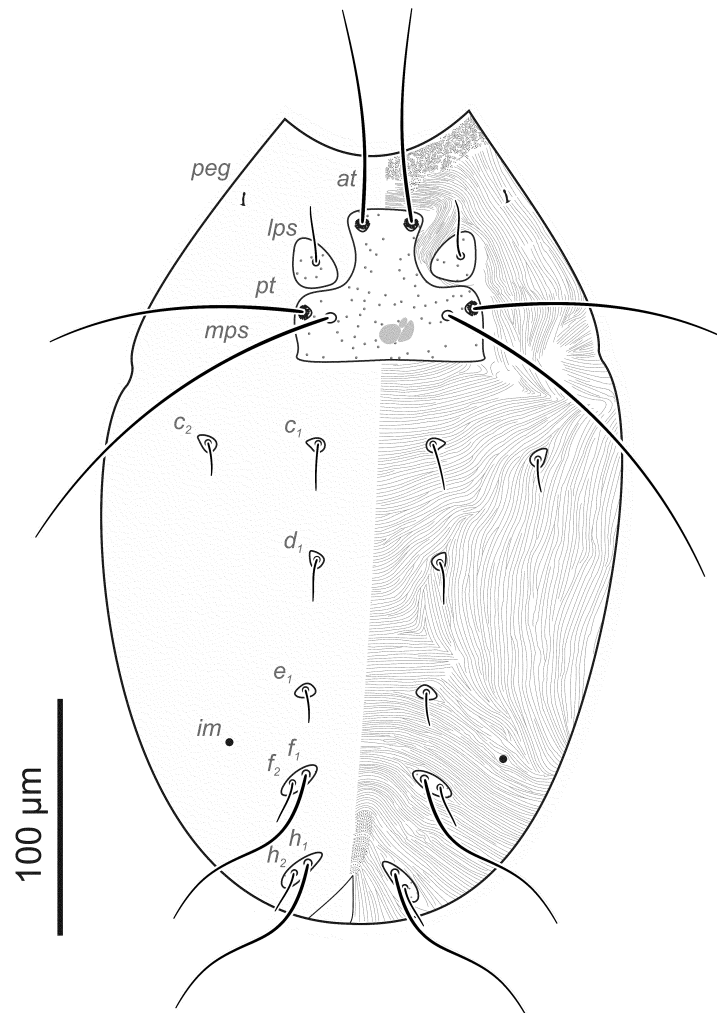
## Description

**Female (n = 15).** Idiosoma 305–389 long, 180–275 wide.

**Dorsum** (Fig. 1, 2). Prodorsal shield an inverted T-shape, 61–69 long, 78–89 wide, sparsely punctate with two pairs of smooth sensilla, *at* (82–94), *pt* (93–117), one pair of simple setae, *mps* (117–163), and a small, medial patch of unsclerotized reticula between setae *mps*. Setae *lps* (19–28) situated on small, subtriangular, smooth platelets narrowly separated from prodorsal shield by soft cuticle. Peg-like epicoxal setae (*peg*) visible on lateral margin of prodorsum. Setae  $c_1$  (15–21),  $c_2$  (14–22),  $d_1$  (12–18),  $e_1$  (10–19) on individual smooth platelets; setae  $f_1$  (85–109) and  $f_2$  (17–22), and  $h_1$  (95–116) and  $h_2$  (13–22) on common, sparsely punctate platelets. Cupule *im* present on cuticle, laterad of seta  $e_1$ . Soft cuticle with striations mostly transverse medially and more or less longitudinal laterad of setae  $c_1$ – $e_1$ ; striations smooth, except anterior of prodorsal shield and anal valves, where they are papillate. Distance between setae: *lps*–*lps* 61–71, *mps*–*mps* 51–60, *lps*–*at* 24–31, *mps*–*pt* 9–14, *lps*–*mps* 23–26. All setae simple.

**Venter** (Fig. 3). Coxisternal plates I–II and III–IV each partially connected by small apodemes, well separated medially; sparsely punctate, with subcuticular ridges near inner margins. Total of 10 pairs of setae anterior of two pairs of pregenital (*ag*) setae (including coxal, propodogastral and hysterogastral setae). Four pairs of genital setae ( $g_{1-4}$ ), short, subequal in length; two pairs of genital papillae, anterior pair larger. Anal region with one pair of anal ( $ps_1$ ) and one pair of paranal ( $ps_2$ )

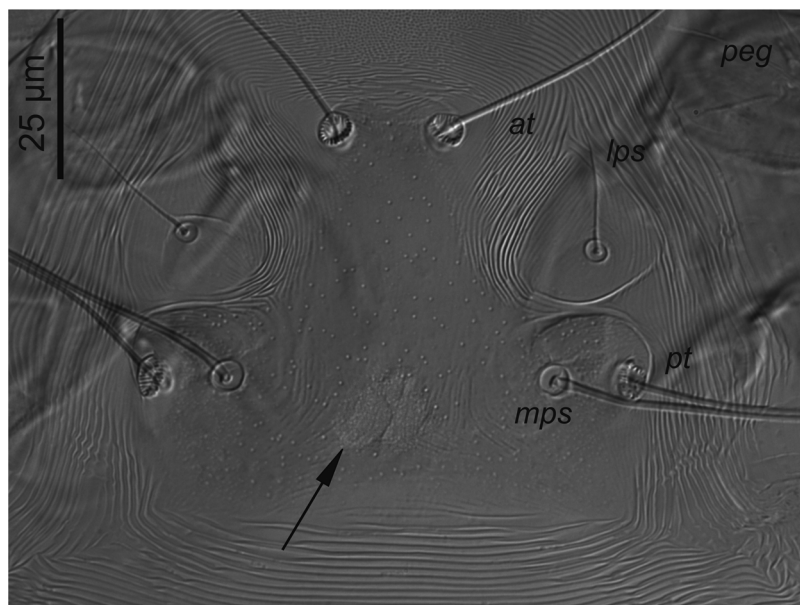
setae, subequal in length. Cupule *ih* on cuticle, laterad of paranal setae. Genital valves with transverse striae. Soft cuticle with mostly smooth striations, except for immediately behind gnathosoma and anterior margin of genital valves, where striations are papillate.



**FIGURE 1.** *Denheyrnaxoides americanus*, female. Dorsal idiosoma.

*Gnathosoma* (Figs. 4A–C). All components sparsely punctate. *Palp* (Fig. 4A) 51–59 long. Three-segmented. Chaetotaxy: trochanter—0; femurogenu—1 inner and 4 outer *sts* (of these, one long seta (87–120)); tibiotarsus—two lateral denticles at distal point and 5 *sts* (of these, one long seta (77–107)), ends in a claw. *Subcapitulum* (Fig. 4B) 87–99 long, 73–106 wide; four pairs of subcapitular setae (*hg*<sub>1</sub> (17–21), *hg*<sub>2</sub> (51–67), *hg*<sub>3</sub> (16–26), *hg*<sub>4</sub> (35–48)) and two pairs of adoral setae present; ventral base with subcuticular ridges. *Chelicera* (Fig 4C) 89–103 long; cheliceral claw 13–14 long; cheliceral seta present.

*Legs* (Fig. 5A–D). Lengths, leg I–IV: 151–165, 141–155, 175–192, 196–218. Chaetotaxy: **coxae I–IV**: 3 *sts*, 1 *peg*—1 *sts*—2 *sts*—2 *sts*; **trochanters I–IV**: 0—0—1 *sts*—0; **femora I–IV**: 4 *sts*—5 *sts*—1 *sts* / 2 *sts*, 1 *ms*—0 / 1 *sts*; **genua I–IV**: 4 *sts*, 4 *asl*—3 *sts*, 2 *asl*—3 *sts*, 2 *asl*—2 *sts*, 1 *ms*, 2 *asl*; **tibiae I–IV**: 4 *sts*, 1 *ms*, 2 *bsl*—4 *sts*, 1 *ms*, 1 *bsl*—4 *sts*, 1 *ms*, 1 *bsl*—3 *sts*, 1 *ms*; **tarsi I–IV**: 14 *sts*, 4 *bsl*, 2 *tsl*, *fam*—12 *sts*, 1 *bsl*, 2 *tsl*—12 *sts*—11 *sts*.



**FIGURE 2.** *Denheyrnaxoides americanus*, female. Prodorsal region, arrow indicates desclerotized reticulation.

**Deutonymph (n = 1).** Idiosoma 241 long, 191 wide.

Similar to adult female, but smaller, and with the following differences in chaetotaxy and structure of idiosoma, gnathosoma and legs: setae  $h_1$  and  $h_2$  on separate punctate platelets (Fig. 6A); femora III and IV only faintly divided, separated by a thin strip of tissue; only one pair of pregenital ( $ag$ ) setae; three pairs of genital setae and two pairs of genital papillae (Fig. 6B). Note that the deutonymph of *D. brevirostris* described by Sergeyenko (2011) may represent a protonymph as the specimen described possesses only one pair of genital setae and lacks a prodorsal shield, although two pairs of genital papillae appear to be illustrated.

**Larva, protonymph, tritonymph and male.** Unknown.

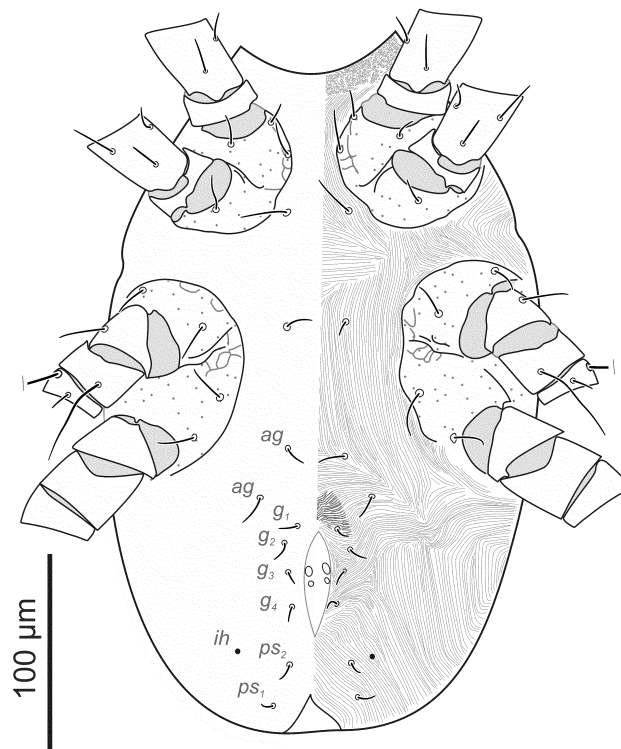
*Material examined*

**Type.** Holotype female, ex. lichens and moss associated with bark of *Ilex paraguariensis* (Aquifoliaceae) collected from yerba-mate agroecosystem, Putinga municipality, Rio Grande do Sul State, Brazil (-28.961722, -52.190417), 19.VIII.2015, coll.: M.S. Rocha *et al.*

**Other material.** 31 females (CNC866621–CNC866626, CNC896645–CNC896647, CNC896670–CNC896671, CNC917708–CNC917710, CNC953093–CNC953100, CNC958855–CNC958863) and one deutonymph (CNC953101), ex. coniferous litter collected from coniferous stand in mixed forest, 113 m, Five Bridge Lakes Wilderness Area, Nova Scotia, Canada (44.64822, -63.76243), 15.VIII.2017, coll.: V. Nowell and E. Sayadi. Three females (CNC915212, CNC915214, CNC953102), ex. moss and soil from boulder collected from mixed woods, 111 m, Five Bridge Lakes Wilderness Area, Nova Scotia, Canada (44.6588, -63.76712), 15.VIII.2017, coll.: V. Nowell and E. Sayadi. One female (CNC896673), ex. mixed litter and soil to 9 cm collected from mixed woods, 111 m, Five Bridge Lakes Wilderness Area, Nova Scotia, Canada (44.6588, -63.76712), 15.VIII.2017, coll.: V. Nowell and E. Sayadi.

**Key to adult female *Denheyernaxoides* species (modified from Rocha *et al.* 2016).**

1. Femur I with 2 *sts*; gnathosoma deeply notched posteroventrally; prodorsal shield absent . . . . . *D. martini* Smiley 1992
- Femur I with 4 *sts*; gnathosoma with only slight indentation posteroventrally; prodorsal shield present . . . . . 2
2. Palp femurogenu with 5 (China) or 6 (Ukraine) *sts*, including 2 long setae; 2 (China) or 3 (Ukraine) pairs of pregenital setae; genu I with 5 *sts*, genua II and III with 4 *sts* . . . . . *D. brevirostris* (Canestrini 1885)
- Palp femurogenu with 5 *sts*, only 1 long seta; 2 pairs of pregenital setae; genu I with 4 *sts*, genua II and III with 3 *sts*. . . . . *Denheyernaxoides americanus* Rocha *et al.* 2016



**FIGURE 3.** *Denheyernaxoides americanus*, female. Ventral idiosoma.

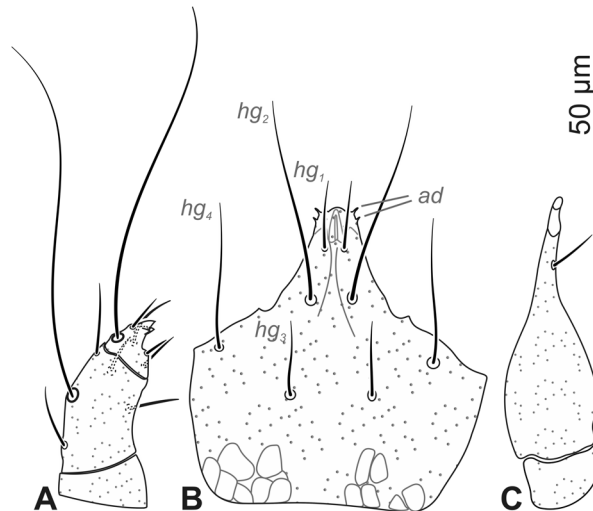
**Remarks**

*Morphological analysis*

Several inconsistencies in the description and diagnosis of *D. americanus* were identified during the present study. Rocha *et al.* (2016) reported differences in the coxal setal count between *D. americanus* and the Chinese and Ukrainian specimens of *D. brevirostris*; however, these can be attributed to how propodogastral and hystergastral setae (see Den Heyer 1981) were treated (i.e., as coxal setae, or not). Illustrations from Lin (2001), Sergeyenko (2011) and Rocha *et al.* (2016), show that *D. brevirostris* and *D. americanus* each bear a total of 10 pairs of ventral setae anterior to pregenital setae (including coxal, propodogastral and hystergastral setae). *D. americanus* was also recorded as having four setae on femur II by Rocha *et al.* (2016), but examination of the holotype reveals that it has five setae, like *D. brevirostris*. Furthermore, *D. americanus* was considered unique in having reticulation on the prodorsal shield and basal segment of the chelicerae in Rocha *et al.* (2016), but reticulation on the prodorsal shield is desclerotized and faint, differing in appearance



from the reticulation on the subcapitulum, which is comprised of obvious, sclerotized, external thickenings. In addition, a patch of unsclerotized reticula were noted in Brazilian specimens at lateral margin of prodorsal plate between *at* and seta *mps*; however, this patch was not observed in Canadian specimens. Reticulation on the cheliceral bases was not observed in either the holotype of *D. americanus* or Canadian specimens of the species in the present study.



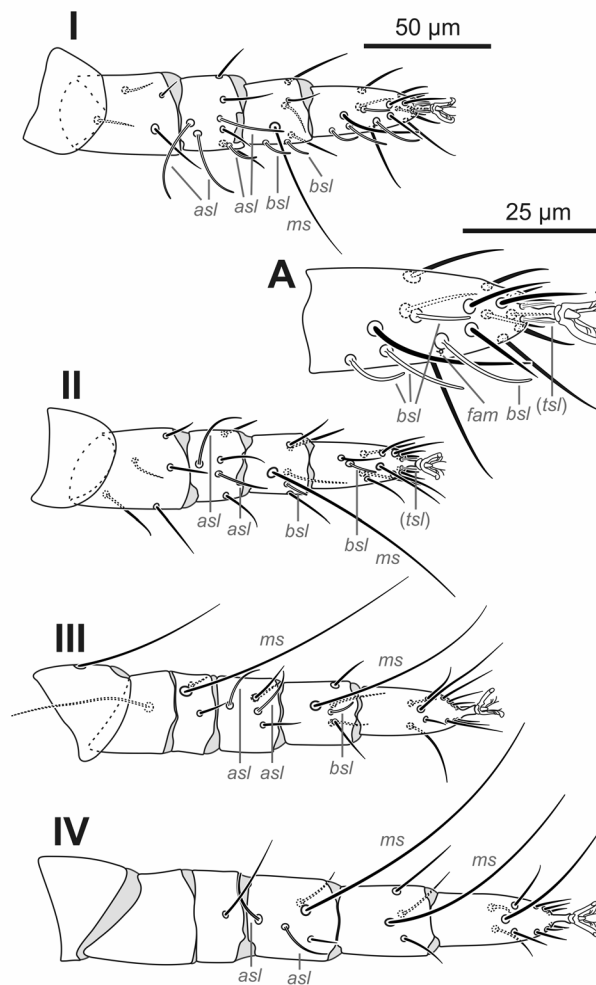
**FIGURE 4.** *Denheyernaxoides americanus*, female, gnathosoma. A. Palp, dorsal; B. Subcapitulum, ventral; C. Chelicera, dorsal.

#### Molecular analysis

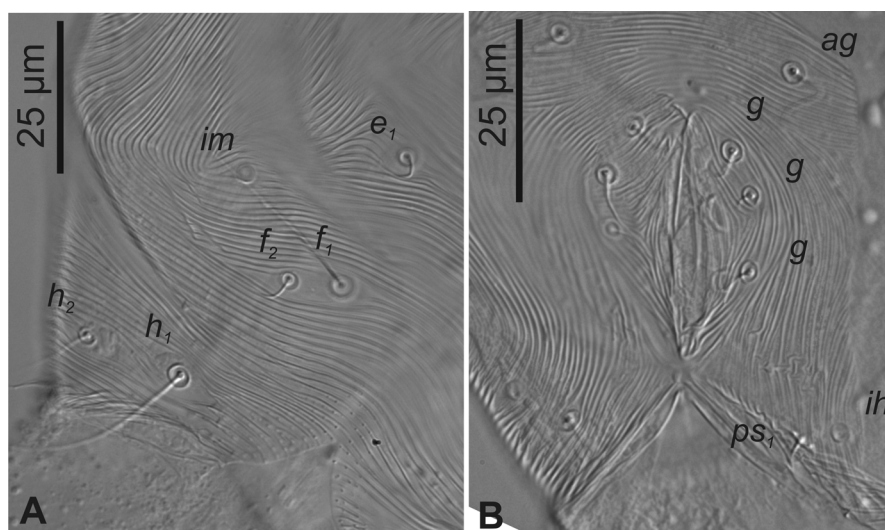
In this paper we presented the first molecular data for *D. americanus*, albeit only for specimens collected in Nova Scotia, Canada. Greater taxonomic and geographic coverage of molecular data offers the potential to clarify relationships within and between species in the genus *Denheyernaxoides*. For example, molecular data would be useful to help ascertain whether the specimens of *D. brevisrostris* described by Lin (2001) from various localities in China represent multiple species, or if the variation in leg chaetotaxy observed is only attributable to intraspecific variation, as suggested by Den Heyer and Castro (2009). Likewise, molecular data could be useful to compare Chinese specimens of *D. brevisrostris* with the Ukrainian specimens described by Sergeyenko (2011), as the morphological differences between these suggest they may be different species. Furthermore, we suspect that the Canadian specimens of *D. americanus* may represent a new species, given the large geographic separation between the Canadian and Brazilian specimens, and because they occupy a different microhabitat than other *Denheyernaxoides*. However, we currently lack molecular and morphological evidence to support this hypothesis.

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**FIGURE 5.** *Denheyernaxoides americanus*, female, legs (dorsal view). A. Tarsus leg I.



**FIGURE 6.** *Denheyernaxoides americanus*, deutonymph. A. Dorsal idiosoma, posterior region. B. Genital area.

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