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## A new species of Opilioacaridae (Parasitiformes: Opilioacarida) from Belize with some observations on life history and behavior

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### Abstract

A new species of opilioacarid mite, *Neocarus belizensis* sp. nov., is described from mid-level elevations in Belize's Maya Mountains. Laboratory observations confirmed that females nearly always deposit only one egg at a time, and that eggs are coated before deposition. Females guard both eggs and larvae. The larvae are non-feeding and molt quickly to protonymphs. Adults appear to facilitate feeding by protonymphs. Actual mating could not be observed, but pre-mating behavior is documented.

**Key words:** *Neocarus*; development; feeding behavior, Central America

### Introduction

Mites in the order or suborder Opilioacarida form a relatively small lineage, currently containing 9 genera and about 24 species (Walter & Harvey, 2009). Even so, Opilioacarida has an outsized importance in acarine systematics, because of its position as presumed intermediate between the two main lineages, the Acariformes and Parasitiformes (Grandjean, 1936). First described in 1902, the group has received relatively little attention for most of the 20<sup>th</sup> century, although there has been a notable increase in the number of studies devoted to descriptions of new species in the last decade (Bernardi *et al.* 2014; Bernardi, Klompen, Zacarias *et al.* 2013; Bernardi, Silva *et al.* 2013; Bernardi *et al.* 2012; Das & Bastawade 2006(2007); Vázquez *et al.* 2014, 2015; Vázquez & Klompen 2002, 2009, 2010, 2015). However, such a strong increase in attention for the group is not reflected in studies on their behavior. A few studies have addressed feeding modes (Vázquez & Palacios-Vargas 1988; Walter & Proctor 1998), or defensive behaviors (Coineau & Legendre 1975; Walter & Proctor 1998), but only a few anecdotal reports exist on egg deposition (Klompen 2000), and none on mating behavior. The availability of a number of live specimens of a new species from Belize has allowed the documentation of some additional data on opilioacarid feeding, as well as providing the first data on reproductive behavior and brood care. Rearing also allowed preliminary data on life tables for the immature instars.

The goal of this study is to describe the specimens observed as a new species and to document various aspects of their behavior.

## Material and Methods

Specimens were collected by the first author on 19 November 2007, 1 November 2008, and 27 November 2010 in Belize (Maya Mountains, near caves at Rio Frio, 16.97°N 88.99°W, 490m elevation), an area is covered by temperate forest. The collecting site was located near a small river. Most specimens were hand collected from under moss and lichens on large standing live pine trees. A few other specimens were collected from litter in the surrounding forest. A total of 28 specimens were collected from this site. An additional seven specimens were reared from larvae born in laboratory cultures.

Three pairs of one male and one female were each placed in small jars with a moistened layer of activated carbon and plaster of Paris (Walter & Krantz 2009). Each jar also contained a small piece of bark and a little moss from the tree on which the specimens were collected. Specimens were fed once a week; surplus food was removed after approximate 30 minutes (if the specimens were feeding immediately) or the next day. Removal of excess food was necessary to prevent fungal growth. Water was provided as needed in the form of small drops of water.

Behavioral observations were made 1–2x daily for 30 minutes (usually late morning and/or late afternoon), using a Zeiss Stemi 2000-c dissecting microscope. Recordings were made using a digital video camera (Sony Exwave HAD) mounted on the dissecting microscope.

Description. Most material was studied as slide-mounted specimens. For this purpose, specimens were dissected, cleared in lactic acid and mounted on slides using Hoyer's medium (Walter & Krantz, 2009). Frequently, parts of single specimens were mounted on multiple slides. Terminology for the palp tarsal sensilla follows Grandjean (1936) as modified by Vázquez and Klompen (2002).

Drawings were prepared using a Zeiss Axioskop A1 phase contrast microscope, connected to a drawing tube; measurements were taken using an ocular micrometer and are presented in micrometers ( $\mu\text{m}$ ). Measurements for individuals are listed in Table 1, the text uses average  $\pm$  standard deviation. It should be noted that measurements of idiosomal length and width are distorted, as they are based on slide-mounted specimens.

Specimen depository abbreviations: CNAC: Acarology Collection of the Universidad Autónoma de Mexico, Mexico City, Mexico; OSAL: Ohio State University Acarology Collection, Columbus, OH, U.S.A.; UQRoo: Universidad de Quintana Roo, Chetumal, Quintana Roo, Mexico.

## Taxonomic section

### Family Opilioacaridae With 1904

#### *Neocarus* Chamberlin & Mulaik 1942

Type species *Neocarus texanus* Chamberlin & Mulaik 1942: 127 by original designation

The genus currently includes 15 recognized species plus 1 subspecies, *N. texanus* Chamberlin & Mulaik 1942, USA and Mexico, *N. platensis* (Silvestri 1905) from Uruguay, Argentina and Brazil, *N. orghidani* (Juvara-Bals & Baltac 1977), Cuba, *N. ojastii* Lehtinen 1980, Venezuela, *N. bajacalifornicus* (Vázquez & Klompen 2002), Mexico, *N. nicaraguensis* (Vázquez & Klompen 2002), Nicaragua, *N. nohbecanus* (Vázquez & Klompen 2002), Mexico, *N. siankaanensis* (Vázquez & Klompen 2002), Mexico, *N. calakmulensis* Vázquez & Klompen 2009, Mexico, *N. bajacalifornicus chamelaensis* Vázquez & Klompen 2009, Mexico, *N. veracruzensis* Vázquez & Klompen 2009, Mexico, *N. potiguar* Bernardi *et al.* 2012, Brazil, *N. proteus* Bernardi *et al.* 2013,

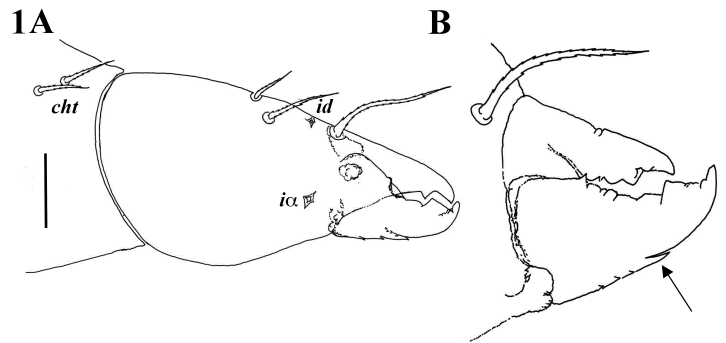
Brazil, *N. caipora* Bernardi *et al.* 2014, Brazil, *N. chactemalensis* Vázquez & Klompen 2015, Mexico, *N. comalensis* Vázquez & Klompen 2015, Mexico.

***Neocarus belizensis* Vázquez, May, Alamilla & Klompen sp. nov.**

(Figs. 1–9)

**Diagnosis.** Palp tarsus with 5 pairs of foliate sensilla, each of which with 4 broad lobes with blunt tips; palp tibia with a large number (16–30) of modified *r*-type setae; setae *st2* with tapering tip, distinct from other sternal setae, setae *st3* stout with a rounded tip, similar to remaining sternal setae; female without setae in pregenital or genital area, but with two small structures vaguely resembling setal bases; males with 2–3 pregenital and 4–5 genital setae, all short, thick and ribbed, with blunt tips; ovipositor without terminal sensilla or extensive internal sclerites, but with a pair of membranous projections near the base. Glands in males distinct.

**Description.** Available specimens: 5 females, 6 males, 1 larva, 6 protonymphs, 6 deutonymphs, and 11 tritonymphs. Description based on adults unless otherwise noted.



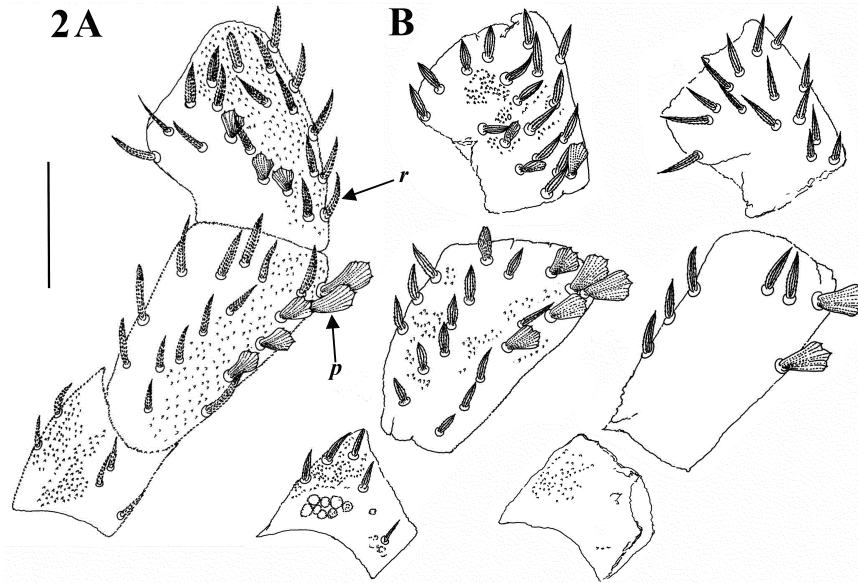
**FIGURE 1.** *Neocarus belizensis* sp. nov., chelicera. A. Female, holotype; B. Male. Arrow: largest ventral denticle. Scale bar = 50  $\mu$ m.

*Gnathosoma.*

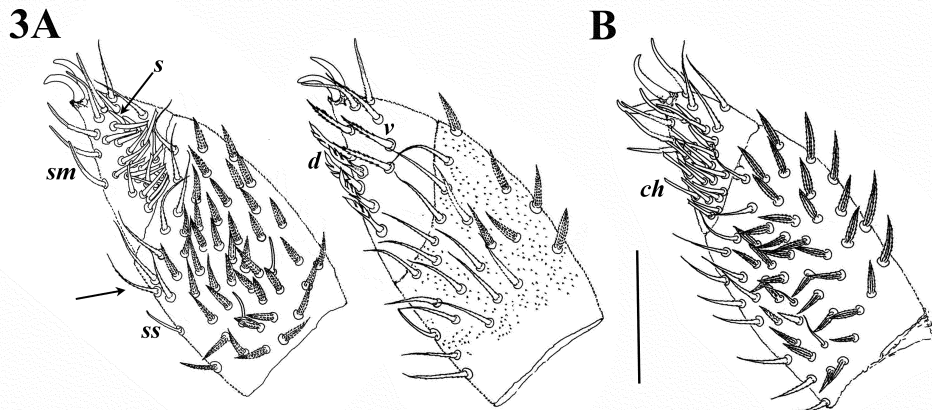
**Chelicera** (Fig. 1) chelate. Fixed digit with 1 tooth and a secondary, slightly offset, protrusion, movable digit with 1 distinct large tooth, a small proximal ridge-like structure, and a well-developed terminal hook. Fixed digit with 3 setae in all postlarval instars. Basal segment with seta *cht* present (rarely (1/14) duplicated but see Fig. 1A). All setae lightly barbed. Dorsal (*id*) and antiaxial (*iα*) lyrifissures on fixed digit well developed. Three or more ventral denticles on movable digit in adults, but all, except the first one, extremely small (Fig. 1B, arrow). Axial scale-like process on movable digit absent.

**Palp** (Figs. 2–4). Trochanters with 5 +/- 1 spine-like, ribbed, tapering setae (= *r*-type); femora with 8 +/- 1 papilliform (= *p*-type) and 18 +/- 4 *r*-type setae (numbers of *p*-type setae slightly higher in males, number of *r*-type setae distinctly higher in females); genua with 4 +/- 3 *p*-type and 30 +/- 4 *r*-type setae (number of *p*-type setae distinctly smaller (average 2 vs. 5) in males vs. females); tibiae with 39 +/- 9 *r*-type setae and 23 +/- 5 setae of a new type: longer, thinner, and more rapidly tapering to a tip than “standard” *r*-type setae (Fig. 3A, arrow), plus 2–3 smooth sensilla (Fig. 3A, *ss*). Tibia and tarsus partially fused. Tarsus well developed, at least half as large as the tibia. Sensillar set including: 5–6 (5 +/- 1) foliate (*d*) (each with 4 lobes with rounded, not filiform, tips; Fig. 4A), 5 +/-

- 1 *v*, 19 +/- 2 *ch*, 6 +/- 1 *sm*, and 3 *s* sensilla (solenidia). Sexual dimorphism in palp tarsus setation minor, females often with one more *v* sensillum than males. Tarsal lyrifissures not observed. Pretarsus in shape of a pair of well-developed sessile claws, positioned apically. Variability in sensillar numbers within adults and across development listed in Table 2.

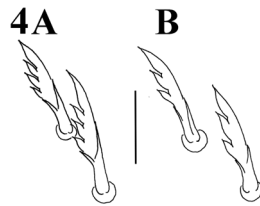


**FIGURE 2.** *Neocarus belizensis* sp. nov., palp trochanter, femur and genu. A. Female, holotype, antiaxial view; B. Male, antiaxial (left) and axial (right) views of individual segments. Scale bar = 100  $\mu$ m.

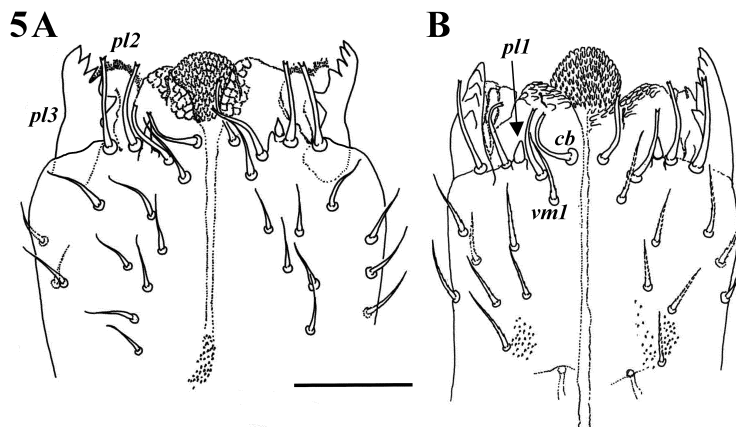


**FIGURE 3.** *Neocarus belizensis* sp. nov., palp tibiotarsus. A. Female, antiaxial (left) and axial (right) views; B. Male, antiaxial view. Arrow: modified *r*-type seta. Scale bar = 100  $\mu$ m

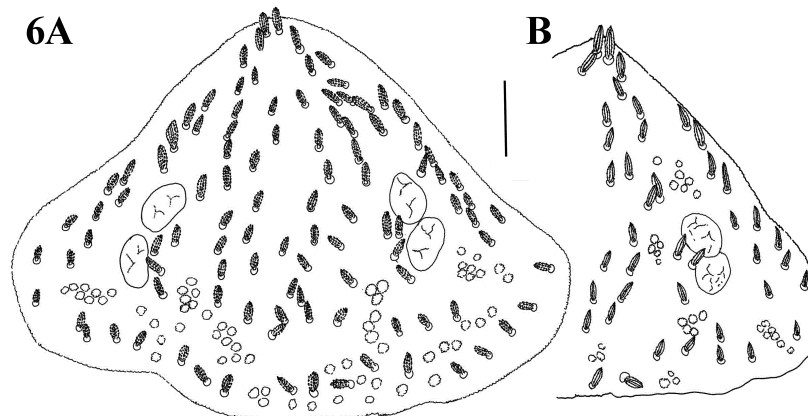
**Subcapitulum** (Fig. 5). All 4 pairs of paralabial setae present: *pl1* small, conical; With's organ (*pl2*) membranous, discoid; rutellum (*pl3*) with one row of five teeth, inserted dorso-lateral; *pl4* tiny, inserted dorsal. With 4 pairs of circumbuccal (*cb*) and, respectively, 10 +/- 1 and 8 +/- 1 pairs of median and subcapitular (*vm*, *lvm*, *ldm*, *vp*, *lvp*) setae in the females and males. Setae *vm1* with an attenuate tip (similar to other median and subcapitular setae), unlike *cb* setae. Number of median and subcapitular setae in nymphs increases over development (protonymph 2; deutonymph 4; tritonymph 7) (Table 2). Lateral lips with distinct canals in all instars examined.



**FIGURE 4.** Comparative structure of *d*-sensilla. A. *Neocarus belizensis* sp. nov.; B. *N. siankaanensis* Vazquez & Klompen 2002. Scale bar = 25  $\mu$ m



**FIGURE 5.** *Neocarus belizensis* sp. nov., subcapitulum. A. Female, holotype; B. Male. Scale bar = 100  $\mu$ m



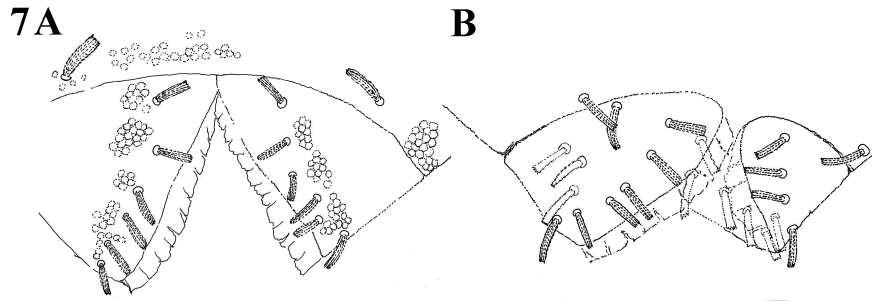
**FIGURE 6.** *Neocarus belizensis* sp. nov., prodorsal shield. A. Female, holotype; B. Male. Scale bar = 100  $\mu$ m.

*Idiosoma.*

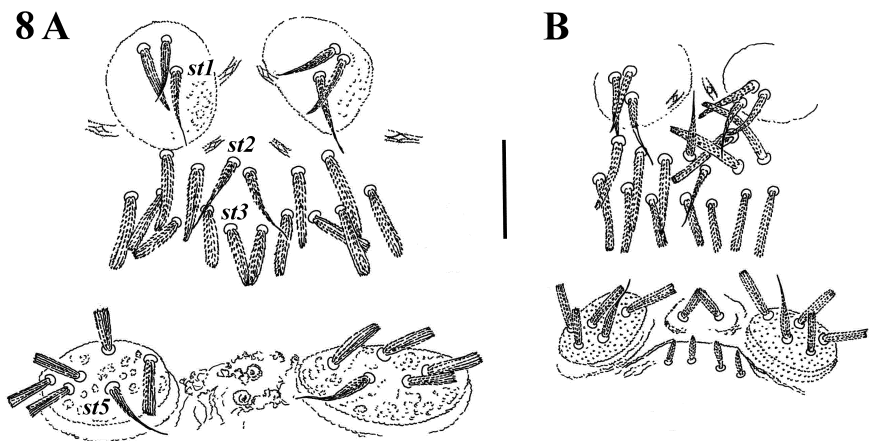
Color of the body variable. Morph 1: with light blue bands and patches alternating with a brownish to orange base color (the latter possibly affected by food content); legs with a light blue bands on most segments. Morph 2: much darker throughout (video clip 1: <https://youtu.be/ey6SNu516GM>).

Dorsum (Figs. 6–7). Prodorsal shield in adults with two pairs of eyes, and 88 (males) to 120 (females) stout ribbed setae. Setae slightly wider and shorter in females than in males (Fig. 6). Dorsal

idiosoma between prodorsal shield and the preanal segment without setae but with numerous lyrifissures arranged in transverse rows. Preanal segment with 1 dorsal and 2 ventro-lateral setae in all instars. Anal plates in adults each with 8–10 stout, ribbed setae (Fig. 7).



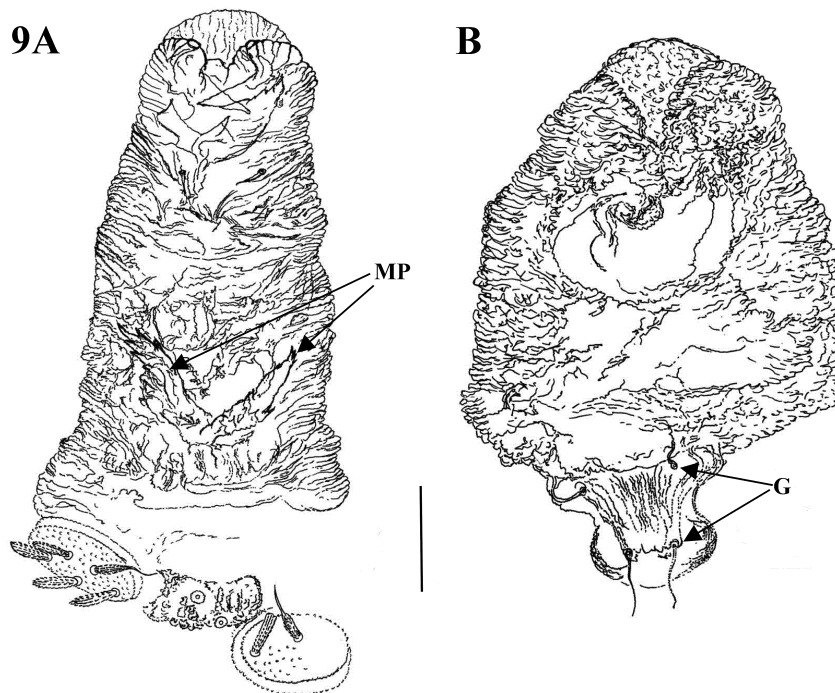
**FIGURE 7.** *Neocarus belizensis* sp. nov., anal valves, ventral view. A. Female, holotype, dorsal setae only; B. Male, including ventral setae. Scale bar = 100  $\mu$ m.



**FIGURE 8.** *Neocarus belizensis* sp. nov., sternitogenital region. A. Female; B. Male. Scale bar = 100  $\mu$ m.

**Sternitogenital region** (Fig. 8). Sternapophyses present, bases not fused, each with 2 setae. Sternal verrucae, each with 2 medium long, ribbed, setae and 1 distinctly longer, barbed and tapering seta (*st1*). Remaining sternal region with 7  $\pm$  1 pairs of stout, ribbed setae with a blunt tip and centrally, 1 pair of barbed and tapering setae (*st2*). Setae *st3* barbed, stout, with a rounded tip, barely distinguishable from surrounding setae. With 3 pairs of lyrifissures, 2 large and distinct near the sternal verrucae, and 1 smaller pair anterolateral to setae *st2*. Pregenital capsules each with 1 long, tapering setae (*st5*) and, respectively, 4  $\pm$  1 and 3  $\pm$  1 stout, ribbed setae with a blunt tip in females and males.

Pregenital and genital areas in female nude, although pregenital area in some specimens with 2 structures that resemble setal bases (Fig. 8A). Pregenital and genital areas in male with, respectively, 2.0  $\pm$  1.0 and 3.0  $\pm$  1.4 short, ribbed, and blunt-tipped setae (Fig. 8B). Ovipositor (Fig. 9) without terminal setae, but terminating in 3 lobes. With a pair of distinctive membranous projections (MP) at the basis of the ovipositor and two pairs of glands-like structures (G), one of them connected with a tiny channel. Glands in males distinct.



**FIGURE 9.** *Neocarus belizensis* sp. nov., female, ovipositor. A. Evaginated, based on molted cuticle, holotype.; B. Invaginated, holotype.. Abbreviations: G: gland-like structure; MP: membranous projections. Scale bar = 100  $\mu$ m.

#### *Legs.*

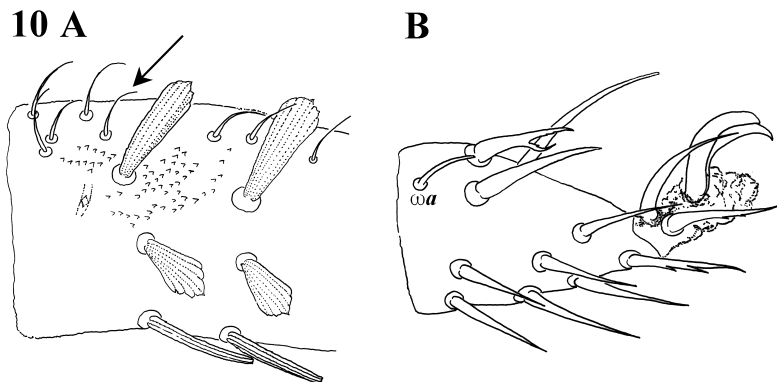
Sensilla on anterior portion of tarsus I: including one sensillum below surface and another emerging. Crown-like sensillum on tarsus I inserted in dorsal sensory field. Solenidion  $\alpha$  basal on acrotarsus II, absent on acrotarsi III–IV. Acrotarsus II also with an unpaired, forked seta. In addition to standard set of 1 pair of ventro-lateral and 1 pair of dorso-lateral setae, acrotarsi II–IV with 2 pairs of lateral and 3 pairs of ventral setae. Distal setae on ambulacra II–IV in adults distinctly barbed (Fig. 10B). Solenidion  $\alpha$  on basitarsi II–IV present, sunk into segment. Coronidia (Fig. 10A, arrow) limited to basitarsi, absent on tibiae and genua II–IV. Smooth setae on tibia, genu and femur I in males absent. Modified setae on base of male telofemur I, or base of female tibia I, absent.

*Deposition of types.* Holotype female at UQROO; one paratype female and one paratype male at CNAC; one paratype female and one paratype male at OSAL; all other paratypes at UQROO.

*Material examined (preserved as slides).* Belize: Cayo, Rio Frio Caves, 16.9705° N 88.9956° W, elevation 490m, coll. M. M. Vázquez, 27-XI-2010, ex under moss and bark on standing tree in pine-oak forest (holotype female, 2 females). Same locality & collector, 17-XI-2007, ex under moss and bark on standing tree in pine-oak forest (1 female, 1 male); 19-XI-2007, ex litter in pine-oak forest, (2 males, 10 tritonymphs); 1-XI-2008, ex under moss on standing tree in pine-oak forest (1 female, 3 males, 1 deutonymph, 1 tritonymph); 2-XI-2008, ex medium high pine-oak forest (5 deutonymphs). Lab culture from holotype female (1 larva, 6 protonymphs).

*Etymology.* The specific name is derived from the country where the material was collected, Belize. Belix is a Mayan word which means muddy water.





**FIGURE 10.** *Neocarus belizensis* sp. nov., female. A. Basitarsus II; B. Acrotarsus II, holotype. Arrow: coronidium.

*Taxonomic remarks.* *Neocarus belizensis* differs from previously described Mexican and Central American taxa by the combination of 5 *d* sensilla on the palp tarsus (4 in *N. chactemalensis*, *N. nohbecanus*, and *N. orghidani*), the absence of terminal sensilla on the female ovipositor (present in *N. bajacalifornicus*, *N. chactemalensis*, *N. comalensis*, *N. nohbecanus*), sternal setae *st3* similar in shape to surrounding setae (shared with *N. calakmulensis*, *N. chactemalensis*, *N. nicaraguensis*, *N. nohbecanus*, and *N. siankaanensis*), and the absence of setae in the pregenital area of the female (shared with *N. chactemalensis*, *N. nohbecanus*, *N. orghidani*, and *N. siankaanensis*). Based on these characters, the new species is most similar to *N. siankaanensis*. *Neocarus belizensis* differs from *N. siankaanensis* by the presence of 4, rather than 3 lobes on the *d* sensilla of the palp tarsus (Fig. 4), the presence of the unusual type of *r*-type setae on the palp tibia, and the presence of distinct membranous projections in the ovipositor (both absent in *N. siankaanensis*).

#### *Ecology and behavior*

**Food source.** Specimens were offered a range of food options, from pollen grains to crushed insects (Vázquez & Palacios-Vargas 1988; Walter & Proctor 1998). The best feeding occurred with pollen grains. Initially the mites were fed on pollen from *Coccinia* sp. (Cucurbitaceae), a decorative plant found in the university gardens (video clip 2: <https://youtu.be/cOYmCry84FI>). The relatively small pollen were ingested whole. When feeding on this food source appeared to slow (after approximately 6 months), pollen of *Hibiscus rosa-sinensis* L. (Malvaceae) were provided. These pollen grains are substantially larger than those of *Coccinia* sp. The mites fed on them by breaking the outer covering and feeding on the softer content (video clip 3: <https://youtu.be/pPTAAmg3I0Q>). Occasionally, the mites subsequently started feeding on the hard pollen cuticle. Co-feeding on single anthers was repeatedly observed, with no signs of aggression.

**Development.** Specimens were kept alive for periods of 5–8 months. During this period reproduction took place for two pairs, resulting in eggs, larvae (video clip 4: <https://youtu.be/Qii1O2CjE84>), protonymphs, and deutonymphs. Molting to tritonymphs was not observed. For one pair, the female produced at least 8 eggs over a period of about 8 months, for an egg-laying frequency of approximately one per 3–4 weeks. This time period corresponds fairly well to the 3-week time period between the 1<sup>st</sup> and 2<sup>nd</sup> egg laying period reported for *N. texanus* (Klompen, 2000). In most cases eggs are deposited one at the time, but we have at least one observation where two similar sized protonymphs emerged in a container with a single female, suggesting twin births are possible. Eggs hatch after about two weeks.

Larvae molted to protonymphs in a few (2) days, while protonymphs molted to deutonymphs in about two weeks. Family groups of a pair of adults, larvae, protonymphs and a deutonymph were observed together. This may be artificial, because of the confines of the rearing containers, but it may also explain the observation that Opilioacaridae are often found in small groups.

Larvae were not observed to feed, consistent with previous hypotheses (Klompen *et al.* 2015). All nymphal instars were observed feeding, usually on the same pollen grains as the adults. Protonymphs were also observed feeding on female excreta. These excreta were examined and contain partially digested pollen grains. Notably, these observations were isolated, disallowing conclusions on whether this type of feeding is common or maybe even required for protonymphal development.

One female molted after about four months in culture, confirming the hypothesis that post-imaginal molts occur in Opilioacaridae (Bernardi, Klompen, & Ferreira 2013; Coineau & Legendre 1975; Vázquez & Palacios-Vargas 1988). Moreover, this molt was not tied to earlier autotomy of legs, a common defense mechanism in these mites (Klompen 2000; Thaler & Knoflach 2002; Vázquez & Palacios-Vargas 1988), suggesting post-imaginal molts are not just for recovery of limbs. Neither does it appear to correlate with egg-deposition, as this female deposited eggs, molted, and continued depositing eggs, with little interruption.

Reproduction. Earlier observations suggested that females coat their eggs (Klompen 2000), a hypothesis confirmed during the current study. Females held the eggs close to the gnathosoma with the help of their chelicera, rutella and palps, generating a rolling motion, presumably used to coat the egg (video clip 5: <https://youtu.be/8V-KwvMzzM8>). The appearance of the egg changed after this treatment from white or yellowish white to a much darker color. The nature of the coating material and its function is still unclear, although some “waterproofing”, as observed in eggs of Ixodidae (Sonenshine, 1991), seems likely. Only when finished treating the egg did she deposit it under a small piece of bark or similar. The female remained near the egg, barely moving until the egg hatched.

Mating behavior (1 pair). A major question regarding Opilioacarida concerns their mating behavior and specifically their method for sperm transfer. We have not been able to observe the latter, but can provide some observations on some aspects of pre-mating behavior. Three phases of pre-mating behavior could be distinguished. First female and male approach head to head. Their legs I touch at the telotarsus and both individuals maintain this position for a few seconds. Next the male touches the dorsum of the female, running the tips of his legs I all over her back. During this action she remains motionless. Next he turns 180° and the posterior parts of their bodies contact. Both hold this position for a brief period. As noted, actual mating was not observed, but at one time the male was seen holding some yellowish structure (oval and larger than an egg) in its mouthparts. Unfortunately he subsequently disappeared under a piece of bark, preventing further observations. It is possible that this was a spermatophore.

Parental care (2 pairs). There are some indications of basic parental care. This includes putative guarding of the egg and larva by the female (noted above). The male may also be involved, as it remains very close to the female and egg/larva. Both female and male appear to facilitate feeding, or at least tolerate co-feeding, by the developing protonymphs (video clip 6: <https://youtu.be/1yh-cA44QaY>). In one case the female was eating pollen from a provided anther, when a protonymph appeared from under her (they are much smaller than the adults), and started to eat from the same site. The female immediately stopped feeding, but remained in place while the nymph was feeding. On another occasion adults were seen bringing anthers to protonymphs or guiding protonymphs to food.

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### Supplemental data

Video clip 1. *Neocarus belizensis* sp. nov. Adults interacting, general habitus and color. <https://youtu.be/ey6Snu5l6GM>

Video clip 2. *Neocarus belizensis* sp. nov. Feeding on *Coccinia* sp. pollen. <https://youtu.be/cOYmCry84FI>

Video clip 3. *Neocarus belizensis* sp. nov. Feeding on *Hibiscus rosa-sinensis* pollen. <https://youtu.be/pPTAAmg3I0Q>

Video clip 4. *Neocarus belizensis* sp. nov. Newly born larva. <https://youtu.be/Qii1O2CjE84>

Video clip 5. *Neocarus belizensis* sp. nov. Female manipulating egg. <https://youtu.be/8V-KwvMzzM8>

Video clip 6. *Neocarus belizensis* sp. nov. Female co-feeding with nymph. <https://youtu.be/1yh-cA44QaY>