Two new species of *Bonnetina* tarantulas (Theraphosidae: Theraphosinae) from Mexico: contributions to morphological nomenclature and molecular characterization of types

David Ortiz* and Oscar F. Francke

Colección Nacional de Arácnidos, Instituto de Biología, Universidad Nacional Autónoma de México, México DF, Mexico

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Two new species of tarantulas from Mexico are described and illustrated: *Bonnetina tenuiverpis* and *Bonnetina juxtantricola*, from the states of Mexico and Guerrero, respectively. Male palpal bulbs, tibial apophyses and spermatheca are among the most taxonomically informative characters. Male bulb microstructure is revealed from scanning electron microscopy; both new and homologous structures to other Theraphosinae genera are identified and described. Nomenclatural changes for male tibial apophyses are also proposed. The holotype male and allotype female of one of the species are molecularly characterized and matched from CO1 partial sequence.

http://zoobank.org/urn:lsid:zoobank.org:pub:BBDBBEDE-6983-4F5C-B34C-4622DF494C84

Keywords: Mygalomorphae; tibial apophyses; ciliate hairs; bulbal keels; sperm pore

Introduction

*Bonnetina* Vol 2000 is one of the most recently described genera of Mexican tarantulas and is also endemic to the country. Six species have been described since its original report: *Bonnetina cyaneifemur* Vol 2000 (type species), from the state of Colima, *Bonnetina rudloffi* Vol 2001 (Michoacán), *Bonnetina alagoni* Locht and Medina 2008 (Morelos), *Bonnetina aviae* Estrada-Alvarez and Locht 2011 (Estado de México), *Bonnetina papalutlensis* Mendoza-Marroquín 2012 (Guerrero) and finally *Bonnetina tanzeri* Schmidt 2012 (unknown).

Here we present the description and wide illustration of two new species of *Bonnetina*, the initial results of author DO’s PhD project on phylogenetic systematics of the genus.

We also propose corrections to the nomenclature of the males leg I tibial spurs introduced in preceding papers on *Bonnetina* and we make a deep examination of male palpal bulbs (from scanning electron microscope [SEM] images), that reveal structures apparently homologous to those of other Theraphosinae genera, but also others not previously recorded and that are thus described and named here for the first time.

To expand the descriptions from the traditional morphological only approach to potentially useful molecular characterisation, we include a cytochrome c oxidase subunit 1 (CO1) $\approx$ 960 bp partial sequence of the holotype and allotype of one of the new species.
(no fresh material of the other was available). Beyond the widely discussed potential utility of molecular barcodes to identify species in several groups, this fragment has been found to be useful for this purpose in *Aphonopelma* Pocock 1901 tarantulas (Hamilton et al. 2011, 2014; Hendrixson et al. 2013) and can help match conspecific males and females in sympatric closely related species, which is often problematic as most taxonomically valuable characters in mygalomorph spiders are sexually dimorphic.

**Materials and methods**

**Morphological study**

Diagnoses were based in the examination of type and non-type specimens and bibliographical data. The male holotype and female paratype of *B. alagoni*, *B. aviae* and *B. papalutlensis* were examined, as well as male and female paratypes of *B. tanzeri*. These specimens are deposited at Colección Nacional de Arácnidos, Instituto de Biología, Universidad Nacional Autónoma de México, México DF, Mexico (CNAN). For *B. cyaneiferum* and *B. rudolphi*, data were taken from their original descriptions. The following non-type material (from close to type locality) was also examined:

*B. alagoni*. MEXICO: Morelos state: Tepoztlán municipality. ♂ a (CNAN-Ar003650A) and 2 ♀♀ a (CNAN-Ar003650B and CNAN-Ar003650C): 1 km E of San Juan Tlacatenco town: 19.0178°, −99.0811°; 2420 m asl. 13 April 2012. David Ortiz, Diego Barrales, Jorge Mendoza, Gerardo Contreras, Carlos Santibáñez, Laura Olguín, Natalia Zepeda, cols. Under stones. ♂ a (CNAN-Ar004099A) and ♀ a (CNAN-Ar004099B): Tepoztlán: 19.0172°, −99.0663°; 2200 m asl. 11 March 1993. George Odell, Rick C. West, cols. Under stones.

*B. aviae*. MEXICO: state of Mexico. ♂ a (CNAN-Ar003729A) and 2 ♀♀ a (CNAN-Ar003729B and CNAN-Ar003729C): Ecatepec de Morelos municipality: NE slope of Sierra de Guadalupe: 19.6069°, −99.0734°; 2370 m asl. 15 August 2012. Gerardo Contreras & Diego Barrales, cols. Under stones. ♀ a (CNAN-Ar003543): Coacalco de Berriozábal municipality: main entrance to Sierra de Guadalupe Urban Park: 19.6080°, −99.0942°; 2415 m asl. 13 August 2011. Diego Barrales, col. Under a stone.

All measurements were taken along the central axis of structures, with an ocular micrometer on a stereomicroscope and are given in millimetres; the extension of the metatarsal scopulae relative to the length of the segment was evaluated at plain view.

The photographs of specimens in ethanol were taken with a Nikon Coolpix S10 digital camera coupled to a stereomicroscope. In most instances, several images were taken at different focal planes and then mounted to obtain a single optimally focused image with software Helicon Focus 5.3.7 (http://www.heliconsoft.com/). Additional processing of images was performed using the software Adobe Photoshop CS4 (http://www.adobe.com/) and plates were composed using Corel Draw X6 (http://www.corel.com/).

Palpal bulbs of males were cleaned in an E/MC 250 Ultrasonic Cleaner (EMC Global Technologies, Quakertown, PA, USA) submerged in biological detergent for five minutes, coated with a layer of gold in a Q150R ES Sputter Coater (Quorum Technologies, Lewes, East Sussex, UK) and examined and photographed in a SU-1510 SEM (Hitachi, Chiyoda City, Tokyo, Japan).
Spermathecae were cleaned by submerging them in absolute lactic acid from one to several hours.

The term allotype is here considered for the fully described paratype female, which assures an easy identification of the specimen, although it is a term not regulated by the current International Code of Zoological Nomenclature (1999).

Terminology for tibial apophyses (or spurs) follows the general usage in Theraphosidae. It includes the prolateral apophysis (or apophysis branch) and retrolateral apophysis (e.g. Bertani 2001; Kaderka 2007; Fukushima et al. 2008; Ortiz 2008; Pérez-Miles et al. 2008). A third apophysis, present only in Bonnetina, is herein considered non-homologous to any of the remaining genera and thus we introduce the new term ‘accessory apophysis’. Accessory apophysis substitutes the term ‘retrolateral apophysis’ sensu Vol (2000, 2001), Locht and Medina (2008), Estrada-Alvarez and Locht (2011), Mendoza-Marroquin (2012) and Schmidt (2012). Retrolateral apophysis is the correct term for the ‘ventral apophysis’ introduced by Estrada-Alvarez and Locht (2011) and followed by Mendoza-Marroquin (2012) and Schmidt (2012).

Pedipalpal bulbs terminology follow Bertani (2000) in general terms. Nevertheless, there are some keels found in the species treated in this paper that do not seem to be homologous to any of those described before and are thus named here for the first time (Figures 4, 11). Sperm pore keels are two structures located at the ventral apex of the embolus that fold onto each other, shaping the sperm outlet as a sperm pore. The dorsal keel is a structure located subapically, opposite to the sperm pore, in one of the species treated in this paper. The prolateral sub-apical keel, in the other species, is similar in shape and position to the dorsal keel, but is located between prolateral superior keel and sperm pore, so should not be considered a priori homologous to the dorsal keel. Future thorough exploration of several species of Bonnetina and related genera is expected to help clarify the homology of bulbal structures. The position of the structures is considered discarding the torsions that the embolus presents from base to apex, using its base as a reference.

Terminology for urticating hairs was taken from Cooke et al. (1972); for tarsal scopulae from Pérez-Miles (1994); for spination patterns from Petrunkevitch (1925) and for barbed hairs from Font Quer (1953) (i.e. if barbs are at least two times longer than hair width, it is plumose; if shorter, it is ciliate).

Abbreviations used in the text are as follows. Morphology. Palpal bulbs: PS, prolateral superior keel; PI, prolateral inferior keel; D, dorsal keel; PSA, prolateral sub-apical keel; and SP, sperm pore keel. Ocular patterns: AME, anterior median eyes; ALE, anterior lateral eyes; PME, posterior median eyes; and PLE, posterior lateral eyes. PLS, posterior lateral spinnerets.

Material examined will be deposited in the following collections: CNAN and American Museum of Natural History, New York, USA (AMNH).

**Molecular protocol**

Immediately after the specimens were sacrificed, right leg III was removed and preserved in 96% ethanol at –20°C. DNA extraction was performed with Qiagen DNeasy Tissue Kit (Qiagen, Venlo, Limburg, Netherlands) and its products were stored at –20°C until amplification.

A partial fragment (= 960 bp) of Cytochrome c oxidase subunit 1 (CO1) was amplified from the holotype and allotype of one of the species that is herein described, using the forward primer C1-J-1751 ‘SPID’ (5′-GAG CTC CTG ATA TAG CTT TTC
C-3') and reverse primer C1-N-2776 (5'-GGA TAA TCA GAA TAT CGT CGA GG-3') (Hedin and Maddison 2001). The 25 µl reaction mixtures using reagents from Qiagen Taq PCR Core Kit contained: 17.75 µl of water, 2.5 µl of 10X PCR buffer, 1.5 µl of 25mM MgCl₂, 0.5 µl of 10 mM dNTP Mix, 0.25 µl (1.25 U) of Taq DNA polymerase, 0.75 µl of each 10 pmol primer and 1 µl of DNA. The following thermal cycle was followed in GeneAmp® PCR System 9700 (Life Technologies, Foster City, CA, USA): initial denaturation at 95°C for 2 min; 30 cycles of denaturation at 96°C for 30 s, annealing at 48°C for 30 s and extension at 72°C for 1 min; final extension at 72°C for 5 min. PCR products were purified using Amicon Ultra-0.5 centrifugal filters (Millipore, Billerica, MA, USA).

Sequencing was performed at Instituto de Biología (UNAM) in a 3500 XL Genetic Analyzer (Life Technologies), using the same manufacturer’s BigDye® Terminator v3.1 cycle sequencing kit.

Sequences were edited using Chromas 2.4 (http://technelysium.com.au/) and BioEdit 7.1.3 (Hall 1999). Not properly read bases were scored as ‘n’.

The reading frame was determined by translating to the six possibilities using the Expasy Online Translate Tool (http://web.expasy.org/translate/). The only option that did not have stop codons was then successfully aligned with other theraphosid spiders using GenBank’s Standard Protein Blast, which confirmed the selection. Sequences are also deposited at GenBank (http://www.ncbi.nlm.nih.gov/genbank/).

Systematics

Family **THERAPHOSIDAE** Thorell 1870
Subfamily **THERAPHOSINAE** Thorell 1870
Genus **Bonnetina** Vol 2000
Type species: **Bonnetina cyaneifemur** Vol 2000

**Bonnetina tenuiverpis** sp. nov.
urn:lsid:zoobank.org:act:B1F7E8B8-F542-430B-BEE3-9F1349419DCA (Figures 1–8; Tables 1, 2)

Figure 1. **Bonnetina tenuiverpis** sp. n. Habitus. (A) Male holotype; (B) female allotype. Scale line: 10 mm. Photos by David Ortiz.
Holotype. ♂ (CNAN-T0755). MEXICO: Mexico state: Otzoloapan municipality: 3 km NNE of Zuluapan town: 19.1627°, −100.3085°: 1250 m asl. 7 August 2012. David Ortiz, Carlos Santibáñez, Diego Barrales & Rodrigo Monjaraz, cols. Under a stone. Matured in captivity 11 October 2012.

Allotype. ♀ (CNAN-T0756): same collecting data as holotype.
Figure 3. *Bonnetina tenuiverpis* sp. n. Male holotype, left pedipalpal bulb, stereomicroscope images. (A) Prolateral view; (B) retrolateral view; (C) dorsal view; (D) ventral view. Scale line: 1 mm.

Figure 4. *Bonnetina tenuiverpis* sp. n. Male holotype, left pedipalpal bulb, SEM images. (A) Embolus apical half, prolateral view; (B) embolus apex, ventral–frontal view; (C) apex, prolateral view; (D) apex, frontal view. Arrow colours represent keels: prolateral superior (black), sperm pore (white), dorsal (grey) and prolateral inferior (hatched). Chevron: sperm pore. Scale lines: 10 µm (except A: 100 µm).
Other paratypes. ♀ (AMNH): same collecting data as holotype. ♀ (CNAN-T0757): same locality as holotype. 16 December 2001. Oscar F. Francke and Edmundo González, cols.

Etymology
The specific name is composed by the Latin adjective *tenuis* (slender) and plural noun *verpis* (penes). It makes reference to the attenuate condition of the male pedipalpal bulbs of this species.

Diagnosis
Males differ from those of all known species of the genus by the shape of its palpal bulbs, that are also remarkably slender compared to those of the other species. It additionally has much better developed tibiae I accessory apophyses than *B. alagoni* and *B. aviae*. It is a distinctly smaller species than *B. cyaneifemur*, *B. rudloffii*, *B. papalutensis* and *B. tanzeri*. Females differ from those of other species by having subdigitiform spermatheca instead of domiform (as in *B. alagoni* and *B. aviae*), flattened (*B. tanzeri*), subtriangular (*B. papalutensis*) or digitiform (*B. cyaneifemur*). Females of *B. rudloffii* have not been described.

Description

**Male holotype**
**Morphology**
Some quantitative characters are given in Table 1.
Coloration and pilosity. Carapace covered by dense shiny coppery pubescence, that masks partially the dark brown colour of the integument (Figures 1A, 2A). Posterior area of carapace bears distinctly thick erect setae (Figure 2B). Leg and pedipalpal segments (except femora) with intermixed copper and light brown setae. Femora black, with iridescent blue tones. Abdomen dark brown, with long and thick red setae and dense short dark brown setae.

Figure 6. *Bonnetina tenuiverpis* sp. n. Female allotype. (A) Carapace; (B) posterior margin of carapace, showing thin setae; (C) ocular area, dorsal view; (D) sternum; (E) labium and maxillae; (F) ventral view of metatarsus and tarsus of left leg III. Scale lines: 1 mm.
Carapace with caput nearly flat and fovea broad and procurved. Ocular area: eight eyes disposed in two rows on markedly elevated tubercle (PLE almost perpendicular to carapace) (Figure 2C); anterior eye row procurved; posterior row, recurved (Figure 2D). Ocular quadrangle width, 1.14; length, 0.86. Clypeus, 0.14 wide. AME circular, diameter, 0.28; ALE ovoid, greater diameter, 0.36; PME ovoid, greater

Figure 7. *Bonnetina tenuiverpis* sp. n. Spermathecae. (A) Female allotype, dorsal view; (B) female allotype, ventral view; (C) female paratype AMNH, dorsal view; (D) female paratype CNAN-T0757, dorsal view. Scale lines: 1 mm.

Figure 8. *Bonnetina tenuiverpis* sp. n. (A) Type locality (near Zuluapan, State of Mexico, Mexico); (B) landscape surrounding type locality. Photos by Carlos E. Santibañez.

Carapace with caput nearly flat and fovea broad and procurved. Ocular area: eight eyes disposed in two rows on markedly elevated tubercle (PLE almost perpendicular to carapace) (Figure 2C); anterior eye row procurved; posterior row, recurved (Figure 2D). Ocular quadrangle width, 1.14; length, 0.86. Clypeus, 0.14 wide. AME circular, diameter, 0.28; ALE ovoid, greater diameter, 0.36; PME ovoid, greater
diameter, 0.26; PLE ovoid, greater diameter, 0.26. Row of erect hairs present between fovea and ocular area.

Chelicerae with seven teeth (left appendage), and seven teeth plus one tiny basalmost tooth (right), close and parallel to the promargin on ventral side.
Sternum (Figure 2E) slightly convex to its centre, covered uniformly by erect thick hairs and other hairs much smaller; with three pairs of sigillae, placed opposite to coxae I, II and III. Labium subtrapezoidal (Figure 2F); middle length, 0.90; anterior width, 0.80; posterior width, 1.25.

Appendage segment lengths (left limbs). Palp: femur, 3.9; patella, 2.5; tibia, 3.0; total, 9.4. Leg I: femur, 5.8; patella, 3.5; tibia, 4.1; metatarsus, 3.6; tarsus, 2.6; total, 19.6. Leg II: femur, 5.1; patella, 2.9; tibia, 3.6; metatarsus, 3.3; tarsus, 2.6; total, 17.5. Leg III: femur, 4.1; patella, 2.5; tibia, 3.0; metatarsus, 4.0; tarsus, 2.4; total, 16.0. Leg IV: femur, 5.5; patella, 3.0; tibia, 4.8; metatarsus, 6.1; tarsus, 2.9; total, 22.3 (Leg formula: leg IV > I > II > III).

Retrolateral face of palpal tibiae with prominent, apically inclined conic-shaped nodule near apex, densely covered by long and thick setae (Figure 2G).

Palpal bulbs (Figures 3, 4) with embolus very slender, with its apical half markedly curved and twisted dorsally and retrolaterally (from base to apex) to the point that in the apex, the ventral structures of the bulb become prolateral. Prolateral inferior, prolateral superior, sperm pore and dorsal keels present. PI keel is mostly serrated and extends from the base of the embolus to half of the space between its most apical denticle and the sperm pore. PS keel extends from close to the base of the embolus to sperm pore level. SP keels extend from the bulb apex to the sperm pore and are folded onto each other, except in the basalmost region, forming the sperm pore; they are not completely fused at the apex, allowing a small pore at embolus tip. D keel, about as long as SP keels, is subapical to the embolus.

Legs I holding organ. Three apophyses near the apex of tibiae I, with separated bases (Figure 5A, B). Prolateral apophysis conical and bent prolaterally, bearing a mega-spine on its internal border; length, 0.34/0.36 (left/right). Retrolateral apophysis subdigitiform, parallel to tibial axis; length, 0.80/0.90. Accessory apophysis semicircular and well developed, bearing three subtriangular megaspines at its apex and a spine on the internal border; length, 0.24/0.20. When flexed, the moderately curved metatarsus I (Figure 5C) folds between prolateral and retrolateral apophyses.

Nodule of 17 granules on basal ventro-retrolateral region of both metatarsi I (Figure 5B).

Femora of palps and legs I and II prolaterally and femora of legs IV retrolaterally covered by pad of simple and ciliated hairs.

Palpal coxae and trochanters with non-plumose setae pro- and retrolaterally.

Scopulae Metatarsi. On legs I entire segment except apex; on legs II apical 5/6; on legs III distal half of segment and on legs IV apical 1/5. Tarsi. On legs I and II entire with few dispersed type B hairs; on legs III divided completely by a band of 1–3 fine hairs; on tarsi IV divided full length by band of 3–4 very thick hairs.

Very dense claw tufts on every leg.

Abdominal urticating hairs. Type III, in dorsal oval patch, located mostly in posterior half of abdomen, covering 0.42 of its length.
Spination pattern (left limbs). Palp: femur p0-0-1. Leg I: femur p0-0-1; tibia v2-2-0 p0-1-1; metatarsus v0-0-1. Leg II: femur p0-0-1; tibia v2-2-3 p0-1-1; metatarsus v2-0-1. Leg III: femur p0-1-1 r0-0-1; tibia v1-1-3 p1-0-1 r0-0-1; metatarsus v1-3-4 p1-1-1 r0-1-1. Leg IV: femur r0-0-1; tibia v2-2-3 r1-0-1; metatarsus v1-2-4 p0-0-2 r0-0-1.

Molecular characterization
Mitochondrial CO1 partial sequence (GenBank accession: KC807369):

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g ttg cct cct tcg ttg ttt tta tta gta tta tct tct ttg act gat gta ggg gtt ggg gct ggg tga
act att tat ccg cct tta tct tct ttg atg ggt cat tct gta ggg gat gat ttt gct aat ttt act ttg
ttg cat tta cct tct gct tga aat act atg ata aat atg cgt gca tct gga ata tga atg ggg gct att
cct ttg ttg ttt gta tga tgg att aca act gta tta tgg tta tga ttc tta cct gca atg gta gtt ttt
act gct aat acg cgt gga aat ggt aat ttt act tga cca tgg tgg act gct act atg gta gtt gaa ggt
ttt gta aat acg cgt gga aat ggt gct gct gta tct ttt aat gca cct tta ttt aat gga atg gta
att ttt gta ggb ggg ggt gct ttt gta gtt gga cate ggt act atg ttt gct gct gct gtt ggg gat
ttt act gct gtt gga gta gta tta gta ggg gct ggg gtt ggg ggt ggg ggt ggt ggt ggt ggt ggt
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Preservation state
The specimen is in optimal conditions, stored in a flask of 80% ethanol. Right leg III preserved in 96% ethanol at −20°C for molecular studies. Left pedipalpal bulb is housed apart, coated with gold.

Female allotype
Morphology
Some quantitative characters are given in Table 1

Coloration and pilosity. As for holotype, but the carapace pubescence is less dense and the femora lack blue tones (Figures 1B, 6A). Posterior area of carapace bears distinct setae, considerably thinner than those on the holotype (Figure 6B).

Carapace with caput elevated. Fovea procurved. Anterior eye row procurved; posterior row recurved (Figure 6C). Ocular quadrangle width, 1.56; length, 0.92. Clypeus, 0.22 wide. AME circular, diameter, 0.40; ALE ovoid, greater diameter, 0.52; PME ovoid, greater diameter, 0.42; PLE ovoid, greater diameter, 0.40. Row of erect hairs present between fovea and ocular area.

Chelicerae with nine teeth plus one tiny basalmost tooth (left appendage) and eight teeth plus one tiny basalmost tooth (right), close and parallel to the promargin on ventral side.

Sternum (Figure 6D) slightly convex, covered uniformly by thick erect hairs and other hairs much smaller; with three pairs of sigillae, placed opposite to coxae I, II and III. Labium subtrapezoidal; middle length, 1.25; anterior width, 1.15; posterior width, 2.05 (Figure 6E).
Appendage segment lengths (left limbs). Palp: femur, 5.7; patella, 3.8; tibia, 3.6; tarsus, 3.8; total, 16.9. Leg I: femur, 7.5; patella, 5.0; tibia, 3.9; metatarsus, 4.2; tarsus, 2.9; total, 23.5. Leg II: femur, 6.0; patella, 4.0; tibia, 3.7; metatarsus, 4.3; tarsus, 3.1; total, 21.1. Leg III: femur, 5.7; patella, 4.2; tibia, 3.8; metatarsus, 5.3; tarsus, 3.1; total, 22.1. Leg IV: femur, 8.0; patella, 4.8; tibia, 5.9; metatarsus, 7.4; tarsus, 4.0; total, 30.1 (Leg formula: leg IV > I > III > II).

Scopulae. Metatarsi. On legs I entire; on legs II entire except base; on legs III apical half prolaterally and apical third retrolaterally (Figure 6F); on legs IV apical fourth. Tarsal scopulae and femoral pads as on holotype.

Coxae and trochanters of palps and legs I and II as in holotype, covered by non-plumose hairs.

Abdominal urticating hairs. Type III, in oval dorsal patch (with rear part distinctly narrower), located mostly in posterior half of abdomen, covering 0.43 of its length.

Spination pattern (left limbs). Palp: femur, p0-0-1 r0-0-1; tibia, v0-1-4. p0-1-0 r0-1-0. Leg I: femur, p0-0-1; tibia, v0-1-0 p0-2-0; metatarsus, v1-1-1. Leg II: femur, p0-0-1; tibia, v0-1-1 p0-1-1; metatarsus, v2-0-2. Leg III: femur, p0-0-1 r0-0-1; tibia, v3-2-2 p2-2-1 r1-0-1; metatarsus, v1-1-3 p2-2-2 r0-1-1. Leg IV: femur, r0-0-1; tibia, v2-2-3 r0-1-1; metatarsus, v8 p1-1-3 r1-0-1.

Only one spermatheca subdigitiform (Figure 7A, B), symmetrical (in dorsal and ventral views) with well-defined base, neck and fundus; length, 1.02; base width, 0.72. A wide poorly sclerotized atrium is at its base.

Molecular characterization
Mitochondrial CO1 partial sequence (GenBank accession: KC807370):

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ttg ttg ccc cct tcc ttg ttt tta tta nta tta tct tct ttn act gat gta ggg gtt ggg gct ggg
tga act att tat ccc cct tta tct tct ttt act gat cat tct ggt gga ggg atg gat ttt gct gat ttt
tct ttg cat ttg gct ggg gct tcc tct act att atg ggg ctt gta aat ttt att act act gtt ata aat atg
cgt gca tct gga ata tcc atc gaa cgt att cct tct ttt gta tct gta atg att cca act gta
tta tta tta tct tta ccc ggt ttt gct ggg ggt gct act att atc tta tta tct gta atg cag aat ttt aat
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Variation
The variation of some quantitative characters in the three females examined is summarized in Tables 1 and 2. None of the females has thick erect setae on the posterior margin of carapace, although setation in this area is distinct from the rest of carapace.

All have a row of erect hairs that extends between ocular area and fovea.

The shape of the urticating hairs patch varies, although it is oval for all specimens.

Coxae and trochanters lack plumose setae and hairs in all specimens.

The pubescence of the carapace is considerably denser on the male than on the three females. The coloration is very similar in the two females that were examined alive.

Spermathecae of the three specimens share the same structural pattern (Figure 7), although that of AMNH paratype is wider. The AMNH paratype also has tarsi on all the legs divided, probably due to its smaller size (Pérez-Miles 1994).

The holotype and allotype differ only by one base pair of the CO1 sequenced region (0.1% uncorrected pairwise sequence divergence).

Distribution
*Bonnetina tenuiverpis* is known only from the vicinity of Zuluapan town, situated in the State of Mexico and geographically at 1250 m asl in the Trans-Mexican Volcanic Belt (Eje Neovolcánico), which extends from Pacific to Atlantic coast of Central–Southern Mexico.

Natural history
The specimens looked faded when collected in August 2012, and all moulted in captivity in September and October. The only male matured on 11 October, suggesting that the breeding season might include fall. The only known population lives in an absolutely open, very rocky grassland (Figure 8) that is frequently used by cattle. Dominant vegetation of the area is tropical deciduous forest. The specimens were found mainly on the edges of middle sized rocks (20–150 cm diameter), in shallow depressions bordered by silk, presumably dug by them. Two specimens of the tarantula *Brachypelma* cf. *albiceps*, three of another *Bonnetina* species and few of *Diplocentrus* scorpions were also found syntopically. These three species could be competing for resources or even preying on *B. tenuiverpis*.

*Bonnetina juxtantricola* sp. nov.
urn:lsid:zoobank.org:act:7A6A6CC2-E07C-4CFD-910D-0255D69F7AD4
(Figures 9–13; Table 1)

**Holotype.** ♂ (CNAN-T0758). MEXICO: Guerrero state: Quechultenango municipality: Outside of Juxtlahuaca Cavern: 17.4387°, −99.1595°: 930 m asl. 12 September 2005. Alejandro Valdez & Héctor Montaño, cols. Under a stone.

**Paratype.** ♀ (CNAN-T0759): same locality, collectors and microhabitat as holotype. 19 July 2005.
Etymology

The specific name is composed from the Latin preposition *juxta* (nearby), the noun *antrum* (cavern) and the suffix *cola* (dweller). The name can be translated as ‘nearby-cavern dweller’ and makes reference to the type locality of this species, which is the surroundings of an archaeologically very valuable cave.

Diagnosis

Males differ from those of all known *Bonnetina* species in the shape of the palpal bulbs. Additionally, from *B. rudloffii*, *B. alagoni*, *B. papalutensis*, *B. tenuiverpis* and...
Figure 10. *Bonnetina juxtantricola* sp. n. Male holotype, left pedipalpal bulb, stereomicroscope images. (A) Proximal view; (B) distal view; (C) dorsal view; (D) ventral view. Scale line: 1 mm.

Figure 11. *Bonnetina juxtantricola* sp. n. Male holotype, right pedipalpal bulb, SEM images. (A) Embolus, proximal view; (B) sperm pore; (C) embolus, ventral–distal view; (D) apex, frontal view. Arrow colours represent keels: proximal superior (black), sperm pore (white), proximal sub-apical (grey) and proximal inferior (hatched). Chevron: sperm pore.
B. tanzeri in that PI bulbal keel is not denticulate as on those species. It also differs from males of B. aviae in the lesser retrolateral bend of the embolus, which describes an angle of approximately 30° with the axis of the bulb and about 70° in B. aviae. The male tibia I accessory apophysis is present but considerably less developed than that of B. cyaneifemur, B. rudloffi, B. papalutensis, B. tanzeri and B. tenuiverpis. Females have domiform spermatheca and so differ from the species that have it digitiform (B. cyaneifemur), subdigitiform (B. tenuiverpis), subtriangular (B. papalutensis) or flattened (B. tanzeri). Females differ from those of B. aviae and B. alagoni by presenting very distinctly thick, erect setae on the posterior area of carapace.

Description

Male holotype

Morphology

Some quantitative characters are given in Table 1.

Carapace with caput nearly flat; fovea broad and procurred (Figure 9A). Posterior area of carapace bears distinctly thick erect setae (Figure 9B). Ocular area: eight eyes disposed in two rows on elevated tubercle (PLE oblique to carapace) (Figure 9C); anterior eye row procurred; posterior row recurved (Figure 9D). Ocular quadrangle width, 1.36; length, 0.94. Clypeus, 0.32 wide. AME circular, diameter, 0.36; ALE ovoid, greater diameter, 0.40; PME ovoid, greater diameter, 0.34; PLE ovoid, greater diameter, 0.34.

Chelicerae with seven teeth (left appendage) and seven teeth plus one tiny apical-most tooth (right), close and parallel to the promargin of ventral side.

Sternum (Figure 9E) slightly convex to its centre, covered uniformly by thick erect hairs and other hairs much smaller; with three pairs of sigillae, placed opposite to
coxae I, II and III. Labium subtrapezoidal; middle length, 0.96; anterior width, 0.90; posterior width, 1.70 (Figure 9F).

**Appendage segment lengths (left limbs).** Palp: femur, 4.9; patella, 3.2; tibia, 4.6; total, 12.7. Leg I: femur, 8.1; patella, 4.3; tibia, 6.0; metatarsus, 5.6; tarsus, 4.2; total, 28.2. Leg II: femur, 6.7; patella, 4.1; tibia, 5.4; metatarsus, 5.2; tarsus, 3.8; total, 25.2. Leg III: femur, 6.1; patella, 3.3; tibia, 4.5; metatarsus, 6.3; tarsus, 3.9; total, 24.1. Leg IV: femur, 8.2; patella, 3.8; tibia, 6.6; metatarsus, 9.1; tarsus, 4.5; total, 32.2 (*Leg formula*: leg IV > I > II > III).
Retrolateral face of palpal tibiae with prominent, apically inclined, cone-shaped nodule near apex, covered by long thick setae.

Palpal bulbs (Figures 10, 11) robust with thick embolus, that is moderately curved and twisted dorsally and retrolaterally (from base to apex) so that in the apex, the ventral structures of the bulb become prolateral. Prolateral inferior, prolateral superior, sperm pore and prolateral sub-apical keels present. PI keel is smooth and extends from close to the base of the embolus to near to sperm pore; it ends sharply at its apex, forming a distinct prominence. PS keel extends from embolus base to apex. SP keels extend from the bulb apex to the sperm pore and are folded onto each other, except in the basalmost region; they are fused at the apex, not forming a pore at the embolus tip. PSA keel, about as long as SP keels, is subapical to the embolus and located between PS and sperm pore.

Legs I holding organ. Tibiae I with three apophyses near the apex, all parallel to segment axis (Figure 12A, B). Prolateral apophysis tubular, blunt at its apex and bearing a megaspine on its internal border; length, 0.48/0.48 (left/right). Retrolateral apophysis curved, acute at apex; length, 1.20/1.10. Accessory apophysis very poorly developed, bearing a subtriangular megaspine at its apex and a spine on the internal border; length, 0.14/0.10. When flexed, the moderately curved metatarsus I (Figure 12C) folds between prolateral and retrolateral apophyses. Metatarsi I with nodule of 16 (left) and 19 (right) granules on basal ventro-retrolateral region (Figure 12B). Two (left) and one (right) small rounded structures, presumably modified spines, on the basal ventro-prolateral region of metatarsi I (Figure 12A, C).

Femora of palps and legs I and II prolaterally and femora of legs IV retrolaterally covered by pads of ciliated hairs.

Palpal coxae and trochanters with non-plumose setae pro- and retrolaterally.

Scopulae Metatarsi. On legs I entire segment except the basal 1/5; on legs II apical 2/3; on legs III on apical half (left) and apical 1/3 (right) of segment; on legs IV apical 1/5. Tarsi. On legs I and II entire with few dispersed type B hairs; on legs III divided completely by a band of 1–3 fine hairs; on legs IV divided full length by band of 2–4 very thick hairs.

Claw tufts dense on every leg.

Abdominal urticating hairs. Type III, in patch located dorsally in central-posterior half of abdomen, covering 0.31 of its length. It could have been oval or subrectangular, according to the mark, but only few hairs remain, located on its anterior border.

Spination pattern (left limbs). Palp: femur p0-0-1; tibia p1-2-0. Leg I: femur p0-0-1; tibia v3-2-2 p0-1-1; metatarsus v0-0-1 p0-0-1. Leg II: femur p0-0-1; tibia v3-2-3 p0-1-1 r1-0-0; metatarsus v2-1-3 p0-1-1. Leg III: femur p0-1-1; tibia v1-2-2 p0-2-1 r1-1-1; metatarsus v2-3-3 p1-1-2 r0-1-1. Leg IV: femur r0-0-1; tibia v1-2-3 p0-1-1 r0-2-1; metatarsus v10 p1-2-1 r0-1-1.

Preservation state

The specimen is in good condition, stored in a flask of 80% ethanol. Both pedipalpal bulbs are housed apart, coated with gold. The specimen is homogeneously reddish-brown due to the length of time it has remain preserved.
Allotype female

Morphology

Some quantitative characters are given in Table 1.

Carapace (Figure 13A) with caput elevated. Fovea procurved. Posterior area of carapace bears distinctly thick erect setae (Figure 13B). Anterior eye row clearly procurved; posterior row recurved (Figure 13C). Ocular quadrangle width, 1.44; length, 0.94. Clypeus, 0.22 wide. AME circular, diameter, 0.36; ALE ovoid, greater diameter, 0.52; PME ovoid, greater diameter, 0.30; PLE ovoid, greater diameter, 0.34.

Chelicerae with nine teeth plus one tiny basalmost tooth (left appendage) and eight teeth plus one tiny basalmost tooth (right), close and parallel to the promargin on ventral side.

Sternum (Figure 13D) slightly convex, covered uniformly by erect thick hairs and other hairs much smaller; with three pairs of sigillae, placed opposite to coxae I, II and III. Labium (Figure 13E) subtrapezoidal; middle length, 1.30; anterior width, 0.96; posterior width, 2.02.

Appendage segment lengths (left limbs). Palp: femur, 5.7; patella, 3.5; tibia, 3.9; tarsus, 4.1; total, 17.2. Leg I: femur, 7.8; patella, 5.0; tibia, 5.3; metatarsus, 4.1; tarsus, 2.3; total, 24.5. Leg II: femur, 6.6; patella, 3.7; tibia, 4.7; metatarsus, 4.0; tarsus, 3.1; total, 22.1. Leg III: femur, 6.0; patella, 3.9; tibia, 4.1; metatarsus, 5.3; tarsus, 3.3; total, 22.6. Leg IV: femur, 8.0; patella, 4.3; tibia, 6.0; metatarsus, 7.8; tarsus, 3.7; total, 29.8 (Leg formula: leg IV > I > III > II).

Only one spermatheca, domiform in shape (Figure 13F, G). It is fully sclerotized ventrally, while dorsally apically only, forming a crescent-shaped figure. Length, 0.46; base width, 0.80.

Scopulae Metatarsi. Legs I: entire on proventral half, 3/4 apical on retroventral half. Legs II: 3/4 apical on anterior half, 1/4 apical on posterior half. Legs III: 1/3 apical. Legs IV: 1/5 apical. Tarsi. As in holotype.

Palpal coxae and trochanters with non-plumose setae pro- and retrolaterally.

Femoral pads as in holotype.

Abdominal urticating hairs. Type III, in oval dorsal patch (with rear part moderately narrower), located dorsally on central-posterior half of abdomen, covering 0.33 of its length.

Spination pattern (left limbs). Palp: femur, p0-0-1; tibia, v0-3-3 p0-1-0. Leg I: femur, p0-0-1; tibia, v0-1-0; metatarsus, v0-1-1 p0-0-1. Leg II: femur, p0-0-1; tibia, v0-1-4 p0-1-1; metatarsus, v1-1-2 p0-1-0. Leg III: femur, p0-0-1; tibia, v2-2-4 p1-1-0 r1-1-1; metatarsus, v0-3-5 p1-2-2 r0-1-1. Leg IV: femur, r0-0-1; tibia, v1-2-3 p0-1-1 r1-0-2; metatarsus, v9 p0-2-1 r0-2-1.

Preservation state

The specimen is in good condition, stored in a flask of 80% ethanol. Genital area is housed in a plastic vial inside the flask. The specimen is homogeneously reddish-brown, due to the length of time it has remain preserved.
Distribution

*Bonnetina juxtantricola* is known only from the vicinity of Juxtlahuaca cavern, situated in Guerrero state and geographically at 930 m asl in the Sierra Madre del Sur, a mountain range that extends parallel to the Pacific Ocean coast between Central and Southern Mexico.

Natural history

Very little is known of the natural history of this species. The only known male was collected in September, suggesting that this month could be part of its breeding season. Both male and female were collected under stones in a tropical deciduous forest.

Discussion

One of the main challenges that this study faced was determining the homology and therefore the appropriate nomenclature for the male pedipalpal bulbs’ structures of the new species described. These organs are fundamental in theraphosine spiders’ taxonomy as they are very often used as the cornerstone to differentiate between genera and species in the group.

By using SEM images, it was possible to find a considerable amount of information in the two new *Bonnetina* species. Nevertheless, the genus has never been included in phylogenetic or comparative morphology analyses, and belongs to a subfamily of more than 50 genera (Ortiz 2008) with unclear relationships. That fact, joined to the wide diversity of bulbal structures in the subfamily (Bertani 2000), makes it very hard to apply the congruence test, which is the most decisive homology test as it helps to discriminate homology from homoplasy (Patterson 1982).

In the description of *Bonnetina papalutlensis*, Mendoza-Marroquín (2012) mentioned the presence of a Subapical denticulate keel (*sensu* Bertani 2000). That keel seems to be clearly homologous to the keel that we name here as prolateral inferior, which is also denticulate in *B. tenuiverpis*. Bertani (2000) found that prolateral superior and prolateral inferior keels are present in most of the 27 studied genera of Theraphosinae and he defined them as ‘two parallel keels … in the prolateral area of the embolus’. This is exactly how the two longest keels on the bulbs of *B. tenuiverpis* and *B. juxtantricola* look. Additionally, subapical keel was found always associated to apical keel, which is probably absent in *B. juxtantricola* and definitely absent in *B. tenuiverpis*. After Bertani (2000), other authors have considered prolateral superior and prolateral inferior as the basic keels in Theraphosinae (e.g. Fukushima et al. 2008; Pérez-Miles et al. 2008; Yamamoto et al. 2012).

Concerning the keels that are described for the first time in this contribution, there is no evidence to homologize dorsal and prolateral sub-apical keels to any of the already recognized and named keels. Although prolateral sub-apical keel could be alleged to be positionally similar to apical keel, and dorsal keel to retrolateral keel, in the context of phylogenetic uncertainty, there are important objections to assume their homology: (1) apical keel is not as common in Theraphosinae as prolateral superior and prolateral inferior keels, while retrolateral keel is only present in a small number of genera (Bertani 2000); (2) the low structural complexity of these keels is likely to hide independent acquisitions (homoplasy); and (3) each of these keels is
present in only one of the species of *Bonnetina* herein described, revealing variation and thus remarkable homoplasy even at generic level.

Assigning names to structures based on very weak evidence is equivalent to assuming homology arbitrarily and, for example, can lead to erroneous character scoring and conclusions in morphological phylogenetic analyses. Not giving names to these structures is a serious impediment to their use in the taxonomy of the group; this is not desirable, considering the noticeable differences that are found among both species herein described and other *Bonnetina* species (Ortiz & Francke, unpublished observations). We conclude that the more reasonable choice is to consider them autapomorphic and thus give new names to keels that are especially difficult to homologize to those already described. If in the future Theraphosinae bulbal structures are clarified and it is found that prolateral sub-apical and dorsal keels are homologous to other structures previously named, it will be easy to modify *Bonnetina* bulbal nomenclature in consequence.

On the other hand, we think that there is no doubt of the authenticity of sperm pore keels, as nothing similar to those structures has been described before.

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**References**

Bertani R. 2000. Male palpal bulbs and homologous features in Theraphosinae (Araneae, Theraphosidae). *J Arachnol.* 28:29–42.

Bertani R. 2001. Revision, cladistic analysis, and zoogeography of *Vitalius, Nhandu,* and *Proshapalopus,* with notes on other theraphosine genera (Araneae, Theraphosidae). *Arq Zool.* 36:265–356.

Cooke JAL, Roth VD, Miller FH. 1972. The urticating hairs of theraphosid spiders. *Am Mus Novit.* 2498:1–43.

Estrada-Alvarez J, Locht A. 2011. Descripción de *Bonnetina aviae* sp. n. de México (Araneae: Theraphosidae: Theraphosinae) [Description of *Bonnetina aviae* sp. n. from Mexico (Araneae: Theraphosidae: Theraphosinae)]. Bol SEA. 48:151–155. Spanish.

Font Quer P. 1953. Diccionario de Botánica [Dictionary of botany]. Barcelona: Labor. 1244 pp.

Fukushima CS, Nagahama RH, Bertani R. 2008. The identity of *Mygale brunnipes* C.L. Koch 1842 (Araneae, Theraphosidae), with a redescription of the species and the description of a new genus. *J. Arachnol.* 36:402–410.
