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First description of the male spider *Pacifiphantes magnificus* (Chamberlin & Ivie) (Araneae: Linyphiidae)

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Abstract

The spider *Pacifiphantes magnificus* (Chamberlin & Ivie 1943)(Linyphiidae), originally described as a member of the genus *Bathyphates,* is redescribed and the first description of the male is presented. DNA barcoding was used to test the conspecificity of specimens from different collection events and to help match specimens from different sexes.

Key words: *Pacifiphantes, Bathyphantes, Porrhomma, Kaestneria,* DNA barcoding, COI, species description, taxonomy

Introduction

Originally described only from females, the spider *Pacifiphantes magnificus* (Chamberlin & Ivie 1943) was included as a close relative of *Bathyphantes approximatus* (O. Pickard-Cambridge 1871) by Ivie (1969) in his *Bathyphantes* revision. Eskov & Marusik (1994) moved the species to the genus *Pacifiphantes* Eskov and Marusik 1994 upon the designation of the genus and generic type *P. zakharovi* Eskov & Marusik 1994, however, no comments were made as to what warranted the transfer from *Bathyphantes,* and at that time no male *P. magnificus* had been identified for comparison to *P. zakharovi*.

Linyphiid generic and species identifications can be difficult. The most commonly used method relies on morphological characters which may be homoplasious (Arnedo et al. 2009). However, recent works have shown the utility of DNA barcoding to highlight phylogenetic patterns, which may help to place uncertain species in appropriate genera (Barrett & Hebert 2005, Robinson et al. 2009). These studies have shown that generic placement and genetic divergence appear to be correlated in many spiders. DNA barcoding also has the ability to pair conspecifics from different collection events. With species in which the conspecific morphology is not obvious (e.g. due to sexual dimorphism) the barcoding technique is very powerful in exposing conspecific relations (Barrett & Hebert 2005, Robinson et al. 2009).

Here we describe for the first time the male and internal female genitalia of the linyphiid spider *Pacifiphantes magnificus*. We also produced sequences of the mitochondrial gene COI for *P. magnificus* specimens and used DNA barcoding methods to confirm conspecifics and examine the generic placement of *P. magnificus*.

Methods

Comparison specimens were obtained from the University of Alaska Museum, Fairbanks (Alaska), the Burke Museum of Natural History, Seattle (Washington), and from the American Museum of Natural History (New York). Measurements were made with an optical micrometer and are expressed in mm. Abbreviations: Tm—metatarsus trichobothrium, followed by the leg number. Chaetotaxy patterned as dorsal-prolateral-retrolateral-ventral. A—atrium, BP—bursal plate, CD—copulatory duct, CO—copulatory opening, E—embolus, FD— fertilization duct, L—Lamella, PC—paracymbium, P—parmula, R—radix, S—spermatheae, SPT—suprategulum, T—tegulum.

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DNA Barcode Analysis.—Specimens used for barcode analysis (UAM100046066, UAM100046555) had DNA extracted using the right third leg. DNA was extracted using a Qiagen DNeasy Tissue Kit (www.qiagen.com) following the manufacturers instructions except for; -80C ethanol, and a 100 ul elution twice using 65C AE buffer, which increased DNA yields.

Polymerase Chain Reaction (PCR) amplification of the mtDNA COI gene was conducted at the CORE lab, University of Alaska, Fairbanks. PCR mixture consisted of 12.5 ul GoTaq master mix (www.promegna.com), 2 ul LCO-1490 5'-GGTCAACAAATCATAAAGATATTGG (Folmer et. al. 1994), 2ul C1-N-2776-spider 5'-GGATAATCAGAATANCGNCGAGG (Vink et al. 2005), 1 ul MgCl2, 8.5 ul DNA free water, and 4 ul of DNA template. PCR was conducted using a PTC-255 thermocycler (MJ Research Peltier). The PCR temperature profile had an initial separation stage of 94C for 4 min; 50 cycles of 30 s at 94C, 45s at 48C, 1 min at 72C; and a final elongation stage of 72C for 10 min. PCR clean up and sequencing was conducted at the High Through-Put Genomic Unit at the University of Washington (www.htseq.org). This created a 1180 bp section of the COI gene, which was cut to 680 bp for comparison with shorter publicly available sequences. Assembled sequences were submitted to Genbank, reference numbers JF416293 and JF416294.

Comparison sequences were obtained from the BOLD (The Barcode of Life Data Systems, www.boldsystems.org, Ratnasingham & Hebert 2007) and Genbank (http://blast.ncbi.nlm.nih.gov) for any species mentioned in Ivie (1969), Hormiga (1999) or Arnedo et al. (2009) as being relatives of *Bathyphantes*. An attempt was made to find comparison species represented by at least two sequences which allowed us to assess generic placement and remove the possibility of identification or sequence error. Sequences of 25 species representing 18 pimoid and linyphiid genera were located and used in the analysis (Table 1).

Forward and reverse reads were assembled using 4peaks v 1.7.2 (http://mekentosj.com) to survey the chromatograms, and MESQUITE v 2.72 (Maddison & Maddison 2009, www.mesquiteproject.org) to create contiguous sequences and check for ambiguities. Sequences were aligned using Clustal X v 2.0.12 (Thompson et al. 1997), and then checked for odd gaps and alignment errors against chromatograms when available. Alignment was verified by translation to protein and comparison to protein sequences AAX40574.1 and ABF59654.1 obtained from Genbank. Aligned sequences were used to construct a Neighbor Joining tree using PAUP* v 4.0b10 (Swofford 2002) with K2P distances.

Results

Taxonomy

Family Linyphiidae Blackwall, 1859
Genus *Pacifiphantes* Eskov & Marusik, 1994
***Pacifiphantes magnificus* (Chamberlin & Ivie 1943)**
Bathyphantes magnificus Chamberlin & Ivie 1943; Ivie 1969
P. magnificus Eskov & Marusik 1994

Type material.—Holotype female, CANADA, *British Columbia*, Vancouver Island, Lake Cameron [49.292 N 124.619 W, 189m el.], 13 Sept 1935, Coll: R. V. Chamberlin and W. Ivie. (not examined, the specimen was deposited in the AMNH collection but so far we have not been able to locate it).

Material examined.—CANADA: BRITISH COLUMBIA: 1F, Terrace (54.31N, 128.36W) AMNH-Ivie (1969); 1F, Inverness (54N, 130W) AMNH; USA: ALASKA: 1M, Prince of Wales Island, Forest Rd. 2056100 (55.894N, 133.071W) UAM100046555; 1F, Sumez Island, Port Santa Cruz (55.253N, 133.421W) UAM100046066; 1F, Lituya Bay, N. of Echo Creek (58.709N, 137.702W) Burke230A; 3F, Lituya Bay, N. of Echo Creek (58.714N, 137.694W) Burke245; OREGON: 1F, 5 mi E of Detroit (44.44N, 122.05W) AMNH-Ivie(1969); WASHINGTON: 1F, King Co, N. slope Tiger Mtn. (47.471N, 121.933W) Burke168d; 1F, 5 mi E. of McCleary (47.03N, 123.1W) AMNH-Ivie (1969);

Diagnosis.—Specimens of *Pacifiphantes magnificus* can be separated from all other *Pacifiphantes* species by the embolus forming a single loop, located on the prolateral axis of the palp (Figure 1), and the copulary ducts forming a large anterior area, often appearing folded or twisted (Figures 5 and 6).

TABLE 1. List of comparison sequences used in the Neighbor Joining analysis. Species, sequence database and individual sequence record numbers are listed.

Species	Database	Specimen Record
Agnyphantes arboreus	BOLD	SPIAL070
Agnyphantes arboreus	BOLD	SPIAL195
Agnyphantes arboreus	BOLD	SPIAL271
Australolinyphia remota	GenBank	FJ838649
Bathyphantes brevipes	BOLD	SPIAL198
Bathyphantes brevipes	BOLD	SPIAL210
Bathyphantes brevipes	BOLD	SPIAL222
Bathyphantes brevipes	BOLD	SPIAL281
Bathyphantes brevipes	BOLD	SPICH1097
Bathyphantes brevipes	BOLD	SPICH426
Bathyphantes brevipes	BOLD	SPICH499
Bathyphantes brevipes	BOLD	SPICH796
Bathyphantes brevipes	BOLD	SPICH797
Bathyphantes brevipes	BOLD	SPICH803
Bathyphantes brevipes	BOLD	SPICH812
Bathyphantes brevis	BOLD	SPICH255
Bathyphantes brevis	BOLD	SPICH257
Bathyphantes brevis	BOLD	SPICH263
Bathyphantes brevis	BOLD	SPICH501
Bathyphantes brevis	BOLD	SPICH533
Bathyphantes brevis	BOLD	SPICH546
Bathyphantes canadensis	BOLD	BBCAN388
Bathyphantes canadensis	BOLD	SPICH292
Bathyphantes canadensis	BOLD	SPICH296
Bathyphantes gracilis	GenBank	FJ838650
Bathyphantes gracilis	GenBank	FJ899797
Bathyphantes gracilis	GenBank	FJ899798
Bathyphantes orica	BOLD	SPIAL025
Bathyphantes orica	BOLD	SPIAL037
Bathyphantes orica	BOLD	SPIAL061
Bathyphantes orica	BOLD	SPIAL073
Bathyphantes pallidus	BOLD	BBCAN109
Bathyphantes pallidus	BOLD	BBCAN189
Bathyphantes pallidus	BOLD	BBCAN245
Bathyphantes pallidus	BOLD	BBCAN738
Bathyphantes pallidus	BOLD	BBCAN753
Bathyphantes pallidus	BOLD	SPICH485
Bathyphantes pallidus	BOLD	SPICH799
Bathyphantes pallidus	BOLD	SPISH038
Bathyphantes pallidus	GenBank	AY944732
Bathyphantes tongulensis	GenBank	EF128165
Bathyphantes tongulensis	GenBank	EF128166
Diplostyla concolor	BOLD	BBCAN428
Diplostyla concolor	GenBank	FJ838651
Haplinis diloris	GenBank	FJ838657
Incestophantes washingtoni	BOLD	SPICH222
Incestophantes washingtoni	BOLD	SPICH273
Incestophantes washingtoni	BOLD	SPICH289
Incestophantes washingtoni	BOLD	SPICH290
Incestophantes washingtoni	BOLD	SPICH329
Incestophantes washingtoni	BOLD	SPICH352
Incestophantes washingtoni	BOLD	SPICH369
Incestophantes washingtoni	BOLD	SPICH374
Incestophantes washingtoni	BOLD	SPICH996

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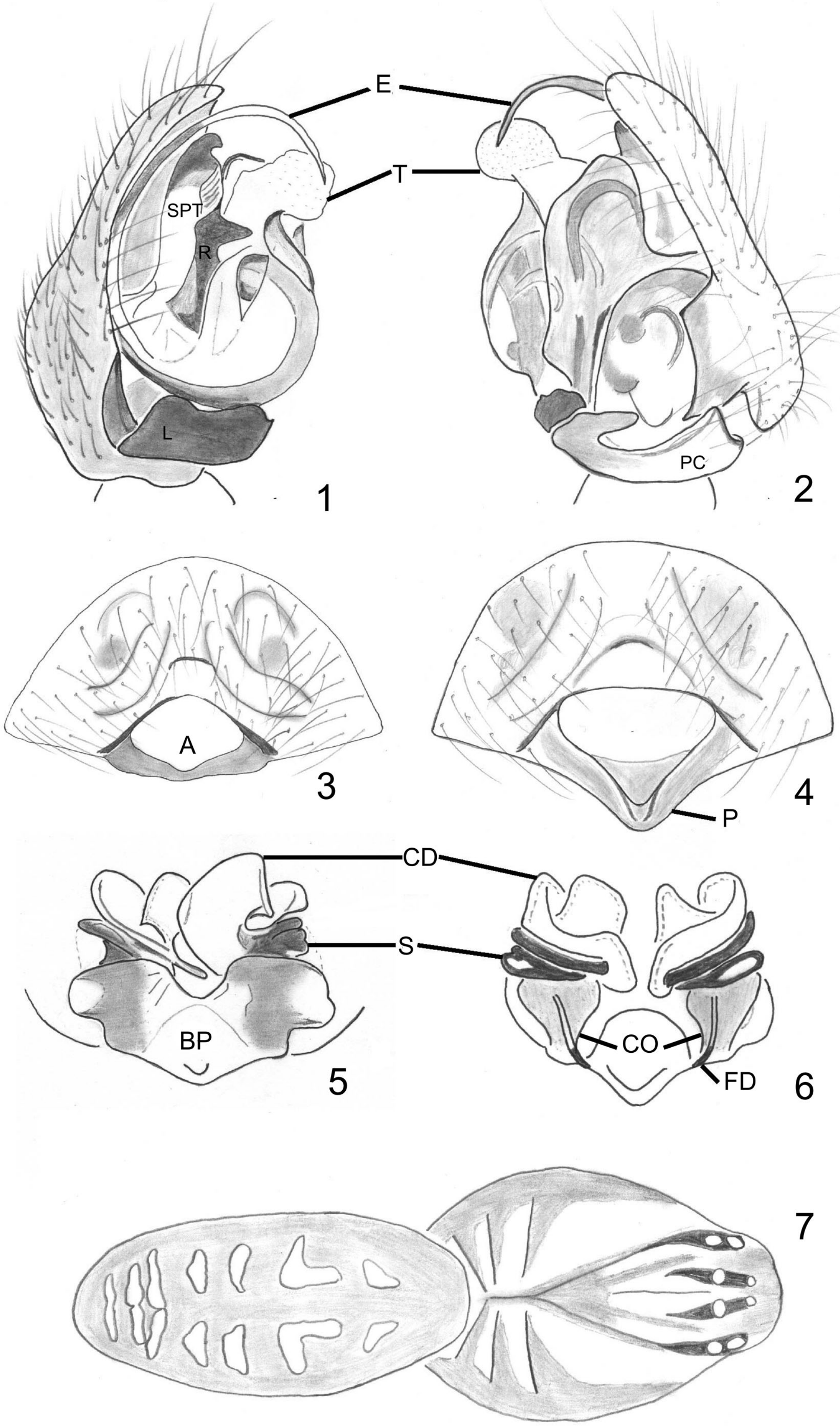
TABLE 1. (Continued)

Species	Database	Specimen Record
Kaestneria pullata	BOLD	BBCAN782
Kaestneria pullata	BOLD	SPICH1084
Kaestneria pullata	BOLD	SPICH271
Kaestneria pullata	BOLD	SPICH480
Kaestneria pullata	BOLD	SPICH483
Kaestneria pullata	BOLD	SPICH489
Kaestneria pullata	BOLD	SPICH491
Kaestneria pullata	BOLD	SPICH496
Kaestneria pullata	BOLD	SPICH503
Kaestneria pullata	BOLD	SPICH530
Kaestneria pullata	BOLD	SPICH581
Kaestneria rufula	BOLD	SPICH574
Kaestneria rufula	BOLD	SPICH885
Labulla thoracica	GenBank	AY078694
Laetesia sp.	GenBank	FJ838659
Lepthyphantes alpinus	BOLD	BBCAN570
Lepthyphantes alpinus	BOLD	SPICH798
Lepthyphantes alpinus	BOLD	SPICH800
Lepthyphantes alpinus	BOLD	SPICH801
Lepthyphantes alpinus	BOLD	SPICH802
Lepthyphantes alpinus	BOLD	SPICH804
Lepthyphantes alpinus	BOLD	SPICH805
Lepthyphantes alpinus	BOLD	SPICH806
Linyphantes orcinus	BOLD	SPIAL026
Linyphantes orcinus	BOLD	SPIAL147
Linyphantes orcinus	BOLD	SPIAL159
Linyphantes orcinus	BOLD	SPIAL201
Linyphantes orcinus	BOLD	SPIAL206
Linyphantes orcinus	BOLD	SPIAL207
Linyphantes orcinus	BOLD	SPIAL212
Linyphantes orcinus	BOLD	SPIAL218
Linyphantes orcinus	BOLD	SPIAL219
Linyphantes orcinus	BOLD	SPIAL230
Linyphantes orcinus	BOLD	SPIAL260
Linyphantes orcinus	BOLD	SPIAL272
Linyphantes orcinus	BOLD	SPIAL284
Notholepthyphantes australis	GenBank	FJ838662
Novafronetta vulgaris	GenBank	FJ838663
Pimoa clavata	GenBank	EF567107
Pimoa clavata	GenBank	EF567108
Pocobletus sp.	GenBank	FJ838665
Porrhomma convexum	BOLD	SPIEU150
Porrhomma convexum	BOLD	SPIEU151
Porrhomma convexum	BOLD	SPIEU152
Porrhomma convexum	BOLD	SPIEU153
Porrhomma convexum	BOLD	SPIEU154
Porrhomma convexum	BOLD	SPIEU158
Porrhomma convexum	BOLD	SPIEU159
Porrhomma convexum	BOLD	SPIEU160
Pseudafroneta incerta	GenBank	FJ838666
Tenuiphantes tenuis	GenBank	AY383539
Tenuiphantes tenuis	GenBank	DQ504374
Tenuiphantes tenuis	GenBank	FJ838669
Tenuiphantes tenuis	GenBank	FJ899827



FIGURES 1–7. *Pacifiphantes magnificus* (Chamberlin & Ivie 1943). 1–2 palp UAM100046555, 1—prolateral aspect, 2—retrolateral aspect; 3—Ventral view epigynum, UAM100046066; 4—Ventral view epigynum AMNH Inverness B.C.; 5—Dorsal view epigynum, Burke245; 6—Dorsal view epigynum, UAM100046066, 7—Dorsal habitus UAM100046555. A—atrium, BP—bursal plate, CD—copulatory duct, CO—copulatory opening, E—embolus, FD—fertilization duct, L—lamella, PC—paracymbium, P—parmula, R—radix, S—spermathecae, SPT—suprategulum, T—tegulum.

Description.—Total body length, 3.50–3.90. Carapace low, lacking cephalothoracic sucli. Anterior median eyes smallest, less than one diameter apart, other eyes approximately equal in size. Lateral eyes adjacent, posterior median eyes less than one diameter apart. Posterior median eyes 1.5 diameters from lateral eye rows. Posterior eye row slightly recurved. Chelicerae with three prolateral and no retrolateral teeth. Leg lengths I, II, IV, III. Male leg I about seven times carapace length, female leg I about five time carapace length. TmI ~0.25, TmIV absent, all tibiae with two dorsal spines and no metatarsal trichobothria. Legs with distinct dusky bands.

Male (n=1): Total length = 3.50, carapace length = 1.70, carapace width = 1.20, TmI = 0.25, TmIV absent. Chaetotaxy: F I, 0-1-0-0; F II-IV, 0-0-0-0; Pt I-IV, 1-0-0-0; Ti I, 2-1-1-0, Ti II, 2-0-1-0, Ti III-IV, 2-0-0-0; Mt I-IV, 0-0-0-0. Carapace smooth, yellow. Dusky lines extending off lateral eye rows to fovea. Dark triangular patches extending anteriorly and posteriorly of all eyes. Lateral margins dusky with lines radiating from fovea for each coxa. Sternum dusky. Legs with terminal and medial dusky bands. Abdomen dark with six light chevrons and two light patches adjacent to the heart mark (Figure 7). Venter dusky. Palp with prominent embolus originating from ventral prolateral edge, making almost a complete loop along the lateral axis, exterior of the radix/suprategular assembly (Figure 1). Radix and suprategulum fused along entire length of radix, without tailpiece. Suprategular apophysis large, hook shaped (Figure 1). Lamella short and wide, located medially on prolateral side of palp. Tegulum smooth with distal globular apophysis, somewhat wrinkled. Paracymbium small, cymbium with ectal rectangular expansion extending ventrally by paracymbium (Figure 2).

Female (n=6): Total length = 2.92–3.90, carapace length = 1.33–1.70, carapace width = 1.20–1.33, TmI = 0.24–0.26, TmIV absent. Chaetotaxy: Same as male except; F I, 1-1(2)-0-0. Coloration similar as male. Pedipalp with tarsal claw. Epigynum lacking a ventral plate scape. Parmula low and wide with pit located along posterior edge, often projecting posteriorly in a "V" shape. Atrium roughly triangular (Figures 3 and 4). Large bursal plate (Figure 5). Copulatory opening located on lateral margin of atrium. Copulatory ducts extend laterally from atrium, ventrally of the spermathecae, and proceed anteriorly, expanding in the anterior region, then often folding as they curve medially to the spermathecae. Fertilization ducts extend posteriorly along widest lateral edge of atrium (Figure 6).

Results of the distance analysis (Figures 8) clearly show the monophyly of the independently collected *P. magnificus* specimens in relation to other linyphiid taxa (specimens UAM100046555 and UAM100046066). Additionally, the low amount of genetic difference between the two *P. magnificus* specimens, 0.3%, is a indicator that these are conspecifics. The species clade sister to *Diplostyla* and *Porrhomma* within the general *Bathyphantes* lineage, which is separate from both the *Lepthyphantes* and *Linyphantes* lineages. Both *Bathyphantes* and *Kaestneria* appear polyphyletic in the NJ analysis limiting the use of these DNA barcode results to direct the generic placement of *Pacifiphantes pacificus*.

Distribution.—Known only from the Pacific Coast region of North America from Oregon north to Alaska, USA (Figure 9).

Habitat.—It would appear from the few specimens collected, the difficulty in collecting the single male specimen, and fairly extensive collecting done along the Pacific Coast (Mann & Gara 1980, Crawford 1988, Bennett 2001, Slowik 2006) that this is a rare species. There is nothing specific known about the preferred habitat in which this species may occur as many of the specimens are known from pitfall traps or casual collections. Based on the locations and descriptions of the specimens examined, this species occurs in closed canopy mature forests. Specimens examined were collected from shrubs along creeks, and overwinter pitfall traps.

Discussion.—This morphological examination has shown that the placement of *P. magnificus* in *Pacifiphantes* is likely incorrect. The generic designation for *Pacifiphantes* comments that males have a "very short embolus and suprategular apophysis" (Eskov & Marusik 1994: 49), in which specimens of *P. magnificus* have a long embolus, and large hook shaped suprategular apophysis. Additionally the characters used for the female designation of *Pacifiphantes* are brief and refer only to the delimited parmula (Eskov & Marusik 1994).

Although the generic designation of *Pacifiphantes* excludes *P. magnificus*, clear placement based on morphological characters alone is not obvious. *P. magnificus* shares many epigynal characters with *Porrhomma*. specifically the shape of the atrium and the location and shape of the copulary ducts and spermathecae, for example compare Figure 6 with Eskov & Marusik 1994 figure 48 of *Porrhomma longjiangensis* Zhu & Wang 1983 (=*P. rakanum* Yaginuma & Saito 1981).

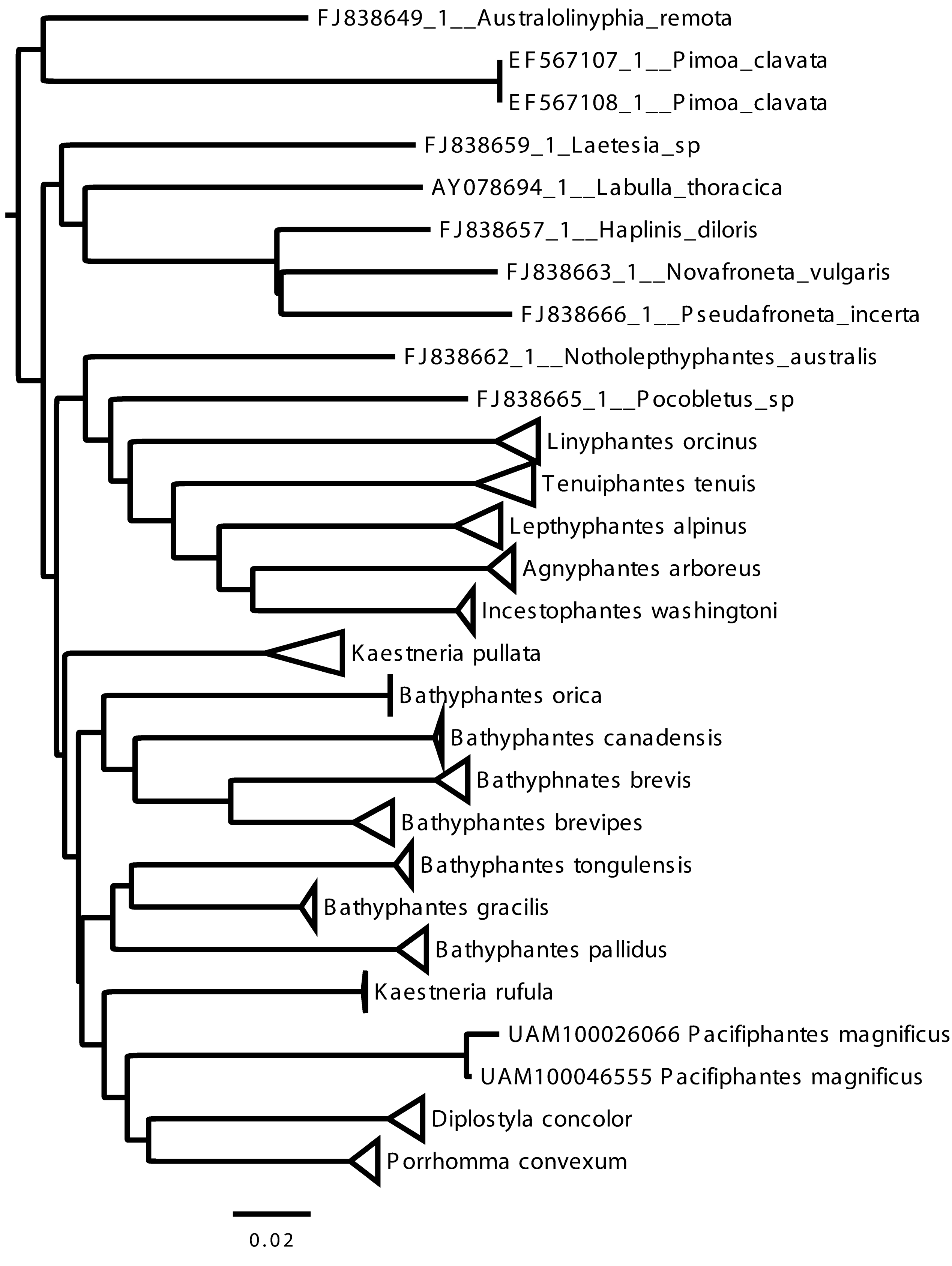


FIGURE 8. Neighbor joining analysis of Kimura 2—parameter (K2P) distances of the barcode region of the COI gene of linyphiid genera and species related to *Pacifphantes magnificus* (Chamberlin & Ivie 1943).

Although the females may show similarities to *Porrhomma,* males of *P. magnificus* have a morphology not typical for the genus, including a reduced radix and a longer embolus free of a terminal sheath (or embolic membrane). Ivie's (1969) hypothesis that *P. magnificus* was sister to *Bathyphantes approximatus* (O. Pickard-Cambridge 1871) is also questioned as the morphology of the palp, particularly the embolus and radix/ suprategulum assembly, differ significantly. Additionally, examination of the internal epigynal structures of the two species fail to support this grouping as *B. approximatus* has highly coiled copulatory ducts and anteriorly placed spermathecae, more typical of the the genus *Microlinyphia* (e.g., see Blauvet 1936: figs. 72 and 77). Additionally *P. magnificus* lacks cephalothoracic sulci, which have been found on *Porrhomma, Bathyphantes,* and *Pacifiphantes* species (Hormiga 1999).

FIGURE 9. Map showing distribution of specimens of *Pacifiphantes magnificus* (Chamberlin & Ivie 1943). Black circles—locations of specimens examined, White circle—type locality (Ivie 1969).

Thus, combined characters from the male and female morphology could be used to associate or dissociate *P. magnificus* to or from many of the other genera contained in the *Porrhomma* group (Millidge 1977), or *Bathyphantes* clade (Hormiga 1999, Arnedo et al. 2009). Our DNA barcode results are insufficient to provide a generic placement for *P. magnificus*. Currently relatively few species from the *Bathyphantes* lineage are available for comparison. Additionally, our results find several genera polyphyletic suggesting that more data are needed to address these taxonomic problems. Given the data at hand, the placement of *P. magnificus* remains elusive and we feel that at this time it is best to leave the spider in *Pacifiphantes* until a more thorough analysis can be done.

Pacifiphantes magnificus males will key out in the Spiders of North America (Ubick et al. 2005) in the Linyphiidae generic key to couplet 25, *Poeciloneta*. However, *Pacifiphanytes magnificus* lack trichobothria on the metatarsus. Continuing with the key leads to couplet 27, in which the genus differs from either *Porrhomma* or *Kaestneria* by the embolus forming almost a complete loop. Females are included in the key under couplet 222, *Pacifiphantes*.

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