

Article

Integrative Systematics Reveals Cryptic Diversity in *Paraphrynus* Whip Spiders (Amblypygi: Phrynidae) from Southwestern North America [†]

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Abstract: Due to their continuous growth, reclusive nature, and low vagility, the distributions and species limits of many whip spiders (Amblypygi Thorell, 1883) remain poorly understood, and much cryptic diversity remains unrecognized. Past attempts to separate the historical “forms” of *Paraphrynus* Moreno, 1940 into morphologically diagnosable species resulted, for example, in the division of *Paraphrynus mexicanus* (Bilimek, 1867) into three species—the nominotypical form, *Paraphrynus cubensis* Quintero, 1983, and *Paraphrynus carolynae* Armas, 2012. Nevertheless, the limitations of conservative morphology continue to hinder progress towards clarifying the diversity of *Paraphrynus*. One such example concerns *P. carolynae*, distributed from Arizona to central Mexico as currently defined. Through the acquisition of new, freshly collected material, the discovery of novel morphological characters, and molecular systematics analyses, it became apparent that *P. carolynae* comprises at least two morphologically diagnosable species. In this present contribution, the northernmost population of *P. carolynae* occurring in Arizona and California is described as a new species, *Paraphrynus tokdod*, sp. nov., raising the number of species in the genus to 22. This investigation also revealed more variation than expected in the secondary spine counts of the pedipalps and the trichobothrial counts of leg IV, previously used for species delimitation in *Paraphrynus*, suggesting that such characters should be used with caution.

Keywords: Arachnida; Mexico; molecular systematics; Phryninae; phylogeny; taxonomy; southwestern USA



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1. Introduction

The order Amblypygi Thorell, 1883 popularly known as whip spiders or tailless whip scorpions, are an ancient group of predatory arachnids inhabiting tropical and subtropical regions across the globe. The morphology of whip spiders has remained largely unchanged since their emergence ca. 315 million years ago [1]. Due to their continuous growth, reclusive nature, and low vagility, the distributions and species limits of many whip spiders remain poorly understood, and much cryptic diversity remains unrecognized. Only a few studies to date have incorporated modern, molecular phylogenetic techniques alongside morphological systematics to delimit the species [2–4].

One group of whip spiders in need of revision is the American genus *Paraphrynus* Moreno, 1940, currently containing 21 currently described species, inhabiting forests, caves, deserts, and scrubland from Southern USA, through Central America and the Greater Antilles to northern South America [3]. Over the past decades, several attempts were made to separate the historical “forms” of *Paraphrynus* into morphologically diagnosable species, resulting, for example, in the division of *Paraphrynus mexicanus* (Bilimek, 1867) into three

species—the nominotypical form, *Paraphrynus cubensis* Quintero, 1983, and *Paraphrynus carolynae* Armas, 2012 [5–7]. Nevertheless, the limitations of conservative morphology continue to hinder progress towards clarifying the diversity in this genus. One such example concerns *P. carolynae*, distributed from Arizona to central Mexico as currently defined. Through the acquisition of new, freshly collected material, the discovery of novel morphological characters, and molecular systematics analyses, it became apparent that *P. carolynae* comprises at least two morphologically diagnosable species. In this present contribution, the northernmost population of *P. carolynae* occurring in Arizona and California (Figure 1) is described as a new species, *Paraphrynus tokdod*, sp. nov. (Figure 2), raising the number of species in the genus to 22.

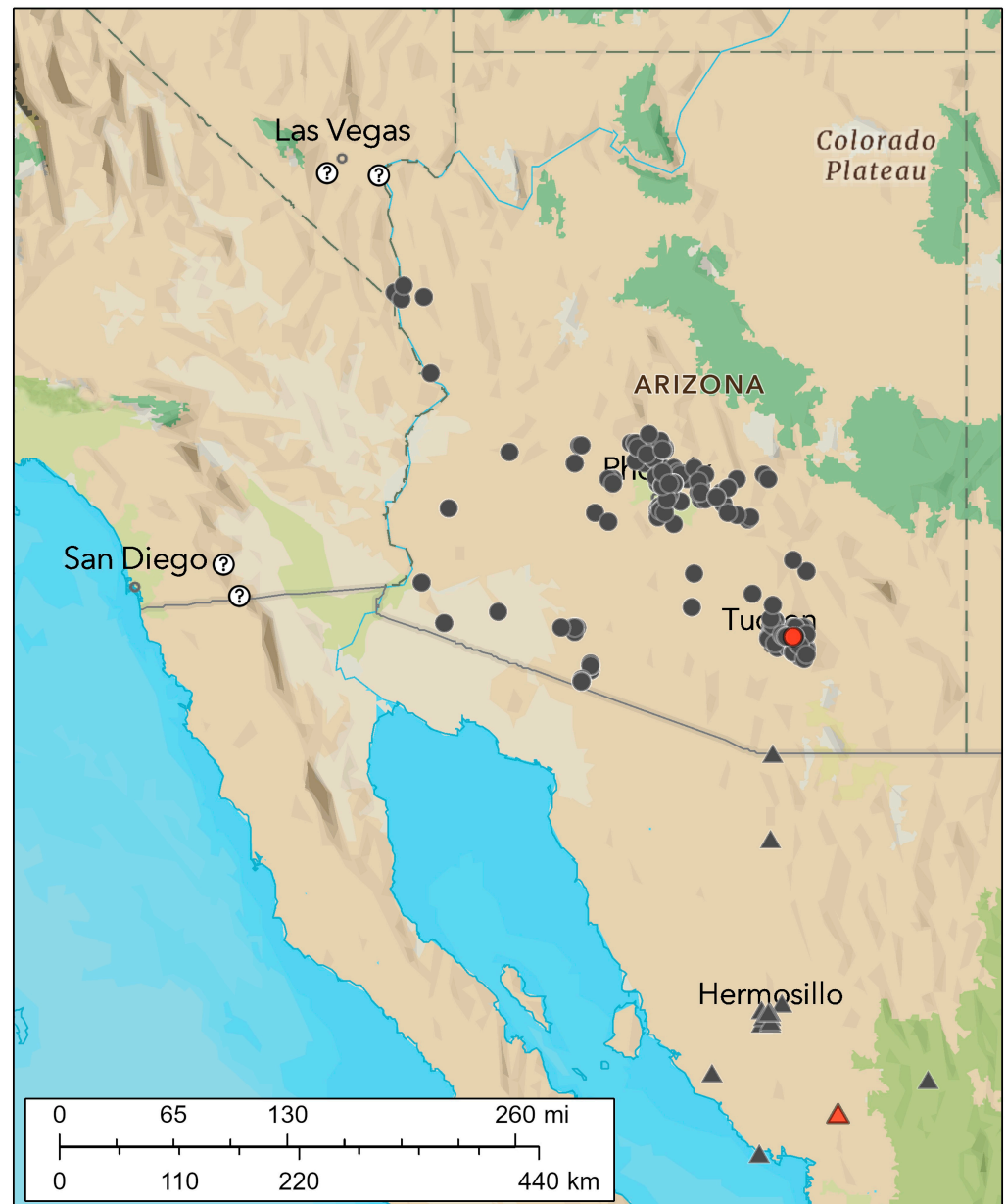


Figure 1. Map of the southwestern USA and northern Mexico, plotting known distributions of the newly revised *Paraphrynus carolynae* Armas, 2012 (triangle) and *Paraphrynus tokdod*, sp. nov. (circle), compiled from records in GBIF and this present study [8]. Red symbols indicate the type localities of each species. Symbols denoted with “?” indicate records of *P. tokdod*, sp. nov. west of the Colorado River that may not be conspecific.

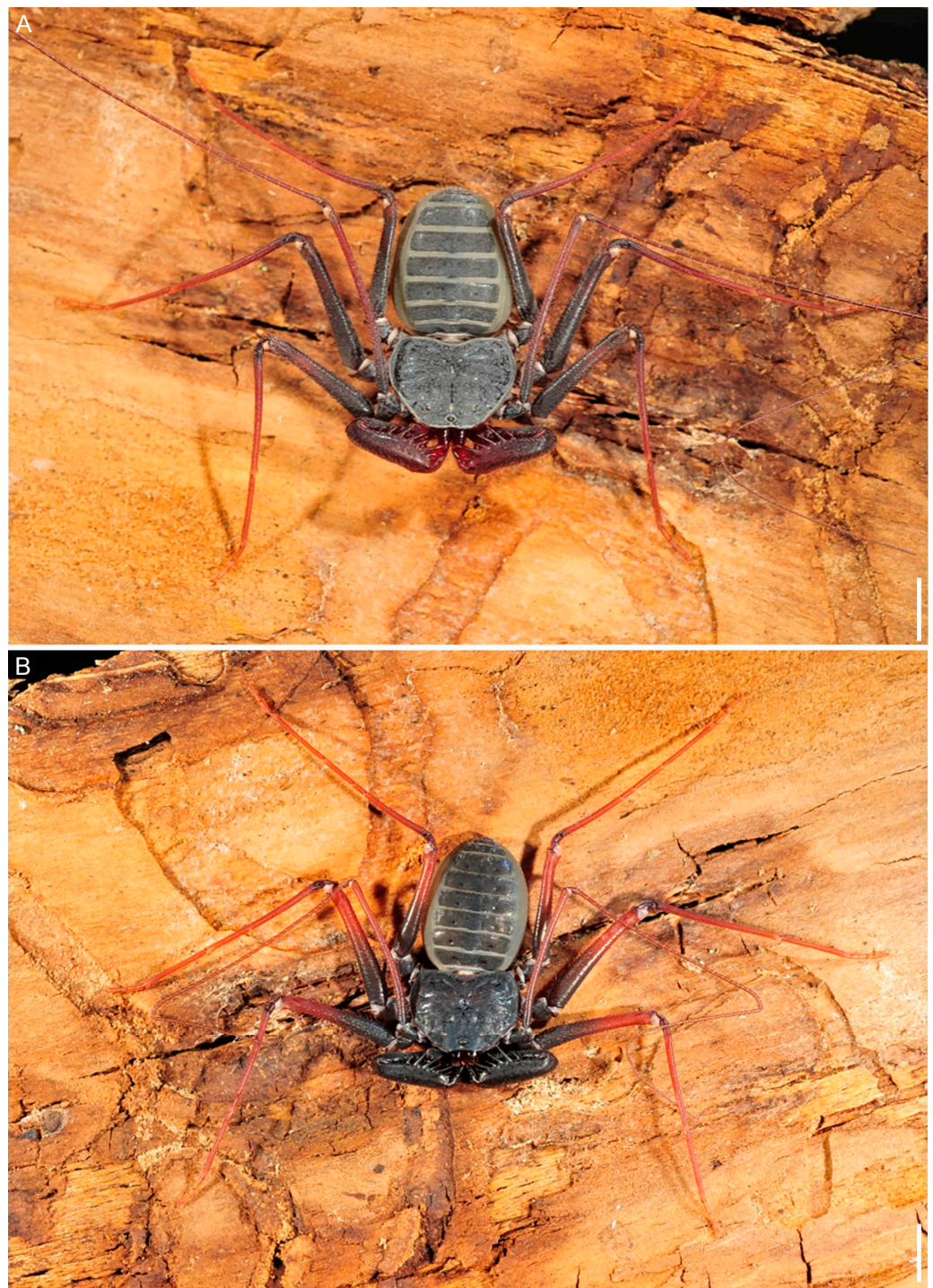


Figure 2. *Paraphrynus* Moreno, 1940 species, habitus in life. (A). *Paraphrynus carolynae* Armas, 2012, ♀ (AMNH), Nogales, Arizona, USA (B). *Paraphrynus tokdod*, sp. nov., ♀ (AMNH), Tucson, Arizona, USA. Scale bars: 5 mm.

This investigation also revealed more variation than expected in the secondary spine counts of the pedipalps and the trichobothrial counts of leg IV, previously used for species delimitation in *Paraphrynus*, suggesting that such characters should be used with caution.

2. Materials and Methods

2.1. Material, Morphology, Microscopy, and Imaging

Newly collected specimens were taken from crevices in cliffside rock outcrops and rodent burrows (Figure 3), between 1 a.m. and 4 a.m. during the new moon in early August, at the start of monsoon season. Material, preserved in 70% ethanol for morphological examination and 95% ethanol for DNA isolation, was deposited in the collections of the American Museum of Natural History (AMNH), New York, and the Ambrose Monell Cryocollection for Molecular and Microbial Research (AMCC) at the AMNH. The new species was compared morphologically with closely related species, *P. carolynae*, *P. mexicanus*, and *P. pococki*, using existing and newly collected material deposited at the AMNH, listed as follows:



Figure 3. *Paraphrynus tokdod*, sp. nov., habitat, petrous hillside. (A). Phoenix, Arizona, USA (B). Tucson, Arizona, USA (type locality).

Paraphrynus carolynae Armas, 2012: **MEXICO:** Sonora: Municipio de Soyopa: Tonichi, 10 km E, dirt road to El Encinal, 28°34'35.48" N 109°25'28.8" W, E. González, 24.v.2006, rock rolling, 1 ♂ (AMCC [LP 6335]), 1 ♀ (AMCC [LP 6340]). **USA:** Arizona: Santa Cruz Co.: Nogales, AZ, USA, rock wall and sidewalk near historic courthouse, 31°20'10.6" N 110°56'16.8" W, 1185 m, 19–20.viii.2023, N. Cazzaniga, 1 ♂, 2 ♀ (AMNH), 1 juv. (AMCC [LP 20029]).

Paraphrynus mexicanus (Bilimek, 1867): **MEXICO:** Guerrero: Municipio de Quechultenango: Grutas de Juxtlaajuaca [17°26'22.5" N 99°09'34.5" W], 17.viii.1966, J. Fish & J. Reddell, 2 ♂, 1 ♀ (AMNH).

Paraphrynus pococki Mullinex, 1975: **MEXICO:** San Luis Potosí: Municipio de Ciudad Valles: Sotano de Yerbaniz (entrance), 22°11'07.4" N 98°59'12.8" W, L. Prendini, 1.viii.2002, under stones outside cave entrance, 2 ♂ (AMNH), 1 ♀ (AMCC [LP 2091]).

Specimens were imaged using a Microoptics™ ML-1000 digital photomicrography system. Morphological examination of specimens was conducted using a Nikon SMZ 1500 stereoscope. Diagnostic characters and morphology follow Seiter et al. [3]. Spine terminology was based on primary spination; i.e., spines which were consistently observed.

The distribution map (Figure 1) was based on records in GBIF [8] and this present study, and it was generated using ArcGis [9].

2.2. Molecular Systematics

The phylogenetic analysis presented herein expanded the dataset of Seiter et al. (2020) [3] by adding sequences for six new samples (three new samples of *P. carolynae* and three samples of the new species). The updated dataset includes 11 terminal taxa, representing six described species of the *aztecus* group, i.e., *Paraphrynus aztecus* (Pocock, 1894), *P. carolynae*, *P. cubensis*, *P. mexicanus*, *P. pococki*, *Paraphrynus pseudomexicanus* Seiter et al., 2020 and the new species; two other species of *Paraphrynus*, i.e., *Paraphrynus robustus* (Franganillo, 1931) and *Paraphrynus viridiceps* (Pocock, 1894); and the outgroups, *Phrynus marginemaculatus* C.L. Koch, 1840 and *Heterophrynus alces* Pocock, 1902.

DNA sequencing followed the methods of Prendini et al. (2005) [2], Seiter et al. (2020) [3] and Schramm et al. (2021) [4]. Genomic DNA was extracted using a Qiagen DNeasy Blood and Tissue extraction kit. Tissue was dissected from leg muscles to best retain intact morphology. PCR amplifications were performed for five gene loci, selected based on their ability to provide resolution at various taxonomic levels, in overlapping fragments using universal eukaryote and arachnid-specific primers—three mitochondrial loci, i.e., Cytochrome *c* Oxidase Subunit I (hereafter, COI), 12S rRNA (12S) and 16S rRNA (16S), and two nuclear loci, i.e., 18S rRNA (18S) and 28S rRNA (28S) [10]. PCRs were performed in a thermocycler, using EmeraldAmp PCR Mastermix. Successful DNA amplifications were assessed by gel electrophoresis using Sybersafe stain. After confirmation, samples were purified using AMPure magnetic beads and suspended in water until sequencing.

Cycle sequencing was conducted using a Big Dye v1.1 Mastermix and automated Sanger dideoxy sequencing of single-stranded DNA performed on an Applied Biosystems Inc. Prism™ 3730x (Applied Biosystems Inc., Waltham, WA, USA). Paired-strand reads were aligned using Sequencher™ and edited by hand. Forty DNA sequences were newly generated such that the final dataset comprises 85 sequences (Table 1). The sequences were complete for all individuals.

Multiple sequence alignments were performed individually by gene using MAFFT v7 online using the Q-INS-i algorithm [11]. Alignments were manually checked and concatenated in SeaView v7 [12]. The concatenated alignment, comprising 3872 base-pairs, was partitioned by gene, run through ModelFinder in IQTree v2, and subsequently analyzed with Maximum Likelihood (ML), using a rapid bootstrap analysis with 1000 replicates [13]. The ModelFinder algorithm in IQTree identified TNe + I, TIM3 + F + I + G4, and TIM2 + F + I + R3 as the best fitting models for the nuclear ribosomal (18S, 28S) partitions, the mitochondrial ribosomal (12S, 16S) partitions, and the mitochondrial COI partition, respectively.

Uncorrected pairwise genetic distances of each mitochondrial locus were calculated using the K2P model in Mega v10.1.7 for fifteen *Paraphrynus* terminals representing six described species in the *aztecus* group and four samples of the new species (Table 2) [14]. Mean intraspecific and interspecific distances were compared with other uncorrected distances, as in Schramm et al. (2021) [4].

Table 1. Terminal taxa, countries, and states or provinces of origin, Ambrose Monell Cryocollection (AMCC) tissue catalog numbers, and GenBank accession codes for 12S rDNA (12S), 16S rDNA (16S), 18S rDNA (18S), 28S rDNA (28S), and Cytochrome *c* Oxidase Subunit I (COI) sequences used in phylogenetic analysis of *Paraphrynus* Moreno, 1940 whip spiders from the following species: *Heterophrynus alces* Pocock, 1902; *Paraphrynus aztecus* (Pocock, 1894); *Paraphrynus carolynae* Armas, 2012; *Paraphrynus cubensis* Quintero, 1983; *Paraphrynus mexicanus* (Bilimek, 1867); *Paraphrynus pococki* Mullinex, 1975; *Paraphrynus pseudomexicanus* Seiter et al., 2020; *Paraphrynus robustus* (Franganillo, 1931); *Paraphrynus viridiceps* (Pocock, 1894); and *Phrynus marginemaculatus* C.L. Koch, 1840. GenBank accession numbers that are underlined were newly generated in this present study.

Species	AMCC	Country: State/Prov.	18S	28S	12S	16S	COI
<i>H. alces</i>	15665	French Guiana: Cayenne Arr.	PQ522187	PQ522195	PQ522179	PQ521908	PQ524108
<i>P. aztecus</i>	2096	Mexico: Veracruz	MT734769	MT734785	MT753014	MT734759	MT738748
<i>P. carolynae</i>	6335	Mexico: Sonora	PQ522190	PQ522198	PQ522182	PQ521911	PQ524111
	6340		<u>PQ522191</u>	<u>PQ522199</u>	<u>PQ522183</u>	<u>PQ521912</u>	<u>PQ524112</u>
	20029		<u>PQ522189</u>	<u>PQ522197</u>	<u>PQ522181</u>	<u>PQ521910</u>	<u>PQ524110</u>
<i>P. cubensis</i>	13883	Cuba: Artemisa	MT734772	MT734788	MT753017	MT734761	MT738751
<i>P. mexicanus</i>	15431	Mexico: Guerrero	MT734773	MT734789	MT753018	MT734762	MT738752
<i>P. pococki</i>	2091	Mexico: San Luis Potosí	MT734774	MT734790	MT753019	MT734763	MT738753
<i>P. pseudomexicanus</i>	14443	Mexico: Morelos	MT734775	MT734791	MT753020	MT734764	MT738754
	14450		MT734776	MT734792	MT753021	MT734765	MT738755
	13872		MT734777	MT734793	MT753022	MT734766	MT738756
<i>P. robustus</i>	14444	Cuba: Guantánamo	MT734771	MT734787	MT753016	MT734760	MT738750
<i>P. tokdod</i>	20026	USA: Arizona	PQ522192	PQ522200	PQ522184	PQ521913	PQ524113
	20027		<u>PQ522193</u>	<u>PQ522201</u>	<u>PQ522185</u>	<u>PQ521914</u>	<u>PQ524114</u>
	20028		<u>PQ522194</u>	<u>PQ522202</u>	<u>PQ522186</u>	<u>PQ521915</u>	<u>PQ524115</u>
	13881		MT734778	MT734794	MT753023	MT734767	MT738757
<i>P. viridiceps</i>	20013	Cuba: Pinar del Río	<u>PQ522188</u>	<u>PQ522196</u>	<u>PQ522180</u>	<u>PQ521909</u>	<u>PQ524109</u>
<i>P. marginemaculatus</i>		USA: Florida					

Table 2. Mean intraspecific (boldface) and interspecific uncorrected pairwise (*p*) distances of three mitochondrial loci, 12S rDNA (12S), 16S rDNA (16S), Cytochrome *c* Oxidase Subunit I (COI), for eight species of the whip spider genus *Paraphrynus* Moreno, 1940: *P. aztecus* (Pocock, 1894); *P. carolynae* Armas, 2012; *P. cubensis* Quintero, 1983; *P. mexicanus* (Bilimek, 1867); *P. pococki* Mullinex, 1975; *P. pseudomexicanus* Seiter et al., 2020; *P. robustus* (Franganillo, 1931); *P. tokdod*, sp. nov.; *P. viridiceps* (Pocock, 1894).

	<i>P. carolynae</i>	<i>P. tokdod</i>	<i>P. aztecus</i>	<i>P. cubensis</i>	<i>P. mexicanus</i>	<i>P. pococki</i>	<i>P. pseudomexicanus</i>	<i>P. robustus</i>
12S								
<i>P. carolynae</i>	0.0681							
<i>P. tokdod</i>	0.123	0.0062						
<i>P. aztecus</i>	0.3115	0.3135						
<i>P. cubensis</i>	0.291	0.3051	0.1648					
<i>P. mexicanus</i>	0.3059	0.2863	0.1811	0.182				
<i>P. pococki</i>	0.2162	0.1909	0.2493	0.2307	0.2539			
<i>P. pseudomexicanus</i>	0.2853	0.2801	0.1808	0.2107	0.1387	0.2234	0	
<i>P. robustus</i>	0.3757	0.3874	0.3673	0.3528	0.3816	0.3356	0.3167	
<i>P. viridiceps</i>	0.3172	0.3243	0.3646	0.3357	0.3173	0.2992	0.3152	0.176
16S								
<i>P. carolynae</i>	0.074							
<i>P. tokdod</i>	0.1402	0.0042						
<i>P. aztecus</i>	0.2282	0.2319						
<i>P. cubensis</i>	0.2414	0.218	0.1683					
<i>P. mexicanus</i>	0.2498	0.2563	0.1772	0.179				
<i>P. pococki</i>	0.1943	0.1637	0.2155	0.2109	0.222			
<i>P. pseudomexicanus</i>	0.2398	0.2213	0.1662	0.1813	0.1361	0.2029	0	
<i>P. robustus</i>	0.2417	0.26	0.2705	0.267	0.2472	0.2528	0.2525	
<i>P. viridiceps</i>	0.2589	0.2605	0.2271	0.2524	0.2625	0.2208	0.2353	0.1608
COI								
<i>P. carolynae</i>	0.0886							
<i>P. tokdod</i>	0.1366	0.0085						
<i>P. aztecus</i>	0.2002	0.2121						
<i>P. cubensis</i>	0.2174	0.2292	0.1972					
<i>P. mexicanus</i>	0.2408	0.2332	0.2389	0.2457				
<i>P. pococki</i>	0.1931	0.1882	0.2127	0.2159	0.2278			
<i>P. pseudomexicanus</i>	0.2324	0.2393	0.2288	0.2154	0.1827	0.251	0	
<i>P. robustus</i>	0.241	0.2357	0.2372	0.2354	0.2601	0.2262	0.2607	
<i>P. viridiceps</i>	0.2455	0.2376	0.2734	0.2167	0.2607	0.2516	0.2387	0.1896

3. Results

3.1. Morphology

Unlike previous studies [3], significant variation was observed among the leg IV trichobothrial counts in the new species and related species, preventing their use as diagnostic characters. Pedipalp secondary spines, which may be diagnostic for species at particular life stages, were also avoided as diagnostic characters due to the variation observed across stages and among conspecifics of comparable life stages. Pedipalp secondary spine counts and leg trichobothrial counts should be used with caution in future diagnoses of *Paraphrynus* species.

Although gonopod shape is a diagnostic character for many species of *Paraphrynus* [3], this was not the case for the new species and its sister species, *P. carolynae*. Gonopod shape was observed to change during development among specimens collected at the same locality and determined to be conspecific based on DNA. Gonopod shape should also be used cautiously as a diagnostic character, with care taken to compare sexually mature specimens of similar size.

3.2. Phylogeny

The phylogenetic analysis recovered the same tree topology (Figure 4) as Seiter et al. (2020) [3] for relationships among the exemplar species of *Paraphrynus*. Except for the group comprising *P. aztecus* and *P. cubensis*, all groups received high bootstrap support values (greater than 50%). High support values (100%) were obtained for the new species, its sister species, *P. carolynae*, and the group comprising both species. A previously misidentified

sample of *P. carolynae* from Tucson, Arizona (AMCC [LP 14444]) [3], was correctly placed within the clade representing the new species.

3.3. Genetic Distances

Uncorrected pairwise genetic distances varied marginally across each mitochondrial locus (Table 2), maintaining general trends within and among species sampled [3,4]. The average intraspecific distances of 0.62% (12S), 0.42% (16S), and 0.85% (COI) recovered among the samples of *P. tokdod*, sp. nov., contrast markedly with the average interspecific distances of 12.3% (12S), 14.02% (16S), and 13.66% (COI) recovered between *P. tokdod* and its sister species, *P. carolynae*. Similar or slightly greater interspecific distances, of 13.87% (12S), 13.61% (16S), and 18.27% (COI), were observed between the sister species, *P. pseudomexicanus* and *P. mexicanus*. The average uncorrected pairwise mitochondrial genetic distance of 13.33%, recovered for *P. tokdod*, also surpasses the average mitochondrial distance of 12.54% for morphologically distinct species in the phrynichid whip spider genus *Damon* C.L. Koch, 1850, established by Schramm et al. [4].

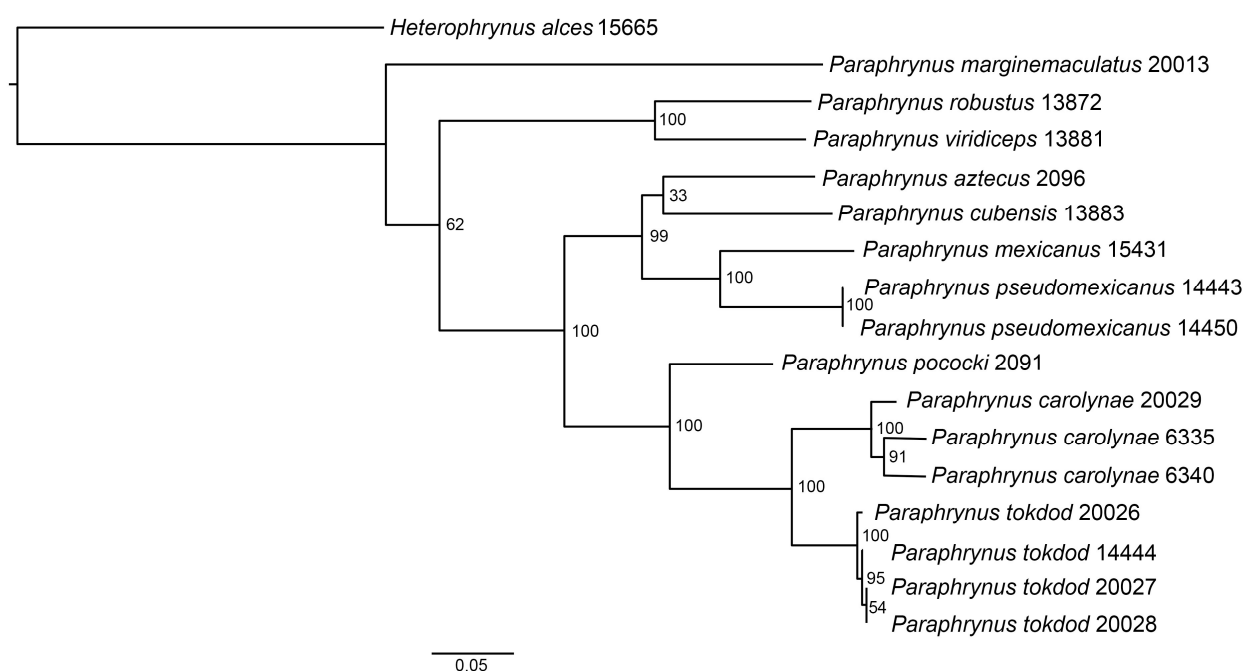


Figure 4. Maximum Likelihood phylogeny of *Paraphrynus* Moreno, 1940 whip spiders, based on 3872 aligned nucleotides of DNA sequence from three mitochondrial and two nuclear gene loci (final optimization likelihood probabilities for Q-INS-i alignment of −14,125.451). Numbers following species refer to tissue samples (Table 1). Bootstrap support values are indicated at nodes.

The average interspecific distances of 6.82% (12S), 7.4% (16S), and 8.86% (COI) recovered between the Nogales and Sonoran samples of *P. carolynae* are noteworthy in being greater than expected between putative conspecifics. Although these distances remain well below the species threshold and are assumed to have resulted from geographical isolation, further investigation of species limits across the distribution of *P. carolynae* is merited.

3.4. Systematics

Genus *Paraphrynus* Moreno, 1940

Paraphrynus tokdod, sp. nov.

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Figures 1–10

Paraphrynus mexicanus (misidentification): Mullinex, 1975: 30–32, figures 29, 30 [5].

Paraphrynus carolynae (misidentification): Armas, 2012: 27, 30, figures 2, 3, tables 1, 2 [7]; Seiter et al., 2020: 268, 278, figures 1–6, 8–13, tables 2, 6, 8 [3].

Type Material. USA: Arizona: Pima Co.: Tucson, Soldier Trail trailhead, along Mt Lemmon Highway, 32°18'32" N 110°44'10.5" W, 995 m, 18–19.viii.2023, N. Cazzaniga & M. Leckbee, petrous slope with deep crevices in rock, holotype ♀, paratype ♂, paratype ♀(AMNH), 1 juv ♂paratype (AMCC [LP 20027]); Tucson, Sabino Canyon [32°18'38.4" N 110°49'21.5" W], 8.viii.1956, V. Roth, 1 ♂[paratype of *P. carolynae*] (AMNH), 32°18'38.4" N 110°49'21.5" W, 834 m, 19.viii.2023, N. Cazzaniga & M. Leckbee, artificial rock wall, paratype ♂(AMCC [LP 20028]). Maricopa Co.: Phoenix, Piastewa Peak, 33°32'21.5"N 112°01'31.1"W, 509 m, 16–17.viii.2023, N. Cazzaniga & M. Leckbee, petrous slope, within rodent burrow, paratype ♂(AMCC [LP 20026]).



Figure 5. *Paraphrynus* Moreno, 1940, dorsal habitus. (A). *Paraphrynus carolynae* Armas, 2012, ♀(AMNH), Nogales, Arizona, USA (B). *Paraphrynus tokdod*, sp. nov., paratype ♀(AMNH), Tucson, Arizona, USA. Scale bars: 5 mm.

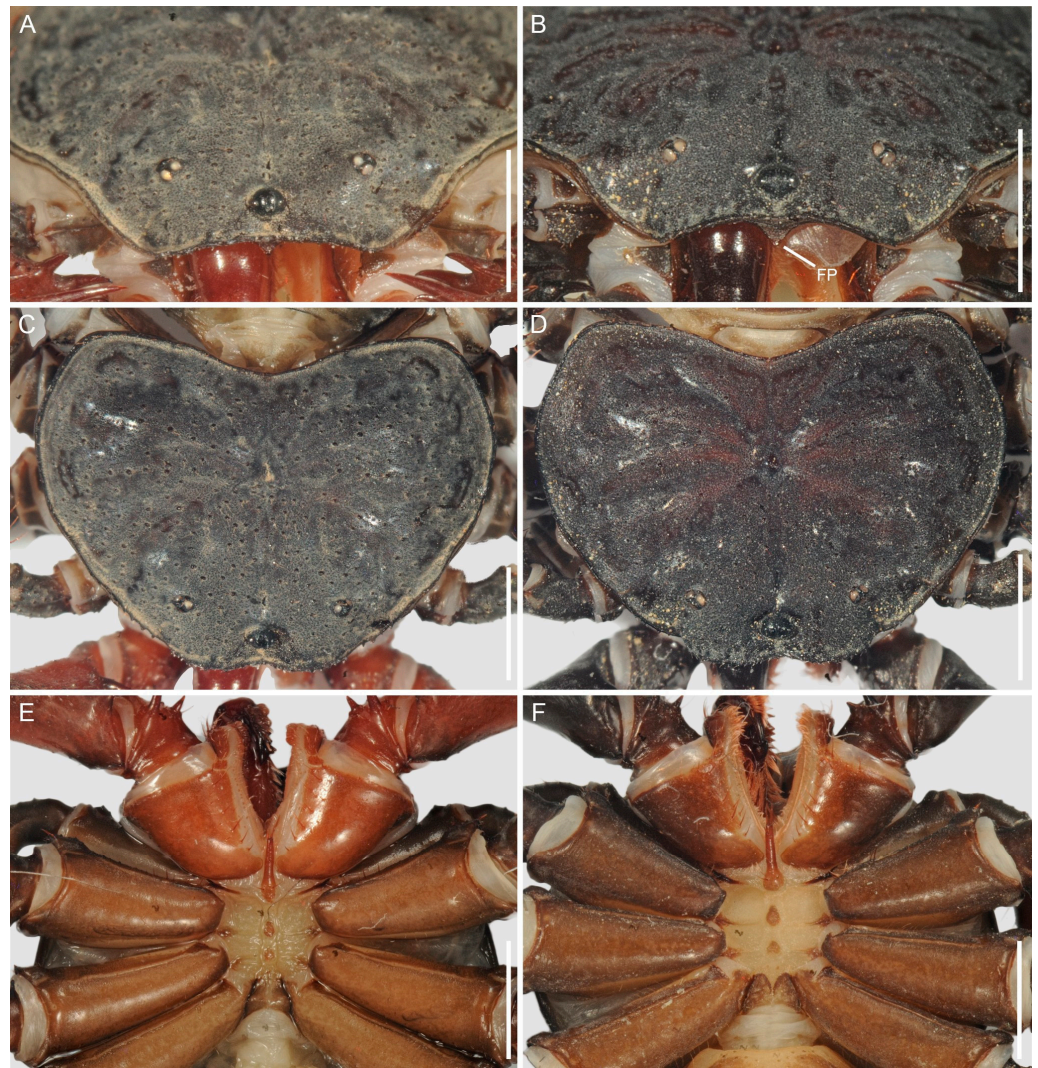


Figure 6. *Paraphrynus* Moreno, 1940, carapace dorsal (C,D) and frontal (A,B) aspects, and tritosternum, ventral aspect (E,F). (A,C,E). *Paraphrynus carolynae* Armas, 2012, ♀(AMNH), Nogales, Arizona, USA (B,D,F). *Paraphrynus tokdod*, sp. nov., holotype ♀(AMNH), Tucson, Arizona, USA. Scale bars: 2 mm.

Diagnosis. *Paraphrynus tokdod*, sp. nov., differs from other *Paraphrynus* species in the following respects (Table 3). Immature stages retain black pedipalps from the juvenile to adult stage, unlike most other *Paraphrynus* species, in which the pedipalps of immature stages are bright red (Figure 5). Additionally, this species retains a distinctly bicolored (red/black) femur of the walking legs from juvenile to adult, which has not been observed at any stage in the sister species (Figure 5). Lastly, as noted by Seiter et al. [3], who misidentified *P. tokdod* as *P. carolynae*, the new species exhibits an enlarged frontal process, unlike its sister species (Figure 6) [3].

Paraphrynus tokdod most closely resembles *P. carolynae*, the sister species with which it was previously confused [3,7]. Both species are similar in size, spination, and adult coloration but may be consistently separated based on the tubercles of the carapace and legs (Figures 6 and 9), the size of the carapace frontal process (Figure 6), and abovementioned characters of coloration (Figure 5; Table 3). Compared with *P. carolynae*, the tubercles are reduced on the carapace and legs and almost absent on the pedipalp (Figures 6, 8 and 9), the frontal process is enlarged (Figure 6), the pedipalps are dark at all life stages, and the leg femora are bicolored in *P. tokdod* (Figures 5 and 9).



Figure 7. *Paraphrynus* Moreno, 1940, cheliceral dentition, prolateral (A,C) and retrolateral (B,D) aspects (dense setae near prolateral denticle row, at cheliceral base, removed). (A,B). *Paraphrynus carolynae* Armas, 2012, ♀(AMNH), Nogales, Arizona, USA (C,D). *Paraphrynus tokdod*, sp. nov., holotype ♀(AMNH), Tucson, Arizona, USA. Numbers (D) refer to teeth. Scale bars: 0.5 mm.

The new species may be further separated from *P. pococki* by the enlarged frontal process; the presence of four denticles on the cheliceral claw (five denticles are present in *P. pococki*); the reduced tubercles on the pedipalps and legs; the dark juvenile pedipalp coloration; and the bicolored leg femora (the femora are variegated in *P. pococki*).

Paraphrynus tokdod may be further separated from *P. mexicanus* by the presence of 62 tibial and 29 tarsal segments in leg I (27 tibial and 59 tarsal are present in leg I in *P. mexicanus*); the enlarged carapace frontal process; the presence of four denticles on the cheliceral claw (five denticles are present in *P. mexicanus*); the reduced tubercles on the carapace; the dark juvenile pedipalp coloration; reduced setation on the tritosternum; and the bicolored leg femora (the femora are consistently dark in *P. mexicanus*).

Etymology. The specific epithet is a noun in apposition taken from “tokdoḍ”, the Tohono O’odham word for spider.

Description. The following description is based on the type material.

Color: Carapace, pedipalps, and opisthosomal tergites black (Figure 5B). Chelicerae black with reddish setae. Leg femur bicolored, proximal half black, distal half red (Figure 5B). Opisthosomal sternites tan to dark olive brown. Intersegmental membranes tan or olive to gray, depending on molt cycle.

Carapace: Frontal process well developed, clearly visible in anterior aspect (Figure 6B). Dorsal surface finely granular with few tubercles to almost smooth, asetose (Figure 6D). Median ocelli and lateral ocular triads well developed.

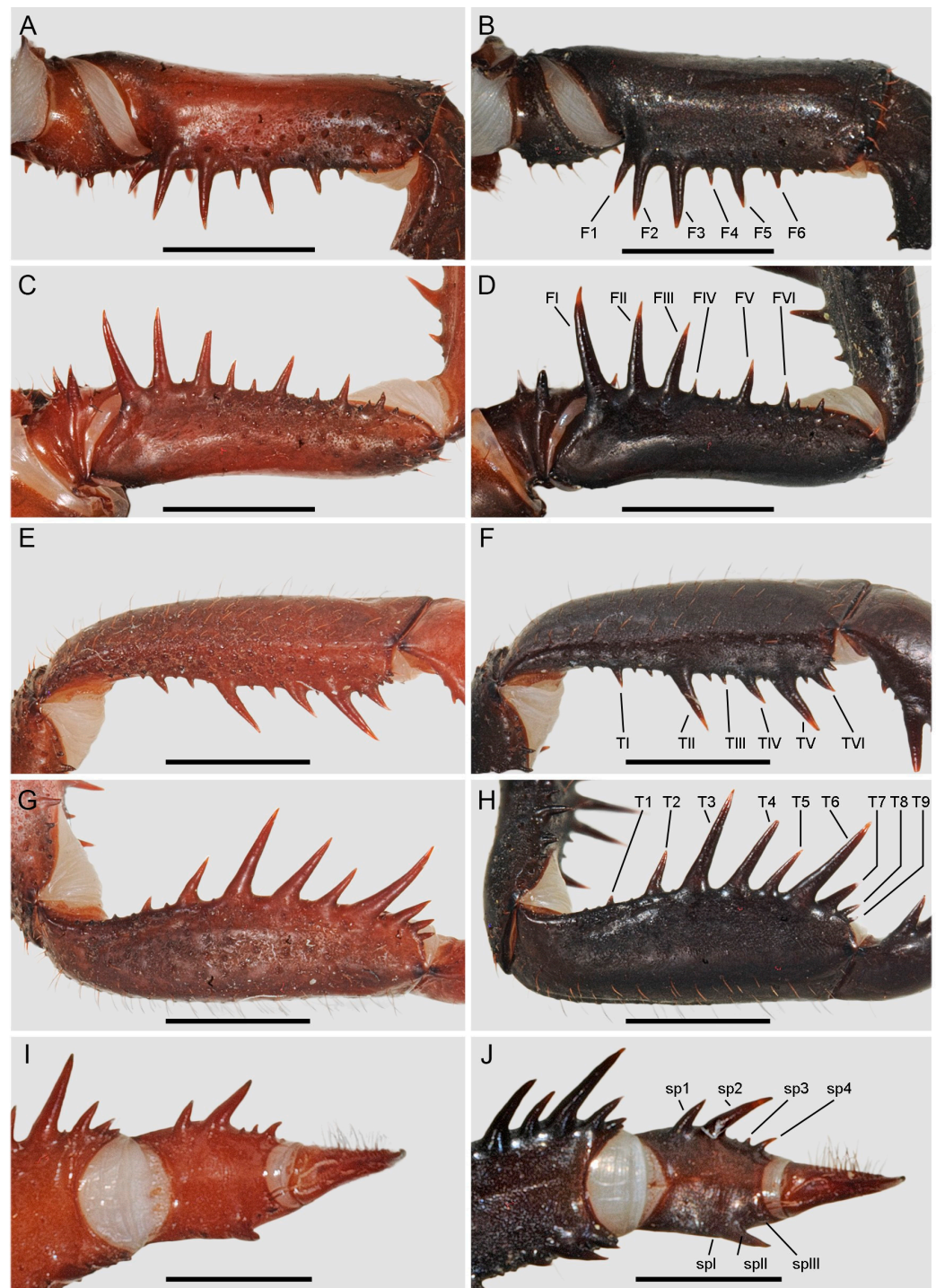


Figure 8. *Paraphrynus* Moreno, 1940, pedipalp trochanter and femur, dorsal (A,B) and ventral (C,D) aspects, pedipalp tibia, dorsal (E,F) and ventral (G,H) aspects, and pedipalp basitarsus and pretarsus, prolateral aspect (I,J). (A,C,E,G,I). *Paraphrynus carolynae* Armas, 2012, ♀(AMNH), Nogales, Arizona, USA (B,D,F,H,J). *Paraphrynus tokdod*, sp. nov., holotype ♀(AMNH), Tucson, Arizona, USA. Annotations (B,D,F,H,J) refer to primary spines. Scale bars: 2 mm.

Chelicerae: Cheliceral base, dorsal surface sparsely setose with few obsolete tubercles; prolateral surface densely setose, becoming asetose prodorsally, with three teeth along margin, first (dorsalmost) tooth tricuspid, second bicuspid, third cuspid (Figure 7C,D). Cheliceral claw, dorsal surface with four cuspid teeth, first (distalmost) tooth half the size of others, prolateral surface densely setose, becoming asetose distally (Figure 7C,D).



Figure 9. *Paraphrynus* Moreno, 1940, legs II–IV, prodorsal aspects. (A). *Paraphrynus carolynae* Armas, 2012, ♀(AMNH), Nogales, Arizona, USA (B). *Paraphrynus tokdod*, sp. nov., holotype ♀(AMNH), Tucson, Arizona, USA. Scale bars: 2 mm.

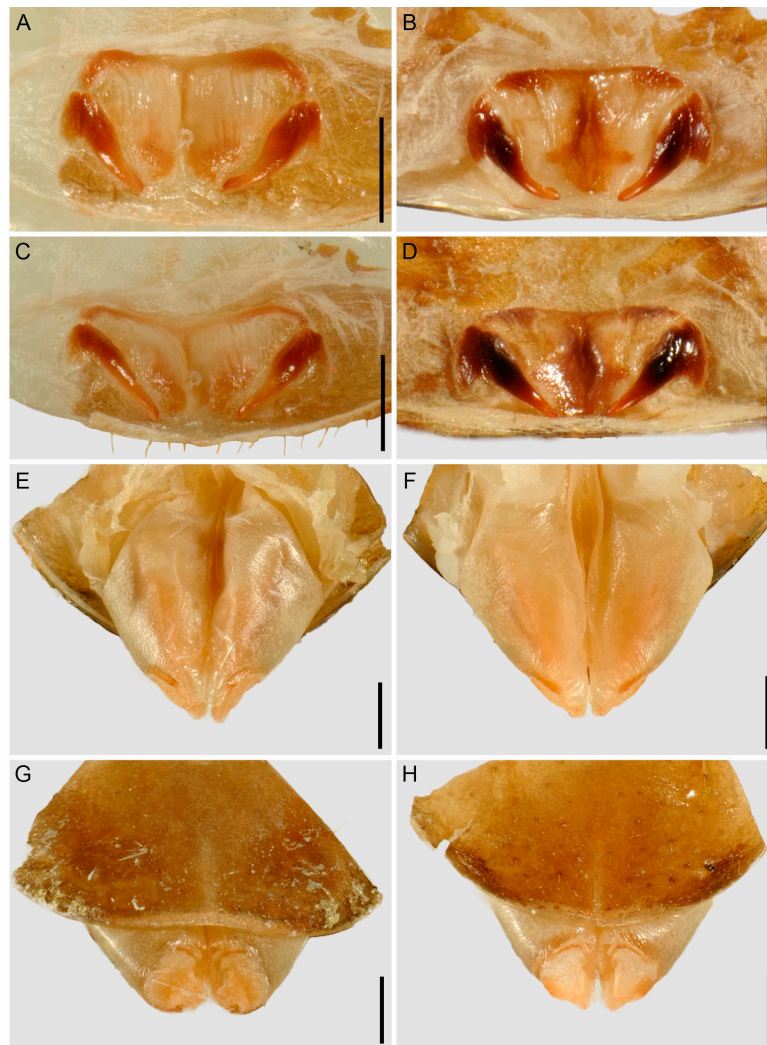


Figure 10. *Paraphrynus* Moreno, 1940, female gonopods, dorsal (A,B) and posterior (C,D) aspects and male gonopods, dorsal (E,F) and ventral (G,H) aspects. (A,C). *Paraphrynus carolynae* Armas, 2012, ♀(AMNH), Nogales, Arizona, USA (B,D). *Paraphrynus tokdod* sp. nov., holotype ♀(AMNH), Tucson, Arizona, USA (E,G). *Paraphrynus carolynae* Armas, 2012, ♂(AMNH), Nogales, Arizona, USA (F,H). *Paraphrynus tokdod* sp. nov., paratype ♂(AMNH), Tucson, Arizona, USA. Scale bars: 0.5 mm.

Table 3. Morphological differences among four species of the whip spider genus *Paraphrynus* Moreno, 1940: *P. carolynae* Armas, 2012; *P. mexicanus* (Bilimek, 1867); *P. pococki* Mullinex, 1975; *P. tokdod*, sp. nov.

	<i>P. carolynae</i>	<i>P. tokdod</i>	<i>P. mexicanus</i>	<i>P. pococki</i>
Carapace tubercles	moderate	fine	moderate	moderate
Frontal process	obsolete	enlarged	obsolete	obsolete
Tritosternum setation	sparsely hirsute	sparsely hirsute	densely hirsute	sparsely hirsute
Cheliceral dentition	3 + 1/4	3 + 1/4	3 + 1/5	3 + 1/5
Cheliceral tubercles	obsolete	obsolete	obsolete	obsolete
Femur spines (D/V)	6/6	6/6	6/6	6/6
Tibial spines (D/V)	9/6	9/6	9/6	9/6
Basitarsal spines (D/V)	4/3	4/3	4/3	4/3
Pedipalp tubercles	coarse	smooth	coarse	coarse
Leg tubercles	moderate	fine	fine	fine
Leg I tibial segments	29	29	27	29
Leg I tarsal segments	62	62	59	62
Leg IV distitibia trichobothria	ave. 22	ave. 20	ave. 21	ave. 27
Immature pedipalp coloration	bright red	black	bright red	bright red
Walking legs femoral coloration	light red distally, mostly dark	bicolored, bright red and dark	entirely dark	entirely dark, striped in immature

Sternum: Sternal plates moderately sclerotized, three primary plates more markedly sclerotized. Anteriormost plate (tritosternum) elongated and sparsely setose, with few setae surrounding ventral surface (Figure 6F).

Pedipalps: Surfaces smooth and glabrous, sparsely tuberculate, mostly on ventral surface, near spines. Femur (F) with six primary dorsal spines and six primary ventral spines (Figure 8B,D); F3 largest (F3 > F2 > F5 > F1 > F6 > F4), with F1 and F2 sharing same base; FI largest (FI > FII > FIII > FV > FVI > FIV). Tibia (T) with nine primary dorsal spines and six primary ventral spines (Figure 8F,H); T3 largest (T3 > T6 > T4 > T5 > T2 > T7 > T8 > T1 > T9); TV largest (TV > TII > TIV > TVI > TI > TIII) with many variable secondary spines. Basitarsus (sp) with four primary dorsal spines and three primary ventral spines (Figure 8J); sp2 largest (sp2 > sp4 > sp1 > sp3), spII largest (spII > spIII > spI) with two larger, thickened setae on prolateral surface of spIII. Tarsus aspinose, with prominent cleaning organ. Pretarsus not separated from tarsus.

Legs: Antenniform legs comprising 29 and 62 tibial and tarsal segments, respectively. Leg II–IV femora finely tuberculate (Figure 9B). Leg IV basitibia with single trichobothrium, distitibia with average of 20 trichobothria.

Gonopods: Male gonopods lobed, blunt distally and covered proximally along ventral side, by plate of genital operculum (Figure 10F,H). Female gonopods comprising pair of sclerotized, hook-like appendages, broadening basally and slightly curved towards ventral surface, terminally, curve more prominent in mature specimens (sometimes straight in immature females); basally with markedly sclerotized area parallel to hook-like appendages and unsclerotized area between them (Figure 10B,D).

Ecology. The type material was collected on petrous hillsides, dominated by bushy scrub and cacti, where bluffs gave way to crevice-filled crags along roads or trails, up to an elevation over 995 m above sea level. Individual specimens were usually found in crevices within vertical rock faces or slopes, but occasionally in old or abandoned rodent burrows in similar habitat. Captive individuals exhibit earthmoving behavior, suggesting the ability to enlarge suitable crevices in the wild. Although common in suitable habitats, all specimens were separated, none occurring in direct contact with one another. No females were observed carrying eggs or protonymphs. Several spiders of the genera *Latrodectus* Walckenaer, 1805, *Loxosceles* Heineken & Lowe, 1832, and *Selenops* Latreille, 1819, and scorpions were found in proximity.

Distribution. Material was examined from populations occurring in two counties (Pima Co. and Maricopa Co.) of Arizona, USA. Museum records and observations from internet databases suggest that the species may extend further north, at least as far as

Bullhead City, in Arizona (Figure 1). Records from west of the Colorado River, in southern Nevada and California may not be conspecific, however. The southern limit of the new species has yet to be determined, but based on the occurrence of *P. carolynae* populations in southern Arizona, it may be endemic to the U.S.

Other Material. USA: Arizona: Maricopa Co.: Mesa [33°24′45.11″ N 111°49′54.8″ W], 1 ♀(AMNH).

4. Discussion

This present study investigated the species limits of *P. carolynae*, a widespread North American whip spider allegedly distributed from Arizona to central Mexico [7]. Through the acquisition of new, freshly collected material, a reassessment of its morphology, and molecular systematics analyses, it became apparent that *P. carolynae* comprises at least two morphologically diagnosable species (Figures 1 and 2).

Novel morphological characters including an enlarged carapace frontal process, greatly reduced cuticular tubercles, and unique coloration of the pedipalps and legs in the adult and immature stages suggested that the northernmost population of *P. carolynae* (which included one of the paratype localities) represented an undescribed species. A molecular phylogenetic reconstruction of two nuclear and three mitochondrial gene loci demonstrated that the northern populations and the typical populations of *P. carolynae* from further south were reciprocally monophyletic with high bootstrap support (Figure 4). Average uncorrected pairwise mitochondrial genetic distances between samples from these populations surpassed the known threshold for distinct species in the order Amblypygi [4], further justifying the recognition of two different species. Based on these independent analyses of morphology and multilocus DNA sequence data, the northernmost population of *P. carolynae*, occurring in Arizona and California (Figures 1 and 3), was described as *P. tokdod*, sp. nov.

Additionally, this is the second study, following Schramm et al. [4], to demonstrate the utility of adult and immature coloration as potential diagnostic characters in Amblypygi. Because coloration degrades in preserved material and is widely considered to be intraspecifically variable and thus inappropriate for species diagnosis, it is often ignored. However, characters of coloration, which are readily observed in live animals, may be more informative than previously thought, as well as being potentially more useful for identification in the field and by the layman, than characters of the internal and external morphology that require microscopy for examination. The inclusion of such characters not only strengthens taxonomic knowledge but facilitates citizen science.

Conversely, the variation observed in leg IV trichobothrial and pedipalp secondary spine counts, both within and among conspecifics, has questioned their utility as potential diagnostic characters, at least in the genus *Paraphrynus*. Whereas average counts and relative positions of trichobothria and spines may be useful for higher-level analyses, these characters may introduce confusion among closely related species and should therefore be used with caution in future taxonomic assessments of *Paraphrynus* and other Amblypygi. This is especially important for poorly collected species, for which sample size may mask the true variability of characters.

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Data Availability Statement: The data presented in this study are openly available in GenBank.

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References

1. Garwood, R.J.; Dunlop, J.A.; Knecht, B.J.; Hegna, T.A. The phylogeny of fossil whip spiders. *BMC Evol. Biol.* **2017**, *17*, 105. [CrossRef] [PubMed]
2. Prendini, L.; Weygoldt, P.; Wheeler, W.C. Systematics of the *Damon variegatus* group of African whip spiders (Chelicerata: Amblypygi): Evidence from behaviour, morphology, and DNA. *Org. Divers. Evol.* **2005**, *5*, 203–236. [CrossRef]
3. Seiter, M.; Lerma, A.; Král, J.; Sember, A.; Divišová, K.; Palacios-Vargas, J.; Colmenares, P.A.; Loria, S.F.; Prendini, L. Cryptic diversity in the whip spider genus *Paraphrynus* (Amblypygi: Phrynidae): Integrating morphology, karyotype, and DNA. *Arthropod Syst. Phylogeny* **2020**, *78*, 265–285. [CrossRef]
4. Schramm, F.D.; Valdez-Mondragón, A.; Prendini, L. Volcanism and palaeoclimate change drive diversification of the world's largest whip spider (Amblypygi). *Mol. Ecol.* **2021**, *12*, 2872–2890. [CrossRef] [PubMed]
5. Mullin, C.L. Revision of *Paraphrynus* Moreno (Amblypygida: Phrynidae) for North America and the Antilles. *Occas. Pap. Calif. Acad. Sci.* **1975**, *116*, 1–80.
6. Quintero, D.J. Revision of the amblypygid spiders of Cuba and their relationships with the Caribbean and continental American amblypygid fauna. *Stud. Fauna Curaçao Other Caribb. Isl.* **1983**, *65*, 1–54.
7. de Armas, L.F. A new species of *Paraphrynus* Moreno, 1940 (Amblypygi: Phrynidae) from Mexico and the south-west of the United States. *Rev. Ibérica Aracnol.* **2012**, *21*, 27–32.
8. GBIF. Available online: <https://www.gbif.org/species/10680736> (accessed on 29 September 2024).
9. ArcGIS. Available online: <https://www.arcgis.com/index.html> (accessed on 10 October 2024).
10. Prendini, L.; Crowe, T.M.; Wheeler, W.C. Systematics and biogeography of the family Scorpionidae Latreille, with a discussion of phylogenetic methods. *Invertebr. Syst.* **2003**, *17*, 185–259. [CrossRef]
11. Katoh, K.; Rozewicki, J.; Yamada, K.D. MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. *Brief. Bioinform.* **2019**, *20*, 1160–1166. [CrossRef] [PubMed]
12. Gouy, M.; Guindon, S.; Gascuel, O. SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol. Biol. Evol.* **2010**, *27*, 221–224. [CrossRef] [PubMed]
13. Minh, B.Q.; Schmidt, H.A.; Chernomor, O.; Schrempf, D.; Woodhams, M.D.; Von Haeseler, A.; Lanfear, R. IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Mol. Biol. Evol.* **2020**, *37*, 1530–1534. [CrossRef] [PubMed]
14. Tamura, K.; Stecher, G.; Kumar, S. MEGA11: Molecular Evolutionary Genetics Analysis version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. [CrossRef] [PubMed]

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