

## Testing species boundaries in *Pardosa sierra* (Araneae: Lycosidae) using female morphology and COI mtDNA

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**Abstract.** The wolf spider *Pardosa sierra* was described and illustrated by Banks in 1898 based on specimens from the Sierra de la Laguna, Baja California Sur. Later, two morphologically similar species, *P. atromedia* Banks 1904 from Claremont, California, and *P. sura* Chamberlin & Ivie 1941, also from California, were described. However, the latter two species were subsequently synonymized with *P. sierra*, due to similarities in male genitalia. In this study we test the species limits within this group. We suggest that the details of the epigynum are different enough among the genitalic morphs studied to consider them different species as originally designated. We conducted a morphological and genetic-distance analysis of a fragment of the cytochrome *c* oxidase subunit I gene sequences of some species of *lapidicina* group, as well as some sequences of *Pardosa astrigera* L. Koch 1878 from the GenBank database. Genetic analysis revealed greater genetic distances (GD) among haplotypes of *P. sierra*, *P. atromedia*, and *P. sura* (GD = 0.053–0.069) than with other species of the *lapidicina* group. Moreover, *P. sierra* was closest to *P. sura* (GD = 0.053), *P. sura* was closest to *P. vadosa* Barnes 1959 (GD = 0.040), and *P. atromedia* was closest to *P. steva* Barnes 1959 (GD = 0.052). Overall, morphological and genetic differences, and disjoint distributions, suggest that the synonymy of *P. sierra*, *P. atromedia*, and *P. sura* was in error, and that these “morphs” do indeed represent different species.

**Keywords:** Epigynal morphs, genetic distances, *lapidicina* group, taxonomy

Araneomorph spider taxonomy is based mainly on phenotypic variation of adult copulatory organs (Huber 2004; Astrin et al. 2006). These structures, the epigyna in females and pedipalps in males, usually have little intraspecific variation and conspicuous interspecific variation (Huber 2004). However, identification of spiders through morphology is not always straightforward. In some taxa, detailed morphological analyses of genitalia have failed to reveal diagnostic traits for one or both genders, and immatures further lack adult genitalia structures and are difficult to identify.

Additionally, some species show striking sexual dimorphism, exhibiting more than one genitalic morph as a result of environmental changes and/or reproductive isolation (Chang et al. 2007). In other cases, these genitalic morphs are so different that it is difficult to determine whether or not they belong to the same species (Huber 2004; Ubick et al. 2004).

These problems are particularly prevalent in the family Lycosidae and have been observed in different genera including *Trochosa*, *Pirata*, and *Pardosa* (Dondale & Redner 1981; Stratton & Uetz 1981; Stratton 1991; Reiskind & Cushing 1996; Milasowszky et al. 1998; Töpfer-Hofmann et al. 2000; Hepner & Milasowszky 2006; Dreyer & Brady 2008) and other genera (Scheffer et al. 1996; Parri et al. 1997; Miller et al. 1998). In the genus *Pardosa* this topic, mainly with regard to European groups, has received special attention from several authors (Tongiorgi 1966a; Tongiorgi 1966b; Holm & Kronstedt 1970; Hollander & Dijkstra 1974; Kronstedt 1981; Wunderlich 1984; Barthel & Helvesen 1990; Kronstedt 1990, 1992, 2007; Chang et al. 2007). The American groups also contain species with closely similar genitalia (Barnes 1959; Lowrie & Dondale 1981; Dondale & Redner 1984). Thus,

traditional taxonomy of the genus *Pardosa* has limitations for classifying many of its species.

Determining species limits can be facilitated through the use of molecular markers. In particular, genetic information derived from mitochondrial DNA is increasingly being used to supplement morphological data in taxonomy (Brower 1994; Hebert et al. 2003; Segraves & Pellmyr 2001; González et al. 2003; Froufe et al. 2003).

The wolf spider *Pardosa sierra* Banks 1898 (Araneae, Lycosidae) belongs to the *lapidicina* group of *Pardosa*. It is considered a widespread species from the southwestern region of the United States to Oaxaca and Veracruz in Mexico (Barnes 1959; Vogel 2004). These diurnal, ground-dwelling spiders are 5–9 mm. long and live in wetland boundaries of rocky places (Lowrie 1973; Punzo & Farmer 2006). Originally, *P. sierra* was described based on specimens from the Sierra de la Laguna in the southern part of the Baja California Peninsula illustrating a single female morph (Banks 1898). Later, Barnes (1959) revised the *lapidicina* group of *Pardosa* and synonymized *P. atromedia* Banks 1904 from Claremont, California, and *P. sura* Chamberlin & Ivie 1941 from California (36°16'N, 121°56'W) with *P. sierra*. Barnes (1959) also described another female morph from Sierra City, California, noting that this morph and one described by Banks (1898) were, in males, slightly different, but not enough to justify recognition as different species. However, no proof was provided that these morphs were all the same species.

To address this problem, we examined the genitalia of adult spiders collected from the Baja California Peninsula and the northern part of Mexico, in addition to specimens from several museum collections. Also, the cytochrome *c* oxidase subunit I (COI) gene was sequenced to detect differences at the molecular level. In the present study, we attempt a morphological and genetic separation of the different species included in *P. sierra*.

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Table 1.—Individuals sequenced in this work and information on sequences of *Pardosa astrigera* used to compare values of intraspecific and interspecific distances.

| Species                  | Sampling location                  | Accession numbers | Voucher numbers | Individuals used |
|--------------------------|------------------------------------|-------------------|-----------------|------------------|
| <i>Pardosa valens</i>    | Sinaloa, Mexico                    | FJ546474          | CAECIBG 1614    | 1♀ 1♂            |
|                          | Chihuahua, Mexico                  | FJ546475          | CAECIBG 1615    | 2♀               |
| <i>Pardosa steva</i>     | Sonora, Mexico                     | FJ546470          | CAECIBG 1610    | 2♀ 2♂            |
|                          | Nuevo León                         | FJ546471          | CAECIBG 1611    | 2♀ 2♂            |
| <i>Pardosa sura</i>      | Chihuahua, Mexico                  | FJ546468          | CAECIBG 1608    | 2♀ 1♂            |
|                          | Durango, Mexico                    |                   | CAECIBG 1616    | 2♀ 1♂            |
|                          | Nuevo León, México                 | FJ546469          | CAECIBG 1609    | 2♀ 1♂            |
| <i>Pardosa vadosa</i>    | Sonora, Mexico                     | FJ546472          | CAECIBG 1612,   | 2♀ 1♂            |
|                          | Chihuahua, Mexico                  | FJ546473          | CAECIBG 1613    | 2♀ 1♂            |
| <i>Pardosa sierra</i>    | Ensenada, B. C., Mexico            |                   | CAECIBG 1617    | 2♀ 1♂            |
|                          | Cadejé, B. C. S., Mexico           | FJ546465          | CAECIBG 1605    | 2♀ 1♂            |
|                          | Sierra la Laguna, B. C. S., Mexico | FJ546464          | CAECIBG 1604    | 3♀ 3♂            |
| <i>Pardosa atromedia</i> | Rio Osos, California, USA          | FJ546466          | CAECIBG 1606    | 2♀               |
|                          | Rio Osos, California, USA          | FJ546467          | CAECIBG 1607    | 1♂               |
| <i>Pardosa astrigera</i> | China                              | AY836055.1        | -               | -                |
|                          | China                              | AY836072.1        | -               | -                |

## METHODS

**Spider specimens.**—We examined adults of “*P. sierra*” wolf spiders in four collections as follows: American Museum of Natural History, 60 specimens: 15 of *P. sierra*, 30 of *P. atromedia* and 15 of *P. sura*; California Academy of Sciences, 20 specimens: 10 of *P. sierra*, 5 of *P. atromedia* and 5 of *P. sura*; Darrell Ubick’s personal collection: seven specimens of *P. sierra*; Centro de Investigaciones Biológicas del Noroeste: 150 specimens of *P. sierra*. Moreover, specimens of *P. sierra*, *P. atromedia*, and *P. sura* were collected from Baja California Sur, California and Chihuahua for morphological and molecular analysis; specimens of *Pardosa vadosa* Barnes 1959, *Pardosa valens* Barnes 1959, and *Pardosa steva* Lowrie & Gertsch 1955 were collected from Sonora, Chihuahua, and Sinaloa for molecular analysis (Table 1).

**Voucher specimens and morphology.**—Spiders, or parts of spiders, used for DNA extraction were stored in the Arachnological Collection at Centro de Investigaciones Biológicas del Noroeste (Table 1). Somatic measurements were made following the protocols described by Brady (1979) and Dreyer & Brady (2008). The measurements are reported here as mean  $\pm$  standard deviation and maximum and minimum values in tables indicating the variability among species. We dissected and cleaned the genitalia of each specimen under a dissection microscope (60 $\times$ ). Epigynal characteristics include the following: hood, anterior limit of the epigynum, middle field, tissue between the hood and the transverse piece, copulatory openings, orifices for the male copulatory organ, transverse piece, tissue below the copulatory openings, which includes the crescent-shaped troughs, spermathecae, organs that produce germinal cells, copulatory ducts, and tubes connecting spermathecae with the copulatory openings. The main characteristics used for morphological comparison of individuals were the shape of the transverse piece, the middle field and the spermathecae (Figs. 2–4). For the male pedipalpal structure, the characters included total length and distal part of the cymbium, bulb size, embolus, conductor, and median and terminal apophysis. The characteristics used for morphological comparison among individu-

als were embolus, conductor and median accessory process, median and terminal apophysis (Figs. 5, 6, 7).

**Abbreviations.**—Body: sternum width (WE), sternum length (LE), posterior median eye width (PMEW), posterior lateral eye width (PLEW), posterior ocular quadrangle (POQ). Male palpal structures: embolus (E), median apophysis (MA), terminal apophysis (TA). Tibia (TP), femur (FP), total length of cymbium (BT), bulb length (Bx), distal part of the cymbium (AB). Female epigynal structures: fertilization ducts (FD), middle field length (MF), transverse piece (TP), spermathecae (SP), epigynum length (EpL), epigynum wide (EpW). Political units: County (Co.). Institutions: American Museum of Natural History, New York (AMNH); Museum of Comparative Zoology, Cambridge, Massachusetts (MCZ); Centro de Investigaciones Biológicas del Noroeste, La Paz (CARCIB); California Academy of Science, San Francisco (CAS).

**Electron microscopy.**—Genitalia of three males and three females of each species were dissected and processed for scanning electron microscopy (SEM). Genitalia were processed overnight to degrade soft tissue with 100  $\mu$ l DNA extraction buffer, 20  $\mu$ l SDS, and two  $\mu$ l proteinase K in 0.2 ml tubes at 56° C. Subsequently, we stopped the enzymatic process by dehydrating the epigyna in 200  $\mu$ l absolute alcohol, and prepared them for critical point drying. We coated samples with vanadium, examined them under SEM (Hitachi S-3000N), and digitalized images with Quartz PCI 5.0 software.

**DNA extraction.**—All specimens were preserved in 96% ethanol immediately after collection. The total genomic DNA (at least  $n = 3$  in each species) was extracted from legs and sometimes half of the prosoma tissue of individual spiders as described by Aljanabi & Martinez (1997). The next step was to break down the tissue by placing it in a 1.5-ml Eppendorf tube with 410  $\mu$ l extraction buffer (100 mM NaCl, 10 mM EDTA, 10 mM Tris at pH 8.0) and 90  $\mu$ l 10% SDS, and macerating it with a plastic pestle. We added proteinase K (Sigma, #P2308, St. Louis, MO) to a final concentration of 10 U/ml and then incubated the mixture overnight at 56° C. Next we centrifuged

the tubes at 14000 rpm in a microcentrifuge (model 5414, Eppendorf) for 5 min. The supernatant was collected; 180  $\mu$ l 5 M NaCl was added. After mixing and centrifuging again, we recovered the supernatant in a clean tube and cleaned it twice with a chloroform-isoamyl alcohol (24:1) solution. Finally, we precipitated DNA in the supernatant with absolute ethanol and washed it with 80% ethanol.

**Polymerase chain reaction.**—A 710-bp fragment of the COI gene was amplified by PCR with the following primers: COIP-L (5'-TAG AAA TAG GGG TTG GTG-3') and COIP-R (5'-AAT GAA AAT GAG CTA CAA CA-3). These primers were designed from COI sequence of *Pardosa milvina* (Hentz 1844) previously reported (Greenstone et al. 2005 – GenBank sequence DQ072280). We performed PCR amplification in a 15  $\mu$ l reaction volume containing approximately 50 ng genomic DNA, 0.40 mM each primer, 2.5 mM MgCl<sub>2</sub>, 0.2 mM of each of the dNTP, 1 $\times$ PCR buffer (Invitrogen, #Y02028, Carlsbad, California), and 0.5 U Taq polymerase (Invitrogen, #18038-042). Next, we ran PCR with initial denaturing step at 94° C for 4 min, followed by 35 cycles at 94° C for 30 s, 30 s at 52° C, and 30 s at 72° C, and a final step at 72° C for 10 min, in a programmable thermal cycler (BioRad Laboratories, Hercules, California).

**DNA sequencing.**—PCR amplification products were submitted for single-strand sequencing, using the ABI dye termination method in an ABI 377 automatic sequencer (Macrogen, Seoul, Korea). We translated each sequence in the amino acids, and no stop internal codons were found. We submitted all sequences to GenBank (Table 1). For comparative purposes, COI sequences of the three species were compared at two levels (intraspecific and interspecific) with sequences of some other *lapidicina* group species. We also used COI sequences of *P. astrigera* L. Koch 1878, because it has the most diverse dataset of haplotypes reported for a single species in the genus *Pardosa* (Chang et al. 2007).

**Sequence analysis.**—We made DNA sequence alignments of COI gene fragments from various spiders in the Chromas Pro program and ClustalX Windows interface v. 1.8 (Thompson et al. 1997), using these default parameters: gap opening cost = 15; gap extension cost = 6.66; delay divergent sequences = 30%; and DNA transition = 0.50). Sequences were truncated to 630 bp to avoid any bias in sequence alignment. We visually checked alignments using the BioEdit program (Hall 2007); final alignments were exported to a Nexus file using ClustalX Windows interface v. 1.8 (Thompson et al. 1997).

**Molecular taxonomy.**—We estimated taxa separation by calculating the interval between the lowest interspecific and the highest intraspecific observations (Astrin et al. 2006). A negative value indicates the numerical extent of overlap of both categories (intraspecific vs. interspecific distances). In this way, the gap range has to be zero or a negative value to consider an overlap that could be the degree of overlap between categories (intraspecific vs. interspecific) and is expressed by the proportion of data outside the gap range.

We used PAUP\* ver. 4.0b10 (Swofford 2000) to calculate *p*-distance matrices as well as for construction of a neighbor-joining (NJ, Saitou & Nei 1987) tree, chosen to build a species identification tree (distinct from a tree chosen when striving for phylogenetic accuracy). As an exclusively algorithmic, phenetic procedure, NJ is fast at processing datasets, but

produces only a single uncontested tree, often applied in molecular taxonomy (Paquin & Hedin 2004; Barrett & Hebert 2005; López-Legentil & Turon 2005; Markmann & Tautz 2005; Vences et al. 2005; Ward et al. 2005; Hajibabaei et al. 2006; Astrin et al. 2006; Smith et al. 2006). We used the Shapiro-Wilk test implemented in STATISTICA vs. 6 to test for a normal distribution of *p*-distance values.

## SYSTEMATICS

Family Lycosidae Sundevall 1833  
Subfamily Pardosinae Simon 1898  
Genus *Pardosa* C.L. Koch 1847

**Type species.**—*Lycosa alacris* C.L. Koch 1833, designated by Charitonov (1932).

**The *lapidicina* group.**—This group presents a great many taxonomic difficulties because of its extreme homogeneity. All members of the *lapidicina* group possess essentially the same markings, although each species exhibits a considerable range in coloration from very pale to very dark individuals (Barnes 1959). The carapace is highest between the second and third eye row and slopes slightly to the posterior declivity in the posterior one-fifth of the carapace. The anterior median eyes are separated by approximately four-fifths of a diameter; the anterior laterals are three-fourths to one-half of the diameter of the anterior median eyes in size and separated from the latter by one-fifth of their diameter. The eyes of the second row are two to two and one-half times the diameter of the anterior median eyes. The eyes of the third row are only very slightly smaller than the eyes of the second row and are separated from the latter by one to two times the diameter of the eyes of the second row. The second eye row is one and one-half times the length of the first; the third eye row, twice the length of the first. The ocular area is wider than long. Order of leg length: 4:1:2:3 (Barnes 1959).

The structure of the male pedipalpal organ is the most valuable character for separating the species of the *lapidicina* group (Barnes 1959), but not in the case of our three species where the female genitalia showed the main differences among species.

After morphological study of specimens from collections, we found three morphs designated as *P. sierra* described by Banks 1898:374, pl. XVI, fig. 20 as *P. sierra*, *P. atromedia* described by Banks 1904:355, pl. XXXIX, fig. 32, and *P. sura* (with epigynum illustrated by Chamberlin & Ivie 1941:10, pl. V, fig. 61) and Barnes 1959, fig. 36 as a morph of *P. sierra*. *Pardosa sierra* was described from a collection from the Sierra de la Laguna (Banks 1898), but also was collected from other localities on the Baja California Peninsula. *Pardosa atromedia* was collected from California. *Pardosa sura* was collected in Mexico (Distrito Federal, Estado de Mexico, Chihuahua, and Puebla) and in the southwestern USA (Utah, Colorado, Arizona, California, and Texas) (Fig. 1 and Table 1).

We designated a neotype for *Pardosa sura* following the statements in the International Code of Zoological Nomenclature, based principally that it will clarify the taxonomic status of the species of interest; data and description are sufficient to ensure recognition of the specimen designated. Additionally, type specimens (holotype and lectotype) have been lost, though we made the necessary steps to trace them.

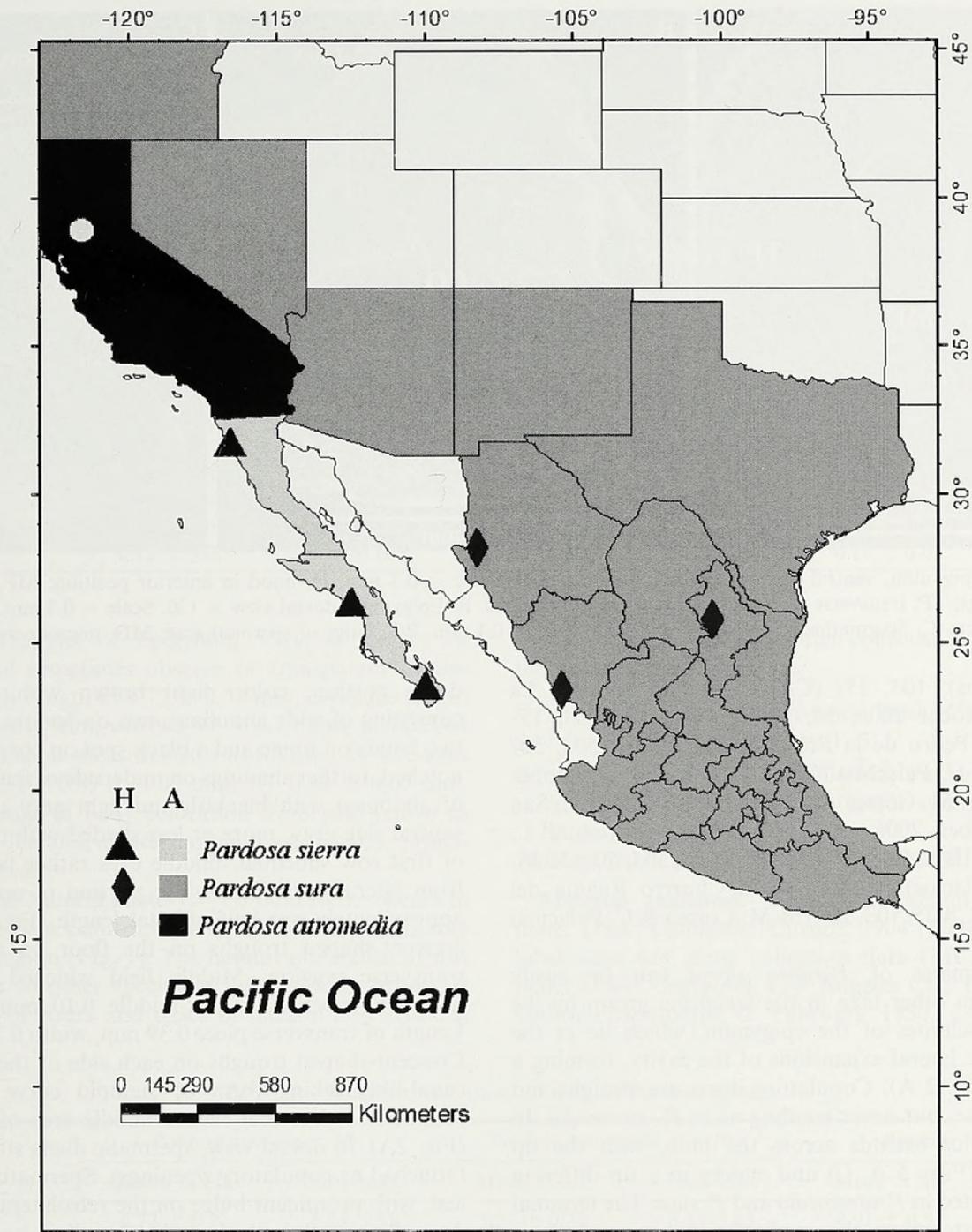


Figure 1.—Distribution of the *Pardosa sierra* species complex. Symbols represent haplotype origins (H); gray and black areas (A) represent potential distribution of the species based on location of specimens in collections. *P. sierra* = triangles and light gray; *P. atromedia* = circle and black area; *P. sura* = diamonds and dark gray.

*Pardosa sierra* Banks 1898

Figs. 1, 2, 5

*Pardosa sierra* Banks 1898:274; Petrunkevitch 1911:575; Gertsch, 1934:19; Roewer, 1954:194; Bonnet, 1958:3422; Barnes 1959:14; Vogel 2004:72; Platnick 2009.

**Material examined.**—Lectotype (present designation) female: MEXICO: *Baja California*: Sierra Laguna: 1898 (Nathan Banks Coll.), label does not show collection record (MCZ). Paralectotype male: MEXICO: *Baja California Sur*: collected from Sierra de la Laguna 2–4 November 2006 (M.M. Correa & C. Palacios) (CARCIB). Holotype female

deposited in CAS was destroyed by the earthquake and fire of 1906.

**Other material examined.**—MEXICO: *Baja California*: Isla Cedros, 22 February 1945, 8♀ (B. F. Osorio & M. T. H. Tafall) (AMNH); Idem Gran Cañón, 10 March 1945, 6♀ (B.F. Osorio & M.T.H. Tafall) (AMNH); Tajo Branch of Cantil Canyon east side of Laguna Salada, 2♀ (T. Briggs) (CAS); Ensenada, April 2008, 3♂, 5♀ (García de León) (CARCIB); El Rosarito, April 2008, 1♂, 3♀ (García de León) (CARCIB); Arrollo Cataviña, 2♀ (CAS). *Baja California Sur*: Sierra la Laguna, 1898, 1♀; idem 2–4 November 2006, 50♂, 50♀ (M.M. Correa & C. Palacios) (CARCIB); San José de Comondú, 29 October

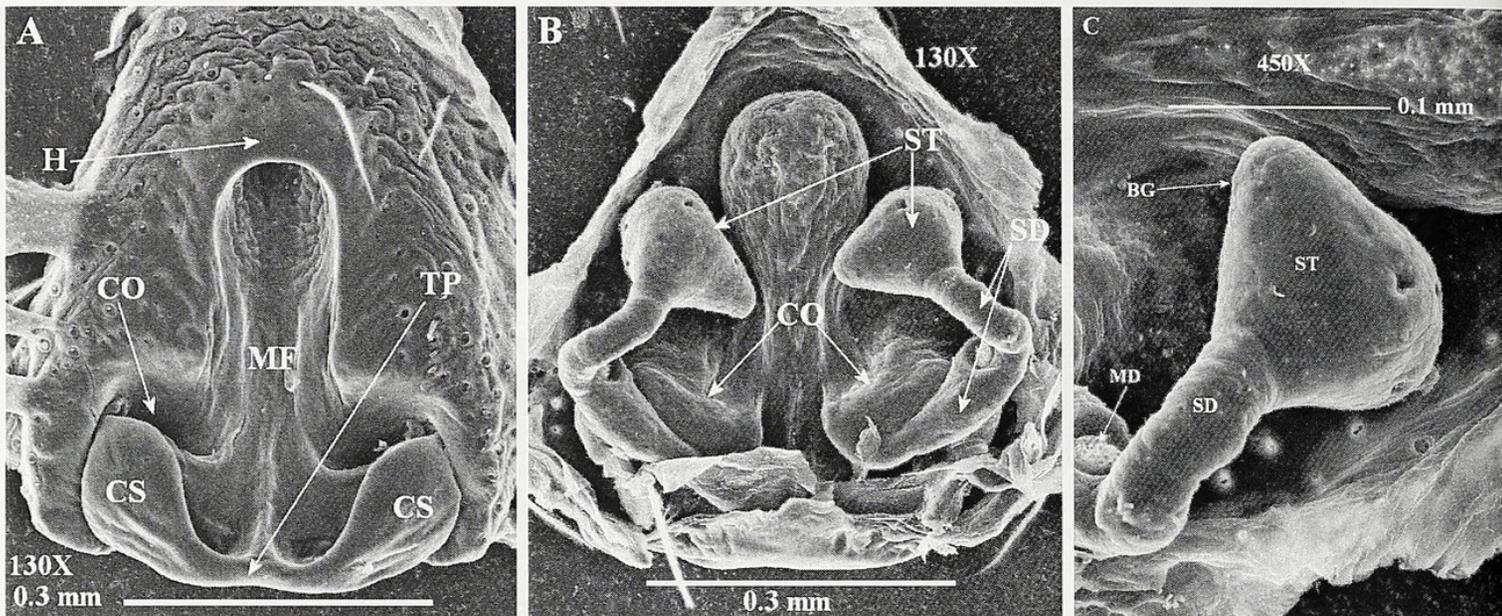


Figure 2.—A. Epigynum, ventral view of *Pardosa sierra*  $\times 130$ . Scale = 0.3 mm. H, hood in anterior position; MF, middle field; CO, copulatory openings; TP, transverse piece; CS, crescent-shaped troughs. B. Epigynum, dorsal view  $\times 130$ . Scale = 0.3 mm. ST, spermathecae; SD, copulatory ducts. C. Spermathecae, dorsal view  $\times 450$ . Scale = 0.1 mm. BG, bulge of spermathecae; MD, microducts.

2006 (C. Palacios); 10♂, 15♀ (CARCIB); San Isidro - La Purísima, 28 October 2006, 5♂, 20♀ (C. Palacios); 5♂, 15♀ (CARCIB); San Pedro de la Presa, 19 June 2008, 50♂, 50♀ (M.M. Correa & C. Palacios) (CARCIB); Cadejé, 5 October 2006, 50♂, 50♀ (M.M. Correa & C. Palacios) (CARCIB); San Ignacio, 3 October 2006, 50♂, 50♀ (M.M. Correa & C. Palacios) (CARCIB); Mulegé, 4 October 2006, 50♂, 50♀ (M.M. Correa & C. Palacios) (CARCIB); El Chorro Región del Cabo, 23 October 2005, 10♂, 20♀ (M.M. Correa & C. Palacios) (CARCIB).

**Diagnosis.**—Females of *Pardosa sierra* can be easily distinguished from other taxa in the *lapidicina* group by the crescent-shaped sclerites of the epigynum, which lie at the apical edge of the lateral expansions of the cavity, forming a sigmoid curve (Fig. 2 A). Copulatory ducts are straight and wider at their base, but never winding as in *P. atromedia*. In males, the embolus extends across the bulb, with the tip curving apically (Figs. 5 A, C) and ending in a tip differing from those presented in *P. atromedia* and *P. sura*. The terminal apophysis is shorter than the median apophysis, which is thumb-like and straight. *Pardosa sierra* differs from other closely related species on the basis of the following unique mtDNA nucleotide substitutions at the following reference alignment positions: C (51), G (54), G (63), G (102), T (264), G (267), G (279), T (226), G (390), C (477), A (480), C (489), G (543) and T (606).

**Description.**—*Female* (lectotype): Total length 5.8 mm, carapace length 2.75 mm, width 2.31 mm. Prosoma light brown, eye region black, an irregular broad band on each upper side, which indents in the middle area before the groove; clypeus with white and black hairs, large marginal spots and a patch across the clypeus. Sternum brownish black, covered with hairs, with pale margins. Chelicerae brown, with some dark hairs. Endites dusky brown with pale tips; labium dusky brown with a pale tip. Legs slender, hind pair very long; tibiae I and II with three subequal pairs of spines and a short pair on

distal portion; color, light brown with black markings, consisting of wide annulling, two on femora, one on patellae, two bands on tibiae and a black spot on coxae, all trochanters notched, further shadings on underside of femora. Dorsal view of abdomen with blackish and light gray spots and specks, ventral side gray, more or less shaded with darker gray. Eyes of first row subequal, middle eyes rather farther apart than from lateral eyes. Transverse portion of epigynum occupies approximately one-half of total length (Fig. 2), with pair of crescent-shaped troughs on the floor on each side of the transverse portion. Middle field widened anteriorly, with narrowest portion in the middle 0.10 mm long (Fig. 2A). Length of transverse piece 0.39 mm, width 0.39 mm (Fig. 2A). Crescent-shaped troughs on each side of the transverse piece canal-like, taking form of sigmoid curve with wide and rounded borders that reduce middle area of transverse piece (Fig. 2A). In dorsal view, spermathecae straight at the base (attached to copulatory openings). Spermathecae semi-spherical, with prominent bulge on the retrolateral sides (Fig. 2C). As well as in the other species described here, the epigynum has a rounded structure located in the middle part of the spermathecae that appears to be a series of microducts (MD in Fig. 2C).

**Male** (Paralectotype): Total length 4.25 mm, carapace length 2.18 mm, width 1.76 mm. Color and body shape similar to female, but darker. Embolus short, with thin tip. Conductor of the male pedipalpal structure sword-shaped and projecting upward from the bulb. This process is sufficiently sclerotized and conspicuous to be distinctly visible in the unexpanded pedipalp. Conductor rounded. Median accessory process (Fig. 5A) much less conspicuous and concealed or partially concealed by the conductor in unexpanded pedipalp.

**Variations.**—Females have average body length of  $7.87 \pm 0.52$  mm, carapace length averaging  $2.96 \pm 0.25$  mm, width  $2.52 \pm 0.21$  mm. Epigyna vary as follows: MF with average length of  $0.12 \pm 0.02$  mm; EpL  $0.39 \pm 0.06$  mm and EpW  $0.39$

Table 2.—Measurements (mm) of female of *Pardosa* species.

|                 | <i>Pardosa sierra</i> n = 10 |           | <i>Pardosa atromedia</i> n = 10 |           | <i>Pardosa sura</i> n = 10 |           |
|-----------------|------------------------------|-----------|---------------------------------|-----------|----------------------------|-----------|
|                 | Mean ± SD                    | Min – Max | Mean ± SD                       | Min – Max | Mean ± SD                  | Min – Max |
| Total Length    | 7.87 ± 0.52                  | 7.08–8.63 | 6.81 ± 0.64                     | 6.00–7.50 | 7.22 ± 0.77                | 5.58–8.25 |
| Carapace Length | 2.96 ± 0.25                  | 2.48–3.25 | 2.93 ± 0.19                     | 2.48–3.25 | 3.04 ± 0.33                | 2.58–3.55 |
| Carapace Width  | 2.52 ± 0.21                  | 2.24–2.85 | 2.51 ± 0.17                     | 2.27–2.90 | 2.56 ± 0.34                | 2.09–3.15 |
| WE              | 1.43 ± 0.10                  | 1.29–1.57 | 1.42 ± 0.09                     | 1.27–1.57 | 1.40 ± 0.12                | 1.14–1.61 |
| LE              | 1.57 ± 0.11                  | 1.33–1.69 | 1.55 ± 0.09                     | 1.37–1.71 | 1.53 ± 0.13                | 1.27–1.76 |
| PMEW            | 0.96 ± 0.04                  | 0.86–1.02 | 1.00 ± 0.08                     | 0.86–1.10 | 1.05 ± 0.10                | 0.88–1.18 |
| PLEW            | 1.29 ± 0.08                  | 1.14–1.39 | 1.36 ± 0.10                     | 1.18–1.47 | 1.39 ± 0.14                | 1.18–1.59 |
| POQ Length      | 0.96 ± 0.06                  | 0.84–1.06 | 1.00 ± 0.08                     | 0.86–1.12 | 1.05 ± 0.10                | 0.90–1.18 |
| Femur I         | 2.93 ± 0.24                  | 2.50–3.30 | 3.00 ± 0.16                     | 2.70–3.20 | 2.80 ± 3.20                | 2.21–0.34 |
| Femur II        | 2.89 ± 0.28                  | 2.45–3.35 | 2.95 ± 0.15                     | 2.65–3.15 | 2.74 ± 0.33                | 2.18–3.15 |
| Tibia I         | 2.59 ± 0.22                  | 2.20–2.90 | 2.69 ± 0.18                     | 2.40–2.90 | 2.51 ± 3.35                | 1.82–0.44 |
| Tibia III       | 2.30 ± 0.18                  | 2.00–2.55 | 2.45 ± 0.14                     | 2.20–2.60 | 2.51 ± 0.25                | 2.27–3.10 |
| Tarsus I        | 1.15 ± 0.07                  | 1.04–1.24 | 1.15 ± 0.06                     | 1.02–1.24 | 1.07 ± 0.10                | 0.90–1.29 |
| Trochanter IV   | 0.52 ± 0.04                  | 0.49–0.59 | 0.58 ± 0.03                     | 0.53–0.63 | 0.55 ± 0.07                | 0.45–0.65 |
| EpL             | 0.39 ± 0.06                  | 0.33–0.55 | 0.51 ± 0.04                     | 0.41–0.55 | 0.50 ± 0.07                | 0.39–0.61 |
| EpW             | 0.39 ± 0.03                  | 0.33–0.47 | 0.49 ± 0.04                     | 0.43–0.53 | 0.47 ± 0.55                | 0.41–0.04 |
| MF              | 0.12 ± 0.02                  | 0.10–0.16 | 0.13 ± 0.01                     | 0.12–0.16 | 0.14 ± 0.03                | 0.10–0.18 |

± 0.03 mm. Troughs of epigynum vary in degree of sclerotization and sometimes obscure or transparent. Males have average body length of  $4.72 \pm 0.32$  mm, carapace length averages  $2.40 \pm 0.14$  mm, width  $1.97 \pm 0.13$  mm, pedipalpal structures vary in some measurements as follows: TP averages  $0.50 \pm 0.06$  mm, FP  $0.87 \pm 1.02$  mm, BT  $0.80 \pm 0.07$  mm. (Tables 2, 3). Range of body coloration from pale yellow to dusky brown in females; males darker than females, principally in ocular area.

**Distribution and Natural History.**—*Pardosa sierra* occurs in most parts of the Baja California Peninsula and is presumably endemic to this region (Fig. 1). The habitat preference of this species is similar to other species in the *lapidicina* group. It prefers the edge of rivers and oases or natural and artificial

rocky wetlands, where it is often collected by hand and/or with the use of pitfall traps.

*Pardosa atromedia* Banks 1904

Figs. 1, 3, 6

*Pardosa atromedia* Banks 1904:355; Petrunkevitch 1911:571 (in part); Roewer 1954:194 (junior synonymy of *P. sierra* Banks 1898).

*Pardosa sierra* Banks 1898; Barnes 1959:14.

**Material examined.**—Lectotype (present designation) female: USA: California: Curtice, 1904 (Nathan Banks Coll.), label does not show collection data (MCZ). Paralectotype male: USA: California: Los Angeles Co., Fish Canyon, San Gabriel Mountains, 2 February 1950, 1♂ (E.I. Schlinger)

Table 3.—Measurements (mm) of male of *Pardosa* species.

|                 | <i>Pardosa sierra</i> n = 10 |           | <i>Pardosa atromedia</i> n = 10 |           | <i>Pardosa sura</i> n = 10 |           |
|-----------------|------------------------------|-----------|---------------------------------|-----------|----------------------------|-----------|
|                 | Mean ± SD                    | Min–Max   | Mean ± SD                       | Min–Max   | Mean ± SD                  | Min–Max   |
| Total Length    | 4.72 ± 0.32                  | 4.25–5.33 | 5.57 ± 0.22                     | 5.33–5.83 | 4.91 ± 0.51                | 4.35–5.50 |
| Carapace Length | 2.40 ± 0.14                  | 2.18–2.64 | 2.62 ± 0.22                     | 2.30–2.88 | 2.50 ± 0.19                | 2.33–2.73 |
| Carapace Width  | 1.97 ± 0.13                  | 1.76–2.18 | 2.21 ± 0.14                     | 2.09–2.36 | 2.04 ± 0.20                | 1.82–2.27 |
| WE              | 1.15 ± 0.08                  | 1.04–1.24 | 1.19 ± 0.07                     | 1.12–1.27 | 1.11 ± 0.08                | 1.02–1.22 |
| LE              | 1.26 ± 0.07                  | 1.18–1.37 | 1.36 ± 0.03                     | 1.31–1.39 | 1.31 ± 0.11                | 1.18–1.47 |
| PMEW            | 0.80 ± 0.03                  | 0.75–0.86 | 0.97 ± 0.03                     | 0.94–1.02 | 0.86 ± 0.05                | 0.80–0.94 |
| PLEW            | 1.06 ± 0.05                  | 0.94–1.14 | 1.25 ± 0.06                     | 1.18–1.33 | 1.17 ± 0.07                | 1.08–1.24 |
| POQ             | 0.78 ± 0.03                  | 0.75–0.84 | 0.94 ± 0.01                     | 0.92–0.94 | 0.85 ± 0.06                | 0.80–0.94 |
| Femur I         | 2.20 ± 0.18                  | 1.91–2.52 | 2.73 ± 0.20                     | 2.55–3.00 | 2.37 ± 0.25                | 2.10–2.70 |
| Femur II        | 2.14 ± 0.16                  | 1.85–2.45 | 2.67 ± 0.17                     | 2.50–2.85 | 2.31 ± 0.23                | 2.05–2.60 |
| Tibia I         | 2.22 ± 3.20                  | 1.76–0.48 | 2.55 ± 0.38                     | 2.05–3.10 | 2.14 ± 0.19                | 2.00–2.45 |
| Tibia III       | 2.13 ± 0.47                  | 1.57–2.80 | 2.41 ± 0.23                     | 2.15–2.75 | 1.95 ± 0.19                | 1.80–2.25 |
| Tarsus I        | 0.95 ± 0.03                  | 0.90–1.00 | 1.10 ± 0.04                     | 1.06–1.14 | 0.99 ± 0.09                | 0.86–1.12 |
| Trochanter IV   | 0.41 ± 0.03                  | 0.37–0.45 | 0.49 ± 0.00                     | 0.49–0.49 | 0.44 ± 0.05                | 0.39–0.49 |
| TP              | 0.50 ± 0.06                  | 0.41–0.63 | 0.59 ± 0.02                     | 0.57–0.63 | 0.57 ± 0.06                | 0.49–0.65 |
| FP              | 0.87 ± 1.02                  | 0.82–0.06 | 0.99 ± 0.06                     | 0.92–1.06 | 0.94 ± 0.11                | 0.82–1.08 |
| BT              | 0.80 ± 0.07                  | 0.73–0.94 | 0.99 ± 0.05                     | 0.94–1.06 | 0.86 ± 0.05                | 0.82–0.94 |
| Bx              | 0.48 ± 0.07                  | 0.41–0.65 | 0.60 ± 0.02                     | 0.59–0.63 | 0.53 ± 0.04                | 0.49–0.59 |
| AB              | 0.33 ± 0.02                  | 0.29–0.37 | 0.39 ± 0.04                     | 0.35–0.43 | 0.33 ± 0.02                | 0.29–0.35 |

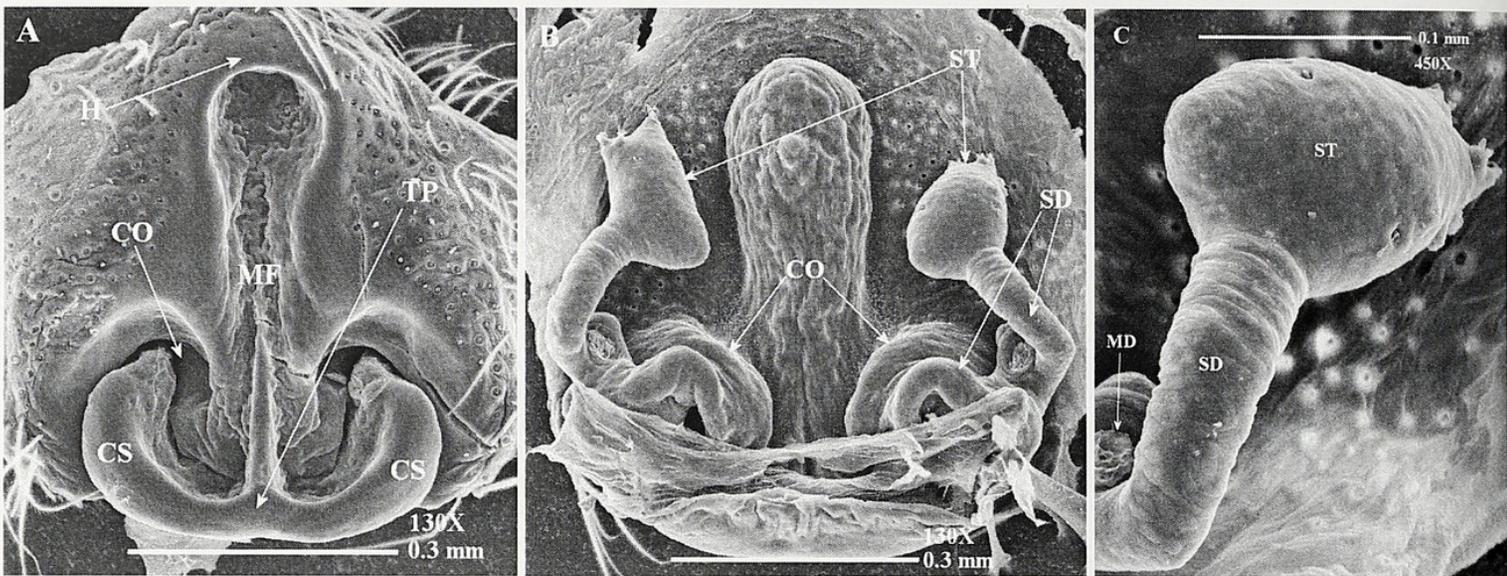


Figure 3.—A. Epigynum, ventral view of *P. atromedia*  $\times 130$ . Scale = 0.3 mm. H, hood in anterior position; MF, middle field; CO, copulatory openings; TP, transverse piece; CS, crescent-shaped troughs. B. Epigynum dorsal view  $\times 130$ . Scale = 0.3 mm. ST, spermathecae; SD, copulatory ducts. C. Spermathecae, dorsal view  $\times 450$ . Scale = 0.1 mm. BG, bulge of spermathecae; MD, microducts.

(AMNH). Holotype female deposited in CAS was destroyed by the earthquake and fire of 1906.

**Other material examined.**—USA: *California*: Curtice, 1904, 1♀ (Nathan Banks Coll.) (MCZ); 40°N, 111°W (AMNH); Calaveras Co., 5 mi west of Durrington, Stanislaus River, 5 August 1953, 2♀ (W. Gertsch & J. Gertsch) (AMNH); Toulumne Co., Pinecrest, 6 July 1947, 1♀ (P. H. Arnaud) (CAS); Contra Costa Co., Diablo, 25 March 1947, 6♀ (B. Malkin & D.G. Kelley) (AMNH); Monrovia Canyon, 26 July 1931, 34°10'N, 117°5'W, 2♀ (Chamberlin & Ivie) (AMNH); San Diego River near mouth, 12 July 1931, 32° 46'N, 117°10'W, 1♀ (Chamberlin & Ivie) (AMNH); San Diego Co., Indian Canyon, 25 May 1948, 1♂, 1♀ (M.A. Pearce) (AMNH); Siskiyou Co., Wildcat Creek, 1 mi NW Callahan, 31 July 1968, 1♀ (H.B. Leech) (CAS); San Juan Creek, near mountains, 18 July 1931, 33°27'N, 117°40'W, 2♀ (Chamberlin & Ivie) (AMNH); Palm Springs, 5 April 1925, 33° 55'N, 116° 40'W, 2♀ (Chamberlin & Ivie) (AMNH); San Diego, 2♀; Lower End Indian Canyon, 14 July 1948, 1♀ (M.A. Pearce) (AMNH); Santiago, 33°45'N, 117°45'W, 29 December 1930, 1♀ (Chamberlin & Ivie) (AMNH); Los Angeles Co., Valyermo, Los Angeles Big Rock Creek, 4200 ft, 12 June 1943, 1♀ (K. Cowles) (AMNH); Mono Co., Montgomery Canyon, 13 July 1941, 5♀ (M.A. Pearce) (AMNH); Los Angeles Co., Fish Canyon, San Gabriel Mountains, 2 February 1950, 1♂ (E.I. Schlinger) (AMNH), Idem, Los Angeles Co., 2 October 1944, 1♀ (E.I. Schlinger) (AMNH); Idem, Los Angeles Co., 29 April 1945, 3♂, 5♀ (E. I. Schlinger) (AMNH); Roads End, Kern River, 3 July 1956, 2♀ (V. Roth & W. Gertsch) (AMNH); Claremont, California (R.V.C.) (R-4) 1♀ (Chamberlin) (AMNH); Idem 34°3'N, 117°48'W, 1♀ (Chamberlin); Yosemite Park (Wawona Camp), 17 September 1941, 37°32'N, 119°39'W, 1♂ (Ivie) (AMNH); Mariposa Co., Idem, 14 July 1952, 1♂, 3♀ (W. Gertsch, Schrammel & M. Cazier) (AMNH); Irwins, near Santa Ana Park, 17 July 1931, 33°40'N, 117°48'W, 1♂ (Chamberlin & Ivie) (AMNH); Near San Diego Mission, 12 July 1931, 5♀ (Chamberlin Det. Ivie) (AMNH); San Diego, 2 June 1948, 2♂, 3♀ (M.A. Pearce) (AMNH); Eatons Canyon,

March 1913, Quad. 34°N, 118°W, 2♂ (Chamberlin) (AMNH); Idem, March 1913, Quad. 34°N, 118°W, 2♂ (Chamberlin) (AMNH); Inyo Co., Olanca, 18 July 1952, 1♀ (W. Gertsch, Schrammel & M. Cazier) (AMNH); San Juan Hot Spring, 3 July 1931, 33°36'N, 117°33'W, 2♀ (Chamberlin & Ivie) (AMNH); Idem, 3 July 1931, 33°36'N, 117°33'W, 4♀ (Chamberlin & Ivie) (AMNH); Santa Monica, 12♀ (Det. Gertsch) (AMNH); Idem, 9♀ (Det. Gertsch) (AMNH); San Diego Co., Houser Creek, 29 June 1948, 4♀ (M.A. Pearce) (AMNH); Pine Forest, 21 November 1927, 1♀ (W.G. Dietz); Riverside Co., Magnesia Canyon, 21 April 1951, 1♀ (E.I. Schlinger) (AMNH); Riverside Co., Idyllwild, 7 July 1953, 1♀ (W. Gertsch & J. Gertsch) (AMNH); Palm Spring, Andreas Canyon, 3 March 1956, 3♂, 5♀ (V. Roth) (AMNH); San Diego Co., Boulder Creek, 6 May 1948, 1♂, 8♀ (M.A. Pearce) (AMNH); Tulare Co., Kawea River, 5 mi E. Treerivers, 1258 ft 17 July 1952, 2♀ (W. Gertsch) (AMNH); Los Angeles Co., Tanbark Flats, San Gabriel Mountains, 20 June 1952, 1♂, 6♀ (W. Gertsch) (AMNH).

**Diagnosis.**—Females of *Pardosa atromedia* can be differentiated from *P. sierra* and *P. sura* by the crescent-shaped sclerites of the epigynum which are curved and slender, almost with the same thickness as in the apical lateral parts and in the middle part of the transverse piece (Fig. 3A). The thin septal ridge extends apically to the hood. Copulatory ducts winding at their base and never are straight as in *P. sierra* and *P. sura*. In males, the embolus extends across the bulb with the tip curving apically, but ends in a blunt tip (Fig. 6C), not as in *P. sierra*. The terminal apophysis is nearly half as long as the median apophysis. It is thumb-like and straight; the median accessory process is indistinguishable with respect to *P. sierra* and *P. sura*. *Pardosa atromedia* differs from other closely related species on the basis of the following unique mtDNA nucleotide substitutions at the following reference alignment positions: C (42), G (66), A (81), G (114), G (129), G (288) and A (423).

**Description.**—*Female* (Lectotype): Total length 7.03 mm., width 2.39 mm. Cephalothorax pale yellow with median band

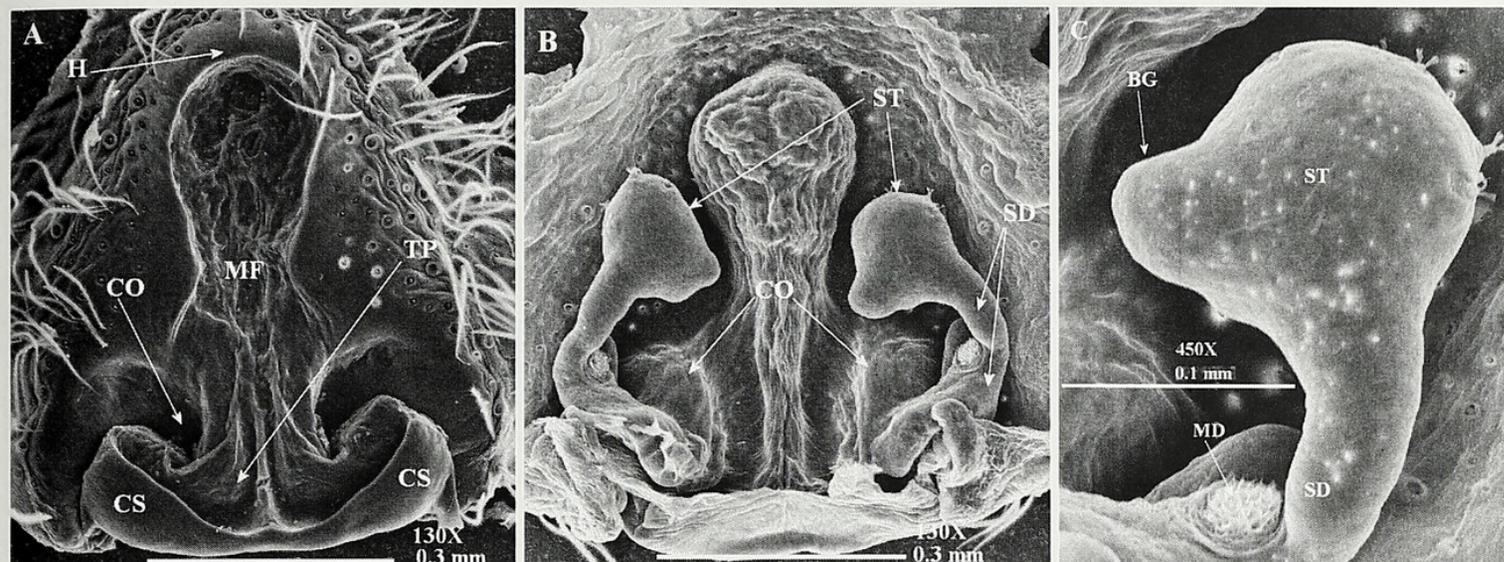


Figure 4.—A. Epigynum, ventral view of *P. sura*  $\times 130$ . Scale = 0.3 mm. H, hood in anterior position; MF, middle field; CO, copulatory openings; TP, transverse piece; CS, crescent-shaped troughs. B. Epigynum dorsal view  $\times 130$ . Scale = 0.3 mm. ST, spermathecae; SD, copulatory ducts. C. spermathecae dorsal view  $\times 450$ . Scale = 0.1 mm. BG, Bulge of spermathecae; MD, microducts.

light brown, eye region black, irregular broad band on each upper side, which indents in the middle area before the groove; clypeus with white and black hairs, large marginal spots and patch across it. Sternum brown, covered with hairs, with pale margins. Chelicerae light reddish to dark brown, shaded with dusky brown. Endites brown with pale tips, labium brown with pale tip. Legs slender, hind pair very long; tibiae I and II with 4 pair of subequal spines, legs pale yellowish with dusky brown markings, consisting of wide annulling, two on femora, one on patellae, two bands on tibiae and a black spot in coxae, further shadings underside of the femora. Dorsal view of abdomen marked with gray and light yellow spots and specks; ventral side light gray, more or less shaded with darker gray. Eyes of first row subequal, middle eyes rather farther apart than from lateral eyes. Transverse piece of epigynum 0.55 mm wide, 0.53 mm long (Fig. 3A). Crescent-shaped troughs on each side of transverse piece curved and slender, with almost equally thick in middle part of transverse piece. In dorsal view, spermathecae winding at base and straight at anterior part, with same thickness (Fig. 3B). Spermathecae almost spherical, with apical small bulges (Fig. 3C).

**Male** (Paralectotype): Total length 5.83 mm, carapace length 2.88 mm, width 2.09 mm. Appearance similar to female. Conductor of the male pedipalp sword shaped and projecting upward from the bulb, conductor truncated, embolus little winding with blunt tip, median accessory process, median and terminal apophysis similar to those presented in *P. sierra* and *P. sura*.

**Variation.**—Females have average body length of  $6.81 \pm 0.64$  mm, carapace length averages  $2.93 \pm 0.19$  mm, width  $2.51 \pm 0.17$  mm; epigynum varying as follows: MF with average length of  $0.13 \pm 0.01$  mm; EpL  $0.51 \pm 0.04$  mm and EpW  $0.49 \pm 0.04$  mm. Troughs of epigynum vary in degree of sclerotization and sometimes obscure or transparent. Males have average body length of  $5.57 \pm 0.22$  mm, carapace length averages  $2.62 \pm 0.22$  mm, width  $2.21 \pm 0.14$  mm, pedipalps vary in some measurements as follows: average TP of  $0.59 \pm 0.02$  mm, FP  $0.99 \pm 0.06$  mm, BT  $0.99 \pm 0.05$  mm. Body color

ranges from pale yellow to dusky brown in females and sometimes reddish; males darker than females, principally in ocular area.

**Distribution and Natural History.**—*Pardosa atromedia* occurs in major parts of California and apparently is restricted to the state (Fig. 1). The habitat preference of this species is similar to other species in the *lapidicina* group. It prefers the edge of rivers, generally with rocks, where it is often collected by hand.

*Pardosa sura* Chamberlin & Ivie 1941

Figs. 1, 4, 7

*Pardosa sura* Chamberlin & Ivie 1941:10; Roewer 1954:194.

*Pardosa sierra* Banks 1898; Barnes 1959:14.

**Material examined.**—Neotype, (present designation) female: USA: *California*: West Sierra County, Sierra City, 7 mi, 8 July 1952 (W. Gertsch) (AMNH). Paratype male: USA: *Utah*: Beaver Canyon, 6 August 1927 (R.V. Chamberlin & W. Ivie) (AMNH). Holotype female and paratype female deposited in Museum of Natural History at the University of Utah, are lost; the arachnological collection of this institution has changed its location several times until finally it was integrated into the collection of the AMNH, but we did not find these specimens in that collection.

**Other material examined.**—USA: *Oregon*: Corvallis, Kiger Isl., on rocky shore, 18 July 1951, 1♂ (V.Roth) (AMNH); Robinette, 18 June 1938, 1♀ (Hatch) (CAS). *Utah*: Beaver Canyon, 6 August 1927, 5♂, 5♀ (R.V. Chamberlin & W. Ivie) (AMNH). *California*: West Sierra County, Sierra City, 7 mi, 8 July 1952, 2♀ (W. Gertsch) (AMNH). *Arizona*: Sabino Canyon Sta., Catalina Mountains, 26 July 1948, 2♀ (W. Gertsch & J. Gertsch) (AMNH); Roosevelt Lake, 23 August 1923, 1♀ (R. Flock) (CAS); Grand Canyon, Indian Gardens, 24 July 1934, F340724, 1♂, 1♀ (Lutz Det. Gertsch) (AMNH); Coyote Mountains, 4–7 August 1916, 1♂, 1♀ (Lutz Det. Gertsch) (AMNH); 5 mi west Portal, Southwestern Research Station, 6–20 July 1955, 2♀ (W. Gertsch) (AMNH); Oak Creek Canyon, Manzanita Camp, 26 July 1950, 2♀ (M. A. Cazier)

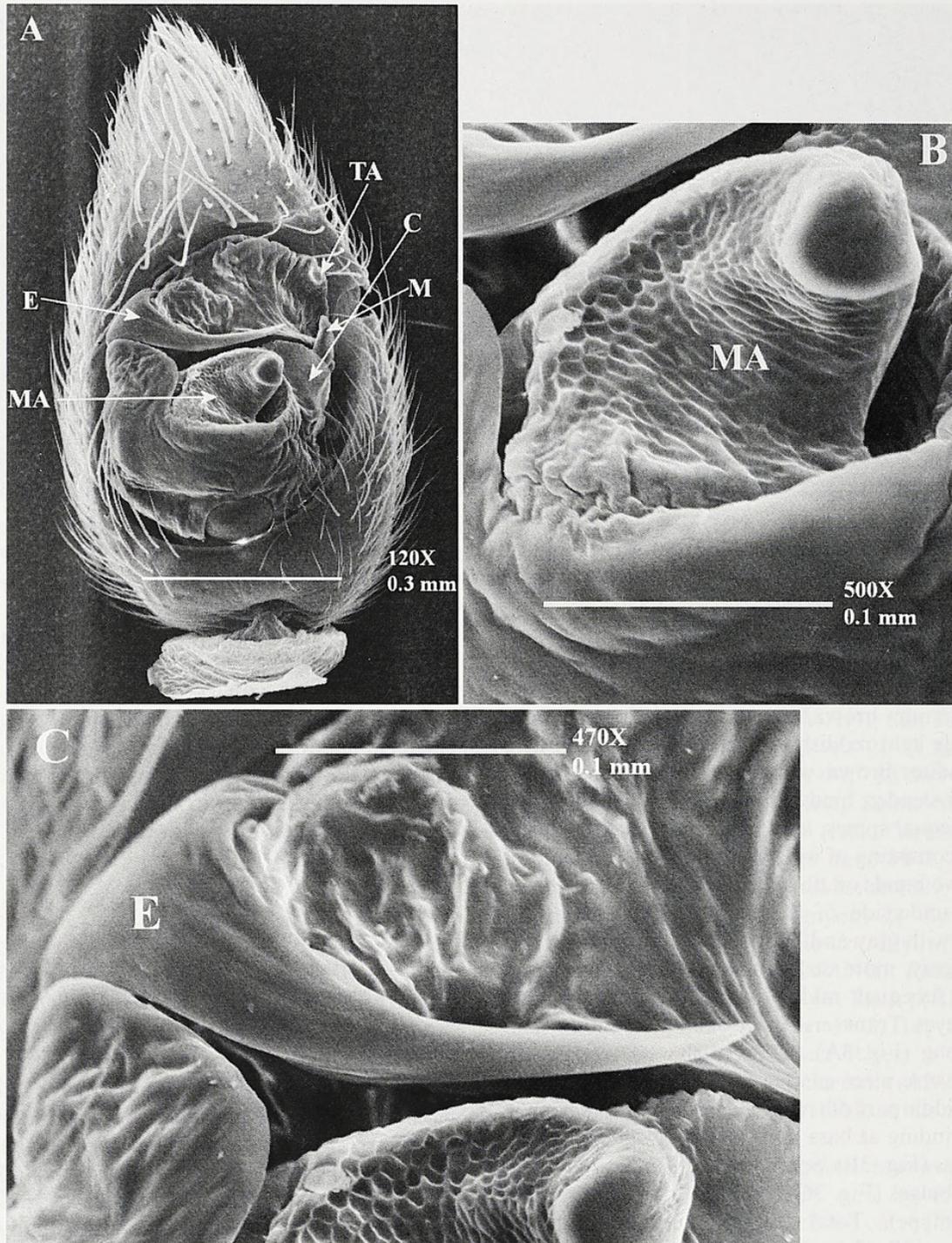


Figure 5.—A. Male pedipalpal ventral view of *Pardosa sierra*  $\times 120$ . Scale = 0.3 mm. E, embolus; MA, median apophysis; TA, terminal apophysis; M, median accessory process; C, conductor. B. Close-up median apophysis  $\times 500$ . Scale = 0.1 mm. C. Close-up embolus  $\times 470$ . Scale = 0.1 mm.

(AMNH); Huachuca Mts., Carr Canyon, 5000 ft, 1 August 1952, 2 $\text{f}$  (W. Gertsch, Schrammel & M. Cazier) (AMNH); Cochise Co., Chiricahua Mountains, Cave Creek, 5500 ft., 16 June 1958, 1 $\text{f}$  (MacNeill) (CAS); Moenocupi, 24 July 1952, 1 $\text{f}$  (W. Gertsch, Schrammel & M. Cazier) (AMNH); Chiricahua National Monument, 15 July 1948, 3 $\text{f}$  (C. Vauries & P. Vauries) (AMNH) Catalina Mountains, 10 mi S Oracle Station, 25 July 1949 (W. Gertsch & J. Gertsch) (AMNH); Huachuca Mts., Carr Canyon, 5000 ft, 3 June 1952, 15 $\text{f}$  (W. Gertsch, Schrammel & M. Cazier) (AMNH); Bottom Walnut Canyon, 18 August 1934, 1 $\text{f}$  F340818 (Lutz Det. Gertsch)

(AMNH); 5 mi W Portal, Southwestern Research Station, 15 June 1955, 1 $\text{m}$ , 1 $\text{f}$  (M. Statham) (AMNH); Oak Creek Canyon, Manzanita Camp, 27 July 1950, 2 $\text{m}$ , 5 $\text{f}$  (M. A. Cazier) (AMNH); Graham Mountains near Safford, 14 July 1955, 2 $\text{f}$  (V. Roth & W. Gertsch) (AMNH); Oak Creek Canyon, 22 July 1949, 3 $\text{f}$  (W. Gertsch & J. Gertsch) (AMNH); White River, 9 July 1940, 3 $\text{f}$  (Gertsch & Hook) (AMNH); Baboquivari Mts., Browns Canyon, 29–30 June 1952, 3 $\text{m}$  (H. B. Leech & J. W. Green) (CAS); Strawberry, 15 May 1939, 2 $\text{f}$  (R. H. Crandall) (AMNH); Tucson, Sabino Canyon, 5 June 1952, 1 $\text{m}$ , 2 $\text{f}$  (W. Gertsch, Schrammel & M. Cazier) (AMNH);

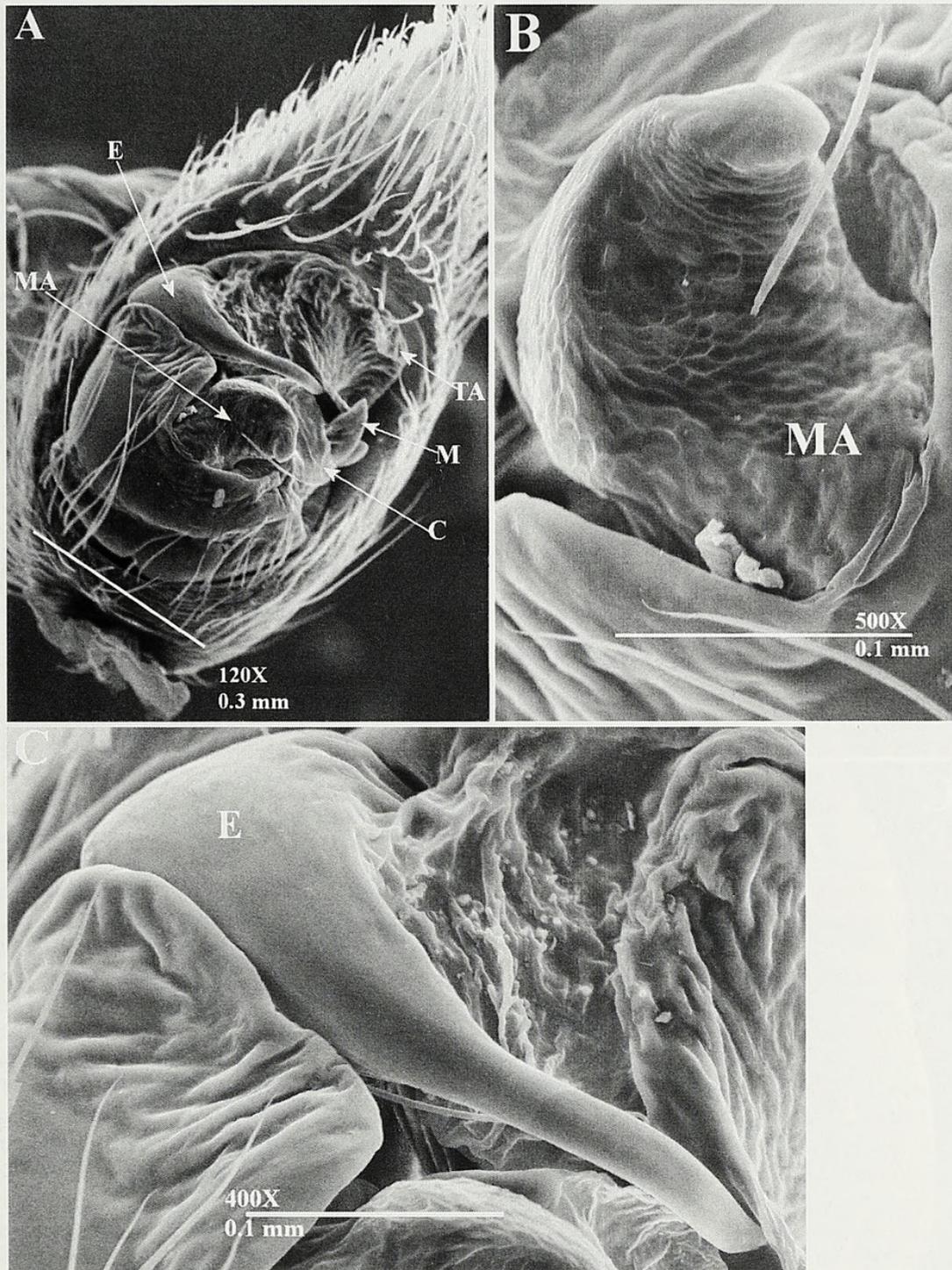


Figure 6.—A. Male pedipalpal ventral view of *Pardosa atromedia*  $\times 120$ . Scale = 0.3 mm. E, embolus; MA, median apophysis; TA, terminal apophysis; M, median accessory process; C, conductor. B. Close-up median apophysis  $\times 500$ . Scale = 0.1 mm. C. Close-up embolus  $\times 400$ . Scale = 0.1 mm.

10 mi NE White River, 8–11 July 1940, 1♂, 3♀ (Gertsch & Hook) (AMNH); Miami, 12 May 1938, 1♀ (R. H. Crandall) (AMNH). *Texas*: Sanderson, 26 May 1952, 1♀ (W. Gertsch, Schrammel & M. Cazier) (AMNH). *Idaho*: 10 mi S Swan Valley town, 6 July 1935 (W. Ivie) (AMNH). *Colorado*: Montrose, near Water, 25 July 1941 1♀ (C. Goodnight & M. Goodnight). *MEXICO*: *Chihuahua*: San Francisco Mesa near Santa Barbara, 8 July 1948, 1H (W. Gertsch) (AMNH); 44 mi N Chihuahua, 13 June 1939, 1♂, 1♀ (A.M. & L.I. Davis) (AMNH); Cañón Prieto near Primavera, 30 June 1947, 1♀ (W.

Gertsch) (AMNH); Puente Bravo, 9 October 2007, 2♀ (M.M. Correa & F. J. García de León) (CARCIB); Cerocahui, 25–26 June 1979, 2♀ (G. J. Millick) (CAS). *Coahuila*: 5 mi W Saltillo, 5 July 1936, 2♂ 3♀ (L.I. Davis) (AMNH). *Nuevo León*: Monterrey, 23 May 1952, 1♂, 1♀ (W. Gertsch, Schrammel & M. Cazier) (AMNH); 25 mi W of Monterrey, 6 July 1936, 1♂, 1♀ (L.I. Davis) (AMNH); Chipinque, 15 July 1942, 1♂, 1♀ (Bonet, Osorio & Pelaez) (AMNH); Morelos, 23 May 1952, 1♂, 1♀ (W. Gertsch) (AMNH). *Tamaulipas*: Victoria, 12 June 1936, 1♂, 2♀ (L.I. Davis) (AMNH). *Durango*: 10 mi E El

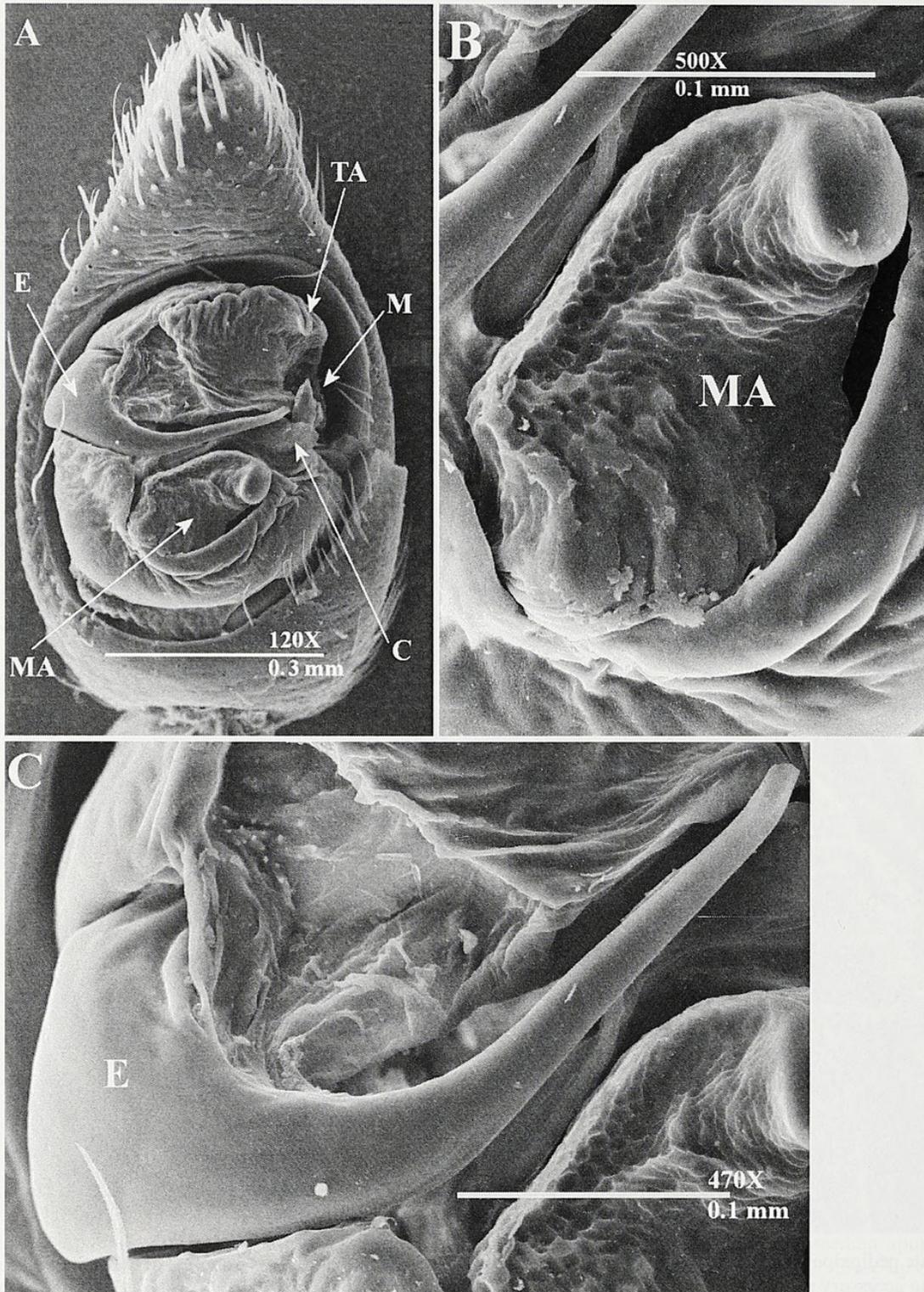


Figure 7.—A. Male pedipalpal ventral view of *Pardosa sura*  $\times 120$ . Scale = 0.3 mm: E, embolus; MA, median apophysis; TA, terminal apophysis; M, median accessory process; C, conductor. B. Close-up median apophysis  $\times 500$ . Scale = 0.1 mm. C. Close-up embolus  $\times 470$ . Scale = 0.1 mm.

Salto, 8 August 1947, 4♀ (W. Gertsch) (AMNH); Palos Colorados, 5 August 1947, 1♂, 3♀ (W. Gertsch) (AMNH). *San Luis Potosí*: Picolo, 21 May 1952, 2♂, 12♀ (W. Gertsch, Schrammel & M. Cazier) (AMNH). *Hidalgo*: 5 mi S Zimapan, 20 July 1956, 2♀ (V. Roth & W. Gertsch) (AMNH); Ixmiquilpan, 6 July 1944, 1♀ (L.I. Davis) (AMNH); 10–25 mi S of Jacala, 20 July 1956, 1♀ (V. Roth & W. Gertsch) (AMNH). *Distrito Federal*: Almoloya del Rio, 7 April 1944,

1♂, 1♀ (Hernandez & Mercado) (AMNH). *Jalisco*: Chapala, 22 June 1941, 2♀ (A.M. Davis) (AMNH). *Michoacán*: 10 mi W Tizapán, 11 July 1972, 1♀ (A.R. Brady & A. Jung) (AMNH). *Morelos*: Cuernavaca, October 1944, 1♀ (N.L.H. Krauss) (AMNH). *Puebla*: Tehuacán, 24 July 1956, 7♀ (V. Roth & W. Gertsch) (AMNH). *Gerrero*: Ixtapan de la Sal, 21–28 August 1946, 2♀ (H. Wagner) (AMNH). *Oaxaca*: near Oaxaca, 12 April 1941, 1♀ (H. Wagner) (AMNH); Base San Felipe

Mountains, 16–17 September 1947, 2♀ (B. Malkin) (AMNH).  
Veracruz: Cordava 492, 1♀ (AMNH).

**Diagnosis.**—Females of *Pardosa sura* differ from *P. sierra* and *P. atromedia* by the crescent-shaped sclerites of the epigynum that are canal-like in shape with the apical edge of the lateral crescent sclerites of the cavity forming an angle of 45°. The thin septal ridge extends apically to the hood. Copulatory ducts are straight at their base and never winding as in *P. atromedia*, subequal in thickness, and not wider at their base as in *P. sierra*. In males, the pedipalpal structure has a long and thin embolus, which extends across the bulb with a curved and truncated tip that turns toward of the conductor; the base is wider than in *P. sierra* and *P. atromedia*. The conductor is short and truncated and opposite the embolus (Fig. 7C). *Pardosa sura* differs from other closely related species by the unique mtDNA nucleotide substitutions at the following reference alignment positions: G/A (18), G (57), C (72), G (78), T (114), T (129), G (216), G (237), G (249), A/G (291), G (303), A (333), G (336), G (342), T (387), G (399), T (426) and C (613).

**Description.**—*Female* (Neotype): Total length 8.25 mm, carapace length 3.55 mm, width 3.15 mm. Prosoma dusky brown; eye region black, irregular broad band on each upper side, which indents in the middle area before the groove; clypeus with white and black hairs, large marginal spots with patch across it. Sternum brownish-black, covered with hairs, with pale margins. Chelicerae dark brown, shaded with dusky brown. Endites and labium brown with pale tips. Legs slender, hind pair very long; tibiae I and II with 3 subequal spines and pair of short spines on distal part, legs dark brown with black markings, consisting of wide annulling. Dorsal side of abdomen marked with blackish and gray spots and specks; ventral side gray, more or less shaded with darker gray. Eyes of first row subequal, middle eyes rather farther apart than from lateral eyes. Transverse portion of epigynum occupies approximately one-half the total length (Fig. 4). Middle field of epigynum widened anteriorly, with narrowest portion in middle averaging 0.14 mm. (Fig. 4A). Transverse piece 0.47 mm long (Fig. 4A). Crescent-shaped troughs on each side of transverse piece also canal-like, but narrower with borders almost straight in 45° angle that reduces thickness in the middle area of transverse piece. In dorsal view, ducts have same thickness along it (Fig. 4B). Bulge of spermathecae stretched at base (Fig. 4C). In ventral view, epigynum has rounded structure in middle part of spermathecae that appears to be a series of microducts (MD in Fig. 4C).

*Male* (Paratype): Total length 4.35 mm, carapace length 2.33 mm, width 1.82 mm. Coloration and body shape as female. Male pedipalpal organ with small and truncated conductor, similar to that of *P. atromedia*. Embolus long and thin, extending across the bulb with curved and truncated tip; median accessory process, median and terminal apophysis very similar to *P. sierra* and *P. atromedia* (Fig. 7).

**Variation.**—Females with average body length of  $7.22 \pm 0.77$  mm, carapace length averages  $3.04 \pm 0.33$  mm; epigynum with variation as follows: MF with average length of  $0.14 \pm 0.1$  mm; EpL  $0.50 \pm 0.07$  mm and EpW  $0.47 \pm 0.55$  mm. Troughs of epigynum vary in degree of sclerotization; sometimes obscure or transparent. Males with average body length of  $4.91 \pm 0.51$  mm, carapace length averages  $2.50 \pm$

Table 4.—Pairwise *p*-distance values (in percent) for each category: median inter-quartile range and the smallest and largest observations compared with the median and standard deviation.

|         | Distances between species category (%) | Distances within species category (%) |
|---------|--|---------------------------------------|
| Median  | 7.29 (6.18)                            | 0.63 (0.16)                           |
| Min–Max | 2.46–8.15                              | 0.0–2.06                              |
| Mean    | 7.29 (0.70)                            | 0.65 (0.35)                           |

0.19 mm, width  $2.04 \pm 0.20$  mm, male pedipalps vary in some measurements as follows: TP averages  $0.57 \pm 0.06$  mm, FP  $0.94 \pm 0.11$  mm, BT  $0.86 \pm 0.05$  mm. Body coloration ranges from pale yellow to reddish-brown in females. Males darker than females, principally in ocular area.

**Distribution and Natural History.**—*Pardosa sura* occurs from Oregon, northeastern parts of California, Utah, Colorado, Arizona, and Texas in USA; in Mexico from Chihuahua to Veracruz. It is not found or reported from Sonora, Sinaloa, or Baja California Peninsula in Mexico (Fig. 1). Habitat preferences of this species are similar to other species in the *lapidicina* group. It prefers the edge of rivers, generally with rocks, where it is often collected by hand.

**General Remarks.**—Twenty specimens per taxon were measured (females  $n = 10$  and males  $n = 10$ ) and analyzed. The somatic characteristics (Tables 2 and 3) show that in both sexes the different somatic parts measured are useless as a means to distinguish among species, because in most parameters a prominent area of overlap exists. *P. sura* shows the largest variation in all measurements, covering the range ( $P > 0.05$ ) of the other two species (Tables 2, 3). Genitalia differ among females. Male pedipalpal structure, especially that of the pedipalpal bulb, is similar from species to species (Table 3); differences among males lie in the shape of the median apophysis, terminal apophysis, and embolus (Figs. 5B, 6B, 7B), which vary slightly. The median accessory process does not appear to vary among species.

**Molecular data.**—The average nucleotide composition in these three species of *Pardosa* (*P. sierra*, *P. atromedia*, and *P. sura*) and other species (including the *lapidicina* group) indicates that the nucleotide composition among *Pardosa* species is homogeneous (data not shown). The data included 548 invariant sites and 82 variable sites (consisting of 75 parsimony-informative sites and seven singleton sites). As observed in other spiders (Astrin et al. 2006), there is an A+T bias in the third codon position (data not shown) of the *lapidicina* group of *Pardosa* COI.

The Shapiro-Wilk test rejected the hypothesis of normal distribution of distance for this molecular marker. Distances between individuals were arranged in two categories: intra-species and interspecies. We did not encounter haplotype-sharing among taxa. Genetic divergence (measured by *p*-distances) for any species ranging from 0.16 to 1% were less than those between individuals of different species, ranging from 2.46 to 6.9% (Tables 4, 5). The largest divergences were observed in comparisons involving *P. astrigera*, which was chosen because it represents the major haplotype collection of a species in the genus *Pardosa*, where the differences were higher (Table 4, Fig. 8).

Table 5.—Distance matrix (*p*-distance or uncorrected) among *Pardosa* species category; distances within species category in diagonal.

| Species               | 1      | 2      | 3      | 4      | 5      | 6      | 7      |
|-----------------------|--------|--------|--------|--------|--------|--------|--------|
| 1 <i>P. valens</i>    | 0.0032 |        |        |        |        |        |        |
| 2 <i>P. steva</i>     | 0.0246 | 0.0016 |        |        |        |        |        |
| 3 <i>P. vadosa</i>    | 0.0571 | 0.0500 | 0.0032 |        |        |        |        |
| 4 <i>P. sura</i>      | 0.0397 | 0.0405 | 0.0593 | 0.0016 |        |        |        |
| 5 <i>P. atromedia</i> | 0.0635 | 0.0516 | 0.0683 | 0.0582 | 0.0032 |        |        |
| 6 <i>P. sierra</i>    | 0.0643 | 0.0571 | 0.0659 | 0.0526 | 0.0690 | 0.0016 |        |
| 7 <i>P. astrigera</i> | 0.0766 | 0.0655 | 0.0719 | 0.0815 | 0.0733 | 0.0738 | 0.0065 |

Using *p*-distance data, we found that these lycosid spiders have a between-species genetic divergence from 2.46–6.90% with respect to the *lapidicina* group and within-species from 0.0–2.06% in this fragment of the COI gene (Table 5). The gap range was +0.4, indicating no overlap between categories (intraspecific vs. interspecific). Additionally, box-and-whisker plots appear suited for DNA taxonomy for interspecific purposes because they displayed variations simultaneously at intraspecific and interspecific levels (Fig. 8).

Finally, the topology of the COI NJ species identification tree (Fig. 9) showed that morphological conspecifics grouped together whereas between species, obvious segregation could be discerned (Fig. 9; see Table 5 for quantitative results).

Splits within species were slight (Fig. 9, Table 4) in all cases, but conspicuous among species (Fig. 9, Table 5). This is in contrast to the absence of detectable morphological variation

in somatic characteristics and the slight differences in male but marked differences in female genitalia.

## DISCUSSION

The morphological differences in females of the *P. sierra* complex are more conspicuous than those of the males. This situation probably led Barnes (1959) to synonymize *P. atromedia* and *P. sura* with *P. sierra*. Nevertheless, he identified differences between females of *P. sierra* and *P. sura*, but probably did not find differences between *P. atromedia* and *P. sura*, registering just two morphs of "*P. sierra*."

These three genitalic morphs have conspicuously distinct characteristics that can be used for taxonomic purposes, such as the shape of transverse piece, the spermatic conduct, and spermathecae in females and conductor, embolus, median apophysis, terminal apophysis, and median accessory process in males. These genital structures provide sufficient evidence to suggest that these morphological variations correspond to interspecific differentiation, as reported for other species (Dondale & Redner 1984; Chang et al. 2007; Wiemers & Fiedler 2007; Dreyer & Brady 2008).

Because the type material of *P. sura* was lost, a neotype was designated. The original material consisted of one holotype and one paratype deposited in the Utah Museum of Natural History (Chamberlin & Ivie 1941), which was later transferred to the American Museum of Natural History. This material was not found when we checked the specimens in the AMNH collection.

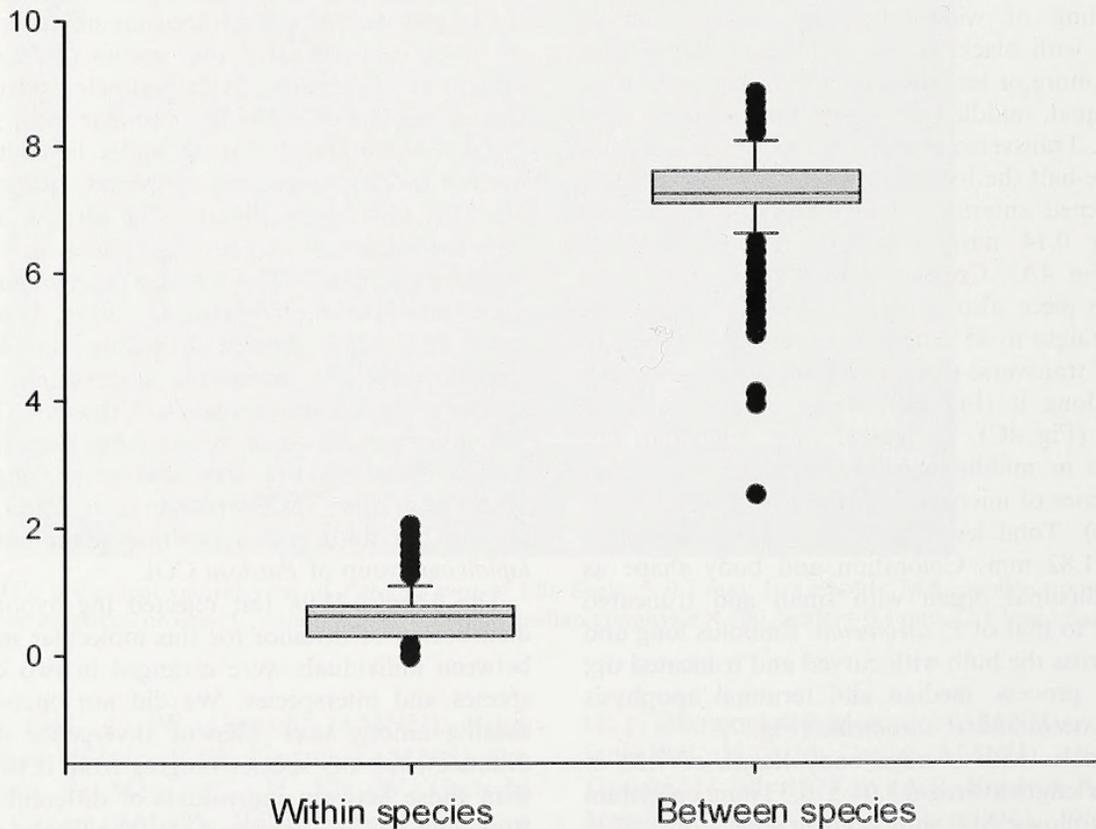


Figure 8.—Box plots of *p*-distances for a fragment of COI. Boxes indicate inter-quartile range (IQR: between upper [Q3] and lower [Q1] quartile). Black bar designates median, whiskers indicate values within 1.5× the IQR beneath Q1 or 1.5× above Q3. 'Mild' outliers (circles): between 1.5× and 3× IQR. Total of 2,216 pair wise comparisons for within-species category and total of 661 pairwise comparisons in between-species category.

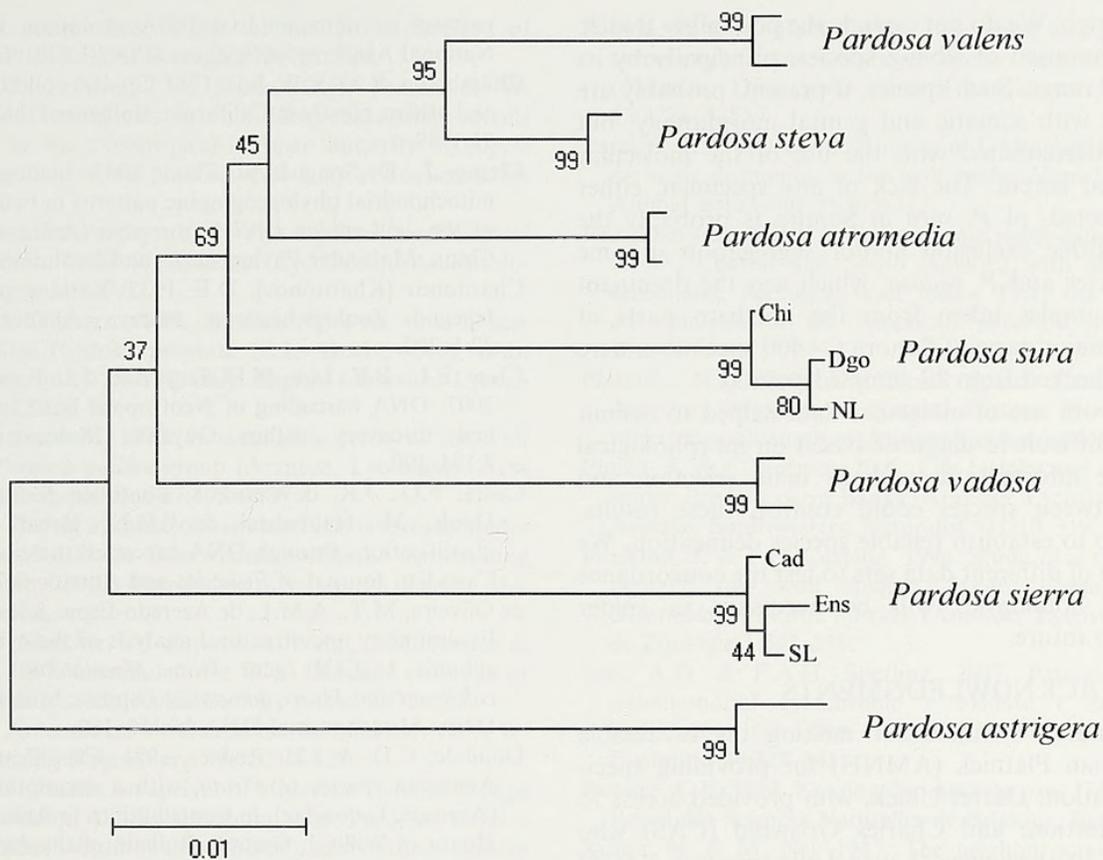


Figure 9.—Topology of neighbor-joining tree (Kimura-2 parameters; 10000 replicates of bootstrap) of COI mtDNA to some species of *lapidicina* group. The number in nodes indicates the statistical support of branches. Chi, Chihuahua; Dgo., Durango; NL, Nuevo León; Cad, Cadejé; SL, Sierra de la Laguna; Ens, Ensenada.

The nucleotide proportions of variable sites indicate that this region (COI) is a good indicator, not just for taxonomic information, but also for phylogenetic information because these species (*lapidicina* group) in this region are not saturated with transversions as in other species of invertebrates (de Oliveira et al. 2005; Yassin 2009). Hence, most changes are silent mutations, and just one change detected in an amino acid sequence corresponds to a transversion (A/G) in the base number 497 at the second position of the codon AAG (K/S) in two species *P. steva* and *P. atromedia*, data not shown. It will be necessary to include other species to corroborate this difference (Roe & Sperling 2007) and make a phylogenetic study of the *lapidicina* group.

The Gap Analysis was positive (+0.4), because it indicates the categories are isolated from each other. In other words they do not overlap between intraspecific and interspecific *p*-distance (Tables 4, 5) because the minimum variation between species is bigger than the maximum variation within species.

Based on morph differentiation, the minimal interspecific *p*-distance value of 2.46% divergence was found between the two morphologically differentiated species, as well as between *P. steva* and *P. valens*. We then confirmed the separation of *P. sierra* into three different species (Fig. 8) by using the value as a “threshold” of COI sequence divergence (Hebert et al. 2003, 2004; Barrett & Heber 2005; Astrin et al. 2006).

The three species’ genetic distances of ~ 5–7% differentiation at the interspecific level indicate that these are genetically differentiated species, so the COI fragment that we selected is a good estimator for identification of species (Hebert et al.

2003, 2004; Paquin & Hedin 2004; Barrett & Hebert 2005; Astrin et al. 2006). Such results principally occur when these species are closely related, e.g., in the same species group, sibling or cryptic species as in other animals (arthropods: Hajibabaei et al. 2006; mammals: Clare et al. 2007; birds: Kerr et al. 2007). The levels of divergence found (Tables 4, 5) suggest that barcodes from COI can be used to distinguish among *Pardosa* species.

Additionally, the NJ tree shows that these species represent different groups, positioning *P. sierra* at the base, close to *P. vadosa* and *P. sura*; but *P. atromedia* is close to *P. valens* and *P. steva*. Finally, the branches indicate that lineage sorting is probably incomplete, although these lineages are separated enough to be considered as different reproductive units, forming a complex of cryptic species (Fig. 9).

Consequently, the use of DNA characteristics in a diagnostic context is entirely compatible with our taxonomic research. In this respect, we agree with Costa et al. (2007) that DNA barcoding is not a substitute for traditional taxonomy, but a good tool that has applications to delineate cryptic and sibling species and to resolve ecological questions. For example, many organisms that disperse by ballooning are immature, and it is almost always impossible to identify them to the species level (Greenstone et al. 1987; Greenstone 2001).

Regarding the distribution of species, *P. sierra* is restricted to Baja California Peninsula, *P. atromedia* is found in southern and central parts of California, and *P. sura* is the most widely distributed species, ranging from Oregon, northeastern parts of California, and Utah, USA to Veracruz

and Oaxaca, Mexico. We do not exclude the possibility that *P. sura* could be composed of sibling species, principally by its wide distribution range. Such species, if present, probably are hard to separate with somatic and genital morphology, but they could be differentiated with the use of the molecular markers employed herein. The lack of any specimen, either reported or collected, of *P. sura* in Sonora is probably the result of competitive exclusion and/or segregation in time between this species and *P. vadosa*, which was the dominant species in our samples taken from the northern parts of Sinaloa and the major parts of Sonora (> 400 specimens were recollected and checked from 13 sampled points).

In conclusion, our use of molecular data helped to delimit species that are difficult to diagnose based on morphological characters. More information, such as mate selection and hybridization between species could confirm these results, which would help to establish reliable species delineation. We think that the use of different data sets to test the concordance between species boundaries will be necessary in spider systematics in the future.

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