Phylogeny of the North American scorpion genus *Diplocentrus* Peters, 1861 (Scorpiones: Diplocentridae) based on morphology, nuclear and mitochondrial DNA

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**Abstract**

The scorpion genus *Diplocentrus* Peters, 1861, endemic to North and Central America, is the most diverse in family Diplocentridae Karsch, 1880. There is considerable morphological variation among the species of *Diplocentrus*. It is necessary to test the monophyly and phylogenetic position of *Diplocentrus* in order to revise its diagnosis and taxonomic limits. The present contribution provides a phylogenetic analysis of 29 species of *Diplocentrus*, five exemplar species representing the three putatively most closely related diplocentrid genera, and an exemplar of a more distantly related diplocentrid genus. The analysis was based on 95 morphological characters and 4202 aligned nucleotides from DNA sequences of five markers in the nuclear and mitochondrial genomes. Separate and simultaneous parsimony analyses of the morphological and DNA sequence data were conducted with equal weighting and six implied weighting regimes. The nuclear and mitochondrial DNA datasets were also analyzed separately and simultaneously with Bayesian inference. The resulting topologies recovered the monophyly of *Diplocentrus*, with the exception of two neobothriotaxic species from central Mexico, for which a new genus *Kolotl* Santibáñez-López et al., 2014, is justified. The *keyserlingii* group, as previously defined, was not monophyletic due to the placement of two species in the *mexicanus* group; the rest of its component species were monophyletic, however. A third clade was recovered that has not been previously recognized: the *zacatecanus* group, comprising four species from northern Mexico and the southwestern U.S.A. New insights are provided concerning relationships among *Diplocentrus* and the diplocentrid genera *Bioculus* Stahnke, 1968 and *Didymocentrus* Kraepelin, 1905, the phylogenetic positions of which were previously ambiguous.

**Key words**

Diplocentridae, *Diplocentrus*, phylogeny, molecular data, morphology.

**1. Introduction**

The scorpion genus *Diplocentrus* Peters, 1861 is the most diverse in the family Diplocentridae Karsch, 1880. Since publication of the Catalog of Scorpions of the World (Sismon & Fet 2000), the number of *Diplocentrus* species increased from 35 to 59 (Santibáñez-López et al. 2013). *Diplocentrus* is endemic to North and Central America, ranging from the southwestern U.S.A. (Arizona, New Mexico and Texas) to northern Honduras (Sismon & Fet 2000), but its greatest diversity (47 described species) and endemism occurs in mainland Mexico. Although most species of *Diplocentrus* are fossorial, these scorpions exhibit considerable morphological variation, from small species such as *Diplocentrus bereai* Armas & Martín-Frias, 2004, with a total adult length of 20–30 mm, to rather large species such as *Diplocentrus tai­beli* (Caporiacco, 1938), total adult length, 80–90 mm.
Hoffmann (1931) was the first to subdivide the morphological diversity within Diplocentrus into two species groups, the whitei group and the keyserlingii group, based largely on differences in size and coloration. Francke (1977) redefined these groups on morphometric criteria. The whitei group, renamed the mexicanus group because it included the type species of the genus, Diplocentrus mexicanus Peters, 1861, revalidated from synonymy with Diplocentrus whitei (Gervais, 1842), comprised species with short cheliceral fingers and the pedipalp femur wider than high. The keyserlingii group comprised species with long cheliceral fingers and the pedipalp femur higher than wide. Francke (1978) realized this distinction was problematic, because the diagnostic characters of the pedipalp femur were also used to separate other genera in subfamily Diplocentrinae Karsch, 1880. Additionally, one of the groups was by definition paraphyletic with respect to the other. Recently, Santibáñez-López et al. (2013) presented an operational diagnosis for the keyserlingii group, but refrained from assuming it was monophyletic, pending further investigation of Diplocentrus phylogeny.

The monophyly and phylogenetic position of Diplocentrus has remained ambiguous since the first and, thus far, only published phylogenetic analysis of diplocentrid relationships, based on exemplar species included in a taxonomically broader analysis of scorpionoid phylogeny (Prendini 2000). Diplocentrus was rendered paraphyletic in most of the analyses, by two other diplocentrid genera, Bioculus Stahnke, 1968 and Didymocentrus Kraepelin, 1905, the validity of which had been disputed by several authors (Williams & Lee 1975; Francke 1978; Sissom 1990; Stockwell 1992). Prendini’s (2000) analyses suggested one or both genera should be synonymized with Diplocentrus, or the generic limits of Diplocentrus redefined, to restore its monophyly. Neither alternative was implemented, however, pending a more comprehensive analysis with a larger and more representative sample of diplocentrid species.

Recently, Diplocentrus poncei Francke & Quijano-Ravell, 2009, the first species of Diplocentrus with accessory trichobothria on the pedipalp chela and patella, was described. Francke & Quijano-Ravell (2009) also discovered accessory trichobothria on the pedipalp patella of Diplocentrus magnus Beutelspacher & López-Forment, 1991. These two species from the central Mexican states of Michoacán and Guerrero, respectively, are unique among diplocentrids in presenting neobothriotaxic pedipalps, raising questions about their phylogenetic placement within Diplocentrus.

A quantitative test of the monophyly and phylogenetic position of Diplocentrus is necessary to revise its diagnosis and taxonomic limits with respect to other diplocentrid genera. The present contribution provides a phylogenetic analysis of 29 species of Diplocentrus, five exemplar species representing the three putatively most closely related diplocentrid genera, and an exemplar of a more distantly related diplocentrid genus. The analysis was based on 95 morphological characters and 4202 aligned nucleotides from DNA sequences of five markers in the nuclear and mitochondrial genomes. Separate and simultaneous parsimony analyses of the morphological and DNA sequence data were conducted with equal weighting and six implied weighting regimes. The nuclear and mitochondrial DNA sequence data were also analyzed separately and simultaneously with Bayesian inference.

2. Material and methods

2.1. Taxa

Thirty-five species of six diplocentrid genera were included in the analysis (Appendix 1). The ingroup comprised 29 species of Diplocentrus, including the type species and representatives of both species groups, selected to cover the geographical distribution and morphological diversity of the genus (Prendini 2001). Based on the phylogeny of Prendini (2000), exemplar species of the three putatively most closely related diplocentrid genera, Bioculus, Didymocentrus and Tarsoporosus Francke, 1978, were included as outgroup taxa, with an exemplar species of a more distantly related diplocentrid genus, hetereonebo Pocock, 1899, as the primary outgroup taxon. Bioculus and Didymocentrus were each represented by the type species and a second species, selected to maximize morphological diversity (Prendini 2001).

2.2. Material examined

Scorpions were collected at night with ultraviolet light detection and during the daytime, by turning rocks and excavating burrows (Santibáñez-López et al. 2013). Tissue samples, mostly taken from immature specimens, were deposited in the Ambrose Monell Collection for Molecular and Microbial Research at the American Museum of Natural History (AMNH), New York (Table 1). Adult voucher specimens, collected from the same populations, were deposited in the AMNH and the Colección Nacional de Arácnidos (CNAN) at the Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City.

2.3. Morphological characters

Ninety-five qualitative characters of adult morphology (Appendix 2) were scored (Table 2) for the 35 terminal taxa in the analysis using freshly collected and/or museum material. Morphological terminology follows Vachon (1974) for trichobothria, Francke (1977) for metasomal carination, Prendini (2000) for pedipalpal carination, and Prendini et al. (2003) for carapacial surfaces.

<table>
<thead>
<tr>
<th>Species</th>
<th>Specimen</th>
<th>AMCC</th>
<th>18S</th>
<th>28S</th>
<th>12S</th>
<th>16S</th>
<th>COI</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Heteronebo jamaicae</em></td>
<td>1♂</td>
<td>LP 5131</td>
<td>KM514559</td>
<td>KM514594</td>
<td>KM514849</td>
<td>KM514524</td>
<td>KM514629</td>
</tr>
<tr>
<td><em>Tarsoporosus kugleri</em></td>
<td>1♀</td>
<td>LP 5204</td>
<td>KM514660</td>
<td>KM514695</td>
<td>KM514940</td>
<td>KM514525</td>
<td>KM514630</td>
</tr>
<tr>
<td><em>Bioculus caboensis</em></td>
<td>1♂</td>
<td>LP 1796</td>
<td>KM514651</td>
<td>KM514696</td>
<td>KM514981</td>
<td>KM514626</td>
<td>KM514631</td>
</tr>
<tr>
<td><em>Bioculus comorensis</em></td>
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<td>LP 2123</td>
<td>KM514652</td>
<td>KM514697</td>
<td>KM514982</td>
<td>KM514627</td>
<td>KM514632</td>
</tr>
<tr>
<td><em>Didymocentrus krausi</em></td>
<td>1 subad.♂</td>
<td>LP 1987</td>
<td>KM514563</td>
<td>KM514598</td>
<td>KM514983</td>
<td>KM514628</td>
<td>KM514633</td>
</tr>
<tr>
<td><em>Didymocentrus lesueurii</em></td>
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<td>LP 3638</td>
<td>KM514564</td>
<td>KM514599</td>
<td>KM514984</td>
<td>KM514629</td>
<td>KM514634</td>
</tr>
<tr>
<td><em>Kolotl magnus</em></td>
<td>1 juv.</td>
<td>LP 7029</td>
<td>KM514655</td>
<td>KM514600</td>
<td>KM514495</td>
<td>KM514530</td>
<td>KM514635</td>
</tr>
<tr>
<td><em>Kolotl gonzalezii</em></td>
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<td>LP 7030</td>
<td>KM514656</td>
<td>KM514601</td>
<td>KM514496</td>
<td>KM514531</td>
<td>KM514636</td>
</tr>
<tr>
<td><em>Diplocentrus anopthalmus</em></td>
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<td>LP 10980</td>
<td>KM514657</td>
<td>KM514602</td>
<td>KM514497</td>
<td>KM514532</td>
<td>KM514637</td>
</tr>
<tr>
<td><em>Diplocentrus bereai</em></td>
<td>1 juv.</td>
<td>LP 6532</td>
<td>KM514658</td>
<td>KM514603</td>
<td>KM514498</td>
<td>KM514533</td>
<td>KM514638</td>
</tr>
<tr>
<td><em>Diplocentrus coddingtoni</em></td>
<td>1 juv.</td>
<td>LP 9169</td>
<td>KM514659</td>
<td>KM514604</td>
<td>KM514499</td>
<td>KM514534</td>
<td>KM514639</td>
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<tr>
<td><em>Diplocentrus coylei</em></td>
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<td>LP 6386</td>
<td>KM514660</td>
<td>KM514605</td>
<td>KM514503</td>
<td>KM514535</td>
<td>KM514640</td>
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<tr>
<td><em>Diplocentrus cozumel</em></td>
<td>1 juv.</td>
<td>LP 7674</td>
<td>KM514661</td>
<td>KM514606</td>
<td>KM514504</td>
<td>KM514536</td>
<td>KM514641</td>
</tr>
<tr>
<td><em>Diplocentrus hoffmanni</em></td>
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<td>LP 3078</td>
<td>KM514662</td>
<td>KM514607</td>
<td>KM514505</td>
<td>KM514537</td>
<td>KM514642</td>
</tr>
<tr>
<td><em>Diplocentrus jaca</em></td>
<td>1 juv.</td>
<td>LP 8518</td>
<td>KM514663</td>
<td>KM514608</td>
<td>KM514506</td>
<td>KM514538</td>
<td>KM514643</td>
</tr>
<tr>
<td><em>Diplocentrus keyserlingii</em></td>
<td>1♂</td>
<td>LP 4102</td>
<td>KM514664</td>
<td>KM514609</td>
<td>KM514507</td>
<td>KM514539</td>
<td>KM514644</td>
</tr>
<tr>
<td><em>Diplocentrus kraepelinii</em></td>
<td>1♀</td>
<td>LP 4707</td>
<td>KM514665</td>
<td>KM514610</td>
<td>KM514508</td>
<td>KM514540</td>
<td>KM514645</td>
</tr>
<tr>
<td><em>Diplocentrus melici</em></td>
<td>1 juv.</td>
<td>LP 5918</td>
<td>KM514666</td>
<td>KM514611</td>
<td>KM514509</td>
<td>KM514541</td>
<td>KM514646</td>
</tr>
<tr>
<td><em>Diplocentrus mexicanus</em></td>
<td>1♀</td>
<td>LP 11034</td>
<td>KM514667</td>
<td>KM514612</td>
<td>KM514510</td>
<td>KM514542</td>
<td>KM514647</td>
</tr>
<tr>
<td><em>Diplocentrus mitlae</em></td>
<td>1 subad.♂</td>
<td>LP 5204</td>
<td>KM514668</td>
<td>KM514613</td>
<td>KM514511</td>
<td>KM514543</td>
<td>KM514648</td>
</tr>
<tr>
<td><em>Diplocentrus motagua</em></td>
<td>1 juv.</td>
<td>LP 5987</td>
<td>KM514669</td>
<td>KM514614</td>
<td>KM514512</td>
<td>KM514544</td>
<td>KM514649</td>
</tr>
<tr>
<td><em>Diplocentrus pelaezii</em></td>
<td>1 juv.</td>
<td>LP 2140A</td>
<td>KM514670</td>
<td>KM514615</td>
<td>KM514513</td>
<td>KM514545</td>
<td>KM514650</td>
</tr>
<tr>
<td><em>Diplocentrus rojoae</em></td>
<td>1 subad.♀</td>
<td>LP 10981</td>
<td>KM514671</td>
<td>KM514616</td>
<td>KM514514</td>
<td>KM514546</td>
<td>KM514651</td>
</tr>
<tr>
<td><em>Diplocentrus sissomi</em></td>
<td>1 juv.</td>
<td>LP 7874</td>
<td>KM514672</td>
<td>KM514617</td>
<td>KM514515</td>
<td>KM514547</td>
<td>KM514652</td>
</tr>
<tr>
<td><em>Diplocentrus thubaram</em></td>
<td>1 subad.♂</td>
<td>LP 11034</td>
<td>KM514673</td>
<td>KM514618</td>
<td>KM514516</td>
<td>KM514548</td>
<td>KM514653</td>
</tr>
<tr>
<td><em>Diplocentrus whitei</em></td>
<td>1 juv.</td>
<td>LP 3078</td>
<td>KM514674</td>
<td>KM514619</td>
<td>KM514517</td>
<td>KM514549</td>
<td>KM514654</td>
</tr>
<tr>
<td><em>Diplocentrus zacatecanus</em></td>
<td>1 juv.</td>
<td>LP 5339</td>
<td>KM514675</td>
<td>KM514620</td>
<td>KM514518</td>
<td>KM514550</td>
<td>KM514655</td>
</tr>
</tbody>
</table>

Twenty-four characters were adopted and variously modified from previous analyses by Prendini (2000), Prendini et al. (2003) and Mattoni et al. (2012). Twenty-one characters had not been studied previously in diplocentrid scorpions (e.g., basitarsal spiniform macrosetae).

Most diplocentrid species are sexually dimorphic, especially with respect to pedipalp shape and carination. Separate characters were defined for sexually dimorphic structures of males (18 characters) and females (16 characters). Adult males are unknown in *Diplocentrus anopthalmus* Francke, 1977 and *Kolotl magnus* (Beutelspacher & López-Forment, 1991), hence questionmarks were inserted for these species, where applicable.

Seventy-nine characters were binary and sixteen multistate. One multistate character was additive (character 47, we consider the states of this character to be a transformation series), and the other fifteen characters nonadditive (unordered). Fifteen characters were uninformative and deactivated in all analyses († in Appendix 2).

#### 2.4. DNA sequencing

DNA isolation, PCR amplification and sequencing were conducted at the AMNH Sackler Institute for Comparative Genomics, using standard protocols (Prendini et al. 2002, 2003, 2005). Five gene markers were sequenced based on previous studies of scorpions and other arachnids (Prendini et al. 2003, 2005): 18S rDNA (18S) and the D3 region of the 28S rDNA (28S), from the nuclear
genome, and 12S rDNA (12S), 16S rDNA (16S) and Cytochrome c Oxidase I (COI), from the mitochondrial genome. The nuclear gene fragments were amplified using primer pairs 18S1/5R, 18S3F/bi, and 18Sa2.0/9R for the 18S rDNA (Wheeler et al. 1993) and 28Sa/bout for the 28S rRNA (Unn et al. 1996). The mitochondrial gene fragments were amplified using primers 12Sai/bi for the 12S rDNA (Kocher et al. 1989), 16Sar/br (Simon et al. 1991) and HCO/LCO (Folmer et al. 1994) or HCOout/LCO and Exta/B (Prenini et al. 2005) for the COI.
Table 3. Statistics for aligned DNA sequences of 5 nuclear and mitochondrial gene markers used for phylogenetic analyses of 35 species in 6 diplocentrid scorpion genera. Aligned length (base-pairs); number and percentage of variable positions; number and percentage of parsimony-informative (PI) positions, including gaps (and percentage of aligned length); number and percentage of conserved (invariant) positions; percentage nucleotide composition; percentage of transitions (ts) and transversions (tv) for each nucleotide combination and overall. Percentages for COI represent total, first and second positions (COI 1 2 3), respectively. Calculations were conducted using the maximum composite likelihood test (mcl) under the Tamura-Nei (2004) model of substitution.

<table>
<thead>
<tr>
<th></th>
<th>Nuclear</th>
<th>Ribosomal</th>
<th>Mitochondrial</th>
<th>Protein-coding</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Length (bp)</strong></td>
<td>1761</td>
<td>520</td>
<td>347</td>
<td>499</td>
<td>1078</td>
</tr>
<tr>
<td><strong>Variable (%)</strong></td>
<td>14 (1)</td>
<td>52 (10)</td>
<td>223 (64)</td>
<td>272 (54)</td>
<td>485 (45)</td>
</tr>
<tr>
<td><strong>PI (%)</strong></td>
<td>10 (1)</td>
<td>28 (5)</td>
<td>181 (52)</td>
<td>233 (47)</td>
<td>370 (34)</td>
</tr>
<tr>
<td><strong>Conserved (%)</strong></td>
<td>1747 (89)</td>
<td>466 (90)</td>
<td>122 (35)</td>
<td>218 (44)</td>
<td>593 (55)</td>
</tr>
<tr>
<td>A (COI 1 2 3) %</td>
<td>25</td>
<td>23</td>
<td>40.21</td>
<td>36.34</td>
<td>20 (19 26 14)</td>
</tr>
<tr>
<td>C (COI 1 2 3) %</td>
<td>23</td>
<td>20</td>
<td>9.96</td>
<td>13.77</td>
<td>13 (5 11 22)</td>
</tr>
<tr>
<td>G (COI 1 2 3) %</td>
<td>28</td>
<td>26</td>
<td>14.57</td>
<td>15.89</td>
<td>23 (20 30 21)</td>
</tr>
<tr>
<td>T (COI 1 2 3) %</td>
<td>24</td>
<td>31</td>
<td>35.36</td>
<td>34</td>
<td>44 (56 33 43)</td>
</tr>
<tr>
<td>ts A→G (COI 1 2 3) %</td>
<td>5</td>
<td>20</td>
<td>25</td>
<td>27</td>
<td>51 (65 38 44)</td>
</tr>
<tr>
<td>C→+T (COI 1 2 3) %</td>
<td>49</td>
<td>50</td>
<td>39</td>
<td>37</td>
<td>12 (11 25 29)</td>
</tr>
<tr>
<td>tv A→+C (COI 1 2 3) %</td>
<td>11</td>
<td>7</td>
<td>9</td>
<td>9</td>
<td>6 (3 7 5)</td>
</tr>
<tr>
<td>A→+T (COI 1 2 3) %</td>
<td>11</td>
<td>7</td>
<td>14</td>
<td>13</td>
<td>12 (9 11 8)</td>
</tr>
<tr>
<td>C→+G (COI 1 2 3) %</td>
<td>12</td>
<td>8</td>
<td>4</td>
<td>5</td>
<td>6 (3 7 6)</td>
</tr>
<tr>
<td>G→+T (COI 1 2 3) %</td>
<td>12</td>
<td>8</td>
<td>9</td>
<td>9</td>
<td>13 (9 12 8)</td>
</tr>
<tr>
<td>ts→tv (COI 1 2 3)</td>
<td>1.11</td>
<td>2.27</td>
<td>1.28</td>
<td>1.43</td>
<td>1.46 (2.29 1.58 2.61)</td>
</tr>
</tbody>
</table>

DNA was isolated from pedipalp, leg, or metasomal tissues dissected from freshly collected specimens fixed in 95 – 100% ethanol using the Qiagen DNeasy Blood and Tissue Kit. PCR amplification was conducted with Ready-To-Go PCR beads (Amersham Pharmacia Biotech) in a 25 μl reaction comprising 21 μl de-ionized water, 1 μl forward primer, 1 μl reverse primer and 2 μl DNA. The PCR program consisted of an initial denaturing step at 94°C for 5 min, 30 – 35 amplification cycles (94°C for 15 s, 49°C for 10 s, 72°C for 15 s), and a final step of 72°C for 7 min, in a GeneAmp PCR System 9700 thermocycler. Specific conditions were optimized for primer pairs (e.g., a lower annealing temperature was used for COI). PCR products were verified on 1% agarose/TBE electrophoretic gels stained with SYBR Safe (Invitrogen, Life Technologies Corporation). PCR products were purified using an AMPure Magnetic Beads Purification System (Agencourt Bioscience) and resuspended in 40 μl de-ionized water using a Biomek NX robot. Double-stranded sequencing of the purified PCR product was conducted by the dideoxy terminase method (SANGER et al. 1977) with AmpliTaq DNA Polymerase FS (Perkin Elmer) and dye-labeled terminators (Applied Biosystems Inc. Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit), in a GeneAmp PCR System 9700 thermocycler. Cycle sequencing was conducted in a 10 μl reaction, comprising 0.5 μl Big Dye, 2 μl Big Dye Terminator Buffer, 1 μl forward or reverse primer, 4 μl de-ionized water, and 2.5 μl purified PCR product. Cycle sequencing program consisted of 35 amplification cycles (94°C for 30 s, 50°C for 1 min, 60°C for 4 min). Cycle sequencing product was cleaned using CleanSeq Clean-Up (Agencourt Bioscience) on the Biomek NX robot. Purified cycle sequencing product was sequenced with an Applied Biosystems Inc. 3730xl automated capillary sequencer.

The accuracy of sequences was verified by independently amplifying and sequencing the complementary strands of all fragments. Primer sequences were removed and complementary strands of DNA assembled into consensus sequences, edited, and checked for quality using Sequencher 5.0 (Gene Codes). If complementary strands disagreed (besides minor mismatches), the sample was replotted and sequenced to resolve discrepancies.

One hundred and seventy five sequences were generated from 38 samples for the study (Table 1). The 16S fragment was the most variable in length among the genetic markers, ranging from 482 – 485 nucleotides (nt) in the outgroup and 481 – 490 nt in the ingroup. The 12S fragment varied from 333 – 335 nt in the outgroup and 332 – 339 nt in the ingroup. The COI fragment was 1078 nt in the outgroup and varied from 1072 – 1078 nt in the ingroup. Length variation was minimal in the nuclear markers: 18S was 1761 nt in all species and 28S was 511 nt in all except Didymocentrus krausi Francke, 1978, which was 516 nt.

2.5. DNA sequence alignment

Static alignments of the length-variable 28S, 12S, 16S and COI gene fragments were generated with MAFFT online version 6 (KATO et al. 2002, 2005). The G-INS-i strategy, which performs a global alignment based on
an FFT approximation (Kato 1999; Bremer 1994), was selected. This method is suitable for large datasets comprising sequences with relatively limited variation in length, i.e., few, short gaps (Kato et al. 2005). The scoring matrix for nucleotide sequences was set to 1/PAM \( k = 2 \), gap opening penalty to 1.53, and offset value to 0. Alignments obtained with MAFFT were analyzed using MEGA 5.05 (Tamura et al. 2011) to calculate genetic content and transition : transversion ratios (Table 3).

### 2.6. Phylogenetic analyses

Separate and simultaneous parsimony analyses of the concatenated DNA sequence alignments (824 informative characters) and the morphological data matrix (80 informative characters) were conducted with equal weighting or implied weighting with six values of the concavity constant (\( k \)) = 1, 3, 10, 30, 60 and 100, using TNT ver 1.1 (Goloboff et al. 2008). In each case, gaps were treated as missing data, uninformative characters deactivated using the \( \text{xnace} \) command, and a driven search, combining three of the new technology algorithms (Nixon 1999; Goloboff 1999) executed using a script file modified from Dimitrov et al. (2013); \text{hold 10000}; \text{rseed1}; \text{xm: noverb}.

The nuclear and mitochondrial DNA datasets were also analyzed separately and simultaneously with Bayesian inference, using MrBayes ver. 3.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). The best fitting model of sequence evolution was selected using jModelTest ver. 1.0 (Posada 2008), according to the Akaike information criterion, on the basis of which the GTR + G + I model was applied to all markers. The analysis comprised two iterations of four Markov chain Monte Carlo models, performed for 5 million generations for all concatenated DNA sequence alignments, and 2 million generations for the separate nuclear and mitochondrial DNA sequence alignments. Trees were sampled every 1000 generations, those sampled before stationarity discarded using the \text{burnin} command.

The relative support for each node in the topology obtained by the parsimony analyses was calculated in TNT using 1000 jackknife pseudoreplicates with heuristic searches, consisting of ten random addition sequences, followed by ten iterations of tree bisection-reconnection, retaining one tree at each iteration (Dimitrov et al. 2013), and Bremer support (Bremer 1994), by searching for suboptimal trees up to ten steps longer (for the separate morphological analyses) or 100 steps longer (for separate analyses of the concatenated DNA sequence alignments and simultaneous analyses of the morphology and DNA), retaining 1000 trees at each iteration. We recognize that Bremer support values do not indicate relative branch support (DeBry et al. 2001). Posterior probabilities are shown for the Bayesian phylogram obtained by simultaneous analysis of the concatenated nuclear and mitochondrial DNA sequence alignments, and branch lengths on the phylograms obtained by separate analyses of the nuclear and mitochondrial DNA sequence alignments.

A preferred hypothesis was selected from among the topologies recovered by the simultaneous parsimony analyses of the morphology and DNA. Morphological characters were optimized unambiguously and with accelerated transformation (Farris 1970; Swoford & Maddison 1987, 1992) in WINCLADA 1.00.09 (Nixon 1999–2002).

### 3. Results

#### 3.1. Morphological parsimony analyses

Separate parsimony analyses of the morphological character matrix with equal weighting or implied weighting with \( k = 1, 3, 10, 30, 60 \) and 100 (Table 4) consistently
recovered the monophyly of *Bioculus*, *Didymocentrus*, *Diplocentrus*, and *Kolotl*, with the following relationships (Fig. 1): (*Diplocentrus* (*Bioculus* (*Didymocentrus* + *Kolotl*))). *Diplocentrus motagua* Armas & Trujillo, 2009 was consistently placed sister to the remaining species of *Diplocentrus*. *Bioculus*, *Didymocentrus*, and *Kolotl* received high jackknife and Bremer support values, whereas *Diplocentrus* received lower support. Jackknife and Bremer support values predictably increased with lower values of *k* (increased weighting against homoplasy). Relationships within *Diplocentrus* were weakly supported and mostly unresolved. The *keyserlingii* group was paraphyletic and its placement, in turn, rendered the *mexicanus* group paraphyletic.

### 3.2. Molecular parsimony analyses

Separate parsimony analyses of the concatenated DNA sequence alignments with equal weighting or implied weighting with *k* values of 1, 3, 10, 30, 60 and 100 (Table 4) consistently recovered the monophyly of *Bioculus*, *Didymocentrus*, and *Kolotl* (Fig. 2) with high jackknife and Bremer support. *Diplocentrus* was monophyletic only in the analysis with implied weighting and *k* = 1, where it received lower support than *Bioculus*, *Didymocentrus*, and *Kolotl*. In all other analyses, a monophyletic group of four species, i.e., *Diplocentrus peloncillensis* Francke, 1975, *Diplocentrus silanesi* Armas & Martín-Frías, 2000, *Diplocentrus whitei* (Gervais, 1844), and *Diplocentrus zacatecana* Hoffmann, 1931, hereafter referred to as the “ Zacatecana group”, was consistently placed sister to *Bioculus*, as follows: (*Didymocentrus* (*Kolotl* (*Diplocentrus* (*Bioculus* + Zacatacana group)))). Although the group comprising *Bioculus* and the Zacatecana group was weakly supported, the group comprising the remaining species of *Diplocentrus* received high support.

The *keyserlingii* and *mexicanus* groups of *Diplocentrus* were consistently paraphyletic. *Diplocentrus coylei* Sissom & Fritts, 1986 and *Diplocentrus formosus* Armas & Martin-Frias, 2003, previously assigned to the *keyserlingii* group, were placed within the *mexicanus* group.

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**Fig. 1.** 50% majority rule consensus of trees obtained by separate parsimony analyses of 95 morphological characters for 35 species in 6 diplocentrid scorpion genera, with equal weighting and implied weighting with 6 *k* values. Percentages less than 100 indicated below branches. * indicates members of the *keyserlingii* group, ° indicates members of the *mexicanus* group, + indicates members of the *zacatecana* group.
The remaining members of the keyserlingii group were placed sister to the group comprising D. coylei, D. formosus and members of mexicanus group, with high jackknife and Bremer support (Fig. 2). The mexicanus group was also rendered paraphyletic by the placement of Diplocentrus anophthalmus Francke, 1977 sister to the more inclusive group comprising the keyserlingii group and other members of the mexicanus group, in the majority of analyses. Only in the analysis with implied weighting and \( k = 10 \), was D. anophthalmus placed sister to the group comprising D. coylei, D. formosus and other members of mexicanus group. As in the separate morphological analyses, jackknife and Bremer support values increased with lower values of \( k \).
cies of the *keyserlingii* group were placed sister to the group comprising *D. coylei*, *D. formosus* and members of *mexicanus* group.

The topology recovered with Bayesian inference of the concatenated nuclear and mitochondrial DNA (Fig. 5) was similar to the topologies obtained by parsimony analyses of this dataset (Fig. 2), especially the analysis with implied weighting and \( k = 1 \). *Bioculus*, *Didymocentrus*, *Diplocentrus* and *Kolotl* were monophyletic. The main difference concerned the position of the *zacatecanus* group, placed sister to *Bioculus*, rendering *Diplocentrus* paraphyletic, in most of the parsimony analyses (Fig. 2), but sister to other *Diplocentrus* exemplars, rendering *Diplocentrus* monophyletic, in the Bayesian analysis (Fig. 5). *Diplocentrus anophthalmus* was placed sister to all other *Diplocentrus* exemplars in the parsimony analyses, whereas it was placed within the *mexicanus* group in the Bayesian analyses. The *zacatecanus* group was monophyletic and placed sister to a group comprising members of the *keyserlingii* and *mexicanus* groups. The *keyserlingii* and *mexicanus* groups were rendered paraphyletic by the placement of *D. coylei* and *D. formosus* within the *mexicanus* group. The remaining members of the *keyserlingii* group formed a monophyletic sister group of the group comprising *D. coylei*, *D. formosus* and members of *mexicanus* group. All Bayesian analyses recovered the monophyly of *Bioculus*, *Didymocentrus*, *Diplocentrus* and *Kolotl* with low posterior probabilities.

3.4. Simultaneous parsimony analyses

Simultaneous parsimony analyses of the concatenated DNA sequence alignments and morphological character matrix with equal weighting or implied weighting and \( k = 1, 3, 10, 30, 60 \) and 100 (Table 4) consistently recovered the monophyly of *Bioculus*, *Didymocentrus*, *Diplocentrus*, and *Kolotl* with the following relationships (Fig. 6): ((*Bioculus* + *Diplocentrus*) (*Didymocentrus* + *Kolotl*)). The four genera received high jackknife and Bremer support values. The *zacatecanus* group was consistently recovered with high jackknife support, and placed sister to a group comprising members of the *keyserlingii* and *mexicanus* groups, which also received high support. The *keyserlingii* and *mexicanus* groups were consistently rendered paraphyletic by the placement of *D. coylei* and *D. formosus* within the *mexicanus* group. The remaining members of the *keyserlingii* group were consistently monophyletic with high support, and placed sister to a monophyletic group comprising *D. coylei*, *D. formosus* and members of *mexicanus* group, which received low jackknife support. *Diplocentrus anophthalmus* was consistently placed sister to all other members of the *mexicanus* group. As in the separate parsimony analyses of the morphology and concatenated nuclear and mitochondrial DNA sequences, jackknife and Bremer support values increased with lower values of \( k \).

The topology obtained by the simultaneous parsimony analysis with implied weighting and \( k = 3 \) is preferred, due to its high tree statistics, jackknife and Bremer support values (Fig. 7, Table 4). The topology recovered by this analysis is congruent with the 50% majority rule consensus of the most parsimonious trees obtained by the simultaneous parsimony analyses with equal weights and implied weights with \( k = 1, 3, 10, 30, 60 \) and 100. *Bioculus*, *Didymocentrus* and *Kolotl* were monophyletic, with high jackknife and Bremer support values, whereas *Diplocentrus* was monophyletic with high jackknife support, but low Bremer support. Three groups were recovered within *Diplocentrus*. The *zacatecanus* group was placed sister to a more inclusive group comprising all species previously assigned to the *keyserlingii* group, except *D. coylei* and *D. formosus*. This group was, in turn, placed sister to a group comprising *D. coylei*, *D. formosus* and members of the *mexicanus* group.

4. Discussion

4.1. Monophyly and relationships among genera

All analyses corroborated the monophyly of *Bioculus* and *Didymocentrus* (each based on two exemplar species per genus) and confirmed the need to redefine the generic limits of *Diplocentrus* by excluding its two neobothriotoxid species. However, the monophyly of *Bioculus* and *Didymocentrus* await further testing with a larger and more representative sample of species, before they can be satisfactorily diagnosed. *Bioculus* was supported by one morphological synapomorphy, i.e., equal development of the pedipalp chela dorsal secondary, digital and retrolateral secondary carinae. *Didymocentrus* was supported by four morphological synapomorphies: orientation of the ventromedian carina of the pedipalp chela manus, with the distal edge directed towards trichobothrium \( V_1 \); concavity on the proventral surface of the chela manus of the male; distal position of chela trichobothrium \( ib \); and rounded laterodistal lobes of the leg telotarsi.

Parsimony and Bayesian analyses consistently placed the two neobothriotoxid diplodentrid species from central Mexico, previously assigned to *Diplocentrus*, in a monophyletic group, sister to *Didymocentrus* (the preferred hypothesis) or the monophyletic group comprising *Bioculus* and *Diplocentrus* (topology obtained by parsimony and Bayesian analyses of the concatenated nuclear and mitochondrial DNA sequences), to the exclusion of all other exemplar species of *Diplocentrus*. This finding justifies removal of the two species from *Diplocentrus* and the creation of a new genus, *Kolotl*, to accommodate them (Santibañez-López et al. 2014). The two species can be distinguished from all other diplodentrids by the following combination of characters. The anteromedian longitudinal sulcus of the carapace is complete. The subdistal denticle of the cheliceral movable finger is equal to the medial denticle and the dorsal distal denticle equal...
Fig. 3. Phylogram obtained by Bayesian analysis of 2277 aligned nucleotides from 2 markers in the nuclear genome for 35 species in 6 diplocentrid scorpion genera. Branch lengths indicated above branches, posterior probabilities higher than 0.90 indicated below branches. * indicates members of the keyserlingii group, ** indicates members of the mexicanus group, + indicates members of the zacatecanus group.
Fig. 4. Phylogram obtained by Bayesian analysis of 1925 aligned nucleotides from 3 markers in the mitochondrial genome for 35 species in 6 diplocentrid scorpion genera. Branch lengths indicated above branches, posterior probabilities higher than 0.90 indicated below branches. * indicates members of the *keyserlingii* group, ° indicates members of the *mexicanus* group, + indicates members of the *zacatecanus* group.
The monophyly of the remaining exemplar species of Diplocentrus (i.e., excluding the two neobothriotaxic species assigned to Koloti by Santibáñez-López et al. 2014) differed among the analyses. Diplocentrus monophyly was recovered by the separate parsimony analyses of the morphology and the concatenated nuclear and mitochondrial DNA, with implied weighting and $k = 1$, the Bayesian analyses of the nuclear DNA and the concatenated nuclear and mitochondrial DNA, and the simultaneous parsimony analyses of the morphology and DNA. However, Diplocentrus was rendered paraphyletic in the Bayesian analyses of the concatenated nuclear and mitochondrial DNA with equal weighting or implied weighting and $k = 3, 10, 30, 60$ and $100$, due to placement of the Zacatecanus group sister to Bioculus. The paraphyly of Diplocentrus in these analyses may be resolved by the inclusion of additional ingroup and outgroup taxa.

In the analyses in which Diplocentrus was monophyletic, the genus was supported by the following three morphological synapomorphies. The median denticle row of the pedipalp chela movable finger is weakly defined in the proximal third, discontinuous, and interrupted by larger denticles. The intercarinal surfaces of the male chela manus are reticulate, with reversals in D. anophthalmus and Diplocentrus mitlae Francke, 1977. A retrolateral median spiniform macroseta is present on the basitarsus of leg II, except in D. motagua, also a reversal.

4.2. Relationships within Diplocentrus

Internal relationships within Diplocentrus differed little among the analyses. Neither the keyserlingii group, as defined by Santibáñez-López et al. (2013), nor the mexicanus group, were monophyletic. Both groups were consistently rendered paraphyletic by the placement of D. coylei and D. formosus, previously assigned to the keyserlingi group, in the mexicanus group. In addition, D. anophthalmus was placed outside the mexicanus group in some topologies. The remaining members of the keyserlingi group (i.e., excluding D. coylei and D. formosus) were consistently monophyletic in the parsimony and Bayesian analyses of the concatenated nuclear and mitochondrial DNA, and in the simultaneous parsimony
analyses of the morphology and DNA. The group comprising *D. coylei*, *D. formosus* and members of the *mexicanus* group was also consistently monophyletic in the Bayesian analyses of the mitochondrial DNA and the concatenated nuclear and mitochondrial DNA, the separate parsimony analyses of the concatenated nuclear and mitochondrial DNA, and the simultaneous parsimony analyses of the morphology and DNA. This result suggests that *D. coylei* and *D. formosus* should be transferred from the *Keyserlingii* group to the *mexicanus* group, restoring the monophyly of each.

Fig. 6. 50% majority rule consensus of trees obtained by simultaneous parsimony analyses of 95 morphological characters and 4202 aligned nucleotides from 5 markers in the nuclear and mitochondrial genomes for 35 species in 6 diplocentrid scorpion genera, with equal weighting and implied weighting with 6 k values. Percentages less than 100 indicated below branches. * indicates members of the *Keyserlingii* group, ° indicates members of the *mexicanus* group, + indicates members of the *zacatecanus* group.

A previously unrecognized group, referred to as the *zacatecanus* group, was recovered by the Bayesian analyses of the mitochondrial DNA and the concatenated nuclear and mitochondrial DNA as well as the separate parsimony analyses of the concatenated nuclear and mitochondrial DNA, and the simultaneous parsimony analyses of the morphology and DNA, but not the separate parsimony analyses of the morphology. No consistent morphological differences, separating species of the *zacatecanus* group from those of the *mexicanus* group, have thus far been identified. The inclusion of more species and morphological characters are necessary to corroborate its validity.

The group comprising *D. coylei*, *D. formosus* and members of the *mexicanus* group was monophyletic in all except the separate parsimony analyses of the concatenated nuclear and mitochondrial DNA sequences, due to the position of *D. anophthalmus*, placed sister to all other species of the genus. All other parsimony and Bayesian analyses placed *D. anophthalmus* sister to the group comprising *D. coylei*, *D. formosus* and members of the *mexicanus* group. The placement of *D. anophthalmus*, a troglobiont from the Yucatan Peninsula, was unexpected. It was not placed sister to *Diplocentrus cozumel* Beutelspacher & Armas, 1998 or *Diplocentrus redelli* Francke, 1977, the only other exemplar species from the Yucatan, in any analysis.
The relationships of other species within the *mexicanus* group were consistent with their geographical distributions, however. A group comprising three species restricted to northeastern Mexico and the southeastern U.S.A., i.e. *Diplocentrus colwelli* Sissom, 1986, *Diplocentrus diablo* Stockwell & Nilsson, 1987, and *Diplocentrus lindo* Stockwell & Baldwin, 2001, was recovered by all analyses, except the separate parsimony analyses of the morphology and the Bayesian analysis of the nuclear DNA. Both groups formed a larger monophyletic group, sister to species from the Yucatan Peninsula (*D. cozumel* and *D. reddelli*) and Guatemala (*Diplocentrus motagua* Armas & Trujillo, 2009) to the exclusion of other species occurring in Oaxaca or Veracruz, in these analyses. The inclusion of additional species of *Diplocentrus* may be necessary to corroborate these relationships.

The positions of four *Diplocentrus* species with punctate pedipalp surfaces are noteworthy: *Diplocentrus bereai* Armas & Martin-Frias, 2004 and *Diplocentrus melici* Armas, Martin-Frias & Berea, 2004, both from Veracruz, Mexico; *D. gertschi* from Nayarit, Mexico; and *D. motagua* from Guatemala. Punctate pedipalps were previously
considered present only in *Didymocentrus*, and cited as justification for synonymizing *Bioculus* with the latter (Williams & Lee 1978). Sissom & Walker (1992) considered *D. gertschi* a “link” to the diplocentrids of the Baja California Peninsula (i.e., *Bioculus*), due to their punctate pedipalps and similar chelal carination. Sissom & Walker’s (1992) hypothesis was tested and falsified by Prendina (2000), who included *D. gertschi* and two exemplar species of *Bioculus* in his analysis of scorpionoid phylogeny. Armas & Trujillo (2009) noticed a similarity between the pedipalp chelal carination and punctate pedipalps of *D. motagua* and those of *Didymocentrus*. However, the orientation of the pedipalp chela ventromedian carina was inconsistent with that of *Didymocentrus*, among other characters considered diagnostic for that genus, e.g., the presence of a retrolateral median spiniform macroseta on the basitarsons of leg II. None of these species formed a monophyletic group in the analyses presented here. Separate parsimony analyses of the morphology did not recover a close relationship between *D. gertschi*, *D. melici*, and *D. motagua*. Separate parsimony and Bayesian analyses of the concatenated DNA sequences and simultaneous parsimony analyses of the morphology and DNA placed these species within the *mexicanus* group, *D. bereai* and *D. melici* as sister species, and *D. gertschi* in a different group from *D. motagua*. The phylogenetic relationships among them may be better resolved by the inclusion of additional species, particularly from Central America.

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6. References


7. Appendix 1

Terminal taxa, specimens and tissue samples used for cladistic analyses of 35 species in 6 diplocentrid scorpion genera. Material examined is deposited in the following collections: American Museum of Natural History (AMNH), New York, U.S.A.; Colección Aracnológica de la Facultad de Biología (CAFUBM), Universidad Michoacana de San Nicolás de Hidalgo; Morelia, Michoacán, Mexico; Colección Aracnológica “Luis de Armas”, Instituto Tecnológico del Valle de Oaxaca (ICAL), Oaxaca, Mexico; Colección Nacional de Arácnidos (CNAN), Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City; Museo de Historia Natural (MHN), Escuela de Biología, Universidad de San Carlos.
de Guatemala, Guatemala City; U.S. National Museum of Natural History (USNM), Smithsonian Institution, Washington, DC, U.S.A. Tissue samples are deposited in the Ambrose Monell Collection (AMCC) at the AMNH.

Outgroup

Heteronebion Franeck, 1899: Sixteen species are currently recognized in this genus, which occurs in the Caribbean and two islets between Somalia and the island of Socotra. Based on previous evidence that Heteronebion is basal to Diplacentrus and the other genera of Diplacentrinae (Prendini 2000), Heteronebion jamaicae Francke, 1978, an exemplar species from the Caribbean, was selected as the primary outgroup for analyses presented here. This species, mistakenly synonymized with Heteronebion scaber (Hesse, 1893) by Teruel (2009), is hereby revalidated.


Tarsoporosus Franck, 1978: Five species are currently recognized in this genus, which is endemic to northern South America and closely related to Diplacentrus and Didymocentrus (Prendini 2000). The genus was represented by the type species in the analyses presented.


Bioculus Stahnke, 1968: Five species are recognized in this genus, which occurs on the Baja California Peninsula and mainland Mexico. Prendini (2000) recovered this genus as monophyletic with two alternative placements, sister to the Caribbean diplocentrid genera, or within Diplacentrus, rendering the latter paraphyletic. The genus was represented in the analyses by the type species and a second species from Baja California.


Didymocentrus Kraepelin, 1905: Ten species are currently recognized in this genus. Francke (1978) considered it distinct from Diplacentrus and recognized two groups, the lesueurii group, from the Caribbean islands, and the nitidus group, from Central America. Although Prendini (2000) recovered the monophyly of Didymocentrus, the phylogenetic placement of the two exemplar species included in the analysis rendered Diplacentrus paraphyletic. In the present analysis, we included two exemplar species of Didymocentrus, the type species, representing the lesueurii group, and a species from Central America, representing the nitidus group.


6. Didymocentrus lesueurii (Gervais, 1844): MARTINIQUE: E of Anses-D’Arlet, 6.5 km W of Le Diamant, 14°29’.627’N 61°04’.267’W, 43 m, 7.xii.2004, J. Huff, scrub forest with little old growth, hand collected under rocks and garbage, 1 ♂, 3 ♀, 19 juv. (AMNH), 1 juv. (AMCC [LP 3638]); Le Diamant, just S, 14°28’.832’N 61°01’.88’W, 355 m, 7.xii.2004, J. Huff, under rocks and garbage at edge of road, 2 ♀, 1 juv. (AMNH), 1 juv. (AMCC [LP 3639]).

Ingroup

Kalotol Santibáñez-López, Francke & Prendini, 2014: Santibáñez-López et al. (2014) created a new genus to accommodate two Mexican diplocentrid species, previously placed in Diplacentrus, which rendered the latter paraphyletic in the analyses presented here.

Diplocentrus, 1861: This genus presently comprises 59 species, although several may eventually be synonymized. Penedini (2000) recovered this genus as paraphyletic with respect to Didymocentrus. Two groups were recognized by Santibañez-López et al. (2013), the keyserlingii group, comprising 10 species, and the mexicanus group, comprising 45. The present study included 9 species from the keyserlingii group and 18 from the mexicanus group, including type species of the genus, *Diplocentrus mexicanus* Peters, 1861.


1 ♂ paratypes (AMNH), 26°23.015′N 98°47.091′W, 25.viii.2006, T. Anton, G. Casper, V. Torti & W.D. Sissom, 3 juv. (AMCC [LP 6386]).


26. Diplocentrus matogata Armas & Trujillo, 2009: GUATEMALA: Departamento Zacapa: Municipio de Río Hondo: Aldea Casas de Pinto, 15°01.403′N 89°36.82′W, 195 m, 26.vi.2008, R. Trujillo & C. Avila, holotype ♂ (MNHN); Aldea Casas de Pinto, near turn off for Zacapa at Río Hondo, 15°01.618′N 89°36.953′W, 77 m, 13.vi.2006, J. Huff, C. Viquez & D. Ortiz, 1 ♀, 1 ♂, 2 subad. ♂, 1 juv. (AMNH), 4 juv. (AMCC [LP 5997]), 1 ♂, 3 juv. (AMCC [LP 5998]).


List of 95 morphological characters scored for cladistic analysis of 35 species in 6 diplocentrid scorpion genera. Characters from previous analyses that correspond partially or entirely to those in the present list (and matrix, Table 2) are indicated in brackets by the following abbreviations P00 (Prendini 2000), PEA03 (Prendini et al. 2003) and MEA12 (Mattoni et al. 2012), followed by the character number from the corresponding publication. 15 uninformative characters (excluded from all analyses) are indicated by †. In characters defined for one sex only, the respective sex symbol follows the character description.

**Pigmentation pattern**

0. Base coloration: dark brown to black (0); reddish (1); orange-brown (2); yellowish (3).

1. Chelicerae, infuscation: absent (0); present (1) [PEA03-90].

2. Metasoma dorsal and lateral carinae, coloration relative to adjacent intercarinal surfaces: darker (0); similar (1).

3. Pedipalp chela manus, dorsal secondary carina, coloration relative to adjacent intercarinal surfaces (♂): darker (0); similar (1).

4. Pedipalp chela manus, digital carina, coloration relative to adjacent intercarinal surfaces (♂): darker (0); similar (1).

5. Pedipalp chela manus, retro lateral secondary carina, coloration relative to adjacent intercarinal surfaces (♂): darker (0); similar (1).

6. Pedipalp chela fingertips, coloration relative to chela manus: similar (0); darker (1); paler (2).

7. Pedipalp chela manus, dorsal secondary carina, coloration relative to adjacent intercarinal surfaces (♂): darker (0); similar (1).

8. Pedipalp chela manus, digital carina, coloration relative to adjacent intercarinal surfaces (♂): darker (0); similar (1).

9. Pedipalp chela manus, retro lateral secondary carina, coloration relative to adjacent intercarinal surfaces (♂): darker (0); similar (1).

10. Legs, coloration relative to mesosomal tergites: similar (0); paler (1).

11. Legs, infuscation: absent (0); present (1) [PEA03-99].

**Chelicerae**

12. Movable finger subdistal tooth, length relative to medial tooth: smaller (0); similar (1).

13. Movable finger ventral distal tooth, length relative to dorsal distal tooth: equal (0); subequal, i.e. >0.5 (1); unequal, i.e. < 0.5 (2) [PEA03-2].

**Carapace**

14. Median ocular tubercle, protrusion: raised (0); level (1) [P00:2].

15. Median longitudinal sulcus, width: narrow (0); broad (1) [P00:4].

16. Anteromedian longitudinal sulcus, length: complete (0); vestigial (1) [MEA12:7].

17†. Lateral ocelli, number of pairs: 3 (0); 2 (1); 0 (2) [P00:1].

18. Nongranular surfaces, punctuation: absent (0); present (1).

**Pedipalp carination and surface macrosculpture**

19. Pedipalp femur intercarinal surfaces: uniformly granular (0); granular only medially (1); smooth (2).

20. Pedipalp femur nongranular intercarinal surfaces, punctuation: present (0); absent (1).

21. Femur dorsal intercarinal surface, shape: flat (0); shallowly convex (1); markedly convex (2) [PEA03:40; MEA12:10].

22. Patella dorsal retrolateral carina, development (♂): distinct, i.e., raised above adjacent intercarinal surfaces (0); obsolete, i.e., not raised above adjacent intercarinal surfaces (evident as difference in texture or pigmentation) (1) [PEA03-42].

23. Patella dorsal retrolateral carina, texture (♂): granular (0); smooth (1).

24. Patella dorsal retrolateral carina, development (♂): distinct (0); obsolete (1) [P00:17].

25†. Patella dorsal retrolateral carina, texture (♂): granular (0); smooth (1).

26. Patella retrolateral median carina, development (♂): distinct (0); obsolete (1).
27. Patella retrolateral median carina, texture (♂): granular (0); smooth (1).
28. Patella retrolateral median carina, development (♂): distinct (0); obsolete (1).
29. Patella retrolateral median carina, texture (♀): granular (0); smooth (1).
30. Patella ventral median carina (♂): absent (0); granular (1); smooth (2).
31. Chela manus, dorsal secondary carina, development (♂): distinct (0); obsolete (1) [P00:20; PEA03:31].
32. Chela manus, dorsal secondary carina, texture (♂): smooth (0); granular to crenulate (1).
33. Chela manus, dorsal secondary carina, development (♀): distinct (0); obsolete (1) [P00:21].
34. Chela manus, dorsal secondary carina, texture (♀): smooth (0); granular to crenulate (1).
35. Chela manus, digital carina, development (♂): distinct (0); obsolete (1) [P00:23; PEA03:32].
36. Chela manus, digital carina, texture (♂): smooth (0); granular (1).
37. Chela manus, digital carina, length (♂): base of manus to tip of fixed finger (0); base of manus to base of fixed finger (1) [PEA03:32].
38. Chela manus, digital carina, development (♀): distinct (0); obsolete (1) [P00:23].
39. Chela manus, digital carina, texture (♀): smooth (0); granular (1).
40. Chela manus, digital carina, length (♀): base of manus to tip of fixed finger (0); base of manus to base of fixed finger (1).
41. Chela manus, dorsal secondary, digital and retrolateral secondary carinae, relative development (♂): digital carina more developed than dorsal secondary and retrolateral secondary carinae (0); dorsal secondary, digital and retrolateral secondary carinae similarly developed (1); dorsal secondary and retrolateral secondary carinae more developed than digital carinae (2) [P00:24].
42. Chela manus, dorsal secondary, digital and retrolateral secondary carinae, relative development (♀): digital carina more developed than dorsal secondary and retrolateral secondary carinae (0); dorsal secondary, digital and retrolateral secondary carinae similarly developed (1); dorsal secondary and retrolateral secondary carinae more developed than digital carinae (2) [P00:24].
43. Chela manus, retrolateral secondary carina, texture (♂): smooth (0); granular (1).
44. Chela manus, retrolateral secondary carina, texture (♀): smooth (0); granular to crenulate (1).
45. Chela manus, dorsal margin, curvature relative to digital carina (♂): convex, not parallel to digital carina (0); subparallel to digital carina (1); parallel to digital carina (2) [MEA12: 15].
46. Chela manus, dorsal margin, curvature relative to digital carina (♀): convex, not parallel to digital carina (0); subparallel to digital carina (1).
47. Chela manus, ventral median carina, orientation of distal edge relative to trichobothria $E_1$ and $V_1$: directed towards $E_1$ (0); directed towards a point less than half the distance from $E_1$ to $V_1$ (1); directed towards a point approximately half the distance from $E_1$ to $V_1$ (2); directed towards a point more than half the distance from $E_1$ to $V_1$ (3); directed towards $V_1$ (4) ADDITIVE [P00:27].
48. Chela manus, dorsal marginal carina length: base of manus to base of fixed finger (0); base of manus to tip of fixed finger (1).
49. Chela manus, intercarinal surfaces (♂): smooth (0); granular (1); reticulate (2) [MEA12:29].
50. Chela manus, intercarinal surfaces (♀): smooth (0); granular (1); reticulate (2) [MEA12:30].
51. Chela manus, nongranular intercarinal surfaces, punctuation: present (0); absent (1).
52. Chela fixed finger, prolateral concavity, proximal to $ib$ and $it$ trichobothria (♂): weakly developed, shallow (0); well developed, deep (1).

**Pedipalp chela finger dentition**

53. Chela movable finger, median denticle row, development: distinct from base to tip of finger (0); weakly defined in basal third of finger, indistinct from prolateral denticle row (1).
54. Chela movable finger, median denticle row: discontinuous, interrupted by larger denticles (0); continuous, not interrupted by larger denticles (1).
55. Chela movable finger, median denticle row, first and second denticles, size relative to other denticles: larger (0); similar (1).
56. Chela movable finger, retrolateral denticle row, disposition: parallel to median denticle row from second large median denticle to tip of finger (0); parallel to median denticle row from base to tip of finger (1).
57. Chela movable finger, prolateral denticle row, disposition: parallel to median denticle row from second large median denticle to tip of finger (0); parallel to median denticle row from base to tip of finger (1).

**Pedipalp trichobothria**

58. Patella, ventral surface, $v$ trichobothria, number: 3 (0); 4, i.e., one accessory (1); 12–18, i.e., 8–14 accessories (2).
59. Patella, retrolateral surface, $et$ trichobothria, number: 3 (0); 4 (1).
60. Patella, retrolateral surface, $est$ trichobothria, number: 2 (0); 3 (1).  
61. Patella, retrolateral surface, $em$ trichobothria, number: 2 (0); 3 (1); 4 (2).
62. Patella, retrolateral surface, $esb$ trichobothria, number: 2 (0); 5 (1).
63. Patella, retrolateral surface, $eb$ trichobothria, number: 5 (0); 6 (1).
64. Chela manus ($\cdots$), trichobothrium $ib$, position relative to articulation between fixed and movable fingers: aligned (0); distal (1).
65. Chela manus ($\cdots$), trichobothrium $it$, position relative to trichobothrium $ib$: aligned (0); distal (1).
66. Chela manus, ventral surface, $F$ trichobothria, number: 4 (0); more than 4, i.e., 5–9 accessories (1).

**Legs**

67. Leg telotarsi, laterodistal lobes: truncate (0); rounded (1) [P00:65].
68. Leg lateral surfaces, punctuation: absent (0); present (1).
69†. Leg basitarsi, prolateral pores (♂): absent (0); present (1) 
[P00:67].
70. Leg I basitarsus, proventral distal spiniform macroseta: ab-
sent (0); present (1).
71. Leg I basitarsus, retroventral distal spiniform macroseta: ab-
sent (0); present (1).
72. Leg I basitarsus, proventral subdistal spiniform macroseta: ab-
sent (0); present (1).
73. Leg I basitarsus, retroventral subdistal spiniform macroseta: ab-
sent (0); present (1).
74. Leg I basitarsus, proventral medial spiniform macroseta: ab-
sent (0); present (1).
75. Leg I basitarsus, retroventral medial spiniform macroseta: ab-
sent (0); present (1).
76. Leg II basitarsus, retrolateral medial spiniform macroseta: ab-
sent (0); present (1).
77. Leg II basitarsus, proventral distal spiniform macroseta: ab-
sent (0); present (1).
78†. Leg II basitarsus, retroventral distal spiniform macroseta: ab-
sent (0); present (1).
79. Leg II basitarsus, proventral subdistal spiniform macroseta: ab-
sent (0); present (1).
80. Leg II basitarsus, retroventral subdistal spiniform macroseta: ab-
sent (0); present (1).
81. Leg II basitarsus, proventral medial spiniform macroseta: ab-
sent (0); present (1).
82. Leg II basitarsus, retroventral subdistal spiniform macroseta: ab-
sent (0); present (1).
83. Leg II basitarsus, retroventral subdistal spiniform macroseta: ab-
sent (0); present (1).
84. Leg II basitarsus, ventral distal spiniform macroseta: absent 
(0); present (1).
85. Leg II basitarsus, retroventral subbasal spiniform macroseta: ab-
sent (0); present (1).
86†. Leg II basitarsus, retrolateral subdistal spiniform macroseta: ab-
sent (0); present (1).
87. Leg II basitarsus, retrolateral medial spiniform macroseta: ab-
sent (0); present (1).
88. Leg II basitarsus, retrolateral subbasal seta: absent (0); spini-
form macroseta present (1); macroseta present (not spini-
form) (2).
89. Legs III and IV basitarsi, retroventral subdistal spiniform macrosetae: absent (0); present (1).
90. Legs III and IV basitarsi, ventral medial spiniform macrosetae: absent (0); present (1).

Mesosoma, metasoma and telson
91. Sternite VII median carina, development: distinct (0); obso-
lete (1).
92. Sternite VII median carina, length relative to submedian cari-
nae: equal (0); less (1).
93. Sternite VII submedian carinae, development: distinct (0); obso-
lete (1) [PEA03:102, 103].
94. Mesosoma, metasoma and telson, nongranular dorsal sur-
faces, punctuation: absent (0); present (1).