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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 (Sissom & 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 (Sissom & 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-Frías, 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, *Heteronebo* 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 1. Genbank accession codes for tissue samples, deposited in the Ambrose Monell Collection for Molecular and Microbial Research (AMCC) at the American Museum of Natural History, New York, from which DNA was extracted and sequenced for phylogenetic analyses of 35 species in 6 diplocentrid scorpion genera, *Bioculus* Stahnke, 1968, *Didymocentrus* Kraepelin, 1905, *Diplocentrus* Peters, 1861, and *Heteronebo* Pocock, 1899, *Kolotl* Santibáñez-López et al., 2014, and *Tarsoporosus* Francke, 1978. Provenance data provided in Appendix 1.

Species	Specimen	AMCC	18S	28S	12S	16S	COI
Heteronebo jamaicae	1 ♂	LP 5131	KM514559	KM514594	KM514489	KM514524	KM514629
Tarsoporosus kugleri	1 ♀	LP 5204	KM514560	KM514595	KM514490	KM514525	KM514630
Bioculus caboensis	1 ♀	LP 1796	KM514561	KM514596	KM514491	KM514526	KM514631
Bioculus comondae	1 juv.	LP 3123	KM514562	KM514597	KM514492	KM514527	KM514632
Didymocentrus krausi	1 subad. ♂	LP 1987	KM514563	KM514598	KM514493	KM514528	KM514633
Didymocentrus lesueurii	1 ♂	LP 3638	KM514564	KM514599	KM514494	KM514529	KM514634
Kolotl magnus	1 juv.	LP 7029	KM514565	KM514600	KM514495	KM514530	KM514635
Kolotl poncei	1 juv.	LP 7030	KM514566	KM514601	KM514496	KM514531	KM514636
Diplocentrus anophthalmus	1 subad. ♀	LP 10980	KM514567	KM514602	KM514497	KM514532	KM514637
Diplocentrus bereai	1 juv.	LP 6532	KM514568	KM514603	KM514498	KM514533	KM514638
Diplocentrus coddingtoni	1 juv.	LP 9169	KM514569	KM514604	KM514499	KM514534	KM514639
Diplocentrus colwelli	1 ♀	LP 6483	KM514570	KM514605	KM514500	KM514535	KM514640
Diplocentrus coylei	1 subad. ♂	LP 7031	KM514571	KM514606	KM514501	KM514536	KM514641
Diplocentrus cozumel	1 ♀	LP 4102	KM514572	KM514607	KM514502	KM514537	KM514642
Diplocentrus diablo	1 juv.	LP 6386	KM514573	KM514608	KM514503	KM514538	KM514643
Diplocentrus formosus	1 ♀	LP 10979	KM514574	KM514609	KM514504	KM514539	KM514644
Diplocentrus gertschi	1 ♀	LP 4707	KM514575	KM514610	KM514505	KM514540	KM514645
Diplocentrus hoffmanni	1 ♂	LP 2036	KM514576	KM514611	KM514506	KM514541	KM514646
Diplocentrus jaca	1 ♀	LP 9518	KM514577	KM514612	KM514507	KM514542	KM514647
Diplocentrus keyserlingii	1 ♀	LP 6517	KM514578	KM514613	KM514508	KM514543	KM514648
Diplocentrus kraepelini	1 subad. ♀	LP 10973	KM514579	KM514614	KM514509	KM514544	KM514649
Diplocentrus lindo	1 ♂	LP 3078	KM514580	KM514615	KM514510	KM514545	KM514650
Diplocentrus melici	1 juv.	LP 6546	KM514581	KM514616	KM514511	KM514546	KM514651
Diplocentrus mexicanus	1 juv.	LP 7674	KM514582	KM514617	KM514512	KM514547	KM514652
Diplocentrus mitlae	1 subad. ♂	LP 11034	KM514583	KM514618	KM514513	KM514548	KM514653
Diplocentrus motagua	1 juv.	LP 5997	KM514584	KM514619	KM514514	KM514549	KM514654
Diplocentrus peloncillensis	1 juv.	LP 2140A	KM514585	KM514620	KM514515	KM514550	KM514655
Diplocentrus rectimanus	1 ♂	LP 2032	KM514586	KM514621	KM514516	KM514551	KM514656
Diplocentrus reddelli	1 ♂	LP 10981	KM514587	KM514622	KM514517	KM514552	KM514657
Diplocentrus sagittipalpus	1 ♀	LP 10975	KM514588	KM514623	KM514518	KM514553	KM514658
Diplocentrus silanesi	1 ♂	LP 2025	KM514589	KM514624	KM514519	KM514554	KM514659
Diplocentrus sissomi	1 ♀	LP 6531	KM514590	KM514625	KM514520	KM514555	KM514660
Diplocentrus tehuacanus	1 ♂	LP 2044	KM514591	KM514626	KM514521	KM514556	KM514661
Diplocentrus whitei	1 juv.	LP 4101	KM514592	KM514627	KM514522	KM514557	KM514662
Diplocentrus zacatecanus	1 juv.	LP 5339	KM514593	KM514628	KM514523	KM514558	KM514663

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 anophthalmus* 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

Table 2. Distribution of states among 95 morphological characters scored for parsimony analyses of 35 species in 6 diplocentrid scorpion genera, *Bioculus* Stahnke, 1968, *Didymocentrus* Kraepelin, 1905, *Diplocentrus* Peters, 1861, and *Heteronebo* Pocock, 1899, *Kolotl* Santibáñez-López et al., 2014, and *Tarsoporosus* Francke, 1978. Character states are scored 0–3, ? (unknown) or – (inapplicable). Refer to Appendix 1 for material examined and Appendix 2 for character descriptions.

	0000000000	**1111111111**	**2222222222**	**3333333333**	**4444444444**	**5555555555**	**6666666666**	**7777777777**	**8888888888**	**99999**
	0123456789	**0123456789**	**0123456789**	**0123456789**	**0123456789**	**0123456789**	**0123456789**	**0123456789**	**0123456789**	**01234**
Outgroup										
Heteronebo jamaicae	2101002101	0002111010	0200111111	0101000000	0000000000	0001----00	0000010000	1110110111	1110000010	10100
Tarsoporosus kugleri	0101010111	1001111000	1211111111	0000000000	0010010010	01011-1100	0000000011	1110000111	0000000000	01010
Bioculus caboensis	3111110111	0001010111	0111111111	0101010110	1110000100	0001----00	0000010010	0011100111	1000000020	10101
Bioculus comondae	1101110111	0001010011	0101110111	0101010110	1110000100	0001----00	0000010010	0011000111	1000000020	10101
Didymocentrus krausi	2100100110	0101110011	0111111111	0001010110	1210011400	0011----00	0000110100	0000100111	0000000020	00001
Didymocentrus lesueurii	2100100110	0101110011	0111111111	0000010110	1220011400	0011----00	0000110100	0000100111	0000000020	01111
Ingroup										
Kolotl magnus	010???2000	1110100011	00??11??11	???10???10	1?2?0?110?	00?11-1110	0100000010	1111000111	1010000020	00101
Kolotl poncei	0100002000	0110100011	0011111111	0101010110	1220011100	00011-1121	1211001010	1111000111	1000000020	00101
Diplocentrus anophthalmus	201???0111	0001-11202	10??11??11	???10???10	1?1?0?130?	01?0000000	0000010010	1111111111	1110000100	11110
Diplocentrus bereai	2011110111	0001111001	1011111111	0101010110	1010011302	0100000000	0000010010	0011110111	1110000100	11100
Diplocentrus coddingtoni	0101110101	0001011000	1011011111	0111100100	1000011302	2100000000	0000010010	1111110111	1110000100	10100
Diplocentrus colwelli	1111110111	0001111000	1011111111	0101011110	1010011302	0100000000	0000010010	0011100111	1110000101	10000
Diplocentrus coylei	2111011111	0102011000	1211111111	0001000110	1000020202	0100000000	0000010010	1001111110	1001110100	00000
Diplocentrus cozumel	0101010101	0101011000	1001111111	2111000100	1000011302	2100000000	0000010010	0011111111	1110000101	10100
Diplocentrus diablo	1101110111	0101111000	1011111111	1011000110	1000011302	0100000000	0000010010	1111111111	1100000101	10100
Diplocentrus formosus	2011110111	1002111000	1211111111	0011101101	1011110201	1100000000	0000010010	1100011111	1010000100	00000
Diplocentrus gertschi	1101110111	1001011000	1111111111	2101010110	1010011302	0100000000	0000010010	1111110111	1110000100	10100
Diplocentrus hoffmanni	2111010111	0102011000	1200111111	1011001101	1011111201	1100000000	0000010010	1100101001	1010100100	00000
Diplocentrus jaca	0101010001	0101111000	1000010001	1111100100	1000011302	2100000000	0000010010	0111110111	1100000101	11000
Diplocentrus keyserlingii	2111010111	0102011000	1201111111	2011000110	1010000202	2100000000	0000010010	1111001111	1010000100	00000
Diplocentrus kraepelini	2111010111	0102011000	1201111111	2001000110	1010011202	0100000000	0000010010	0111010011	1010100100	00000
Diplocentrus lindo	1111110111	0101111000	1001111111	1101000110	1010000302	0100000000	0000010010	0111101111	1110000101	10100
Diplocentrus melici	0101012111	0101111011	0011111111	1101000110	1010021300	0000000000	0000010010	0011110111	1110000100	10101
Diplocentrus mexicanus	0110010001	0101011000	1001000101	1111100100	1000111302	2100000000	0000010010	0111010111	1111010101	10100
Diplocentrus mitlae	2111110111	0102011000	1211111111	0101000110	1010010200	0100000000	0000010010	1110000111	0010000100	00000
Diplocentrus motagua	3011010101	0001111011	0111111111	0101000110	1010011302	0000000000	0000010010	0011000111	1100000021	00001
Diplocentrus peloncillensis	2011110111	0001011000	1010111011	1111000110	1010011202	2100000000	0000010010	1111111111	1110001101	10000
Diplocentrus rectimanus	2110011111	0002011000	1201111111	2001000110	1011010202	0100000000	0000010010	0110010111	1000000100	00000
Diplocentrus reddelli	1100000000	0101011000	1011111111	0101000110	1000020302	0100000000	0000010010	1111000111	1110000101	11000
Diplocentrus sagittipalpus	2110010101	0102011000	1201111111	1011101101	1011121202	1100000000	0000010010	0011011111	0010000100	00000
Diplocentrus silanesi	2111010111	0101111000	1011111111	0101000110	1000020202	0100000000	0000010010	0111010111	1110000101	10100
Diplocentrus sissomi	2111010111	0102011000	1211111111	0001000110	1010011202	0100000000	0000010010	1111011111	1010000100	00000
Diplocentrus tehuacanus	2111112111	0101011000	1011111111	0101000110	1010020302	0100000000	0000010010	0011111110	1110100100	11100
Diplocentrus whitei	0100012001	0101011000	1011111111	0101000100	1000010200	0100000000	0000010010	1111111111	1110000101	10100
Diplocentrus zacatecanus	2011112111	0001111000	1001111111	0001000110	1010000202	0100000000	0000010010	1111011111	1010000101	10000

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 18S1F/5R, 18S3F/bi, and 18Sa2.0/9R for the 18S rDNA (Wheeler et al. 1993) and 28Sa/bout for the 28S rRNA (Nunn 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-out/LCO and ExtA/B (Prendini 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, second and third positions (COI 1 2 3), respectively. Calculations were conducted using the maximum composite likelihood test (mcl) under the Tamura-Nei (2004) model of substitution..

		Nuclear		**Mitochondrial**			**Total**
		Ribosomal				**Protein-coding**	
		18S	**28S**	**12S**	**16S**	**COI**	
	Length (bp)	1761	520	347	499	1078	4202
	Variable (%)	14 (1)	52 (10)	223 (64)	272 (54)	485 (45)	1021 (24)
	PI (%)	10 (1)	28 (5)	181 (52)	233 (47)	370 (34)	824 (20)
	Conserved (%)	1747 (99)	466 (90)	122 (35)	218 (44)	593 (55)	3168 (75)
	A (COI 1 2 3) %	25	23	40.21	36.34	20 (19 26 14)	26
	C (COI 1 2 3) %	23	20	9.96	13.77	13 (5 11 22)	19
	G (COI 1 2 3) %	28	26	14.57	15.89	23 (20 30 21)	24
	T (COI 1 2 3) **%**	24	31	35.36	34	44 (56 33 43)	31
ts	**A ↔ G** (COI 1 2 3) %	5	20	25	27	51 (65 38 44)	35
	C ↔ T (COI 1 2 3) %	49	50	39	37	12 (11 25 29)	22
tv	**A ↔ C** (COI 1 2 3) %	11	7	9	9	6 (3 7 5)	10
	A ↔ T (COI 1 2 3) %	11	7	14	13	12 (9 11 8)	12
	C ↔ G (COI 1 2 3) %	12	8	4	5	6 (3 7 6)	9
	G ↔ T (COI 1 2 3) %	12	8	9	9	13 (9 12 8)	12
	ts : tv (COI 1 2 3)	1.11	2.27	1.28	1.43	1.46 (2.29 1.58 2.61)	1.27

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 (Beckman-Coulter). Double-stranded sequencing of the purified PCR product was conducted by the dideoxy termination 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. The 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 reamplified 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 (Katoh et al. 2002, 2005). The G-INS-i strategy, which performs a global alignment based on an FFT approximation (Katoh et al. 2002), was selected. This method is suitable for large datasets comprising sequences with relatively limited variation in length, i.e., few, short gaps (Katoh 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 *xinact;* 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): *hold 10000; rseed1; xm: noverb nokeep; rat: it 0 up 4 down 4 au 0 num 36 give 99 equa; dri: it 10 fit 1.00 rfi 0.20 aut 0 num 36 give 99 xfa 3.00 equa; sec: mins 45 maxs 45 self 43 incr 75 minf 10 god 75 drift 6 glob 5 dglob 10 rou 3 xss 10- 14+2 noxev noeq; tf: rou 5 minf 3 best ke nochoo swap; xm : level 10 nochk rep 50 fuse 3 dri 10 rss css noxss mult nodump conse 5 conf 75 nogive notarg upda autoc 3 xmix; xm; xmult.*

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. 1.0.1 (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 *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.

Table 4. Tree statistics obtained from parsimony analyses of 35 species in 6 diplocentrid scorpion genera. Length, consistency index (CI), retention index (RI), Fit and adjusted homoplasy (AH) of most parsimonious trees (MPTs) obtained by separate and simultaneous (Simul) analyses of the morphological (Mor) dataset, and the molecular dataset (concatenated aligned DNA sequences of five nuclear and mitochondrial gene markers, Mol), with equal weighting (EW) and implied weighting (IW), with six concavity values (k). Analyses of the molecular dataset were conducted with gaps as missing data.

		MPTs	Length	CI	RI	Fit	AH
Mor	IW: k = 1	2	329	0.292	0.574	43.19	39.81
	IW: k = 3	2	321	0.299	0.589	53.43	29.57
	IW: k = 10	2	318	0.302	0.595	68.11	14.89
	IW: k = 30	3	316	0.304	0.599	76.72	6.28
	IW: k = 60	3	316	0.304	0.599	79.62	3.38
	IW: k = 100	2	316	0.304	0.599	80.91	2.09
	EW	19	363	0.264	0.513	50.82	—
Mol	IW: k = 1	1	4303	0.328	0.47	329.37	494.63
	IW: k = 3	1	4286	0.329	0.473	478.28	345.72
	IW: k = 10	1	4530	0.367	0.475	643.4	180.6
	IW: k = 30	1	4267	0.33	0.476	745.02	78.98
	IW: k = 60	1	4267	0.33	0.476	780.91	43.09
	IW: k = 100	1	4267	0.33	0.476	797.14	26.86
	EW	2	4284	0.33	0.476	476.48	—
Simul	IW: k = 1	1	4682	0.322	0.471	361.3	545.7
	IW: k = 3	1	4682	0.322	0.471	525.43	381.57
	IW: k = 10	1	4665	0.323	0.474	707.51	199.49
	IW: k = 30	1	4663	0.323	0.474	819.74	87.26
	IW: k = 60	1	4663	0.323	0.474	859.39	47.61
	IW: k = 100	1	4663	0.323	0.474	877.32	29.68
	EW	3	4681	0.323	0.474	706.75	—

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; Swofford & 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

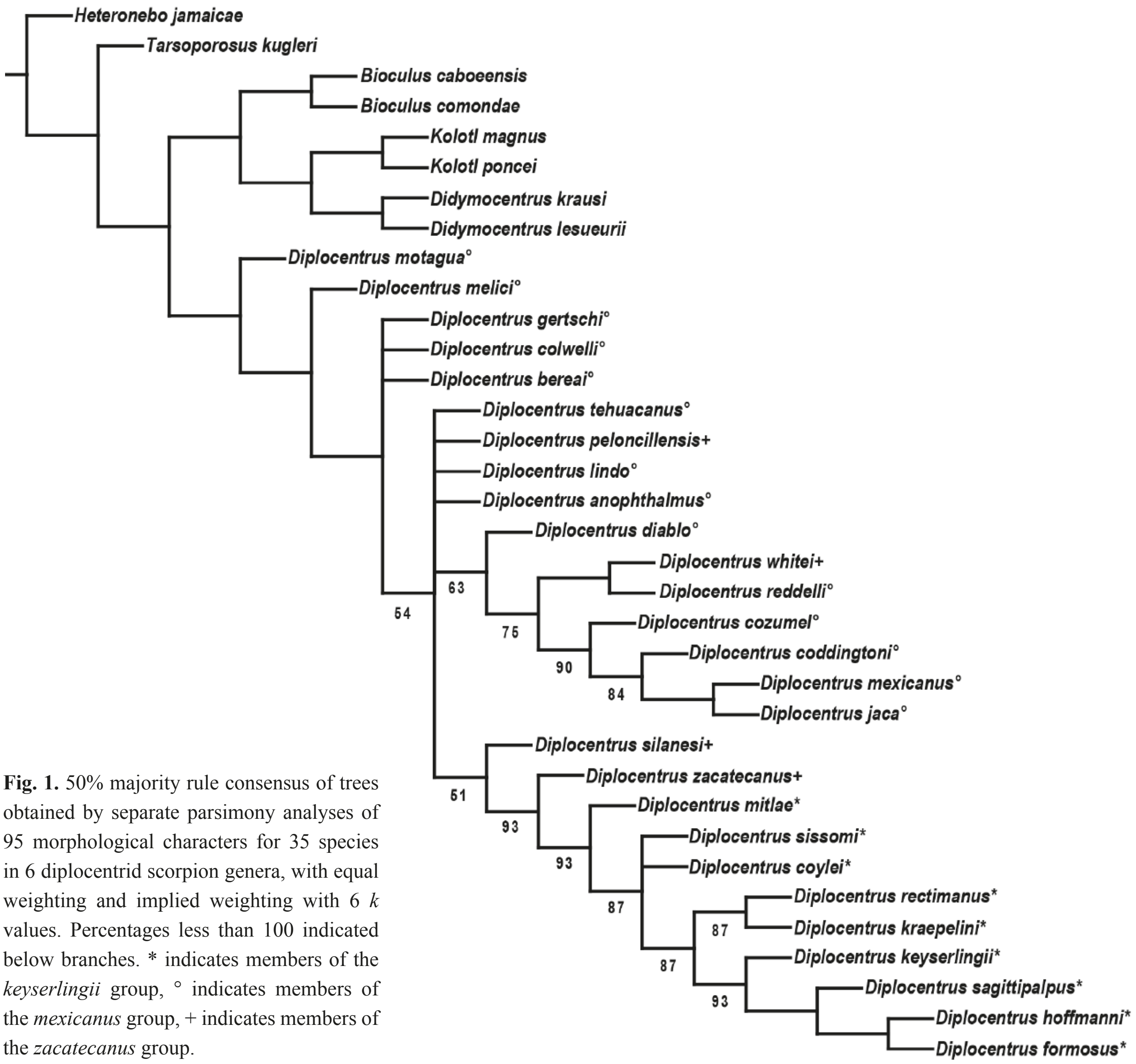

Fig. 1. 50% majority rule consensus of trees obtained by separate parsimony analyses of 95 morphological characters for 35 species in 6 diplocentrid scorpion genera, with equal weighting and implied weighting with 6 *k* values. Percentages less than 100 indicated below branches. * indicates members of the *keyserlingii* group, ° indicates members of the *mexicanus* group, + indicates members of the *zacatecanus* group.

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 = 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 zacatecanus* Hoffmann, 1931, hereafter referred to as the “*zacatecanus* group”, was consistently placed sister to *Bioculus*, as follows: (*Didymocentrus* (*Kolotl* (*Diplocentrus* (*Bioculus* + *zacatecanus* group)))). Although the group comprising *Bioculus* and the *zacatecanus* 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 & Martín-Frías, 2003, previously assigned to the *keyserlingii* group, were placed within the *mexicanus* group.

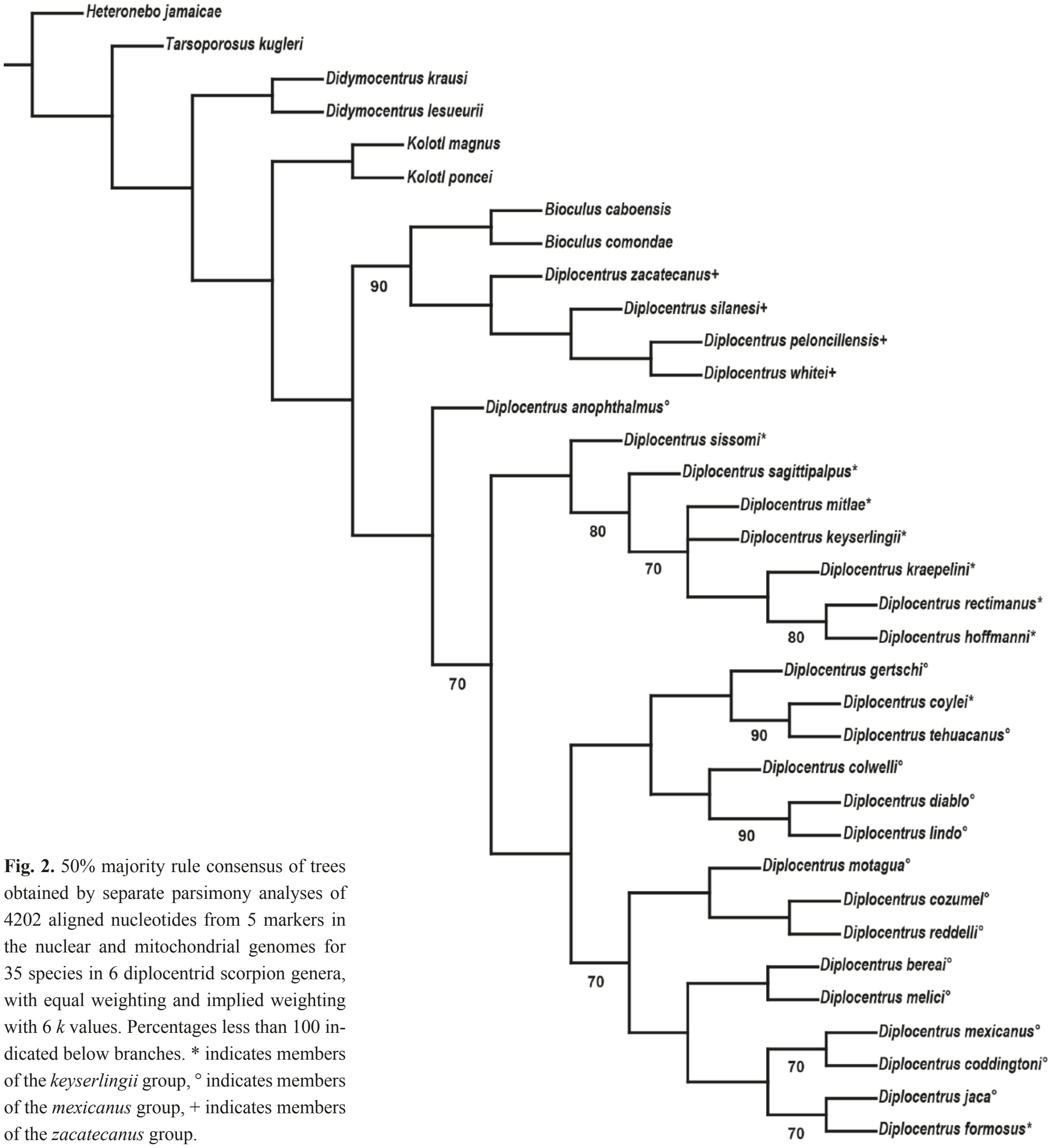

Fig. 2. 50% majority rule consensus of trees obtained by separate parsimony analyses of 4202 aligned nucleotides from 5 markers in the nuclear and mitochondrial genomes for 35 species in 6 diplocentrid scorpion genera, with equal weighting and implied weighting with 6 k values. Percentages less than 100 indicated below branches. * indicates members of the *keyserlingii* group, ° indicates members of the *mexicanus* group, + indicates members of the *zacatecanus* group.

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.

3.3. Molecular Bayesian analyses

Bayesian inference of the nuclear DNA recovered the monophyly of *Bioculus*, *Didymocentrus*, *Diplocentrus*, and *Kolotl* (Fig. 3). Species relationships within *Diplocentrus* were mostly unresolved, and previously recognized species groups were not monophyletic. Bayesian inference of the mitochondrial DNA also recovered the monophyly of *Bioculus*, *Didymocentrus* and *Kolotl*. *Diplocentrus* was rendered paraphyletic by the placement of *Bioculus* sister to the *zacatecanus* group (Fig. 4). The *keyserlingii* and *mexicanus* groups were rendered paraphyletic due to the placement of *D. coylei* and *D. formosus* within the latter. The remaining species 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 neobothriotaxic 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 neobothriotaxic diplocentrid 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 (Santibáñez-López et al. 2014). The two species can be distinguished from all other diplocentrids 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

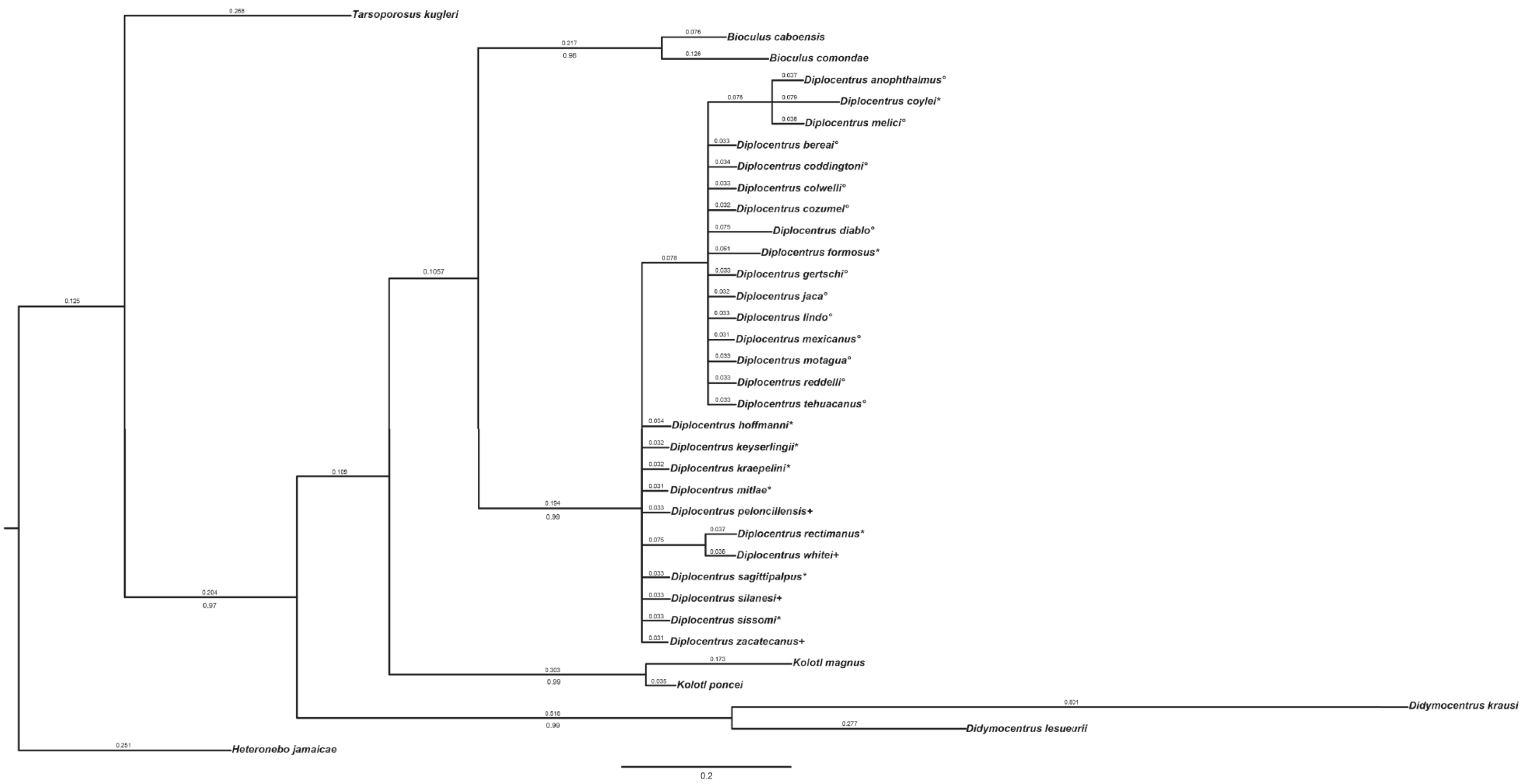

Fig. 3. Phylogram obtained by Bayesian analysis of 2277 aligned nucleotides from 2 markers in the nuclear genome for 35 species in 6 diplocentrid scorpion genera. Branch lengths indicated above branches, posterior probabilities higher than 0.90 indicated below branches. * indicates members of the *keyserlingii* group, ° indicates members of the *mexicanus* group, + indicates members of the *zacatecanus* group.

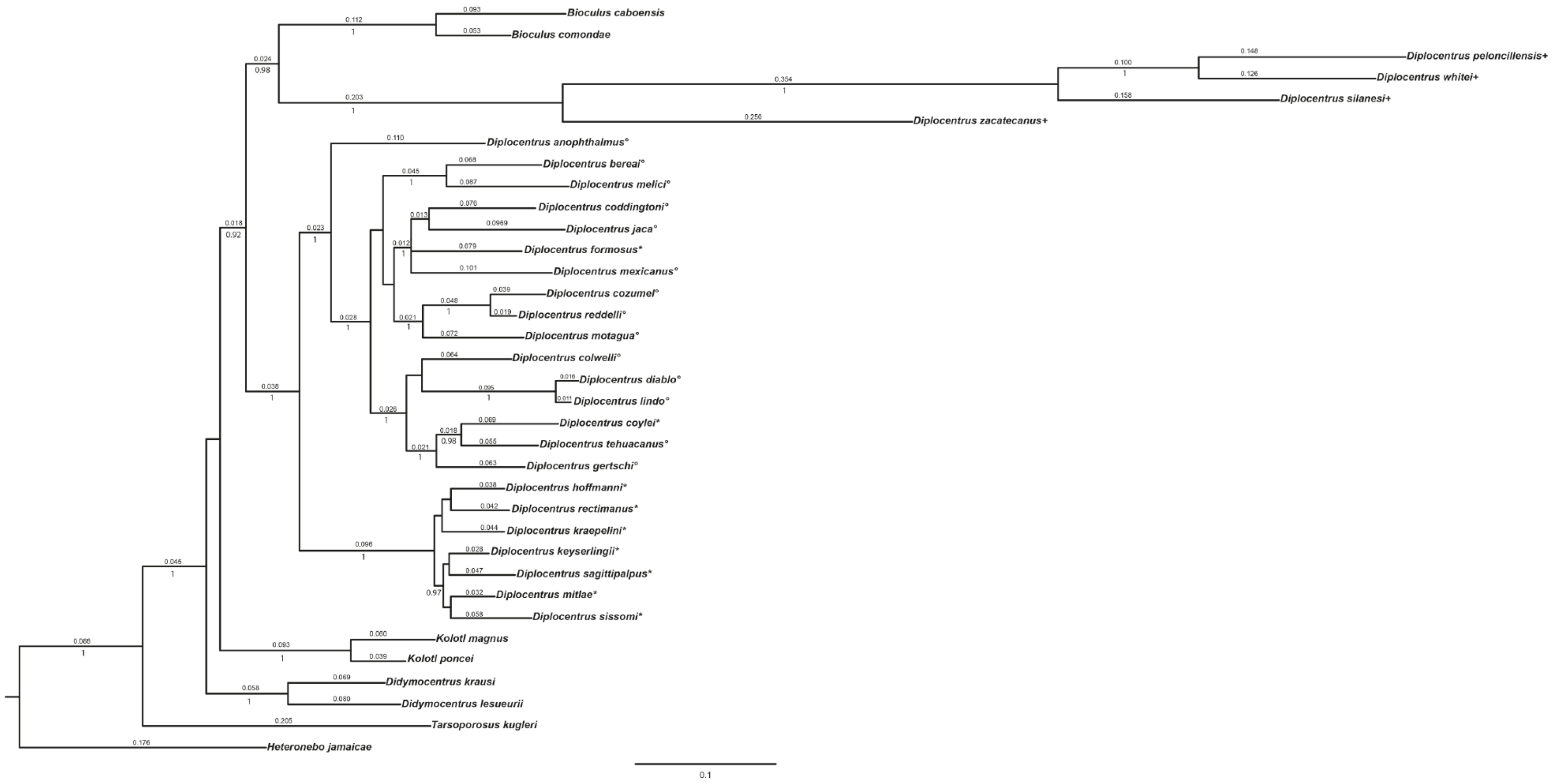

Fig. 4. Phylogram obtained by Bayesian analysis of 1925 aligned nucleotides from 3 markers in the mitochondrial genome for 35 species in 6 diplocentrid scorpion genera. Branch lengths indicated above branches, posterior probabilities higher than 0.90 indicated below branches. * indicates members of the *keyserlingii* group, ° indicates members of the *mexicanus* group, + indicates members of the *zacatecanus* group.

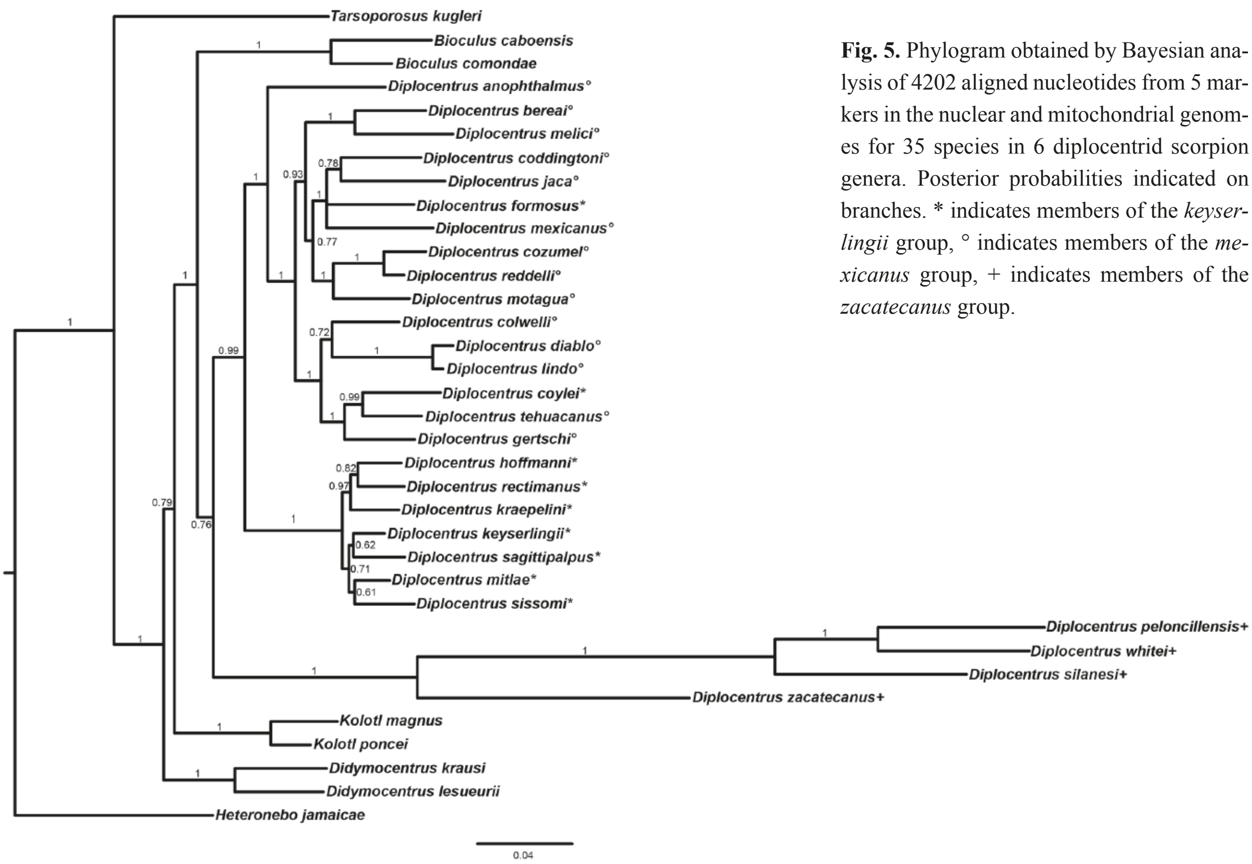

Fig. 5. Phylogram obtained by Bayesian analysis of 4202 aligned nucleotides from 5 markers in the nuclear and mitochondrial genomes for 35 species in 6 diplocentrid scorpion genera. Posterior probabilities indicated on branches. * indicates members of the *keyserlingii* group, ° indicates members of the *mexicanus* group, + indicates members of the *zacatecanus* group.

to the ventral distal denticle. The prolateral, median and retrolateral denticle rows of the pedipalp chela fingers are well defined from the basal quarter to the tip of the finger, and continuous, i.e., not interrupted by larger denticles. The pedipalps are neobothriotaxic, with accessory trichobothria present on the ventral and retrolateral surfaces of the patella. *Kolotl* closely resembles *Didymocentrus* based on the dense punctation of the nongranular pedipalp surfaces, and the similar development of the pedipalp chelal carinae. However, species of *Didymocentrus* differ from *Kolotl* in the presence of a distinct concavity on the prolateral surface of the pedipalp chela in adult males, proximal to trichobothria *ib* and *it*, and in the dentition of the pedipalp chela fingers: the prolateral, median and retrolateral denticle rows are weakly defined in the proximal third, continuous and parallel, not from the base of the finger, but from its proximal third.

The monophyly of the remaining exemplar species of *Diplocentrus* (i.e., excluding the two neobothriotaxic species assigned to *Kolotl* 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 mitochondrial DNA and the parsimony 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-Lopez 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

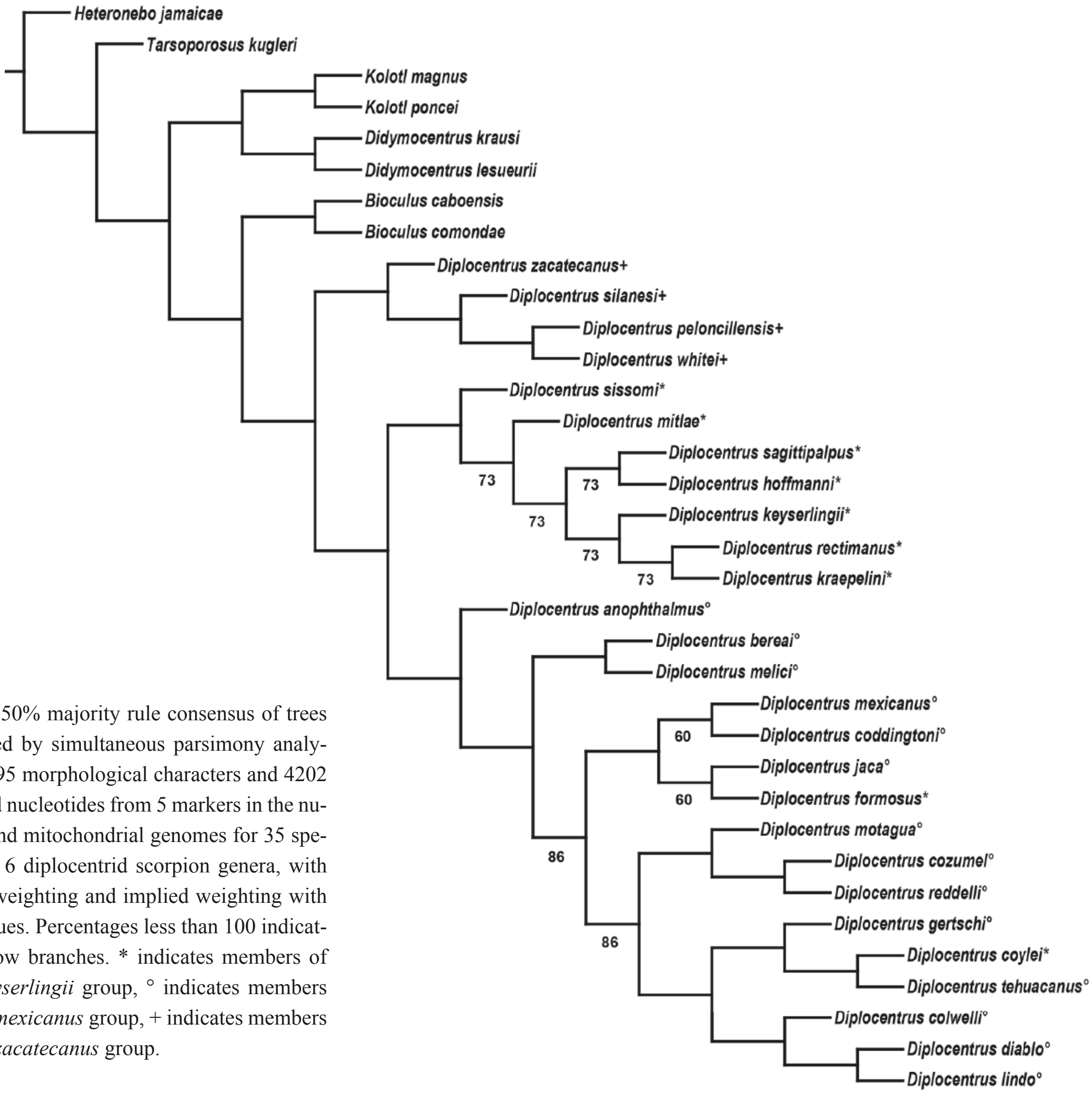

Fig. 6. 50% majority rule consensus of trees obtained by simultaneous parsimony analyses of 95 morphological characters and 4202 aligned nucleotides from 5 markers in the nuclear and mitochondrial genomes for 35 species in 6 diplocentrid scorpion genera, with equal weighting and implied weighting with 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.

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 *keyserlingi* group to the *mexicanus* group, restoring the monophyly of each. *Diplocentrus coylei* and *D. formosus* consistently appeared in different parts of the *mexicanus* group, however: *D. coylei* grouped with *D. tehuacanus*, whereas *D. formosus* grouped with *D. coddingtoni*, *D. jaca*, and *D. mexicanus*.

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 reddelli* Francke, 1977, the only other exemplar species from the Yucatan, in any analysis.

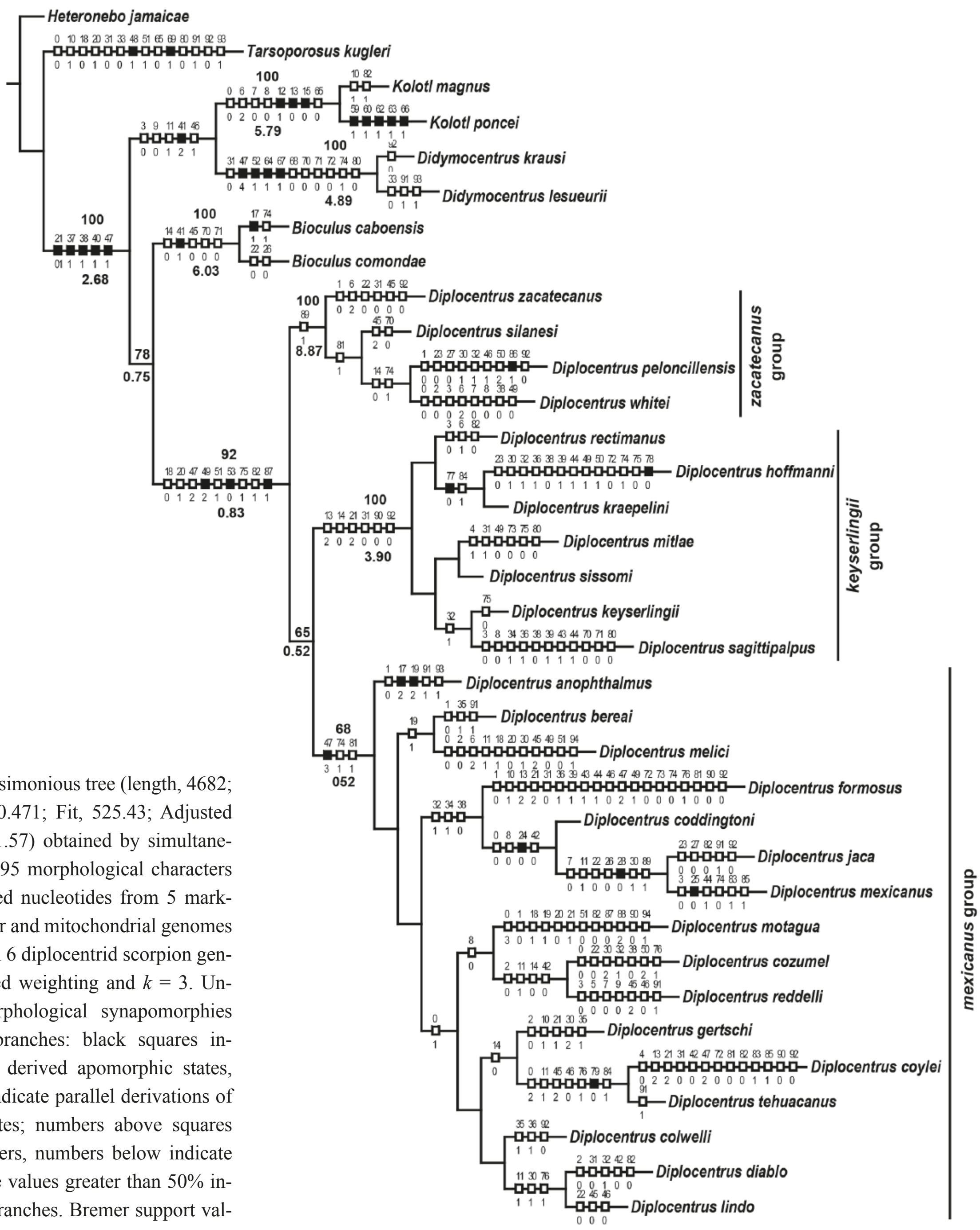

Fig. 7. Most parsimonious tree (length, 4682; CI, 0.322; RI, 0.471; Fit, 525.43; Adjusted Homoplasy, 381.57) obtained by simultaneous analysis of 95 morphological characters and 4202 aligned nucleotides from 5 markers in the nuclear and mitochondrial genomes for 35 species in 6 diplocentrid scorpion genera, with implied weighting and *k* = 3. Unambiguous morphological synapomorphies optimized on branches: black squares indicate uniquely derived apomorphic states, white squares indicate parallel derivations of apomorphic states; numbers above squares indicate characters, numbers below indicate states. Jackknife values greater than 50% indicated above branches. Bremer support values indicated below branches.

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. A group comprising three species restricted to central Mexico, i.e. *D. coylei*, *Diplocentrus gertschi* Sissom & Walker, 1992, and *Diplocentrus tehuacanus* Hoffmann, 1931 was also recovered by these analyses. 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 & Martín-Frías, 2004 and *Diplocentrus melici* Armas, Martín-Frías & 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 Prendini (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 basitarsus 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.

5. Acknowledgements

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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 (CAFBUM), Universidad Michoacana de San Nicolás de Hidalgo; Morelia, Michoacán, Mexico; Colección Aracnológica "Luis de Armas", Instituto Tecnológico del Valle de Oaxaca (CALA), 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 Cryocollection (AMCC) at the AMNH.

Outgroup

Heteronebo Pocock, 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 *Heteronebo* is basal to *Diplocentrus* and the other genera of Diplocentrinae (Prendini 2000), *Heteronebo jamaicae* Francke, 1978, an exemplar species from the Caribbean, was selected as the primary outgroup for analyses presented here. This species, mistakenly synonymized with *Heteronebo scaber* (Pocock, 1893) by Teruel (2009), is hereby revalidated.

1. *Heteronebo jamaicae* Francke, 1978: **JAMAICA**: *St. Andrew's Parish*: Bull Bay, 17°56.508′N 76°40.74′W, 17 m, 29.vii.2005, L. Esposito, hillside, lowland semi-deciduous forest with succulents, UV detection, 1 ♂ (AMCC [LP 5131]); Yallahs, 10 km W, Quarry Lot, 17°55.3908′N 76°38.4288′W, 84 m, 30.vii.2005, L. Esposito, degraded, lowland semi-deciduous forest, UV detection, 1 ♂ (AMCC [LP 5132]). *St. Elizabeth Parish*: Lover's Leap, 1 km E, 17°52.270′N 77°39.114′W, 24.vii.2005, L. Esposito, evergreen drought-deciduous scrubland with succulents, high bluffs, UV detection, 8 ♂, 2 ♀ (AMNH), 1 juv. (AMCC [LP 5133]).

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

2. *Tarsoporosus kugleri* (Schenkel, 1932): **VENEZUELA**: don. E. Ythier, xi.2005, 1 ♂ (AMCC [LP 5204]).

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 *Diplocentrus*, rendering the latter paraphyletic. The genus was represented in the analyses by the type species and a second species from Baja California.

3. *Bioculus caboensis* (Stahnke, 1968): **MEXICO**: *Baja California Sur*: Municipio de La Paz: S of Todos los Santos, 23°25.793′N 110°11.602′W, 81 m, 10.vii.2004, O. Francke, W.E. Savary, E. González & A. Valdez, 1 ♂ (AMCC [LP 3125]). Municipio de Los Cabos: Cabo San Lucas, 15 miles E [23°01.359′N 109°43.491′W], 12.vi.2000, M.E. Soleglad, 1 ♀ (AMCC [LP 1796]); San Bartolo, 6 km SW, 23°41.816′N 109°50.8′W, 100 m, 13.vii.2008, E. González & H. Montaño, 1 ♂, 3 juv. (AMNH), 1 juv. (AMCC [LP 8844]); Santiago, 23°26.408′N 109°43.577′W, 225 m, 9.vii.2004, O. Francke, E. González & A. Valdez, 1 ♂, 1 ♀ (AMCC [LP 3124]). Municipio de San Jose del Cabo: 22°56.143′N 109°48.757′W, 21 m, 1.ii.2005, O. Francke & H. Carmona, 1 ♀ (AMCC [LP 4749]).

4. *Bioculus comondae* Stahnke, 1968: **MEXICO**: *Baja California Sur*: Municipio de La Paz: Isla Espiritu Santo, Playa Bonanza 24°27.364′N 110°18.471′W, 0–50 m, 31.v.2008. I.G. Nieto & E. González, 1 juv. (AMCC [LP 8683]); Las Cruces, 4 km NW, 24°13.511′N 110°07.410′W, 31 m, 24.vi.2008, E. González & H. Montaño, 1 ♂ (AMCC [LP 8679]); La Paz, ca. 10 km SE on BCS 286 to San Juan de los Planes, 24°08.433′N 110°15.333′W, 106 m, 9.vii.2005, L. Prendini & R. Mercurio, 1 ♂ (AMCC [LP 4731]); La Paz, 18 km SE, 24°02.765′N 110°08.865′W, 625 m, 8.vii.2004, O. Francke, E. González & A. Valdez, 1 juv. (AMCC [LP 3123]); Libramiento to Pichilingue marker km 4, by Termoelectrica Punta Prieta, 24°12.843′N 110°16.214′W, 105 m, 23.vi.2008, M. Correa, I.G. Nieto & E. González, 1 ♀ (AMCC [LP 8680]); Microwave antenna, La Paz to Pichilingue, 24°07.872′N 110°16.987′W, 224 m, 9.vi.2008, I.G. Nieto & E. González, 1 ♀ (AMCC [LP 8686]); San Evaristo, 5 km S, 24°52.901′N 110°41.404′W, 5–50 m, 12.vii.2008, E. González & H. Montaño, 1 ♂ (AMCC [LP 8836]). Municipio de Loreto: Loreto, ca. 8 km S along gravel road to San Javier, from junction with Route 1, 24°13.511′N 110°07.410′W, 31 m, 8.vii.2005, L. Prendini, E. González & W.E. Savary, 2 juv. (AMCC [LP 4738]).

Didymocentrus Kraepelin, 1905: Ten species are currently recognized in this genus. Francke (1978) considered it distinct from *Diplocentrus* 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 *Diplocentrus* 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.

5. *Didymocentrus krausi* Francke, 1978: **NICARAGUA**: *Granada*: Municipio de Granada: Domitila [11°42′N 85°55′W], 55 m, 27.v.2002, J.M. Maes, 1 ♀ (AMCC [LP 1987]). *León*: Municipio de El Jicaral: San Rafael, road to Matagalpa, 12°40′35″N 86°25′37″W, 110 m, 29.xi.2007, C. Viquez & J. Mata, on slopes, 1 juv. ♀ (AMCC [LP 8590]). Municipio de La Paz Centro: Volcán Momotombo, El Cardón, 12°24′57″N 86°29′16.8″W, 55 m, 2.xii.2007, C. Viquez & J. Mata, volcanic rock outcrop in forest, 1 juv. ♀ (AMCC [LP 8591]). Municipio de Nagarote: Carretera vieja a León, 12°09′41″N 86°39′35″W, 58 m, 24.xi.2007, C. Viquez & J. Mata, in stockbreeding ranch near road, 1 juv. ♂ (AMCC [LP 8589]).

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

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

7. *Kolotl magnus* (Beutelspacher & López-Forment, 1991): **MEXICO**: *Guerrero*: Municipio de Acapulco de Juárez: Cumbres de Llano Largo, 16°49.505′N 99°49.999′W, 371 m, 19.vi.2006, O. Francke, H. Montaño & A. Ballesteros, 3 juv. (CNAN), 1 juv. (AMCC [LP 7029]); Puerto Marquéz [16°47.689′N 99°49.239′W], W. López-Forment, 1 juv. (CNAN-S00712); Puerto Marquéz, 2 km W [16°48.984′N 99°50.780′W], 28.v.1974, 1 ♀ (CNAN-S00710), 8.vii.1974, W.J. Mautz, at entrance of crevice in granite boulder, holotype ♀ (CNAN-T0122), juv. ♂ paratype (CNAN-T0123), 10.vii.1974, W. López-Forment, 1 ♀ (CNAN-S00713); Puerto Marquéz, 4 km N [16°49.717′N 99°49.255′W], 5.vii.1975, W. López-Forment, 2 juv. (CNAN-S00714); Puerto Marquéz, 5 km W [16°49.400′N 99°51.594′W], 21.vi.1985, W. López-Forment, 1 ♀ (CNAN-S00711).

8. *Kolotl poncei* (Francke & Quijano-Ravell, 2009): **MEXICO**: *Michoacan*: Municipio de La Huacana: El Vado, km 17 road Zicuiran to Churumuco, 18°48.908′N 101°54.976′W, 20.v.2007, O. Francke, J. Ponce, A. Quijano, M. Villaseñor & A. Ballesteros, holotype ♂, 1 ♂, 3 ♀ (1 with 24 offspring), 4 juv. ♂, 4 juv. ♀ paratypes (CNAN-T0392), 1 juv. (AMCC [LP 7030]), 1.xi.2007, J. Ponce, A. Quijano & M. Villaseñor, 1 ♂ (CAFBUM), 30.vi.2008, O. Francke, J. Ponce, A. Quijano & H. Montaño, 1 ♂, 7 juv ♂, 3 ♀ juv. (CNAN), 1 ♂, 1 ♀ (AMNH).

Diplocentrus Peters, 1861: This genus presently comprises 59 species, although several may eventually be synonymized. Prendini (2000) recovered this genus as paraphyletic with respect to *Didymocentrus*. Two groups were recognized by Santibáñez-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 the type species of the genus, *Diplocentrus mexicanus* Peters, 1861.

9. *Diplocentrus anophthalmus* Francke, 1978: **MEXICO**: *Yucatán*: Municipio de Opichen: Actun Chukum [20°33.070′N 89°54.724′W, 93 m], 29.xi.1974, J. Reddell, D. McKenzie, S. Wiley & R.W. Mitchell, holotype ♀ (AMNH); Actun Xpukil, 20°33.070′N 89°54.724′W, 93 m, iii. 2010, M. Paradiz, 1 subad. ♀ (CNAN), 1 subad. ♀ [leg] (AMCC [LP 10980]), cave, under stone.

10. *Diplocentrus bereai* Armas & Martín-Frías, 2004: **MEXICO**: *Veracruz*: Municipio de Actopan: 19°30′N 96°36′W, 5.iii.2003, P. Berea, 2 ♀ (CNAN-S03098), 28.iii.2004, P. Berea, 1 ♂ (CNAN-S03184), 23.iv.2003, P. Berea, 1 ♀ (CNAN-S03074), 24.v.2003, P. Berea, holotype ♂ (CNAN); Buenavista [19°30′N 96°36′W], 15.v.2005, P. Berea, 4 ♀ (CNAN-S03187); El Conejito, road to Jibaro 19°26.197′N 96°36.470′W, 291 m, 6.v.2006, O. Francke, P. Berea & A. Ballesteros, 1 ♂, 1 ♀ (CNAN-S03073), 1 juv. (AMCC [LP 6532]); Los Ídolos, 19°24.154′N 96°31.091′W, 1.x.2003, P. Berea, 1 ♂ (CNAN-S03188), 18.vi.2004, P. Berea, 1 ♂ (CNAN-S03189); Paso de Milpa, 19°26.237′N 96°36.490′W, 280 m, 2.iii.2004, P. Berea, 1 ♂ (CNAN-S03190), 1 ♀ (AMCC [LP 6369]).

11. *Diplocentrus coddingtoni* Stockwell, 1988: **HONDURAS**: *Departamento Atlantida*: La Ceiba [15°41.37′N 86°54.204′W], 1920, W.L. Mann, holotype ♂, 4 ♀ paratypes; La Ceiba, Pico Bonito Lodge, property with cacao close to lodge, 15°41.368′N 86°54.206′W, 124 m, 2.x. 2008, C. Viquez & M. Branstetter, 1 juv. ♀ (AMCC [LP 9169]); Isla Corlum Grande, 2.xii.1997, 1 ♀ (CNAN-S03003); Pico Bonito Natural Park, La Ceiba, 15°37′14″N 86°51′59″W, 28.viii.2013, C. Santibáñez, S. Longhorn, K. Salastume & V. Henriquez, 1 ♂, 1 ♀, 2 subad. ♀, 2 juv. (AMNH).

12. *Diplocentrus colwelli* Sissom & Fritts, 1996: **MEXICO**: *Nuevo León*: Municipio de Aramberri: San Juanito de Jésus, 24°26.25′N 99°57.15′W, 2319 m, 26.vii.2006, O. Francke, W.D. Sissom, B. Hendrixson, K. McWest, S. Grant, C. Durán, A. Jaimes, M. Córdova & A. Ballesteros, 1 ♂ (CNAN), 1 ♂, 1 ♀ (AMNH), 1 ex. [leg] (AMCC [LP 6604]). Municipio de Santa Catarina: Cañon de la Huasteca, 25°36.874′N 100°28.531′W, 728 m, 24.vii.2006, O. Francke, W.D. Sissom, B. Hendrixson, K. McWest, S. Grant, C. Durán, A. Jaimes, M. Córdova & A. Ballesteros, 25 ♂, 3 ♀, 3 juv. (AMNH), 25 ♂, 3 ♀, 2 juv. (CNAN-Sc1538), 13 ♂ (CAS), 1 ♀ (AMCC [LP 6483]); La Huasteca Recreation Park, Santa Catarina Mountains, Monterrey, 723 m, 10.vi.1996, R.C. West, 1 ♂ (AMCC [LP 6218]).

13. *Diplocentrus coylei* Sissom & Fritts, 1996: **MEXICO**: *Estado de México*: Municipio de Malinalco: Chichicasco, 18°51.369′N 99°28.123′W, 1374 m, 25.vii.2002, E. González, 1 ♂, 2 subad. ♂ (AMCC [LP 2236]). Municipio de Tonatico: Tonatico, outside Estrella cave, near viewpoint, 18°44.740′N 99°37.821′W, 1594 m, 15.ix.2011, J. Mendoza, R. Monjaraz, D. Barrales & F. Torres, 2 ♀ (CNAN), 1 ♀ [leg] (AMCC [LP 11474]). *Guerrero*: Municipio de Buenavista de Cuellar: Casino de la Unión, 2 km S, 18°35.53′N 99°28.91′W, 1178 m, 28.viii.2009, O. Francke, T. López, C. Santibáñez & A. Valdez, 2 ♀, 6 juv. (CNAN), 1 juv. [leg] (AMCC [LP 11051]), 1 juv. [leg] (AMCC [LP 11053]). Municipio de Iguala de la Independencia: Iguala de la Independencia [18°21′N 99°33.6′W], vi.1961, 1 ♀ (CNAN); La Cumbre, 18°24.031′N 99°29.120′W, 1212 m, 28.viii.2009, O. Francke, C. Santibáñez & E. Miranda, thorn forest, on the ground, UV light, 1 juv. (CNAN), 1 juv. [leg] (AMCC [LP 11033]), 1 juv. [leg] (AMCC [LP 11050]). Municipio de Ixcateopán de Cuahutemoc: Ixcateopán, 2 km S, 18°30.230′N 99°46.656′W, 1930 m, 21.iv.2012, J. Mendoza, R. Monjaraz, D. Ortiz & G. Contreras, 1 ♀ (CNAN), 1 ♀ [leg] (AMCC [LP 11480]). Municipio de Picaya: Cacahuamilpa [18°24.6′N 99°20.4′W], 1520 m, 11.viii.1984, R. Ríos, 1 ♂, 1 ♀, 2 juv. (CNAN), 15.vii.2001, M. Córdova & A. Burgos, 1 ♀ (CNAN); Gruta de Cacahuamilpa, 8.vi.1982, F. Coyle, 1 juv. paratype (AMNH); Cacahuamilpa, 4 mi. W, summit [18°41′N 99°34′W], 3.ix.1966, J. & W. Ivie, 1 ♂, 1 ♀, 1 juv. paratypes (AMNH). Municipio de Taxco de Alarcon: Las Granadas [18°57′N 99°51′W], 12.vii.1980, E. Martin & R. Garcia, 1 ♂, 1 ♀ paratypes (AMNH). Municipio de Tetipac: Dos Bocas [18°39.6′N 99°30.6′W], vi.1946, 1 ♂, 1 ♀ (CNAN); Iguala 18°21′N 99°33.6′W, vi.1961, 1 ♀ (CNAN). *Morelos*: Municipio de Amacuzac: Huajintlán, 18°36.6′N 99°25.8′W, 1510 m, 4.ix.2004, M. Córdova & A. Gotilla, 1 ♀ (CNAN). Municipio de Coatlán del Rio: El Oyanco, 18°43.8′N 99°25.8′W, 1022 m, 6.viii.2004, M. Córdova & O. Sotelo, 1 ♀ (CNAN-S03005). Municipio de Miacatlán: Palpan, 18°51′N 99°25.2′W, 1587 m, 7.viii.2004, M. Córdova & O. Vázquez, 1 ♂, 4 ♀, 10 juv. (CNAN). Municipio de Tlaquiltenango: El Comal, border between Morelos & Guerrero, 18°27.086′N 99°17.139′W, 1749 m, 13.vi.2007, O. Francke, J. Ponce, M. Córdova, H. Montaño, L. Beltrán & A. Ballesteros, 6 ♂, 4 ♀, 1 subad. ♂, 2 subad. ♀, 3 juv. (AMNH), 6 ♂, 4 ♀, 1 subad. ♂, 3 subad. ♀, 3 juv. (CNAN), 1 ♂, 2 ♀, 2 juv. (CAS), 1 subad. ♂ (AMCC [LP 7031]).

14. *Diplocentrus cozumel* Armas & Beutelspacher, 1998: **MEXICO**: *Quintana Roo*: Municipio de Benito Juárez: Cancun, Hotel Moon Palace, 20°59′17.0″N 86°50′06.4″W, 25 m, 26.iv.2005, H. Carmona, 1 leg (AMCC [LP 4102]); Cozumel, 28.xii.1959, 3 ♂, 1 ♀, 2 juv. (CNAN-Sc1542), 12.xii.1990, C. Beutelspacher, 2 ♀, 3 subad. ♂, 2 subad. ♀, 3 juv. (CNAN-Sc1543); Cozumel Island, San Gervancio, Mayan ruins, 17.iii.2004, J. Huff, 1 ♂, 1 ♀, 4 juv. (AMCC [LP 2672–2676]).

15. *Diplocentrus diablo* Stockwell & Nilsson, 2001: **U.S.A.**: *Texas*: Starr County: Santa Cruz, 18.viii.1985, J.A. Nilsson, holotype ♂ (AMNH); Rio Grande City, 4.vi.1934, S.D. Mulaik, 1 ♂, 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])

16. ***Diplocentrus formosus*** Armas & Martín-Frías, 2003: **MEXICO**: *Oaxaca*: Distrito de Tehuantepec: Municipio de Asunción Ixtaltepec: Chivela [16°42.813′N 94°59.827′W], 210 m, 30.v.1962, J. Martinez, 1 ♂, 1 ♀ (CNAN); Nizanda, 16°39.4902′N 95°00.6342′W, 99 m, 15.ix.2009, R. Paredes, C. Santibáñez & A. Valdez, deciduous forest, in burrow entrance, UV light detection, 9 ♀ (CNAN), 1 ♀ [leg] (AMCC [LP 10979]); Santo Domingo Tehuantepec [16°19.650′N 95°17.273′W], 80 m, 9.vii.2004, P. Berea, 4 ♂, 11 ♀ (CNAN).

17. ***Diplocentrus gertschi*** Sissom & Walker, 1992: **MEXICO**: *Nayarit*: Municipio de Tepic: Jésus María Corte, 4 km N, 2 km along gravel road E of main paved road, ca. 30 km N Tepic, 21°45.203′N 104°51.213′W, 126 m, 29.vii.2005, E. González & R. Mercurio, 1 ♀ (AMCC [LP 4707]). Municipio de Tuxpam: Microwave tower Peñitas, ca. 10 km E Tuxpan, 21°55.843′N 105°12.805′W, 230 m, 31.vii.2005, E. González & R. Mercurio, 2 ♂, 1 ♀ (AMNH), 1 ♂ (AMCC [LP 4706]).

18. ***Diplocentrus hoffmanni*** Francke, 1977: **MEXICO**: *Oaxaca*: Distrito de Etla: Municipio de San Francisco Telixtlahuaca: Telixtlahuaca, 6 mi. N [17°17.667′N 96°54.25′W], 7050 ft, 26.vii.1966, C.M. Bogert, under rocks in moderately dry area with oak and juniper, paratype ♂ (AMNH); Telixtlahuaca, 6 km N, 17°20.367′N 96°56.121′W, 1915 m, 22.vii.2002, O. Francke, E. González & J. Ponce, SE slope, dry scrub, 1 ♂, 1 ♀ (AMCC [LP 2036]), 1 ♂ (CNAN). Municipio de Santiago Tenango: near Tejocote [17°14′N 97°00′W], 7800 ft, summer 1963, C.M. Bogert, under logs, holotype ♂, paratype ♀ (AMNH); Tejocote, 31 mi. NW Oaxaca city, 7600 ft, 9.ix.1962, M.R. Bogert, paratype ♂ (AMNH). Distrito de Tlacolula: Municipio de San Pablo Mitla: Mitla, 15 km E, 16°56.606′N 96°17.114′W, 2081 m, 18.vii.2007, O. Francke, H. Montaño, A. Valdez, A. Ballesteros & C. Santibáñez, pine-oak woodland, 1 ♂ (CNAN), 1 subad. ♂ (AMCC [LP 7615]). Distrito de Zimatlán: Municipio de Magdalena Mixtepec: Magdalena Mixtepec, 16°52.824′N 96°51.056′W, 1710 m, 19–20.vi.2006, O. Francke, G. Villegas, H. Montaño, C. Santibáñez & A. Valdez, 1 ♂, 4 ♀, 4 subad. ♀, 2 juv. (AMNH), 2 ♂, 3 ♀, 4 subad. ♀, 2 juv. (CNAN), 2 ♀ (AMCC [LP 6599]).

19. ***Diplocentrus jaca*** Armas & Martín-Frías, 2000: **MEXICO**: *Oaxaca*: Distrito de Tuxtepec: Municipio de San Jose Rio Manso: Cerro Chango, 6.iii.2009, J. Cruz, holotype ♂ (CNAN), 2 ♀ (CNAN-S03484). Municipio de Santa María Jacatepec: Jacatepec, 21.vi.1977, M. Varela; Vega de Sol, 17°48.759′N 96°12.913′W, 46 m, 5.vi.2008, O. Francke, R. Botero, C. Santibáñez & A. Valdez, 1 ♂, 4 ♀ (CNAN-S03486), 1 ex. [leg] (AMCC [LP 9518]).

20. ***Diplocentrus keyserlingii*** Pocock, 1889: **MEXICO**: *Oaxaca*: Distrito de Etla: Municipio de Santa María Peñoles: Santa Catarina Estetla, 17°01.6′N 97°05.766′W, 20.ix.2007, P. Lara, 1 ♂ (CALA). Municipio de Santiago Tenango: near Tejocote [17°14′N 97°00′W], 7800 ft, summer 1963, C.M. Bogert, under logs, holotype ♂, paratype ♂ [*Diplocentrus reticulatus* Francke, 1977] (AMNH). Distrito de Ixtlán de Juárez: Municipio de Santa Catarina Ixtepeji: El Punto, road to Ixtlán de Juárez [17°13.30′N 96°35.03′W], 19.viii.1961, C.M. & M.R. Bogert, paratype ♂ (AMNH). Distrito de Zaachila: Municipio de Santa Inés del Monte: Santa Inés del Monte, 16°56.442′N 96°51.629′W, 2270 m, 19.ix.2009, R. Paredes-León, C. Santibáñez, A. Valdez & J. Cruz, 1 ♂, 5 juv. (CNAN), 1 juv. [leg] (AMCC [LP 11052]); Santa Inés del Monte, 3 km E, 16°56.445′N 96°51.6312′W, 2665 m, 12.xii.2005, O. Francke, H. Montaño, C. Santibáñez & A. Valdez, 1 ♂, 1 ♀ (CNAN), 2 ♀ (AMNH), 1 ♀ (AMCC [LP 6517]).

21. ***Diplocentrus kraepelini*** Santibáñez-López, Francke & Prendini, 2013: **MEXICO**: *Oaxaca*: Distrito de Coixtlahuaca: Municipio de San Cristóbal Suchixtlahuaca: Km 2 road San Cristóbal Suchixtlahuaca to Santiago Tejupan, 17°42.240′N 97°23.667′W, 2290 m, 28.vi.2006, O. Francke, G. Villegas, H. Montaño & A. Valdez, holotype ♂ (CNAN-T0671), 2 ♂, 3 ♀, 2 subad. ♀, 2 juv. paratypes (AMNH), 1 ♂, 3 ♀, 1 subad. ♂, 2 juv. paratypes (CNAN-T0672), 1 ♀ paratypes (AMCC [LP 6426]); Suchixtlahuaca, 8 km NE, 17°42.124′N 97°23.776′W, 2030 m, 25.iii.2010, O. Francke, A. Valdez, C. Santibáñez & J. Cruz, oak forest, under rock, daytime rock rolling, 1 subad. ♀ paratype (CNAN), 1 subad. ♀ paratype [leg] (AMCC [LP 10973]). Distrito de Teposcolula: Municipio de San Bartolo Soyaltepec: Caballo Blanco [17°35.432′N 97°18.414′W], 12.vii.1963, G. Sludder, under logs, 1 ♂ (AMNH).

22. ***Diplocentrus lindo*** Stockwell & Baldwin, 2001: **U.S.A.**: *Texas*: Jeff Davis County: Davis Mountains State Park, 20.vi.1970, M.A. Cazier, L. Draper, O.F. Francke, 1 ♂, 1 ♀ paratypes (AMNH). Terrell County: Sanderson, 1.viii.2004, B.R. Tomberlin & S.J. Burchfield, 1 ♂ (AMCC [LP 3078]).

23. ***Diplocentrus melici*** Armas, Martín-Frías & Berea, 2004: **MEXICO**: *Veracruz*: Municipio de Actopan: Los Ídolos, 19°24.517′N 96°31.0′W, 6.iii.2004, P. Berea, 1 juv. ♀ (CNAN-S03265), 18.vi.2004, P. Berea, holotype ♂ (CNAN), 1.v.2006, P. Berea, 1 ♂ (AMCC [LP 6380]), 1.viii.2006, P. Berea, 1 ♀ (CNAN-S03538), 4 ♂ 36 ♀, 4 juv. (CNAN-S03191), 5.iv.2006, O. Francke, P. Berea & A. Ballesteros, 23 ♀, 12 subad. ♀, 10 juv. (CNAN-Sc1595), 4 subad. ♂ (CNAN-Sc1593), 1 juv. (AMCC [LP 6546]).

24. ***Diplocentrus mexicanus*** Peters, 1861: **MEXICO**: *Oaxaca*: Distrito de Etla: Municipio de Santiago Nacaltepec: El Moral, 2 mi. N, neotype ♂, 1 ♀ (AMNH). Distrito de Ixtlán de Juárez: Municipio de Ixtlán de Juárez: Guelatao, Hydroelectric plant, Xia, 28.iv.1960, 1 ♀ (CNAN-Sc1578); Xía, Guelatao, 1.vii.1962, 2 ♀ (CNAN-Sc1573); Km 171 road 175 Tuxtepec to Ixtlán de Juárez 17°17.835′N 96°32.577′W, 2006 m, 14.vi.2007, C. Santibáñez & A. Valdez, 2 ♂, 1 ♀, 1 subad. ♀, 1 juv. (CNAN-Sc1582), 16.iii.2008, C. Santibáñez, A. Valdez & H. Montaño, 1 ♀ (CNAN-Sc1577). Municipio de San Juan Atepec: San Juan Atepec, 2 km NW, 17°25.76′N 96°33.31′W, 1849 m, O. Francke, C. Santibáñez & A. Quijano, 1 ♀, 2 juv. ♂ (CNAN-Sc1579). Municipio de San Pablo Macuiltinguis: Campamento recreativo de San Pablo Macuiltinguis, 17°32.981′N 96°34.478′W, 1983 m, 23.vii.2007, O. Francke, C. Santibáñez, H. Montano, A. Valdez & A. Ballesteros, 1 ♂, 2 ♀ (CNAN-Sc1575), 1 ♂, 3 ♀, 1 subad. ♂ (CNAN-Sc1581), 1 juv. (AMCC [LP 7674]).

25. ***Diplocentrus mitlae*** Francke, 1977: **MEXICO**: *Oaxaca*: Distrito de Tlacolula: Municipio de San Pablo Villa de Mitla: Mitla, 6 mi. N [16°55.252′N 96°21.997′W], 1889 m, 1.ix.1962, M.R. Bogert, holotype ♂ (AMNH); San José del Paso, 1 km N, 16°55.935′N 96°17.220′W, 1880 m, 17.i.2007, C. Santibáñez & N. Gómez, 2 ♀, 1 juv. (CNAN); San José del Paso, 2 km N, 16°55.735′N 96°17.867′W, 1937 m, 15.ix.2009, R. Paredes, C. Santibáñez & A. Valdez, 2 ♂, 2 juv. (CNAN), 1 subad. ♂ [leg] (AMCC [LP 11034]); San Juan, 2 km E, 14.ix.2009, A. Valdez, C. Santibañez & R. Paredes, 1 ♂ [leg] (AMCC [LP 11465]).

26. *Diplocentrus motagua* Armas & Trujillo, 2009: **GUATEMALA**: *Departamento Zacapa*: Municipio de Rio Hondo: Aldea Casas de Pinto, 15°01.403′N 89°36.82′W, 195 m, 26.vi.2008, R. Trujillo & C. Avila, holotype ♂ (MHN); Aldea Casas de Pinto, near turn off for Zacapa at Rio Hondo, 15°01.618′N 89°36.953′W, 77 m, 13.vii.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]).

27. *Diplocentrus peloncillensis* Francke, 1975: **U.S.A.**: *New Mexico*: Hidalgo County: Geronimo Pass, Peloncillo Mountains, Coronado National Forest, 31°30.885′N 109°01.510′W, 1754 m, 13–14.ix.2002. L. Prendini, L. Esposito & Y. Rodriguez, oak-pine scrub on rocky hills; collected at night with UV and during day by turning stones, 1 ♂, 1 ♀ (AMNH), 1 juv. (AMCC [LP 2132]), 3 juv. (AMCC [LP 2139B]), 1 juv. (AMCC [LP 2140A]).

28. *Diplocentrus rectimanus* Pocock, 1899: **MEXICO**: *Oaxaca*: Distrito Centro: Municipio de Oaxaca de Juárez: Ejido Guadalupe Victoria, 17°04.006′N 96°43.20′W, 1700 m, 12.vii.2009, J. Cruz, 1 ♂ (CNAN); Oaxaca City, 19.vi.1947, B. Malkin, 1 ♀ (AMNH). Municipio de Santa Cruz Xoxocotlán: Monte Alban [17°02.639′N 96°46.048′W], 30.i.1940, 3 ♀, 1 ♂ (CNAN). Municipio de Soledad Etla: San Gabriel, 7 km N [17°13.65′N 96°44.697′W], 2125 m, 22.vii.2002, O. Francke, E. González & J. Ponce, SW facing slopes with oaks, in burrows in open ground, 2 ♂, 3 ♀ (AMCC [LP 2032]), 1 ♂, 2 ♀ (CNAN); San Miguel Etla, 9.3 km N (road to las Guacamayas), 17°13.438′N 96°44.301′W, 2196 m, 15.x.2005, O. Francke, M. Córdova, A. Jaimes, G. Montiel & C. Santibáñez, 5 ♀ (AMNH), 1 ♀, 2 juv. (AMCC [LP 6540]); San Miguel Etla, 9 km N, 17°13.486′N 96°44.315′W, 2197 m, 26.iii.2010, O. Francke, J. Cruz, C. Santibáñez & A. Valdez, pine forest, under rocks, daytime rock rolling, 1 ♂, 1 ♀ (CNAN), 1 ♀ [leg] (AMCC [LP 11036]). Distrito de Tlacolula: Municipio de San Lorenzo Albarradas: Cerro Guirone, N slope [16°54.383′N 96°16.567′W], 7200–7500 ft, 12.vi.1970, M.R. Bogert, 1 ♀ (AMNH); Mitla, 4.5 km E, 17°15.642′N 96°32.427′W, 23.vii.2002, J. Ponce, in burrow under stone, 1 ♀ (AMCC [LP 2030]). Municipio de Santiago Matatlan: San Pablo Guila, 16°48.1′N 96°26.4′W, S. Luna, 1 ♂ (CALA).

29. *Diplocentrus reddelli* Francke, 1978: **MEXICO**: *Campeche*: Municipio de Calakmul: Calakmul, Reserva Ejidal Ley de Fomento Agropecuario, 17°59.226′N 89°24.907′W, 234 m, 7.vii.2011, G. Montiel, G. Contreras, H. Montaño, R. Paredes & A. Valdez, 1 ♀, 1 subad. ♀ (CNAN-Sc1638). Calakmul, Yaax'Che camping site, 18°29.227′N 89°53.953′W, 199 m, 13.vii.2010, O. Francke, G. Montiel, C. Santibáñez, D. Barrales, G. Contreras & J. Cruz, 9 ♂, 1 ♀ (CNAN-Sc1642). *Quintana Roo*: Municipio de Benito Juárez, road 307 km 304, Hotel Moon Palau, golf camp, 20°59.326′N 86°49.923′W, 25 m, 30.i.2004, H. Carmona, 1 ♀ (CNAN-Sc1637), 22.vi.2005, H. Carmona, 1 ♀ (CNAN-Sc1633). Municipio de Chetumal: ECOSUR, campus Chetumal, 18°32.660′N 88°15.844′W, 11 m, 16.vii.2010, O. Francke, C. Santibáñez, D. Barrales, G. Contreras, J. Cruz, 2 ♂, 6 ♀, 4 juv. (CNAN), 1 ♀ [leg] (AMCC [LP 11460]); Puerto Morelos, Botanical Garden "Alfredo Barrera Martín", 20°50.702′N 86°54.214′W, 38 m, 4.vii.2007, R. Paredes, 1 ♂, 1 juv. (CNAN-Sc1634), 20.vii.2010, O. Francke, G. Montiel, C. Santibañez, J. Cruz, G. Contreras & D. Barrales, 1 ♀ [leg] (AMCC [LP 10982]), 1 juv. [leg] (AMCC [LP 11463]). Municipio de Felipe Carrilo Puerto: Felipe Carrilo Puerto, 28.ix.1965, V. Molina, 1 ♂ (CNAN-Sc1641); Reserva Natural Sian Ka'an, 19°43.343′N 97°48.72′W, 18 m, 22.vii.2010, O. Francke, E. Francke, G. Montiel, C. Santibáñez, D. Barrales, G. Contreras, J. Cruz & M. Paradiz, 1 ♂ (CNAN-Sc1643), 2 juv. (AMCC [LP 11461]); Km 95, 1 km E Chunhuhub, 19°34.192′N 88°35.587′W, 36 m, 16.vii.2010, O. Francke, E. Francke, G. Montiel, C. Santibáñez, D. Barrales, G. Contreras, J. Cruz & M. Paradiz, 1 juv. ♀ (CNAN-Sc1639), 1 juv. ♀ [leg] (AMCC [LP 11464]). *Yucatán*: Municipio de Abalá: Cenote Yak Ha, Cacao, 20°40.365′N 89°13.173′W, 23 m, 18.vii.2010, O. Francke, E. Francke, G. Montiel, C. Santibáñez, D. Barrales, G. Contreras, J. Cruz & M. Paradiz, 1 ♂ (CNAN-Sc1635), 1 ♀ [leg] (AMCC [LP 11459]). Municipio de Hoctún: 3 km W of deviation to Izmal-Hoctun on Mexico Route 180, 20°52.807′N 89°13.173′W, 23 m, 19.vii.2010, O. Francke, E. Francke, G. Montiel, C. Santibáñez, D. Barrales, G. Contreras, J. Cruz & M. Paradiz, 2 ♀ (CNAN-Sc1636), 1 subad. ♀ [leg] (AMCC [LP 11462]). Municipio de Opichen: Actun Xpukil [20°33.070′N 89°54.724′W, 93 m], 3.viii.1973, J. Reddell, holotype ♂ (AMNH), 17.vii.2010, O. Francke, E. Francke, G. Montiel, C. Santibáñez, D. Barrales, G. Contreras, J. Cruz & M. Paradiz, outside cave, 20 ♂, 1 ♀, 5 juv. (CNAN), 1 ♂ [leg] (AMCC [LP 10981]).

30. *Diplocentrus sagittipalpus* Santibáñez-López, Francke & Prendini, 2013. **MEXICO**: *Oaxaca*: Distrito de Ixtlán de Juárez: Municipio de Santa Catarina Ixtepeji: Highway 175, S of Ixtlán, 17°15.642′N 96°32.427′W, 2075 m, 21.vii.2002, L. Prendini, E. González, O. Francke & J. Ponce, in burrows under stones, 2 ♀, 6 juv. paratypes (AMCC [LP 2029]), 7 juv. paratypes (CNAN). Ixtepeji, 8.4 km del Punto, 17°16.059′N 96°35.275′W, 4.vii.2008, O. Francke, A. Quijano & C. Santibáñez, paratype ♀ (CNAN), 1 juv. (AMCC [LP 11466]). El Cumbre, on ridge E Cerro San Felipe, road to Ixtlán de Juárez [17°14.336′N 96°29.486′W], 8000–9000 ft, 28.ix.1961, C. M. & M. R. Bogert, paratype ♂ (AMNH); El Punto, road to Ixtlán de Juárez [17°12.779′N 96°35.176′W], 19.viii.1961, C. M. & M. R. Bogert, 4 ♂ paratypes (AMNH); El Punto, 1–5 mi. NE, road to Ixtlán de Juárez [17°12.779′N 96°35.176′W], 7500 ft, 3.ix.1961, Miller & Bogert, paratype ♂ (AMNH); Ixtlán de Juárez, 2 mi. E [17°19.929′N 96°29.486′W], 7600 ft, 20.vii.1963, G. Sludder, paratype ♂ (AMNH). Municipio de Santa María Jaltianguis: Campamento las Flores, 17°21.036′N 96°31.829′W, 2309 m, 16.vi.2007, C. Santibáñez & A. Valdez, holotype ♂ (CNAN-T0676), 9 ♂, 1 ♀ paratypes (CNAN-T0677), 5 ♂ paratypes (AMNH), 17°21.056′N 96°31.873′W, 2320 m, 22.iv.2010, A. Valdez, C. Santibáñez, J. Cruz & D. Barrales, pine-oak forest, on ground, UV light detection, paratype ♀ (CNAN), paratype ♀ [leg] (AMCC [LP 10975]).

31. *Diplocentrus silanesi* Armas & Martín-Frías, 2000. **MEXICO**: *Estado de México*: Municipio de Tejupilco: Puerto el Rodeo, 900 m, 21.vi.2002, E. González, 1 ♂, 1 subad. ♂, 1 juv. (AMCC [LP 2025]); Tejupilco, 3 km E Pungaracho, 860 m, 16.iii.2002, O. Francke, E. González & S. Reynaud, 2 ♂ (CNAN-Sc1618). *Guerrero*: Teloloapan, Cueva de los 7 salones, 1.5 km NW La Yerbabuena, 18°27.374′N 99°55.38′W, 1724 m, 23.x.2010, A. Valdez, J. Cruz, D. Barrales & G. Contreras, 1 ♂, 4 ♀, 1 ♂ (CNAN-Sc1617; Sc1620), 4.xi.2010, G. Contreras, D. Barrales, J. Mendoza & D. Ortiz, 2 ♂, 2 ♀, 4 juv. (CNAN-Sc1621). *Michoácan*: Municipio de Zitácuaro: Hacienda "La Florida", 19°22.018′N 100°29.27′W, 750 m, 17.i.2007, X. Vázquez, 1 ♂, 1 ♀ (CNAN-Sc1619).

32. *Diplocentrus sissomi* Santibáñez-López, Francke & Prendini, 2013: **MEXICO**: *Oaxaca*: Distrito de Miahuatlan: Municipio de San Cristóbal Amatlán: San Juan Mixtepec, 16°16.6′N 96°17.95′W, iv.2002, E. Aldasoro, 2 ♂ (CALA); San Lorenzo

Mixtepec, 1 km N, 16°17.493′N 96°20.910′W, 2120 m, 23.vi.2006, O. Francke, G. Villegas, H. Montaño, A. Valdez & C. Santibáñez, holotype ♂ (CNAN-T0678), 3 ♂, 8 ♀, 3 juv. paratypes (CNAN-T0679), 3 ♂, 7 ♀, 2 subad. ♀, 5 juv. paratypes (AMNH), 1 ♀, 22 juv. paratypes (AMCC [LP 6531]), 1 ♀, 18 juv. paratypes (AMCC [LP 6538]), 1 ♀, 12 juv. paratypes (AMCC [LP 6539]), 1 ♀, 2 juv. paratypes (AMCC [LP 6541]).

33. ***Diplocentrus tehuacanus*** Hoffmann, 1931: **MEXICO**: *Morelos*: Municipio de Tlaquiltenango: Huautla, 18°26.4′N 99°01.5′W, 945 m, 3.viii.2003, M. Córdova, A. Jaimes & H. Laguna, 1 ♀, 1 ♂, 2 subad. ♀, 2 juv. (CNAN); Quilamula, 18°30.616′N 99°01.183′W, 1070 m, M. Córdova & A. Jaimes, 3 ♂, 1 ♀ (CNAN). *Puebla*: Municipio de Tehuacan: Tehuacan, 2 km E, 18°24.002′N 97°22.867′W, 1435 m, 25.vii.2002, L. Prendini, O. Francke, E. González & J. Ponce, in burrows and under stones, semi-desert scrub, 1 ♂, 1 juv. ♂, 2 juv. ♀ (AMCC [LP 2044]), 1 ♂, 1 ♀, 1 subad. ♂, 1 juv. ♂, 1 juv. ♀ (AMCC [LP 2045]).

34. ***Diplocentrus whitei*** (Gervais, 1844): **MEXICO**: *Coahuila*: Municipio de Cuatrociénegas: Sierra San Marcos II, 26°54.798′N 102°08.25′W, 761 m, 18.vii.2006, E. González, B. Hendrixson, K. McWest & S. Grant, 2 ♂ (AMNH), 2 ♂ (CNAN-Sc1691), 1 ♂ (AMCC [LP 6580]), 1 ex. [leg] (AMCC [LP 6614]); Ojo de Agua, Ejido el Oso, 27°00.056′N 102°00.226′W, 1039 m, 19.vi.2006, O. Francke, W.D. Sissom, K. McWest, B. Hendrixson, S. Grant, E. González, M. Córdova, A. Ballesteros, 3 ♂, 2 ♀ (AMNH), 3 ♂, 1 ♀, 1 juv. (CNAN-Sc1692), 1 ex. (AMCC [LP 6463]). Municipio de Ocampo: Rancho Agua Verde, 29.i.2005, P. Sprouse & C. Savvas, 1 juv. ♀ (AMCC [LP 4101]).

35. ***Diplocentrus zacatecanus*** Hoffmann, 1931: **MEXICO**: *Aguascalientes*: Municipio de Tepezala, [22°13.362′N 102°10.014′W], 2100 m, lectotype ♂ (CNAN-T-0761), 1 ♂, 2 ♀ (CNAN-T0762); Tepezala, 1 km N, 22°14.348′N 102°10.467′W, 2048 m, 4.vii.2005, O. Francke, J. Ponce, M. Córdova, A. Jaimes, G. Francke & V. Capovila, 3 ♂ (AMNH), 3 ♂ (CNAN), 3 juv. (AMCC [LP 5339]).

8. Appendix 2

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, retrolateral 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, retrolateral 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, punctation: 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, punctation: 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 carina (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 carina (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 Et_1 and V_1: directed towards Et_1 (0); directed towards a point less than half the distance from Et_1 to V_1 (1); directed towards a point approximately half the distance from Et_1 to V_1 (2); directed towards a point more than half the distance from Et_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, punctation: 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 (♂), trichobothrium *ib*, position relative to articulation between fixed and movable fingers: aligned (0); distal (1).

65. Chela manus (♂), trichobothrium *it*, position relative to trichobothrium *ib*: aligned (0); distal (1).

66†. Chela manus, ventral surface, *V* 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, punctation: absent (0); present (1).

69†. Leg basitarsi, prolateral pores (♂): absent (0); present (1) [P00:67].

70. Leg I basitarsus, proventral distal spiniform macroseta: absent (0); present (1).

71. Leg I basitarsus, retroventral distal spiniform macroseta: absent (0); present (1).

72. Leg I basitarsus, proventral subdistal spiniform macroseta: absent (0); present (1).

73. Leg I basitarsus, retroventral subdistal spiniform macroseta: absent (0); present (1).

74. Leg I basitarsus, proventral medial spiniform macroseta: absent (0); present (1).

75. Leg I basitarsus, retroventral medial spiniform macroseta: absent (0); present (1).

76. Leg I basitarsus, retrolateral medial spiniform macroseta: absent (0); present (1).

77. Leg II basitarsus, proventral distal spiniform macroseta: absent (0); present (1).

78†. Leg II basitarsus, retroventral distal spiniform macroseta: absent (0); present (1).

79. Leg II basitarsus, proventral subdistal spiniform macroseta: absent (0); present (1).

80. Leg II basitarsus, retroventral subdistal spiniform macroseta: absent (0); present (1).

81. Leg II basitarsus, proventral medial spiniform macroseta: absent (0); present (1).

82. Leg II basitarsus, retroventral medial spiniform macroseta: absent (0); present (1).

83. Leg II basitarsus, retroventral submedial spiniform macroseta: absent (0); present (1).

84. Leg II basitarsus, ventral distal spiniform macroseta: absent (0); present (1).

85. Leg II basitarsus, retroventral subbasal spiniform macroseta: absent (0); present (1).

86†. Leg II basitarsus, retrolateral subdistal spiniform macroseta: absent (0); present (1).

87. Leg II basitarsus, retrolateral medial spiniform macroseta: absent (0); present (1).

88. Leg II basitarsus, retrolateral subbasal seta: absent (0); spiniform macroseta present (1); macroseta present (not spiniform) (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); obsolete (1).

92. Sternite VII median carina, length relative to submedian carinae: equal (0); less (1).

93. Sternite VII submedian carinae, development: distinct (0); obsolete (1) [PEA03:102, 103].

94. Mesosoma, metasoma and telson, nongranular dorsal surfaces, punctation: absent (0); present (1).