

## Variation in the spiniform macrosetae pattern on the basitarsi of *Diplocentrus tehuacanus* (Scorpiones: Diplocentridae): new characters to diagnose species within the genus

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**Abstract.** Spiniform macrosetae have been useful as a taxonomic trait in the genus *Diplocentrus*, such as the telotarsal spiniform macrosetae formula widely used to separate species. Basitarsal spiniform macrosetae have been studied in the family Scorpionidae but not in its sister family (Diplocentridae). In this study, we analyzed the variation in the position and number of spiniform macrosetae on the basitarsus of one species of the genus *Diplocentrus*. We found minimal ontogenetic, intersexual and geographical variation within the species. We also compare the pattern found in *Diplocentrus tehuacanus* Hoffmann 1931 to those of two morphologically similar species, and found that the basitarsal macrosetal pattern is also a good, reliable taxonomic character at the interspecific level.

**Keywords:** Ontogenetic variation, intersexual variation, geographical variation, interspecific variation, diagnostic character

Spiniform setae on scorpion legs have been used as a reliable source of taxonomic information, especially in the superfamily Scorpionoidea Latreille 1802 (Francke 1978; Lamoral 1979; Prendini 2000). For example, species of the family Diplocentridae are partially characterized by the telotarsal spiniform macrosetae formula. This formula (e.g., 4/4: 4/5: 5/5: 6/6) represents the number of spiniform macrosetae present on each face (prolateral/retrolateral) of the ventral aspect of the telotarsus on the four pairs of legs (I: II: III: IV). Basitarsal spiniform macrosetae have been considered only for the family Scorpionidae (Prendini et al. 2003) and have been ignored in the family Diplocentridae.

Recently, the basitarsal spiniform macrosetae pattern for legs III–IV has been used to separate species groups in *Diplocentrus* Peters 1861 (Santibáñez-López et al. 2013) and to diagnose *D. zacatecanus* Hoffmann 1931 (Santibáñez-López & Francke 2013), but no other attempt has been made to analyze its utility as a species-specific diagnostic character, nor as a phylogenetically informative character. In a separate contribution, the basitarsal macrosetal pattern for the genera within the family Diplocentridae has been tested to determine its utility as a generic diagnostic character (Santibáñez-López et al. in prep.).

To study the variation in the position and number of spiniform macrosetae on the ventral face of the basitarsus of the species in this family, we analyzed the degree or extent of intraspecific variation first on one species: *Diplocentrus tehuacanus* Hoffmann 1931, a species that is widely distributed in central Mexico and is well represented in collections (Fig. 1). In the present contribution, we considered four types of variation: a) individual variation (bilateral symmetry), b) ontogenetic variation (three stages of development), c) sexual dimorphism (males versus females) and d) geographical variation (different populations).

### METHODS

Terminology for the leg segmentation follows Couzijn (1976), and for spiniform setae Lamoral (1979), McWest (2009) and slightly modified from Prendini (2000). We

consider the spiniform macroseta as stout, blunt seta, spine-like, with a socketed base and usually dark in color.

**Spiniform macrosetal pattern on the leg basitarsus.**—These setae are found on the ventral face of the basitarsus of the four legs; the arrangement (position and number of setae) is different between them, except for legs III and IV, which present the same pattern (Fig. 2). Macrosetae on the distal margin of the segment are not considered, only those on the ventral face proper. Setae are named according to their relative position on the transverse axis of the ventral face of the basitarsus: p = prolateral side, v = ventral, r = retrolateral; and followed by their position with respect to the longitudinal axis: t = terminal, st = subterminal, m = medial, sb = suprabasal and b = basal. For example, a seta named pt means that it is found on the prolateral side and near the terminal portion of the basitarsus (e.g., Fig. 3). On legs I and II, one spiniform macroseta is also found on the retrolateral face, at the medial portion of the basitarsus (located in the retrolateral face and not in the ventral face; therefore, we use capital R: Rm to designate it; Fig. 3). The presence of the retrolateral median spiniform macroseta on leg II is a diagnostic trait for the genus *Diplocentrus* [the importance of the basitarsal macrosetae for the taxonomy of the family will be presented elsewhere (Santibáñez-López et al. in prep.)].

Observations were made using a stereoscopic microscope, Nikon SMZ 800. All illustrations are ventral views of the corresponding right leg (I, II, III and IV) and the prolateral pedal spur, located in the joint between the basitarsus and the telotarsus, is shown to help the reader understand the relative positions of the macrosetae studied. Illustrations were drawn using the software Adobe Illustrator C3.

Sixty-five specimens from different populations covering a wide range of the geographic distribution of *D. tehuacanus* were studied, including 44 adults (30 males and 14 females), 15 subadults (7 males and 8 females) and 6 juveniles.

In order to analyze the variation in the number and position of the setae present on the basitarsi, we considered the following:



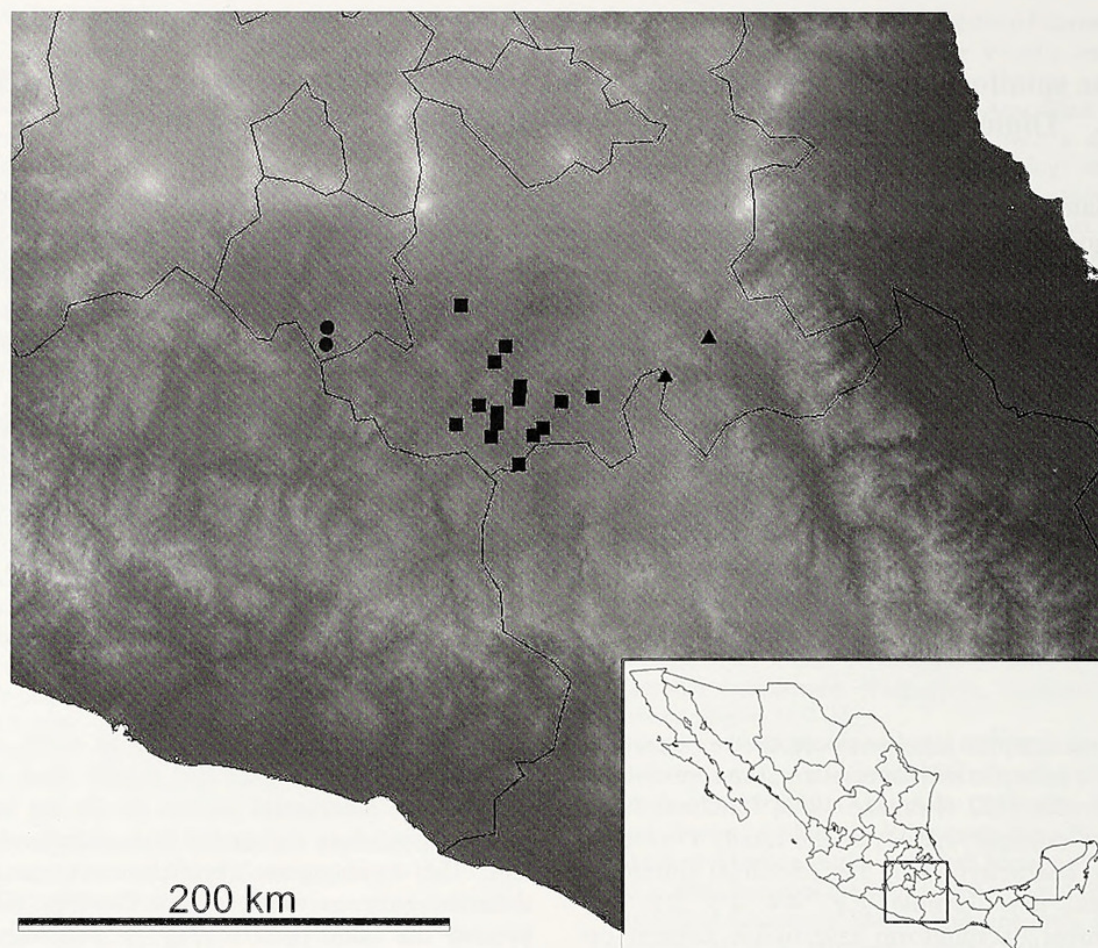


Figure 1.—*Diplocentrus tehuacanus* Hoffmann 1931, known records in central Mexico. Map divided into three regions for analysis of geographical variation in basitarsal macrosetae: Region I (circles), Region II (squares) and Region III (triangles).

- Individual variation. To determine whether asymmetrical variation within a single specimen existed, we compared the position and number of the macrosetae on both legs. We used (randomly selected) 12 males, 12 females, and 6 juveniles.
- Ontogenetic variation. To analyze variation in the position and number of the macrosetae between different developmental stages, we compared their arrangement on all adults against the subadults and juveniles.
- Sexual variation. To determine whether sexual dimorphism in spiniform macrosetal patterns within the species was present, we compared the arrangement of the macrosetae on each leg (I, II, III and IV) in males and females.
- Geographical variation. Because the range of distribution of the species is wide, we divided the populations available into three geographical sections: those found in the northern range of the distributional area (Region I with 10 specimens), those found in the central range (Region II with 43 specimens), and those found in the southeastern range of the distributional area (Region III with 12 specimens, which includes the type locality; see Fig. 1). We compared the ventral basitarsal spiniform macrosetal pattern on each leg (I, II, III and IV) from each region to each other, to determine whether geographical variation was present.

Finally, we propose a generalized pattern for the species, and compare it against the pattern of two morphologically

similar species also found in central Mexico: *Diplocentrus coylei* Fritts & Sissom 1996 and *Diplocentrus longimanus* Santibáñez-López et al. 2011.

Abbreviations of specimen depositories are CNAN – Colección Nacional de Aracnidos, Instituto de Biología, UNAM; CAIMSc – Instituto de Diagnóstico y Referencia Epidemiológicos, Secretaría de Salud, Mexico.

**Specimens studied.**—*Diplocentrus tehuacanus* Hoffmann 1931: MEXICO, REGION I: Morelos, Tlaquiltenango, Huautla 18°26'24"N, 99°01'30"W, 945 m, 3 August 2003, M. Córdova, A. Jaimes and H. Lagunas, 1 ♀, 1 ♂, 2 subadult ♀, 2 juveniles (CNAN-503038); Tlaquiltenango, Quilamula 18°30'37"N, 99°01'11"W, 1070 m, unknown date; M. Córdova and A. Jaimes, 3 ♂, 1 ♀ (CNAN-503213). REGION II: Puebla. Acatlán 18°12'12"N, 98°02'55"W, 1180 m, 21 June 2000, V. Vidal, 2 ♂ (CAIMSc-04249); Acatlán, Rancho Nuevo 17°56'41"N, 98°13'16"W, 1220 m, 10 November 2005, unknown collector, 1 ♀ (CAIMSc-04240); Ahuehuetitla 18°12'44"N, 98°13'16"W, 1200 m, 8 January 2003, unknown collector, 2 ♂, 2 ♀, 1 subadult ♂, 1 subadult ♀ (CAIMSc-04259); Axutla 18°11'21"N, 98°23'24"W, 860 m, 30 September 2004, unknown collector, 2 subadult ♂, 1 subadult ♀ (CAIMSc-04254); Chila de la Sal 18°06'36"N, 98°29'03"W, 940 m, 9 July 2003, unknown collector, 1 ♂, 1 ♀, 1 subadult ♂ (CAIMSc-04271); Chinautla 18°16'03"N, 98°13'11"W, 1200 m, 20 October 2000, unknown collector, 2 ♀ (CAIMSc-04278); Guadalupe 18°05'35"N, 98°07'14"W, 1100 m, 17 April 2006,





Figure 2.—*Diplocentrus tehuacanus* Hoffmann 1931, legs I–III, basitarsus and telotarsus, showing ventral spiniform macrosetae.

unknown collector, 1 ♂ (CAIMSc-04253); Guadalupe, La Providencia 18°03'46"N, 98°09'53"W, 1060 m, 9 August 2006, unknown collector, 1 ♀ (CAIMSc-04247); Izucar de Matamoros 18°36'10"N, 98°27'55"W, 1280 m, 23 June 2004, unknown collector, 1 ♀, 3 subadult ♂, 1 subadult ♀ (CAIMSc-04245); Piaxtla, Tlaxcoapan 18°09'22"N, 98°18'40"W, 980 m, 1 September 2000, unknown collector, 1 ♂ (CAIMSc-04273); San Jeronimo Xayacatlan 18°13'22"N, 97°54'52"W, 1320 m, 6 March 2006, unknown collector, 1 ♂ (CAIMSc-04241); Tecamatlán, Rancho Nuevo 18°03'27"N, 98°20'09"W, 980 m, August 2001, unknown collector, 2 ♂, 2 ♀ (CAIMSc-04246); Tecamatlán, Rancho Nuevo 18°03'27"N, 98°20'09"W, 980 m, 13 March 2006, unknown collector, 1 ♀ (CAIMSc-04243); Tecamatlán 18°06'44"N, 98°18'54"W, 920 m, 1 June 2001, F. Martinez, 1 ♂ (CAIMSc-04239); Tehuiztingo, San Francisco de Asís 18°26'12"N, 98°16'45"W, 1060 m, 7 September 2001, unknown collector, 4 ♂, 1 ♀ (CAIMSc-04261); Tehuiztingo, Tuzantlán 18°22'01"N, 98°19'31"W, 1000 m, 11 September 1999, unknown collector, 1 ♂ (CAIMSc-04269); Xicotlán, Coacalco 18°04'18"N, 98°40'05"W, 800 m, 5 June 2001, M. Sanchez, 5 ♂ (CAIMSc-04257). REGION III: Puebla, Tehuacán, San Lorenzo (18°28'20"N, 97°26'W, 1660m) 22 January 1964, L. Vazquez, 1 ♂, 3 subadult ♀, 4 juveniles (CNAN-500726); Zapotitlán, San Juan Raya 18°18'58"N, 97°36'54"W, 1840 m, unknown date, unknown collector, 4 ♂ (CAIMSc-04250).

*Diplocentrus coylei* Fritts & Sissom 1996: MEXICO: Guerrero, El Comal, Buena Vista de Cuellar 18°27'86"N, 99°17'39"W, 1749 m, 13 June 2007, O. Francke et al., 5 ♂, 4 ♀, 1 subadult ♂, 2 subadult ♀, 3 juveniles (CNAN-503262).

*Diplocentrus longimanus* Santibáñez-López et al., 2011: MEXICO: Puebla, Altepexi 18°22'03"N, 97°17'55"W, 1240 m, 16 October 2000, unknown collector, 1 ♂ (CAIMSc-04308); Ahuehuetitla 18°12'44"N, 98°13'16"W, 1200 m, 1 May 2004, unknown collector, 1 ♂ (CAIMSc-O1147); Chila de la Sal 18°06'36"N, 98°29'03"W, 940 m, 19 June 2000, unknown collector, 1 ♀ (CAIMSc-04271); Piaxtla, Tlaxcoapan

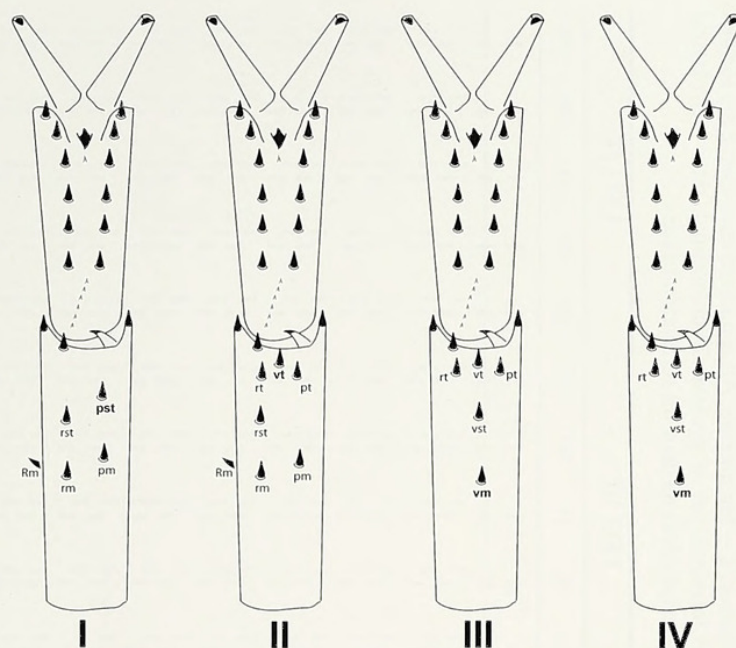


Figure 3.—*Diplocentrus tehuacanus* Hoffmann 1931, basitarsal ventral spiniform macrosetal pattern (differences from the other two species included in this study marked in bold type).

18°09'22"N, 98°18'40"W, 980 m, 1 September 2000, unknown collector, 2 ♂ and 1 ♂ subadult (CAIMSc-04306); Tehuiztingo, Tejalpa 18°21'39"N, 98°21'37"W, 960 m, 6 December 2001, E. Bello, 1 ♂ (CAIMSc-04269); Tulcingo, Aguacatitlán 17°58'43"N, 98°20'02"W, 1100 m, 4 September 2003, M.A. Sanchez and F. Santos R., 1 ♂ (CAIMSc-04297); Xicotlán 18°03'34"N, 98°31'32"W, 1260 m, 17 October 2001, unknown collector, 1 ♂ (CAIMSc-04257).

## RESULTS

The spiniform macrosetal pattern was more variable on leg I than on the others, followed by leg II. The basitarsal spiniform macrosetal pattern for *Diplocentrus tehuacanus* is as follows (see also Fig. 3):

Leg I. Two subterminal spiniform macrosetae (pst and rst) and two median spiniform macrosetae (pm and rm) are found. The presence of a ventral terminal macroseta (vt) was observed in 11 of the 65 specimens, and it was always asymmetrical (present on one side and absent on the other; Table 1) and is not considered part of the generalized species-specific pattern. A retrolateral terminal macroseta (rt) was found on 11 specimens; on 5 of them asymmetrical (present on one side and absent on the other) and on 6 specimens present on both legs; it is nonetheless not considered part of the species-specific pattern because it is missing in the majority of the specimens (Table 1). A retrolateral median spiniform macroseta (Rm) is present on all specimens.

Leg II. Three terminal spiniform macrosetae are found (pt, vt and rt), one subterminal (rst) and two median spiniform macrosetae (pm and rm) are present. On this leg, we found only one case of asymmetry, involving macroseta rm (Table 1). On the retrolateral surface, the median spiniform macroseta (Rm) is present on all specimens.

Legs III and IV. These two legs share the same basitarsal macrosetal pattern: Three terminals (pt, vt and rt), one subterminal (vst) and one median (vm) spiniform macrosetae





Table 1.—Continued.

[illegible]



Table 2.—Ontogenetic variation (noted in bold type, or lack thereof) on the spiniform macrosetae on the ventral (and the retrolateral) face of legs I–IV. *n* = sample size (one juvenile missing both legs II); pt = prolateral terminal, rt = retrolateral terminal, vt = ventrolateral terminal, pst = prolateral subterminal, rst = retrolateral subterminal, vst = ventral subterminal, pm = prolateral medial, rm = retrolateral medial, vm = ventral medial, Rm = Retrolateral medial on retrolateral face; - = inapplicable. On all juveniles, instead of a spiniform macroseta, a stunted macroseta was present on the retrolateral face.

Leg	Stage	<i>n</i>	pt	Rt	vt	pst	rst	vst	pm	rm	vm	Rm
I	Adults	88	-	28	12	86	85	-	86	74	-	88
	Subadults	30	-	<b>6</b>	<b>5</b>	30	30	-	30	30	-	30
	juveniles	12	-	<b>0</b>	<b>0</b>	10	10	-	10	10	-	<b>10*</b>
II	Adults	88	88	88	88	-	86	-	86	80	-	88
	Subadults	30	29	29	29	-	29	-	29	29	-	30
	juveniles	10	10	10	10	-	10	-	10	10	-	10
III	Adults	88	88	88	88	-	-	88	-	-	87	-
	Subadults	30	30	30	30	-	-	30	-	-	30	-
	juveniles	12	12	12	12	-	-	12	-	-	12	-
IV	Adults	88	87	88	87	-	-	87	-	-	88	-
	Subadults	29	29	29	29	-	-	29	-	-	29	-
	juveniles	12	12	12	12	-	-	12	-	-	12	-

Table 3.—Sexual variation on the presence and counts of spiniform macrosetae on the ventral (and retrolateral) face of legs I–IV: *n* = sample size; pt = prolateral terminal, rt = retrolateral terminal, vt = ventrolateral terminal, pst = prolateral subterminal, rst = retrolateral subterminal, vst = ventral subterminal, pm = prolateral medial, rm = retrolateral medial, vm = ventral medial, Rm = Retrolateral medial on retrolateral face; - = inapplicable.

Leg	Stage	Sex	<i>n</i>	pt	rt	vt	pst	rst	vst	pm	rm	vm	Rm
I	adult	♂	74	-	21	8	72	71	-	72	62	-	74
	adult	♀	44	-	13	9	44	44	-	44	42	-	44
II	adult	♂	74	74	74	74	-	74	-	73	66	-	74
	adult	♀	44	44	44	44	-	44	-	44	44	-	44
III	adult	♂	74	74	74	74	-	-	74	-	-	74	-
	adult	♀	44	44	44	44	-	-	44	-	-	43	-
IV	adult	♂	73	73	73	72	-	-	73	-	-	73	-
	adult	♀	44	43	43	44	-	-	43	-	-	44	-

Table 4.—Analysis of geographical variation on the spiniform macrosetae on the ventral face of legs I–IV. *n* = sample size; pt = prolateral terminal, rt = retrolateral terminal, vt = ventrolateral terminal, pst = prolateral subterminal, rst = retrolateral subterminal, vst = ventral subterminal, pm = prolateral medial, rm = retrolateral medial, vm = ventral medial, Rm = Retrolateral medial on retrolateral face, Rt = Retrolateral terminal on retrolateral face; - = inapplicable.

Leg	Region	<i>n</i>	pt	Rt	vt	pst	rst	vst	pm	rm	vm	Rm
I	I	20	-	10	7	18	18	-	18	19	-	20
	II	86	-	24	10	85	84	-	84	73	-	86
	III	24	-	0	0	23	23	-	24	22	-	22
II	I	18	18	18	18	-	18	-	18	18	-	18
	II	86	86	86	86	-	86	-	86	78	-	86
	III	24	24	24	24	-	24	-	23	23	-	24
III	I	20	20	20	20	-	-	20	-	-	20	-
	II	86	86	86	86	-	-	86	-	-	85	-
	III	24	24	24	24	-	-	24	-	-	24	-
IV	I	20	20	20	20	-	-	20	-	-	20	-
	II	85	84	85	84	-	-	84	-	-	85	-
	III	24	24	24	24	-	-	24	-	-	24	-



Table 5.—Interspecific variation on the spiniform macrosetae on the basitarsus of legs I–IV in three species of *Diplocentrus* Peters 1861 (differences highlighted in bold type): n = sample size; pt = prolateral terminal, rt = retrolateral terminal, vt = ventrolateral terminal, pst = prolateral subterminal, rst = retrolateral subterminal, vst = ventral subterminal, pm = prolateral medial, rm = retrolateral medial, vm = ventral medial, rsb = retrolateral suprabasal, Rm = Retrolateral medial on retrolateral face; - = inapplicable.

Leg	Species	n	pt	rt	vt	pst	rst	vst	pm	rm	vm	rsb	Rm
I	<i>D. tehuacanus</i>	130	-	34	17	<b>126</b>	125	-	126	114	-	-	128
	<i>D. longimanus</i>	20	<b>20</b>	18	1	-	20	-	20	20	-	-	18
	<i>D. coylei</i>	30	<b>28</b>	-	-	-	30	-	20	28	-	-	30
II	<i>D. tehuacanus</i>	128	128	128	<b>128</b>	-	128	-	127	<b>119</b>	-	-	128
	<i>D. longimanus</i>	20	18	18	-	<b>18</b>	18	-	18	-	-	-	18
	<i>D. coylei</i>	30	30	30	<b>30</b>	-	29	-	6	-	-	<b>24</b>	30
III	<i>D. tehuacanus</i>	130	130	130	130	-	-	130	-	-	<b>129</b>	-	-
	<i>D. longimanus</i>	20	20	20	20	-	<b>20</b>	20	-	-	<b>20</b>	-	-
	<i>D. coylei</i>	30	28	28	28	-	2	28	-	-	<b>2</b>	-	-
IV	<i>D. tehuacanus</i>	129	128	128	128	-	-	128	-	-	<b>129</b>	-	-
	<i>D. longimanus</i>	20	20	20	20	-	<b>20</b>	20	-	-	<b>20</b>	-	-
	<i>D. coylei</i>	30	28	28	28	-	1	28	-	-	<b>4</b>	-	-

are found on these legs. A single case of asymmetry involving macroseta pt on leg IV was observed (Table 1).

#### Variation.—

- A) Individual variation. From the sample used in this study, only 13 legs out of 236 studied (30 specimens  $\times$  8 legs = 240, less 4 missing legs) showed asymmetry: 11 (4.7%) on leg I (6 on macrosetae vt and 5 on rt); one (0.4%) on leg II (macroseta rm) and one (0.4%) on leg IV (macroseta pt). Six specimens out of 30 (20%) possessed macroseta rt symmetrically on both legs I, and as mentioned above five had it asymmetrically on the same legs I.
- B) Ontogenetic variation. Leg I. Seven adults (4 males and 3 females) and 2 subadults (1 male and 1 female) presented macroseta vt, which was absent in all juveniles; 15 adults and 4 subadults presented rt, which was also absent in all

juveniles. No differences in the number or the pattern of spiniform macrosetae among age groups were found on the other legs (see Table 2).

- C) Sexual variation. No differences in number or pattern of spiniform macrosetae were observed between males and females, although both sexes had a low propensity to present one extra macroseta (either vt or rt) on leg I (as indicated above). No differences between sexes were found on the other legs (see Table 3).
- D) Geographical variation. Regions I and II had eight out of 53 specimens (15.1%) with vt and in 19 specimens (35.9%) rt on leg I. The 12 specimens from region III had no extra macroseta on leg I. No other differences were found in the other legs (see Table 4).

Thus the generalized basitarsal spiniform macrosetae formula for *D. tehuacanus* is Leg I with five: pst, rst, pm, rm

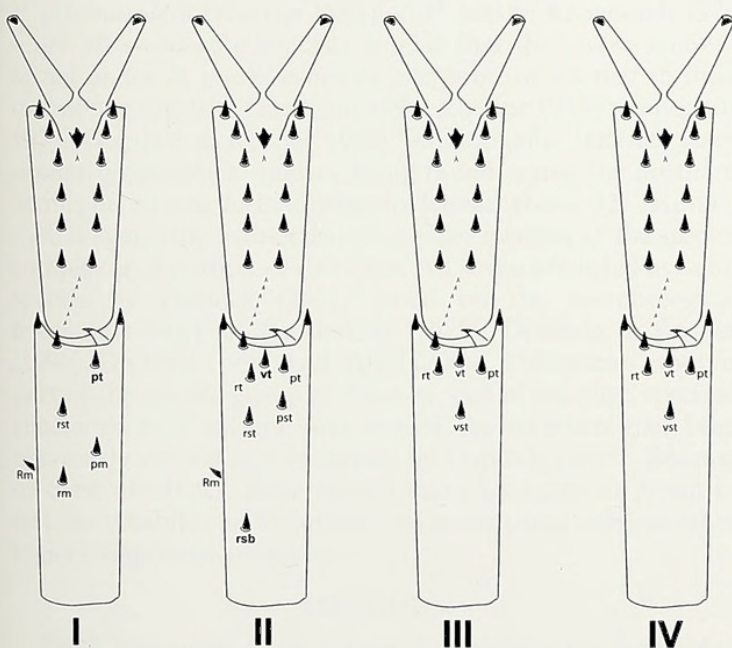


Figure 4.—*Diplocentrus coylei* Fritts and Sissom 1996, basitarsal ventral spiniform macrosetal pattern (differences from the other two species included in this study marked in bold type).

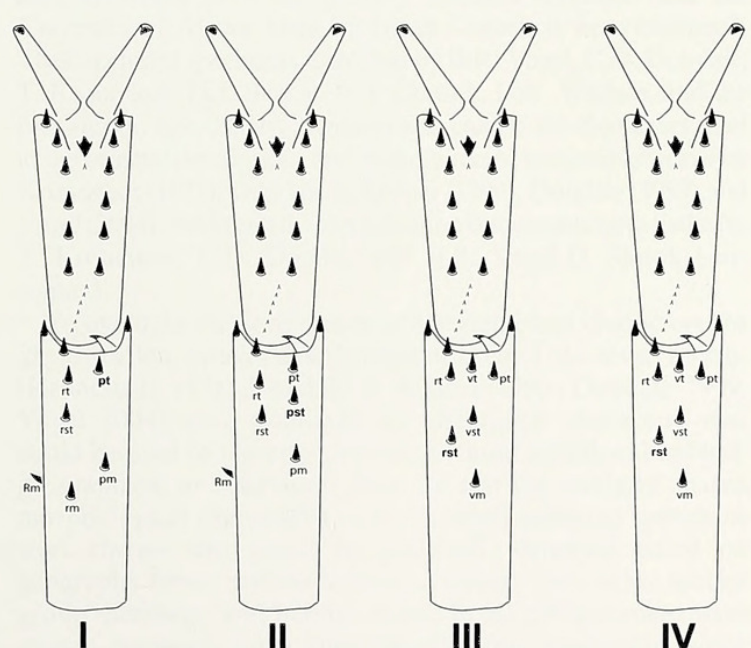


Figure 5.—*Diplocentrus longimanus* Santibáñez-López et al. 2011, basitarsal ventral spiniform macrosetal pattern (differences from the other two species included in this study marked in bold type).



and Rm; Leg II with seven: pt, vt, rt, rst, pm, rm and Rm. Legs III–IV with five: pt, vt, rt, vst and vm (Fig. 3).

**Basitarsal spiniform macrosetal formula as a diagnostic species-specific character in the genus *Diplocentrus* Peters 1861.**—We compared the pattern found on *D. tehuacanus* against the patterns found on two morphologically similar species: *Diplocentrus longimanus* and *Diplocentrus coylei*. Differences between the patterns are as follows (see also Table 5 and Figs. 3–5):

Leg I. The three species share the presence of macrosetae rst, pm, rm, and Rm, but they differ as follows: *D. longimanus* and *D. coylei* present pt, whereas on *D. tehuacanus* that macroseta is absent; *D. longimanus* presents rt, which is absent on the other two species; and *D. tehuacanus* presents pst, which is absent on the other two species.

Leg II. The presence of macrosetae pt, rt, rst and Rm is common to the three species, but their differences are: *D. coylei* and *D. tehuacanus* present vt, which is absent on *D. longimanus*; *D. longimanus* and *D. tehuacanus* present pm, but it is absent on *D. coylei*; pst is present only on *D. longimanus*, rsb (retrolateral suprabasal) is present only on *D. coylei* and rm is present only on *D. tehuacanus*.

Legs III–IV. The patterns for the three species share the three terminal spiniform macrosetae (pt, vt and rt); they also share the presence of vst, but they differ in the presence of rst (only on *D. longimanus*) and the presence of vm (on *D. longimanus* and *D. tehuacanus*).

### CONCLUSIONS

The basitarsal spiniform macrosetal pattern on each of the four legs of species of the genus *Diplocentrus* is rather invariable, showing minimal bilateral asymmetry, predictable ontogenetic changes, lacking sexual dimorphism and presenting minimal geographic variation. Furthermore, there are reliable differences in the basitarsal macrosetal patterns among the three species analyzed. Thus we consider that it is a species-specific diagnostic character and strongly recommend that this

pattern be noted on all future descriptions, along with the telotarsal count of spiniform macrosetae.

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