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LARVAL DEVELOPMENT AND GROWTH RATIO IN *ISCHNURA CRUZI* DE MARMELS, WITH DESCRIPTION OF LAST LARVAL INSTAR (ZYGOPTERA: COENAGRIONIDAE)

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Under stable laboratory conditions larval stages were measured and morphologically compared in order to establish growth ratio and total number of instars through their postembryonic development. Head width, total length, metafemur length, forewing pad length, and length and width of prementum were measured to determine variation between instars, and growth ratio was calculated. By Dyar's Law, 12 larval instars were estimated. Fundamental morphological differences were found in order to distinguish the stages and at the same time to have a record of the morphological development through the stages. Finally, the last larval instar is described and illustrated.

INTRODUCTION

Hemimetabolous insects experience a gradual development by consecutive molts (RUPPERT & BARNES, 2006). *Ischnura cruzi* DeMarmels is a small sized coenagrionid species with hyaline wings. Its body lacks metallic reflections (CORBET, 1999). The males present a variable colouration (green blue) on the terminal abdominal segments, while in females colouration is variable brown-green, orange and predominantly pruinescent. The genus *Ischnura* has the most cosmopolitan distribution in the Coenagrionidae; *I. cruzi* is endemic in the Colombian Andes (Sabana de Bogotá). A specific diagnostic character for Colombian *Ischnura* males is a dorsal posterior process on its tenth abdominal segment (Fig. 1). *Ischnura* species present an important ecological component on swamps and ponds, with dense riparian vegetation (FÖRSTER, 1999).

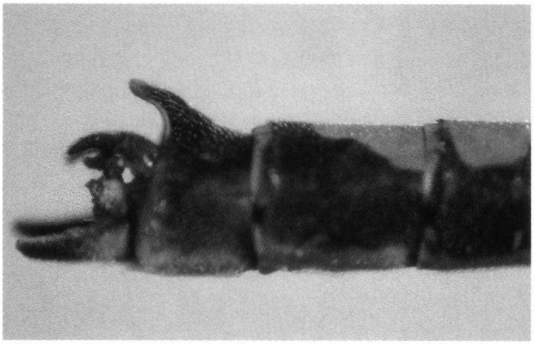


Fig. 1. *Ischnura cruzi*, male abdomen. The dorsal process on the 10th abdominal segment is a diagnostic character, as is colour pattern of abdominal segments 8 and 9.

Occasionally, larval instars are influenced by temperature changes, especially under laboratory conditions, where the number of larval stages increases more than under natural conditions (AOKI, 1999). Generally, temporary pond organisms present fast development (in a short period of time) and short life cycles (CORBET, 1999; SUHLING, 2004). On the contrary, many taxa in permanent ponds present slow growth ratios with long life cycles (DUDGEON & WAT, 1986; SUHLING, 2005).

The purpose of this research is to describe the life cycle of *I. cruzi*, differentiate and recognize larval instars, and to determine their development pattern. Finally, the last instar larva is described and illustrated.

MATERIAL AND METHODS

Locality: COLOMBIA, Cundinamarca Dept., Municipio El Rosal, Vereda El Rodeo (4°50'59.89"N, 74°16'00.48"W), permanent pond, 2400 m.a.s.l, and 9-22°C temperature range.

Larval search was made in aquatic macrophyte samples, which were randomly collected, (SOUZA & TAKEDA, 2002). The collected larvae were placed individually in glass flasks in order to obtain successive moults. Larvae were maintained at the Laboratorio de Zoología y Ecología Acuática (LAZOEA) of the Universidad de los Andes.

From macrophytes, all potential predators were removed (SOUZA & TAKEDA, 2002), viz. Notonectidae (Hemiptera) and Libellulidae. Larvae were fed with Chironomidae and Ephemeroptera larvae, found at the sample site, and with *Tubifex* (Annelida: Oligochaeta).

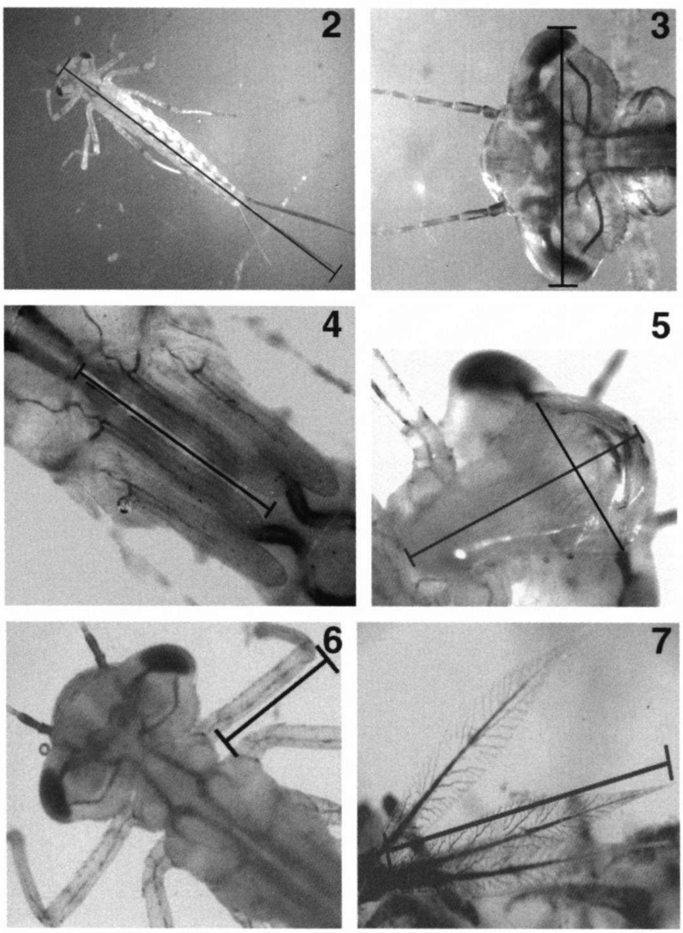
Sixty-three larvae were raised and analyzed. Of all collected larvae, only one presented an F-0 stage at the beginning of this study. After each moult, characters as head width, total length excluding gills, metafemur (maximum length parallel to dorsal margin), internal forewing-pad length, prementum maximum width and length were measured (Figs 2-7; Tab. I). Exuviae were preserved in 70% ethyl alcohol and last instar exuviae were used for larval description. Measurements and illustrations were made using a stereoscope Zeiss Stemi SV6, coupled to a camera lucida and using an objective with micrometric ruler, in order to obtain precision and minimize any possible error.

In order to obtain an F-0 instar reference, the last exuviae obtained was used, and labeled as last instar. Dyar's Law was used for data analysis to determine a larval development pattern, which increases in lineal dimensions from one instar to another in a constant ratio along its development (CHAPMAN, 2006). Finally 23 exuviae were used for description (16 ♂, 7 ♀).

RESULTS

All morphological combination analyses showed that only internal forewing--pad length, presents allometric growth (GORETTI, 2001), and could be taken as a distinctive variable along all instars (Figs 8-11). A clear example is observed on internal forewing-pad length – head width (Fig 8). Additionally, a logarithmic x-axis graph is presented to minimize data dispersion and thus to be able to differentiate each preimaginal stage (RODRIGUES, 1983).

Dyar's law allowed to make an estimate number from preimaginal instars that



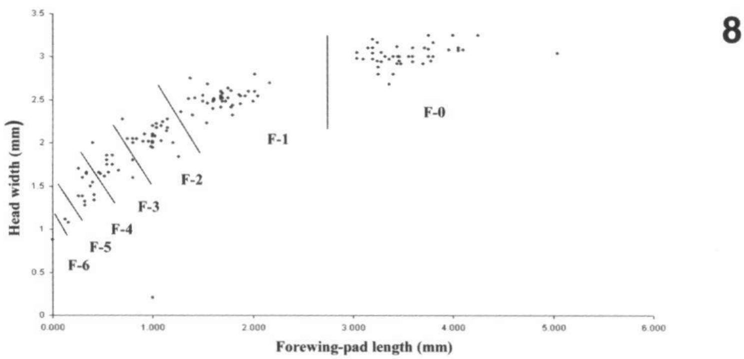
Figs 2-7. *Ischnura cruzi*, larval characters measured: (2) total length (head to median gill), dorsal view; – (3) head width, dorsal view; – (4) forewing-pad, dorsal view; – (5) labium width and length, ventral view; – (6) metafemur length, dorsal view; – (7) median gill length, lateral view.

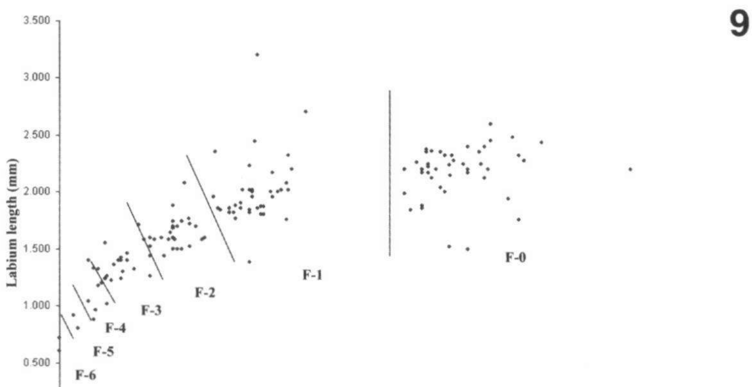
were not observed (Tab. I). It can be observed that forewing-pad length growth ratio is greater, due to allometric growth.

DESCRIPTION OF LAST LARVAL INSTAR

Figures 13-23

H e a d (Fig. 13) widest across eyes; brown oval spots along head maximum diameter. Concave occipital margin; hind lobe rounded and slightly prominent with 28 setae. Seven-segmented antenna (0.4:0.6:1:0.5:0.4:0.3:0.2); first two segments pigmented. Prementum triangular (Fig. 14) almost 0.67 times as wide as long, reaching caudad to 1st coxa; median lobe little prominent, uncleft, finely serrated; sides with 8 short spinules. Premental setae 4 on each side. Labial palp (Fig. 15) with 5 setae, movable hook slightly curved, anterior margin with well



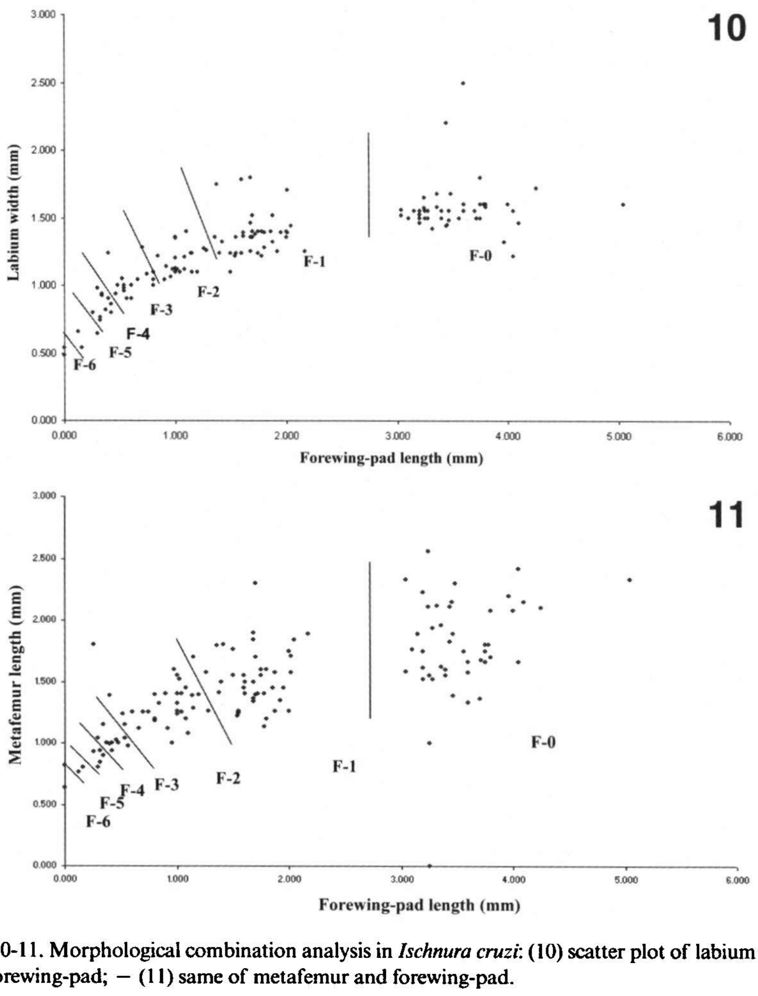


Figs 8-9. Morphological combination analysis in *Ischnura cruzi*: (8) scatter plot of head and forewing-pad length; — (9) same of labium length and forewing-pad.

developed end hook and 4 teeth, with 3 denticles; inner margin finely serrated. Mandibular formula : I 1+2 3 4 5 y a b / D 1 2 3 4 y a (Fig. 16) (sensu WATSON, 1956).

T h o r a x. – Prothorax and pterothorax without dark markings. Forewing-pads reaching to anterior margin of the 4th abdominal segment. Legs relatively long and pale. Femur with dark band near tibiae union.

A b d o m e n. – Cylindrical, long and slender, without dark markings. Dorsal surface of segments with uniformly distributed setae. Male cerci in lateral view as in Figure 17. Caudal lamellae (Figs 18-19) broadly lanceolate with acuminate tips. Nodus not well marked. Dorsal margin of median caudal lamella with 37



Figs 10-11. Morphological combination analysis in *Ischnura cruzi*: (10) scatter plot of labium width and forewing-pad; – (11) same of metafemur and forewing-pad.

stiff and rigid setae, extending to 0.37 of length of lamella; ventral margin with 19 setae, extending to 0.27 its length. Lateral caudal lamella with 42 ventral setae, extending to 0.45 of its length. Dorsal margin of lateral caudal lamella with 8-10 stiff setae, extending to 0.17 of its length. Tracheae dark. Ovipositor and male gonapophyses as in Figures 20-23.

Measurements (mm) N = 23 (all last intar exuviae). – Total length 17.8; – maximum width of head 3.1; prementum maximum length (through median line) 2.3, maximum width 1.6. – Length of forewing pad: inner margin 3.7, external 3.5; length of femora: I = 3.0, II = 3.6, III = 5.0; tibiae: I = 3.1, II = 3.7, III = 4.6; caudal lamellae (maximum and minimum length): median 6.4-6.1; lateral 7.1-6.7.

DISCUSSION

An adequate Zygoptera life cycle study depends on larval instars analysis (HAWKING & NEW, 1996). Morphological character biometry allows understanding and interpretation of life cycle (GORETTI et al., 2001). It is estimated that *I. cruzi* displays a multivoltine developmental cycle (completed generations in a year) of 12 larval stages throughout the year in the study area (nonseasonal climate and permanent pond habitat conditions) (CORBET, 1999). Probably the environment had some effects on larval development (growth, moulting and emergence) but those are little understood (LUTZ, 1974b). Some environmental factors are day and night cycles, fluctuating temperatures and seasonal progression (LUTZ, 1974a).

Growth ratio varies irregularly between instars and organisms (JONES et al., 1981; CALVERT, 1934). In spite of this feature, approximated size ranks can be obtained for each instar, but ambiguous data between two consecutive instars always exist because ranks show superposition (Tab. I).

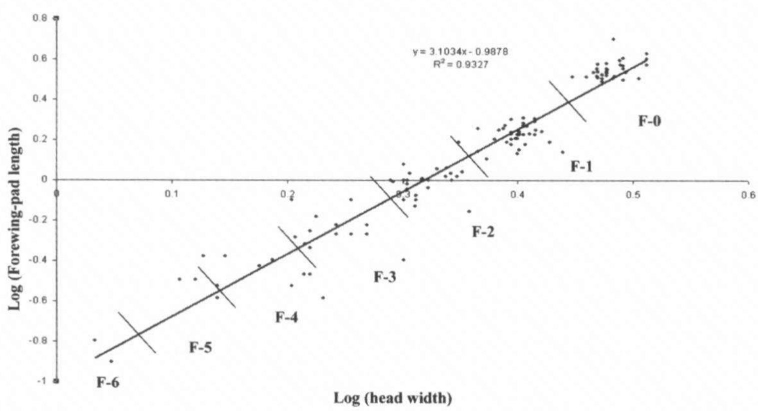


Fig. 12. Morphological combination ananlysis in *Ischnura cruzi*: logarithmic scale with data on forewing-pad length and head width.

Table I
Average, standard deviation, maximum and minimum value in biometric characters measured, relative coefficient in development ratio and derivative measure with Dyar law*

Instar	N	Head width (mm)		Metafemur		Forewing-pad (mm)		Labium width (mm)		Labium length (mm)		Total length	
		prom±s.d	min.-max.	prom±s.d	min.-max.	prom±s.d	min.-max.	prom±s.d	min.-max.	prom±s.d	min.-max.	prom±s.d	min.-max.
F-12	1	0.234*		0.163*		Absent		0.170*		0.193*		1.248*	
F-11	1	0.292*	0.209*	Absent	0.204*	0.237*	1.586*						
F-10	1	0.364*	0.269*	Absent	0.245*	0.291*	2.016*						
F-9	1	0.454*	0.345*	Absent	0.294*	0.357*	2.563*						
F-8	1	0.566*	0.443*	Absent	0.354*	0.438*	3.257*						
F-7	1	0.706*	0.569*	Absent	0.425*	0.538*	4.140*						
F-6	8	0.880±0	0.880-0.905	0.730±0.127	0.64-0.82	Absent	Absent	0.51±0.0424	0.48-0.54	0.66±0.085	0.6-0.72	5.262±0.224	5.103-5.42
F-5	4	1.098±0.025	1.080-1.116	0.780±0.028	0.76-0.8	0.143±0.0247	0.125-0.16	0.6±0.0848	0.66-0.54	0.86±0.085	0.8-0.92	6.308±0.704	5.81-6.806
F-4	5	1.356±0.0477	1.28-1.4	0.922±0.0736	0.8-1	0.344±0.0727	0.26-0.42	0.772±0.082	0.64-0.89	1.032±0.142	0.88-1.26	7.636±0.287	7.47-7.968
F-3	17	1.695±0.094	1.540-1.860	1.375±0.995	0.9-5.16	0.514±0.156	0.26-0.8	0.95±0.075	0.8-1.05	1.335±0.085	1.18-1.46	9.102±1.548	4.73-10.836
F-2	31	2.062±0.096	1.840-2.280	1.718±2.008	1-12.5	0.971±0.171	0.4-1.26	1.221±0.175	1-1.7	1.573±0.211	1-1.922	11.489±1.491	5.805-15.48
F-1	33	2.493±0.106	2.232-2.75	1.510±0.254	1.08-2.3	1.662±0.276	0.7-2.17	1.372±0.158	1.1-1.9	1.946±0.244	1.38-2.709	14.275±1.545	9.675-18.576
F-0	35	3.006±0.183	2.2-3.25	1.872±0.473	1.328-2.418	3.587±0.644	1.08-5.04	1.554±0.159	1.21-2.2	2.197±0.287	1.52-2.6	17.512±2.182	12.13-21.027
	Σ 133												
Fn/F(n+1)		1.247±0.166	1.034-2.307	1.284±0.191	1-1.744	2.111±0.607	1.095-4.624	1.201±0.127	1-1.705	1.227±0.151	1.008-1.806	1.271±0.165	1-1.986

A proportional increase in the size of any body part exists after moulting, known as growth ratio (GR). In hemimetabolous insects such as Odonata, this ratio corresponds to 1.26 (WIGGLESWORTH, 1972). Our results approach this value (Tab. I).

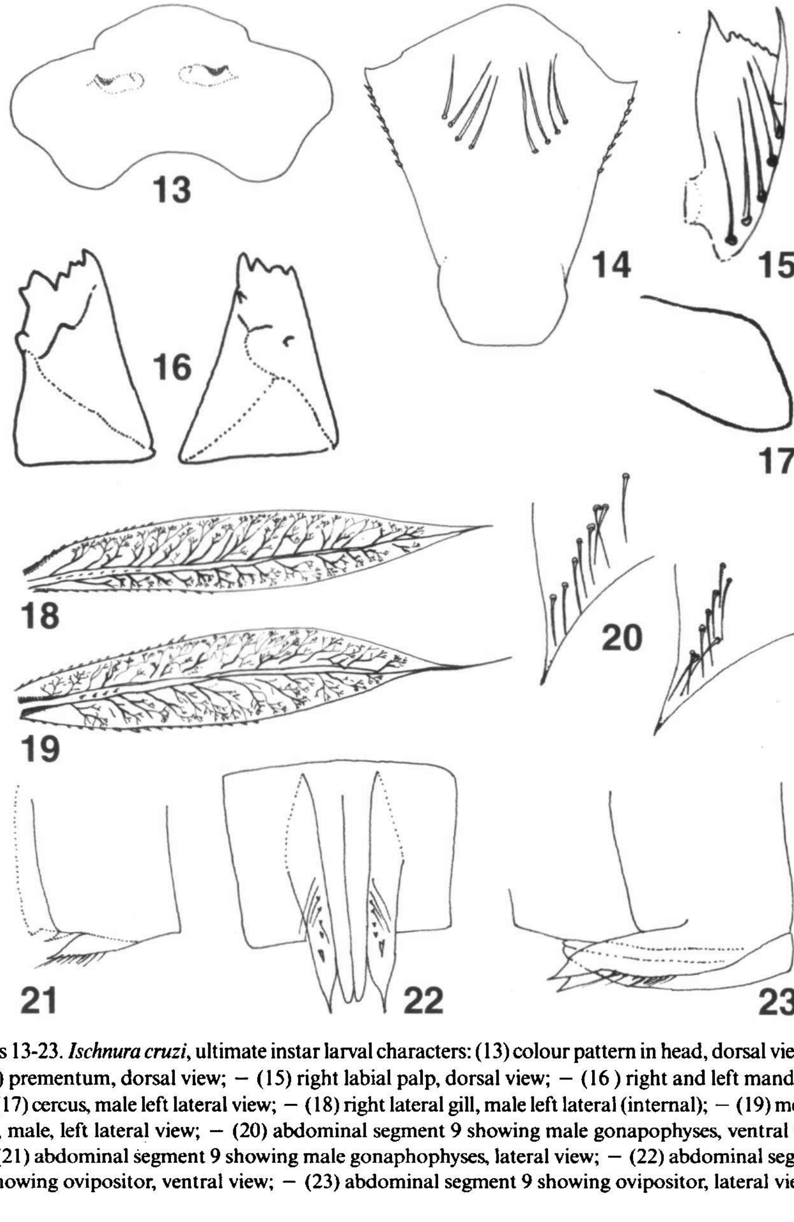
In Table II the larva of *I. cruzi* is compared with *I. ramburii* (GEIJSKES, 1941), *I. capreolus* (GEIJSKES, 1941) and *I. ultima* (MUZON et al., 2005).

In early larval instars, the number of palpal and premental setae best reflects the changes among instars, i. e. only second and third instars have 2 premental and 3 palpal setae. Nevertheless, the appearance of these setae is not strictly obligatory, since some individuals were observed in intermediate instars with different combinations in the number of premental and palpal setae. The definitive formula (4 premental and 5 palpal setae) is reached in the 7th instar.

Pigmentation of the first two antennal segments is present in *I. capreolus* and *I. ramburii*, but in these species pigmentation reaches up to the third segment. Caudal lamellae are similar in *I. cruzi* and *I. ram-*

burii, viz. lanceolated with abundant tracheation.

However, *I. cruzi* can be easily distinguished from the other species because the lamellae present longer acuminate tips. In *I. ramburii* and *I. capreolus* ligula



Figs 13-23. *Ischnura cruzi*, ultimate instar larval characters: (13) colour pattern in head, dorsal view; – (14) prementum, dorsal view; – (15) right labial palp, dorsal view; – (16) right and left mandibles; – (17) cercus, male left lateral view; – (18) right lateral gill, male left lateral (internal); – (19) median gill, male, left lateral view; – (20) abdominal segment 9 showing male gonapophyses, ventral view; – (21) abdominal segment 9 showing male gonaphophyses, lateral view; – (22) abdominal segment 9 showing ovipositor, ventral view; – (23) abdominal segment 9 showing ovipositor, lateral view.

Table II
Morphological character comparison between last larval instar in different *Ischnura* species

Species	Total length	Palpal setae	Mentonian setae	External wing-pads	Lateral ridge of prementum	Internal palp teeth	Antenal segments
cruzi	17.7	5	4	Posterior ridge IV-S	9 setae	4	0.4:0.6:1:0.5:0.4:0.3:0.2
ramburii	19	6	5	median III-S	8- 9 setae	3	0.5:0.8:1:0.7:0.4:0.4:0.2
capreolus	11	5	3	Anterior ridge V-S	4- 5 setae	3	0.4:0.7:1:0.7:0.4:0.4:0.2
ultima	****	6	5	****	7- 8 setae	4	0.4:0.8:1:0.5:0.5:0.4:0.2

of the labial mask is relatively more convex than in *I. cruzi.* The number of premental and palpal setae in *I. cruzi* differs from the other species mentioned.

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