

SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

New Morphological Aspects and Phylogenetic Considerations of *Cicindis* Bruch (Coleoptera: Carabidae: Cicindini)

S ROIG-JUÑENT, S SALLENAVE, F AGRAIN

Lab de Entomología, Instituto Argentino de Investigaciones de las Zonas Áridas – IADIZA, CCT-CONICET, Mendoza, Argentina

Keywords

Cladistics, *Cicindis horni*, morphology, phylogeny

Correspondence

FEDERICO ALEJANDRO AGRAIN, Lab de Entomología, Instituto Argentino de Investigaciones de las Zonas Áridas – IADIZA, CCT-CONICET Mendoza, CC 507, 5500 Mendoza, Argentina; saroig@mendoza-conicet.gov.ar

Edited by Roberto A Zucchi – ESALQ/USP

Received 27 October 2010 and accepted 13 December 2010

Abstract

Cicindis Bruch is a monospecific genus of carabid beetles endemic to Argentina. In this contribution, *Cicindis horni* Bruch is re-described, with addition of new morphological features of male internal sac, female genital tract and elytral closure. New information on the species' habitat and distribution is also provided. The phylogenetic placement and relationships of *Cicindis* within the family Carabidae are discussed on the basis of a cladistic analysis. Terminal taxa included representatives of all subfamilies of Carabidae and supertribes of Carabinae, with a major sampling of those taxa considered to be closely related to Cicindini by previous authors. The phylogenetic analysis shows the basal position of *Cicindis* in a clade that includes Ozaeninae, Omphronini, Scaritinae and Conjuncta. A close relationship of *Cicindis* with Ozaenini + Metriini is supported by the particular closure of the procoxa and the ventral position of the oviduct with respect to the spermatheca.

Introduction

The monotypic genus *Cicindis* Bruch constitutes one of the several enigmatic carabid beetles endemic to the southern regions of South America. It is classified within the tribe Cicindini together with the genus *Archaeocicindis* Chaudoir. Southern southamerican carabid beetles (as other austral American insects groups) are phylogenetically related with the carabid fauna from other regions of the world. Southern southamerican carabids such as zolines, migadopines, and broscines are related to groups occurring in other austral continents (Jeannel 1938, 1967, Darlington Jr 1965, Roig-Juñent & Cicchino 2001). Other members of the southern South America fauna such as trachypachids (*Systolosoma* Solier) and omiines (the genus *Pycnochila* Motschulsky of the tribe Megacephalini) are relictual lineages related to groups also occurring in North America (Roig-Juñent *et al* 2008). In southern South America there are also Pangean taxa

such as Cnemalobini (Roig-Juñent 1993), Notiokasini (Kavanaugh & Nègre 1983), and Cicindini (Kavanaugh & Erwin 1991) which are related to holarctic or tropical carabids.

Beyond the particular pattern of distribution of the tribe Cicindini, with one species in South America and other in Iran, the unusual combination of morphological characters exhibited by *Cicindis* and *Archaeocicindis* had led taxonomists to propose appreciably different hypotheses about its relationship with other carabid groups. When Bruch (1908) described the genus *Cicindis*, he considered it to be related to Nebriini and Omphronini. Other classification schemes considered this genus to be a unique taxon within the tribe Cicindini, related to Ozaenini and Metriini (Bänninger 1925, Bruch 1927, Kryzhanovsky 1976, Reichardt 1977). Erwin & Sims (1984) classified Cicindini within the supertribe Nebriitae, subfamily Carabinae, along with the tribes Nebriini, Notiokasini, Opisthiini, and Notiophilini. Later,

Erwin (1985) hypothesized that Cicindini were closely related to the tribe Notiophilini. Finally, Kavanaugh & Erwin (1991) modified Kryzhanovsky's classification scheme by elevating Cicinditae to the supertribe level and placing it taxonomically between Nebriitae and Elaphritae.

Kavanaugh (1998) presented a phylogenetic analysis including both genera of the tribe Cicindini, and proposed that the tribe is the sister group of a clade comprising *Omophron* Latreille (Omophronini), *Cicindela* L. (Cicindelini), *Omus* Eschscholtz (Megacephalini), *Scaphinotus* Latreille (Cychrini), and *Carabus* L. (Carabini). Because representatives of Ozaeninae and other carabid subfamilies such as Psydrinae were not included in Kavanaugh's analysis, the relationships of Cicindini with these taxa were not tested.

Liebherr & Will (1998) in a phylogenetic analysis using characters from female genitalia found *Cicindis* as part of a polytomy with Migadopini, Amblytelina, Carabidae Limbata, and a monophyletic group conformed by Siagonini, Cychrini, Pamborini, Carabini, and Cicindelini. Liebherr & Will (1998) considered Cicindini in a middle level grade because it possesses gonocoxal rami, but lacks harpalidian type of abdomen. These authors also pointed out the absence of accessory spermathecal gland.

Representatives of the tribe Cicindini have not been included in other phylogenetic analyses using morphological (e.g. Beutel 1998, Kavanaugh 1998), or molecular data (Maddison *et al* 1998, 1999, 2009, Balke *et al* 2005).

The main objectives of this paper are to describe new morphological features of *Cicindis horni* Bruch, such as male and female internal structures and the particular closure of the elytra, and to perform a preliminary cladistic analysis based on adult morphology in order to explore the phylogenetic placement of *Cicindis* within the family Carabidae.

Material and Methods

The description of the morphological variability of *C. horni* is based on examination of 25 males and 14 females. Several specimens of 25 other carabid and trachypachid species were studied for the cladistic analysis (See *Online Supplementary Material 1*). Material for this study was borrowed from entomological collections of the following institutions: Instituto Argentino de Investigaciones de Zonas Áridas Mendoza, Argentina (Sergio Roig-Juñent) (IAZA), Museo Argentino de Ciencias Naturales, "Bernardino Rivadavia," Buenos Aires, Argentina (Arturo Roig- Alsina) (MACN), Museo de Ciencias Naturales de La Plata, La Plata, Argentina (Alberto Abramovich) (MLPA), University of Nebraska State Museum, USA (Brett Ratcliffe) (UNSM), National Museum of Natural

History, Smithsonian, Washington D.C, USA (Terry Erwin) (USNM).

Dissections were made following the techniques used in previous contributions of Carabidae (Roig-Juñent 2000). Drawings were made with camera lucida adapted to a stereomicroscope. Elytral structures were examined and photographed under a compound microscope. A transverse section of the elytron was made using a microtome after inclusion of the elytron in paraplast solution. Scanning electron microscope pictures were taken using a LEO 1450 VP microscope. Terminology used follows previous revisions (Deuve 1988, 1993, Liebherr & Will 1998, Roig-Juñent 1998, 2000, **Roig-Juñent & Cicchino 2001**).

Cladistic analyses

In our analyses we included representatives of all the supertribes of Carabinae and of the other carabid subfamilies, especially those for which previous authors proposed closer phylogenetic relationships with *Cicindis*.

For the cladistic analysis, a total of 50 adult morphological characters (*Online Supplementary Material 2*) were scored for 27 species belonging to six subfamilies and 20 tribes. These species represent all the subfamilies proposed by Erwin & Sims (1984) and 20 of the 86 tribes. Characters in the text are referred to by number and their states appear in superscript (*i.e.* 10¹).

A representative species of the family Trachypachidae, regarded as the sister taxon of Carabidae in previous works (Erwin 1985, Beutel 1998, Kavanaugh 1998, Roig-Juñent 1998), was used to root the tree.

Morphological characters used in this analysis correspond to those proposed for the higher classification of Carabidae in previous studies (Sloane 1923, Jeannel 1941, 1955, Bell 1967, Erwin 1985, Nichols 1985, Deuve 1993, Baehr 1998, Liebherr & Will 1998, Roig-Juñent & Cicchino 2001). All characters were considered to be non-additive (unordered). The data matrix is presented as the *Online Supplementary Material 3*.

Data analysis

Phylogenetic analyses were performed using parsimony software TNT (Goloboff *et al* 2003). The data set was analyzed using two procedures: (a) equally weighted character analysis, and (b) implied weighting method (Goloboff 1993), exploring the topologies obtained with different K (constant concavity) values from K = 1 to K = 6. All analyses were conducted using a traditional heuristic search on the base of Wagner trees, 100 random addition sequences, followed by the tree-bisection reconnection (TBR) swapping algorithm, saving 100 trees per replicate. Branch robustness was assessed by standard Bootstrapping and Jackknifing (removal

probability = 36), with 500 replicates, searching among trees with traditional search for the equally weighted analysis. Bremer support and symmetric resampling (change probability = 33) were used as support values for implied weighting analyses since neither of these two measures is distorted by weight. All support numbers are given as relative values.

Redescription

Characters not described in Kavanaugh & Erwin (1991) are provided.

Systematic remarks

The new material of *C. horni* shows some interesting morphological differences from that described by Kavanaugh & Erwin (1991) and from the material preserved in MACN and MLPA. The most remarkable difference is in the color pattern (the frontal and central part of the pronotum, and the tarsi have a darker coloration with red tones). Other characters showing variation are the number of glossal sclerites and the number of parameral setae. New features previously not noticed are antennomeres 5-11 with a lateral relief colored and asetose, the special closure of the elytra, a post-orbital furrow and the ventral position of oviduct with respect to the spermatheca.

Male

Color. Base of head, body, and appendages pale yellowish-white. Apex of mandibles reddish-black to black (Fig 1). Elytra with distinct dark pattern, reddish-brown, with median maculae covering almost the whole surface of the elytra (Figs 1, 16). Elytral lateral margin and first interneur pale yellowish (nearly white).

Head: Mouthparts. Glossal sclerite with apical margin concave with four setae, two apical and two medial on the midline (Fig 2); paraglossa not fused to the glossal sclerite, equal in length to the glossal sclerite. Mentum with epilobes rounded, narrowly toothed antemedially (Fig 3), with two paramedian setae, nine pairs of marginal setae, and three pairs of central and basal setae. Maxillary palpi long and slender (Figs 4-5). Frons with sulcus obsolete. **Antennae.** Antennomeres 5-11 with a lateral relief extending from base to preapex (Figs 6-7). **Eyes.** Ocular furrow behind the eyes (Figs 8-11) covered with pronotum setae, these setae with a particular brush-like shape. Another particularity are the setae on the basal pronotum margin.

Legs. Fore tarsomeres 1-4 not expanded laterally, pads of articulo-setae with straight cylindrical shafts bearing

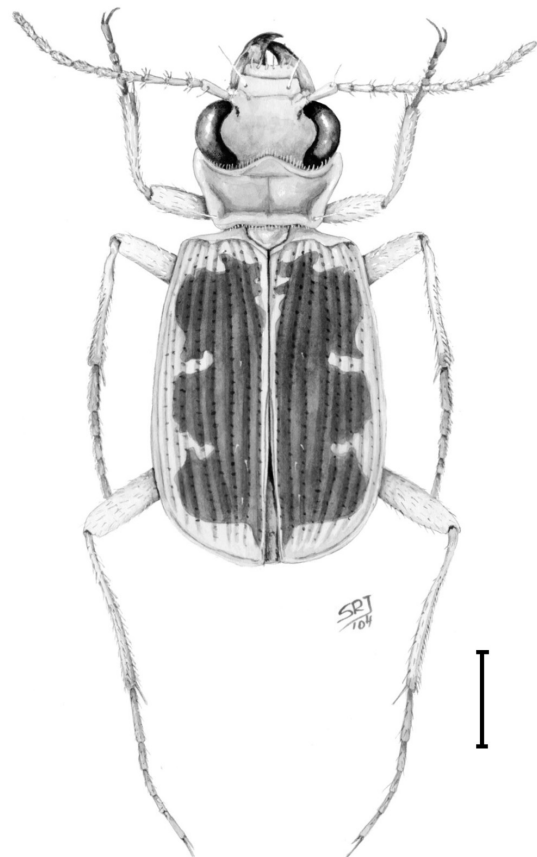
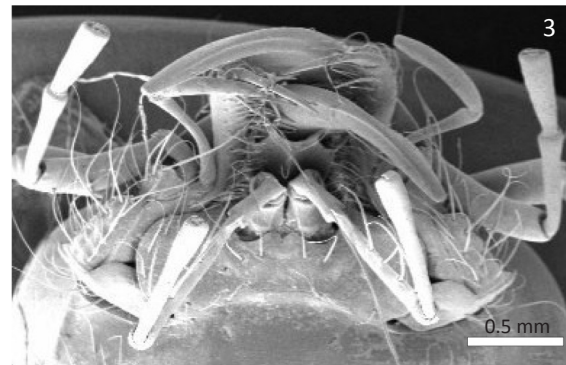
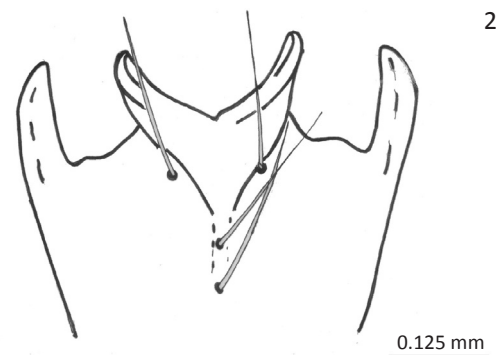
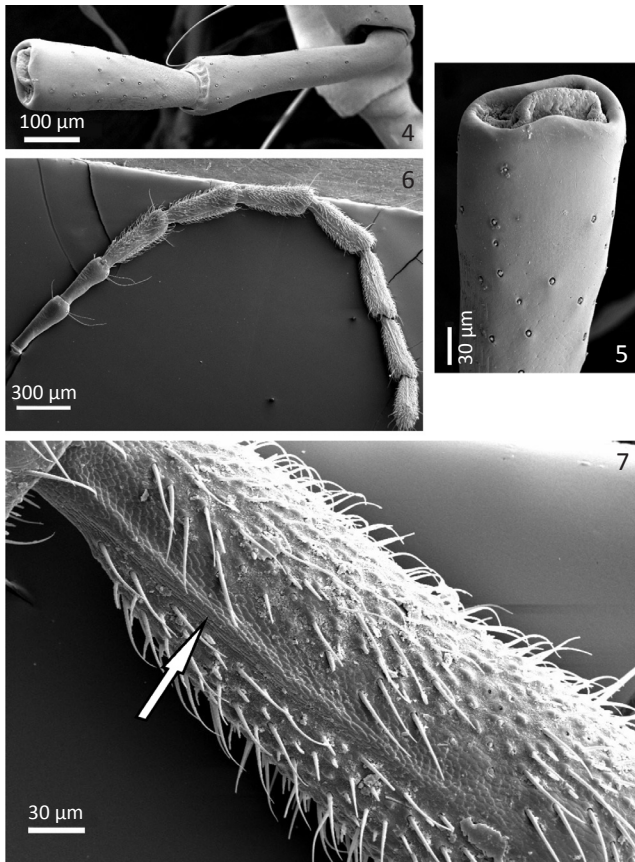


Fig 1 Dorsal habitus of *Cicindis horni* Bruch, male. Scale 1 mm.



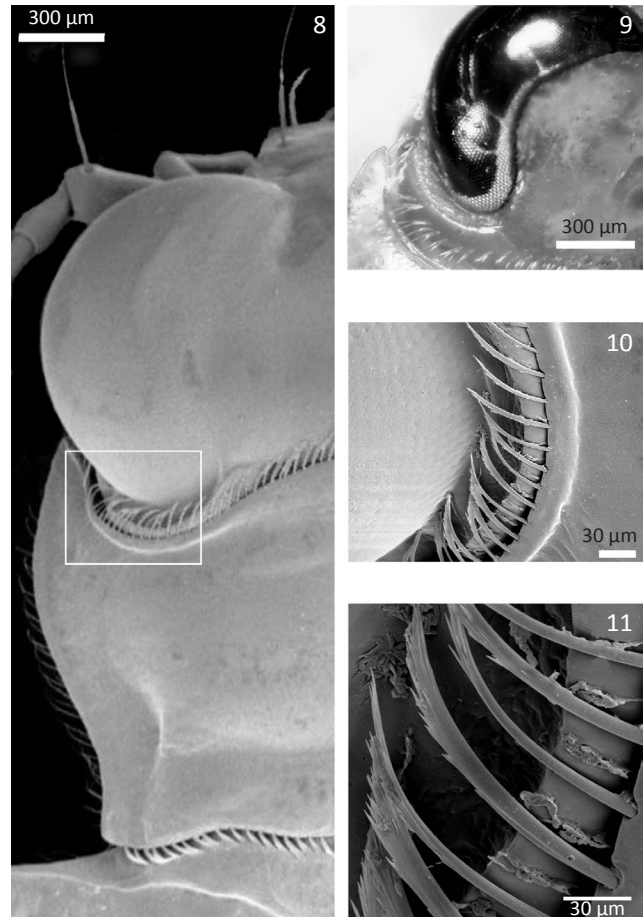
Figs 2-3 *Cicindis horni*. 2) Glossa and paraglossa, scale 0.125 mm; 3) Mouthparts, ventral view.



Figs 4-7 *Cicindis horni* 4) Right maxillary palpi; 5) Right maxillary palpomere; 6) *Cicindis horni*, antenna; 7) *Cicindis horni*, antenna relief.

regular round plates (Figs 14-15). Posterior procoxal closure with proepimeron fitted into the lateral arm of the prosternal process (Fig 12). Posterior claws of anterior and posterior tarsi equal to 0.66 the length of anterior claw, posterior claw of middle tarsi 0.5 the length of anterior claw. Cleaning organ with curved clamp setae (Fig 13).

Elytra. Humeral region well developed, rounded; elytra apex rounded (Fig 16). Interneurs 1 to 7 striatopunctate, intervals slightly convex. *Cicindis* adults have two different elytral closure systems; the first one goes from the elytral base to about 0.5 mm after the scutellum (Fig 17) and consists of a thickened fusiform structure, a transverse cut of this region (Fig 18) shows that this structure is hollow, and presents several rows of spicules on the inner side which, together with the metanotal furrow (Fig 19) seem to be responsible for the strong elytral locking. This helps confirm the observation presented in **Erwin & Aschero (2004)** that these beetles carry air bubbles under the elytra for diving. The second closure system extends between the end of the first one and the elytral apex. This system is formed by the two sutural borders of each elytron, the right elytron (Figs 20-21)

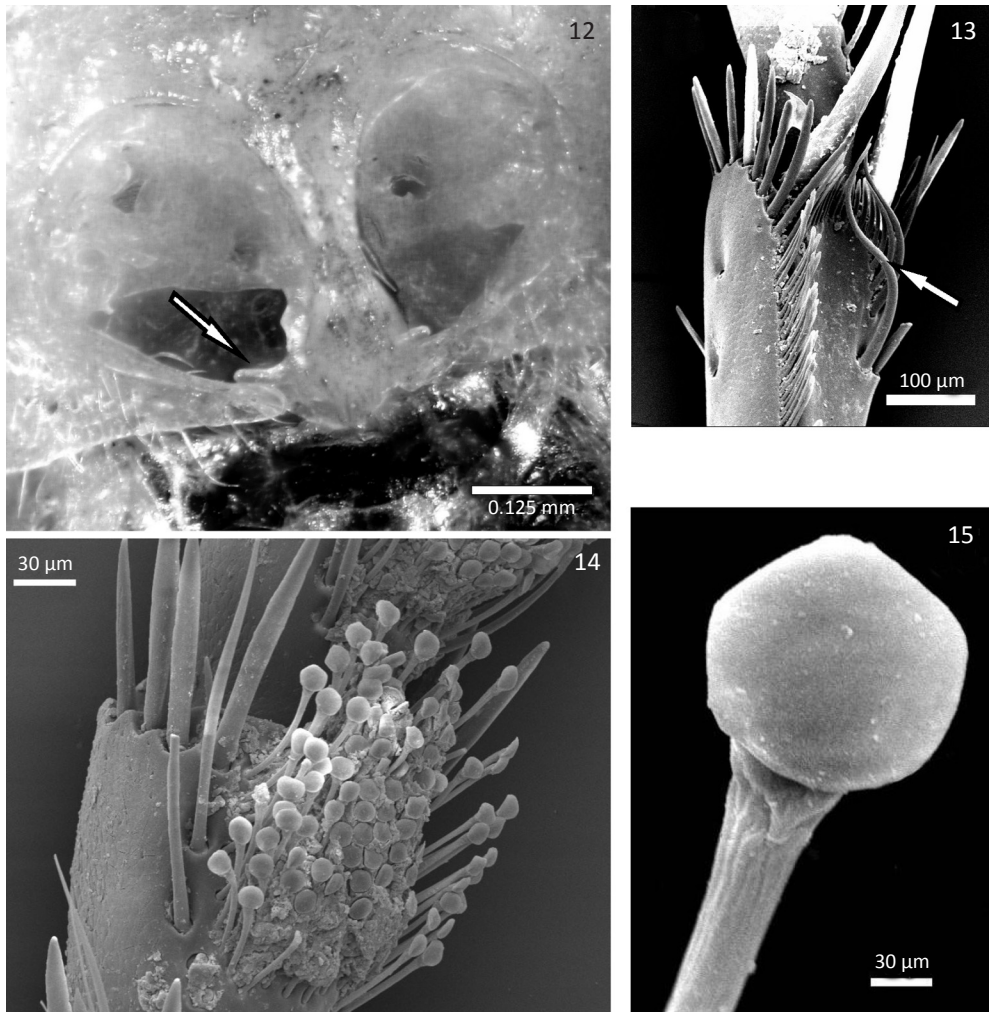


Figs 8-11 *Cicindis horni* 8) Sagittal cut of left region of eye and pronotum; 9) Ocular furrow; 10) Detail of ocular furrow; 11) Pronotum setae over ocular furrow.

has a furrow into which fits a membranous region of the left elytron (Fig 22). Both elytral margins have several rows of tapered spines (Fig 23) that could help to a better joining between both elytra. The relevance of this elytral closure-system is both functional and physiological, we suggest that this system might aid the maintenance of an air bubble during submergence for short period of time as observed by Erwin & Aschero (2004). Although the dense vestiture of the abdomen (Fig 24) seems appropriate to act as a plastron, we could not observe air trapped into it, neither did Erwin & Aschero (2004), therefore the bubble trapped by the elytral closure might aid underwater respiratory function.

Abdomen. First visible sternite of abdominal sternum with lateral concavity weakly impressed.

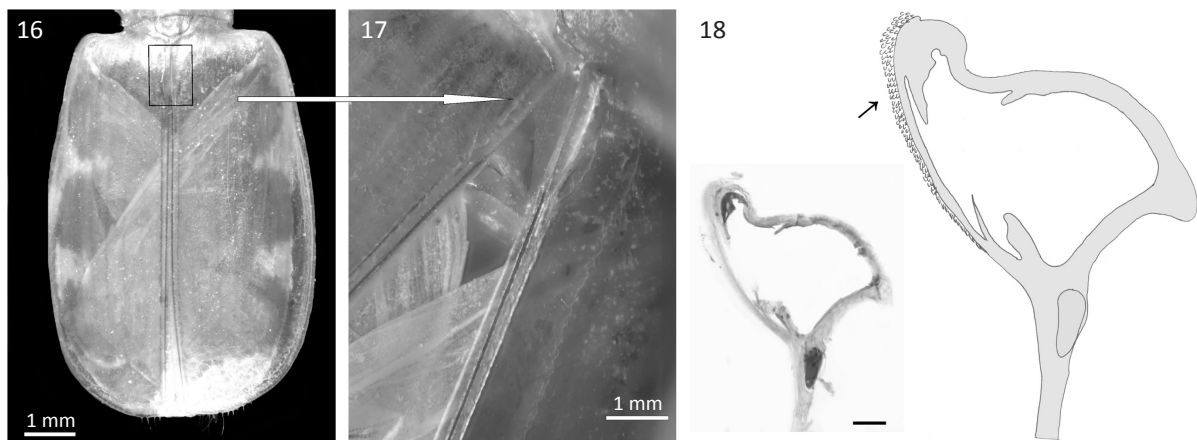
Male genitalia. Median lobe (Figs 25-28) short, with broad apical orifice. Parameres similar in shape, with two-four **on?** apical setae on the left paramere (Fig 26), and two or three on the right paramere (Fig 27). Internal sac (Figs 29-30). Apical orifice opened dorsally in the central



Figs 12-15 *Cicindis horni* 12) Procoxal closure; 13) Curved clamp setae; 14) First and second male protarsomeres with pads; 15) Ventose pads.

region, a series of grouped spicules is visible in cleared material of internal sac. Figs 29-30 show progressive eversion of internal sac. The internal sac is composed of two groups of non-fused spicules. There is a group of

apical spicules (Figs 29-30 *ap*) surrounding the gonopore, and another cluster of spicules forming an incomplete ring (Figs 29-30 *r*) which is basal to the other group. In cleared non-everted internal sac a group of spicules (Fig



Figs 16-18 *Cicindis horni* 16) Elytra, dorsal view; 17) Basal thickness of the elytra. First elytral closure system; 18) Transverse cut of the first elytral closure system. Scale 25x.

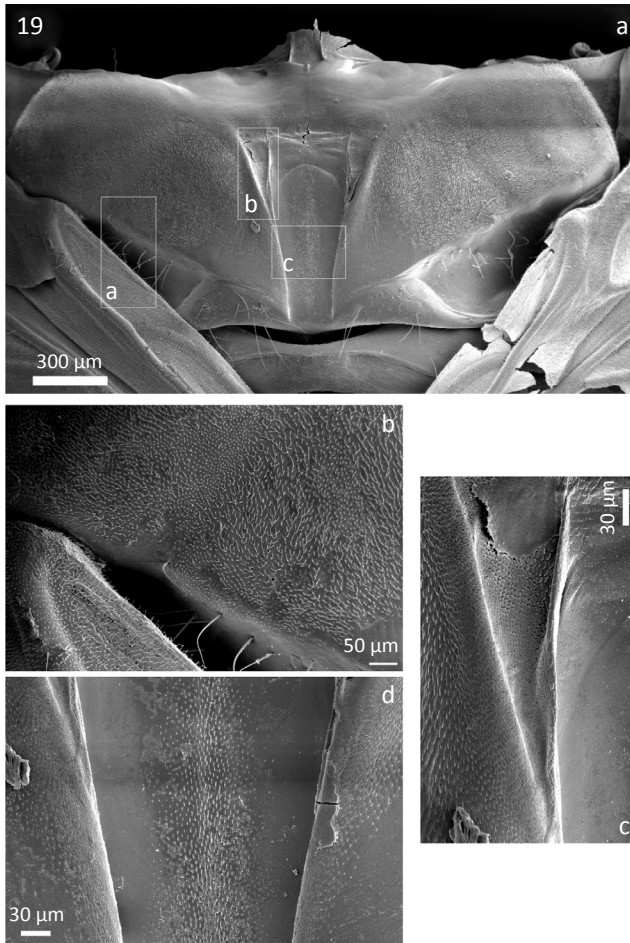
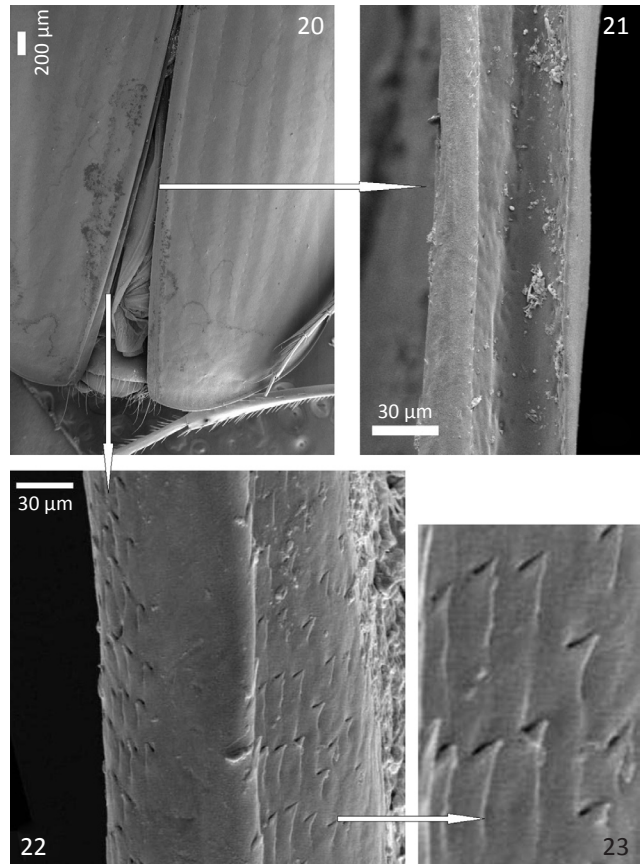


Fig 19 Metanotum: a. Complete metanotum, b. Surface detail of metanotum and membranous wing, c. detail of superior left furrow, d. detail of middle furrow.

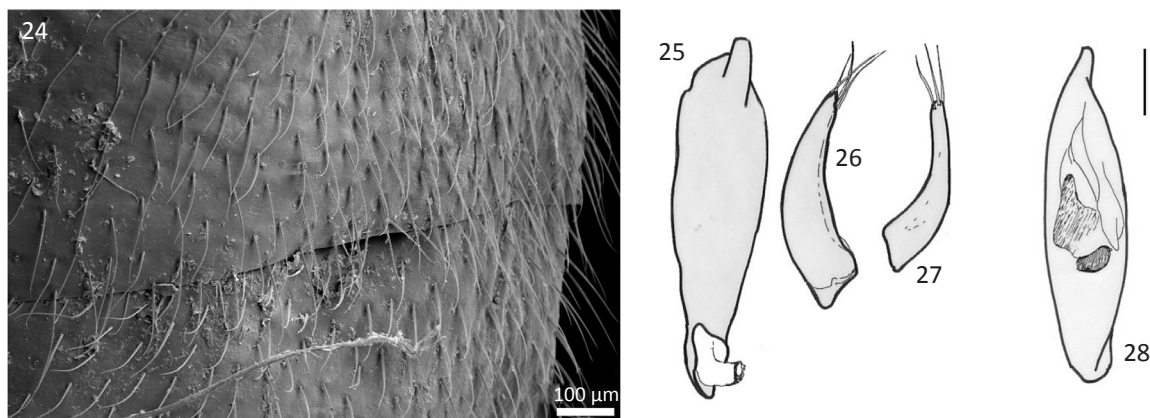
30a, *sp*) is visible. These spicules are in a position similar to the X sclerite present in other carabid groups, such as Broscini and Paussinae (Roig-Juñent 1998). However, in the fully everted internal sac, it is easy to see that it is not



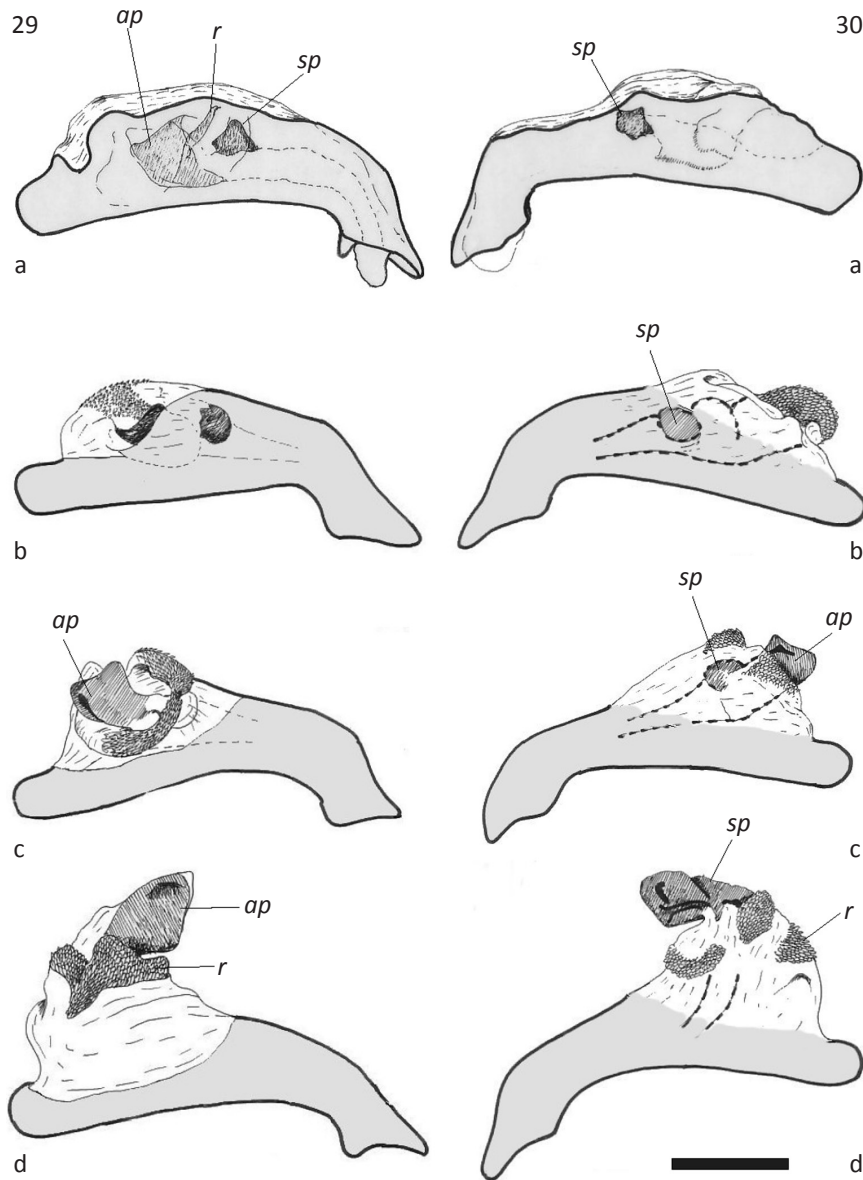
Figs 20-23 *Cicindis horni* 20) Apical region of the elytra, dorsal view; 21) Second elytral closure system: Groove on the right elytron 22) Second elytral closure system: Membrane on the left elytron; 23) Elytral closure system (spicules).

a sclerotized plate, but a group of free non-fused spicules immersed in a folded region of the membrane, and this is part of the group of apical spicules (*ap*).

Female genitalia. Kavanaugh & Erwin (1991) described the female genitalia with monomerous gonopods, with a sub-



Figs 24-28 *Cicindis horni* 24) Abdomen vestiture; 25) Male genitalia, ventral view. Scale 1 mm; 26) Left paramere. Scale 1 mm.; 27) Right paramere. Scale 1 mm.; 28) Male genitalia, dorsal view. Scale 1 mm.



Figs 29-30 *Cicindis horni*, internal sac eversion at different steps from a. (rest position) to d. (completely everted): 29) right lateral view; 30) left lateral view. *ap* (group of apical spicules), *r* (ring of spicules), *sp* (spicules). Scale 1 mm.

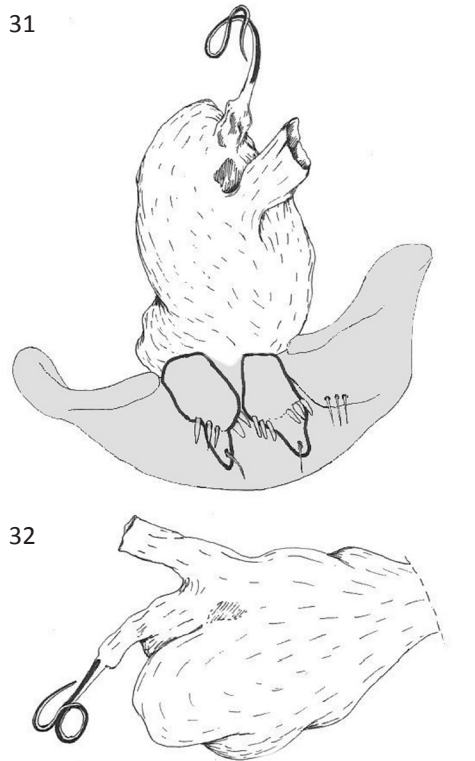
apical setiferous organ with one nematiform seta. In the present study we report the presence of the helminthoid sclerite, which is present in the spermatheca of several groups of basal carabids (Deuve 1988). The gonopod VIII (ramus coxae in Liebherr & Will 1998) is absent. The latter authors considered this structure as present for *Cicindis* in their data matrix. Another important character is the relative position of the spermatheca, which is ventral with respect to the oviduct (Figs 31-32). Deuve (1988) mentioned that this character is present in Paussinae, constituting an apomorphy of the group.

Material Examined

Holotype. *Cicindis horni* Bruch, female at MACN, Córdoba, Argentina.

Type locality. Bruch (1927) mentioned that the specimen was captured by Ernesto Piotti in light trap. Bruch did not know the type locality; he supposed that this species inhabits the mountain regions near Cordoba city, although Kavanaugh & Erwin (1991) designated Guanaco Muerto as the type locality. We argue that the type locality remains unknown, since specimens from Guanaco Muerto are not the type material.

Other material examined. **Catamarca:** two males and one female, 30 km S of Recreo, intersection of Routes 167 and 60, 3-12-2003, Gómez, Ocampo & Roig-Juñent coll. 29° 30' 29" S 64° 55' 38" W (IAZA) (one male preserved in 100% ethanol at -20C° at IAZA) (**New record**); 20 males and ten females from the same locality, 25/2-5/3-2006, Roig-Juñent, Erwin, Sallenave & Agrain colls. (IAZA, MLPA) (13 males and eight females in ethanol). **Córdoba:**



Figs 31-32 *Cicindis horni* 31) Female genitalia. Scale 1 mm.; 32) Bursa and spermatheca. Scale 1 mm.

two females from Route 60 km 895.5, 30° 00' S, 64° 30' W, 13-I-2004, Erwin & Aschero colls.; two males from the same locality, 25/2-5/3-2006, Roig-Juñent, Erwin, Sallenave & Agrain colls. (IAZA) (one in ethanol); one male from Guanaco Muerto 30° 28' 60" S, 65° 2' 60" W 12/1979, Ronderos coll. (MLPA). One female, San Luis between Agro Candelaria and Mina Los Dos Buhos (31° 59' 09" S, 65° 58' 00" W), 378 m. 7-IV-2010, Ocampo & Roig-Juñent Coll. (IAZA) (in ethanol).

Geographical distribution. New specimens were collected from two different provinces. The first is from Catamarca (30 km S of Recreo). This locality is about 100 km away from Guanaco Muerto (previously known locality of *C. horni*), and about 40 km N of the locality cited by Erwin & Aschero (2004). This is the first record for Catamarca province in Argentina. The second new material was collected in San Luis province at night with normal + UV lights, in a locality characterized by dry forest vegetation, in the Espinal Biogeographical Province. This locality is about 59.6 km W from the nearest salt area: Pampa de Las Salinas, more than 200 km S from the southern known locality (Guanaco Muerto) and 138 km from the southern tip of Salinas Grandes. The latter new record could support the idea that this species is distributed in other Salines in Argentina, and future explorations are needed to know if it is present in other northern

or southern salines areas. Furthermore, the presence of *Cicindis* in dry areas, far from Salines may indicate that this species have a higher dispersion power than previously thought.

Habitat

The information that follows is supplemental to that provided by Erwin & Aschero (2004). The new collected material of *C. horni* was found in the saline habitat of "Jumadales" (*sensu* Ragonesse 1951). Water analyses for the two collection areas are presented in Table 1 and shows a great concentration of salt. This is important because this enigmatic carabid beetle swing and prey in these high concentrate salt water (Erwin & Aschero). This habitat is a halophytic community that covers large areas of salty soils. The two dominant plant species are *Allenrolfea patagonica* (jume), and *Atriplex argentina* (zampa), both succulent Chenopodiaceae, between 0.3 and 0.8 m high, adapted to high salinity soils of Argentinean deserts. These plants grow in clumps forming an open low shrub community. In the place where *C. horni* was found, vegetation cover is about 60%. There were also other shrub-like plants in the area such as *Heterostachys hritteriana* (jumecillo) (Chenopodiaceae), *Grahamia bracteata* (Portulacaceae), and *Prosopis reptans* (retortuño) (Fabaceae), the latter being a species endemic to the Argentinean "Salinas" (Ragonesse 1951). Among herbaceous plants there are *Monantochloe littoralis* (Gramineae) and *Helitdropum johnstonii* (Boraginaceae). The area where the specimens of *C. horni* were collected forms part of "Salinas Grandes", one of the areas studied by Ragonesse (1951, p. 46).

The climate in the area is characterized as continental, semiarid, and mesothermal, with dry winters and extreme temperatures. Average annual humidity is 58% and annual precipitation in localities close to the area is between 305 mm and 491 mm, usually occurring between November and March. The pool where *Cicindis* was collected has about 6% salt concentration. Some specimens were collected swimming on the water. It is interesting to note that when some collected specimens were placed in fresh water, they sank, losing their ability to float.

Cladistic Analyses

For the equally weighted analysis the program analyzed a total of 7,614,223 rearrangements, and the best score hit 87 times out of 100. Four optimal trees of 176 steps were obtained. The strict consensus tree is shown in Fig 33. The cladogram shows *C. horni* as related to Scaritinae, Paussinae, and Carabidae Conjuncta. Paussinae and Carabidae Conjuncta are monophyletic groups, but not

Table 1 Water chemical analyses results for each studied site.

Variables / ions	Córdoba		Catamarca	
	Meq/l	Ppm	Meq/l	Ppm
Ca ⁺⁺	80.00	1600.00	64.00	1280.00
Mg ⁺⁺	20.00	243.00	16.00	194.40
Na ⁺	4000.00	92000.00	750.00	17250.00
K ⁺	10.53	411.72	2.46	96.19
CO ₃ ⁼	0.00	0.00	0.00	0.00
HCO ₃ ⁺⁺	1.60	97.60	2.00	122.00
Cl ⁻	4260.00	151017.00	760.00	269.42
SO ₄ ⁼	120.00	5760.00	160.00	7680.00
NO ₃ ⁻	Negative	Negative	Negative	Negative
B	Negative	Negative	Negative	Negative
Conductivity: microsiemens/cm at 25°C)	208000		66400	
pH	7.49		7.14	
Dry residuum (ppm)	277340		56648	
Sodium adsorption ratio (SAR)	565.00		118.67	
Total ions (Mg/l)	252720.32		26649.52	

*Percentage between electric conductivity and salt residuum equal to 22% of total saline material for Cordoba site, and 6.15% for Catamarca site (expressed as NaCl).

Scaritinae, where Migadopini (*Migadops* Waterhouse and *Antarctonomus* Chaudoir) and Loricerini (*Loricera* Latreille) constitute a polytomy, and Elaphrini (*Elaphrus* Fabricius) is more closely related to Carabidae Conjuncta than the other Scaritinae. Paussinae is the first group splitting from this clade, the second is *Cicindis*, and finally Scaritinae + Carabidae Conjuncta. This consensus tree is highly congruent with the general classification of Carabidae proposed by Erwin & Sims (1984), except for the position of *Cicindis*. Erwin & Sims (1984) placed *Cicindis* within the Carabinae, a group that after this analysis turned out to be polyphyletic. Carabidae Conjuncta constitutes a monophyletic group (Fig 33), which is congruent with the divisions Melaeniforms plus Psydriforms presented by Erwin & Sims (1984). The conjuncta condition (character 22¹) was parallelly acquired by *Notiophilus* Dumeril. The analysis shows that the group defined by Jeannel (1941) as Styliifera is currently separated into two subfamilies, Broscinae and Psydrinae (Roig-Juñent & Cicchino 2001), and is paraphyletic in this analysis. Support values to the different clades are relatively low. For this reason a second analysis using implied weighting to reduce homoplasy was performed.

Analyses using implied weighting method resulted in all cases (tested K values = 1 to 6) in only one tree. There were no differences in the proposed phylogenetic relationships of *Cicindis* between the cladograms obtained

with K = 2 to K = 6, but the topology is different for the phylogenetic relationships of the remaining Carabinae when K = 1 is applied (Fig 34). With K = 1, Carabinae constitute a paraphyletic group, where Nebriini (*Nebria* Latreille and *Leistus* Frölich) is the basal group of Carabidae, and of the following sequence: Notiophilini, Opisthiini, Carabini + Ceroglossini + Cychrini, and Cicindelini + Megacephalini. Omophronini is more closely related to Scaritinae + Carabidae Conjuncta. With K = 3 (Fig 35) or higher, Opisthiini is the basal clade of Carabidae, followed by Nebriini, and the remaining Carabinae (except *Cicindis* and *Omophron*) constitute a monophyletic group.

In the analysis under equal weight, *Cicindis* + Scaritinae + Paussinae + Carabidae Conjuncta constitute a monophyletic group. In the analyses using implied weighting method this clade is also recovered, but including the tribe Omophronini. This clade is separated from the rest of the Carabinae, and is supported by synapomorphies 0¹, 10¹, and 30¹². Support values are still very low (Fig 35). Anyhow the relationships inside the generated clades are the same and the support values for this alternative topology are also very low (5). In all cases *Cicindis* constitutes a monophyletic group with the Paussinae, supported by two exclusive characters, the particular condition of the procoxal closure (19²) and the position of the oviduct with respect to the spermatheca (49¹).

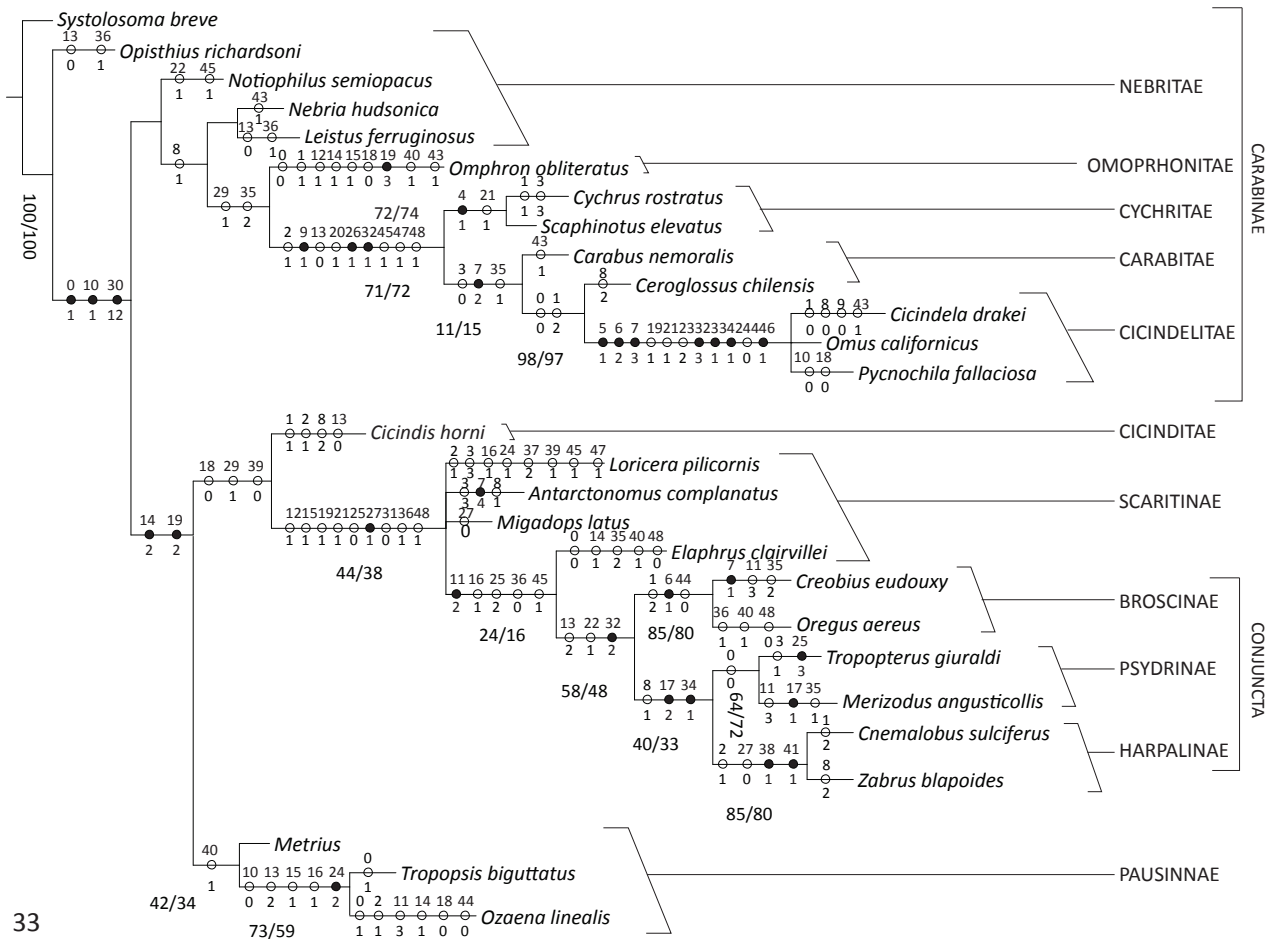


Fig 33 Strict consensus tree of four trees. Analysis under equal weights. With Jackknife (left) and Bootstrap (right) of tree resulting from equally weighted analysis (relative values).

Discussion

The study of a longer series of *Cicindis* specimens yields new characters such as those of male and female genitalia and the closure of the elytra, and also the recognition of color variation previously unknown.

Results of the cladistic analyses, based on adult morphological characters, show differences from the current classification and from recent proposals on the relationships of *Cicindis*. Based on the phylogeny obtained, *Cicindis* should be regarded as a member of, or as related to, the subfamily Paussinae instead of Carabinae as suggested by Kavanaugh & Erwin (1991). Kavanaugh (1998) pointed out that the inclusion of *Cicindis* and *Archaeocindis* in the clade that contains Carabinae is supported by 11 synapomorphies. But he did not list the 153 characters used in his analysis, so it is not possible for us to compare his characters with the characters used herein.

As mentioned previously Liebherr & Will (1998) considered Cicindini in a middle grade group of Carabidae together with Migadopini, Amblytelina and

a monophyletic group of tribes (Siagonini, Cychrini, Pamborini, Carabini and Cicindelini). These latter authors only used characters from female genitalia, highlighting the importance of the gonocoxal rami in Cicindini as to include it in this group of Carabidae. Nevertheless, after the dissection of several females we did not found the gonocoxal rami in the female track.

Relationships between *Cicindis* and Paussinae (*Metrius*, *Ozaena* and *Tropopsis* in our analysis) were also previously proposed by Bänninger (1925) and followed by several other authors (Bruch 1927, Kryzhanovsky 1976, Reichardt 1977). Bänninger (1925) considered *Cicindis* to be related to Metriini because of its particular protibial structure (mainly the antenna cleaning organ) and procoxal closure. The presence of curved clamp setae in the cleaning organ (14^2) supports the group that includes *Cicindis* + Paussinae + Scaritinae + Carabidae (Conjuncta). Additional characters shared with other members of the clade are: spurs equal in size, both apical (11^0), the disjunct condition of the mesocoxae (22^0), the left paramere with few setae (37^1), and the right paramere setose (39^0) and plesiomorphic, and thus not

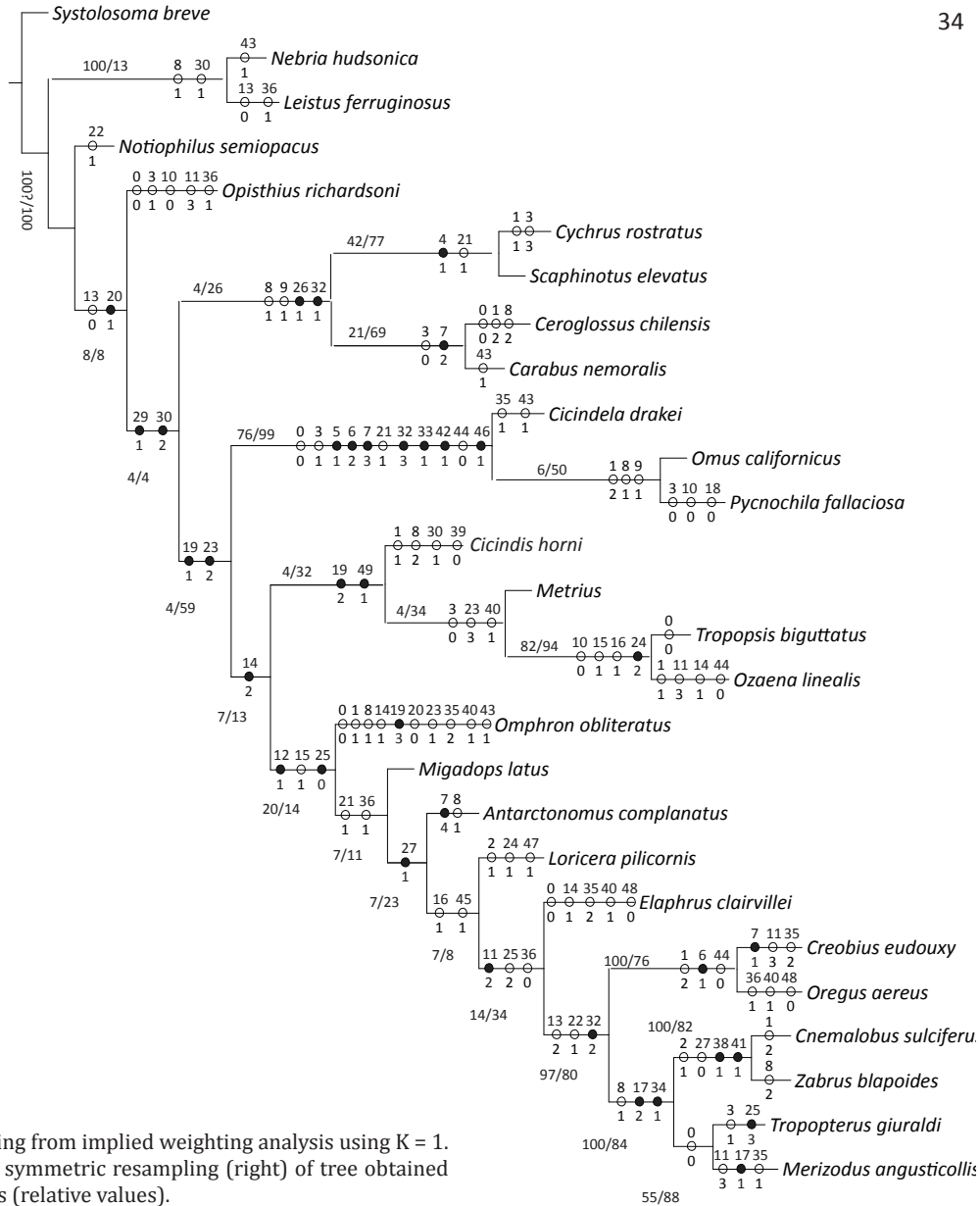


Fig 34 Tree resulting from implied weighting analysis using K = 1. Bremer (left) and symmetric resampling (right) of tree obtained with K = 1 analysis (relative values).

useful for grouping *Cicindis* with other carabids. The only two apomorphies which are exclusive characters of Paussinae and *Cicindis*, and support this relationship under K = 1, are the particular condition of the procoxal closure (19²) and the position of the oviduct with respect to the spermatheca (49¹). Confidence in the phylogenetic value of these two characters is provided by Nichols (1985), who made an analysis of the different kinds of coxal closure, and pointed out that the proepimeron overlapping the prosternum is exclusive to Paussinae, and by Deuve (1988), who described the female genital tract of almost all Carabidae and noticed that only in Paussinae is the oviduct dorsal with respect to the spermatheca (49¹). Our finding that *Cicindis* has the same character condition as described for the Paussinae is worth knowing.

A further difference from the classification by Erwin & Sims (1984) is that the subfamily Carabinae does not constitute a monophyletic group in our cladograms where Omophronini (*Omophron* Latreille) is more closely related to Carabidae Conjuncta, and Opisthiini (*Opisthius* Kirby) and Nebriini could be considered to be the adelphotaxon of all Carabidae. The only group of Carabinae recovered as natural by our analysis consists of the supertribes Cychritae and Cicindelitae.

Scaritinae do not constitute a natural group in our analysis. Kavanaugh (1998), in his analysis of basal groups of Carabidae, also found Scaritinae as non-monophyletic, with *Blethisia* (Elaphrini, Scaritinae) being the sister group of *Loricera* (Loricerini, Scaritinae) + *Calathus* Bonelli (Platynini, Harpalinae). Nevertheless,

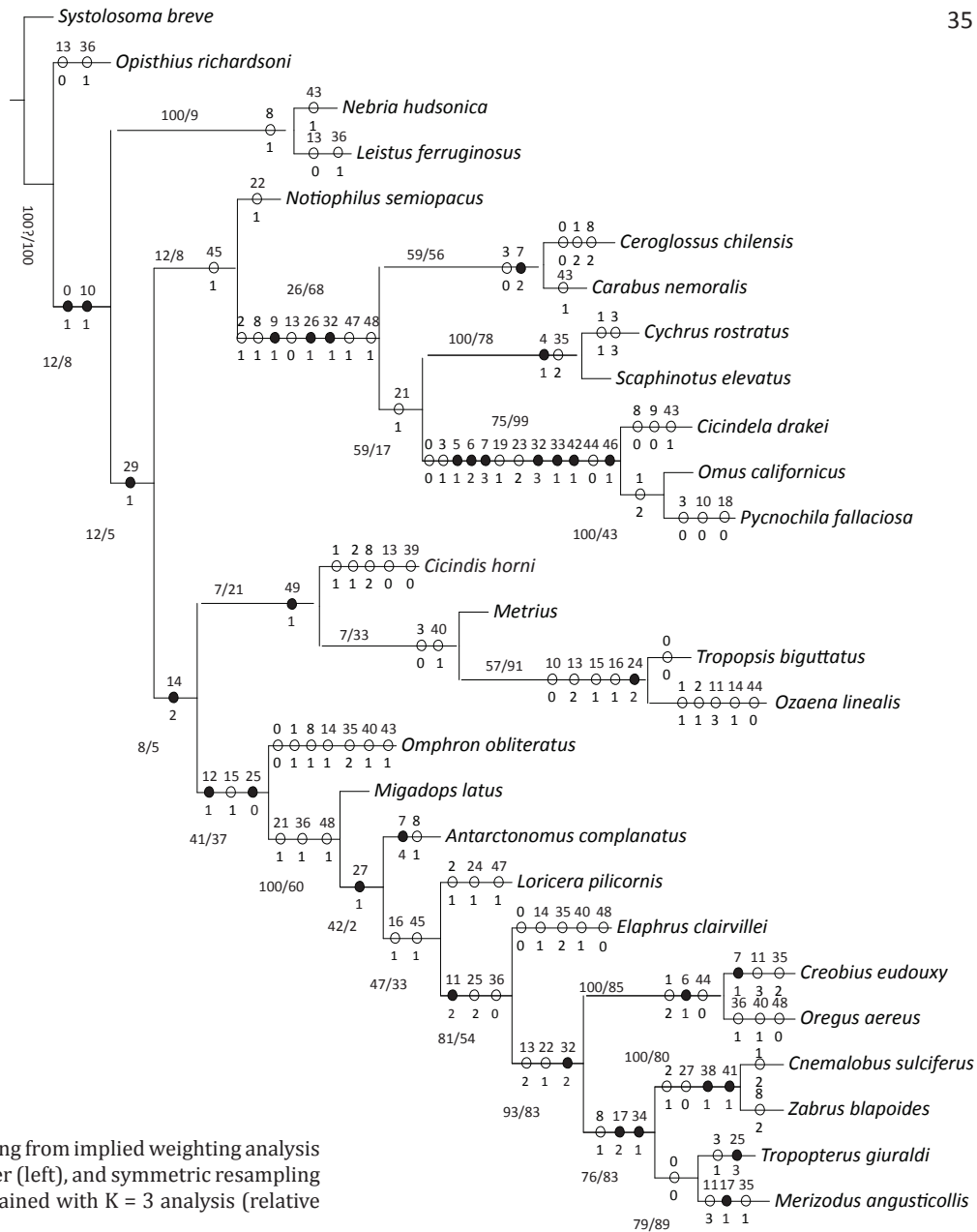


Fig 35 Tree resulting from implied weighting analysis using K = 3. Bremer (left), and symmetric resampling (right) of tree obtained with K = 3 analysis (relative values).

there is a significant difference between Kavanaugh’s 1998 analysis and ours. In the former, Scaritinae + Carabidae (Conjuncta) is the sister group of Carabinae (including *Cicindis*) and in the present analysis this group is more closely related to Omophronini + Paussinae + *Cicindis*.

In summary, although weakly supported, the obtained topologies show important differences from previous classifications, since in our study *Cicindis* always forms a monophyletic group with the Paussinae. Additional, independent sets of data, such as molecular or larval characters, will help us to get a better knowledge of the evolutionary history of *Cicindis*.

Acknowledgments

We express our gratitude to Dr Terry Erwin for his invaluable professional help during the field trip and for his hospitality and support during our stay at Smithsonian Institution in Washington DC. We are grateful to Adriana Marvaldi for her comments on a previous version of the manuscript and for her critical review and two other anonymous reviewers for the useful comments. We also thank Rodrigo Gómez for his help during the first collecting trip. To Nelly Horak for useful English advice, finally to Ana Scollo and María Auxiliadora Lazzaro for technical support. We acknowledge the Consejo Nacional

de Investigaciones Científicas y Técnicas (CONICET, Argentina) for support through grant PIP 5766. This contribution is part of the project "La región austral del Chaco, su evolución histórica a través de reconstrucciones de los patrones biogeográficos y evolutivos de los componentes de su artropofauna" CONICET, PIP N°: 1869.

References

- Baehr M (1998) A preliminary survey of the classification of Psydrinae (Coleoptera: Carabidae), p.359-368. In Ball GE, Casale A, Vigna A, **Taglianti** (eds) Phylogeny and classification of Caraboidea (Coleoptera: Adephaga). Torino, Mus Reg Sci Nat, **total number of pages**.
- Balke M, Ribera I, Beutel RG (2005) The systematic position of Aspidytidae, the diversification of Dytiscoidea (Coleoptera, Adephaga) and the phylogenetic signal of third codon positions. *J Zool Syst Evol Res* 43: 223-242.
- Bänninger M (1925) Neunter Beitrag zur kenntnis der Carabinae: die Nebriini. *Mitt Muench Entomol Ges* 14: 180-195, 256-281.
- Bell RT (1967) Coxal cavities and the classification of the Adephaga (Coleoptera). *Ann Entomol Soc Am* 60: 101-107.
- Beutel RG (1998) Trachypachidae and the phylogeny of Adephaga (Coleoptera), p.81-106. In Ball GE, Casale A, Vigna A, Taglianti (eds) Phylogeny and classification of Caraboidea (Coleoptera: Adephaga), Torino, Mus Reg Sci Nat, **total number of pages**.
- Bruch C (1908) Eine neue Carabidengattung aus Argentinien, *Cicindis horni* (Col.). *Dtsche Entomol Z* 1908: 497-500.
- Bruch C (1927) Tres interesantes coléopteros. *Rev Soc Entomol Argent* 1: 15-22.
- Darlington Jr PJ (1965) Biogeography of the southern end of the world. Distribution and history of far southern life and land, with an assessment of continental drift. **City**, Harvard University Press, 236 p.
- Deuve T (1988) Etude phylogénétique des coléoptères Adephaga: redéfinition de la famille des Harpalidae, sensu novo, et de la position systématique des Pseudomorphidae et Brachinidae. *Bull Soc Entomol Fr* 92: 161-182.
- Deuve T (1993) L'abdomen et les genitalia des femelles de Coléoptères Adephaga. *Mem Mus Natl Hist Nat* 155: 1-184.
- Erwin TL (1985) The taxon pulse: a general pattern of lineage radiation and extinction among carabid beetles, p. 437-472. In Ball GE (ed) Taxonomy, phylogeny and zoogeography of beetles and ants. Dordrecht, Dr. Junk W. **total number of pages**.
- Erwin TL, Aschero V (2004) *Cicindis horni* Bruch (Coleoptera: Carabidae, Cicindini): the fairy shrimp hunting Beetle, its way of life on the Salinas Grandes of Argentina. *Zootaxa* 553: 1-16.
- Erwin TL, Sims LL (1984) Carabid beetles of the West Indies (Insects: Coleoptera): a synopsis of the genera and checklist of Caraboidea, and of the West Indian species. *Quaest Entomol* 20: 351-466.
- Goloboff P (1993) Estimating character weights during tree search. *Cladistics* 9: 83-91.
- Goloboff P, Farris JS, Nixon K (2003) TNT (tree analysis using new technology), Program and documentation available from the authors, and at www.zmuc.dk/public/phylogeny. Access date: August 2009.
- Hlavac TF (1971) Differentiation of the Carabid antenna cleaner. *Psyche* 78: 51-66
- Jeannel R (1938) Les Migadopides (Coleoptera, Adephaga), une lignee subantarctique. *Rev Fr Entomol* 5: 1-55
- Jeannel R (1941) Coléoptères Carabiques, première partie. Paul Lechevalier et fils (eds) Faune de France 39: 1-571. Paris, France.
- Jeannel R (1955) L'édèage. Initiation aux recherches sur la systématique des Coléoptères. *Publ Mus Nat Hist Natur Paris* 16: 1-155.
- Jeannel R (1967) Biogéographie de l'Amérique Australe. In Delamare CD, Deboutteville, Rapoport E (eds). *Biologie de l'Amérique Australe*. 3: 401-460.
- Kavanaugh DH (1998) Investigations of phylogenetic relationships among some basal grade Carabidae (Coleoptera): a report on work in progress, p.329-342. In Ball GE, Casale A, Vigna A, Taglianti (eds) Phylogeny and classification of Caraboidea (Coleoptera: Adephaga). Torino, Mus Reg Sci Nat, **total number of pages**.
- Kavanaugh DH, Erwin TL (1991) The tribe Cicindini Bänninger (Coleoptera: Carabidae) comparative morphology, classification, natural history, and evolution. *Proc Entomol Soc Wash* 93: 356-389.
- Kavanaugh DH, Nègre J (1983) Notiokasiini - a new tribe of Carabidae (Coleoptera) from southeastern South America. *Coleopt Bull* 36: 549-566.
- Kryzhanovsky OL (1976) An attempt at a revised classification of the family Carabidae (Coleoptera). *Entomol Rev* 90: 56-64. (English translation from Russian).
- LeConte JL, Horn GH (1883) Classification of the coleoptera of North America. *Smithsonian misc coll (Washington)* 26 (507): 1-567.
- Liebherr JK, Will KW (1998) Inferring phylogenetic relationships within Carabidae (Insecta, Coleoptera) from characters of the female reproductive tract, p.107-170. In Ball GE, Casale A, Vigna A, Taglianti (eds) Phylogeny and classification of Caraboidea (Coleoptera: Adephaga). Torino, Mus Reg Sci Nat, **total number of pages**.
- Maddison DR, Baker MD, Ober KA (1998) A preliminary phylogenetic analysis of 18s ribosomal DNA of carabid beetles (Coleoptera), p.229-250. In Ball GE, Casale A, Vigna A, Taglianti (eds) Phylogeny and classification of Caraboidea (Coleoptera: Adephaga). Torino, Mus Reg Sci Nat, **total number of pages**.
- Maddison DR, Baker MD, Ober KA (1999). Phylogeny of carabid beetles as inferred from 18s ribosomal DNA (Coleoptera: Carabidae). *Syst Entomol* 24: 103-138.

- Maddison DR, Moore W, Baker MD, Ellis TM, Ober KA, Cannone JJ, Gutell RR (2009) Monophyly of terrestrial adepghan beetles as indicated by three nuclear genes (Coleoptera: Carabidae and Trachypachidae). *Zool Scr* 38: 43-62.
- Nichols SW (1985) *Omophron* and the origin of Hydradephaga (Insecta: Coleoptera: Adepgha). *Proc Acad Nat Sci Phila* 137: 182-201.
- Putzeys J (1868) Les Broscides. *Ent Zeit* 29: 305-379.
- Ragonesse AE (1951) La vegetación de la República Argentina. II Estudio fitosociológico de las Salinas Grandes. *R Inv Agr* 5: 1-233.
- Reichardt H (1977) A synopsis of the genera of Neotropical Carabidae. *Quaest Entomol* 13: 346-493.
- Roig-Juñent SA (1993) Cnemalobini, una tribu de Carabidae (Coleoptera) endémica de América del Sur. *Acta Entomol Chil* 18 7-18.
- Roig-Juñent SA (1998) Cladistic relationships of the tribe Broscini (Coleoptera: Carabidae), p.343-358. In Ball GE, Casale A, Vigna A, Taglianti (eds) *Phylogeny and classification of Caraboidea* (Coleoptera: Adepgha). Torino, Mus Reg Sci Nat, **total number of pages.**
- Roig-Juñent SA (2000) The subtribes and genera of the tribe Broscini (Coleoptera: Carabidae): cladistic analysis, taxonomic treatment, and biogeographical considerations. *Bull Am Mus Nat Hist* 255: 1-90.
- Roig-Juñent SA, Cicchino A (2001) A new genus and species belonging to a new subtribe of Zolini (Coleoptera: Carabidae) from southern South America. *Can Entomol* 133: 651-670.
- Roig-Juñent SA, Tognelli M, Morrone JJ (2008) Aspectos biogeográficos de los insectos de la Argentina. In Debandi G, Claps L, Roig-Juñent S (eds) *Biodiversidad de artrópodos argentinos II*. Mendoza, Sociedad Entomológica Argentina, **total number of pages.**
- Sloane TG (1890) Studies in Australian entomology. III. On *Promecoderus* and closely allied genera (Carabidae). *Proc Linn Soc N S W* 5 (2nd ser): 189-242.
- Sloane TG (1923) The classification of the family Carabidae. *Trans R Entomol Soc Lond*: 234-250.
- Stork NE (1980) A scanning electron microscope study of tarsal adhesive setae in the Coleoptera. *Zool J Linn Soc* 68: 173-306
- Ward RD (1979) Metathoracic wing structures as phylogenetic indicators in the Adepgha (Coleoptera), p.181-191. In Erwin TL, Ball GE, Whitehead DR, Halpern AL (eds) *Carabid beetles, their evolution, natural history and classification*. The Hague, Dr. Junk W., **total number of pages.**
-

Online Supplementary Material - 1

Roig-Juñent S, Sallenave S, Agrain F (2011) New Morphological Aspects and Phylogenetic Considerations of *Cicindis* Bruch (Coleoptera: Carabidae: Cicindini)

List of species representing the tribes and subtribes included as terminals in the cladistic analysis.

Cited species are those examined herein for scoring the characters. Two wingless species were assigned the state present in related genera that belong to the same tribe, as follows: data for the *Merizodus* Solier wing were taken from *Chaltenia* Roig-Juñent & Cichino (Zolini), and data for the *Carabus* L. wing were taken from *Calosoma* Weber (Carabini). Tribes are arranged in subfamilies and supertribes according to Erwin & Sims (1984).

	Trachypachidae (outgroup)	
1	Systolosomini	<i>Systolosoma breve</i> Solier (IAZA)
	Carabidae	
	Carabinae	
	Cicinditae	
2	Cicindini	<i>Cicindis horni</i> Bruch (IAZA)
	Nebriitae	
3	Nebriini	<i>Nebria hudsonica</i> LeConte (USNM)
4		<i>Leistus ferruginosus</i> Mannerheim (USNM)
5	Notiophilini	<i>Notiophilus semiopacus</i> Eschscholtz (USNM)
6	Opisthiini	<i>Opisthius richardsoni</i> Kirby (USNM)
	Carabitae	
7	Ceroglossini	<i>Ceroglossus chilensis</i> (Eschscholtz) (IAZA)
8	Carabini	<i>Carabus nemoralis</i> Müller (IAZA)
	Cychritae	
9	Cychrini	<i>Scaphinotus elevatus</i> Fabricius (USNM)
10		<i>Cychrus rostratus</i> Linnaeus (IAZA)
	Omophronitae	
11	Omophronini	<i>Omophron obliteratedus</i> Horn (USNM)
	Cicindelitae	
12	Cicindelini	<i>Cicindela drakei</i> Horn (IAZA)
13	Megacephalini	<i>Omus californicus</i> Eschscholtz (USNM)
14		<i>Pycnochila fallaciosa</i> Motschusky (IAZA)
	Scaritinae	
	Elaphritae	
15	Elaphrini	<i>Elaphrus clairvillei</i> Kirby (USNM)
16	Loriccerini	<i>Loricera foveata</i> LeConte (USNM)
17	Migadopini	<i>Antarctonomus complanatus</i> (Blanchard) (IAZA)
18		<i>Migadops latus</i> (Guérin-Ménéville) (IAZA)
	Psydrinae	
	Psydritae	
19	Psydrini	<i>Tropopterus giuraldi</i> Solier (IAZA)
	Trechitae	
20	Zolini	<i>Merizodus angusticollis</i> Solier (IAZA)

Continue

	Broscinae	
21	Broscini	<i>Creobius eydouxii</i> (Guérin-Ménéville) (IAZA)
22		<i>Oregus aereus</i> (White) (IAZA)
	Harpalinae	
23	Cnemalobini	<i>Cnemalobus sulciferus</i> Philippi (IAZA)
24	Zabrini	<i>Zabrus spinipes</i> Creutz (IAZA)
	Paussinae	
25	Ozaenini	<i>Ozaena linealis</i> Bänninger (IAZA)
26		<i>Tropopsis biguttatus</i> (IAZA)
27	Metriini	<i>Metrius constrictus</i> Esch. (Smithsonian)

Online Supplementary Material - 2

Roig-Juñent S, Sallenave S, Agrain F (2011) New Morphological Aspects and Phylogenetic Considerations of *Cicindis* Bruch (Coleoptera: Carabidae: Cicindini)

Characters used in the cladistic analyses

Note: Characters marked (*) were previously used by Kavanaugh & Erwin (1991) to study the phylogenetic relationships of Cicindini.

0*. *Supraorbital anterior setae*: present (0), absent (1).

1*. *Supraorbital posterior setae*: present (0), absent (1), two or more punctures each with one seta (3).

Supraorbital setae (characters 0 and 1) were considered to be independent. Jeannel (1941) considered two main groups of tribes (families) within Limbata Styliifera based on the presence of supraorbital setae anteriorly and posteriorly to the eye. According to Jeannel, one of these groups has two supraorbital setae, whereas the other has only the posterior seta. Nevertheless, the anterior and posterior setae are independent and sometimes the anterior seta is present while the posterior is absent. For this reason, we regard these setae as two independent characters.

2*. *Mandibular scrobal seta*: present (0), absent (1).

3*. *Number of labral setae*: twelve (0), eight, (1), six (2), four (3).

4. *Shape of labrum*: anterior margin not bilobed (0); anterior margin strongly bilobed (1).

5. *Clypeus*: narrower than distance between antennal sockets (0), longer than distance between antennal sockets (1).

6*. *Temporal ridge*: absent (0), incomplete, when the central or central-anterior part is obsolete (1), complete, when this structure is distinct on the entire lateroventral surface of the head (2).

The temporal ridge is a more or less distinct suture on both sides of the head that extends backwards below and behind the eyes (Sloane 1890). This is the "suture temporale" for Putzeys (1868), and the "sub-ocular ridge" for LeConte & Horn (1883), or genal flange for Kavanaugh & Erwin (1991).

7. *Apical margin of glossal sclerite*: bisetose (0), tetrasetose (1), six or more (2), asetose (3), one seta (4).

8. *Maxillary palpomere 4*: longer than penultimate (0), as long as penultimate (1), shorter than penultimate (2).

9. *Shape of last maxillary palpomere*: similar to penultimate (0), dissimilarly enlarged (1).

10. *Scape*: with numerous umbilicate setae (0), one setiferous puncture (1).

11. *Pubescence of antennomeres*: glabrous antennomeres (0), 1-4 glabrous antennomeres (1), 1-3 glabrous antennomeres (2), 1-2 glabrous (3).

12*. *Antenna cleaner on apical spurs of front tibia* (Jeannel 1938, 1941, Hlavac 1971): with spurs equal in size, apical (isochaeta) (0), with posterior tibial spur longer than anterior and displaced medially (anisochaeta) (1).

13. *Antenna cleaner, setal band* (Hlavac 1971): short, almost entirely horizontal, as a simple and transverse setal band across the concave tibial apex between spurs (sulcate) (0); long, with distinct vertical section and confluent zone (1); long, divided into a large distal region and a proximal cleaning arc (2).

14. *Antenna cleaner, clamp setae*: absent (0); present, straight (1); present, curved (2).

15*. *Antenna cleaner, antennal channel*: longitudinal (0); oblique (1).

16. *Antenna cleaner, medial expansion of front tibia*: absent (0); present (1).

17. *Adhesive setae of male front tarsi* (Stork 1980): spongiose for Sloane (1923) articulo-setae, arranged in a more or less hexagonal pattern, with straight cylindrical shafts bearing regular round or oval plates (0); squamo-setae in one row on long axis of tarsus (1); squamo-setae in two rows on long axis of tarsus (2).

18. *Mesotibial brush on the lateral surface*: absent (0), present (1).

19*. *Posterior procoxal closure* (Nichols 1985; Bell 1967): open posteriorly (0); closed by medial extension of propleuron (1); proepimeron fitted into the lateral arm of the prosternal process (2); tip of the lateral arm of the prosternal process shallowly fitted into the proepimeron (3).

20*. *Frontal coxal cavities* (Nichols 1985; Bell 1967): conjoined, not separated medially (0); separated medially by sclerotized internal septum (1).

21*. *Frontal coxal cavities* (Nichols 1985, Bell 1967): the dorsal unsclerotized opening is divided by a narrow sclerotized bridge (biperforate or bridged) (0); without sclerotized bridge (unbridged or uniperforate) (1).

22. *Pleural contact with mesocoxa* (Nichols 1985): mesepimeron forming part of the mesocoxal cavity [disjunct condition for Sloane (1923), Jeannel (1941) and Bell (1967)] (0); mesepimeron excluded from the mesocoxal cavity [conjunct condition for Sloane (1923), Jeannel (1941) and Bell (1967)] (1).

23*. *Pleural contact with metacoxa* (Nichols 1985): metacoxa expanded laterally to the elytral epipleuron [incomplete for Bell (1967)] (0); metepimeron absent [conjunct condition for Bell 1967] (1); metepimeron in contact with the metacoxa [disjunct for Bell 1967] (2); metepimeron forms a shelf that overlaps abdominal sternum II (the first visible abdominal sternite) [disjunct lobate characteristic of Lobopleuri (Bell 1967)] (3).

24. *Elytral plica*: absent (0), present (1), turned forward (2).

25*. *Parascutellar striole* (Jeannel 1938): one stria complete, between stria 1 and stria 2 (0); short, between striae 1 and 2 (1); short and joined to the medial portion of stria 1 (2); absent (3).

26. *Supernumerary striae*: absent (0), present (1).

27. *Parascutellar seta (basal seta of the second stria)*: absent (0); present (1).

28*. *Metathoracic wings, shape of the oblongum cell (OC)* (Ward 1979): transversely rectangular (0); narrowed posteriorly (1).

29. *Metathoracic wings*: 4mcu and 5mcu separated distally (0); 4mcu and 5mcu joined before the Cu vein, forming a stalk (1).

30*. *Metathoracic wings, point of insertion of M4*: on the anterior distal wall of the OC (0); M4 inserted in the middle (1); M4 inserted posterior to the middle (2).

31*. *Metathoracic wings, relative sizes of the third radial (3RC) and posterior sector (SAC) cells*: 3RC subequal in size to SAC (0); 3RC larger than SAC (1)

32. *Type of abdomen* (Deuve 1993): nebridian, the last apparent tergum is the unmodified VIII (0); the IX invaginated (Carabidean) (1); tergum VIII partially invaginated, with anterior margin without apophysis (1); harpalidian, the last apparent tergum is VIII, anterolateral apodemes present (2); last one corresponds to VIII (not apparent), deeply invaginated and telescoped (3).

33. *Last visible sternite of male*: apical border right (0); apical border distinctly emarginated (1).

34. *Attachment of pygidial glands* (Deuve 1993): anterolateral margin of abdominal tergum IX (0), near abdominal tergum VIII (1).

35. *Base of median lobe* (Jeannel 1955, Erwin 1985): base closed dorsally (0); base partially closed (1); base open dorsally (2).

36. *Base of median lobe, basal keel*: absent (0); present (1).

37*. *Left paramere* (Jeannel 1941, 1955, Erwin 1985): densely setose (0); with few setae (1); glabrous (2).

38*. *Shape of left paramere* (Jeannel 1941, 1955, Erwin 1985): styliiform (similar in shape to the right paramere) (0); conchiferous (1).

39*. *Right paramere* (Jeannel 1941, 1955, Erwin 1985): setose (0); glabrous (1).

40. *Internal sac, sclerite X and Y* (Roig-Juñent 1998, 2000): absent (0); present (1).

41. *Internal sac, copulatory piece* (Jeannel 1941, 1955): absent (0); present (1).

42. *Dorsal sclerification joining left and right parameres*: absent (0); present (1).

43. *Gonopod VIII (Deuve 1993) or ramus gonocoxae* (Liebherr & Will 1998): absent (0); present (1).

44. *Nematiform setae of subapical setose organ* (Deuve 1993): absent (0); present (1).

45. *Stylomere 1 or gonopod IX* (Deuve 1993): monomeric (0) (Cicindini); dimerous (1).

46. *Gonocoxite 2*: without any basal dentiform process, simple, formed by only one part (0); formed by two parts with basal dentiform process (1).

47. *Ligular apophysis*: absent (0), present (1).

48. *Helminthoid sclerite* (Deuve 1993): present (0), absent (1).

49. *Oviduct position*: ventral respect to spermathecae (0), dorsal respect to spermathecae (1).

Online Supplementary Material - 3

Roig-Juñent S, Sallenave S, Agrain F (2011) New Morphological Aspects and Phylogenetic Considerations of *Cicindis* Bruch (Coleoptera: Carabidae: Cicindini)

Morphological data matrix (missing data are coded as “?”).

	0	1	2	3	4	5
	0123456789	0123456789	0123456789	0123456789	0123456789	0
<i>Systolosoma breve</i>	000000000	000110000	000000200	000000100	000000000	0
<i>Cicindis horni</i>	1112002010	110000011	1002110200	111100001	0000001000	1
<i>Nebria hudsonica</i>	1002000010	1101000010	0001110200	1011000002	0100011000	0
<i>Leistus ferruginosus</i>	1002000010	1100000010	0001010200	1011000012	0100001000	0
<i>Notiophilus semiopacus</i>	1002000000	1101000010	0011010200	????000002	0100001100	0
<i>Opisthius richardsoni</i>	0001000000	0300000010	1001100200	1001000012	0100001000	0
<i>Ceroglossus chilensis</i>	0210000221	1100000010	1001100?10	????100102	0100001101	1
<i>Carabus nemoralis</i>	1010000211	1100000010	1001100?10	1121100102	0100011101	1
<i>Cychnus rostratus</i>	1113100011	1100000010	1101100?10	????100202	0100001101	1
<i>Scaphinotus elevatus</i>	1012100011	1100000010	1101100?10	????100202	0100001101	1
<i>Omphron oblitteratum</i>	0102000010	1111110003	0001100000	1121000202	0110011000	0
<i>Cicindela drakei</i>	0011012300	1100000011	1101100??0	?12?310102	0100110111	1
<i>Omus californicus</i>	0211012311	1100000011	1101100??0	????310002	0100100111	1
<i>Pycnochila fallaciosa</i>	0210012311	0100000001	1101100??0	????310002	0100100111	1
<i>Elaphrus clairvillei</i>	0002000000	1211111001	1102100201	1110000200	0010001100	0
<i>Loricera foveata</i>	1013000000	1111211002	1102111001	1110000012	0100001101	1
<i>Antarctonomus complanatus</i>	1003000410	1111210001	1102000001	????000011	0000001000	1
<i>Migadops latus</i>	1002000000	1111210001	1102000000	????000011	0000001000	1
<i>Tropopterus giuraldi</i>	0001000010	1212211201	1112111301	????201001	0000011100	1
<i>Merizodus angusticollis</i>	0002000010	1312211101	1112101201	1110201101	0000011100	1
<i>Creobius eydouxii</i>	1202001100	1312211001	1113100201	????200200	0100010100	1
<i>Oregus aereus</i>	1202001000	1212211001	1113100201	????200010	0110010100	0
<i>Cnemalobus sulciferus</i>	1212000010	1212211201	1113000200	????201002	1101001100	1
<i>Zabrus spinipes</i>	1012000020	1212211201	1113011200	????201002	1101001100	1