Larval morphology and biology of oxycorynine weevils and the higher phylogeny of Belidae (Coleoptera, Curculionoidea)

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Accepted: 9 April 2004

Marvaldi, A. E. (2005). Larval morphology and biology of oxycorynine weevils, and the higher phylogeny of Belidae (Coleoptera, Curculionoidea). — *Zoologica Scripta*, *34*, 37–48.

Phylogenetic relationships among members of the family Belidae (Curculionoidea) were reconstructed through cladistic analysis using 58 characters and 17 terminals. The characters were from larval morphology (30), adult morphology (25) and biology regarding larval host-plants and feeding habits (three). They were scored for exemplar taxa in 17 genera, representing different belid subfamilies and tribes, plus two outgroup taxa in Megalopodidae and Nemonychidae. The sampled genera included all those for which larval and adult information is available, and two known only from adults. New information on the larvae and biology of two oxycorynines is provided. These are the Chilean *Oxycraspedus cribricollis*, whose larvae live in decayed female strobili of the gymnosperm *Araucaria araucana*, and *Hydnorobius hydnorae* from Argentina, whose larvae, described and illustrated in the present paper, develop inside the flower and fruit bodies of *Prosopanche americana* (Hydnoraceae), a root-parasitic angiosperm. The relationships proposed by the single optimal cladogram resulting from simultaneous analysis of all taxa and characters are recovered by one of three optimal cladograms based on the larval data set alone. The cladogram justifies a revised classification of Belidae in two sister subfamilies: Belinae (with tribes Pachyurini, Agnesiotidini and Belini) and Oxycoryninae (with tribes Oxycorynini and Aglycyderini). It summarizes larval and adult synapomorphies defining the family Belidae, subfamilies and tribes. Based on the phylogenetic tree, the evolution of biological traits is traced. Larval development in vegetative organs of conifers is ancestral in Belidae. A shift to reproductive structures characterizes the Oxycorynini, a habit which was conserved while several shifts to distantly related host-plant groups occurred.

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Introduction

The family Belidae is a monophyletic group of relatively basal weevils (Curculionoidea), according to evidence from morphology (of larvae and adults) and 18S rDNA sequences (Marvaldi *et al*. 2002). Kuschel (1995a) provided the first cladistic analysis supporting the monophyly of Belidae, and defined three, putatively monophyletic, constituent subfamilies: Belinae, Aglycyderinae and Oxycoryninae. These have family rank in other classificatory schemes (i.e. Thompson 1992; Zimmerman 1994; see also Alonso-Zarazaga & Lyal 1999). The monophyly of each belid subfamily has not yet been tested. Nor has a phylogenetic study of relationships within each subfamily been considered, with the exception of Belinae (Kuschel & Leschen 2003). Their cladistic study on generic relationships of Belinae is based almost exclusively on adult morphology, with only eight larval characters scored for the eight genera for which larvae are known. Based on the results, the three recognized tribes of Belinae (Belini, Agnesiotidini and Pachyurini) are re-delimited and defined, but uncertainty remains because the Pachyurini appear to be paraphyletic with respect to a monophyletic Belini or (if some characters are weighted differently) to a monophyletic Agnesiotidini. As Kuschel & Leschen (2003) pointed out, additional larval characters scored for more terminals would be helpful to resolve these problems.

The aims of the present paper are to: (1) explore the significance of larval characters for recognizing natural groups and estimating phylogenetic relationships of the weevil family Belidae; (2) clarify evolutionary aspects on shifts in larval habits and host-plant associations in this group of weevils. The monophyly and relationships of the major taxa (i.e. subfamilies and tribes) of Belidae are explored via cladistic analysis based on larval morphological evidence and via simultaneous analyses by adding data on adult anatomy, as well as biological data. In

this paper, the larval comparative morphology of Belidae is reviewed and expanded by adding new information, particularly for the subfamily Oxycoryninae, as larvae of species in two critical genera are now available. These are the larvae of the Chilean *Oxycraspedus cribricollis*, recently discovered living in female fallen strobili of the gymnosperm *Araucaria araucana* (Mol.) Koch (Marvaldi *et al*. 2003; in preparation), and the larvae of *Hydnorobius hydnorae* from Argentina, described and illustrated in the present paper, along with a summary on the biology of the species.

Materials and methods

Specimens examined

Taxon sampling. For the phylogenetic analysis, characters were scored for species representing 15 belid genera (13 for which larval and adult information is available, and two known only from adults), plus two outgroup taxa. The placement in subfamilies and tribes of the sampled taxa, listed below, is according to Kuschel (1995a) and Kuschel & Leschen (2003) (see also Alonso-Zarazaga & Lyal 1999). The three belid subfamilies were represented in the sample, with more than one representative in different tribes, thus allowing the cladistic analysis to test monophyly and to indicate relationships among and within subfamilies.

List of species examined. Larval material from Australia and New Zealand was borrowed from the New Zealand Arthropod Collection (NZAC) and corresponds to species studied by May (1993, 1994). The larvae of species from Argentina and Chile, together with adult voucher specimens, are held at the Instituto Argentino de Investigaciones de Zonas Aridas, Mendoza, Argentina (IADIZA). Adult specimens of two oxycorynines were borrowed from the Museo Argentino de Ciencias Naturales, Bernardino Rivadavia, Buenos Aires (MACN). Character states of some species were taken from the literature and references are given accordingly.

Ingroup. Belinae, Pachyurini: *Pachyurinus sticticus* (Broun) [New Zealand (NZAC)]; *Rhicnobelus rubicundus* (Broun) [New Zealand (NZAC)]; *Sphinctobelus niger* Zimmerman [Australia (May 1994)]; *Hadrobelus undulatus* (Zimmerman) [Australia (May 1994)].

Belinae, Agnesiotidini: *Agathinus tridens* (Fabricius) [New Zealand (NZAC)]; *Cyrotyphus blandus* [Australia (NZAC)].

Belinae, Belini: *Rhinotia* spp. [Australia (May 1994)].

Aglycyderinae: *Aralius wollastoni* (Sharp) [New Zealand (NZAC)]; *Proterhinus* spp. [Hawaii (Anderson 1941)].

Oxycoryninae, Oxycorynini: *Oxycraspedus cribricollis* (Blanchard) [Chile (IADIZA)]; *Hydnorobius hydnorae* (Pascoe) [Argentina (IADIZA)]; male and female adult specimens of *Alloxycorynus bruchi* (Heller) [Argentina (MACN)], and *Oxycorynus missionis* Kuschel [Argentina (MACN)].

Oxycoryninae, Allocorynini: *Parallocorynus* sp. [Mexico (May 1993)]; *Rhopalotria* spp. [Central America (Emden 1938; May 1993)].

Outgroup. Chrysomeloidea, Megalopodidae, Palophaginae: *Palophagoides vargasorum* Kuschel [Argentina and Chile (IADIZA)].

Curculionoidea, Nemonychidae, Rhinorhynchinae: *Rhynchitomacerinus kuscheli* (Voss) [Argentina and Chile (IADIZA)].

Preparation, terminology and illustration of larvae. The techniques for preservation, dissection and slide mounting of larvae follow May (1993, 1994). Illustrations were made with camera lucida associated with stereo and compound microscopes. The terminology employed in larval descriptions generally follows May (1994) and is explained and illustrated in Marvaldi (1999).

Phylogenetic analysis

Characters and data matrix. A total of 58 characters (30 from larval morphology, 25 from adult morphology and three from larval feeding habits) were scored for exemplar taxa in 17 genera. The characters and states are described and listed in the Appendix. The data matrix is shown in Table 1.

Cladistic analyses. Trees were reconstructed using the parsimony program NONA (Goloboff 1998). The search was performed through tree bisection reconnection (TBR) branch swapping on random addition replicates (commands hold*; hold/100; mult*20; max*). The program WINCLADA (Nixon 2002) was used for visualizing character changes and to prepare the cladogram figure. Characters were equally weighted and treated as unordered (except character 24, additive). The support to tree topology was evaluated by means of bootstrap and jackknife values, using default commands in WINCLADA (via NONA). The data matrix (Table 1) was analysed simultaneously. The larval morphological data set (30 characters × 15 taxa) was also analysed separately, to evaluate the contribution of this source of evidence to belid phylogeny. Then, an expanded data matrix of larval plus adult morphology (55 characters × 15 taxa) was analysed, followed by the addition of biological data (58 characters × 15 taxa), and finally the two terminals known only from adults were also included in the simultaneous analysis (58 characters × 17 taxa).

Results and discussion

Phylogeny of Belidae

The simultaneous analysis, including all characters and taxa, resulted in a single most parsimonious tree (Fig. 1) of 125 steps, consistency index (CI) = 0.68, and retention index (RI) = 0.81. The cladistic analysis of the larval morphological

Table 1 Data matrix. Characters 1–30 (larva), 31–55 (adult), 56–58 (biology). Character 24 = ordered.

	1	2	3	4	5	
	1234567890	1234567890	1234567890	1234567890	1234567890	12345678
Palophagoides	0400000000	0000001001	0000?00000	0000000000	0000010001	00000000
Rhynchitomacerinus	0020000000	0010000000	0000000000	1000001000	0000100?00	00000000
Sphinctobelus	1101111010	1000000?0?	0111210001	0100000000	0100100000	10000011
Hadrobelus	1111110011	1100000?0?	0111200101	0100000000	0100100100	1000003?
Pachyurinus	1101110012	1001000000	0101000002	0100000000	0100100100	10000331
Richnobelus	1101110012	1001000000	0101000002	0100000000	0100100100	10000431
Agathinus	1001130022	0101000011	0112000001	0100001000	0101100100	10000?31
Cyrotyphus	1001120022	0001000011	0112000000?	0100001000	0101100100	10000531
Rhinotia	100111?001	2101000001	011202?0?1	0100000000	0101101100	10000231
Aralius	1011120000	0010110201	1110111002	2010101111	1110011011	10011631
Proterhinus	1011120000	0010110201	1110?11002	2010101111	1110011011	10011731
Oxycraspedus	1311111130	1000010101	1000011011	2101101101	0100011001	10000011
Hydnorobius	1211110130	1000010101	1010011111	2101111001	0111010001	20100821
Parallocorynus	1210110?30	1000111201	1000111111	1100101001	01?1010001	21100111
Rhopalotria	1210110130	1010111201	10?0111111	1100101001	0101010001	21100111
Alloxycorynus	??????????	??????????	??????????	1101111001	0111010001	20100921
Oxycorynus	??????????	??????????	??????????	2101101001	0111010001	20100921

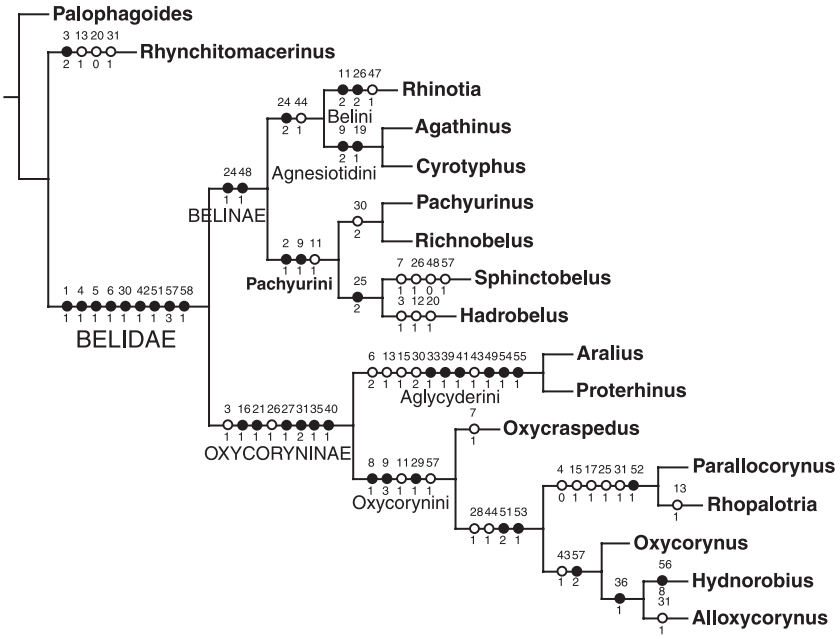

Fig. 1 Most parsimonious tree for the Belidae, obtained from an analysis of the matrix in Table 1. Unambiguously placed character changes are mapped on the cladogram. Unique and homoplasious changes are indicated by black and white dots, respectively.

data set resulted in three most parsimonious trees (69 steps, CI = 0.65, RI = 0.78). One of these cladograms has the same topology as the one in Fig. 1; the other two differ in the position of *Oxycraspedus*, as sister taxon to *Hydnorobius* or to (*Parallocorynus* + *Rhopalotria*), respectively. The analysis of larval plus adult morphological data resulted in a single most parsimonious tree (110 steps, CI = 0.65, RI = 0.79), with the same topology as in Fig. 1, and identical relationships are recovered by the optimal tree obtained when biological data were added (123 steps, CI = 0.68, RI = 0.79). When *Alloxycorynus* and *Oxycorynus*, with unknown larvae, were included in the simultaneous analysis, the resulting cladogram (Fig. 1) shows that the data available place them in a clade with *Hydnorobius*.

As simultaneous analysis combines all available data, and thus maximizes information content and explanatory power, the resulting cladogram was chosen as the reference tree (Fig. 1). Based on the results, it is justified (see remarks below) to consider some changes in the classification of Belidae: by uniting the former 'Aglycyderinae' and 'Oxycoryninae' in a single subfamily, the monophyletic clade that is sister to the Belinae receives a formal name and corresponds to the subfamily Oxycoryninae with two monophyletic tribes,

Aglycyderini and Oxycorynini (which includes 'Allocorynini'). Monophyletic groups and apomorphies (of unambiguous optimization) supporting them are listed below (see Fig. 1). The numbers in parentheses are bootstrap/jackknife values (>50%), respectively.

Belidae (= Belinae + Oxycoryninae) (100/100)
Larva. Head permanently retracted into thorax (1.1), antenna with retractable basal membrane (4.1), frontal lines not distinct (5.1), endocarina bifid, V-shaped (6.1), posterior ventriculus of alimentary canal with gastric caecae present, arranged randomly (30.1).

Adult. Fore tibia with grooming area of dense vestiture on face opposite to tarsal articulation (42.1), spermatheca very reduced in size and/or submembranous (51.1).

Biology. Larval development in branch or twig (57.3), in decaying or dead plant tissue (58.1).

Belinae (= Pachyurini + Agnesiotidini + Belini) (71/76)
Larva. Posterior margin of pronotum enlarged and extended back (24.1).

Adult. Aedeagus with basal sclerite in internal sac (48.1).

Pachyurini
Larva. Occipital foramen open, divided, with dorsal emargination ridged (2.1), labrum-epipharynx with paired paramesal, divergent sclerotizations (9.1), mandibular apex tridentate (11.1).

Agnesiotidini + Belini (63/61)
Larva. Posterior margin of pronotum enlarged and sloping back to a grossly thickened hind margin (24.2).

Adult. Laterodorsal surface of middle and hind femora and tibiae crenulated with a longitudinal row of denticles (44.1).

Agnesiotidini (79/72)
Larva. Labrum-epipharynx with paired paramesal, curved sclerotizations (9.2), maxillary mala densely setose (19.1).

Belini (as represented by* Rhinotia*)
Larva. Mandibular apex adentate (11.2), spiracles on thorax and abdomen without air-tubes (26.2).

Adult. Aedeagal pedon and tectum largely fused (47.1).

Oxycoryninae (Oxycorynini + Aglycyderini) (96/98)
Larva. Antenna one-segmented, lacking apical cylindrical segment, sensorium mounted on convex basal segment (3.1), apical segment of maxillary palp without seta (16.1), labium without lateral strut (21.1), spiracles on thorax and abdomen with one air-tube (26.1), spiracular air-tubes simple (27.1).

Adult. Antenna with distinct club and articles 9–10 loosely connected and 10–11 joined or compact (31.2), elytron lacking erect sensory setae (35.1), tarsal segment 1 shorter than 2 and 3 combined (40.1).

Oxycorynini (68/74)
Larva. Labrum with two basal and two anteromedian sensilla (8.1), labrum-epipharynx with single mesal sclerotization (9.3), mandibular apex tridentate (11.1), body recurved terminally, anus ventral (29.1).

Biology. Larvae consume vegetative tissues of reproductive organs, strobili (57.1).

(Hydnorobius, Alloxycorynus, Oxycorynus) + (Parallocorynus, Rhopalotria) ***(67/69)***
Larva. Body widest at mid-abdomen (28.1).

Adult. Laterodorsal surface of middle and hind femora and tibiae crenulated with a longitudinal row of denticles (44.1), spermatheca absent (51.2), spermathecal duct inserted on common oviduct (53.1).

(Hydnorobius, Alloxycorynus, Oxycorynus) ***(78/81)***
Adult. Tibial spurs absent (43.1).

Biology. Larvae consume flower or fruit vegetative tissues (57.2).

(Parallocorynus + Rhopalotria) ***(99/97)***
(This clade, now included in the tribe Oxycorynini, corresponds to the tribe 'Allocorynini' of previous classifications).

Larva. Antenna lacking retractable basal membrane (4.0), maxillary palp two-segmented (15.1), maxillary mala entire, with no trace of incision or lobes (17.1), pedal area with setae only, no sensilla (25.1).

Adult. Antenna with club articles 9–10 and 10–11 loosely connected (31.1), spermathecal gland forming a common tube with the duct (52.1).

Aglycyderini (100/100)
Larva. Endocarina only minutely divided in front (6.2), maxilla without distinct sclerotized palpifer (13.1), maxillary palp two-segmented (15.1), posterior ventriculus of alimentary canal with gastric caecae in two clusters on either side (30.2).

Adult. Prementum large, concealing maxilla in ventral view (33.1), tarsi four-segmented, pseudotrimerous (39.1), tarsal segment 2 rounded at apical angles (41.1), tibial spurs absent (43.1), tergite 9 of female entirely membranous (49.1), alimentary canal proventricular blades well developed, with sharp ridges on external face (54.1), alimentary canal hind gut with rectal loop (55.1).

Remarks on the classification of Belidae (see Fig. 1). The significance of larval characters is important, as the family Belidae and major clades can be diagnosed by synapomorphies from this source of evidence alone. The use of larval characters also has some implications for the classification into tribes. Within Belinae, larval evidence allows the definition of the three tribes (*sensu* Kuschel & Leschen 2003): Pachyurini, Agnesiotidini and Belini. Kuschel & Leschen (2003) propose to recognize the tribe Pachyurini only for convenience, as the results of their cladistic analysis of genera of Belinae show Pachyurini to be paraphyletic. In the present study, there are at least three larval apomorphies that may actually be evidence of the monophyly of this tribe. Also, the present proposal of Belini and Agnesiotidini forming a clade is novel (and, of course, in need of further testing by expanding the sampling of taxa and characters). The close relationship between 'Aglycyderinae' and 'Oxycoryninae', proposed by Kuschel (1995a), is strengthened herein by adding several larval synapomorphies, and their amalgamation into a single subfamily is justified (Fig. 1). Also, the larval evidence is fundamental in supporting the monophyly of an enlarged concept of the tribe Oxycorynini (Fig. 1). The 'Oxycorynini' of previous classifications, as represented by *Oxycraspedus*, *Hydnorobius*, *Alloxycorynus* and *Oxycorynus*, is paraphyletic in the present analysis, with respect to a monophyletic 'Allocorynini' (*Parallocorynus* + *Rhopalotria*). Based on the results (Fig. 1) the family Belidae is classified into two subfamilies, Belinae (with tribes Pachyurini, Agnesiotidini, and Belini) and Oxycoryninae (with tribes Oxycorynini and Aglycyderini).

Remarks on the evolution of larval feeding habits (see Fig. 1). The larvae of the outgroups, deemed as basal curculionoids and chrysomeloids, develop in living reproductive tissues (i.e. pollen sacs of strobili) (Kuschel & May 1990, 1996; Marvaldi *et al*. 2002). Based on the phylogenetic tree (Fig. 1), the Belidae are proposed to have shifted to larval development in decaying or dead vegetative tissues (i.e. branches). Association with conifers (i.e. Araucariaceae) is ancestral. A further important shift in the evolution of Belidae is proposed to have occurred in Oxycorynini: to larval development in (vegetative tissues of) reproductive organs (strobili, and fruits in those that have colonized angiosperms). This feeding habit is conserved throughout Oxycorynini, while several shifts to distantly related host-plant groups occurred. The basal position of *Oxycraspedus* suggests that their association with the conifer *Araucaria* is preserved from the oxycorynine ancestor.

Description of larva and biology of an oxycorynine weevil

Subfamily OXYCORYNINAE
Genus *Hydnorobius* Kuschel
Hydnorobius Kuschel, 1959: 268.
Type species *Oxycorynus hydnorae* Pascoe

Mature larva. Body (Fig. 2A) robust, widest at mid-abdomen; ampullae present on abdominal segments AI–AV, paired (at each side of mid-dorsum). Head (Fig. 2B,E,F) longer than wide, deeply emarginate behind both dorsally and ventrally (occipital foramen open), with dorsal excavation occupying one third to one quarter distance from hind margin to front; pigmented on exposed anterior part, darker on frons and epistoma, hialine behind ridged line at cuticle attachment; stemmata comprising one conspicuous convex unit close to antenna and one dark ocellar spot behind these; endocarina V-shaped, with distance between apices as wide as clypeus; attachment of heavy musculature at the junction of endocarina with epicranial line. Antenna (Fig. 2G) with retractable membrane, basal segment very convex, as long as wide, bearing a narrow conical sensorium and six much smaller sensorial structures (four setiform and two round). Frontal horn absent, except in first instar larvae. Labrum-epipharynx (Fig. 3A) with mesal sclerotization rounded posteriorly and incised anteriorly, lateral labral sclerotizations extended below clypeus; epipharynx (Fig. 3B) with three anterolateral setae, two anteromedian setae, shorter than labral setae, one pair of sensillum clusters behind median epipharyngeal setae. Mandibles (Fig. 3C) tridentate. Maxillae (Fig. 3D,E) with palp three-segmented and sclerotized palpiger; stipes with two setae; maxillary mala incised at apex and bilobate. Labium (Fig. 3D) with premental sclerite wide; postmentum pigmented. Thorax (Figs 4A,B and 5A): pronotal shield (Fig. 4A) lacking a median ecdysial line. Spiracle (Figs 4B, 5A) on thoracic segment TII, subcircular, unicameral, with air-tube simple; vestigial spiracle distinct on TIII. Pedal areas with numerous setae and clusters of few minute sensilla. Abdomen (Figs 4B, 5B): spiracles (Figs 4B, 5B) similar to thoracic one, lateral, air-tube posteriorly directed. Anus T-shaped (Fig. 2C). Alimentary canal (Fig. 2D) as described for Belidae (May 1994: 437), anterior ventriculus bulky, posterior ventriculus with vermiform gastric caecae.

Note. May (1993) provided a larval diagnosis and figures of an undetermined species of *Hydnorobius* which were used to formulate the present generic description.

Hydnorobius hydnorae *(Pascoe) (Figs 2–5)*

Oxycorynus hydnorae Pascoe, 1868: xiv.
Hydnorobius hydnorae Kuschel, 1959: 268.

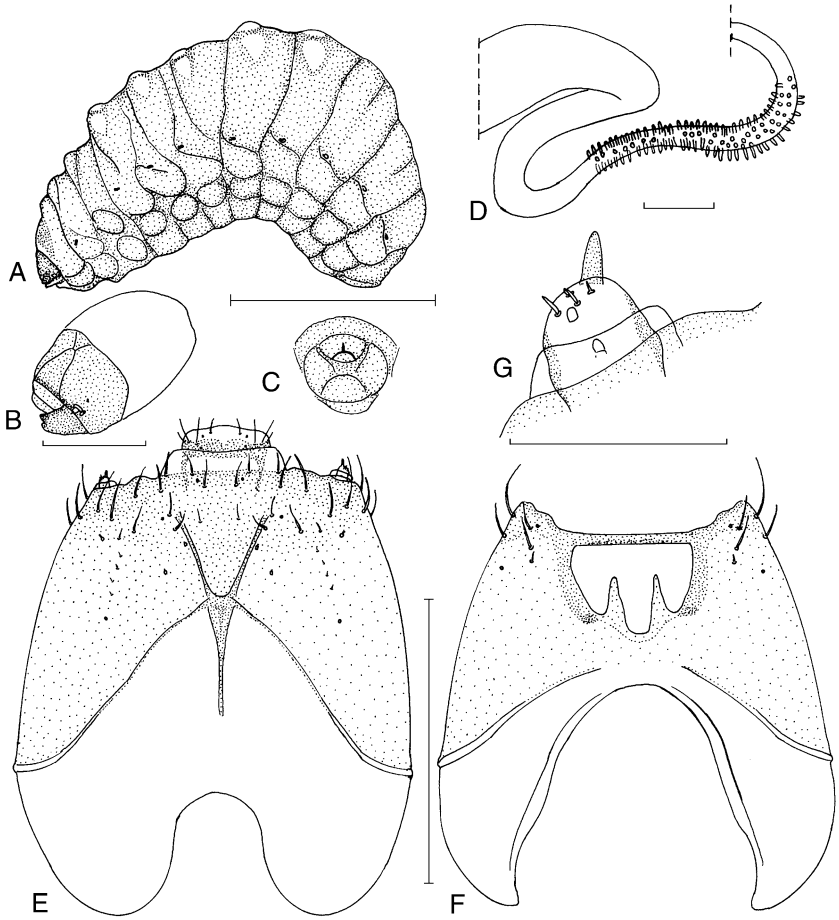

Fig. 2 A–G. *Hydnorobius hydnorae* (Pascoe), larva. —A. Habitus, lateral. —B. Head extracted, anterolateral. —C. Abdominal apex, abdominal segments AIX, X, ventral. —D. Alimentary canal, portion of ventriculus showing gastric caecae. —E. Head, dorsal. —F. Head, ventral. —G. Antenna. Scales: A, C = 5 mm; B, D–F = 1 mm; G = 0.1 mm.

Mature larva. Maximum dimensions 12.50 × 5.00 mm. Head width 1.35 mm. Cuticle minutely spiculate on anterior portions of folds. Setae pallid, inconspicuous, more numerous on thoracic segment TI. Pigmented areas of head and pronotum dark red–brown. Head (Fig. 2E) with frons indistinctly separated from clypeus; setae on pigmented anterior portion; three clypeal setae and one sensilla actually on epistoma; five frontal setae and two sensilla; five dorsal epicranial setae and a compound sensilla near dorsal epicranial seta 2 and posterior epicranial setae; two lateral epicranial setae; four minute posterior epicranial setae plus two sensilla; ventrally (Fig. 2F) three ventral cranial setae and two sensilla. Hypopharyngeal bracon (Fig. 2F) pigmented. Epipharynx (Fig. 3B) with one median epipharyngeal seta and clusters of three sensilla. Mandibles (Fig. 2C) with mandibular setae 1, 2 subequal, aligned longitudinally. Maxillary mala (Fig. 3D,E), six dorsal malar setae (two on basal lobule), four ventral malar setae (two minute) and one sensilla. Labium (Fig. 3D), premental sclerite with broad anteromedian and posterior extensions; labial seta 2 longer and less separated than labial setae 1, 3. Thorax (Figs 4, 5A): pronotum (Fig. 4A) bearing setae and sensilla on pigmented shield and several setae along the area behind it. Spiracle (Fig. 4B) with air-tube round, as long as peritremal width. Abdomen (Figs 4B, 5B) with very short setae on dorsal ampullae (Fig. 5B), postdorsum with postdorsal setae 1–5 discernible. Spiracles (Fig. 5B) similar to thoracic one. Alimentary canal (Fig. 2D) with numerous gastric caecae arranged randomly on lower coil.

Material examined. All material obtained from flower and fruit bodies of *Prosopanche americana* (R. Br.) Baillon (Hydnoraceae) retrieved from soil beneath trees of *Prosopis flexuosa* De Candolle (Fabaceae). Specimens and microscope slides are deposited at IADIZA. Collection data: ARGENTINA, Mendoza Province, Department Lavalle, 'Reserva Forestal' Telteca, September 2000, six larvae from floral stalks; October 2000, eight larvae from floral stalks; 13 September 2001, one larva from fruit wall; same area at 'El jagüel', 13 September 2001, six larvae from fruit wall and stalk; January 2002, about 20 adults from flowers burst with pollen; 27 October 2002, one larva from stalk and one dead adult from dry perigynium tube; same area at 'Médanos Altos Limpios', 4 May 2003,

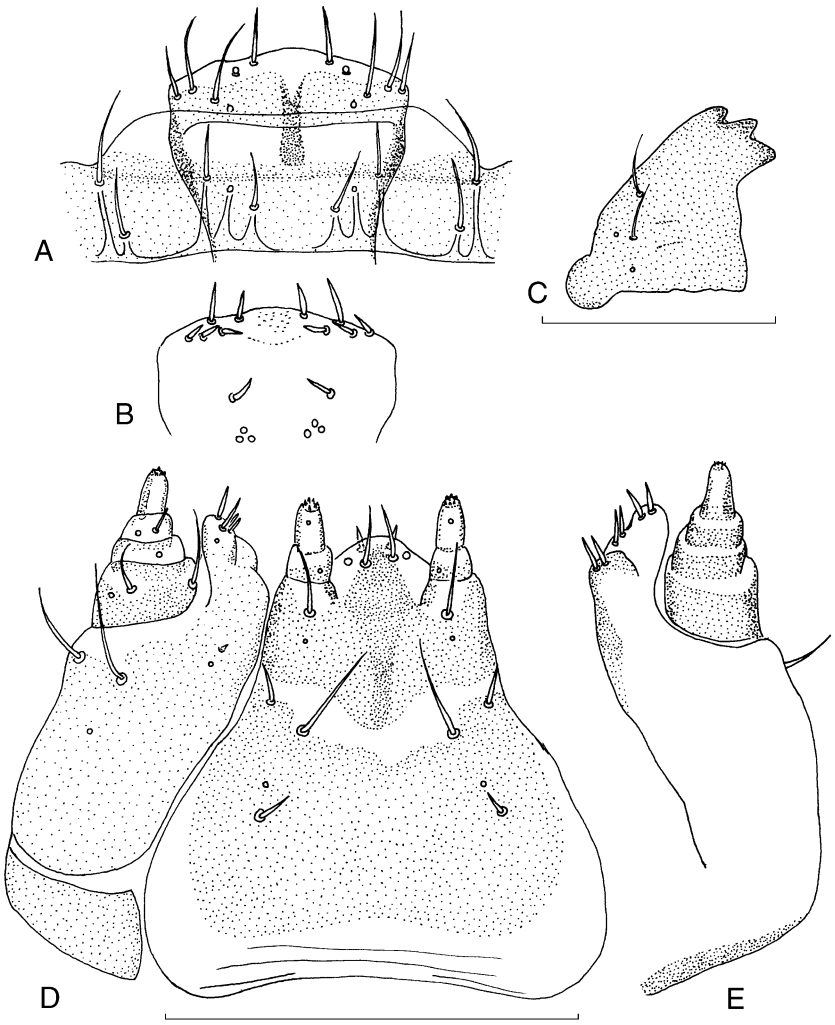

Fig. 3 A–E. *Hydnorobius hydnorae* (Pascoe), larva. —A. Epistoma, clypeus and labrum. —B. Epipharynx. —C. Mandible. —D. Maxilla and labium, ventral. —E. Maxilla, dorsal. Scales = 0.5 mm.

about 16 early instar larvae (some first instar) from tepals and anther column, one dead adult in perigynium tube, four larger larvae from fruit wall and stalk; 25 June 2003, about 30 larvae (in cells) from walls and stalks of dried fruits, four pupae and two adults reared on 19 November 2003.

Comparative notes. The larva of *Hydnorobius* shares with those in 'Allocorynini' (*Parallocorynus* and *Rhopalotria*) the body shape expanded at the mid-abdomen, and the occipital foramen deeply emarginate both dorsally and ventrally. Also, the line at cuticle attachment is distinctly ridged on the epicranium of *Hydnorobius* and *Parallocorynus*. Characters shared between the larvae of *Hydnorobius* and *Oxycraspedus*, like the antennae with the retractable basal membrane, three-segmented maxillary palps, bilobated maxillary mala, and pedal areas with both setae and sensillae, are regarded as plesiomorphies (which are modified, by reduction, in the larvae of 'Allocorynini'). The mature larva of *Oxycraspedus* is diagnosed by its occipital foramen with bisinuate dorsal emargination, and by having a frontal horn between the arms of bifurcate endocarina (this feature is present in all larval instars). A similar frontal horn, in the same position, is observed in the first instar larva of *Hydnorobius* (although absent in later instars), suggesting that this feature is probably ancestral in Oxycorynini, and subsequently lost (first instar larvae of *Rhopalotria* and *Parallocorynus* not seen). Frontal horns are also present in some larvae of Belinae (i.e. *Sphinctobelus niger*, *Rhinotia bidentata*) (May 1994), but May's figures show them as situated on the epistoma (anterior margin of frons) rather than between the endocarinal arms. Thus, frontal horns may have originated independently in Belinae and Oxycoryninae. The presence of a vestigial metathoracic spiracle (the functional one is on the mesothorax) is reported herein for both *Oxycraspedus* and *Hydnorobius* (Oxycoryninae), and May (1993: 39) also mentions its presence in larvae of some Belinae. Again, this is a relictual feature, deemed as simplesiomorphic for Curculionoidea, as

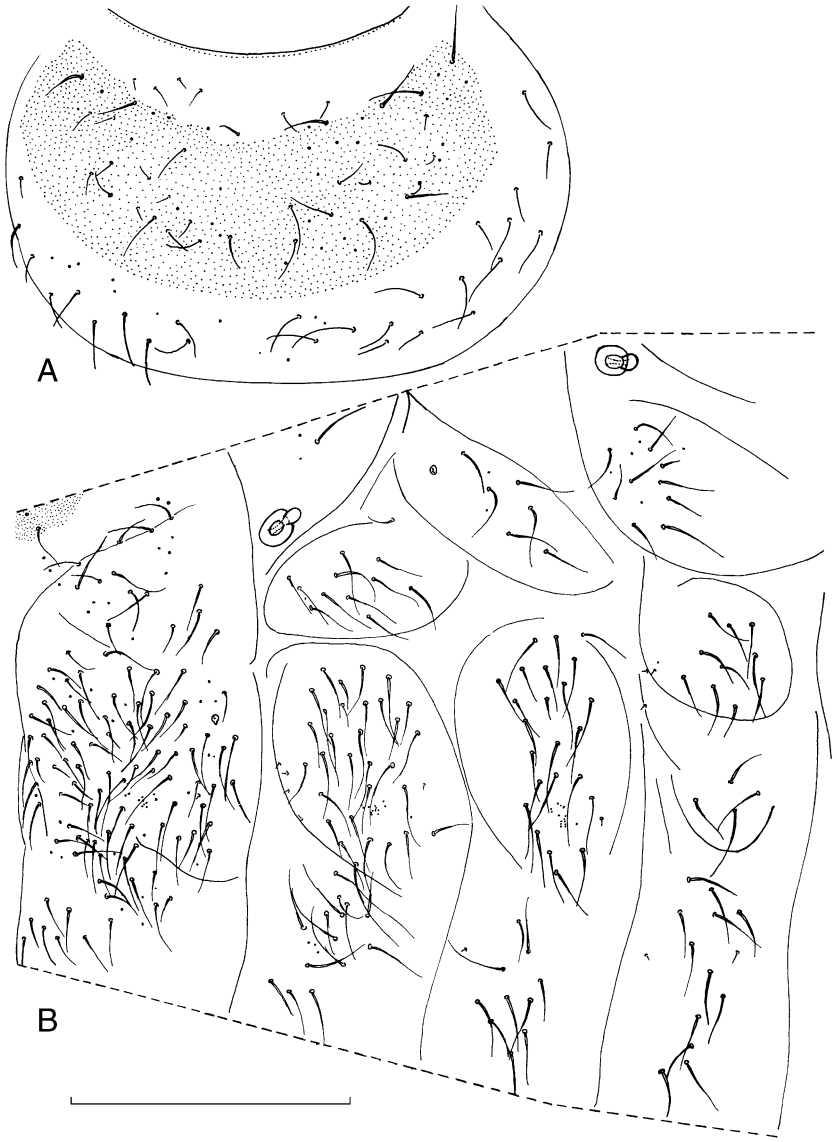

Fig. 4 A, B. *Hydnorobius hydnorae* (Pascoe), larva. —A. Thoracic segment TI, pronotum, dorsal. —B. Thoracic segments TI–III, abdominal segment AI, one side, latero-ventral. Scale = 1 mm.

vestigial metathoracic spiracles are present in larvae of basal weevils, like Nemonychidae and Anthribidae, and among chrysomeloids (May 1994: 392, 411), including both species used as outgroups in the present study.

Biology of Hydnorobius hydnorae

The host-plant. The weevil *Hydnorobius hydnorae* is host-specific to the plant *Prosopanche americana*, in the angiosperm family Hydnoraceae. It is an aclorophylous, hypogean, parasitic plant on the roots of 'algarrobo' in the genus *Prosopis* (Fabaceae) (Ruiz-Leal 1972: 128; Boelcke & Vizinis 1987). The vegetative body of the plant is constituted by subterranean rhizomes with austorial filaments attached to the host-roots. From the rhizomes, reproductive bodies grow to the surface, the perigynium or 'flower' being the only epigeal part of the plant. The floral or fruit body consists of a subterranean floral stalk (its length depends on the profundity of the rhizome), an inferior ovary, and the perigynium comprising a short perigynium tube, three lignified tepals and a central anther column (with pollen sacs appressed on the outer surface) [see Cocucci (1975) for details on floral anatomy].

The weevil biology. A brief account of the biology of the species is given by Bruch (1923), and it is completed herein on the basis of observations made in Mendoza (Argentina) in the field and in the laboratory since 2000. Early in summer the adults emerge from decayed fruit bodies from the previous year, and congregate (mainly in January) on new flowers that have

Fig. 5 A, B. *Hydnorobius hydnorae* (Pascoe), larva. —A. Thoracic segment TII, one side, dorsolateral. —B. Abdominal segment AIII, one side, dorsolateral. Scale = 1 mm.

reached the surface and are open and releasing pollen. There they mate, feed and oviposit. The weevils, dusted with pollen, may play a role in pollination contributing to cross-fertilization when visiting different flowers. Single eggs are laid into holes chewed in the perigynium, mostly in the tepals or floral lobules, but apparently they also oviposit in the anther column, as early larvae are also found there. The larvae consume parenchymatous tissue, of the perigynium first, and then larval development continues down inside the wall of the fruit and the floral stalk (where most mature larvae are found). Pupation, observed in November in the laboratory, takes place *in situ*, in an oval, smooth-walled cell built by the larva where it has overwintered. Adult emergence coincides with the appearance of the new flowers in early summer, usually after rain has softened the sandy soil. There appears to be one generation a year.

Adults of *Hydnorobius hydnorae* are more abundant in the field during January and can be collected throughout the summer until early autumn. Fruit bodies of *Prosopanche* with larvae, collected in May (autumn), were maintained in the laboratory for more than 6 months, inside a container with soil from the collecting site. The fruits reached maturity a few days after being collected, and their rapidly degrading tissues were allowed to dry out, as they would in nature, with the larvae developing inside to finally pupate *in situ*. Four pupae and two adults came by mid-November (spring).

Remarks on the biology of oxycorynine weevils. A common pattern emerges from what is known on oxycorynine biology. Adult weevils feed on pollen and oviposit in strobili or flowers, and larval development takes place inside parenchymatous tissues of such structures as they disintegrate and dry out. It is important to note that oxycorynine larvae consume vegetative tissue of the reproductive structure (sporophylls or axes of strobili, parts of the wall and stalk of fruits); they do not damage the sporangia or seeds (reproductive tissues) as do those of nemonychids or of the chrysomeloid Palophaginae.

Comparative note on host-plants of Hydnorobius *and allies.* The host-plants known for (two) species in the genus *Hydnorobius* Kuschel are in the family Hydnoraceae. Species in three genera close to *Hydnorobius* [*Oxycorynus* Chevrolat, *Alloxycorynus* Voss, and a new genus recently discovered (Anderson, in preparation)] have been collected in plants of the family Balanophoraceae. The Hydnoraceae and Balanophoraceae are both root-parasitic angiosperms, but they are not close phylogenetically. According to recent molecular studies (Nickrent *et al.* 2002), Hydnoraceae are near or within Aristolochiaceae (Piperales); the position of Balanophoraceae is more uncertain, but evidence places them within the eudicots, thus quite distant from Hydnoraceae (Nickrent *et al.* 2002).

Other Coleoptera associated with Prosopanche americana. A species of the beetle family Nitidulidae, identified by Bruch (1923) as *Neopocadius nitiduloides* Grouvelle, also develops in flower and fruit bodies of *Prosopanche americana*. Nitidulid larvae are found in large numbers, mingled with those of the belid *Hydnorobius hydnorae*, and are easily distinguished from those of *Hydnorobius* by their smaller size, the presence of legs and urogomphi. In addition, adults, pupae, and larvae (very hairy and expanded at the thorax) which correspond to a species in the beetle family Anobiidae have been collected from dry remains of *Prosopanche*. Also, a single adult specimen of an undetermined species of Anthribidae: Anthribiinae has been collected from a dry flower of *Prosopanche* in Telteca. Voucher specimens (larvae and adults) of all these beetles are deposited in the collection of IADIZA.

Conclusions

This study provides support for the monophyly of Belidae, their two main lineages, and subclades within them. It emphasizes the value of a detailed study of larval morphology for resolving weevil phylogenetic relationships. Increased taxon sampling and improvement of the morphological knowledge of adult and immature stages, as well as the use of molecular data, will be most important for further clarification of belid relationships at lower levels. This study also shows the significance of phylogenetic estimation for the clarification of the evolution of bio-ecological traits. The

diversification in Belidae is coupled with shifts in larval feeding habits.

Acknowledgements

I thank R. Leschen for the loan of material from NZAC, and for sending a copy of the paper on the genera of Belinae by G. Kuschel and himself. I also thank A. Bachmann for the loan of oxycorynine specimens from MACN. G. Debandi, S. Roig and G. San Blas assisted in several collecting trips to Telteca. G. Kuschel provided clues to find the larvae of *Oxycraspedus*, and enthusiastic collaboration by C. Ruiz and D. Lanfranco was invaluable for their discovery. Special thanks are extended to R. Anderson, A. Lanteri, C. Lyal, R. Oberprieler and J. Wenzel for encouragement along the way. R. Anderson provided constructive comments on the manuscript. This work was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina).

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Appendix

Characters used in the cladistic analysis of Belidae

Morphological data

Larva. Particularly important publications for interpreting and scoring the following larval characters are: May (1993, 1994) and Kuschel & May (1990, 1996). Characters previously used by Kuschel & Leschen (2003) are indicated by an asterisk, although there are differences in the character states recognized for characters 2, 9 and 26, in order to reflect accurately the observations for the taxa studied.

1 Head: (0) exposed or extrusible; (1) permanently retracted into thorax (Fig. 2A), ridged posteriorly for muscle attachment (Figs 2B,E).

2* Occipital foramen: (0) closed, not divided; (1) open, divided, with dorsal emargination ridged; (2) open, divided, with dorsal emargination simple (Fig. 2E); (3) open, divided, with dorsal emargination bisinuate; (4) widely open and extended to the apex of frons (the epicranial line is absent). [When the occipital foramen is open, the head is (usually deeply) emarginate behind both dorsally (Fig. 2E) and ventrally (Fig. 2F)].

3 Antenna: (0) three- or two-segmented, with apical cylindrical segment accompanying the sensorium, both mounted on convex segment; (1) one-segmented, lacking apical cylindrical segment, sensorium mounted on convex basal segment (Fig. 2G); (2) one-segmented, lacking apical cylindrical segment, sensorium mounted on flat basal segment.

4 Antenna: (0) without retractable basal membrane; (1) with retractable basal membrane (Fig. 2G).

5 Frontal lines: (0) visible; (1) not visible (Fig. 2E).

6 Endocarina: (0) simple; (1) bifid, V-shaped (Fig. 2E); (2) only minutely divided in front; (3) absent.

7* Frontal horn: (0) absent (Fig. 2E); (1) present.
(The condition is considered as observed in the mature larva, on the basis of which most descriptions are made).

8 Labral sensilla: (0) two basal sensilla; (1) two basal and two anteromedian sensilla (Fig. 3A).

9* Labrum-epipharynx: (0) without mesal or paramesal sclerotizations; (1) with paired paramesal, divergent sclerotizations; (2) with paired paramesal, curved sclerotizations; (3) with single mesal sclerotization (Fig. 3A).
[In addition there are labral basal sclerotizations extended laterally below clypeus (Fig. 3A)].

10 Epipharynx, anterolateral setae on each side: (0) three (Fig. 3B); (1) more than three in a row; (2) more than three in two or more rows.

11* Apex of mandible: (0) bidentate; (1) tridentate (Fig. 3C); (2) adentate.
(It can be supplementary teeth below the apical ones, which are not taken into account in this study).

12 Stipes: (0) with two setae (Fig. 3D); (1) multisetose.

13 Maxilla, palpifer: (0) distinct, sclerotized at least laterally (Fig. 3D); (1) not distinct (except by the palpiferal setae), not sclerotized.

14 Maxilla, palpifer: (0) with two setae (Fig. 3D); (1) multisetose.

15 Maxillary palp: (0) three-segmented (Fig. 3D); (1) two-segmented.

16 Apical segment of maxillary palp: (0) with seta; (0) without seta (Fig. 3D).

17 Maxillary mala: (0) subtruncate, expanded on the inner side (Fig. 3D); (1) cylindrical, parallel-sided.

18 Maxillary mala: (0) incised at apex; (1) incised at apex and bilobated (Fig. 3D,E); (2) entire, with no trace of incision or lobes.

19 Maxillary mala: (0) with discrete number of setae (Fig. 3D,E); (1) densely setose.

20 Maxilla, tooth-like projection on inner margin of mala; (0) present; (1) absent (Fig. 3D).

21 Labium: (0) with a lateral strut or postlabial bracon; (1) without a lateral strut (Fig. 3D).

22 Postmentum: (0) pigmented on the area including labial seta 1 (Fig. 3D); (1) unpigmented.

23* Pronotal shield: (0) with a medial (ecdysial) line; (1) entire, without a median line (Fig. 4A).

24 Posterior margin of pronotum: (0) simple (Figs 2A, 4A); (1) enlarged and sloping back to a slightly thickened hind margin; (2) enlarged and sloping back to a grossly thickened hind margin.
[This character is ordered. The conditions (1) and (2) both imply a modified pronotum, and it seems more likely a change from a simple, unmodified margin (0) to a slightly thickened one (1), or from the latter condition (1) to a grossly thickened margin (2). It would be logical to consider a higher cost to change from (0) to (2)].

25 Pedal area: (0) with a subcircular cluster of sensilla combined with setae (Fig. 4B); (1) only with setae, no sensilla; (2) only with sensilla, no setae.

26* Spiracles on thorax and abdomen: (0) with two air-tubes, bicameral; (1) with one air-tube, unicameral (Figs 4B, 5); (2) without air-tubes.
(The air-tubes are one or two lateral accessory chambers connected to the main opening of the spiracle).

27 Spiracular air-tubes: (0) annulated; (1) simple (Figs 4B, 5).
(When rings are present on air-tubes they are considered 'annulated air-tubes').

28 Body, at the area of typical thoracic (TII–III) and abdominal segments (AI–VII): (0) of even width or slightly widest at thorax; (1) widest at mid-abdomen (Fig. 2A).

29* Body shape and anus: (0) body straight or slightly curved, anus terminal or subterminal; (1) body recurved terminally, anus ventral (Fig. 2A).

30 Alimentary canal, posterior ventriculus: (0) without gastric caecae; (1) with gastric caecae arranged randomly (Fig. 2D); (2) with gastric caecae in two clusters on either side.

A*dult*. Particularly important publications for interpreting and scoring the following adult characters are: Kuschel (1959), Calder (1990), Thompson (1992), Zimmerman (1994), Kuschel (1995a,b), Kuschel & May (1990, 1996), Kuschel & Leschen (2003).

31 Antenna: (0) with club indistinct; (1) with distinct club (last articles expanded), articles 9–10 and 10–11 loosely connected; (2) with distinct club (last articles expanded), articles 9–10 loosely connected but articles 10–11 tightly joined or compact.
(The antenna has 12 articles, the first is the 'scape', followed by seven 'funicular' articles, and the last four constitute the 'club', which appears three-articulated because the last 11–12 are tightly fused).

32 Mandible: (0) plurisetose, with numerous setae; (1) paucisetose, with up to three to four setae.

33 Prementum: (0) small, leaving maxilla exposed in ventral view; (1) large, concealing maxilla in ventral view.

34 Lateral margin of prothorax: (0) not carinate; (1) with sharp carina.

35 Elytron: (0) with erect sensory setae; (1) lacking such setae.
36 Elytra: (0) not costate; (1) costate.
37 Fore coxal cavity: (0) partially open laterally; (1) completely closed laterally.
38 Fore coxae: (0) contiguous; (1) separate.
39 Tarsi: (0) five-segmented, pseudotetramerous; (1) four-segmented, pseudotrimerous.
(In weevils, the fourth tarsite is called 'cryptotarsite' because it is usually very reduced and hidden between the lobes of the third).
40 Tarsal segment 1: (0) at least as long as 2 and 3 combined; (1) shorter than 2 and 3 combined.
41 Tarsal segment 2: (0) with (at least slightly) projecting apical angles; (1) rounded at apical angles.
42 Grooming area of dense vestiture on fore tibia, on face opposite tarsal articulation: (0) absent; (1) present.
43 Tibial spurs: (0) present; (1) absent.
44 Laterodorsal surface of middle and hind femora and tibiae: (0) lacking a crenulated ridge; (1) crenulated, with a longitudinal row of denticles.
45 Tergite 8 of male: (0) concealed under tergite 7; (1) exposed beyond tergite 7.
46 Sternite 8 of male: (0) with distinct apodeme; (1) lacking distinct apodeme.
47 Aedeagal pedon and tectum: (0) completely free; (1) largely fused.
48 Aedeagus, basal sclerite in internal sac: (0) absent; (1) present.
49 Tergite 9 of female: (0) sclerotized; (1) entirely membranous.
50 Female genitalia, proximal hemisternites: (0) with pigmentation reduced to struts; (1) entirely pigmented.
51 Spermatheca: (0) falciform, well pigmented; (1) not falciform, very reduced in size and/or submembranous; (2) absent. [States (1) and (2) are associated with a relatively large spermathecal gland].
52 Spermathecal gland: (0) tapering to the spermathecal duct; (1) forming a common tube with the duct.
53 Spermathecal duct: (0) inserted on bursa; (1) inserted on common oviduct.
54 Alimentary canal, proventricular blades: (0) not developed; (1) well developed, with sharp ridges on external face.
55 Alimentary canal, hind gut: (0) with rectal ring: (1) with rectal loop.

Biological data. Information on host-plants and larval feeding habits was gathered from the collection data of the specimens studied and/or the literature, mainly from Bruch (1923), Anderson (1941), May (1993, 1994), Zimmerman (1994), Howden (1995).
56 Host-plant taxon used for larval development [G, gymnosperms; A, angiosperms]: (0) G, Araucariaceae; (1) G, Cycadaceae; (2) A, Fabaceae; (3) A, Nothofagaceae; (4) A, Proteaceae; (5) A, Myrtaceae; (6) A, Araliaceae; (7) A, various; (8) A, Hydnoraceae; (9) A, Balanophoraceae.
57 Tissue consumed by larvae: (0) male strobili, pollen sacs; (1) strobili (male or female), vegetative tissues (sporophylls or axes); (2) flower or fruit, vegetative tissues; (3) trunk, branch or twig.
58 State of host-plant tissue at moment of consumption (by larva): (0) living, healthy; (1) dying, decaying or dead.