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Phylogenetic Relationships of the Genus *Hoplia* Illiger (Scarabaeidae: Hopliinae)

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Abstract

Results of phylogenetic analysis based on 34 morphological characters of 24 species of 11 genera of Hopliinae from Europe, Japan, South Africa, Madagascar, North and Central America, indicates that the genus *Hoplia* is a monophyletic group with species distributed in Europe, Japan and America. Based in this analysis the Asiatic genus *Ectinohoplia* is the closest relative of the genus *Hoplia*, and the South American genus *Barybas* (Melolonthinae: Macrodactylini) is the sister group of Hopliinae.

Introduction

The genus *Hoplia* Illiger is one of 62 genera belonging to the subfamily Hopliinae (Carrillo-Ruiz & Morón 2006). Most part of the species richness of the group belongs to this genus, including 297 of 397 species of the hopliids in the world (Dalla-Torre 1912-1913, Boyer 1940, Hardy 1977, Lacroix 1998, Micó 2001). Species of *Hoplia* are widely distributed in the Middle East, Asia, Europe, Northern Africa and Madagascar, as well as in America North of Panamá (Dalla-Torre 1912-13, Hardy 1977, Baraud 1981, Lacroix 1998, Evans 2003, Micó *et al* 2003). The type species of the genus is *Scarabaeus farinosus* L., subsequently designated by Medvedev in 1952 (Evans 2003), includes small species (0.4-0.7 mm) that are characterized by the wide body, slightly depressed and covered with abundant scales and setae. The clypeus is short and the antennal club is usually short and oval. Pygidium protrudes at the end of the elytra, without apical spurs of metatibia and a single metatarsal claw (Morón *et al* 1997).

Little is known about the biology of the species of this genus. Ritcher (1949) described the larvae of three North American species: *Hoplia equina* LeConte, *H. oregona* LeConte and *H. cazieri* Boyer (but following Evans (2003) the last two names were synonymized under *H. callipyge* LeConte). The adults of *Hoplia sackeni* LeConte and *H. callipyge* have been observed feeding on foliage and flowers (Boyer 1940) and adults of *H. squamifera* Burmeister occasionally have been collected on flowers of *Hibiscus rosa-sinensis* (Morón 1996). Adults of the Iberian species of *Hoplia* feed mainly on pollen of Gramineae, Rosaceae, Plantaginaceae, Asteraceae, Malvaceae, Umbeliferae and numerous fruit trees (Micó 2001).

Available publications on the genera of Hopliinae mainly are regional works on taxonomy (Péringuey 1902, Boyer 1940, Baraud 1981, Lacroix 1998, Hardy 1977, Micó *et al* 2003), but works on the phylogenetic relationships of these genera are scarce (Carrillo-Ruiz & Morón 2006). The objectives of the present study were: a) to confirm the monophyly of the American species of hopliids and their inclusion into the genus *Hoplia*; b) to obtain

a hypothesis of the phylogenetic relationships among some species of the genus *Hoplia* distributed in Asia, Europe and America, with representative species of the genera *Madahoplia* Lacroix, *Odontoplia* Fairmaire, *Echyra* Erichson, *Gymnoloma* Burmeister, *Hoplocnemis* Harold, *Peritrichia* Burmeister, *Lepithrix* Serville, *Heterochelus* Burmeister and *Scelophysa* Burmeister (Hopliinae), distributed in South Africa and Madagascar.

Material and Methods

Taxa selection

In this study, we selected taxa based on the hypothesis proposed by Carrillo-Ruiz & Morón (2006), in which hopliids are considered a subfamily of the Scarabaeidae. The out-group was formed with representative species of five genera of Melolonthinae (Melolonthini and Macrodactylini). The in-group was formed with 24 representative species of 11 genera of Hopliinae (Table 1). We studied 160 males deposited in the collections of Instituto de Ecología A. C., Xalapa (IEXA), Canadian National Collection, Ottawa (CNC), University of Nebraska State Museum, Lincoln (UNSM), National Museum of Natural History, Washington D. C. (NMNH), The Natural History Museum, London (NHML), and the private collections of M. A. Morón, Xalapa, México (MXAL), H. F. Howden (HAHC), and Bruce Gill (BDGC) Ottawa, Canada. Also we examined the type material of *Hoplia argyritis* Bates, *Hoplia asperula* Bates and *Hoplia surata* Bates. Dried specimens were softened with water vapor in order to dissect the genitalia and mouthparts. These structures were extracted using microforceps and insect pins, card-mounted, and then pinned beneath each specimen. The left elytron were separated to treat with KOH (5%) and then dry-mounted for scanning with an electron microscope (Jeol JSM-5600LV, 1,000x to 2,000x), to obtain better definition of the morphology of the setae and scales. We then proceeded to score each of the 34 morphological characters (*See Online Supplementary Material - Appendix 1*).

Table1 Selected taxa for this study based on the phylogenetic hypothesis proposed by Carrillo-Ruiz & Morón (2006). O.G. = out-group; I. G. = in-group.

Taxonomic rank studied	Species	No. specimens
Melolonthinae		
Melolonthini		
Phyllophaga lenis Horn	O. G.	10
Macrodactylini		
Macrodactylus silphys Bates	O. G.	10
Isonychus ocellatus Burmeister	O. G.	10
Ceraspis pilatei Harold	O. G.	10
Barybas aurita Bates	O. G.	5
Hopliinae		
Madahoplia nodipennis (Burmeister)	I. G.	1
Echyra oberthuri Lacroix	I. G.	1
Odontoplia alluandi Fairmaire	I. G.	1
Dichelus detritus Burmeister	I. G.	1
Scelophysa pruinosa Burmesiter	I. G.	1
Peritrichia vansoni Schein	I. G.	1
Lepithrix fulvipes Thunberg	I. G.	1
Hoplocnemis mutica Burmeister	I. G.	3
Gymnoloma femorata Burmeister	I. G.	5
Ectinohoplia obducta Motschulsky	I. G.	10
Hoplia festiva Burmeister	I. G.	10
Hoplia asperula Bates	I. G.	5
Hoplia subcostata Bates	I. G.	10
Hoplia squamifera Burmeister	I. G.	10
Hoplia equina LeConte	I. G.	5
Hoplia modesta Haldeman	I. G.	5
Hoplia trivialis Harold	I. G.	5
Hoplia dispar LeConte	I. G.	5
Hoplia surata Bates	I. G.	10
Hoplia argyritis Bates	I. G.	10
Hoplia pollinosa Kryn	I. G.	2
Hoplia farinosa L.	I. G.	2
Hoplia chlorophana Erichson	I. G.	4
Hoplia coerulea (Drury)	I. G.	5
Hoplia moerens Waterhouse	I. G.	2

Phylogenetic methods

Phylogenetic analyses were performed using 34 morphological characters. The matrix of characters employed for the analysis (Table 2) was built and analyzed using Winclada ver. 1.00.08 (Nixon 2002) and NONA ver. 2.0 (Goloboff 1999). The data were analized with a heuristics search routine (1,000 replications). Characters states for the cladistic analysis were polarized by out-group comparison (Maddison *et al* 1984, Nixon & Carpenter 1993). Characters are scored only for males, and all the characters were discrete rather than continuous values. Characters were coded as either binary or multistate (0-4). Multistate characters were treated as unorderer and with equal weight. The tree was rooted with the Melolonthinae, *Phyllophaga lenis* Horn. To evaluate the characters of the matrix we obtain the bootstrap values (1000 replications).

Table 2 Characters states of taxa used in the phylogenetic analysis.

	11111111112222222222333333
	12345678901234567890123456789012345
Phyllophaga lenis	01101102112113200110002111102000100
Isonychus ocellatus	01110301010013211011102111100100010
Barybas aurita	01111301004111001011011101102100100
Ceraspis pilatei	11111201204010011111102101102122110
Macrodactylus silphys	11110301010013211011102111102022010
Madahoplia nodipennis	01110301004111000100011000002001000
Echyra oberthuri	01110201004011000001111000000011100
Odontoplia alluandi	11130102004012100001121000012011000
Dichelus detritus	01130010102003200101121110012010000
Scelophysa pruinosa	00130001122001000011101100012010000
Peritrichia vansoni	00130001012001000111101000002010000
Lepithrix fulvipes	00130001112001000111101000012010000
Hoplocnemis mutica	00130201122100000101101000000010000
Gymnoloma femorata	00100311122111000101001000000010200
Ectinoplia obducta	01100301104112100100000010010011000
Hoplia moerens	01020301221111000100000000010010100
Hoplia pollinosa	01021301123112100100000010010010100
Hoplia farinosa	01021301122112100100000000010010100
Hoplia clorophana	01021301122112100101000000010010100
Hoplia coerulea	01021301121112100100000000010010100
Hoplia argyritis	01120002121112100100010000000011110
Hoplia surata	01120012121112100100010000000001110
Hoplia asperula	01121002101112000100010000000211110
Hoplia subcostata	01121212121111000100010000001001010
Hoplia squamifera	01121202121111000100010000001101110
Hoplia festiva	01121212121112100100010000000001110
Hoplia trivialis	01121201122111000101010000001001110
Hoplia equina	01121201122111000101010010010000010
Hoplia modesta	01121001122111000101010010010011110
Hoplia dispar	01121101121112100101010000001001110

Results and Discussion

The phylogenetic analysis resulted in five equally parsimonius trees with a total length (TL) of 159, consistency index (CI) of 0.410, and retention index (RI) of 0.71. The strict consensus tree is shown in the Fig 1. In this phylogenetic hypothesis, two main clades were obtained. The first clade (1), including three representative species of Macrodactylini (*Ceraspis pilatei* Harold, *Isonychus ocellatus* Burmeister and *Macrodactylus sylphis* Bates), is supported by a bootstrap value of 63% and two synapomorphies: sixth abdominal sternite well development and disc of the mentum furrowed.

The relationships among *Isonychus ocellatus* and *Macrodactylus sylphis* (clade 3) is supported by three synapomorphies: short, abundant, piliform setae at the pronotum, protibia with two well- defined teeth and distal part of left mandible truncated.

The second main clade (2), support the relationships among *Barybas aurita* Bates and the clade 4, joins all the representative species of Hopliinae. The clade 2 is supported by a bootstrap value of 60% and two synapomorphies: oval squamiform setae on elytra and one metatibial spur.

The clade of the Hopliinae (4) is supported by a bootstrap value of 71% and five synapomorphies: one metatarsal claw (character state that changes in *Dichelus detritus* Burmeister and in *Scelophysa pruinosa* Burmeister), widely retractile metatarsal claws, metatarsal claws without onychia, basis of the *spiculum gastrale* truncated and distal part of left mandible truncated. The Madagascar species *Madahoplia nodipennis* (Burmeister) is related with the clade 5 that maintains the relationships of the rest of the Hopliinae and is supported by one synapomorphy: basis of the *spiculum gastrale* widened. Two species from Madagascar appears closely related *Echyra oberthuri* Lacroix and *Odontoplia alluandi* Fairmaire (clade 6), this relationship is supported by three synapomorphies: lateral edge of the pronotum entire, protibia with two well defined teeth and mesocoxae separated.

The clade 7 joins South African genera and the clade (9) that maintains the relationships among the representative species of the genus *Ectinoplia* and the representative species of the genus *Hoplia* is supported by a bootstrap value of 51% and three synapomorphies: clypeal apex rounded, posterior edge of pronotum with piliform setae and metatibial external border entire.

The clade of the six South African species (clade 8) is supported by a bootstrap value of 60% and two synapomorphies: body depressed and pronotum with large, abundant piliform setae. The clade of *Hoplocnemis mutica* Burmeister, *Peritrichia vansoni* Schein, *Lepithrix fulvipes* Thunberg, *D. detritus* and *S. pruinosa* (clade 10) is supported by a bootstrap value of 52% and two synapomorphies: clypeal apex ornate and mesoepimera dorsally exposed. The relationships of *D. detritus*, *S. pruinosa*, *Peritrichia vansoni* and *L. fulvipes* (clade 12) is supported by two synapomorphies: protibia with apical spurs and apex of the parameres acute.

Ectinohoplia obducta Motschulsky appears as the sister taxon of the genus *Hoplia* (clade 9), is supported by a bootstrap value of 53% and three synapomorphies: metatibiae without spurs, short distance between the protibial teeth and parameres large. The relationships among all species of *Hoplia* (clade 11) are supported by a bootstrap value of 61% and two synapomorphies:

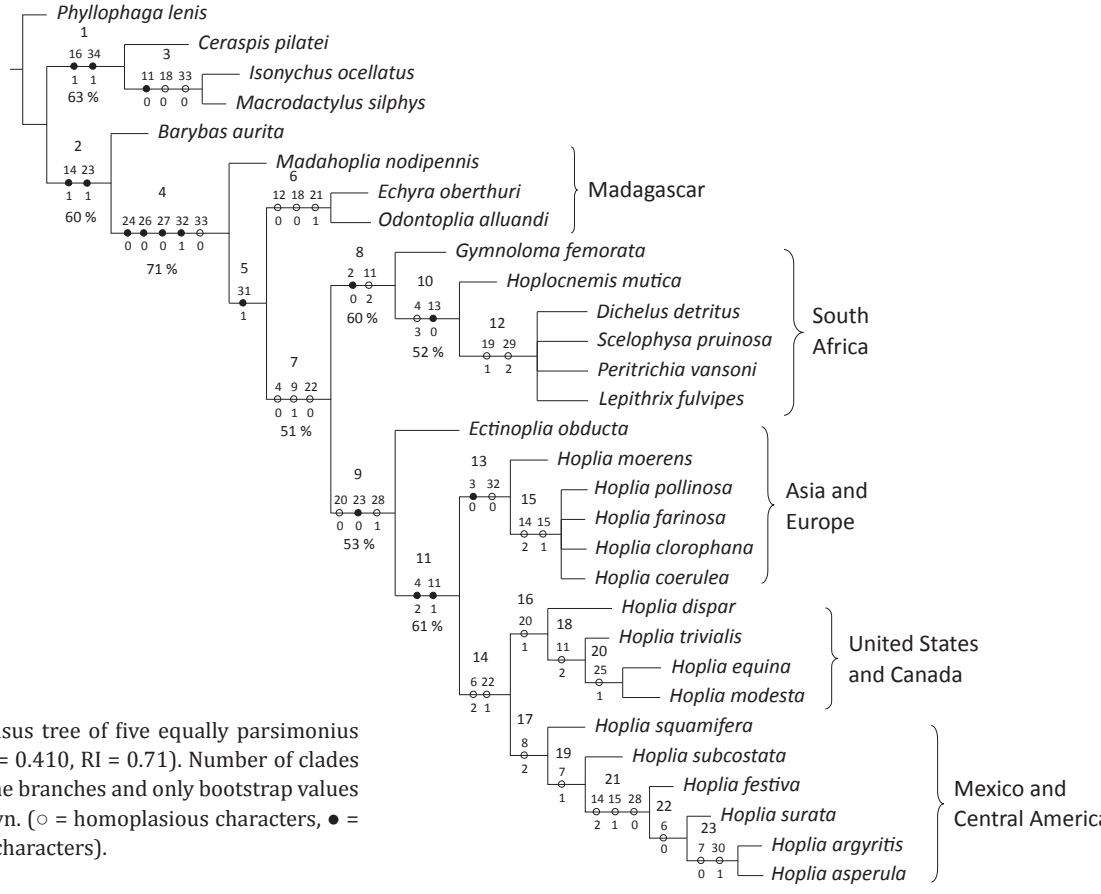

Fig 1 Strict consensus tree of five equally parsimonius trees (TL = 159, CI= 0.410, RI = 0.71). Number of clades are shown upper the branches and only bootstrap values over 50% are shown. (○ = homoplasious characters, ● = nonhomoplasious characters).

anterior clypeal apex entire and pronotum with short, scarce, piliform setae and distal part of the left mandible curved.

The clade of the genus *Hoplia* is formed by two main clades: the first, clade 13 that maintains the relationships among European and Asiatic species [*Hoplia moerens* Waterhouse, *H. pollinosa* Kryn, *H. farinosa* (L.), *H. clorophana* Erichson and *H. coerulea* (Drury)], is supported by two synapomorphies: short clypeus and apex of the *spiculum gastrale* rounded, the relationships among Euro-asiatic species are not resolved at all; we obtained a polytomy among *H. pollinosa*, *H. farinosa*, *H. clorophana* and *Hoplia coerulea*, species with rounded squamiform setae without stalk.

The second, clade 14 joins the North American species (*H. dispar* LeConte, *H. trivialis* Harold, *Hoplia equina* LeConte and *H. modesta* Haldeman) and North and Central American species is supported by two synapomorphies: metatibial external border sinuate and without frontoclypeal suture.

The clade of the *Hoplia* species from the United States and Canada (clade 16) is supported by one sinapomorphy: distance between the protibial teeth large. In this clade *H. equina* and *H. modesta* are very closely related, the two species present the external metatarsal claw cleft.

The clade joining the species of North and Central America (clade 17), is supported by one synapomorphy: ten antennal segments. In this clade two species are very close, *H. argyritis* and *H. asperula*, the relationship is supported by two synapomorphies: monochromatic antenna and apex of the parameres with piliform setae.

With our analysis the monophyly of Hopliinae is not rejected; the species of *Barybas* is the closest relatives of the hopliids, in fact *Barybas* is the sister group of the Hopliinae, these results are congruent with the phylogenetic hypothesis proposed by Carrillo-Ruiz & Morón (2006).

This phylogenetic hypothesis supports the genus *Hoplia* closely related to the species of the genus *Ectinohoplia* as it sister group. Both genera present the metatibiae without spurs, however in the genus *Hoplia* the distal part of the left mandible is curved while in *Ectinoplia* the distal part of the left mandible is shortened; moreover in all species of *Hoplia* the clypeal apex is entire and in the species of *Ectinohoplia* represented in this study the clypeal apex is sinuate.

The results obtained in this work show the Madagascaran genera as paraphyletic group with a basal position in the topology with respect to the others hoplines. It is possible that the species distributed in

Madagascar constitute the sister group of the subfamily Hopliinae and perhaps its position is out of this subfamily (Lacroix 1998). By the other hand this hypothesis maintains the South African genera very close to the Hopliinae genera distributed in Asia (*Ectinoplia*) and the representative species of the genus *Hoplia*. There is evidence that the genus *Hoplia* is a Holarctic group and is necessary to continue exploring the relationships among the genus *Hoplia*, other species of the genus *Ectinoplia*, and other genera of the subfamily to confirm the internal relationships of the Hopliinae.

Based on the present analysis we confirm that: a) the American species of hopliids belong to the genus *Hoplia*; b) the monophyly of the genus *Hoplia* is not rejected; c) *Ectinohoplia* is the closest relative of the genus *Hoplia*.

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Online Supplementary Material - Appendix I

Carrillo-Ruiz H, Morón MA (2011) Phylogenetic Relationships of the Genus *Hoplia* Illiger (Scarabaeidae: Hopliinae)

List of the characters used for the phylogenetic analysis. Number in parentheses indicate the state assigned for each character.

1. Body form, determined as the total length measured from the apex of clypeus to the apex of elytra divided among the maximum elytral width. Rounded (0) (two times longer than wide), elongate (1) (three times longer than wide)

2. Dorso-ventral body shape. Depressed (0), not depressed (1)

3. Proportions of the clypeus. Short (0) (wider than long), large (1) (longer than wide)

4. Clypeal apex. Rounded (0), sinuate (1), entire (2), ornate (3)

5. Anterior region of the clypeal surface. Not raised (0) (Fig 2), raised (1) (Fig 3)

6. Frontoclypeal suture. Complete-entire (0), complete-sinuate (1), printed only at sides (1), absent (2)

7. Coloration of the antenna. Monochromatic (0), dichromatic (1)

8. Number of the antennal segments. 8 (0), 9 (1), 10 (2)

9. Posterior edge of the pronotum. Without piliform setae (0), with piliform setae (1), with piliform setae only at sides

10. Squamiform setae at the pronotum. Only squamiform setae (0), only piliform setae (1), piliform and squamiform setae (2)

11. Piliform setae at the pronotum. Short-abundant (0), short-scarce (1), large-abundant (2), large-scarce (3), absent (4)

12. Lateral edge of pronotum. Entire (0), crenate (1)

13. Mesoepimera. Dorsally exposed (0), not dorsally exposed (1)

14. Squamiform setae on elytra. Large and narrow (0) (Figs 10, 15), Oval (1) (Figs 11,13), rounded (2) (Figs 12-14), absent (3)

15. Insertion of the squamiform setae at the elytral surface. With stalk (0) (Figs 13, 15), without stalk (1) (Fig 14), not apply (2)

16. Sixth abdominal sternite. Reduced (0), Well development (1)

17. Length of fifth abdominal sternite two times longer than preceeding. Absent (0), present (1)

18. Number of well defined teeth on protibial external border. 2 (0), 3 (1)

19. Apical spurs of protibiae. Absent (0), present (1)

20. Distance among the first protibial teeth and the second protibial teeth: Small (0) (less or same to 0.04 mm), large (1) (more than 0.04 mm)

21. Mesocoxae. Not separated (0), separated (1)

22. Metatibial external border. Entire (0) (Fig 4), sinuate (1) (Fig 5), curve (2) (Fig 6)

23. Metatibial spurs. Absent (0), 1(1), 2 (2)

24. Number of metatarsal claws. 1(0), 2 (1)

25. External metatarsal claw. Entire (0), cleft (1)

26. Movement of metatarsal claws. Widely retractile (0), scarcely retractile or moveless (1)

27. Metatarsal onychia. Absent (0), present (1)

28. Length of parameres. Short (0) (less length than the genital capsule), large (1) (more length than the genital capsule)

29. Apex of the parameres. Rounded (0) (Fig 7), capitate (1) (Fig 8), acute (2) (Fig 9)

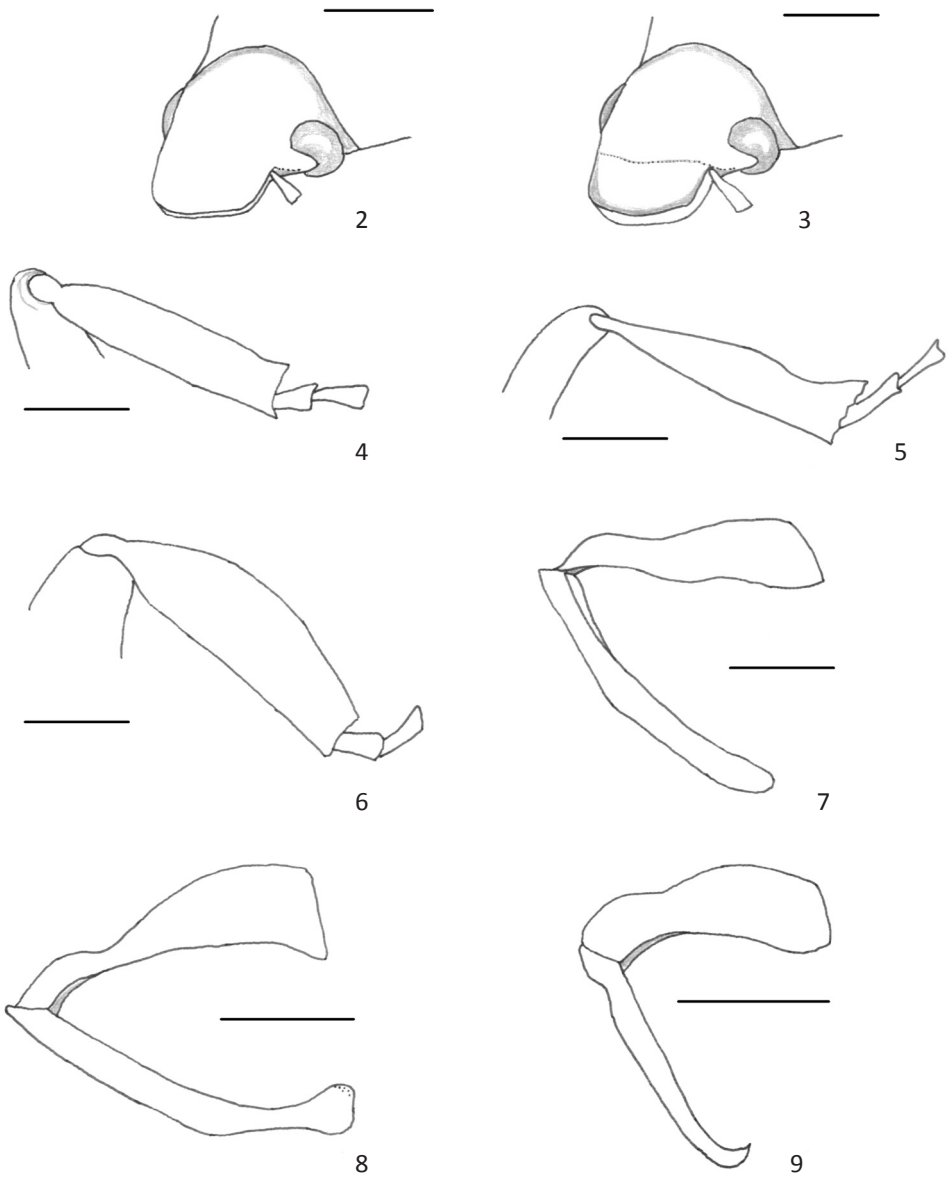
30. Piliform setae on the apex of parameres. Absent (0), present (1)

31. Basis of the *spiculum gastrale*. Narrowed (0), widened (1), absent (2)

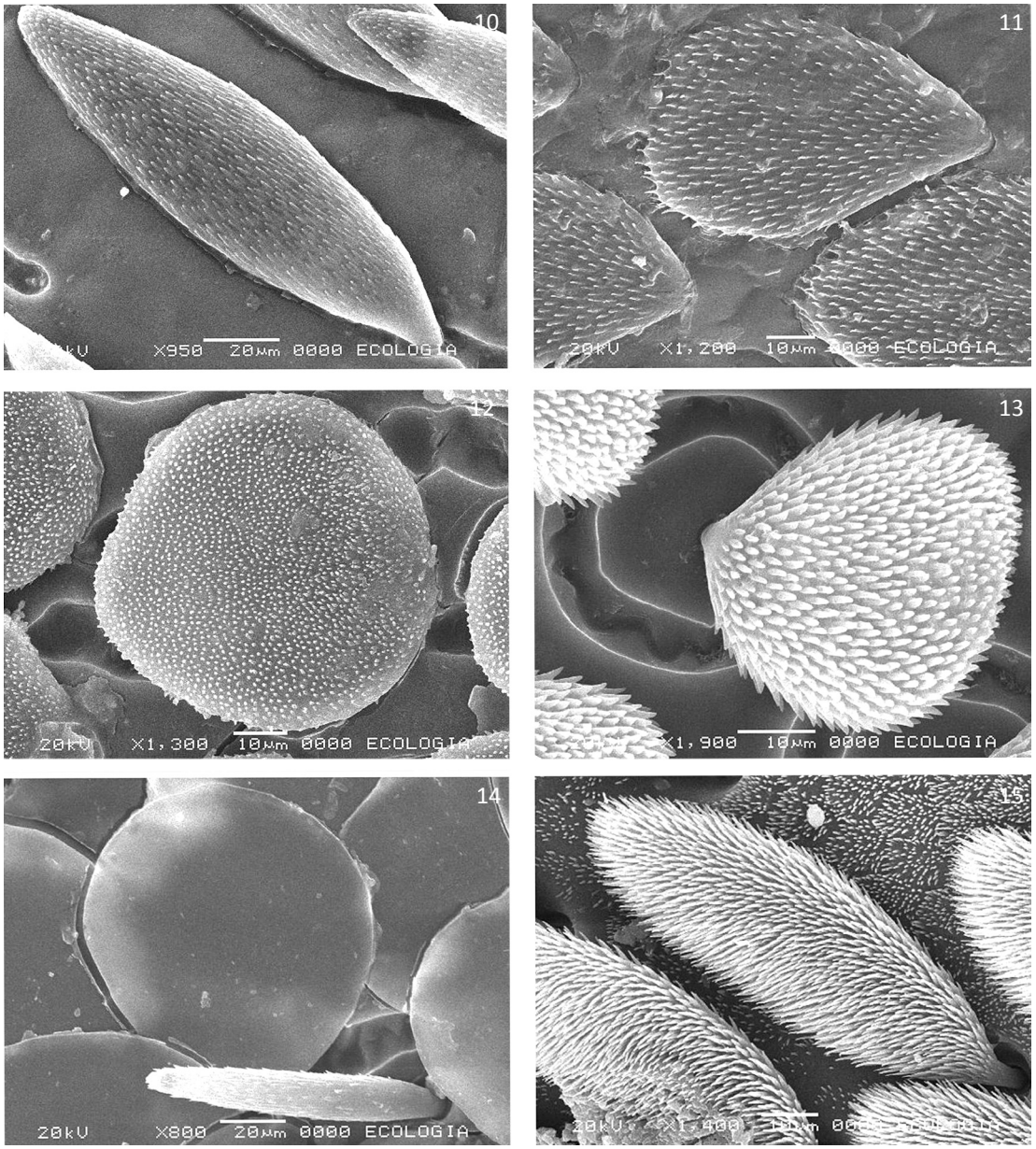
32. Form of the basis of the *spiculum gastrale*. Rounded (0), truncate (1), not apply (2)

33. Form of distal part of left mandible. Truncate (0), Curved (1), elongated (2)

34. Disc of mentum. Longitudinally without furrow (0), furrowed (1)



Figs 2-9 Structures of Hopliinae. 2. Head of *Hoplia argyritis* with anterior region of clypeus not raised; 3. Head of *Hoplia farinosa* with anterior region of clypeus raised; 4. Metatibia of *Madahoplia nodipennis* with external border entire; 5. Metatibia of *Echyra oberthuri* with external border sinuate; 6. Metatibia of *Scelophysa pruinosa* with external border curved; 7. Aedeagus of *Hoplia coerulea* with apex of parameres rounded; 8. Aedeagus of *Hoplia squamifera* with apex of parameres capitate; 9. Aedeagus of *Hoplia asperula* with apex of parameres acute. Scale lines = 1 mm, except Figs 4, 7, 15 = 0.5 mm.



Figs 10-15 Squamiform setae of: 10. *Ceraspis pilatei*. 11. *Scelophysa pruinosa*. 12. *Hoplia argyritis*. 13. *Hoplia asperula*, setae with stalk. 14. *Hoplia coerulea*, setae without stalk. 15. *Lepithrix fulvipes*, setae with stalk.