
Comparative morphology of the venom apparatus in the braconid wasp subfamily Rogadinae (Insecta, Hymenoptera, Braconidae) and related taxa

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The morphology of the venom apparatus intima in representatives of 38 genera of the problematic braconid wasp subfamily Rogadinae and other cyclostome braconids was investigated and a preliminary phylogenetic analysis for the group was performed with the information obtained. Despite the limited number of characters, the data suggest several relationships at various taxonomic levels. The venom apparatus in the Clinocentrini and the Stiropiini is relatively unmodified and similar to that found in other genera previously placed within a broader concept of the Rogadinae (e.g. genera of Lysitermini, Pentatermini, Tetratermini, Hormiini) and also to that of the Betylobraconinae. The presence of a cone of filaments located inside the secondary venom duct near to its insertion on the venom reservoir/primary venom duct is proposed as a synapomorphy for the tribe Rogadini to the exclusion of Stiropiini, Clinocentrini and Yeliconini. Other features of the secondary venom duct and its insertion on the venom reservoir/primary venom duct support a number of relationships between the genera of the Rogadini and also within the large genus *Aleiodes*. A clade containing 15 Rogadini genera (*Bathoteca*, *Bathotecoides*, *Bulborogas*, *Canalirogas*, *Colastomion*, *Conspinnaria*, *Cystomastacoides*, *Macrostomion*, *Megarhogas*, *Myocron*, *Pbolicchora*, *Rectivena*, *Rogas*, *Spinaria* and *Tiraphis*) is supported by the presence of a thickened and short secondary venom duct, whereas the different members of *Aleiodes* (excluding members of the subgenus *Heterogamus*) and *Cordylorhogas* are distinguished by having a recessed secondary venom duct with well-defined and numerous internal filaments. New World *Rogas* species exhibit a unique venom apparatus and may not be closely related to the Old World ones. Features of the venom apparatus of the enigmatic genus *Telengaia* and the exothecine genera *Shawiana* and *Colastes* suggest that the Telengainae and Exothecinae are both closely related to the Braconinae, Gnamptodontinae, and possibly to the Opiinae and Alysinae. An unsculptured venom reservoir was found in one specimen of the type species of *Avga*, *A. choaspes*, which is consistent with it occupying either a very basal position within the cyclostome braconids or belonging to a recently recognized 'Gondwanan' clade that also includes the Aphidiinae.

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Introduction

In recent years, attempts to discover evolutionary relationships between different groups of parasitic braconid wasps have made increasing use of novel characters and character systems. Among these are morphological studies of the final

instar larval head capsule (e.g. Capek 1970), male genitalia (e.g. Belokobylskij 1987; Quicke 1998), internal and external ovipositor features (Quicke *et al.* 1992a,b, 1994; Rahman *et al.* 1998a,b), and venom apparatus (Edson & Vinson 1979; Quicke *et al.* 1992c, 1997). Information derived from such studies has

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proved useful in phylogenetic analyses at a range of taxonomic levels, from subfamily (e.g. Quicke & van Achterberg 1990; Whitfield 1992; van Achterberg & Quicke 1993; Quicke & Belshaw 1999; Dowton *et al.* 2002) to genus level (e.g. Belshaw *et al.* 2001; Belokobilskiy *et al.* in preparation).

As regards venom apparatus morphology in the Braconidae, Edson & Vinson (1979) first noted a major difference between that of the cyclostome group of subfamilies which have a heavily muscularized (type 1) venom reservoir whose intima is strongly spirally sculptured, and that of the non-cyclostomes which is far less muscularized and has a smooth intima. Quicke & van Achterberg (1990) provided additional data and scored additional features in their phylogenetic analysis of braconid subfamilies. Different component structures of the venom apparatus were subsequently shown to vary greatly both within and between two large groups of cyclostome braconid wasps, the Doryctinae (Quicke *et al.* 1992c) and the Opiinae + Alysinae (Quicke *et al.* 1997), in each case providing indications of previously unsuspected relationships. Moreover, although in these two works no variation was encountered in the venom apparatus of the large subfamily Braconinae, they suggested that it might provide phylogenetic information concerning the members of an ill-defined group of subfamilies that has been referred to as the Rogadinae *sensu lato*. The Rogadinae *sensu lato* constitutes one of the most problematic groups of braconids in terms of its taxonomy due to the considerable morphological and biological heterogeneity shown by its members (Shaw & Huddleston 1991). More recently, some authors have split the Rogadinae *sensu lato* into several small subfamilies, e.g. Exothecinae, Hormiinae, Lysiterminae, Mesostoinae (in part, namely Hydrangeocolini), Pambolinae, Rhyssalinae, and Rhyssipolinae (see Quicke & van Achterberg 1990; van Achterberg 1995; Belshaw & Quicke 2002), although some of these are still morphologically heterogeneous and also dubiously monophyletic because of the absence of clear synapomorphies.

We recognize the subfamily Rogadinae, hereinafter referred to as the Rogadinae *sensu stricto*, to include only four tribes composed exclusively of koinobiont endoparasites of larval Lepidoptera which mummify their hosts: Clinocentrini van Achterberg 1991, Stiropiini van Achterberg 1993, Yeliconini van Achterberg 1991, and Rogadini Foerster 1862 (cf. van Achterberg 1991, 1993, 1995). Although mummification of the host larva is regarded as a synapomorphy that distinguishes members of the Rogadinae *sensu stricto*, the lack of morphological synapomorphies among its members has not allowed a proper definition for it (although most taxa have fairly distinctive Gestalt). In the present work, we describe several previously unreported features of venom apparatus morphology in various genera of the Rogadinae *sensu lato* and other potentially related cyclostome braconids. Based on the information obtained, we performed a preliminary phylogenetic analysis

for this group. Despite the limited amount of information, we show that this character system seems to provide valuable phylogenetic information at a variety of taxonomic levels.

Methods

Material examined

The venom apparatus intimae (the chitinous part that is visible after the soft tissue is removed) of 122 specimens belonging to 100 species were examined. These comprised 24 genera representing each of the four Rogadinae *sensu stricto* tribes, including a large number of species of the cosmopolitan genus *Aleiodes* Wesmael. Furthermore, specimens of some genera belonging to the Rogadinae *sensu lato* (e.g. *Allobracron* Gahan, *Lysitermus* Foerster, *Pentatermus* Hedqvist, *Hormius* Blanchard), and specimens from other potentially related subfamilies (e.g. Telengainae) whose venom apparatus was not previously known, were incorporated and discussed. Two of the venom apparatus preparations included in this study [*Hormius moniliatus* (Nees) and *Aleiodes ruficornis* (Herrich-Schaeffer) = *A. dimidiatus* (Spinola)] were those previously examined by Falco Gari (1991). Voucher specimens are deposited in the Natural History Museum, London, UK and in the National Museums of Scotland, Edinburgh, UK.

The venom apparatus from dry and 100% alcohol preserved specimens were prepared by sinking the metasoma in a c. 10% w/v KOH solution for at least 2 days at room temperature to dissolve all the soft tissue, followed by dissection in water, staining with Chlorazole Black E, dehydration through graded alcohols, and mounting on microscope slides using Histo-mount™ via HistoClear™ (National Diagnostics, New Jersey, USA) (see Quicke *et al.* 1992c for details). This technique makes possible a detailed observation of the fine sculpture and individual secretory ductules of the venom glands and reservoir (Quicke *et al.* 1992c). Terminology of the different venom apparatus structures follows Quicke *et al.* (1992c, 1997). A list of the specimens examined together with their subfamilial and tribal placements follows.

Rogadinae *sensu stricto*

Tribe Clinocentrini. *Clinocentrus cunctator* (Haliday), UK; *C. exsertor* (Nees), UK, two specimens; *C. vestigator* (Haliday), UK.

Tribe Rogadini. *Aleiodes albitibia* (Herrich-Schaeffer), UK; *A. aciculatus* Cresson, USA (two specimens); *A. aestuosus* (Reinhard), Cyprus; *A. alternator* (Nees), UK; *A. apicalis* (Brullé), Cyprus; *A. assimilis* (Nees), France; *A. aterrimus* (Ratzeburg), UK; *A. borealis* (Thomson), UK, two specimens; *A. breviradialis* (Granger), Zambia; *A. burrus* Cresson, USA; *A. circumscriptus* (Nees), UK; *A. compressor* (Herrich-Schaeffer), UK; *A. coxalis* (Spinola), France; *A. dispar* (Haliday), UK; *A. dissector* (Nees), UK; *A. flavistigma* Shaw, Brazil; *A. gasterator* (Jurine), France; *A. cf. gastritor* (Thunberg), agg., UK [two

specimens, one reared from *Tberia primaria* (Haworth), univoltine, delayed ovigenesis; the other one reared from *Eupithecia assimolata* Doubleday, plurivoltine]; *A. grassator* (Thunberg), UK; *A. malacosomatos* (Mason), USA, two specimens; *A. melanopterus* (Erickson), Argentina, two specimens; *A. modestus* (Reinhard), UK; *A. nigricornis* Wesmael, UK; *A. pallidator* (Thunberg), UK; *A. parasiticus* Norton, USA (two specimens); *A. pictus* (Herrich-Schaeffer), UK; *A. plurilineatus* (Cameron), South Africa; *A. praetor* (Reinhard), UK; *A. politiceps* (Gahan), USA, two specimens; *A. pulchripes* Wesmael, UK; *A. ruficornis* (Herrich-Schaeffer) no data; *A. rugulosus* (Nees), UK; *A. scrutator* (Say), USA, two specimens; *A. seriatus* (Herrich-Schaeffer), UK; *A. similis* (Curtis), UK; *A. simillimus* (Ashmead), USA; *A. sp. 1*, Turkey (two specimens); *A. sp. 2* [*bicolor* (Spinola) group], no data; *A. sp. 3* [*tricolor* (Haliday) group], Australia; *A. sp. 4*, Thailand; *A. sp. 5*, Thailand; *A. sp. 6* (*bicolor* group), USA; *A. stigmator* (Say), USA (three specimens); *A. testaceus* (Telenga), France; *A. terminalis* Cresson, USA (two specimens); *A. unipunctator* (Thunberg), UK; *Batotheca* sp., Singapore; *Batothecoides* sp., India; *Bulborogas compressifemur* van Achterberg, Panama (two specimens); *Canalirogas balgooyi* van Achterberg & Chen, Malaysia; *Colastomion concolor* (Szépligeti), Benin; *Conspinaria* sp., Thailand; *Cordylorhogas trifasciatus* Enderlein, Kenya; *Cystomastacoides* sp. Papua New Guinea; *Cystomastax* sp. 1, Ecuador; *Cystomastax* sp. 2, Brazil; *Cystomastax* sp. 3, Costa Rica (two specimens); *Hemigyroneurone* sp., Gambia; *Macrostromion gnathoblibi* Shaw, Papua New Guinea; *Megarhogas* sp., Malaysia; *Myocron antefurcalle* van Achterberg, Kenya (two specimens); *Pholichora bipanna* van Achterberg, Kenya; *Rectivena* sp., Sierra Leone; *Rogas luteus* Nees, the Netherlands; *Rogas* sp. 1, Peru; *Rogas* sp. 2, Peru; *Rogas* sp. 3, Costa Rica; *Rogas* sp. 4, Costa Rica; *Rogas* sp. 5, Costa Rica; *Rogas* sp. 6, Tanzania; *Scoporogas* sp., Mali; *Spinaria* sp., no data; *Triraphis* sp., Brazil; *Xenobolus* sp., Nyasaland.

Tribe Stiropiini. *Stiropius longicarinatus* van Achterberg, Panama (two specimens).

Tribe Yeliconini. *Yelicones belokobylskiji* Quicke, Chishti, Chen & Kruft, Thailand; *Yelicones contractus* Papp, Nepal; *Yelicones delicatus* (Say), USA; *Yelicones glabromaculatus* Belokobylskij, Taiwan.

Subfamily Lysiterminae

Tribe Lysitermini. *Acanthormius* sp., Madagascar; *Aulosaphes* sp., South Africa; *Afrotritermus capensis* (Hedqvist), South Africa.

Tribe Tetratermini. *Tetratermus* sp., Australia; *Katytermus palmicola* van Achterberg, Malaysia.

Subfamily Betylobraconinae

Mesocentrus sp. 1, Australia; *Mesocentrus* sp. 2, Australia.

Subfamily Exotbecinae

Sbarwiana catenator (Haliday) no data, Mongolia (two specimens).

Subfamily Horminae

Tribe Hormiini. *Allobracon* sp., Brazil; *Hormius moniliatus* (Nees), no data; *Hormius* sp., Thailand; *Parabormius* sp., Madagascar; *Parabormius* sp., no data.

Tribe Avgini. *Avga choaspes* Nixon, no data; *Avga* sp., Madagascar.

Subfamily Pambolinae

Cedria africana Belokobylskij, South Africa.

Subfamily Telengainae

Telengia ventralis Tobias, Turkmenia.

Unplaced taxa

Tribe Pentatermini. *Pentatermus striatus* (Szépligeti), Nigeria (three specimens); *Pentatermus striatus*, no data; *Pentatermus* sp., Thailand (two specimens).

Phylogenetic analysis

A preliminary phylogenetic analysis for the included Rogadinae and related taxa was conducted based on those characters of the venom apparatus that varied consistently. In order to discuss the variation found at different taxonomic levels, the analysis included species, genera, and subfamilies as terminal taxa. In addition to the material examined here, the subfamilies Doryctinae and Braconinae, and the genera *Colastes* Haliday (Exothecinae), *Betylobracon* (Betylobraconinae), *Gnamptodon* Haliday (Gnamptodontinae), and *Rhysipolis* Foerster (Rhysipolinae) were also included as terminal taxa, with the Doryctinae selected as the outgroup. Information on these additional taxa was extracted from previous published studies (Quicke *et al.* 1992c, 1997).

A maximum parsimony analysis followed by successive approximation weighting (Farris 1969) was performed with PAUP* 4.0b10 (Swofford 2001). The initial search with equally weighted, unordered characters used 10 000 replicates with tree bisection reconnection swapping, holding only one tree from each addition. Subsequent iterative searches, employing the maximum retention index (RI) as the reweighting function and setting the maxtrees at 30 000, were performed until weights became stable. Owing to the scarce amount of information used to construct this phylogeny, support for the clades was not evaluated. Instead, characters that appeared to support a clade with RI = 0.3 were mapped on to the topology obtained.

Table 1 Characters and character states from the venom apparatus used in this study.

Character number	Morphological characters and states
1	Venom reservoir: (0) essentially unsculptured (e.g. Fig. 1J); (1) totally covered with spiral sculpture (e.g. Fig. 2A–I); (2) with spiral sculpture but unsculptured in the anterior part of the reservoir (e.g. Fig. 1H).
2	Venom reservoir: (0) undivided (e.g. Fig. 3A–H); (1) divided into two sections (e.g. Fig. 1D).
3	Posterior end of venom reservoir: (0) without a neck (e.g. Fig. 2A,C,F); (1) with a distinct, very narrow neck (e.g. Fig. 2B,E).
4	Venom reservoir: (0) less than 2 × longer than wide (e.g. Fig. 1D); (1) 2–4 × its width (e.g. Fig. 1A,B); (2) 4–6 × its width (e.g. Fig. 1C,E); (3) more than 6 × its width (e.g. Figs 1H, 2D).
5	Primary venom duct: (0) narrow (e.g. Fig. 2B,I); (1) wide (e.g. Figs 1A,B, 2F–H).
6	Primary venom duct: (0) without sculpture or with only poorly defined sculpture (e.g. Figs 1H,J, 2B); (1) with spiral or annular sculpture (e.g. Figs 1A,B, 2A); (2) with hexagonal-like sculpture (e.g. Figs 2C, 4D); (3) globular with reticulate sculpture (e.g. Fig. 6E); (4) with evident flask-shaped protuberances (e.g. Fig. 1I); (5) with evident secretory ductules (e.g. Quicke <i>et al.</i> (1992c: figs 3–5).
7	Insertion of secondary venom duct: (0) anteriorly or medially on reservoir (e.g. Figs 1B, 2E,G); (1) posteriorly on venom reservoir (e.g. Fig. 3A–H); (2) distinctly on primary venom duct (e.g. Figs 1E, 6E).
8	Base of secondary venom duct: (0) without internal filaments (e.g. Figs 1C,K, 4A); (1) with internal filaments (e.g. Fig. 4B–D).
9	Secondary venom duct insertion on to reservoir: (0) not recessed (e.g. Fig. 4A,E,F); (1) recessed, with well-defined and numerous internal filaments (e.g. Fig. 4B–D).
10	Secondary venom duct: (0) soft (e.g. Fig. 4A–D); (1) hard, with a distinct flange (e.g. Fig. 4E,F).
11	Secondary venom duct: (0) simple or with no evident sculpture (e.g. Fig. 4E,F); (1) with spiral sculpture (e.g. Fig. 4B,C); (2) with hexagonal-type sculpture (e.g. Fig. 4D); (3) with flask-shaped protuberances; (4) with evident ductules (e.g. Figs 2I, 6F).
12	Secondary and following venom ducts: (0) tubular and distinctly narrow, terminating abruptly in globular glandular sacks (e.g. Fig. 1F,G); (1) tubular and swollen, terminating abruptly in globular glandular sacks (e.g. Fig. 3E–H); (2) swollen, more or less gradually developing into gland filaments (e.g. Fig. 1I).
13	Secondary venom duct: (0) considerably long, at least 2 × as long as the venom reservoir length (e.g. Fig. 3A,B,F); (1) moderately long, not more than 0.5 × length of the venom reservoir (e.g. Fig. 1E–G); (2) distinctly short, not more than 2 × as long as wide (e.g. Figs 1A,B,H, 3C–E).
14	Secondary venom duct: (0) branched into tertiary and subsequent gland ducts, without insertion of venom glands (e.g. Fig. 1A–C); (1) with numerous venom gland sacks inserted along its length and bifurcating it into the tertiary ducts near to its end (e.g. Fig. 3A,B); (2) not branched, with two venom gland bunches, one at its middle and the other at its end (e.g. Fig. 3F).
15	Tertiary venom duct: (0) parallel-sided (e.g. Fig. 3A–H); (1) medially expanded (e.g. Figs 1I, 6F).

Results and discussion

Variation in a number of qualitative and quantitative venom apparatus features was noted at different taxonomic levels, including between currently recognized subfamilies, both between and within the four tribes of the Rogadinae *sensu stricto*, and between species of the genus *Aleiodes* (Tables 1 and 2). Among the variable features that could be scored as discrete character states were: (i) sculpture (e.g. Fig. 1A–H cf. Fig. 1J), size (e.g. Figs 2D, 3H cf. Fig. 2A–C), and form (e.g. Fig. 2B cf. Fig. 2C) of the venom reservoir; (ii) width (e.g. Fig. 2A cf. Fig. 3E,G) and sculpture (e.g. Fig. 2C,F cf. Fig. 3C,E) of the primary venom duct; and (iii) width and length (e.g. Fig. 3A–H cf. Fig. 1E,F), insertion site (e.g. Figs 1B, 2E, 4F, cf. Fig. 2A–C), and structure (e.g. Fig. 4B–D cf. Fig. 4E,F) of the secondary venom duct.

In total, 15 characters were recorded (Table 1), of which 14 were parsimony informative. The initial heuristic search resulted in 1714 most parsimonious trees with length = 69, RI = 0.76, and consistency index (CI) = 0.39. The final analysis using the successive approximation weighting approach reached the limit imposed of 30 000 most parsimonious trees with length = 40.62, RI = 0.85, and CI = 0.47. The strict

consensus of these trees is shown in Fig. 5. The most relevant features observed in the venom apparatus within each of the taxa included are described below and their phylogenetic relationships based on the analysis performed are discussed.

Rogadinae sensu stricto

Within the Rogadinae *sensu stricto* there are considerable differences in the venom apparatus among its four recognized tribes, with no evident synapomorphies that help to define this subfamily with respect to the other cyclostome subfamilies and in particular the Doryctinae (Quicke *et al.* 1992c). Nevertheless, there are some characters that distinguish members of the Rogadini from the remaining tribes of the Rogadinae *sensu stricto*. The Rogadini, which is by far the most diverse tribe in the Rogadinae *sensu stricto*, currently comprises two subtribes, the Rogadina and the Spinariina, the first being much more diverse and widespread than the second. Members of the Rogadini are defined by having the inner sides of the eyes emarginate, the second tergite usually with a triangular or semicircular mediobasal area, and a short ovipositor sheath (van Achterberg 1991). The venom apparatus in the specimens of the Rogadini examined usually

Table 2 Distribution of character states from the venom apparatus characters. See Table 1 for character descriptions. Polymorphism coded as a = 01; b = 12.

Characters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
<i>Clinocentrus</i>	1	0	0	2	0	1	b	0	0	0	0	0	1	0	0
<i>Aleiodes</i> type A	1	0	0	1	0	1	1	1	0	0	0	1	2	0	0
<i>Aleiodes</i> type B	1	0	1	2	0	0	1	1	1	0	1	1	2	0	0
<i>Aleiodes</i> type C	1	0	0	1	1	2	1	1	1	0	2	1	2	0	0
<i>Aleiodes</i> type D	1	1	0	3	0	2	1	1	1	0	1	1	2	0	0
<i>Aleiodes</i> type E	1	0	1	2	0	1	1	1	1	0	1	1	2	0	0
<i>Aleiodes</i> type F	1	0	1	2	1	2	0	1	1	0	0	1	2	0	0
<i>Batotheca</i>	1	0	0	1	1	1	0	1	0	1	0	1	2	0	0
<i>Batothecoides</i>	1	0	0	2	1	1	0	1	0	1	0	1	2	0	0
<i>Bulborogas</i>	1	0	0	1	0	2	1	1	0	1	0	1	0	2	0
<i>Canalirogas</i>	1	0	0	1	0	1	1	1	0	1	0	1	2	0	0
<i>Colastomion</i>	1	0	0	1	1	2	1	1	0	1	0	1	2	0	0
<i>Conspinaia</i>	1	0	0	1	1	2	1	1	0	1	0	1	2	0	0
<i>Cordylorhogas</i>	1	0	1	2	1	2	1	1	1	0	1	1	2	0	0
<i>Cystomastacoides</i>	1	0	0	1	1	1	1	1	0	1	0	1	2	0	0
<i>Cystomastax</i>	1	0	0	1	1	1	1	1	0	0	0	1	2	0	0
<i>Hemigroneurone</i>	1	0	0	2	1	2	0	1	0	0	1	1	2	0	0
<i>Macrostomion</i>	1	0	0	1	1	2	1	1	0	1	0	1	2	0	0
<i>Megarhogas</i>	1	0	0	1	1	1	1	1	0	1	0	1	2	0	0
<i>Myocron</i>	1	0	0	1	1	2	1	1	0	1	0	1	2	0	0
<i>Pholichora</i>	1	0	0	1	1	3	2	1	0	1	0	0	1	0	0
<i>Rectivena</i>	1	0	0	?	1	1	?	1	0	1	0	1	2	0	0
<i>Rogas</i> New World	1	0	0	2	1	2	1	1	0	1	0	1	0	1	0
<i>Rogas</i> Old World	1	0	0	2	1	1	1	1	0	1	0	1	2	0	0
<i>Scoporogas</i>	1	0	0	2	1	2	1	1	0	0	0	1	2	0	0
<i>Spinaria</i>	1	0	1	2	1	1	0	1	0	1	0	1	2	0	0
<i>Triraphis</i>	1	0	0	2	1	1	1	1	0	1	0	1	2	0	0
<i>Xenolobus</i>	1	0	0	1	1	2	1	1	0	0	2	1	2	0	0
<i>Stiropius</i>	1	0	0	2	0	0	2	0	0	0	0	0	1	0	0
<i>Yelicones</i> New World	1	0	0	1	0	0	1	0	0	0	4	0	1	0	0
<i>Yelicones</i> Asia	1	0	0	1	0	0	1	0	0	0	4	1	1	0	1
<i>Acanthormius</i>	1	0	0	1	0	1	1	0	0	0	1	0	1	0	0
<i>Afrotritermus</i>	1	0	0	1	0	?	1	0	0	0	?	0	1	0	0
<i>Aulosaphes</i>	1	0	0	1	0	1	1	0	0	0	2	0	1	0	0
<i>Katytermus</i>	1	0	0	2	0	1	?	0	0	0	?	0	2	0	0
<i>Tetratermus</i>	1	0	0	2	0	2	2	0	0	0	3	0	2	0	0
<i>Betylobracon</i>	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0
<i>Mesocentrus</i>	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0
<i>Rhysipolis</i>	0	1	0	3	0	0	0	0	0	0	1	0	1	0	0
<i>Allobracon</i>	2	0	0	3	0	1	1	0	0	0	0	0	2	0	0
<i>Avga choaspes</i>	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0
<i>Avga</i> sp.	1	0	0	1	0	0	1	0	0	0	0	0	2	0	0
<i>Hormius</i>	1	0	0	2	0	1	1	0	0	0	0	0	1	0	0
<i>Parahormius</i>	1	0	0	2	0	1	1	0	0	0	1	0	1	0	0
<i>Cedria</i>	1	0	0	2	0	1	?	0	0	0	0	0	1	0	0
<i>Pentatermus</i>	1	0	0	2	0	0	1	a	0	a	a	a	1	0	0
<i>Telengaia</i>	1	0	0	1	0	4	1	0	0	0	0	2	1	0	1
<i>Colastes</i>	1	1	0	2	0	4	0	0	0	0	1	2	1	0	0
<i>Shawiana</i>	1	0	1	2	0	4	1	0	0	0	1	2	1	0	0
<i>Braconinae</i>	1	0	0	2	0	4	1	0	0	0	0	2	?	?	0
<i>Gnamptodon</i>	1	0	0	2	0	5	1	0	0	0	1	2	1	0	0
<i>Doryctinae</i>	1	1	0	3	0	5	2	0	0	0	1	0	1	0	0

consists of a wide venom reservoir (e.g. Figs 2F–H, 3A–H), a wide primary venom duct (e.g. Figs 2F–H, 6E; except some *Aleiodes*, e.g. Fig. 2A,B), a short and wide secondary venom duct (e.g. Fig. 1A,B; except *Pholichora* van Achterberg and

some *Rogas* Nees, see below), and glands well differentiated and often numerous (e.g. Figs 2A–H, 3A–H). Although a short and wide secondary venom duct is also present in most species of the Opiinae and Alysiinae, the gland ducts in these

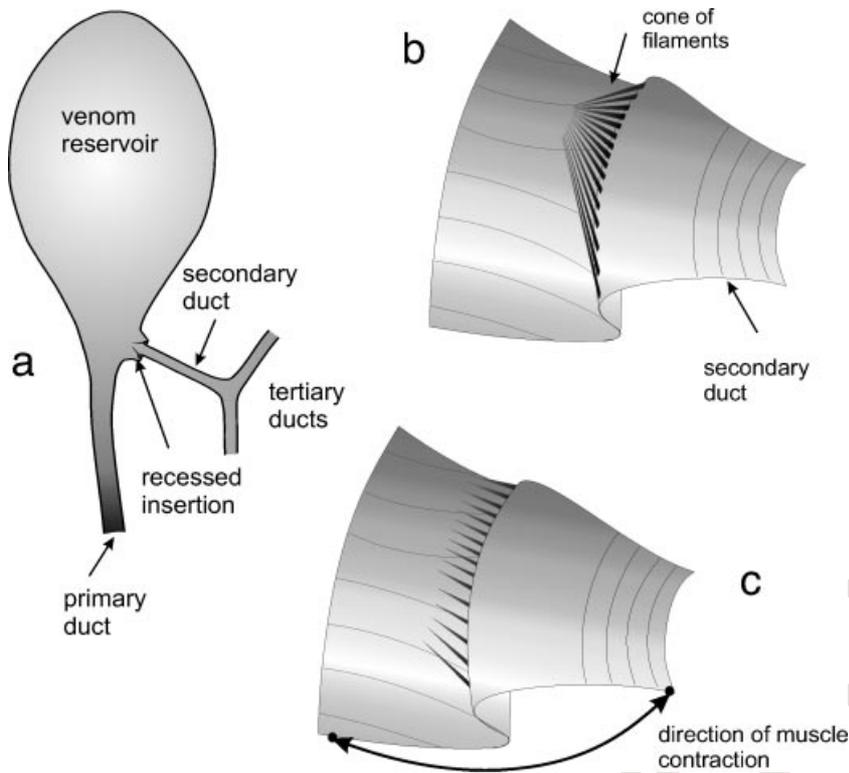


Fig. 1 A–K. Interpreted drawings of the chitinous intimas of venom glands and reservoirs of Rogadini, Tetratermini, Betylobraconinae, Stiropiini, Pentatermini, Hormiinae, Telengainae and Avgini. —A. *Megarhogas* sp. —B. *Batobteca* sp. —C. *Tetratermus* sp. —D. *Mesocentrus* sp. —E. *Stiropius longicarinatus*. —F. *Pentatermus striatus*. —G. *Hormius moniliatus*. —H. *Allobracon* sp. —I. *Telengaiia ventralis*. —J. *Avga choaspes*. —K. *Avga* sp.

taxa are not tubular and the venom glands are often undifferentiated with respect to their ducts (Quicke *et al.* 1997).

Despite the above differences, the synapomorphy that consistently supports the monophyly of the Rogadini in our analysis, apart from the presence of a swollen secondary venom duct (RI = 0.9), is the presence of a ring of distally directed filaments within the secondary venom duct just at or before its insertion into the venom reservoir (RI = 1.0; Fig. 4B–F). These filaments are arranged in a cone which we suggest acts as a valve for controlling the flow of venom from the glands to the reservoir, potentially limiting back-flow when the reservoir muscles contract to expel venom during host manipulation (Fig. 7A–C). The presence of thick muscle around the secondary venom duct (Fig. 7B) suggests that the wasp may be able to control the opening of the filament cone. No similar structure has been observed in any other group of braconids and therefore in our phylogenetic hypothesis it appears as a synapomorphy that defines this tribe.

Some relationships within the Rogadini can be proposed based on features of the secondary venom duct. A number of genera of the subtribe Rogadina (*Bulborogas* van Achterberg, *Canalirogas* van Achterberg & Chen, *Colastomion* Baker, *Conspinarina* Schulz, *Cystomastacoides* van Achterberg, *Myocron* van Achterberg, *Macrostomion* Szépligeti, *Pholichora*, *Rectivena* van Achterberg, *Rogas*, and *Triraphis* Ruthe) and the three

examined species representing the subtribe Spinariina (*Spinaria* Brullé, *Batobteca* Enderlein, and *Batobtecoides* Watanabe) are nested within a clade supported by the presence of a thickened, apparently rigid, and distinctly differentiated secondary venom duct with a distinct flange (RI = 1.0; e.g. Fig. 4E,F). Although *Pholichora* has a narrow and moderately large secondary venom duct (Fig. 6E), its modification is essentially similar. On the other hand, the six remaining examined genera of the Rogadina (*Aleiodes*, *Cordylorhogas* Enderlein, *Cystomastax* Szépligeti, *Hemigyronneurone* Baker, *Scoporogas* van Achterberg, and *Xenobolus* Cameron) lack the above feature, instead having a soft, undifferentiated secondary venom duct (Fig. 4B–D).

The relationships recovered between the Rogadini genera are also supported by some external morphological characters. A setal comb on the inner side of the apex of the hind tibia is found in most of those genera with a thickened and short secondary venom duct, and is absent in those with a soft secondary venom duct and also in *Pholichora*, which has a distinctive duct (Fig. 6E). The only exception is *A. seriatus*, which also has a setal comb but has a soft secondary venom duct as in the other examined species of *Aleiodes*. Furthermore, in two subsets of genera, two other external morphological characters show a high degree of congruence with the thickened secondary venom duct, these being a basally widened first

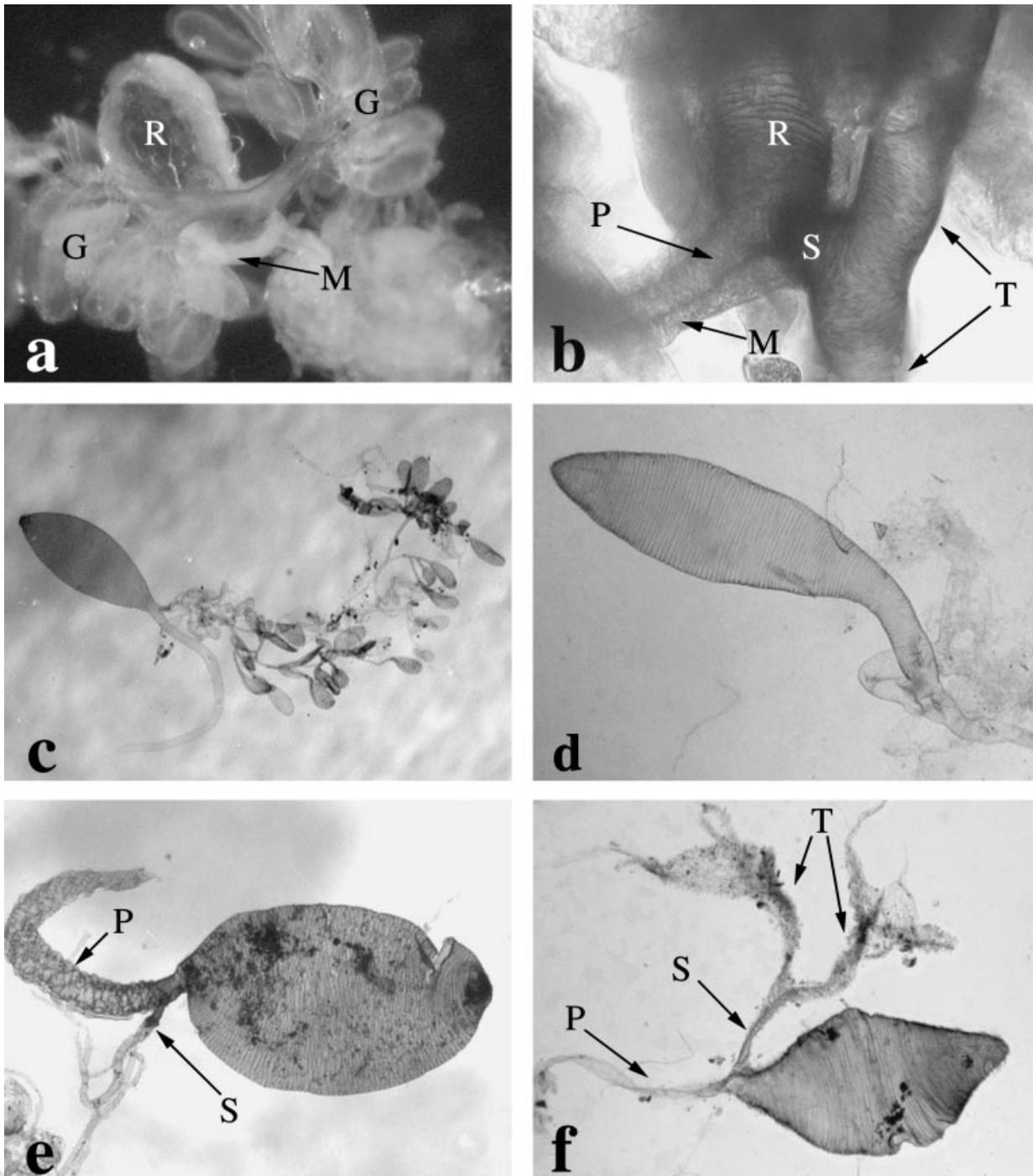


Fig. 2 A–I. Interpreted drawings of the chitinous intimas of venom glands and reservoirs of Rogadini and Yeliconini. —A. *Aleiodes dispar*. —B. *A. circumscriptus*. —C. *A. unipunctator*. —D. *A. albitibia*. —E. *A. parasiticus*. —F. *Scoporogas* sp. —G. *Hemigyronneurone* sp. —H. *Xenolobus* sp. —I. *Yelicones delicatus*.

metasomal tergite which is present in *Canalirogas*, *Colastomion*, *Macrostromion*, and *Myocron*, and curved and largely glabrous hind tibial spurs in *Colastomion*, *Cystomastacoides*, *Macrostromion*, *Megarhogas*, *Myocron*, and *Rectivena*. Finally,

although not recovered as monophyletic in our analysis, members of the Spinariina, which are characterized by a disproportionately small and transverse head and by the metasomal tergites being united to form a carapace and

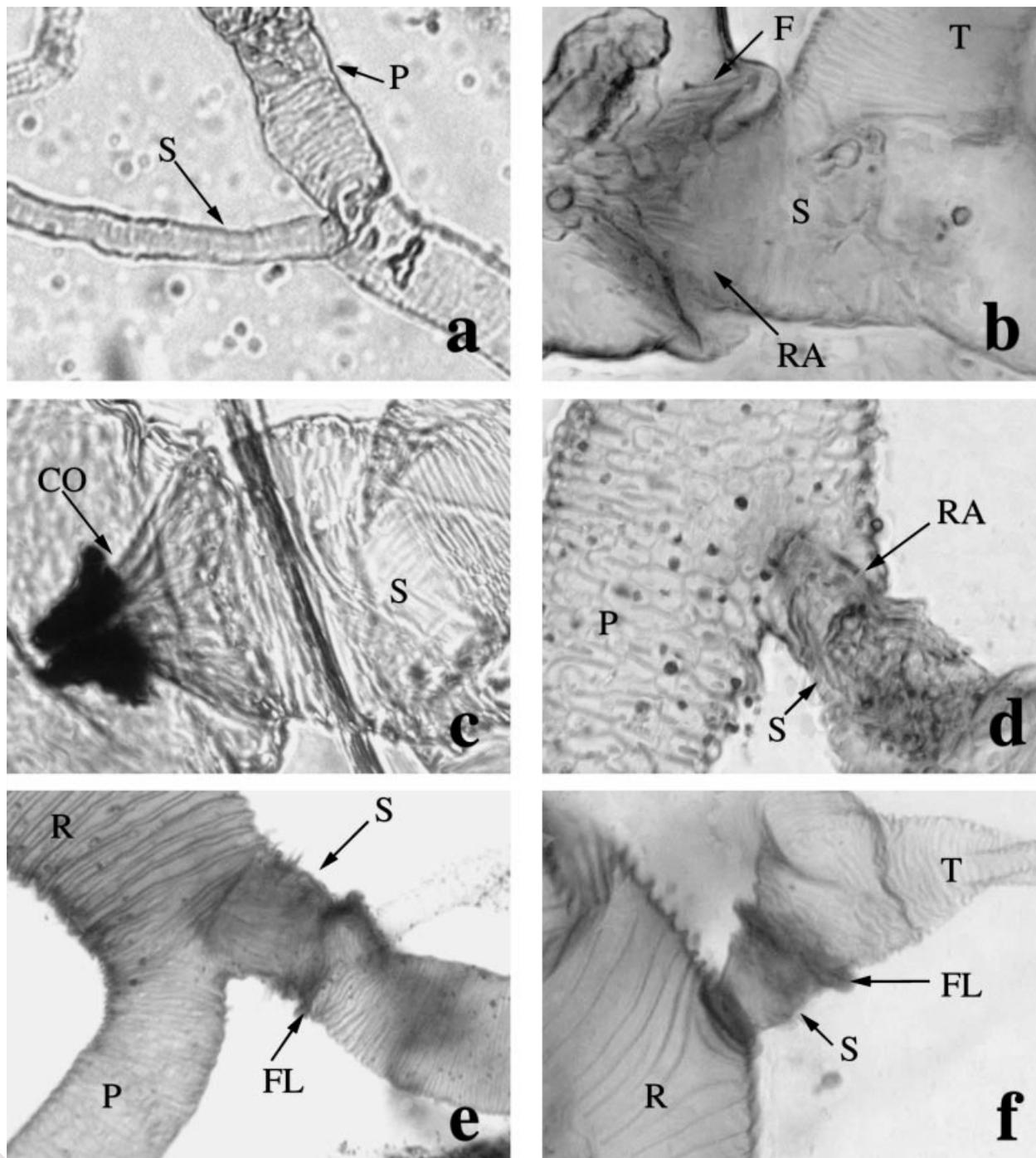


Fig. 3 A–H. Interpreted drawings of the chitinous intimas of venom glands and reservoirs of Rogadini. —A. *Rogas* sp. 1. —B. *Rogas* sp. 4. —C. *R. luteus*. —D. *Colastomion concolor*. —E. *Cystomastacooides* sp. —F. *Bulborogas compressifemur*. —G. *Canalirogas balgooyi*. —H. *Macrostomion gnathoblibi*.

bearing spines (Chen & He 1997), are the only genera which have a thickened secondary venom duct inserted at the midlength of the reservoir (Fig. 1B; see also Quicke *et al.* 1992c: fig. 8C). However, because these genera share the

probably derived thickened hard secondary venom duct with several other Rogadini genera, it seems that the Spinariina is just a derived genus group within a larger group of genera.

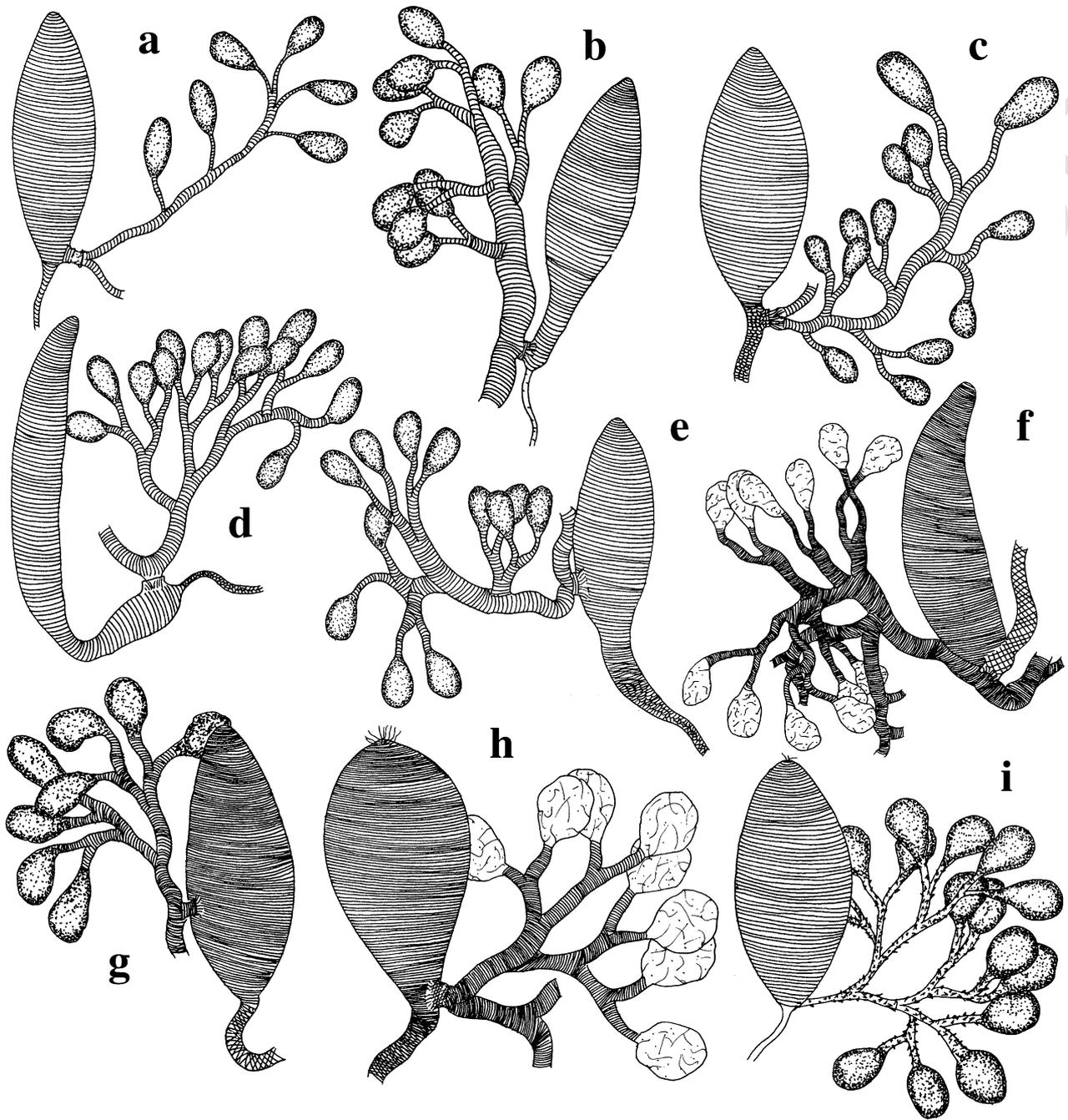


Fig. 4 A–F. Automontage® light photomicrographs of the secondary venom duct. —A. *Clinocentrus cunctator*. —B. *Aleiodes albitibia*. —C. *Cordylorhogas* sp. —D. *A. pulchripes*. —E. *Rogas* sp. 1. —F. *Batotheca* sp. CO, cone of filaments; F, filaments; FL, flange; P, primary duct; R, reservoir; RA, recessed area; S, secondary duct; T, tertiary duct.

With more than 300 described species, the cosmopolitan genus *Aleiodes* is the largest genus of the Rogadinae *sensu stricto* (Chen & He 1997). Members of *Aleiodes* are known usually to oviposit into second or early third instar larvae of several macrolepidopteran families and kill the host before it

becomes fully grown (Shaw 1983; Shaw & Huddleston 1991). Although many species of *Aleiodes* have in the past been placed in *Rogas* (e.g. Shaw 1983), the latter name is correctly applied to a different group and both generic names are considered as valid (van Achterberg 1991; Shaw 1997).

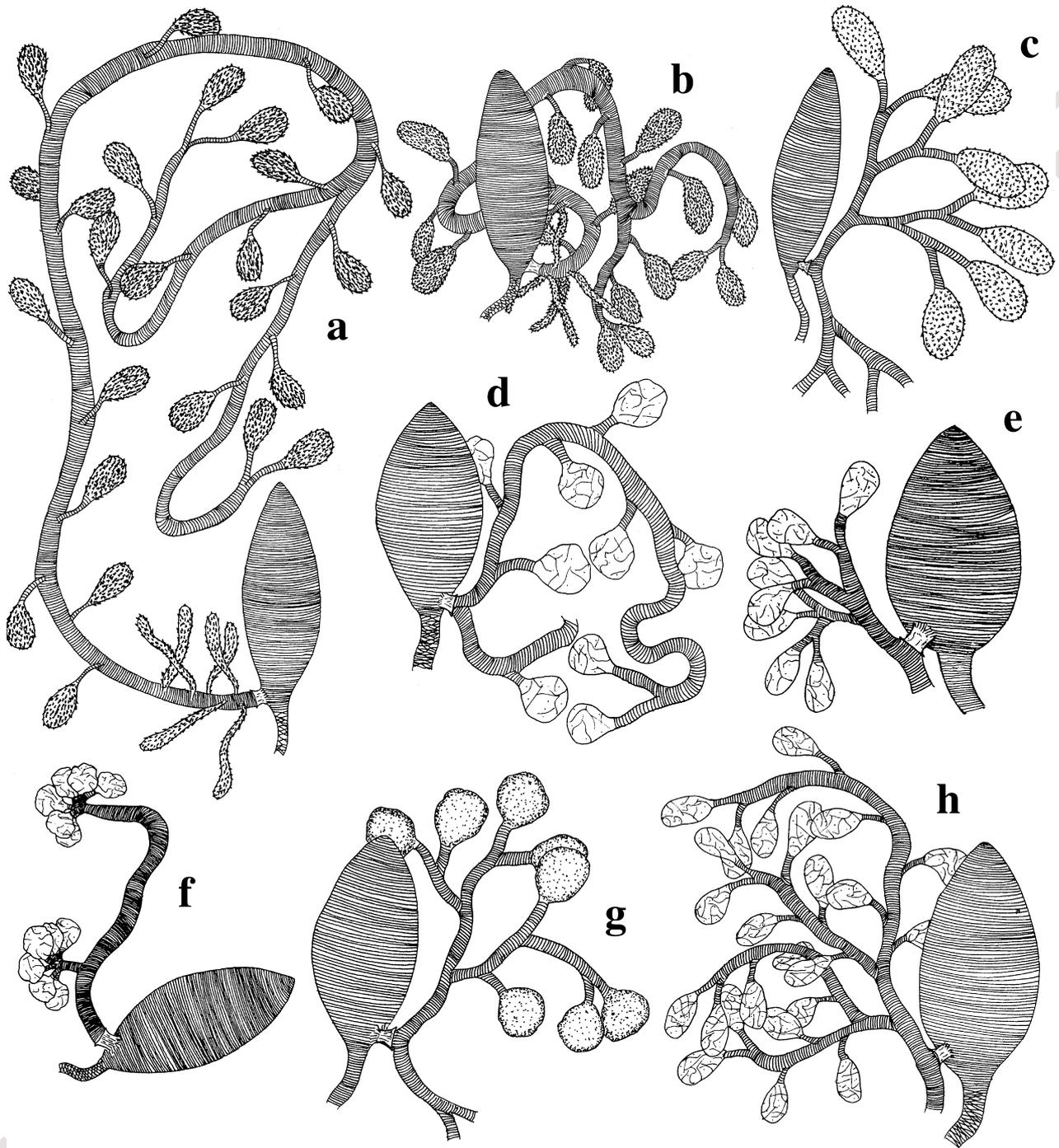


Fig. 5 Strict consensus of the 30 000 most parsimonious trees recovered using a successive approximation approach for the venom apparatus characters examined in this study. The numbers refer to characters supporting recovered clades with a retention index = 0.33 (see Table 1 for character descriptions).

However, owing to the occurrence of aberrant character states within *Aleiodes*, the distinction between even the true *Rogas* and *Aleiodes* can be problematic, with few consistent or convincing characters to separate them, such that keys to

genera rely heavily or entirely on the presence of a large pointed basal lobe to the claws in *Rogas*. From our study, based on the differences in the secondary venom duct mentioned above, it seems that *Aleiodes* and *Rogas* are each more closely

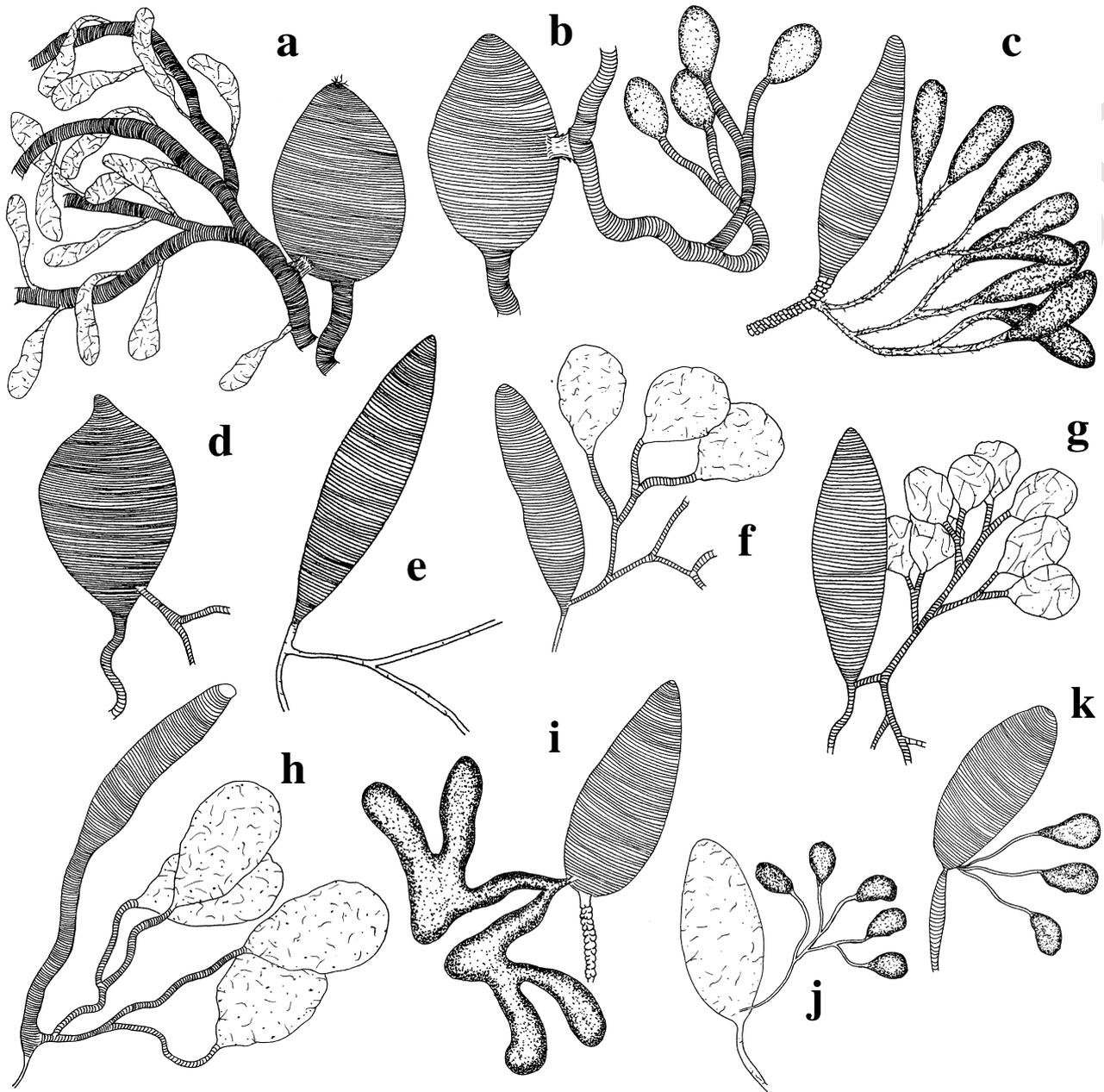
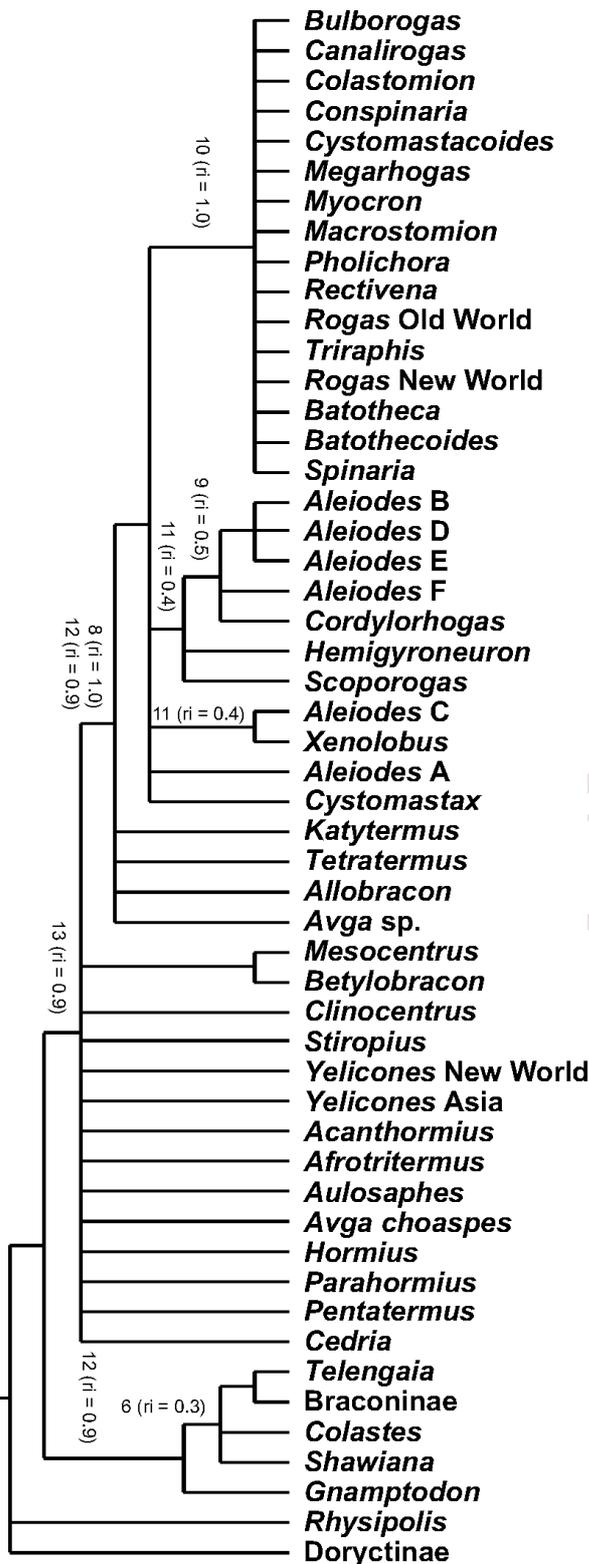


Fig. 6 A–F. Automontage® light photomicrographs of Rogadinae venom apparatus. —A. Muscularized venom apparatus of *Aleiodes borealis*, dark field illumination showing the thick muscle layer over a brown pigmented reservoir intima, and symmetrical branching of the venom glands. —B. Muscularized secondary venom duct and part of the venom reservoir of *A. borealis*. —C–F. Chlorazol Black stained venom reservoir and part of the gland ducts of *A. pulcristipes*, *A. coxalis*, *Pbolichora bipanna*, and *Yelicones contractus*, respectively. G, venom glands; M, muscle layer; P, primary duct; R, reservoir; S, secondary duct; T, tertiary duct.

related to other genera of Rogadini than to each other. Unlike all other Rogadini, *Cordylorbogas* and all species of *Aleiodes*, except for *A. dispar* and *A. spp.* 4 and 5 (see below), have the secondary venom duct evidently recessed and with numerous, well-defined internal filaments (Fig. 4B–D).

Despite the fact that one of the terminal taxa with a recessed secondary venom duct is nested outside the clade comprising the rest of the taxa with this condition (species of *Aleiodes* with type C venom apparatus, see below), this arrangement is apparently unique within the Braconidae and probably



represents a synapomorphy for these two genera. Although not recovered in our phylogenetic hypothesis, variation in the venom apparatus between the species of *Rogas* from Old and New Worlds has also revealed a marked dichotomy. Whereas the gland ducts in European and Afrotropical *Rogas* are symmetrically branched in a similar way to those of the other Rogadini genera (Fig. 3C), those of all five New World *Rogas* species examined possess an extremely long tubular secondary venom duct that has numerous venom gland sacks inserted along its length and which only bifurcates into the tertiary ducts near to its end (Fig. 3A,B). The relative length of the secondary venom duct varies among the New World species, being relatively longer in larger species.

Examination of the venom apparatus in 46 species of *Aleiodes* from different continents resulted in the recognition of six different venom apparatus types (Tables 2 and 3), although most of the species displayed either type B or type C. It is notable that the type B species all belong to the subgenus *Aleiodes* (*sensu* van Achterberg 1991), while those of type C all fall into the subgenera *Chelonorhogas* Enderlein or *Neorhogas* Szépligeti. Furthermore, our species groupings based on venom apparatus type are generally congruent with the phylogeny proposed by Fortier & Shaw (1999), where the 208 examined species of *Aleiodes* were placed in 18 species groups distributed among three sections (basal, intermediate and derived). Our observations revealed that: (1) the only described species we examined with type A venom apparatus, *A. dispar* (Fig. 2A), and the species with type E venom apparatus are present in the intermediate *A. dispar* species group; (2) species with type B venom apparatus (Figs 2B, 6D) fall within basal groups or within the intermediate *coxalis* species group; and (3) species with types C, D, and F venom apparatus (Fig. 2C,D,E, respectively) are placed in derived species groups.

Although our data are not sufficient to resolve fully the relationships within *Aleiodes*, it is interesting that in *A. dispar* and related species, the venom apparatus differs from the others in being least modified. Although type A venom apparatus possesses a series of filaments at the insertion of the secondary venom duct, these are not so well defined in this group and lack a distinctly recessed part of the secondary duct. Van Achterberg (1991) synonymized four small genera with *Aleiodes*, including *Heterogamus* Wesmael of which *dispar* is the type species, because he considered that recognition of

Fig. 7 A–C. Diagrammatic representations of venom apparatus features in the Rogadinae. —A. Showing terminology of venom ducts. —B, C. Detail of the internal structure of the recessed insertion of the secondary duct on to the reservoir, showing filaments in a cone arrangement (B) and indicating the hypothesized result of the contraction of muscles associated with insertion causing an increase in the degree of recess and consequent separation of filaments (C).

Table 3 Classification of the species of *Aleiodes* examined in this study and their respective venom apparatus types. Character states for the venom apparatus types are listed in Table 2.

Venom apparatus types	<i>Aleiodes</i> species
Type A	<i>A. dispar</i> , <i>A. sp. 4</i> , <i>A. sp. 5</i>
Type B	<i>A. aciculatus</i> , <i>A. alternator</i> , <i>A. assimilis</i> , <i>A. borealis</i> , <i>A. circumscriptus</i> , <i>A. compressor</i> , <i>A. coxalis</i> , <i>A. cf. gastritor</i> , <i>A. malacosomatos</i> , <i>A. modestus</i> , <i>A. nigricornis</i> , <i>A. pallidator</i> , <i>A. pictus</i> , <i>A. scrutator</i> , <i>A. seriatus</i> , <i>A. similis</i> , <i>A. simillimus</i> , <i>A. sp. 1</i> , <i>A. sp. 2</i> (<i>bicolor</i> group), <i>A. sp. 6</i> (<i>bicolor</i> group), <i>A. stigmator</i> , <i>A. testaceus</i>
Type C	<i>A. aestuosus</i> , <i>A. apicalis</i> , <i>A. aterrimus</i> , <i>A. burrus</i> , <i>A. dissector</i> , <i>A. flavistigma</i> , <i>A. gasterator</i> , <i>A. grassator</i> , <i>A. melanopterus</i> , <i>A. politiceps</i> , <i>A. praetor</i> , <i>A. pulchripes</i> , <i>A. ruficornis</i> , <i>A. rugulosus</i> , <i>A. sp. 3</i> (<i>tricolor</i> group), <i>A. terminalis</i> , <i>A. unipunctator</i>
Type D	<i>A. albitibia</i>
Type E	<i>A. breviradialis</i> , <i>A. plurilineatus</i>
Type F	<i>A. parasiticus</i>

all of them as genera would leave the remainder of *Aleiodes* paraphyletic. The differences in the venom apparatus between *A. dispar*, with its closely related species, and the other *Aleiodes* studied suggest that these species represent a basal lineage within *Aleiodes*, and in the future it may be desirable to resurrect the generic name *Heterogamus* for them (Mori *et al.* in preparation).

5

Based on their morphological and venom action characteristics, the tribes Clinocentrini and Stiropiini have been proposed as basal members within the Rogadinae *sensu stricto* (Shaw 1983; Whitfield 1988; Shaw & Huddleston 1991). Species of these taxa are known as endoparasitoids of web-inhabiting or semiconcealed well-grown microlepidoptera (Shaw 1983; Shaw & Huddleston 1991), and of leaf-mining Lyonetiidae and Gracillariidae larvae (Whitfield 1988; Shaw 1997), respectively (cf. van Achterberg 1991, 1995). The unmodified venom apparatus morphology in the examined specimens of these tribes compared with members of the Rogadini suggests a basal placement of them within the Rogadinae *sensu stricto*, although their relationships were not recovered here. Typically, the venom apparatus in these taxa consists of an undivided and sculptured venom reservoir, a narrow primary venom duct, a narrow secondary venom duct without any traces of filaments at its insertion with the venom reservoir, and well-differentiated and not numerous venom glands (Figs 1E, 4A).

Yelicones Cameron, the only genus of the tribe Yeliconini, is a moderately extensive, cosmopolitan group whose species are known as solitary endoparasites of lepidopteran larvae which they also mummify (Capek 1970; Quicke & Kruft 1995). Furthermore, some morphological features present in *Yelicones* (e.g. short, robust tarsi; longitudinal row of pegs on the fore tibia; form of hypoclypeal depression) resemble those seen in members of other groups (e.g. Betylobraconinae, various Doryctinae such as *Stenocorse* Marsh, and Opiinae). The venom apparatus in the four examined species of *Yelicones* does not show any obvious relationship with other taxa

(Figs 2I, 6F; see also Quicke *et al.* 1992c). However, our observations do suggest a possible biogeographical trend within *Yelicones*. In the Asian species of *Yelicones* examined, the venom ducts show evident ductules and have a very distinctive form with the secondary ducts medially expanded, which could terminate either in a few or no distinct venom glands (Fig. 6F). In contrast, the only *Yelicones* examined from the New World possesses tubular and strongly branched ducts with numerous, well-differentiated glands that are more typical of members of the Rogadini (Fig. 2I).

Other taxa

Considerable confusion has existed with respect to the placement of some groups of cyclostome genera that exhibit intermediate morphological characteristics between the Doryctinae and the Rogadinae *sensu lato* (Wharton 1993). Most of these genera were originally grouped by Shenefelt (1975) in four tribes of the subfamily Exothecinae (namely Exothecini, Pambolini, Hormiini, and Lysitermini). Currently, despite extensive taxonomic work on these genera, their placement has not been adequately solved, mainly because of the vast amount of homoplasy present in their external morphological characters.

In general, the venom apparatus in Betylobraconinae and in most of the examined genera that were previously in the Rogadinae *sensu lato* and are now excluded from the Rogadinae *sensu stricto* (i.e. in the Hormiini, Lysitermini, Pambolini, Pentatermini, Tetratermini) resemble those seen in the examined specimens of the Clinocentrini and Stiropiini (e.g. Fig. 1C,D,F,G), showing no derived features. The only exception involves one specimen of *Pentatermus striatus*, which differs from the rest of the specimens assigned to this genus by having a hard secondary venom duct with filaments, as in some members of the Rogadina. Currently, six species of *Pentatermus* have been described, *P. austrini* Belokobylskij, *P. carinatus* Hedqvist, *P. concolor* Szépligeti, *P. medvedewi* Belokobylskij, *P. parnarae* He & Chen, and *P. striatus*

Szépligeti. Because they cannot be readily differentiated morphologically, *P. carinatus* had previously been considered as a synonym of the widespread Afrotropical and oriental *P. striatus* (van Achterberg 1991). However, in the last revision of the genus, Belokobylskij (2002) considered *P. striatus* and *P. carinatus* as separate species, and *P. medvedewi* and *P. parnarae* as junior synonyms of *P. striatus*. Although, unfortunately, our specimen with a hard secondary venom duct has no locality data, this finding emphasizes the necessity of more detailed taxonomic studies on this poorly known genus and poses the possibility that it is not monophyletic as currently constituted. Indeed, this genus is not defined by any obvious autapomorphy.

Wharton (1993) placed the genus *Allobracon* Gahan within the Hormiinae based mainly on the presence of a desclerotized metasomal tergite and the narrow separation of the submedius from the hind wing margin. However, differences in the petiole and propodeum with respect to the Hormiinae noted by Wharton leave the placement of *Allobracon* still uncertain. As in some members of the Doryctinae, Alysiniinae and Opiinae (the last two not included in this study), the venom reservoir of *Allobracon* is divided and, as in some opiines and alysiines, has a distinctly unsculptured posterior area (Fig. 1H). However, the secondary and tertiary ducts in *Allobracon* are tubular and with well-differentiated glands, whereas in the Opiinae they are swollen and more or less gradually developing into gland filaments (Quicke *et al.* 1997). Moreover, although some alysiines have similar secondary and tertiary gland ducts, they never occur in combination with a sculptured anterior bulb to the reservoir (Quicke *et al.* 1997).

The Telengainae and the Exothecinae are two small braconid subfamilies, the first one represented only by the enigmatic genus *Telengaia* Tobias, which is restricted to Central Asia (Quicke & van Achterberg 1990; van Achterberg 1993), whereas the second one comprises few genera which contain idiobiont ectoparasites of mining larvae of holometabolous insects (Shaw 1983; van Achterberg 1983, 1993). Although Quicke & van Achterberg (1990) stated that the Telengainae lacks any clear morphological synapomorphy with other braconid subfamilies, they hypothesized that it may be closely related to the Gnamptodontinae and to the Braconinae, mainly because of the presence of posteriorly diverging grooves on the third metasomal tergite. Moreover, in this work, the authors demonstrated that the group currently referred to as the Exothecinae does not form part of the Rogadinae *sensu stricto*. These suggestions are consistent with the features of the venom apparatus observed for the specimens examined belonging to the Telengainae and the Exothecinae. *Telengaia* and the two members of the Exothecinae (*Shawiana* van Achterberg and *Colastes*) were recovered in a clade containing the Braconinae and *Gnamptodon* of the Gnamptodontinae (Fig. 5). The synapomorphy that supports

this clade is the presence of swollen secondary and following venom ducts, more or less gradually developing into gland filaments (RI = 0.33; e.g. Fig. 1I). This type of gland duct also resembles those observed in most members of the Opiinae and Alysiniinae (Quicke *et al.* 1997). Furthermore, within the latter clade there is a subclade containing the Braconinae, Telengainae and Exothecinae. This clade is supported by the presence of a primary duct densely supplied with ductules that open into a fairly large dome or vase-shaped chamber (RI = 0.33; e.g. Fig. 1I). This same apomorphic condition has also been observed in some species of the Alysiniinae (Quicke *et al.* 1997).

The presence of an unsculptured venom reservoir is a feature apparently fixed in the non-cyclostome braconids with the marked exception of the putatively basal Trachypetinae (Quicke & van Achterberg 1990; Quicke *et al.* 1992c, 1999; Jiménez *et al.* 1997), although it has also been seen in some species of the Aphidiinae, which has proved to be part of a 'Gondwanan' clade that is the sister group of the cyclostome braconids (Belshaw *et al.* 2000; Dowton *et al.* 2002). In contrast, all the cyclostome braconids present a pattern of spiral annulation on the venom reservoir which seems to be a derived characteristic that distinguishes this group (Quicke & van Achterberg 1990; Quicke *et al.* 1992c, 1997). In this study, all the taxa included present a venom reservoir with spiral sculpture except one of the two examined species of *Avga*, the type species *A. choaspes* (Fig. 1J). Our analysis did not include non-cyclostome taxa, and hence this feature was uninformative. However, it can be observed that the overall morphology of the venom apparatus in the two examined species of *Avga* (Fig. 1J,K) is more similar to those seen in the Aphidiinae than to those of the Hormiinae. Thus, the possibility that *Avga* does not form part of the cyclostome group needs to be considered. This finding seems to agree with Wharton's (1993) review of the Hormiini, who observed that *A. choaspes* and closely related species present some morphological differences with respect to the rest of hormiines. Future critical taxonomic studies are needed to test the monophyly of *Avga* and the actual taxonomic position of *A. choaspes* and related species.

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