

## The phylogenetic position of parasitoids of spiders within Pimplinae (Hymenoptera, Ichneumonidae)

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### Abstract

Within the Ichneumonidae, the Pimplinae is one of the subfamilies whose patterns of parasitism are the most diversified, being associated with a larger host range than any other ichneumonid subfamilies. The Pimplinae taxonomy has been studied to a greater extent than other subfamilies. Hypotheses on Pimplinae phylogeny have been proposed, based on morphological characters only. These indicated that the hypothetical pimpline ancestor was a solitary ectoparasitoid idiobiont on weakly concealed immature Hymenoptera. Different shifts are investigated in this paper, both toward endoparasitism of Lepidoptera pupae, and toward koinobiont ectoparasitism of spiders. A new morphology-based hypothesis on the spider parasitoid genera relationships is proposed, and evolutionary trends are discussed.

**Key words:** evolution of patterns of parasitism, idiobiont, koinobiont, morphology, phylogeny

### INTRODUCTION

The Hymenoptera are one of the largest insect order after the Coleoptera, with approximately 145000 described species. Within the suborder Parasitica (105000 species), the Ichneumonidae is the largest family with about a fifth of the described species (22000 species) (Yu & Horstmann 1997). The Pimplinae subfamily is nested within an informal monophyletic grouping of subfamilies: the Pimpliformes (Wahl 1990).

The biology of the Pimplinae is one of the most diverse within the Ichneumonidae. Thus, they are an ideal group within which to investigate the evolution of patterns of parasitism. However the evolutionary biology may be discussed only once a rigorous and falsifiable hypothesis of relationships is established.

The intention of this paper is a review of the Pimplinae phylogeny, and to discuss the principal evolutionary trends within an explicit phylogenetic framework. From this an original morphology-based phylogeny of the spider parasitoids is presented, and the evolution of the host-parasitoid relationships is discussed.

### Synopsis of the classification

Pimplinae classification and nomenclature have undergone major changes during the last forty years. Nomenclatural changes were due, principally, to the fact that Henry Townes, one of the most well-known ichneumonid workers of the last century, did not follow the International Code on Zoological Nomenclature (hereafter ICZN). He preferred to follow a strict priority principle, despite the

opinion 159 (Hemming 1945) on Pimplinae nomenclature (Townes 1969, p.17). Changes are summarized in Table 1. Thereafter we will use the names recommended by the ICZN only.

In 1969, Townes proposed a new classification of the world genera of Ichneumonidae, dividing the current Pimplinae (=Ephialtinae *sensu* Townes) into 7 tribes: Pimplini, Ephialtini, Polysphinctini, Theroniini, Diacritini, Rhyssini and Poemeniini. In the post-Townes literature, the limits of the tribes Theroniini and Polysphinctini were uncertain: the composition of the Theroniini was heterogeneous, and united by symplesiomorphies only. The limit between the Ephialtini (=Pimplini *sensu* Townes) and the Polysphinctini was ill-defined; some authors grouped all the parasitoids of spiders within the Polysphinctini (Gupta & Tikar 1976), whilst others excluded the *Tromatobia* genus-group, restricting the Polysphinctini to strict koinobiont ectoparasitoids of spiders (Fitton et al. 1988).

Since these publications, Eggleton (1989) and Gauld (1991) divided the Pimplinae in four distinct subfamilies (Pimplinae *sensu stricto*, Diacritinae, Poemeniinae and Rhyssinae). They highlighted the paraphyly of the Ephialtini with respect to the Polysphinctini. Wahl & Gauld (1998) made the first rigorous phylogenetic analyses of the Pimpliformes. Their results led these authors to propose a new tribal classification of the Pimplinae, including the synonymy of the Polysphinctini with the Ephialtini. However, this group of genera is of particular interest since they exhibit the same mode of parasitism, all are strict koinobiont ectoparasitoids of spiders. For practical reasons, the *Polysphincta* group of genera are referred to as the polysphinctine. Recently, Gauld et al. (2002) refined the relationships within the Pimplinae, and highlighted the problems with the status of some existing genera. These two last works will serve us as

backbone to present the evolutionary biology of the Pimplinae.

### Overview of the Pimplinae biology

The Pimplinae is not only one of the largest subfamilies of Ichneumonidae (with approximately 1500 species; Yu & Horstmann 1997) but also one of the most diverse biologically. They are associated with a larger host range than any other Ichneumonidae subfamilies (Aubert 1969). All species of

**Table 1.** Comparison of two major Pimplinae classifications of Townes (1969) and Gauld et al. (2002). Superscripts are: *Pimpla*<sup>1</sup> incorrect usage of generic name for group correctly called *Ephialtes*<sup>2</sup>; *Coccygomimus*<sup>3</sup> junior synonym of *Pimpla*<sup>4</sup>; *Pimpla*<sup>4</sup> correct usage of generic name; *Ephialtes*<sup>5</sup> incorrect usage of generic name for group correctly called *Apechthis*; \* incorrect tribal names – the Pimplini of Townes should correctly be called Ephialtini, and the Ephialtini of this author should be called the Pimplini.

Townes 1969 EPHIALTINAE	Gauld et al. 2002 PIMPLINAE
PIMPLINI*	EPHIALTINI
<i>Alophosternum</i>	<i>Alophosternum</i>
<i>Pseudopimpla</i>	<i>Camptotypus</i>
<i>Camptotypus</i>	<i>Pseudopimpla</i>
<i>Pimpla</i> <sup>1</sup>	<i>Ephialtes</i> <sup>2</sup>
<i>Tromatobia</i>	<i>Tromatobia</i>
POLYSPHINCTINI	
<i>Polysphincta</i>	<i>Polysphincta</i>
EPHIALTINI*	PIMPLINI
<i>Coccygomimus</i> <sup>3</sup>	<i>Pimpla</i> <sup>4</sup>
<i>Ephialtes</i> <sup>5</sup>	<i>Apechthis</i>
THERONIINI	
<i>Theronia</i>	<i>Theronia</i>
	DELOMERISTINI
<i>Delomerista</i>	<i>Delomerista</i>
<i>Pseudorhyssa</i>	
POEMENIINI	POEMENIINAE
<i>Poemenia</i>	<i>Poemenia</i>
	<i>Pseudorhyssa</i>
RHYSSINI	RHYSSINAE
<i>Rhyssa</i>	<i>Rhyssa</i>
DIACRITINI	DIACRITINAE
<i>Diacritus</i>	<i>Diacritus</i>

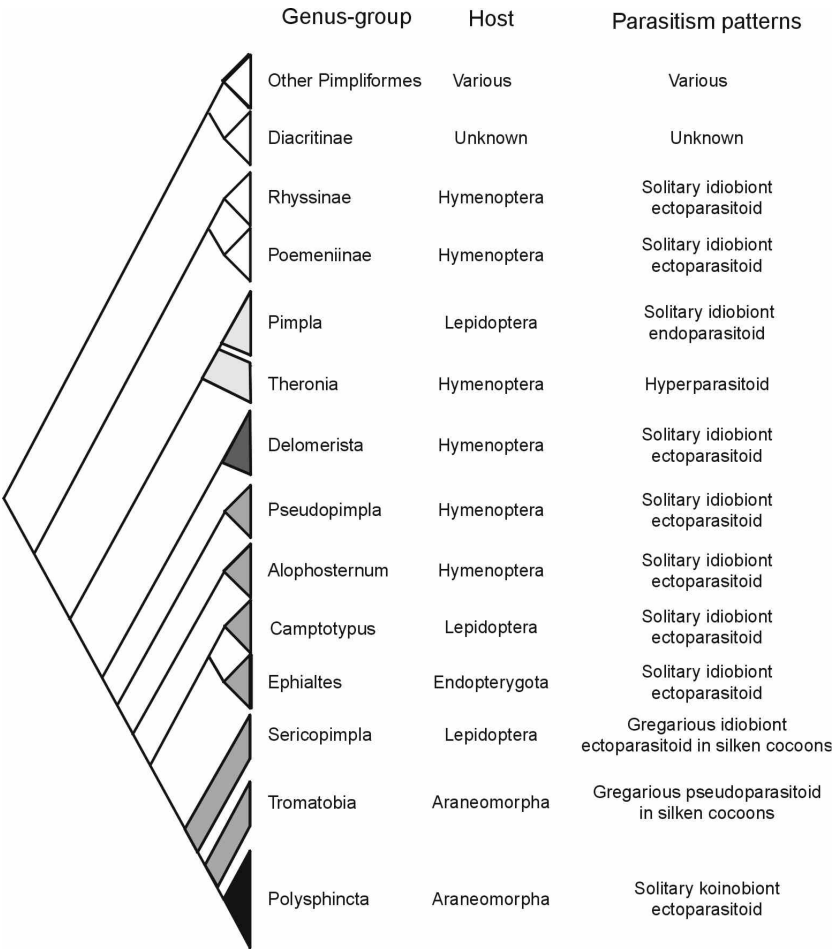
Pimplinae are parasitoid: the larvae deriving their entire sustenance feeding on or within an immobilized arthropod. Subsequent feeding and growth of the larvae almost invariably led to the death of the host at some stage (Gauld & Bolton 1988).

Pimplinae may be primary or secondary parasitoids (hyperparasitoids). The larvae may be ecto- or endoparasitoid of phytophagous or xylophagous holometabolous insects (Coleoptera, Hymenoptera, Lepidoptera, Diptera), but they may also be found feeding within the spider egg sac as egg predators

(pseudoparasitoids) or on active spiders (only Araneomorpha). The idiobiont parasitoids block any further development of the host after the egg-laying sting, while the koinobiont parasitoids permit their host to resume its development, and even to moult (sensu Askew & Shaw 1986).

**PIMPLINAE PHYLOGENY: THE CURRENT SITUATION**

Wahl & Gauld (1998) and Gauld et al. (2002) proposed the first rigorous phylogenies of the Pimplinae based on morphological characters



**Fig. 1.** Simplified phylogeny of the Pimplinae. Only the genus-groups are specified and the ground plan biology including host nature and parasitism patterns are given.

only (Fig. 1). They analysed a matrix of 162 taxa and 166 characters. The details of the analyses are not given here; the character list, the data matrix, and the phylogenetic analyses are found in the papers themselves. The biology of the different groups is shown in Fig. 1.

### Monophyly of the Pimplinae

These two studies (Wahl & Gauld 1998; Gauld et al. 2002) demonstrated the monophyly of the subfamily, and its relationships with the other subfamilies within the Pimpliformes. The clade of the Poemeniinae + Rhyssinae is the sister-group of the Pimplinae. The Diacritinae (whose biology is unknown) may be related with the Oxytorinae (Fitton et al. 1988) or Helictinae (Gauld 1991), but further investigations are needed to confirm this relationship.

### Tribal relationships and common ancestor

The Pimplinae are composed of three tribes: the Delomeristini (including the Perithoini *sensu* Wahl & Gauld 1998), the Pimplini and the Ephialtini (including the former tribe Polysphinctini) (Wahl & Gauld 1998; Gauld et al. 2002). The most parsimonious hypothesis about the evolution of the patterns of parasitism states that the common ancestor of the Pimplinae was a solitary idiobiont ectoparasitoid of phytophagous Hymenoptera. This pattern persists in the Delomeristini and in the basal members of the Pimplini and Ephialtini.

### Pimplini and the appearance of the endoparasitism

The Pimplini is the sister group of a clade uniting Delomeristini + Ephialtini. The optimisation of the parasitism patterns on the phylogeny indicates that the derived primary endoparasitism has evolved from secondary parasitism. Indeed, the species of the *Theronia* complex are mainly hyperparasitoids of primary parasitoids in pupae of Lepidoptera. From this paraphyletic grade arose the higher

Pimplini, idiobiont endoparasitoids. This transition toward endoparasitism allows the use of hosts that form little or no cocoon, the ectoparasitoids attacking pupae in thick cocoons.

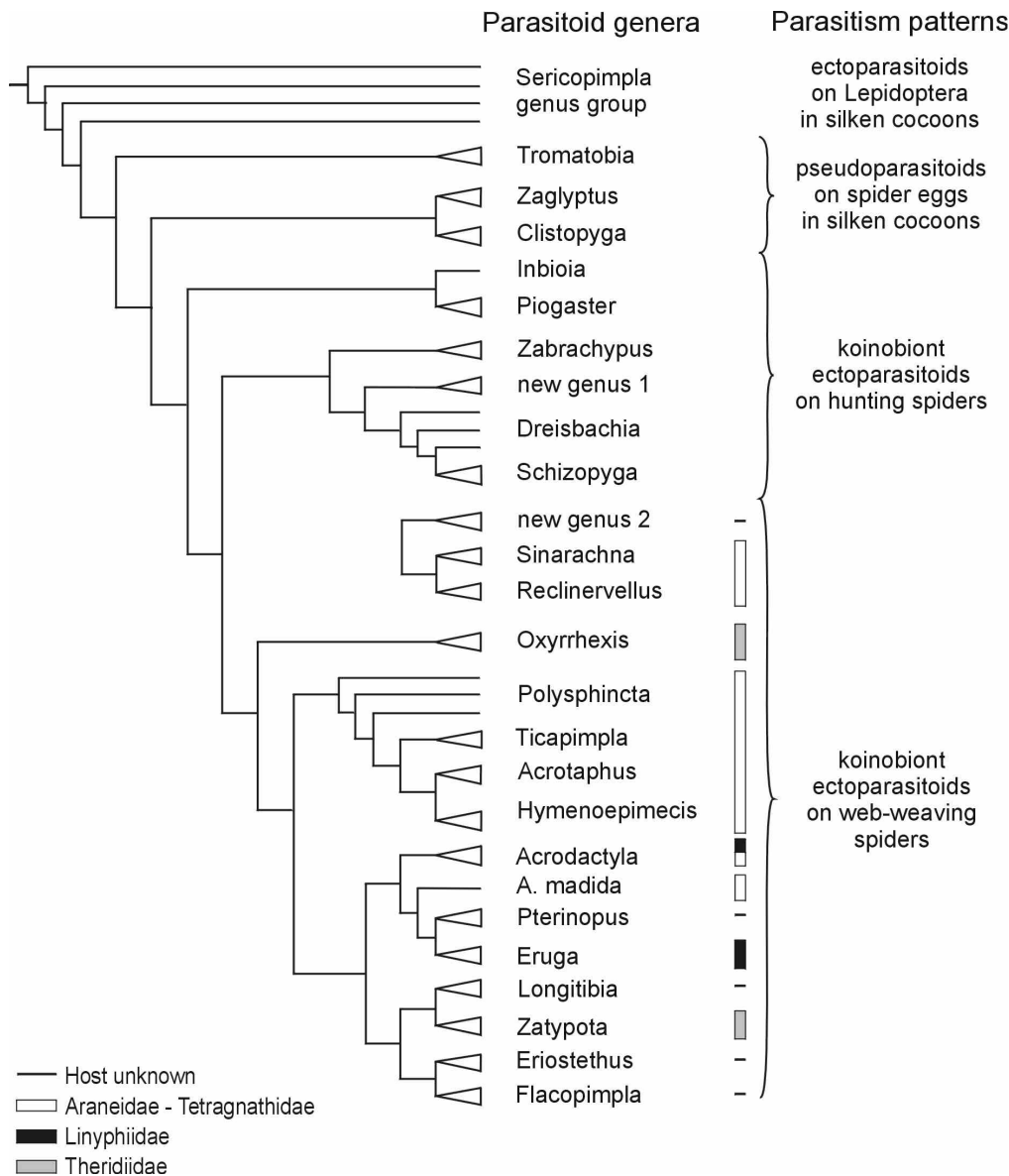
### Ephialtini diversification and parasitoids of spiders

Within the Ephialtini, the basal clades (*Pseudopimpla* and *Alophosternum* genus-group) are associated with Symphyta as solitary idiobiont ectoparasitoids. In the higher clade, two main lineages are differentiated. The first clade (the *Camptotypus* and *Ephialtes* genus-group) exhibit less diverse patterns of parasitism than other tribes but a larger host range; they are solitary idiobiont ectoparasitoids of holometabolous pupae (Coleoptera, Diptera, Hymenoptera, and Lepidoptera) concealed in plant tissues.

In the second lineage, several transitions have occurred. A first one is from parasitism of phytophagous insects toward that of pupae in silken cocoons within the *Sericopimpla* genus-group. The association with a silken cocoon is linked to the appearance of the gregarious behaviour. The second shift concerns the host nature, from Lepidoptera to Araneomorpha within the *Tromatobia* genus-group and the *Polysphincta* group of genera.

### PHYLOGENY AND EVOLUTION OF PARASITIDS OF SPIDERS

This study is based on the current working hypothesis of I.D. Gauld & J. Dubois (unpublished data) (Fig. 2). The matrix is composed of 96 characters based on the adult morphology coded for 77 taxa. Sixty-five species of 19 genera of polysphinctine were selected to represent the diversity of the 190 or so described species. The 12 outgroup taxa were chosen within the *Sericopimpla* and *Tromatobia* genus-groups, based on previous studies (Wahl & Gauld 1998; Gauld et al. 2002). The tree presented here is the result of a sensitivity analysis (Wheeler 1995). It has been



**Fig. 2.** Cladogram resulting from the sensitivity analysis: relationships within the *Polysphincta* group of genera.

prepared using eight weighting schemes: three successive weighting (Farris 1969) using the three possible indices and five implied weighting (Goloboff 1993) using five concavity parameter values. The details of the character list, matrix and analyses are not given here.

Careful attention must be given to the parasitoid host ranges, particularly when the available literature is used, in order to avoid misleading host records. Shaw (1994) gave a comprehensive review of this problem, listing several objections to reliance on records within

the literature. Host records biases are both qualitative and quantitative (e.g. the misidentification of either the parasitoid or the host; equal importance given to regular and freak association). Shaw (1994, p.119) stated that "the behavioural relationship between polysphinctines and spiders is clearly highly specialized, and *a priori* it might be expected that host ranges would be rather narrow". He compared the literature records with 422 reliable rearing records. This study concluded that 70% of the literature records remained unconfirmed and the rearing records indicated much narrower host ranges than those in the literature. Thirteen of the 105 spider families were found in the literature (Agelenidae, Araneidae, Clubionidae, Dictynidae, Gnaphosidae, Linyphiidae, Miturgidae, Philodromidae, Salticidae, Segestridae, Tetragnathidae, Theridiidae and Thomisidae). Shaw (1994) restricted the host ranges of polysphinctines occurring in Britain to five families of spiders (Clubionidae, Theridiidae, Tetragnathidae, Araneidae and Linyphiidae). For example, *Oxyrrhexis carbonator* was considered to have a large host range including Araneidae, Linyphiidae, Tetragnathidae, Theridiidae and Thomisidae; but it has been reared from Theridiidae only (Shaw 1994).

The host data used here come from Shaw's (1994) study, or from identified voucher specimens in museum collections. Spider nomenclature has been checked using the world catalogue of spiders (Platnick 2003).

### The basal lineages of parasitoids of spiders

The pimpline parasitoids of spiders have arisen from gregarious parasitoids of the *Sericopimpla* genus-group, which develop in silken cocoons of Lepidoptera. The transition toward parasitism of spider occurred within the *Tromatobia* genus-group (*Tromatobia*, *Zaglyptus* and *Clistopyga*), still associated with silken cocoons. These pseudoparasitoids attack the unguarded cocoons of different families (Araneidae, Clubionidae,

Linyphiidae, Philodromidae and Tetragnathidae) (Fitton et al. 1988), their larvae developing on spider eggs within the egg sacs. The females of some species of *Zaglyptus* kill the guarding spider female before oviposition [e.g. *Cheiracanthium erraticum* (Walckenaer, 1802)]. The larvae feed on the spider, whether it has laid eggs or not (Nielsen 1935). These three genera are paraphyletic at the base of the clade of the *Polysphincta* group of genera (the former Polysphinctini).

With the appearance of the *Polysphincta* group of genera, three changes occurred. There are two reversals: a switch from gregarious to solitary parasitoids, and another from pseudoparasitoids to ectoparasitoids. The last transition is the switch from idiobiosis to koinobiosis. These transitions mean that all the members of the *Polysphincta* group of genera have only solitary larvae developing ectoparasitically on an active spider.

### The *Polysphincta* group of genera

This group of genera is composed of six main monophyletic lineages (Fig. 2):

- 1) the *Piogaster* genus-complex (*Inbioia* and *Piogaster*),
- 2) the *Schizopyga* genus-complex (*Dreisbachia*, *Schizopyga*, *Zabrachypus* and an undescribed genus),
- 3) the *Reclinervellus* genus-complex (*Reclinervellus*, *Sinarachna* and an undescribed genus),
- 4) the *Oxyrrhexis* complex,
- 5) the *Polysphincta* genus-complex *sensu stricto* (*Acrotaphus*, *Hymenopimecis*, *Polysphincta* and *Ticapimpla*),
- 6) the *Eruga-Zatypota* genus-complex (*Acrodactyla*, *Eriostethus*, *Eruga*, *Flacopimpla*, *Longitibia*, *Pterinopus*, *Zatypota*).

The *Polysphincta* and the *Eruga-Zatypota* genus-groups form a clade, *Oxyrrhexis* being its sister group. This monophyletic group belongs to a trifurcation with *Schizopyga* and *Reclinervellus* genus-groups. The *Piogaster* genus-complex is the sister group of all other

polysphinctines. When some biological characters are added to the matrix such as the host nature, the larval position on the host, and the form of the parasitoid cocoon, the trifurcation is resolved. The *Schizopyga* complex becomes then the sister group of a clade comprising *Reclinervellus*, *Oxyrrhexis*, *Polysphincta* and *Eruga-Zatypota* genus-complexes, the *Piogaster* genus-complex remaining at the base of all the genus-groups (J. Dubois & I.D. Gauld, unpublished data).

Within this group of genera, two transitions occurred. The first change concerns the host ecology and the second the larvae position on the host. Members of the *Piogaster* - *Schizopyga* genus-groups attack wandering spiders (Clubionidae, Miturgidae, Salticidae) in their silken retreats. The four other genus-groups limit their host range on web weaver spiders (Araneidae, Linyphiidae, Tetragnathidae, Theridiidae). This transition is coupled with the change of the larval position on the host. The larvae of the *Piogaster* - *Schizopyga* genus-groups are located on the cephalothorax of the spider, while those of the second clade are attached to the mesosoma of the host.

Another transition may occur at the same node. A species of one of the most specialized genera, *Hymenoepimecis* has been observed to expel the egg from the base of the ovipositor, instead of ovipositing down the lumen of the ovipositor, as do most of the Ichneumonidae. The ovipositor is used as a stinging weapon to paralyse the host only (Fincke et al. 1990; Eberhard 2000a). The members of the *Reclinervellus*, *Oxyrrhexis*, *Polysphincta* and *Eruga-Zatypota* genus-groups have the same ovipositor with a modified base. However data is lacking about the ovipositing behaviour of other species, rendering it impossible to make any evolutionary inferences.

The web-weaving spiders attacked by polysphinctines belong to the three main types: orb web-weaving spiders (Araneidae), sheet web-weaving spiders (Linyphiidae) and

irregular space web-weaving spiders (Theridiidae). Even if the orb-web weaving spider parasitoid is the plesiomorphic condition under different optimisations, and the use of theridiid and linyphiid host is derived (J. Dubois & I.D. Gauld, unpublished data), it is not possible to suggest any hypothesis on the evolution of the use of these different web-weaving spiders. The main difficulties are the low number of host records and the lack of their reliability. The number of missing data highly increases the number of equi-parsimonious hypotheses.

### Host behaviour manipulation

The larvae of *Hymenoepimecis argyraphaga* (Gauld, 2000) attack the spider *Leucauge argyra* (Walckenaer, 1842) (Eberhard 2000b). The wasp female attacks the spider at the hub of its orb, stinging and causing temporary paralysis and lays an egg on the spider's abdomen. The spider recovers from the sting and resumes a normal activity, whilst the larva feeds and grows on the spider's back. The night before the larva kills its host, it induces a change in the spider's weaving behaviour, causing it to build a cocoon web to serve as a durable support for the wasp larva's cocoon. Then it kills and consumes the spider and pupates, hanging its own cocoon by a line from the cocoon web. The changes in the spider's behaviour are apparently induced chemically rather than by direct physical interference (Eberhard 2000a).

### CONCLUSIONS AND PERSPECTIVES

This original study on the phylogeny of parasitoids of spiders has given us a better understanding of the evolution of the spider-parasitoid relationships and of the different transitions in the parasitism patterns. The use of spider as a host has originated from a single event, as the use of web-weaving spiders. However several questions remain, mainly on the evolution of the use of linyphiid and theridiid hosts and the impact of the web architecture on the parasitoidism as suggested

by Blackledge (2003). Such questions require more biological information. Re-investigation of host records and new, reliable, data are needed. It would be also interesting to test the present morphological hypothesis with molecular data, in order to suggest a more robust hypothesis or new alternatives of phylogeny of parasitoids of spiders. This would also permit more precise investigation of the pattern of Pimplinae appearance and diversification.

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