

Inferring life history from ovipositor morphology in parasitoid wasps using phylogenetic regression and discriminant analysis

ROBERT BELSHAW¹, ALAN GRAFEN² and DONALD L. J. QUICKE^{1,3}

¹Department of Biological Sciences, Imperial College at Silwood Park, Ascot, Berks SL5 7PY, UK

²Zoology Department, University of Oxford, Oxford OX1 3PS, UK

³Department of Entomology, The Natural History Museum, London SW7 5BD, UK

Received April 2002; accepted for publication February 2003

Our understanding of the critical shift in life history among insect parasitoids from ectoparasitism to endoparasitism is hindered by the fact that this particular life history trait is not known for many phylogenetically important taxa. One method of coping with this problem is to seek correlations between the ovipositor structure and this life history trait among taxa whose life history is known, and then to use this to infer the trait in species whose life history is unknown. In one group of parasitoid wasps, the Ichneumonoidea (Hymenoptera), we scored a total of 20 morphometric and morphological characters of the ovipositor for 41 species whose life histories are known – representing all the main clades in which there have been independent transitions to endoparasitism plus a broad range of the ectoparasitoid groups; we then used phylogenetic regression and discriminant analyses to infer the life history of four species whose life histories are unknown. To allow for the effect of phylogenetic non-independence in the discriminant analysis, we carried out analyses using different randomly chosen representatives of the endoparasitoid clades (phylogenetic regression controls for phylogeny). These two methods gave congruent results from which we conclude that *Megalohelcon* and *Gnamptodon* are endoparasitoids, and *Aspilodemon* and *Allobracon* are ectoparasitoids. We discuss the consequences of these inferences for our understanding of the evolution of endoparasitism in the Ichneumonoidea. © 2003 The Linnean Society of London. *Zoological Journal of the Linnean Society*, 2003, **139**, 213–228.

ADDITIONAL KEYWORDS: Braconidae – Ichneumonidae – independent contrasts – koinobiosis – morphometrics.

INTRODUCTION

A parasitoid may be defined as ‘an organism which develops on or in another single (“host”) organism, extracts nourishment from it, and kills it as a direct or indirect result of that development’ (Eggleton & Gaston, 1990). Perhaps 10% of all described insect species are parasitoids and the life history is found among many insect groups (Eggleton & Belshaw, 1992). The parasitoid Hymenoptera (also known as parasitic Hymenoptera, or parasitoid/parasitic wasps) account for approximately three-quarters of the total number of parasitoid species. Typically laying their eggs on to or into the immature stages of other insects, the par-

asitoid Hymenoptera represent a single origin of the parasitoid life history, from which there have been later shifts to other life histories such as phytophagy found in Cynipidae (gall wasps) and sporadically elsewhere, and the ‘provisioning predator’ one found in many Aculeate Hymenoptera (Godfray, 1994).

Among parasitoid wasps a major change in life history has been that from an ancestral ectoparasitism, in which the egg is laid on the host and the larva develops externally, to endoparasitism, in which the egg is laid inside the host and the parasitoid larva develops internally (Quicke, 1997). Whitfield (1998) reviews the distribution of endoparasitism among different groups of parasitoid Hymenoptera.

Several studies have used phylogeny estimates to infer the historical occurrence and nature of this tran-

*Corresponding author. E-mail: r.belshaw@ic.ac.uk

sition in lineages of parasitoids (Whitfield, 1992; Downton & Austin, 2001), and there has been additional discussion of the selective pressures causing the transition (Gauld, 1988; Quicke, Le Ralec & Vilhelmsen, 1999b). In addition, Mayhew & Blackburn (1999) used phylogenetic regression to examine the relationship between life history traits and mode of development. More specifically, they tested whether a range of traits such as size and fecundity could be predicted by proposed fundamental dichotomies between endoparasitoids and ectoparasitoids, and between the developmental modes of koinobiosis and idiobiosis (discussed below).

However, a major problem that all these studies face is that the life histories of many taxa – often phylogenetically important ones – are unknown. Typically, such taxa are also rare and/or have a restricted geographical range, which makes it difficult to obtain direct observations. It would therefore be valuable to be able to infer whether species were ectoparasitic or endoparasitic from the morphology of the adult females in museum collections. It would also be preferable to use a non-destructive method because typically such species are rare and dissection of museum specimens is unlikely to be permitted. We expect that the structure of the ovipositor is adaptive and hence likely to be useful to infer life history. This assumption is supported by our earlier detection of evidence for convergence among morphological characters of the female reproductive system (including the ovipositor) in several phylogenetically independent lineages of Braconidae (Quicke & Belshaw, 1999). There are some clear associations of structure with function, e.g. the large teeth on the ovipositor valves in species that ‘drill’ through wood to reach their host (Vincent & King, 1996), and the high concentrations of zinc or manganese in the ovipositors of some such species – presumably for cuticular hardening (Quicke *et al.*, 1998). However, the correlations are not always obvious, e.g. in some endoparasitoid species attacking hosts that are concealed within fruits, Kimani-Njogu & Wharton (2002) recently found character states of the ovipositor that were previously thought to be indicative of ectoparasitism.

In this paper we focus on a single superfamily of parasitoid wasps (the Ichneumonoidea). This is for several reasons: (1) we have estimates of their phylogeny, (2) there appear to have been many independent transitions from ectoparasitism to endoparasitism and (3) we can homologize structures within the group (Belshaw *et al.*, 1998; Belshaw & Quicke, 2002; Downton *et al.*, 2002). The final point refers to the apparent functional shifts in ovipositor morphology between parasitoid superfamilies: when ovipositor teeth are present, ichneumonoids have them on the lower valves whereas nearly all the other

parasitoid superfamilies have them on the upper valve (Gerling, Quicke & Orion, 1999; Quicke *et al.*, 1999b). We examine the ovipositor morphology of a range of genera whose life histories are known, and use this to infer the history of certain taxa whose life history is not known. In an earlier study of ovipositor morphology, Le Ralec, Rabasse & Wajnberg (1996) used correspondence analysis to look for adaptive convergence among the parasitoids that attack hosts with similar morphologies. However, a major problem with such an approach is phylogenetic non-independence (Harvey & Pagel, 1991). The results are likely to be biased by differences in the relative strength of representation of different clades; therefore, characters that are clade dependent, and potentially not associated with the life history trait in question, may be given disproportionate weight. To overcome this problem we need to take account of phylogeny, and in our study we did this using two techniques (phylogenetic regression and a specifically modified discriminant analysis).

Phylogenetic regression (Grafen, 1989) offers the hypothesis-testing facilities of General Linear Models in a phylogenetic context; thus it is possible to control for one set of variables while testing for another (both sets may include continuous and categorical variables, and interactions). The method automatically fits by maximum likelihood a parameter (‘rho’) that stretches or shrinks lengths low down in the phylogeny compared with high up, as indicated by the data. Whereas phylogenetic regression is designed for a continuous y -variable, Grafen & Ridley (1996) compared methods for discrete data and found that it was the most satisfactory of those tested, and therefore is suitable for our study where the life history variable is a binary character.

Previous studies have used discriminant analysis to predict group membership based on similarities between morphological characters (e.g. Senturia, 1995; Reig, Daniels & Macdonald, 2001; Riga *et al.*, 2001). In our study, we allowed for phylogenetic non-independence among our data in the following manner. We repeated the discriminant analyses using randomly selected single representatives of clades in which the derived trait endoparasitism has arisen independently; thus, for each transition from ectoparasitism to endoparasitism, only a single endoparasitoid species will be represented in the model.

The four genera for which we wish to infer life history are as follows.

1. *Megalohelcon* Turner (Trachypetinae). There has been a debate over the timing of the shift to endoparasitism within the Braconidae (Downton, Austin & Antolin, 1998). This genus is recovered at the base of the Braconidae in analyses of both molecular (Belshaw *et al.*, 1998; Belshaw & Quicke, 2002) and

morphological data (Quicke *et al.*, 1999a) and knowing its life history would help resolve this.

2. *Aspilodemon* Fischer. The sister group of the largely Northern Hemisphere aphid-parasitoid clade Aphidiinae has recently been shown to be a Southern Hemisphere gall-associated clade, which is called the Mesostoinae (Belshaw *et al.*, 2000; Belshaw & Quicke, 2002). Some of the members of the Mesostoinae are phytophagous (Austin & Dangerfield, 1998), but the life history of parasitoid members of the clade, such as *Aspilodemon*, is not known with certainty (Oda, de Macedo & Quicke, 2001).

3. *Gnamptodon* Haliday. Another important dichotomy among parasitoid Hymenoptera is between idiobiosis and koinobiosis (Askew & Shaw, 1986). The distinction here is whether the host (a) is not allowed to develop further after the female parasitoid lays her egg(s), being either permanently paralysed or killed (= idiobiosis), or (b) is allowed to continue development and is only killed later by the developing parasitoid larva(e) (= koinobiosis). There is a very strong correlation between idiobiosis/koinobiosis and ectoparasitism/endoparasitism: idiobionts tend to be ectoparasitic while koinobionts tend to be endoparasitic (Mayhew & Blackburn, 1999). The genus *Gnamptodon* is known to be a koinobiont, but it has not been confirmed as an endoparasitoid (Shaw & Huddleston, 1991). If it were, it might represent an origin of the trait within the cyclostome clade of the Braconidae independent of that in the Opiinae/Alysiinae and Rogadinae. However, if it were ectoparasitic, as indicated by the papilliform antennae of the final instar larvae (Čapek, 1970), then it would be only the second known ectoparasitic koinobiont in the Braconidae (see below).

4. *Allobracon* Gahan. Although there are many undescribed species, all known reared specimens of *Allobracon* are from leaf-mining Lepidoptera and Coleoptera (Wharton, 1993), and we are unaware of any observations of the larvae. Although the genus has been treated as ectoparasitic (Čapek, 1970), this was based on the assumption that this genus was closely related to the Braconinae and this is now thought to be incorrect (D. L. J. Quicke, unpubl. data).

MATERIAL AND METHODS

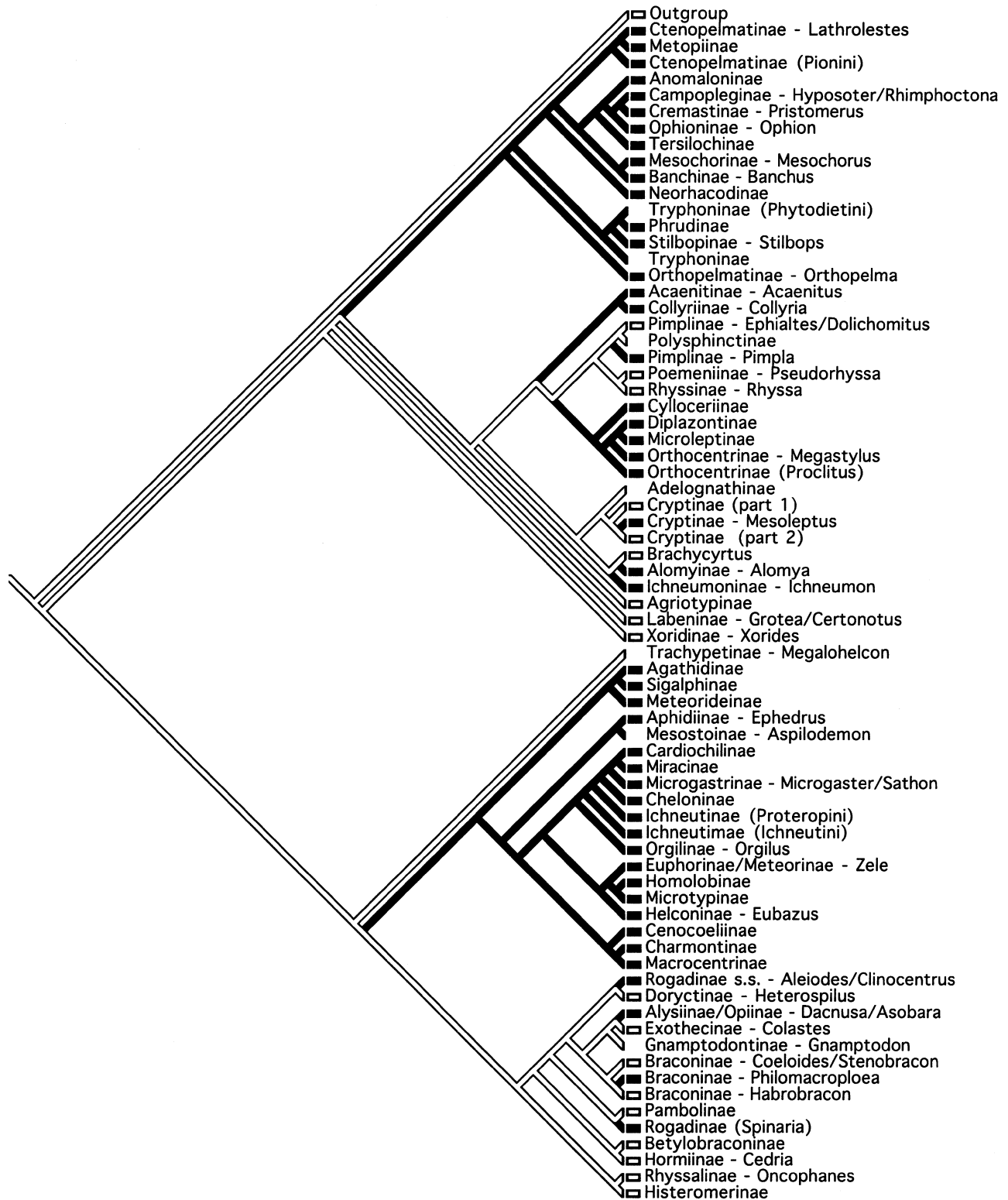
First, we need a phylogeny estimate both for the phylogenetic regression analysis and to enable us to select taxa for the discriminant analysis that represent independent origins of endoparasitism. The phylogeny estimate of the Ichneumonoidea that we use here is derived from previous publications. The Ichneumonoidea may be divided into three clades following Belshaw & Quicke (2002: fig. 4): (a) the

estimate for the non-cyclostome Braconidae is from the above study (figs 5–7); (b) the estimate for the cyclostome Braconidae is from Belshaw *et al.* (2000: fig. 1); (c) the estimate for the Ichneumonidae is from Quicke *et al.* (2000: fig. 3). These three estimates were simply melded together, i.e. there was no reconciling of conflicting tree topologies to create a supertree.

We then used maximum parsimony (MP), as implemented in MACCLADE 4.0 (Maddison & Maddison, 1992), to estimate the transitions to endoparasitism on this phylogeny estimate. Where this MP estimate of the transitions to endoparasitism was ambiguous, we resolved the character evolution using the DELTRAN transformation in MACCLADE, which forces changes on trees to be as far away from the root as possible (i.e. it prefers independent origins of derived states over early origin with subsequent reversal). Our phylogeny estimate contained three polytomies and, although we could use this for the phylogenetic regression (which is designed to handle incompletely resolved phylogenies), resolving ambiguous character estimation using the above transformation requires a fully resolved tree. All possible random resolutions had the same effect on the estimate of character evolution, and the result of one resolution is shown in Figure 1. In the legend to this figure we discuss at length several modifications we made to the basic analysis using other sources of data, and other issues of categorization, etc.

Our analysis therefore gave us 12 independent evolutions of endoparasitism, and within these 12 clades we selected 27 representative species whose life history is known (Table 1). To represent the ancestral trait of ectoparasitism we selected 14 species that covered as broad a range of phylogenetic and life history variation as possible; these included species from ten subfamilies, including parasitoids of hosts in varying degrees of concealment within plant tissue (Table 2).

We found a wide range of ovipositor morphologies among species both where the life history is known and where it is unknown (Fig. 2), showing that the inference of life history from morphology is not a trivial exercise. We scored a total of 20 morphological characters described in Table 3 (comprising seven linear measurements, seven ratios and six discrete characters), some of which are shown in Figure 3. The character definitions and data are given in Table 3. Our selection of characters is based upon hypotheses about the mode of functioning of ovipositors (Vincent & King, 1996; Quicke *et al.*, 1999b). Most of our characters relate to the following main ovipositor features. A protruding nodus on the upper valve is believed to play an important role in wood-boring taxa, locking the upper valve in place so that the lower valves can



be pushed into the substrate. Similarly, lower valve teeth are expected to be involved in rasping or breaking wood fibres (although the precise mechanism is not yet understood and may vary between taxa). A

strong nodus and strong serrations are not expected in endoparasitoids as they are likely to cause an unnecessarily large degree of damage to the host, which in most cases needs to survive oviposition and continue

Figure 1. Maximum parsimony estimation of the transition from ectoparasitism to endoparasitism in the Ichneumonoidea on our estimate of phylogeny, with the position of analysed species shown. Note, some higher taxa are not recovered as monophyletic. Tree shown is one of the random resolutions of the original phylogeny estimate, which has the nodes under Agriotypinae and Labeninae collapsed, and a trichotomy involving *Banchus* and *Mesochorus*. Coding: Open = Ectoparasitoid; Closed = Endoparasitoid. Taxa whose life history is not given are either under investigation here or their life history is inapplicable to our coding (see below). We assume that *Mesoleptus* and *Philomacroploea* were not basal taxa within the Cryptinae and Braconinae, respectively, and have added additional branches accordingly (relationships within Braconinae drawn arbitrarily). The affinities of *Allobracoon* are not known so it is not included here (see text). We made the following modifications to the result of the character estimation on the above tree. (a) We treated the Aphidiinae as an independent origin of endoparasitism to that in the other non-cyclostome Braconidae. This entirely endoparasitic subfamily forms a well-supported clade together with the Mesostoinae, which are phytophages or whose biology is poorly known – such as *Aspilodemon* investigated in this study. This clade has been recovered both as the sister group of the non-cyclostomes (Belshaw *et al.*, 2000) and within the non-cyclostome clade (Belshaw & Quicke, 2002) in neither case with much branch support. (b) Within the cyclostome Braconidae, we did not treat *Spinaria* Brullé as representing an independent origin of endoparasitism to that represented by *Aleiodes* Wesmael (the Rogadinae *s.s.*); there is evidence from both morphology and life history (the shared mummification of the host larvae) that – despite weak molecular evidence – these form a single clade (van Achterberg, 1991). (c) In the Ichneumonidae, we excluded the endoparasitoid *Euceros* Gravenhorst, which lays its eggs on leaves and uses planidial larvae to contact the host (Gauld & Bolton, 1988). We also treated *Alomya* Panzer as a separate origin of endoparasitism to that in the Ichneumoninae. Although they are placed together in our analysis, and on purely morphological grounds (Wahl & Mason, 1995), more recent analysis of a larger molecular dataset separates them (Laurenne, Broad & Quicke, 2002). In addition, we also note that our analysis did not include *Sericobracon* Shaw & Edgerly, which is very poorly known (although reputed to be an endoparasitoid and placed in the otherwise entirely ectoparasitic doryctine Braconidae) and we have ignored the (few) phytophagous taxa within the Ichneumonoidea. Our analysis includes some idiobiont endoparasitoids (one of the exceptions to the correlation between endoparasitism and koinobiosis among the Ichneumonoidea). Some pimpliform and ichneumoniform ichneumonids are such idiobionts endoparasitoid, attacking the pupal stages of their host, and we grouped these together with the koinobiont endoparasitoids because their life history as regards oviposition appeared similar. However, we excluded from our analysis (= treated as 'missing data') the four unrelated koinobiont ectoparasitoid clades (the other exceptions) because of their highly aberrant oviposition habits, which we suspect are subject to very different selection pressures from those dealt with in this study. (a) The Tryphoninae (Ichneumonidae) have anchored eggs whose main part is carried external to the ovipositor during oviposition, and the morphologically diverse egg anchors are inserted through the host cuticle in various ways. (b) Similarly, in at least some species of *Adelognathus* Holmgren (Ichneumonidae), the egg does not pass down the ovipositor, but rather emerges near the ovipositor base and is glued onto the host (M. R. Shaw, pers. comm.). (c) The Polyphinctinae (Ichneumonidae) are unique in being parasitoids of spiders. (d) *Rhysipolis* Foerster (Braconidae) has been hypothesized as having a biology that is consistent with it being at an intermediate stage in the evolution of both koinobiosis and endoparasitism (Shaw, 1983).

development. The presence of a pre-apical notch in the upper valve is tentatively assumed to be associated with moderating penetration of the host cuticle; an analogous notch has been shown to act as a clip for securing the host cuticle in the cynipoid *Leptopilina* Foerster (van Lenteren, Isidoro & Bin, 1998). Similar arguments can be put forward concerning the internal characters: the internal division of the upper valve lumen by a septum is likely to be involved in strengthening it (Quicke *et al.*, 1994); valvilli features may be associated with venom or egg manipulation within the ovipositor; the extent of the olistheter mechanism is likely to be connected to the need to maintain the upper and lower valves together as a single functional unit at the tip during envenomation and/or oviposition.

In order to determine if absolute size of the parasitoid has an effect on the way characters reflect life history we needed a measure of wasp size. In the par-

asitoid literature, hind tibia length has been widely used as a measure of size, e.g. it can be measured in wingless species, it is easy to measure accurately and is not affected by drying (Godfray, 1994).

Ovipositor measurements were taken manually from scanning electron microscope (SEM) images using Adobe Photoshop. We used an ISI ABT55 variable-pressure SEM with a Robinson back-scattered electron detector. Exceptions were *Ephedrus plagiator* and *Habrobracon juglandis*, for which ovipositor measurements were taken from Le Ralec (1991) and Bender (1943), respectively. One aspect of the ovipositor that needs to be noted is that it lacks the landmarks necessary to reduce measurements to sets of interlandmark Euclidean distances (Reig, Daniels & Macdonald, 2001).

For phylogenetic regression we used the single, largely resolved, tree obtained above and all morphological measurements from all taxa. The charac-

Table 1. Species representing ichneumonoid clades (numbered 1–12) in which endoparasitism appears to have arisen independently. All species are koinobionts except where otherwise indicated

Clade	Species	Reference
Braconidae		
1. Aphidiinae	<i>Ephedrus plagiator</i> Froggatt	Gärdenfors (1989) E
2. Non-cyclostomes	Meteorinae <i>Zelee albiditarsus</i> Curtis	van Achterberg (1979). E ¹
	Helconinae <i>Eubazus semirugosus</i> (Nees)	Haeselbarth (1962). C ²
	Microgastrinae <i>Microgaster tibialis</i> Nees	Vance (1932). E
	Microgastrinae <i>Sathon falcatus</i> (Nees)	Shenefelt (1972). SC ³
	Orgilinae <i>Orgilus lepidus</i> Muesebeck	Oatman, Platner & Greany (1969). C ⁴
Cyclostomes		
3. Rogadinae	<i>Aleiodes pictus</i> Evik-Shaeffer	M. R. Shaw (pers. comm.). E
	<i>Clinocentrus gracilipes</i> (Thomson)	Shaw (1983). SC
4. Opiinae/Alysiinae	<i>Asobara tabida</i> (Nees)	Commonly cultured. E ⁵
	<i>Dacnusa sibirica</i> Telenga	Commonly cultured. SC
5. Braconinae	<i>Philomacroploea basimaculata</i> Cameron	D. L. J. Quicke (pers. observ.). E ⁶
Ichneumonidae		
6. Ophioniformes	Campopleginae <i>Hyposoter carbonarius</i> Ratzeberg	M. R. Shaw (pers. comm.). E
	Campopleginae <i>Rhimphoctona</i> sp.	Wahl (1991). C ⁷
	Cremastrinae <i>Pristomerus vulnerator</i> Panzer	Rosenberg (1934). SC ⁸
	Ophioninae <i>Ophion minutus</i> Kriechbaumer	Brock (1982). E ⁹
	Banchinae <i>Banchus hastator</i> (Fabricius)	Van Veen (1982). E ¹⁰
	Mesochorinae <i>Mesochorus</i> sp.	Inferred from genus. E
	Ctenopelmatinae <i>Lathrolestes nigricollis</i> Thomson	Quednau & Guevremont (1975). SC
	Orthopelmatinae <i>Orthopelma mediator</i> Thunberg	Gauld & Mitchell (1977). SC ¹¹
	Stilbopinae <i>Stilbops ruficornis</i> (Gravenhorst)	Fitton (1984). C ¹²
Pimpliformes		
7. Orthocentrinae	<i>Megastylus</i> sp.	inferred from genus. E ¹³
8. Collyriinae/Acaenitinae	<i>Collyria coxator</i> (Villers)	Salt (1931). SC ¹⁴
	<i>Acaenitus dubitator</i> Panzer	Shaw & Wahl (1989). SC ¹⁵
9. Pimplinae	<i>Pimpla turionellae</i> (Linnaeus)	Fitton, Shaw & Gauld (1988). E ¹⁶
Ichneumoniformes		
10. Cryptinae	<i>Mesoleptus angustulus</i> (Först.)	Beaver (1972). E ¹⁷
11. Alomyinae	<i>Alomya semiflava</i> Stephens	Hinz & Short (1983). E ¹⁸
12. Ichneumoninae	<i>Ichneumon caloscelis</i> Wesmael	Hinz (1983). E ¹⁶

Host location: E = exposed; SC = semi-concealed (e.g. leaf-miners and web-spinners); C = concealed (e.g. wood and seed borers). Other notes: 1 – all hosts are nocturnally feeding lepidopteran larvae; 2 – genus parasitizes weevil eggs via the egg tube made by the host female, but species taxonomy within the genus is problematic (Kenis, Hulme & Mills, 1996); 3 – hosts are semi-concealed; 4 – ovipositor is inserted into the frass hole of the host; 5 – host is commonly just under the surface of very soft decaying fruit; 6 – we found uneclosed adults within host pupae in the collection of The Natural History Museum, London; 7 – not known how the female contacts her concealed host (from the delicacy of the ovipositor, Wahl (1991) suggests that it probes rather than bores for its host); 8 – attacks young *Cydia pomonella* larvae through the apple skin when they are near the surface; 9 – reared from *Agriopsis* spp. (Geometridae), all of which are all exposed feeders (S.-H. Yen pers. comm.); 10 – as *Banchus femoralis*; 11 – biology not known for certain, the host *Diplolepis rosae* is a gall former; 12 – has a longer ovipositor than the rest of the genus; observed probing flower heads of *Knautia arvensis* to oviposit in eggs of *Nemophora metallica*. 13 – all known hosts of orthocentrines are Diptera larvae (Wahl, 1990); another species of *Megastylus* is a known koinobiont endoparasitoid (Wahl, 1996); 14 – As *C. calcitrator*; attacks eggs of stem borers in grasses; 15 – attacks stem gall-forming *Cleonis* larvae in *Cirsium*; 16 – idiobiont parasitoid of lepidopteran pupae; 17 – an unidentified species in the same genus probes decaying snails with its ovipositor for fairly mature larvae of *Sarcophaga nigriiventris*; 18 – female enters the host burrow to attack the larva.

ters were all treated as continuous except for I, which was treated as categorical because it is easy to imagine that notches and nodules could evolve independently from or to a smooth state, or a notch could transform directly into a nodule or vice versa

(Table 3, Fig. 3). It is necessary to make assumptions about branch lengths, and in our case 14 phylogenetic levels were recognized, and assumed to be equidistant in the initial branch lengths (although the fitting of rho allows a degree of flexibility in

Table 2. Ectoparasitoid species used in the analyses

Rhyssalinae	<i>Oncophanes laevigatus</i> (Ratzeburg)	Shaw (1983). E ¹
Exothecinae	<i>Colastes braconus</i> Haliday	Shaw (1983). SC
Braconinae	<i>Coeloides scolyticida</i> Wesmael	Shaw & Huddleston (1991). CS
	<i>Stenobracon deesae</i> Cameron	Venkatraman & Subba Rao (1954). CP
	<i>Habrobracon juglandis</i> (Ashmead)	Commonly cultured. E.
Doryctinae	<i>Heterospilus prosopidis</i> Viereck	Commonly cultured. CS
Hormiinae	<i>Cedria paradoxa</i> Wilkinson	Mathur (1959). E
Labeninae	<i>Grotea anguina</i> Cresson	Rau (1928). SC ²
	<i>Certonotus fractinervis</i> Vollenhoven	Gauld & Wahl (2000). CS
	<i>Xorides brachylabris</i> Kriechbaumer	Chrystal & Skinner (1931). CS
Xoridinae	<i>Rhyssa persuasoria</i> (Linnaeus)	Fitton, Shaw & Gauld (1988). CS
Rhyssinae	<i>Pseudorhyssa alpestris</i> Holmgren	Fitton <i>et al.</i> (1988). CP
Poemeninae	<i>Ephialtes manifestator</i> (Linnaeus)	Fitton <i>et al.</i> (1988). CP ³
Pimplinae	<i>Dolichomitus populneus</i> Ratzburg	Fitton <i>et al.</i> (1988). CS

Host location: E = exposed, SC = semi-concealed (leaf-miners and stem-borers); CP = concealed (wasp probes existing holes to locate the host); CS = concealed (wasps bores through plant tissue). Other notes: 1 – wasp stings the host through the leaf but then enters the mine to oviposit; 2 – attacks bee larvae in stems, and another member of the genus (*G. californica*) has been observed to bore through the stem wall to oviposit (Slobodchikoff, 1967). 3 – hosts not certain but wasp reared from dead wood and adult females are seen probing host emergence holes.

this). The life history trait was treated as the response variable and each other explanatory variable was fitted in turn. Having found the explanatory variable that explained the largest variance (F value), it was controlled for and the remaining variables fitted in turn. This was repeated until F values became small and non-significant. Predicted values of the response variable for each taxon were obtained for each of the best models (that for *Allobracon*, which was not included in the phylogeny estimate, was calculated by hand from the models). Analyses were also performed with $\log(\text{size})$ to see whether that transformation changed conclusions.

We carried out standard discriminant analyses as implemented in STATISTICA version 4.1, using the 20 independent variables derived from the morphology plus one binary grouping variable (ecto- vs. endoparasitism). We recorded the classification of cases (species) by the program (either ecto- or endoparasitoid) together with the posterior probabilities, and the partial lambdas for each independent variable. Only 1.0% of our data were missing, and values for these were imputed using means. We performed 30 discriminant analyses, each of which contained all 14 ectoparasitoid species, the four species of unknown life history, but only a single randomly selected species from each of the 12 endoparasitoid clades.

RESULTS

In the phylogenetic regression, the largest $F_{1,15}$ value (39.62) in the uncontrolled analysis was for variable U (extent of olistheter). Controlling for U and refitting

each variable in turn showed that variable P (relative height of nodus) had the largest $F_{1,13}$ value (27.66). Controlling for U and P, and again fitting the other variables in turn, gave variable H (absolute height of nodus) the highest $F_{1,25}$ value (3.96). Repeating this, the next highest was 3.455, which was not significant. In subsequent analyses, we therefore tested the predictive powers of models based on U, U + P, and U + P + H. As shown in Figure 4, model U correctly classified all but two of the taxa with known biology. Model U + P + H correctly classified all but one of the taxa (*Mesoleptus*), but at the expense of less confidence for the others (U + P was intermediate). For this reason we prefer the simple phylogenetic regression model with just U as the predictor. The inclusion of $\log(\text{size})$ in the model made no difference to the results as it did not beat any of the 'winning' variables. The predicted values for the species whose life history is unknown are shown in Table 4. All models classified *Megalohelcon* and *Gnamptodon* as endoparasitoids, and *Aspilodemon* as an ectoparasitoid. Our preferred model classified *Allobracon* as an ectoparasitoid, but the more complex models give an ambiguous result.

The discriminant analyses found no more than one incorrect classification of the species used to create the model in any replicate, and found none in 26 of the 30. For the four species whose life history is unknown, their classifications together with the associated posterior probabilities are shown in Table 5. This analysis gives strong support for *Aspilodemon* being an ectoparasitoid, and weak to moderate support for *Megalohelcon* being an endoparasitoid

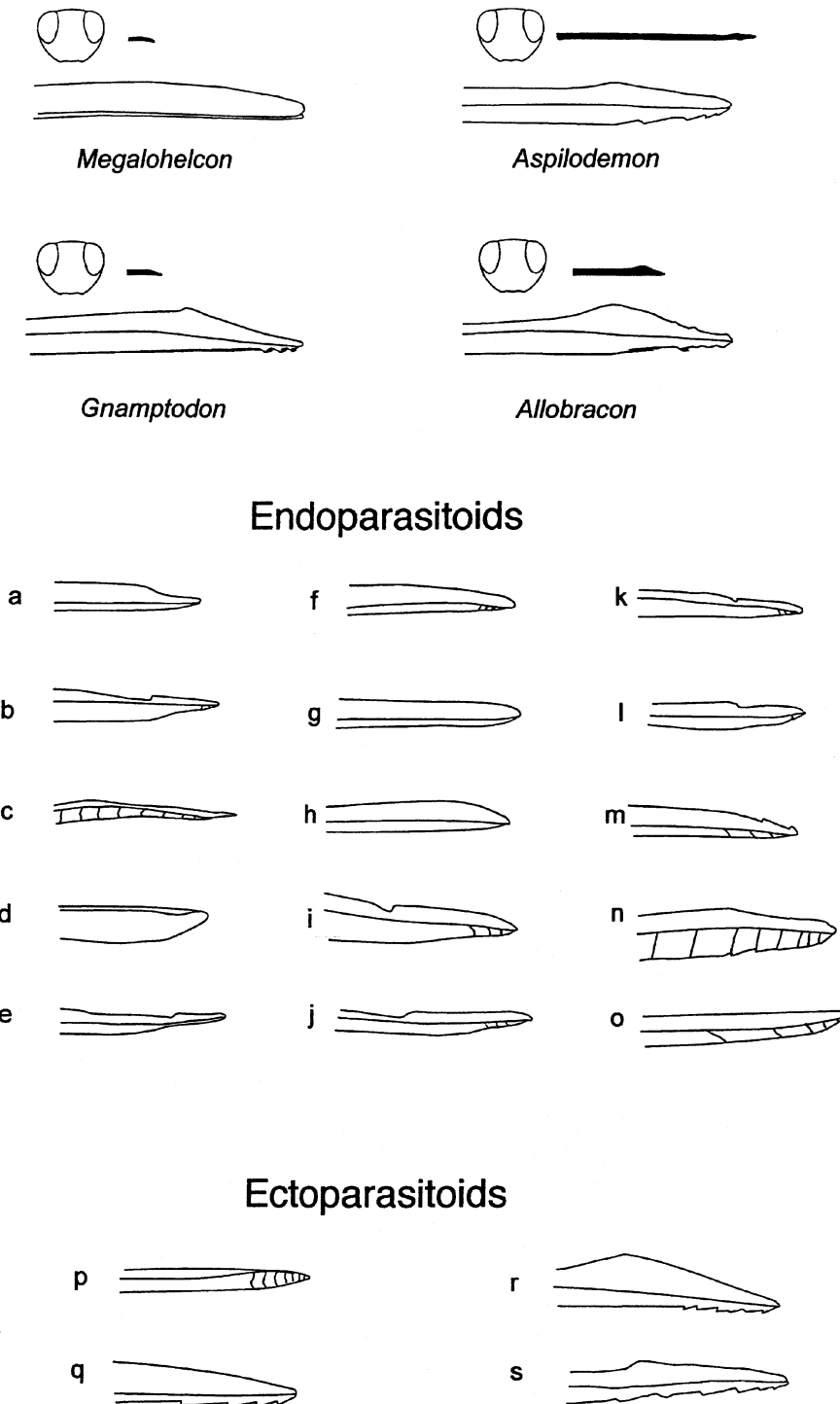


Figure 2. Distal part of ovipositor of the four parasitoid species whose life history is unknown, plus a selection of species whose life history is known. The complete ovipositor of the four species is also shown in profile, drawn relative to the width of the head of the species. Species with known life history are as follows (see Tables 1 and 2 for full names and classification). Endoparasitoids: host is exposed – a *Aleiodes*, b *Zele*, c *Mesoleptus*, d *Megastylus*, e *Ophion*, f *Microgaster*; host is leaf-miner – g *Dacnusa*, h *Sathon*; host is under fruit skin – i *Pristomerus*; host is in decaying fruit – j *Asobara*; wasp probes for deeply concealed host – k *Orgilus*, l *Rhimphoctona*, m *Eubazus*; host is stem-borer – n *Collyria*; host is gall-former – o *Orthopelma*. Ectoparasitoids: wasp probes for deeply concealed host – p *Pseudorhyssa*, q *Stenobracon*; host is leaf-miner – r *Colastes*; wasp bores for deeply concealed host – s *Coeloides*.

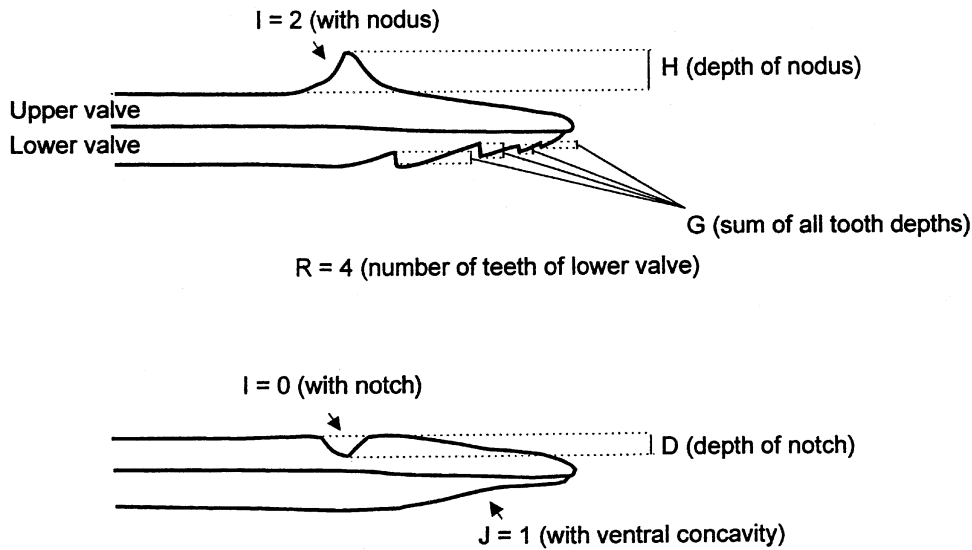


Figure 3. Measurements used in the analyses. See Table 3 for further explanation of characters.

and for *Allobracon* being an ectoparasitoid. Regarding *Gnamptodon*, the result is inconclusive; we can only say that it is more likely to be an endoparasitoid than an ectoparasitoid.

In Table 6, characters are ranked by their partial lambda in the discriminant analyses, averaged across the 30 analyses. Both methods found character U (whether or not the rhachis of the olistheter mechanism extends to the tip of the ovipositor) to be the most useful one for predicting whether species are endoparasitoids or ectoparasitoids. In Table 5 we also show the results of excluding from our discriminant analyses the three internal ovipositor characters (S, T and U in Table 3). Unlike the other characters, these require disarticulation of the ovipositor and hence would be considered as destructive and unlikely to be permitted for rare museum specimens. Excluding these characters has the expected effect of merely reducing the significance level of the results for three of our test species, but in *Allobracon* it has the opposite effect, with all replicates classifying it as an ectoparasitoid (and with a lower *P* value). This appears to be the result of one character: in *Allobracon* the dorsal valve of the ovipositor is undivided ($T = 1$) as in many endoparasitoids, but unlike in all the included ectoparasitoids – and in all of the many other ectoparasitoids examined but not included in our analysis (Quicke & van Achterberg, 1990; Quicke *et al.*, 1994).

DISCUSSION

Both phylogenetic regression and our, phylogenetically adjusted, discriminant analyses gave similar

predictions of life history for our four genera where it is unknown. We therefore conclude that *Megalohelcon* and *Gnamptodon* are probably endoparasitoids, and *Aspilodemon* and *Allobracon* are probably ectoparasitoids. For the latter two species, however, we find that only one of the methods gives a robust classification. Neither method gave a perfect classification of life history of our taxa where it was already known, but we attribute this largely to the misclassification of *Mesoleptes*, a taxon with a particularly unusual ovipositor morphology (Fig. 2c), and *Philomacroploea*, both of which have the olistheter mechanism ending somewhat before the apex of the upper valve. These may well represent relatively recent origins of endoparasitism as both are nested deeply within subfamilies (Cryptinae and Braconinae, respectively) that are dominated by ectoparasitoids. Inclusion of additional information, as readily done by discriminant analysis and by inclusion of variables $U + P + H$ in phylogenetic regression, allow *Philomacroploea* to be correctly classified. The fact that the most important predictive variable (U) was the same in both methods, but that the next best variable in phylogenetic regression was 15th in terms of its partial lambdas in the discriminant analysis (Table 6), is because many of the characters with lower partial lambda (better predictive power) were strongly correlated with variable U.

Our inference that *Megalohelcon* is an endoparasitoid (ovipositor morphology appears to be effectively constant within its subfamily the Trachypetinae) makes the unweighted MP estimation of the ancestral life history of the Braconidae ambiguous rather than ectoparasitoid as previously found (note, DELTRAN

Table 3. Morphological and life history data (all absolute measurements are in millimetres)

Taxon	A	B	C	D	E	F	G	H	I	J
<i>Acaenitus</i>	1	3.12	7.680	0	0.0951	0.0624	0.0351	0.0229	1	1
<i>Aleiodes</i>	1	1.78	0.036	0	0.0300	0.0137	0	0	1	0
<i>Allobracon</i>	?	0.57	0.360	0	0.0170	0.0096	0.0040	0.0180	2	0
<i>Alomya</i>	1	2.24	0.677	0	0.0636	0.0270	0.1065	0	1	0
<i>Asobara</i>	1	0.74	0.550	0.0048	0.0204	0.0128	0.0035	0	0	0
<i>Aspilodemon</i>	?	0.81	1.625	0	0.0431	0.0283	0.0086	0.0086	2	1
<i>Banchus</i>	1	4.20	0.518	0.0218	0.0772	0.0415	0.0090	0	0	0
<i>Cedria</i>	0	0.54	0.285	0	0.0160	0.0150	0.0020	0.0066	2	1
<i>Certonotus</i>	0	4.56	27.20	0	0.2100	0.2260	0	0.0440	1	1
<i>Clinocentrus</i>	1	1.36	1.940	0	0.0267	0.0148	0.0002	0.0101	2	0
<i>Coeloides</i>	0	1.60	5.600	0	0.0532	0.0272	0.0346	0.0058	2	1
<i>Colastes</i>	0	0.92	0.425	0	0.0359	0.0186	0.0079	0.0041	1	1
<i>Collyria</i>	1	2.72	1.870	0	0.0836	0.0437	0.0122	0	1	0.5
<i>Dacnusa</i>	1	0.74	0.121	0	0.0091	0.0060	0	0	1	0.5
<i>Dolichomitus</i>	0	3.32	11.84	0	0.1160	0.0615	0.0829	0	1	1
<i>Ephedrus</i>	1	0.63	0.100	0.0019	0.0071	0.0029	0.0016	0	0	1
<i>Ephialtes</i>	0	2.88	20.32	0	0.0995	0.0422	0.0733	0.0035	2	1
<i>Eubazus</i>	1	1.40	1.540	0.0031	0.0287	0.0140	0.0031	0	0	1
<i>Gnamptodon</i>	?	0.56	0.203	0	0.0250	0.0110	0.0040	0.0030	1	0
<i>Grotea</i>	0	2.68	5.920	0	0.0638	0.0360	0.0616	0.0204	2	1
<i>Habrobracon</i>	0	0.83	0.519	0	0.0111	0.0095	0.0206	0.0048	2	1
<i>Heterospilus</i>	0	1.08	0.800	0	0.0292	0.0168	0.0452	0.0052	1	1
<i>Hyposoter</i>	1	1.86	1.690	0.0136	0.0970	0.0347	0	0	0	0
<i>Ichneumon</i>	1	2.72	1.471	0	0.0963	0.0578	0.1213	0	1	1
<i>Lathrolestes</i>	1	1.00	0.683	0	0.0489	0.0320	0.0045	0	1	0
<i>Megalohelcon</i>	?	6.00	0.847	0	0.0520	0.0339	0	0	1	0.5
<i>Megastylus</i>	1	2.02	0.213	0	0.0084	0.0078	0	0.0031	1	0.5
<i>Mesochorus</i>	1	1.58	0.605	0	0.0215	0.0077	0.0037	0.0035	1	0
<i>Mesoleptus</i>	1	2.04	0.939	0	0.0704	0.0287	0.0296	0	1	0
<i>Microgaster</i>	1	1.14	0.438	0	0.0263	0.0165	0	0	1	0
<i>Oncophanes</i>	0	0.74	0.447	0	0.0212	0.0082	0	0.0091	1	0
<i>Ophion</i>	1	2.52	1.020	0.0135	0.1020	0.0390	0.0093	0	0	0
<i>Orgilus</i>	1	1.44	3.550	0.0041	0.0440	0.0265	0	0.0029	0	0
<i>Orthopelma</i>	1	0.86	1.160	0	0.0278	0.0136	0	0	1	1
<i>Philomacropyl.</i>	1	0.89	0.408	0	0.0170	0.0270	0	0.0260	2	1
<i>Pimpla</i>	1	1.60	5.360	0	0.2017	0.1140	0.0535	0	1	1
<i>Pristomerus</i>	1	1.76	2.150	0.0079	0.0550	0.0305	0.0050	0	0	0
<i>Pseudorhyssa</i>	0	4.28	25.00	0	0.1420	0.0862	0.0606	0.0050	1	1
<i>Rhimphoctona</i>	1	2.52	4.290	0.0099	0.0414	0.0238	0	0.0028	0	0
<i>Rhyssa</i>	0	5.68	24.50	0	0.2060	0.1600	0.0901	0.0360	2	1
<i>Sathon</i>	1	1.34	1.420	0	0.0326	0.0191	0	0	1	1
<i>Stenobracon</i>	0	3.60	13.10	0	0.0514	0.0288	0.02	0.0044	1	0
<i>Stilbops</i>	1	1.60	2.254	0	0.0648	0.0287	0	0	1	0.5
<i>Xorides</i>	0	4.40	10.24	0	0.0746	0.0374	0.0802	0.0007	2	1
<i>Zelee</i>	1	3.96	2.200	0.0137	0.1440	0.5840	0	0	0	0

Our terminology comes from Quicke *et al.* (1999b). A = ectoparasitic (0) or endoparasitic (1). B = length of hind tibia. C = length of ovipositor excluding basal expansions. D = depth of notch (Fig. 3). E = depth of ovipositor at its mid-point. F = depth of ovipositor at a distance from the tip equal to E (a measure of 'pointedness'). G = sum of depths of all teeth (Fig. 3) (a measure of overall tooth development). H = height of nodus (or swelling in the posterior half of ovipositor) above that of E (Fig. 3). I = ovipositor with dorsal notch (0), at least one dorsal nodus (2) or neither (1) (Fig. 3). J = lower valve with deep concavity (0), faint concavity (0.5) or no concavity (0) in profile (Fig. 3); K = C/B (relative length of ovipositor). L = D/E (relative depth of notch). M = E/C (relative thickness of ovipositor). N = F/E (relative 'pointedness'). O = G/E (relative tooth

K	L	M	N	O	P	Q	R	S	T	U
2.462	0	0.012	0.656	0.369	0.241	0.031	12	2	0	?
0.020	0	0.833	0.457	0	0	0	2	1	0	1
0.629	0	0.047	0.565	0.235	1.059	0.045	3	1	1	0
0.302	0	0.094	0.425	1.675	0	0	10	2	1	1
0.743	0.235	0.037	0.627	0.173	0	0.090	3	1	0	1
2.019	0	0.027	0.657	0.200	0.200	0.125	4	2	0	0
0.123	0.282	0.149	0.538	0	0	0.070	4	2	1	1
0.531	0	0.056	0.938	0.125	0.413	0.130	2	1	0	0
5.965	0	0.008	1.076	0	0.210	0	12	2	0	?
1.426	0	0.014	0.554	0.007	0.378	0.060	3	1	0	?
3.500	0	0.010	0.511	0.650	0.109	0.200	7	1	0	0
0.462	0	0.084	0.518	0.220	0.114	0.090	3	1	0	0
0.688	0	0.045	0.523	0.146	0	0.030	4	2	0	?
0.164	0	0.075	0.662	0	0	0	0	1	0	1
3.566	0	0.010	0.530	0.715	0	0.080	12	2	0	0
0.159	0.265	0.071	0.408	0.225	0	0.003	5	0	1	1
7.056	0	0.005	0.424	0.737	0.035	0.070	14	2	0	0
1.100	0.106	0.019	0.488	0.109	0	0.050	3	1	1	1
0.361	0	0.123	0.440	0.160	0.120	0.060	3	1	1	1
2.209	0	0.011	0.564	0.966	0.320	0	10	2	0	0
0.624	0	0.021	0.856	1.856	0.432	0.110	6	1	0	?
0.741	0	0.037	0.575	1.548	0.178	0.180	10	1	0	0
0.909	0.140	0.057	0.358	0	0	0	6	1	1	1
0.541	0	0.065	0.600	1.260	0	0.070	8	2	0	?
0.683	0	0.072	0.654	0.092	0	0.030	3	?	?	1
0.141	0	0.061	0.652	0	0	0	0	0	?	1
0.105	0	0.039	0.921	0	0.369	0	2	2	1	1
0.383	0	0.036	0.358	0.172	0.163	0.050	8	1	0	1
0.460	0	0.075	0.408	0.420	0	0.040	10	2	0	0
0.384	0	0.060	0.627	0	0	0	3	2	1	1
0.604	0	0.047	0.387	0	0.429	0	0	3	0	0
0.405	0.132	0.100	0.382	0.091	0	0.030	4	1	1	1
2.465	0.093	0.012	0.602	0	0.066	0.025	3	1	1	1
1.349	0	0.024	0.489	0	0	0.050	4	4	0	1
0.456	0	0.042	1.588	0	1.529	0.040	3	1	0	0
3.350	0	0.038	0.565	0.265	0	0.040	12	2	0	1
1.222	0.144	0.026	0.555	0.091	0	0.020	3	1	0	1
5.841	0	0.006	0.607	0.427	0.035	0.050	10	1	0	0
1.702	0.239	0.010	0.575	0	0.068	0.040	1	1	0	1
4.313	0	0.008	0.777	0.437	0.175	0.070	10	3	0	0
1.060	0	0.023	0.586	0	0	0	3	2	1	1
3.639	0	0.004	0.560	0.389	0.086	0.080	4	1	0	0
1.409	0	0.029	0.443	0	0	0	2	2	0	1
2.327	0	0.007	0.501	1.075	0.009	0.040	21	1	0	0
0.556	0.095	0.065	4.056	0	0	0	3	1	1	1

development). P = H/E (relative height of nodus). Q = depth of largest tooth/E (relative tooth development). R = number of teeth on each lower valve. S = number of valvilli. T = dorsal valve divided (0) or undivided (1) (equivalent to lumen paired or single). U = rhachis of the olistheter mechanism does not (0) or does (1) extend to the tip of the ovipositor. All measurements are taken from a single individual and are seen in lateral view except in *Grotea*, where the ovipositor and its teeth in cross-section extend further laterally than dorso-ventrally (ovipositor measurements in this species were taken in dorsal view).

Table 4. Values of the response variable (= life history) predicted by the phylogenetic regression models for the four species whose life history is unknown (1 = endoparasitoid; 0 = ectoparasitoid)

Species	Model		
	U	U + P	U + P + H
<i>Megalohelcon</i>	0.99	1.03	0.98
<i>Gnamptodon</i>	0.99	1.05	1.03
<i>Allobracon</i>	0.11	0.19	0.60
<i>Aspilodemon</i>	0.11	0.06	0.06

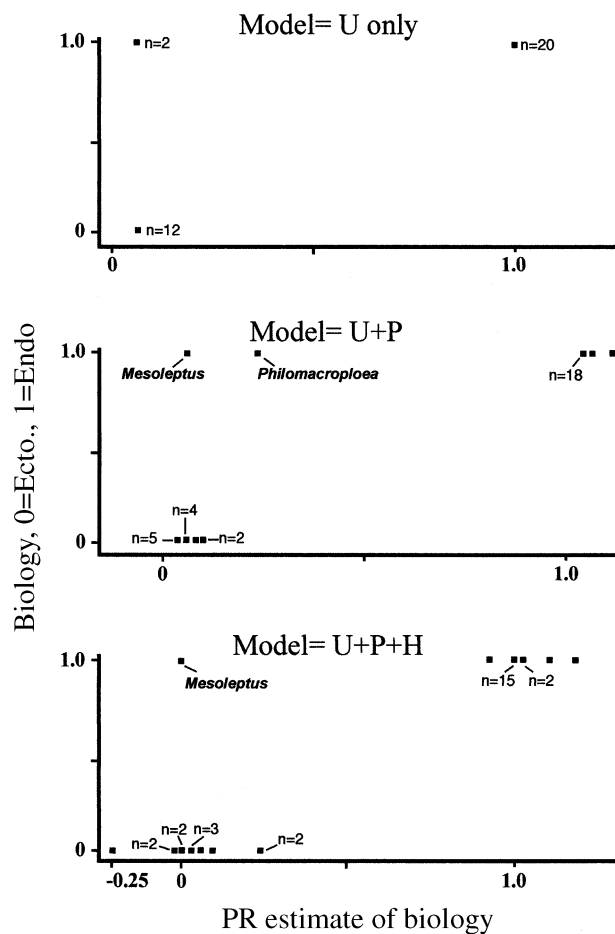


Figure 4. Plots of biology vs. phylogenetic regression (PR) estimates of biology for the taxa where the biology is known; results from the three best PR models shown. State 0 = taxa are ectoparasitoids and state 1 = taxa are endoparasitoids. *Philomacroploea* and *Mesoleptus*, which are wrongly classified with PR, are indicated. The characters used in the models (U, P and H) are explained in Table 3. Estimates are derived as follows: PR (U) = $(0.5281 + 0.8821) \times (U - 4730)$. PR (U + P) = $0.5144 + 1.001 \times (U - 0.4617) + 0.1531 \times (P - 0.1531)$. PR (U + P + H) = $0.4948 + 0.9818 \times (U - 0.4456) + 0.7428 \times (P - 0.1571) - 10.17 \times (H - 0.0054)$.

resolution of this ambiguity makes it ectoparasitoid with an additional independent transition to endoparasitism in the Trachypetinae). The possibility, raised by Dowton *et al.* (1998), of an early shift to endoparasitism in the family followed by a reversal thus has less weight against it than previously assumed (although we suspect that the rooting they obtained in their molecular analysis with *Meteorus* Haliday (Euphorinae) appearing as a basal braconid may be artefactual, perhaps due to base compositional bias). *Allobracon* is within the predominantly ectoparasitoid cyclostome clade, so its being ectoparasitoid has no effect on the estimation of trait evolution. As previously discussed, the phylogenetic placement of the Mesostoinae (which includes *Aspilodemon*) and its sister group the Aphidiinae is uncertain. If we are correct in our inference here that *Aspilodemon* is an ectoparasitoid, then this also may be interpreted as further evidence for a basal position of this clade within the Braconidae; otherwise, an unweighted MP estimation of character change would infer a reversal to ectoparasitism in *Aspilodemon*, which is not supported by larval morphology – there is no reduction in its cephalopharyngeal skeleton as found with such reversals in other parasitoid lineages (Oda, de Macedo & Quicke, 2001; G. R. Broad, R. Belshaw & D. L. J. Quicke, unpubl. data).

We should also mention that species in the ichneumonid subfamily Labeninae that bore through hard wood, such as the *Certonotus* species examined here, tend to have many extremely small teeth on the lower valve of the ovipositor rather than the more typical fewer and larger teeth (Gauld & Wahl, 2000), and these are not reflected in our characters. The possession of very small teeth also occurs in a few Cryptinae and Braconinae (see, for example, van Achterberg & Quicke, 1991), and probably represents a different mechanism for wood penetration (J. Vincent, pers. comm.).

Both phylogenetic regression and discriminant analysis identified character U (extent of olistheter) as the morphological variable most strongly corre-

Table 5. Classification by discriminant analysis of the four species whose life history is unknown. Figures in parentheses show the result if the three internal ovipositor characters are excluded from the analyses (characters S, T and U in Table 3)

Species	No. of analyses (out of 30) where species classified as an endoparasitoid	No. of analyses (out of 30) where species classified as an ectoparasitoid	Mean <i>P</i> -value for the classification	No. of analyses (out of 30) where <i>P</i> < 0.05
<i>Megalohelcon</i>	28 (27)	2 (3)	0.078 (0.106)	26 (25)
<i>Gnamptodon</i>	23 (15)	7 (15)	0.205 (0.436)	23 (4)
<i>Allobracon</i>	2 (0)	28 (30)	0.087 (0.009)	25 (29)
<i>Aspilodemon</i>	0 (0)	30 (30)	0.001 (0.022)	30 (23)

Table 6. Morphological characters ranked in descending order of usefulness in classifying taxa as endoparasitoids or ectoparasitoids. The partial lambda for each character averaged across the 30 analyses is shown. See Table 3 for further details of characters

Character (name and code)	Mean partial lambda
Olistheter U	0.773
Lower valve concavity J	0.790
Relative ovipositor depth near tip N	0.845
Relative length of teeth O	0.845
Depth of notch D	0.863
Ovipositor depth near tip F	0.864
Notch or nodus I	0.868
Ovipositor length C	0.879
Relative depth of notch L	0.885
Total length of teeth G	0.893
Teeth on lower valve R	0.899
Nodus height H	0.902
Ovipositor depth at mid-point E	0.908
Dorsal valve T	0.921
Relative nodus height P	0.956
Relative ovipositor depth at mid-point M	0.960
Relative ovipositor length K	0.961
Hind tibia length B	0.967
Number of valvilli S	0.973
Relative depth of largest tooth Q	0.987

lated with biology. Considering the usefulness of individual characters for classification, we suspect that the importance of the development of this rha-chis reflects whether the egg is extruded from near the ovipositor tip, or considerably anterior to the tip. Neither the length of the ovipositor nor its length relative to that of the tibia are good classifiers by themselves (eighth and seventeenth most useful, respectively, in the discriminant analyses). We know of no published observations of the detailed workings of ovipositors, which would be extremely

valuable and should be a high priority in future research.

ACKNOWLEDGEMENTS

We thank Chris Jones and Alex Ball for help with the SEM, and Gavin Broad, Lex Kraaijeveld, Patricia Reader and Michael Sharkey for specimens, and two referees for useful comments on the manuscript.

REFERENCES

- van Achterberg C. 1979.** A revision of the subfamily Zelinae auct. (Hymenoptera, Braconidae). *Tijdschrift voor Entomologie* **122**: 241–479.
- van Achterberg C. 1991.** Revision of the genera of the Afrotropical and W. Palaearctical Rogadinae Foerster (Hymenoptera: Braconidae). *Zoologische Verhandelingen* **273**: 1–102.
- van Achterberg C, Quicke DLJ. 1991.** A new genus of Braconinae with depressed ovipositor-tip from the Oriental Region (Hymenoptera: Braconidae). *Zoologische Mededelingen, Leiden* **64**: 199–202.
- Askew RR, Shaw MR. 1986.** Parasitoid communities: their size, structure and development. In: Waage J, Greathead D, eds. *Insect parasitoids*. London: Royal Entomological Society, 225–264.
- Austin AC, Dangerfield PC. 1998.** Biology of *Mesostoa kerri* Austin and Wharton (Insecta: Hymenoptera: Braconidae: Mesostoinae), an endemic Australian wasp that causes stem galls on *Banksia marginata* Cav. *Australian Journal of Botany* **46**: 559–569.
- Beaver RA. 1972.** Ecological studies on Diptera breeding in dead snails. 1. Biology of the species found in *Cepaea nemoralis* (L.). *The Entomologist* **105**: 41–52.
- Belshaw R, Dowton M, Quicke DLJ, Austin AD. 2000.** Estimating ancestral geographic distributions: a Gondwanan origin for aphid parasitoids? *Proceedings of the Royal Society of London (Series B)* **267**: 491–496.
- Belshaw R, Fitton M, Herniou E, Gimeno C, Quicke DLJ. 1998.** A phylogenetic reconstruction of the Ichneumonidea (Hymenoptera) based on the D2 variable region of 28S ribosomal RNA. *Systematic Entomology* **23**: 109–123.

- Belshaw R, Quicke DLJ. 2002.** The robustness of ancestral state estimates: evolution of life history strategy in ichneumonoid parasitoids. *Systematic Biology* **51**: 450–477.
- Bender JC. 1943.** Anatomy and histology of the female reproductive organs of *Habrobracon juglandis* (Ashmead) (Hymenoptera, Braconidae). *Annals of the Entomological Society of America* **36**: 537–545.
- Brock JP. 1982.** A systematic study of the genus *Ophion* in Britain (Hymenoptera, Ichneumonidae). *Tijdschrift voor Entomologie* **125**: 57–97.
- Čapek M. 1970.** A new classification of the Braconidae (Hymenoptera) based on the cephalic structures of the final instar larva and biological evidence. *Canadian Entomologist* **102**: 846–875.
- Chrystal RN, Skinner ER. 1931.** Studies in the biology of *Xylonomus brachylabris* Kr. & *X. irrigator* F., parasites of the larch longhorn beetle, *Tetropium gabrieli* Weise. *Forestry (London)* **5**: 21–33.
- Dowton M, Austin AD. 2001.** Simultaneous analysis of 16S, 28S, COI and morphology in the Hymenoptera: Apocrita – evolutionary transitions among parasitic wasps. *Biological Journal of the Linnean Society* **74**: 87–111.
- Dowton M, Austin AD, Antolin MF. 1998.** Evolutionary relationships among the Braconidae (Hymenoptera: Ichneumonoidea) inferred from partial 16S rDNA gene sequences. *Insect Molecular Biology* **7**: 129–150.
- Dowton M, Belshaw R, Austin AD, Quicke DLJ. 2002.** Simultaneous molecular and morphological analysis of braconid relationships (Insecta: Hymenoptera: Braconidae) indicates independent mt-tRNA gene inversions within a single wasp family. *Journal of Molecular Evolution* **54**: 210–226.
- Eggleton P, Belshaw R. 1992.** Insect parasitoids: an evolutionary overview. *Philosophical Transactions of the Royal Society of London (Series B)* **337**: 1–20.
- Eggleton P, Gaston KJ. 1990.** ‘Parasitoid’ species and assemblages: convenient definitions or misleading compromises? *Oikos* **59**: 417–421.
- Fitton MG. 1984.** A review of the British Collyriinae, Eucerotinae, Stilbopinae and Neorhacodinae (Hymenoptera: Ichneumonidae). *Entomologist's Gazette* **35**: 185–195.
- Fitton MG, Shaw MR, Gauld ID. 1988.** Pimpline ichneumon-flies. Hymenoptera, Ichneumonidae (Pimplinae). *Handbooks for the Identification of British Insects* **7** (1): 1–110.
- Gärdenfors U. 1989.** Taxonomic and biological revision of Palearctic *Ephedrus* Haliday (Hymenoptera: Braconidae, Aphidiinae). *Entomologica Scandinavica, Suppl.* **27**: 1–95.
- Gauld ID. 1988.** Evolutionary patterns of host utilization by ichneumonoid parasitoids (Hymenoptera: Ichneumonidae and Braconidae). *Biological Journal of the Linnean Society* **35**: 351–377.
- Gauld I, Bolton B. 1988.** *The Hymenoptera*. London and Oxford: British Museum (Natural History) and Oxford University Press.
- Gauld ID, Mitchell PA. 1977.** Ichneumonidae, subfamilies Orthopelmatinae and Anomaloniinae. *Handbooks for the Identification of British Insects* **7** (2b): 1–32.
- Gauld ID, Wahl DB. 2000.** The Labeninae (Hymenoptera: Ichneumonidae): a study in phylogenetic reconstruction and evolutionary biology. *Zoological Journal of the Linnean Society* **129**: 271–347.
- Gerling D, Quicke DLJ, Orion T. 1999.** Oviposition mechanisms in the whitefly parasitoids, *Encarsia transvena* and *Eretmocerus mundus*. *Biocontrol* **43**: 289–297.
- Godfray HCJ. 1994.** *Parasitoids: behavioural and evolutionary ecology*. Princeton, NJ, USA: Princeton University Press.
- Grafen A. 1989.** The phylogenetic regression. *Philosophical Transactions of the Royal Society of London (Series B)* **326**: 119–157.
- Grafen A, Ridley M. 1996.** Statistical tests for discrete cross-species data. *Journal of Theoretical Biology* **183**: 255–267.
- Haeselbarth E. 1962.** Zur Biologie, Entwicklungsgeschichte und ökologie von *Brachistes atricornis* Ratz. (Hym. Brac.) als eines Parasiten von *Pissodes piceae* (Ill.) (Col., Curc.). *Zeitschrift für angewandte Entomologie* **49**: 233–289.
- Harvey PH, Pagel MD. 1991.** *The comparative method in evolutionary biology*. Oxford: Oxford University Press.
- Hinz R. 1983.** The biology of the European species of the genus *Ichneumon* and related species (Hymenoptera: Ichneumonidae). *Contributions of the American Entomological Institute* **20**: 151–152.
- Hinz R, Short J. 1983.** Life-history and systematic position of the European *Alomya* species (Hymenoptera: Ichneumonidae). *Entomologica Scandinavica* **14**: 462–466.
- Kenis M, Hulme MA, Mills NJ. 1996.** Comparative developmental biology of populations of three European and one North American *Eubazus* spp. (Hymenoptera: Braconidae), parasitoids of *Pissodes* spp. weevils (Coleoptera: Curculionidae). *Bulletin of Entomological Research* **86**: 143–153.
- Kimani-Njogu SW, Wharton RA. 2002.** Two new species of Opiinae (Hymenoptera: Braconidae) attacking fruit-infesting Tephritidae (Diptera) in Western Kenya. *Proceedings of the Entomological Society of Washington* **104**: 79–90.
- Laurenne NM, Broad GR, Quicke DLJ. 2002.** Preliminary molecular phylogenetic analysis of Cryptinae and related taxa based on 28S D2+D3 rDNA analysed using POY. In: Melika G, Thuroczy C, eds. *Parasitic wasps: evolution, systematics, biodiversity and biological control*. Budapest: Agroiinform, 229–233.
- Le Ralec A. 1991.** Les hyménoptères parasitoïdes: Adaptations de l'appareil reproducteur femelle. Morphologie et ultrastructure de l'ovaire, de l'oeuf et de l'ovipositeur. Unpublished PhD Thesis, University of Rennes, France.
- Le Ralec A, Rabasse JM, Wajnberg E. 1996.** Comparative morphology of the ovipositor of some parasitic Hymenoptera in relation to characteristics of their hosts. *Canadian Entomologist* **128**: 413–433.
- van Lenteren JC, Isidoro N, Bin F. 1998.** Functional anatomy of the ovipositor clip in the parasitoid *Leptopilina heterotoma* (Thompson) (Hymenoptera: Eucolidae), a structure to grip escaping host larvae. *International Journal of Insect Morphology and Embryology* **27**: 263–268.
- Maddison WP, Maddison DR. 1992.** *MacClade: analysis of phylogeny and character evolution*, Version 3.0 Sunderland, MA, USA: Sinauer Associates.

- Mathur RN. 1959.** Breeding technique and utilisation of *Cedria paradoxa* Wilk. (Hym., Braconidae) for biological control of teak defoliator. *Entomologist's Monthly Magazine* **95**: 248–250.
- Mayhew PJ, Blackburn TM. 1999.** Does development mode organise life-history traits in the parasitoid Hymenoptera? *Journal of Animal Ecology* **68**: 906–916.
- Oatman ER, Platner GR, Greany PD. 1969.** The biology of *Orgilus lepidus* (Hymenoptera; Braconidae), a primary parasitoid of the potato tuberworm. *Annals of the Entomological Society of America* **62**: 1407–1414.
- Oda RAM, de Macedo MV, Quicke DLJ. 2001.** First biological data for *Aspilodemon* Fischer (Hymenoptera: Braconidae: Hydrangeocolinae): parasitoids of cecidomyiid fly galls on Asteraceae in Brazil. *Journal of Hymenoptera Research* **10**: 126–130.
- Quednau FW, Guevremont H. 1975.** Observations on mating and oviposition behaviour of *Priopoda nigricollis* (Hymenoptera: Ichneumonidae), a parasite of the birch leaf-miner, *Fenusa pusilla* (Hymenoptera: Tenthredinidae). *Canadian Entomologist* **107**: 1199–1204.
- Quicke DLJ. 1997.** *Parasitic wasps*. London: Chapman & Hall.
- Quicke DLJ, Basibuyuk HH, Fittton MG, Rasnitsyn AP. 1999a.** Morphological, palaeontological and molecular aspects of ichneumonoid phylogeny (Hymenoptera, Insecta). *Zoologica Scripta* **28**: 175–202.
- Quicke DLJ, Belshaw R. 1999.** Incongruence between morphological data sets: an example from the evolution of endoparasitism among parasitic wasps (Hymenoptera: Braconidae). *Systematic Biology* **48**: 436–454.
- Quicke DLJ, Fittton MG, Notton DG, Broad GR, Dolphin K. 2000.** Phylogeny of the Ichneumonidae (Hymenoptera): a simultaneous molecular and morphological analysis. In: Austin AD, Downton M, eds. *Hymenoptera: evolution, biodiversity and biological control*. Canberra: CSIRO Publishing, 74–83.
- Quicke DLJ, Fittton MG, Tunstead JR, Ingram SN, Gaitens PV. 1994.** Ovipositor structure and relationships within the Hymenoptera, with special reference to the Ichneumonidae. *Journal of Natural History* **28**: 635–682.
- Quicke DLJ, Le Ralec A, Vilhelmsen L. 1999b.** Ovipositor structure and function in the parasitic Hymenoptera with an exploration of new hypotheses. *Atti dell'Accademia Nazionale di Italiana di Entomologia Rendiconti* **47**: 197–239.
- Quicke DLJ, van Achterberg C. 1990.** Phylogeny of the subfamilies of the family Braconidae (Hymenoptera: Ichneumonoidea). *Zoologische Verhandelingen* **258**: 1–95.
- Quicke DLJ, Wyeth P, Fawke JD, Basibuyuk HH, Vincent JFV. 1998.** Manganese and zinc in the ovipositors and mandibles of hymenopterous insects. *Zoological Journal of the Linnean Society* **124**: 387–396.
- Rau P. 1928.** The nesting habits of the little carpenter-bee *Ceratina calcarata*. *Transactions of the Entomological Society of America* **21**: 380–397.
- Reig S, Daniels MJ, Macdonald DW. 2001.** Craniometric differentiation within wild-living cats in Scotland using 3D morphometrics. *Journal of Zoology (London)* **253**: 121–131.
- Riga F, Trocchi V, Randi E, Toso S. 2001.** Morphometric differentiation between the Italian hare (*Lepus corsicanus* De Winton, 1898) and the European brown hare (*Lepus europaeus* Pallas, 1778). *Journal of Zoology (London)* **253**: 241–251.
- Rosenberg HT. 1934.** The biology and distribution in France of the larval parasitoids of *Cydia pomonella*, L. *Bulletin of Entomological Research* **25**: 201–256.
- Salt G. 1931.** Parasites of the wheat-stem sawfly, *Cephus pygmaeus* Linnaeus, in England. *Bulletin of Entomological Research* **22**: 479–545.
- Senturia SJ. 1995.** Morphometry and allometry of the primate humerus. *Primates* **36**: 523–547.
- Shaw MR. 1983.** On evolution of endoparasitism: the biology of some genera of Rogadinae (Braconidae). *Contributions of the American Entomological Institute* **20**: 307–328.
- Shaw MR, Huddleston T. 1991.** Classification and biology of Braconid wasps (Hymenoptera: Braconidae). *Handbooks for the Identification of British Insects* **7** (11): 1–126.
- Shaw MR, Wahl DB. 1989.** The biology, egg and larvae of *Acaenitus dubitator* (Panzer) (Hymenoptera, Ichneumonidae: Acaenitinae). *Systematic Entomology* **14**: 117–125.
- Shenefelt RD. 1972.** Braconidae 4: Microgastrinae; *Apanteles*. *Hymenopterorum Catalogus (Nova Editio)* **7**: 429–668.
- Slobodchikoff CN. 1967.** Bionomics of *Grotea californica* Cresson, with a description of the larva and pupa (Hymenoptera: Ichneumonidae). *Pan-Pacific Entomologist* **43**: 161–168.
- Van Veen JC. 1982.** Notes on the biology of *Banchus femoralis* Thomson (Hym., Ichneumonidae) an endoparasitoid of *Panolis flammea* (D. & S.) (Lep., Noctuidae). *Zeitschrift für Angewandte Entomologie* **94**: 300–311.
- Vance AM. 1932.** *Microgaster tibialis* Nees as a hymenopterous parasite of *Pyrausta nubilalis* Hubn. in Europe. *Technical Bulletin of the Entomological Society of America* **25**: 121–134.
- Venkatraman TV, Subba Rao BR. 1954.** The mechanism of oviposition in *Stenobracon deesae* (Cam.) (Hymenoptera: Braconidae). *Proceedings of the Royal Entomological Society of London (Series A)* **2**: 1–9.
- Vincent JFV, King MJ. 1996.** The mechanism of drilling by wood wasp ovipositors. *Biomimetics* **3**: 187–201.
- Wahl DB. 1990.** A review of the mature larvae of Diplazoninae, with notes on larvae of Acaenitinae and Orthocentrinae and proposal of two new subfamilies (Insecta: Hymenoptera, Ichneumonidae). *Journal of Natural History* **24**: 27–52.
- Wahl DB. 1991.** The status of *Rhimphoctona*, with special reference to the higher categories within Campopleginae and the relationships of the subfamily (Hymenoptera: Ichneumonidae). *Transactions of the American Entomological Society* **117**: 193–213.
- Wahl DB. 1996.** Two new species of *Megastylus* from the New World (Hymenoptera: Ichneumonidae; Orthocentrinae). *Journal of the New York Entomological Society* **104**: 221–225.
- Wahl DB, Mason WRM. 1995.** The family-group names of the

Ichneumoninae (Hymenoptera: Ichneumonidae). *Journal of Hymenoptera Research* **4**: 285–293.

Wharton RA. 1993. Review of the Hormiini (Hymenoptera: Braconidae) with a description of new taxa. *Journal of Natural History* **27**: 107–171.

Whitfield JB. 1992. The polyphyletic origin of endoparasitism

in the cyclostome lineages of Braconidae (Hymenoptera). *Systematic Entomology* **17**: 273–286.

Whitfield JB. 1998. Phylogeny and evolution of host-parasitoid interactions in Hymenoptera. *Annual Review of Entomology* **43**: 129–151.