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Combining morphological, molecular, and biological characters to sort out taxonomical problems in parasitic Hymenoptera. The case of *Eubazus* spp. (Hymenoptera: Braconidae)

Marc Kenis

ABSTRACT

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A study was carried out to separate four closely related species of *Eubazus* (Hymenoptera: Braconidae), parasitoids of *Pissodes* spp. (Coleoptera: Curculionidae) in conifer trunks and cones. A combination of several methods was needed to provide diagnostic characters. Variations were found in developmental biology, morphometrics, isoenzyme patterns, fecundity, and host preference in the laboratory and in the field. Crosses were made between *Eubazus* species and populations to ascertain their behavioural and genetic compatibility or incompatibility. This study exemplifies the need for an integrated approach in parasitoid taxonomy, including the use of morphological, molecular and biological characters.

Introduction

Parasitoids are insects whose larvae feed exclusively on the body of a single arthropod. Although parasitoids are found in several insect orders, most species are Hymenoptera. Because of their ability to kill and control pests, parasitoids are beneficial insects and are often used in biological control and integrated pest management programmes. Parasitic Hymenoptera form a very large group, with about 50–60'000 species described, which accounts for 7% of all insect species and 3% of all metazoans. However, compared to other insects groups, parasitic wasps are rather poorly known. Estimates suggest that there could be up to 1 or 2 million species, which would represent up to 20% of all insect species (Gaston 1991, 1993; Godfray 1993). The poor knowledge in parasitoid systematics is partly due to a complex of taxonomic problems that are not specific to, but particularly critical in this group, such as high intraspecific variation, large numbers of sibling species, paucity of informative morphological characters of use to the taxonomist, frequent occurrence of convergent evo-

lution, parallel evolution and character reversal, etc. (Godfray 1993). This has led to much confusion in the taxonomic literature.

Morphological studies are often not sufficient to sort out taxonomic problems in parasitic wasps. An approach combining several methods is usually more desirable. Here I present a summary of a long-term study on the biosystematics of sister species of the genus *Eubazus* (Hymenoptera: Braconidae: Helconinae), subgenus *Allodorus*, parasitoids of wood- and cone-boring weevils of the genus *Pissodes* (Coleoptera: Curculionidae). This work was carried out in Europe as part of a biological control programme against the white pine weevil, *Pissodes strobi* (PECK, 1817) in Canada.

Larvae of *Pissodes* spp. live under conifer bark or in pine cones. Pupation occurs in a pupal chamber in the wood or in the cone. Adults live externally and lay their eggs in the bark or in the cone, in a feeding hole covered with frass. Eight species occur in Europe. *P. castaneus* (DE GEER, 1775), *P. pini* (LINNAEUS, 1758), and *P. piniphilus* (HERBST, 1795) attack pine trunks, *P. piceae* (ILLIGER, 1807) is a frequent pest of fir trunk, *P. harcyniae* (HERBST, 1795), *P. scabricollis* MILLER, 1859 and *P. gyllenhali* (SAHLBERG, 1834) feed in spruce trunk and *P. validirostris* (SAHLBERG, 1834) is the only species of the genus living in pine cones. Other species occur in Asia and North America, including the Nearctic pine and spruce pest *P. strobi*. In Europe, the main parasitoids of *Pissodes* spp. are braconids of the genus *Eubazus* (Kenis & Mills 1994). All species lay their eggs in *Pissodes* eggs and kill their host when the latter is in its last larval instar. Then they build a cocoon under the bark or in the cone, in which they pupate.

At the beginning of the study, the taxonomy of the genus *Eubazus* was confusing. Several species had been described in the past, in several genera, but the most recent studies on *Pissodes* parasitoids had suggested that a single species attacks all European *Pissodes* spp. (Haeselbarth 1962; Annala 1975; Roques 1975; Alauzet 1982; Mills & Fisher 1986). Our first biological observations, however, suggested the possible occurrence of several sibling species, leading to a larger research programme on the taxonomy of this group. Finally, our studies revealed the existence of at least three European species, each of them being largely specialised in different hosts and microhabitats (Table 1) (Kenis & al. 1996; Kenis & Mills 1998). In addition to these European species, a single North American species was included in the study, *E. strigitergum* (CUSHMAN, 1930) (referred to as *E. crassigaster* [PROVANCHER, 1886] in Kenis & al. [1996] and Kenis & Mills [1998]) since this latter is a parasitoid of *P. strobi* in North America. The taxonomy of the group was revised in van Achterberg & Kenis (2000). *E. semirugosus* (NEES, 1816) is a parasitoid of *Pissodes* spp. which develop in pine and spruce trunks, *E. robustus* (RATZEBURG, 1844) attacks

essentially *P. validirostris* in pine cones and is very occasionally found attacking *Pissodes* spp. in pine trunks, and *E. abieticola* VAN ACHTERBERG & KENIS, 2000 attacks only *P. piceae* in fir trunks. *E. strigitergum* parasitises *P. strobi* in spruce and pine leaders in North America, and several other closely related species occur in North America and Asia (van Achterberg & Kenis 2000).

A combination of several methods was needed to ascertain the co-existence of three *Eubazus* spp. on *Pissodes* spp. in Europe and to provide a diagnostic separation with the North American *E. strigitergum*. Variations were found in the following traits: developmental biology, morphometrics, isoenzyme patterns, fecundity and host preference. Crosses were made between *Eubazus* spp. populations to assess the general and behavioural compatibility of these populations.

	Europe	Hosts	Microhabitats
Table 1. <i>Eubazus</i> spp. investigated during our studies, with their respective <i>Pissodes</i> hosts and microhabitats (from Kenis and Mills 1994, 1998; Kenis & al. 1996). Hosts and microhabitats in brackets are occasional records.	<i>E. semirugosus</i>	<i>Pissodes pini</i>	Pine trunks
		<i>P. castaneus</i>	Pine trunks
		<i>P. piniphilus</i>	Pine trunks
		<i>P. harcyniae</i>	Spruce trunks
	<i>E. robustus</i>	<i>P. validirostris</i>	Pine cones
		(<i>P. castaneus</i>)	(Pine trunks)
		(<i>P. piniphilus</i>)	(Pine trunks)
	<i>E. abieticola</i>	<i>P. piceae</i>	Fir trunks
	<u>North America</u>		
	<i>E. strigitergum</i>	<i>P. strobi</i> and others (?)	Spruce and pine leaders

Developmental biology

Observations on variation in developmental responses among *Eubazus* populations were the first signs suggesting that several species exist in Europe, with specialised hosts and habitats (Kenis 1994; Kenis & al. 1996). *Eubazus* spp. strongly differ in their development time when reared on a standard host species, both in a laboratory and under natural conditions, *E. semirugosus* being the quickest developing species, and *E. robustus* the slowest (Kenis & al.

1996). Intraspecific variations were found in *E. semirugosus* and *E. robustus*. Populations of both species collected at high altitudes in the Alps and reared on a non-diapausing *Pissodes* host developed an obligatory diapause in the early larval stage in the host larva. The diapause was broken by a cold period of at least three months. In contrast, populations collected in the lowland developed without diapause when reared in the laboratory. Variations in development time and diapause characteristics were maintained in the offspring generation, suggesting a genetic basis. These variations were regarded as adaptations to the phenology of their respective hosts in their respective environments (Kenis & al. 1996).

Morphometrics

Since no simple morphological character was sufficient to separate *Eubazus* spp., morphometric measurements of over 40 characters were performed on 25 populations of the four investigated species as well as on their offspring emerged from a standard host species (Kenis & Mills 1998). Univariate, bivariate and multivariate analyses were made and morphometric measurements were compounded into canonical discriminant analyses. The ratio of the length of the ovipositor sheath to the fore wing length provided a diagnostic character between *E. robustus* and *E. abieticola*, but there were overlaps with the other species. Canonical discriminant functions including 15 measurements were needed to separate females of the three European species, whereas there were slight overlaps between males of *E. semirugosus* and of the two other species. The North American *E. crassigaster* could be separated from *E. semirugosus* in males only, whereas females overlapped. Canonical discriminant functions were used to assess the identity of doubtful populations. This method showed that *E. semirugosus* also attacks the spruce species *P. harcyniae*. It also revealed that *E. robustus* occasionally emerges from pine *Pissodes* spp. We failed to separate the mountain, diapausing biotypes of *E. semirugosus* and *E. robustus* from their lowland, non-diapausing counterparts, suggesting that the geographic biotypes belong to the same species.

Isoenzyme analyses

Several populations of the four *Eubazus* spp. were compared using isoenzyme starch gel electrophoresis (Kenis and Mills 1998). The banding patterns of two enzymes, Hexokinase and Esterase, provided a diagnostic separation between

E. robustus and *E. abieticola*, but *E. semirugosus* shared common bands with both species. No significant difference was found between the mountain and lowland populations of *E. semirugosus*. The isoenzyme analysis was particularly useful in separating the North American *E. strigitergum* from the European species. The banding pattern of Phosphogluconate Dehydrogenase provided the only diagnostic character found during the study to separate both sexes of *E. strigitergum* from its most closely related species, *E. semirugosus*.

Fecundity

The potential fecundity was compared by counting the number of ovarioles per female. *E. abieticola* females had almost twice as many ovarioles as females of the three other species. These variations were explained by the distribution of their respective hosts, *P. piceae* being more gregarious than the other species (Kenis & Mills 1998).

Host preference

Preference for host species was tested with *E. semirugosus* and *E. abieticola* only. Naive females were given the choice between ovipositing in *P. castaneus* eggs in pine logs and *P. piceae* eggs in fir logs. Both species showed a strong preference for their original host. When reared on *P. castaneus* in pine logs for one generation, the offspring of both species still showed a significant difference in host preference, but the acceptability of *E. abieticola* for pine had significantly increased. This suggested that host preference is partly genetically based and partly influenced by the host or host habitat experienced during the pre-emergence period.

Crosses

Crosses were made between different populations of the three European *Eubazus* species (Kenis & Mills 1998). When a crossing was successful, two offspring generations were reared to monitor the fertility of the progeny. In intra-specific crosses, mating was very frequent and fertile offspring generations were easily produced. The mountain, diapausing biotype and the lowland, non-diapausing biotype of *E. semirugosus* interbred as well as specimens from the same biotype, suggesting again that they belong to the same species. Howev-

	<i>E. semirugosus</i>	<i>E. robustus</i>	<i>E. abieticola</i>	<i>E. strigitergum</i>
<i>E. semirugosus</i>	—			
<i>E. robustus</i>	DEV; MOR^f; ISO; CRO; HOS	—		
<i>E. abieticola</i>	DEV; MOR^f; ISO; FEC; PRE; CRO; HOS	DEV; MOR; ISO; FEC; CRO; HOS	—	
<i>E. strigitergum</i>	DEV; MOR^m; ISO;	DEV; MOR; ISO;	MOR; ISO; FEC	—

Table 2. Traits in which variations were found among *Eubazus* spp.: DEV = Development time; MOR = Morphometrics; ISO = Isoenzyme patterns; FEC = Fecundity; PRE = host preference in the laboratory; CRO = crosses; HOS = Natural host. Traits in bold provided diagnostic characters. Crosses were not performed with *E. strigitergum*. Host preference was assessed in *E. semirugosus* and *E. abieticola* only. Variations in natural hosts are relevant for European species only. Source: Kenis & al. (1996); Kenis & Mills (1998). MOR^m: Morphometrics provide diagnostic characters in males only. MOR^f: Morphometrics provide diagnostic characters in females only.

er, the diapause characteristic is genetically based because it is transmitted from father to offspring (Kenis & Mills 1998).

Very few matings were observed in inter-specific crosses, but two out of 10 crosses between *E. semirugosus* and *E. abieticola*, and one out of seven crosses between *E. semirugosus* and *E. robustus* resulted in a fertile offspring. *E. abieticola* and *E. robustus* appeared to be totally reproductively isolated (Kenis & Mills, 1998).

Conclusions

This study established the existence of three sibling *Eubazus* species in Europe, each of them being largely specialised in a different microhabitat. Although no cladistic analysis was performed, observations in morphometrics, isoenzyme analyses and cross-mating experiments suggest that *E. robustus* and *E. abieticola* are most different, whereas *E. semirugosus* occupies an intermediate position in the evolution of the group. Speciation is probably not complete because *E. semirugosus* is still genetically compatible with the two other European species. Discussions on the implications of this study on ecological,

evolutionary and pest management issues are found in Kenis & al. (1996) and Kenis & Mills (1998).

These investigations also exemplify the need for an integrated approach in parasitoid taxonomy. Indeed, none of the methods provided sufficient diagnostic characters for both sexes of all *Eubazus* species (Table 2). The systematic research programme was initiated because observations on developmental biology suggested the existence of sibling species. Morphometrics separated – with difficulty – females of the European species and males of *E. strigitergum* from the European species but failed to separate males of European species and females of *E. strigitergum* and *E. semirugosus*. Isoenzyme analysis was the only method providing a clear separation between *E. strigitergum* and the European species, but separation among the European species was less satisfactory. Morphometrics, isoenzyme analyses and crosses revealed a clear separation and reproductive isolation between *E. robustus* and *E. abieticola*, and an intermediate position of *E. semirugosus*. Crosses, morphometrics and isoenzyme analyses showed that the mountain and lowlands biotypes of *E. semirugosus* and *E. robustus*, albeit showing very different and genetically based developmental responses, belonged to the same species. Finally, observations on fecundity and host preference confirmed the other data.

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