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First host records for the rogadine genus *Conspinaria* (Hymenoptera: Braconidae), and notes on Rogadinae as parasitoids of Zygaenidae (Lepidoptera)

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The parasitic wasp genus *Conspinaria* is recorded as parasitizing two species of chalcosiine zygaenid moths, *Chalcosia thaivana owadai* Wang and *Erasmia pulchella hobsoni* Butler in Taiwan. Parasitism of Zygaenidae and of Limacodidae by rogadine braconids is discussed in the light of possible phylogenetic relationships.

Introduction

The past few years have seen a considerable resurgence in taxonomic interest in the Rogadinae *sensu stricto* (van Achterberg, 1991; Chen and He, 1997; Fortier and Shaw, 1999), and as a result it is now easier to place species from most of the world to genus and also to access and interpret information on their biology. Under the most favourable of circumstances an abnormal degree of reliability can be accorded to host records in the case of the Rogadinae s. str. because they mummify their host lepidopteran larvae in which they pupate (Shaw and Huddleston, 1991) and therefore, if the host mummy is retained with the emerged parasitoids, this provides an absolute record of the rogadine's host association. Considerable effort has therefore been afforded to collecting and analysing the host associations of the commonest rogadine genus, *Aleiodes*, but there are still numerous tropical genera for which no host data are available. One of these is *Conspinaria*, a frequently

collected taxon in China and South-East Asia (Chen and He, 1997), and here we report the first two rearings of it, both from chalcosiine Zygaenidae.

Molecular protocols

DNA was extracted from single mid-legs preserved in absolute ethanol using an ethanol precipitation method with final elution into 30 μl of water. Polymerase chain reactions (PCR) were carried out in a GeneAmp9600 thermal cycler in 20 μl reactions containing 1.0 μl of DNA extract, 10 pmol of primers (forward: 5′ GCG AAC AAG TAC CGT GAG GG 3′; reverse: 5′ TAG TTC ACC ATC TTT CGG GTC 3′), 10 nmol of dNTPs (Amersham Pharmacia Biotech: APB), 1.0 U of Taq polymerase (Bioline) and 2 μl of $10 \times$ reaction buffer (2.0 mM $MgCl_2$). PCR conditions were 94°C for 30 s; 50°C for 30 s and 72°C for 60 s (35 cycles with an initial denaturation for 2 min and a final extension for 7 min). PCR products were purified using GFX gel band purification kit (APB) and sequenced directly using BigDye terminators.

Observations

Living larvae of two species (in two genera) of chalcosiine zygaenids were collected in Taiwan. These subsequently constructed their cocoons in a slightly folded host plant leaf and were mummified at the prepupal stage. The emerging adult *Conspinaria* specimens chewed their way out of the host larva via an irregular posterodorsal hole and then chewed a large hole out of the host cocoon. In one rearing, a larva of *Chalcosia thaivana owadai* Wang (figure 1A, C, E) was collected by Shih-Fu Huang at Taitung County, Tien-Chih, Lanyu Island (=Orchid Island), Taiwan in May 2001 feeding on its host plant, *Symplocus cochinchinensis* (Loureiro) S. Moore var. *philippinensis* (Brand) Nooteboom (Symplocaceae). In the second, a larva of *Erasmia pulchella hobsoni* Butler (figure 1B, D, F) feeding on *Helicia formosana* Hemsley (Proteaceae) was collected by one of us (S.H.Y.) on 6 June 1996, at an altitude of 250 m at the Jinglong Temple, Neihu Area, Taipei City, Taiwan. The two *Conspinaria* specimens are both males. Although they are morphologically very similar, there are several differences in the distribution of dark areas, particularly on the hind leg, that suggest they may not be conspecific.

Molecular data

The D2–D3 expansion region of the nuclear 28S ribosomal DNA gene was sequenced for both of the reared *Conspinaria* adults (see Belshaw and Quicke, 2002 for details). The sequences are deposited in the EMBL/GenBank database under the accession numbers AJ509014 and AJ509015 for the specimens from *Chalcosia* and *Erasmia*, respectively. The two sequences differ in two bases in what are normally quite conserved regions. In general, in the cyclostome Braconidae investigated to date, all 28S D2–D3 rDNA sequences from conspecific individuals have been found to be identical whereas congeneric taxa often differ by one to a few bases though, occasionally, even morphologically quite different species have been found to have identical sequences. It seems likely, therefore, that the two *Conspinaria* individuals represent different species despite being morphologically very similar.
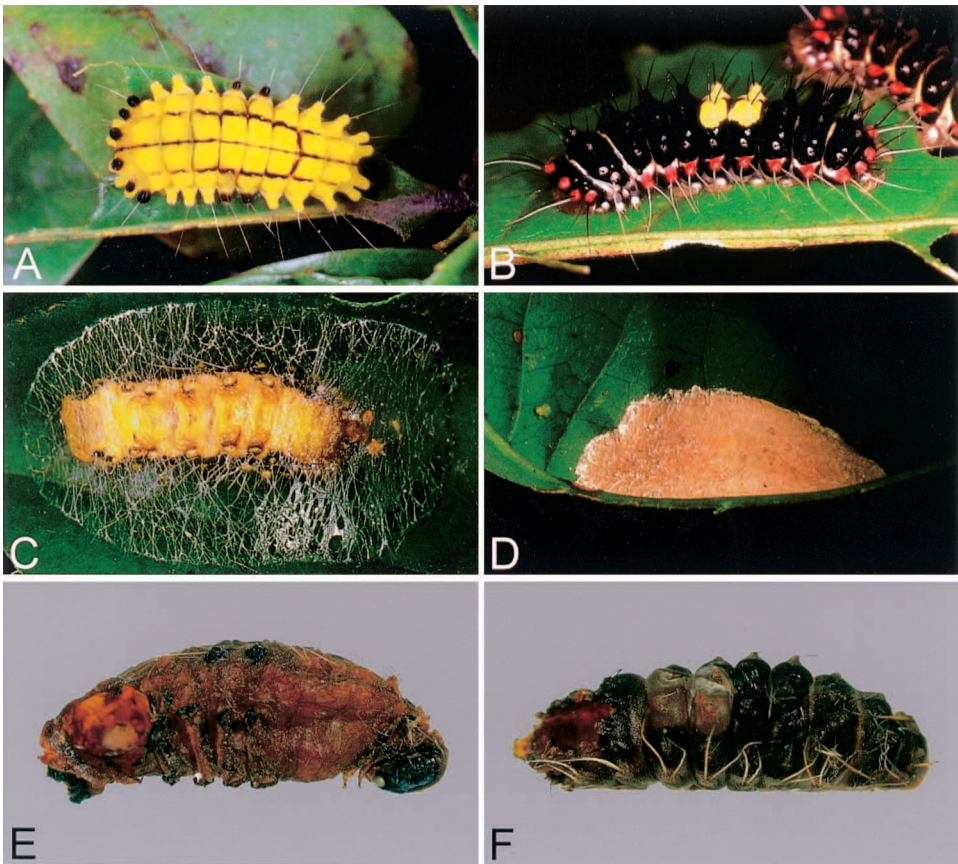
FIG. 1. Chalcosiine zygaenids and their parasitism by rogadine braconids. (A, C, E) *Chalcosia thaivana owadai* Wang, larva, final instar larva attaching itself to inner surface of cocoon viewed from below and mummy produced by parasitization with *Conspinaria* sp. 1, respectively. (B, D, F) *Erasmia pulchella hobsoni* Butler, larva, cocoon and mummy produced by parasitization with *Conspinaria* sp. 2, respectively.

Discussion

Careful investigation of various Rogadinae s. str. has invariably shown that at least a small amount of fluid is expelled at the time of mummification from the end of the host mummy opposite that from which the adult will emerge. This applies to all investigated (Shaw, 1983; Shaw, unpublished) species of *Clinocentrus* and *Aleiodes* species, whether or not the mummy is also stuck to the substrate by the expelled fluid. In *Clinocentrus* (tribe Clinocentrini *sensu* van Achterberg, 1991) the mummy forms in the host's cocoon and is never stuck down, adult emergence is always from the head end, and the fluid is expelled from an incision at the caudal end; though no trace of this having happened is usually detectable once the mummy has hardened and dried. *Aleiodes* (tribe Rogadini *sensu* van Achterberg, 1991) is well known for causing partly grown hosts to mummify in more or less exposed situations, sticking the mummies firmly to the substrate by an initially thin fluid expelled ventrally from the contracted thoracic region of the forming mummy that

soon gels then darkens and hardens to a rigid cement of remarkable strength and durability. Adult emergence takes place dorsally at the caudal end of the mummy (up to 10–11 months later in many temperate species). However, in several unrelated species of *Aleiodes*, e.g. *pallidator* (Thunberg), *compressor* (Herrich-Schaefer), *modestus* (Reinhard), *dissector* (Nees) and *albitibia* (Herrich-Schaefer), the mummy is not stuck down to the substrate, forming usually in precocious cocoon-like structures or (*albitibia*) loosely on a leaf, but fluid is still expelled from the venter of the thoracic region of the forming mummy (in *albitibia* the whole front end of the host detaches filled with fluid) and, as in *Clinocentrus*, it is often difficult to detect that this has happened once the mummy has hardened and dried. In the case of *Conspinaria* we have been unable to detect signs of a ventral thoracic opening through which fluid had been expelled but this, and statements (Smith *et al.*, 1955) that it does not occur in *Triraphis* (as *Pelecystoma*), which mummifies its hosts over a wide developmental span, may indicate only that observations were not made at the best time. Indeed, it seems likely that all Rogadinae mummifying hosts need to reduce overall fluid content and that expulsion through a small prepared opening in the host integument is a plesiomorphic mechanism common to the group, which in many *Aleiodes* has been taken further as a means to deal successfully with mummification of partly grown hosts not protecting themselves in cocoons by sticking them down to substrates (the hosts often having first crawled away from areas of high pseudohyperparasitoid activity or other danger). Acute and deliberate observations of the mummification process in genera such as *Rogas* and *Conspinaria*, which, like *Clinocentrus* (though with differing orientation), mummify prepupal hosts protected by cocoons, are needed to elucidate the supposition that fluid expulsion through a prepared opening does always take place.

In general, Zygaenidae have only extremely specialized parasitoids (see Naumann *et al.*, 1999; Askew and Shaw, 2001) and this is widely presumed to be because they are highly protected chemically by cyanogenic compounds that are stored by the zygaenid larva in subcuticular spaces (Frenzl and Naumann, 1984, 1985; Naumann and Feist, 1987). To date only two other rodagine genera and a handful of species have been reared from zygaenid larvae, and none previously from Chalcosiinae. This supports the idea that the Zygaenidae is a very difficult host group to colonize. Previous records are the Nearctic *Triraphis harrisinae* (Ashmead) from *Harrisina* and *Acoloithus* (Smith *et al.*, 1955, as *Pelecystoma*) and the Eastern Palaearctic *T. fuscipennis* Chen and He from *Artona funeralis* Butler (Chen and He, 1997). In both these cases the hosts belong to the subfamily Procridinae, a group which may be relatively easy to colonize because their larvae do not produce any sticky cyanide fluid from the cuticle and do not have any cuticular cavities in which to store cyanide compounds (Naumann *et al.*, 1999). *Triraphis* is also associated with Limacodidae (Watanabe, 1962; van Achterberg, 1991; Chen and He, 1997). The other genus is *Aleiodes*, in which a single Western Palaearctic species, *A. assimilis* (Nees), is a specialist parasitoid of *Zygaena* and has been reared from several species (M. R. Shaw, unpublished). Unfortunately, *A. assimilis* was not distinguished by Shaw (1983) from the very closely related *A. bicolor* (Spinola), a specialist parasitoid of polyommatine Lycaenidae, a mistake that has misled some subsequent authors (e.g. Ockenfels *et al.*, 1993). It is evident that *A. assimilis* arose as a result of a relatively recent ecologically driven speciation event (see Shaw, 1994, 2003) and it is clearly not indicative of any particular (or

ancestral) relationship of *Aleiodes* with Zygaenidae. Literature references to *Rogas* (as opposed to *Aleiodes*) species attacking Zygaenidae (e.g. Shaw, 1997) result from failure to recognize *Triraphis* as a distinct genus (see van Achterberg, 1991; Chen and He, 1997).

The generic relationships of the Rogadinae are only just being investigated seriously, based on both morphology (Zaldivar-Riveron *et al.*, in press) and DNA sequence data (Mori and Quicke, in preparation). Nevertheless, these data support the hypothesis that *Conspinaria* belongs to the same clade as *Triraphis*, *Rogas* and the Spinariina (namely *Spinaria*, *Batotheca* and *Batothecoides*). Of these, *Triraphis*, *Rogas*, *Spinaria* and *Batotheca* are all parasitoids of Limacodidae (see Shenefelt, 1975; Austin, 1987). Amongst the 12 families of Zygaenoidea, Zygaenidae+ Heterogynidae is possibly the most basal clade, and Aididae plus (Limacodidae+ Dalceridae) is the most advanced group (Holloway, 1986; Miller, 1994; Epstein, 1996, 1997, 1999); thus zygaenids and limacodids cannot be described as particularly closely related. Further, the textures of limacodid larvae and of zygaenid larvae are generally quite different. Many limacodid and dalcerid larvae have 'gelatine'-containing cuticle which is not present in other members of the Zygaenoidea (Cock *et al.*, 1987). In the absence of other rearing records for *Conspinaria* it is impossible to distinguish between two possibilities: that this genus has arisen in complete association with Zygaenidae; or that a secondary colonization of Zygaenidae has occurred within it. In either case, however, a limacodid-parasitizing ancestor seems the most likely. It is interesting to note that *Triraphis* species collectively attack Limacodidae and Zygaenidae (as well as Lymantriidae), although some other genera apparently belonging to the *Rogas*+Spinariina group are apparently specialists on Limacodidae alone (e.g. *Rogas*, *Rectivena*, see van Achterberg, 1991).

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