Zootaxa 1846: 35–46 (2008)
www.mapress.com/zootaxa/

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

The systematics and biology of *Cotesia nonagriae* (Olliff) stat. rev. (Hymenoptera: Braconidae: Microgastrinae), a newly recognized member of the *Cotesia flavipes* species complex

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Abstract

The Australian species *Cotesia nonagriae* Olliff stat. rev. (Hymenoptera: Braconidae) is redescribed and formally removed from synonymy with *C. flavipes* based on molecular, morphological and biological differences. The taxonomic history and phylogenetic relationships of *C. nonagriae* with other members of the *C. flavipes* complex are presented and underscore the importance of molecular-based identification within this group. The biology of *C. nonagriae* on the native noctuid stemborer host, *Bathytricha truncata* (Walker), is compared with previously recorded *C. flavipes* life history traits. The implications of this taxonomic study relative to biological control and importation of stemborer parasitoids into Australia are discussed.

Key words: parasitoid, taxonomy, biology, stemborer, Australia, biological control

Introduction

The *Cotesia flavipes* complex of parasitioid wasps are natural enemies of lepidopterous stemboring pests associated with sugarcane and cereal crops (Walker 1994). Since these are staple crops in many countries, the complex is economically important worldwide as biological control agents. The complex currently consists of three species, *Cotesia flavipes* Cameron, *C. sesamiae* (Cameron) and *C. chilonis* (Matsumura), of uncertain taxonomic validity and relationships. Identifying the various species within the *flavipes* complex has been problematic in the past and has been usefully summarized by Kimani-Njogu and Overholt (1997).

The monophyly of the complex is well supported by molecular (Smith & Kambhampati 1999; Michel-Salzat & Whitfield 2004; Muirhead *et al.* 2006) and morphological characters, such as a dorsoventrally compressed mesosoma (Watanabe 1965; Walker 1994). However, the species within the complex are morphologically similar, and many of the characters that have been used to separate species have proven unreliable due to intraspecific variation (Polaszek & Walker 1991; Smith & Kambhampati 1999). As a result, their use in biological control has been confounded by inaccurate identification, as well as the existence of host specific populations (Kimani-Njogu & Overholt 1997).

The species of the *C. flavipes* complex are thought to be endemic to the following areas: *C. flavipes* to the Indo-Australian region; *C. sesamiae* to central and southern Africa; and *C. chilonis* to eastern Asia, including Japan (Polaszek & Walker 1991; Kimani-Njogu & Overholt 1997). However, all three species have been utilized for classical biological control of stemboring pests, resulting in their much broader inter-continental distribution (Polaszek & Walker 1991). In some cases, a species of the complex has been introduced into an area indigenous to one of the other two species (Smith & Kambhampati 1999). For example, *C. flavipes* has been

Accepted by R. Kula: 11 Jul. 2008; published: 11 Aug. 2008

introduced several times into various countries of Africa (Overholt *et al.* 1994) and is now established in several parts of sub-Saharan Africa (Omwega *et al.* 1995; Overholt *et al.* 1997) where it co-exists with the native *C. sesamiae*. Although *C. flavipes* and *C. sesamiae* can occupy a similar ecological niche, it has been shown that they prefer different host species and are not likely to compete (Rajabalee & Govendasamy 1988; Sallam *et al.* 2001; Sallam *et al.* 2002). Similarly, certain populations of the same species within the complex have differences in host range (Mohyuddin 1971; Shami & Mohyuddin 1992; Zhang & Hewitt 1996; Potting *et al.* 1997b; Ngi-Song *et al.* 1998), an indication of genetic divergence among strains (Muirhead *et al.* 2006) and the possible existence of cryptic species.

There has been ongoing confusion regarding the status and presence of *C. flavipes* in Australia, and this has the potential to impact the future importation of biological control agents. Over 80 years ago, the Australian native species *Apanteles nonagriae* Olliff, 1893 was synonymized with *A. flavipes* (Cameron, 1891) (Wilkinson 1929; Austin & Dangerfield 1992), thus indicating the presence of *C. flavipes* in Australia. However, recent molecular work suggests that the Australian populations represent a ‘cryptic’ species different from *C. flavipes* and other members of the species group (Muirhead *et al.* 2006). Records of *A. nonagriae* in Australia extend back to its original description when it was first recorded as a parasitoid of the native noctuid stemborer *Nonagria exitiosa* Olliff (= *Bathytricha truncata* (Walker)) in sugarcane in the Richmond and Clarence River Districts of north-eastern New South Wales (Olliff 1893). It was subsequently reared from *Phragmatiphila truncata* Walker (= *Bathytricha truncata*) in sugarcane at South Mulgrave, south of Cairns, Queensland (Jarvis 1927). The same report also indicated that the parasitoid had been previously recorded parasitising 50% of *B. truncata* larvae infesting rice in New South Wales. *Bathytricha truncata* is a stemborer recorded from sugarcane, rice, maize and a range of other plants (Sallam 2003). It has a distribution from Cairns to South Australia and Tasmania (Common 1990) and is considered a minor pest that rarely causes substantial damage (Jones 1966). Bell (1934) recorded *Apanteles nonagriae* on *B. truncata* larvae at Mackay, Queensland. Similarly, Li (1970) recorded “*A. flavipes* (*A. nonagriae*)” from *Chilo suppressalis* (Walker) and *Chilo polychrysa* (Meyrick) (Pyralidae) in rice fields in the Northern Territory, but no voucher material was deposited in any collection to confirm this finding.

Apanteles nonagriae was originally described by Olliff (1893) along with *Tetrastichus howardi* (Olliff), a eulophid pupal parasitoid reared from *B. truncata* (Boucěk 1988). In his study of Indo-Australian *Apanteles* s.l., Wilkinson (1928a, b) noted the strong similarity between *A. nonagriae* Olliff and *A. flavipes* but did not synonymize them until the following year (Wilkinson 1929). However, he did synonymize a second species of the same name, *A. nonagriae* Viereck, 1913, with *A. flavipes* that had been reared from *Sesamia* (*Nonagria*) *inferens* Walker from Taiwan (Wilkinson 1928a). Unfortunately, Olliff (1893) did not designate any type specimens in the original description of *C. nonagriae* and did not refer to any depository that might hold syntypes.

Based on morphological examination and biological data, supplemented by the previous molecular study (Fig. 1) (Muirhead *et al.* 2006), we formally recognize *Cotesia nonagriae* stat. rev. as a distinct species. In so doing, we redescribe the species and discuss its taxonomic history, relationships within the *flavipes* complex, its biology compared to *C. flavipes* and the implications of this taxonomic study to future biological control programs and importation of stemborer parasitoids into Australia.

Materials and methods

Taxonomy

Specimens of *C. nonagriae* used in this study were reared from *B. truncata* collected from three sugarcane-growing localities in Queensland, while specimens of *C. flavipes* (India, Thailand, Japan, Papua New Guinea, Kenya, Mauritius), *C. sesamiae* (west Kenya, east Kenya, Tanzania) and *C. chilonis* (China, Japan) were accessed from the voucher material from Muirhead *et al.* (2006) deposited in the Waite Insect and Nematode Collection, Adelaide. Morphological terminology follows Sharkey and Wharton (1997) for body structures and venation, Eady (1968) and Harris (1979) for sculpturing, and Kimani-Njogu and Overholt (1997) for male genitalia. Specimens were imaged using a Philips XL30 FEGSEM scanning electron microscope at the Adelaide Microscopy and Microanalysis Research Facility, The University of Adelaide. Male genitalia were dissected from the metasoma of several specimens and mounted on carbon conductive adhesive tabs after overnight digestion in 140µl of lysis buffer and 7µl of proteinase K (20mg/ml) at 55°C.

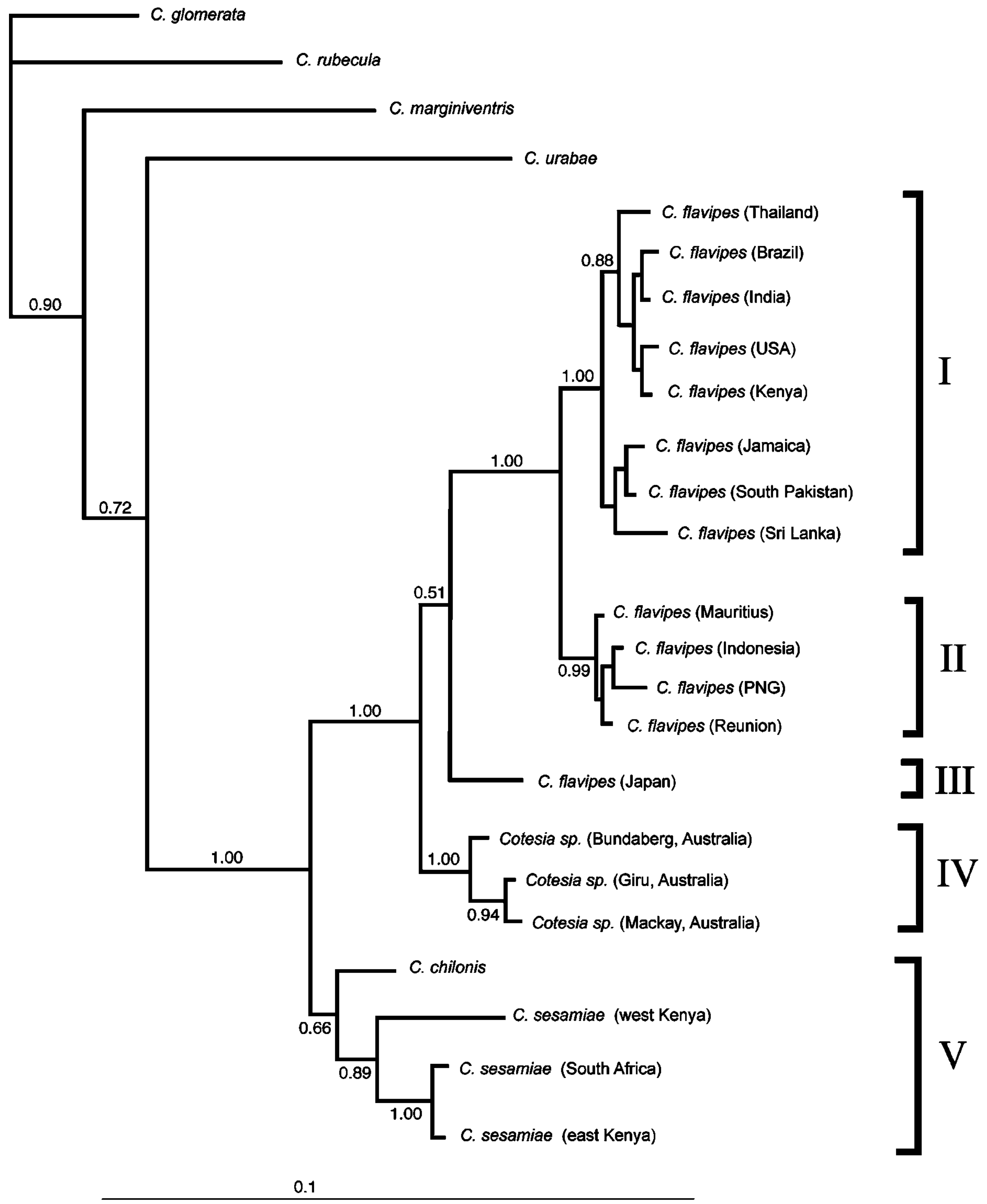

FIGURE 1. Bayesian tree derived from partial *16S rRNA* and *COI* mtDNA nucleotide sequence data from geographic populations of the *Cotesia flavipes* complex (Clades I-V) and four outgroups. Australian *Cotesia nonagriae* populations are shown in clade IV. The numbers represent Bayesian posterior probabilities ≥ 50% (from Muirhead *et al.* 2006).

Abbreviations for collections in the text are: AM, Australian Museum, Sydney; ANIC, Australian National Insect Collection, Canberra; ASCT, Agricultural Scientific Collections Trust, Orange Agricultural Institute, Orange; QDPI, Queensland Department of Primary Industries, Brisbane; and WINC, Waite Insect and Nematode Collection, Adelaide.

Biology

Insect colonies. We maintained two colonies of *C. nonagriae* originating from field parasitized larvae of *B. truncata* infesting sugarcane in Mackay and Bundaberg, Queensland. Parasitoids were maintained on laboratory reared fourth instar *B. truncata* larvae in a temperature controlled room at 25ºC, 60–70% RH under a 12L:12D photoperiod. Mated females were offered one host larva with some fresh larval frass to stimulate oviposition. Wasp cocoons were collected from host larvae and transferred to emergence cages where they were provided honey as a food source.

Field collected *B. truncata* were reared to the pupal stage within cut sugarcane stems, whereas subsequent lab generations were maintained on an artificial diet adopted from Onyango and Ochieng-Odero (1994), replacing maize leaf powder with sugarcane leaf powder. See Songa *et al.* (2001) and Macqueen (1969) for more details on stemborer rearing procedures and the life history of *B. truncata*. Adult moths were kept in oviposition cages with waxed paper tubes to provide suitable oviposition sites. Egg masses were cut from the paper daily and transferred to a closed Petri dish containing moist cotton wool to maintain high humidity. Egg masses at the blackhead stage were transferred to 2.55 mm diameter containers with artificial diet. Larvae used in the experiments were removed from the artificial diet as fourth instars and fed 5 cm cuts of sugarcane stems.

Life history traits. To study the life history of *C. nonagriae* on the native host *B. truncata*, we employed the procedure used by Sallam *et al*. (2002) on *C. flavipes* and *C. sesamiae*. Thirty fourth instar host larvae were parasitised by newly emerged, mated female parasitoids. Adult female parasitoids were kept in individual vials and exposed to one host larva each for oviposition. Parasitised larvae were kept in vials containing cut sugarcane stems until the mature parasitoid progeny emerged and pupated. Ten parasitised hosts were dissected one to two days after oviposition to determine the number of parasitoid progeny allocated to each host. Cocoon masses from the remaining 20 larvae were counted, weighed and placed in vials. Duration of the parasitoid's immature stages, percent emergence, number of adult progeny, adult longevity and sex ratio were recorded. Three females from each progeny (n=60) were chosen randomly and dissected to count the number of eggs contained in the ovaries.

Descriptive taxonomy

Cotesia nonagriae (Olliff) stat. rev.

(Figs. 2–4)

Apanteles nonagriae Olliff, 1893: 376 [original description]; Wilkinson (1928b): 136 [type data, biology, taxonomic status].

Apanteles flavipes (Cameron, 1891); Wilkinson (1928a): 93 [synonymy of *A. nonagriae* Viereck]; Wilkinson (1929): 108 [synonymy *A. nonagriae* Olliff]; Shenefelt (1972): 509 [complete taxonomic bibliography].

Cotesia flavipes Cameron, 1891: 185 [original description]; Mason (1981): 113 [resurrected the genus with *C. flavipes* as type]; Austin and Dangerfield (1992): 21 [status and hosts for Australia].

Material examined. Queensland: 21♀ 3♂ Bundaberg, 12-30.xi.2004, K. Muirhead (10♀ 1♂ ANIC, 11♀ 2♂ WINC); 16♀ 2♂ Mackay, 12-30.xi.2004, K. Muirhead (6♀ 1♂ ANIC, 10♀ 1♂ WINC); 14♀ 3♂ Giru [via Townsville], 5.x.2003, M. Sallam (9♀ 1♂ QDPI, 5♀ 2♂ WINC): 3♀ 1♂ '*parasite larva sugar-cane moth*' '*Apanteles nonagriae Olliff*', no date or locality (1♀ AM, 2♀ 1♂ ASCT).

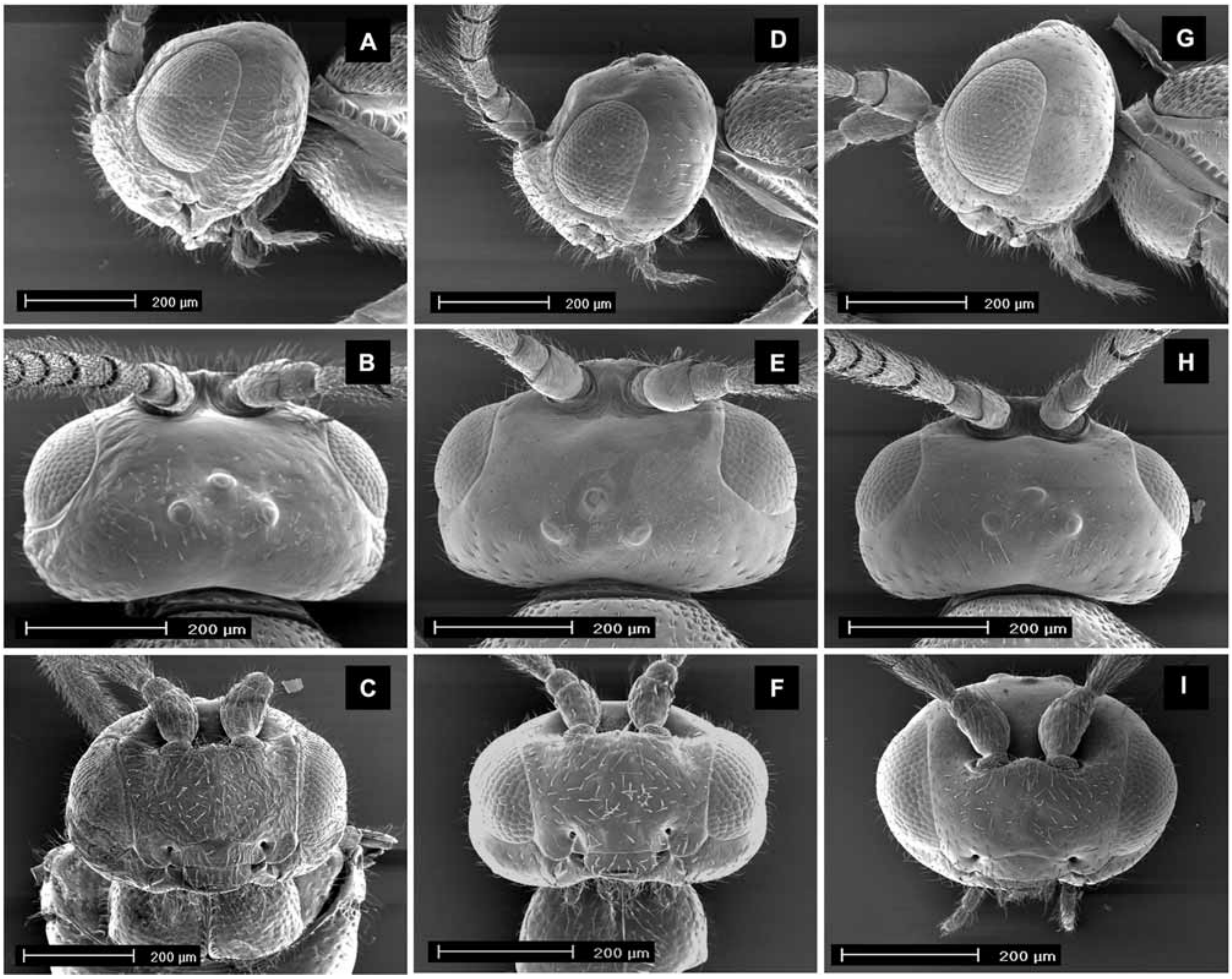

FIGURE 2. A–C: *Cotesia nonagriae* (Bundaberg, Australia), head. (A) lateral view, (B) dorsal view, (C) anterior view. D–F: *C. nonagriae* (Mackay, Australia), head. (D) lateral view, (E) dorsal view, (F) anterior view. G–I: *C. flavipes* (India), head. (G) lateral view, (H) dorsal view, (I) anterior view.

Female. *Length*. Body 2.1–2.4 mm

Colour. Body black, metasomal sterna including hypopygium dark brown to brown, antenna dark brown with scape lighter, palps yellow; legs yellow brown with tarsus slightly darker, mesocoxa pale brown, metacoxa dark brown to black basally grading to brown apically; forewing stigma brown, venation slightly lighter.

Head. In anterior view oval in shape, substantially wider than high, eyes slightly converging ventrally, face slightly rugulose-punctate to punctuate; in lateral view oval (globular) in shape, only slightly higher than long, gena and temples rugulose-punctate to punctuate, slightly more striate along posterior eye margin; in dorsal view vertex and occiput moderately smooth except for scattered fine punctures associated with sparse short setae, frons usually smooth but sometimes with faint striations along eye margin.

Mesosoma. Strongly flattened dorsoventrally so that posterior two-thirds of scutum, scutellum, anterior part of propodeum and ventral margin of mesopleuron horizontal and parallel; in dorsal view scutum punctate anteriorly, mostly smooth posteriorly and along midline, notauli indicated by posterior extension of anterior punctuate area and smooth areas on either side but disappearing before reaching posterior margin; medial scutellum smooth with sparse setae, posterior margin broad; propodeum coarsely rugose-punctate, often with indistinct carina around spiracle and oblique lateral carina converging posteriorly; in lateral view mesopleuron smooth, sternaulus faintly indicated along dorsal margin by sparse punctures; metapleuron rugose-punctate in posterior part, smooth in anterior part; dorsal and outer surfaces of hind coxa punctuate; forewing veins r and

2RS usually meeting at distinct angle, sometimes with small stub of 3RS present; 2M 0.5 to almost 1.0X as long as 2RS.

Metasoma. Tergum 1 almost as wide at posterior margin as long, lateral margins strongly diverging posteriorly; longitudinally striate-rugulose, often with incomplete medial longitudinal carina; tergum 2 longitudinally striate-rugulose with smoother longitudinal area medially and laterally; remaining terga smooth with sparse longish setae.

Male. As for female except: antenna slightly longer and lighter in colour; punctuate sculpturing on scutum, particularly in anterior part, slightly denser; genitalia very similar to *C. flavipes*; aedeagal-volsella shaft elongate; volsella more than 4.0X as long as wide, digital (apical) teeth minute; aedeagus barely protruding past apex of parameres and volsella.

Comments. The description above is largely based on specimens from Bundaberg. For specimens from Mackay and Giru, the degree of sculpturing on the face and gena is less pronounced, and the frons and temples are completely smooth. These populations also have the propodeum less coarsely sculptured and rugulose rather than rugulose-punctate and tergum 1 lacking a medial longitudinal carina. As such they are more similar to *C. flavipes.* The specimens in AM and ASCT have identical labels and are clearly very old. We initially considered that they were part of Olliff's original material and therefore a likely syntype series. This was based on the age of the material and that there are several lectotypes of Olliff species in the AM, including that of *T. howardi* (designated by Bouček 1988) which was described by Olliff in the same paper as *C. nonagriae*. However, comparison of the labels on Olliff specimens in the AM shows that the handwriting is different to the AM and ASCT specimens, and so they cannot be directly associated with that used in the original description of *C. nonagriae*. The specimens in AM and ASCT have the face and gena smooth and are therefore more similar to the recently collected material from Mackay and Giru.

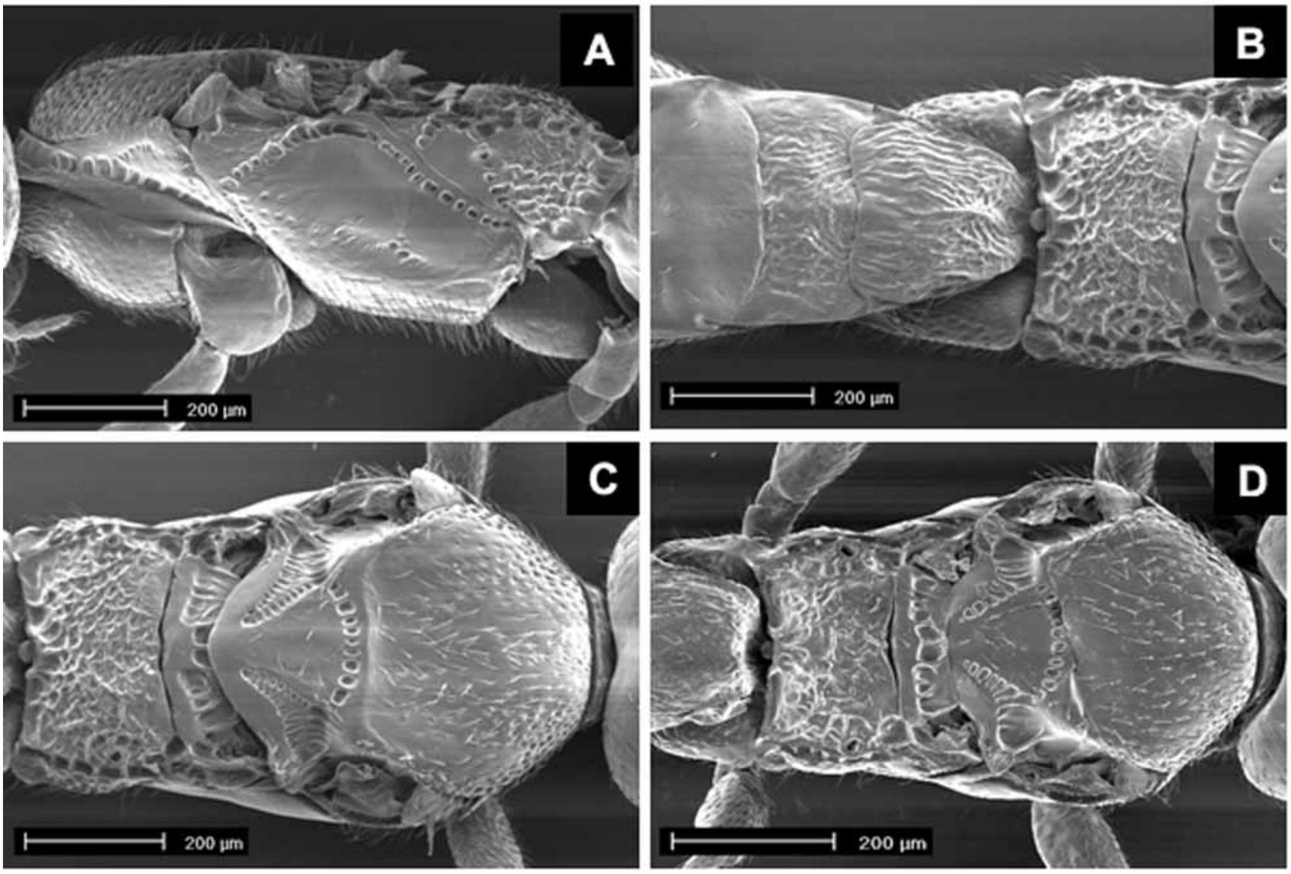

FIGURE 3. A–C: *Cotesia nonagriae* (Bundaberg, Australia): (A) mesosoma, lateral view, (B) posterior mesosoma and terga 1–3, dorsal view, (C) mesosoma, dorsal view. D: *C. flavipes* (India), mesosoma, dorsal view.

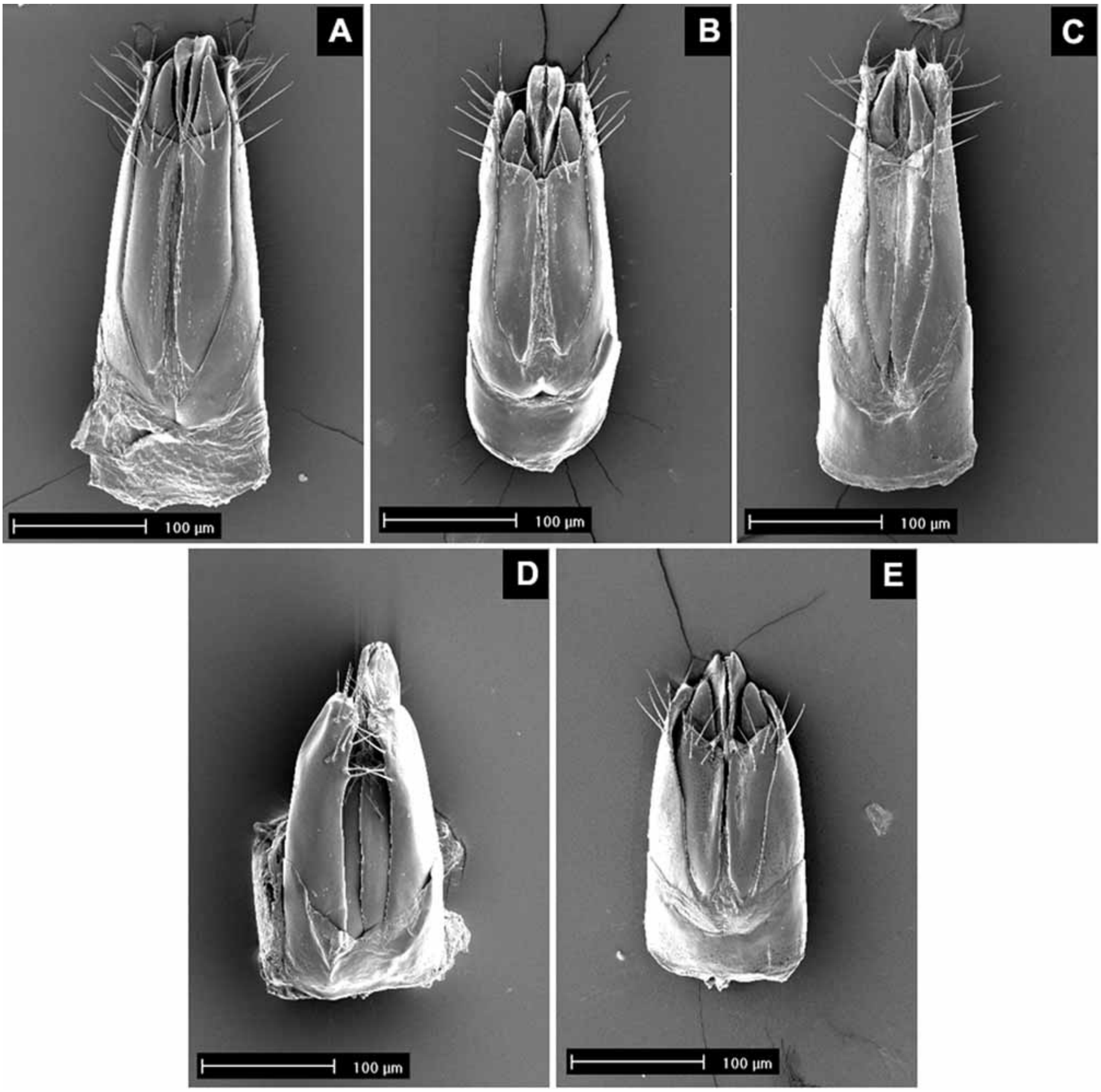

FIGURE 4. A–E: Male genitalia of *Cotesia flavipes* complex species. (A) *C. nonagriae* (Australia), (B) *C. flavipes* (India), (C) *C. flavipes* (Japan), (D) *C. sesamiae* (Kenya), (E) *C. chilonis* (China).

Results and discussion

Species recognition

Based on the mitochondrial gene phylogeny of Muirhead *et al.* (2006), there are clearly two pairs of sister species within the *flavipes* complex: *C. sesamiae/C. chilonis* and *C. flavipes/C. nonagriae*. Although there are a number of morphological differences that distinguish these two pairs of species (e.g., form of the scuto-scutellar sulcus and propopeal sculpturing), they also display relatively high levels of intraspecific variation making it difficult to interpret these characters. Without doubt, the definitive difference between these species pairs is the structure of the male genitalia. In *C. sesamiae/C. chilonis* the major elements of the genitalia are relatively short and broad, while in *C. flavipes/C. nonagriae* they are more elongate (Kimani-Njogu & Overholt 1997; Fig. 4). Distinguishing between *C. nonagriae* and *C. flavipes* is more difficult if geographic location is not taken into account. The more sculptured head of *C. nonagriae* and to a lesser degree the courser sculpturing on the propodeum will distinguish most populations. However, levels of intraspecific variation that occur in both species will at times render identification difficult. Because of this, and until *C. flavipes* can be shown definitively not to occur in Australia, we advocate the use of molecular diagnostic techniques using the phylogenetic framework generated by Muirhead *et al.* (2006) in cases where accurate identification is critical.

Biology

Life history traits for *C. nonagriae* assessed as part of this study are summarized and compared with published data for *C. flavipes* in Table 1. The potential fecundity of *C. nonagriae* females was similar to *C. flavipes* with an initial load of ~ 200 eggs. However, *C. nonagriae* females allocated an average of 111.6 (SD ± 25.32) eggs into each host, whereas *C. flavipes* is known to allocate a maximum of 30–40 eggs into at least two different hosts (Sallam *et al.* 2002). This high egg allocation suggests that *C. nonagriae* females will deplete their egg load after just two oviposition events, while *C. flavipes* females are depleted of eggs after they have parasitized four to five hosts (Potting *et al.* 1997a). In spite of the higher number of *C. nonagriae* progeny that emerged from *B. truncata,* cocoon weight was not very different from that produced by *C. flavipes* parasitizing *Sesamia calamistis* Hampson (Noctuidae) in Africa based on studies by Sallam *et al.* (2002). The total life cycle of *C. flavipes* is about 20 days but is longer for *C. nonagriae* at 24 days. This is due to a longer duration of the larval stages (17 versus 21 days), which may also be influenced by the higher number of larvae competing for food. After 14–15 days *C. nonagriae* larvae emerged from the host and formed small white silken cocoons, which usually surrounded the host cadaver within its tunnel. Like *C. flavipes* adults, *C. nonagriae*, generally lives for one to three days without food; however *C. flavipes* adults can live up to six days when provided honey (Potting *et al.* 1997a).

TABLE 1. Number of cocoons, cocoon weight, adult progeny, duration of immature stages, sex ratio, adult longevity, emergence rate and potential fecundity (mean (± SD)) of *C. nonagriae* on the native stemborer host *Bathytricha truncata* compared with the same biological traits for *C. flavipes* on *S. calamistis* (from Sallam *et al.* 2002).

Species	Number of cocoons/host	Cocoon Weight (mg)	Adult progeny/host	Duration of immature stages (days)	Sex ratio (% female/ total progeny)	Adult longevity (days)	% Emergence	Potential fecundity(egg load)
C. nonagriae	99.28 (21.8)	0.101 (0.023)	91.56 (20.9)	21.07 (1.2)	52.1 (5.8)	2.92 (0.35)	91.97 (4.92)	196.56 (12.2)
C. flavipes (Sallam *et al.* 2002)	34.300 (17.2)	0.106 (0.011)	32.00 (17.6)	17.20 (3.0)	53.00 (0.26)	3.60 (0.7)	92.97 (8.9)	203.60 (8.7)

Relevance to biological control

Accurate identification of both natural enemies and pests is vital for research, quarantine and successful biological control (Clausen 1942; Debach 1960; Compere 1969; Danks 1988; Debach & Rosen 1991; Schauff & LaSalle 1998; Beard 1999). However, biocontrol programs are often confounded by intraspecific variation within complex taxonomic groups. Overlapping intraspecific variation in hymenopteran parasitoids is well documented and has been reported for ecological, behavioural and physiological traits such as climatic adaptability, diapause, host selection and virulence (Hopper *et al.* 1993; Unruh & Messing 1993) Ruberson *et al.* (1989) alone listed over 65 studies that deal with intraspecific variation in hymenopteran parasitoids, predominantly revealed through biological control introductions. Species that are seemingly widespread and abundant in reality can represent several cryptic species. This may well be the case for the *C. flavipes* complex, where numerous authors have recorded geographic variation among *C. flavipes* populations in ecology, host-searching behaviour and host-parasitoid compatibility (Mohyuddin 1971; Mohyuddin *et al.* 1981; Inayatullah 1983; Polaszek & Walker 1991; Ngi-Song *et al.* 1995; Potting *et al.* 1997b; Ngi-Song *et al.* 1998; Mochiah *et al.* 2001). The ability to discriminate between biotypes on different hosts is crucial for biological control. Moreover, from an evolutionary perspective, it is important to identify the forces that structure genetic differences among parasitoid populations relative to their host insects (Vaughn & Antolin 1998; Heraty 2004)

Whereas this study underscores the need for molecular diagnostic techniques (e.g., Dupas *et al.* 2006; Muirhead *et al*. 2006) for reliable identification of near cryptic species, it also emphasizes the need for detailed comparative morphology and supplemental biological data to support critical taxonomic decisions. The Cohesive Species Concept stresses the importance of establishing species boundaries by examining phylogenetically distinct entities for reproductive incompatibility or ecological, behavioural or morphological differences (Templeton 1989). The mtDNA sequence data of Muirhead *et al.* (2006) provided the first evidence for the monophyly of the Australia populations, and likewise, our results support the conclusion that *C. nonagriae* is a distinct species based on morphological and biological traits.

Although there are subtle morphological differences between *C. nonagriae*, *C. flavipes* and the other members of the complex, it is not surprising that earlier authors confused these species given their close similarity and intrinsic variability (Wilkinson 1928a; Watanabe 1932, 1965; Alam *et al.* 1972; Ingram 1983; Polaszek & Walker 1991). Male genitalia are certainly the most reliable character system (Polaszek & Walker 1991) and clearly separate the two morphospecies groups: *C. sesamiae*/*C. chilonis* and *C. flavipes/C. nonagriae* (Fig 4). Despite biological variation between *C. nonagriae* and *C. flavipes*, there is limited phenotypic diversity. Their similarity probably reflects not only recent common ancestry but also stabilizing selection arising from ecological selection, while diversification within the complex is probably linked to biogeographic barriers and host use.

Independent of the conclusion that *C. nonagriae* is a distinct species associated with the native sugarcane pest *B. truncata*, we were unable to discern whether or not *C. flavipes* also occurs on the continent. Previous researchers reporting the occurrence of *C. flavipes* over the last century have failed to deposit voucher specimens in recognized insect collections (e.g., Jarvis 1927; Macqueen 1969; Li 1970) Thus, no reliable specimens are available to verify the identity of *C. flavipes* referred to in the literature. In several cases these are very likely to be *C. nonagriae* when associated with *B. truncata* (e.g., Bell 1934). However, reference to *C. flavipes* associated with *C. suppressalis* and *C. polychrysa* in rice (Li 1970) is more problematic given that true *C. flavipes* have been reared from these hosts in southeast Asia (Kajita & Drake 1969; Hattori & Siwi 1986; Khoo 1986; van Verden & Ahmadzabidi 1986). We were unable to access populations of either *Chilo* species in Australia to rear parasitoids for comparison. Thus, the question of whether *C. flavipes* occurs in Australia still needs to be addressed. This is crucial for future biological control projects in Australia because if *C. flavipes* is not native, it will need to undergo pre- and post-release studies in order to assess its interaction with *C. nonagriae* and impact on non-target species (Howarth 1991; Messing 1992; Samways 1997; Sands 1997; Henneman & Memmott 2001).

Perhaps a more central issue for potential stemborer pest incursions into Australia is the host range of *C. nonagriae* and whether it will successfully parasitize host species not encountered during its evolutionary history. Interestingly, this was the case for *C. flavipes*, which formed a novel association with *Diatraea saccharalis* (F.) when introduced into the New World for biological control purposes (Simmonds 1969; Polaszek & Walker 1991). Thus, future work could profitably be directed towards the testing of *C. nonagriae* on high threat stemborer species from Indonesia and Papua New Guinea.

Acknowledgments

We wish to thank David Britton for sending us examples of Olliff's handwriting and Lachlan Farrington, Michelle Guzik, John Jennings, Lars Krogmann, Nick Murphy, Gary Taylor and Jim Whitfield for helpful discussions. We are particularly grateful to the following agencies for funding this project: the Australian Research Council, the Bureau of Sugar Experiment Stations, the South Australian Museum, the Sugar Research and Development Corporation and The University of Adelaide.

References

Alam, M.M., Beg, M.N. & Ghani, M.A. (1972) Introduction of *Apanteles* spp. against graminaceous borers into Pakistan. *Technical Bulletin, Commonwealth Institute Biological Control*, 15, 1–10.

Austin, A.D. & Dangerfield, P.C. (1992) Synopsis of Australasian Microgastrinae (Hymenoptera: Braconidae) with a key to genera and description of new species. *Invertebrate Taxonomy*, 6, 1–76.

Beard, J.J. (1999) Taxonomy and biological control: *Neoseiulus cucumeris* (Acari: Phytoseiidae), a case study. *Australian Journal of Entomology*, 38, 51–59.

Bell, A.F. (1934) *Annual Report Bureau of Sugar Experiment Stations*, 50–74 pp.

Boucĕk, Z. (1988) *Australasian Chalcidoidea (Hymenoptera): A Biosystematic Revision of Genera of Fourteen Families, with a Reclassification of Species*. CAB International, Wallingford, 832 pp.

Clausen, C.P. (1942) The relation of taxonomy to biological control. *Journal of Economic Entomology*, 35, 744–748.

Common, I.F.B. (1990) *Moths of Australia*. Melbourne University Press, Melbourne, 535 pp.

Compere, H. (1969) The role of systematists in biological control: a backward look. *Israel Journal of Entomology*, 4, 5–10.

Danks, H.V. (1988) Systematics in support of entomology. *Annual Review of Entomology*, 33, 271–296.

Debach, P. (1960) The importance of taxonomy to biological control as illustrated by the cryptic history of *Aphytis holoxanthus* n. sp. (Hymenoptera: Aphelinidae), a parasite of *Chrysomphalus aonidium* and *Aphytis coheni* n. sp., a parasite of *Aonidiella aurantii*. *Annals of the Entomological Society of America*, 53, 701–705.

Debach, P. & Rosen, D. (1991) *Biological Control by Natural Enemies*. Cambridge University Press, Cambridge, 440 pp.

Dupas, S., Gitau, C., Le Ru, B. & Silvain, J.-F. (2006) Single-step PCR differentiation of *Cotesia sesamiae* (Cameron) and *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) using polydnavirus markers. *Annales de la Société Entomologique de France*, 42, 319–324.

Eady, R.D. (1968) Some illustrations of microsculpture in the Hymenoptera. *Proceedings of the Royal Entomological Society of London Series A*, 43, 66–72.

Harris, R.A. (1979) A glossary of surface sculpturing. *California Department of Food and Agriculture, Bureau of Entomology, Occasional Papers*, 28, 1–31.

Hattori, I. & Siwi, S.S. (1986) Rice stemborers in Indonesia. *Japan Agricultural Research Quarterly*, 20, 25–30.

Henneman, M.L. & Memmott, J. (2001) Infiltration of a Hawaiian community by introduced biological control agents. *Science*, 293, 1314–1316.

Heraty, J.M. (2004) Molecular systematics, chalcidoidea and biological control. *In*: Ehler, L.E., Siorza, R. & Mateille, T., (Eds.). *Genetics, Evolution and Biological Control*. CAB International, London, pp. 39–71.

Hopper, K.R., Roush, R.T. & Powell, W. (1993) Management of genetics of biological control introductions. *Annual Review of Entomology*, 38, 27–51.

Howarth, F.G. (1991) Environmental impacts of classical biological control. *Annual Review of Entomology*, 36, 489–509.

Inayatullah, C. (1983) Host selection by *Apanteles flavipes* (Cameron) (Hymenoptera: Braconidae): Influence of host and host plant. *Journal of Economic Entomology*, 76, 1086–1087.

Ingram, W.R. (1983) Biological control of graminaceous stemborers and pod-borers. *Insect Science and its Application*, 4, 205–209.

Jarvis, E. (1927) Notes on Insects Damaging Sugar Cane in Queensland. Division of Entomology. Bureau of Sugar Experiment Stations, 9–11 pp.

Jones, E.L. (1966) Rice stem borer *Bathytricha truncata*. Annual Report 1965–66. New South Wales Department of Agriculture, Entomology Branch, 14–15 pp.

Kajita, H. & Drake, E.F. (1969) Biology of *Apanteles chilonis* and *A. flavipes* (Hymenoptera: Braconidae), parasites of *Chilo suppressalis*. *Mushi*, 42, 163–179.

Khoo, S.G. (1986) Pest outbreaks in the tropics. *Journal of Plant Protection in the Tropics*, 3(1), 13–24.

Kimani-Njogu, S.W. & Overholt, W.A. (1997) Biosystematics of the *Cotesia flavipes* species complex (Hymenoptera:

Braconidae), parasitoids of the gramineous stemborers. *Insect Science and its Application*, 17(1), 119–130.

Li, C.S. (1970) Some aspects of the conservation of natural enemies of rice stem borers and the feasibility of harmonizing chemical and biological control of these pests in Australia. *Mushi*, 44, 15–23.

Macqueen, A. 1969. Notes on the large moth borer, *Bathytricha truncata* (Walker). *Proceedings of the Queensland Society of Sugar Cane Technologists*. Queensland, Australia. pp. 57–67.

Mason, W.R.M. (1981) The polyphyletic nature of *Apanteles* Foerster (Hymenoptera: Braconidae): a phylogeny and reclassification of Microgastrinae. *Memoirs of the Entomologcial Society of Canada*, 115, 1–47.

Messing, R.H. (1992) Biological control in island ecosystems: cornerstone of sustainable agriculture or threat to biological diversity. *Pacific Science*, 46, 387–388.

Michel-Salzat, A. & Whitfield, J.B. (2004) Preliminary evolutionary relationships within the parasitoid wasp genus *Cotesia* (Hymenoptera: Braconidae: Microgastrinae): combined analysis of four genes. *Systematic Entomology*, 29, 1–12.

Mochiah, M.B., Ngi-Song, A.J., Overholt, W.A. & Botchey, M. (2001) Host suitability of four cereal stem borers (Lepidoptera: Crambidae, Noctuidae) for different geographic populations of *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) in Kenya. *Biological Control*, 21, 285–292.

Mohyuddin, A.I. (1971) Comparative biology and ecology of *Apanteles flavipes* (Cam) and *A. sesamiae* Cam. as parasites of graminaceous borers. *Bulletin of Entomological Research*, 61, 33–39.

Mohyuddin, A.I., Inayatullah, C. & King, E.G. (1981) Host selection and strain occurrence in *Apanteles flavipes* (Cameron) (Hymenoptera: Braconidae) and its bearing on biological control of graminaceous stem-borers (Lepidoptera: Pyralidae). *Bulletin of Entomological Research*, 71, 575–581.

Muirhead, K.A., Murphy, N.P., Sallam, M.N., Donnellan, S.C. & Austin, A.D. (2006) Mitochondrial DNA phylogeography of the *Cotesia flavipes* complex of parasitic wasps (Hymenoptera: Braconidae). *Annales de la Société Entomologique de France*, 42, 309–318.

Ngi-Song, A.J., Overholt, W.A. & Ayertey, J.N. (1995) Suitability of African graminaceous stemborers for development of *Cotesia flavipes* and *C. sesamiae* (Hymenoptera: Braconidae). *Environmental Entomology*, 24, 978–984.

Ngi-Song, A.J., Overholt, W.A. & Stouthammer, R. (1998) Suitability of *Busseola fusca* and *Sesamia calamistis* (Lepidoptera: Noctuidae) for the development of two population of *Cotesia sesamiae* (Hymenoptera: Braconidae) in Kenya. *Biological Control*, 12, 208–214.

Olliff, A.S. (1893) Report on a visit to the Clarence River District for the purpose of ascertaining the nature and extent of insect ravages in the sugar cane crops. *Agricultural Gazette of New South Wales*, 4, 373–387.

Omwega, C.O., Kimani, S.W., Overholt, W.A. & Ogol, C.K.P.O. (1995) Evidence of the establishment of *Cotesia flavipes* (Hymenoptera: Braconidae) in continental Africa. *Bulletin of Entomological Research*, 85, 525–530.

Onyango, F. & Ochieng-Odero, J. (1994) Continuous rearing of the maize stem borer *Busseola fusca* on an artificial diet. *Entomologia Experimentalis Applicata*, 73, 139–144.

Overholt, W.A., Ngi-Song, A.J., Omwega, C.O., Kimani-Njogu, S.W., Mbapila, J., Sallam, M.N. & Ofomata, V. (1997) A review of the introduction and establishment of *Cotesia flavipes* Cameron in East Africa for biocontrol of cereal stemborers. *Insect Science and its Application*, 17, 79–88.

Overholt, W.A., Ochieng, J.O., Lammers, P. & Ogedah, K. (1994) Rearing and field release methods for *Cotesia flavipes* Cameron (Hym.: Braconidae), a parasitoid of tropical gramineous stemborers. *Insect Science and its Application*, 15, 253–259.

Polaszek, A. & Walker, A.K. (1991) The *Cotesia flavipes* species-complex: parasitoids of cereal stem borers in the tropics. *Redia*, 74, 335–341.

Potting, R.P.J., Overholt, W.A., Danso, F.O. & Takasu, K. (1997a) Foraging behavior and life history of the stemborer parasitoid *Cotesia flavipes* (Hymenoptera: Braconidae). *Journal of Insect Behavior*, 10, 13–29.

Potting, R.P.J., Vet, L.E.M. & Overholt, W.A. (1997b) Geographic variation in host selection behaviour and reproductive success in the stemborer parasitoid *Cotesia flavipes* (Hymenoptera: Braconidae). *Bulletin of Entomological Research*, 87, 515–524.

Rajabalee, M.A. & Govendasamy, M. (1988) Host specificity and efficacy of *Apanteles flavipes* (Cam.) and *A. sesamiae* (Cam.) (Hymenoptera: Braconidae) parasites of sugarcane moth borers in Mauritius. *Revue Agricole et Sucriere*, 67, 78–80.

Ruberson, J.R., Tauber, M.J. & Tauber, C.A. (1989) Intraspecific variability in hymenopteran parasitoids: comparative studies of two biotypes of the egg parasitoid *Edovum puttleri* (Hymenoptera: Eulophidae). *Journal of the Kansas Entomological Society*, 62, 189–202.

Sallam, M.N. (2003) *A Review of Sugarcane Stemborers and Their Natural Enemies in Asia and Indian Ocean Islands: An Australian Perspective*. Bureau of Sugar Experiment Stations, 62 pp.

Sallam, M.N., Overholt, W.A. & Kairu, E. (2002) Intraspecific and interspecific competition between *Cotesia flavipes* and *Cotesia sesamiae* (Hymenoptera: Braconidae), gregarious larval endoparasitoids of lepidopteran stemborers. *Biocontrol Science and Technology*, 12, 493–506.

Sallam, M.N., Overholt, W.A., Kairu, E. & Guofa, Z. (2001) Will *Cotesia flavipes* drive its African homologue, *Cotesia* *sesamiae*, to extinction? *Insect Science and its Application*, 21, 297–302.

Samways, M.J. (1997) Classical biological control and biodiversity conservation: what risks are we prepared to accept? *Biodiversity and Conservation*, 6, 1309–1316.

Sands, D.P.A. (1997) The "safety" of biological control agents: assessing their impact on beneficial and other non-target hosts. *Memoirs of the Museum of Victoria*, 56, 611–616.

Schauff, M.E. & La Salle, J. (1998) The Relevance of systematics to biological control: Protecting the Investment in Research. *In*: Zalucki, M.P., Drew, R.A.I. & White, G.G., (Eds). *Pest Management - Future Challenges.* Proceeding of the Sixth Australasian Applied Entomological Research Conference. Brisbane. pp. 425–436.

Shami, S. & Mohyuddin, A.I. (1992) Studies on host plant preference of *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) an important parasitoid of graminaceous stalk borers. *Pakistan Journal of Zoology*, 24, 313–316.

Sharkey, M.J. & Wharton, R.A. (1997) Morphology and terminology. *In*: Wharton, R.A., Marsh, M.J. & Sharkey, M.J., (Eds.). *Manual of the New World Genera of the Family Braconidae (Hymenoptera)*. International Society of Hymenopterists, Washington DC, pp. 19–37.

Shenefelt, R.D. (1972) *Braconidae 4, Microgasterinae, Apanteles. Part 7*. Gravenhage, Junk, 429–668 pp.

Simmonds, F.J. (1969) *Report of Work Carried Out During 1969*. Commonwealth Institute of Biological Control, 103 pp.

Smith, P.T. & Kambhampati, S. (1999) Status of the *Cotesia flavipes* species complex (Braconidae: Microgastrinae) based on mitochondrial 16S, rRNA and NADH 1 dehydrogenase gene sequence. *Journal of the Kansas Entomological Society*, 72, 306–314.

Songa, J.M., Bergvinson, D. & Mugo, S. 2001. Mass rearing of the maize stem borers *Chilo partellus*, *Busseola fusca, Sesamia calamistis, Chilo orichalcociliellus* and *Eldana saccharina*. *Seventh Eastern and Southern Africa Regional Maize Conference.* Nairobi, Kenya. pp. 120–124.

Templeton, A.R. (1989) The meaning of species and speciation: a genetic perspective. *In*: Otte, D. & Endler, J.A., (Eds.). *Speciation and its Consequences*. Sinauer Associates, Sunderland, Mass., pp. 3–27.

Unruh, T.R. & Messing, R.H. (1993) Intraspecific biodiversity in hymenoptera: Implication for conservation and biological control. *In*: LaSalle, J. & Gauld, I.D., (Eds.). *Hymenoptera and Biodiversity.* CAB International, Wallingford, pp. 27–54.

Van Verden, G. & Ahmadzabidi, A.L. (1986) *Pests of Rice and Their Natural Enemies in Peninsular Malaysia*. PUDOC, Wageningen, pp. 230 pp.

Vaughn, T.T. & Antolin,, M.F. (1998) Population genetics of an opportunistic parasitoid in an agricultural landscape. *Heredity*, 80, 152–162.

Walker, A.K. (1994) Species of Microgastrinae (Hymenoptera: Braconidae) parasitizing lepidopterous cereal stem borers in Africa. *Bulletin of Entomological Research*, 84, 421–434.

Watanabe, C. (1932) A revision of Braconid-species parasitic in the injurious insects of rice-plant and sugar-cane in Japan and Formosa. *Transactions of the Sapporo Natural History Society*, 12, 63–72.

Watanabe, C. (1965) Notes on the *Apanteles flavipes* complex (Hymenoptera: Braconidae). *Mushi*, 38, 111–116.

Wilkinson, D.S. (1928a) A revision of the Indo-Australian species of the genus *Apanteles* (Hymenoptera: Braconidae). Part A. *Bulletin of Entomological Research*, 19, 79–105.

Wilkinson, D.S. (1928b) A revision of the Indo-Australian species of the genus *Apanteles* (Hymenoptera: Braconidae). Part B. *Bulletin of Entomological Research*, 19, 109–146.

Wilkinson, D.S. (1929) New parasitic Hymenoptera and notes on other species. *Bulletin of Entomological Research* 20, 103–114.

Zhang, D.X. & Hewitt, G.M. (1996) Nuclear integrations: challenges for mitochondrial DNA markers. *Trends in Ecology and Evolution*, 11, 247–251.