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Morphometric Evidence for Three Sibling Species in *Sycophila mellea* (Hymenoptera: Chalcidoidea: Eurytomidae) in Britain, Parasitoids of *Tetramesea* sp. (Hymenoptera: Chalcidoidea: Eurytomidae) in Poaceae

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Abstract: The taxonomic status of *Sycophila mellea* (Curtis) species complex (Hymenoptera: Eurytomidae), reared from *Tetramesa linearis* (Walker), *T. brevicornis* (Walker) and *T. exemia* (Girauld) feeding in the grasses: *Elymus repens* (Viv), *Festuca rubra* (L.) and *Ammophila arenaria* (L.) respectively, were investigated using morphometric study. Morphometric analysis of *S. mellea* showed significant differences among those individuals reared from *T. linearis, T. brevicornis* and *T. eximia*. The *S. mellea* complex also exhibited morphological variation in size. The results of discriminant analysis showed that in males and females, *S. mellea* (ex: *T. linearis, T. eximia* and *T. brevicornis*) populations were significantly different from each other, although it remains debatable whether they have yet reached separate species status.

Key words: Biological species, morphometric, hymenoptera, eurytomidae, *Tetramesa*, *Sycophila*, poaceae

INTRODUCTION

In many organisms, changes in the ecology of a species may be followed by changes in morphological characters. Taxonomists have used variable morphological characters as one of the primary data for separation and identification of species and population variation in thousands of different kinds of insects. Since the chalcidoids show cryptic morphological variability, their identification is very difficult and the application of fine-scale morphometric analysis to reveal subtle differences in shapes is necessary. The term morphometric comes from the Greek words morph (form) and metrien (to measure) and involves the analysis of morphological structures. This approach enables the summarization of morphological data and the numerical and graphical expression of results. Statistical analysis can then be used to investigate possible relationships between populations (Daly, 1985). Using these methods population data can be measured, interpreted and compared in terms of shapes and structures for better quantitative analysis of morphological diversity (Pimentel, 1979). Morphometry measures the distances between 'landmarks' which have been taken directly from the specimens and analyses may be performed to reveal species and character differences (Daly, 1985; Pungerl, 1983).

Since insects have a hard exoskeleton, which retains the body form throughout adult life and after death, they are especially suitable for morphometric investigation (Sneath and Sokal, 1973) and indeed such approaches have been used in a wide diversity of studies and species. Typical examples are: morphometric variation between populations of the sibling species of *D. melanogaster* (McNamee and Dytham, 1993); confirmation of studies on acoustic signals of three sibling species of leafhopper

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Oncopsis flavicollis (L.) (Homoptera: Cicadellidae) (Claridge and Nixon, 1986); morphometrics of selected allopatric populations of *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) (Kimani-Njogu *et al*., 1997); intraspecific morphometric variability between populations of the beetle, *Erichsonius tuberculatus* Schuster and Grigaric (Coleoptera: Staphylinidae); (Masch and Plotner, 1993) and morphometric variation between populations of the diving beetle, *Hydroporous glabriusculus* (Coleoptera: Dytiscidae) (Bilton, 1993).

Insects of the genus *Sycophila mellea* (Curtis, 1831) are small (2.0-2.5 mm in length), winged, generally orange, propodeum distinctly pale with dark T-shaped marking (Claridge, 1959), orange-black in colour and feed on nectar as adult wasps. In the larval stage, they are parasitic on various gall-forming *Tetramesa* species (= isosoma Walker, = Harmolita Motsch) (hymenoptera: Eurytomidae) (Claridge, 1959).

The taxonomy of this complex is in a state of confusion. It is regarded as a single biological species (Fitton *et al*., 1978), or different biological species (Anga, 1991).

The taxonomic status of *S. mellea* reared from *T. eximia*, *T. linearis* and *T. brevicornis*, feeding on three host plants, *Ammophila arenaria*, *E. repens* and *F. rubra*, respectively has been studied by Anga (1991). He used enzyme electrophoresis and morphometric techniques and stated that a high degree of morphological distinctiveness was apparent amongst females Sycophila reared from *T. eximia*, *T. linearis* and *T. brevicornis*.

The purpose of this experiment is to investigate morphological variation within and between members of *S. mellea*, more especially to determine whether there are significant measurable changes in the morphology of the species attacking *Tetramesa* on different host grasses. The null hypothesis for this experiment was therefore that no morphological differences for exist *S. mellea* on attacking *Tetramesa* on three host grasses.

MATERIALS AND METHODS

Collection Sites

Grasses were collected from 1999 to 2000 from the following numbered localities in South Wales and England. Sites were identified according to their Counthy (Local government Act, 1972) and National Grid Reference (NGR). (National Grid Reference shown in brackets). Wales: 1. Gwent, Wentlooge, Peterston (ST 268 801), 2. Cleppa Park, Gwent (ST 285 844), 3. Fairwater, City and county of Cardiff (ST 133 788), 4. Merthyr Mawr Dunes, Brigend County Borough Council (SS 860 770), 5. Kenfig Dunes, Brigend County Borough Council (ST 794 816), 6. Mumbles, Aberthaw, (SS 031 661) 7. Cosmeston lake park, Vale of Glamorgan (ST 174 613), 8. Rhoose, Vale of Glamorgan (ST 067664), 9. Cold Knap Point, Vale of Glamorgan (ST 104 660), England, 10. Aindale Dunes, Merseyside (SD 353 198), 11. Cornwall (Cape Cornwall) (SW 349 319). Detailed locality data are given in Ghajarieh (2003).

Rearing Individual Larvae from Stems

Rearing techniques of *Tetramesea* and *Sycophila* species larvae employed here was described by Dawah (1987). Grasses were collected in November 2000, when the larvae within had completed feeding (Graham and Claridge, 1965). The stems were dissected by scalpel without damaging the larvae when the larvae were fully fed and in diapause and placed in individual gelatine capsules and labelled. They were placed in a shed where they were constantly checked until *Sycophila* larvae were reared to adulthood. All males and females of *Sycophila* and *Tetramesa* used in all experiments were fed with 10% (w/v) honey solution. Grasses were identified using Hubbard (1954). Larvae were identified to genus using Dawah and Rothfritz (1996) and host plant association. Reared specimens of *S. mellea* complex, together with host, host food-plant and collection sites are given in Table 1.

Table 1: Reared populations from the *S. mellea* complex, together with host, host food-plant and collection sites

Host	Host food plant	Collection sites
T. exemia	*A. arenaria*	4, 5, 10
T. brevicornis	*F. rubra*	5, 6
T. linearis	*E. repens*	1, 2, 7, 8, 9

List of Specimens and Number of Individuals Used

S. mellea were sampled during 1999 and 2000 at three sites in South Wales and two sites in England. For this particular study, three populations of *S. mellea* were reared from three *Tetramesa* species (*T. linearis*, *T. eximia* and *T. brevicornis*) feeding on the grasses *E. repens, A. arenaria* and *F. rubra*. The insects were sexed. Accordingly, for each sex, 84 specimens were used to measure morphological characters (64 from *E. repens*, 10 from *A. arenaria* and 10 from *F. rubra*). Also three *S. mellea* populations from one grass (*E. repens*) from three locations (48 individuals from Rhoose, 10 from Cosmeston Lake and 6 from Cold Knap point in Wales) were used.

Choice of Characters

The characters were chosen based on one of two criteria traditionally used in taxonomic studies of Chalcidoidea and used to identify *Sycophila* species and others which could be reliably measured that could be potentially useful (Dawah, 1986; Anga, 1991). Fifty-five characters where chosen for females and 53 for males. Fourteen measurements were taken from the head and antenna, three from the forewing and 24 from the legs (i.e., foreleg, midleg and hindleg, eight characters each).

Morphological Characters Used

In this experiment from fifty-three characters for males and 55 for females only 24 characters were significant (Fig. 1a-f). These characters will be presented using abbreviations as seen below:

Adult Male and Female

Head

- CO: Distance between compound eye and oculus (Fig.1a).
- AO: Distance between two top ocelli (Fig. 1a).
- BO: Distance between top and median ocelli (Fig. 1a).
- OF: Oral fossa (Fig. 1a).
- AM: Distance between antennal socket and proximal oral point (Fig. 1a).
- TM: Distance between upper compound eye and median ocellus (Fig. 1a).
- ANA: Width of antennal segment 1 (Fig. 1b).
- ANB: Width of antennal segment 2 (Fig. 1b).
- ANI: Angle of antennal segment 2 (Fig. 1b).
- ANK: Angle of antennal segment 4 (Fig. 1b).
- ANM: Angle of antennal club (Fig. 1b).

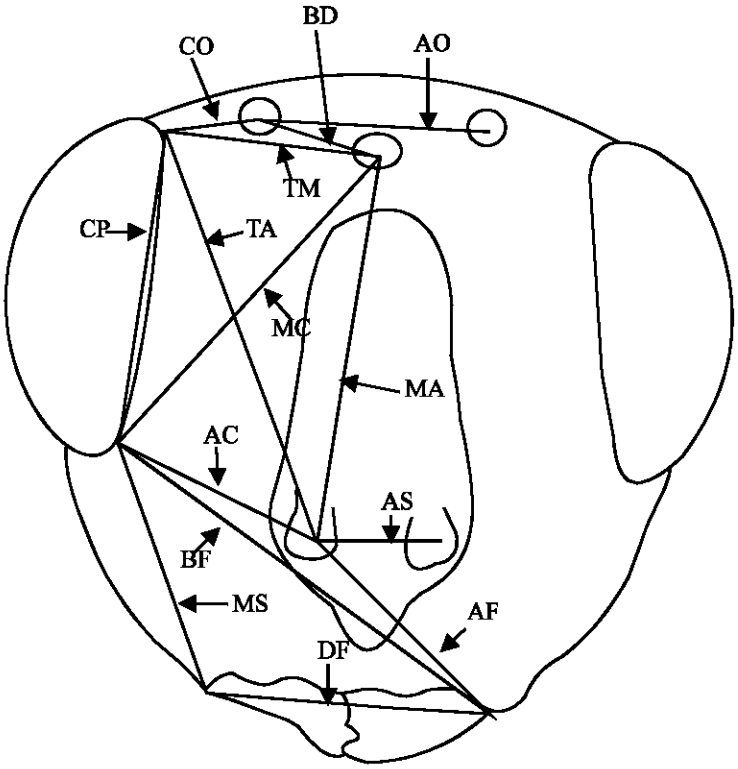
Forewing

- VB: Veinal break (Fig. 1c).
- MV: Marginal vein (Fig. 1c).
- V: Stigmal vein (Fig. 1c).

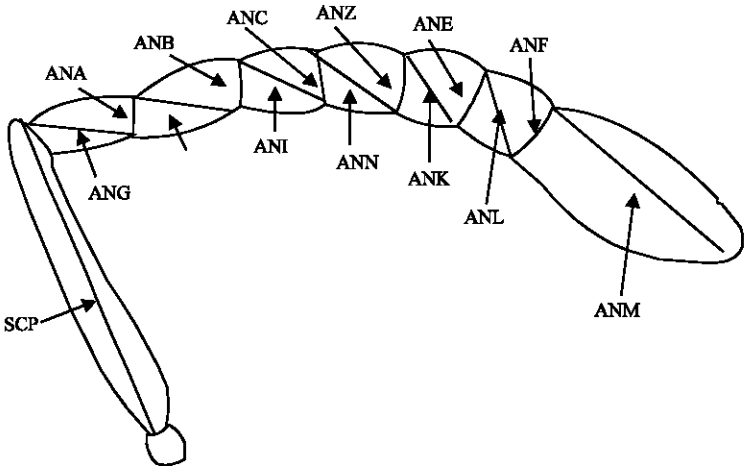
Legs

- FLG: Tarsal segment 4 of foreleg (Fig. 1d).
- FLH: Tarsal segment 5 of foreleg (Fig. 1d).
- MLC: Tibia spur of midleg (Fig. 1e).
- MLD: Tarsal segment 1 of midleg (Fig. 1e).
- MLE: Tarsal segment 2 of midleg (Fig. 1e).

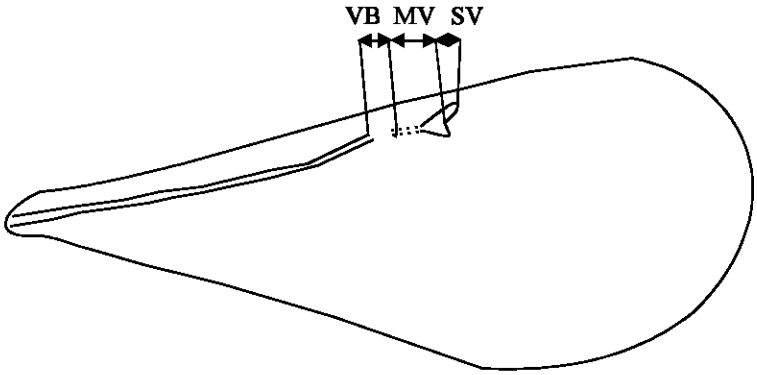
- HLA: Femur of hindleg (Fig. 1f).
- HLB: Tibia of hindleg (Fig. 1f).
- HLC: Tibia spur of hindleg (Fig. 1f).
- HLG: Tarsal segment 4 of hindleg (Fig. 1f).
- HLH: Tarsal segment 5 of hindleg (Fig. 1f).



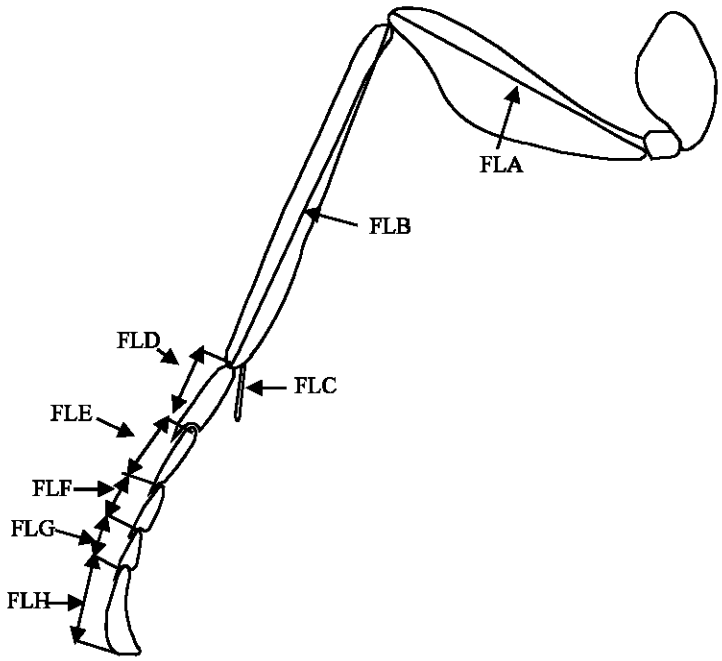
(a) Head



(b) Antenna



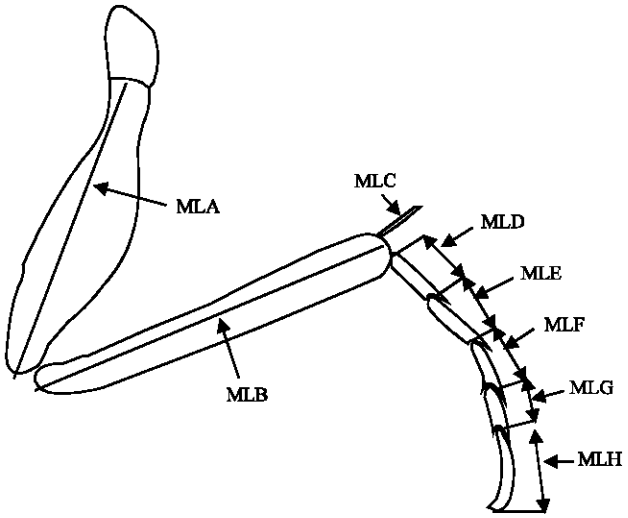
(C) Fore wing



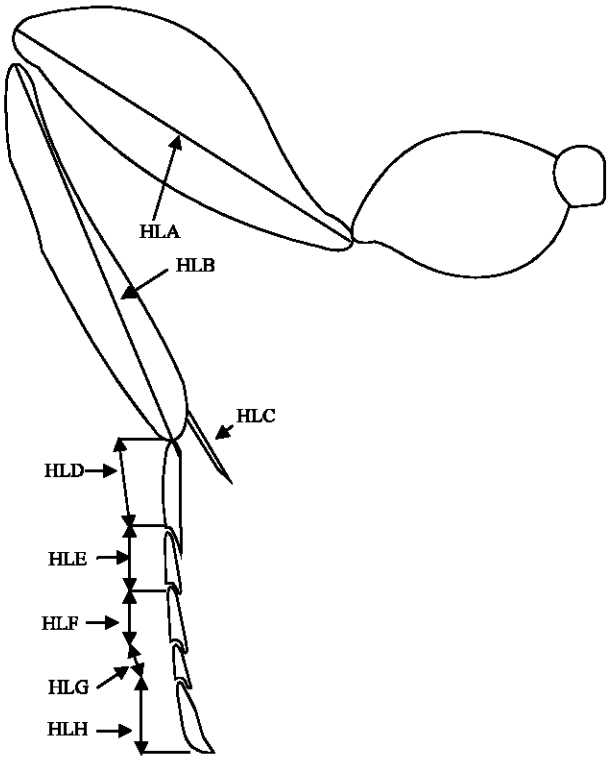
(d) Fore leg

Preparation of Species for Measurement

In order that the insects used in this study had, after emergence, a hard skeleton, they were fed with honey solution (10% w/v) for three days where after they were killed by freezing for 10 min and put in 70% alcohol for 24 h. Individuals were then dissected into different body parts for measurement, with antennae, legs and wings pulled off the body using an entomological pin. All the body parts were fixed with water-soluble adhesive onto a white rectangular insect card (10 mm long, 5 mm wide). There were 84 such cards for both males and females (64 cards for insects from *E. repens*, 10 for insects from *A. arenaria* and 10 for *F. rubra*). For the male and female populations, 4452 and 4620 measurements were made, respectively.



(e) Mid leg



(f) Hind leg

Fig. 1: Morphometric characters of *Sycophila*

Measurment Procedure

Morphological distance measurements were made using a SigmaScan Image Analyser. Structures to be measured were magnified to the appropriate size using a binocular microscope, superimposed by a *camera lucida* on to a digitizing table and viewed on a television monitor. Before measurement, the microscope magnification was calibrated and kept unchanged throughout the measurements. The morphological characters used had very clear measuring points and were made from the left-hand side of the body. Each character was measured on the same day for all the populations of each sex to avoid error, since calibration might be slightly changed otherwise when new measurement were taken.

RESULTS AND DISCUSSION

Data analysis was performed using SPSS (Statistical Package for the social Science), Base 10.0 and Minitab ver. 13.0.

The ANOVA (Univariate Analysis of Variances) showed that the difference between members of *S. mellea* complex means is significant for some characters. ANOVA tests revealed: For males 14/29 characters (AO, CO, BO, TM, DF, AM, ANA, ANB, ANI, VB, MV, SV, HLB and HLC) and for females 13/23 characters, (ANB, ANK, ANM, NB, FLG, FLH, MLC, MLD, MLE, HLA, HLB, HLG, HLH) showed significant differences. The MANOVA (Multivariate Analysis of variances) tests showed significant population differences ($p = 0.001$) for males and females Table 2 and 3.

Table 2 and 3 summaries the (DFA) for each character used, respectively, including the levels of variance significant for functions 1 and 2. Figure 2 and 3 are graphical presentations of group centroids for males and females separately, showing the degree of variation between members of *S. mellea* complex. For *S. mellea* female (according to host grass), all were highly separated from each other (Table 3). For male there is a big overlap between populations in relation to host grass (Fig. 2).

Results of reclassification tests of individuals of *S. mellea* using the original discriminant functions, derived with a prior specified group membership for both males and females, are shown in Table 6 and 7, respectively. The results based on grasses for males (Table 6) shows 100% for *F. rubra*, 98.4% for *E. repens* and 90% for *A. arenaria* reclassified into their original groups. The results based on grasses for females (Table 7) shows 100% for *F. rubra* 95.3% for *E. repens* and 80% for *A. arenaria* reclassified into their original groups.

Relatively few studies have carried out morphometric analysis on Eurytomidae. Al-Barrak *et al*. (2004) used morphometric technique to confirm the status of four closely related species of *Tetramesa*: studied different *Tetramesa* species and sibling species within *T. hyalipennis*: *T. longicornis T. petiolata T. calamagrostidis* and *T. hyalipennis*. However, he found that *T. hyalipennis* reared from Elymus repens and E. farctus are conspecific. Despite the fact that many species complex are exists within Eurytomidae (Claridge and Dawah, 1994). Very few stuudies have been carried out on Eurytomidae.

The present results for *S. mellea* in relation to host grass show that for males, 29/53 morphological characters and for the females, 23/55 morphological characters, displayed significant differences among members of *Sycophila*. The similar results for *S. mellea* in relation to location show that for males, 42/53 of such characters and for females 46/55 of characters showed significant differences among members of, *Sycophila*.

The pair-wise group comparison (Table 4 and 5) showed that morphologically the members of *Sycophila* are significantly different from each other.

Results of reclassification tests of individuals of *S. mellea* using the original discriminant functions, derived with a prior specified group membership for both males and females, are shown in Table 6 and 7, respectively. The high reclassification values indicate that the three groups of *S. mellea* showed high degrees of differentiation according to the associated grass of its *Tetramesa* host. In both

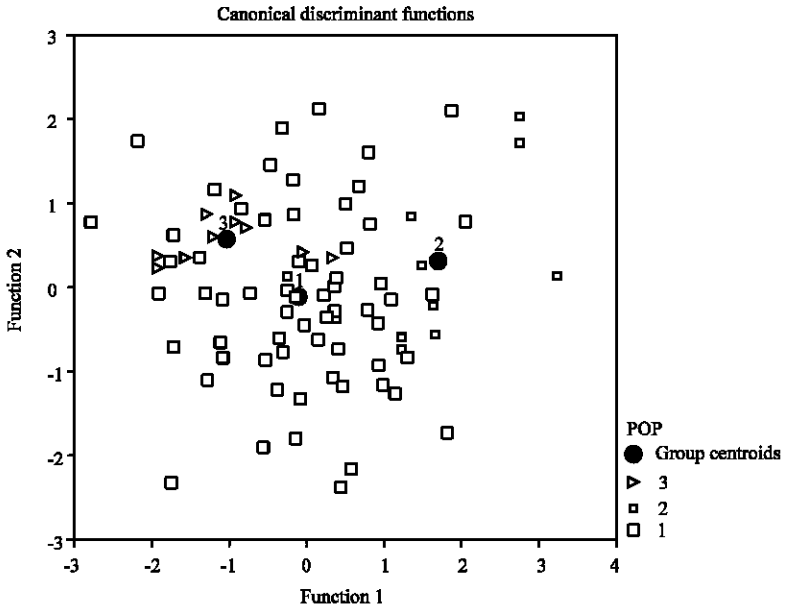


Fig. 2: Group centroids of the first two discriminant functions for males according to host grass. Plot of the first two discriminant functions of three groups of *S. mellea* collected from three grass species (1-*E.repens*, 2-*A.arenaria*, 3-*F.rubra*), showing the position of group centroids, where all the groups are morphologically separated from each other

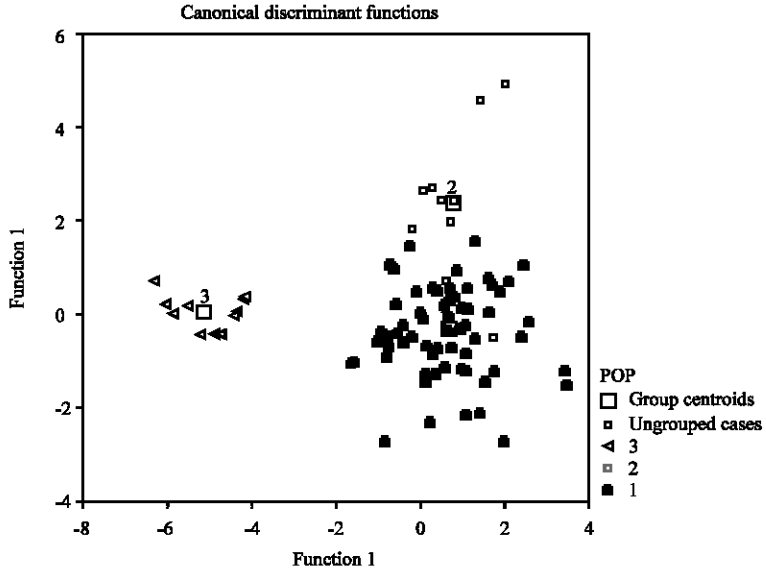


Fig. 3: Group centroids of the first two discriminant functions for females according to host grass. Plot of the first two discriminant functions of three groups of *S. mellea*, collected from three grasses species (1- *E. repens*, 2-*A. arenaria*, 3-*F. rubra*), showing the position of group centroids, where all the groups are morphologically separated from each other

Table 2: Summary of canonical discriminant functions for male *S. mellea*

Function	Eig.Val	%Var	%Cum	Can.Cor	Wilk's L
1	1.506	69.7	69.7	0.775	0.241
2	0.656	30.3	100.0	0.629	0.604

Functions 1 shows significant difference with greatest variance

Table 3: Summary of canonical discriminant functions for female *S. mellea* according to host grass

Function	Eig.Val	%Var	%Cum	Can.Cor	Wilk's λ
1	4.049	81.4	81.4	0.896	0.103
2	0.927	18.6	100.0	0.694	0.519

Functions 1 and 2 show significant difference with greatest variance, The meaning of the above statistical abbreviations are as following: Eig.Val =Eigen Value, %Var. =Percentage variance, %Cum. = Percentage cumulative, Can.Cor. = canonical correlation and Wilks L. = Wilks Lambda

Table 4: Pair-wise group comparisons for males of *S. mellea* according to host grass

Host plant	*E. repense*	*A. arenaria*	*F. rubra*
E. repense	-	14.6***	5.8***
A. arenaria	-	-	18.7***
F. rubra	-	-	-

***p<0.001

Table 5: Pair-wise group comparisons for females of *S.mellea*

Species	*E. repense*	*A. arenaria*	*F. rubra*
Repense	-	5.9***	19.9***
A. arenaria	-	-	15.2***
F. rubra	-	-	-

***p<0.001

Table 6: Results of a classification of individual males from three species of grasses according to discriminant function

	Predicted Group Membership			
Population	*E. repens*	*A. arenaria*	*F. rubra*	Total
Original Count				
E. repens	63.0	1.0	0.0	64.0
A. arenaria	1.0	9.0	0.0	10.0
F. Rubra	0.0	0.0	10.0	10.0
% E. Repens	98.4	1.6	0.0	100.0
A. Arenaria	10.0	90.0	0.0	100.0
F. Rubra	0.0	0.0	100.0	100.0

97.6% of original grouped cases were correctly classified

Table 7: Results of a classification of individual females from three species of grasses according to discriminant function.

	Predicted Group Membership			
Population	*E. repens*	*A. arenaria*	*F. rubra*	Total
Original Count				
E. repens	61.0	3.0	0.0	64.0
A. arenaria	2.0	8.0	0.0	10.0
F. Rubra	0.0	0.0	10.0	10.0
% E. Repens	95.4	4.7	0.0	100.0
A. Arenaria	20.0	80.0	0.0	100.0
F. Rubra	0.0	0.0	100.0	100.0

94.0% of original grouped cases were correctly classified

sexes *F. rubra* with 100% reclassification showed the highest level of differentiation. Variation between the members of the *S. mellea* complex was investigated using DFA. Table 2 and 3 show summaries of two Canonical Discriminant Functions. The highest variation was found in female populations according to host grass which showed 81.1% variation (Table 3) and male populations according to location which showed 69.7% variation for function one (Table 2). These results indicate a high degree of intra-population variation. Since, according to Reyment *et al.* (1984), such variation > 80% may be biologically significant. This evidence is highly suggestive of significant genetic divergence between members of the *S. mellea* complex and hence for the existence of sibling species

or incipient differentiation in host associated memberss of this parasitoid from (ex: *E. repens, A. arenaria* and *F. rubra*). The extent of variation in males was less than in females. Yet so, before relying solely on the results of the morphometric analysis, it would be necessary to determine the degree to which the phenotypic variability in *S. mellea* (ex *T. linearis, T. brevicornis* and *T. exemia*) is genetically induced. In conclusion, the results from the morphometrics studied reveal that members of the *S. mellea* complex (ex: *T. linearis, T. brevicornis* and possibly *T. exemia*) represent good biological taxa that have either already speciated or which are currently doing so. Any decision is to its exact status depends on whether or not they are within the same gene pool in nature. Since investigations into sibling species are very important for pest management strategies, the ability to identity biological species with unique genetic heritage is a fundamental necessity (Lawton *et al*., 1998; Basset, 2001). From the results of the present study, further morphological investigation based on abdomen and thorax characters among members of *S. mellea* complex is recommended.

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