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Geometric morphometric analysis of wings variation between two populations of the *Scythris obscurella* species-group: geographic or interspecific differences? (Lepidoptera: Scythrididae)

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Abstract

The wings of scythridids of two population from Valle d'Aosta and Maritime Alps were examined through geometric morphometrics analysis. All the specimens belong to the the"*Scythris obscurella* group". Currently, 10 species are assigned to the group, and the uncertain identification of many specimens is due mainly to their marked similarity in external features. Aim of the work is to evaluate the overall shape variation, and thence to test if the specimens could be divided at population level, or at specific level employing both the pairs of wings. Size and shape variation of each wing were examined separately through GPA. The left-right asymmetry of scythridids wings was examined by *t*-test, and the shape variation of forewings was compared to the one of hindwings. For each wing, shape variation was correlated to size variation.
KEY WORDS: Lepidoptera, Scythrididae, geometric morphometrics, generalized procrustes analysis, forewings, hindwings

Análisis morfo-geométrico de la variación de las alas entre dos poblaciones del grupo de especies de *Scythris obscurella*: ¿diferencias geográficas o inter-específicas? (Lepidoptera: Scythrididae)

Resumen

Fueron examinadas las alas mediante un completo análisis morfo-geométrico, de dos poblaciones de escítridos del Valle de Aosta y de los Alpes Marítimos. Todos los especímenes pertenecen al grupo de "*Scythris obscurella*". Normalmente, 10 especies son asignadas al grupo, y la incertidumbre en la identificación de algunos especímenes se debe a su marcada similitud en sus aspectos externos. El ánimo de este trabajo es evaluar la diferencia de las formas en conjunto y si los especímenes podrían estar separados a nivel de población desde allí, o empleando ambos pares de alas, al nivel específico. Se revisó por separado la forma de cada ala a través del GPA. Se examinó la asimetría del ala izquierda de los escítridos por el *t*-test, y se fue comparando la variación de la forma de las alas anteriores con las posteriores. Para cada ala, se correlacionó la variación de la forma y la del tamaño.
PALABRAS CLAVE: Lepidoptera, Scythrididae, morfo-geométrico, análisis intolerante generalizado, alas anteriores, alas posteriores.

Introduction

The widespread genus *Scythris* Hübner, [1825] (Lepidoptera, Gelechioidea, Scythrididae) includes almost three hundred species, which are further arranged in groups of species on the basis both of genitalia and external features (BENGTSSON, 1997; JÄCKH, 1977; PASSERIN D'ENTRÈVES, 1995, 1996). Besides, scythridids are yet poorly known altogether, except for Palaearctic and Nearctic species, the more recent papers dealing mainly with regional faunas as FALKOVITSH paper on Russian fauna (1981), LANDRY work on Nearctic species (1991), and BENGTSSON revision on western Palaearctic Scythrididae (1997).

Furthermore, the marked resemblance of external features complicates more and more the studies on these species, and at times a great number of synonymies were proposed and discussed (PASSERIN D'ENTRÈVES & ROGGERO, 2005), although we can usually identify specimens by the features of genitalia for both the sexes (BENGTSSON, 1997; LANDRY, 1991).

The *S. obscurella*-species group includes at present 10 species (BENGTSSON, 1997; BENGTSSON & LIŠKA, 1996), showing sometimes a so uniform appearance that the individuals can not be assigned unquestionably to a species.

As it was established by BENGTSSON (1997), the species belonging to the *S. obscurella*-group clearly share many characters, i.e. the greenish or olive brown forewings, often with cream-coloured or yellow markings. The wing length varies from 9.5 mm to 21 mm, and the males are larger than females. Some species display different features in forewings. The hindwings are even, and lighter than the fore ones. Abdomen is dark, sometimes with an anal tuft, and can be larger in females than in males.

In regard of the biology, the life cycle (i.e., the larval and pupal stages) is currently unknown for all the species of *obscurella* species-group; besides, the majority of them were recorded feeding on Caryophyllaceae and Cistaceae, but also on Lamiaceae, Fabaceae, Poaceae, Rosaceae and Saxifragaceae (PASSERIN D'ENTRÈVES & ROGGERO 2005).

Although the genitalia examination is traditionally the most reliable method to identify the specimens, in the past wings characters were also employed in scythridid species recognition as well as in the species of many other lepidopteran families (BRAUN, 1919, 1949; BROHMER *et al.*, 1927-36), since they keep an invariant pattern within each taxon.

In regard of their taxonomical importance, wings have been already employed in a great number of studies treating various topics of variation among species and populations (BAYLAC *et al.*, 2003), both by traditional morphometrics, and, more recently, by geometric morphometrics (ADAMS *et al.*, 2004; MARCUS *et al.*, 1996; ROHLF & MARCUS, 1993). Morphometric analysis of wings has been employed to clarify the relationships among closely related taxa in Hymenoptera (KLINGENBERG *et al.*, 2001; KÖLLIKER-OTT *et al.*, 2003), Diptera (DE LA RIVA *et al.*, 2001; DUJARDIN *et al.*, 2003; GILCHRIST *et al.*, 2000; HAAS & TOLLEY, 1998; HOFFMANN & SHIRRIFFS, 2002; KLINGENBERG & ZAKLAN, 2000; KLINGENBERG *et al.*, 1998; MORAES *et al.*, 2004; ROHLF & ARCHIE, 1984) and Hemiptera (GUMIEL *et al.*, 2003; JARAMILLO *et al.*, 2002; VILLEGAS *et al.*, 2002).

Wing morphometrics can help in identifying populations within a species, as it was showed by the analysis of geographic variation in three populations of *Drosophila lummei* (HAAS & TOLLEY, 1998), and in many female populations of *D. serrata* (HOFFMANN & SHIRRIFS, 2002). Morphological variation was often compared to the genetic one, and wings showed themselves very useful to study complexes of species, as in Diptera Psychodidae (DE LA RIVA *et al.*, 2001), or to examine the effects of hybridization, as in *Apis mellifera* subspecies (SMITH *et al.*, 1997), or also to test genetic variation in the developmental control of wing patterning for *D. melanogaster* (BIRDSALL *et al.*, 2000). Studies of asymmetry through morphometrics methods pointed mainly on effects of stress (as variation of temperature, or percentage of CO_2) during development (DEBAT *et al.*, 2003; KLINGENBERG & MCINTYRE, 1998; KLINGENBERG *et al.*, 2001; WEBER, 1992).

Here, we examined morphological variation in scythridid wings through geometric morphometrics analysis. Aim of the work is to evaluate the amount of shape variation within the *S. obscurella*-species group, testing the possible use of wings patterns to study differences within the species. From two separate populations, we selected the specimens that were previously identified at first sight as *S. obscurella*-group by external morphology and genitalia.

Materials and methods

During 1998 and 1999 summers, scythridid specimens of both the sexes were collected from Colle di Tenda (Maritime Alps) and Valtournenche (Valle d'Aosta). The collection areas are geographically well separated, although fairly close (Fig. 1). The study areas in Maritime Alps lies between southwestern Piemonte (Cuneo, Italy) and southern France (Provence-Alpes-Côte d'Azur), namely at the Colle di Tenda boundary. The Valle d'Aosta study area is located in NE part of the region, in Pennine Alps, south to Cervinia.

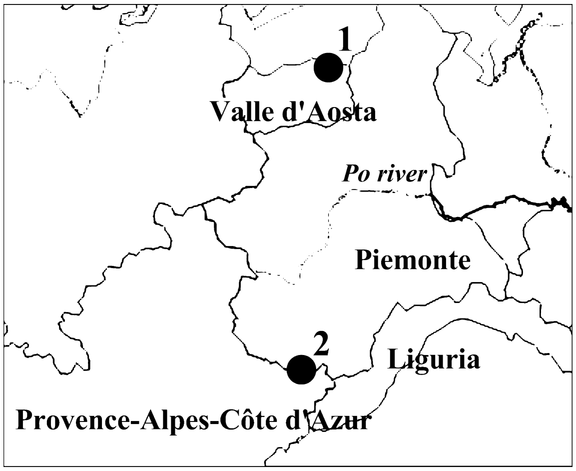

Fig. 1.– Map of the collection areas. The localities of Valtournenche (1), and Maritime Alps (2) are indicated by black dots.

On the whole, 223 scythridids were collected from Maritime Alps, and 452 from Valtournenche. About one third of the specimens collected from both the sites were identified as *S. obscurella* group, the sample being composed by 205 specimens, 156 from Valtournenche, and 49 from Colle di Tenda.

Some of the specimens collected in both the sites (N = 64) were studied by geometric morphometrics analysis. The sample was composed by 34 specimens from Valtournenche (N_V), and 30 from Colle di Tenda (N_T). Although the sample sizes could be considered quite small, they are well representative of the usual taxonomic study constraints, as BAYLAC *et al.* (2003) appropriately pointed in their study on parasitoid Hymenoptera wings.

Slide preparation

Each wing was cut off pointing on the articulation with thorax by a scalpel constituted by a vise holding a sharpened insect pin. To remove the scales on the surface and free the veins, the wings were put in glass microvials and cleared by 5% KOH watery solution for almost 10 minutes, then washed with distilled water prior to be transferred in 70% ethanol.

The scales were removed by passing lightly over the wing surface with a bent pin on a vise. The cleared wing was placed in 90% ethanol, then in Euparal essence, and mounted in Euparal mounting medium.

Once fixed, the wing slides were examined under a microscope to describe venation of both the wings.

Besides, if the wings were damaged by preparation, they were excluded from the analysis, so we could examined the complete set of the four wings for 52 specimens solely.

Wing structure

The nomenclature used for the description of wing venation (Fig. 2) follows BRAUN (1919; 1949), and BENGTSSON (1984).

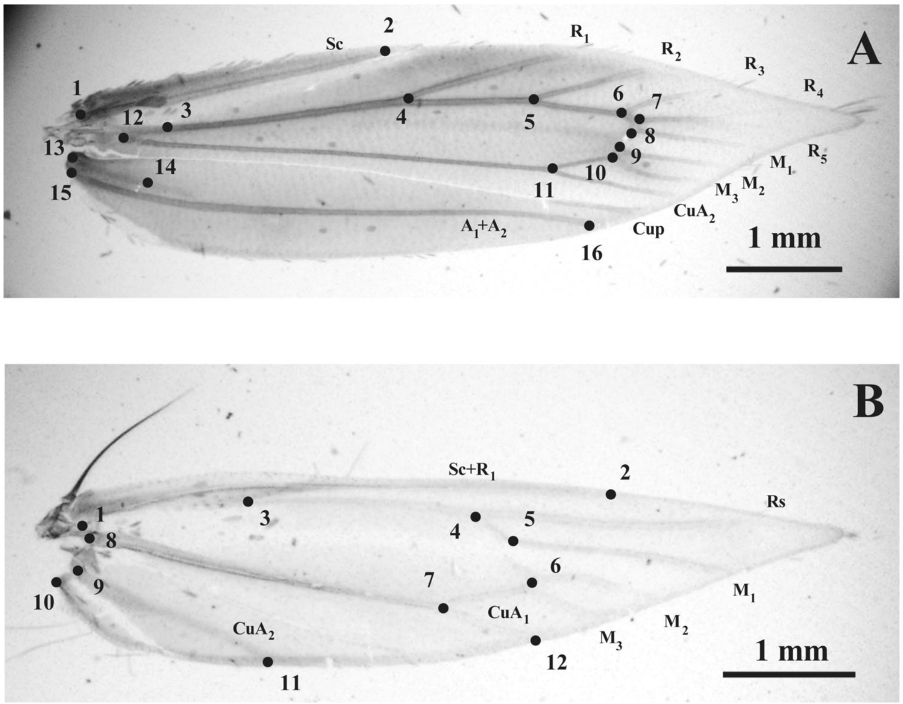

Fig. 2.– A. Landmarks collected on the forewing (N = 16). **B.** Landmarks collected on the hindwing (N = 12). For landmarks nomenclature, see Material and methods.

Forewing. The subcosta (Sc) reaching costa before middle, radial (r) five-branched, R_1 arising after middle of wing, R_1-R_4 to costa, R_5 to termen, R_4 and R_5 stalked; median (M) three-branched, M_3 coalescent with CuA_1; analis (A) weak, A1 and A_2 coalescent.

Hindwing. The $Sc+R_1$ and Rs are parallel, the former extending beyond middle of costa; M three-branched.

Geometric morphometric analysis

Images were captured by a digital camera Olympus DP11 attached to a stereoscopic microscope Leica MZ8, and stored in a jpg format using the software Camedia Olympus 1.11 (Olympus Optical Co., 1999). The right wings were reflected, so both sides could be analysed together, although by choose we also examined severally each set, i.e. the left and right sides in fore and hindwings, then compared the two pairs of wings.

We collected 16 landmarks on the venation of the forewing, and 12 landmarks on the hindwing

(Fig. 2) through tpsDig 1.40 (ROHLF, 2004a). To estimate the measurement error, coordinates were recorded twice on each digitalized image. All the landmarks are at the intersections of wing veins, or at the wing edge, and can be considered type I landmarks (BOOKSTEIN, 1991). The dimension of our sample wholly met with the BOOKSTEIN (1996) advice on being preferable to have the number of individuals four times at least the number of landmarks (GUMIEL *et al.*, 2003).

The landmarks were chosen for their relative easy identification, and their ability to capture the general shape of the wing (BOOKSTEIN, 1991). Besides, insects wings are widely employed in morphometrics analysis because they are basically 2-dimensional, and the venation provides many well-defined morphological landmarks (GUMIEL *et al.*, 2003).

For each wing, we examined size and shape variation separately, and all the analyses were repeated for the forewings and the hindwings of both body sides.

Size was computed as centroid size (or, the square root of the sum of squared distances from the landmarks to their centroid). In the absence of allometry the centroid size is the only size measure uncorrelated with all the shape variables (BOOKSTEIN, 1991). Centroid size was used to test the presence of directional asymmetry (KLINGENBERG *et al.*, 1998) through *t*-test (*two-groups*, *one-sample* and *paired*) using the statistical package Systat 8.0 (SPSS Inc., 1998). The centroid size values were computed for both the wings, as a measure of overall size variation of wings, and are far more sensitive than conventional measurements (KLINGENBERG *et al.*, 1998).

Then, the overall size variation between sexes and between populations was tested for each wing with a *two-groups t*-test on centroid size values, employing Systat 8.0 (SPSS Inc., 1998).

Shape variation was obtained by GPA (Generalized Procrustes Analysis), removing the effects of size, location and rotation (ROHLF & SLICE, 1990). The Relative warps scores were estimated by the software tpsRelw 1.39 (ROHLF, 2004b), these values explaining variance only if they summarize enough variance. Ordination of the Relative warps can reveal clusters of individuals, which can be interpreted as naturally affiliated groups. We created a scatterplot of the first two Relative warps (RW1 and RW2) to summarize the results for each wing.

A minimum-length spanning tree (MST) was obtained by NTSYS 2.11 (ROHLF, 1998-2002) from the Procrustes distances matrix, computed by the software tpsSmall 1.20 (ROHLF, 2003). Each MST was then superimposed onto the respective RWs scatterplot, to connect the points (i.e., the specimens) more closely related.

To corroborate the data from the former analysis, a multivariate regression analysis was then applied running the software tpsRegr 1.28 (ROHLF, 2004c) on the two groups. The collection locality data were set as independent variable.

To estimate the significance of the separation between the two groups, the discriminant function was tested through Hotelling's T^2 using PAST 1.10 (HAMMER & HARPER, 2003) from the weight matrix (i.e., the Partial warps), computed by tpsRelw.

We then compared the two sets of wings (the right forewings and the left hindwings) which share the major number of individuals (N = 59). The covariation between the two wings was tested through tpsPLS 1.13 (ROHLF, 2004d), since the software can easily investigate covariation between the shapes of the two different configurations of points. We performed a Partial least-squares analysis of the covariation between the two sets of partial warps, this resulting in two set of vectors (linear combinations) which are paired – the first partial warp vector of forewings is paired with the first partial warp vector of the hindwings, the second with the second, and so on (ROHLF, 2004d). In the analysis the correlations (here expressed as D1, D2, etc…) between the paired vectors are computed, and the scatterplots corresponding to the paired vectors can be displayed. Besides, ROHLF (2004d) warns to check also what percentage of the covariation is explained by each pair of vectors: even though a correlation for a pair is quite high, it can yet be unimportant since it explains very little of the covariation.

Statistics of the amount of covariation between the wings were calculated, and the scatterplot of the correlation between the two sets of shape vectors is displayed.

Results of the former analysis were then compared to the results of the Mantel Test on the Procrustes distances matrices of both the wings using NTSYS 2.11 (ROHLF, 1998-2002).

To verify the contribution of size variation to the overall shape variation in the two populations, a linear multivariate regression of the shape variables was performed against the centroid size values for each wing through tpsPLS 1.13 (ROHLF, 2004d). The values retained from each analysis were showed in a scatterplot by NTSYS 2.11 (ROHLF, 1998-2002).

After carefully checking the results of the former analyses, the data from geometric morphometrics analysis were compared with the identification data obtained employing genitalia features. The accuracy of identification, and the precision of the geometric morphometrics analysis to capture the overall shape variation of the scythridid wings were therefore tested.

Results

Wing size showed non significant directional asymmetry both for the forewings (N_F= 119) and the hindwings (N_H = 117) employing the *two-groups t*-test, the *one-sample t*-test (with $\overline{m}_F = 1465.07 \pm 108.028$, and $\overline{m}_H = 966.780 \pm 105.665$), and the *paired t*-test (Table I).

Table I. Results of the analysis of directional asymmetry for the two pair wings, with the number of specimens of each sample (N_{Tot}= total number of specimens, N_R= right wing specimens, N_L= left wing specimens)

	N_{Tot}	N_R	N_L	two-groups *t*-test		one-sample *t*-test		paired *t*-test	
				t	p	t	p	t	p
Forewings	119	61	58	-0.27990	0.78	0.000170	0.99	-0.669724	0.51
Hindwings	117	57	60	-0.13989	0.89	-0.000007	0.99	-0.593298	0.56

A significant difference in wing size between the two populations was revealed by *t*-test for the hindwings, but not for the forewings. The analysis by pooling sexes was also non significant for both the wings (Table II). The results were corroborated by ANOVA, with a significant result solely for the hindwings, when grouped by geographical area (Table II).

Table II. Results of *t*-test and analysis of variance (ANOVA) between groups for Centroid size values of forewings (F) and hindwings (H). The samples comprehend N_F= 119 (95 males and 24 females; 55 from Colle di Tenda, and 64 from Valle d'Aosta), and N_H = 117 (95 males and 22 females; 55 from Colle di Tenda, and 62 from Valle d'Aosta)

	AREA				SEX			
	two-groups *t*-test		ANOVA		two-groups *t*-test		ANOVA	
	t	p	F	p	t	p	F	p
Forewing	-1.71592	0.09	2.78436	0.09	1.88773	0.07	4.44095	0.37
Hindwing	4.69864	0.001	23.4635	>0.001	-0.89456	0.38	0.78207	0.38

As for the overall shape variation, the first two Relative warps accounted together for 51.54% of the total variation (RW1 = 31.52%, and RW2 = 20.02%) in the right forewings, and respectively for the 38.73%, and 12.39% of overall shape variation (51.12% total) in the left forewings. More than the 80% of the observed variation of both the forewings is explained by Relative warps 1-6 together. Although the variation expressed by ordination plot is scattered to more than two Relative warps, the plot of the Relative warps 1-2 for the right forewings (Fig. 3) shows a separation of the two populations, except for some specimens from the Valle d'Aosta which are grouped with the Maritime Alps ones. This close relationship is corroborated also by the MST, projected onto the scatterplot.

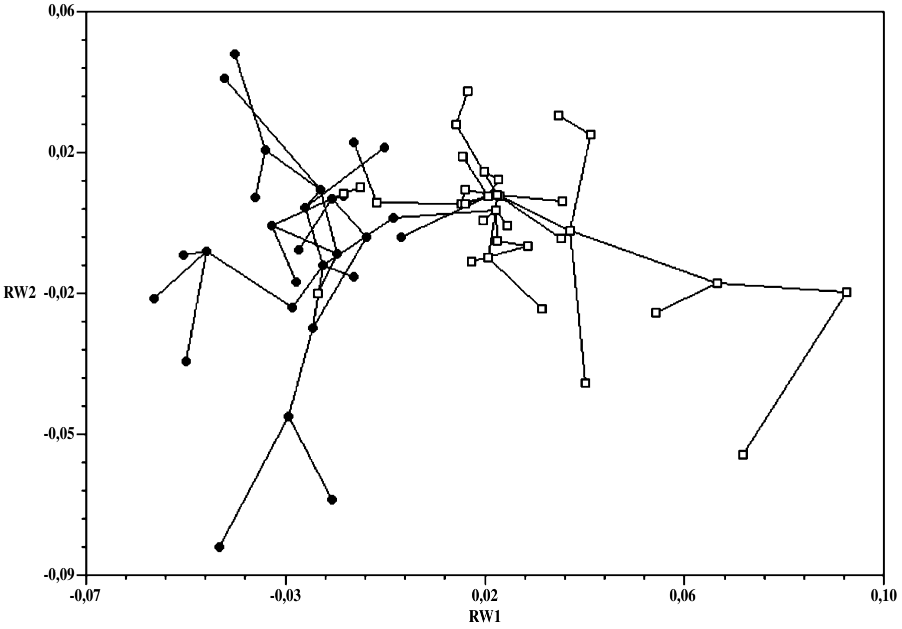

Fig. 3.– Scatterplot of the RW1 and RW2 for the right forewings (percentage of variance explained = 51.12%), with the MST from the Procrustes distances overimposed. Black circles = Maritime Alps population, white squares = Valtournenche population.

The Multivariate test (Wilks's Lambda) on weight matrix was significant for both the forewings, with p = 0.0000003784 for the right set, and p = 0.000006051 for the left set.

In the left hindwings, the first two Relative warps accounted for 80.74 % of overall shape variation (RW1 = 73.03 %, and RW2 = 7.71 %), and for 66.40% (RW1 = 55.30% e RW2 = 11.10%) in the right ones. Moreover, about the 80% of the observed variation of the right hindwings is explained by Relative warps 1-4 together. Although both the scatterplot of Relative warps 1 and 2 for the hindwings showed two groups, they are less clearly distinguishable than in the two forewings plots, and are partially superimposed.

The Multivariate tests (Wilks's Lambda) are significant both for the left (p = 0.00002314) and right (p = 0.00004813) forewings sets.

These results confirm that there are non significant differences between the left-right axis both for the forewings and the hindwings, although the percentage of overall shape variation explained by the first two Relative warps is greater in the latter. Since variation in wing shape is probably related to flight movements, little changes in wing shape and size could deeply influence fly mechanism and movements (KÖLLIKER-OTT *et al.*, 2003).

Using the Partial warps values to test the specimens group assignment, the discriminant factor allowed an almost perfect reclassification of the specimens to their pertaining population, resulting in 98.36% of correctly assigned specimens for the right forewings (Hotelling's T^2 P = 0.00000038), and 93.33% for the left hindwings (Hotelling's T^2 P = 0.000023), thus the results of Multivariate test of significance were confirmed. Moreover, when the specimens were grouped by sex instead than by geographic origin, the same analysis gave a non significant result.

Since the forewings and the hindwings showed significant differences in shape variation analysis, we used the Partial least squares analysis (ROHLF, 1999) to identify the shape covariation between the two wings, employing the software tpsPLS 1.13. The Cross set analysis gave a significant result, with 75.22% of covariance in D1 and 20.87% in D2 (cumulative covariance >96%). The correlation index (r) between the two shapes vectors was respectively r = 0.55753 for D1, and r = 0.71611 for D2, while the Permutations test (with 1000 random permutations) showed that the covariation was concentrated in one dimension (D1 = 53.85%), and the correlations were 0.40% for D1, and 0.10% for D2. The percentages are significantly small, and the results imply that the correlations between the first two pairs of dimensions are stronger than one would expect due to chance, evidencing a significant correlation in the forewings and hindwings shape covariation.

Both the scatterplots of the two shapes paired vectors D1 and D2 gave significant values, although we presented here only the plot of D2 (Fig. 4), where the samples differently coloured by locality form two distinct groups.

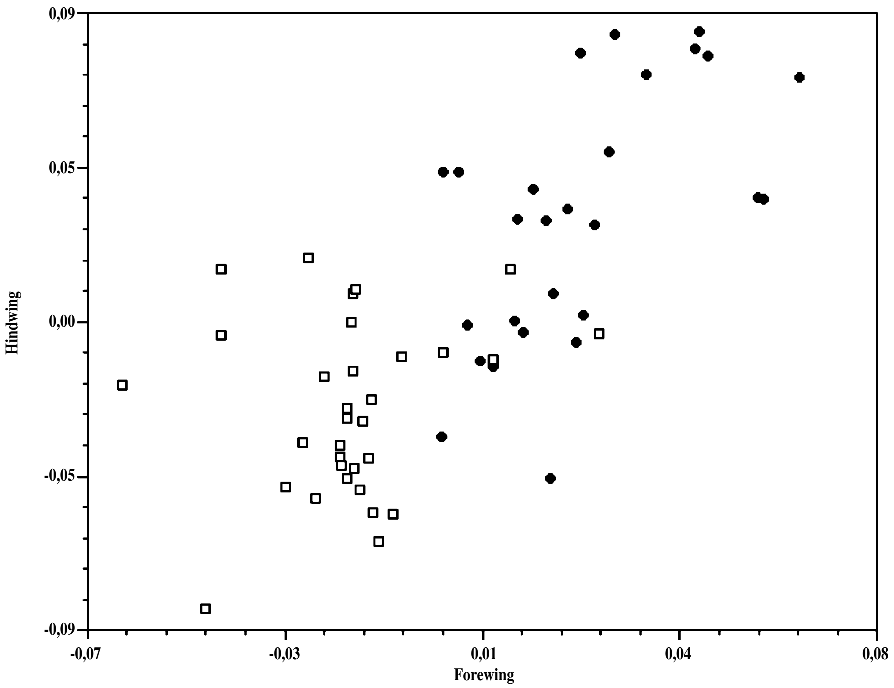

Fig. 4.– Scatterplot of shapes vectors of forewings and hindwings D2 from PLS analysis. Cumulative covariance >96%, r = 0.71611, Correlations = 0.10%. Black circles = Maritime Alps population, white squares = Valtournenche population.

The correlation between the two wings shape was also analysed by the Mantel test (1000 random permutations) on the Procrustes distances matrices. Unlike the former analysis, here the correlation between the two pairs of wings is non significant with r = 0.16784, t = 3.3542, and P = 0.9996 from the test for association.

To examine whether there was a significant correlation between size and shape for each pair of wings separately, we employed again Partial least squares analysis. The results of multivariate analysis of covariance on overall shape variation, with localities and Centroid size as independent variables, show a significant difference for the two localities.

For the right forewings the percentage of covariation for D1 was 95.56%, and r_{D1} = 0.81592 in the Cross set analysis. The correlation gave a significant result (0.10% for D1, and 4.70% for D2) in the Permutations test (N permutations = 1000), although the covariation was unusually concentrated in D2 (88.01%). While the two samples are well-separated in the scatterplot from the former analysis, employing the Centroid size alone as independent variable, the analysis gave a non significant result, with r = 0.32161 in the Cross set analysis, and 53.35% of observed correlations in the Permutations test.

Likewise, for the left hindwings the results were significant, giving in the Cross set analysis 78.83% of covariation for D1, with r_{D1} = 0.36864 and r_{D2} = 0.54211 respectively. The Permutations test (with 1000 random permutations) showed that the covariation was concentrated in one dimension (D1 = 71.33%), and the percent of observed correlations were 3.00% for D1, and 0.20% for D2.

The Cross set analysis (r = 0.37111 of correlation between variables and shape vectors) and Permutations test (2.80% of observed correlations) are both non significant, and in the scatterplot the two populations are mingled, as it would be predictable by the statistical results.

Comparing the results of geometric morphometric analysis on wings and the specimens identification on the basis of genitalia (mainly the characters of aedeagus and gnathos), we drew some interesting observations. All the specimens from Maritime Alps belong to *S. cuspidella*, but in the sample from Valle d'Aosta the majority of them were identified as *S. speyeri*, and there are only few specimens of *S. cuspidella*. By comparison of the data of geometric morphometrics analysis, we gained that the few *S. cuspidella* from Valtournenche were the same specimens which were already comprised in the group of Maritime Alps in the scatterplot of Relative warps (Fig. 3).

Conclusions

Geometric morphometrics revealed itself a valuable tool to examine the overall variation in Scythrididae wings as well as hitherto it was usefully employed to study the wings of various taxa. Since the presence of directional asymmetry in insects was proved many times, the potential asymmetry in Scythrididae wings was carefully tested, but in our sample the left and right sides do not differ significantly from each other, unlike clearly evinced for *Drosophila* wings (KLINGENBERG *et al.*, 1998; KLINGENBERG & ZAKLAN, 2000). The presence of directional asymmetry for size could not be demonstrated therefore in forewings nor in hindwings. Besides, although the developmental mechanism of directional asymmetry was elucidated for vertebrates, some authors rejected the presence of right-left axis in insects due to developmental constraint (TUINSTRA *et al.*, 1990).

Conversely, size gave a significant result for the hindwings, and only grouping the specimens by population, but the result was ostensibly non significant for both the wings when the individuals were grouped by sex. The failure to assess the sexual dimorphism in Scythrididae wings is anyway a questionable result, even more looking how dissimilar is the flight behaviour in the sexes. Males fly briefly, but the females usually do not fly at all, and move on ground through the grasses.

The overall shape variation analysis gave a significant result for both the pairs of wings, which yet show dissimilar pattern, as previously assessed for size variation. The groups evinced for the forewings do not correspond to the hindwings ones. The scatterplot of forewing shows two well separated groups, corresponding to two species (*S. cuspidella* and *S. speyeri*), and the relationship among the specimens within each group is corroborated by the MST (Minimum-length Spanning Tree) projected onto its scatterplot, and showing the closest neighbours of specimens.

Since both the two pairs of wings showed a significant difference for shape variation, we analysed the amount of covariation between the shapes of forewing and hindwing. Besides, correlations gave a non significant result, which could be explained by hypothesis that different effects work on forewings and hindwings overall shape variation, or the same forces may affect unlikely the wings.

Many questions are evinced by the analysis of scythridid wings, and it seems likely to be verified also the amount of population variation, how much both specific variation and population variation affect the overall variance, the potential sexual dimorphism in wings, and the influence of asymmetry in flight mechanism.

Moreover, also in analysis of covariation between size and shape we obtained a significant result when the specimens were grouped by species.

As formerly reported for sand flies by DUJARDIN *et al.* (2003), the wing shape can reveal for scythridids a far more reliable character in species identification than commonly believed, provided that the correct tools were employed to capture the overall shape variation, as the Geometric morphometrics analysis seems to be.

The fascinating hypothesis of different pattern in the two pairs of wings which is outlined through the scythridid data must surely be corroborated by careful observation on other Lepidoptera wings. Meanwhile, the results of scythridid wings have to be also compared with other morphological structures of scythridids, as the genitalia. A study of shape variation was performed on male genitalia (valva and uncus) for *S. obscurella* species-group (ROGGERO, NEGRO & PASSERIN D'ENTRÈVES, *unpublished data*). The specimens were identified mainly on the basis of aedeagus and gnathos characters, regarding that the valva reveal itself a lesser reliable character for specific recognizing, but nevertheless very useful to test the individual variability degree in Scythrididae species. Moreover, the use of Geometric morphometrics methods on insect genitalia has many possibility, although it was applied very few times (MONTI *et al.*, 2001; ROGGERO, 2004) till now, in spite of the diffusely employment of genitalia in species identification.

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