

## Multivariate analysis of male genital structures in the *Hipparchia semele-muelleri-delattini* complex (Nymphalidae, Satyrinae) from the Balkans: how many taxa?

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**Abstract.** Two closely related *Hipparchia* taxa, *Hipparchia delattini* Kudrna, 1975 and *H. semele muelleri* Kudrna, 1975 have been described from the Balkans based on differences in male genital structure, compared to each other and to another nominal European taxon (*H. semele* Linnaeus, 1758). Subsequently, Kudrna (1977) synonymised both *H. delattini* and *H. muelleri* with *H. volgensis* (Mazochin-Porshnjakov, 1952). Application of multivariate statistical techniques on male genital data indicates a cline in several aspects of genital morphology linking these three taxa across Europe. Although clusters are repeatedly found that correspond with the three taxa, it is not possible to ascribe every individual specimen to one of the three *Hipparchia* taxa. *Hipparchia muelleri* is shown to occupy an intermediate position between *H. semele* and *H. delattini*. Generally, *H. delattini* is present in an area covering part of northern Greece and the central Balkans. *H. semele* is present in western Europe, the Balkans and down the western side of Greece. However, individual specimens that classify to *H. delattini* in the current analyses may occur much further west, where historically only *H. semele* has been, and there appears to be a correlation between putative taxa and altitude with *H. delattini* occurring at higher altitudes. It is suggested that genetic differentiation between these taxa has been maintained and enhanced during glacial-interglacial cycles. The results of this study are discussed in relation to other morphological characters and biogeography and require further testing with molecular data.

**Key words.** Lepidoptera, Nymphalidae, Satyrinae, *Hipparchia semele*, *Hipparchia delattini*, *Hipparchia muelleri*, *Hipparchia volgensis*, genitalia, Balkans, biometrics, numerical taxonomy.

### Introduction

Kudrna (1975), through comparison of male genital structures in a relatively small number of specimens, described two *Hipparchia* taxa from Greece and the Balkans, namely *Hipparchia muelleri* (type locality: Mt. Chelmos, southern Greece) and *H. delattini* (type locality: Pristina, Kosovo), differentiating them from each other and from *H. semele* (Linnaeus, 1758; type locality: Sweden). Later, Kudrna (1977) synonymised both *H. delattini* and *H. muelleri* with *H. volgensis* (Mazochin-Porshnjakov, 1952). According to Kudrna (1977), the *H. semele* male valve has a prominent terminal dorsal process, which is triangular in shape, and a well-pronounced distal termination, while in *H. delattini* the dorsal process is poorly pronounced. *H. muelleri* has a valve dorsal process that is intermediate between *H. semele* and *H. delattini* (Kudrna 1975; Coutsis 1983).

In a study to test the validity of these nominal taxa (Wakeham-Dawson et al. 2003), discriminant function analysis (DFA) was used to compare *Hipparchia* specimens

captured in Greece and the Balkans against specimens of nominal taxa from type localities (topotypes). This analysis indicated the presence of specimens consistent with one or other of the topotypical concepts of *H. semele*, *H. muelleri* and *H. delattini* in the area. We did not incorporate topotypical *H. semele* material from Sweden, but our sample of this species included specimens from southern England, Spain, France and various localities to the north of the Balkans from Italy to Romania. However, in the same study, cluster analysis of a sample of these data without preconceptions of the validity of the taxa cast doubt on the presence of three taxa (Wakeham-Dawson et al. 2003). The cluster analysis indicated two main groups: a Balkan cluster (incorporating the majority of the nominal *H. muelleri* and *H. delattini* specimens of the DFA analysis) and an extra-Balkan cluster (incorporating mainly nominal *H. semele* specimens of the DFA analysis). There was some, albeit limited, overlap between the clusters that could be the result of errors in measurement, or represent hybrids, or a continuum between the two clusters, which might become more apparent with additional data.

The results were thus inconclusive. To test these hypotheses further, and supplied with additional data from the second author that extended through areas of the Balkans not represented in the first analysis, the current paper returns to the question: is it possible to recognise more than one discrete taxon in the Balkan area? Such issues in taxonomy require several distinct approaches: (i) the investigation of dimensionality and relationships in and among variables and the placement of individuals and putative taxa within axes describing these dimensions; (ii) the degree to which putative taxa can be discriminated within the space described by the variables; (iii) an exploration of natural clusters (putative taxa) using information on the specimens representing them. This latter approach makes no prior assumptions about the allegiance of individuals and works either by determining whether a fixed number of clusters exist or by allowing numbers of clusters (taxa) to be generated by the data.

Preliminary analysis by the second author of an initial data set including 91 specimens and using Lorkovic's total and partial transitions method (see Sijarić 1980) failed to show a clear separation between Balkan specimens subjectively classified as *H. delattini* and *H. semele*, when uncus length was plotted against valve length. It should be noted that the current study does not include specimens of the more distantly related Balkan species *Hipparchia senthes* (Fruhstorfer, 1908) (see Olivier & Coutsis 1997; Wakeham-Dawson et al. 2003).

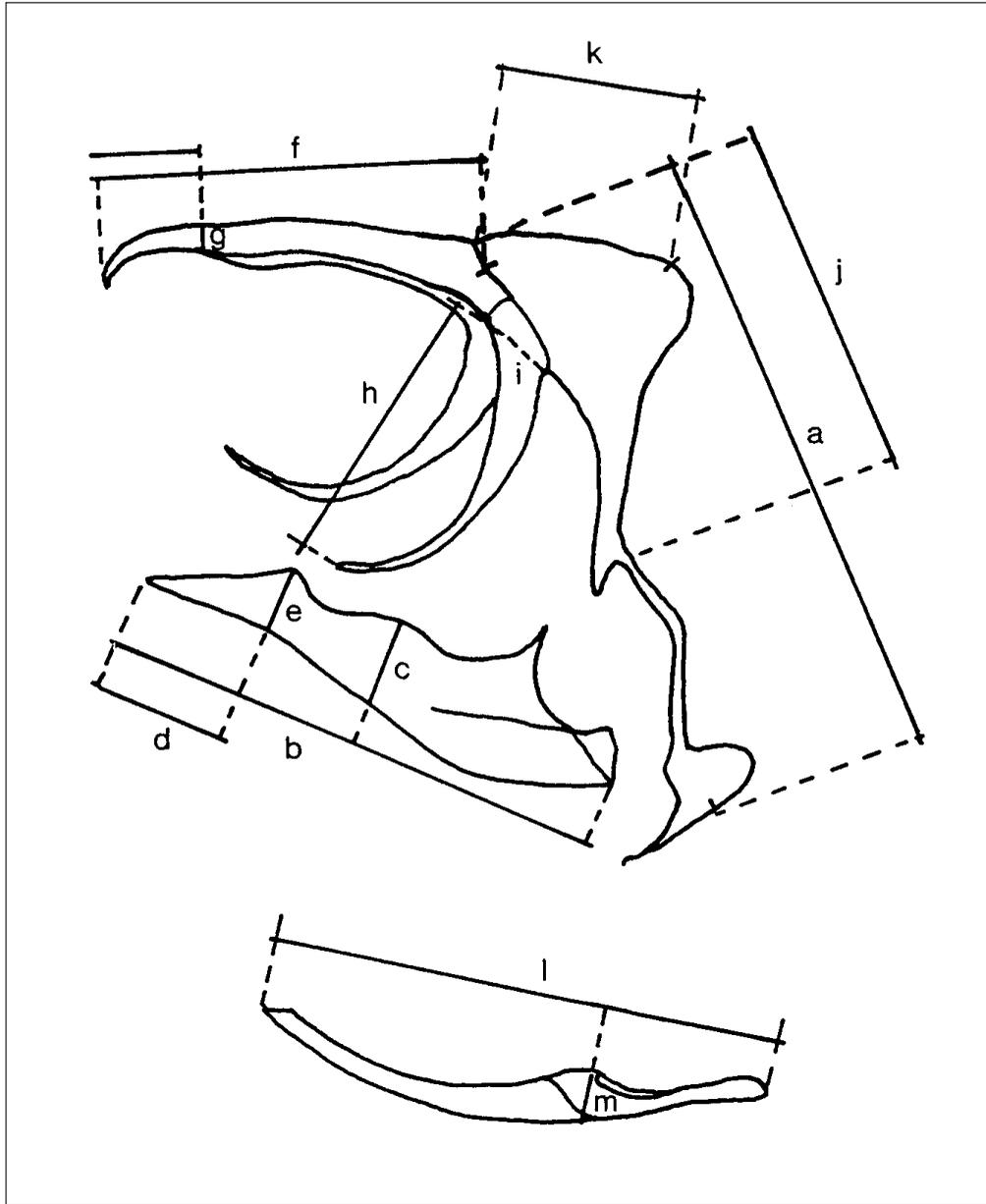
## Methods

**Sources of data and measurements.** The genitalia data used in the current study are taken from 82 male butterfly specimens captured in the Balkans and other areas in Europe (see Appendix). These include measurements from 20 specimens in the second author's collection added to the data set used for  $B_k$  analysis in Wakeham-Dawson et al. (2003), as well as further specimens from the northern Balkans included in that analysis but not clustered. Of these, 54 specimens have been identified by subjective comparison of genitalia (see Kudrna 1977; Jakšić 1998) and capture locality in relation to type locality for the nominal taxa; a further 28 specimens are not assigned to a

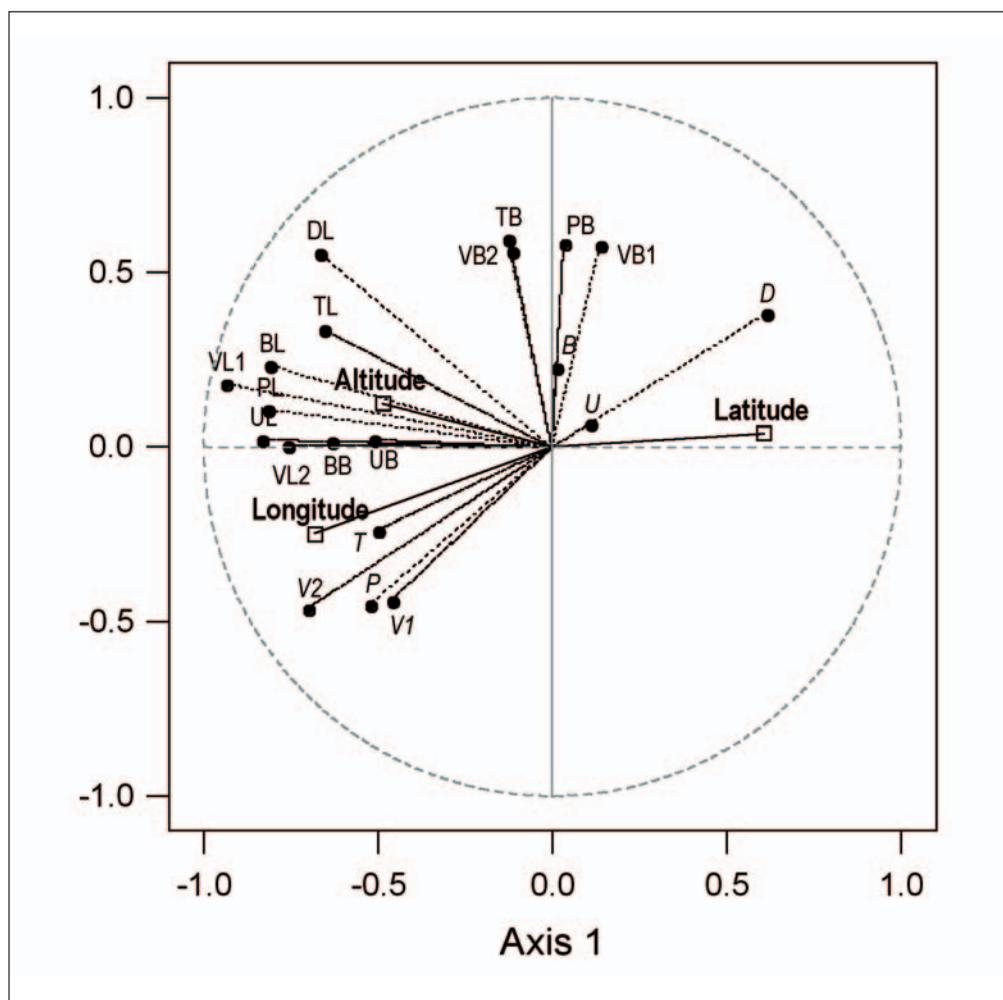
taxon. The first author made all the measurements using the methods described in Wakeham-Dawson (1998) (see Fig. 1). Diagonal length (DL) is divided by valve length (VL1) to produce a unitless ratio  $D$ , which measures overall proportion (shape) of the genitalia independently of size variation between individuals in a taxon. Similarly, valve length (VL1) is divided by valve breadth (VB2) to produce a ratio  $V1$ , representing overall valve shape. Posterior valve length (VL2) is divided by posterior valve breadth (VB1) to produce a ratio  $V2$ , representing valve shape at the posterior end of the valve. Uncus length (UL) is divided by uncus breadth (UB) to produce a ratio  $U$ , representing uncus shape. Brachium length (BL) is divided by brachium breadth (BB) to produce a ratio  $B$ , representing brachium shape. Tegumen length (TL) is divided by tegumen breadth (TB) to give a ratio  $T$ , representing tegumen shape, and phallus length (PL) is divided by phallus breadth (PB) to give a ratio  $P$ . This provides 20 variables (13 measurements and 7 ratios) for analysis.

**Statistical analysis.** We first investigated the dimensionality and relationships among the genitalia variables using principal components analysis (PCA). This also allows the examination of specimens, and the taxa to which they putatively belong, in a reduced space accounting for key amounts of the variance, typically the first two dimensions. We entered key geographical variables (latitude, longitude and altitude) as supplementary to the analysis allowing trends in variables and taxa to be determined.

Second, we determined the degree to which the *Hipparchia* specimens can be discriminated and classified to taxa by using discriminant function analysis (DFA). DFA, like PCA, also produces linear combinations of the original variables, but DFA constructs these new variables (discriminant functions) to maximize differences between groups allocated a priori to analysis. To be completely effective as a technique the groups need to be allocated on different criteria (variables) from those used in the DFA. We have not been able to apply such rigour here. But, a number of the individuals ( $N = 54$ ) were previously allocated to taxa on the basis of geography and visual inspection of the genitalia, as indicated above, independently of this analysis. DFA is particularly useful in the current case for identifying significant discriminatory variables and for classifying specimens without labels. For DFA, variables were first selected as being significant discriminators by applying ANOVA. We have chosen non-metric multidimensional scaling (NMMS) based on Mahalanobis's distance ( $D^2$ ) for the placement of individuals in the multidimensional discriminant space; this allows distances on all discriminant axes to be adequately portrayed instead of their placement on just the first two discriminant axes. DFA produces Mahalanobis's  $D^2$  between taxa and groups. The closer a particular specimen's discriminant score is to a particular taxon's mean location (centroid) in the discriminant space (measured by Mahalanobis's  $D^2$ ) compared to the mean location of other taxa, the more likely it is that it belongs to that taxon. Similarly, the closer a taxon (represented by group centroids) is to another in discriminant space, the more similar the two are morphologically. Wilks's lambda ( $\lambda$ ) measures the discriminatory power of the model. Its value ranges from 0 (perfect discriminatory power) to 1 (no discriminatory power). The



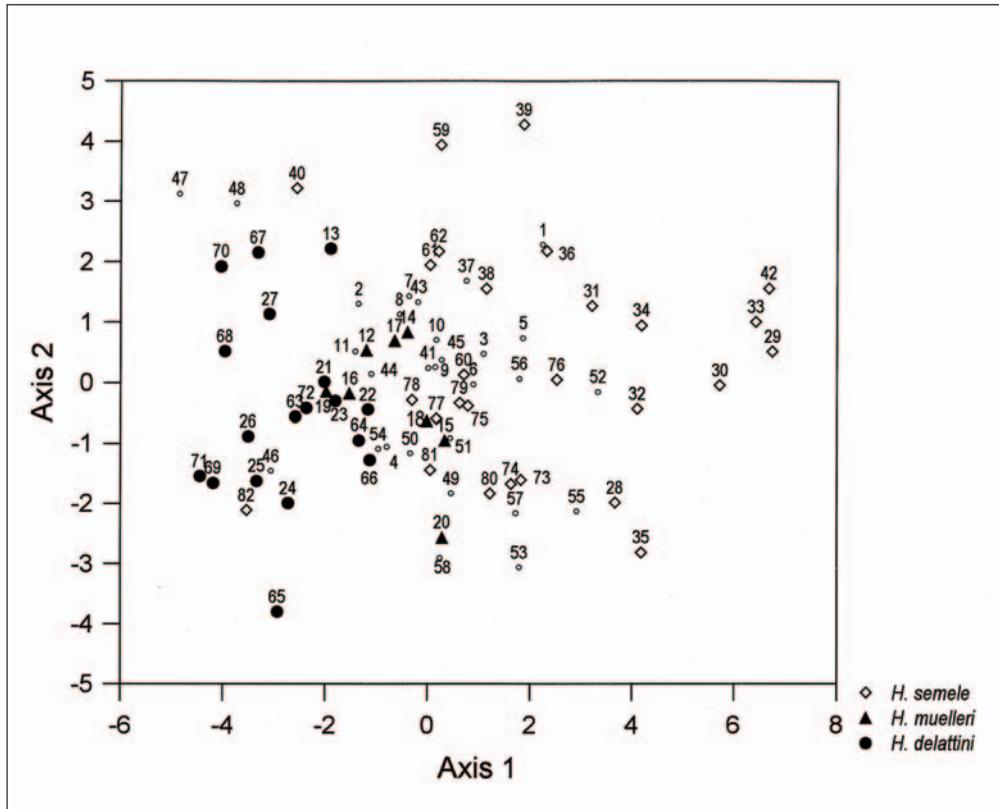
**Fig. 1.** Diagram of male genitalia, including measurements made on male genitalia, of *Hipparchia* butterflies. Terminology based on Higgins 1975. a = diagonal length (DL), measured from dorsal junction of tegumen and uncus to base of saccus (the line running at the same angle as the vinculum); b = valve length (VL1); c = valve breadth (VB2), measured across the widest part of the central valve process and at 90° to the line b; d = posterior valve length (VL2), measured from valve apex to line e; e = posterior valve breadth (VB1), measured across the widest part of the terminal valve process and at 90° to the line b; f = uncus length (UL), measured from uncus apex to mid-point between junction of tegumen and uncus; g = uncus breadth (UB), measured at 0.5 mm from uncus apex and at 90° to line f; h = brachium length (BL), measured from apex of brachium to dorsal junction of tegumen and brachium; i = brachium breadth (BB), measured across junction of tegumen and brachium; j = tegumen length (TL), measured from dorsal junction of tegumen and uncus to junction of apex angularis and vinculum, at same angle as line a; k = tegumen breadth (TB); l = phallus length (PL); m = phallus breadth (PB).



**Fig. 2.** Principal components analysis of 20 genitalia variables (13 measurements and 7 ratios) in *Hipparchia* (N = 82). Geographical variables (latitude, longitude and altitude) were entered as supplementary to the analysis. See Tables 2 and 3 for eigenvalues and loadings for axes.

success of DFA is determined by the percentage of specimens classified correctly as predicted, which itself requires prior assumptions of the validity of the taxa involved and of material that can be referred definitely to them.

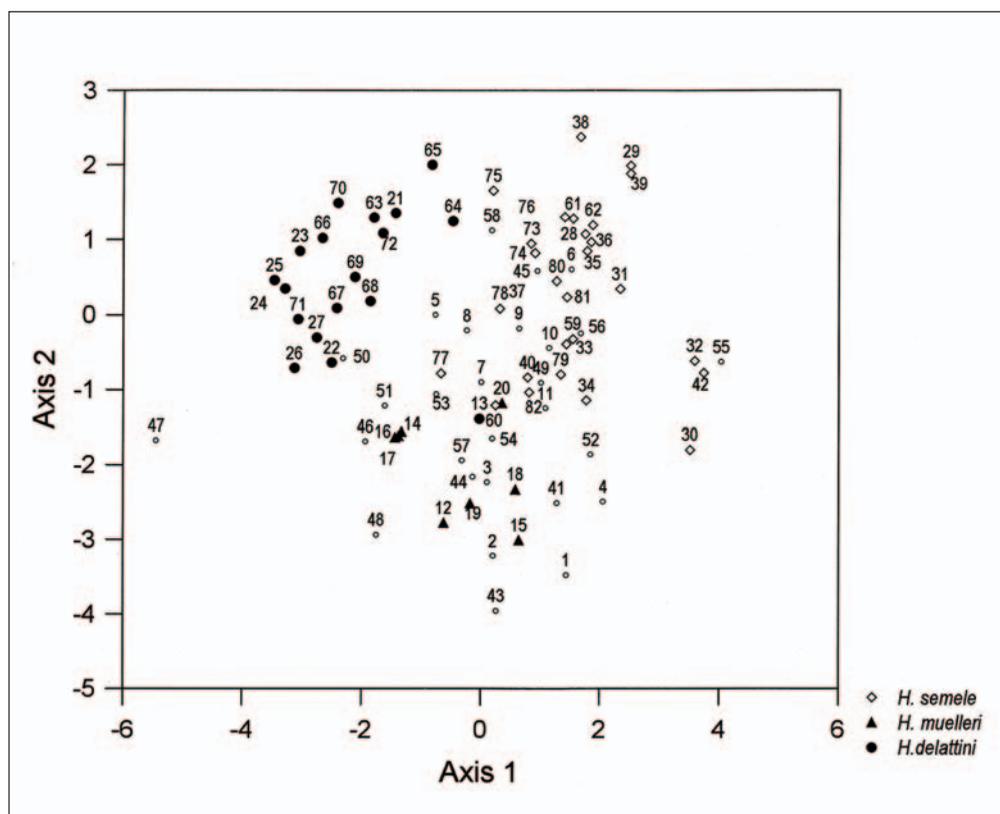
Third, we subjected the data on the 82 specimens for the 20 variables to k-means clustering (Legendre & Legendre 1998). This technique starts with  $k$  random clusters and then moves objects between those clusters with the goal to (i) minimize variability within clusters and (ii) maximize variability between clusters. The technique is related to ANOVA, and the success of the operation is determined from the F statistics associated with each dimension (variable). It is a suitable technique for situations when a certain number of groups is suspected to exist and allows the testing of *a priori*



**Fig. 3.** Placement of *Hipparchia* specimens (N = 82) in the first two axes of a principal components analysis for 20 genitalia variables (see Fig. 2 and Tables 2 and 3). Undesignated specimens prior to analysis indicated by small dots.

classifications. Here, we tested for 3 groups as three putative taxa occur but also discuss results for a  $2k$  solution. The approach we used is to ‘seed’ the analysis with three ‘types’ for *H. semele* (awd281 (case 30) from Eastbourne, Sussex, UK), *H. muelleri* (awd127 (case 12) from Mount Chelmos, S. Greece) and *H. delattini* (pj117 [kos-5/6293] (case 65) from Pristina, Serbia), the latter two coming from the type localities and chosen independently of the DFA.

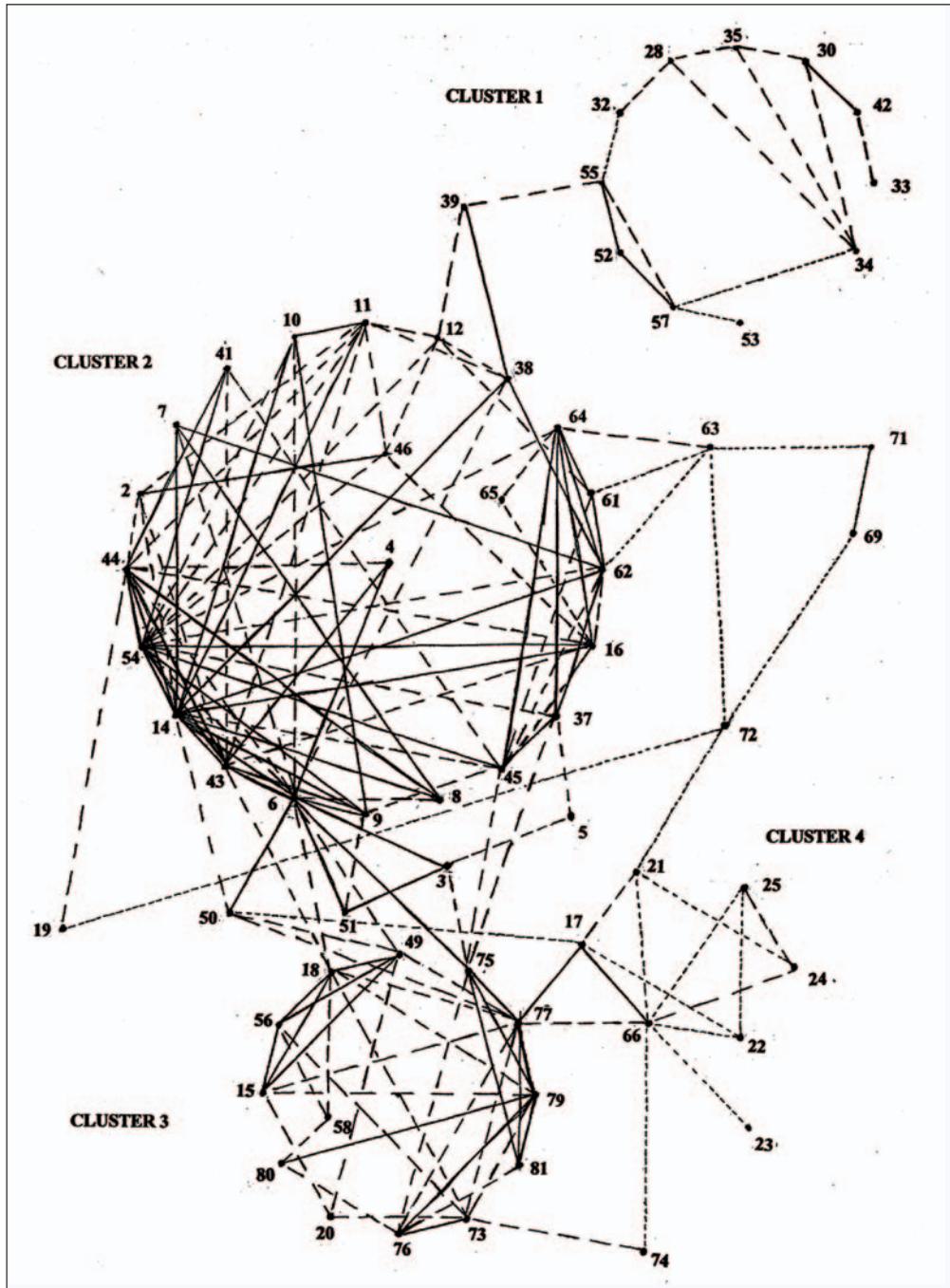
Fourth, non-hierarchic (permitting clusters to overlap)  $B_k$  clustering procedure of Jardine & Sibson (1968) is used to explore clustering structure in data sets, using association coefficients (Euclidean distance measures) derived from all variables. This method is independent of *a priori* classifications or hypotheses of the number of groups expected.  $B_k$  clustering is used to build up linkage diagrams from low to progressively greater distance levels and assessing them for clustering structure. In  $B_k$  clustering, as values of  $k$  increase, the number of links required below a certain distance level for an operational taxonomic unit (OTU) to join a cluster is equal to  $k$ , but  $k-1$  OTUs can also fall into an overlap between clusters under certain circumstances



**Fig. 4.** Non metric multidimensional scaling plot (2 dimensions) of Mahalanobis's  $D^2$  distances for the 82 *Hipparchia* specimens based on 20 variables (measurements and ratios). Kruskal stress and coefficient of alienation < 1%. See Appendix for specimen locality data and text for explanation. Undesignated specimens prior to analysis indicated by small dots.

without those clusters being thereby united. Single linkage is the first in the sequence, with  $k=1$  and therefore no overlap, and represents the only situation where a hierarchic classification is derived. In the approach of assessing linkage diagrams under  $B_k$  rules, the clusters forming as one moves from a low to a high dissimilarity (distance) level are examined for the extent of linkage amongst their component OTUs in relation to linkages with other clusters. In the situation of discriminating taxa, evidence is sought for strong within-cluster linkage and of only limited between-cluster linkage and few OTUs in cluster overlap.

The method has been applied also in biogeographic and ecological analyses to explore commonality of distribution patterns and associations of species across samples (e.g. Holloway 1977, 1979; Davis et al. 2001). It offers a more sensitive method of recapturing information on clustering structure in data than other approaches such as averaging methods which are usually favoured because the results produced appear more clear-cut, but sometimes unjustifiably so because of their tendency to break chaining in the data structure rather than identify where it occurs.



**Fig. 5.** Linkage diagram showing the links clustering 64 of the 82 specimens to a dissimilarity level of 0.40, although for clarity, only links to 0.35 are shown for Clusters 2 and 3. The additional links to 0.40 are predominantly within each of these two clusters, though 75 and 77 (two links) and 18 (seven links) in Cluster 3 show further affinity to Cluster 2 and could also be considered intermediate. Links to 0.30 are shown as solid lines, those to 0.35 as broken lines, and those to 0.40 outside clusters 2 and 3 as lines of small dashes. 18 of the next 22 links for Cluster 1 (except OTU 53) are interior to the cluster, supporting its distinctiveness and homogeneity.

**Tab. 1.** Measurements (mm) (means  $\pm$  standard errors) of male genitalia in three *Hipparchia* taxa from six geographical localities. N = number of specimens.

Taxon & Locality	<i>H. semele</i> (UK) N=9		<i>H. semele</i> (France/ Spain) N=9		<i>H. muelleri</i> (S. Greece) N=8		<i>H. delattini</i> (Kosovo) N=7		<i>H. delattini</i> (N. Greece) N=8	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
DL	3.24	0.03	3.76	0.08	3.48	0.07	3.75	0.07	3.58	0.05
VL1	2.47	0.05	2.99	0.06	3.01	0.04	3.19	0.06	3.14	0.03
VL2	0.54	0.02	0.63	0.02	0.71	0.02	0.74	0.03	0.74	0.02
VB1	0.46	0.02	0.48	0.02	0.47	0.02	0.42	0.03	0.42	0.02
VB2	0.51	0.02	0.51	0.01	0.51	0.01	0.47	0.03	0.58	0.01
UL	1.76	0.02	2.13	0.05	2.22	0.05	2.50	0.05	2.53	0.06
UB	0.16	0.01	0.15	0.01	0.23	0.01	0.20	0.01	0.23	0.01
BL	1.33	0.04	1.71	0.04	1.61	0.05	1.95	0.05	1.82	0.05
BB	0.26	0.01	0.31	0.02	0.35	0.01	0.38	0.02	0.34	0.01
TL	1.91	0.05	2.28	0.06	2.08	0.06	2.36	0.07	2.13	0.05
TB	1.28	0.03	1.43	0.02	1.24	0.03	1.34	0.04	1.24	0.04
PL	2.77	0.07	3.24	0.07	3.30	0.03	3.33	0.07	3.43	0.05
PB	0.29	0.02	0.34	0.01	0.31	0.01	0.29	0.01	0.30	0.01

All procedures, but the  $B_k$  cluster analysis, have been carried out in STATISTICA version 5.5 (Statsoft 1999). It was unnecessary to normalise the variables. Variables have been standardised for the PCA, DFA and k-means clustering and analysis has been carried on all 20 measurements and the seven ratios separately for the three (*H. semele*, *H. muelleri* and *H. delattini*) and two taxa (*H. semele* and *H. delattini*).

## Results

Summary statistics are provided in Table 1 for geographical units with adequate samples rather than the taxa as these concepts of taxa may prove to be unsupported. Principal components analysis. Table 2 and 3 record the eigenvalues and loadings for the 20 variables. The first two axes accounted for 47% of the variance; the distribution of variables and specimens in the axes are provided in Figs 2 and 3. Axis 1 primarily distinguished the majority of variables from *D*, the ratio of diagonal length (DL) to valve length (VL1). All but three variables (UB, *U* and *B*) have their highest loadings on the first two axes. Latitude increases in the same direction as *D*, whereas the majority of other variables are related to increasing longitude and altitude (Fig. 2). Latitude correlates significantly with 13 of the genitalia variables, longitude with 14 and altitude with 10 variables at  $P < 0.05$  (11, 12 and 5 respectively with Bonferroni correction at  $P < 0.0025$ ). The distribution of putative taxa correspond with this pattern; a clear gradient is established from *H. semele* through *H. muelleri* to *H. delattini*, the latter increasingly located in the direction of higher values (increased size) for most genitalia variables and with increasing longitude and altitude (Fig. 3). Principal components of the 7 ratios produced much the same results (not tabulated). The first two axes accounted for 53% of the variance. All variables had their highest loadings on the first two axes. Axis 1 separated *VI*, *V2*, *T* and *P* (loadings  $-0.60$  to

**Tab. 2.** Eigenvalues and extracted variables for the first 10 components of a principal components analysis on 20 genitalia variables (13 measurements and 7 ratios).

Component extracted	Eigenvalue	% Total variance	% Cumulative variance
1	6.715	33.58	33.58
2	2.727	13.63	47.21
3	2.394	11.97	59.18
4	1.769	8.85	68.03
5	1.308	6.54	74.57
6	1.213	6.07	80.64
7	1.065	5.32	85.96
8	0.976	4.88	90.84
9	0.518	2.59	93.43
10	0.470	2.35	95.78

**Tab 3.** Loadings for the first six axes of a principal components analysis of 20 genitalia variables (13 measurements and 7 ratios) in *Hipparchia* specimens (\* supplementary to analysis. *Hipparchia* specimens include all 82 individuals in the study).

Variable	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5	Axis 6
DL	-0.672	0.555	0.109	-0.219	-0.049	0.252
VL1	-0.941	0.180	-0.033	0.032	-0.065	-0.032
VL2	-0.760	0.002	-0.154	0.159	0.059	-0.161
VB1	0.137	0.576	-0.274	0.131	0.111	0.258
VB2	-0.117	0.558	-0.496	0.478	0.162	-0.065
UL	-0.837	0.022	0.095	0.211	0.139	-0.204
UB	-0.515	0.020	-0.687	-0.026	-0.219	0.012
BL	-0.812	0.232	0.184	0.079	0.023	-0.031
BB	-0.637	0.017	-0.172	-0.394	0.593	-0.040
TL	-0.656	0.339	0.311	-0.228	-0.042	0.380
TB	-0.132	0.594	0.548	0.116	0.184	-0.060
PL	-0.818	0.106	0.027	0.045	-0.175	-0.065
PB	0.030	0.581	-0.090	-0.518	-0.316	-0.491
D	0.609	0.381	0.163	-0.274	0.036	0.349
V1	-0.461	-0.441	0.443	-0.439	-0.199	0.062
V2	-0.706	-0.463	0.095	0.018	-0.075	-0.322
U	0.106	0.064	0.804	0.095	0.294	-0.172
B	0.008	0.229	0.393	0.533	-0.678	0.045
T	-0.504	-0.241	-0.209	-0.316	-0.228	0.425
P	-0.524	-0.450	0.096	0.496	0.171	0.416
*Latitude	0.607	0.038	0.297	0.134	0.050	0.071
*Longitude	-0.682	-0.247	-0.398	0.037	0.052	-0.064
*Altitude	-0.488	0.123	0.100	0.194	-0.032	-0.162

–0.83) from *D* (+0.76), and axis 2 distinguished variables *U* and *B* (+0.70 and +0.77) from the remainder (<0.20). Longitude, altitude and latitude are again polarised on Axis 1, with longitude (-0.66) and altitude (-0.34) relating to *V1*, *V2*, *T* and *P* and latitude (+0.50) to *D*. The distribution of taxa is similar to that from PCA on all variables, but *a priori* designated *H. muelleri* are offset from *H. semele* and *H. delattini* – a distribution reappearing in the NMMS plot of Mahalanobis's  $D^2$  distances (Fig. 4) – rather than sandwiched between them, and linking up more with variable *T* rather than *V1*, *V2* and *P*.

**Discriminant function analysis.** ANOVA identified 17 of the 20 variables that significantly distinguished *a priori* labelled *Hipparchia* to the three taxa ( $F_{(3,50)} = 2.82$  to  $22.70$ ,  $P = 0.048$  to  $< 0.00001$ ), 13 variables at  $P < 0.0025$  (Bonferroni correction); the exceptions were VB2, TB and *V1*. Stepwise DFA of the 54 specimens that had been labelled (the others had not been assigned to a taxon prior to the classification; see Wakeham-Dawson et al. 2003) selected six variables (UB, UL, VB1, BB, BL and PL) that contributed to the discrimination. This results in 94% correct classification of specimens (Wilks's lambda = 0.136,  $F_{(12,90)} = 12.81$ ,  $P < 0.001$ ), with only three specimens being incorrectly classified. The parameters for variables are given in Table 4, the distances in Table 5 (lower diagonal) and the classification matrix in Table 6. An NMMS plot of Mahalanobis's  $D^2$  distances between labelled specimens, including those not labelled, shows three clusters, each overlapping to a degree but with '*semele*' and '*delattini*' separated more than '*muelleri*' and '*semele*' (Fig. 4). The predicted classification of individuals not previously tagged is given in the Appendix together with a marker for those misclassified. Specimens classified as *H. delattini* are restricted to the Balkans but those determined to be *H. semele* occur as far east as 23°E and down the west side of the Balkans.

Stepwise discriminant function analysis for the three taxa based only on the seven ratios selected three variables that contributed to the discrimination of *a priori* designated individuals (*V2*, *U*, *B*). Significant discrimination was achieved (Wilks's lambda: 0.32,  $F_{(6,98)} = 12.443$ ,  $P < 0.00001$ ) with 85.2% correct classification of designated individuals. *H. muelleri* was intermediate in distance between *H. semele* and *H. delattini* with all Mahalanobis's  $D^2$  distances being significant (see Table 5, upper diagonal).

Further DFA for just two taxa (*H. semele* and *H. delattini*) based on the seven ratios selected three variables (*V2*, *V1*, *T*) that discriminated between individuals. Significant discrimination was achieved (Wilks's lambda = 0.35,  $F_{(3,42)} = 25.52$ ,  $P < .00001$ ) with 93.48% correct classification. *H. muelleri* specimens were regarded as unclassified prior to the analysis. Mahalanobis's  $D^2$  between the two groups is = 7.65,  $F_{(3,42)} = 25.40$ ,  $P < 0.00001$ . Individuals classified as *H. delattini* occurred as far west as 14°E and those classified as *H. semele* occurred as far east as 23°E. An interesting outcome is that of the collection of eight *H. muelleri* specimens from the same locality (22°E, 38°N), six were classified as *H. semele* (cases 12, 14–17, 19) and two as *H. delattini* (cases 18, 20).

**K-means clustering.** This technique resolved three main clusters based on three type individuals, group 1 *H. semele* (n = 15), group 2 *H. muelleri* (n = 42) and group 3

**Tab. 4.** Parameters for variables entered into stepwise (forward) discriminant function analysis of 54 specimens designated a priori to three *Hipparchia* taxa (Wilks's Lambda: 0.13632.  $F_{(12,90)}=12.813$   $P < .00001$ ).

Variable	Wilks' lambda	Partial lambda	$F_{(2,45)}$ to remove	P level
VB1	0.190	0.718	8.82	0.0006
UL	0.167	0.817	5.04	0.0106
UB	0.235	0.579	16.35	0.0000
BL	0.177	0.769	6.75	0.0027
BB	0.166	0.821	4.90	0.0119
PL	0.162	0.841	4.25	0.0204

**Tab. 5.** Mahalanobis's  $D^2$  between taxa for the 54 designated *Hipparchia* specimens (Lower triangle based on all genitalia variables; for comparison, the upper triangle is based on 7 ratio variables).

Taxa	<i>H. semele</i>	<i>H. muelleri</i>	<i>H. delattini</i>
<i>H. semele</i>	0.00	4.67	6.20
<i>H. muelleri</i>	10.27	0.00	4.27
<i>H. delattini</i>	14.70	10.25	0.00

**Tab. 6.** Classification matrix from discriminant function analysis of 54 specimens designated a priori to three *Hipparchia* taxa (Rows: observed classifications; columns: predicted classifications).

Group identity	Percent Correct	<i>H. semele</i>	<i>H. muelleri</i>	<i>H. delattini</i>
<i>H. semele</i>	92.86	26	2	0
<i>H. muelleri</i>	100.00	0	8	0
<i>H. delattini</i>	94.44	0	1	17
Total	94.44	26	11	17

*H. delattini* (n = 25). The distances between the groups and the accompanying ANOVA results are given in Tables 7 and 8. In phenetic (Euclidean) distances, group 2 is intermediate to groups 1 and 3, mirroring the PCA and DFA results. Fifteen variables have significant F values at  $P < 0.05$ ; this number is still 14 with a Bonferroni correction. Nearly 65% of individuals were correctly assigned to the presumptive taxa. However this figure varied considerably among taxa, respectively 42.9% for *H. semele*, 75.0% for *H. muelleri* and 94.4% for *H. delattini* (Table 9). *H. semele*

**Tab. 7.** K-means clustering for three groups, Analysis of Variance

Variable	Between SS	Within SS	F <sub>(2,79)</sub>	P
DL	28.564	52.436	21.517	<0.00001
VL1	53.195	27.805	75.571	<0.00001
VL2	35.114	45.886	30.227	<0.00001
VB1	4.737	76.263	2.454	0.093
VB2	6.434	74.566	3.408	0.038
UL	51.421	29.579	68.669	<0.00001
UB	17.968	63.032	11.260	0.00005
BL	45.110	35.890	49.648	<0.00001
BB	28.991	52.009	22.018	<0.00001
TL	27.136	53.864	19.899	<0.00001
TB	1.693	79.307	0.843	0.434
PL	45.402	35.598	50.378	<0.00001
PB	1.949	79.051	0.974	0.382
D	23.194	57.806	15.849	<0.00001
V1	12.340	68.660	7.099	0.0015
V2	39.150	41.850	36.952	<0.00001
U	0.940	80.060	0.464	0.631
B	1.227	79.773	0.608	0.547
T	19.601	61.399	12.610	0.00002
P	27.071	53.929	19.828	<0.00001

**Tab. 8.** K-means clustering for three groups, Euclidean distances between clusters (Distances below diagonal; squared distances above diagonal).

Groups	No. 1 ( <i>H. semele</i> )	No. 2 ( <i>H. muelleri</i> )	No. 3 ( <i>H. delattini</i> )
No. 1 ( <i>H. semele</i> )	0.000	0.760	2.366
No. 2 ( <i>H. muelleri</i> )	0.872	0.000	0.539
No. 3 ( <i>H. delattini</i> )	1.538	0.734	0.000

tended to be misclassified to *H. muelleri*, *H. muelleri* to *H. delattini* and *H. delattini* to *H. muelleri*. The groups were significantly different for geography and altitude with group number corresponding to the seeding by *H. semele*, *H. muelleri* and *H. delattini* respectively; specimens classified to *H. semele* occur to the north and west, and at the lower altitudes, those classified to *H. delattini* occur to the south and east, and at higher altitudes and specimens classified to *H. muelleri* occur at intermediate altitudes and geographical locations (Table 10). Even so, there is substantial geographical

**Tab. 9.** Summary (means and standard errors) for geographical variables of clusters from *k*-means clustering (ANOVA on normalized data; latitude:  $F_{(2, 79)}=19.23$ ,  $P < 0.00001$ ; longitude:  $F_{(2, 79)}=29.84$ ,  $P < 0.00001$ ; altitude:  $F_{(2, 79)}=11.79$ ,  $P < 0.00003$ ).

Groups	No. 1 ( <i>H. semele</i> )	No. 2 ( <i>H. muelleri</i> )	No. 3 ( <i>H. delattini</i> )	Total	% correct classification
<i>H. semele</i> *	12	14	2	28	42.9
<i>H. muelleri</i> *	0	6	2	8	75.0
<i>H. delattini</i> *	0	1	17	18	94.4
Total	12	21	21	54	64.8

**Tab. 10.** Summary (means and standard errors) for geographical variables of clusters from *k*-means clustering (ANOVA on normalized data; latitude:  $F_{(2, 79)}=19.23$ ,  $P < 0.00001$ ; longitude:  $F_{(2, 79)}=29.84$ ,  $P < 0.00001$ ; altitude:  $F_{(2, 79)}=11.79$ ,  $P < 0.00003$ ).

CLUSTER	Latitude		Longitude		Altitude	
	Mean	SE	Mean	SE	Mean	SE
No. 1 ( <i>H. semele</i> )	47.83	1.12	4.83	1.96	453.33	163.55
No. 2 ( <i>H. muelleri</i> )	42.88	0.51	15.70	1.01	683.33	80.43
No. 3 ( <i>H. delattini</i> )	41.59	0.39	20.53	0.82	1102.00	97.83
All groups	43.39	0.42	15.18	0.89	768.90	63.84

**Tab. 11.** The geographic distribution of the *Hipparchia* specimens in each  $B_k$  cluster (see text for explanation).

Area	1	2	Intermediate 2/3	3	4	Loose outlying cluster 5	Outliers
Southern England (9)	7	0	1	0	0	0	1
Spain to French Alps (9)	0	7	1	0	0	1	0
Hungary, Romania (5)	0	5	0	0	0	0	0
Bavaria, Austria, Slovenia (10)	0	0	1	8	0	0	1
Croatia (Istria, Dalmatia [Croatia]) (10)	1	4	3	2	0	0	0
Serbia (10)	0	3	0	0	1	4	2
Corfu & Albania (5)	2	2	0	0	1	0	0
N.W. Greece (Epiros) (6)	0	4	1	1	0	0	0
N. Greece (Naoussa) (8)	0	1	0	0	6	1	0
S. Greece (Mt. Chelmos) (8)	0	4	0	3	1	0	0
Greece (Kevi) (2)	0	0	0	0	0	1	1
Total (82)	10	30	7	14	9	7	5

overlap based on all 82 specimens with *H. delattini* designated specimens being found as far west as 2° East and *H. semele* designated specimens being found as far east as 22° East.

Running *k*-means clustering for two groups, seeded with the *H. semele* and *H. delattini* specimens, rather than three is highly instructive. Sixteen variables provide significant group differentiation at  $P < 0.05$  and 14 variables at  $P < 0.0025$ . There is 91.3% correct classification of *H. semele* and *H. delattini*, the two taxa used to seed the two groups. All *H. delattini* are correctly classified and all but four of the 24 *H. semele*. The eight *H. muelleri* are allocated to group 2 (*H. delattini*). In the  $k = 2$  solution, there is as much geographical overlap of all 82 specimens as in the  $k = 3$  means clustering solution, despite the fact that they again differ for longitude, altitude and latitude (t test;  $t_{(80)}$ : latitude 6.14, longitude -6.60, altitude -4.90; all  $P < 0.00001$ ). Group 1 (seeded with *H. semele*) occur eastwards to 20°E and Group 2 (seeded with *H. delattini*) occur west to 2°E.

$B_k$  analysis. This produces four main clusters, and a number of clusters that are not so well defined (Fig. 5). Cluster 1 includes nominal *H. semele* specimens from southern England and three from the Balkans. These specimens are: awd279 (28), awd281 (30), awd283 (32), awd284 (33), awd285 (34), awd286 (35), awd287 (42), bmnh13377 (52), bmnh13374 (55), and bmnh29808 (57). See Appendix for capture locations. Numbers in parenthesis are those allocated to each specimen during cluster analysis and used in Figs 3 & 4. Cluster 2 largely contains Balkan specimens, including most of the *H. muelleri* topotypes and some unidentified specimens. These specimens are: bmnh29865 (2), bmnh29867 (4), bmnh29872 (6), bmnh29869 (7), bmnh29874 (8), bmnh29875 (9), bmnh29871 (10), bmnh29868 (11), awd127 (12), awd382 (14), awd386 (16), awd316 (37), awd288 (38), and awd289 (39). Cluster 2 has some peripheral specimens; these are: bmnh11994 (1), awd247 (13), awd392 (19), awd427 (59), awd428 (60), and pj115 (63). Cluster 2 links through intermediates with Cluster 3; this includes *H. muelleri* topotypes and nominal *H. semele* specimens. These are specimens: awd383 (15), awd391 (18), awd393 (20), jdh60 (49), bmnh29807 (56), bmnh29809 (58), pj61 (73), pj62 (74), pj63 (75), pj64 (76), pj65 (77), pj67 (79), pj68 (80), pj70 (81). Specimens: bmnh29866 (3), bmnh29870 (5), awd282 (31), awd315 (36), jdh 59 (50), bmnh13378 (51), and pj66 (78) are those intermediate between Clusters 2 and 3. Cluster 4 contains nominal *H. delattini* specimens from North Greece and an *H. delattini* topotype from Pristina, Kosovo. These are specimens: awd387 (17), awd394 (21), awd396 (22), awd398 (23), awd399 (24), awd400 (25), and pj118 (66). Specimens: awd401 (26) and bmnh13375 (53) are peripheral to Cluster 4. An additional loose cluster (Cluster 5) contains a mix of specimens that includes *H. delattini* topotypes from Pristina, Kosovo. These are specimens: pj121 (69), pj123 (71), pj124 (72), awd402 (27), awd292 (40), awd356 (48), and pj122 (70). Specimens: awd280 (29), awd355 (47), pj119 (67), pj120 (68), and pj71 (82) are extreme outliers. These clusters show some geographical segregation, although the sample size for each geographic region in Table 11 is small.

A DFA (based on the genitalia measurements and shape ratios) of the specimens identified in the five main  $B_k$  clusters confirms the integrity of these clusters by showing

segregation of the clusters (Wilks's lambda = 0.008,  $F_{(32,185)} = 15.45$ ,  $p < 0.001$ ) and 95% correct classification of specimens within clusters (variables UL, DL, PL, *U*, BL, TB, BB and *B* retained in the stepwise forward entry analysis). However, there is some overlap between clusters, especially if only ratios are used in a DFA (Wilks's lambda = 0.387,  $F_{(12,145)} = 5.25$ ,  $p < 0.001$ ; 55% correct classification of specimens within clusters; ratios *D*, *U* and *T* retained in the stepwise forward entry analysis), so size is an important factor in segregating specimens. PCA and DFA on the clusters failed to identify just which variables might be used to distinguish individuals to groups as Clusters 1 and 2 did not separate neatly from Clusters 3 and 4. However, PCA suggests that *H. semele* specimens from southern England (Cluster 1) are largely distinct from the three southern European *H. semele* (also included in Cluster 1) because of genital shape differences as well as size difference.

When  $B_k$  clusters are presented visually (Fig. 5), the nominal *H. semele* specimens from southern Europe are grouped into Clusters 2 & 3 with the nominal *H. muelleri* specimens. The nominal *H. semele* specimens from southern England (Cluster 1) are segregated to one side of Cluster 2 and nominal *H. delattini* specimens (Cluster 4) from North Greece and Kosovo are segregated to the other side.

## Discussion

This study was generated by questions concerning the number of *Hipparchia* taxa in the Balkans, particularly on the existence of one or two *Hipparchia* species closely related to *H. semele*. A number of findings emerge. First, clusters for the three taxa, *H. semele*, *H. muelleri* and *H. delattini*, are repeatedly found in all analyses based on genital morphology; in the PCA, DFA, NMMS of Mahalanobis's  $D^2$  distances, the *k*-means clustering and the  $B_k$  clustering. Second, even so, the clusters merge and are not so distinct that every individual can be categorised unequivocally to one of the three taxa, certainly not on individual genital morphology and with even less success for genital ratio variables which control for differences in absolute size. Overlap occurs for the putative taxa in PCA, the NMMS plot of Mahalanobis's  $D^2$  distances and  $B_k$  clustering and incorrect classification occurs in DFA and *k*-means clustering. Third, from the highly significant associations of genital morphology with longitude, latitude and altitude, there is a strong suggestion of a gradient (cline) in genital morphology. In this, *H. muelleri* largely adopts an intermediate position between *H. semele* and *H. delattini*, but not exactly an intermediate position for all genital attributes as evident in the PCA of ratio data. Just whether *H. muelleri* is more closely associated with *H. semele* or *H. delattini* depends on the analysis being applied and which variables are used. For example, DFA on two groups for ratios links *H. muelleri* more with *H. semele*, but *k*-means clustering for  $k = 2$  on ratios allocates *H. muelleri* to *H. delattini*.

Fourth, despite the apparent cline for many variables in genital morphology, classification (DFA, *k*-means clustering) reveals a geographical overlap of taxa, regardless of whether two or three groups are considered. The overlap is greater for *k*-means clustering than for DFA; the reason for this is that, in the absence of distinct morphological

boundaries between the taxa being considered, *k*-means clustering has shifted the morphological boundary between them into *H. semele*; that is, more *a priori* designated *H. semele* are misclassified than *H. delattini* and the latter has grown in number and geographical range (expanding westwards) at the expense of the former. A more conservative picture is provided by DFA on two groups for ratios. This shows that *H. delattini* extends no further west than 14°E but that *H. semele* extends eastwards to 23°E. It is a more reliable picture since complications associated with *H. muelleri* are removed and genital comparisons are based on ratios, not purely on size aspects on genital morphology.

Arising, then, from this analysis is the possibility of two *Hipparchia* taxa present in the Balkan study area, a finding greatly in need of confirmation from more extensive sampling and DNA markers. Broadly, these are *H. semele* and *H. delattini* (which includes *H. muelleri*-like forms). Kudrna (1975) originally described *H. muelleri* as a subspecies of *H. semele*; on the whole, we find *H. muelleri* to be closer to *H. delattini* than to *H. semele* in male genital morphological space. The two species may have broader distributions (geographical ranges) than previously considered. *H. delattini*, which is probably conspecific with Russian *H. volgensis* (see Kudrna, 1977), appears to be largely confined to the Balkans and Northern Greece. *H. semele* is present in western Europe, but also into the Balkans. The complexity of some clusters (clusters 2 and 3) in  $B_k$  clustering may point towards hybridisation between the two taxa.

The suggested relationship between taxa and altitude accords with the findings of Pamperis (1997), who recorded *H. muelleri* at low to intermediate altitudes and *H. delattini* (synonymised with *H. volgensis*) at intermediate to higher altitudes in Greece. However, it should be noted that the validity of Pamperis' wing-pattern based method of taxa identification has been questioned by Wakeham-Dawson & Kudrna (2000).

The two apparent taxa of the current study may be the product of speciation during isolation in ice-age refugia; other species conform to this east versus west European pattern (see Dennis et al. 1991; Dennis 1993; Hewitt 1999, 2000; Schmitt & Seitz 2001a, b). This model would describe the different taxa evolving in western (*H. semele*) and eastern (*H. delattini* or *H. volgensis*) regions at Mediterranean latitudes and recolonising northern areas in Europe and coming into contact following each glacial stage. Subsequent glacial advances tend to erase populations north of the Alps in which case genetic differentiation is maintained and enhanced during glacial-interglacial cycles (Dennis et al. 1991). Examples of similar 'cryptic' taxa are provided by sibling species groups of *Maniola jurtina* (Linnaeus, 1758) (Thomson 1987), *Pontia daplidice* (Linnaeus, 1758) and *P. edusa* (Fabricius, 1777) (Geiger et al. 1988) and *Leptidea reali* Reissinger, 1989 and *L. sinapis* (Linnaeus, 1758) (Mazel 2001), the last of which comprises components that extensively overlap in geographical range. The two taxa hypothesis produced by the current study is supported by a study of female genitalia (Coutsis 1983), which shows a clear disjunction in morphology between *H. semele* and *H. delattini*. Coutsis (1983) groups *H. muelleri* with *H. delattini* based on female genital morphology, corresponding with the closer relationship found here for *H. muelleri* and *H. delattini* than that for *H. muelleri* and *H. semele* based on male

genital attributes. No significant difference was found in the morphology of *H. muelleri* and *H. delattini* androconia (Wakeham-Dawson 1998). Even so, in all analyses of male genitalia, there is failure to discriminate absolutely between the three taxa investigated here, and *H. muelleri* emerges as being approximately equidistant from *H. delattini* and *H. semele* in phenetic distances.

The issue arises that variation in male genitalia may not be a reliable taxonomic characteristic. Any structure that is not critical during copulation will probably not be under powerful sexual selection and so will not be a reliable indicator of reproductive isolation between taxa. If this is the case, genital shape may be neutral to selection and subject only to random processes, or to pleiotropic effects. In another satyrine butterfly, *Maniola jurtina* Linnaeus, 1785, there is an absence of an apparent relationship between valve shape and either mating success or strength of the male-female bond (Goulson 1993). However, both valve shape and uncus are much larger in species with a sphragis (e.g., *Heteronympha penelope* Waterhouse (Lepidoptera: Satyridae), and this may be an adaptation to sphragis removal during mating (Orr 2002).

Specimens, which are as yet unmeasured, from Bulgaria, appear to exhibit two clear forms of male genitalia (valve shape) that associate more definitely with *H. semele* or *H. delattini* (S. Abadijev, loan material) than do most specimens from the Balkans. The next stage in this research is: (1) to test the two-taxa (*semele* and *volgensis*) hypothesis with further specimens from across Europe including the Balkans and eastwards into Bulgaria and Russia, and (2) to assess the morphological data against molecular data.

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**Appendix.** Label data of the 82 *Hipparchia* specimens included in the current study. Sp. no. = specimen number; Case no. = cluster number used in cluster diagrams and Figs 3 & 4. Taxon: ? (specimens of uncertain taxonomic attribution) determined by discriminant function analysis; classification noted as 's' *semele*, 'm' *muelleri* or 'd' *delattini*. The three individuals misclassified and their reclassification are indicated by appropriate letter. Specimens deposited at The Natural History Museum, London (BMNH), the collection of A. Wakeham-Dawson (AWD), the collection of P. Jakšić (PJ), the collection of J. D. Holloway (JDH), and the Booth Museum, Brighton (BMB).

sp. no. (Case no.)	Taxon	Country	Location	Notes
bmnh11994 (1)	? m	Italy	Gorizia, La Logua	BMNH
awd127 (12)	muelleri	S. Greece	Mount Chelmos, Pelopónnisos	1300 m, 15.vii.1995, AWD
bmnh13374 (55)	? s	N. Greece	Corfu	BMNH
bmnh13375 (53)	? m	N. Greece	Corfu	BMNH
bmnh13376 (54)	? m	N. Greece	Corfu	BMNH
bmnh13377 (52)	? s	Albania	Tepelenë	16.vi.1933, leg. A.H.G Alston & N.Y. Sandwith, BM 1932-383, BMNH
bmnh13378 (51)	? m	Croatia	Perkovic	12.vi.(19)08, H. Stauder, Fruhstorfer, Coll., BM 1987 285, BMNH
awd247 (13)	delattini m	Macedonia	Séli, Náoussa	1500-1600 m, 21.vi.1996, AWD
awd279 (28)	semele	England	Eastbourne, Sussex	200 m, 4.viii.1996, AWD
awd280 (29)	semele	England	Eastbourne, Sussex	200 m, 4.viii.1996, AWD
awd281 (30)	semele	England	Eastbourne, Sussex	200 m, 4.viii.1996, AWD
awd282 (31)	semele	England	Eastbourne, Sussex	200 m, 4.viii.1996, AWD
awd283 (32)	semele	England	Eastbourne, Sussex	200 m, 4.viii.1996, AWD
awd284 (33)	semele	England	Eastbourne, Sussex	200 m, 4.viii.1996, AWD
awd285 (34)	semele	England	Eastbourne, Sussex	200 m, 4.viii.1996, AWD
awd286 (35)	semele	England	Eastbourne, Sussex	200 m, 4.viii.1996, AWD
awd287 (42)	semele	England	Eastbourne, Sussex	200 m, 4.viii.1996, AWD
awd288 (38)	semele	France	Mount Canigou, E. Pyrenees	2200 m, 28.vii.1993, AWD
awd289 (39)	semele	France	Les Arcs, Alps	2600 m, 1.viii.1993, AWD
awd292 (40)	semele	France	Prades, E. Pyrenees	1000 m, 17.vii.1990, AWD
bmnh29807 (56)	? s	Croatia	Knin	July (19)08, Fruhstorfer, Coll., BM 1987-285, BMNH
bmnh29808 (57)	? m	Croatia	Dernis	10.vi.(19)08, H. Stauder, Fruhstorfer, Coll., BM 1987-285, BMNH
bmnh29809 (58)	? s	Croatia	Perkovic	12.vi.(19)08, H. Stauder, Fruhstorfer, Coll., BM 1987-285, BMNH
bmnh29865 (2)	? m	Italy	Venezia Giulia, Costa Trieste	13.vii.1926, BMNH
bmnh29866 (3)	? m	Italy	Littorale austr.	H. Stauder, 17.vi.1912, BMNH
bmnh29867 (4)	? m	Croatia	St Kriz	12.vii.1912, BMNH
bmnh29868 (11)	? s	Romania	Biharfüred, Bihar Mts	800-1200 m, K. Jordan, 20.vii.1911, BMNH
bmnh29869 (7)	? m	Romania	Herkulesbad	W. R. & E. H., 25.vii.1907, BMNH
bmnh29870 (5)	? d	Croatia	Senjska drega	17.vii.1912, BMNH
bmnh29871 (10)	? s	Hungary	Szovata, Maros-Torda c.	27.vii.1910, BMNH
bmnh29872 (6)	? s	Croatia	Zengg	1913, BMNH
bmnh29874 (8)	? s	Romania	Herkulesbad	21.vii.1907, BMNH
bmnh29875 (9)	? s	Romania	Herkulesbad	W. R. & E. H., 25.vii.1907, BMNH
awd315 (36)	semele	Spain	Albaraccin	1000 m, 20-23.vi.1995, AWD
awd316 (37)	semele	Spain	Albaraccin	1000 m, 20-23.vi.1995, AWD

sp. no. (Case no.)	Taxon	Country	Location	Notes
awd355 (47)	? d	N. Greece	Kevi	22.vi.1981, P.W. Cribb Coll. (Acc. no. 094732) BMB
awd356 (48)	? m	N. Greece	Kevi	22.vi.1981, P.W. Cribb Coll. (Acc. no. 094733) BMB
awd382 (14)	muelleri	S. Greece	Mount Chelmos, Pelopónnisos	1350-1600 m, 14.vi.1997, AWD
awd383 (15)	muelleri	S. Greece	Mount Chelmos, Pelopónnisos	1350-1600 m, 14.vi.1997, AWD
awd386 (16)	muelleri	S. Greece	Mount Chelmos, Pelopónnisos	1300 m, 13.vi.1997, AWD
awd387 (17)	muelleri	S. Greece	Mount Chelmos, Pelopónnisos	1350-1600 m, 14.vi.1997, AWD
awd391 (18)	muelleri	S. Greece	Mount Chelmos, Pelopónnisos	1300 m, 13.vi.1997, AWD
awd392 (19)	muelleri	S. Greece	Mount Chelmos, Pelopónnisos	1350-1600 m, 14.vi.1997, AWD
awd393 (20)	muelleri	S. Greece	Mount Chelmos, Pelopónnisos	1350-1600 m, 14.vi.1997, AWD
awd394 (21)	delattini	N. Greece	Séli, Náoussa, Macedonia	1500-1600 m, 25-26.vi.1997, AWD
awd396 (22)	delattini	N. Greece	Séli, Náoussa, Macedonia	1500-1600 m, 25-26.vi.1997, AWD
awd398 (23)	delattini	N. Greece	Séli, Náoussa, Macedonia	1500-1600 m, 25-26.vi.1997, AWD
awd399 (24)	delattini	N. Greece	Séli, Náoussa, Macedonia	1500-1600 m, 25-26.vi.1997, AWD
awd400 (25)	delattini	N. Greece	Séli, Náoussa, Macedonia	1500-1600 m, 25-26.vi.1997, AWD
awd401 (26)	delattini	N. Greece	Séli, Náoussa, Macedonia	1500-1600 m, 25-26.vi.1997, AWD
awd402 (27)	delattini	N. Greece	Séli, Náoussa, Macedonia	1500-1600 m, 25-26.vi.1997, AWD
awd427 (59)	semele	France	Lozère	W.G. & S.M. Tremewan, 1000 m, 15.vii.1998, AWD
awd428 (60)	semele m	France	Lozère	W.G. & S.M. Tremewan, 1000 m, 15.vii.1998, AWD
awd429 (61)	semele	France	Lozère	W.G. & S.M. Tremewan, 1000 m, 15.vii.1998, AWD
awd430 (62)	semele	France	Lozère	W.G. & S.M. Tremewan, 1000 m, 15.vii.1998, AWD
cfu350 (41)	? m	Greece	Corfu	Ex coll. D & S. Howell, AWD
jdh20 (46)	? m	N.W. Greece	4 km w. of Spartos (e. of Vonitsa)	26.viii.1963, JDH
jdh23 (43)	? m	N. Greece	Dodoni, sw. of Konitsa	JDH
jdh24 (44)	? m	N. Greece	Aristi, SW. of Konitsa	13.vi.1977, JDH
jdh51 (45)	? s	N. Greece	Mesovounion, between Konitsa and Ioánnina	June 1984, JDH
jdh59 (50)	? d	N. Greece	Ioánnina	open hillside, June 1984, JDH
jdh60 (49)	? s	N. Greece	Ioánnina	open hillside, June 1984, JDH
pj61 [BDR-365] (73)	semele	Germany	Bavaria, Fohrenheide Gebiet	15.7.1973, H. Weigel, PJ
pj62 [BDR-363] (74)	semele	Germany	Bavaria mer., Eching	28.7.1951, P. Hotler, PJ
pj63 [BDR-364] (75)	semele	Germany	Bavaria mer., Eching	28.7.1951, P. Hotler, PJ
pj64 [BDR-362] (76)	semele	Germany	Bavaria mer., Eching	28.7.1951, P. Hotler, PJ

sp. no. (Case no.)	Taxon	Country	Location	Notes
pj65 [AUS-1274] (77)	semele m	Austria	Marchfeld, Oberweiden	date?, O. Wagner, PJ
pj66 [slov-412] (78)	semele	Slovenia	Slavnik	500m, 29.7.1976, B. Mihljevic, PJ
pj67 [slov-432] (79)	semele	Slovenia	Postojna	9.8.1931, M. Hafner, PJ
pj68 [slov-430] (80)	semele	Slovenia	Sorsko polje	9.8.1931, M. Hafner, PJ
pj70 [slov-422] (81)	semele	Slovenia	Opatija	25.6.1948, S. Michieli, PJ
pj71 [slov-428] (82)	semele	Slovenia	Ankaran	20-30.6.1959, S. Michieli, PJ
pj115 [kos-6/7277] (63)	delattini	Serbia	Pristina, Grmija	700 m, 25.6.1979, P. Jaksic, PJ
pj116 [kos-2249] (64)	delattini	Serbia	Prizen	950 m, 22.8.1994, P. Jaksic, PJ
pj117 [kos-5/6293] (65)	delattini	Serbia	Pristina, Grmija	700 m, 19.9.1979, P. Jaksic, PJ
pj118 [kos-6/7709] (66)	delattini	Serbia	Pristina, Grmija	700 m, 9.9.1979, P. Jaksic, PJ
pj119 [kos-6/7340] (67)	delattini	Serbia	Pristina, Grmija	700 m, 6.7.1979, P. Jaksic, PJ
pj120 [kos-6/7341] (68)	delattini	Serbia	Pristina, Grmija	700 m, 6.7.1979, P. Jaksic, PJ
pj121 [kos-1185] (69)	delattini	Serbia	Sar-planina, Piribeg	1700m, 3-4.8.1987, P. Jaksic, PJ
pj122 [kos-439 b] (70)	delattini	Serbia	Pristina, Grmija	700 m, 15.6.1979, P. Jaksic, PJ
pj123 [kos-5637] (71)	delattini	Serbia	Pristina, Grmija	700 m, 5.9.1976, P. Jaksic, PJ
pj124 [kos-1189] (72)	delattini	Serbia	Kopaonik, Sipacine	1200 m, 19.7.1985, P. Jaksic, PJ