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Studies in *Ophrys* L. sectio *Pseudophrys* Godfery - II. *Andrena flavipes* Pz. pollinated taxa

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Summary

Lowe, M. R. (2011): Studies in *Ophrys* L. sectio *Pseudophrys* Godfery - II. *Andrena flavipes* Pz. pollinated taxa.- J. Eur. Orch. 43 (3): 455-497.

In the second of a planned series of investigations of *Ophrys* sectio *Pseudophrys*, taxa known to be pollinated by *Andrena flavipes* are studied. *Ophrys subfusca* is considered to be a valid name and applies to the small and early flowering plants of the *O. fusca* group pollinated by *A. flavipes* from North Africa, Sicily, Malta, Iberia and the western Mediterranean area of France. A lectotype and epitype are designated. The names *O. caesiella* and *O. gazella* are treated as synonyms.

Ophrys murbeckii is also considered to be a valid name for a small flowered member of the *O. lutea* group from North Africa and Iberia. A lectotype is designated. The name *O. numida* is treated as a synonym. The western group of *O. murbeckii* is treated as a subspecies under the new combination *O. murbeckii* subsp. *quarteirae*, creating the autonym *O. murbeckii* subsp. *murbeckii*.

Biometric analysis of the various disjunct sub-populations of *O. subfusca* demonstrates that the discontinuity between the sub-populations is no greater than would be expected within a widely distributed species. The variation between the sub-populations is explained as resulting from genetic drift and a possible bottleneck event causing a slight separation of the Iberian and French sub-populations. *Ophrys subfusca* is shown to be clearly distinct from

O. leucadica and *O. bilunulata*. As expected from their morphological characters, two other *A. flavipes* pollinated taxa, *O. israelitica* and *O. zonata* are also shown to be distinct species. *Ophrys funerea* and *O. zonata* are shown to be related, whilst the difference in flowering time and distribution, together with substantial biometric differences, indicates they should be treated as different biological species.

The recent demonstration of genetic differences between *O. leucadica* from western and eastern populations is investigated with samples from Kefalonia, Athens and Rhodes. The distinction between these plants is confirmed. The recently named *O. fusca* subsp. *sancti-isidorii* is provisionally considered to be the eastern taxon pollinated by *A. flavipes*.

Overall, it is therefore apparent that adaptation to *A. flavipes* as a pollinator has occurred independently not only in the *O. fusca* and *O. omegaifera* groups, but several times within the wider *O. fusca* group. Finally, the interpretation of biometric analysis is discussed in relation to pollinator shift and progenitor-derivative speciation.

Zusammenfassung

Lowe, M. R. (2011): Untersuchungen von *Ophrys* L. sectio *Pseudophrys* Godfery - II. *Andrena flavipes* Pz. bestäubte Taxa.- J. Eur. Orch. 43 (3): 455-497.

Im Rahmen der Untersuchungen über die Gattung *Ophrys* sectio *Pseudophrys* werden hier die von *Andrena flavipes* bestäubten Taxa behandelt. Für kleinblütige, frühblühende, von *A. flavipes* bestäubte Pflanzen der *O. fusca*-Gruppe aus Nordafrika, Sizilien, Malta, der Iberischen Halbinsel und aus dem westlichen Teil der französischen Mediterraneis steht als valider Name *Ophrys subfusca* zur Verfügung. Für diesn werden hier ein Lectotypus und Epitypus ausgewählt; *O. caesiella* und *O. gazella* werden als Synonyme dazu gestellt.

Der korrekte Name für einen ebenfalls kleinblütigen nordafrikanisch-iberischen Vertreter der *O. lutea*-Gruppe ist *Ophrys murbeckii*, für den hier ein Lectotypus bestimmt wird; *O. numida* wird als sein Synonym eingestuft. Die als *Ophrys lutea* subsp. *quarteirae* bekannte westmediterrane Sippe wird als Unterart zu *O. murbeckii* gestellt, die neue Kombination *O. murbeckii* subsp. *quarteirae* schafft das Autonym *O. murbeckii* subsp. *murbeckii*.

Biometrische Analysen der verschiedenen disjunkten Subpopulationen von *O. subfusca* ergeben, daß die Differenzen zwischen diesen Subpopulationen nicht größer sind als sie für eine weit verbreitete Art zu erwarten wären. Die Variation zwischen diesen Subpopulationen wird als Resultat genetischer Drift und eines möglichen Flaschenhalsereignisses erklärt, die zusammen zu einer leichten Trennung der iberischen und südwestfranzösischen Subpopulationen geführt haben. Weiter kann gezeigt werden, daß *Ophrys subfusca* sich deutlich

von *O. leucadica* und *O. bilunulata* unterscheidet. Wie entsprechend ihrer Morphologie zu erwarten, erweisen sich auch zwei andere von *A. flavipes* bestäubte Taxa, *O. israelitica* and *O. zonata*, als eigenständige Arten.
Trotz naher Verwandschaft sollten *Ophrys funerea* und *O. zonata* aufgrund unterschiedlicher Blütezeit und Verbreitung, zusammen mit substantiellen biometrischen Differenzen als verschiedene biologische Arten eingestuft werden.
Die kürzlich anhand von Stichproben von *O. leucadica* aus Kefalonia, Athen und Rhodos aufgezeigten genetischen Unterschiede zwischen westlichen und östlichen Populationen kann hier biometrisch bestätigt werden. Vorläufig werden die ostägäischen von *A. flavipes* bestäubten Pflanzen zur jüngst beschriebenen *O. fusca* subsp. *sancti-isidorii* gestellt.
Insgesamt ist es augenscheinlich, daß eine Anpassung an *A. flavipes* als Bestäuber nicht nur in den Gruppen von *O. fusca* und *O. omegaifera*, sondern unabhängig voneinander bei anderen Taxa einer weiter gefassten *O. fusca*-Gruppe erfolgte. Abschließend wird die Interpretation biometrischer Analysen im Hinblick auf durch Bestäuberanpassung und Abstammung beeinflusste Artbildung diskutiert.

* * *

1. Introduction

This paper continues the biometric investigations (LOWE 2010) into the variation and taxonomic structure of *Ophrys* L. sectio *Pseudophrys* Godfery. Over 1000 floret samples are analysed by univariate and multivariate methods to investigate the similarity and discontinuity of colonies of *Pseudophrys* taxa known to be pollinated by *Andrena flavipes* Pz.

2. Methods

Biometric analyses were conducted on material gathered from France, Spain, Portugal, Italy, Morocco, Tunisia, Malta, Greece and Cyprus. The sampling points for the *A. flavipes* pollinated taxa are shown in Fig. 21 and the site locations are given in Table 3, or otherwise indicated in LOWE (2010). Details of the method of data collection and methods of statistical analysis are set out in LOWE (2010). Summary data of the main taxa considered is shown in Tables 1 and 2. The twelve floral characters selected are shown in Fig. 37.

3. Nomenclature

3.1. *Ophrys subfusca*

The identity of *Ophrys lutea* var. *subfusca* Rchb.f. (REICHENBACH 1851a: 76) and, at specific rank, *O. subfusca* (Rchb.f.) Hausskn. has hitherto been problematic (see for example GÖLZ & REINHARD 2000). The plant was described with the diagnosis "fascia velutina prope marginem attingente. Haec varietas forsan nos coget, ut utramque conjungamus. An hybrida existimanda? Reperi inter flores Ophrydis luteae a cl. Durieu explantos." Further details are provided under the heading of *Ophrys fusca* (REICHENBACH 1851a: 74) and in the German language version (REICHENBACH 1851b: 95). The critical factors are that the taxon has a labellum with a pilosity that extends almost to the edges and that the margin of the labellum is yellow. Two illustrations are provided (REICHENBACH 1851a: 99 tab, 165, figs. 1-2) and reproduced here in Fig. 3. The material upon which the illustrations were made was collected by DURIEU from near Oran in Algeria (REICHENBACH 1851a: 74 & 1851b: 95), however no matching material has been found within REICHENBACH'S herbarium at Vienna (BAUMANN & KÜNKELE 1986: 567) or other sources. The clearly defined characters of REICHENBACH'S description led BAUMANN & KÜNKELE (1986) to place *O. subfusca* as a synonym of *O. fusca* rather than as the name for the hybrid *O. fusca* × *O. lutea*.

In the twentieth century it was common practice to refer to the small forms of the *O. fusca* group as *O. fusca* var. *funerea* Viv. Under this name GODFERY (1925) noted that in Algeria there are two varieties of *O. fusca* distinguished by the flower size and time of flowering. Later (GODFERY 1930) reported the findings of M. POUYANNE that the pollinators of the early small flowered *O. fusca* was *A. fulvicrus* (= *A. flavipes*) from near Alger. More recently, a small flowered *O. fusca* group taxon with a narrow yellow margin to the labellum has been reported from Tunisia (VALLES & VALLES-LOMBARD 1988, GÖLZ & REINHARD 2000, DEVILLERS & DEVILLERS-TERSCHUREN 2000b and FOELSCHE & FOELSCHE 2001) and from eastern Algeria (DE BÉLAIR, VÉLA & BOUSSOUAK 2005) resulting in the names *O. africana* and *O. gazella*. A further similar taxon from Malta was named as *O. caesiella* (DELFORGE 2000). The pollinator for the plants from Malta and Tunisia has been identified as *A. flavipes* (PAULUS 2001a).

The identity of the material collected by DURIEU from Oran, Algeria is assisted by material in the Muséum national d'Histoire Naturelle, Paris. In particular P00428830 collected by M. DE MARSILLY on 18 February 1847 'audessu de Christel, Oran' and shown here in Fig. 10. The early flowering date is consistent with GODFERY (1925: 38) who noted a flowering time of January - February for the small form of *O. fusca*.

REICHENBACH'S illustrations show the clear affinity of *O. subfusca* to the western Mediterranean *A. flavipes* pollinated plants, in comparison with the *O. lutea* group plants from Tunisia upon which DEVILLERS & DEVILLERS-TERSCHUREN (2000b) based their interpretation of *O. subfusca*.

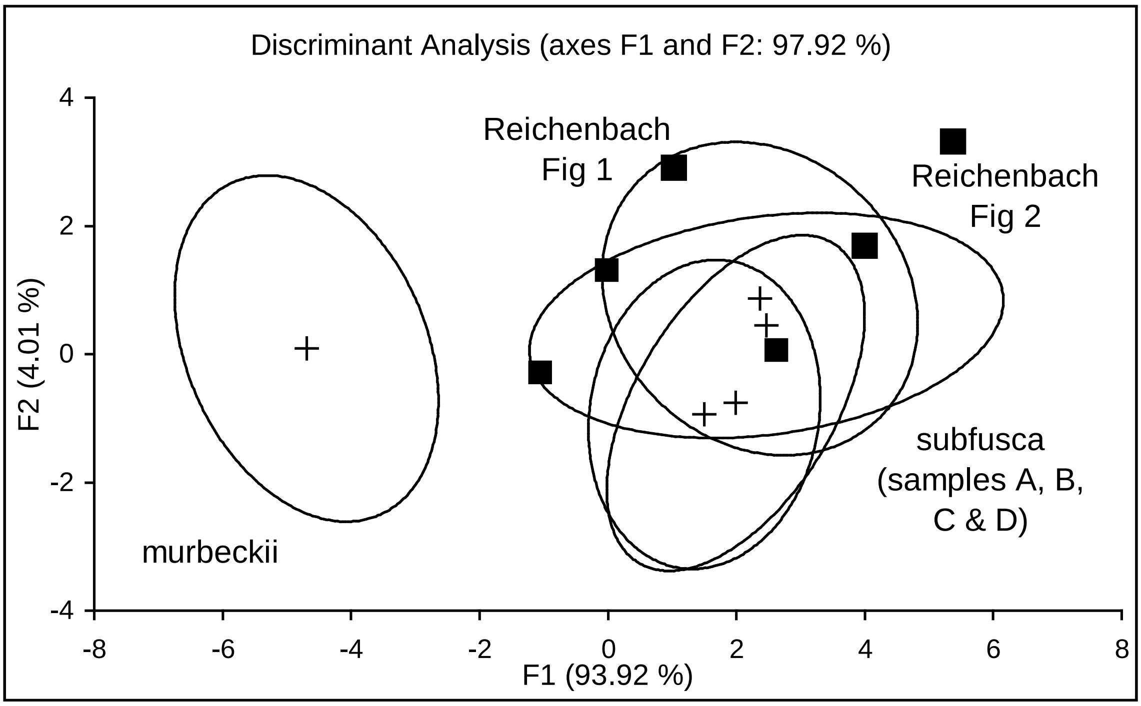

Fig. 1: Discriminant analysis of labellum characters with 95% confidence limits of *Ophrys subfusca* and *O. murbeckii* colonies from Tunisia and Morocco. Labellum characters for Reichenbach's figures cross-validated at 11, 12 & 13 mm for labellum length shown by bold squares.

The two illustrations by REICHENBACH are the only original material available. Fig. 2 is chosen as the lectotype. For the avoidance of further doubt the holotype of *O. africana* is designated as the epitype. The material of FOELSCHE has been chosen, in preference to the type specimens of *O. gazella* or *O. caesiella*, as it is conserved in a public herbarium in accordance with recommendation 7A. 1. of the ICBN (Vienna Code).

Ophrys subfusca (Rchb.f.) Hausskn., Mitt. Thür. Bot. Ver. N. F. 13-14: 25 (1899).

Basionym: *Ophrys lutea* var. *subfusca* Rchb.f., Icon. fl. germ. helv. 13-14: 76 (1851).

Synonyms: *Ophrys subfusca* (Rchb.f.) Batt., Fl. Algerie, Suppl. Phan. 84 (1910). *Ophrys lutea* subsp. *subfusca* (Rchb.f.) Murb., Acta Reg. Soc. Physiogr. Lund 10: 21 (1899). *Ophrys africana* G. Foelsche & W. Foelsche, J. Eur. Orch. 33(2): 656 (2001). *Ophrys gazella* Devillers-Tersch. & Devillers, Natural. belges 81(3): 322 (2000). *Ophrys fusca* subsp. *gazella* (Devillers-Tersch. & Devillers) Kreutz, Kompend. Eur. Orchid., 94 (2004). *Ophrys caesiella* P. Delforge, Natural. belges 81(3): 233 (2000). *Ophrys fusca* subsp. *caesiella* (P. Delforge) Kreutz, Kompend. Eur. Orchid. 93 (2004).

Lectotypus (hic designatus): Rchb.f., Icon. fl. germ. helv. 13-14: Tab 165 DXVII, fig. 2. 1851 (Latin edition).

Epitypus (hic designatus): Tunisia, Promontorium Mercurii (Cap Bon), Nabeul, SW El Haouaria, UTM PF 79.04, circa 40 m 16.02.2000 leg. W Foelsche; in herb GJO, Graz, sub Inv.-Nr. 26.700.

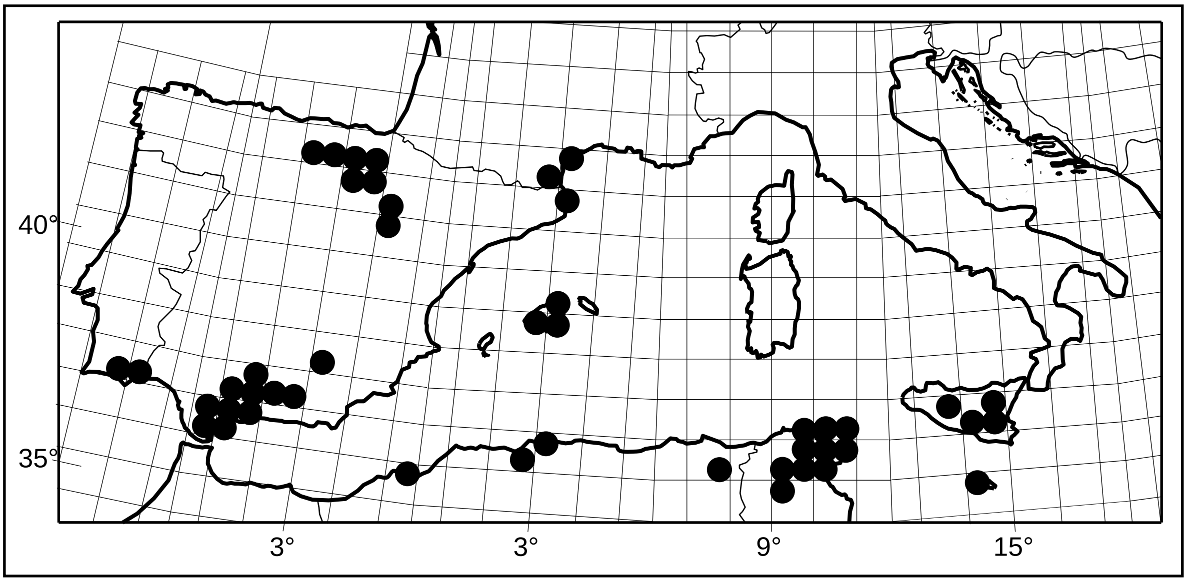

Fig. 2: UTM$_{WGS84}$ 50 × 50 km distribution of *Ophrys subfusca*.

3.2. *Ophrys murbeckii*

From his tours of Tunisia, MURBECK (1899: 21) described a plant intermediate between *O. lutea* and *O. fusca*, which he recognised as a species, distinct from the hybrid and REICHENBACH'S *O. lutea* var. *subfusca*. He sought to name the plant as a new subspecies, but the name *O. lutea* subsp. *subfusca* (Rchb.f.) Murb., although a valid name, is only effective in changing the rank of

REICHENBACH'S taxon. MURBECK'S Latin diagnosis is, however, the basis for FLEISCHMANN'S (1925: 183) *O. murbeckii*, the reference to MURBECK'S previously published description providing the necessary effective publication of the description. The name *O. murbeckii* H. Fleischm. is therefore a valid name, as accepted by BAUMANN & KÜNKELE (1986: 359).

MURBECK'S (1899: tab. XII 4) illustration of his intended subspecies is reproduced in Fig. 3 in comparison with REICHENBACH'S illustrations. Material in the herbarium of the Muséum national d'Histoire Naturelle, Paris (P00428487) includes MURBECK'S gathering from Dj. Serdj collected on 9 June 1896 with the title '*Ophrys subfusca* (Reichb.) Murb.' and referenced under the description (MURBECK 1899: 21). The voucher at P is therefore selected as the lectotype, as it is original material explicitly cited by MURBECK in the protologue.

Ophrys murbeckii H. Fleischm., Österr. Bot. Z. 74: 183 (1925).
Lectotypus (hic designatus): Tunisia, Dj. Serdj, leg. S. Murbeck; in herb P, P00428487.

DEVILLERS & DEVILLERS-TERSCHUREN (2000b), considered MURBECK'S plant, from high altitudes in the Dorsal Mountains of Tunisia, to be the same as their interpretation of REICHENBACH'S *O. lutea* var. *subfusca*. They also identified a similar plant, distinguished by being more robust and larger flowered, as a new species, *O. numida* (DEVILLERS & DEVILLERS-TERSCHUREN 2000a). However, a multivariate analysis by AMICH et al. (2009) found it was not possible to accept *O. numida* as distinct from *O. subfusca* sensu DEVILLERS & DEVILLERS-TERSCHUREN. My own field studies at the two sites visited by DEVILLERS & DEVILLERS-TERSCHUREN, at Dj. Skarna and near Teboursouk, and at which they identified both *O. numida* and *O. subfusca* do not indicate two identifiable forms. In my experience there is no discontinuity between plants of variable stature, flower size or labellum pilosity to indicate that there are two taxa at any rank. A discriminant analysis of 25 colonies of these plants from Tunisia, Morocco and Iberia is shown in Fig. 34. Whilst there is a considerable amount of variation between the eastern and western colonies, there is no basis upon which to find any discontinuity within the Tunisian colonies.

The name *Ophrys murbeckii* can therefore be appropriately applied to describe the widespread member of the *O. lutea* group from the mountains of Tunisia and the plants already well documented in Algeria by STEBBINS & FERLAN (1956) in their classic study of hybridisation. I have found the same plants in Morocco, which together with populations from The Algarve, Portugal and Andalusia previously described as *O. lutea* subsp. *quarteirae* (KREUTZ et al.

2007) indicates the plant is widespread in the western Mediterranean. Throughout this range, *O. murbeckii* flowers slightly later than *O. lutea* s. s. when present in the same localities and is frequently found at high altitudes. The variation in the eastern and western colonies of *O. murbeckii*, indicated in Figs. 34 & 35, is sufficient for the time being and in the absence of pollinator or genetic data, to continue the treatment of the western population as a distinct subspecies. The new combination is given below and creates the autonym *O. murbeckii* subsp. *murbeckii*.

Ophrys murbeckii **subsp.** ***quarteirae*** (Kreutz, M. R. Lowe & Wucherpfennig) M. R. Lowe comb. nov.
Basionym: *Ophrys lutea* subsp. *quarteirae* Kreutz, M. R. Lowe & Wucherpfennig, J. Eur. Orch. 39 (3/4): 627 (2007).

4. Genes and morphometric variation

A small number of genes are expected to be responsible for the differential pollinator attraction leading to floral isolation and rapid speciation by pollinator shift (SCHLÜTER et al. 2011). Pollinator mediated progenitor-derivative speciation results in the derivative species being closely related to the progenitor. The derivative will contain a genetic subset of the progenitor whilst the progenitor species remains unchanged. The derivative species will be geographically restricted compared to its more widespread progenitor (SCHLÜTER 2006 and SCHLÜTER et al. 2011).

The colour of the perianth segments has been shown to be of importance in pollinator attraction in *O. heldreichii* (SPAETHE et al. 2007) but not the labellum pattern, the variation of which may be involved in aversive learning by the male bee and benefit the orchid as a strategy to increase outcrossing (STREINZER et al. 2010). Similarly, VEREECKEN & SCHIESTL (2009) found that colour polymorphism in *O. arachnitiformis* is not subject to selection by the pollinator. The key to the attraction of the pollinator is floral scent (see for example PAULUS 2006 and VEREECKEN et al. 2011).

In the case of progenitor-derivative speciation, floral scent is likely to be the primary change. There will be selective pressure upon morphological characters, such as the size of the labellum, the length of the speculum and the pilosity of the labellum. However, it is argued that the selective pressure upon other morphological floral characters, such as the size of perianth segments, the length of lateral lobes and width of the mid lobe, will be small or insignificant. In particular it is difficult to envisage how changes such as the relative size of perianth segments could result other than by a non-selective process. A pollinator shift involving a different size of pollinator is, therefore, likely to be strongly reflected in a change in labellum and speculum length, but reflected to a lesser extent in change in labellum or perianth shape, as such floral characters are correlated and not subject to any direct selection pressure. Indeed such characters are expected to be a subset of the progenitor species.

Morphometric studies of the floral components may, therefore, be a useful tool to detect pollinator shift. It is argued that morphometric characters may act as markers for the small number of genes initially responsible for pollinator shift. It is axiomatic that discriminant analysis will detect morphological change resulting from genetic drift within a species and may also highlight bottleneck events due to the founder effect between disjunct populations. Morphological discontinuity detected by discriminant analysis of floral characters can be used to infer discontinuity of gene flow between operational taxonomic units, and may also infer phylogenies between species of hybrid origin. The weakness of morphometric studies is that convergent evolution of different lineages to the same pollinator, or pollinators of similar size, may lead to morphometric similarity despite a distant phylogenetic relationship, and with respect to hybridization the resultant characters will not necessarily be intermediate. Morphometric studies are therefore particularly well suited to studies within a clade diversified to different pollinators.

Recent studies by SCHLÜTER (2006), STÖKL et al. (2009) and SCHLÜTER et al. (2011) have shown hitherto elusive genetic differences between reputed *Ophrys* species. As morphometric studies are expected to detect recent pollinator shift events, combined morphometric and genetic studies would be complimentary, and preferable, to subjective assessment of morphological characters such as colour and labellum patterns that are unreliable or, at best, should only be used with caution.

Ophrys species are generally, but not necessarily always, pollinated by one species of insect (PAULUS & GACK 1990b and PAULUS 2006). Nonetheless, a minor attraction to a second pollinator can occur and provide the starting point for pollinator shift. The distribution and abundance of the pollinator will limit the geographical spread of *Ophrys* species. In a scenario in which several *Ophrys* species are established on one island, or otherwise isolated geographical area, and spread to another island through long or middle distance dispersion, it is a matter of chance as to which species first colonises the new area. If the new area provides both the established pollinator and a diversity of other potential pollinators there will be the opportunity for pollinator shift; the relative abundance of the potential pollinators providing some selective pressure. It is therefore plausible, in a complex geographical region in which there are strong physical barriers to population dispersal, for *Ophrys* evolution to the same pollinator to have arisen independently, but from closely related taxa. It should not, therefore, be assumed that disjunct populations of *Ophrys* that have the same pollinator are the same biological species.

Andrena nigroaenea and *A. flavipes* both have a pan-Mediterranean distribution (GUSENLEITNER & SCHWARZ 2002). Within *Pseudophrys* taxa it is evident that adaptation to the same pollinator has occurred independently, for example *O. forestieri* and *O. sitiaca* adapted to *A. nigroaenea* pollination and with *O. israelitica* and *O. subfusca* to *A. flavipes*. These pairs of *Ophrys* species are readily identifiable as being from different lineages by comparison of major morphological characters. However, independent lineages evolving to the same pollinator within more closely related taxa are more difficult to detect both by genetic analyses and biometric analysis. Any differences between such potential cryptic species could be due to genetic drift between populations that have become geographically isolated or due to their independent origin. Convergent evolution may also lead to similarities of morphological characters adding to the problem of species delimitation. However, the extent of variation due to genetic drift and the founder effect within a widespread species give a measure of variation to compare to the differences between potential cryptic species.

Furthermore, as indicated by SCHLÜTER et al. (2011), progenitor-derivative speciation may lead to a widespread species having a greater genetic diversity than the derivative species and the derivative species will contain only a subset of the progenitor’s variation. Cryptic derivative species may, therefore, be inferred when they are more different than would be expected from a widespread progenitor species and are more similar to a geographically co-occurring candidate progenitor species, despite the pollinator discrepancy. It should also be recognised that pollinator shift is a reversible process. If the origin of a species starts with pollinator A, shifts to pollinator B and then reverts back to pollinator A in a different location, it will be expected that the resulting characters of the final species will be different from the species at the start of the process. As progenitor-derivate speciation does not result in any change in the progenitor population from which the derivate originated, the original taxa and the final derivative taxon may exist in different geographical areas, both old and new taxa will have similar characters, but will have different characters from that which would have resulted from genetic drift or the founder effect. In particular, it may have acquired adaptive characters from the intermediate stage.

5. Biometric analysis and discussion

Andrena flavipes is known to be the pollinator of

- *O. israelitica* in Cyprus and Israel (BAUMANN & HALX 1972 and PAULUS & GACK 1990a);
- *O. bilunulata* in SE France (PAULUS & GACK 1999);
- *O. obaesa* in Sicily (PAULUS & GACK 1990c);
- *O. zonata* in Sardinia (PAULUS & GACK 1995);
- *O. subfusca* from Tunisia and Malta (PAULUS 2001a), from Andalusia (PAULUS & GACK 1980), from Mallorca (STÖKL et al. 2009), from The Algarve (PAULUS 2001a) and
- *O. leucadica* with both western and eastern populations from Greece (PAULUS & SALKOWSKI 2008; PAULUS 2001b and SCHLÜTER et al. 2011).

The affinities of populations from Greece, Tunisia, Malta, Spain and France from which the types of *O. leucadica*, *O. bilunulata*, *O. caesiella* and *O. gazella* originate have been controversial (for example PAULUS 2001a).

Fig. 22 compares the labellum dimensions of individual plants from the Languedoc-Roussillon and Catalonian sample of *O. subfusca*, the *O. bilunulata* samples from SE France and *O. leucadica* from Kefalonia. Clearly there is a high level of variation within these samples, although the consistent difference is size of *O. bilunulata* is apparent and the difference in the ratios between the labellum length, width and length of the outer lobes between *O. subfusca* and *O. leucadica* is visible. It is also apparent that all of the characters are highly correlated. Fig. 23 shows the labellum characters of all the colonies of *O. subfusca* (sampled from Malta, Tunisia, The Algarve, mainland Spain, Mallorca and SW France) compared to *O. bilunulata* and *O. leucadica* colonies. Three data sets compare the primary data, standardized data and three ratios. The differences between the three taxa now become more pronounced with the reduced ‘fogging’ of the within colony variation, and some differences between the *O. subfusca* sub-populations from Malta, Tunisia and The Algarve and the sub-populations in Spain and SW France become apparent. Figures 24 & 25 compare the labellum and perianth characters of six *A. flavipes* pollinated taxa and that of *O. funerea* from Aude, France. *Ophrys leucadica*, *O. zonata* and *O. funerea*, together with *O. calocaerina*, all have large perianth segments relative to the labellum size and larger inner perianth segments relative to the dorsal and lateral segments.

The results of a discriminant analysis of the 7 sub-populations of *O. subfusca* are shown in Fig. 26, which indicates some differences between the Malta, Tunisia and The Algarve samples and the remainder. The samples from Malta and Tunisia are almost identical and the samples from Mallorca and La Rioja form sub-sets within the sample from Andalusia, whilst overall there is a wide overlap between the 95% confidence limits of the colonies sampled. Fig. 27 compares the *O. subfusca* sub-populations with *O. bilunulata*, indicating the complete separation of *O. bilunulata* from *O. subfusca*. Figures 28 & 29 compare *O. subfusca*, *O. bilunulata* and *O. leucadica* using standardized data. The separation of *O. subfusca* and *O. bilunulata* is maintained indicating the difference is not only in size but also in shape. Fig. 30 shows the clustering of Mahalanobis Distances, obtained from discriminant analysis, of the colonies of *O. subfusca, O. bilunulata* and *O. leucadica* together with *O. israelitica*, as an out group to the assumed clade.

Fig. 31 uses multidimensional scaling to compare the extent of variation between the sub-populations of *O. subfusca* with other *A. flavipes* pollinated taxa and some other possibly related species, and Fig. 32 adds a wide range of species from within the *O. fusca* group. Overall, these illustrate a wide difference between the various *A. flavipes* pollinated taxa and suggest that adaptation to pollination by *A. flavipes* has occurred several times within the *O. fusca* group and once within the *O. omegaifera* group. This presentation should be treated with caution as similarities may result from convergent evolution or pure chance with multiple lineages. Nonetheless, it is notable that taxa from the western Mediterranean generally cluster together with relatively little variation, consistent with an early diversification in the eastern Mediterranean from which a limited number of taxa reached the western Mediterranean, followed by extensive pollinator shift. Progenitor-derivative speciation may be inferred with *O. decembris* having evolved from *O. forestieri* on Mallorca, whilst *O. frabrella* may also have evolved sympatrically from *O. subfusca*. Fig. 33 compares the level of intra-specific variation of some species over a wide geographical area. The data is not standardized thus retaining the maximum differences, but the differences of size becomes the main distinguishing factor between the taxa, rather than any clustering of shape that would be more indicative of evolutionary similarity. Fig. 36 compares the *A. flavipes* pollinated taxa from the eastern Mediterranean. The *A. flavipes* pollinated taxon from Rhodes, named as *O. fusca* subsp. *sancti-isidorii* with a type from Chios (SALIARIS et al. 2010), is provisionally considered to be the taxon that was treated as ‘eastern *leucadica*’ by SCHLÜTER et al. (2011).

Whilst the data is capable of wide interpretation, it provides cogent evidence for treating all the disjunct sub-populations of *O. subfusca* as a single species. The names *O. caesiella* and *O. gazella* become synonyms. The variation between the sub-populations from Malta around the western Mediterranean to SW France are of the same order of magnitude to the differences between sub-populations of *O. cinereophila* from Cyprus, Rhodes, Crete and the Greek mainland and less than the variation within *O. iricolor* and *O. eleonorae* sampled throughout a wide part of their distributional range. The difference between the North African / The Algarve sub-populations and the Spanish and French sub-populations may indicate a bottleneck event within the dispersion.

Ophrys bilunulata is readily separable from *O. subfusca* and from *O. leucadica*, albeit that a common ancestor lies at the root. The difference between *O. subfusca* and *O. leucadica* is of a magnitude greater than would be expected from isolation and genetic drift or from the founder effect. The similarity of *O. bilunulata* and *O. leucadica* is such that they are likely to be within the same clade, but their complete separation by discriminant analysis, reflecting the considerable difference in size and shape, indicates that other possibilities need to be considered before treating the two taxa as geographical sub-species. In particular, material from Italy and the Adriatic, such as *O. lucifera*, would need to be assessed.

Ophrys zonata from Sardinia and Corsica is well defined by morphological characters and by biometric analysis, so as to be distinguished from *O. subfusca*, *O. bilunulata* and *O. leucadica*. It is related to *O. funerea* (syn. *O. sulcata*) but the difference in phenology and biometric properties would suggest that *O. funerea* has a different pollinator. It seems likely that VIVIANI'S description of *O. funerea* included the type of *O. zonata* from Corsica, but the lectotypification of *O. funerea* by SOCA (2001) of material from northern Italy attaches the name *O. funerea* to the late flowering plants from Italy and France. *Ophrys obaesa* from Sicily, pollinated by *A. flavipes*, has some similarities to *O. zonata* from Sardinia and Corsica, but further investigations are required in Sicily.

Ophrys leucadica was, until recently, considered to be widespread throughout Greece. However, genetic investigations by SCHLÜTER et al. (2011) have shown surprising differences between the eastern and western populations. Although based upon small samples and from a limited number of locations, the biometric data indicates the separation of *A. flavipes* pollinated plants from Kefalonia, the locus typicus of *O. leucadica*, and plants from Rhodes.

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Bibliography

AMICH, F., GARCÍA-BARRIUSO, M., CRESPÍ & S. BARNARDOS (2009): Taxonomy, morphometric circumscription and karyology of the Mediterranean Africa representatives of *Ophrys* sect. *Pseudophrys* (*Orchidaceae*).- Plant Biosystems 143 (1): 47-61.

BAUMANN, H. & G. HALX (1972): *Ophrys* - die Planze mit "sex". Kosmos 68: 78-80.

DE BÉLAIR, G., VÉLA, E. & R. BOUSSOUAK (2005): Inventaire des orchidées de Numidie (N-E Algérie) sur vingt années.- J. Eur. Orch. 37 (2): 291-401.

DELFORGE, P. (2000): *Ophrys caesiella* sp. nova, une espèce maltaise du groupe d’*Ophrys fusca*, présente aussi en Sicile.- Natural. belges 81 (orchid 13): 232-236.

DEVILLERS, P. & J. DEVILLERS-TERSCHUREN (2000a): Observations sur les *Ophrys* du groupe d'*O. subfusca* en Tunisie.- Natural. belges 81 (Orchid. 13): 283-297.

DEVILLERS, P. & J. DEVILLERS-TERSCHUREN (2000b): Notes phylogénétiques sur quelques *Ophrys fusca* s.l. en Méditerranée centrale.- Natural. belges 81 (Orchid. 13): 298-322.

FLEISCHMANN, H. (1925): Beitrag zur Orchideenflora der Insel Kreta.- Österr. Bot. Zeitschr. 74: 180-195.

FOELSCHE, G. & W. FOELSCHE (2001): *Ophrys africana* spec. nov., ein früh blühendes Taxon der *Ophrys fusca*-Gruppe in Tunesien.- J. Eur. Orch. 33 (2): 637-672.

GASC, G. (1990): Pollinisation des *Ophrys* (pollinisation conjointe d'*Ophrys araneola* et *O. fusca*).- L'Orchidophile 25 (110): 8-13.

GODFERY, M. J. (1925): The fertilisation of *Ophrys speculum, O. lutea* and *O. fusca*.- J. Bot., Lond. 63: 33-40.

GODFERY, M. J. (1930): Further notes on the fertilisation of *Ophrys fusca* and *O. lutea*.- J. Bot., Lond. 68: 237-238.

GÖLZ, P & H. R. REINHARD (2000): Beitrag zur Kenntnis der Orchideenflora Tunesiens, insbesondere des *Ophrys lutea-fusca*-Aggregates.- J. Eur. Orch. 32 (1): 3-68.

GUSENLEITNER, F. & M. SCHWARZ (2002): Weltweite Checkliste der Bienengattung *Andrena* mit Bemerkungen und Ergänzungen zu paläarktischen Arten (Hymenoptera, Apidae, Andreninae, *Andrena*).- Entomofauna, supplement 12: 1-1280.

KREUTZ, C. A. J., LOWE, M. R. & W. WUCHERPFENNIG (2007): *Ophrys lutea* subsp. *quarteirae* Kreutz, M. R. Lowe & Wucherpfennig, eine neue *Ophrys*-Sippe aus Südportugal (Algarve) und Südspanien (Andalusien).- J. Eur. Orch. 39 (3/4): 625-636.

LOWE, M. R. (2010): Studies in *Ophrys* L. sectio *Pseudophrys* Godfery - I. *Ophrys forestieri* and *O. malacitana* spec. nov.- J. Eur. Orch. 42 (3/4): 541-562.

MURBECK, S. V. (1899): Contributions a la connaissance de la Flore du nord-ouest de l'Afrique et plus spécialement de la Tunisie. III. *Plumbaginacea* - *Graminaceae*.- Malmström, Lund.

PAULUS, H. F. (1998): Der *Ophrys fusca* s. str. - Komplex auf Kreta und anderer Ägäisinseln mit Beschreibung von *O. blitopertha, O. creberrima, O. cinereophila, O. cressa, O. thriptiensis* und *O. creticola* ssp. nov. (*Orchidaceae*).- J. Eur. Orch. 30: 157-201.

PAULUS, H. F. (2001a): Material zu einer Revision des *Ophrys fusca* s.str. Artenkreises 1. *Ophrys nigroaenea-fusca*, *O. colletes-fusca*, *O. flavipes-fusca*, *O. funerea*, *O. forestieri* oder was ist die typische *Ophrys fusca* Link 1799 (*Orchidaceae*).- J. Eur. Orch. 33 (1): 121-177.

PAULUS, H. F. (2001b): Daten zur Bestäubungsbiologie und Systematik der Gattung *Ophrys* in Rhodos (Griechenland) mit Beschreibung von *Ophrys* parvula, *Ophrys persephonae*, *Ophrys lindia*, *Ophrys eptapigiensis* spec. nov. aus der *Ophrys fusca* s. str. Gruppe und *Ophrys cornutula* spec. nov. aus der *Ophrys oestrifera*-Gruppe (Orchidaceae und Insecta, Apoidea).- Ber. Arbeitskrs. Heim. Orch. 18 (1): 38-86.

PAULUS, H. F. (2006): Deceived males - pollination biology of the Mediterranean orchid genus *Ophrys* (*Orchidaceae*).- J. Eur. Orch. 38 (2): 303-353.

PAULUS, H. F. & C. GACK (1980): Beobachtungen und Untersuchungen zur Bestäubungsbiologie südspanischer *Ophrys*-Arten.- Jber. Naturwiss. Ver. Wuppertal 33: 55-68.

PAULUS, H. F. & C. GACK (1990a): Pollination of *Ophrys* (*Orchidaceae*) in Cyprus.- Plant Syst. Evol. 169: 177-207.

PAULUS, H. F. & C. GACK (1990b): Pollinators as prepollinating isolation factors: Evolution and speciation in *Ophrys* (*Orchidaceae*).- Israel J. Bot. 39: 43-79.

PAULUS, H. F. & C. GACK (1990c): Zur Pseudokopulation und Bestäuberspezifität der Gattung *Ophrys* in Sizilien und Süditalien.- Jber. Naturwiss. Ver. Wuppertal 43: 119-141.

PAULUS, H. F. & C. GACK (1995): Zur Pseudokopulation und Bestäubung in der Gattung *Ophrys* (*Orchidaceae*) Sardiniens und Korsikas.- Jber. Naturwiss. Ver. Wuppertal 48: 188-227.

PAULUS, H. F. & C. GACK (1999): Bestäubungsbiologische Untersuchungen an der Gattung *Ophrys* in der Provence (SO-Frankreich), Ligurien und Toscana (NW-Italien) (*Orchidaceae* und *Insecta*, *Apoidea*).- J. Eur. Orch. 31: 347-422.

PAULUS, H. F. & H.-E. SALKOWSKI (2008): Bestäubungsbiologische Untersuchungen an Winterorchideen auf der Ägäis-Insel Kos (*Orchidaceae* und *Insecta*, *Hymenoptera*, *Apoidea*).- Ber. Arbeitskrs. Heim. Orchid. 24 (2): 4-29. "2007".

REICHENBACH, H. G. fil. (1851a): Orchideae in Flora Germanica Recensitae additis Orchideis Europae Reliquae, Reliqui Rossic Imperi, Algerii ergo Tentamen Orchidiographiae Europeae.- Lipsiae.

REICHENBACH, H. G. fil. (1851b): Die Orchideen der deutschen Flora nebst denen des übrigen Europa, des ganzen russischen Reichs und Algiers also ein Versuch einer Orchideographie Europas.- Leipzig.

SALIARIS, A., SALIARIS, P. & A. ALIBERTIS (2010): *Ophrys fusca* subsp. *sancti-isidorii*, a new subspecies from Chios (Greece).- J. Eur. Orch. 42 (2): 327-332.

SCHLÜTER, P. M. (2006): Pollinator-driven evolution in *Ophrys fusca* s.l. (*Orchidaceae*): Insights from molecular studies with DNA fingerprint and sequence markers. Doctoral dissertation. Fakultät für Lebenwissenschaften der Universität Wien.- Wien.

SCHLÜTER, P. M., RUAS, P. M., KOHL, G., RUAS, C. F. R., STUESSY, T. F. & H. F. PAULUS (2011): Evidence for progenitor-derivative speciation in sexually deceptive orchids.- Annals of Botany (in-press).

SOCA, R (2001): Typification d'*Ophrys funerea* Viviani.- Le Monde des Plantes 2001 (No. 471): 29-31.

STEBBINS, G. L. & L. FERLAN (1956): Population variability, hybridization, and introgression in some species of *Ophrys*.- Evolution 10: 32-46.

STÖKL, J., SCHLÜTER, P. M., STUESSY, T. D., PAULUS, H. F., FRANCKE, W., ASSUM, G. & M. AYASSE (2009): Speciation in sexually deceptive orchids: pollinator-driven selection maintains discrete odour phenotypes in hybidizing species.- Biol. J. Linn. Soc. 98: 439-451.

SPAETHE, J., MOSER, W. H. & H. F. PAULUS (2007): Increase of pollinator attraction by means of a visual signal in the sexually deceptive orchid, *Ophrys heldreichii* (*Orchidaceae*).- Plant Syst. Evol. 264: 31-40.

STREINZER, M., ELLIS, T., PAULUS, H. F. & J. SPAETHE (2010): Visual discrimination between two sexually deceptive *Ophrys* species by a bee pollinator.- Arthropod-Plant Interactions 4: 141-148.

VALLES, V. & A.-M. VALLES-LOMBARD (1988): Orchidées de Tunisie.- Librairie de la Renaissance, Toulouse.

VEREECKEN, N. J., & F. P. SCHIESTL (2011): On the roles of colour and scent in a specialised floral mimicry system.- Annals of Botany 104: 1077-1084.

VEREECKEN, N. J., STREINZER, M., AYASSE, M., SPAETHE, J., PAULUS, H. F., STÖKL, J., CORTIS, P. & F. P. SCHIESTL (2011): Integrating past and present studies on *Ophrys* pollination - a comment on Bradshaw et al.- Bot. J. Linn. Soc. 165: 329-335.

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Table 1: Summary data of *Ophrys subfusca* samples indicating mean ($\bar{x}$) dimensions and standard deviation (σ).

mm		Malta	Tunisia	The Algarve	Andalusia	Mallorca	La Rioja & Euskadi	Languedoc & Catalonia
N		68	80	90	82	24	42	63
Labellum characters								
1.	Length (L)	12,4	12,9	12,9	12,1	12,0	11,7	12,0
	σ	0,96	1,03	0,94	0,86	0,87	0,91	0,72
2.	Width (W)	10,2	10,5	10,4	9,9	10,0	9,6	9,8
	σ	0,79	0,87	0,85	0,78	1,03	0,93	0,78
3.	Outer lobe length (OLL)	9,2	9,5	9,6	8,9	8,9	8,8	9,3
	σ	0,87	0,90	0,81	0,79	0,63	0,69	0,72
4.	Speculum length (SP)	7,8	8,2	8,1	7,5	7,5	7,3	7,3
	σ	0,68	0,70	0,76	0,80	0,54	0,59	0,62
5.	Mid lobe width max. (MA)	6,5	6,4	6,3	6,0	5,8	5,7	5,9
	σ	0,74	0,81	0,69	0,74	0,77	0,75	0,75
6.	Mid lobe width min. (MI)	4,9	4,9	4,9	4,5	4,5	4,5	4,6
	σ	0,49	0,60	0,59	0,47	0,56	0,62	0,61
Perianth characters								
7.	Dorsal outer length (DL)	8,3	8,5	8,2	8,3	8,1	8,2	8,4
	σ	0,81	0,76	0,72	0,65	0,85	0,80	0,57
8.	Dorsal outer width (DW)	6,1	6,1	5,7	5,4	5,4	5,1	5,5
	σ	0,79	0,74	0,59	0,66	0,66	0,54	0,63
9.	Lateral outer length (LL)	9,4	9,6	9,1	9,3	9,0	9,1	9,6
	σ	1,00	0,84	0,74	0,67	0,95	0,83	0,66
10.	Lateral outer width (LW)	5,7	5,8	5,3	5,1	5,1	5,0	5,4
	σ	0,63	0,61	0,49	0,57	0,55	0,47	0,54
11.	Inner length (IL)	6,0	6,2	6,0	5,9	5,8	5,7	6,3
	σ	0,71	0,66	0,59	0,54	0,59	0,51	0,50
12.	Inner width (IW)	2,0	2,0	2,0	1,7	1,8	1,9	1,9
	σ	0,27	0,26	0,19	0,35	0,24	0,21	0,26

Table 2: Summary data of *Andrena flavipes* pollinated taxa samples indicating mean ($\bar{x}$) dimensions and standard deviation (σ).

mm		*O. subfusca*	*O. bilunulata*	*O. leucadica*	*O. zonata*	*O. israelitica*	*O. fusca* subsp. *sancti-isidorii*
N		449	80	40	70	35	78
Labellum characters							
1.	Length (L) σ	12,4 1,00	14,1 0,86	12,6 0,80	11,3 0,79	13,5 1,09	13,2 0,84
2.	Width (W) σ	10,1 0,89	11,2 0,90	10,8 0,86	9,3 0,78	11,7 0,94	11,7 0,86
3.	Outer lobe length (OLL) σ	9,2 0,84	11,2 0,97	9,4 0,83	8,8 0,65	9,6 0,98	9,7 1,06
4.	Speculum length (SP) σ	7,7 0,78	8,6 0,67	7,5 0,51	7,5 0,58	8,0 0,85	7,9 0,55
5.	Mid lobe width max. (MA) σ	6,2 0,79	6,1 0,62	6,0 0,53	4,9 0,49	8,0 0,82	7,6 0,91
6.	Mid lobe width min. (MI) σ	4,7 0,59	5,0 0,53	4,6 0,43	4,1 0,43	5,5 0,66	5,6 0,57
Perianth characters							
7.	Dorsal outer length (DL) σ	8,3 0,81	9,6 0,72	9,3 0,83	8,8 0,73	10,5 0,86	9,3 0,85
8.	Dorsal outer width (DW) σ	5,7 0,75	5,9 0,60	5,8 0,67	5,0 0,59	7,0 0,69	6,3 0,69
9.	Lateral outer length (LL) σ	9,4 0,82	10,5 0,80	10,4 0,89	9,6 0,82	11,9 0,83	10,5 0,88
10.	Lateral outer width (LW) σ	5,4 0,61	5,9 0,60	5,5 0,51	4,9 0,45	7,0 0,63	6,0 0,58
11.	Inner length (IL) σ	6,0 0,62	7,4 0,57	7,8 0,94	7,0 0,70	7,9 0,64	7,3 0,70
12.	Inner width (IW) σ	1,9 0,28	2,0 0,29	2,1 0,23	1,9 0,27	2,3 0,32	2,2 0,28

Fig. 3: Left to right: Reichenbach’s figs. 1 & 2 (1851); *Ophrys subfusca* (Tunisia); *Ophrys murbeckii* (Tunisia) and Murbeck’s illustration (1899) (not to scale).

Fig. 4: *Ophrys subfusca*, Tunisia, Djebel bou Kornine, $\mathrm{UTM}_{\mathrm{WGS84}}$ 33SPF184646, 120 m asl, 19.02.2007 (part sample 07TnF034).

Fig. 5: *Ophrys subfusca*, Portugal, The Algarve, Santa Margarida, $\mathrm{UTM}_{\mathrm{WGS84}}$ 29SNB710232, 270 m asl, 25.03.2007 (part sample 07LuF019).

Fig. 6: *Ophrys subfusca*, Spain, La Rioja, Las Lagunillas, $\mathrm{UTM}_{\mathrm{WGS84}}$ 30TWN021127, 525 m asl, 22.04.2004 (part sample 04HsF026).

Fig. 7: *Ophrys bilunulata*, France, Var, St-Paul-en-Forêt, UTM$_{WGS84}$ 32TLP125283, 270 m asl, 04.04.2006, (part sample 06GaF058).

Fig. 8: *Ophrys leucadica*, Greece, Kefalonia, Fragkata, UTM$_{WGS84}$ 34SDH616255, 485 m asl, 09.04.2010, (part sample 10IoF042).

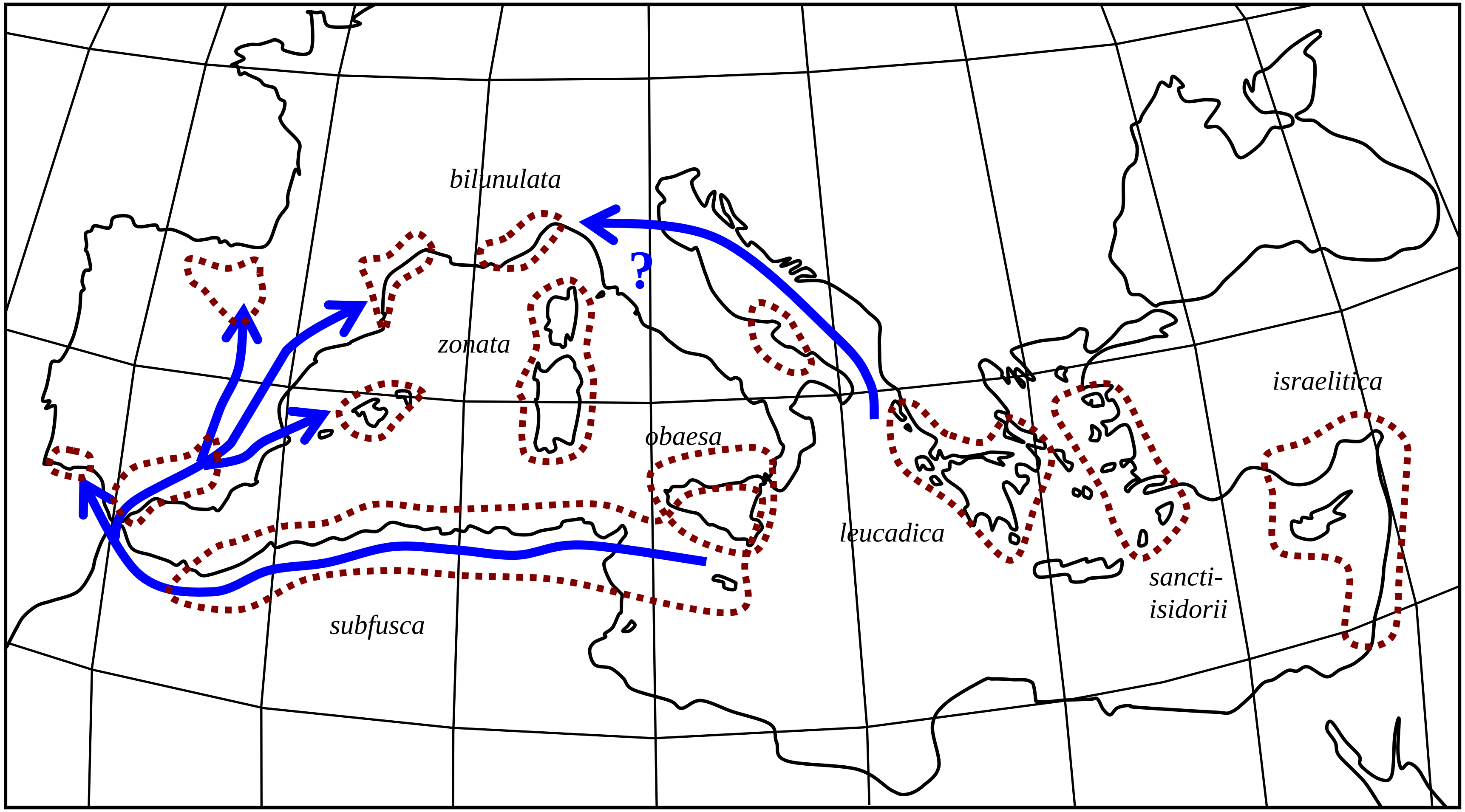

Fig. 9: Distribution of *Andrena flavipes* pollinated taxa and possible direction of migration.

Fig. 10: Herbarium sheet P00428830, M. de Marsilly, 18 February 1847, audessu de Cristel, Oran (Algeria). Above enlarged.

Colour plate page 480

11	12
13	14
15	16

Fig. 11: *Ophrys subfusca*, Spain, Granada, Cubillas, $\mathrm{UTM}_{\mathrm{WGS84}}$ 30SVG397264, 660 m asl, 13.03.2002, (sample 02HsF024) MRL.

Fig. 12: *Ophrys subfusca*, Spain, La Rioja, Las Lagunillas, $\mathrm{UTM}_{\mathrm{WGS84}}$ 30TWN021127, 525 m asl, 22.04.2004 (sample 04HsF026) MRL.

Fig. 13: *Ophrys subfusca*, France, Aude, La Clape, $\mathrm{UTM}_{\mathrm{WGS84}}$ 31TEH074756, 60 m asl, 23.03.2002, (sample 02GaF029) MRL.

Fig. 14: *Ophrys subfusca*, Tunisia, Djebel Amar, $\mathrm{UTM}_{\mathrm{WGS84}}$ 33SNF961813, 275m asl, 25.03.1996, MRL.

Fig. 15: *Ophrys murbeckii* subsp. *quarteirae*, Spain, Cádiz, El Bosque, $\mathrm{UTM}_{\mathrm{WGS84}}$ 30STF776711, 605m asl, 04.05.2000, MRL.

Fig. 16: *Ophrys murbeckii*, Tunisia, El Krib, $\mathrm{UTM}_{\mathrm{WGS84}}$ 33SNF102239, 650m asl, 05.04.2011, (sample 11TnL069) MRL.

Colour plate page 481

17	18
19	20

Fig. 17: *Ophrys bilunulata*, France, Var, St-Paul-en-Forêt, $\mathrm{UTM}_{\mathrm{WGS84}}$ 32TLP125283, 270m asl, 04.04.2006, (sample 06GaF058), MRL.

Fig. 18: *Ophrys leucadica*, Greece, Kefalonia, Fragkata, $\mathrm{UTM}_{\mathrm{WGS84}}$ 34SDH616255, 485m asl, 09.04.2010, (sample 10IoF042) MRL.

Fig. 19: *Ophrys murbeckii* subsp. *quarteirae*, Morocco, Volubilis, $\mathrm{UTM}_{\mathrm{WGS84}}$ 30STC642732, 370m asl, 14.03.2007, (sample 07MaL038) MRL.

Fig. 20: *Ophrys murbeckii* subsp. *quarteirae*, Morocco, Volubilis, $\mathrm{UTM}_{\mathrm{WGS84}}$ 30STC642732, 370m asl, 14.03.2007, MRL.

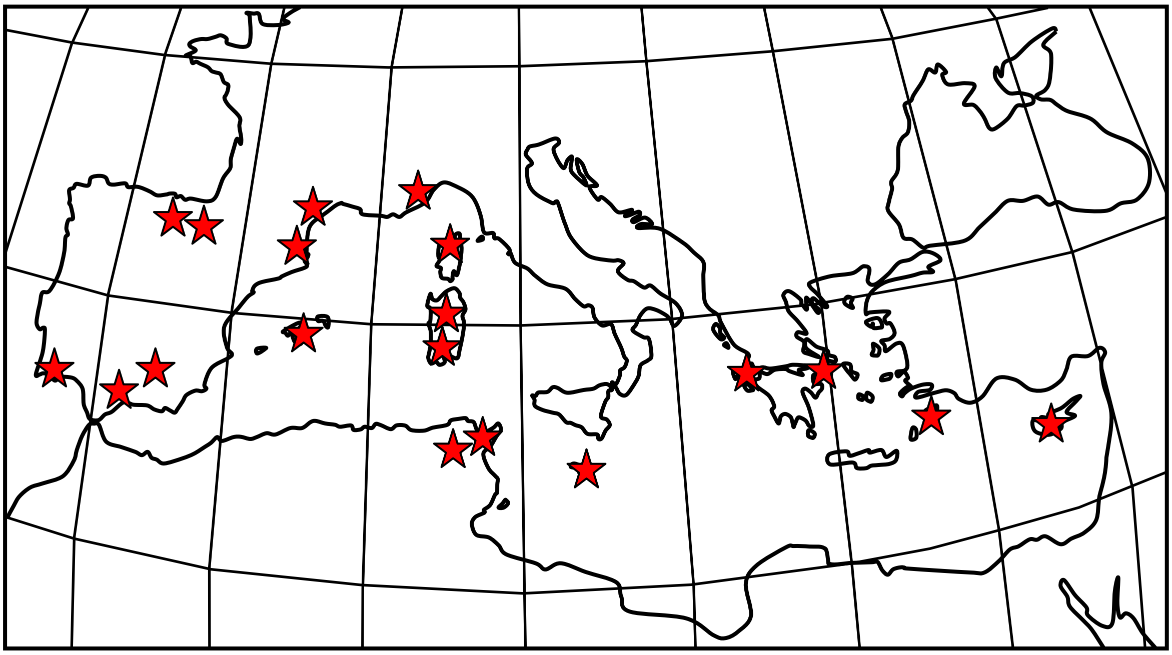

Fig. 21: *Andrena flavipes* pollinated taxa - sampling locations ★.

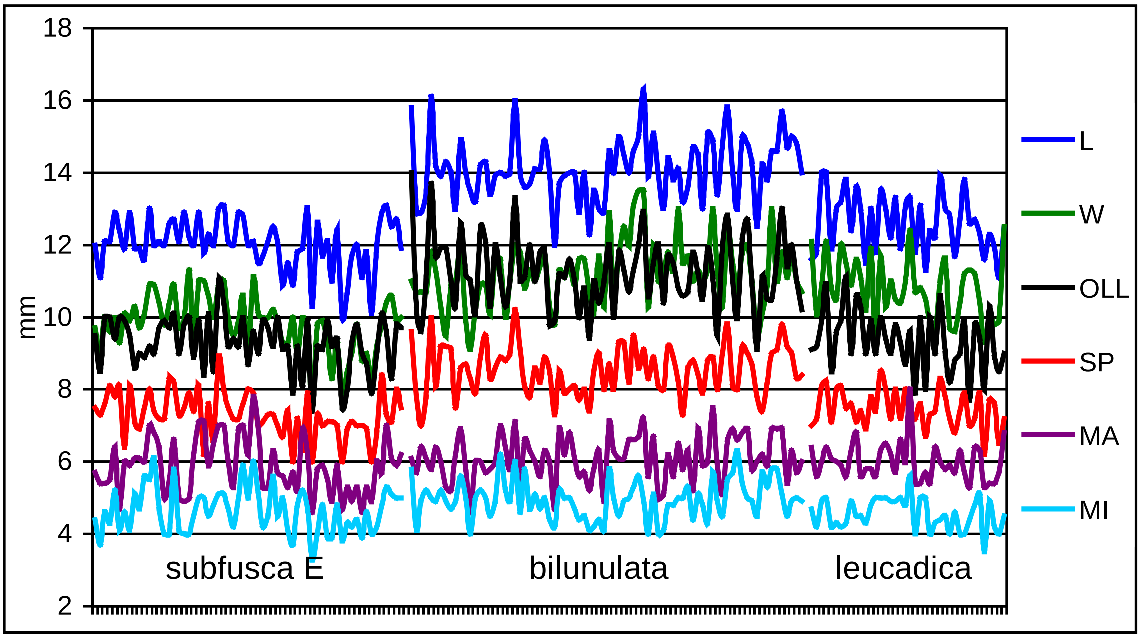

Fig. 22: Labellum characters of individual florets (for details of characters see Fig. 37).

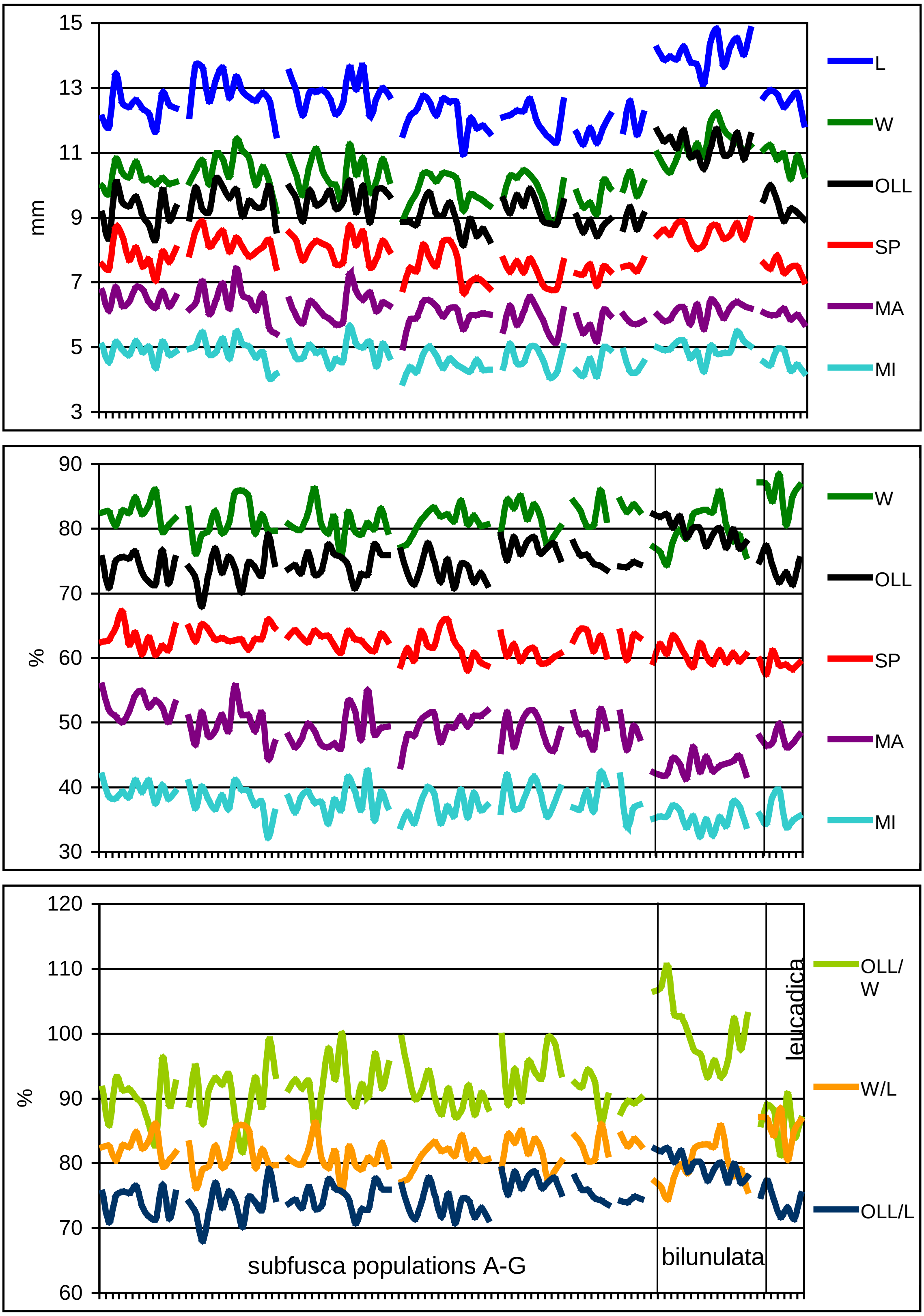

Fig. 23: Labellum characters of colony data; Above, primary data; Centre, standardized data; Below, ratio data.

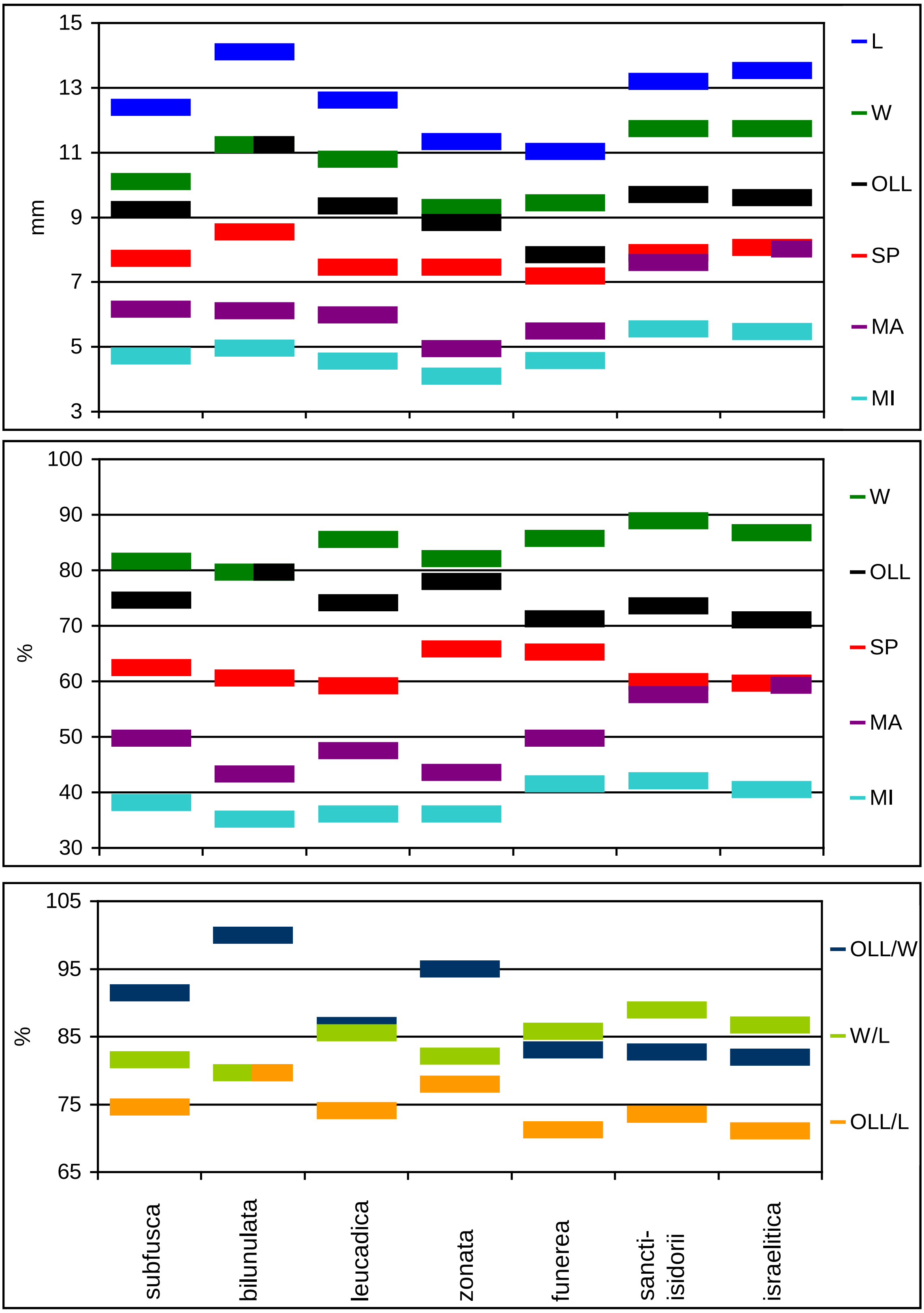

Fig. 24: Labellum characters: Above, primary data; Centre, standardized data; Below, ratio data.

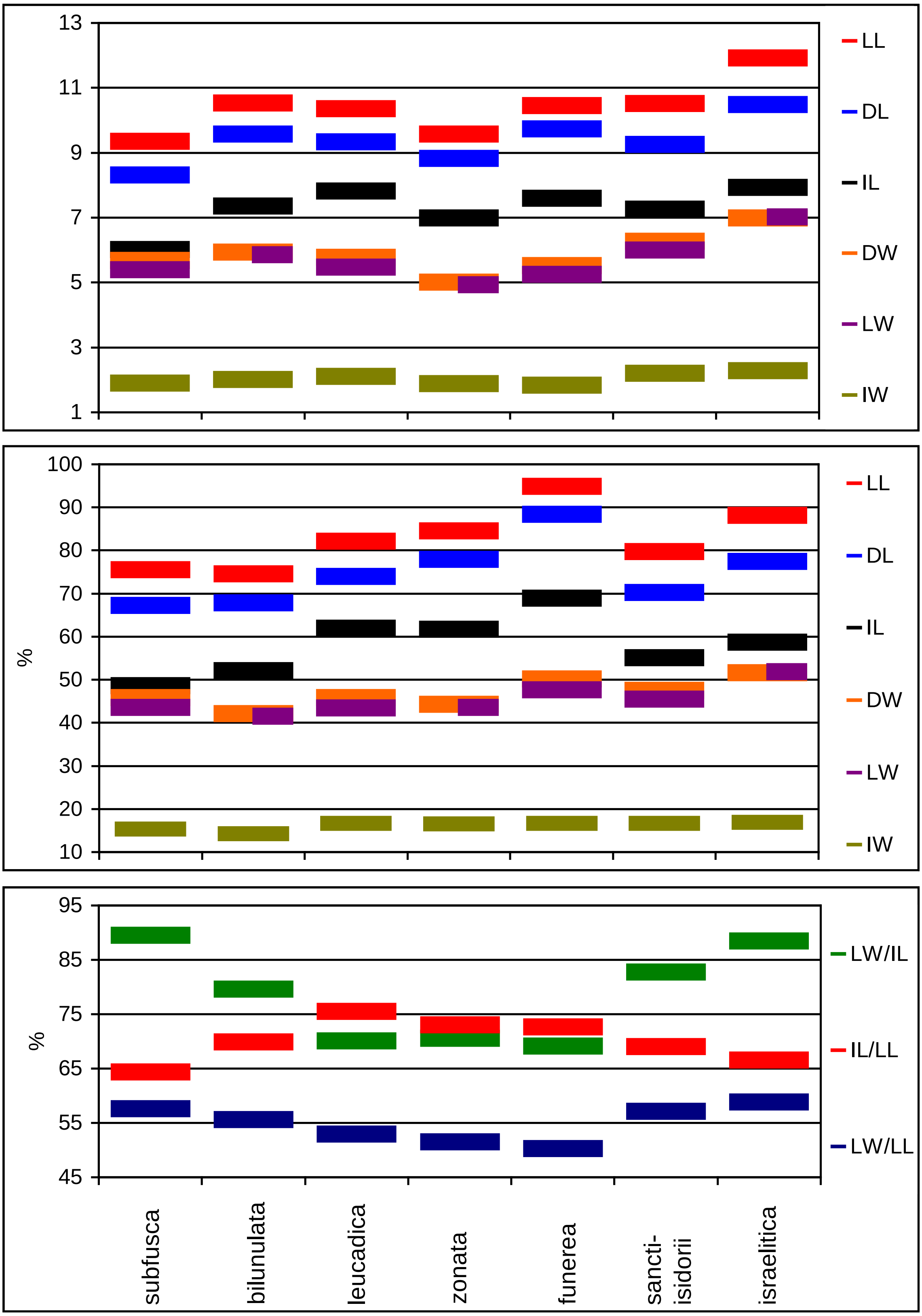

Fig. 25: Perianth segments characters: Above, primary data; Centre, standardized data; Below, ratio data.

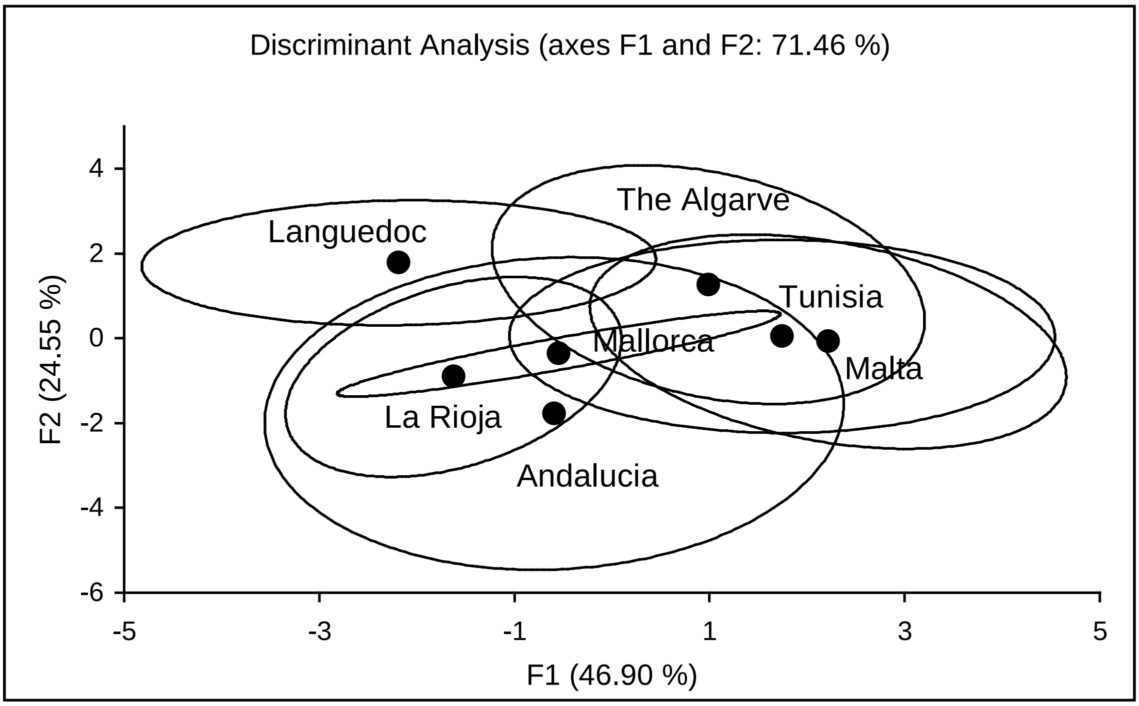

Fig. 26: Discriminant analysis with 95% confidence limits of *Ophrys subfusca* colonies indicating differences between disjunct sub-populations.

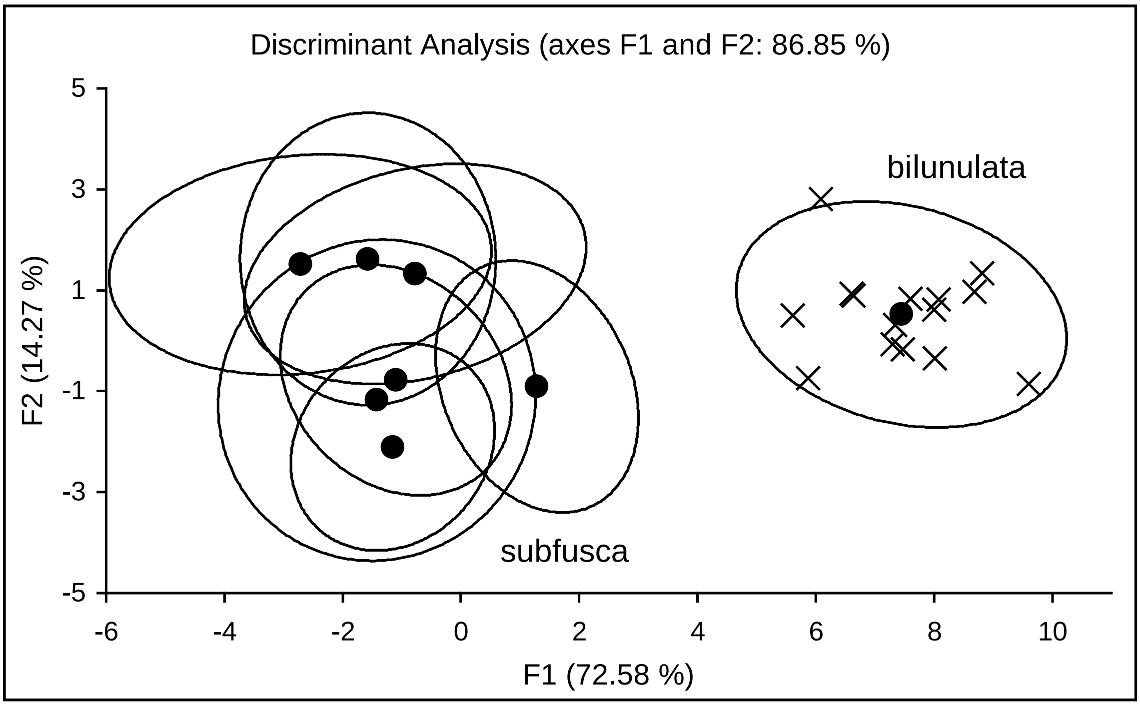

Fig. 27: Discriminant Analysis with 95% confidence limits of *Ophrys subfusca* and *O. bilunulata* colonies.

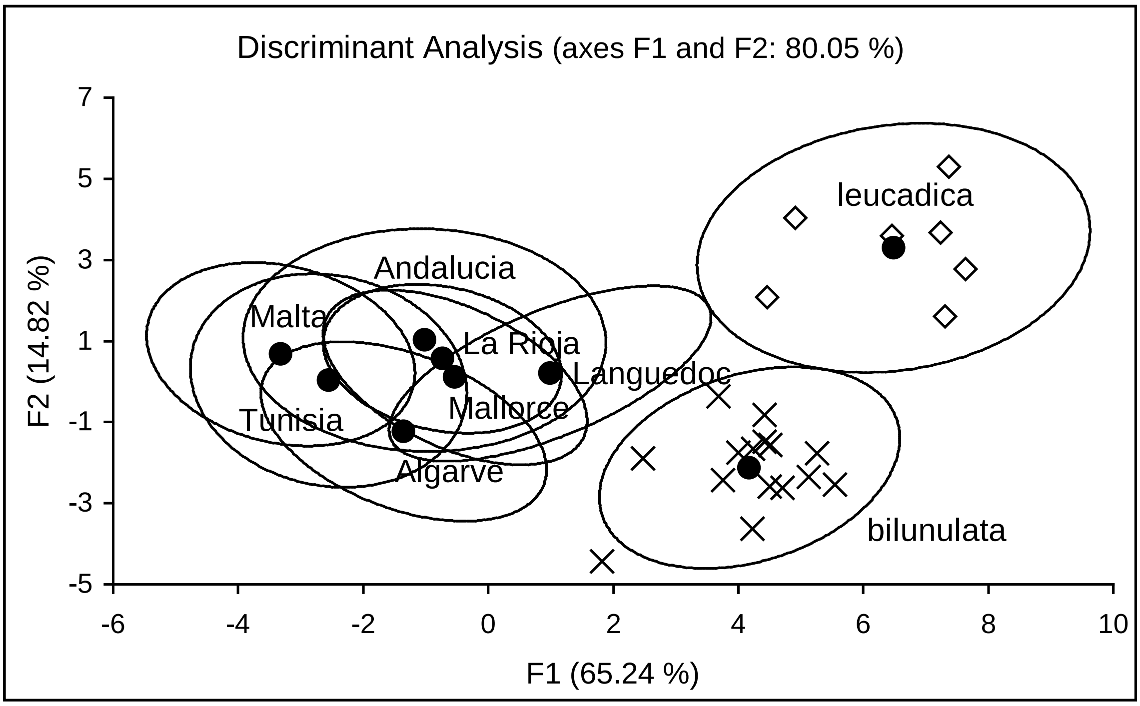

Fig. 28: Discriminant analysis with 95% confidence limits of *Ophrys subfusca, O. bilunulata* and *O. leucadica* colonies. Standardized data.

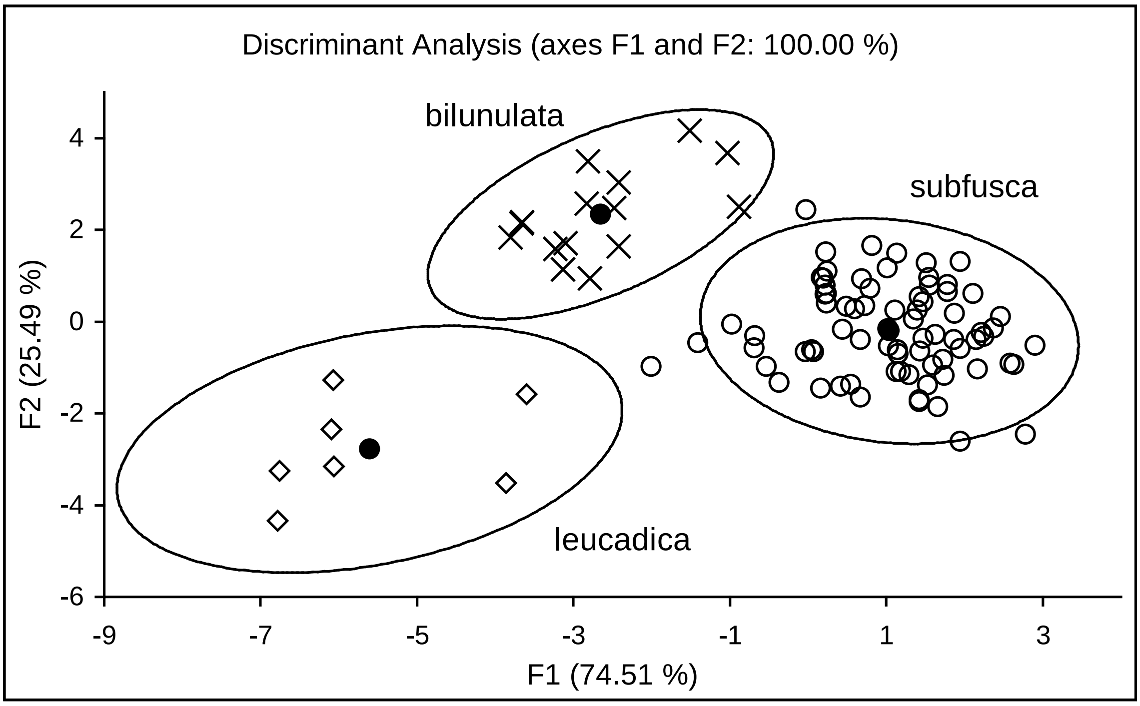

Fig. 29: Discriminant analysis with 95% confidence limits of *Ophrys subfusca, O. bilunulata* and *O. leucadica* colonies. Standardized data.

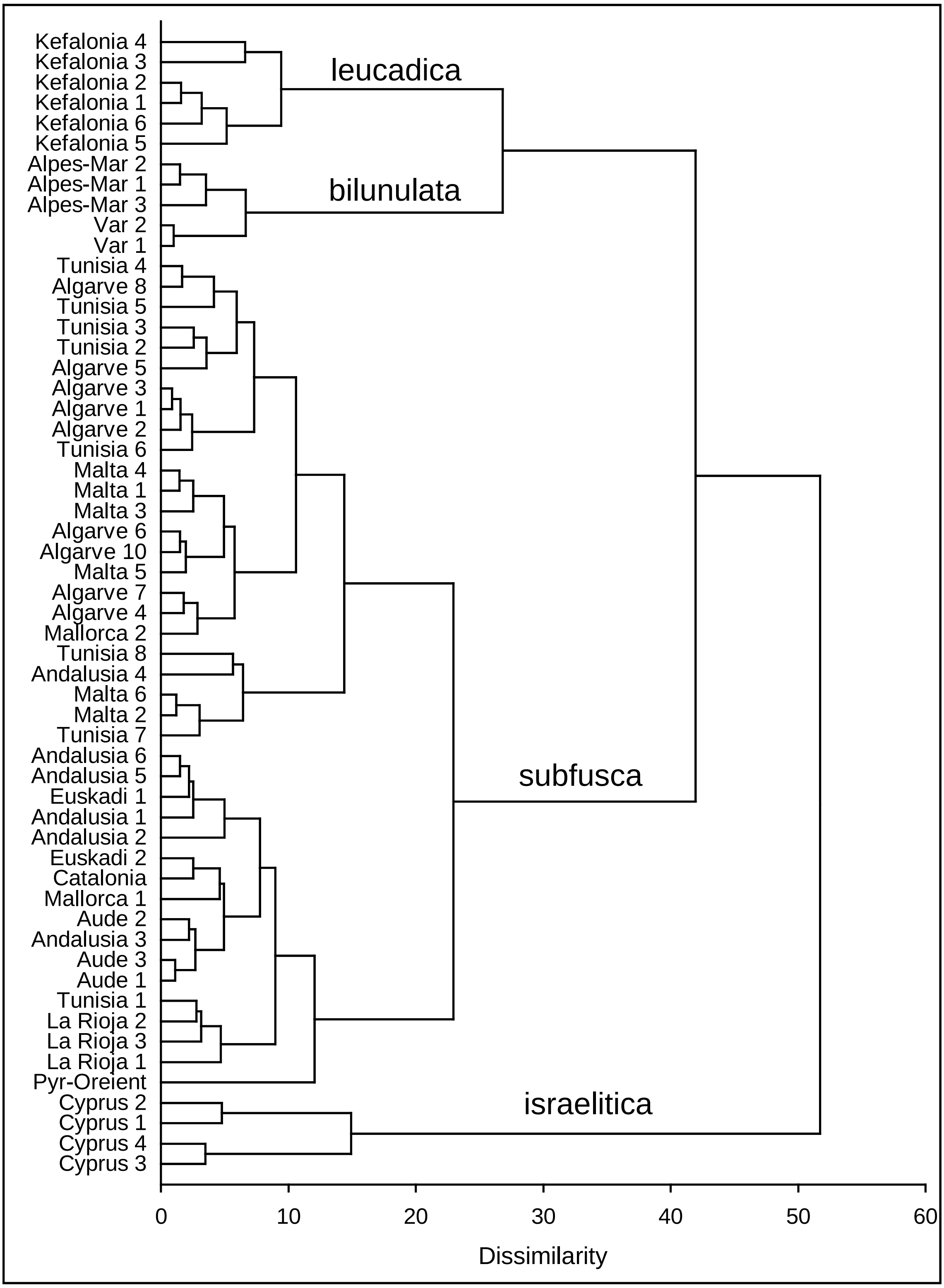

Fig. 30: Agglomerative Hierarchical Clustering (AHC) of Mahalanobis Distances indicating variation between colony samples of *Ophrys subfusca, O. leucadica*, *O. bilunulata* and *O. israelitica*.

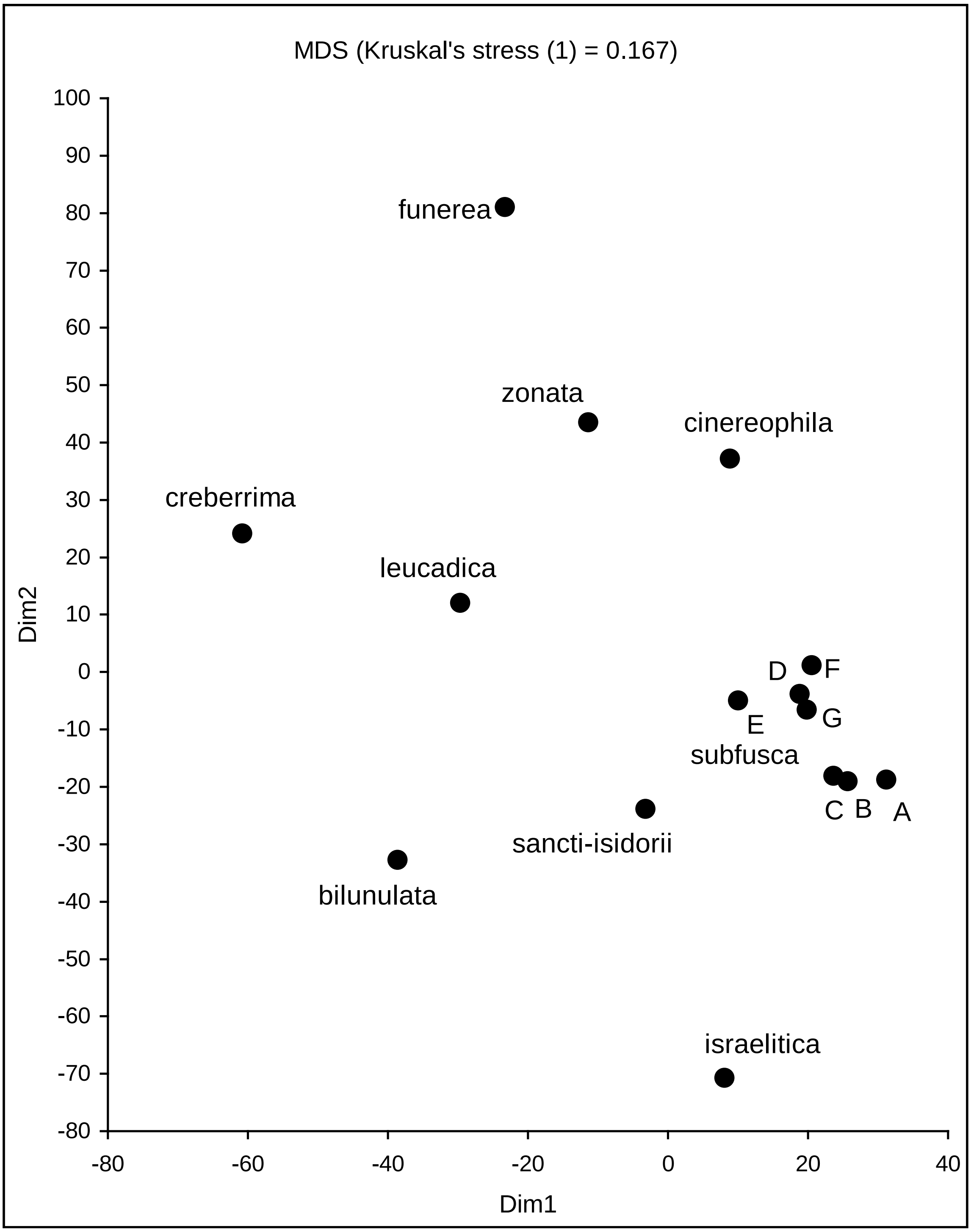

Fig. 31: Multidimensional Scaling of Mahalanobis Distances of *Ophrys subfusca* sub-populations, other *Andrena flavipes* pollinated taxa and possibly related taxa.

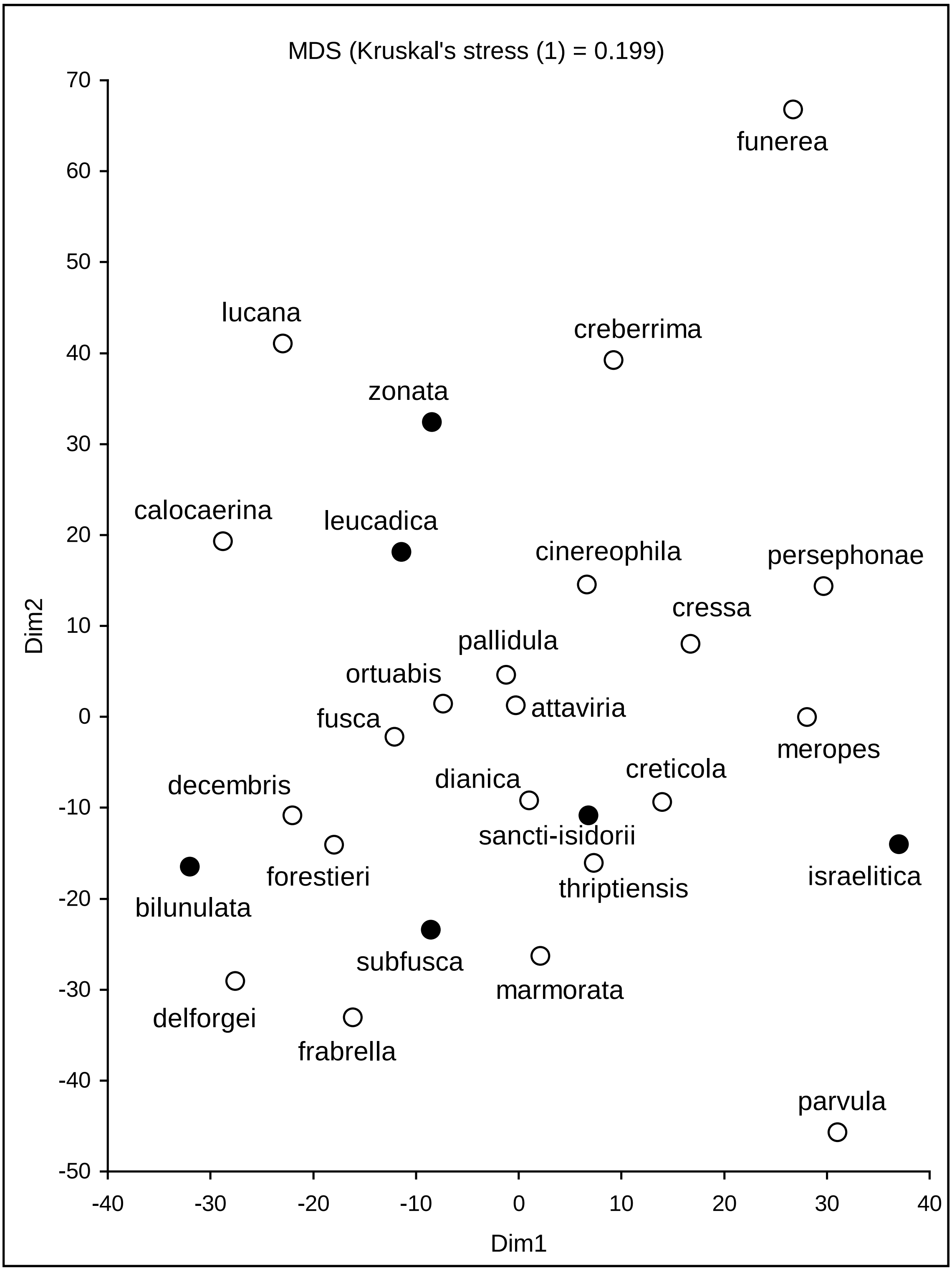

Fig. 32: Multidimensional Scaling of Mahalanobis Distances of *Andrena flavipes* pollinated taxa (●) and other *Ophrys fusca* group taxa. Standardized data.

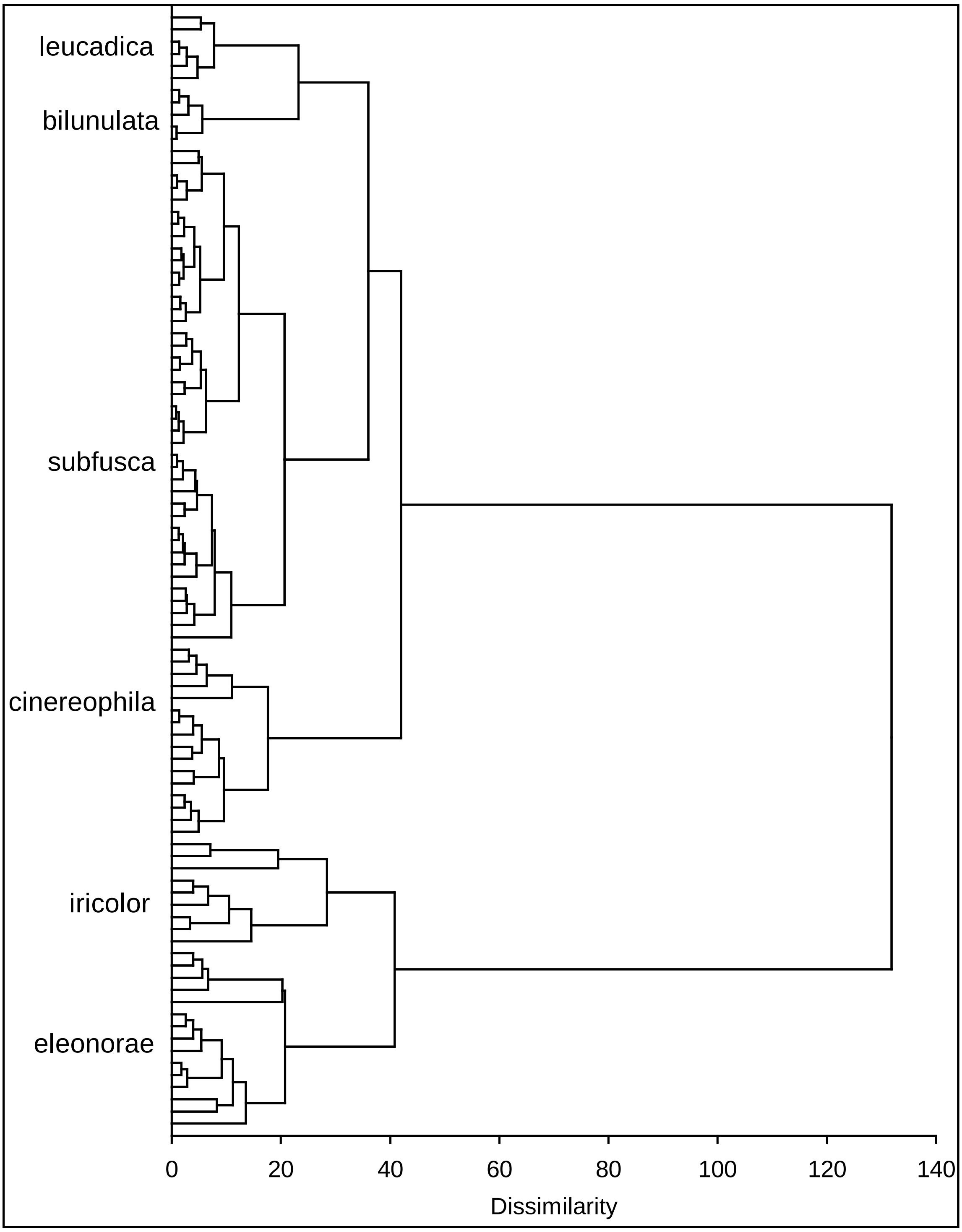

Fig. 33: Agglomerative Hierarchical Clustering (AHC) of Mahalanobis Distances indicating comparative intra-specific variation within *Ophrys subfusca* sub-populations A-E; *O. iricolor* from Crete, Rhodes & Cyprus; *O. eleonorae* from Sardinia, Malta & Tunisia; and *O. cinereophila* from Crete, Rhodes, Cyprus, Attica & Evia.

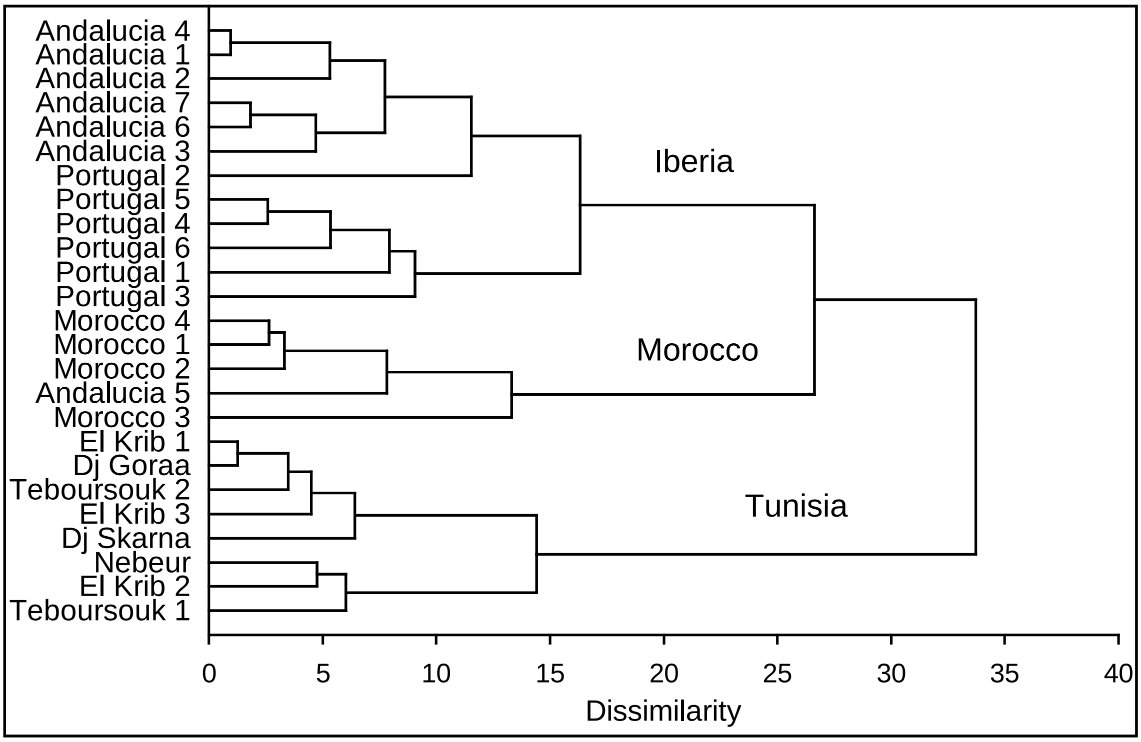

Fig. 34: Agglomerative Hierarchical Clustering (AHC) of Mahalanobis Distances indicating geographical discontinuity within *O. murbeckii*.

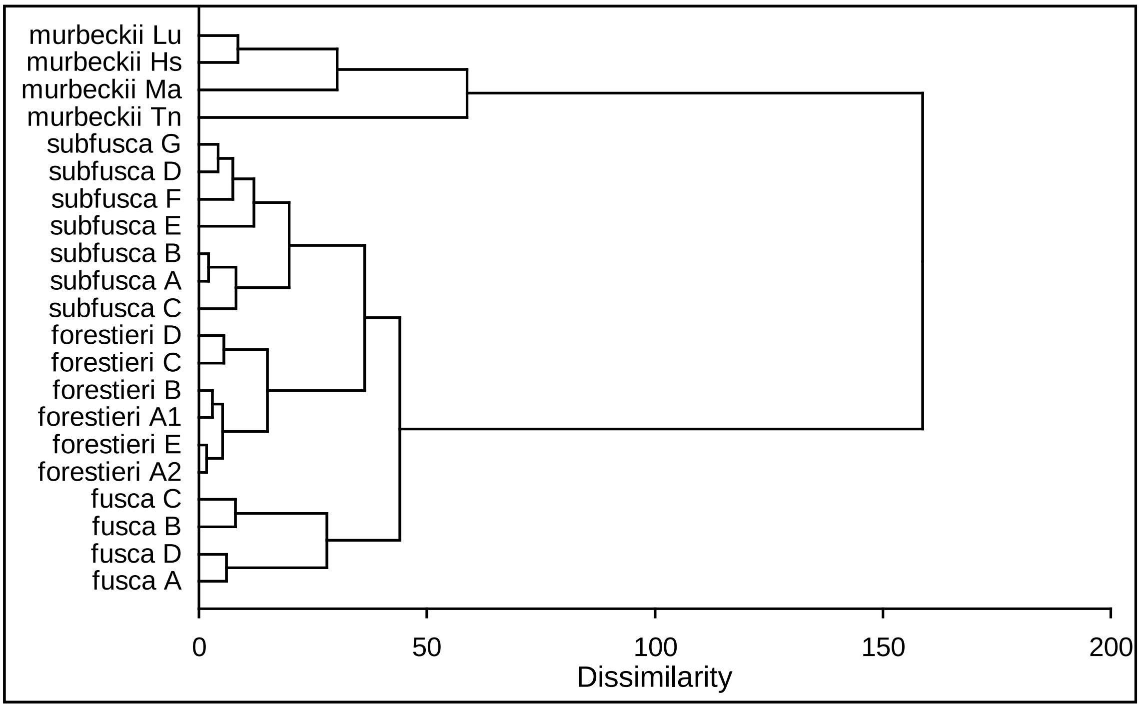

Fig. 35: Agglomerative Hierarchical Clustering (AHC) of Mahalanobis Distances indicating relative variation within *O. murbeckii*. Standardized data.

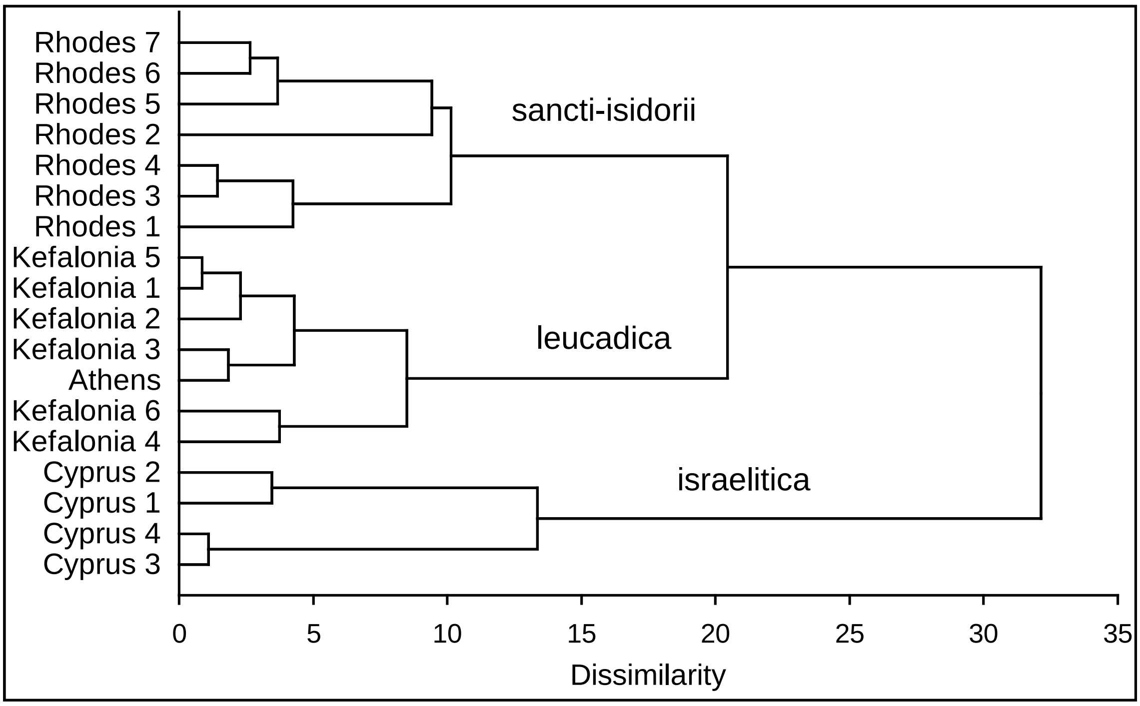

Fig. 36: Agglomerative Hierarchical Clustering (AHC) of Mahalanobis Distances indicating discontinuity of eastern Mediterranean taxa.

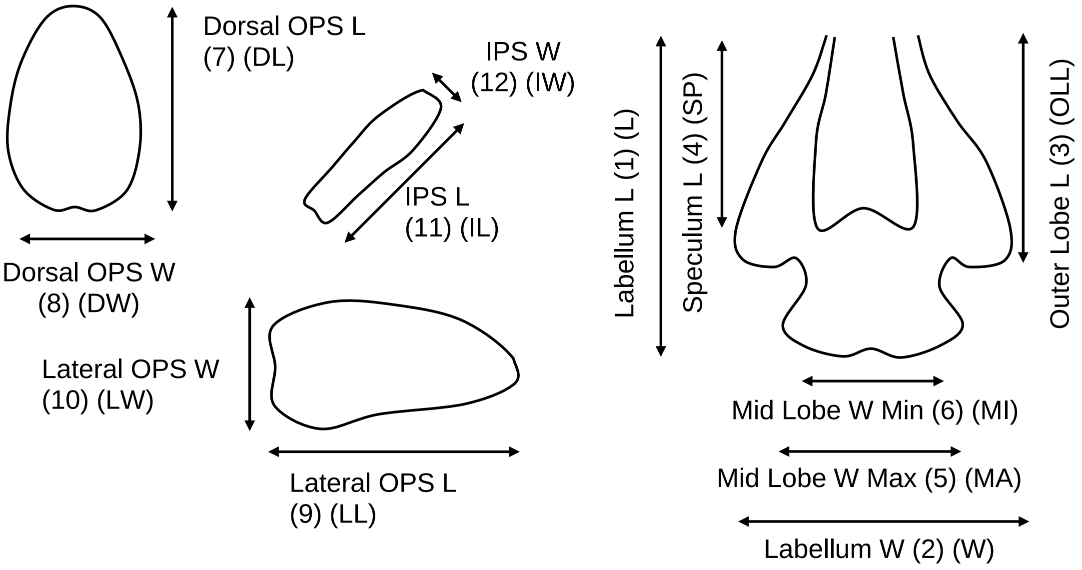

Fig. 37: Explanatory diagram for the 12 morphological characters.

Table 3: List of *Ophrys* populations sampled for biometric studies.

Sample identifier	n	Colony locality	UTM grid WGS 84		altitude	date
Ophrys subfusca N = 449						
Sample A - Malta (*Ophrys caesiella*)						
07MtF026	13	Malta, Red Tower, Marfa Ridge	33SVV	406 816	100	11.03.2007
07MtF027	16	Malta, Id-Dahar, Marfa Ridge	33SVV	401 816	90	11.03.2007
07MtF028	21	Malta, Id-Dahar, Marfa Ridge	33SVV	395 817	90	11.03.2007
07MtF029	12	Malta, Marfa Ridge	33SVV	404 816	75	11.03.2007
07MtF030	6	Malta, Bajda Ridge	33SVV	436 787	45	11.03.2007
Sample B Tunisia (*Ophrys gazella*)						
07TnF031	6	Tunisia, Djebel Amar, Djebel Rorof	33SNF	961 813	275	16.02.2007
07TnF032	13	Tunisia, Djebel Nahali, Djebel el Ayari	33SPF	031 834	55	16.02.2007
07TnF033	5	Tunisia, Djebel Amar, Djebel Rorof	33SNF	974 814	235	16.02.2007
07TnF034	16	Tunisia, Djebel bou Kornine	33SPF	184 646	120	19.02.2007
07TnF035	17	Tunisia, Djebel bou Kornine	33SPF	183 644	140	19.02.2007
07TnF036	6	Tunisia, Djebel Nahli, Dj Sidi Amor	33SPF	029 873	75	19.02.2007
07TnF037	7	Tunisia, Djebel Nahli, Ennahli Park	33SPF	031 828	50	19.02.2007
11TnF060	10	Tunisia, Kesra Forest	32SNE	324 648	995	04.04.2011
Sample C - The Algarve						
07LuF017	26	Portugal, Algarve, Morgado	29SNB	937 118	260	24.03.2007
07LuF018	6	Portugal, Algarve, Boliqueime - Paderne	29SNB	727 122	160	25.03.2007
07LuF019	16	Portugal, Algarve, Santa Margarida - Serro	29SNB	710 232	270	25.03.2007
07LuF020	12	Portugal, Algarve, Pena - Salir	29SNB	821 221	225	25.03.2007
07LuF021	12	Portugal, Algarve, Fonte de Benémola	29SNB	880 182	150	25.03.2007
07LuF022	6	Portugal, Algarve, Marco - Prego	29SPB	126 123	100	27.03.2007
07LuF023	6	Portugal, Algarve, Parque Almeida Matias	29SPB	031 127	235	27.03.2007
07LuF025	6	Portugal, Algarve, 4 Estradas	29SPB	137 117	90	27.03.2007
Sample D - Andalusia						
02HsF024	12	Spain, Granada, Parque del Cubillas	30SVG	397 264	660	13.03.2002
02HsF025	6	Spain, Córdoba, El Portachelo, Río Genil	30SUG	631 241	320	12.03.2002
02HsF026	19	Spain, Málaga, Río del Burgo	30SUF	238 724	600	11.03.2002
02HsF027	20	Spain, Málaga, Sierra de la Pizarra	30SUF	395 884	405	12.03.2002
02HsF028	5	Spain, Córdoba, Puerto El Mojón, Santa Rita	30SUG	803 472	795	12.03.2002
11HsF052	10	Spain, Jaén, SW Carrasco	30SWH	244 353	730	25.03.2011
11HsF053	15	Spain, Jaén, NW Hornos	30SWH	239 312	675	25.03.2011
Sample E - Languedoc-Rousillon / Catalonia						
02GaF029	13	France, Aude, La Clape, Maison Forestière	31TEH	074 756	60	22.03.2002
02GaF030	18	France, Aude, La Clape, S Maison Forestière	31TEH	073 753	55	22.03.2002
02GaF031	13	France, Aude, La Clape, la Garde	31TEH	093 766	65	22.03.2002
02GaF032	14	France, Pyrénées-Orientales, Rivesaltes	31TDH	892 379	35	23.03.2002
02HsF033	5	Spain, Girona, Cala de Mongó	31TEG	140 605	25	24.03.2002
Sample F - La Rioja / Euskadi						
04HsF026	8	Spain, La Rioja, E Las Lagunillas	30TWN	021 127	525	22.04.2004
04HsF027	9	Spain, La Rioja, W Las Lagunillas	30TWN	021 127	525	22.04.2004

Sample identifier	n	Colony locality	UTM grid WGS 84		altitude	date
04HsF028	6	Spain, La Rioja, El Puerto, E Villalba de Rioja	30TWN	109 176	540	23.04.2004
04HsF029	6	Spain, Euskadi, Alava, El Sotillo	30TWN	309 137	600	24.04.2004
04HsF030	5	Spain, La Rioja, Rivas de Teresco - Peñacerrada	30TWN	204 173	710	24.04.2004
08HsF088	8	Spain, Euskadi, Alava, Nanclares de la Oca	30TWN	155 391	510	10.04.2008
Sample G - Mallorca						
05BlF033	7	Majorca, Can Picafort, Platja de Muro	31SEE	111 040	5	14.03.2005
06BlF052	17	Majorca, El Mal-Pas	31SEE	094 200	200	18.03.2006
Ophrys bilunulata N = 80						
05GaF036	30	France, Alpes-Maritimes, Grasse	32TLP	314 369	540	13.04.2005
06GaF058	33	France, Var, Baudisset, St-Paul-en-Forêt	32TLP	125 283	270	04.04.2006
06GaF059	17	France, Alpes-Maritimes, Biot	32TLP	438 327	95	19.04.2006
Ophrys leucadica N = 47						
10IoF042	18	Kefalonia, NW Fragkata	34SDH	616 255	485	09.04.2010
10IoF044	12	Kefalonia, NW Fragkata	34SDH	621 271	405	09.04.2010
10IoF045	5	Kefalonia, NE Sami	34SDH	705 342	205	09.04.2010
10IoF046	5	Kefalonia, W Sami	34SDH	671 349	10	09.04.2010
11GrF024	7	Athens, Mt. Hymettus, W Asterious Monestry	34SGH	464 059	520	14.03.2011
Ophrys zonata N = 76						
11SaF081	8	Sardinia, Dormusnovas, Sa. Duchessa	32SMJ	646 588	395	12.04.2011
11SaF082	37	Sardinia, Thiesi, Lake Bidighinzu	32TMK	729 866	335	13.04.2011
11SaF083	10	Sardinia, Perdasdefogu	32SNJ	403 941	640	17.04.2011
11SaF084	5	Sardinia, Osini	32SNK	410 093	885	18.04.2011
11SaF085	5	Sardinia, NE Sadali	32SNK	236 084	780	18.04.2011
11SaF086	5	Sardinia, Ortuabis	32SNK	110 149	835	18.04.2011
11CoF108	6	Corsica, Ogliastro	32TNN	053 222	20	12.04.2011
Ophrys funerea (*Ophrys sulcata*) N = 21						
05GaFu030	11	France, Aude, N le Mas, Bugarach	31TDH	444 481	440	21.05.2005
05GaFu031	5	France, Aude, SE le Mas, Bugarach	31TDH	449 476	460	21.05.2005
05GaFu032	5	France, Aude, W Bugarach	31TDH	459 472	460	21.05.2005
Ophrys fusca* subsp. *sancti-isidorii N = 86						
10DoF026	8	Rhodes, Kalithes	35SPA	041 238	160	02.04.2010
11DoF003	6	Rhodes, S Faliraki, Ladiko	35SPA	077 198	55	10.03.2011
11DoF004	5	Rhodes, N Faliraki	35SPA	074 243	55	10.03.2011
11DoF005	16	Rhodes, NW Kalithies	35SPA	032 241	225	10.03.2011
11DoF006	6	Rhodes, NE Empona	35SNA	789 114	370	11.03.2011
11DoF007	16	Rhodes, NE Empona	35SNA	787 113	400	11.03.2011
11DoF008	10	Rhodes, Kattivia marsh	35SNV	795 782	40	12.03.2011
11DoF009	19	Rhodes, S Kattivia	35SNV	697 761	60	12.03.2011

Sample identifier	n	Colony locality	UTM grid WGS 84	altitude	date
Ophrys forestieri					
Sample E - Sardinia N = 45					
11SaF090	5	Sardinia, S Dorgali	32TNK 482 547	565	16.04.2011
11SaF091	15	Sardinia, S Dorgali	32TNK 478 543	600	16.04.2011
11SaF092	10	Sardinia, S Dorgali	32TNK 477 542	600	16.04.2011
11SaF093	10	Sardinia, S Dorgali	32TNK 471 527	655	16.04.2011
11SaF094	5	Sardinia, Triei, Genna Ramene	32TNK 532 342	570	16.04.2011
Ophrys fusca					
Sample A - Lisboa and central Portugal N = 144					
04LuF001	8	Portugal, Estremadura, Aldeia de Irmãos	29SMC 972 616	90	10.03.2004
04LuF002	11	Portugal, Estremadura, Serra de S. Francisco	29SNC 018 649	130	11.03.2004
04LuF003	14	Portugal, Estremadura, Moinho da Cruz	29SNC 014 645	175	11.03.2004
04LuF004	14	Portugal, Estremadura, Palmela	29SNC 076 689	180	11.03.2004
09LuF026	10	Portugal, Estremadura, Sesimbra, Santana	29SMC 917 567	220	22.03.2009
09LuF027	10	Portugal, Estremadura, Sesimbra Castelo	29SMC 908 562	185	22.03.2009
09LuF028	5	Portugal, Estremadura, Sa. da Arrábida	29SNC 053 605	130	22.03.2009
09LuF029	5	Portugal, Estremadura, Cascais, Charneca	29SMC 603 870	65	23.03.2009
09LuF030	6	Portugal, Estremadura, Bucelas	29SMD 892 049	90	23.03.2009
09LuF031	5	Portugal, Estremadura, Santiago dos Velhos	29SMD 904 066	160	23.03.2009
09LuF032	5	Portugal, Estremadura, Santiago dos Velhos	29SMD 905 081	185	23.03.2009
09LuF033	5	Portugal, Estremadura, Ericeira	29SMD 643 152	90	23.03.2009
09LuF037	6	Portugal, Estremadura, Mato de Cruz	29SMD 929 080	315	31.03.2009
09LuF034	5	Portugal, Ribatejo, Ind. Estate do Campores	29SNE 518 177	300	25.03.2009
09LuF035	5	Portugal, Beira Littoral, Rabaçal - Pombalinho	29TNE 460 306	205	25.03.2009
09LuF036	7	Portugal, Estremadura, Alvorge	29SNE 470 249	260	29.03.2009
09LuF038	6	Portugal, Estremadura, Sa. de Montejunto	29SMD 929 362	440	24.03.2009
09LuF039	17	Portugal, Estremadura, Sa. de Montejunto	29SMD 926 363	400	24.03.2009
Sample B - The Algarve N = 32					
07LuF003	6	Portugal, Algarve, Marco - Prego	29SPB 124 123	100	27.03.2007
07LuF004	12	Portugal, Algarve, 4 Estradas	29SPB 137 117	90	27.03.2007
07LuF005	8	Portugal, Algarve, Bensafrim - Lagos	29SNB 250 114	20	28.03.2007
07LuF006	6	Portugal, Algarve, Silves	29SNB 506 159	50	28.03.2007
Sample C - Cádiz N = 20					
08HsF001	5	Spain, Cádiz, San Ambrosio	30STF 304 108	140	19.03.2008
08HsF002	10	Spain, Cádiz, Pinar de Barbate	30STF 310 104	105	19.03.2008
08HsF003	5	Spain, Cádiz, Los Caños de Meca	29SQA 685 093	20	19.03.2008
Sample D - Badajoz N = 39					
07HsF001	21	Spain, Badajoz, Alconera	29SQC 195 510	520	26.03.2007
07HsF002	18	Spain, Badajoz, Los Santos de Maimona	29SQC 309 590	560	26.03.2007

Sample identifier	n	Colony locality	UTM grid WGS 84	altitude	date
Ophrys israelitica N = 35					
11CyO031	6	Cyprus, Gerasa	36SVD 999 509	350	15.03.2011
11CyO032	10	Cyprus, S Apesia	36SVD 969 475	320	15.03.2011
11CyO033	19	Cyprus, Akamas, Ag. Minas	36SVD 397 758	275	16.03.2011
Ophrys murbeckii N = 82					
Tunisia sample					
11TnL066	4	Tunisia, Dj. Skarna	32SNE 072 643	1060	04.04.2011
11TnL067	10	Tunisia, S Nebeur	32SMF 813 157	555	05.04.2011
11TnL068	21	Tunisia, N El Krib	32SNF 108 233	650	05.04.2011
11TnL069	13	Tunisia, N El Krib	32SNF 102 239	745	05.04.2011
11TnL070	6	Tunisia, N El Krib	32SNF 120 269	595	05.04.2011
11TnL071	13	Tunisia, Dj. Goraa, W Teboursouk	32SNF 144 336	645	05.04.2011
11TnL072	10	Tunisia, Dj. Goraa, W Teboursouk	32SNF 172 369	550	05.04.2011
11TnL073	5	Tunisia, Dj. Goraa, W Teboursouk	32SNF 174 371	550	05.04.2011
Ophrys murbeckii* subsp. *quarteirae N = 185					
Morocco sample					
07MaL038	30	Morocco, Volubilis	30STC 642 732	370	14.03.2007
07MaL039	5	Morocco, Jbel Zerhoun	30STC 757 668	755	14.03.2007
07MaL040	6	Morocco, Oued-el Kell, Meknes	30STC 537 490	535	15.03.2007
07MaL041	18	Morocco, Oued-el Kell, Meknes - Oulmés	30STC 533 476	425	16.03.2007
Algarve sample					
09LuL008	10	Portugal, Algarve, Portela	29SNB 669 220	190	31.03.2009
09LuL009	6	Portugal, Algarve, Tunes	29SNB 670 131	85	31.03.2009
09LuL010	7	Portugal, Algarve, Malhão	29SNB 727 121	160	01.04.2009
09LuL011	16	Portugal, Algarve, Castelo de Paderne	29SNB 712 128	75	01.04.2009
09LuL012	6	Portugal, Algarve, Castelo de Paderne	29SNB 709 128	55	01.04.2009
09LuL013	5	Portugal, Algarve, Monte Seco	29SNB 792 144	280	01.04.2009
Andalusia sample					
09HsL014	17	Spain, Málaga, Rio del Burgo	30SUF 248 727	570	25.04.2009
09HsL015	6	Spain, Málaga, Rio del Burgo	30SUF 214 709	675	25.04.2009
09HsL016	7	Spain, Málaga, Pico del Jarro	30SUF 230 682	850	25.04.2009
09HsL017	11	Spain, Cádiz, Zahara	30STF 866 762	710	26.04.2009
09HsL018	5	Spain, Sevilla, Cerro de El Mojón	30STF 846 922	420	26.04.2009
09HsL019	15	Spain, Cádiz, Sa. del Pinar	30STF 820 707	810	27.04.2009
09HsL020	5	Spain, Málaga, Sa. del Oreganol	30SUF 100 603	975	28.04.2009