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Systematics of *Primula veris* (*Primulaceae*)

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Abstract: Primarily, 50 characters taken from herbarium specimens, cultivated plants, and whole populations of *Primula veris* L. were critically evaluated. Only 24 characters were found to be suitable for taxonomic use. The geographical and multivariate analysis of about 1000 herbarium specimens from the whole area lead to a reduction of the number of subspecies. Only a less restricted subsp. *veris* (incl. subsp. *canescens*, subsp. *inflata* and probably including subsp. *macrocalyx*) and subsp. *columnae* (incl. subsp. *suaveolens*) can be distinguished considering the variability of characters within an individuum, under different culture conditions, and during the growth period. The status of the taxon "*macrocalyx*" cannot be cleared presently.

The crude drug Radix Primulae (Österr. Arzneibuch 1990) originates from both *Primula veris* L. and *Primula elatior* (L.) HILL. Dried underground parts are used in medical practice as a secretomotoric and secretolytic expectorans because of their saponin content (WICHTL 1989). Rhizomes and roots of *P. veris* and *P. elatior* can be differentiated anatomically (KOFLER 1922, LÄNGER 1983) as well as by means of saponin analysis (HARTKE & MUTSCHLER 1986). There are different saponin patterns in *P. veris* plants of different origins (WAGNER & REGER 1986). It is unknown whether these differences relate to different taxa or to different collecting time of the respective samples. The taxonomic division of these species into subspecies has not yet been considered as a possible background for variations in the secondary metabolism.

Unfortunately, the unambiguous identification of subspecies of *P. veris* is very difficult. In the most recent taxonomic revision, SCHWARZ (1968) defined three major taxa, each divided into two subspecies (the "pure" type and a hybridogenous type with another species):

1. *P. veris* L. (British Islands, Portugal, N Spain, France, C Europe to S Scandinavia, S Finland, NW Russia, Poland, Czech Republik, Slovakia, and Austria). a) *P. veris* subsp. *veris*. b) *P. veris* subsp. *canescens* (OPIZ) HAYEK: intermediate between 1 a and 3 a (S Poland, C Bohemia and in a continuous area from Lower Austria through Hungary to Rumania and W Ukraine).

2. *P. columnae* TEN. (S France, Italy, Submediterranean part of the Balcan peninsula, NW Anatolia). a) *P. columnae* subsp. *columnae*. b) *P. columnae* subsp. *suaveolens* (BERTOL.) SCHWARZ: intermediate between 2 a and 1 b (xerothermic

valleys of Switzerland, the Rhine valley, C France, Spain, border of the Eastern Alps to the western parts of the Balcan peninsula).

3. *P. inflata* LEHM. (S Siberia, region of Wolga-Kama, E Ukraine, Turkestan, N Iran, Caucasus, N Anatolia and Crimea). a) *P. inflata* subsp. *macrocalyx* (BUNGE) SCHWARZ. b) *P. inflata* subsp. *inflata* (= *P. pannonica* KERNER): intermediate between 3 a and 2 a (Rumania, Hungary) or 3 a and 2 b (western parts of the Balcan peninsula to Lower Austria).

The validity of *P. columnae* and *P. inflata* in the rank of a species was revised by GUTERMANN & al. (1973), *P. veris* L. is now divided into six subspecies. However, we have found that generally it is very difficult to ascribe specimens to a certain subspecies, as the plants often present character combinations different to those cited in the keys of SCHWARZ (1968), LÜDI (1927) or VALENTINE (1972), even in the pannonic part of Lower Austria (e.g., Mödling, locus classicus of *P. pannonica* KERNER).

The name "subsp. *canescens* (OPIZ) HAYEK" is used by SCHWARZ (1968), LÜDI (1927), and VALENTINE (1972). In the sense of LÜDI and VALENTINE it relates to plants from continental parts of Europe, in the sense of SCHWARZ only to a northern type, while types from eastern continental parts are named as subsp. *inflata*. One sheet deposited in WU had been determined by HAYEK (without date) with the name *P. pannonica* KERNER. He recognized no distinguishable types and denoted this name as a synonym to subsp. *canescens* (HAYEK 1928); in the sense of SCHWARZ (1968) it is a synonym to subsp. *inflata*.

Material from the locus classicus of *P. pannonica* with comments of KERNER (1886) was available in the herbarium WU. His main statement is, that the illustration of LEHMANN (1817) given for his *P. inflata* does not correspond to plants growing in Hungary. He supposes a mistake and that this plant refers to the type "*macrocalyx*". The considerable differences in several character states between the two plants on this sheet examined are noteworthy (e.g., indumentum on abaxial side of leaves: one plant length up to 380 µm, most hairs unbranched, the other 650 µm, nearly all hairs branched). In the sense of KERNER these differences are insignificant, while at the consequent application of the key of SCHWARZ (1968) these plants belong to two different subspecies.

Additionally there are divergations in descriptions of taxa between several keys (SCHWARZ 1968, LÜDI 1927, VALENTINE 1972, PIGNATTI 1982), undoubtedly caused by the lack of the precise definition of the diagnostic characters and their measurements. The key of SCHWARZ makes use of several characters which can be measured in different ways [e.g., corolla diameter: determination on the fresh plant (value depends on the corolla shape), measuring of separately fixed corolla limbs (only few vouchers are prepared in this way) and the method proposed in the present paper (see Material and methods, the only one which is suitable for working with herbarium material)]. There are also no comments on the determination of the hair length. PIGNATTI (1982) proposes the application of a magnifying glass, in this way, however, the thickness of the indumentum but not the actual length of the often curved hairs is measured. In our opinion the hair length can only be determined correctly with a microscope. SCHWARZ (1968) emphasizes that all characters of leaves and calyx cited in his key are true for summer plants only. Apart from the hair length PIGNATTI (1982) uses the same values of characters for dif-

ferentiation of subspecies but he lays emphasis on the fact that all data are true for plants during the flowering period.

The main characters cited for discrimination of the subspecies are listed in Table 1. But at the beginning of a revision numerous characters have to be evaluated for their taxonomic significance by comparison of several leaves and flowers of an individuum, by observation during the growth period and, above all, by cultivation under different conditions. *P. veris* had been the object of cultivation experiments previously (MESTENHAUSER 1961, DRODZDZ 1964, JENTZSCH & al. 1973). These tests, however, were dominated by questions of propagation and saponin content. Up to now nothing is known about modificative variability of characters.

Consequently a revision must be based on a geographical analysis but not on the different estimations in systematics and not on the nomenclature on herbarium specimens. Therefore, the selection of specimens for analysis regards the whole area of *P. veris*.

Material and methods

Plant material. About 1000 herbarium specimens from the whole distribution area (Table 2) were examined. The vouchers are largely loans from W, WU, KL, TSB, G, and PD; in Lower Austria and in Italy (Alpi Apuane; Rovereto) extensive personal collections were made, the material is deposited in the herbarium of the Institute of Pharmacognosy, University of Vienna. From the 53 localities observed in Lower Austria and from the eight origins in Italy up to 30 specimens were examined. The complete list of specimens is available from the authors. Sampling sites which are cited in the text are coded with reference numbers which relate to the map in Fig. 8. Samples of four populations from Lower Austria (about 30 plants each) have been cultivated under different culture conditions.

Definition of characters. Dry material (herbarium specimens) was used for measuring the dimensions of leaves. Small parts of leaves and flowers were warmed with a 60%

Table 1. Discrimination characters between the subspecies of *Primula veris* according to SCHWARZ (1968)

Character	subsp. *veris*	subsp. *canescens*	subsp. *columnae*	subsp. *suaveolens*	subsp. *macrocalyx*	subsp. *inflata*
Base of lamina	decurrent	cuneate	cordate	cordate	decurrent	cuneate-subcordate
Petiole	alated	narrow alated	scarcely alated	small alated	denticulate alated	broad alated
Leaf hair length	0.1–0.3 mm	??	− 2 mm	− 2 mm	0.4–0.5 mm	0.5–1 mm
Branched hairs	no	yes	yes	yes	no	yes
Corolla-diameter	6–12 mm	< 10 mm	16–20 mm	12–15 mm	< 10 mm	10–15 mm
Calyx length	< 15 mm	12–18 mm	< 15 mm	??	??	??
Capsule length	= calyx	< calyx	> calyx	??	< calyx	= calyx

Table 2. Number of examined *Primula veris* agg. specimens per country

Albania	10	France	15	Rumania	10
Austria	542	Great Britain	3	Spain	7
Belgium	1	Greece	8	Sweden	7
Bulgaria	4	Hungary	9	Switzerland	5
CSFR	7	Iran	3	Turkey	4
Denmark	2	Italy	247	"USSR"	7
Fed. Rep. Germ.	14	Norway	1	Yugoslavia	56
Finland	15	Poland	5	Total	982

solution of chlorale hydrate. The flowers reach the original size for exact measurements in this way. In addition chlorophyll and other components of the cells are destroyed and the slides are cleared for anatomical observation.

A) Leaf characters. (All measurements were taken from the largest leaf of the rosette):

1. Width of leaf 1 cm below the leaf tip.
2. Maximum width of leaf.
3. Width of petiole at the transition of lamina into petiole.
4. Distance between position of maximal width and leaf tip.
5. Length of lamina (in the case of cordate leaves the maximal length of the lamina).
6. Length of petiole.
7. Shape of base of the lamina. 6 categories: 1 = "cordate", 3 = "truncate", 6 = "cuneate", other categories intermediate.
8. Margin of leaf: comparison of microscopical drawings prepared with a drawing apparatus.
9. Amount of pubescence on abaxial side of the leaf (subjectively defined). 5 categories, with 5 being the densest. The density of the small glandular trichomes is not ascertainable if the leaf is pubescent or tomentose.
10. Max. length of articulate glandular trichomes. A small piece of the leaf was bent back after clearing with chlorale hydrate. The longest hair found was measured with a calibration ocular in the microscope. All bends of twisted hairs were considered.
11. Degree of ramification of articulate glandular trichomes. Observation in microscope. 6 categories: 0 = not branched, 5 = most hairs branched.
12. Shape and dimension of epidermis cells (comparison of drawings prepared with a drawing apparatus).
13. Number of stomata per mm^2. Ten counts were made in the visual field of the microscope both from adaxial side and abaxial side of the leaf. The means were converted to the plane of 1 mm^2.
14. Diameter of palisade cells. The diameter was measured from the drawings of palisade cells and the means compared.

Ratios were computed from the following characters:

15. Lamina length/maximum width of leaf.
16. Lamina length/distance between position of maximal width of leaf and tip of leaf.
17. Maximum width of leaf/width 1 cm behind tip.
18. Lamina length/width of petiole.
19. Total width of leaf/width of petiole.
20. Lamina length/length of petiole.
21. Number of stomata per mm^2 abaxial side/number of stomata per mm^2 adaxial side.

B) Flower characters:

22. Length of peduncle from place of attachment on the rhizome to the circle of bracts.

23. Anatomy of the cross section through the peduncle.
24. Anatomy of the cross section through the pedicel.
25. Number of flowers incl. buds.
    A fully developed flower was warmed with chlorale hydrate for measurement of dimensions of calyx and corolla.
26. Length of calyx tube, measured from the insertion of the ovary to the beginning of the teeth of the calyx.
27. Length of corolla tube.
28. Length of corolla lobes.
29. Width of corolla lobes.
30. Corolla diameter. The limb of the corolla was removed from the corolla tube. The petals were spread and the diameter was measured. The shape of the corolla (concave or flat) is not determinable on a herbarium specimen.
31. Length of calyx teeth (= mean of all 5 calyx teeth).
32. Circumference of calyx. The calyx was sliced and spread on a slide. Division by 5 = width of calyx teeth.
33. Total length of calyx = length of calyx tube + length of calyx teeth.
34. Max. length of hairs on the abaxial surface of the calyx. All measurements were taken on the midrib of the sepals in the height of the basis of calyx teeth.
35. Degree of ramification of hairs on the outside of the calyx. 2 categories: 1 = branched hairs present, 2 = branched hairs absent.
36. Number of stomata on both abaxial and adaxial side of calyx. The counts were taken symmetrically on both sides of the midrib of calyx teeth. The connection zone of two calyx teeth is free of stomata.
37. Max. length of hairs on the pedicel. A region of about 1 cm was examined.
38. Degree of ramification of hairs on the pedicel (similar to no. 35).
39. Diameter of pollen grains = mean of 30 measurements from a preparation in chlorale hydrate.
40. Capsule: Ratio of capsule length to total calyx length.
41. Shape and anatomy of seeds.
    The following ratios were computed:
42. Length of calyx teeth/width of calyx teeth.
43. Length of calyx tube/length of calyx teeth.
44. Length of calyx tube/width of calyx teeth.
45. Length of corolla tube/total length of calyx.
46. Density of stomata on the adaxial surface of calyx/density of stomata on the abaxial surface of calyx.
47. Diameter of corolla/diameter of calyx (circumference divided by π).
    C) Underground parts:
48. Anatomy of the cross section through the rhizome.
49. Anatomy of the cross section through the roots.
50. Size and shape of starch.

Methods of numerical analysis. Beside uni- and bivariate methods (frequency plots, scatterplots) several multivariate methods were used: cluster analysis (CA, WARD-algorithm, cited in FAHRMEIR & HAMERLE 1984), factor analysis (Centroid method, Varimax-rotation, cited in HARTUNG & ELPELT 1986), principal component analysis (PCA, cited in BORTZ 1985) and MOBCENTR analysis (MA, SAUKEL & LÄNGER 1989).

The data were standardized for CA and PCA (Z-transformation) or normed over the range of a character for MA.

The result of a multivariate classification depends on the chosen algorithm and on the selected characters. A priori it is not known which combination of features leads to an interpretable result in multivariate classification. Therefore, different data sets were ana-

lyzed: 1) characters employed in the classification of SCHWARZ (1968); 2) the highest loading character from each factor after factor analysis of characters; 3) accentuation of ratios; 4) all characters except ratios; 5) individual combinations; 6) all characters. The results of all analyses were graphically compared (like in SAUKEL & LÄNGER 1992) and a final result created.

Results

Evaluation of characters. In numerical analysis the only useful characters are those which vary from population to population or from individual to individual but remain ± unchanged under environmental modification.

For example, the values of diameter of pollen grains are nearly equal for all pin and all thrum plants. Therefore, this character is not suitable for classification. The variability of characters within one plant organ is sufficiently low for all characters, but some characters are not suitable for systematic use because of their considerable variability within a single individual. Especially the density of stomata on leaves as well as on calyx is variable within one plant (differences up to 460%), whereas the dimensions of calyx and corolla are nearly equal for an individuum (Table 3). During the growth period the leaves become enlarged, the base of lamina becomes more decurrent, but the overall shape remains ± unchanged. The hair length on the abaxial side of the leaves may increase considerably, especially immediately after the flowering period (on an average – for a population in Lower Austria – from 321 µm to 433 µm). Likewise the degree of ramification of the hairs increases, but in the natural habitat no change from unbranched to branched trichomes was observed. To avoid this variation only flowering specimens were considered in numerical analysis. Cultivation experiments with about 120 plants

Table 3. Minimal, mean, and maximal difference of the character states on two leaves or two flowers of one individual observed in 62 *Primula veris* spp. specimens from Lower Austria. 0% = no difference

Character	minimal	mean	maximal
Hair length of leaf	2%	26%	68%
Degree of ramification of leaf hair	0 cat.	0.7 cat.	3 categories
Density of hairs of leaf	0 cat.	0.3 cat.	2 categories
Density of adaxial leaf stomata	1.5%	30%	153%
Density of abaxial leaf stomata	1%	59%	240%
Ratio density of leaf stomata	5%	94%	458%
Length of calyx tube	0%	5.3%	20%
Width of calyx teeth	0%	11%	48%
Length of calyx teeth	0%	7%	25%
Length of corolla tube	0%	5%	21%
Corolla diameter	0%	14%	33%
Hair length outside calyx	0%	11%	27%
Density of stomata outside calyx	2%	23%	76%
Density of stomata inside calyx	4%	36%	114%
Pollen grain diameter	0%	2.3%	9.8%
Hair length of pedicel	0%	11%	41%

of four samples originating from Lower Austria demonstrated the dependence of leaf characters on light conditions, whereas characters of flowers were not influenced. Leaves and petioles of plants in sunlight are considerably smaller than leaves from shaded plants during the whole vegetative period. This is in agreement with observations on natural populations. Plants competing with tall vegetation (under trees, not mown meadows) produce larger leaves than plants with small surrounding vegetation. Compared with the population in the natural location the cultivation conditions lead to slightly smaller hairs and a smaller degree of ramification of hairs. Thus the great morphological variability of leaves found in the original locations disappears.

Consequently small differences in leaf shape and hair length on the abaxial side of leaves are not significant for differentiation of taxa especially from Lower Austria.

Important to remember is the fact that plants from the Alpi Apuane remain unchanged in leaf shape as well as in indumentum at least after cultivation in Vienna for one year. Further cultivation experiments failed. After two growth periods only one plant of 30 was still alive.

The anatomy of rhizomes and roots is uniform (examined on about 200 specimens), plants from the Mediterranean region (typical subsp. *columnae*) do not differ from those from Lower Austria. The starch content is very low during the flowering period (MESTENHAUSER 1961). If possible, the maximal and the mean size of starch grains was measured. The maximal size is between 5 µm and 20 µm, the mean between 4 µm and 10 µm, the mean values differ within a population considerably (up to 100%). The shape of all examined starch grains is similar. Therefore the origin of the crude drug "Radix Primulae" is not determinable using anatomical criteria based on starch grains.

By chance the existence of stolons in *P. veris* was found (LÄNGER & SAUKEL 1986). It was, therefore, possible to compare the character states of genetically identical plants. Considerable differences occur in the diameter of the corolla (40%) and in the ratio of corolla tube length/total calyx length (23%). These are two characters important for the identification of subspecies of *P. veris* by SCHWARZ (1968).

On account of the great variability of some characters (even genetically identical plants are different) possible subspecific taxa must differ considerably in their character states.

After elimination of highly correlated characters [hair length on the abaxial side of the leaf (correlated with the length on the adaxial side); length of corolla lobes, width of corolla lobes (correlated with the corolla diameter)] only those listed in Table 4 were used. Nevertheless, characters like the length of the petiole were included in numerical analysis because of their importance in the key of SCHWARZ (1968), but little value was attached to the results based on such character combinations.

Geographical analysis. Lower Austria. Because of the numerous specimens from Lower Austria (421 plants from 53 populations including plants from the locus classicus of *P. pannonica* were considered in the numerical analysis) this climatically diversified region was taken as a starting point. The sampling sites were chosen from the main climatic regions of Lower Austria (for example from the mesic "Dunkelsteiner Wald", a part from the Herzynicum with granite as

Table 4. Characters used in the numerical analysis of *Primula veris*

Width of petiole	Lamina length of leaf/max. width of leaf
Shape of lamina base of leaves	Lamina length of leaf/distance tip of leaf to position of max. width
Length of petiole	
Hair length on abaxial side of leaves	Max. width of leaf/width 1 cm behind tip
Degree of ramification of hairs of leaves	Lamina length of leaf/width of petiole
Density of hairs on abaxial side of leaves	Max. width of leaf/width of petiole
Length of calyx tube	Lamina length of leaf/length of petiole
Length of calyx teeth	Length of calyx teeth/width of calyx teeth
Width of calyx teeth	Length of calyx tube/width of calyx teeth
Hair length on the outside of calyx	Length of calyx tube/length of calyx teeth
Length of corolla tube	Length of corolla tube/total length of calyx
Corolla diameter	Circumference of calyx
Hair length on pedicle	Corolla diameter/circumference of calyx

substratum; or from the Pannonic part with east-continental and submediterranean influence).

All characters have unimodal distributions. The comparison of the minimal, mean, and maximal value of each population shows, however, a continuous transition between the extremes. Considering the variability of characters the differences in character states among the populations are insignificant. A grouping of material using frequency plots or scatterplots is impossible.

Because of the great variability of some characters within populations, specimens from one site are classified to different classes in multivariate analysis independent of the chosen combination of characters. Therefore classifications were done using only the mean of the character states from each population.

The compromise of the different results of ten classifications (hierarchical and non-hierarchical, different combinations of characters) are five groups of populations. In Figs. 1–4 the minimal, mean, and maximal value of some characters are shown for each classification group. In the illustrations "A" the means of the populations, in "B" the data of single specimens are used for drawing. Although the variance is smaller, the means of character states of populations do not allow an unambiguous identification of a single group. The mean of most character states increases or decreases from group 1 to group 5 continuously (Fig. 1). The means of hair lengths on the abaxial side of leaves more or less characterize the groups 1 and 5; data of single specimens do not so (Fig. 2). The means of the lengths of the calyx tube characterize groups 1 and 2 versus the others (Fig. 3 A), the means of the diameters of the corolla the groups 2 and 4 versus the groups 1, 3, and 5 (Fig. 4 A). The intrapopulation variability of these characters leads to an indistinctness of the classes (Figs. 3 B, 4 B).

Hypothetical "average plants" based on the mean character states of each classification group are shown in Fig. 5. All proportions are reproduced correctly, only the corolla must be drawn in plane because of the kind of measurement. There are only insignificant differences between the classification groups. Plants from classification group 1 are in agreement with the description of subsp. *veris*, those

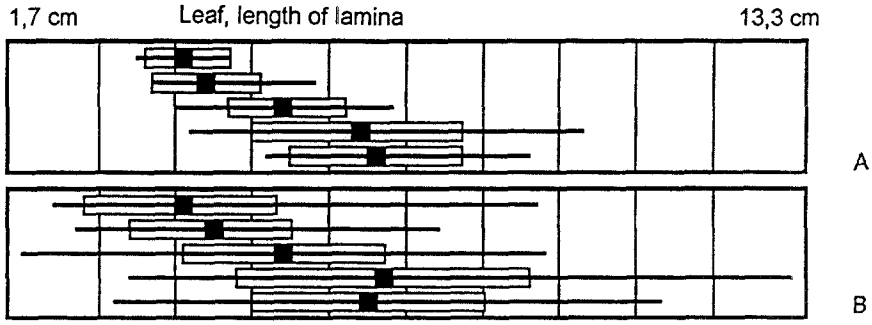

Fig. 1

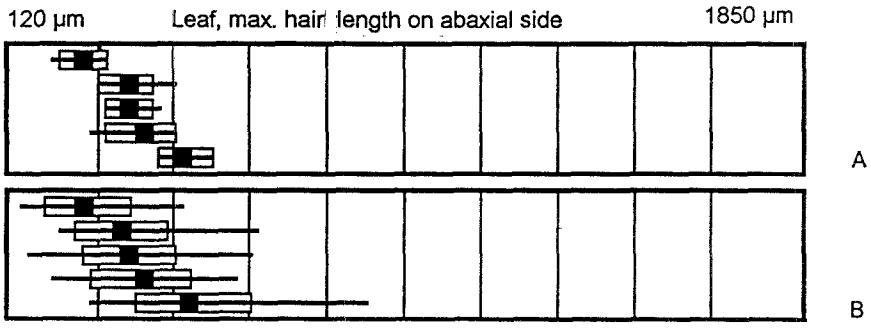

Fig. 2

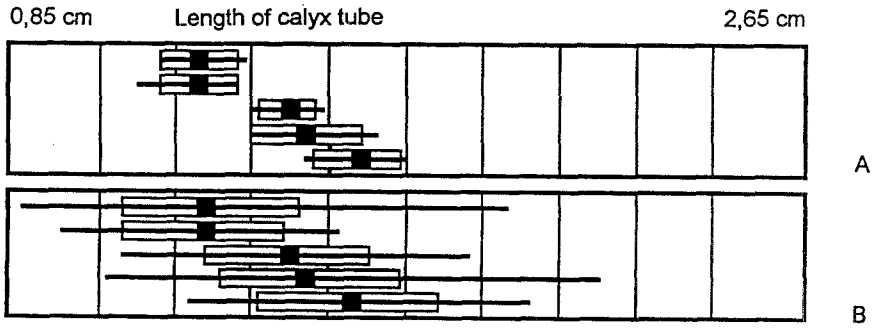

Fig. 3

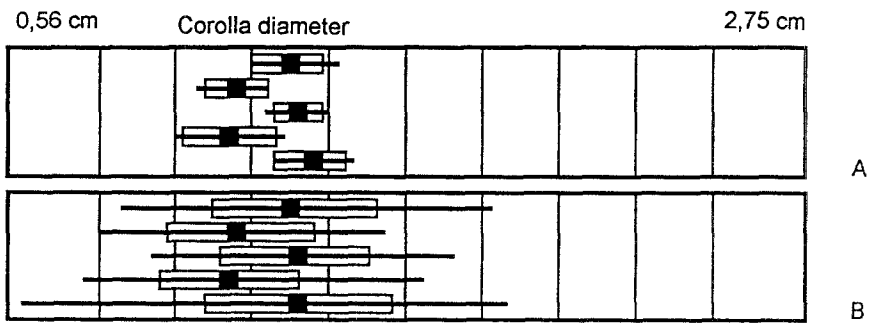

Fig. 4

Figs. 1–4. Comparison of the character states of the five classification groups of specimens of *Primula veris* from Lower Austria. – Minimal to maximal value; ■ mean; □ standard deviation. Groups 1–5 from top to base of the respective figures. *A* Means of populations. *B* Data of all specimens. – Fig. 1. Length of leaf lamina. – Fig. 2. Hair length of abaxial side of leaf. – Fig. 3. Length of calyx tube. – Fig. 4. Corolla diameter

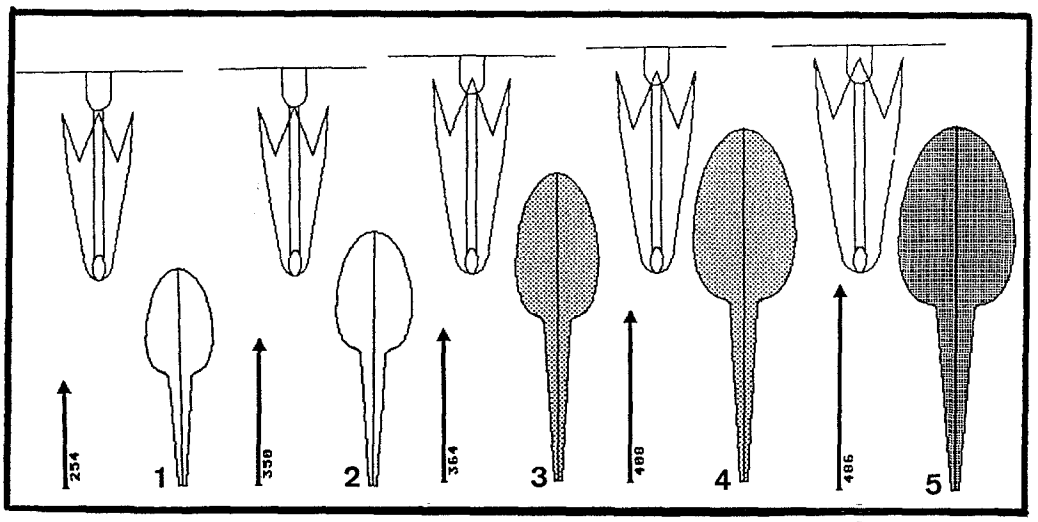

Fig. 5. "Average plants" reconstructed according to the mean values of characters of the five classification groups from Lower Austria. Length of arrow according to hair length on abaxial side of leaves. Increasing density of the gray colour of leaves indicates a higher degree of ramification of hairs

from classification group 5 which resemble "*P. pannonica*". The other classification groups are intermediate in their character states and not typical of a taxon.

Connections between climatic factors and the morphological results are evident (Fig. 6). Plants from the Pannonic part of Lower Austria (on the whole the region southwards and eastwards from Vienna, following the Danube westwards, characterized with a small amount of rainfall, mean yearly temperature higher than 8 °C) are reflected mainly in groups 4 and 5. The populations of the classification groups 1 and 2 are localized in mesic parts. But the differences in the character states between the marginal groups 1 and 5 are insignificant following the results of common garden experiments.

Austria. *P. veris* occurs in the whole territory of Austria. 75 specimens, which include plants from lower altitudes (370 m s.m.) up to 1200 m s.m., were analysed in addition to those of Lower Austria. Most of the specimens are single vouchers from a sampling site. To avoid an unbalanced classification when combining all data from Austria (421 specimens from Lower Austria versus 75) similar and neighbouring origins from Lower Austria were summarized into one data set (means of all character states). The combined classification of this data demonstrates the similarities between specimens of both data sets.

Ten different classifications were computed and the results were combined. Only three classification groups can be established. As in the analysis of specimens from Lower Austria there is no single character or a simple combination of characters which defines one group unambiguously. The ranges of the main characters are shown in Figs. 17, 18. The computer graphs of the "average plants" of each classification group (Fig. 7) show no significant differences, many characters increase continuously from group 1 to group 3.

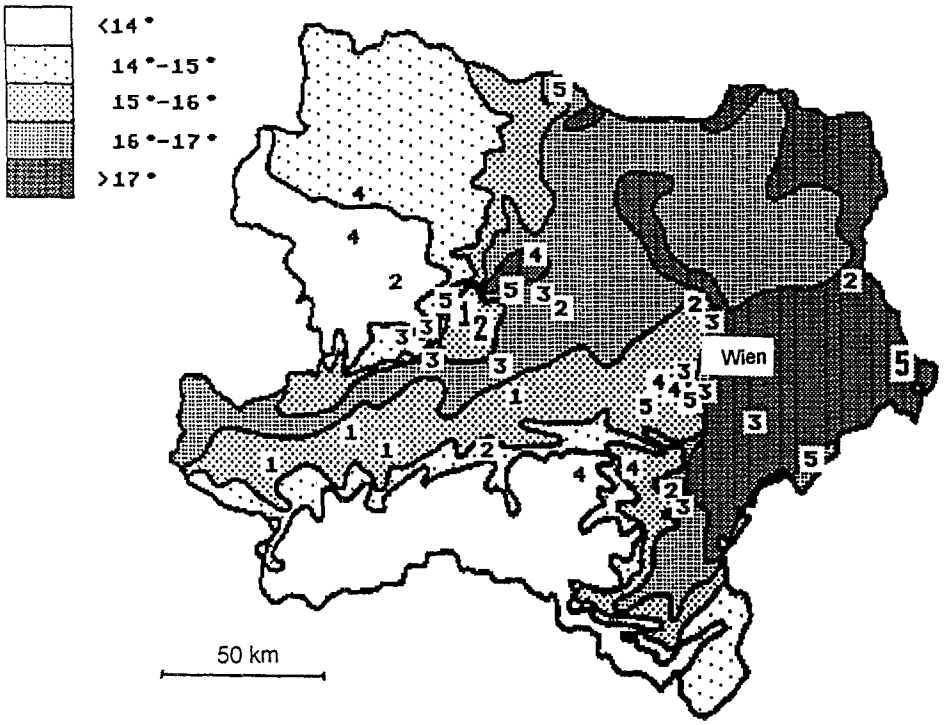

Fig. 6. Zones of mean temperature during the growth period in Lower Austria (data from HASSINGER & al. 1952). Populations of *Primula veris* are coded with their group number of classification

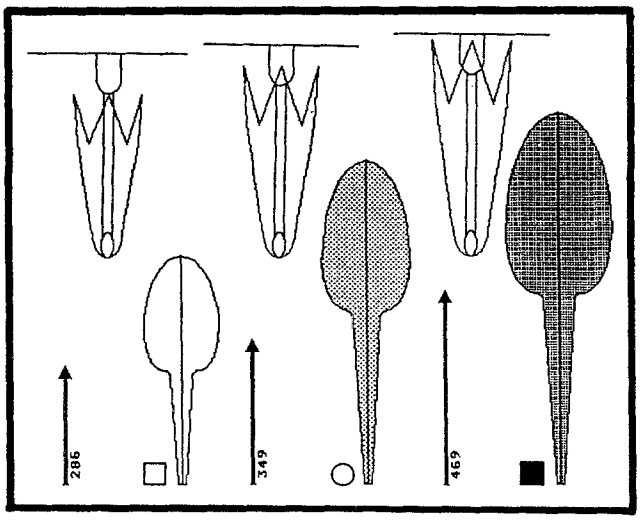

Fig. 7. "Average plants" reconstructed according to the mean values of characters of the three classification groups from Austria. Length of arrow according to hair length on abaxial side of leaves. Increasing density of the gray colour of leaves indicates a higher degree of ramification of hairs. □ Group 1, ○ group 2, ■ group 3

The sampling sites are marked in Fig. 8 according to the result of the classification. Specimens which are similar to those from the Pannonic part of Lower Austria (classification group 3) are present in the whole area. This type can be found especially in Carinthia and in climatically subcontinental valleys (e.g., Mur

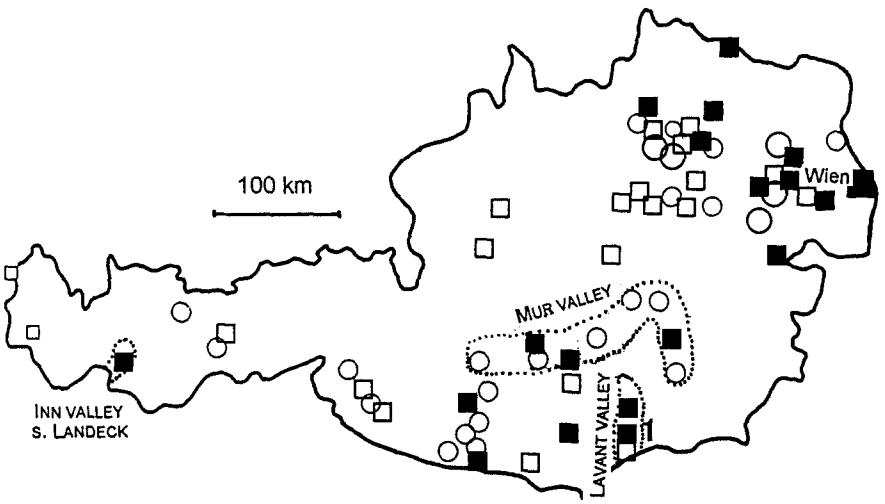

Fig. 8. Map of Austria. Origins of *Primula veris* are coded according to the results of multivariate classifications. Large symbols indicate several populations grouped together. *1* St. Paul. Group symbols as in Fig. 7

valley, Inn valley south of Landeck). In the Lavant valley in Carinthia specimens from classification group 1 and 3 are present. Several mean character states from plants of St. Paul im Lavanttal (no. 1 in Fig. 8) are the maximum observed in Austria (e.g., hair length both on leaves and peduncle, density of hairs).

Italy, "Yugoslavia", Albania, and Greece. The summation of the results of 10 different classifications leads to a division of the 321 specimens of this region into two groups only with the intraclass variability small. The partition of material in different multivariate classifications is in a wide range equally independent of the chosen combination of characters. Only few specimens are intermediate. The main differential characters (Figs. 17, 18) between these two classes are the hairlength on abaxial side of leaves (group 2 in the mean about 1000 µm), the degree of ramification of hairs (nearly all hairs branched in group 2), the density of hairs (specimens of classification group 2 in most cases with leaves wite tomentose beneath) and the shape of the lamina base of leaves (classification group 1 truncate-decurrent, group 2 cordate). The differences of these characters are considerably higher than the variability observed in common garden experiments. All character states of flowers have a higher mean in group 2 than in group 1 but an overlap in the range of these characters exists between the two groups.

However, the "average plants" constructed with the means of each classification group (Fig. 9) are quite different. Most plants from group 1 are in agreement with the description of subsp. *veris*, and those from group 2 with that of subsp. *columnae*. A separate classification of the members of each group does not allow the differentiation between further distinct subclasses even with an accentuation of the differential characters used by SCHWARZ (1968) for discrimination of subsp. *suaveolens* and subsp. *canescens*.

In Italy the two groups are allopatric; only in the region around Udine do they come into contact (Fig. 10). Plants of the "*columnae*"-type are found from Piemonte

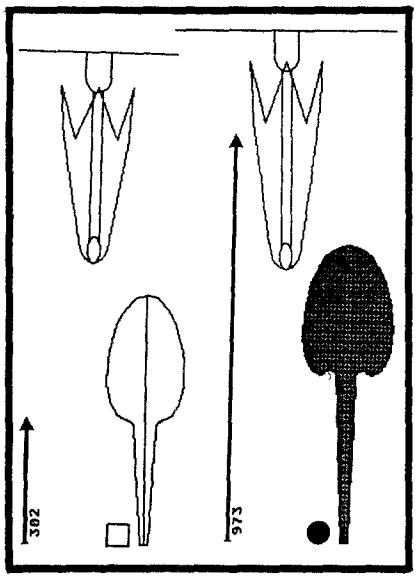

Fig. 9. "Average plants" reconstructed according to the mean values of characters of the two classification groups from Southern Europe. Length of arrow according to hair length on abaxial side of leaves. Increasing density of the gray colour of leaves indicates a higher degree of ramification of hairs. □ Group 1, ● group 2

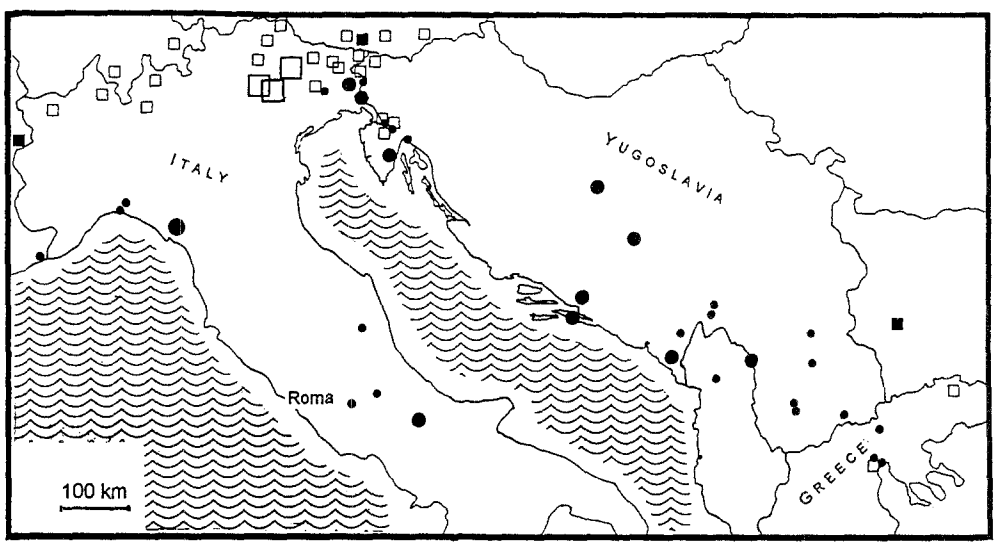

Fig. 10. Map of Southern Europe. Origins of *Primula veris* are coded according to the result of multivariate classifications. Large symbols indicate several populations grouped together. Group symbols as in Fig. 9

in the Northwest along the Appennin chain to the Abruzzi Mountains in the South as well as in the region of Udine in the Northeast. The "*veris*"-type is restricted to the southern margin of the Alps. No material was available from the great plains along the Po (free from *P. veris*?). Around the border between Italy and "Yugoslavia" the two areas overlap, but only a few specimens are intermediate with respect to their character states. The situation is unclear in the Alpi Giulie and in the region in the north and east of Trieste. Along the Alpi Carniche only the "*veris*"-type is found. The northernmost sites of the "*columnae*"-type are from the Gran Monte

(Alpi Giulie) and from Gemona. Intermediate types occur on the Mte Mataiur and Mte Bernardia (both Alpi Giulie). In the immediate vicinity of Udine and Gorizia only subsp. *columnae* is found among the herbarium material, but near the "Yugoslavian" village of Komen (north of Trieste) subsp. *veris* and intermediate plants do occur.

A separate multivariate classification of the data of plants from the border area Carinthia – Italy – "Yugoslavia" was done. Except for plants from St. Paul im Lavanttal and Nötsch (both intermediate type) all specimens from Carinthia are placed together with subsp. *veris* from Italy. In the north of Gemona there is a strict border, introgression does not occur.

In the western and southern parts of the Balcan Peninsula plants from the "*veris*"-type only occur in northern "Yugoslavia" and in the mountains of Greece (Fig. 10). Plants of the "*columnae*"-type are not so uniform as in Italy. Especially in the interior parts of the Balcan Peninsula the typical "*columnae*" characters are aberrant (leaves not white tomentose beneath, truncate lamina base, increasing width of the petiole). Nevertheless, the classification of the whole data set from Southern Europe is undoubtedly correct. Homogeneous populations of subsp. *columnae* are on Ucka Gora (Istria), Peljesac, Mostar, Orjen (near Dubrovnik) and the mountains at the border Albania – "Yugoslavia". Plants from Trebevic near Sarajevo resemble subsp. *columnae*, but the degree of hair ramification is less and the shape of the lamina base of leaves is often not cordate. Plants from the south of Nis and from Makedonija are grouped within group 2, although many character states deviate from typical subsp. *columnae*. In Greece typical subsp. *columnae* (e.g., from the Olymp, island Thassos) and typical subsp. *veris* (Pindhos mountains) do occur; near Thessaloniki both types are present. The differences between typical "*columnae*" and aberrant plants are insignificant, they do not allow a taxonomic separation.

Switzerland (incl. surrounding area of the Bodensee), France, and Spain. Multivariate grouping of the material leads to three classification groups. Plants of classification group 1 (mainly in Switzerland, but also in the Alpes Maritimes and the Pyrenees) resemble subsp. *veris*. The length of mostly unbranched hairs on the abaxial side of leaves, the dimensions of corolla and calyx confirm their status (Figs. 17, 18). Plants of group 2 are similar to subsp. *columnae* as in the interior parts of the Balcan Peninsula (leaf shape not so typical as in Italy, Fig. 11, average plants); they are present from the Alpes Maritimes along the Pyrenees to the Gulf of Biscaya and Madrid (Fig. 12); plants of the third group (e.g., Herault in S France) are intermediate in some character states (e.g., leaf shape, hair length on abaxial side of leaves and the dimensions of flowers), but the mean values of corolla- and calyx-parameters contradict a potential introgression of "*veris*" and "*columnae*" (Figs. 17, 18, average plants in Fig. 11).

Northern Central Europe, Great Britain, Scandinavia. In this region we expect the "pure" subsp. *veris* in the sense of SCHWARZ (1968). Nearly all plants available from Germany resemble this type (classification group 1, small unbranched hairs on the abaxial side of leaves), their character states are similar to the "*veris*"-type like in Austria or Italy (Figs. 17, 18). This type can also be found in the northern parts of the Czech Republic, in Poland, and in the region around London (Fig. 13). Most plants from Scandinavia (even from S Finland and the

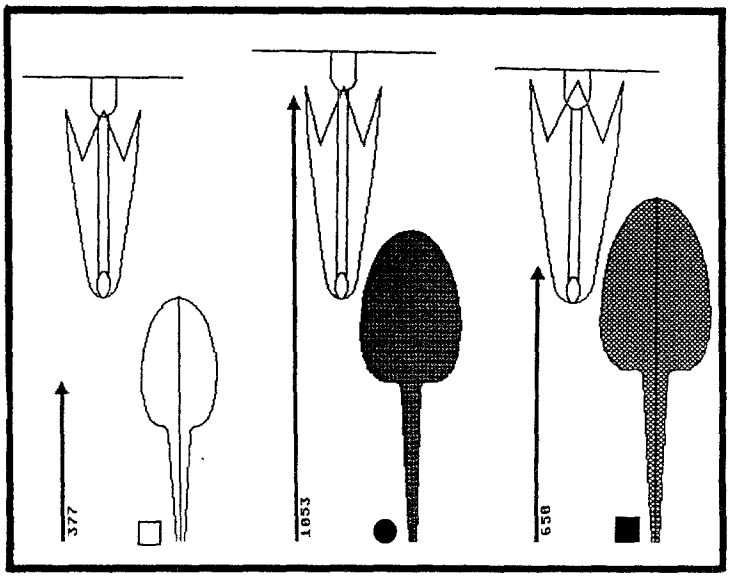

Fig. 11. "Average plants" reconstructed according to the mean values of characters of the three classification groups from Western Europe. Length of arrow according to hair length on abaxial side of leaves. Increasing density of the gray colour of leaves indicates a higher degree of ramification of hairs. □ Group 1, ● group 2, ■ group 3

Åland-island) are grouped together with such from the pannonic part of the Czech Republic and of Slovacia (classification group 2), which is in agreement with nomenclature on the herbarium sheets and with comments to the subsp. *canescens* by LÜDI (1927). In the sense of SCHWARZ (1968) such plants from Scandinavia must be placed to subsp. *suaveolens* but they are not distinguishable from the specimens of *P. pannonica* of KERNER. In addition this type (hair length about 500 µm, large flowers) can be found in Germany, Belgium, and Great Britain. Plants of the third classification group are intermediate in all character states (Fig. 14, average plants).

Eastern Europe, Caucasus, Turkey, Iran. The specimens from the Caucasus and Iran should represent the typical *P. veris* subsp. *macrocalyx*, which is defined primarily by the campanulate-enlarged calyx (BUNGE 1829). Plants which are in agreement with this description (diameter of calyx in the mean about 1 cm) can actually be found in this region (Fig. 15), they are clearly separated in multivariate classification. In contrast to the description given by SCHWARZ (1968) these plants are characterized by small hairs and large flowers (Figs. 16–18). In addition specimens were available which are similar to the "pure" subsp. *veris* in C Europe. Therefore, the Caucasus and Iran are not the central area of the pure, geographically defined type *P. inflata* as claimed by SCHWARZ (1968). From the Crimea to the Pannonic lowlands in Hungary plants with longer and branched hairs on the abaxial side of leaves do occur, there seems to be no continuous introgression with the type "*columnae*" from the western parts of the Balcan Peninsula.

Combined multivariate classification of specimens of the whole area. The concept of SCHWARZ (1968) with three combinations of characters with divergent geograph-

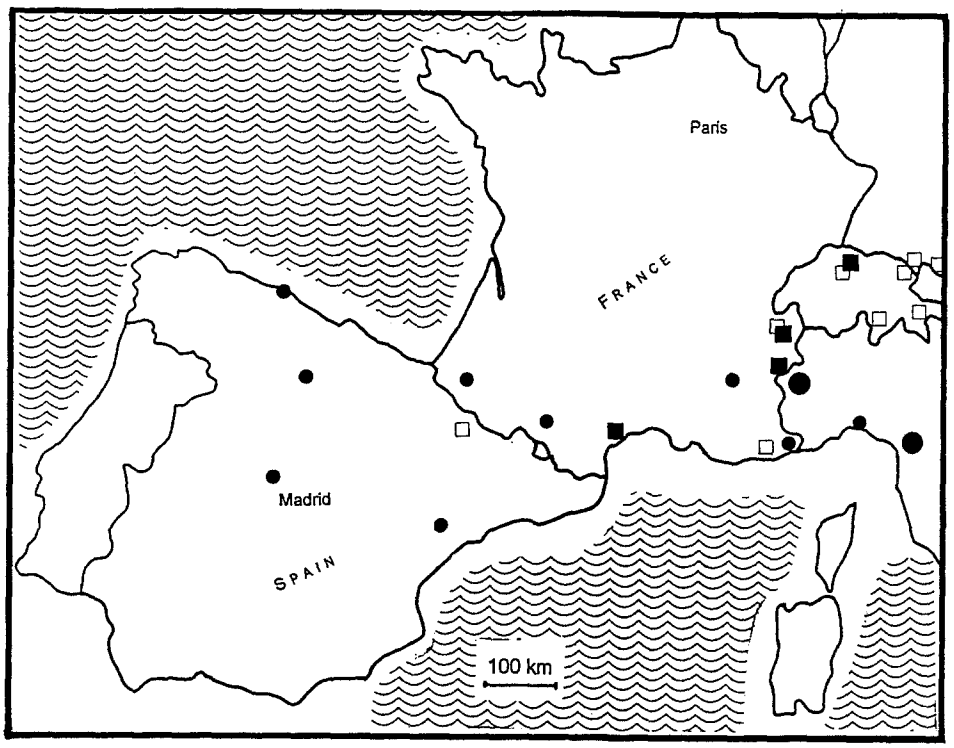

Fig. 12. Map of western Europe. Origins of *Primula veris* are coded according to the result of multivariate classifications. Large symbols indicate several populations grouped together. Group symbols as in Fig. 11

ical distribution must be refuted ["*inflata*"-types in France and Great Britain (Liverpool), "pure *veris*" in the central area of "*macrocalyx*"]. Therefore, the only way to achieve a well-founded systematic revision is the simultaneous classification of all specimens. To avoid an unbalance in the data set (nearly half of the plants are from Lower Austria, about 100 from the Alpi Apuane in Italy) neighbouring populations which are similar in their character states are combined. Thus, 247 OTU's were analysed with the different methods.

Generally the MA-algorithm recognizes well defined groups in a data set, even in the polyploid *Achillea millefolium* complex (SAUKEL & LÄNGER 1992). The differences within the *P. veris* data set are too insignificant for automatic discrimination of material, which means that the quite different types "*veris*" and "*columnae*" found in Italy can be subspecies at best. In six classifications all plants of the "*columnae*"-type are grouped together, while the other are combined depending on the chosen characters.

A scatterplot constructed with data from PCA demonstrates the relative individuality of the type "*columnae*" (Fig. 19). A second distinct group is built by plants of typical "*macrocalyx*" pattern. In the third cloud all other types are connected, even with multivariate methods it is impossible to discriminate subunits. The validity of the type "*macrocalyx*" as an independent taxon is uncertain. The width of the calyx is the only character for differentiation, moreover, specimens with small calyces do occur in the main area of "*macrocalyx*". The examination of more

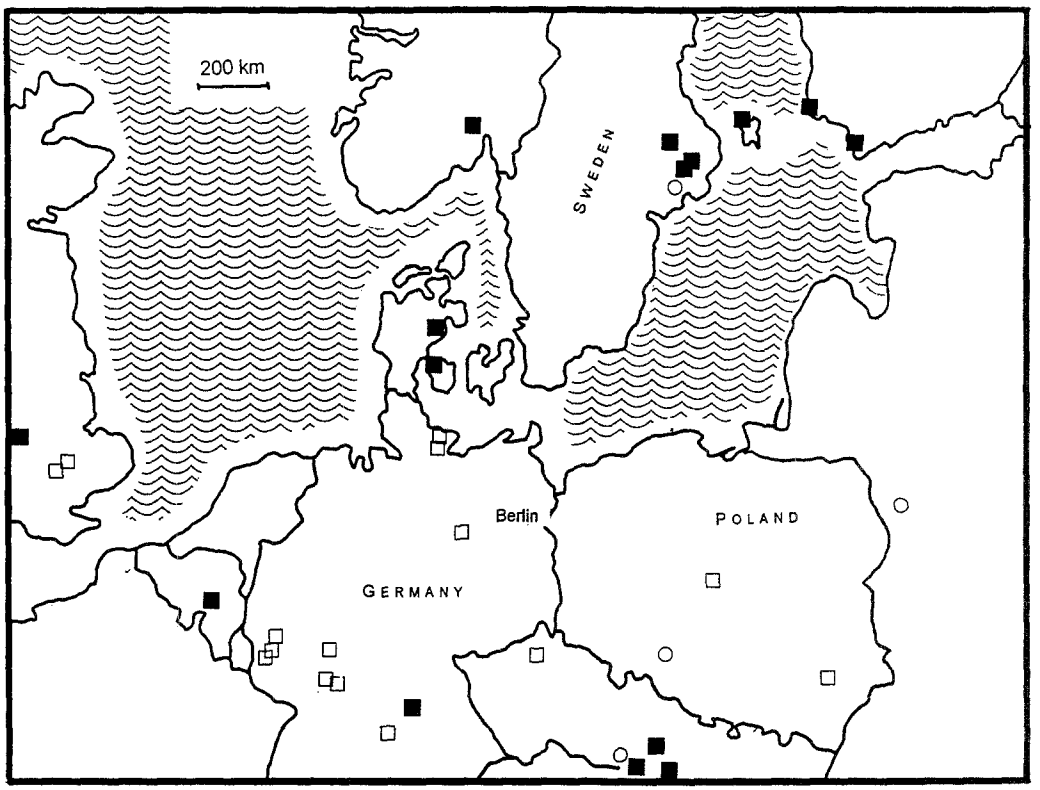

Fig. 13. Map of Nouthern Central Europe and Southern Scandinavia. Origins of *Primula veris* are coded according to the results of multivariate classifications. Large symbols indicate several populations grouped together. □ Group 1, ■ group 2, ○ group 3

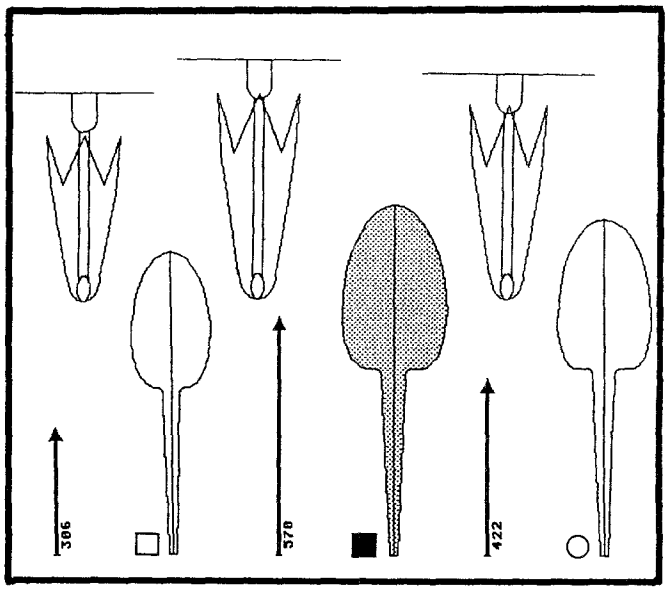

Fig. 14. "Average plants" reconstructed according to the mean values of characters of the three classification groups from Nouthern Central Europe and Southern Scandinavia. Length of arrow according to hair length on abaxial side of leaves. Increasing density of the gray colour of leaves indicates a higher degree of ramification of hairs. Group symbols as in Fig. 13

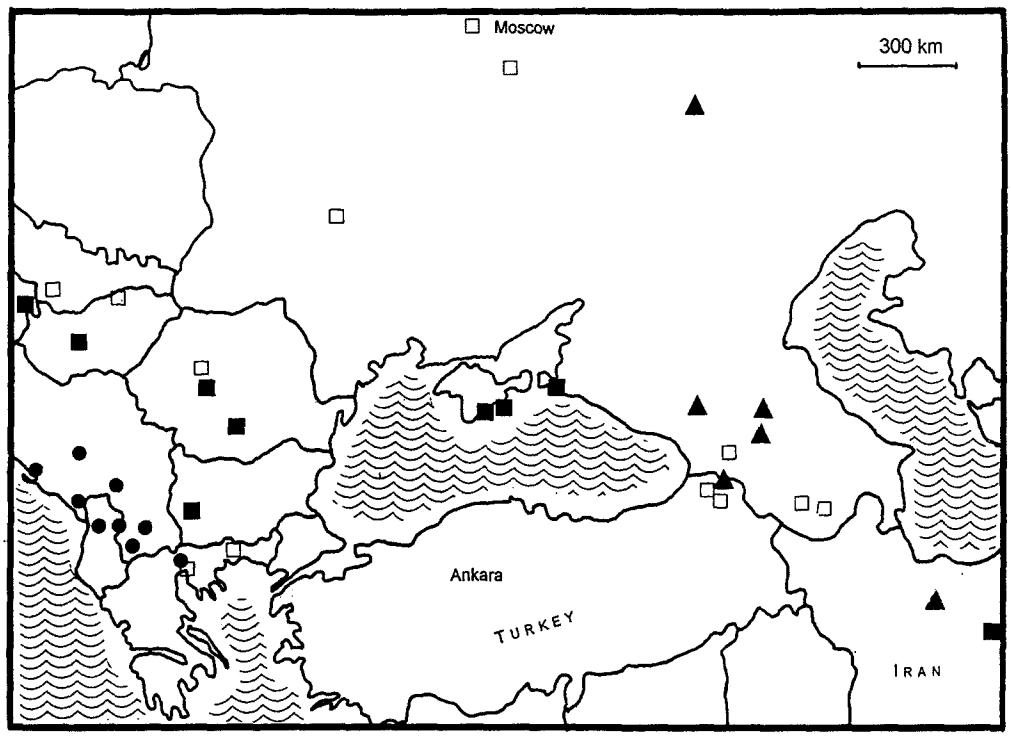

Fig. 15. Map of eastern Europe and of the Caucasus. Origins of *Primula veris* are coded according to the results of multivariate classifications. Large symbols indicate several populations grouped together. □ Group 1, ▲ group 2, ■ group 3

material of this region is necessary. At the moment the only significant subunit within *P. veris* is the type "*columnae*".

Discussion

Already in the beginning nineteenth century botanists recognized the variability of *Primula veris*. Many subspecies, varieties, and forms were described and recent identification literature is controversial. Therefore, the present investigation of *P. veris* is based on a geographical analysis of character states supported with modern methods of data analysis. The screening for systematically essential characters (sufficiently invariable within one individuum, during the growth period and under different environmental conditions) reduced the primary list to those in Table 4 [including variable characters which are cited in literature; for example, the ratio lamina length/length of petiole depends on light conditions, it cannot be used for discrimination of subsp. *columnae* and subsp. *suaveolens* as in the keys of SCHWARZ (1968) and PIGNATTI (1982)].

Infraspecific groups of *P. veris* cannot be detected in the analysis of 53 populations of Lower Austria (421 plants, including typical "*P. pannonica*"), all characters show a continuous transition between the extremes observed in Lower Austria. In general many specimens from the Pannonic part of Lower Austria relate to the circumscription of subsp. *canescens*, and such from mesic parts to subsp.

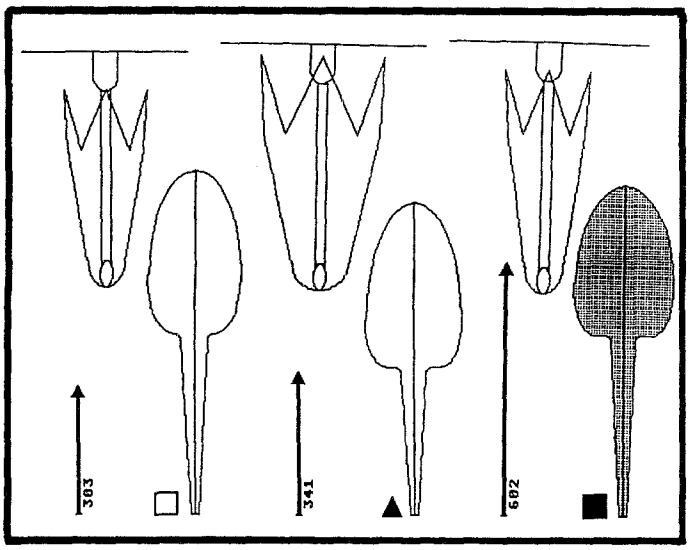

Fig. 16. "Average plants" reconstructed according to the mean values of characters of the three classification groups from eastern Europe and of the Caucasus. Length of arrow according to hair length on abaxial side of leaves. Increasing density of the gray colour of leaves indicates a higher degree of ramification of hairs. Group symbols as in Fig. 15

veris. But in most specimens the character states are intermediate. The differences in the character states of the extremes are not significant enough (considering the variability of characters) for discrimination of subspecific units. The analysis of specimens from the whole area of Austria indicates no strict connection between geography and character states. In southern Europe (Italy, Slovenia, Croatia), however, two morphologically distinct types, the one resembling subsp. *veris*, the other subsp. *columnae*, can be found. The latter, characterized by leaves white-tomentose beneath and large flowers, is restricted to an area from Spain (specimens from Santander, Madrid) along the Pyrenees, the mediterranean coast of France, the Appenin chain, Istria, the Adriatic coast of Slovenia and Croatia and Montenegro, in the interior parts of the western Balcan Peninsula (Sarajevo, the mountains of Albania, Macedonia) as far as Greece. In Italy subsp. *veris* and subsp. *columnae* are separated geographically. A further division of the type "*columnae*" into distinguishable subunits (type "*suaveolens*") is not possible.

Plants with small and unbranched hairs and small flowers like the Italian "*veris*"-type can be found within the whole area. So can plants with longer, partly branched hairs (until now called subsp. *canescens*) but in no single area they can be distinguished from the "*veris*"-type even with multivariate analysis. The status of the "*macrocalyx*"-type cannot be clarified presently. It is sure that this type cannot be an only geographically defined main subspecific taxon of *P. veris* because of the sympatric occurrence of pure "*veris*". The examination of more material is necessary. The comparison of plants reconstructed with the mean values of several classification groups indicates subsp. *columnae* as the only subspecific taxon which is clearly defined.

The shape of the terminal cell of the hairs on the abaxial side of leaves is connected with the degree of ramification of hairs and is, therefore, suitable for

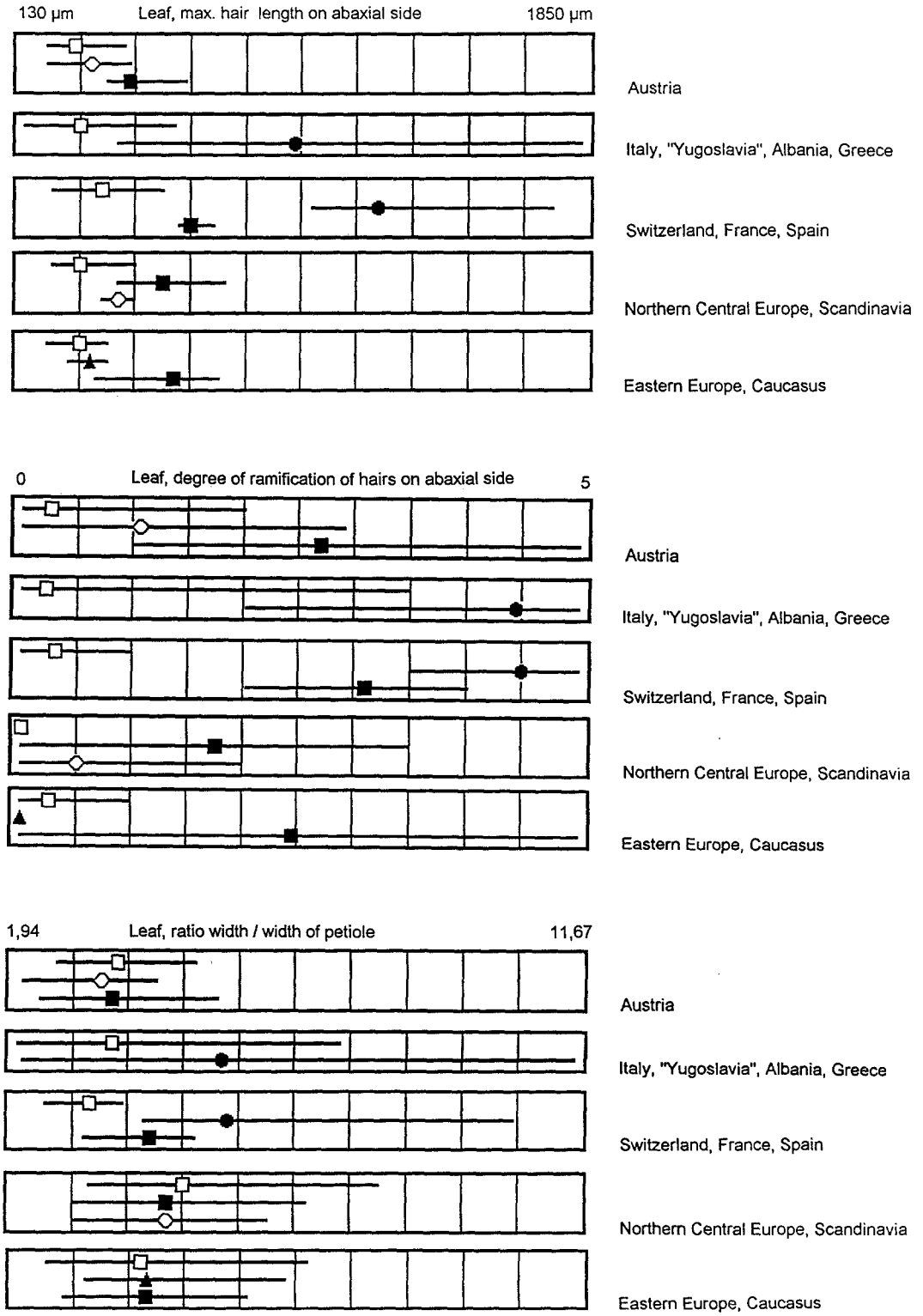

Fig. 17. Mean values and ranges of the main diagnostical characters in the *Primula veris* agg. Line: minimal to maximal value; symbol: mean. Symbols corresponding with maps and "average plants"

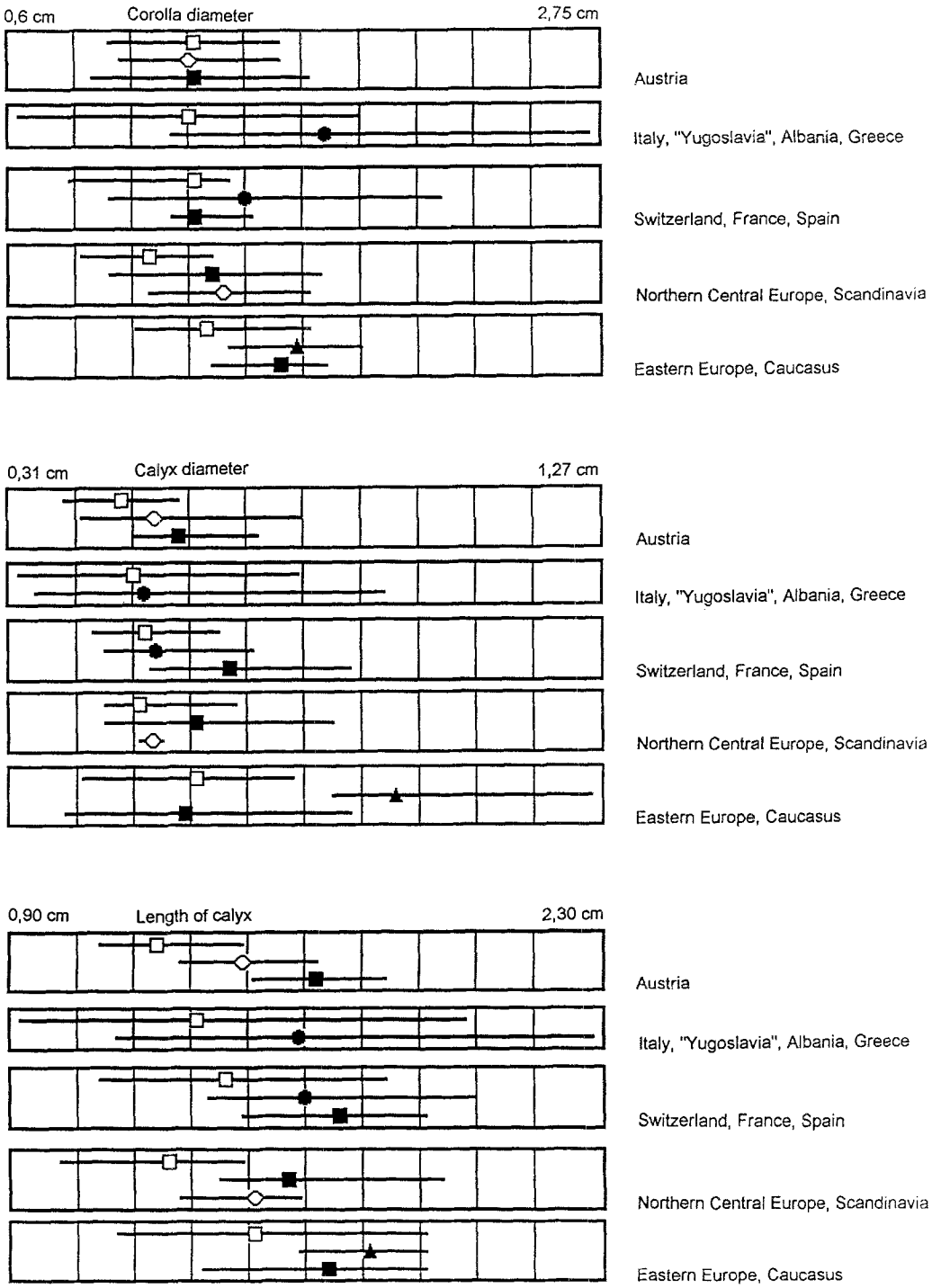

Fig. 18. Mean values and ranges of the main diagnostical characters in the *Primula veris* agg. Line: minimal to maximal value; symbol: mean. Symbols corresponding with maps and "average plants"

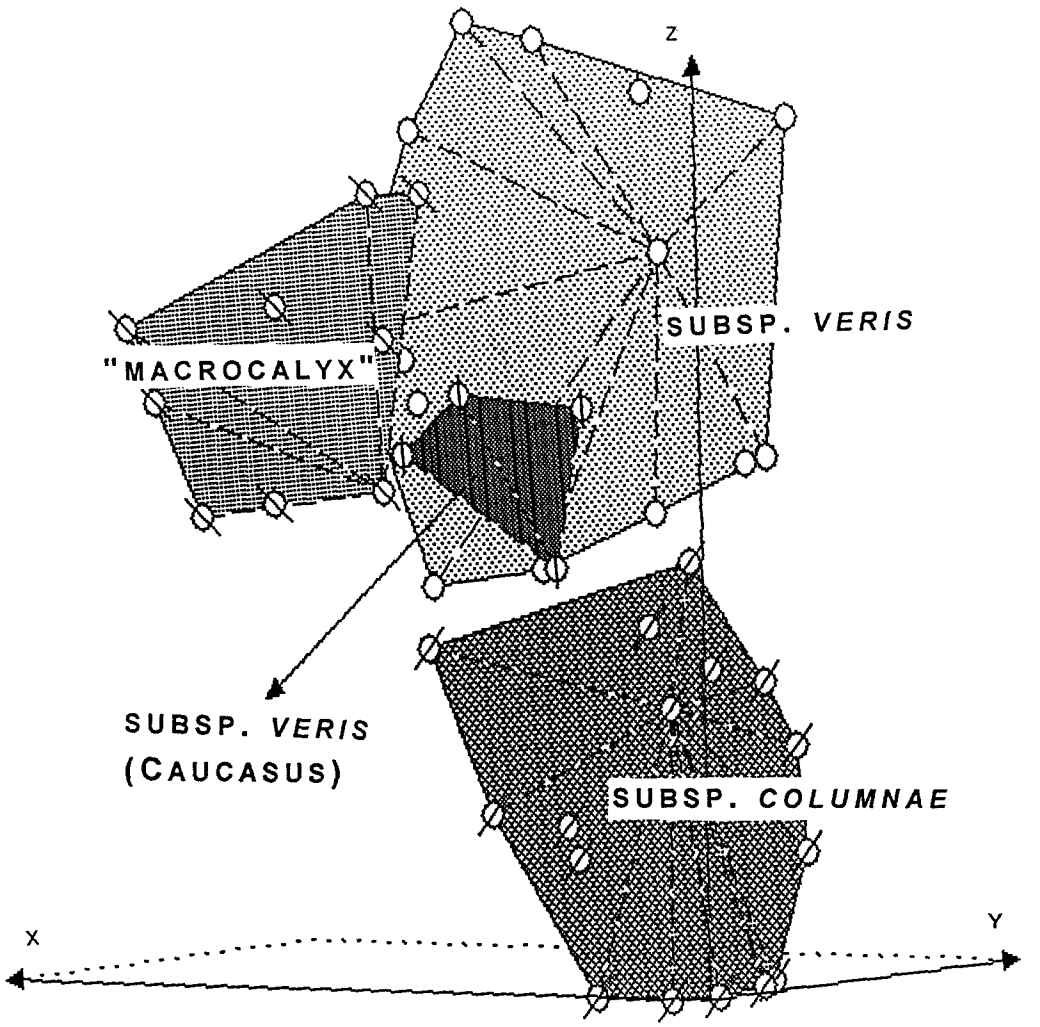

Fig. 19. Result of principal component analysis of the *Primula veris* agg. OTU's belonging to the same taxon enclosed in a polygon. x-axis: PC 1; y-axis: PC 2; z-axis: PC 3

an estimation of the state of this character. The drawings of hairs in PIGNATTI (1982) are quite incorrect. The terminal cell of typical subsp. *veris* is inverse pear-shaped, of typical subsp. *columnae* slightly ventricose (Fig. 20). If the leaf indumentum consists only of unbranched hairs, these generally have an inverse pear-shaped terminal cell. With an increasing amount of ramificated hairs in the indumentum the terminal cells are less inflate.

From the six published subspecies only two (*veris, columnae*) could be unambiguously verified, subsp. *macrocalyx* needs further examination, the other cannot be distinguished from the "*veris*"-type (subsp. *canescens*, subsp. *inflata*) or from the "*columnae*"-type (subsp. *suaveolens*).

In a revised taxonomic frame the "*veris*"- and "*columnae*"-types must be placed as equivalent taxa with less restricted descriptions. Such an approach was done by HAYEK (1928), who placed the "*canescens*"-type as a subunit to "*officinalis*",

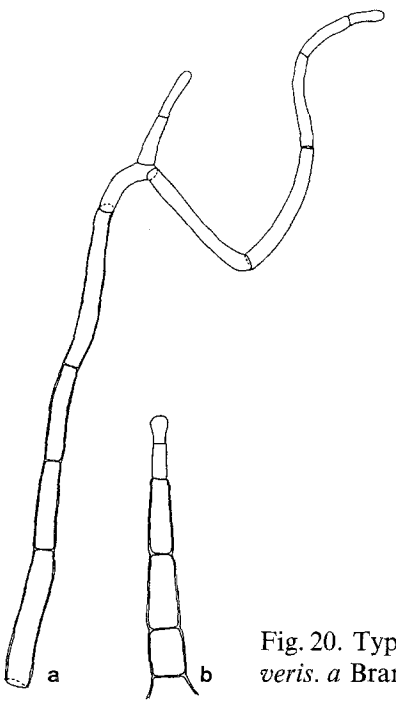

Fig. 20. Typical hairs from the abaxial side from leaves of *Primula veris*. *a* Branched hair; *b* unbranched hair

whereas "*suaveolens*" is demoted as a synonym to "*columnae*". Although some populations show typical "*canescens*"-characters plants with this character combinations must be considered as marginal types from the subspecific taxon "*veris*".

The subspecific classification of *P. veris* L. must be as follows [descriptions according to HAYEK (1928)]:

P. veris L.: Folia ovata in petiolum cito attenuata vel decurrentia subtus in tota lamina puberula vel tomentosa viridia vel alba. Flores in umbellam longe pedunculatum unilateraliter nutantem dispositi. Calyx tota superficie puberulus. Corolla limbo concavo, vitellina fauce maculis 5 aurantiacis notata.

subsp. *veris* [incl. subsp. *canescens* (OPIZ) HAYEK, *P. pannonica* KERNER, *P. inflata* LEHM. subsp. *inflata* sensu SCHWARZ, probably incl. subsp. *macrocalyx* (BUNGE) KOCH]

Folia subtus viridia vel canescenti-tomentosa, subtus pilis simplicibus vel parum ramosis 180 µm–800 µm longis.

Table 5. Characters for discrimination of *Primula veris* subsp. *veris* and subsp. *columnae*

	subsp. *veris*	subsp. *columnae*
Leaf, max. hair length on abaxial side	130 µm–750 µm	420 µm–1850 µm
Leaf, indumentum on abaxial side	canescent-tomentose	white tomentose
Leaf, shape of lamina base	truncate-cuneate	cordate (-truncate)
Ratio leaf width/width of petiole (mean)	3.3–5	5.5–5.7
Corolla diameter (in plane)	6 mm–19 mm	9.5 mm–27.5 mm
Shape of corolla lobes	concave	± spreading

subsp. *columnae* (TEN.) MAIRE & PETITM. [incl. subsp. *suaveolens* (BERTOL.) GUTERM. & EHREND.)]

Folia ± cordata in petiolum subito constricta subtus dense albo tomentosa pilis (400 µm–)600 µm–1850 µm longis ramosis dense contextis.

The main diagnostical characters are listed in Table 5. For a correct identification the investigation of a representative sample of a population is recommended.

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