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STUDY OF THE MULTIVARIATE STRUCTURE OF THE ESTONIAN *Alchemilla* L. (ROSACEAE) MICROSPECIES: AN EXAMPLE OF THE STRUCTURAL INDICES APPROACH

Silvia SEPP[a*], Tatjana NAHTMAN[b], Tõnu MÖLS[b], and Jaanus PAAL[a]

[a] Institute of Botany and Ecology, University of Tartu, Lai 40, 51005 Tartu, Estonia
[b] Institute of Mathematical Statistics, University of Tartu, J. Liivi 2-411, 50409 Tartu, Estonia

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Abstract. The structural indices approach was used in the taxonomy of 23 microspecies of *Alchemilla* L. to overcome the problem of the statistical incorrectness caused by testing the objectivity of taxa applying the same morphometric variables as those used to define them. We tried to find answers to the following questions: How distinct are the microspecies according to the metric and count variables? How do the structural indices distinguish microspecies? What are the most stable proportions between the characters? Which characters are most informative in microspecies distinction? The structural indices proved to be better for taxon discrimination than the first principal components and single variables. The pairs of indistinct microspecies found in discriminant analysis were confirmed by structural indices analysis, but additionally many indistinct species pairs appeared. The hairiness characters were effective for microspecies discrimination while flower measurements were the poorest discriminators; all the metric variables and counts together were the most effective of all.

Key words: morphological variation, plant taxonomy, principal components analysis.

INTRODUCTION

The genus *Alchemilla* L. (Fam. Rosaceae Juss., subfam. Rosoidae Focke) consists of more than 1000 taxa (Fröhner, 1995), about 300 of which have been described in Europe. Because of its agamospermy and large variation, the genus has been an object of widespread scientific interest since the last century. Most authors rank *Alchemilla* microspecies on a species level; some suggest that only a

* Corresponding author, silvias@ut.ee

few collective species should be recognized. Numerical methods have been used quite rarely (Turesson, 1956; Glazunova & Myatlev, 1990; Sepp & Paal, 1998) for the analysis of this genus, though they have proved to be useful in analogous agamic complexes (e.g., *Amelanchier*, Dibble et al., 1998; *Antennaria*, Chmielewski, 1995; *Rosa*, Nybom et al., 1997; *Rubus*, Kraft & Nybom, 1995). Until recently nobody had investigated whether the morphological characters used in identification of these taxa really work and whether the microspecies are clearly distinct. From the first investigations of that kind (Sepp & Paal, 1998) it may be pointed out that sections are distinguished rather well, while among microspecies some are significantly distinct but some pairs or even bigger groups are taxonomically continuous.

A difficult problem in taxonomy is associated with the statistical incorrectness caused by testing the distinctness and objectivity of taxa using the same morphometric variables that are the basis of defining these taxa. To overcome this problem is a rather complicated task: it is equivalent to testing the reality of clusters in cluster analysis. The difference between taxonomy and cluster analysis is that the empirical definition of species is based on a visual univariate or bivariate analysis, whereas cluster analysis is mainly a multivariate technique, taking all the characters into consideration.

In component analysis eigenvectors corresponding to minimal eigenvalues were calculated instead of the standard principal components. These express stable proportions between variables, and are called structural indices (Möls & Paal, 1998). We argue that the eigenvector-based structural indices as multivariate tools are less dependent on the descriptive characters of taxa and give more objective criteria for taxon differentiation than other methods.

In the current paper 23 microspecies belonging to the collective species *Alchemilla vulgaris* L. (coll.) are analysed. All these microspecies are high polyploids and apomicts (apospory + parthenogenesis, Gustafsson, 1947). The questions to be answered in the current paper are:

1. How distinct are the microspecies according to the metric and count variables, and what is the error rate of classification based on these variables?
2. What are the most stable proportions between the variables according to the structural indices?
3. Which variables are the most informative for microspecies distinction?
4. How do the structural indices distinguish microspecies and by what measure is the pattern different from the one obtained by other methods?

MATERIAL AND METHODS

Altogether 598 specimens of 23 *Alchemilla* microspecies occurring in the Estonian flora (Laasimer et al., 1996) were analysed (cf. list of microspecies, notations and number of specimens in Table 1). Herbarium material from

the Herbarium of the University of Tartu and the Herbarium of the Institute of Zoology and Botany, as well as material collected by the authors, was used. The microspecies were identified using the key compiled by Tikhomirov (in Laasimer et al., 1996), in doubtful cases they were checked and/or reidentified by K. P. Glazunova.

Only metric variables and counts were taken into account, mainly for mathematical reasons, but also to see if the same pattern appears as with the whole set of variables, including qualitative ones (as used by previous researchers and also in Sepp & Paal, 1998). In all, 28 variables – 15 metric ones and 13 counts – of 43 assessed parameters (Sepp & Paal, 1998) were used in the analysis (Table 2). As a rule, each variable was measured three times on every specimen, and in data processing the means of these measurements were used. Metric variables were log or log+1 transformed to approximate their distribution to the normal distribution.

Microspecies assignments and the values of different variables in microspecies discrimination were checked by linear discriminant analysis, realized in SAS/DISCRIM release 6.12 (SAS Institute Inc., 1998). For each microspecies the misclassification probability, or error rate, was estimated by the cross-validation method using three different sets of predictor variables: only metric, only counts, metric and counts together. The mutual proximity of microspecies was detected from the discriminant analysis as the proportion of "erroneously" classified microspecies – if more than 5% of the specimens of one microspecies were classified as another certain microspecies, these two were considered to be close.

The covariance structure of variables was studied by principal component analysis using SAS/PRINCOMP procedure. Here, instead of the standard principal components, eigenvectors of small eigenvalues, or structural indices, were calculated. The eigenvalue of an eigenvector of the covariance matrix is equal to the variance of the corresponding linear combination of logarithmic morphometric variables. Consequently, the structural indices represent the most stable proportions of variables (Möls & Paal, 1998). For a given taxon, these proportions remain constant regardless of environment and age, being at the same time less influenced by single variables, including those used for defining the taxon.

The structural indices that correspond to the last five eigenvalues of the covariance matrix were used to test the difference between microspecies. Mean values of structural indices were estimated and compared for different microspecies by the SAS/GLM/LSMEANS option. If at least one index was significantly different, the two microspecies were declared to be "distinct by ANOVA", otherwise "indistinct by ANOVA". The Bonferroni adjustment for the significance level was requested because of multiple comparisons.

For each pair of "indistinct by ANOVA" microspecies, the five structural indices were additionally analysed as variables with the multivariate SAS/GLM/MANOVA procedure. Depending on the result of this test, the microspecies were considered to be either "distinct" or "indistinct by MANOVA".

Table 1. Value of different sets of variables (metric, counts, and both together) in *Alchemilla* species discrimination and their error rates

Species	Notation	No. of specimens	No. of observations	Metric only		Counts only		Metric and counts together	
				Error rate, %	Reclassified into >5%	Error rate, %	Reclassified into >5%	Error rate, %	Reclassified into >5%
A. glaucescens	GLC	80	240	39	HIR	32	HIR, PLI	21	HIR
A. hirsuticaulis	HIR	20	60	44	GLC	15	GLC, PLI, OBT	17	GLC
A. acutiloba	ACU	81	243	38	SCR, SAR, MIC, BAL	70	MIC	35	MIC, BAL
A. wichurae	WIC	9	27	67	SAR, BAL, MUR	39	MIC, SCR, SAR, GLO, GLA, FIL, CYM, OBT, XAN	22	SAR, CYM, BAL, XAN
A. subcrenata	SCR	27	81	67	ACU, WIC, SAR, HEP, MIC, SGL	85	SAR, CYM, BAL, SGL	59	SAR, MON, HEP, MIC, CYM, SGL
A. sarmatica	SAR	22	66	67	ACU, SEM, MON, MIC, SGL	49	SCR, MIC, CYM	40	SCR, MON, MIC, SGL
A. semilunaris	SEM	10	30	28	PLI, HEP, SGL	50	LIN	23	PLI
A. propinqua	PRO	22	66	70	HIR, MON	24	SAR, MON, GLO	25	HIR, MON
A. plicata	PLI	29	87	40	GLC, PRO	31	GLC, HIR, MUR	21	GLC, HIR, PRO
A. monticola	MON	57	171	71	PRO, PLI, MIC, SGL	52	SCR, MIC, FIL, BAL, MUR	41	PRO, PLI, MIC
A. lindbergiana	LIN	10	30	23	HIR, MIC, SEM, SGL	44	CYM	20	CYM
A. heptagona	HEP	25	75	43	WIC	39	LIN, GLO, CYM, SGL	21	
A. micans	MIC	35	105	62	ACU, SAR, LIN, SGL	43	ACU, OBT, MUR	36	ACU, SAR

Table 1 continued

Species	Notation	No. of specimens	No. of observations	Metric only		Counts only		Metric and counts together	
				Error rate, %	Reclassified into >5%	Error rate, %	Reclassified into >5%	Error rate, %	Reclassified into >5%
A. glomerulans	GLO	13	39	69	HIR, SAR, CYM	54	WIC, PRO, PLI, BAL, OBT, XAN	40	SAR, BAL
A. glabricaulis	GLI	14	42	49		27	HEP, FIL, CYM, SGL	7	OBT
A. glabra	GLA	23	69	84	SAR, GLI, BAL, OBT	77	MIC, WIC, MIC, BAL, OBT, XAN, MUR	51	SAR, GLI, BAL, OBT
A. filicaulis	FIL	11	33	69		24	HIR, PRO, PLI, MIC, BAL, OBT	16	BAL, OBT
A. cymatophylla	CYM	30	90	79	MIC, SCR, SAR, PRO, HEP, MIC, GLA, SGL	93	SCR, LIN, HEP, MIC, GLI	55	SAR, PRO, HEP, MIC, MUR, SGL
A. baltica	BAL	27	81	67	WIC, SAR, OBT, MUR	72	OBT, XAN	38	OBT, MUR
A. obtusa	OBT	20	60	68	WIC, MIC, GLA, BAL, MUR	7	WIC, GLA, FIL, BAL, SGL	45	MIC, GLA, BAL, MUR
A. xanthochlora	XAN	10	30	40	MIC, SCR, LIN, HEP	46	MIC, GLO	27	
A. murbeckiana	MUR	10	30	60	WIC, CYM, OBT	69	HIR, PLI, LIN, FIL, XAN	27	WIC
A. subglobosa	SGL	12	36	56	SAR, LIN, HEP, MIC, XAN	81	SCR, SAR, MON, HEP, GLI	52	SAR, LIN

Table 2. Variables used in analysis

Notation	Variable	Type	Precision
STNR	Number of (flowering) stems per plant	Count	
LENR	Number of basal leaves per plant	Count	
LBCOR	Angle between basal lobes of basal leaf	Metric	±5°
STLN	Length of stem	Metric	±5 mm
STLHR	Number of hairs per 1 mm length of lower part of stem (first internodes)	Count	
STUHR	Number of hairs per 1 mm length of upper part of stem (just below inflorescence)	Count	
PETHR	Number of hairs per 1 mm length of petiole (of basal leaf)	Count	
SLELN	Length (radius) of stem leaf	Metric	±1 mm
PETLN	Length of petiole (of basal leaf)	Metric	±5 mm
LBNR	Number of lobes per leaf	Count	
LEUHR	Number of hairs per 1 mm² area on upper surface of basal leaf	Count	
LELHR	Number of hairs per 1 mm² area on lower surface of basal leaf	Count	
VNHR	Number of hairs per 1 mm length of vein on lower surface of basal leaf	Count	
LELN	Length (radius) of basal leaf	Metric	±1 mm
LEWD	Width of basal leaf	Metric	±1 mm
LBLN	Length of apical lobe of basal leaf	Metric	±1 mm
LBWD	Width of apical lobe of basal leaf	Metric	±1 mm
THNR	Number of teeth of apical lobe of basal leaf	Count	
STHLN	Length of the tooth next to apical (of apical lobe of basal leaf)	Metric	±0.1 mm
TTHLN	Length of apical tooth (of apical lobe of basal leaf)	Metric	±0.1 mm
STHWD	Width of the tooth next to apical (of apical lobe of basal leaf)	Metric	±0.1 mm
PEDHR	Number of hairs per 1 mm length of pedicel	Count	
HYHR	Number of hairs per one side of hypanthium	Count	
HYLN	Length of hypanthium	Metric	±0.1 mm
HYWD	Width of hypanthium	Metric	±0.1 mm
CALN	Length of sepal	Metric	±0.1 mm
CAHR	Number of hairs per sepal	Count	
OCALN	Length of lobe of epicalyx	Metric	±0.1 mm

RESULTS

The mean error rate for the set of metric variables in discriminating microspecies was 56%; for count variables it was 51%. Using all the variables together, the mean error rate was reduced to 32%. Consequently, the set of only metric variables is the poorest option for discriminating between microspecies, group membership can be predicted considerably more accurately if the metric and count variables are used together (Table 1).

According to ANOVA, only 24% of all possible pairs of microspecies were statistically distinct (at least by one character); MANOVA distinguished an additional 21% of species pairs, which were indistinct by ANOVA (Table 3).

Table 3. Distinctness of the analysed *Alchemilla* species. A, species are distinct by ANOVA at least by one structural index; M, indistinct by ANOVA, but distinct by MANOVA of five structural indices; I, species are indistinct by both ANOVA and MANOVA; d, species are distinct according to the discriminant analysis (not reclassified into one another); i, clearly indistinct (both reclassified into one another); (i), indistinct (only one species reclassified into another, but not vice versa)

Species[a]																						
HIR	Ii																					
ACU	Ad	Id																				
WIC	Id	Md	Id																			
SCR	Ad	Ad	Ad	Md																		
SAR	Ad	Ad	Ad	M(i)	Ai																	
SEM	Id	Md	Id	Id	Id	Id																
PRO	Ad	A(i)	Ad	Md	Ad	Md	Ad															
PLI	I(i)	I(i)	Id	Id	Ad	Id	M(i)	A(i)														
MON	Md	Ad	Ad	Md	I(i)	A(i)	Ad	Ii	I(i)													
LIN	Id	Md	Id	Id	Md	Md	Id	Ad	Id	Md												
HEP	Ad	Ad	Ad	Ad	A(i)	Ad	Ad	Ad	Ad	Ad	Ad											
MIC	Ad	Ad	Mi	Id	A(i)	Ii	Id	Md	Id	M(i)	Id	Ad										
GLO	Md	Ad	Id	Id	Id	I(i)	Id	Id	Id	Id	Id	Ad	Id									
GLI	Ad	Ad	Ad	Ad	Md	Ad	Ad	Ad	Ad	Ad	Ad	Ad	Ad	Ad								
GLA	Id	Id	Id	Id	Id	A(i)	Id	Ad	Id	Id	Id	Ad	Id	Id	A(i)							
FIL	Id	Md	Ad	Ad	Id	Md	Ad	Md	Ad	Md	Ad	Id	Ad	Ad	Ad	Ad						
CYM	Id	Id	Ad	M(i)	A(i)	A(i)	Id	A(i)	Id	Md	I(i)	A(i)	A(i)	Ad	Ad	Id	Id					
BAL	Ad	Ad	A(i)	M(i)	Md	Ad	Ad	Id	Ad	Md	Ad	Ad	Ad	I(i)	Md	A(i)	M(i)	Ad				
OBT	Ad	Md	Md	Md	Ad	Ad	Md	Md	Ad	Id	Ad	Ad	A(i)	Id	I(i)	Ii	M(i)	Ad	Ii			
XAN	Md	Ad	Ad	I(i)	Ad	Ad	Md	Ad	Md	Ad	Md	Ad	Md	Md	Ad	Id	Id	Md	Ad	Md		
MUR	Id	Md	Id	I(i)	Md	Id	Id	Ad	Id	Md	Id	Ad	Id	Id	Ad	Id	Id	I(i)	A(i)	A(i)	Id	
SGL	Id	Md	Ad	Md	A(i)	Ai	Md	Ad	Md	Md	M(i)	Id	Ad	Ad	Ad	Md	Id	I(i)	Ad	Md	Id	Id
	GLC	HIR	ACU	WIC	SCR	SAR	SEM	PRO	PLI	MON	LIN	HEP	MIC	GLO	GLI	GLA	FIL	CYM	BAL	OBT	XAN	MUR

[a] See Table 1.

Thus, by linearly combining structural indices, a new structural index can be constructed, which discriminates between microspecies most effectively.

The characters that have larger coefficients in a structural index (for example, >0.2) are considered to be the main components of the respective structural index. The most stable combination in our case is the ratio of leaf width and leaf length (V_1, Table 4), which describes the general shape of the leaf. Stable combinations also exist between dimensions of the leaf and leaf lobe (V_2: LELN, LEWD, LBLN, LBWD), dimensions of the flower (V_3 and V_4: HYLN, HYWD, CALN, OCALN) and leaf teeth (V_5: STHLN, TTHLN, STHWD). An orthogonal rotation of the five-dimensional space of the indices V_1–V_5 could generate other structural indices, which might emphasize different stable proportions between the same metric variables.

The best numerical characters for distinguishing microspecies according to GLM are mainly hairiness characters: VNHR (distinguishes 167 species pairs, 66% of possible), STUHR, PETHR (both 160, 63%), STLHR (145, 57%), LEUHR (137, 54%), LELHR (136, 54%), and THNR (129, 51%). The least important (distinguish less than 25% of species pairs) are flower characters (FPTHR, HYLN, OCALN, CALN, HYWD), number of leaves and flowering stems (LENR, STNR), and width of the side teeth of leaf lobes (STHWD).

Table 4. The structural indices defined by the five last eigenvectors V_1–V_5 of the covariance matrix of 15 variables

Variable	Eigenvectors (with their eigenvalues)				
	V_1 (0.002)	V_2 (0.008)	V_3 (0.011)	V_4 (0.013)	V_5 (0.014)
LBCOR	0.005	0.029	0.007	0.006	–0.001
STLN	–0.002	0.017	–0.009	–0.057	–0.004
SLELN	–0.004	0.020	0.012	0.044	0.028
PETLN	–0.005	0.049	–0.006	0.0003	0.024
LELN	–0.694	–0.467	–0.060	–0.016	0.013
LEWD	0.720	–0.433	–0.053	–0.037	0.014
LBLN	0.002	0.214	0.010	0.101	–0.028
LBWD	–0.018	0.710	0.156	–0.006	–0.070
STHLN	–0.004	0.026	0.018	–0.092	0.783
TTHLN	0.005	–0.059	–0.069	0.180	–0.528
STHWD	–0.009	–0.063	–0.056	–0.062	–0.214
HYLN	–0.009	0.004	0.162	–0.697	–0.052
HYWD	0.010	0.042	–0.293	0.592	0.198
CALN	0.004	–0.143	0.827	0.301	–0.032
OCALN	–0.001	0.103	–0.407	–0.109	–0.105

DISCUSSION

Comparison of the ordinary discriminant analysis and the analysis of the variance of structural indices (Tables 1 and 3) revealed that the results agree in certain points. In both cases some results are in good concordance with the opinions of taxonomists about the similarity of microspecies, for example, the indistinctness of *A. acutiloba* and *A. micans*, *A. glaucescens* and *A. hirsuticaulis.* But some of the indistinct pairs in both cases are rather surprising, for example *A. lindbergiana* and *A. cymatophylla*, *A. plicata* and *A. semilunaris*, since these microspecies are generally considered to belong to different sections.

As a rule, the results of the discriminant analysis were in relatively good concordance with classical systematics. One reason is that the structural indices approach uses only metric variables while the discriminant analysis also takes into consideration the count variables. According to discriminant analysis, microspecies from different sections are seldom indistinct while microspecies of the same section are more often statistically continuous. For example, in addition to the "classically" continuous species pairs mentioned above, several species pairs in the section *Coriaceae* are not well separable in the discriminant analysis. As a rule, if the two microspecies are indistinct, and one or some authors have placed them into different sections, by other author(s) these microspecies are included into the same section. For example, according to Yuzepchuk (1941), Rothmaler (1962), Walters & Pawlowski (1968), and Plocek (1982) *A. plicata* and *A. monticola* belong to the separate sections or series *Pubescentes* and *Hirsutae*, but Fröhner (1995) merged them in the section *Plicatae*. Still, there are also some "odd" indistinct pairs, whose similarity has not been stated before (cf. Table 1).

The structural indices approach and discriminant analysis also differ if we take into account that it is a mathematical nonsense to use a variable both for the taxon definition and to show that the taxa are well defined and distinct. Split, for example, a homogenous population into two parts, according to whether the value of the variable x is smaller or larger than its mean value. Declare, thereafter, the two parts to be two taxa and check their difference using Student's test. The test will definitely confirm the distinctness of these taxa. Unlike single variables, structural indices depend simultaneously on many variables and cannot be easily followed on certain specimens. For the latter reason, the network of indistinct pairs from the structural index analysis can not always be directly biologically interpreted. Nevertheless, from a statistical point of view, it is safer to test distinctness of taxa using structural indices.

In our case, pairs of indistinct microspecies found in discriminant analysis were mostly confirmed by structural indices analysis. Besides that, according to structural indices numerous indistinct species pairs appeared, often from different sections. It is noteworthy that according to structural indices *A. heptagona* is indistinct from *A. filicaulis* and *A. subglobosa.* The systematic position of the last one is doubtful and it is indistinct from many other microspecies, but Yuzepchuk

(1941) segregated *A. heptagona* and *A. filicaulis* into a separate group: *Exuentes*. No other authors have agreed with him, but the preliminary genetic data (Sepp et al., 2000) also suggest that *A. heptagona* is exceptional in the section *Ultravulgares* Fröhner.

We can also use structural indices for descriptive purposes, to get a general idea of taxon separation according to each structural index. Visual inspection of Fig. 1 indicates that, for example, V_4 and V_5 account for separation between *A. xanthochlora* and *A. propinqua*, but separation between *A. propinqua* and *A. subglobosa* by the same indices appears to be slightly less clear (Fig. 2). The

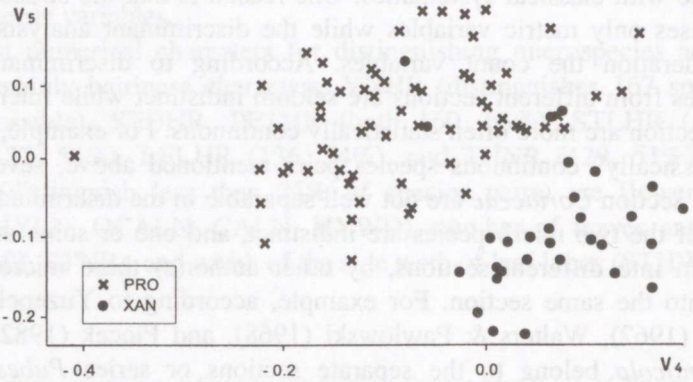

Fig. 1. Ordination plot of *Alchemilla propinqua* and *A. xanthochlora* by the structural indices V_4 and V_5 (see Table 4).

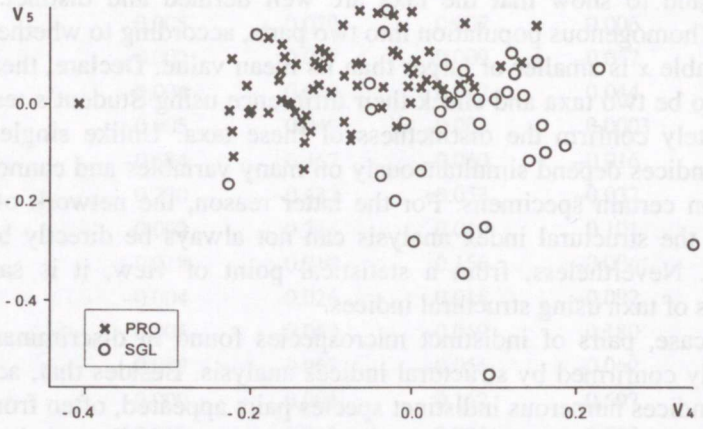

Fig. 2. Ordination plot of *Alchemilla propinqua* and *A. subglobosa* by the structural indices V_4 and V_5 (see Table 4).

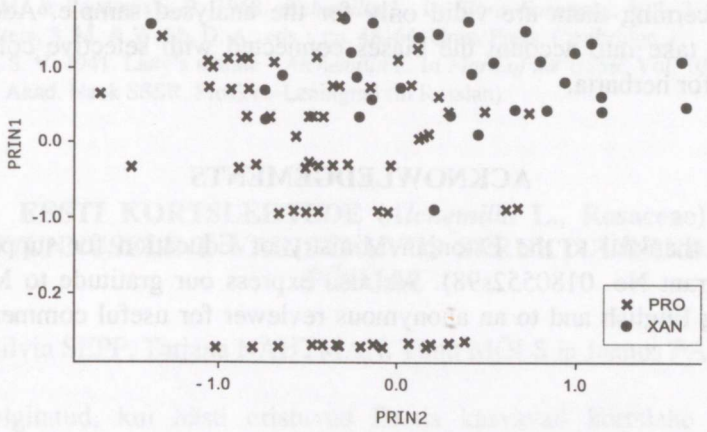

Fig. 3. Ordination plot of *Alchemilla propinqua* and *A. xanthochlora* by the first two principal components (proportions of eigenvalues are 0.63 and 0.22, respectively).

first principal components (Fig. 3, for *A. propinqua* and *A. xanthochlora*) discriminate between microspecies much less clearly. One must certainly be wary of drawing conclusions based only on the visual analysis of ordination plots. If there are many observations for each taxon the plots can show rather undifferentiated groups.

Concerning the variables, general patterns obtained using metric and count variables are similar to those revealed by all kinds of different variables (Sepp & Paal, 1998); for example, they agree in showing the hairiness variables as the most effective and flower measurements as the least effective discriminators. Still, as it appeared from discriminant analysis, the metric variables and counts together are more effective than the hairiness or metric variables alone. However, although the metric variables distinguish a minority of microspecies, these can be just the ones which hair characters are not capable of discriminating.

The most stable combinations of variables reflected in structural indices are in good agreement with the main correlation groups of variables (Sepp & Paal, 1998). The stability of leaf variables, for example, also shows that it is probably better to use ratios of metric variables in taxon discrimination, as already suggested by some authors (e.g., Fröhner, 1995). If we use the structural indices or ratios instead of metric variables as such, there will probably be less individual differences between specimens of the same taxon.

As a concluding remark, we should be cautious with extrapolation of the results to the microspecies as a whole. For more abundant microspecies, the material originated from different populations all over Estonia, and the conclusions are more or less trustful for this part of their distribution area. However, the material is not representative for rare microspecies, and thus the

results concerning them are valid only for the analysed sample. Additionally, one should take into account the biases connected with selective collection of specimens for herbaria.

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EESTI KORTSLEHTEDE (*Alchemilla* L., Rosaceae) PALJUTUNNUSELINE VARIEERUVUS STRUKTUURIINDEKSITE PÕHJAL

Silvia SEPP, Tatjana NAHTMAN, Tõnu MÖLS ja Jaanus PAAL

On selgitatud, kui hästi eristuvad Eestis kasvavad kortslehe mikroliigid meetriliste ja loendustunnuste alusel, samuti hinnatud, missugused on stabiilseimad tunnuste kombinatsioonid ja missugused tunnused on liikide eristamiseks kõige informatiivsemad. Arvestades seda, et matemaatiliselt on ebakorrektne kasutada taksonite objektiivsuse hindamiseks samu tunnuseid, mille põhjal toimub nende defineerimine, rakendati taksonoomiliseks analüüsiks uudset struktuuriindeksite meetodit. Struktuuriindeksid eristasid mikroliike paremini kui esimesed peakomponendid või üksikud tunnused. Samas osutusid diskriminantanalüüsiga selgitatud indistinktsed liigipaarid kontinuaalseteks ka struktuuriindeksite alusel; lisaks tuvastati viimaste abil veel mõned teineteisest halvasti eristatavad liigipaarid. Mikroliikide eristamisel osutusid kõige efektiivsemaks taimede karvasust iseloomustavad tunnused, õite parameetrid aga kõige kehvemateks tunnusteks. Parim tulemus saavutati nii meetriliste kui ka loendustunnuste koos kasutamisel.