Two ivy (*Hedera* L., Araliaceae) species from the classic and geometric morphometrics points of view

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Introduction

Two ivy species from Russia and Ukrainia, *Hedera helix* L. (**Fig. 1**) and *H. colchica* (C. Koch) C. Koch (**Fig. 2**), are noticeably different in ploidy and micromorphological characters (stellate or plate-like hairs, respectively: Ackerfield, 2000), but hardly distinguished in field conditions if only macromorphological characters used. This uncertainty relates with wide diversity of vegetative characters (such as the length of leaf blade, number of lobes etc.) along with heteromorphism of ivy shoots.

Our research is based on the previous morphometric approach (Kost et al., 2003) that tested if there is the differences between these species in morphology and which methods are most suitable for taxonomic purposes. We were trying to employ so-called "geometric morphometrics" which is relatively new technique that has generated valuable results in many fields of taxonomy. The major difference from classical methods is the ability to understand the form of an object directly, as a whole, rather than via fragmentary measurements. Two different kinds of geometric morphometry are most widely used: Fourier analysis of shape curves, and landmark-based methods such a Thin Plate Splines (TPS) analysis. Fourier analysis calculates several "shape curves" from the object outline and then derives "Fourier coefficients" that represent these curves; the coefficients can be used as variables for multivariate analysis of the objects investigated (Jensen, Ciofani & Miramontes, 2002). The landmark approach is based on placing on the shape image several so-called "landmarks": points that locate the most important places of the object. These points are assumed to be homologous, at least in a geometric sense, because landmark-based methods operate only with coordinates of these reference points, so the objects studied should be directly comparable (Pavlinov, 2001). TPS reveals the degree of "bending energy" necessary to transform a rectangular grid superimposed on one shape to fit the consensus configuration (Rolf & Slice, 1990, developing much earlier qualitative work by Thompson, 1917). Multivariate methods can also be applied to the results of TPS. Both geometric approaches imply the preliminary transformation of object (e.g., Procrustes fit). Unfortunately, botanical investigations using TPS are sparse, despite the fact that many plant structures (e.g., leaves) fit well with geometric morphometry conditions.

Material and methods

Material was collected during 2001–2004 on West Caucasus and Crimea. In all, we have investigated 810 shoots. Ivy has at least three types of shoots: (a) vegetative, ground-based; (b) vegetative, trunk-based and (c) generative. Shoots with long and short internodes also exist. Our previous work (Kost et al., 2003) showed that only vegetative, trunk-based shoots with long-internodes are useful for morphometry. On each shoot, leaves periodically change their size from the shoot base upwards: at the beginning of each "period" they are small, than they become bigger and at the end of the period smaller again. Such period we called "series" according to Krenke (1933) point of view. We detected the type of hairs, measured and outlined the biggest leaf in each terminal series.

All the contours was processed by graphic utilities to improve the contrast and to remove defects. For the outlines, we filled contour with black color, and then run the tpsDig program (Rohlf, 2004) where 50 node points for each outline were used. Then we used two programs: EFAWin (Isaev, 1995) and Morpheus et al. (Slice, 1998). The first requires more handwork and did not use many transformations; in fact, it only rotates the outlines (**Fig. 3**). In contrary, Morpheus uses standardizations in size, location, starting point and orientation of outlines (**Fig. 4**) and also chooses the 8 Fourier harmonics (we used 14 harmonics in EFAWin).

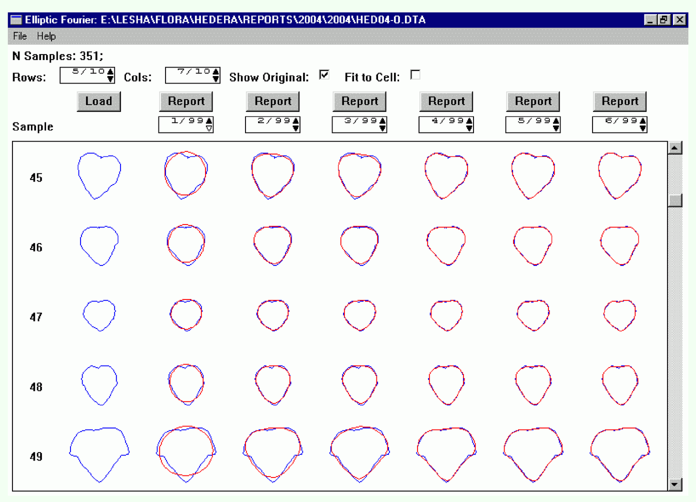

Fig. 3: The window of EFAWin program with the leaf outlines.

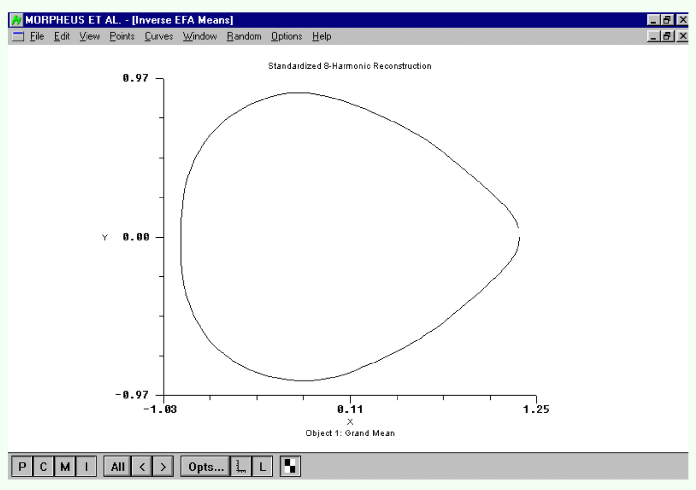

Fig. 4: The window of Morpheus et al. program with the average standardized outline.

The leaves of ivy is the relatively good source for the detection of outlines, but placing the landmarks is complex because the leaves could be entire or 3–5-lobed. We applied the **pseudo-landmarks** (Pavlinov, 2001) that could locate in same place in the case of the absense of given structure (in our case, when the leaf lobe(s) are absent), see **Fig. 5**. We tested also method of "artificial landmarks" where 50 landmarks were allocated along with the contour on the same distance. The last method, however, did not take into account the homology so we refused it. For the TPS analysis, we used the tpsRelw software (Rohlf, 2004). In all three cases (TPS and two types of EFA), the **R** statistical environment (R Development Core Team, 2004) was used for the PCA ordination of the set of Fourier coefficients and TPS weight scores.

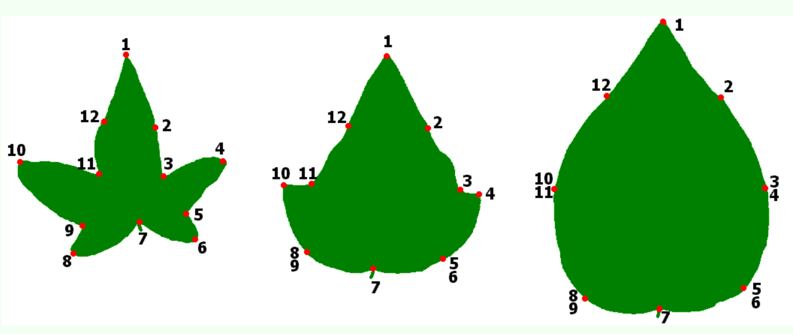

Fig. 5: The placements of landmarks.

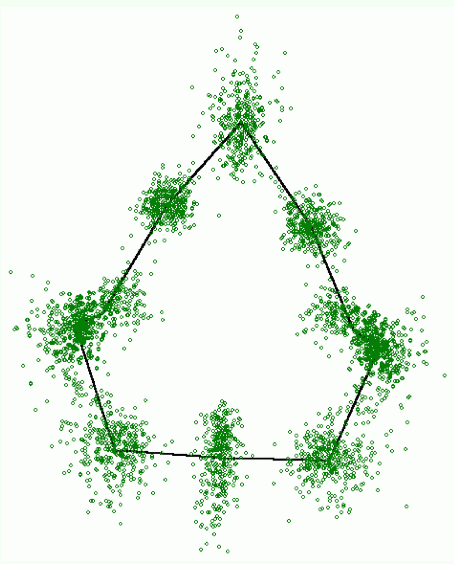

Fig. 6: The TPS consensus configuration.

Fig. 1: *Hedera helix*

Fig. 2: *Hedera colchica*

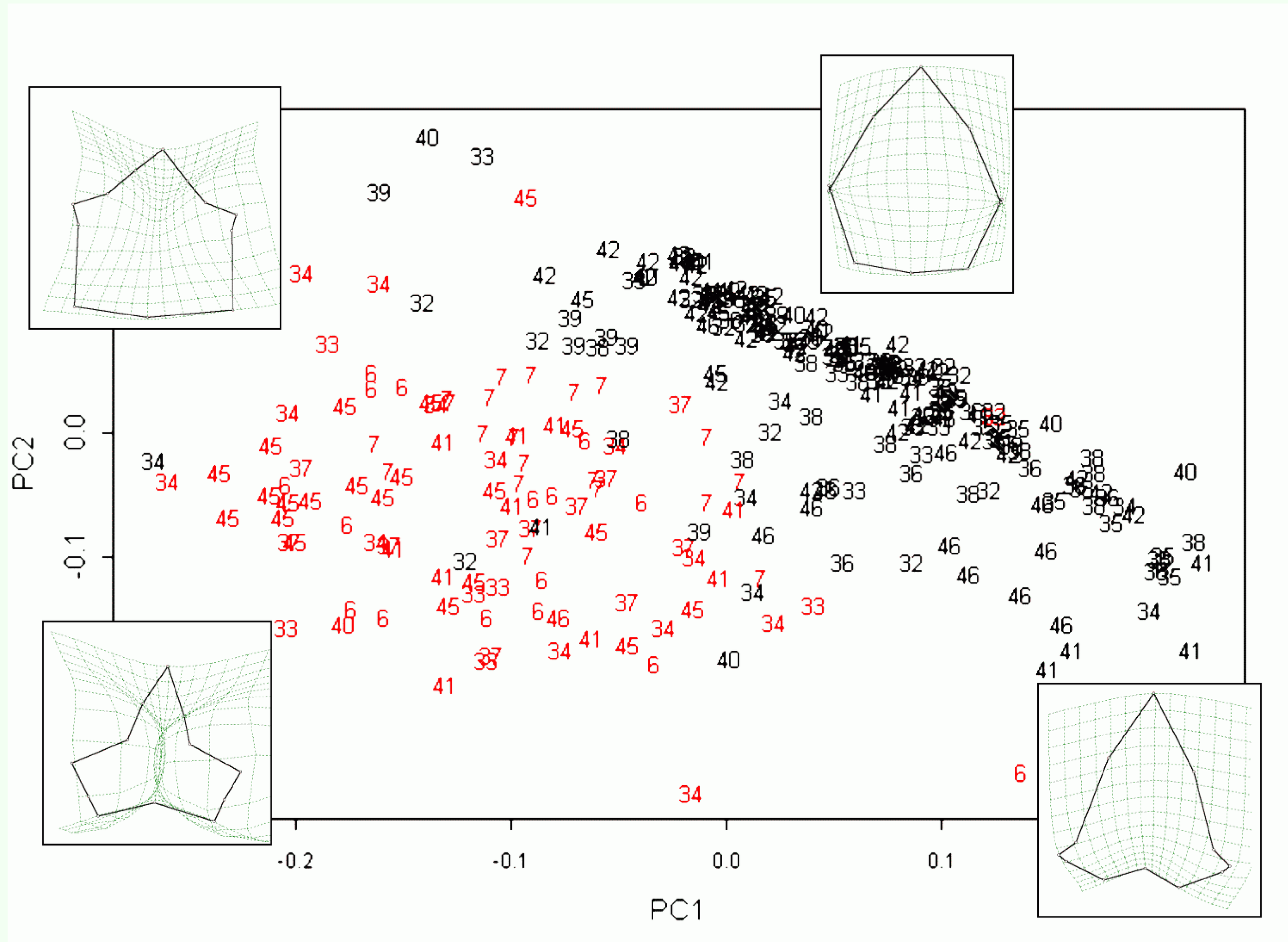

Fig. 7: PCA ordination of the results of TPS analysis. Cartesian transformation grids illustrating the average landmark configuration for the extreme points of ordination are placed in the corners of the graph. Numbers correspond to localities, red color—to the *H. helix*, black—to the *H. colchica*.

Results

The TPS consensus configuration (**Fig. 6**) shows that the most variation of leaf form is located in the first third of the ivy leaf (landmarks 3,4,10,11). The PCA analysis of TPS weight matrix (produced by tpsRelw program) shows that different species (marked by type of hair) is more or less clearly distinguished by overall leaf form. The "clouds" correspond with different types of hairs (and hereby with the species) are slightly interlaced (**Fig. 7**), and some points are located not in proper place. But in general the "clouds" matched the two typical configurations, which are shown in the top right (*H. colchica*) and bottom left (*H. helix*) corners of the graph. There are only some points near to other two configurations (combined the features of two species). Leaves from some locations (32, 34, 40) belong to different clouds. Here could be two explanations: (a) the mistakes of the hair type identification and (b) the introgression between these species.

The EFA analyses return more ambiguous structure. The unscaled outlines from EFAWin analysis (**Fig. 8**) produce the "fish-like" structure in the PCA, where the clouds correspond with different types of hairs (e.g., species) are not clearly separated, whereas Morpheus analysis returns the circular structure (**Fig. 9**) with no clear clouds. Thus, the PCA visualization shows the little "classification power" for two different types of EFA analysis. This ambiguity does not depend from the number of harmonics, if their number is more than 7. Moreover, Morpheus with standardizations turned off returns the picture very similar to EFAWin.

In addition, we have also investigated the type of smell. The smell of melted leaves is reported as pungent (for *H. helix*) and nutmeg-like (for *H. colchica*). We offered leaves from 10 shoots to 10 experts, but the statistical concordance between them is too low (Cohen $\kappa << 0.5$). Thus, the smell could not be used for distinguishing these species in natural habitats.

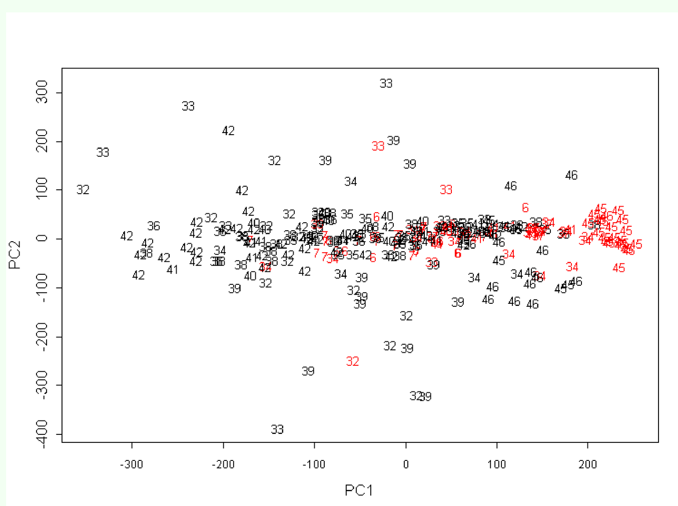

Fig. 8: PCA ordination of the results from EFAWin program. Marks are the same as on Fig. 7.

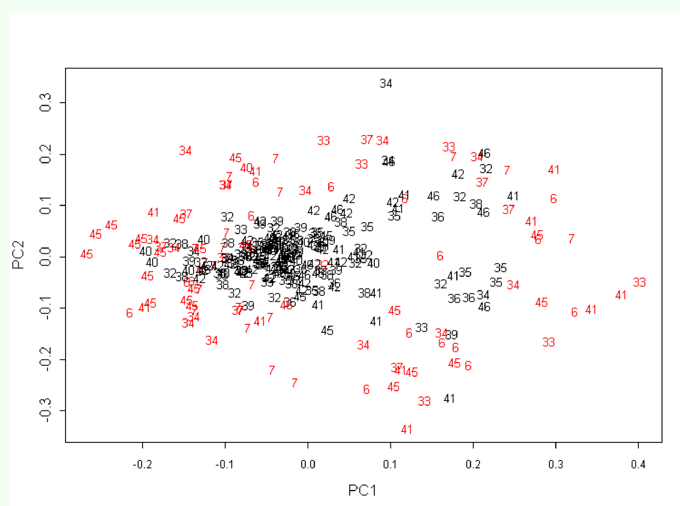

Fig. 9: PCA ordination of the results from Morpheus et al. program. Marks are the same as on Fig. 7.

Conclusions

- The geometric morphometrics methods based on landmarks (TPS) are able to distinguish the leaves of two ivy species whereas the methods based on the outlines (EFA) and classic morphometrics demonstrates the small classification power;
- The most leaves belong to one of two typical configurations (1-lobed, rounded leaves of *H. colchica* vs. 3–5-lobed, angular leaves of *H. helix*), although in the classical morphometrics analysis the number of lobes is unable to distinguish species (Kost et al., 2003);
- "Pseudo-landmarks" are useful in TPS analysis of complex form despite of their questionable morphological status;
- The leaves from Crimean ivy (sometimes considered as separate species, *H. taurica* Carr.) have no differences from typical *H. helix*;
- Several of localities contain the forms with intermediate characters; this could be evidence of introgression between these sympatric species;
- The overall variability of *H. colchica* leaf form is lower than of *H. helix*.

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