

Geographic morphological variation in parapatric Western Palearctic tree frogs, *Hyla arborea* and *Hyla savignyi*: are related species similarly affected by climatic conditions?

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Tree frogs *Hyla arborea* and *Hyla savignyi* are similar, closely-related species distributed in Europe and the Middle East. We investigated geographic variation in body shape within and between these species, and tested its relationships to macroclimatic conditions. We used morphometric distances (based on size corrected external measurements) to construct phenetic trees (unweighted pair-group method of arithmetical averages, Neighbour-joining), and to test correlations between morphology, geography, and climate by the partial Mantel test. Regardless of their specific affiliation, the parapatric populations of both species from the eastern Mediterranean, where they occupy comparable habitats, are closer to each other in morphospace than to conspecific populations from distal regions. This local interspecific similarity is probably driven by the common response to environment, expressed here as macroclimatic conditions. In support, the geographically close but ecologically vicariant populations of both species from the Caucasus region differ quite substantially in body shape. We suggest that climate-provoked phenotypic variation in closely-related parapatric species should be taken into account as a potential complication to character displacement in morphology. Contrariwise, morphological diversification between related species or their populations could be enhanced by habitat shifts resulting in occupation of different environmental space. © 2008 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2008, **95**, 539–556.

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INTRODUCTION

Although controversy still exists with respect to the extent of individual geographic modes of speciation, it is commonly accepted that most species originated in

allopatry (Coyne & Orr, 2004). As a result, ranges of the most of newly-formed species are not in contact, and recently observed parapatric or sympatric occurrence of closely-related species is ordinarily secondary. Related species inherit most of their traits from their common ancestor. Consequently, they are usually very similar in ecology as well as in phenotype. After the contact of their ranges, we can thus

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expect fierce competition for resources between these reproductively isolated, but ecologically largely equivalent entities. The most likely stable evolutionary outcomes of such competition is either exclusion of one of the competitors from the overlaying part of ranges leading to parapatry, in a long view, potentially even to total extinction of the inferior competitor, or the differentiation of ecological niches enabling long-term coexistence in sympatry. The differentiation of niches is often accompanied by the selection for divergent phenotypes that further minimize interactions between competitors. This process, termed character displacement (Brown & Wilson, 1956), generates adaptive shifts in phenotypes of one or both competing species reflecting resource division (Schluter, 2000). Because character displacement is thought to be an important mechanism for promoting adaptive diversification and consequently biodiversity (Schluter, 2000; Coyne & Orr, 2004), a number of studies has focused on identifying the conditions that encourage character displacement (Losos, 2000). However, many closely-related species have not differentiated their niches and live in parapatry (tree frogs *Hyla arborea* and *Hyla savignyi* in southern Anatolia being an example; Schneider, 2001). It seems that a process exists making phenotypic diversification between members of some species pair difficult (i.e. a process that prevents character displacement).

A candidate for such process could be climate-provoked phenotypic variation. Climatic conditions as an important agent influencing animal morphology have been documented in many ectothermic and endothermic animals (Rosso, Castellano & Giacoma, 2004; Schäuble, 2004; Davis, 2005; Kutrup, Bulbul & Yilmaz, 2006). Convergent emergence of ecomorphs during iterative climatic cycles suggests a strong potential of climatic conditions to form similar phenotypes even among distantly-related animals (Martin & Meehan, 2005). We should thus expect convergent evolution driven by common macroclimatic conditions still more likely in related species that share most of their genetic background. Closely-related species might also react to common environment by uniform plastic response because it is highly probable that they inherited the norm of reaction from the ancestor. To sum up, we can thus assume that shared environmental conditions might induce phenotypic resemblance of closely-related species in the common or near parts of their ranges, which could complicate species differentiation necessary for character displacement.

In the present study, we focus on the morphological variation in two parapatric species of tree frogs, *H. arborea* (Linnaeus, 1758) and *H. savignyi* Audouin [1827] '1809'. According to the immunological research, *H. arborea* and *H. savignyi* are closely-

related species, which diverged probably by the end of the Miocene, approximately 5–6 Mya (Maxson & Wilson, 1975; Maxson, 1978; Riehl, Lell & Maxson, 1995). Fossil records of *H. arborea* and *H. savignyi* are known from the Pleistocene and Holocene, and the first species is likely recorded also from the Pliocene (Sanchiz, 1998). The known range of *H. arborea* extends from Iberian Peninsula in the west to Asia Minor and the Caucasus region in the east (Glandt, 2004). *Hyla savignyi* is widely-distributed in southern and eastern Turkey, eastern Transcaucasia, northern and western Iran, Iraq, Levant, and the north-eastern part of Sinai. Two isolated populations live in Cyprus and south-western Arabian Peninsula (Glandt, 2004). The ranges of *H. arborea* and *H. savignyi* are known to be in contact in two areas: in southern Anatolia, where both species live in parapatry (Kaya, 2001; Schneider, 2001, 2004), and in the Caucasus region, where parapatry was also documented in Armenia (Egiasarian & Schneider, 1990, 1991), but where a local sympatric occurrence was anticipated in other studies (Aleksperov, 1978; Kuzmin, 1999; Tarkhnishvili & Gokhelashvili, 1999). However, sympatry was recently rejected by Litvinchuk *et al.* (2006).

Hyla savignyi was originally distinguished from *H. arborea* on the basis of colour pattern, and for a long time, it was understood as a subspecies of *H. arborea* (Duellman, 1977). Nevertheless, bioacoustic studies revealed substantial differences in male advertisement calls between *H. arborea* and *H. savignyi* (Schneider & Nevo, 1972; Schneider, 1974; Kaya & Simmons, 1999). Within the species, male advertisement calls tend to be stable across ranges (Schneider, 2004). Male advertisement calls are important components of courtship behaviour in anurans in general and in the Palearctic tree frogs in particular (Brzoska, Schneider & Nevo, 1982; Schneider *et al.*, 1984), and usually form interspecific premating reproductive barrier. Later authors revealed cytological and osteological traits further supporting the specific status of *H. savignyi* (Anderson, 1991; Kaya, 1997). By contrast, the differentiation between *H. arborea* and *H. savignyi* in external morphology appears to be minimal across populations in southern Anatolia (Zaloğlu, 1972; Kaya, 2001), although their advertisement calls considerably differ also in that region (Kaya & Simmons, 1999; Schneider, 2001). However, the studies focussing on morphology did not include material from other parts of species ranges, and do not allow morphological variation and differentiation to be compared among distant versus near populations of both species. Recently, a new species of tree frog, *Hyla heinzsteinitzi* Grach, Plesser & Werner (2007), was described from the range of *H. savignyi*. The new species is

known only from a restricted area in the surroundings of Jerusalem, Israel, and occurs in sympatry and apparently syntopy with *H. savignyi*. It was distinguished from *H. savignyi* by differences in head shape, coloration and advertisement call (Grach *et al.*, 2007).

The aim of the present study was to document variation in external morphology across the whole range of *H. savignyi* and eastern part of the range of *H. arborea*. We were interested in the degree of morphological differentiation between both species and, specifically, in morphological (dis)similarity in the regions near the contacts of their ranges (southern Anatolia and Caucasus). Furthermore, we tested whether climatic conditions influence the morphological variation across populations of both species, and whether similar environment leads in both species to comparable morphotypes, which would support the above-described scenario of climate-driven prevention of character displacement in morphology in closely-related species of organisms.

MATERIAL AND METHODS

MATERIAL

The material examined consisted of 308 preserved museum adult specimens of *H. savignyi* from the whole range of its distribution (195 males, 89 females, and 24 adults of undetermined sex), 238 preserved voucher adults of *H. arborea* from the central and eastern parts of its range (173 adult males, 50 adult females, and 15 adults of undetermined sex), and 20 preserved museum adult males of *Hyla meridionalis* from Canary Islands/Tenerife (for details, see Appendix). The Mediterranean species *H. meridionalis* was recently shown to be basal within the Western Palearctic clade (Smith, Stephens & Wiens, 2005), and we used it as the outgroup. With the exception of the Iberic subspecies *Hyla arborea molleri* Bedriaga, 1890 and recently described subspecies *Hyla arborea gumilevskii* Litvinchuk, Borkin, Rosanov, Skorinov, 2006, our material covers all other traditionally recognized subspecific taxa of *H. arborea* (Kuzmin, 1999; Valakos *et al.*, 2008), which have been distinguished on the basis of external morphology: *Hyla arborea arborea*, *Hyla arborea schelkownikowi* Chernov, 1926 (including 23 topotypes) and *Hyla arborea kretensis* Ahl, 1931 (including 34 syntypes). Although, some studies do not approve the validity of these subspecies (Schneider, 2004; Frost, 2007). The recently described *H. heinzsteinitzii* was not included in our study since the formal description was published after our analyses. Nevertheless, no tree frogs from the restricted range of the new species were included into our dataset; thus, any taxonomic confusion should not occur.

The material was divided into 13 operational taxonomic units (OTUs) defined according to the species identity and biogeographical division (for the map, see Fig. 1). The individuals of *H. savignyi* form seven OTUs: (1) Arabian Peninsula, (2) Iranian Highlands and Kurdistan, (3) Mesopotamia, (4) Levant, (5) Cyprus, (6) southern Anatolia, and (7) Transcaucasia. *H. arborea* was assorted into six OTUs: (8) Caucasus and adjacent regions, (9) western Anatolia and adjacent islands, (10) Crete, (11) Balkan Peninsula and adjacent islands, (12) Danube Delta, and (13) Central Europe. Six individuals from six localities outside the ranges of defined OTUs were used only in the interspecific comparisons. An extraordinary OTU (14) was established for the outgroup, *H. meridionalis* from Tenerife Island (the type locality).

Sex and sexual maturity were determined according to the presence of vocal sac in males and enlarged abdomen (presence of eggs) in females. The snout-urostyle length of the smallest adult males and females (excluding several miniature individuals considered extreme) was taken as an arbitrary limit of sexual maturity for individuals in nonreproductive phase of life (29.0 mm for males and 31.8 mm for females). The sex of individuals collected during non-reproductive phase was determined by dissection (which was possible only for the material deposited in the National Museum, Prague).

SPECIFIC DETERMINATION: COLOUR PATTERN

The colour pattern of the dark side strip (*linea marginalis*) and the inguinal region was examined in all individuals of *H. arborea* and *H. savignyi*. *Hyla meridionalis* does not possess the dark strip along the body. The coloration of the inguinal region has been suggested to be the key morphological trait discriminating *H. arborea* and *H. savignyi* (Baran & Atatür, 1998), with the exception of the subspecies *H. arborea gumilevskii* from south-eastern Azerbaijan and presumably northern Iran with reduced inguinal loop (Litvinchuk *et al.*, 2006). The specific determination of the material from the region of parapatry was confirmed by molecular evidence (mitochondrial and nuclear DNA sequences; V. Gvoždík, J. Moravec, P. Kotlík *et al.*, unpubl. data). Three types of inguinal colour pattern were distinguished: (1) *linea marginalis* continuously builds a regular inguinal loop, (2) spot(s) instead of the loop, or a thin loop is separated from *linea marginalis*, and (3) loop or spot(s) are entirely absent. Photographs of representatives of the three groups are provided in the Supporting Information (Fig. S1). In six specimens, the condition of the skin colour did not allow an evaluation of the inguinal colour pattern.

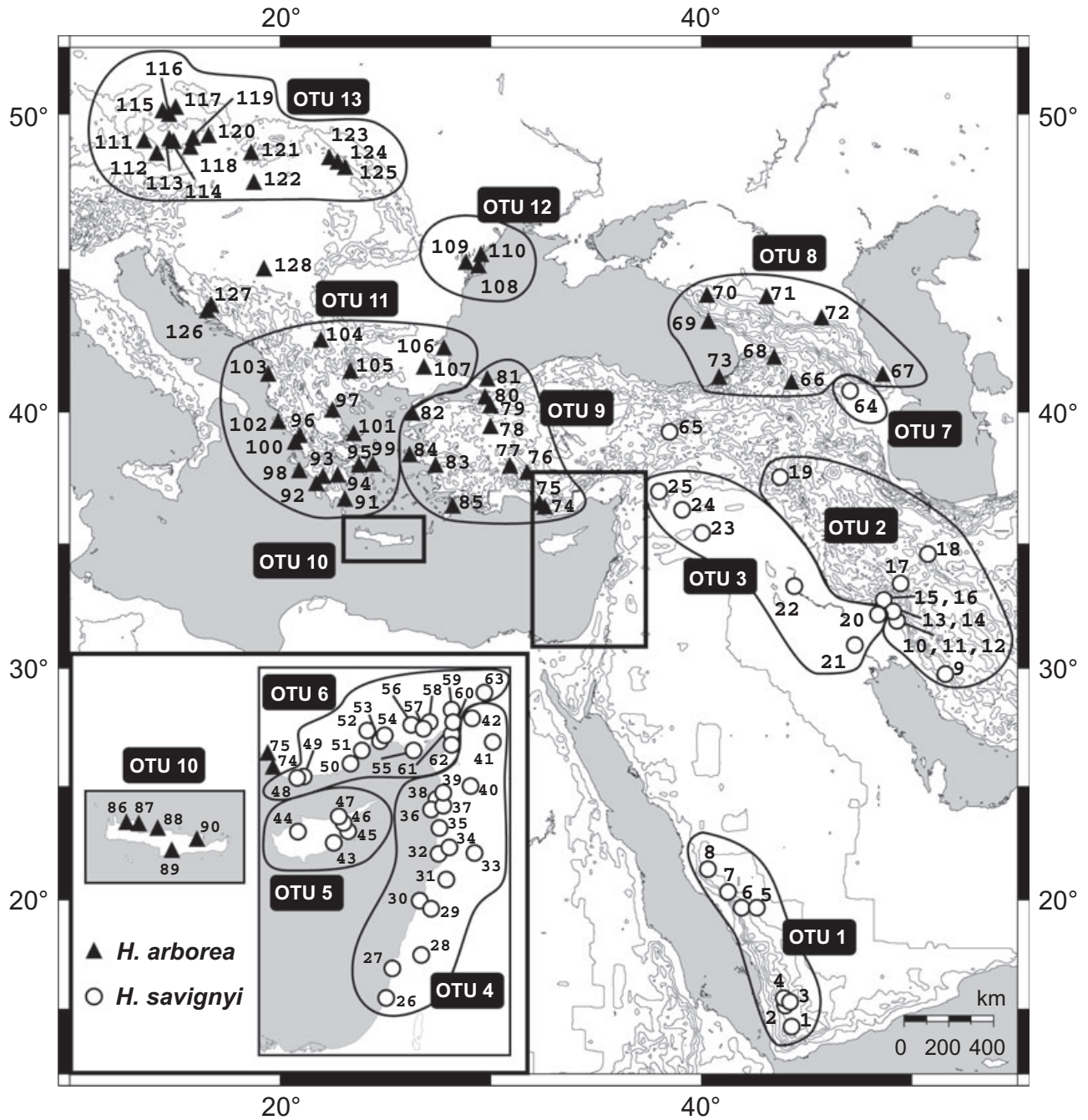


Figure 1. Map of localities. For further details, see Appendix.

MORPHOMETRICS

Seventeen external measurements were taken with the callipers in each adult specimen by V.G. to the nearest 0.1 mm in the standardized manner (Fig. 2): SUL (snout–urostyle length: from the tip of snout to the posterior margin of urostyle); FmL* (femur length: from the middle of cloacal gap to the external

margin of knee joint, when thighs and shins are in perpendicular position to body axis); TbL* (tibia length: from the external margin of knee joint to the external margin of heel articulation); TrL* (tarsus length: from the external margin of heel articulation to the proximal edge of inner metatarsal tubercle); HW (head width: the largest head width); HLT* (head length: from the tip of head to the posterior margin of

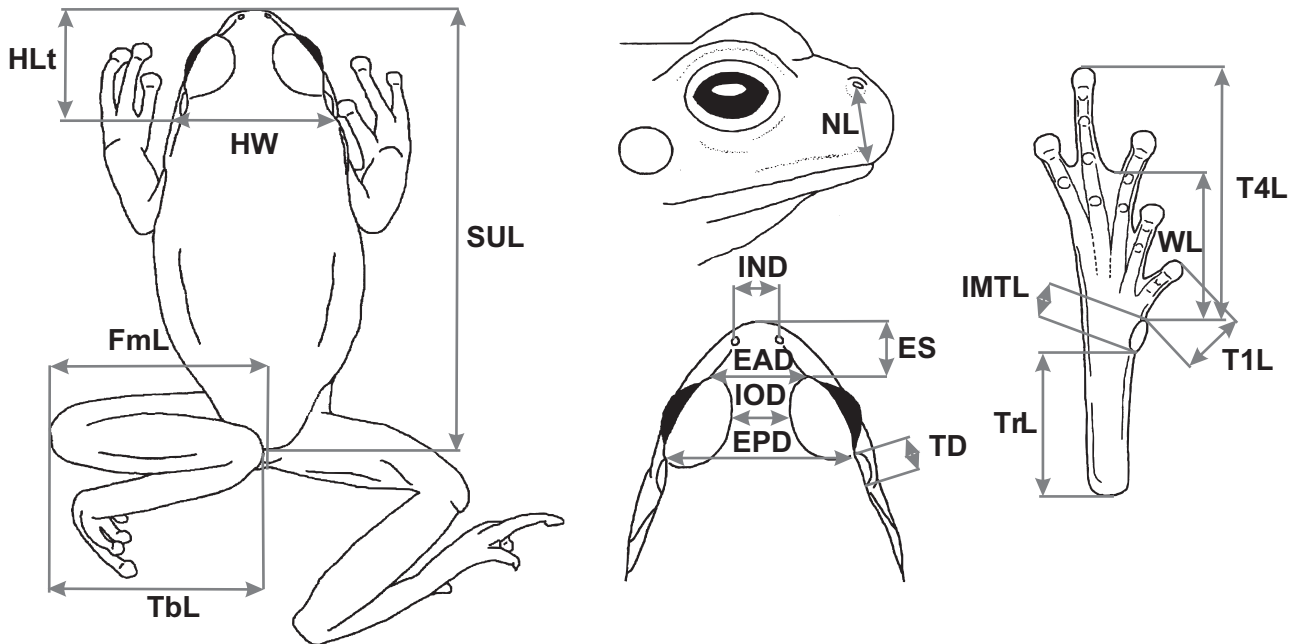


Figure 2. Morphological measurements. SUL, snout–urostyle length: from the tip of snout to the posterior margin of urostyle; FmL, femur length: from the middle of cloacal gap to the external margin of knee joint, when thighs and shins are in perpendicular position to body axis; TbL, tibia length: from the external margin of knee joint to the external margin of heel articulation; TrL, tarsus length: from the external margin of heel articulation to the proximal edge of inner metatarsal tubercle; HW, head width: the largest head width; HLt, head length: from the tip of head to the posterior margin of tympanum; ES, eye–snout distance: from the tip of head to the anterior corner of eye; TD, horizontal tympanum diameter; EAD, distance between the anterior corners of eyes; IOD, interorbital distance: the shortest distance between upper eye lids; EPD, distance between the posterior corners of eyes; IND, internarial distance: the distance between the midpoints of nostrils; NL, nostril–upper lip distance: the distance from the midpoint of nostril to the margin of the upper lip; IMTL, inner metatarsal tubercle length: the length of the base of tubercle; T1L, first toe length: from the distal edge of the inner metatarsal tubercle to the tip of the first toe; T4L, fourth toe length: see T1L; and WL, webbing length: from the distal margin of the inner metatarsal tubercle to the margin of the webbing in the middle between the third and the fourth toe.

tympanum); ES⁺ (eye–snout distance: from the tip of head to the anterior corner of eye); TD (horizontal tympanum diameter); EAD (distance between the anterior corners of eyes); IOD (interorbital distance: the shortest distance between upper eye lids); EPD (distance between the posterior corners of eyes); IND (internarial distance: the distance between the midpoints of nostrils); NL (nostril–upper lip distance: the distance from the midpoint of nostril to the margin of the upper lip); IMTL* (inner metatarsal tubercle length: the length of the base of tubercle); T1L* (first toe length: from the distal edge of the inner metatarsal tubercle to the tip of the first toe); T4L* (fourth toe length: see T1L); and WL* (webbing length: from the distal margin of the inner metatarsal tubercle to the margin of the webbing in the middle between the third and the fourth toe). The means of left and right sides were computed for bilateral traits to reduce effects of fluctuating asymmetry and measurement error (asterisks). The measurements denoted by cross

(⁺) were measured in parallel to body axis using calliper modified according to Goren & Werner (1993). One or more measurements were missing in 21 individuals due to their damage. These missing values (representing less than 0.3% of all measurements) were replaced with the means of values predicted from regressions of traits involved against SUL, FmL, TbL, and HLt in individuals from a given OTU. Predicted values obtained using different independent variables were subsequently averaged to produce a single estimate of missing values (for an analogous method, see Merilä, 1997).

ANALYSIS OF THE PATTERN OF MORPHOMETRIC VARIATION

The original measurements were natural log-transformed before the further analyses. Every comparative analysis has to take into account the growth pattern of studied organisms. Tree frogs typically

mature at small sizes relative to their asymptotic size, and continue growing extensively after maturity (Moravec, 1990; Friedl & Klump, 1997; Kyriakopoulou-Sklavounou & Grumiro, 2002). Ignoring this fact during morphometric comparisons, the discovered variation could reflect differences in age composition among samples rather than factual differences among populations. Therefore, we decided to focus on quantitative morphological variation in body shape after statistical control for body size. The traditionally used method for computing size-adjusted data (residuals of linear regressions) was recently subjected to severe criticism (Smith, 1999; García-Berthou, 2001) and, due to the structure of our data (nonparallel regression lines of many variables against SUL among groups), it would be obviously misleading in our case. Therefore, to remove the effects of size we used the geometric means method of Mosimann (1970), which does not distort group configuration (Butler & Losos, 2002; Losos & Miles, 2002). We defined the individual index of body size as the arithmetic mean of all 17 log-transformed variables (equivalent to the geometric mean of original variables if computed before log-transformation). Each individual was then size-adjusted by taking the difference of each log-variable with this body size index. To obtain linearly independent shape variables for successive multivariate analyses, we arbitrarily omitted one variable (size-adjusted ES). Further on we refer to size-adjusted variable as a variable/S (e.g. size-adjusted femur length as FmL/S).

Principal component analysis (PCA) on 16 size-adjusted measurements of all individuals was used to estimate the pattern of correlation and covariation among variables. The number of interpretable PCA axes was determined using broken-stick model as recommended by Jackson (1993). Retained individuals' scores on the significant principal components were then treated by general linear models (GLM) with sex and OTU as factors to test sexual and geographical differences in our dataset within the ingroup species, *H. arborea* and *H. savignyi*. Next, we performed two canonical discriminant function analyses (DFA): the first DFA with all individuals including the outgroup, *H. meridionalis*, for investigation of general pattern, and the second DFA restricted to the ingroup species, *H. arborea* and *H. savignyi*, to obtain morphological distance matrix for further testing of geographic variation causality. The differences among all groups were tested for reliability by establishing the percentages of individuals correctly classified by classification functions. Further, we calculated squared Mahalanobis distances (D^2) as a measure of morphometric distances among groups of different geographic origin. Morphological phenograms were constructed from the distances between all OTUs (i.e.

including *H. meridionalis*) by unweighted pair-group method of arithmetical averages (UPGMA) cluster analysis and the Neighbour-joining method.

The program STATISTICA, version 6.0 (StatSoft) was used for all morphometric calculations. Phenetic trees were constructed by PHYLIP, version 3.65 (Felsenstein, 2005) and depicted in TREEVIEW, version 1.6.6 (Page, 2001).

ANALYSIS OF MORPHOMETRIC/GEOGRAPHIC/ CLIMATIC CAUSALITY

The causes of geographic variation between and within the species were tested by the partial Mantel test of matrix association (Smouse, Long & Sokal, 1986). We used the morphometric matrix (i.e. the matrix of squared Mahalanobis distances between particular *H. arborea* and *H. savignyi* OTUs) as a dependent matrix. The independent matrices were the matrix of geographic distances and the climatic matrix. Mantel tests were run on 10 000 randomizations. Geographic distances between approximate centres of the ranges of OTUs were taken from the Lambert azimuthal projection map. The macroclimatic data [i.e. 12 average monthly temperatures (°C) and 12 monthly precipitations (mm month⁻¹) for each single locality] were obtained from the International Water Management Institute Climate Atlas Web Query service (IWMI, 2006). These values show the annual climate cycle experienced by a population through a year. The climatic matrix was constructed as the matrix of Euclidean distances between-OTUs based on the weighted means of all 12 average monthly temperatures and all 12 monthly precipitations for particular OTU. The means for each climatic trait were weighted with the number of individuals from each locality to control for biases caused by uneven representation of individual localities within OTUs (for the weighted means of all climatic traits, see Supporting Information, Table S1). The program IBD, version 1.52 (Bohonak, 2002) was employed for the Mantel tests.

RESULTS

COLOUR PATTERN

Most specimens of *H. savignyi* ($N = 207$; 68.1%), lacked any dark inguinal loop or spot(s). The dark spot separated from *linea marginalis* was present in 95 specimens (31.2%), and the loop of irregular shape on the lateral strip was developed in only two specimens (0.7%) from areas in the middle of the range of *H. savignyi*. Interestingly, the populations of *H. savignyi* from Iranian Highlands and Kurdistan possess high rate of absence of both spot(s) and loop ($N = 39$; 97.5%). On the other hand, a loop was present in

Table 1. Factor loadings of size-adjusted external measurements for the first two principal components

	PC 1	PC 2
SUL/S	-0.42	0.54
FmL/S	-0.84*	0.17
TbL/S	-0.82*	0.31
TrL/S	-0.77*	0.14
HW/S	-0.04	0.04
HLt/S	0.09	0.75*
TD/S	0.22	0.14
EAD/S	0.44	0.23
IOD/S	0.04	0.23
EPD/S	0.41	0.17
IND/S	0.64	-0.24
NL/S	0.71	-0.09
IMTL/S	0.32	-0.52*
T1L/S	-0.44	-0.48*
T4L/S	-0.59	-0.56*
WL/S	-0.46	-0.66*

*Characters most strongly correlated with respective principal component. For abbreviations, see Fig. 2.

220 (93.2%) of the examined adult specimens of *H. arborea*. The colour pattern in the form of a separated loop was observed in 13 individuals (5.5%), and the absence of any shaped pattern was noticed in three (1.3%) specimens of *H. arborea* only. All 34 examined individuals of *H. arborea* from western Anatolia (OTU 9) possess well-developed loop of a regular shape. For a graphic expression of inguinal colour pattern distribution, see the Supporting Information (Fig. S2).

GEOGRAPHIC VARIATION OF BODY SHAPE

The broken-stick model revealed that only the first two principal axes summarizing 27.1%, respectively 15.6% of the total variance in body shape are statistically significant. PC1 catches mostly variation in measurements concerning hind limbs (FmL/S, TbL/S, TrL/S), whereas PC2 reflects variation in head length (HLt/S) and in foot characters (IMTL/S, T1L/S, T4L/S, WL/S); factor loadings summarized in Table 1. The studied species differ significantly in factor scores of PC1 as well as PC2 [analysis of variance (ANOVA), $P < 0.0001$], although post-hoc tests revealed that only *H. arborea* and *H. savignyi* differ significantly in both principal components (Tukey HSD tests for unequal N , $P < 0.0001$). *Hyla meridionalis* does not differ significantly from neither *H. arborea*, nor *H. savignyi* in PC1 factor scores, and differs only from *H. arborea* in PC2 factor scores (Tukey HSD for unequal N test, $P < 0.05$). Nevertheless, *H. arborea* and *H. savignyi* show an extensive overlap in the morphometric space, and *H. meridionalis* is imbedded

directly between the two other species (Fig. 3A, B, C). For descriptive statistics of morphological characters, see the Supporting Information (Table S2).

GLM ANOVA of PC1, respectively PC2 scores, performed separately for each ingroup species, revealed substantial geographic intraspecific variation in body shape (factor OTU: *H. savignyi*: PC1: $F = 27.271$, $P < 0.001$; PC2: $F = 13.081$, $P = 0.003$; *H. arborea* PC1: $F = 8.313$, $P = 0.018$; PC2: $F = 15.538$, $P = 0.005$). Compared with OTU, the variation explained by the sex factor is trivial and statistically nonsignificant after Bonferroni correction for multiple tests: *H. savignyi*: PC1: $F = 5.822$, $P = 0.027$; PC2: $F = 0.467$, $P = 0.497$; *H. arborea*: PC1: $F = 2.428$, $P = 0.155$; PC2: $F = 0.063$, $P = 0.808$; as well as the sex \times OTU interactions, nonsignificant, $P > 0.072$ in all cases. Therefore, we pooled data on both sexes and added data on 39 adults of unknown sex in our further analyses.

DFA further confirmed the differences in body shape among individual OTUs (Wilks' $\lambda = 0.028$, $F = 10.99$, $P < 0.0001$; Fig. 4A). However, the success of classification functions in classification of individuals with respect to their origin was moderate (62.8% correctly classified individuals on average; range = 25.0–90.9%; Table 2); for discriminant coefficients of classification functions, see the Supporting Information (Table S3). Classification into incorrect species was relatively low for all OTUs of *H. savignyi* (10.1% specific misclassifications on average, range = 3.4–17.1%; 8.0% into *H. arborea*, 2.1% into *H. meridionalis*), and three OTUs of *H. arborea* [only 2.3% specific misclassifications within the OTU 8 (Caucasus), OTU 12 (Danube Delta), and OTU 13 (Central Europe), on average; range = 0–4.3%; all misclassified as *H. savignyi*]. On the other hand, a relatively high number of misclassification into different species occurred within the southern OTUs of *H. arborea*: OTU 9 (western Anatolia), OTU 10 (Crete), and OTU 11 (Balkan); 24.6% erroneous on average; range = 17.6–35.3%; 21.2% into *H. savignyi*, 3.4% into *H. meridionalis*. The individuals of these populations were often misclassified into the Mediterranean and Mesopotamian populations of *H. savignyi*. *H. meridionalis* was incorrectly classified in 45% (30% as *H. savignyi*, 15% as *H. arborea*).

Initially, the phenetic trees based on the squared Mahalanobis distances between OTUs calculated by alternative computational methods (UPGMA and NJ) appear to be rather different (Fig. 4B, C). However, the main information included in both of them does not differ. Both trees demonstrate low morphometric distances among the eastern Mediterranean populations of *H. arborea* and the Mediterranean and Mesopotamian populations of *H. savignyi*. On the other hand, OTUs with the largest geographic distances from the Anatolian zone of parapatry, and/or with the

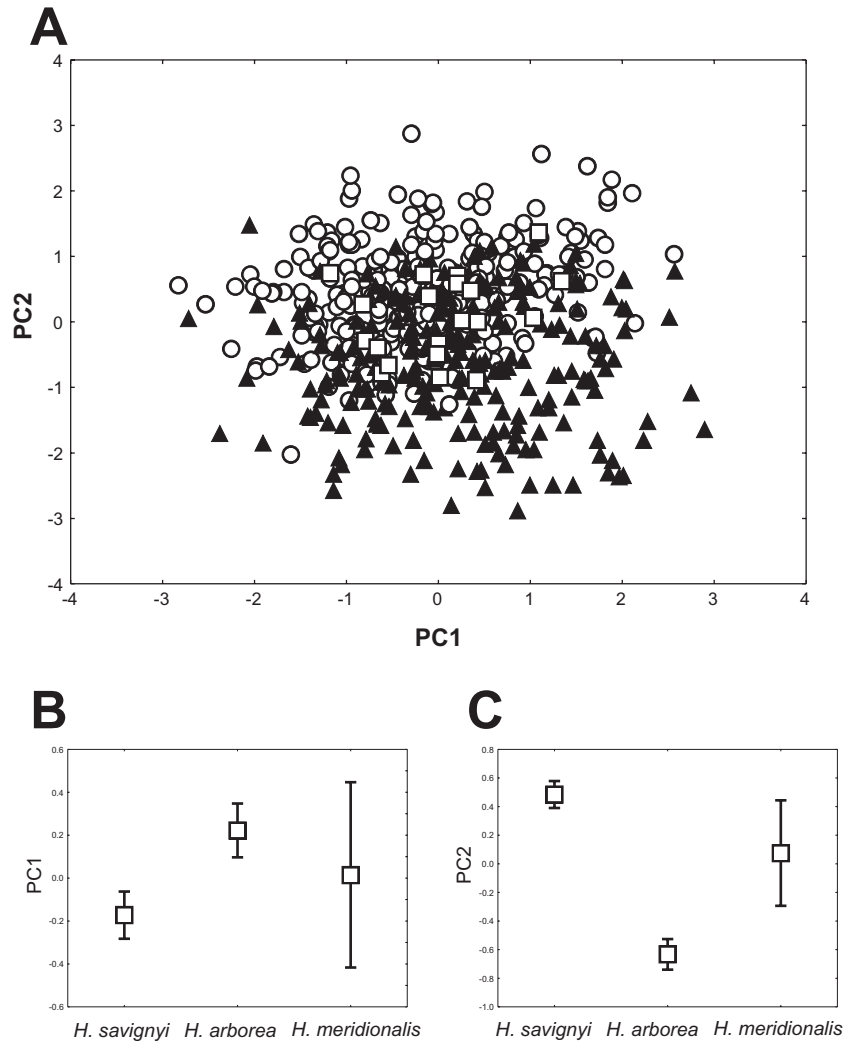


Figure 3. Factor scores of the first two principal components shown as (A) scatterplot for all cases (triangles, *Hyla arborea*; circles, *Hyla savignyi*; squares, *Hyla meridionalis*) and mean values with 95% confidence intervals of (B) PC1 and (C) PC2 for each species separately. The large confidence intervals of *H. meridionalis* mirror smaller sample size in comparison to *H. arborea* and *H. savignyi*.

most outermost climatic conditions (Central European *H. a. arborea*, Caucasian *H. a. schelkownikowi*, Danube Delta populations of *H. arborea*, and Arabian, Iranian and Transcaucasian populations of *H. savignyi*) are relatively dissimilar to each other as well as to the cluster of the Mediterranean and Mesopotamic tree frogs.

EFFECT OF GEOGRAPHY AND CLIMATE

The Mantel tests performed on distances among populations of *H. arborea* and *H. savignyi* revealed significant correlation between body shape and geography ($r = 0.635$, $P < 0.0002$), as well as between body shape and climate defined by temperature and

precipitation ($r = 0.373$, $P = 0.0037$) (Fig. 5A, B). Although the correlation between body shape and geography is stronger, several outliers contradicting this correlation are present (Caucasus versus Transcaucasia, Caucasus versus Iran, and Caucasus versus Mesopotamia). These interspecific outliers reflect deep morphometric differences across relatively short geographic distances. On the other hand, the geographically close Anatolian *H. arborea* and *H. savignyi* populations are close also in morphospace.

Because geographical and climatical matrices are significantly correlated (Mantel test: $r = 0.266$, $P = 0.0242$), the partial Mantel tests were used to analyse their independent association with body

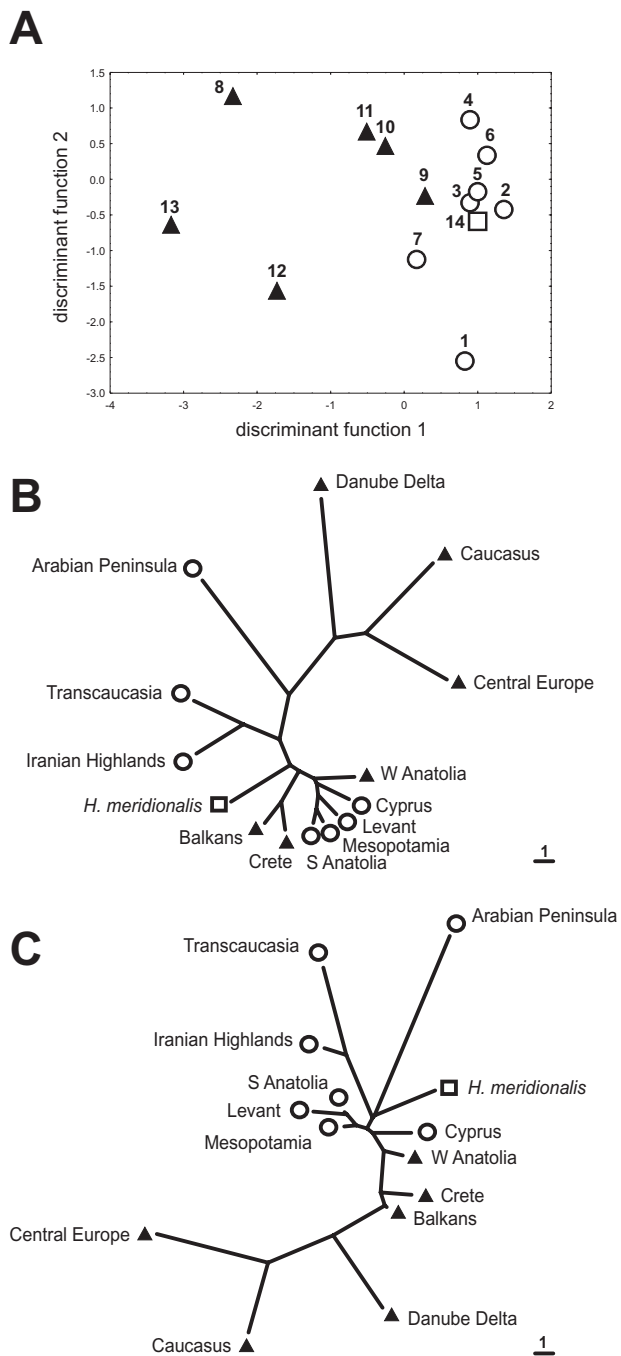


Figure 4. Results of the discriminant analysis presented as (A) median values of the canonical scores of the first two discriminant functions (for OTU identification, see text) and unrooted phenetic trees based on morphometric distances among OTUs using (B) unweighted pair-group method of arithmetical averages cluster analysis, and (C) the Neighbour-joining method. Triangles, *Hyla arborea*; circles, *Hyla savignyi*; squares, *Hyla meridionalis*.

shape. After statistical control of the covariation with geography, climate still showed significant association with body shape ($r = 0.274$, $P = 0.0227$).

DISCUSSION

In closely-related species, we can expect considerable phenotypic similarity caused by their shared ancestry. After speciation, the reproductive barriers prevent interbreeding among populations between species, while individual populations within species are essentially free to interbreed. Consequently, after a longer period of independent evolution, each species should possess its own species-specific morphotype. None or only small interspecific overlap in many morphological traits usually allows us to find key traits useful for determination of even closely-related species. The colour pattern of the inguinal region (either the absence or presence, respectively, of the shape of the dark anterodorsally-oriented loop) has been considered to be the main morphological difference between *H. arborea* and *H. savignyi* since the description of *H. savignyi* (Audouin, [1827] '1809'). Our material more or less confirms the coloration as the key morphological differential diagnostics. However, some individuals of both species do not follow the coloration typical for their species, which warns against determination of tree frogs solely on the base of coloration. This view is also supported by recent description of a subspecies of *H. arborea* with reduced inguinal loop (Litvinchuk *et al.*, 2006). Therefore, we strongly recommend the use of molecular markers or bioacoustics for the verification of the determination of these two species of tree frogs.

We found a statistically significant interspecific differentiation between *H. arborea* and *H. savignyi* in measurements concerning hind limbs and head length, which corresponds to a general historical approach to taxonomy of the Palearctic tree frogs (Terentjev & Chernov, 1949). However, these two species do not form well-separated clusters in the morphospace delimited by external measurements (Fig. 3A). Multivariate analyses revealed that individuals from the populations of the Mediterranean and adjacent regions (the Balkans, Crete, Anatolia, Levant, Cyprus, Mesopotamia) are very similar in body shape regardless of specific affiliation. This means that the individuals of *H. arborea* and *H. savignyi* from these areas are mutually more similar in body shape to each other, irrespective of the sharp difference in their advertisement calls (Schneider, 2001, 2004), than to conspecific individuals (with identical advertisement calls; Schneider, 2000, 2004) from more distal populations.

Significant portion of variation in body shape among particular OTUs can be explained by two basic

Table 2. A posteriori classification of the discriminant function analysis. Percentage of correctly classified individuals for respective OTU in bold.

		Predicted group														
		<i>Hyla savignyi</i>							<i>Hyla arborea</i>							<i>Hyla meridionalis</i>
OTU		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
<i>Hyla savignyi</i>	1	26	0	0	0	0	1	1	0	0	0	0	0	1	0	
	<i>N</i> = 29	89.8%					3.4%	3.4%						3.4%		
	2	0	25	0	3	3	1	2	2	0	0	5	1	0	1	
	<i>N</i> = 41		61.0%		7.3%	7.3%	2.4%	4.9%	4.9%			12.2%	2.4%		2.4%	
	3	1	1	5	1	2	7	0	0	0	2	0	0	0	1	
	<i>N</i> = 20	5.0%	5.0%	25.0%	5.0%	10.0%	35.0%				10.0%				5.0%	
	4	0	6	0	51	4	6	1	1	0	0	3	5	0	2	
	<i>N</i> = 78		7.7%		65.4%	5.1%	7.7%	1.3%	1.3%			3.8%	6.4%		2.6%	
	5	0	1	1	8	47	3	0	0	0	0	2	2	0	1	
	<i>N</i> = 65		1.5%	1.5%	12.3%	72.3%	4.6%					3.1%	3.1%		1.5%	
	6	1	3	0	13	11	25	4	4	0	1	0	1	0	2	
	<i>N</i> = 61	1.6%	4.9%		21.3%	18.0%	41.0%	6.6%	6.6%		1.6%		1.6%		3.3%	
7	0	3	0	0	0	1	7	7	0	0	0	0	1	0		
<i>N</i> = 12		25.0%				8.3%	58.3%	58.3%					8.3%			
8	1	0	0	0	0	0	0	0	28	1	3	3	0	0		
<i>N</i> = 39	2.6%								71.8%	2.6%	7.7%	7.7%				
9	0	1	1	3	3	5	1	0	4	15	1	2	0	1		
<i>N</i> = 34		2.9%	2.9%	8.8%	8.8%	14.7%	2.9%		11.8%	44.1%	2.9%	5.9%		2.9%		
10	0	1	0	3	3	0	0	1	1	2	29	4	1	2		
<i>N</i> = 48		2.1%		6.3%	6.3%			2.1%	2.1%	4.2%	60.4%	8.3%	2.1%	4.2%		
11	0	1	0	3	1	0	0	0	3	3	8	12	2	1		
<i>N</i> = 34		2.9%		8.8%	2.9%				8.8%	8.8%	23.5%	35.3%	5.9%	2.9%		
12	1	0	0	0	0	0	0	0	1	0	0	0	20	0		
<i>N</i> = 23	4.3%								4.3%				87.0%			
13	0	0	0	0	0	0	0	0	4	0	1	0	0	0		
<i>N</i> = 55									7.3%		1.8%			4.3%		
														90.9%		
<i>Hyla meridionalis</i>	14	0	3	0	0	0	3	0	1	2	0	0	0	0	11	
	<i>N</i> = 20		15.0%				15.0%		5.0%	10.0%					55.0%	

OTU, operational taxonomic unit.

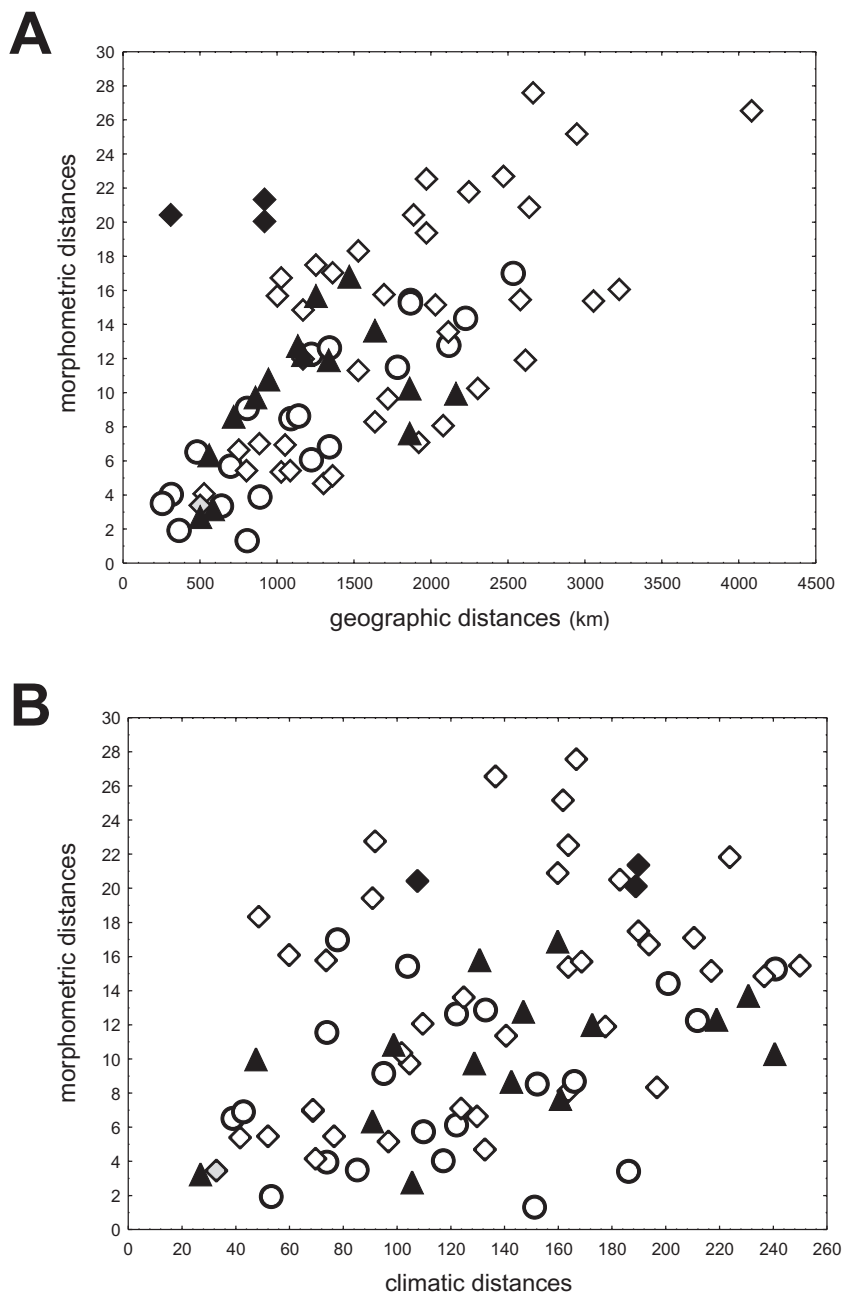


Figure 5. Morphometric distances among operational taxonomic units (OTUs) plotted against (A) geographic and (B) climatic distances. Triangles, distances within *Hyla arborea*; circles, distances within *Hyla savignyi*; rhomboids, distances between OTUs of *H. arborea* versus *H. savignyi* (black rhomboid, Caucasian *H. arborea* versus Transcaucasian, Iranian and Mesopotamian *H. savignyi*; grey rhomboid, Anatolian *H. arborea* versus Anatolian *H. savignyi*).

climatic factors (temperature and precipitation) even after statistical control of the covariation between climate and geography. It suggests that climatic conditions were an important factor in the formation of morphological variation. It appears that they lead to dissimilar morphotypes in populations living in areas with outermost climatic conditions, but to similar

body shape in populations of both species occupying areas with comparable climate.

Most remarkably for the present study, *H. arborea* and *H. savignyi* are more or less undistinguishable in body shape in southern Anatolia, but substantially differ in the Caucasus regions, despite their ranges are in contact in both areas. The Anatolian

populations thus appear to support the scenario of climate-driven geographic morphological variation, whereas the Caucasian populations appear to ostensibly contradict it. This apparent discrepancy could be explained by the very diverse patterns of coexistence of tree frogs in these two zones of parapatry. *H. arborea* and *H. savignyi* inhabit similar habitats on the southern coast of Anatolia (Schneider, 2001; V. Gvoždík, unpubl. data), and they hence face comparable environmental conditions there. On the other hand, the high morphological distance between the populations of *H. savignyi* from Transcaucasia and *H. arborea* from the Caucasus area (but short geographic distance, approximately 300 km in average; Fig. 5A) could reflect substantial ecological vicariance of both species in this second zone of parapatry. In accordance with what we can infer from their ecophysiological differences (*H. savignyi* possess on average 1 °C higher tissue thermal resistance; Egiasarian & Andronnikov, 1986), in the Caucasus, *H. arborea* inhabits relatively humid and cold localities generally placed in the higher elevations, whereas *H. savignyi* prefers drier and warmer places (Aleksperov, 1978; Egiasarian & Schneider, 1990, 1991; Kuzmin, 1999; Tarkhnishvili & Gokhelasvili, 1999). We can conclude that the two species of tree frogs occupy fairly different environmental space in the Caucasian area, and their morphological divergence thus supports, rather than denies, the hypothesis of climate-driven morphological variation.

The results obtained by Kaya (2001) on morphometric differences between the populations of *H. arborea* from the northeastern part of Turkey and the other Anatolian populations of both *H. arborea* and *H. savignyi* fit the hypothesis as well. Environmental conditions of north-eastern Turkey (Ponto-Caspian zoogeographic area) are different from those in southern Anatolia (Sindaco *et al.*, 2000). Tree frogs from the north-eastern part of Turkey used to be assigned as the Caucasian subspecies *H. a. schelkownikowi* (Kuzmin, 1999). This taxon is extensively distant from the western Anatolian populations of *H. arborea* in body shape also in our analyses.

Thus, only the morphometric sovereignty of Kurdistanian-Iranian (OTU 2) and Transcaucasian (OTU 7) populations of *H. savignyi* and their mutual similarity somewhat contradict the climate-provoked phenotypic variation in the tree frogs. The Iranian tree frogs occupy mostly highlands, the Transcaucasian populations are rather a lowland form. But we shall notice that, in contrast to the OTU 7, the OTU 2 has been composed relatively unnaturally. OTU 2 covers the extensive area with both high and low elevations. Almost half of the individuals are from the southern foot of the Zagros Mountains in Khuzestan

Province from low elevations of approximately 300 m a.s.l. (Fig. 1; see also Appendix).

We ascribed the morphological similarity of southern Anatolian parapatric populations of *H. arborea* and *H. savignyi* to an equivalent response of both species to a shared environment. Alternatively, the similarity between species near the contact of their ranges could be explained by interspecific hybridization and subsequent introgression of morphotype-mediated genes (Grant & Grant, 2002). Nevertheless, this explanation is not likely to be applicable to *H. arborea* and *H. savignyi*. These species appear to be isolated by a strong reproductive barrier as a result of substantial differences in the species-specific advertisement call and, to our knowledge, no natural hybridization between them has ever been demonstrated despite several studies performed on tree frogs in this area. Molecular data, both mitochondrial and nuclear markers, further support the non-existence or maximal rarity of hybridization between *H. arborea* and *H. savignyi* near the Anatolian zone of parapatry. All the Anatolian specimens of either species determined by the characteristic colour pattern are well-nested within their respective species-specific molecular clade (V. Gvoždík J. Moravec, P. Kotlík, unpubl. data). Also, the hybridization hypothesis, as opposed to equivalent response to environmental conditions, cannot explain the morphological similarity of tree frogs of both species in the isolated populations from the Mediterranean area (Crete, Cyprus), or in the other populations with similar climate distant from the actual zone of parapatry (the Mediterranean coast of Levant).

Although the correlation between body shape and climatic variables among OTUs of *H. arborea* and *H. savignyi* indicates an important role of climatic conditions in the incitement of morphological variation in these taxa, many interpretations of our results suffer from the obvious problems of the correlational framework. Future experimental work (e.g. common environment or reciprocal transplant experiments in conjunction with quantitative genetics) may identify the evolutionary and ecological processes responsible for the observed matching between climate and morphology in examined tree frogs. Such experimental approaches could decide whether similar morphotypes in Mediterranean populations of *H. arborea* and *H. savignyi* living in more or less the same environmental conditions are caused by convergent evolution or shared phenotypically plastic response to environmental conditions. Nevertheless, the case of two species of tree frogs described in the present study suggests that climate-provoked morphological variation in closely-related parapatric species should be taken into account as a potential process complicating character displacement in morphology. On the other

hand, morphological diversification between closely related species or their populations could be enhanced by habitat shifts resulting in the occupation of different environmental space as in the Caucasian and Transcaucasian tree frogs. Recent knowledge (Streelman & Danley, 2003) indicates that habitat shifts regularly precede morphological diversification during evolutionary events encompassing character displacements in morphology and adaptive radiations. Further studies should investigate how frequently the climate-driven variation can serve as an obstacle to morphological differentiation between close relatives and, conversely, how often the shifts in habitat are connected to changes in environmental space, which could potentially directly drive morphological differentiation and, consequently, facilitate coexistence after secondary contact.

Only after knowing the extent of the contribution of phenotypic plasticity versus genetic evolution to the variation in body shape among particular OTUs, and after estimation of the rate of morphological evolution driven by climatic conditions, will we be able to judge, whether our phenetic trees contain also certain information on the biogeography of the populations within the studied species. Because the morphology in anurans is particularly well-known to be highly sensitive to environmental conditions (Emerson, 1986; Emerson, Travis & Blouin, 1988; Blouin & Loeb, 1991; Castellano & Giacoma, 1998; Blaustein *et al.*, 1999; Castellano, Giacoma & Dujsebayaeva, 2000; Rosso *et al.*, 2004; Schäuble, 2004; Kutrup *et al.*, 2006; Loughheed *et al.*, 2006; Lind & Johansson, 2007), the biogeographic signal in morphometric data in tree frogs can be completely concealed by the plastic response to environmental conditions or rapid climate-provoked evolutionary changes. Several lines of indirect evidence suggest that it is indeed the case. First, as we have documented, some populations of the Middle Eastern and south-eastern European tree frogs belonging to different species have probably been genetically isolated for a few millions years, but are almost uniform in body shape. *Hyla savignyi* has been specified as an Irano-Turanian zoogeographical element with later Mediterranean penetration (Bodenheimer, 1944), whereas *H. arborea* occurs across almost whole Europe from west to east, with Anatolian populations on the eastern distributional margin. Accordingly, the populations of both species in southern Anatolia are probably younger than the populations in the middle of specific ranges, but possess a very similar body shape. Their body shape is also similar to the Canarian population of *H. meridionalis*, which is bioacoustically and genetically more distant (Schneider, 1974, 2004; Smith *et al.*, 2005). Next, the colonization of the Arabian Peninsula by *H. savignyi* is probably more recent than 5 Mya

because, until this period Arabia was connected to Eastern Africa (Braithwaite, 1987) where tree frogs or their fossils are completely absent (Duellman, 1977; Sanchiz, 1998). The isolation of the Arabian population restricted to the Asir Mountains in south-western Arabia started approximately only 5000–6000 years ago, when a period of aridization began in the Middle East (Klüttsch *et al.*, 2004). A rather recent isolation is supported by low immunological divergence between the Saudi Arabian and Israeli tree frogs and no immunological distinction between Yemeni and Israeli populations of *H. savignyi* (Riehl *et al.*, 1995). The substantial morphological distinction of the Arabian population thus presumably mirrors the extraordinary environmental conditions of this climatically extreme part of the species area.

The independence of genetic and morphological variation is relatively well known in frogs. In some cases, morphology is 'conserved' whereas genetic evolution goes forward (Borkin *et al.*, 2004; Camargo, de Sá & Heyer, 2006). For example, in the Far East, *Hyla japonica* Günther, [1859] '1858' and *Hyla suweonensis* Kuramoto, 1980 are morphologically very similar but genetically distant (Kuramoto, 1980; Lee *et al.*, 1999). In other cases, a significant geographic morphological variation exists but does not match genetic relationships among populations. For example, Nevo & Yang (1979) demonstrated the independence of genetic and morphological variation in Israeli populations of *H. savignyi* and, likewise, Kyriakopoulou-Sklavounou, Karakousis & Alexiou (1992) and Kyriakopoulou-Sklavounou (2000) demonstrated the same in Greek populations of *H. arborea*. Thus, within the species complex of the tree frogs, it is impossible to put definitive taxonomic implications and biogeographic scenarios based solely on the external morphology. An investigation of variation of bioacoustic parameters and mainly molecular markers using phylogeographic methods will be necessary to uncover taxonomy and phylogenetic relationships of the tree frogs' population systems.

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APPENDIX

MATERIAL EXAMINED

Hyla savignyi: OTU 1 – ARABIAN PENINSULA:
Yemen: 1. 130 km S of Sana'a (14°13'N; 44°16'E) – 1 ex. (ZFMK 32272); 2. 31 km from Sana'a in direction to Hodeida (15°11'N; 43°59'E) – 4 ex. (ZFMK 42847-42849, 42852); 3. Sana'a (15°21'N; 44°12'E) – 4 ex. (ZMH A4130-A4131, ZFMK 37039-37040); 4. Shibam (15°31'N; 43°54'E) – 1 ex. (ZFMK 43108); **Saudi**

ex. (NMP P6V 34732/1-4); **Turkey:** 41. Kilis (36°43'N; 37°07'E) – 2 ex. (NMP P6V 70775/1-2); 42. Sendschirli (= Zencirli) (37°08'N; 36°40'E) – 7 ex. (ZMB 11034, 14464, 22487, 63385-63388). **OTU 5 – CYPRUS:** 43. Larnaca (34°55'N; 33°38'E) – 2 ex. (MTKD D18594, D18597); 44. Lefka (35°07'N; 32°51'E) – 2 ex. (MHNG 1393.81-82); 45. Famagusta (35°08'N; 33°57'E) – 27 ex. (NMP P6V 71570/1-16, 72539/1-2, ZFMK 14399-14407); 46. Sımrüstü (35°16'N; 33°51'E) – 4 ex. (NMP P6V 71571/1-4); 47. Yalı (35°24'N; 33°45'E) – 14 ex. (NMP P6V 71572/1-7, 71572/9-15); 'Cyprus' – 16 ex. (ZMB 11698, 18142, 63389-63392, 63394-63396, 63398-63402, ZMH A04132-A04133). **OTU 6 – SOUTHERN ANATOLIA:** **Turkey:** 48. Anamur (36°05'N; 32°50'E) – 20 ex. (NMP P6V 71573/1-3, 71574/1-9, 72537/1-8); 49. Bozyazı (36°06'N; 32°58'E) – 1 ex. (NMP P6V 72538); 50. Kurtuluş (36°20'N; 34°00'E) – 1 ex. (NMP P6V 71575/1); 51. Lamaskalesi (36°34'N; 34°15'E) – 9 ex. (NMP P6V 70795/1-9); 52. Fundukbunar (= Findikpinari), Taurus Mts. (36°55'N; 34°22'E) – 1 ex. (ZMB 32227); 53. Mersin (36°44'N; 34°39'E) – 2 ex. (ZMB 21265, MTKD D25225); 54. Kazanlı (36°50'N; 34°45'E) – 3 ex. (NMP P6V 71576/1-3); 55. Karataş (36°34'N; 35°23'E) – 6 ex. (NMP P6V 70772/1-3, 70772/6, 70774/1-2); 56. Adana (37°01'N; 35°20'E) – 4 ex. (NMP P6V 70776/1-4); 57. between Adana and Ceyhan (36°57'N; 35°36'E) – 1 ex. (ZFMK 7703); 58. Yılanlikale (37°04'N; 35°44'E) – 1 ex. (ZFMK 48062); 59. Karatepe (37°17'N; 36°13'E) – 1 ex. (ZMH A03038); 60. Osmaniye (37°04'N; 36°15'E) – 2 ex. (NMW 18536:3-4); 61. Dörtöyl (36°51'N; 36°13'E) – 2 ex. (CAS 105299-105300); 62. Sariseki (36°40'N; 36°13'E) – 5 ex. (CAS 105307-105308, 105317-105319); 63. Kahramanmaraş (37°35'N; 36°56'E) – 2 ex. (NMW 18537:14, 18537:33). **OTU 7 – TRANSCAUCASIA:** **Azerbaijan:** 64. Mingaçevir (40°46'N; 47°03'E) – 12 ex. (NMP P6V 70773/1-6, 70777/1-2, 70778/1-2, 70792/1-2). **Not assigned to any OTU:** **Turkey:** 65. Kemaliye (39°16'N; 38°29'E) – 1 ex. (ZMH A03021); 'Turkey' – 1 ex. (MTKD D33909).

Hyla arborea: **OTU 8 – CAUCASUS REGION** (*H. arborea schelkownikowi*): **Armenia:** 66. Kuybyshev, Stepanavan (**topotypes**) (41°01'N; 44°17'E) – 23 ex. (MTKD D12058-D12067, D12070, D12073-D12074, D12267-D12269, D12272, NMW 24779:1-3, ZFMK 18721-18723); **Azerbaijan:** 67. Nukadi, Kara-Çaj River (41°19'N; 48°35'E) – 3 ex. (ZFMK 39104); **Georgia:** 68. Akhaldaba (41°55'N; 43°29'E) – 2 ex. (ZFMK 70385-70386); 69. Pizunda (43°09'N; 40°21'E) – 1 ex. (ZFMK 38378); **Russia:** 70. Malenkij Sakhray River, Republic of Adygeya (44°02'N; 40°18'E) – 2 ex. (ZMB 57374, 57379); 71. Pjatigorsk (44°03'N; 43°04'E) – 1 ex. (ZMB 18960); 72. Groznyj (43°19'N; 45°42'E) – 3 ex. (NMW 14868:1-3);

Turkey: 73. Pazar (41°11'N; 40°53'E) – 4 ex. (CAS 105577-105580). **OTU 9 – WESTERN ANATOLIA AND ADJACENT ISLANDS:** **Turkey:** 74. Gazipaşa (36°16'N; 32°19'E) – 4 ex. (NMP P6V 72536/1-4); 75. Syedra, 15 km SE of Alanya (36°29'N; 32°07'E) – 2 ex. (NMP P6V 72535/1-2); 76. Beyşehir (37°41'N; 31°44'E) – 2 ex. (NMP P6V 33320/1, 33320/3); 77. Mahmatlar (37°56'N; 30°56'E) – 1 ex. (NMP P6V 70793); 78. Kütahya (39°25'N; 29°59'E) – 1 ex. (MHNG 908.34); 79. Bilecik (40°09'N; 29°59'E) – 2 ex. (NMP P6V 70789/1-2); 80. Nicaea Lake, Iznik (40°26'N; 29°43'E) – 2 ex. (ZMH A03029, A03030); 81. 10 km SW of Ağva (41°06'N; 29°48'E) – 1 ex. (ZFMK 56746); 82. Troy, Bursa (39°56'N; 26°16'E) – 1 ex. (ZFMK 16331); 83. Selçuk – Ephesos (37°57'N; 27°22'E) – 8 ex. (NMP P6V 71098/1-4, 72534/1-3, ZFMK 56677); **Greece:** 84. Chios Is. (38°22'N; 26°08'E) – 8 ex. (NMP P6V 70794/1-8); 85. Maritsa, Rhodes Is. (36°26'N; 28°13'E) – 2 ex. (NHMC 80.2.7.2, 80.2.7.3). **OTU 10 – CRETE** (*H. arborea kretensis*, **syntypes in bold**): 86. Chania (35°31'N; 24°01'E) – 7 ex. (NMW 18413:1-4, ZMB 31575, 63407, NHMC 80.2.7.4); 87. Almiros (35°27'N; 24°12'E) – 1 ex. (NMP P6V 34173); 88. Skopelos, Panormos (35°25'N; 24°42'E) – 1 ex. (ZFMK 61476); 89. Kapetaniana, Asterousia Mts. (34°58'N; 25°02'E) – 1 ex. (NMW 5833:5); 90. Lasithi Plateau (35°11'N; 25°36'E) – 11 ex. (NHMC 80.2.7.5-80.2.7.8, 80.2.7.12, ZFMK 18697-18700, NMP P6V 70780/2, NMW 18413:5); 'Crete' – 27 ex. (ZMB 31569, 63408-63430, 63432-63434). **OTU 11 – BALKANS:** **Greece:** 91. 8 km S of Monemvasia, Peloponnese (36°41'N; 23°03'E) – 1 ex. (ZFMK 40803); 92. 7 km E of Kalon Neron, Peloponnese (37°17'N; 21°42'E) – 3 ex. (ZMB 49823-49824, MTKD D33150); 93. Gortys, Peloponnese (37°33'N; 22°03'E) – 1 ex. (NMW 29159:5); 94. Argos, Peloponnese (37°38'N; 22°44'E) – 2 ex. (NMP P6V 70796/1-2); 95. Athens (37°59'N; 23°44'E) – 1 ex. (ZMUC R14500); 96. between Arta and Salaora (39°05'N; 20°55'E) – 1 ex. (MHNG 1010.65); 97. Platamon (40°02'N; 22°28'E) – 3 ex. (ZFMK 37703, ZMB 49825-49826); 98. SE of Zakynthos Is. (37°47'N; 20°54'E) – 1 ex. (ZFMK 27186); 99. Karystos, Evia Is. (38°01'N; 24°25'E) – 1 ex. (ZMB 13511); 100. Levkas, Levkas Is. (38°50'N; 20°42'E) – 3 ex. (MHNG 1186.53-55); 101. Skiathos Is. (39°10'N; 23°29'E) – 1 ex. (ZFMK 24082); 102. Corfu Is. (39°37'N; 19°53'E) – 2 ex. (ZFMK 24081, ZMB 32332); **Albania:** 103. Durrës (41°19'N; 19°27'E) – 1 ex. (MTKD D3131); **Serbia:** 104. Vranje (42°33'N; 21°54'E) – 1 ex. (ZMUC R14544); **Bulgaria:** 105. Dolno Spachevo (41°25'N; 23°23'E) – 1 ex. (NMP P6V 34163); 106. Primorsko (42°16'N; 27°46'E) – 3 ex. (NMP P6V 7669, 70771/1-2); **Turkey:** 107. Havsa, Edirne (41°33'N; 26°49'E) – 9 ex. (NMP P6V 72533/1-9). **OTU 12 – DANUBE DELTA:** **Romania:** 108. Tuldscha (= Dulcea)

(45°10'N; 28°48'E) – 1 ex. (NMW 5805:13); 109. Periplava (45°24'N; 29°32'E) – 1 ex. (NMP P6V 70783/1); 110. Caraorman (45°05'N; 29°24'E) – 21 ex. (NMP P6V 70784/1-20, 70781/4). **OTU 13 – CENTRAL EUROPE: Czech Republic:** 111. Kašperské Hory (49°09'N; 13°34'E) – 1 ex. (NMP P6V 70813); 112. Černá v Pošumaví (48°44'N; 14°07'E) – 1 ex. (NMP P6V 70820); 113. Veselí nad Lužnicí (49°11'N; 14°42'E) – 19 ex. (NMP P6V 70785/1-2, 70806, 70808, 70812/1-15); 114. Stráž nad Nežárkou (49°04'N; 14°54'E) – 2 ex. (NMP P6V 70804/1-2); 115. Praha (50°04'N; 14°24'E) – 2 ex. (NMP P6V 70807/1-2); 116. Říčany (49°59'N; 14°39'E) – 8 ex. (NMP P6V 33703/1-8); 117. Nymburk (50°11'N; 15°02'E) – 1 ex. (NMP P6V 33001); 118. Bítov (48°57'N; 15°43'E) – 2 ex. (NMP P6V 70786/1-2); 119. Třebíč (49°13'N; 15°53'E) – 2 ex. (NMP P6V 70816/1-2); 120. Ořešín, Brno (49°17'N; 16°36'E) – 1 ex. (NMP P6V 71523); **Slovakia:** 121. Prievidza (48°46'N; 18°38'E) – 1 ex. (NMP P6V 70814); 122. Kováčovské kopce (47°50'N; 18°44'E) – 1 ex. (NMP P6V 32457); **Ukraine:** 123. Uzhgorod (48°37'N; 22°18'E) – 5 ex. (NMP P6V 70805/1-5); 124. Mukachevo (48°27'N; 22°43'E) – 7 ex. (NMP P6V 70811/1-4, 6-8); 125. Irshava (48°19'N; 23°03'E) – 2 ex. (NMP P6V 70810/1-2). **Not assigned**

to any OTU: Croatia: 126. Salona (43°33'N; 16°30'E) – 1 ex. (ZMB 22673); 127. Sinj (43°42'N; 16°38'E) – 1 ex. (NMP P6V 70823); 'Croatia' – 1 ex. (ZMB 23729); **Serbia:** 128. Morovic (45°00'N; 19°13'E) – 1 ex. (ZMUC R14545).

***Hyla meridionalis*: OTU 14 – CANARY ISLANDS:** Tenerife Is. (28°22'N; 16°43'W) – 20 ex. (NMW 5822, 5825:1-2, 5852:1-2, 5857:3, 5858:2, 5861:2, 5869:2, 5871:1-4, 5873:2, 5874:1-2, 5877:1-2, 5880:1-2).

Museum abbreviations: CAS, California Academy of Sciences, San Francisco, CA, USA; MHNG, Muséum d'Histoire Naturelle Genève, Switzerland; MTKD, Museum für Tierkunde Dresden, Germany; MZLU, Museum of Zoology Lund, Sweden; NHMC, Natural History Museum of Crete, Irakleio, Greece; NMP, National Museum Prague, Czech Republic; NMW, Naturhistorisches Museum Wien, Austria; ZFMK, Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany; ZMB, Museum für Naturkunde Berlin, Germany; ZMH, Zoologisches Institut und Zoologisches Museum der Universität Hamburg, Germany; ZMUC, Zoological Museum, Copenhagen, Denmark.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Representatives of three categories according to the types of inguinal colour pattern.

Figure S2. Distribution of three categories of inguinal colour patterns in *Hyla savignyi* and *Hyla arborea*.

Table S1. Average monthly temperature and monthly precipitation values for each single operational taxonomic unit of *Hyla savignyi* and *Hyla arborea*.

Table S2. Descriptive statistics of characters examined.

Table S3. Discriminant coefficients of classification functions.

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SUPPLEMENTARY MATERIAL

Gvoždík V, Moravec J, Kratochvíl L. Geographic morphological variation in parapatric Western Palearctic tree frogs, *Hyla arborea* and *Hyla savignyi*: Are related species similarly affected by climatic conditions? *Biological Journal of the Linnean Society*.

Figure S1. Representatives of three categories according to the types of inguinal colour pattern.

Figure S2. Distribution of three categories of inguinal colour patterns in *H. savignyi* and *H. arborea*.

Table S1. Average monthly temperature and monthly precipitation values for each single OTU of *H. savignyi* and *H. arborea*.

Table S2. Descriptive statistics of characters examined.

Table S3. Discriminant coefficients of classification functions.

Figure S1. Representatives of three categories according to the types of inguinal colour pattern: (1) *linea marginalis* continuously builds a regular inguinal loop, (2) spot(s) instead of the loop, or a thin loop is separated from *linea marginalis*, (3) loop or spot(s) are entirely absent. Photo: Václav Gvoždík

(1) *H. arborea*, Selçuk, western Anatolia, OTU 9

(2) *H. savignyi*, Anamur, southern Anatolia, OTU 6

(3) *H. savignyi*, Anamur, southern Anatolia, OTU 6



Figure S2. Distribution of three categories of inguinal colour patterns in *H. savignyi* and *H. arborea*. See text for more details.

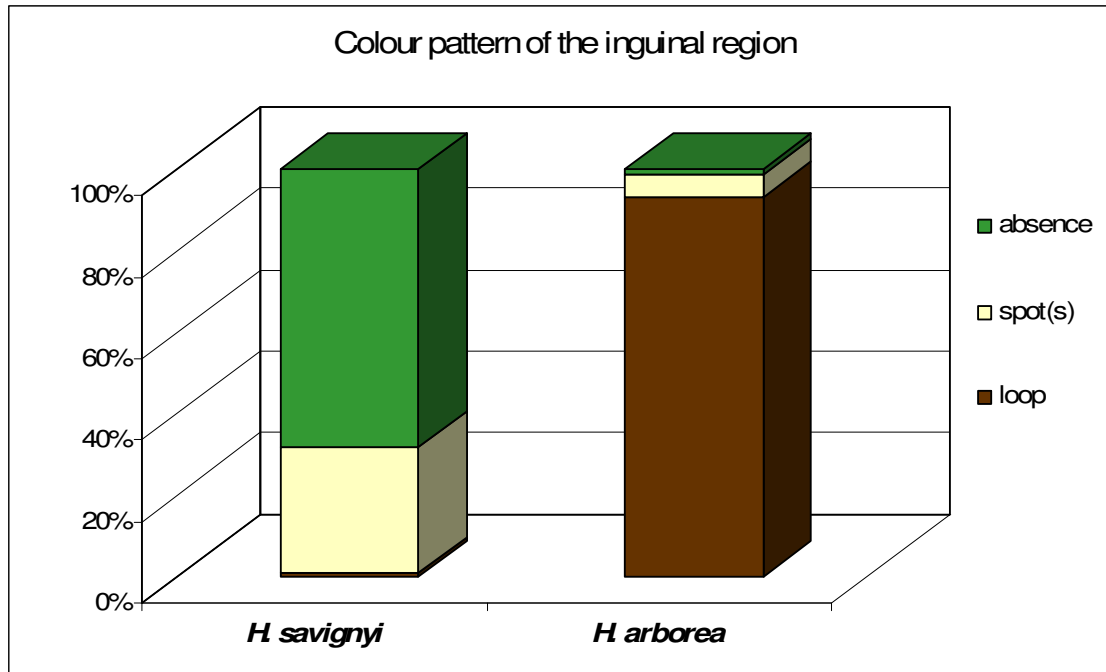


Table S1. Average monthly temperature and monthly precipitation values for each single OTU of *H. savignyi* and *H. arborea*, calculated as weighted means for each single OTU in respect to the number of individuals from each locality. These values were used for calculation of a climatic distance matrix.

OTU		<i>Hyla savignyi</i>							<i>Hyla arborea</i>					
		1	2	3	4	5	6	7	8	9	10	11	12	13
temperature (°C)	January	14.5	5.2	8.1	9.2	11.3	8.6	3.1	-6.2	6.6	8.5	5.6	-0.1	-2.6
	February	16.1	7.1	10.1	10.3	11.6	9.5	4.4	-5.0	7.3	8.6	6.7	0.9	-0.6
	March	18.7	11.4	14.0	12.8	13.5	12.2	8.4	0.0	9.5	9.8	8.9	4.3	3.5
	April	20.2	16.8	19.3	16.4	17.0	15.9	15.0	7.0	13.3	12.8	12.5	10.3	8.5
	May	22.9	22.6	25.0	20.0	20.8	19.7	19.8	11.6	17.3	16.4	17.3	16.0	13.4
	June	25.2	27.4	29.7	23.4	24.8	23.7	24.0	15.4	21.4	20.4	21.3	20.2	16.5
	July	25.8	30.4	32.5	26.0	27.4	26.6	27.3	19.1	23.9	22.5	23.7	22.1	18.0
	August	25.4	29.7	31.8	26.4	27.4	26.8	26.1	18.8	23.7	22.4	23.5	21.8	17.5
	September	23.9	25.9	28.1	24.7	25.2	24.3	22.3	14.7	20.8	20.1	20.5	18.1	13.9
	October	19.8	19.9	22.0	20.7	21.5	19.9	15.8	8.3	16.3	16.6	15.7	12.8	8.8
	November	16.9	13.1	14.8	15.4	16.9	14.7	10.3	2.7	12.0	13.3	11.2	7.4	3.4
	December	14.8	7.4	9.6	11.0	13.0	10.4	5.4	-3.0	8.5	10.3	7.4	2.7	-0.7
precipitation (mm month ⁻¹)	January	3.4	64.6	36.5	137.8	67.7	120.4	16.6	39.9	109.2	166.5	81.2	19.5	33.0
	February	3.2	49.8	30.2	97.7	52.0	97.1	22.2	39.4	85.9	116.2	68.6	20.1	29.7
	March	16.6	48.9	30.0	83.9	37.9	72.3	26.2	47.6	66.6	94.0	59.0	15.9	33.7
	April	24.7	42.2	19.1	41.8	15.3	42.3	37.5	64.3	39.8	35.8	45.0	20.8	41.5
	May	19.0	18.0	6.9	17.5	8.5	26.6	51.5	93.5	25.8	14.4	35.7	28.0	65.8
	June	5.6	3.2	0.3	1.7	0.9	8.7	45.0	93.8	12.2	1.7	23.9	33.5	80.7
	July	19.2	0.9	0.0	0.2	0.0	2.5	21.8	63.7	5.4	0.1	13.2	26.6	70.9
	August	21.8	0.4	0.0	0.4	0.0	2.2	20.8	52.6	4.9	0.0	13.6	24.0	69.0
	September	10.2	0.7	0.1	2.4	0.6	6.1	18.5	50.1	10.5	4.5	25.8	25.3	43.8
	October	3.0	13.0	5.9	42.4	16.3	45.9	46.1	58.5	39.6	72.7	63.4	15.7	30.8
	November	7.3	27.3	17.0	75.6	35.7	74.9	24.6	49.2	71.9	80.2	91.7	22.1	40.4
	December	3.5	58.2	35.7	135.6	79.1	131.1	18.7	44.8	122.3	137.0	101.2	26.2	38.5

Table S2. Descriptive statistics of characters examined.

Loop = qualitative character, see text or Fig. S1 for code explanation; other characters are measurements in mm; mean ± SD / min. - max.; asterisk denotes bilateral characters measured on both sides and averaged.

<i>H y l a s a v i g n y i</i>															
OTU	1 - Arabian Peninsula			2 - Iran and Kurdistan		3 - Mesopotamia		4 - Levant		5 - Cyprus		6 - southern Anatolia		7 - Transcaucasia	
	males (n=3)	females (n=7)	undetermined (n=17)	males (n=30)	females (n=10)	males (n=16)	females (n=4)	males (n=49)	females (n=25)	males (n=47)	females (n=17)	males (n=47)	females (n=13)	males (n=3)	females (n=9)
Loop	2,3	2,3	2,4	3	3	2,7	2,8	2,6	2,4	2,7	2,8	2,6	2,5	3	3
SUL	36,2±1.4	41,5±2.4	37,6±2.4	36,8±3.0	40,7±3.0	37,9±3.6	41,8±5.1	37,9±2.5	41,7±3.3	33,5±2.0	36,5±2.4	37,4±2.8	40,1±3.2	37,2±3.6	40,3±2.9
FmL*	16,5±0.7	19,9±1.1	17,8±1.3	17,7±1.8	19,0±2.4	18,4±2.0	20,2±2.5	18,7±1.4	20,5±1.8	16,1±1.1	17,9±1.5	18,4±1.5	19,4±1.8	18,1±1.7	19,6±1.7
TbL*	15,8±1.2	18,1±2.1	15,8±2.0	15,2±2.4	16,2±2.5	15,6±2.2	17,2±2.3	15,2±2.1	16,6±2.4	13,6±1.7	15,4±1.9	14,3±2.1	15,9±2.7	16,4±1.7	16,2±2.6
WL*	8,6±0.4	10,0±0.5	9,1±0.8	9,0±0.9	9,8±1.0	9,4±1.1	10,5±0.8	9,6±0.8	10,6±1.0	8,1±0.6	8,8±0.8	9,3±0.9	10,1±0.9	9,6±1.0	10,0±0.6
T4L*	14,5±0.6	17,2±0.9	15,6±1.2	15,0±1.6	16,3±1.9	16,3±1.9	17,8±1.3	16,4±1.4	18,2±1.6	14,2±1.0	15,6±1.2	16,0±1.4	17,2±1.7	15,6±2.0	16,7±1.3
T1L*	3,9±0.1	4,8±0.2	4,5±0.4	4,0±0.5	4,4±0.6	4,5±0.5	4,8±0.2	4,4±0.4	4,8±0.5	3,8±0.3	4,1±0.4	4,3±0.4	4,7±0.4	4,4±0.4	4,7±0.4
IMTL*	1,8±0.2	2,0±0.3	1,8±0.1	1,8±0.2	2,0±0.2	1,9±0.3	2,1±0.2	1,8±0.2	1,9±0.2	1,6±0.1	1,8±0.2	1,8±0.2	1,9±0.2	1,8±0.2	2,0±0.3
TrL*	9,3±0.5	10,9±0.6	10,1±0.8	9,5±1.1	10,3±1.2	10,1±1.0	10,8±1.4	10,4±0.8	11,3±0.9	8,7±0.6	9,7±0.8	10,0±0.8	10,8±1.0	9,8±0.5	10,8±0.7
HW	12,9±0.4	15,1±0.9	13,4±0.9	12,7±1.4	13,6±1.3	12,9±1.4	14,3±1.7	13,0±1.0	14,2±1.1	11,4±0.7	12,5±1.1	12,6±1.1	13,6±1.1	13,2±1.1	14,1±1.1
HtL	10,6±0.5	12,2±0.6	11,0±0.7	10,4±0.8	11,3±0.9	10,6±1.2	11,8±1.5	10,5±0.7	11,4±0.9	9,5±0.5	10,2±0.7	10,3±0.9	11,2±0.9	10,7±1.4	11,3±0.7
ES	4,3±0.4	5,0±0.4	4,6±0.5	4,4±0.5	4,6±0.5	4,5±0.6	5,0±0.9	4,5±0.3	4,8±0.5	4,1±0.3	4,1±0.3	4,3±0.4	4,7±0.6	4,4±0.6	4,6±0.3
NL	3,2±0.2	3,7±0.3	3,2±0.3	2,9±0.2	3,2±0.3	3,0±0.3	3,2±0.3	2,9±0.3	3,2±0.3	2,7±0.2	3,0±0.2	2,9±0.2	3,1±0.3	3,0±0.4	3,2±0.3
IND	3,2±0.2	3,3±0.2	3,1±0.2	3,0±0.3	3,3±0.3	3,1±0.3	3,2±0.5	2,9±0.3	3,2±0.3	2,7±0.2	3,0±0.2	3,0±0.2	3,1±0.3	3,3±0.2	3,6±0.3
EAD	6,3±0.3	7,5±0.5	6,5±0.4	6,8±0.7	7,3±0.8	6,8±0.7	7,4±0.7	6,8±0.6	7,4±0.6	6,2±0.4	6,7±0.5	6,7±0.5	7,2±0.5	7,2±0.5	7,5±0.9
IOD	3,7±0.3	3,7±0.3	3,5±0.3	3,5±0.4	3,8±0.5	3,4±0.3	3,7±0.2	3,5±0.3	3,8±0.4	3,0±0.3	3,5±0.5	3,3±0.3	3,5±0.4	3,6±0.0	4,2±0.4
EPD	11,2±0.6	12,5±0.6	11,0±0.6	10,6±0.8	11,3±1.1	10,8±1.0	11,8±1.0	10,8±0.8	11,7±1.0	9,8±0.6	10,6±0.8	10,6±0.8	11,4±1.0	11,2±1.0	11,3±1.1
TD	2,4±0.3	2,6±0.3	2,4±0.2	2,3±0.2	2,4±0.2	2,5±0.4	2,7±0.5	2,3±0.2	2,6±0.3	2,0±0.2	2,2±0.2	2,3±0.2	2,6±0.3	2,4±0.2	2,7±0.2

<i>H y l a a r b o r e a</i>															
OTU	8 - Caucasus			9 - western Anatolia		10 - Crete		11 - Balkans		12 - Danube Delta		13 - Central Europe		14 - Tenerife Is.	
	males (n=31)	females (n=2)	undetermined (n=6)	males (n=25)	females (n=7)	males (n=33)	females (n=13)	males (n=18)	females (n=13)	males (n=21)	females (n=2)	males (n=41)	females (n=13)	males (n=20)	
Loop	1,1	1	1	1	1	1,1	1,2	1,1	1,2	1,1	2	1	1,1	-	
SUL	38,6±2.2	35,8±2.8	39,5±1.1	37,0±2.4	36,5±2.2	35,2±2.5	37,8±4.2	35,7±3.2	37,5±4.0	29,2±2.8	32,4±5.2	36,9±3.0	37,7±2.7	36,1±2.2	
FmL*	19,5±1.3	17,6±0.8	20,2±0.7	18,5±1.7	18,0±1.2	17,1±1.4	18,2±2.3	17,7±1.7	19,2±1.9	14,2±1.4	16,2±1.9	17,8±1.5	18,5±1.7	17,2±1.3	
TbL*	16,7±2.0	17,0±1.8	19,6±2.1	15,8±2.1	15,9±1.9	13,7±1.9	14,9±2.1	14,4±2.0	15,2±2.1	12,4±1.8	14,8±1.7	14,8±2.0	15,5±2.1	15,2±1.9	
WL*	10,3±0.8	9,5±0.3	10,6±0.5	9,3±1.1	9,0±0.8	9,1±0.9	9,7±1.5	9,4±1.2	10,0±1.1	7,3±0.9	8,6±1.4	10,1±0.9	10,4±0.9	9,1±0.7	
T4L*	15,0±2.0	15,3±1.6	17,1±1.8	13,4±1.9	13,9±1.7	12,4±1.7	13,5±2.1	12,2±1.8	13,3±1.9	11,0±1.7	13,3±1.9	13,4±2.0	15,1±1.9	13,1±1.8	
T1L*	4,7±0.3	3,8±0.1	4,9±0.2	4,4±0.4	4,3±0.3	3,9±0.4	4,2±0.7	4,2±0.5	4,5±0.5	3,4±0.4	3,8±0.6	4,5±0.4	4,8±0.4	4,1±0.4	
IMTL*	1,5±2.4	2,0±2.1	1,9±2.3	1,5±2.6	1,5±2.2	1,4±2.0	1,5±2.4	1,5±2.1	1,7±2.5	1,4±2.3	1,6±2.0	1,7±2.5	1,9±2.5	1,7±2.1	
TrL*	10,8±0.7	10,1±0.4	11,1±0.6	10,2±0.9	10,0±0.6	9,4±0.7	9,9±1.2	9,7±1.0	10,5±1.0	7,9±0.8	9,2±1.2	9,8±0.9	10,2±0.9	9,8±0.7	
HW	11,8±1.6	12,1±1.3	13,3±1.5	10,5±1.0	11,2±1.3	10,2±1.4	11,0±1.7	10,9±1.4	13,3±1.3	9,1±1.4	10,1±1.2	11,3±1.5	11,8±1.4	12,0±1.6	
HtL	10,3±0.6	9,9±0.4	10,1±0.6	10,0±0.7	10,0±0.6	9,7±0.7	10,0±0.9	9,8±1.0	10,2±1.0	8,4±0.8	8,7±0.8	10,1±0.6	10,3±0.8	10,2±0.5	
ES	4,4±0.3	4,1±0.3	4,5±0.4	4,4±0.4	4,4±0.5	4,2±0.3	4,0±0.4	4,2±0.4	4,2±0.2	3,8±0.3	3,4±0.2	4,6±0.3	4,3±0.5	4,3±0.2	
NL	2,6±3.6	3,1±3.1	2,7±3.4	2,7±3.6	2,4±3.3	2,3±3.3	2,7±3.6	2,7±3.6	2,6±3.5	2,4±3.4	2,8±3.0	2,8±3.8	3,1±3.8	2,7±3.2	
IND	3,3±0.2	3,1±0.1	3,4±0.2	3,1±0.3	2,9±0.4	2,8±0.2	2,9±0.4	2,9±0.2	3,1±0.2	2,7±0.2	2,9±0.1	3,4±0.2	3,3±0.2	3,0±0.1	
EAD	7,0±0.4	6,8±0.5	7,2±0.3	6,8±0.5	6,6±0.5	6,5±0.4	6,7±0.6	6,7±0.5	6,7±0.5	5,8±0.6	6,6±0.0	7,1±0.5	7,1±0.4	6,4±0.3	
IOD	3,1±4.6	3,6±3.8	3,8±4.0	2,9±4.1	3,0±3.7	2,6±4.4	2,8±4.6	2,7±4.4	2,6±3.9	2,4±3.9	3,4±3.7	2,8±4.4	3,0±3.7	2,9±3.8	
EPD	11,6±0.7	10,8±0.4	10,8±0.6	10,9±0.8	10,6±0.7	10,5±0.7	11,0±1.0	10,7±0.9	11,2±1.0	9,2±0.8	10,0±0.9	11,2±0.8	11,5±0.9	10,3±0.5	
TD	2,3±0.2	2,2±0.4	2,3±0.1	2,3±0.2	2,2±0.3	2,1±0.2	2,2±0.3	2,2±0.3	2,3±0.2	1,9±0.2	2,1±0.1	2,4±0.2	2,5±0.2	2,2±0.2	

Table S3. Discriminant coefficients of classification functions as summarized in the discriminant analysis. Highest and lowest values per character are given in bold. *H. m.* = *Hyla meridionalis*.

OTU	<i>Hyla savignyi</i>							<i>Hyla arborea</i>						<i>H. m.</i>
	1 p = 0.052	2 p = 0.073	3 p = 0.036	4 p = 0.140	5 p = 0.116	6 p = 0.109	7 p = 0.021	8 p = 0.070	9 p = 0.061	10 p = 0.086	11 p = 0.061	12 p = 0.041	13 p = 0.098	14 p = 0.036
SUL/S	1930.1	1976.3	1952.8	1944.6	1958.9	1958.4	1950.4	1907.2	1934.1	1946.6	1915.2	1886.2	1899.6	1960.3
FmL/S	133.2	157.9	132.6	155.7	163.9	164.1	154.4	218.8	164.8	154.4	186.3	162.2	172.8	91.7
TbL/S	776.7	800.6	815.9	776.9	783.5	796.3	804.8	664	781.2	765.1	757.7	709.3	658.3	830.7
WL/S	-136.8	-88.2	-121.9	-112.3	-142.6	-108.5	-91.8	-92.8	-132	-84.1	-90.8	-140.3	-75.5	-103.2
T4L/S	778.1	742.8	797.1	814.9	824.1	798	754.6	814.8	788.5	802.6	792.8	817.7	820.3	795.6
T1L/S	-176.8	-212.8	-196.2	-209.3	-199.4	-200.1	-190.5	-208.1	-196.5	-216.9	-206.9	-214.7	-202.1	-209.9
IMTL/S	-99.5	-87.1	-93.5	-107.9	-93.4	-94.5	-85	-90.3	-95.6	-96.3	-92.5	-108.5	-95.2	-78
TrL/S	-453.9	-502.9	-496.4	-475.3	-484.9	-478.5	-481.3	-442.3	-462	-475.6	-462.3	-451.4	-459.3	-456.3
HW/S	188.6	142.6	127.4	135.2	132.4	128.5	154.7	170.3	141	165.9	144.2	136.1	159	124.1
HLt/S	254.2	239.5	229	224.2	237.1	231.7	237.2	185.7	199.4	207.2	207.9	187	186	247.7
NL/S	-159.8	-199.5	-197.1	-200.4	-187.4	-195.9	-205.9	-202.8	-183.8	-177.9	-177.5	-172.5	-182.5	-186.6
IND/S	78.6	92.9	79.9	70.3	83.4	91	125.3	90	87.3	70.2	76.9	87	88.8	94.8
EAD/S	246.8	289.1	278.8	288.6	291.3	290.9	294.1	270.8	275.9	290.2	279.2	273.9	282.3	269
IOD/S	19.8	23.4	11.9	15.7	19.1	10.7	32	22.4	9.1	23.4	20.8	10.7	8.2	17.7
EPD/S	297.1	261.1	271.7	270.4	282.2	273.6	264.8	293.2	287.1	288	293.9	295	282.7	272.6
TD/S	-44.4	-56.4	-44.9	-54.2	-59.4	-46.3	-35.4	-65.9	-57.3	-58.5	-55.1	-55.3	-51.5	-51.3
Constant	-2512.3	-2585.7	-2571.2	-2612.2	-2608.8	-2596	-2501.2	-2510	-2543.8	-2587.3	-2525.5	-2435.3	-2424.1	-2549.6