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Discordance between ventral colour and mtDNA haplotype in the water frog *Rana* (*ridibunda*) *caralitana*, 1988 Arıkan

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Abstract. The water frog form *caralitana* was first described as a subspecies of *Rana ridibunda* by Arıkan (1988) from southwestern Turkey. Its orange ventral colour has been used as a diagnostic character since its description. After testing for a correlation between body size and ventral colour, we compared mtDNA and venter colour of adult specimens from 27 localities to assess the validity of this character for systematics of Anatolian water frogs. We mapped the distribution of each category and tested whether there is concordance between mtDNA haplotype and ventral colour of sampled individuals at the watershed level. Furthermore, we analyzed relationships between ventral colour and altitude. Size and ventral colouration were found to be significantly correlated. The distribution of orange ventral colour exhibited a complex clinal variation especially west of the Lake District where different coloured individuals are seen syntopically. In other regions, there were abrupt changes, presumably because of geographic barriers such as mountains. Our results indicate that although there is significant concordance between *caralitana*-specific mtDNA and orange venter colour, there are certain watersheds where the majority of sampled individuals exhibits discordance in mtDNA and ventral colouration. In all periphery regions, some degree of genetic introgression is indicated. These patterns clearly indicate gene flow between the *caralitana* lineage and non-*caralitana* lineages and is supported by lack of evidence for habitat-specific selection, the assignment of individuals from the same population into distinct clades, and the occurrence of an intermediate character between different forms in transition zones.

Keywords: character discordance, introgression, mtDNA, *Rana* (*ridibunda*) *caralitana*, Turkey, ventral colour.

Introduction

In amphibians, body colour is an important signal and physiological regulator in several mechanisms, such as aposematism, especially in the poison frog family (Dendrobatiade) (Cott, 1940; Summers, 2003), crypsis (Endler, 1978), thermoregulation (Duellman and Trueb, 1986; Garcia et al., 2003; Tattersall et al., 2006), and sexual selection for mate choice, a crucial selective factor leading to divergence in colouration between populations (Summers et al., 2003; Summers et al., 2007), especially between conspecific populations (Summers et al., 1999). Siddiqi et al. (2004) showed that visual signals coming from ventral body parts are very important for conspecific communication.

Water frogs are prolonged breeders (Wells, 1977) that rely not only on colour pattern but also other traits during mate choice. The role of visual signals, however, is not yet investigated. Females choose their possible mates using visual, olfactory, tactile and mating calls (Abt and Reyer, 1993; Roesli and Reyer, 2000). However, female choice for mates can be overridden due to high levels of male competition and sexual coercion at crowded breeding sites (Bergen et al., 1997). Therefore, species-specific and population-specific mating behaviour patterns (Well, 1977), various environmental factors and their complex interactions determine type and level of introgression among different species (Lamb and Avise, 1986). For example, Plötner et al. (2008) found that unidirectional introgression of mtDNA from *Rana lessonae* to *Rana ridibunda* occurs predominantly rather than the reverse situation because of the hybridogenetic mode of inheritance in *R. esculenta* system.

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The systematic status of Turkish water frogs has recently been reviewed (Jdeidi et al., 2000; Plötner and Ohst, 2001; Akın, 2007) and the presence of several forms was suggested. Among these forms, *Rana* (*ridibunda*) *caralitana* is one of the most distinct. It was first described as a new subspecies because of the orange colour maculation on the white ventral part of frogs in Beyşehir Lake (Konya) (Arıkan, 1988). In later studies, the systematic status of this form has been subjected to many debates based on different methods. Karyologically, Alpagut and Falakalı (1995, 2006) showed that one Lake District population (of the *caralitana* form) is different from one Aegean population (the non-*caralitana* form) in the location of centromers, the presence of secondary constriction on chromosome in mitosis, and the number of rod-shaped bivalents on meiosis. Jdeidi (2000) showed that morphologically, apart from ventral colour, the *caralitana* form was clearly larger than the other forms in almost all external characters and that some body proportions (first toe and inner metatarsal tubercle size vs. overall body size) were different (Jdeidi et al., 2001); for allozymes, the *caralitana* group had higher genetic variation than the others (Jdeidi et al., 1998), and for bioacoustic parameters *caralitana* populations differed prominently in mating call structure, characterized by long means of pulse group duration, call duration and call period (Jdeidi, 2000). Plötner and Ohst (2001) indicated the presence of the *caralitana* specific mtDNA haplotypes. Therefore, Jdeidi (2000), Plötner and Ohst (2001), and Jdeidi et al. (2001) considered the *caralitana* form a distinct group and suggested raising this form to species status. In contrast, Sinsch et al. (2002) suggested on the basis of morphometric data that Anatolian Lake District population should be designated as *Rana bedriagae caralitana*. In all cases, its orange spotted venter has been used as a diagnostic character for the identification of the *caralitana* form in the field. However, up to now it has been not tested whether a degree of consistency exists between ventral colour and any other character.

This study evaluates the level of concordance between orange spotted venter and *caralitana*-specific mtDNA haplotypes and explores the reliability of ventral colour as a diagnostic character. Firstly, we tested whether there is any association between the ventral colour and age of frogs or not. Secondly, we measured the level of concordance between ventral colour and haplotype. Lastly, we examined the relationship between ventral colour and altitude.

Materials and methods

Study area and sampling

The study area is focused on the Turkish Lake District, which basically encompasses the region surrounding the Beyşehir Lake (Konya), the terra typica of *Rana ridibunda caralitana*. During 2004-2006, we collected a total of 183 tissue samples in the form of toe clips from 27 different localities (fig. 1), from the provinces Konya, Karaman, Burdur, Antalya, Muğla, Denizli, Uşak, and Afyon as listed in Appendix 1. Sampling was mainly carried out along three transects radiating from Beyşehir Lake toward east, northwest, and south, respectively (Akın, 2007). Specimens were caught from different types of habitats such as streams, rivers, lakes, ponds, reservoirs, marshy areas, and irrigation canals. Tissue samples were preserved in 80% ethanol for molecular studies. The ventral colour of each specimen was recorded, photographed, and the animal released back into its natural habitat. Lastly, for each locality, geographic coordinates were recorded to construct maps, drawn using ArcMap software (http://www.esri.com/) and MapInfo (http://www.mapinfo.com/).

Ventral colour pattern analysis

To determine the extent of morphological variation and correlation between morphological and molecular parameters, a single qualitative character, the pattern of the ventral colour of specimens, was used. Therefore, the pattern of ventral colour was scored for each specimen as one of three classes during field studies based on the amount of orange, orange-brown, or black-brown spots, and later checked also with the photographs. Three classes were defined:

Class 1: consists of individuals whose ventral parts are either white without spots coded as 1 or white with black-brown spots, coded as 2 (Photo 1/Photo 2 shown in fig. 3).

Class 2: includes individuals having white with orange coloured maculations on their ventral parts, coded as 3 (Photo 3 shown in fig. 3).

Class 3: contains individuals having white with orange-brown colour spots on their venter, coded as 4 (Photo 4 shown in fig. 3).

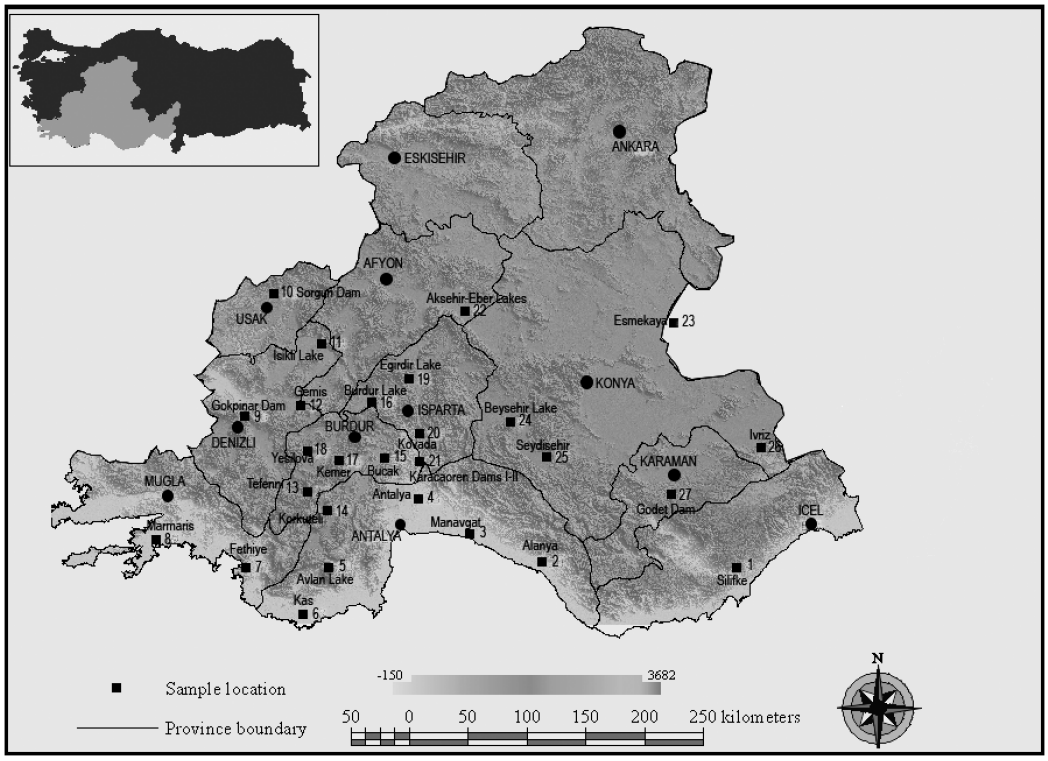

Figure 1. Map of study area with sample localities referred by numbers indicated in Table 1.

All individuals were grouped into adult ($n = 60$) or juvenile ($n = 123$) classes according to SVL (Snout-Vent Length), where SVL $>$ 65 mm for females and SVL $>$ 60 mm for males were accepted as adults (Tarkhnishvili and Gokhelashvili, 1999; Ayaz et al., 2004). Then they were used to test the relation between the ventral colour maculation and SVL (as a substitute of age) using Fisher's exact test (http://faculty.vassar.edu/lowry/webtext.html). It was assumed that colour pattern and age are independent variables. Based on Fisher's exact test results, only adult individuals were used for further analysis.

Molecular techniques and analysis

DNA extraction. A urea based nucleic acid extraction procedure (M. Bilgin et al., unpublished) was used to extract total genomic DNA from clipped toe samples. Briefly, ground tissues were extracted with Urea Extraction Buffer (3 M urea, 20 mM EDTA, 1% N Lauryl-sarcosine-Na-salt, 50 mM Tris HCL pH: 8.0, 125 mM Na_2SO_4, 1% PVP, 20 mg/ml proteinase K) followed by phenol/chloroform (1 : 1 v/v) extraction. DNA from the aqueous phase was precipitated with 2-propanol and the pellets were washed with 70% ethanol. Air-dried DNA pellets were resuspended and stored in TE buffer.

PCR and direct sequencing. The primers (ND3F and ND3R) designed by Plötner et al. (2001) were used for amplification and sequencing of a 340-bp segment of the mitochondrial protein coding ND3 gene corresponding to sites 11562-904 in the mt genome of *Xenopus laevis* (Roe et al., 1985). The PCR program for amplifications (Plötner et al., 2001) had a first cycle at 94°C for 5 min, followed by 50 cycles at 96°C for 30 s, 50°C for 30 s, 74°C for 90 s, with a 7 min final extension. PCR were carried out in 50 μl volume consisting of 2 μl of template DNA, 1 μl of each primer (20 μM), 1 μl dNTP (10 mM), 4 μl of $MgCl_2$ (25 mM), 0.5 μl of JMR Super Therm DNA polymerase (5 U/μl), 5 μl of 10$\times$ PCR buffer, and 35.5 μl of H_2O. PCR products were separated on agarose gels and amplified fragments were extracted from the gel using a gel extraction kit (QIAGEN, Genmed or GENE MARK, Medicor, Ankara, Turkey). Sequencing was then performed with an automatic DNA sequencer (ABI Prism 310 Genetic Analyzer) using a DYEnamic ET Terminator Cycle Sequencing Kit (Amersham). Sequences were aligned using the CLUSTAL W algorithm tool of MEGA version 4 (Tamura et al., 2007) to find all possible *caralitana* and non-*caralitana* type haplotypes. Sequences were deposited in the EMBL Nucleotide Sequence Database under the accession numbers listed in Appendix 1. *Rana cretensis* and *Rana ridibunda* were taken from Genbank with accession numbers AJ310336 (Plötner et al., 2001) and AM90066 (Plötner et al., 2008), respectively.

Initially, we mapped the distribution for both ventral colour and mtDNA haplotypes separately to see the geographic boundaries and variations of characters. Later, we

tested whether there is spatial correlation between mtDNA haplotypes and ventral colour maculation of sampled individuals at the regional level by means of Mapinfo software. For this reason, the study area was divided into 12 separate watersheds according to topographical features. For each watershed, data were pooled and percentage of concordance (81-100% very high, 61-80% high, 41-60% moderate, 21-40% low, 1-20% very low) between mtDNA haplotypes and ventral colour was calculated, and the direction of introgression was estimated.

For phylogenetic analysis among the studied 19 populations (only for adult individuals) the software packages MEGA 4.0 (Tamura et al., 2007) and PAUP* version 4.0b10 (Swofford, 2001) were used. The choice for the model of sequence evolution was performed using Modeltest 3.7 (Posada and Crandall, 1998) and Modeltest Web-Server (http://darwin.uvigo.es) with all options set to default. A neighbour joining tree was constructed with the substitution model proposed by Modeltest using MEGA 4.0. In the maximum-parsimony analysis, we performed heuristic searches with initial trees obtained by simple stepwise addition, followed by branch-swapping algorithm (tree bisection-reconnection, TBR) with 100 addition replicates for each analyis using PAUP* version 4.0b10 (Swofford, 2001). Tree robustness was evaluated by bootstrapping (Felsenstein, 1985) for both NJ and parsimony trees with 1000 replicates.

Lastly, we performed a linear regression analysis with Minitab 13.1 (http://www.minitab.com) to analyse the relationship between venter colour and altitude, where we used the percentage of orange coloured maculation (class 2) as a dependent variable and altitude as an independent variable.

Results

Correlation between ventral colour and age

Fisher's exact test showed that correlation between ventral colour and age was highly significant ($p < 0.001$). 70% of juveniles had white venters (generally) and black spotted venters (less frequently), and more than half of those individuals had SVL $<$ 40 mm. The remaining 30% of juveniles had orange or orange-brown venters and more than 60% of them had SVL $>$ 40 mm. Most important, juveniles from Beyşehir Lake (the terra typica of *caralitana*) had only barely visible orange spots on their venter.

Geographic variation in ventral colour and haplotype

For both ventral colouration and haplotype, geographical heterogeneity was evident. The geographic range of *caralitana* specific orange coloured maculation (coded as 3) extended from Eşmekaya in the north, Kemer and Tefenni in the west, and to Avlan Lake and the Mediterranean coast in the south. Specimens with a white coloured venter (coded as 1) were predominantly recorded in the coastal parts of the Mediterranean region except for some populations from Antalya, but also rarely in populations where individuals with black-brown venters exist. Furthermore, black-brown spotted specimens (coded as 2) were found substantially widespread in many populations apart from the Lake District and the Konya plain (fig. 2, on the right).

Individuals with orange-brown spots, an intermediate colour between the *caralitana* and the non-*caralitana* forms (coded as 4), were recorded in Yeşilova, Tefenni and Işıklı Lake which are transitional regions between orange and black-brown or white coloured populations, as well as in Bucak and Karacaören Dams I-II.

The geographic range of *caralitana* specific haplotypes extend from Akşehir-Eber Lakes to the western borders of the Burdur province (Yeşilova, Tefenni), the Işıklı Lake to the Avlan Lake in the south, including the eastern coastal region of Antalya (fig. 2, on the left).

Concordance between mtDNA haplotypes and ventral colours

Of the 12 different watersheds studied, four watersheds showed a moderate, four of them a high, and two of them a very high level of concordance for these two parameters (fig. 3). Only one watershed (no. 2) is entirely discordant, i.e., all individuals carry *caralitana* specific haplotype but have white or white with black coloured spots on their ventral parts. Introgression seems to occur unidirectionaly from the *caralitana* maternal lineage to the non-*caralitana* lineages. Moderately concordant watersheds are no. 3, and no. 10 (50%), and no. 4 (60%) where individuals similarly have white or black spotted venters with *caralitana* specific haplotypes; no. 11 (50%) in which reciprocal introgression is probably seen, but in different

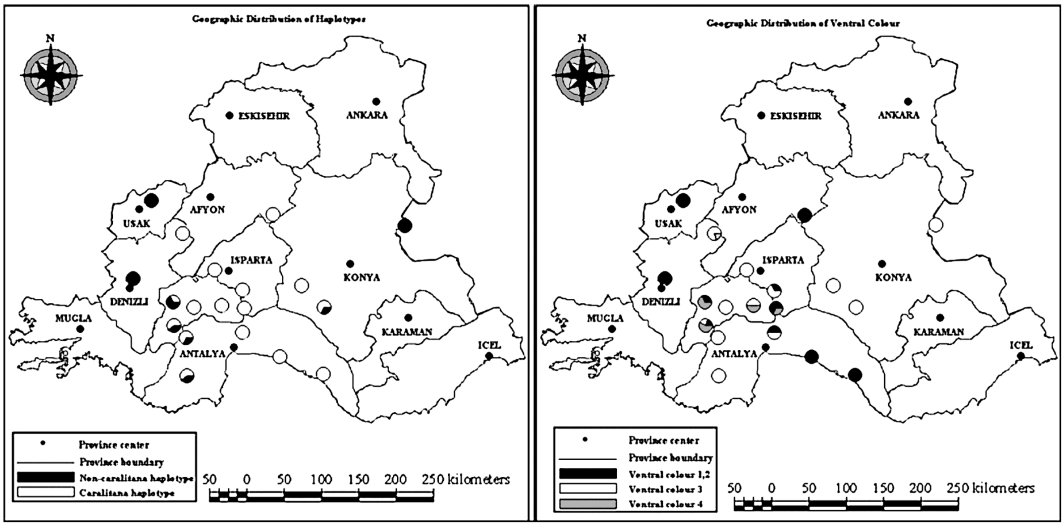

Figure 2. (Left) Map showing the geographical distribution of haplotypes. In the pie chart, black represents the non-*caralitana* haplotype, white representing the *caralitana* haplotype. (Right) Map showing geographical distribution of ventral colours. Black represents black-brown spotted or white coloured venter, coded as 1 and 2; white represents orange spotted venter, coded as 3, and gray represents orange-brown spotted venter, coded as 4.

populations; in the Akşehir-Eber population individuals have *caralitana* haplotypes with white or black spotted venters while in Eşmekaya individuals carry non-*caralitana* haplotypes with orange spotted venters.

Highly concordant watersheds are no. 7 (67%) and no. 12 (80%) in which specimens carrying non-*caralitana* haplotypes have orange spotted venters, suggesting introgression of mtDNA from the non-*caralitana* to the *caralitana* lineage; no. 6 (80%) and no. 9 (78%), where reciprocal introgression takes place again between two forms especially in the marginal populations (Tefenni and Yeşilova) without any introgression in the central population (Kemer in no. 9). Lastly, watersheds no. 5 and no. 8 are entirely concordant (100%) in their haplotype and ventral colour pattern. No. 1 was excluded from analysis since all of 16 samples were juveniles (fig. 3).

Phylogenetics

Among the 63 aligned 340 bp sequences, *Rana cretensis* was used as an outgroup. Of these, 271 were constant, 45 were variable and parsimony uninformative, and 24 were variable and parsimony informative. Modeltest proposed the Tamura-Nei substitution model (TrN + G) (Posada and Crandall, 1998) and a gamma distribution shape parameter of 0.48750, with empirical base frequencies (A: 0.1960; G: 0.1566; C: 0.3085; T: 0.3390) estimated from the data set.

NJ tree was constructed with the TrN + G model (fig. 4). The maximum parsimony analysis resulted in twelve equally parsimonious trees (104 steps in length, CI = 0.8000, RI = 0.9476). The topologies of the strict consensus tree (not shown) and NJ tree revealed two major groups, one exclusively made up of *caralitana*-specific haplotypes (bootstrap value 90-97%), the other composed of paraphyletic populations with non-*caralitana* haplotypes. Individuals from the same populations were clustered into different groups because some populations had haplotypes of both the *caralitana* and the non-*caralitana* groups especially at transitional areas such as Tefenni, Korkuteli, Yeşilova, and Seydişehir. The mean genetic divergence between the *caralitana* and the non-*caralitana* groups was 3.1% (SD = 0.8%).

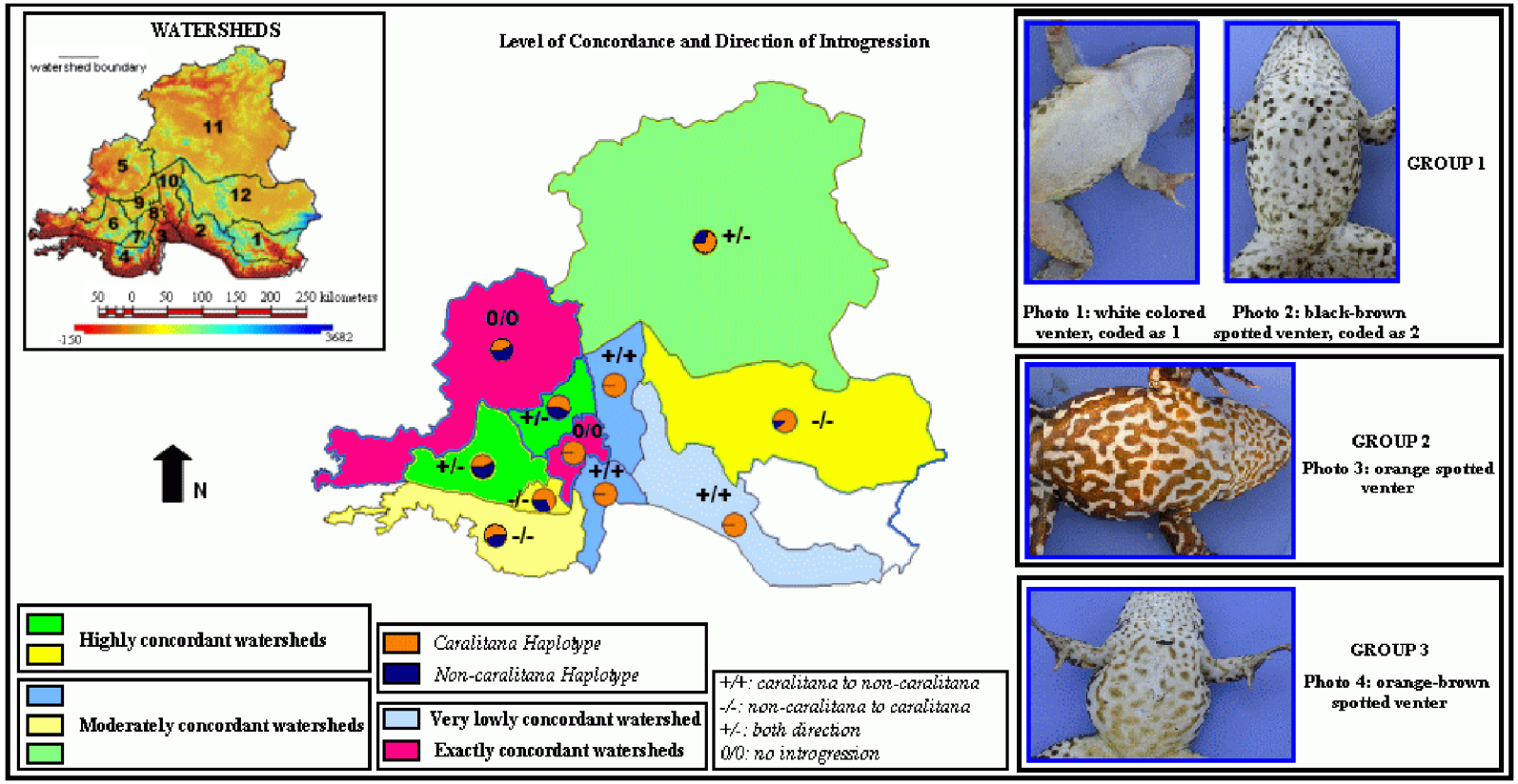

Figure 3. (Inset) Altitude map showing the borders of each watershed in the study area. (Main map) Levels of concordance between ventral colour and mtDNA haplotype, and direction of introgression between the *caralitana* and the non-*caralitana* forms in each watershed: plus/minus and different colours indicating direction of introgression (+/+, blue colours: *caralitana* to non-*caralitana*; −/−, yellow colour: non-*caralitana* to *caralitana*; +/−, green colour: both direction; 0/0, pink colour: no introgression), and light or dark form of same colour reflecting low or high level of concordance between two characters, respectively. Pie charts in each watershed denote percentage of *caralitana* (orange colour) and non-*caralitana* (blue colour) haplotypes. On the right, photos demonstrate three distinct ventral colour groups.

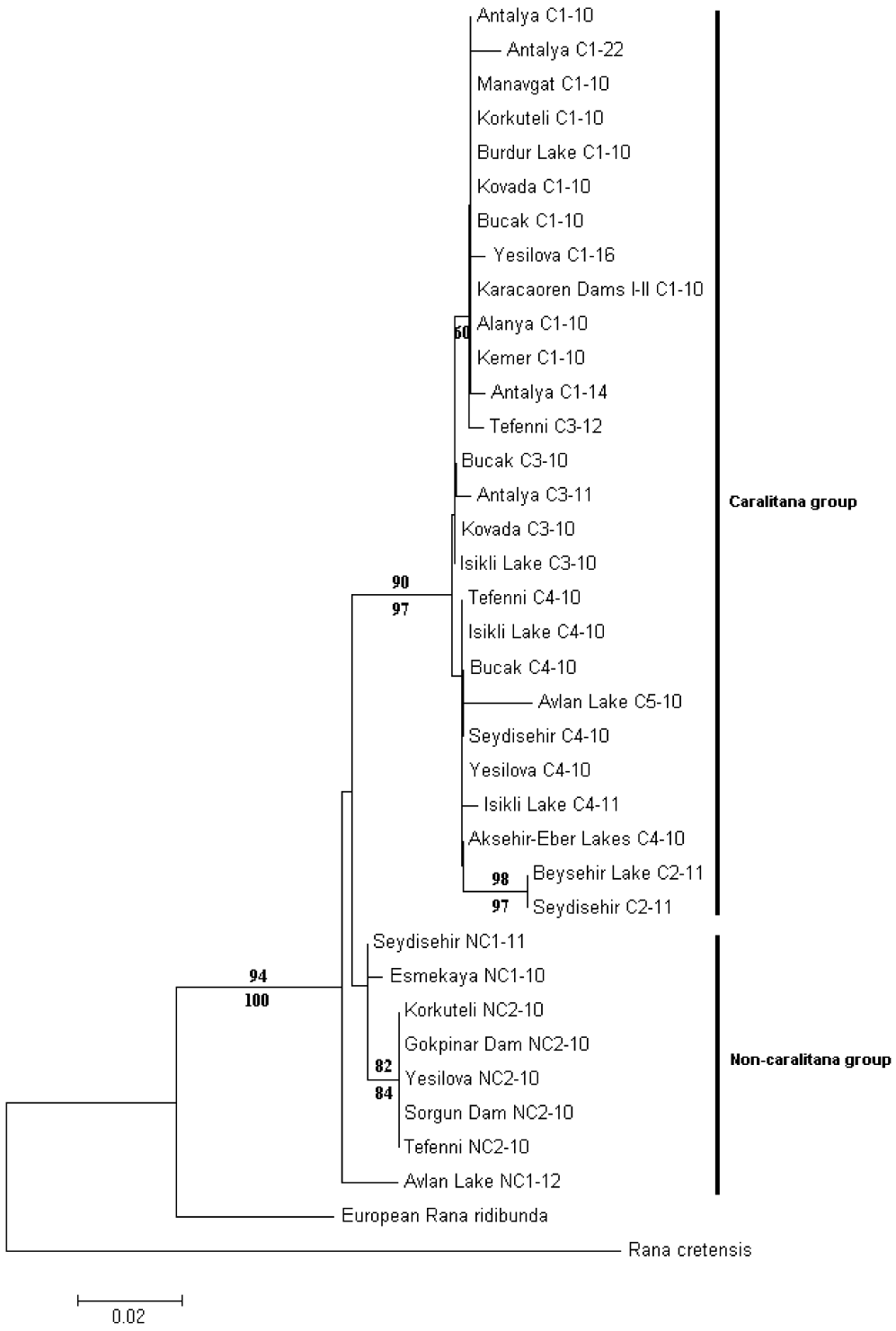

Figure 4. Neighbour-joining phylogram (TrN + G substitution model with gamma shape factor 0.48750) showing phylogenetic relationship among *caralitana* and non-*caralitana* haplotypes from the studied 19 populations. Numbers are bootstrap values above for NJ and below for MP analyses. No values are given for nodes which received support below 50% in both analyses.

Effect of altitude on ventral colour

Linear regression analysis showed no significant relationship between percentage of orange coloured maculation and altitude ($p > 0.05$) and the descriptive power of the relationship is very low ($R^2 = 0.089$). The proportion of individuals with orange coloured spots ranges between 30% to 100% at a wide range of altitudes from 277 to 1391 meters.

Discussion

According to highly significant results of Fisher's exact test, the development of ventral colour maculation is dependent on age during water frog life. This finding indicated that venter colour maculation becomes obvious as the age of frogs increases. In the case of the *caralitana* form, paler or insignificant orange spots in juveniles become prominent and darker in adults. In previous studies, it was suggested that colour change is age-related and occurs in one direction (Hoffman and Blouin, 2000; Summers et al., 2003). Therefore, our analysis was restricted to adult frogs.

The distribution of ventral colour maculation in the study area exhibits a complex clinal variation, and since there is not a clear barrier, a gradual change is observed, especially in the western Lake District where different coloured individuals are seen syntopically. In other transitional regions, however, there are abrupt changes in ventral colour presumably because of effective mountain barriers such as Western and Middle Taurus Ranges, some of which reaches more that 2000 meters above sea level (a.s.l.). The specimens with orange-brown maculation (Class 3) show an intermediate colouration, mainly found at transitional regions, and could probably represent hybrids as supported by previous studies (Arıkan, 1990; Arıkan et al., 1994; Jdeidi at al., 2001).

Generally, the level of concordance is high in many watersheds except in the Eastern Mediterranean coastal region, and the provinces Antalya and Isparta which are fully or moderately discordant areas. At the population level we determined that discordant specimens carrying *caralitana* haplotypes with white or black spotted venter are more frequent (in six populations) than those carrying non-*caralitana* haplotypes with orange spotted venters (in four populations). Nevertheless, the former type was found predominantly in Eastern Mediterranean coastal areas as well as in Antalya and Isparta provinces, while the latter type was detected particularly west of Antalya and in the transitional area between the Konya plain and Eşmekaya.

Areas where both types of discordance were found coincided with populations having intermediate coloured individuals, particularly in the Lake District. In addition, within the same watershed (no. 11), distinct populations have different types of discordance, which could be a result of unidirectional hybridization, but in different directions for particular populations. Additionally, one population in watershed 9 has both types of discordance, indicating reciprocal hybridization while others show complete concordance. This area-specific directionality was shown in other studies as well (Wayne and Jenks, 1991; Wirtz, 1999; Hotz et al., in prep.).

Phylogenetic analysis also showed that individuals from the same populations were not always clustered within the same group, especially in populations at the transitional areas; this also supports introgression among different forms (Slatkin and Maddison, 1989).

Although Sinsch and Schneider (1999) suggested the presence of a positive correlation between size and altitude, and a continuous size distribution among lowland and highland specimens, our more detailed regression analysis did not support this assumption for *caralitana*.

Possible explanations for the discordance between haplotype and ventral colour

Traditionally, systematists have relied on morphological data to identify and delineate taxa, but genetic (especially mtDNA) data are in-

creasingly utilized to test morphology-based systematic hypotheses and finding both concordance and discordance (Wiens and Penkrot, 2002 and references therein). In our case, we found significant discordance of mtDNA haplotypes and "diagnostic" ventral colour at the periphery of the known range of the form *caralitana*. There might be several potential explanations for this observed pattern of discordance.

One explanation is that the gene tree was incorrectly estimated (Wiens and Penkrot, 2002) or a single marker is inadequate to evaluate the historical relationships among populations. In our study, the *caralitana* group is well supported in both types of phylogenetic analyses (bootstrap values $> 90\%$); the non-*caralitana* group has lower bootstrap values most probably due to small sample size, especially because not all non-*caralitana* haplotypes were included as data on associated venter colour was not always present. A Bayesian analysis with a larger data set (unpubl. data) yields a tree where non-*caralitana* haplotypes form another (monophyletic) clade with a posterior probability of 0.80. Thus, incorrect estimation of gene tree seems to be unlikely. Furthermore, not only the ND3 marker but also other markers such as allozymes (Jdeidi, 2000) and ND2 (Akın et al., subm.) support this assessment.

Another explanation could be the incomplete lineage sorting of an ancestral polymorphism, a likely situation in very recently diverged populations (Neigel and Avise, 1986). However, average divergence time between the *caralitana* and the non-*caralitana* groups is between 1.5-1.9 million years based on the ND3 + ND2 genes (Akın et al., subm.; Plötner et al., 2001) and the small effective population size of mtDNA (Moore, 1995) would make this explanation even more unlikely.

Wiens and Penkrot (2002) and Lougheed et al. (2006) suggested that discordance can be a result of high within-species variation relative to between-species variation. A high degree of variation in ventral colour in some of our study populations with identical mtDNA haplotypes suggests that this could be the case. If females in different habitats prefer to mate with males with a particular venter colour (Endler, 1992; Boughman, 2002; Summers et al., 2003), then sexual selection or an interaction between sexual selection and habitat features (Wiens et al., 1999) may lead to significant diversification in ventral colour within *caralitana* populations. Such a scenario would predict a geographical pattern of discordance that (at least in part) reflects the observed habitat or climate diversity. However, the most discordant sites identified in this study lie in regions highly differing in terms of climate, topography or vegetation. It is highly unlikely that the same selective pressures are at work at sites with so different environments, thus leading to an advantage of white venters. Moreover, individuals from the same site (i.e., same habitat) commonly displayed variable venters in disagreement with the idea of selective advantage of a particular venter colour at a specific habitat.

The most plausible explanation of the observed spatial pattern of haplotypes and ventral colour is genetic introgression caused by hybridizations between *caralitana* and non-*caralitana* individuals. The present distribution of the *caralitana* haplotype is centered at the Lake District and expands towards the Mediterranean coast and further inland in all directions. Both the discordance values and the frequency of putative hybrid individuals were highest in the periphery regions of its range, and were lowest near the centre, indicating the existence of hybrid (transition) zones where *caralitana* and non-*caralitana* individuals co-occur. Historically, following the spread of the *caralitana* form, adjoining populations belonging to other forms might have received the *caralitana* mitochondrial genome through gene flow but might have remained otherwise distinct as their white venters indicate.

Conclusion

Although it has been used widely as a diagnostic character for the *caralitana* form, according

to our results orange coloured venter character does not always match *caralitana* mtDNA haplotypes within the geographic range of that lineage. Therefore, it is not reliable by itself to identify the *caralitana* form alone on the basis of ventral colour or mtDNA because of character discordance. This is caused by frequently occurring hybrids between the *caralitana* and the non-*caralitana* lineages which is unidirectional in some watersheds or reciprocal in others, and is the most plausible explanation for the observed patterns of variation. Therefore, a combination of other characters, possibly including morphometric ratios (Jdeidi et al., 2001), mating call parameters (Jdeidi, 2000) or nuclear markers and a knowledge of the geographic extent of hybridization with other forms (Wiens and Penkrot, 2002) is required for reliable taxonomic identification for understanding full the evolutionary history of relevant lineages.

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Appendix 1. Water frog sample location names, represented by numbers in fig. 1, exact GPS co-ordinates in latitude and longitude (all points are in geodetic WGS84), sample size both used in ventral colour versus age and ventral colour versus haplotype comparisons, and corresponding watershed for each locality shown in fig. 3. Sequences submitted to genebank were given with accession numbers.

Water-shed	Locality	Map no	Lati-tude	Longi-tude	N for age vs. ventral color	N for ventral color vs. haplotype	Sample-ID	Accession	Haplotype ID
1	Silifke	1	36.3130	33.9595	16	0			
2	Alanya	2	36.6031	32.0694	2	1	MTHTCA07177	GQ902081	C1-10
2	Manavgat	3	36.7603	31.4515	1	1	CBCASTO702	GQ902104	C1-10
3	Antalya	4	37.2119	30.9415	18	6	CBCASTO705	GQ902085	C3-11
							HKHSBKCA07111/2	GQ902084	C1-22
							HKHSBKCA07118/9	GQ902082	C1-10
							HKHSBKCA07124	GQ902083	C1-14
4	Avlan Lake	5	36.5825	29.9482	5	5	HS07165-169	GQ902086	C5-10
								GQ902087	NC1-12
4	Kaş	6	36.2769	29.6839	3	0			
4	Fethiye	7	36.6288	29.1196	4	0			
4	Marmaris	8	36.8467	28.2879	4	0			
5	Gökpınar Dam	9	37.7851	29.1306	5	3	CCYZCA20153-155	GQ902094	NC2-10
5	Sorgun Dam	10	38.6555	29.3386	3	3	CBCA6444-46	GQ902108	NC2-10
5	Işıklı Lake	11	38.2350	29.9605	6	5	CBCA2047-49	GQ902096	C4-10
							CBCA2050/52	GQ902095	C3-10
								GQ902097	C4-11
5	Gemiş	12	37.7902	29.8708	11	0			
6	Tefenni	13	37.2333	29.7113	9	5	YECA15145/146	GQ902110	C4-10
							KYECA15149/150	GQ902111	NC2-10
							MAHACA15152	GQ902109	C3-12
7	Korkuteli	14	36.9918	29.5279	7	3	VDBKCA07126/127	GQ902100	C1-10
							VDBKCA07131	GQ902101	NC2-10
8	Bucak	15	37.3500	30.5393	10	4	MTAECA1571	GQ902089	C1-10
							MTAECA1574/75	GQ902091	C4-10
							MTAECA1577	GQ902090	C3-10
9	Burdur Lake	16	37.8371	30.3854	4	2	CBCAST1517/20	GQ902092	C1-10
9	Kemer	17	37.4627	30.1118	7	1	MHSACA15138	GQ902099	C1-10
9	Yeşilova	18	37.5348	29.6473	12	6	ISCA1578/79	GQ902114	NC2-10
							ISCA1586-89	GQ902113	C4-10
								GQ902112	C1-16
10	Eğirdir Lake	19	38.1393	30.7588	4	0			
10	Kovada	20	37.6325	30.8641	11	3	CBCAST3214/16	GQ902103	C3-10
							OAOBBKCA1596	GQ902102	C1-10
10	Karacaören Dams I-II	21	37.4031	30.8703	15	3	OBBKCA32106-108	GQ902098	C1-10
11	Akşehir-Eber Lakes	22	38.4544	31.4546	8	3	CBCAST4240	GQ902080	C4-10
							CBCA03187/190		
11	Eşmekaya	23	38.2296	33.4876	4	1	TBOECA68170	GQ902093	NC1-10
12	Beyşehir Lake	24	37.6802	31.7180	4	2	MEFUCA42197/198	GQ902088	C2-11
12	Seydişehir	25	37.4557	31.8156	4	3	CBCAST421	GQ902107	NC1-11
							SGCA42201/202	GQ902105	C2-11
								GQ902106	C4-10
12	İvriz	26	37.4408	34.1705	1	0			
12	Gödet Dam	27	37.1076	33.2918	5	0			