Zoologischer Anzeiger 288 (2020) 43–52

Contents lists available at ScienceDirect

Zoologischer Anzeiger

journal homepage: www.elsevier.com/locate/jcz

Research paper

Syntopy of two species of rock lizards (*Darevskia raddei* and *Darevskia portschinskii*) may not lead to hybridization between them

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ARTICLE INFO

Article history:
Received 19 February 2020
Received in revised form
22 June 2020
Accepted 30 June 2020
Available online 14 July 2020

Corresponding Editor: Alexander Kupfer

Keywords:
Darevskia
Hybridization
Reticulate evolution
Species coexistence

ABSTRACT

The two species of rock lizards, *Darevsia raddei* and *Darevskia portschinskii,* belong to two different phylogenetic clades of the same genus. They are supposed ancestors for the hybrid parthenogenetic, *Darevskia rostombekowi*. The present study aims to identify morphological features of these two species and the potential gene introgression between them in the area of sympatry. External morphological features provided the evidence of specific morphology in *D. raddei* and *D. portschinskii*: the species differed in scalation and ventral coloration pattern, however, they had some proportional similarities within both sexes of the two species. Males of both species had relatively larger heads and shorter bodies than females. Males of *D. raddei* were slightly larger than males of *D. portschinskii* and had longer hindlimbs. Microsatellite genotyping revealed no traces of hybridization or gene flow among these species. We suggest that the absence of individuals with combined morphological and genetic features of the studied species reflects existing reproductive barriers. The hybridization between two bisexual rock lizard species from distant clades is rare and only takes place under very specific environmental conditions.

1. Introduction

Most parthenogenetic vertebrates result from the hybridization between two bisexual species of the same genus (Cuellar 1974; Kearney et al. 2009). This is particularly true for rock lizards of the genus *Darevskia* (Darevsky & Kupriyanova 1985; Fu et al. 2000a; b), whiptail lizards, *Aspidoscelis* (Cole & Townsend 1990; Cole et al. 2014), butterfly lizards, *Leiolepis* (Grismer & Grismer 2010), in addition to other species of reptiles (Kearney et al. 2009). Modern populations of the parthenogenetic rock lizard, *Darevskia rostombekowi* (Darevsky 1957), are comprised of the descendants between the hybrids of *Darevskia raddei* (Boettger 1892) and *Darevskia portschinskii* (Kessler 1878) (Fu et al. 2000b; Murphy et al. 2000). Recent publication verifies the presence of some clonal diversity among distinct populations of this parthenogenetic species, but suggests its uniclonal origin (Ryskov et al. 2017). Hence, the successful hybridization event probably had occurred only once. This is no surprising considering the parental species do not coexist in nature in modern time (Darevsky 1967; Uzzel & Darevsky 1973; Arakelyan et al. 2011).

Natural gene exchange has been documented between coexisting species in *Podarcis* (Pinho et al. 2009; Schulte et al. 2012) and in *Aspidoscelis* (Dessauer et al. 2000), as well among closely related species of *Darevskia* (Tarkhnishvili et al. 2013), but not between the species from different phylogenetic clades, whose alleles are combined in known parthenogenetic species. Triploid hybrids, between bisexual and parthenogenetic species, occur in areas of sympatry of parthenogenetic and bisexual species (Danielyan et al. 2008). Several specimens from the syntopy area of Goshavank (Armenia) have been marked as hybrids between *D. raddei* x *D. portschinskii* by I.S. Darevsky based on the analysis of the phenotype (collection of the Zoological Museum of the Russian Academy of Science, Saint Petersburg, ZISP-22625, 27.06.2001).

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https://doi.org/10.1016/j.jcz.2020.06.007
0044-5231/

However, the study of allozyme markers revealed no trace of hybridization between *D. raddei* and *D. portschinskii* in the present area (Uzzel & Darevsky 1973). Another vast secondary contact zone for these species is known from the northern part of Republic of Artsakh, where these two rock lizards occupy river valleys in the vicinity of Karvachar City (Arakelyan et al. 2011). Hence, the question of whether there are hybridization events at these locations is still unanswered. In the present study, we have tried to clarify the possibility of hybridization between *D. raddei* and *D. portschinskii* in the area of their sympatry. Here, we present data on scalation, morphometry, ventral coloration and microsatellite composition of the individuals of two rock lizard species: *D. raddei* and *D. portschinskii* to find out, if a potential ongoing hybridization is present in the syntopy area.

2. Material and methods

2.1. Study species

According to Arribas (1999) and Murphy et al. (2000), *D. portschinskii* (Kessler 1878) belongs to the phylogenetic clade "*rudis*", while *D. raddei* (Boettger, 1892) belongs to the clade "*caucasica*" within the genus *Darevskia* (Arribas 1999). Both species are small diurnal lizards found throughout the central and eastern part of the Lesser Caucasus (Armenia, Artsakh, Azerbaidjan, Georgia and Turkey) at elevations up to 1500 m a.s.l. for *D. portschinskii* and 3200 m for *D. raddei*. While *D. portschinskii* inhabits rocky habitats along rivers and in deciduous forests, it also occurs in the rocky steppes, together with *D. raddei*. *Darevskia raddei* usually prefers dryer and more open habitats than *D. portschinskii,* which is more dependent on forest habitats (Arakelyan et al. 2011). The maximum body size, snout vent length (SVL) – of adult *D. portschinskii* is 67 mm, while in *D. raddei* it can reach 69 mm (Darevsky 1967; Arakelyan et al. 2011). Although these species belong to two different clades within *Darevskia*, they commonly coexist (Darevsky and Kupriyanova, 1985).

2.2. Study area

The survey area was located in the vicinity of Zuar Village (N40.0692; E462433, WGS 84) in the south-easternmost region of the Lesser Caucasus Mountains at 1500 m a.s.l. on the southern rocky slopes. This is the easternmost region of the range of both *D. portschinskii* and *D. raddei.* The area features deep river valleys between watersheds, where the rock lizards occupy the rocky outcrops along the roads and trails within the gorges, as well as rock and stone piles on the slopes and edges of the watersheds, which are covered by deciduous oak forests and grass meadows. Abandoned villages are common in the study area. They are also suitable for the lizards, which occupy stone walls, and remains of old, abandoned buildings. Sparse trees occur in such areas, namely, *Betula sp., Carpinus orientalis, Mespilus germanica, Prunus armeniaca*. The lizards use the walls around them, sheltering in the cracks between the stones, within wall debris and the rock piles. They also move from one rock pile to another, occasionally crossing into grassland habitat.

2.3. External morphology

Morphological analysis was performed using 16 different scalation characteristics as well as ten separate morphometric values (Arnold et al. 2007; Darevsky 1967). The following pholidosis characteristics were used: MBS – medium body scales, (the number of dorsal scales at mid-body); VSN – ventral scale number, (taken in the central region of the body); CSN - collar scale number; GSN - gular scale number (from the angle between the maxillar scales to the collar); FPNr – femoral pore number; SDLr – number of subdigital lamellae (from the 4th toe of the right forelimb); SCSr – number of supraciliar scales (on the right side of the body); SCGr – number of supraciliary granules (on the right side of the body); SMr – number of scales between the masseteric shield and the supratemporal scale on the right side of the body; MTr – number of scales between masseteric and tympanum shields on the right side of the body; PA – preanal scale number; PTMr–postemporal scale number on the right side of the body; aNDSr – average (from 10 counted scales) number of dorsal scales along the abdominal scale near the limb end on the right side of the body.

The morphometric measurements used were: SVL – length of body from tip of snout to cloaca; TrL – trunk length between fore- and hindlimbs; HH - head height near the occipital plate; HW – maximum width of head; HL – head length, measured ventrally from the tip of the snout to the posterior margin of the collar; MO – mouth opening, measured laterally from the tip of the snout to the end of the mouth; PL – pileus length, measured dorsally from the tip of the snout to the posterior margin of the parietal + occipital scales; ESD - length of the posterior half of the pileus, measured from the anterior margin of the 3rd supraocular scale to the posterior margin of the parietal + occipital scales; FFL – total forelimb length, from the base to the tip of the longest toe; HFL – total hindlimb length, from the base to the tip of the longest toe.

We noted the variation in the color of the throat and venter for adult and sexual matured (SVL > 52 mm, who survived more than two winters) 24 males and 15 females of *D. raddei*; 19 males and 13 females of *D. portschinskii*. The lizards were captured during the period 2016–2019 yrs. We categorized the coloration according to presence or absence of white (W); pale yellow (PY); bright yellow (BY) and green–yellow (GY) colors using color sliding scale in the field. Then we described ventral color phenotype of each lizard. For example, W–PY is a lizard with white gular and pale yellow venter. In this study, we didn't differ between age and social categories, though we understand, that coloration changes with age and social position (Tsellarius & Tsellarius 2002). Venter coloration of each lizard was evaluated once, even if it has been captured in several years. The received proportion of color morphs doesn't reflect the real proportion of these morphs in the studied population. The pictures of the ventral side of the lizards (12 females and 19 males of *D. portschinskii*; 7 females and 23 males of *D. raddei*) were used to receive RGB color code using Capture One 20 software. The values for RGB values were measured by the pointer in the middle part of the gular and venter for each lizard we had a picture of good-enough quality.

The morphometric study was performed on live individuals captured during field work in 2005, 2006, 2017, and 2019. In total, we measured 56 males and 24 females of *D. raddei*; 60 males and 21 females of *D. portschinskii* (Tables S1 and S2). Subadult lizards, whose SVL was less than 50 mm, were excluded from the morphometric analysis so as to not negatively effect the size on body proportions.

2.4. Statistical analysis

The measured variables were tested for normality and then parametric or nonparametric tests were applied, depending on the results of those tests. For each individual, we calculated geometric mean as the *nth* root product of *n* morphometric measurements (SIZE-transformed value). Then we divided each morphometric measurement on the SIZE-transformed value to produce the shape ratios and reduce allometric effects of the individuals (Mosimann 1970; Butler & Losos 2002) and log-transformed the obtained values. We performed general principle component analysis (PCA)

based on the numerical scale variables and transformed morphometric measurements to identify the main components reflecting the overall morphological variation in the studied individuals (males and females of two studied species). Broken stick regression has been used to evaluate the significant principle components. We used one-way analysis of variance (ANOVA) to test the differences in scalation features and log-transformed geometric means of the measurements. Linear discriminant analysis was estimated for the reclassification of the species and the sex. Multivariate analysis of variance (MANOVA) was applied to calculate Wilk's lambda for estimating the most significant values.

T-tests for two samples were used to reveal the differences between scalation and SIZE-transformed values of the measurements that were distributed normally; the Mann–Whitney U test was used, if the distribution differed from normal. Between-group PCA based on species (sexes pooled) was done based on scalation features and SIZE-transformed morphometric values to identify the features, which differentiate the four groups (males and females *D. raddei*; males and females *D. portschinskii*). Non-parametric MANOVA (Cooley & Lohnes 1971) on significant PCs was applied to test if these four groups were significantly different. Post hoc tests (for checking individual differences between the groups) and Bonferonni correction were applied to the p-values. Inter-group differences in venter and throat coloration were identified using Chi–Square test (χ^2). Samples of males with different ventral color phenotypes (bright and pale) were compared by Mann–Whitney U test to reveal the differences in SVL of brighter and paler males. The received RGB color values for gular and venter areas were transformed before the statistical analysis. We divided each value on the geometric mean to minimize the effect of the differences between brightness, white balance and contrast between the pictures. Then we applied between-group PCA segregate ventral coloration between species and sexes. The means of the measured values are presented with standard deviations (±SD) and the minimum and maximum for each value are given as well. The adopted significance level was $P < 0.05$. We used software PAST (Hammer et al. 2001) for PCA calculations and R for basic statistical analysis and boxplots.

2.5. Microsatellite analysis

Muscle tissue (tail tips) samples were collected and stored in 96% ethanol. For genetic studies, we used tail tips of 31 individuals identified as *D. raddei,* and 24 individuals identified as *D. portschinskii*, which were collected between 23 May and 27 June, 2017 (27 days). They occurred in the same study area; their home ranges overlapped and they interacted with each other regularly (Galoyan et al. 2019). DNA extraction was performed using a Qiagen tissue kit, following the manufacturer's instructions (QIAamp DNA Mini and Blood Kit Handbook).

Microsatellite genotypes at five tetranucleotide repeat loci were scored for each sampled individual. We used the primer pairs Du161 developed by Malysheva et al. (2012), Du183, Du231 and Du365 developed by Omelchenko et al. (2009a) and Du255 by Omelchenko et al. (2009b). The PCR was performed using QIAGEN Multiplex PCR kits with one set of the multiplex reaction, following the protocol recommended by the manufacturer. The exact protocols, including primer concentrations, and the amplification protocols were optimized during this study. The fragments were separated on an ABI 3130 Gene Analyzer, using the size standard LIZ 500 (Applied Biosystems Inc., Foster City, CA, USA). Genotypes were scored using Genemapper v3.5 software (PerkinElmer, Waltham, MA, USA). Every locus of each individual was repeated two to four times to control for allelic dropout and false allele amplification.

The obtained microsatellite profiles were analysed using STRUCTURE 2.3.4 (Pritchard et al. 2000), with the purpose of inferring individuals with a shared ancestry. The algorithm is commonly used for inferring hybrids from the datasets, even including few polymorphic loci, since it helps to subdivide a mixed "population" into panmictic groups (Pritchard et al. 2010). We applied the admixture model, correlated allele frequencies, without *a priori* information on the location or population origin (no LOCPRIOR option). Markov Chain Monte Carlo (MCMC) parameters were set with a burn-in period of 100 000 and 100 000 post-burn-in replicates. K was set to 2, and of ten repeats the output of that with the highest log likelihood value was used.

Molecular diversity indices (number of gene copies; number of alleles; observed and expected heterozygosity and Garza-Williamson index (Garza & Williamson 2001)), including observed and expected heterozygosity for individuals of each species were calculated. Significant deviations from Hardy–Weinberg equilibrium and linkage disequilibria were inferred using Arlequin 3.5.1 (Excoffier & Lischer 2010). The significance values were adjusted with Bonferoni sequential tests (Rice, 1989) applied across the loci.

All applicable international, national and institutional (Yerevan State and Moscow State Universities) guidelines for the care and use of live animals were followed.

3. Results

3.1. External morphology

Minimum and maximum values of scalation features overlap among sexes and species, however, means of these values allow to distinguish between the species, but not between sexes of the same species. The results are presented on the plot of between-group PCA (Fig. 1A). The first PC describes 72.93% of variance between the species. On average, males and females of *D. raddei* have larger numbers of MBS, FPNr, SDLr, SCSr, MTr and PA, but lower number PTMr than those of *D. portschinskii* (Tables 1 and 2). In contrast, the number of VSN helps to distinguish between the sexes of the conspecific lizards, but not between the different species; number of GSN works well for separating males and females of *D. portschinskii* (Table 2).

Between-group principle component analysis demonstrated that SVL, and trunk length (TRL) allow to distinguish between groups on the first component scale (Table 3); hindlimb length (HFL) and head length (HL) on the second one (Fig. 1B; Table 3). Unlike the scalation features, the morphometric values better represent intersexual rather than interspecific differences (Fig. 1B; Table 4). The females of both species had longer trunk and greater relation of SVL to SIZE than this in males; the males had longer hindlimbs (HFL) and head lengths than the females. Interspecific differences in body proportions are stronger in males, males of *D. raddei* had longer HFL than those of *D. portschinskii* (Fig. 2; Table 4) and, in general, males and females of *D. raddei* had larger SVL than males and females of *D. portschinskii* (Table 4). Six morphometric values remained significant in Discriminant function for distinguishing between species and sexes (Fig. 2): MTr, PTMr, FPNr, SVL/SIZE, TRL/SIZE (Wilk's lambda = 0.028; 0.036; 0.031; 0.026; 0.040; 0.035 respectively, $P < 0.05$).

Venter coloration morphs differed between species of the same sex ($\chi^2 = 54.00$, for males and $\chi^2 = 22.00$ for females, $P < 0.001$): all adult males and females *D. raddei* had greenish-yellow throat color, which was absent in *D. portschinskii* (Figs. 3 and 4). Venter color in *D. raddei* could be PY or GY. Larger males in *D. raddei* (SVL = 56–63 mm, N = 13) had brighter GY venter, than this in smaller males with SVL = 53–58 mm ($U = 10.5$, $P < 0.001$, N = 12), which had PY venter. Females of *D. portschinskii* had white gular. Their venter varied from white to bright yellow (Figs. 3 and 4). The differences between sexes in their ventral coloration morphs were

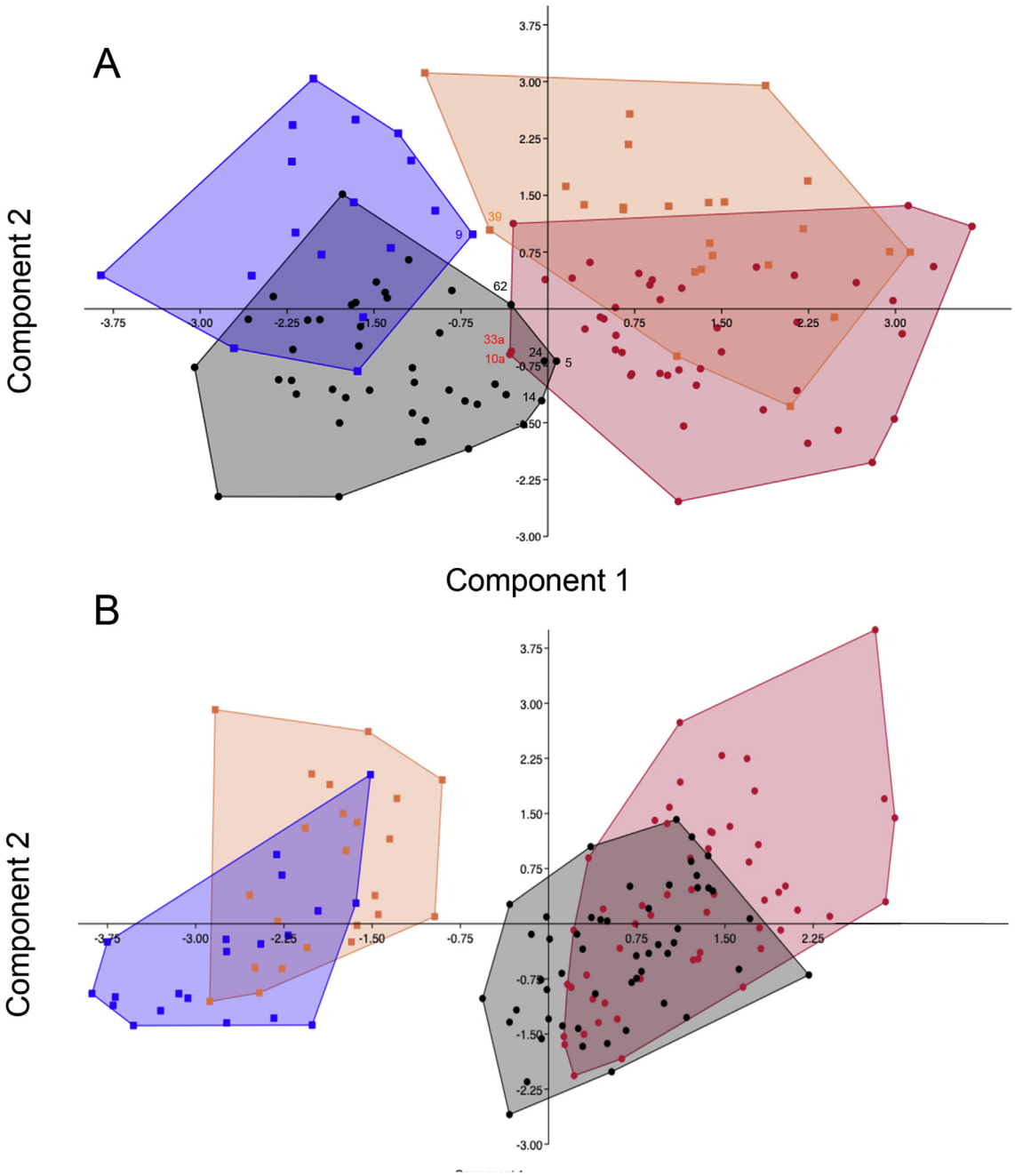

Fig. 1. A – Scatter plot of the first two principle components based on the pholidosis values. Dark blue squares – females *D. portschinskii* (N = 17); black dots – males *D. portschinskii* (N = 43); orange squares – females *D. raddei* (N = 24); red dots – males *D. raddei* (N = 44). Eigenvalue for PC1 = 2.930, 73.38% of variance; eigenvalue for PC2 = 0.977, 24.47% of variance. Numbers near points indicate field number of the individual. B – Scatter plot of the first two principle components based on the SIZE-modified and log-transformed body measurements of *D. portschinskii* and *D. raddei*. Dark blue squares – females *D. portschinskii* (N = 20); black dots – males *D. portschinskii* (N = 50); orange squares – females *D. raddei* (N = 23); red dots – males *D. raddei* (N = 57). Eigenvalue for PC1 = 3.72, 90.62% of variance; eigenvalue for PC2 = 0.339, 8.28% of variance. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

significant for both species ($\chi^2 = 12.76$, for *D. raddei* and $\chi^2 = 25.30$ for *D. portschinskii*, $P < 0.001$). Larger males of *D. portschinskii* (SVL = 54.5–58 mm, N = 5) had BY gular color. Smaller males (SVL = 52–57 mm, N = 17) had W of PY gular, however, the differences were not significant ($U = 19.5$, $P = 0.075$).

3.2. Analysis of microsatellite genotypes

The outcome of the microsatellite genotyping is presented in Table 5. In both *D. raddei,* and *D. portschinskii*, after Bonferroni sequential correction applied across loci, two loci (Du255 and Du161, respectively) showed significant deviation from Hardy Weinberg equilibrium ($P < 0.05$). In general, *D. raddei* was almost twice more genetically diverse than *D. portschinskii* (Table 5), indicating a higher effective size of its population relative to *D. portschinskii*. The STRUCTURE simulation unambiguously separated the individuals of *D. portschinskii* and *D. raddei* (Fig. 5). No individuals that could be considered of mixed ancestry were presented in the studied samples.

Table 1
Factor loadings for the first two PCs of based of shape pholidosis values.

	PC 1	PC 2
MBS	0.337	−0.248
VSN	−0.070	**0.770**
CSN	0.185	−0.190
GSN	0.125	−0.240
FPNr	0.350	−0.148
SDLr	0.288	0.073
SCSr	0.306	0.193
SCGr	−0.045	−0.034
SMr	0.030	0.123
MTr	**0.454**	0.139
PA	0.331	**0.378**
PTMr	**−0.461**	0.032
aNDSr	−0.038	0.087

MBS – medium body scales; VSN – ventral scale number; CSN - collar scale number; GSN - gular scale number; FPNr – femoral pore number; SDLr – number of subdigital lamellae; SCSr – number of supraciliar scales; SCGr – number of supraciliary granules; SMr – number of scales between the masseteric shield and the supratemporal scale on the right side of the body; MTr – number of scales between masseteric and tympanum shields on the right side of the body; PA – preanal scale number; PTMr–postemporal scale number on the right side of the body; aNDSr – number of dorsal scales along the abdominal scale near the limb end on the right side of the body.

Table 3
Factor loadings for the first two PCs of based of shape SIZE-modified and log-transformed values of measurements.

	PC 1	PC 2
SVL	**−0.606**	0.300
TRL	**−0.582**	−0.054
HL	0.120	**0.384**
PL	0.260	0.295
ESD	0.212	−0.235
HW	0.232	−0.050
HH	0.104	−0.292
MO	0.107	−0.354
FFL	0.181	0.022
HFL	0.242	**0.635**

SVL – snout-vent length; TrL – trunk length; HH - head height; HW – width of head; HL – head length; MO – mouth opening; PL – pileus length; ESD - length of the posterior half of the pileus; FFL – total forelimb length; HFL – total hindlimb length.

4. Discussion

Our attempt to find hybrids between *D. raddei* and *D. portschinskii* in the vicinity of their syntopy failed. This result coincides with earlier conclusions by Uzzel and Darevsky (1973) concerning other sympatric population of the studied species from central Armenia. The individuals in the overlapping zone of Fig. 1A are irrespective to the genetic profiles. Poras-Hurtado et al. (2013) suggest that some problems may arise when using small number of markers, as a result of presence of less differentiated alleles in distant groups (in our case, homoplasies in the number of repeats at some short tandem repeat loci are very much probable and should not be interpreted as hybrid origin of the individuals). Moreover, the estimated proportion of alleles also existing in a different species is never above 5% (Fig. 5); hence we prefer to avoid overinterpretation of these minor allele overlaps.

Although minimum and maximum values of the body measurements and scalation traits overlap in the studied species, their means allow to distinguish between species and sexes on the well-sampled data (Fig. 1A and B; Tables 2 and 4). Average values of most of the scalation traits were higher in *D. raddei* (Fig. 1A; Table 2), including number of femoral pores. Besides, scale shape is helpful in distinguishing between the studied species, *D. raddei* have square preanal and temporal scales and this feature allows differentiation between the species of different clades within the genus *Darevskia* (Gabelaia et al., 2017). Body proportion is more sufficient for discrimination between sexes, not between species: females have longer trunks and larger number of ventral scales (Figs. 1D and

Table 2
Summary table for pholidosis values (mean ± SD, range) for males and females of *D. portschinskii* and *D. raddei*.

	D. raddei		*D. portschinskii*		F	P
	Males (N = 44)	Females (N = 24)	Males (N = 43)	Females (N = 17)		
MBS	***	***	***/○ **50.1 ± 2.18 (46–55)**	***/○	**12.2**	**<0.01**
	52.4 ± 2.94 (48–60)	**51.3 ± 2.53 (46–56)**		**48.1 ± 2.60 (43–52)**		
VSN	○○○	○○○	○○○	○○○	**25.43**	**<0.01**
	23.4 ± 1.35 (21–26)	**25.6 ± 1.86 (21–30)**	**23.3 ± 1.32 (21–27)**	**25.9 ± 1.43 (23–28)**		
CSN	***9.7 ± 1.29 (7–13)**	9.2 ± 1.02 (7–11)	***9.1 ± 0.80 (7–11)**	8.7 ± 0.99 (7–10)	**5.49**	**<0.01**
GSN	24.7 ± 1.93 (21–30)	○ **24.0 ± 1.84 (21–28)**	24.2 ± 1.58 (21–28)	○ **23.3 ± 1.72 (21–27)**	2.32	0.08
FPNr	***	***	***/○	***/○	**11.73**	**<0.01**
	18.6 ± 1.35 (15–21)	**18.3 ± 1.62 (16–22)**	**17.4 ± 1.27 (15–21)**	**16.5 ± 1.18 (15–19)**		
SDLr	***	***	***	***	**9.86**	**<0.01**
	25.3 ± 1.65 (21–28)	**25.6 ± 2.02 (20–28)**	**23.9 ± 1.61 (20–27)**	**23.8 ± 1.75 (21–26)**		
SCSr	***	***	***	***	**12.43**	**<0.01**
	6.2 ± 0.93 (5–9)	**6.5 ± 0.78 (5–8)**	**5.5 ± 0.74 (4–6)**	**5.6 ± 0.71 (4–7)**		
SCGr	11.0 ± 1.55 (7–15)	10.5 ± 1.69 (8–14)	10.8 ± 1.38 (6–14)	11.1 ± 1.27 (8–13)	1.032	0.381
SMr	1.3 ± 0.57 (0–3)	1.5 ± 0.66 (1–3)	1.3 ± 0.65 (0–3)	1.4 ± 0.51 (1–2)	0.797	0.498
MTr	***	***	***	***	**34.01**	**<0.01**
	3.4 ± 0.87 (2–6)	**3.6 ± 0.97 (1–5)**	**2.0 ± 0.64 (1–4)**	**2.1 ± 0.66 (1–3)**		
PA	***	***/○	***	***/○	**33.64**	**<0.01**
	2.0 ± 0.40 (1–3)	**2.0 ± 0.20 (2–3)**	**1.2 ± 0.43 (1–2)**	**1.6 ± 0.49 (1–2)**		
PTMr	***	***	***	***	**27.64**	**<0.01**
	3.1 ± 0.78 (2–5)	**3.1 ± 0.61 (2–4)**	**4.3 ± 0.87 (3–7)**	**4.7 ± 0.92 (3–6)**		
aNDSr	2.2 ± 0.32 (2–3)	2.3 ± 0.39 (2–3)	2.2 ± 0.28 (2–3)	2.3 ± 0.29 (2–3)	0.217	0.884

F and *P* values correspond to the results of the ANOVA comparisons between the species and sexes. MBS – medium body scales; VSN – ventral scale number; CSN - collar scale number; GSN - gular scale number; FPNr – femoral pore number; SDLr – number of subdigital lamellae; SCSr – number of supraciliar scales; SCGr – number of supraciliary granules; SMr – number of scales between the masseteric shield and the supratemporal scale on the right side of the body; MTr – number of scales between masseteric and tympanum shields on the right side of the body; PA – preanal scale number; PTMr–postemporal scale number on the right side of the body; aNDSr – number of dorsal scales along the abdominal scale near the limb end on the right side of the body.
*interspecies differences within sexes, supported by t-test, $p < 0.05$; ***, $p < 0.05$.
○intersexual differences within species, supported by t-test, $p < 0.05$; ○○○, $p < 0.001$.

Table 4
Summary table for morphometric values. Measured SIZE-modified morphometric values (mean ± SD, maximum value) for males and females of *D. portschinskii* and *D. raddei*.

	D. raddei		*D. portschinskii*		F	p
	Males (N = 43)	Females (N = 23)	Males (N = 52)	Females (N = 23)		
SVL, mm	58.4 ± **3.18; max = 64.2**	**58.5 ± 3.07, max = 65.0**	**54.7 ± 2.09, max = 60**	**55.6 ± 2.44, max = 59.0**		
SVL/SIZE	***/○○○	○○○	***/○○○	○○○	**283.40**	**<0.01**
	3.64 ± 0.093	4.11 ± 0.106	3.69 ± 0.069	4.14 ± 0.097		
	30.0 ± **3.08, max = 35,3**	**32.8 ± 2.53, max = 37.8**	**28.8 ± 2.14, max = 33.6**	**33.3 ± 2.76, max = 38.2**		
TRL, mm						
TRL/SIZE	***/○○○	***/○○○	***/○○○	***/○○○	**164.20**	**<0.01**
	1.87 ± 0.127	2.30 ± 0.109	1.95 ± 0.116	2.48 ± 0.148		
	20.5 ± **1.98, max = 29.0**	17.7 ± **0.98, max = 19.4**	18.3 ± **0.94, max = 19.9**	16.3 ± **0.81, max = 18.0**		
HL, mm						
HL/SIZE	***	*	***	*	**4.025**	**<0.01**
	1.27 ± 0.109	1.25 ± 0.036	1.24 ± 0.043	1.22 ± 0.050		
	13.7 ± **1.05, max = 15.2**	11.8 ± **0.63, max = 13.8**	12.5 ± **0.59, max = 13.9**	10.8 ± **0.40, max = 11.8**		
PL, mm						
PL/SIZE	○	○	○○○	○○○	**8.56**	**<0.01**
	0.86 ± 0.054	0.83 ± 0.034	0.84 ± 0.031	0.80 ± 0.029		
	7.4 ± 0.93, max = 9.4	**6.0 ± 0.59, max = 7.6**	**6.8 ± 073, max = 8.2**	**5.8 ± 0.75, max = 7.4**		
ESD, mm						
ESD/SIZE	○○○	*/○○○	○	*/○	**5.88**	**<0.01**
	0.46 ± 0.047	0.42 ± 0.036	0.46 ± 0.045	0.43 ± 0.051		
	8.4 ± 0.91, max = 10.0	**7.1 ± 0.50, max = 8.0**	**7.8 ± 0.61, max = 9.2**	**6.6 ± 0.54, max = 7.7**		
HW, mm						
HW/SIZE	○○○	○○○	○○○	○○○	**6.92**	**<0.01**
	0.52 ± 0.045	0.50 ± 0.024	0.53 ± 0.041	0.49 ± 0.036		
	5.3 ± 0.73, max = 7.1	**4.4 ± 0.60, max = 5.3**	**4.8 ± 0.44, max = 5.7**	**4.3 ± 0.52, max = 5.2**		
HH, mm						
HH/SIZE	○	○	0.32 ± 0.025	0.32 ± 0.035	**2.20**	**<0.01**
	0.33 ± 0.036	0.31 ± 0.039				
	11.3 ± **1.09, max = 14.0**	9.8 ± **0.72, max = 11.2**	10.9 ± **0.93, max = 13.0**	9.4 ± **0.97, max = 11.6**		
MO, mm						
MO/SIZE	*	0.69 ± 0.045	*/○	○	**3.85**	**0.01**
	0.70 ± 0.061		0.73 ± 0.056	0.70 ± 0.070		
	19.6 ± **1.32, max = 22.0**	16.8 ± **1.05, max = 18.8**	17.9 ± **0.86, max = 20.0**	15.7 ± **1.22, max = 17.0**		
FFL, mm						
FFL/SIZE	○	○	○	○	**3.67**	**0.01**
	1.22 ± 0.068	1.18 ± 0.077	1.21 ± 0.056	1.17 ± 0.077		
	32.9 ± 1.91, max = 37.0	**28.3 ± 1.55, max = 31.5**	**29.3 ± 1.8, max = 32.4**	**25.2 ± 1.51, max = 28.3**		
HFL, mm						
HFL/SIZE	***/○	***/○	***/○○○	***/○○○	**13.11**	**<0.01**
	2.05 ± 0.107	1.99 ± 0.127	1.98 ± 0.106	1.87 ± 0.112		
SIZE	***/○○○	***/○○○	***/○○○	***/○○○	**85.73**	**<0.01**
	16.07 ± 0.912	14.23 ± 0.589	14.81 ± 0.545	13.44 ± 0.497		

F and *P* values correspond to the results of the ANOVA comparisons between the species and sexes. SVL – snout-vent length; TrL – trunk length; HH - head height; HW – width of head; HL – head length; MO – mouth opening; PL – pileus length; ESD - length of the posterior half of the pileus; FFL – total forelimb length; HFL – total hindlimb length.
*interspecies differences within sexes, supported by t-test, $p < 0.05$; ***, $p < 0.05$.
○intersexual differences within species, supported by t-test, $p < 0.05$; ○○○, $p < 0.001$.

2; Tables 2 and 4). This is the rule for the females in lizards due to female reproduction role (Kratochvíl et al. 2003; Kaliontzopoulou et al. 2006). Males also had longer hindlimbs (Fig. 1B), what is reported previously for *D. raddei* (Dehghani et al., 2014). We suggest that the observed overlap of body size and body proportions within the same sexes of both studied species in the study area (Fig. 1A) indicate similar ecological adaptations rather than genetic introgression or the presence of hybrids. Males of *D. raddei* had slightly longer hindlimbs than those of *D. portschinskii*, what may reflect specifics in mating behaviour: copulation takes up to 50 min in *D. portschinskii* and approximately only six minutes in *D. raddei* (Galoyan et al. 2019). It may also reflect a more arboreal lifestyle for *D. raddei*. We often observed these lizards on the branches of trees and shrubs, which differ in behavior from individuals of *D. portschinskii*. In general, climbing species tend to possess longer limbs than phylogenetically related terrestrial species (Vanhooydonk & VanDamme 2001).

Coloration of the gular area and venter seems to be one of the most suitable distinguishing characteristics for discrimination between the studied species. The gular area is green-yellow in *D. raddei*, even in juveniles. Venter is usually paler, but in adult males it also green-yellow (Figs. 4 and 5). Venter coloration in *D. portschinskii* is white, pale- or bright-yellow in adults (the color of egg yolk). The differences between adult males and females reflect the differences in their social roles, which may help to determine individual behaviors, something that these lizards do quite well (Galoyan et al. 2019). The variety of color patterns within the same sex and species might also reflect reproduction strategies, which are described in other lizards (Sinervo & Lively 1996). Larger and older individuals of rock lizards have brighter ventral coloration; males are brighter than females. According to Tsellarius & Tsellarius (2002), this is also true for *D. brauneri* (Méhely 1909) and very possible for the studied species (Fig. 5). The parthenogenetic *D. rostombekowi* inherited it's coloration from *D. raddei*: "The venter including that of the head and throat is greenish-yellow" (Darevsky 1967). According to our experience, it is difficult to distinguish between young *D. raddei* and adult *D. rostombekowi* (SVL of adult individuals 44–60 mm (Arakelyan et al. (2011)), when locality is unknown, so the main discrimination feature for this species is three small preanal shields (Arakelyan et al. 2011) and not

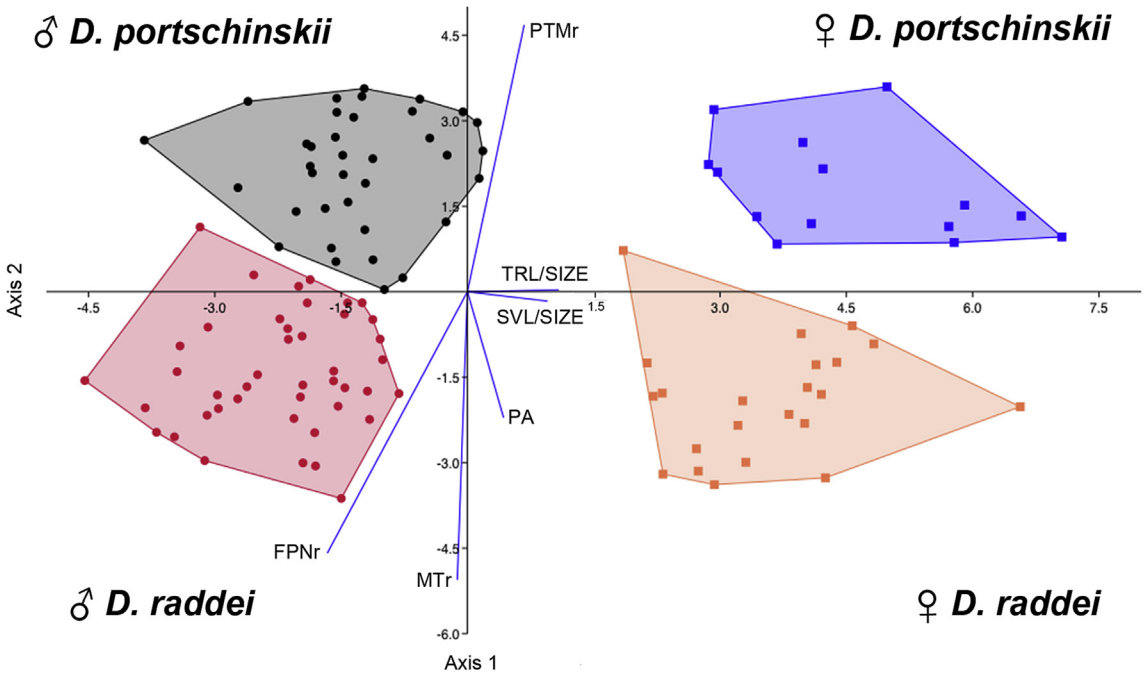

Fig. 2. Scatter plot of the discriminant analysis based on the pholidosis values and SIZE-modified body measurements. Dark blue squares – females *D. portschinskii* (N = 17); black dots – males *D. portschinskii* (N = 43); orange squares – females *D. raddei* (N = 24); red dots – males *D. raddei* (N = 44). Eigenvalue for Axis 1 = 2.930, 68.19% of variance; eigenvalue for Axis 2 = 0.977, 30.40% of variance. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

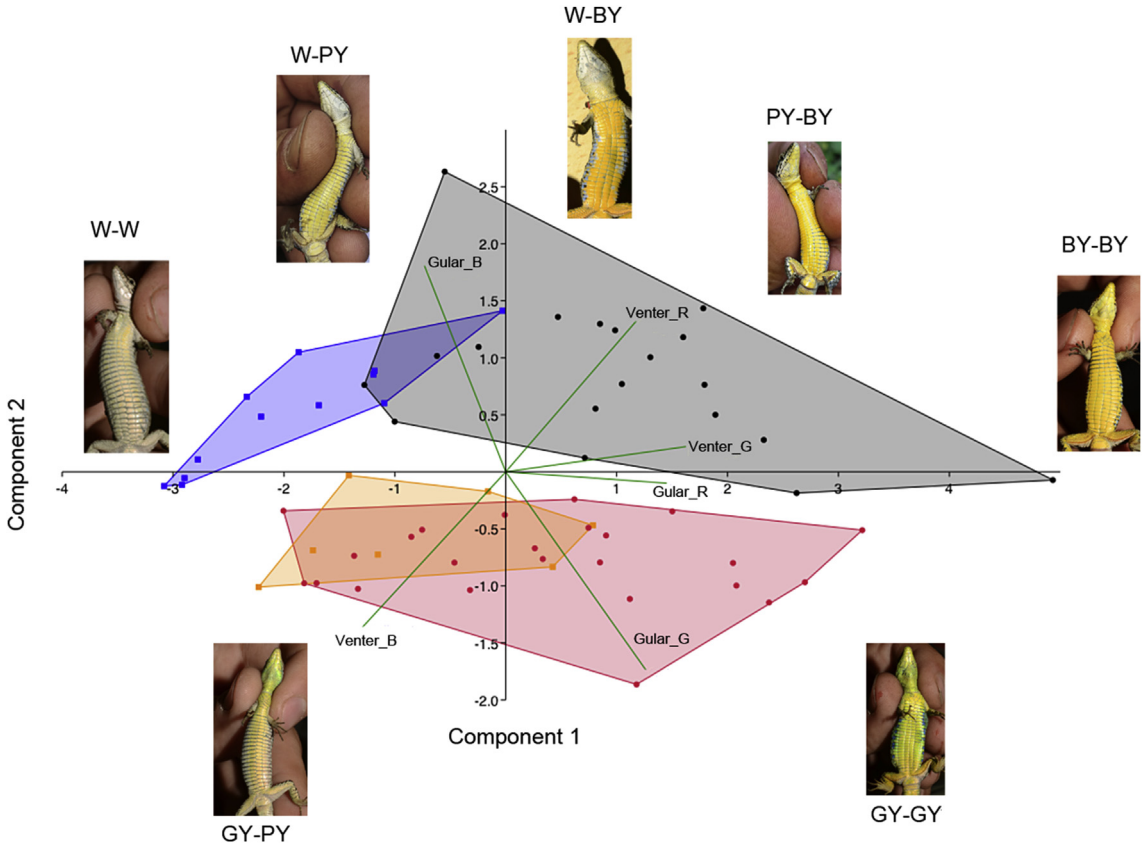

Fig. 3. Scatter plot of the first two principle components based on the modified RGB measurements of the gular and venter coloration of males and females. Dark blue squares – females *D. portschinskii* (N = 12); black dots – males *D. portschinskii* (N = 19); orange squares – females *D. raddei* (N = 7); red dots – males *D. raddei* (N = 23).*D. raddei* and *D. portschinskii*. W – white; PY – pale yellow; BY – bright yellow; GY – green-yellow. Eigenvalue for PC1 = 1.713, 70.20% of variance; eigenvalue for PC2 = 0.641, 26.24% of variance. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

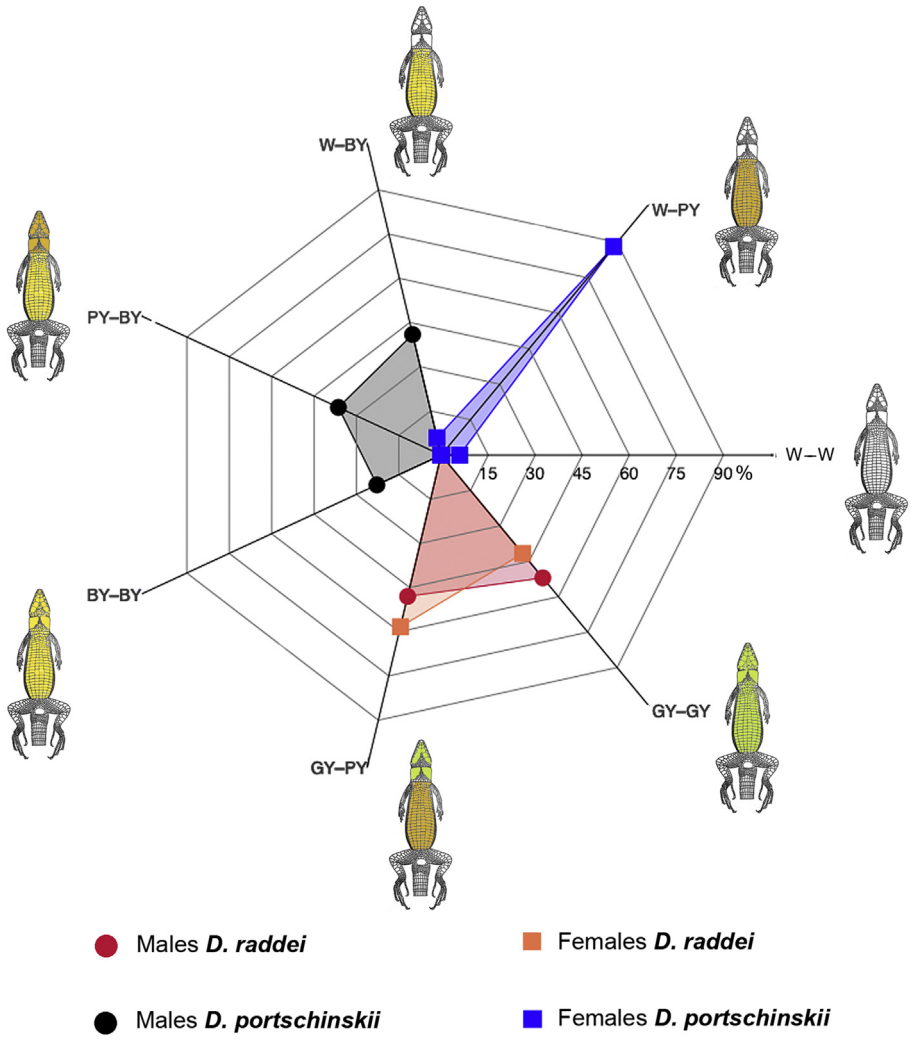

Fig. 4. Radar plot representing the percentage of ventral color morphs in males and females of *D. raddei* (N = 25; 12) and *D. portschinskii* (N = 22; 17). BY, bright yellow; GY, green-yellow; PY, pale yellow; W, white. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 5
Means of genetic diversity indices (±SD) in the studied populations of *D. raddei* and *D. portschinskii*.

Species	Gene copies	No Alleles	Observed heterozygosity	Expected heterozygosity	Garza-Williamson Index	N
D. raddei	71.6 ± 6.99	10.0 ± 2.90	0.71 ± 0.10	0.81 ± 0.09	0.38 ± 0.09	31
D. portschinskii	62.0 ± 5.66	4.8 ± 2.30	0.33 ± 0.29	0.42 ± 0.33	0.37 ± 0.16	24

N – number of individuals.

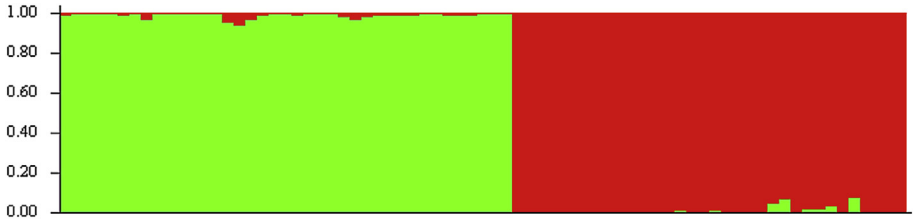

Fig. 5. Output of the STRUCTURE simulations (K = 2) for a mixed sample of *D. portschinskii* (left; N = 34) and *D. raddei* (right; N = 23). Vertical columns represent single individual. The outcome of the simulation with the highest maximum likelihood value was selected.

one like in *D. portschinskii* or two like in *D. raddei* (Table 2). We found no individuals, which were similar in scalation of coloration to individuals with *D. rostombekowi*. Combining all the morphological characters allowed us to identify the species of each studied individual. In general, *D. raddei* is morphologically (Darevsky 1967) and genetically (Fu et al. 2000b; Grechko et al.

2007; Omelchenko et al. 2016) a more variable species inhabiting vast areas and types of habitats, comprising at least four subspecies, including *D. raddei nairensis* (Freitas et al. 2016), which is considered by some to be a separate species (Arakelyan et al. 2011). Such a variability may be confusing and might lead to a false conclusion as to the presence of hybrid individuals in the areas of sympatry.

The origin of parthenogenetic species in vertebrates is considered to be the result of population disturbance (Kearney 2005). Most researchers agree, that parthenogenetic rock lizards appeared less than 200 kyr ago during the glacial period, when the existing isolation boundaries were, most likely, destroyed (Darevsky and Kupriyanova 1985; Freitas et al. 2016). Prezygotic isolation is a result of mating choice based on the morphological, physiological, and behavioral specifics, while postzygotic isolation is a result of incompatibility of less related genomes. Both mechanisms can explain the absence or rarity of hybridization between studied species. Our behavioral study revealed the presence of behavioral mating barriers; males of both studied species are able to distinguish between the females of their own from those of different species and will not mate with non-conspecific individuals (Galoyan et al. 2019). Recent study of meiotic chromosomes of *D. portschinskii* and *D. raddei* from the same studied locality showed, that they look similar in length and general structure (Spangenberg et al. 2019), still it doesn't prove the absence of postzygotic limitations of hybridization. Although it is still unclear whether origin of *D. rostombekowi* occurred several times (Ryskov et al. 2017), it is more likely that it happened only once (Fu et al. 1998). In *Darevskia dahli* (Darevsky 1957), three hybridization events between two sexual species, *D. portschinskii* and *D. mixta* (Méhely 1909), have recently been hypothesized (Vergun et al. 2014). Initially, a multiple origin theory was supposed for *Darevskia armeniaca* (Méhely 1909). Tarkhnishvili et al. (2017), based on the analysis of microsatellite profiles of samples from Georgia, suggest that both *D. dahli* and *D. armeniaca* descent from a single initial hybridization between *D. mixta* and, most likely, *D. portschinskii*. Further genetic and morphological differentiation resulted from interbreeding between *Darevskia valentini* (Boettger 1892) and a parthenogenetic form rather than from repeated hybridization between two sexually breeding species.

We suggest that the hybridization events leading to the origin of parthenogenetic species were extremely rare. It is almost impossible to "catch" the hybridization event that produces a parthenogenetic species in nature. The vitality of the hybrids between *D. portschinskii* and *D. raddei* may be tested in the laboratory in the future. To date, successful attempt to create a parthenogenetic species in the laboratory has been done only in *Aspidoscelis* (Cole et al. 2014). Still, revealing the proximal mechanisms of interspecies mating and breeding isolation remains one of the most intriguing parts of the study of reticulate evolutional process in vertebrates.

Declaration of Competing Interest

None.

Acknowledgments

We are thankful to Paul Freed for his editing work and valuable advises during the preparing of the present manuscript. We also thank two anonymous reviewers for their helpful comments. The work was supported by the research project of Russian Foundation for Basic Research RFBR 17-00-0430 (RFBR 17-00-0425; RFBR 17-00-0429). The research was approved by the Armenian Ministry of Nature Protection (N5/22.1/51043).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcz.2020.06.007.

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