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Research Article

Morphological and morphometric variations of the water frogs (genus *Pelophylax*) in Iran

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Abstract

Despite the efforts conducted on the identification of species of the genus *Pelophylax* from Iran, recent studies revealed that the taxonomy and identification of local populations within this genus have been still unresolved. Here, the morphological and morphometric variations of water frog populations (*Pelophylax*) from different localities of Iran were studied. To assess suitable morphological and morphometric diagnosis for taxonomic identification of the genus, 13 morphological and 23 morphometric characters of 160 specimens were analyzed. Our result indicated *P.* cf. *bedriagae* and *Pelophylax* sp. show cryptic variation in morphology and melanistic diversity. In water frogs, this character is much more influenced by compatibility to special habitat, so its value is less to be considered as a taxonomic trait or key character for identification. However, the morphometric variables of *Pelophylax* sp. are larger than *P.* cf. *bedriagae* from the west of Iran. Following generalized Gloger's rule, a comparison of character states of 12 biological variables revealed that dorsal coloration of water frog populations become paler from north to south in eastern Iran, while the ventral part shows low variation in color. This pattern indicates the low selective value of ventral coloration in the viability of water frogs in eastern Iran. Moreover, natural selection stabilized olive coloration on the populations of water frogs living in the green habitats of north Iran while compromising light grey and dark brown in contrasting ecosystems through the semi-desert and humid oases of south Iran via a disruptive selection mechanism.

Keywords: *Pelophylax*, Morphological characters, Polymorphism, Iran

Introduction

Taxonomy of water frogs of the genus *Pelophylax* Fitzinger, 1843 in Iran has been subjected to long debates. Indeed, species of the genus *Pelophylax* are the most widespread anurans in Iran (Balutch and Kami, 1995; Rastegar-Pouyani et al., 2008; Mohammadi et al., 2015; Pesarakloo et al., 2017). For a long time, *P. ridibundus* (Pallas, 1771) was the only species of the genus *Pelophylax* mentioned in the checklists and published texts on the amphibians of Iran (Balutch and Kami, 1995; Anderson, 1963; Rastegar-Pouyani et al., 2008).

The most recent study related to the phylogeny of water frogs from Iran, conducted by Pesarakloo et al. (2017), reported new species of frogs from the northern, eastern, and central parts of Iran. They proposed the presence of two lineages of *P.* cf. *bedriagae* (Camerano, 1882) in western Iran although the presence of *P.* cf. *bedriagae* is controversial due to lack of molecular comparison to the specimens from the type locality. Moreover, Safaei-Mahroo and Ghaffari (2020) in the synopsis of "Amphibians of Iran" stated that water frogs from the eastern parts of Iran are belonging to *P. gigas* (Gmelin, 1789) but did not clarify the shreds of evidence for their claim. Therefore, the presence of three species of the genus *Pelophylax* in Iran including *P. ridibundus*, *P.* cf. *bedriagae* and *P. gigas* has been still controversial. This may be due to taxonomic problems which have been remaining to these species.

Pelophylax ridibundus is a medium-sized species compared to other anurans inhabiting Iran. The water frogs are semi-aquatic amphibians living in steppes; forest habitats grasslands and semi-desert areas (Düşen and Öz, 2013). The exact type locality of the first described specimens of *P. ridibundus* has not been mentioned by Pallas (1771) only two type localities (Gurvev, Ural River, Kazakhstan, and Volga River) were mentioned later (Mertens and Müller, 1928). Therefore, it is proposed that the neotype should be provided from the type localities for comparison (Dubois and Ohler, 1996). The Eurasian water frog *P. ridibundus* is a species complex that has been previously known as *Rana ridibunda* distributed from Central Europe to the North Baltic Sea, south Mediterranean Sea, and also Western Asia (Frost, 2021). However, due to the non-monophyly of the genus *Rana* (Chen et al., 2005; Frost et al. 2006; Che et al. 2007), the *ridibundus* group was assigned to the genus *Pelophylax* (Frost et al., 2006). The range of *P. ridibundus* is extended to eastern Kazakhstan and China (Ye et al., 1993; Fei et al., 1999; Berezovikov et al., 2001). Its northern boundary is Siberia where it has been introduced (Bannikov et al., 1977; Kuzmin, 1999) and the southern known boundary of the species has been supposed to be the sub-tropical region of southeast Iran (Mohammadi et al., 2015). The taxonomy of Mediterranean populations of the species which are widespread through Turkey has been subjected to many debates (Akin et al., 2010; Bülbül et al., 2011). It has been recorded from all over Iran except the central deserts (Balutch and Kami, 1995; Anderson, 1963; Rastegar-Pouyani et al., 2008). Morphological variations within water frog populations of *P. ridibundus* sensu lato in Iran resulted in taxonomic disparities and described many morpho-species. On the other hand, recent advances in molecular methods and applying interdisciplinary approaches have changed the traditional taxonomy to the modern taxonomy which resulted in the taxonomic revision of water frog morphospecies and synonymized most of them (Sinsch and Schneider, 1999; Bülbül et al., 2011; Pesarakloo et al., 2017). There have been some efforts accomplished to elucidate different aspects of biology and also variations among different populations of *P. ridibundus* including karyology, morphometry, and habitat surveys of the water frogs from different localities in Iran (Nemati, 1998; Molavi, 2000; Hazaveh, 2006; Hashemi Nejad, 2009; Mohammadi et al., 2015, Pesarakloo et al., 2018). Various morphs of *P. ridibundus* were assigned to different subspecies including *P. ridibundus susana* described from Shush, Khuzestan Province, Iran (Boulenger, 1905), and *P. ridibundus ridibundus* from Esfahan, Fars, and Tehran provinces (Schmidt, 1939). However, *P. ridibundus ridibundus* was the only subspecies validated to be present in Iran (Rastegar-Pouyani et al., 2008).

Morphological and morphometric characters can be applied in taxonomy for the identification of different species and intraspecies comparative studies. Variation in morphological and

morphometric characters within populations also can be used for the investigation of the impacts of biotic and abiotic factors on biological populations over time (Ballinger, 1977). In this survey, we aim to investigate morphological traits and morphometric variables of different populations of the genus *Pelophylax* in Iran. For geographical division, we followed and applied the distribution data of the two proposed separate taxa recognized based on mitochondrial data in Pesarakloo et al. (2017) and Safaei-Mahroo and Ghaffari (2020). Additionally, frequencies of qualitative characters within different geographical deme of the water frogs in Iran were evaluated. We also compared the variation of these character states between western and eastern populations of water frogs in Iran to assess suitable morphological characters for taxonomic identification.

Materials and methods

In this survey, 134 adult specimens were collected from different ecological habitats in Iran using hand-nets (Fig. 1 and Table 1). Sampling was done from different localities in Iran including the central and eastern parts of Iran, the type locality of *P. ridibundus susana*, and some localities in west Iran which were proposed as the distributional range of *P.* cf. *bedriagae* by Pesarakloo et al. (2017). The 26 museum samples were also included in our studies (Fig. 1 and Table 1). In total, the morphological variations of *Pelophylax* sp from different localities of Iran were studied based on 160 specimens, and 13 morphological and 24 morphometric characters.

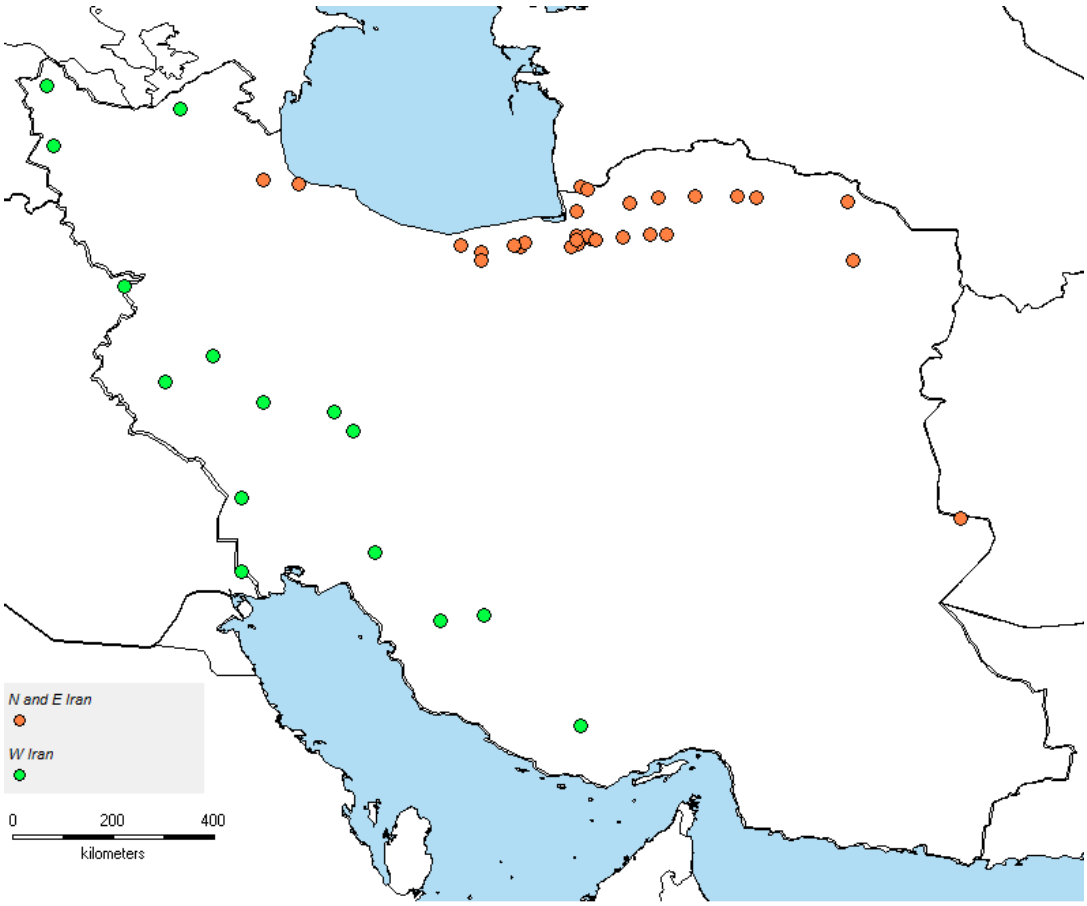


Figure 1. Map of localities and water frog populations in the west (green) and the north-east (red) of Iran

Table 1. List of specimens, numbers, localities, and geographic coordinates.

No.	Localities	Number	Longitude	Latitude
1	Kaleybar, E Azarbayejan	**4**	47.04	38.85
2	Maku, W Azarbayejan	**5**	44.6	39.27
3	Salmas, W Azarbayejan	**1**	44.71	38.19
4	Marivan, Kurdistan	**2**	46.01	35.62
5	Hamil Kermanshah	**1**	46.76	33.88
6	Sarabeleh, Kermanshah	**5**	47.62	34.36
7	Garmabeleh Olia, Lorestan	**4**	48.55	33.52
8	Sang-e Sefid, Lorestan	**1**	49.83	33.34
9	Barf Anbar, Isfahan	**7**	50.19	32.99
10	Bostan, Khuzestan	**4**	48.14	31.78
11	Khorramshahr, Khuzestan	**1**	48.16	30.43
12	Deh Dasht, Kohgiluyeh and Boyer-Ahmad	**2**	50.58	30.78
13	Ab Zangi Fars	**2**	52.56	29.63
14	Kazerun Fars	**1**	51.77	29.54
15	Tang-e Khor Fars	**1**	54.33	27.63
16	Lisar, Gilan	**2**	48.54	37.57
17	Masal, Gilan	**2**	49.18	37.5
18	Vostakola, Mazandaran	**1**	52.53	36.25
19	Mehrabanrud, Mazandaran	**8**	53.24	36.36
20	Behshahr**,** Mazandaran	**12**	53.32	36.42
21	Abshar Gazu, Mazandaran	**1**	52.51	36.1
22	Dashtenaz, Mazandaran	**2**	53.11	36.38
23	Mahmoodabad, Mazandaran	**1**	52.16	36.37
24	Torogh, Razavi Khorasan	**8**	59.31	36.1
25	Daregaz, Razavi Khorasan	**2**	59.2	37.16
26	Ashkhaneh, North Khorasan	**5**	56.43	37.26
27	Bojnord, North Khorasan	**1**	57.18	37.28
28	Shirvan, North Khorasan	**2**	57.54	37.24
29	Ramiyan1, Golestan	**7**	55.91	36.56
30	Ramiyan2, Golestan	**2**	55.11	36.52
31	Ramiyan3, Golestan	**3**	55.74	37.25
32	Ramiyan4, Golestan	**2**	55.6	36.57
33	Aq Qala1, Golestan	**12**	54.33	37.45
34	Aq Qala2, Golestan	**4**	54.27	37
35	Aq Qala3 , Golestan	**5**	54.45	37.4
36	Ziarat**,** Golestan	**2**	54.28	36.41
37	Alangdareh**,** Golestan	**3**	54.27	36.47
38	Jahan nama**,** Golestan	**7**	54.17	36.36
39	Mohammadabad, Golestan	**2**	54.25	36.54
40	Minoodasht, Golestan	**3**	55.22	37.14
41	Bandartorkman**,** Golestan	**3**	54.5	36.5
42	Aliabad**,** Golestan	**6**	54.46	36.54
43	Toshan**,** Golestan	**2**	54.26	36.47
44	Kordkuy**,** Golestan	**2**	54.6	36.48
45	Zabol, Sistan and Balouchestan	**7**	61.27	31.4

Analyses of morphological characters

Morphological characters, including the situation of the dorsolateral fold, ventral coloration, pigmentation pattern of ventral parts, dorsal coloration, dorsal spot pattern, marking on the dorsal surface of the hindlimb, pigmentation pattern of ventral parts of the thighs, lateral parts, internal metatarsal tubercle, coloration of manus, relative length of forelimb digits, relative length of hindlimb digits, and lateral view of the hindlimb (Table 2) were extracted from different publications (Balutch and Kami, 1995; Sinsch and Schneider, 1999; Krizmanić, 2008; Disi and Amr, 2010; Mohammadi et al., 2015; Pesarakloo et al., 2011, 2018; Bülbül et al., 2011; Plötner et al., 2012; Lukanov et al., 2018) including key characters described for different species of the genus *Pelophylax* e.g., *P. ridibundus, P. bedriagae*, *P. caralitanus* distributed in the Middle East and also the taxon *gigas*.

Analyses of morphometric characters

23 morphometric variables including snout-vent length (L), length of the head (LC); head width (HW); length of the tympanic membrane (L.tym); eye length (LO); distance from eye to the tip of snout (DRO); Eye-tympanum length (ETL); Eye-nostril distance (END); distance between lids (SP.P); Upper eyelid width (LT.P); interorbital distance (IO); width of rostrum (SP.C.R); distance between the nostrils (DN); inter naris length (InNar); length of the femur (LF); length of 1st digit of the forelimb (1stDF); length of the hindlimb (LH); length of the femur (F); length of the tibia (T); length of the metatarsal tubercle (C.int); width of 1st digit of the hindlimb (W1st D.h); length of the tibia (LTA); and length of the first toe (1stD.P). We followed character definitions and acronyms from Balutch and Kami, 1995; Krizmanić, 2008; Disi and Amr, 2010; Pesarakloo et al., 2011, 2018; Bülbül et al., 2011; Plötner et al., 2012; Mohammadi et al., 2015; Lukanov et al., 2018. The descriptive analyses have been performed in R v3.4.1 (R Core Team, 2019).

Table 2. List of morphological characters, character states and abbreviation of character states used in this study.

Character	Character state	Abbreviation of character state
The situation of dorsolateral fold	Bulged	I: 1
	Flat	I: 2
Ventral coloration	Dirty white	II: 1
	Milky white	II: 2
Pigmentation pattern of ventral parts	Spotted	III: 1
	Without any spot	III: 2
Dorsal coloration	Olive-green	IV: 1
	Gray	IV: 2
	Brown	IV: 3
Dorsal spot pattern	Jagged solid spots	V: 1
	Solid spots with smooth margins	V: 2
	Hollow spots	V: 3
Marking on dorsal surface of hindlimb	Tuberculate	VI: 1
	Not tuberculate	VI: 2
Pigmentation pattern of ventral parts of the thighs	Spotted	VII:1
	Without any spot	VII: 2
Lateral parts	Densely spotted	VIII: 1

	Not densely spotted	VIII: 2
Internal metatarsal tubercle	Oval	IX:1
	Rounded	IX: 2
Coloration of manus	Green olive	X: 1
	Brown	X: 2
	Gray	X: 3
Relative length of forelimb digits	3>4>1>2	XI: 1
	3>4>2=1	XI: 2
	3>4>2>1	XI: 3
Relative length of hindlimb digits	4>3>5>2>1	XII: 1
	4>3=5>2>1	XII: 2
Lateral view of hindlimb	Thick stripes on the lateral portion of the thighs and pes are along each other	XIII: 1
	Stripes do not meet and are not along each other	XIII: 2

Results

The comparison of 13 morphological and 23 morphometric characters of 160 *Pelophylax* samples (Tables 3 and 4) revealed extreme variation between character stats of examined samples from 45 sampling stations of different geographical localities in Iran.

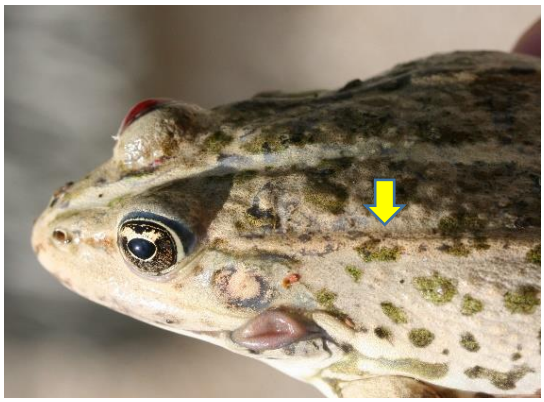
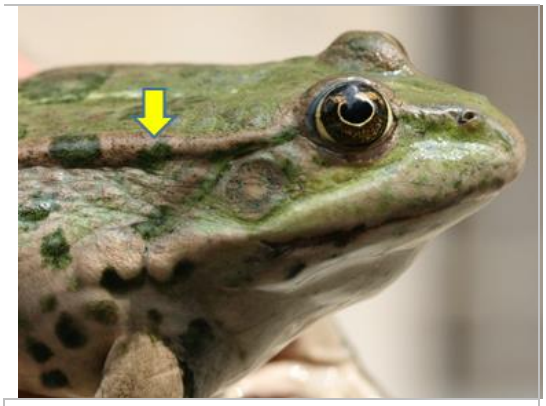
Morphological variations among different deme

Dorsal patterns show high variation even among individuals collected from the same locality e.g., different morphs; gray, green, with and without mid-dorsal lines were observed. Large dark dorsal spots and light mid-dorsal lines vary considerably in size. The dorsolateral fold is mostly bulged with the frequency of 65% in populations of *Pelophylax* in eastern Iran compared with %78 in populations of water frogs in west Iran. All specimens from Lorestan, Isfahan, and Kohgiluyeh, and Boyer-Ahmad in the west and also specimens from Gilan and North Khorasan from the east represents prominent dorsolateral fold. It is noteworthy that the specimens from southeast Iran, Zabol, Sistan, and Baluchestan demonstrated flat dorsolateral fold in all specimens except one. Dominant ventral coloration was milky white in 66% specimens from east vs. 71% in specimens from west Iran whereas the specimens of Kermanshah and most of the specimens from Khuzistan, west Iran were dirty white with pigmentation associated with throat and chest. The ventral coloration of all specimens from Eastern Azarbaijan, Western Azarbaijan, Kurdistan, Kohgiluyeh and Boyer-Ahmad, and Fars in the west and specimens from Gilan, North Khorasan, and Sistan from the eastern populations were completely milky white (Table 3). The pigmentation pattern of ventral parts are nearly similar (83% vs. 80% without any spot in eastern and western populations, respectively). Dorsal coloration is 66% green olive in the specimens from the east vs. 24% green olive in the western specimens. It is also 42% gray in western specimens while the frequency of gray dorsal color is 11% in eastern specimens. Dorsal color is 34% brown in the western populations and 24% brown in the eastern populations. It is noteworthy that 100% of specimens collected from Gilan and northern Khorasan represent olive-green dorsal color. The olive-green coloration is also dominated ≥75% specimens from Golestan and Khorasan Razavi while 100% specimens from Kermanshah have gray dorsal color and all specimens from Kohgiluyeh and Boyer-Ahmad and Fars have brown dorsal pigmentation. Dorsal spot pattern in 15% specimens of water frogs from the eastern parts is hollow spots but 61% is jagged solid spots and 23% is just solid spots with smooth margins while there are no hollow spots in populations of *Pelophylax* from western Iran. Lateral parts are densely spotted in the western populations of *Pelophylax* (76%) while it is just 52% densely spotted in the eastern populations. Internal metatarsal tubercles are oval (46%) and rounded (54%) in the western

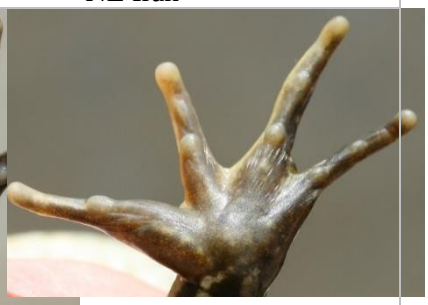
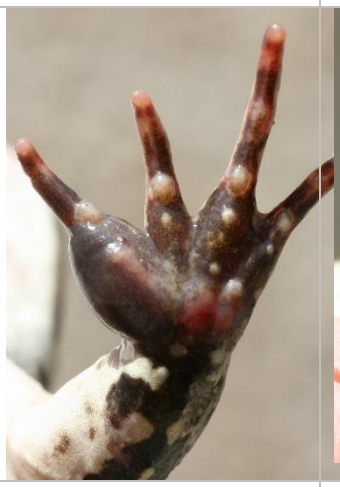
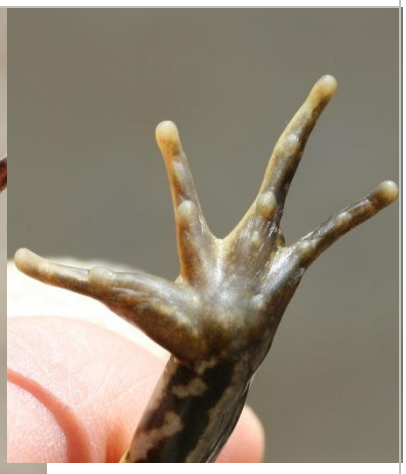
populations while they are oval (55%) and rounded (45%) in the eastern populations of water frogs from Iran. The dorsal surfaces of hind limbs tuberculate (32%) and not tuberculate (68%) in the western populations. In the eastern populations of the water frogs, the dorsal surfaces of hind limbs are 47% tuberculate and 53% not tuberculate. When metatarsals are under the abdomen (frog in the squatting position), thick stripes in the dorsal portion of the thighs and metatarsal stripes are along with each other (61%) while the stripes do not meet and are not along with each other (39%) in the western populations while they meet each other (53%) and are not along with each other (47%) in the eastern populations (Table 3). Relative length comparison of forelimb digits in the eastern populations of water frogs from Iran indicates that in 44% of the specimens third digit is longer than all other digits of the forelimb and the second digit is the shortest one (3>4>1>2), and in 48% specimens the first and the second digits are the same size (3>4>2=1) and just in 8% specimens the first digit is the shortest digit (3>4>2>1). In the western populations 71% of specimens represent the second digit as the shortest one on the forelimb (3>4>1>2) and in 22% of specimens the first and the second digits are the same size (3>4>2=1) while 7% specimens have the first digit of the forearm as the shortest one (3>4>2>1; Table 3).

Morphometric variations

Morphometric data analyses revealed *Pelophylax* sp. from east Iran is larger than the size of the specimens from west Iran in all morphometric variables (Table 4). Tukey's test revealed significant differences between most of the transformed metric morphometric variables (except for L.tym, LO, ETL, LT.P, DN, InNar, W1st D.h) were significant between two populations ($P \leq 0.05$), implying that *Pelophylax* sp. is morphologically different from the specimens from west Iran by its larger body (Table 4).

I	 **Flat:** Ziarat Gorgan, NE Iran	 **Bulged:** Kermanshah, W Iran	
II	 **Milky white:** Kermanshah, W Iran	 **Dirty white:** Ashkhaneh, North Khorasan, NE Iran	
III	 **Spotted:** Kermanshah, W Iran	 **Without any spot:** Toroq Dam, Razavi Khorasan, NE Iran	
IV	 **Gray:** Ziarat Gorgan, NE Iran	 **Brown:** Kermanshah, W Iran	 **Olive-green:** AlangDareh, Gorgan, NE Iran

			
V	**Hollow spots:** Toroq Dam, Razavi Khorasan, NE Iran	**Solid spots with smooth margins:** Toroq Dam, Razavi Khorasan, NE Iran	**Jagged solid spots:** Ziarat Gorgan, NE Iran
			
VI	Not tuberculate: **Kermanshah, W Iran**	Tuberculate**: Ashkhaneh, North Khorasan, NE Iran**	
			
VII	**Without any spot**: Ashkhaneh, North Khorasan, NE Iran	**Spotted:** AlangDareh, Gorgan, NE Iran	
			
VIII	**Not densely spotted:** Toroq Dam, Razavi Khorasan, NE Iran	**Densely spotted:** Kermanshah, W Iran	

		
IX	**Oval:** Kermanshah, W Iran	**Rounded:** Toroq Dam, Razavi Khorasan, NE Iran
		 
X	**Brown:** Ashkhaneh, North Khorasan, NE Iran	**Gray:** Toroq Dam, Razavi Khorasan, NE Iran	**Green olive:** Kermanshah, W Iran
		 
XI	**3>4>2>1:** Ashkhaneh, North Khorasan, NE Iran	**3>4>2=1:** Toroq Dam, Razavi Khorasan, NE Iran	**3>4>1>2:** Kermanshah, W Iran

		
XII	**4>3=5>2>1:** Kermanshah, W Iran	**4>3>5>2>1:** Toroq Dam, Razavi Khorasan, NE Iran
		
XI II	**Stripes do not meet and are not along each other:** Kermanshah, W Iran	**Thick stripes on the lateral portion of the thighs and pes are along with each other:** Kermanshah, W Iran

Fig. 2. Variation in shapes and positions of the 13 morphological characters analysed in 160 specimens of Water frogs from Iran (photos by H.G.K).

Table 3. Percentage of morphological variations based on 13 characters and 160 adult specimens of the Iranian frogs in the different localities.

	West										East						
locality	**EAz**	**WAz**	**Kor**	**Ker**	**Lor**	**Isf**	**Khu**	**Koh**	**Far**	**Total**	**Gil**	**Maz**	**Gol**	**NKh**	**RKh**	**Sis**	**Total**
NO.	**4**	**6**	**2**	**6**	**5**	**7**	**5**	**2**	**4**	**41**	**4**	**25**	**65**	**8**	**10**	**7**	**119**
I:1	75	83	50	66	100	100	60	100	50	78.0	100	75	60	100	70	15	64.7
I:2	25	16	50	33	0	0	40	0	50	22.0	0	25	40	0	30	85	35.3
II:1	0	0	0	100	40	0	80	0	0	29.3	0	10	55	0	20	0	33.6
II:2	100	100	100	0	60	100	20	100	100	70.7	100	90	45	100	80	100	66.4
III:1	0	50	0	66	0	0	20	0	0	19.5	50	25	10	0	0	70	16.8
III:2	100	50	100	33	100	100	80	100	100	80.5	50	75	90	100	100	30	83.2
IV:1	25	33	50	0	40	28	40	0	0	24.4	100	30	75	100	80	14	65.6
IV:2	25	33	50	100	40	71	0	0	0	41.5	0	0	15	0	0	43	10.9
IV:3	50	33	0	0	20	0	60	100	100	34.1	0	70	10	0	20	43	23.5
V:1	50	83	100	83	40	42	100	50	25	63.4	100	90	60	75	10	0	60.5
V:2	50	16	0	16	60	57	0	50	75	36.6	0	0	30	12.5	50	30	24.4
V:3	0	0	0	0	0	0	0	0	0	0	0	10	10	12.5	40	70	15.1
VI:1	75	83	100	83	80	85	100	50	25	78.0	100	65	85	100	100	70	83.2
VI:2	25	16	0	16	20	14	0	50	75	22.0	0	35	15	0	0	30	16.8
VII:1	25	50	50	66	20	28	20	0	0	31.7	75	70	50	0	0	60	47.1
VII:2	75	50	50	33	80	71	80	100	100	68.3	25	30	50	100	100	40	52.9
VIII:1	75	83	100	83	60	100	100	0	25	75.6	75	55	55	40	20	70	52.1
VIII:2	25	16	0	16	40	0	0	100	75	24.4	25	45	45	60	80	30	47.9
IX:1	50	50	100	66	40	28	40	50	25	46.3	50	65	45	90	100	30	55.5
IX:2	50	50	0	33	60	71	60	50	75	53.7	50	35	55	10	0	70	44.5
X:1	50	33	50	0	20	71	40	0	0	31.7	50	55	70	90	50	0	62.2
X:2	50	66	50	100	80	28	60	100	100	68.3	50	20	25	0	50	100	30.3
X:3	0	0	0	0	0	0	0	0	0	0	0	25	5	10	0	0	7. 6
XI:1	75	66	100	33	100	85	100	0	50	70.7	50	30	55	0	20	40	43.7
XI:2	0	16	0	66	0	14	0	50	50	22	50	55	35	100	70	60	47.9
XI:3	25	16	0	0	0	0	0	50	0	7.3	0	15	10	0	10	0	8.4
XII:1	100	100	100	66	100	71	80	50	50	80.5	100	90	85	87.5	100	100	89.1
XII:2	0	0	0	33	0	28	20	50	50	19.5	0	10	15	12.5	0	0	10.9
XIII:1	50	66	50	82	80	56	60	50	25	61.0	0	60	50	50	100	0	52.9
XIII:2	50	33	50	16	20	42	40	50	75	39.0	100	40	50	50	0	100	47.1

Table 4. Univariate summaries of morphometrical characters for the genus Pelophylax from west and east Iran. Abbreviations: snout–vent length (L), length of the head (LC); head width (HW); length of the tympanic membrane (L.tym); length of eye (LO); distance from eye to tip of snout (DRO); Eye-tympanum length (ETL); Eye-nostril distance (END); distance between lids (SP.P); Upper eyelid width (LT.P); interorbital distance (IO); width of rostrum (SP.C.R); distance between the nostrils (DN); inter naris length (InNar); length of the femur (LF); length of 1st digit of the forelimb (1stDF); length of the hindlimb (LH); length of the femur (F); length of the tibia (T); length of the metatarsal tubercle (C.int); width of 1st digit of the hindlimb (W1st D.h); length of the tibia (LTA); length of the first toe (1stD.P); characters (Ch.); standard deviation (Sd); minimum (min); and maximum (max)

	N & E Iran (n=119)			W Iran (n=41)			
Ch.	**Mean±Sd**	**min**	**max**	**Mean±Sd**	**min**	**max**	**Tukey test: P value**
L	70.44±11.97	50.18	106.46	64.25±١١,٣٥	50.24	91.11	***
LC	24.61±3.46	17.33	35.65	23.02± 3.78	17.85	31.11	*
HW	21.34± 3.62	14.55	32.82	19.70±3.45	15.57	28.17	*
L.tym	5.69±0.90	4.01	8.88	5.19±1.16	3.80	9.14	-
LO	6.51±1.06	3.73	9.00	6.29±1.19	4.49	9.09	-
DRO	10.50±1.54	7.32	15.79	9.81±1.53	7.40	14.05	*
ETL	3.05± 0.89	1.43	6.03	2.61±0.81	1.16	4.81	-
END	4.47±0.82	2.63	6.83	3.86±0.83	2.36	6.21	*
SP.P	4.07±1.07	2.21	7.93	3.19±0.86	1.81	6.19	*
LT.P	4.83±0.95	2.36	7.79	4.35±0.96	3.15	6.88	-
IO	12.45±2.05	8.26	19.19	11.01±1.91	7.22	15.77	*
SP.C.R	9.17±1.42	6.15	13.76	8.60±1.36	6.60	12.51	*
DN	3.55±0.73	1.66	5.89	3.13±0.69	2.05	4.77	-
InNar	6.53±0.97	4.24	8.89	6.13±1.06	4.71	9.46	-
LF	18.04±3.07	9.21	28.73	16.05±3.17	10.68	24.31	***
1stDF	9.04±1.79	5.27	13.87	8.21±1.91	5.60	14.25	*
LH	36.99±6.04	25.57	56.77	33.50±5.40	25.23	48.54	***
F	33.79±6.39	20.97	53.50	31.16±5.78	22.85	47.06	**
T	37.05± 6.22	25.08	54.36	33.27±5.80	25.80	48.37	***
C.int	4.00±0.91	2.24	6.81	3.48±0.78	2.56	5.89	*
W1st D.h	1.35±0.41	0.60	2.93	1.07±0.42	0.57	2.13	-
LTA	20.31±3.64	6.92	31.25	18.12±3.27	13.54	25.47	**
1stD.P	9.52±1.60	5.63	13.45	8.25±1.54	5.78	13.57	*

***P<0.001; **P<0.01; *P<0.05

Discussion

Water frogs from Iran have been assigned to *P. ridibundus* sensu lato based on classical taxonomy, however recent advances in modern molecular methods led to taxonomic revisions of the water frogs from Iran and divided them into two separate groups including western Iranian frogs *P.* cf. *bedriagae* and eastern Iranian water frogs *Pelophylax* sp (Pesarakloo et al., 2017; Pesarakloo et al., 2018). Nevertheless, the taxonomy of these two groups has still encountered many challenges due to applying just mitochondrial marker, and morphological variation which cannot make any conclusion regarding possible hybridization. Indeed, these morphological variations cause uncertainties in the species determination in the genus *Pelophylax* (Dufresnes, et al. 2017; Kolenda et al., 2017). Polymorphism in the water frogs has been reported before in Iran (Pesarakloo et al., 2011; Delavar Sheyda Jalali et al., 2017). Jazayeri and Saberi (2018) analyzed the polymorphism of *P. ridibundus* sensu lato in Khuzistan and concluded that the frequency of olive-green morph is higher than other morphs in the region however it was not consistent with our results.

Morphological and morphometric analyses revealed discordance in *Pelophylax* populations from east and west Iran. In concordance to Mohammadi et al. (2015), morphometric results showed the adult specimens of *Pelophylax* sp. from east Iran represent a higher average in all morphometric characters compared with the size of the specimens from west Iran.

The morphological traits and coloration in the genus *Pelophylax* have been resulted from adaptation to various ecological habitats and also introgression and demonstrated high variability and have low taxonomic values (Papežík et al., 2021). Described characters for *P.* cf. *bedriagae* and *P. ridibundus* sensu stricto are very similar and the taxon *gigas* is still controversial due to its assignment in the genus *Pelophylax* (see Smith et al. (1977), Dubois and Ohler, (1996) and all references reviewed therein). Our result revealed that melanistic diversity in water frogs is much more influenced by compatibility to special habitat and is not very valid as a taxonomic trait or key characters to be used for identification. Our analyses indicated that interspecies morphological variations within western Iranian water frogs *P.* cf. *bedriagae* may have been caused by environmental conditions, level of humidity, habitat temperature, vegetation, and probable hybridization.

In addition, different species of water frogs may produce hybrid zones so-called population systems while they are in contact or close geographical proximity (Dedukh et al., 2015; Hoffmann et al., 2015; Doležálková-Kaštánková et al., 2018; Dedukh et al., 2019). Also, some studies confirmed the presence of hybrid forms between *P. ridibundus* and *P.* cf. *bedriagae* (Holsbeek et al., 2008; Vershinin et al., 2019) which caused disparity in morphological characters.

Following generalized Gloger's rule, comparison of character states and climatic conditions of different geographical regions inhabited by water frogs under study revealed that dorsal coloration of water frog populations become paler from north to south Iran, while the ventral part shows low variation in color which indicates the low selective value of ventral coloration in the viability of water frog in Iran. Gloger's rule indicates that in animals living in much more humid habitats there is a tendency for heavy pigmentation (Gloger, 1833). Although the rule implies endothermic animals the generalized rule has also been applied on ectotherms and insects (Zheng et al., 2015; Goldenberg, 2021). Moreover, natural selection stabilized olive coloration on the populations of water frogs living in the green habitats of north Iran while compromising light

grey and dark brown in contrasting ecosystems through the semi-desert and humid oases of south Iran via a disruptive selection mechanism.

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