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RESEARCH ARTICLE **Open Access**

Assessment of Population Status and Diurnal Behaviour of White-rumped Vulture, *Gyps bengalensis* (Gmelin, 1788), in Ghachowk, Kaski, Nepal

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Abstract
Vulture Safe Feeding Sites (VSFS) provide diclofenac-free food and create an ideal environment for long-term ecological research on vultures. This study determines the population status and investigates the diurnal behavior of White-rumped Vulture (*Gyps bengalensis*) at the Ghachowk VSFS in Kaski, Nepal. We deployed the absolute count method to determine population of White-rumped Vulture (*Gyps bengalensis*), while scan sampling was applied to investigate their diurnal behaviour. Throughout the daytime, a total of 1651 instances of 11 distinct activities were recorded for the species in 729 separate observations. The most frequent daytime behaviour observed was perching (28%) followed by preening (14%), basking (13%), scratching (13%) flapping (10%), flying (9%) and disturbance (7%). Other behaviors, like feeding, sleeping (roosting), fighting (competition), and bathing, were observed less frequently (<2% each). Perching occurred most frequently during heavy rain, accounting for 66% of the observations, and in the late afternoon, representing 36% of the instances. Basking was most commonly observed during partly sunny conditions, comprising 26% of the observations, and in the noon hours, representing 20% of the instances. Scratching behavior was most frequently observed during clear weather, accounting for 16% of the observations, and during cloudy conditions and in the morning, accounting for 14% of the instances. In the Ghachowk VSFS, other associated species with White-rumped Vulture were Red-headed Vulture *Sarcogyps calvus*, Egyptian Vulture *Neophron percnopterus*, and Slender-billed Vulture *Gyps tenuirostris*.

Keywords: *behavioral study, critically endangered species, vulture feeding sites*

INTRODUCTION

Vultures found in Nepal are obligatory scavengers that belongs to Accipitride family and the Falconiformes order (Ward et al., 2008, Purohit & Saran, 2013). Vultures have unique adaptation to extremely low pH levels in their stomachs (Ogada et al., 2012) and digest carrion; both fresh and rotten

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dead animals easily (Harris, 2013). Among the nine species of vultures viz. White-rumped Vulture (*Gyps bengalensis*), Slender-billed Vulture (*Gyps tenuirostris)*, Red-headed Vulture (*Sarcogyps calvus*), Indian Vulture (*Gyps indicus*), Egyptian Vulture (*Neophron percnopterus*), Bearded Vulture (*Gypaetus barbatus*), Himalayan Griffon (*Gyps himalayensis*), Cinereous Vulture (*Aegypius monachus*) and Griffon Vulture (*Gyps fulvus*) recorded from Nepal. White-rumped Vulture (WRV) is a resident species which is categorized as critically endangered at both national and global level (Inskipp et al., 2016, IUCN, 2023). The earlier studies on the population status of WRV depicts extremely high decline rate of the species in Nepal. For example, Chaudhary et al., (2012) reported 91% decreases in populations of the White-rumped Vulture in between 2000 and 2011 from the lowlands of Nepal. The primary cause for such catastrophic decline of vultures including WRV was the use of veterinary drug diclofenac for curing pain and inflammations in livestock (Oaks et al., 2004, Harris, 2013).

To provide diclofenac free food to vulture, Government of Nepal in collaboration with local communities, Bird Conservation Nepal have established seven Community Based Vulture Safe Feeding Sites (VSFS), which are also referred to as Jatayu restaurants or Vulture restaurants. The primary objective of these establishments is to offer Diclofenac-free food to vultures and create a suitable environment for conducting long-term ecological research on vultures. These VSFS acquire elderly and unproductive livestock from nearby communities, carefully storing them for a minimum of seven days to ensure they are free from diclofenac. Subsequently, the vultures are provided with these livestock after their natural demise (Paudel, 2012, Bhusal, 2018, DNPWC and DoFSC, 2023). In addition to this ban on the use of diclofenac for curing livestock and promotion of the vulture safe drug i.e., Meloxicam; declaration of vulture safe zone, captive breeding and release into the wild of WRV were the other major efforts intervened in Nepal for the conservation of this ecologically crucial species. Consequently, the wild population of this species seems to be partially recovered as illustrated by the monitoring data between 2013 to 2018 (Galligan et al., 2018). However, the species requires continuous monitoring in its prehistoric and potential habitats to ensure this partial recovery is not destroyed as there are other many emerging threats recognized as vital for the destruction of this population. For example, the use of six NSAIDs, namely ketoprofen, nimesulide, aceclofenac, carprofen, phenylbutazone, and flunixin, has been examined in various studies, including those focused on habitat loss and destruction, poisoning through baits and pesticides, electrocution, and collisions (Margalida et al., 2014; Rana et al., 2019; Bhusal et al., 2020; Galligan et al., 2020). This study illustrates the population status and examine the daily habits of the WRV at the Ghachowk Vulture Safe Feeding Site in Kaski, Nepal which is also the historical habitat for feeding, roosting and nesting of WRV.

MATERIAL AND METHODS

Study area

The Ghachowk Vulture Safe Feeding Site is situated in Ghachowk, specifically in Ward no. 3 of the Machhapuchhre Rural Municipality (Figure 1). It is located within the Annapurna Conservation Area, approximately 12 kilometers away from center of Pokhara city. The site's geographical coordinates are 28.304488° N Latitude and 83.945792° E Longitude, with an average elevation of 1074 meters above sea level. It was established in 2010 encompassing an area of 100 ropani (BCN, 2011). The site falls under the subtropical zone, with typical minimum and maximum temperatures ranging from -2 to 15 °C and 25 to 35 °C, respectively, with an annual rainfall of 3951.5 millimeters. In the study area, various tree species such as *Cedrela toona*, *Engelherdia spicata*, *Pinus roxburghii*, *Sapium insigne*, *Dalbergia sissoo*, *Bombax ceiba*, *Alnus nepalensis*, *Schima wallichii* and *Aegle marmelos* are commonly found.

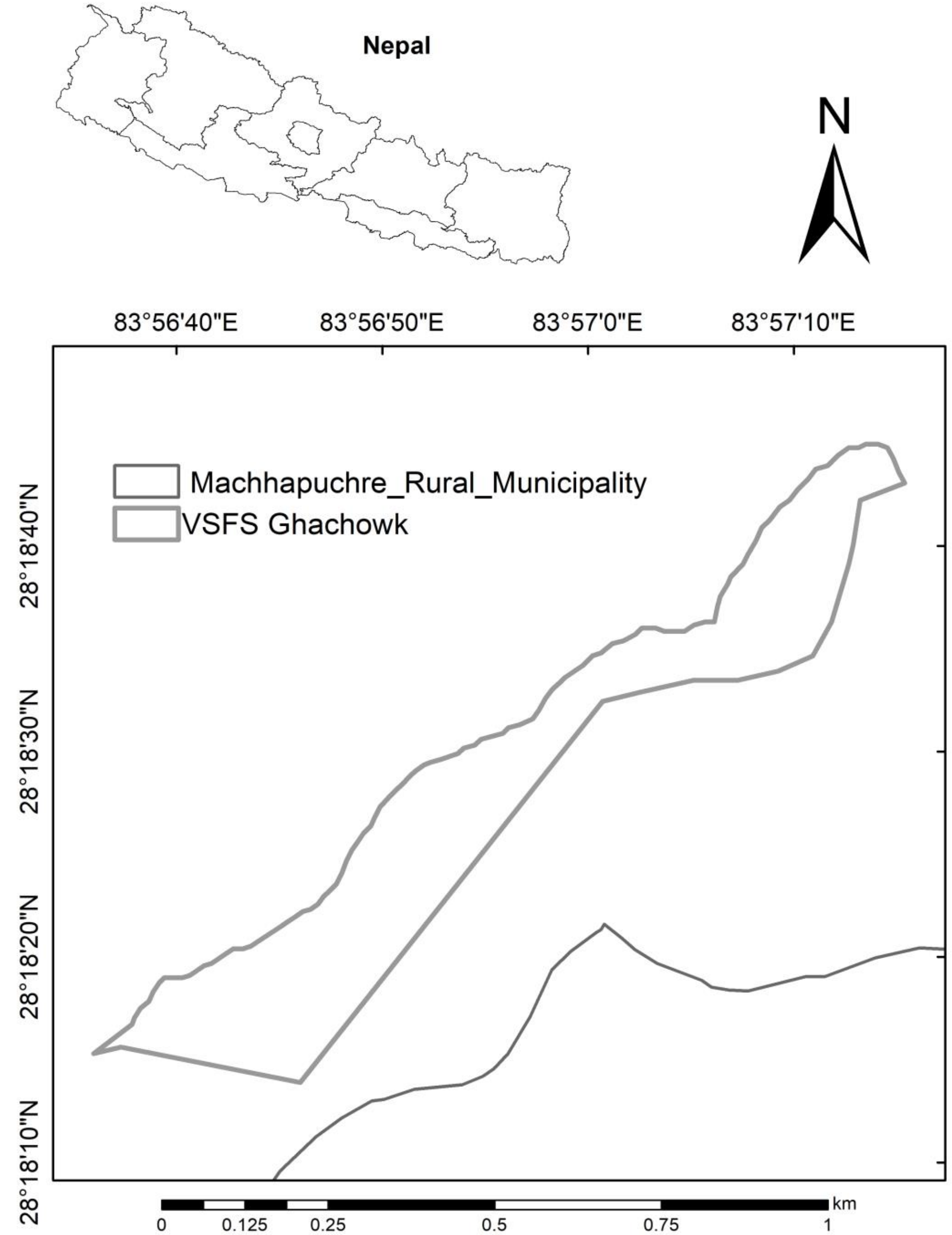

FIGURE 1. Location map of study area.

Methodology of diurnal behaviour

Systematically developed scan sampling method was used to acquire generic diurnal behaviour data (Altmann, 1974). A distinct flock of WRV was selected for behavior observation, which could be subsequently recorded on a data sheet. If the observed vulture was out of sight, another vulture from the same flock was observed and the recording continued in the same data sheet (Manandhar et al., 2019). Scan sampling consisted of two minutes of scanning activity followed by five minutes of break before the next scan was started. The vulture's general daytime behaviour was recorded for 15 days, with a total scan length of 82 hours and 48 minutes. The behaviour data was collected for a total of 729 times of observation, 538 times for adult WHV and 191 times for sub-adult WHV. A timer was used to measure the activity, and weather conditions were also observed and recorded.

Data analysis

The frequency of 11 different behaviours (Table 1) was computed by dividing the number of observations of each behaviour by the total number of observations (Table 1). To better comprehend weather-related activities, weather conditions were classified as sunny, partly sunny, clear weather (neither sun nor cloud), cloudy, sunny with light rain, light rain and heavy rain. To understand the activities in relation to the time of day, the day was divided into five parts: early morning (6:30 hrs to 8:30 hrs), morning (8:30 hrs to 11:00 hrs), noon (11:00 hrs to 13:00 hrs), afternoon (13:00 hrs to 15:30 hrs), and late afternoon (15:00 hrs to 17:30 hrs). Behavior records were included in the analysis which were observed for 2 minutes.

For data analysis, the Statistical Package for Social Sciences Statistics (SPSS) 20.0 edition was used. Chi square testings were performed to determine whether differences in observed vulture behaviour were likely to be associated with changes in weather conditions.

TABLE 1. Description of the daytime behaviours (activities) of vultures.

SN	Behaviour	Description
1	Feeding	Eat dead and decaying bodies
2	Resting	Wings wrapped around
3	Basking (sunning)	Stretch the neck and spread the wings fully (sometimes wing spread to only one side)
4	Scratching	Slightly scratching the body parts with the help of beak
5	Disturbance	By other vultures/chasing
6	Fighting/aggression	Any action with another vulture (competition)
7	Flight (flying)	Within the vulture safe feeding site
8	Flap	Fanning of wings
9	Sleeping (roosting)	Eyes closed
10	Bathing	Immerse wings, submerge head, stood straight in water and beaten
11	Preening	Use of the beak to position feathers

RESULTS

Diurnal behavior of WRV

In total, 1651 events of of White-rumped Vulture were observed during the daytime (6:30 hrs to 17:30 hrs) in 729 observations. The most frequent day time behaviour observed was resting (28%) followed by preening (14%), basking (13%), scratching (13%) flapping (10%), flying (9%) and disturbance (7%). Other behaviours, like feeding, sleeping, fighting, and bathing, were observed less frequently (<2% each) (Figure 2).

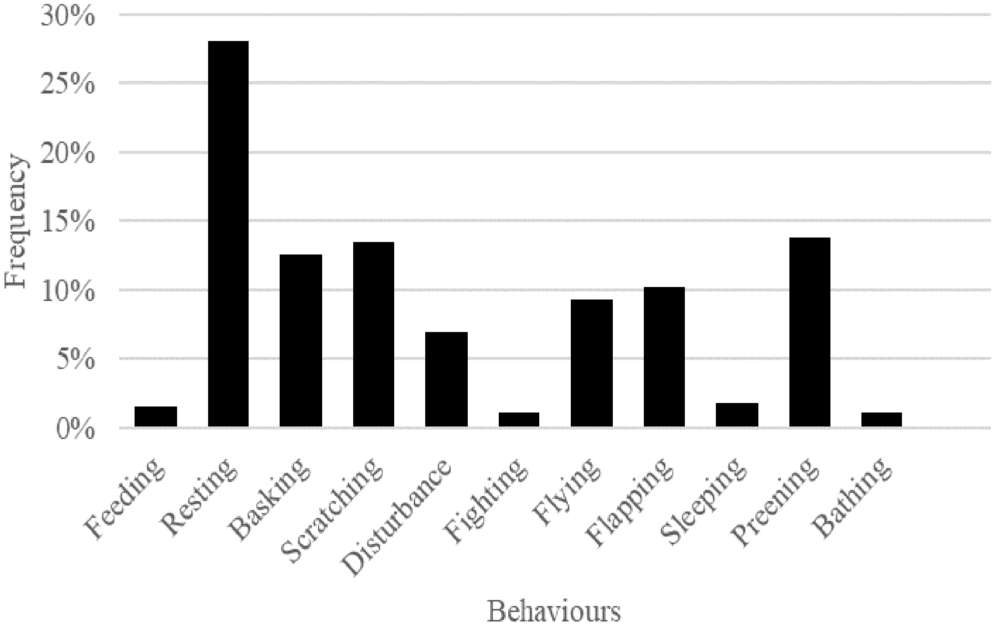

FIGURE 2. Daytime behaviour of WRV.

Behaviour pattern of WRV in response to weather

According to the Chi-square test, all ages of WRV's ($\chi2$ cal.=121.35 $p < 0.05$) behaviour depended on weather conditions, including adults (adult; $\chi2$ cal.=156.53) and sub-adult ($\chi2$ cal.=81.65 $p < 0.05$).

Sub-adult and adult WRV behaviour in response to weather

Resting was recorded in all-weather conditions and was most frequent in heavy rain (66%) followed by light rain (46%) and they were resting less during partly sunny (13%). Basking was most frequently (26%) recorded during partly sunny followed by sunny, with light rain (18%) and no basking (0%) during heavy rain. Scratching was the most frequent and equal during clear weather and cloudy (16%) followed by partly sunny. During clear weather conditions, disturbance behaviour was reported the most (10%) followed by cloudy, sunny and sunny, with light rain (7%). Disturbance was less frequent (1%) during light rain. Flight was most frequent during sunny with light rain (14%), followed by partly sunny and clear weather (13% each). There was no flight recorded during heavy rain. Flapping was the most frequent (13%) during light rain and least frequent (5%) during heavy rain. Preening was most frequent (18%) during light rain and less frequent during heavy rain (11%). Other behaviours such as feeding, fighting, sleeping, and bathing were almost the same with very less frequency (Figure 3a).

Adult WRV behaviour in response to weather

Resting was again the most frequent in adult WRV with 64%, followed by sunny with light rain (49%) and light rain (47%). They took less rest (12%) during clear weather. Basking was most frequent (21%) during partly sunny and less (0%) during heavy rain. Most frequent scratching (17%) was during clear weather and cloudy and less frequent (0%) during sunny with light rain. Disturbance was most frequent (9%) during clear weather and less frequent (0%) during sunny with light rain. Flight was most frequent (22%) during sunny with light rain and no flight (0%) during heavy rain. Flap was most frequent (13%) during cloudy and non (0%) during sunny with light rain. Preening was frequent (22%) during light rain and less frequent (12%) during heavy rain. Preening was almost similar in all weather conditions. Other activities were most frequent during clear weather (Figure 3b).

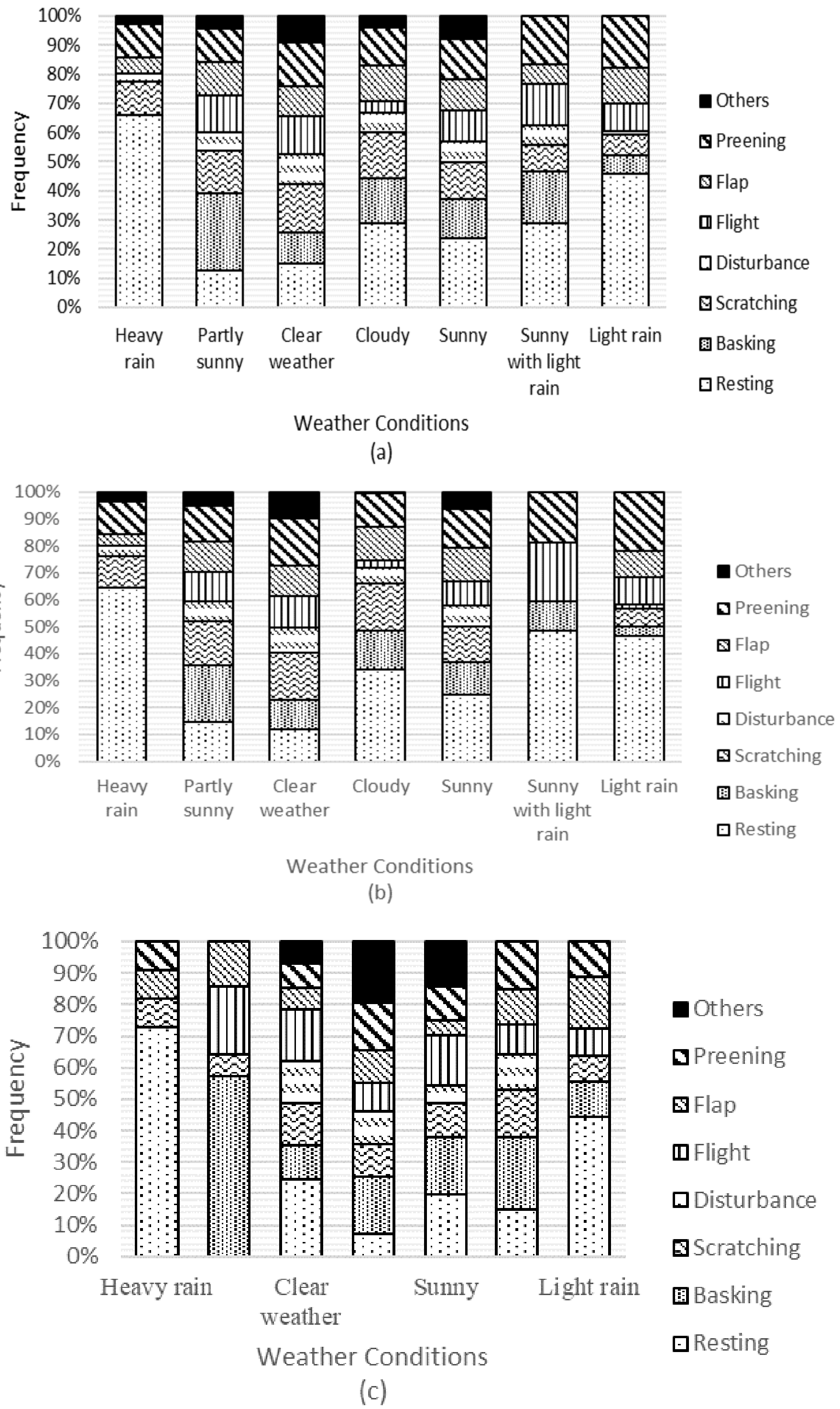

FIGURE 3. WRV behaviour in response to weather condition (a) adult & sub-adult frequency (b) Adult frequency & (c) Sub-adult frequency.

Sub-adult WRV behaviour in response to weather

Resting was again the most frequent in sub-adult WRV with 66% followed by light rain (46%). They took less rest (13%) during partly sunny. Basking was most frequent (26%) during partly sunny and less (0%) during heavy rain. Most frequent scratching (16%) was equal during clear weather and cloudy and less frequent (7%) during light rain. Disturbance was most frequent (10%) during clear weather and less frequent (1%) during light rain. Flight was most frequent (14%) during sunny with light rain and no flight (0%) during heavy rain. Flap was most frequent (13%) during light rain and less (5%) during heavy rain. Preening was frequent (18%) during light rain and less frequent (11%) during heavy rain. Preening was almost similar in all weather conditions the same as in adult. Other activities were most frequent (9%) during clear weather (Figure 3c).

WRV behaviour pattern in response to the time of day

Adult and Sub-adult WRV behaviour in response to Time of day

WRV was observed resting most frequently (36%) in the late afternoon and (35%) during the early morning and least (22%) in noon. Basking was most frequent (20%) during noon and least (9% each) in early morning and late afternoon. Scratching was observed the most (14%) in the morning and noon and least during the late afternoon (12%). Other vultures disturbed the WRV the most (9%) in the afternoon and least (6% each) in the morning and late afternoon. Flights were more frequent (11% each) at noon and late afternoon and less frequent (6%) in the early morning. Maximum (12%) flapping occurred during early morning, morning and late afternoon and minimum flap (8%) were recorded between noon and late afternoon. Preening was frequent (17%) during the early morning and less frequent (11% each) during noon and late afternoon. Other activities were most frequent during the morning session (Figure 4a).

Adult WRV behaviour in response to time of the day

Adult WRV was observed resting most frequently (37%) in the late afternoon, followed by (33%) in early morning and least (25%) in morning. Basking was most frequent (18%) during noon and least (8%) in the early morning. Scratching was observed the most (16%) in early morning and least (13% each) in the morning and late afternoon. Other vultures disturbed the WRV the most (9%) in the afternoon and least (5% each) in the early morning. Flights were more frequent (12%) at noon and less frequent (6% each) in the early morning and late afternoon. Maximum (13%) flapping occurred during the late afternoon and minimum flaps (8%) were recorded in afternoon. Preening was frequent (19%) during the early morning and less frequent (12%) during the late afternoon. Other activities were most frequent during the morning session (Figure 4b).

Sub-adult WRV behaviour in response to time of the day

Sub-adult WRV was observed resting most frequently (38%) in the early morning, followed by (34%) in the late afternoon and least (6%) in noon. Basking was most frequent (29%) during the afternoon and least (8%) in the morning. Scratching was observed the most (14%) in the morning and noon and least (3%) in the late afternoon. Other vultures disturbed the WRV the most (10%) in the afternoon and non (0% each) in the late afternoon. Flights were more frequent (41%) at the late afternoon and less frequent (5%) in the early morning. Maximum (13%) flapping occurred during the early morning and minimum flap (3%) were recorded in the late afternoon. Preening was frequent (19%) during the morning and less frequent (5%) during afternoons. Other activities were most frequent during noon (Figure 4c).

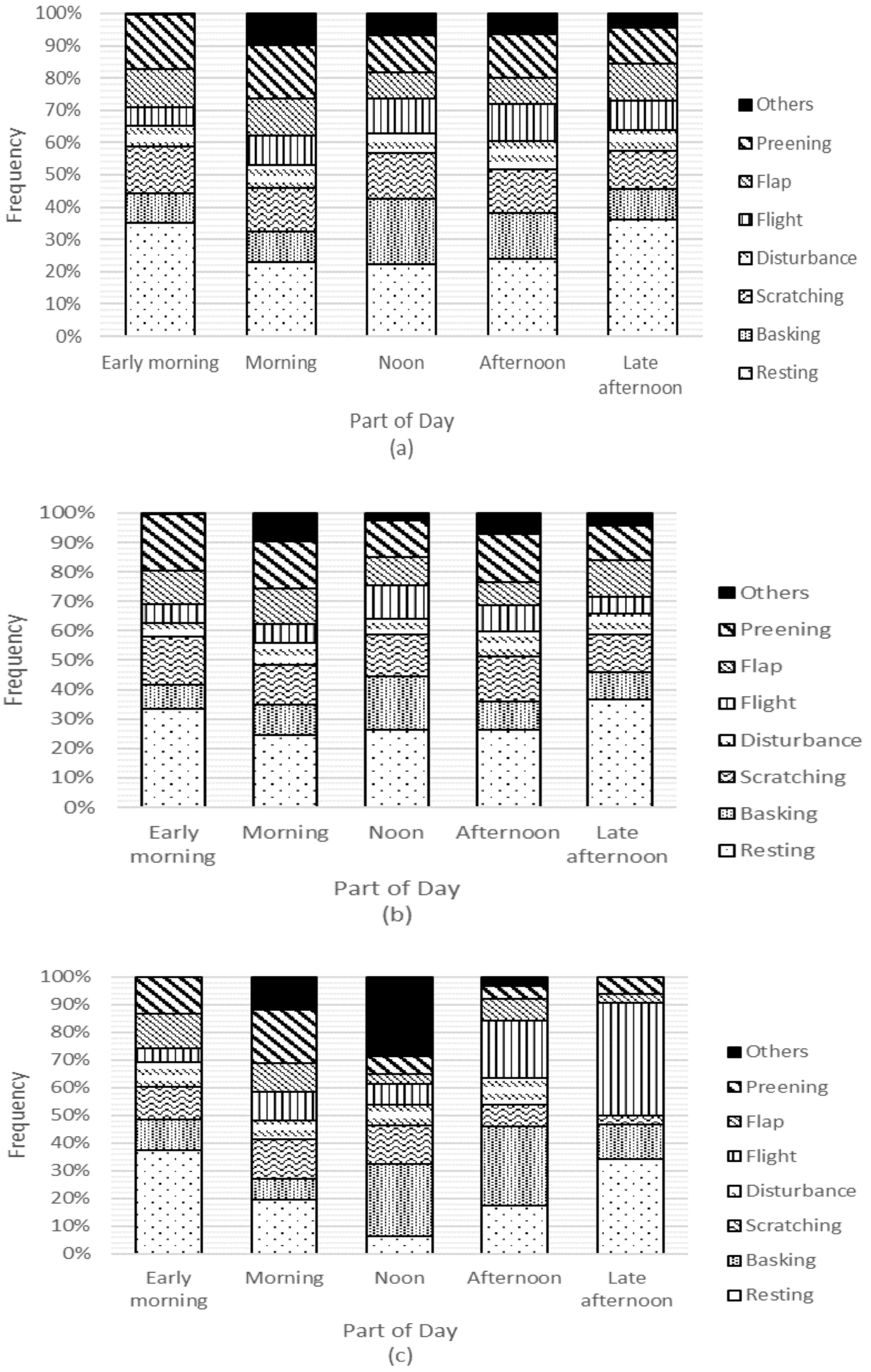

FIGURE 4. Frequency of WRV behaviour (a) adult and sub-adult, (b) adult, and (c) sub-adults at different times of the day

DISCUSSION

Daytime behaviour of WRV

During the day, the WRV displayed a variety of behaviours with varying frequencies. Resting, preening, scratching, basking, flying, flap, and disturbance were all common in the study area. Resting was observed as one of the most common behaviours in WRV (Khatri, 2016). As per Khatri's research in 2016, it was found that basking accounted for the drying of approximately 70-80 percent of the moisture, while the remaining 20-30 percent was evaporated through the exposure to direct sunlight in the air. Our research found basking behaviour in trees, cliffs, and on the ground (bank of Seti River). All raptors appear to like sunbathing, not only for the warmth it gives, but also for the likely additional therapeutic effects. They frequently turn their back to the sun, fan their tails, and partially or completely spread their wings. In our study, WRV expanded their wings entirely or partially to warm up their bodies most of the time, but they did not flap their wings during rains.

Resting was observed in all weather conditions and times of the day. Similarly, scratching and preening behaviours were predominantly noticed during resting time. Raptors preen on a regular basis, not just after a bath, and will frequently interrupt a preening session by "rousing" or rising and vigorously shaking the entire plumage, or by stretching the legs and wings, usually on one side and then the other. The WRV was found disturbed by its competitor vultures while feeding and resting. Vultures wait for thermals to aid in their foraging behaviour, yet Red-headed Vultures were reported waiting near the carcass to feed it after the WRV and Slender-billed Vulture had left it (Gbogbo & Awotwe-pratt, 2008).

Information about behaviours such as drinking, bathing, and sunbathing does not have a high merit to be included in a literature. However, these behaviours are critical for maintaining the hygiene and health of birds (Tributsch, 2016), and several aspects of vulture behaviour remain a mystery. Bathing occurs on warm days (up to 34°C) as well as on cold days (down to 16°C). Vultures circle above the bathing area. According to earlier research, vultures have a unique ability to regulate their body temperature through urohydrosis (Houston, 1994; Snyder & Snyder, 1991) . Because vultures' lives depend on the efficacy of their wings, feather upkeep is critical for these birds (Houston, 1994). Vultures bathe in streams, shallow ponds, or in the rain, which washes their feathers and may aid in their upkeep, particularly during rainy seasons (Ward et al., 2008).

CONCLUSION

Gyps bengalensis were recorded in the highest numbers. Resting (perching), preening, basking, scratching, and flapping were the most frequent day-time behavior observed in *Gyps bengalensis* among 11 different types of activities. Day time behavior of different ages was significantly depending on the weather conditions and part of the day time. WRV rest most frequently in the late afternoon, basking was most frequent at noon, and scratching was most frequent at morning and noon.

WRV was observed resting most frequently (36%) in the late afternoon and (35%) during the early morning and least (22%) in noon. Basking was most frequent (20%) during noon and least (9% each) in early morning and late afternoon. Scratching was observed the most (14%) in the morning and noon. Studying the population and behavior of white-blended vultures is of utmost importance for conserving the vulture species and safeguarding their habitat. This type of research plays a critical role in ensuring their long-term survival and the preservation of their natural environment.

AUTHOR'S CONTRIBUTION

A.K. and T.K.S. designed the research. A.K. collected data and A.K., T.K.S. and R.J. analyzed data. T.K.S., R.J., G.P., K.R.G and S.G. prepared the manuscript. All authors gave approval for publication. There is no conflict of interests among the authors.

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RESEARCH ARTICLE **Open Access**

Prevalence of Avian Haemosporidian Parasites: A Comparative Study between Resident and Migratory Birds of Iraq

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Abstract
Haemosporidians are a diverse group of vector-borne parasitic protozoa that have a global distribution and some of them live in the avian body as a benign parasite, with no symptoms. Avian Haemosporidians, include three main genera, *Plasmodium*, *Haemoproteus*, and *Leucocytozoon*. These blood parasites are transmitted across different regions through migratory birds. We studied the prevalence of avian haemosporidian parasites in both resident and migratory birds in the central and southern regions of Iraq. Our study represents pioneering research on Haemosporidian parasites in a wide range of avian species, including 248 samples from 47 species of both resident and migratory birds, collected from five cities in central and southern Iraq. We identified and determined the prevalence of three distinct haemosporidian genera, *Haemoproteus* spp., *Leucocytozoon* spp., and *Plasmodium* spp. Overall parasite infection was higher in resident hosts than in migrants. The prevalence of *Plasmodium* spp. was found to be highest among resident birds, with a rate of 20.1%, while the highest rate of *Leucocytozoon* spp. was observed in migratory birds, with a rate of 8.2%. Our study holds promising opportunities for understanding the impact of migratory and resident hosts on the dynamics of infectious diseases in wildlife.

Key words: *Haemosporidian, Haemoproteus* spp.*, Plasmodium spp., Leucocytozoon* spp.*, Resident birds, Migratory birds, microscopic examination.*

INTRODUCTION

Birds exhibit the highest diversity of haemosporidian parasites, with more than 250 species classified into three genera: *Plasmodium* spp., *Haemoproteus* spp., and *Leucocytozoon* spp. (Harl et al., 2020). These parasites are commonly transmitted by dipterans and are commonly referred to as "avian malaria" parasites due to their ability to cause malaria-like symptoms in infected birds. Haemosporidian infections are widespread among bird populations globally, affecting a significant number of bird species each year (Clark et al., 2014).

Numerous studies have demonstrated that haemosporidian infections can impose different costs on life-history traits, including impaired body condition (Valkiūnas et al., 2006), reduced reproductive success (Marzal et al., 2005; Knowles et al., 2010), and decreased survival rates (Sol et al., 2003; Bunbury et al., 2007; Lachish et al., 2011). The consequences and symptoms of infection may vary

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depending on factors such as the avian species, the age and immunity of the bird, and the parasite species and lineage, ranging from asymptomatic to severe and potentially fatal disease. Severe outbreaks of infection can lead to significant declines in wild bird populations and, in some cases, even the extinction of bird species (Meister et al., 2021). The roles of migratory and resident hosts in infectious disease dynamics have been hypothesized to be distinct, but the contribution of these hosts to wildlife infectious disease has largely remained unstudied.

FIGURE 1. The study area.

The geological diversity of Iraq plays a crucial role in supporting its rich biodiversity, with bird diversity being a prime example. Furthermore, Iraq's privileged location between the hemispheres makes it a vital stopover site for waterbird migration between Eurasia and Africa (Boere & Stroud, 2006). The diversity of habitats and landscapes represents an added value in harboring large numbers of migratory birds during each migration season, in addition to resident breeding bird species (Salim et al., 2006). The marshes located in central and southern Iraq are considered wintering and resting areas for migratory water birds and other migratory birds, and are well-known for their biodiversity. These marshes also serve as a permanent habitat for millions of birds and as a flight path for millions of other birds migrating between Siberia and Africa, and numerous bird species depend on these wetlands for feeding, resting, and nesting purposes (Salim & Porter, 2009). According to recent studies, a total of 395 bird species, including 130 resident species and 265 migratory species have been recorded (Salim & Porter (2009); Salim et al., 2010).

Despite the high diversity of habitats, a limited number of studies have been conducted on the prevalence of bird infection in Iraq (Shamsuddin & Mohammad 1980; Mohammad, 1990; Al-Hiyali et al., 1998). Interestingly, no studies have focused specifically on the role of resident and migratory hosts in the dynamics of infection. Furthermore, in most of these studies, no distinction was made between resident and migratory host birds. However, many bird species are composed of resident and migratory individuals, known as partial migrants (Lack, 1943). These Individuals that belong to the same species but use distinct migratory strategies, may differ in morphology and behavior (e.g. body size, dominance; Chapman et al., 2011). As a result, it leads to potential differences in their contribution to local and global blood infection dynamics. The few existing studies conducted on individual species or groups of different species in Iraq (e.g. Shamsuddin & Mohammad, 1980; Mohammad, 1990, 2002; Al-Hiyali et al., 1998), have reported a low rate of blood parasite infection, less than 10%. However, domestic birds such as pigeons or doves, which are considered resident birds, have shown a higher infection rate with *Haemoproteus* spp**.** (Wahhab et al., 2017).

Previous studies conducted in other regions have investigated the roles of migratory and resident hosts in the dynamics of blood parasite infections, revealing differences in the prevalence of these infections between migratory and resident populations (Waldenstro et al., 2002; Altizer, 2011; Altizer, 2011). Migratory birds are often considered potential introducers of blood parasites from their breeding areas to their wintering grounds, while resident birds may serve as reservoirs, facilitating the year-round circulation of these infections (Altizer, 2011). However, the specific roles of migratory and resident hosts and their contributions to local and global blood infection dynamics have not been extensively studied.

Therefore, the objective of this study is to assess the prevalence, infection rate, and distribution of blood parasites among birds in Iraq, with a particular focus on the central and southern regions. Additionally, we aim to investigate the contributions of migratory and resident hosts to the local dynamics of blood parasite infections.

MATERIAL AND METHODS

Sampling

This study was performed in the Sedimentary Plain Area of southern and central Iraq (Figure 1) which is characterized by great geodiversity, as it contains semi-desert lands, and vast areas of agricultural land, in addition to the existence of marshes that made it a suitable environment for most of the resident and migratory birds. After determining the appropriate localities in Najaf, Karbala, Qadisiyah, Babel, and Basra, (Table 1), the necessary permits for sampling were obtained from the relevant organizations. All birds were caught with mist nets from January to December 2021.

The blood sample was taken from the brachial vein using an insulin syringe and capillary tube based on bioethical methods, and the bird was returned to the natural environment unharmed. From each bird, 1 or 2 blood films were prepared on microscope glass slides. The residential status of the bird species was determined according to BirdLife International (2023), and Salim et al., (2010).

TABLE 1. Longitude, latitude, and number of migratory and resident captured species in each study area.

Study area	Longitude	Latitude	Total	Resident birds	Migratory birds	Time
Najaf	44° 19' 48"	32° 00' 00"	60	45	15	February--January
Karbala	44°01'30"	32° 36' 50"	43	41	2	May -April
Qadisiyah	45° 04' 59.99"	31° 55' 0.01"	69	28	41	October -September
Babel	44°37'0.01"	32° 31' 59.99"	32	23	9	August -July -June
Basra	47° 48' 59"	30° 30' 03"	44	39	5	December November -March

Preparation and examination of blood smears

The blood films were air-dried and fixed in absolute methanol for 1 minute in the field. In the laboratory, blood smears were stained in a 10% working solution of a stock solution of Giemsa's stain, pH 7.0–7.2, for 20-25 minutes, as described by Valkiūnas, Iezhova et al. (2008). A light microscope equipped with a digital camera was used to examine blood films and prepare illustrations. Finally, about 100 fields were studied at high magnification (1000 x). Morphological identification of parasite species in blood smears was performed according to the method presented by Valkiūnas (2005).

Statistical Analysis

A blood sample was categorized as 'positive' if at least one endoparasite species was found. Prevalence was calculated as the number of individuals infected out of the total sample size, along with the corresponding 95% confidence intervals (95% CI). The normality of the data was tested using one-sample Kolmogorov-Smirnov test. Differences between types of endoparasites were compared using the chi-square test. P values <0.050 were considered significant. Differences between resident and migratory bird groups were compared using an ANOVA test.

RESULTS

In this study, we examined the prevalence of haemosporidian infections in 248 birds belonging to 47 species, 27 families, and 14 orders. Among the sampled birds, 23 species were migratory, while the remaining 24 species were resident birds. We found that Passeriformes species had the highest rate of haemosporidian infections, accounting for 23.3 % of all infections observed. Other orders, such as Anseriformes (18%), Charadriiformes (9%), Coraciiformes (6.5%), Gruiformes (5%), Pelecaniformes (3.3%), and Columbiformes (2%), exhibited lower infection rates compared to Passeriformes. The remaining orders showed even lower infection rates, accounting for less than 1% of the total analyzed samples. Specifically, Galliformes and Phoenicopteriformes had an infection rate of 1.25%, Pterocliformes, and Carimulgiformes had an infection rate of 0.8%, and orders Bucerotiformes, Accipitriformes, and Suliformes had an infection rate of 0.4% (Figure 2).

Microscopic examination

We prepared a total of 294 thin blood smears from 248 birds from south and central Iraq to detect avian blood parasites from three genera *Haemoproteus* spp, *Plasmodium* spp. and *Leucocytozoon spp.* Our finding revealed the presence of haemosporidian parasites which were only identified by microscopy in

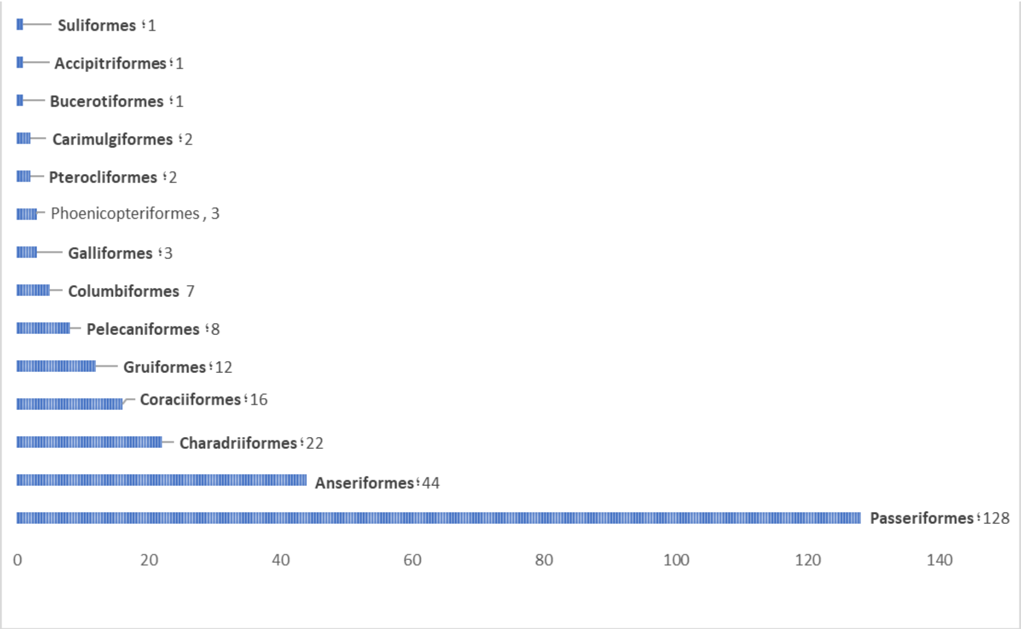

FIGURE 2. The number of birds examined in each order to determine the prevalence of haemosporidian infections.

TABLE 2. The prevalence of blood parasites in wild passerine birds was shown separately for each geneus: H: *Haemoproteus* spp., L: *Leucocytozoon* spp., and P: *Plasmodium* spp.

Order-Family	**Genus**	**State**	**Positive/ examined**	**Blood parasite infection**	**Prevalence (%)**			
					H	**P**	**L**	**(H+L) OR (P+L)**
Passeriformes- Alaudidae	*Calandrella brachydactyla*	Migratory	7/14	0.50	14.2	21.4	7.1	(H+L) 7.1
Passeriformes- Alaudidae	*Emberiza calandra*	Migratory	0/3	0.0	0.0	0.0	0.0	0.0
Passeriformes- Alaudidae	*Eremophila alpestris*	Migratory	0/6	0.0	0.0	0.0	0.0	0.0
Passeriformes- Alaudidae	*Galerida cristata*	Resident	12/21	57.1	4.7	52.3	0.0	0.0
Passeriformes- Cisticolidae	*Prinia gracilis*	Resident	0/1	0.0	0.0	0.0	0.0	0.0
Passeriformes- Corvidae	*Corvus corone*	Resident	0/1	0.0	0.0	0.0	0.0	0.0
Passeriformes- Corvidae	*Pica pica*	Resident	0/6	0.0	0.0	0.0	0.0	0.0
Passeriformes- Fringillidae	*Carduelis carduelis*	Resident	0/2	0.0	0.0	0.0	0.0	0.0
Passeriformes- Motacillidae	*Motacilla alba*	Migratory	1/6	16.6	0.0	16.6	0.0	0.0
Passeriformes- Muscicapidae	*Erithacus rubicola*	Migratory	1/10	10	0.0	10	0.0	0.0
Passeriformes- Muscicapidae	*Oenanthe isabellina*	Migratory	1/3	33.3	0.0	33.3	0.0	0.0
Passeriformes- Passeridae	*Passer domesticus*	Resident	15/23	78.2	60.8	8.6	0.0	(P+L) 4.3
Passeriformes- Phylloscopidae	*Phylloscopus collybita*	Migratory	0/1	0.0	0.0	0.0	0.0	0.0
Passeriformes- Prunellidae	*Prunella modularis*	Migratory	1/6	16.6	0.0	16.6	0.0	0.0
Passeriformes- Pycnonotidae	*Argya altirstris*	Resident	3/12	25	16.6	0.0	0.0	(P+L) 16.6
Passeriformes- Pycnonotidae	*Pycnonotus leucotis*	Resident	1/6	16.6	0.0	0.0	16.6	0.0
Passeriformes- Sturnidae	*Acridotheres tristis*	Resident	0/1	0.0	0.0	0.0	0.0	0.0
Passeriformes-Sturnidae	*Sturnus vulgaris*	Migratory	1/5	20.0	0.0	0.0	20.0	0.0
Anseriformes- Anatidae	*Anas acuta*	Migratory	0/3	0.0	0.0	0.0	0.0	0.0
Anseriformes- Anatidae	*Anas clypeata*	Migratory	2/6	33.3	0.0	16.6	16.6	(P+L) 16.6
Anseriformes- Anatidae	*Anas crecca*	Migratory	0/3	0.0	0.0	0.0	0.0	0.0
Anseriformes- Anatidae	*Anas platyrhynchos*	Migratory	0/5	0.0	0.0	0.0	0.0	0.0
Anseriformes- Anatidae	*A. p. domesticus*	Resident	0/9	0.0	0.0	0.0	0.0	0.0
Anseriformes- Anatidae	*Aythya ferina*	Migratory	2/7	28.5	0.0	14.2	14.2	0.0
Anseriformes- Anatidae	*Netta rufina*	Migratory	0/1	0.0	0.0	0.0	0.0	0.0
Anseriformes- Anatidae	*Tadorna ferruginea*	Migratory	0/5	0.0	0.0	0.0	0.0	0.0
Anseriformes- Anatidae	*Tadorna tadorna*	Migratory	0/5	0.0	0.0	0.0	0.0	0.0
Charadriiformes- Charadriidae	*Vanellus indic*	Resident	1/6	16.6	0.0	16.6	0.0	0.0
Charadriiformes- Charadriidae	*Vanellus leucurus*	Resident	0/8	0.0	0.0	0.0	0.0	0.0
Charadriiformes- Recurvirostridae	*Himantopus himantopus*	Resident	0/8	0.0	0.0	0.0	0.0	0.0
Coraciiformes- Alcedinidae	*Alcedo atthis*	Resident	1/2	50	0.0	50	0.0	0.0
Coraciiformes- Alcedinidae	*Ceryle rudis*	Resident	2/3	66.6	0.0	33.3	0.0	(P+L)33.3
Coraciiformes- Alcedinidae	*Halcyon smyrnensis*	Resident	0/1	0.0	0.0	0.0	0.0	0.0
Coraciiformes- Coraciidae	*Coracias benghalensis*	Resident	1/3	33.3	0.0	0.0	33.3	0.0
Coraciiformes-Meropidae	*Merops persicus*	Resident	0/6	0.0	0.0	0.0	0.0	0.0
Gruiformes- Rallidae	*Fulica atra*	Migratory	0/5	0.0	0.0	0.0	0.0	0.0
Gruiformes- Rallidae	*Gallinula chloropus*	Resident	1/7	14.2	0.0	0.0	0.0	(p+L)14.2
Pelecaniformes- Ardeidae	*Ardea alba*	Migratory	0/8	0.0	0.0	0.0	0.0	0.0
Columbiformes- Columbidae	*Streptopelia turtur*	Resident	0/5	0.0	0.0	0.0	0.0	0.0
Columbiformes- Columbidae	*Streptopelia risoria*	Resident	0/2	0.0	0.0	0.0	0.0	0.0
Galliformes- Phasianidae	*Francolinus francolinus*	Resident	1/3	33.3	0.0	33.3	0.0	0.0
Phoenicopteriformes- Phoenicopteridae	*Phoenicopterus roseus*	Migratory	0/3	0.0	0.0	0.0	0.0	0.0
Pterocliformes- Pteroclidae	*Pterocles exustus*	Resident	1/2	50	50	0.0	0.0	0.0
Carimulgiformes- Caprimulgidae	*Caprimulgus aegyptius*	Migratory	2/2	100	50	50	0.0	0.0
Bucerotiformes- Upupidae	*Upupa epops*	Resident	0/1	0.0	0.0	0.0	0.0	0.0
Accipitriformes- Accipitridae	*Hieraaetus pennatus*	Migratory	0/1	0.0	0.0	0.0	0.0	0.0
Suliformes- Phalacrocoracidae	*Phalacrocorax nigrogulari*	Migratory	0/1	0.0	0.0	0.0	0.0	0.0
Total			**58/248**	**23.3%**	**7.2%**	**12.9%**	**3.2%**	**4%**

TABLE 3. The number of migratory and resident birds, along with the corresponding percentage of infection by blood parasites (H: *Haemoproteus* spp., L: *Leucocytozoon* spp., P: *Plasmodium* spp.).

	Positive / examined	**Blood parasites infection**	**Prevalence (%)**		
			H	**P**	**L**
Resident birds	40/139	28.7%	14.2%	20.1%	2.4%
Migratory birds	18/109	16.5%	2.1%	6.2%	8.2%

58 birds. Of which, 27 were infected with *Plasmodium* spp., 18 with *Haemoproteus* spp., six with *Leucocytozoon* spp. and 7 showed mixed infection. Among the mixed infections, one bird was infected with both *Haemoproteus* spp. and *Leucocytozoon* spp., while six birds were infected with both *Plasmodium* spp. and *Leucocytozoon* spp. Observed differences in terms of parasite species were statistically significant. (*P* = 0.003).

Gametocytes of haemosporidians have sexually dimorphic characteristics, which can be easily distinguished using a light microscope. Haemosporidian macrogametocytes possess compact nuclei and bluish-stained cytoplasm, whereas microgametocyte nuclei are diffuse and the cytoplasm stains appear paler than macrogametocytes. It is important to note that in the Plasmodiidae family, merogony occurs within blood cells, and blood stages contain malarial pigment, also known as hemozoin. On the other hand, in the family Haemoproteidae, merogony does not take place within blood cells.

Statistical analysis showed a significant difference (*P* = 0.007) between resident and migratory bird groups in terms of overall infection (28.7% vs. 16.5%). Our study revealed that out of 27 individuals that were infected with *Plasmodium* (Figure 3 A-B), 18 samples belonging to six species of resident host birds including *Passer domesticus, Galerida cristata, Francolinus francolinus, Alcedo atthis, Vanellus indic and Ceryle rudis*. Furthermore, 9 samples were obtained from migratory host birds, representing 6 different species including *Oenanthe isabellina, Erithacus rubicola, Prunella modularis, Calandrella brachydactyla, Aythya ferina, and Caprimulgus aegyptius*.

The infection with *Haemoproteus* spp, was also relatively severe (Figure 3 C-D), and was observed in 14 samples from resident host birds belonging to 4 species; *P. domesticus, G. cristata, Pterocles alchata, and Argya altirstris*) and four samples from migratory host birds in two species, *C. brachydactyla* and *C.* aegyptius. In contrast, fewer infections with *Leucocytozoon* spp., (Figure 3 E-F) were recorded in migratory host birds, four samples from four migratory species host birds including *C. brachydactyla*, Anas *acuta*, *A. clypeata* and Ardea *alba* were infected, and two samples from resident host birds, *Gallinula chloropus* and *Pycnonotus leucotis*.

Leucocytozoon spp. was recorded in all seven cases of mixed infections, among these, one mixed infection involving *Haemoproteus* spp. and *Leucocytozoon* spp. was recorded in the migratory host bird (*C. brachydactyla*). While six mixed infection including *Plasmodium* spp. and *Leucocytozoon* spp. were observed in four samples obtained from resident host birds belonging to three species (*P. domesticus*, *A. altirstris* and, *C. rudis*), and four samples from migratory host birds representing 4 species (*A. ferina*, *C. aegyptius*, *Anas clypeata*, and, *Motacilla alba*).

The results of examining the blood of the studied birds are summarized in Table 2. It was found that 58 out of 248 (23.3 %) of examined birds were infected with Haemosporidians. Among these, *Plasmodium* spp., *Haemoproteus spp.*, and *Leucocytozoon* spp. were detected in 12.9%, 7.2%, and 3.2% of the birds, respectively. Mixed infections were also observed, with 4% of the birds showing a co-infection of *Plasmodium* spp. and *Leucocytozoon* spp.*,* while 3.2% and 0.8% of the birds had mixed infections of *Haemoproteus* spp. and *Leucocytozoon* spp., respectively. The total numbers of positive samples, prevalence values, and 95% CI are summarized in Table 2.

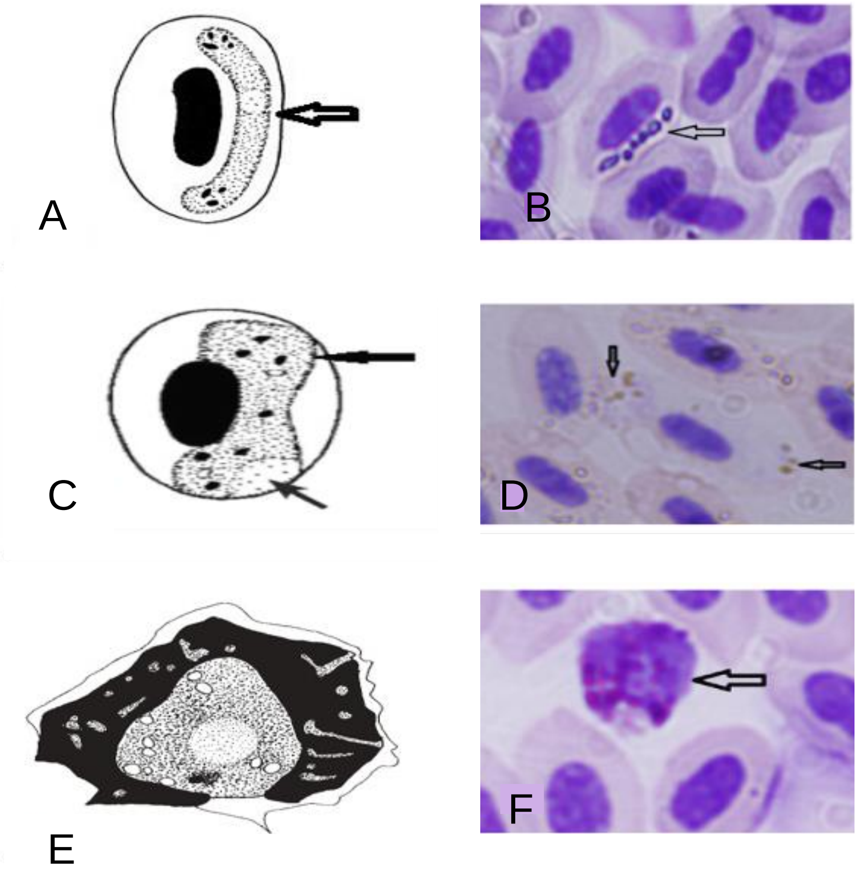

FIGURE 3. Avian haemosporidian parasite, A-B: *Plasmodium* of *Galerida cristata*, C-D: *Haemoproteus* spp. of *Passer domesticus*, and E-F: *Leucocytozoon* of *Anas acuta.*

DISCUSSION

In this study, we report the prevalence and distribution of avian haemosporidian infections in both resident and migratory bird species. Our study reveals an increase in the level of infection within Iraqi birds compared to previous studies that recorded a lower prevalence of blood parasites in the past years.

This could be due to the high vector population (biting mites) in Iraq as well as the limited use of insecticides. Alternatively, it could be due to the more extensive sampling conducted in the present study compared to previous ones.

Initially, we expected a higher prevalence of infections in migratory birds since they are exposed to a larger variety of vector and parasite species during their annual cycle (Waldenstro et al., 2002). However, our results were different with high levels of parasitemia recorded in resident birds, indicating a prevalence of nearly 36.6%. Among the blood parasites, *Plasmodium* spp. demonstrated the highest infection rate at 20.1%, followed by *Leucocytozoon* spp. at 8.2%. (Table 3, Figure 3) This observation could potentially be attributed to the heavy contamination of the study area with dipteran insect vectors, especially black flies (Simuliidae), which are active during summer (Greiner, 1991). Furthermore, we know that birds migrate to Iraq during the spring and winter seasons when temperatures decrease, inhibiting the emergence of suitable vector insects. This reduced exposure to vectors could explain the lower prevalence of blood parasites in migratory birds (Johnston&Janiga,1995). The low prevalence in migratory birds may also be due to migration, which may have a protective effect as migratory behavior allows the host to escape from environments with a high risk of infection (Altizer, 2011, Poulin, 2012; Satterfield, 2015; Fecchio et al., 2020).

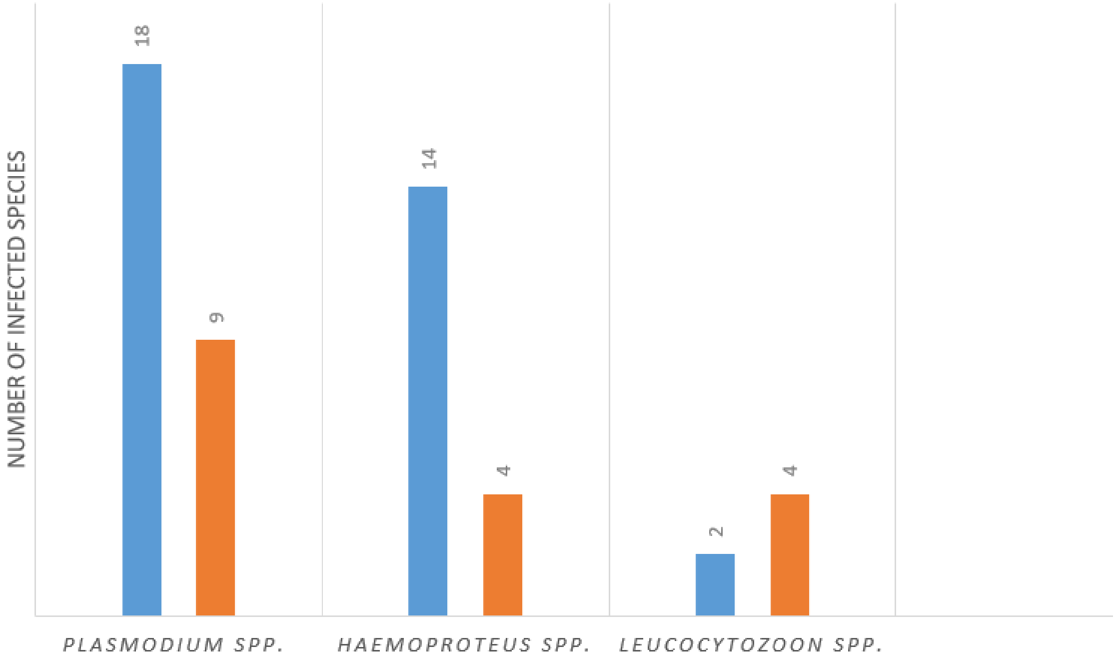

FIGURE 4. Comparisons between the number of resident (blue) and migratory (orange) bird species infected with each genus of blood parasites.

Our research results align with the overall infection rate reported in a study conducted in Turkey, which examined 565 specimens of migratory and resident birds and recorded 34.2% infection rate (Ciloglu et al., 2020). However, they found that overall parasite prevalence was higher in migratory birds compared to resident birds which is contrary to what we found here. In contrast, a study in Brazil examining birds from the Atlantic Forest reported higher infection prevalence in migratory species compared to resident ones (24% vs. 9%) (Anjos et al., 2021). Similarly, our research differs from a study conducted in eastern Tennessee, United States, which reported an overall prevalence of 44% in resident and migratory bird species. Similarly, our research differs from a study conducted in eastern Tennessee, United States, which reported an overall prevalence of 44% in resident and migratory bird species (Matthews et al., 2016).

Previous studies have consistently demonstrated that *Leucocytozoon* species have a wide range of avian host species and are commonly found in wild birds (Bensch et al., 2009; Clark et al., 2014; Valkiūnas & Iezhova, 2017). In our study, we observed a lower prevalence of *Leucocytozoon* species in resident birds compared to migratory birds, which is consistent with previous studies (Anjos, et al., 2021) suggesting a higher prevalence of this parasite in higher latitudes and colder area. Accordingly, it is reasonable that birds living in the tropical regions of Iraq show a lower *Leucocytozoon* prevalence than migratory birds that come from higher altitudes. This finding is also in agreement with what Ciloglu et al., 2020 reported from Sultan Marshes National Park, Turkey, which suggested insufficient habitat conditions for active transmission of *Leucocytozoon* species.

Another explanation for these observations can be resulted from studies conducted on vector species. Several studies have shown that black fly species are distinguishable as either mammalophilic or ornithophilic, in which ornithophilic black fly species preferentially choose large and abundant host species for feeding. (Malmqvist et al., 2004. Chakarov et al., 2021). In this context our results are reasonable, as most migratory birds in our study are large waterfowl. This result is also consistent with the results reported by Anjos et al. (2021) from Brazil, where all positive samples for *Leucocytozoon* were detected in *Elaenia albiceps*.

Plasmodium, on the other hand, has a global distribution and infects various bird hosts (Meister et al., 2021). In our study, microscopic examination revealed a higher prevalence of *Plasmodium* infection compared to the other two parasite genera, with resident birds exhibiting a higher prevalence than migratory birds (20.1% vs 6.2%). In contrast, several studies conducted in Iran on wild passerines have consistently identified *Haemoproteus* as the most common parasite among birds (Nourani et al., 2017a, 2017b, 2018a, 2018b; Djadid, 2019; Noorani et al., 2020a, 2020b). This difference in parasite prevalence may be attributed to favorable environmental conditions that support both the vector and the parasite in the Iranian region.

It is worth mentioning that the current study is the first large-scale description of avian haemosporidian infections in Iraq providing insights into the infection rates of blood parasites between migratory and resident birds in central and southern regions of the country. The high infection rate observed in resident Iraqi birds is significant, as these birds serve as reservoirs for blood parasites that can immediately infect migrant birds upon their arrival in Iraqi migration areas.

However, it is important to note that in morphological studies like the present study, rely on the identification of blood parasites mainly based on morphology and morphometry and, which can be challenging in certain stages of their life cycle (Anjos, et al., 2021). Therefore, further examination of these parasites with advanced methods such as molecular tools is necessary to clarify their taxonomic status and infection rate more accurately (Fallon et al., 2003). Host-parasitic relationships vary widely depending on host species or the geographical regions, and therefore detailed analyses which reflect these dynamics are promising.

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RESEARCH ARTICLE **Open Access**

Evaluation of the effect of ecological factors on cannibalism in *Pelophylax bedriagae* (Pallas, 1771) (Amphibia: Anura)

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Abstract
Marsh frog, *Pelophylax bedriagae* tadpoles habituate in temporary wetlands where intraspecific aggression has been observed. In this study, we examined the independent and interactive effects of predator cues, water level and density on head width and head, tail and whole-body cannibalism of larval *P. bedriagae*. We designed a 2×2×3 factorial experiment, by crossing two levels of predatory cues (presence/absence), two levels of density (low/ high) and three levels of water (low/ high/ decreasing) that were carried out within 29 weeks. Despite using conditions that might facilitate cannibalism (i.e., the presence of predator cues, low water level, and high density), a low rate of cannibalism was observed in all treatments. Independently**,** the highest percentage of total cannibalism was indicated in the presence of predator cues (2.26 %), high water level (2.04 %) and high density (2.24 %). Interactively, the highest rate of total cannibalism was recorded for larvae reared in the high density/absence of predator cues/low water level treatment (3.55%). In contrast, the lowest rate of total cannibalism was observed in the both the low density/presence of predator cues/decreasing water level and low-density/presence of predator cues/high water level treatments (0.44%). The results of the MANOVA indicated that predator cues, water level and density independently and interactively had not significant effect on rate of total cannibalism. At the end of experiment, the maximum size of head width was measured in low-density/absence of predator cues/high water level treatment (9.60±0.39mm), and there was no significant relationship between the maximum head width and the highest rate of cannibalism. According to the results of this experiment, it seems that *P. bedriagae* larvae may function to avoid the risk of cannibalism by conspecific.

Key words*: Amphibia, Intraspecific predation, Predatory cues, Density, Tadpole, Water level, Marsh frog*

INTRODUCTION

Cannibalism or intraspecific predation represents the interactions of animals with individuals of the same species in response to environmental conditions (Polis, 1981; Wakano et al. 2002). This phenomenon is observed in diverse animal taxa including amphibians and is suggested to be a predictable evolutionary consequence of crowding, low availability of food resources, and starvation (Nishimura & Isoda, 2004). In amphibian species that reproduce a large number of offspring (Relyea, 2004; Richter-Boix et al. 2007),

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cannibalism is common during the hatching to metamorphosis stages (larval-larval cannibalism) (Wakano et al. 2002; Gibbons et al. 2003; Zhang et al. 2003). Similar to interspecific predator-prey interactions, cannibalistic interactions contribute to the regulation of population structure and to the evolution of antipredator behavioral responses (Dong & Polis, 1992).

Climate change influence on amphibian community and their habitats (Walther et al. 2002; Brooks, 2004; Blaustein et al. 2010). These changes can lead to alterations in precipitation and temperature regimes, particularly in aquatic environments. Agonistic behaviors in amphibians can be influenced by a variety of factors including desiccation, population density, competition for food, temperature, and the presence of predator (Blaustein et al.2010). In particular, pond desiccation and high temperature can increase cannibalism rates and have negative impacts on survival and reproduction in populations (Crumrine, 2010). As temporary wetlands gradually disappear, larval population density can increase (Wildy, 2001). Jefferson et al. (2014) have suggested that climate changes could alter population density for larval salamanders, leading to increased cannibalism rates. Maneti et al. (2015) found that aggression among larval *Salamandra salamandra* increased with density. Under such conditions, limitations to food resources may restrict their ability to metamorphosis (Enriquez-Urzelai et al. 2013) and intraspecific predation may avoid (Claessen & De Roos, 2003; Persson et al. 2004). Food limitations can also play a significant role in size variations of individuals which can facilitate cannibalism, although typically the cannibals are the largest individuals in the population (Fox, 1975; Polis, 1981; Crump, 1992). Pizzatto and Shine (2008), have demonstrated *Bufo marinus* tadpoles eat eggs, and large adults consume smaller conspecifics.

The effects of predator cues on animal behavior and ecology are well documented, both in amphibian and non-amphibian taxa (Kats & Dill 1998; Fraker, 2009; Kerby et al. 2011). For example, Sih (1986) found that mosquito larvae (*Culex pipiens*) reduce their activity when exposed to *Notonecta undulata* predators. Cannibals often exhibit faster growth, larger body size and broader head morphology compared to non- Cannibals (Fox, 1975; Polis, 1981). Cannibal larvae also tend to attack and consume the tails and developing limbs of non-cannibal larvae (Crump, 1986). Kishida et al. (2011) examined how the presence or absence of predation risk from larval dragonflies (*Aeshna nigroflava*) affected cannibalism dynamics in their prey, larval salamanders (*Hynobius retardatus*). They suggested that the positive feedback dynamics between size structure and cannibalism, and their modification by predation risk may also operate in other systems to shape the population dynamics of cannibalistic prey species, as well as overall community dynamics.

Amphibian tadpoles have been observed to exhibit anti-predator behavior in response to natural predators (Mogali et al. 2011, 2012). Such behaviors include a reduction in movement, avoidance of the predator compartment and an increase in shelter use (Lima & Dill, 1990; Mogali et al. 2015, 2019, 2020). Invasive predators, such as mosquitofish, have been introduced into various freshwater habitats, often resulting in declines in native amphibian populations due to predation on eggs, embryos, and tadpoles (Pyke, 2008). The mosquitofish, *G. holbrooki*, is a small viviparous fish that was introduced to Iran in the 20th century (1922–1930) from Italy and Azerbaijan as part of a mosquito control program (Patimar et al. 2011).

The Middle East is one of the most vulnerable regions to the impacts of climate change, largely due to water scarcity (Elasha, 2010)**.** Iran located in an arid and semi-arid region is particularly susceptible to the effects of global climate change on its ecosystems (Amiri & Eslamian, 2010). The IPCC estimates an increase of 1.5-4 °C in mean temperatures by the year 2100, and predicts a steady decline in annual rainfall (~30%) in Iran (Nazaripour & Daneshvar, 2014). Subtropical species may be more sensitive to temperature increases than temperate species (Deutsch et al. 2008; Dillon et al. 2010; Duarte et al. 2012). The Marsh frog ***P.** bedriagae* (Pallas, 1771) is the most common amphibian species in Iran, often found in aquatic environments such as ponds, streams, and wetlands, and rarely straying far from water bodies (Baloutch & Kami, 1995). In nature, Marsh frog tadpoles are dependent on aquatic environments and frequently face ecological challenges, including desiccation threats and crowding. The aim of this study is to evaluate the independent and interactive effects of three ecological factors- predator cues, water level and density- on cannibalism of *Pelophylax bedriagae* larvae.

MATERIAL AND METHODS

Marsh frog (*Pelophylax bedriagae*) eggs were collected from Sarab-e-Barnaj, a freshwater wetland, Kermanshah Province in Iran (N 34° 28' E 47° 22'). Egg masses were transported to Razi University, and maintained in glass aquarium filled with de-chlorinated water. From this stock, when the eggs reached to Gosner 26 stage (Gosner, 1960), start of the larval stage, individuals (N=540) of similar size and developmental stage were randomly chosen for the experimental containers. *Gambusia holbrooki* specimens were collected from Sarab-e-Youan (N 34° 38' E 46° 35'), 35 km to northwest Kermanshah city, Kermanshah Province in Iran.

The Experimental Design

The experiment consisted of 2×2×3 factorial design incorporating two levels of density (low, n =5 and high, n =25), two levels of predatory (present of predatory cues and without of predatory cues), and three levels of water (low: 300 cc, high 1500 cc, and decreasing 150 cc of water, 10 days once) including: high density/ high water level/ no predator (HD/HW/NP); 2) high density/ low water level/ no predator (HD/LW/NP); 3) high density/ decreasing water level/ no predator (HD/DW/NP); 4) low density/ high water level/ no predator (LD/HW/NP); 5) low density/ low water level/ no predator (LD/LW/NP); 6) low density/ decreasing water level/ no predator (LD/DW/ NP); 7) high density/ high water level/ predator (HD/HW/P); 8) high density/ low water level/ predator (HD/LW/P); 9) high density/ decreasing water level/ predator (HD/DW/P); 10) low density/ high water level/ predator (LD/HW/P); 11) low density/ low water level/ predator (LD/LW/P); low density/ decreasing water level/ predator (LD/DW/P).

In order to prevent the direct contact of the *G. holbrooki* with larvae of *P. bedriagae*, the meshed cages (11.5 cm diameter × 6 cm depth) were designed, and were placed inside glass containers (12.5 cm diameter and 14 cm depth) during experiment. Within each meshed cage, five females *G. holbrooki* were placed at an average total length and head width 31.29 mm ± 8.33 and 5.12mm ± 1.43, respectively.

Monitoring

The experiment was performed under natural photoperiod at air and water temperature of approximately 24°C ± 1.40 and 21°C ± 1.60, respectively. Water in rearing containers was renewed each week. Larvae were fed daily with boiled spinach ad libitum (0.1 g per larva in the first month and 0.2 g per larva in the next months). Predators were fed daily with *P. bedriagae* tadpoles (each with one larva). The experiment was terminated when all tadpoles of all groups metamorphosed (Gosner stage 46).

Measurement

Photos of consumed larvae were taken with a digital camera (SONY, DSC-HX9V, 3.6V) on a tripod at a fixed height (30 cm) (Figs. 1-2). All larvae were measured for pre-ocular head width (width across the head through bisecting line through the external nares) by Digimizer version 4.6.0. Also, the morphological variables of larvae were surveyed daily including head (biting or eating all or part of head), tail (tail shortening, tail narrowing and eating all part of tail), whole body (larva disappearance in container) and total (head, tail and whole body) cannibalism. In *G. holbrooki* total length was calculated by drawing a line from the tip of snout to the end of caudal fin and head width were calculated by drawing a line between left and right jaw angles. Measurements were performed by Digimizer version 4.6.0.

Statistical Analysis

We calculated the average values of each variable for statistical analyses. All data are expressed as mean ± SD. After checking for normality, data were analyzed using multivariate analysis of variance (MANOVA) for testing independently and interactively effects of predatory cues, water level and density on tail, head, whole and total cannibalism and head width. All statistical tests were performed using SPSS software (ver. 22.0) and Stata (ver. 16.0).

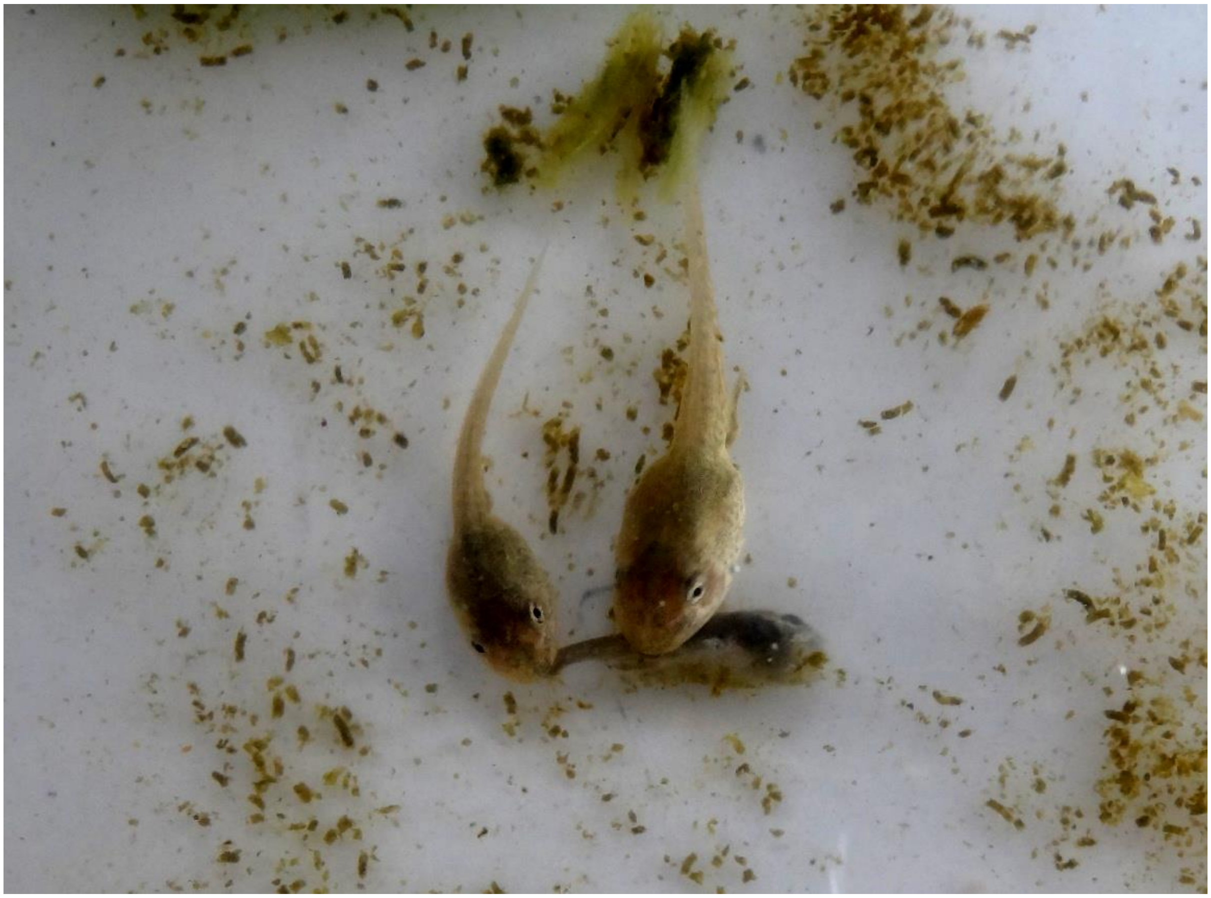

FIGURE 1. Cannibalistic larval *P. bedriagae* consume smaller conspecifics in the 17th week of experiment.

FIGURE 2. An example of larval *P. bedriagae* cannibalism in the 28th week of experiment.

RESULTS

The results of the experiment indicate that cannibalism rates were generally very low across all treatments. However, the highest percentage of cannibalism was observed in the presence of predator cues (2.26 ± 0.89%), high water levels (2.04 ± 1.10%), and high density (2.24 ± 0.72%). In contrast, the lowest percentage of cannibalism was recorded in the absence of predators (1.16 ± 0.66%), decreasing water levels (1.26 ± 0.77%), and low density (1.18 ± 0.87%) (Fig. 3 A-C).

The independent and interactive effects of three factors (predator cues, water level and density) on head cannibalism surveyed in experimental treatments. The percentage of head cannibalism demonstrated from high to low in HD.NP.LW (0.17 ± 0.46 %), HD.P. HW (0.17 ± 0.68 %), HD.NP.DW (0.08 ± 0.34 %) and HD.P. DW (0.08 ± 0.34 %) treatments, respectively. No head cannibalism was observed in the other treatments (Fig. 4). The results of MANOVA indicated that the factors of predatory cues, water level and density, independently and interactively had not significant effect on percentage of head cannibalism (Table 1).

The percentage of tail cannibalism recorded from high to low in LD.NP.LW (1.33 ± 2.16 %), HD.NP.LW (0.44 ± 0.96 %), LD.NP.DW (0.44 ± 1.71 %), HD.NP.HW (0.35 ± 0.60 %), HD.P. LW (0.35 ± 0.60 %), HD.NP.DW (0.17 ± 0.68 %), HD.P. DW (0.17 ± 0.68 %) and HD.P. HW (0.17 ± 0.46 %) treatments, respectively. In other treatments was not observed tail cannibalism (Fig. 4). The results of MANOVA indicated that predator cues, water level and density factors independently and interactively had not significant effect on total rate of tail cannibalism (Table 2).

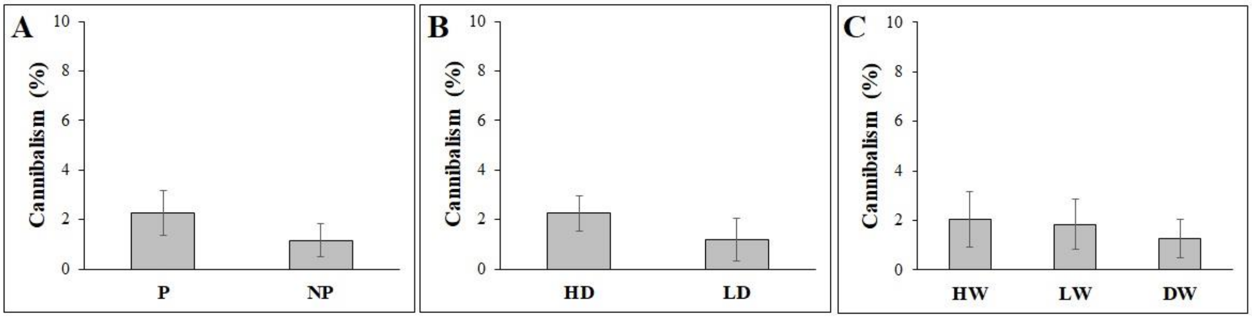

FIGURE 3. The percentage of cannibalism in A) the presence of predator cues (P), the absence of predator's cues (NP); B) the high density (HD), the low density (LD); C) the high-water level (HW), the low water level (LW), the decreasing water level (DW).

TABLE 1. Summary statistics of multivariate analysis of variance (MANOVA) for predator cues, water level and density effects on head cannibalism.

Head cannibalism	**Wilks' lambda**	***df***	***F***	***p-value***
Predator cues	0.99	1	0.35	0.55
Water level	0.99	2	0.77	0.46
Density	0.99	1	1.61	0.20
Predator cues × density	0.99	1	0.61	0.43
Predator cues × water level	0.99	1	0.44	0.63
Water level × density	0.99	2	0.65	0.52
Predator cues × water level × density	0.99	2	0.52	0.59
Predator cues × time	0.98	1	2.03	0.16
Water level × time	0.97	2	1.40	0.14
Density × time	0.99	1	0.11	0.63
Predator cues × density × time	0.98	1	2.03	0.15
Predator cues × water level × time	0.98	1	0.89	0.43
Water level × density × time	0.97	2	1.86	0.15
Predator cues × water level × density × time	0.98	2	0.89	0.41

The percentage of whole body cannibalism observed from high to low in HD.NP.LW (2.93 ± 0.54 %), HD.NP.DW (2.22 ± 0.38 %), LD.NP.DW (2.22 ± 0.65 %), HD.NP.HW (1.86 ± 0.30 %), HD.P.LW (1.59 ± 0.34 %), HD.P.DW (1.50 ± 0.51 %), HD.P.HW (1.15 ± 0.57 %), LD.NP.HW (0.88 ± 0.14 %), LD.P.LW (0.88 ± 0 %), LD.NP.LW (0.44 ± 0.29 %), LD.P.DW (0.44 ± 0 %) and LD.P.HW (0.44 ± 0 %) treatments, respectively (Fig. 4). The results of MANOVA indicated that water level factor independently had significant effect on total rate of whole-body cannibalism in ***P. bedriagae*** ($P \leq 0.04$) (Table 3). During the time, except significant effect of predator cue ($P \leq 0.05$), water level and density factors independently and interactively had not significant effect on rate of whole-body cannibalism (Table 3).

TABLE 2. Summary statistics of multivariate analysis of variance (MANOVA) for predator cues, water level and density effects on tail cannibalism.

Tail cannibalism	**Wilks' lambda**	***df***	***F***	***p-value***
Predator cues	0.99	1	0.39	0.53
Water level	0.99	2	0.33	0.72
Density	0.98	1	2.05	0.15
Predator cues × density	0.99	1	1.35	0.24
Predator cues × water level	0.99	1	0.33	0.65
Water level × density	0.99	2	0.42	0.65
Predator cues × water level × density	0.98	2	1.49	0.22
Predator cues × time	0.99	1	0.17	0.68
Water level × time	0.99	2	0.05	0.94
Density × time	0.99	1	0.04	0.83
Predator cues × density × time	0.99	1	0.69	0.40
Predator cues × water level × time	0.99	1	0.02	0.97
Water level × density × time	0.99	2	0.05	0.94
Predator cues × water level × density × time	0.99	2	0.13	0.88

TABLE 3. Summary statistics of multivariate analysis of variance (MANOVA) for predator cues, water level and density effects on whole body cannibalism.

Whole body cannibalism	**Wilks' lambda**	***df***	***F***	***p-value***
Predator cues	0.99	1	0.03	0.86
Water level	0.96	2	3.15	**0.04**
Density	0.99	1	0.63	0.43
Predator cues × density	0.99	1	0.48	0.49
Predator cues × water level	0.97	1	2.08	0.12
Water level × density	0.99	2	0.16	0.85
Predator cues × water level × density	0.99	2	0.28	0.75
Predator cues × time	0.96	1	5.55	**0.05**
Water level × time	0.99	2	1.09	0.73
Density × time	0.99	1	0.11	0.77
Predator cues × density × time	0.99	1	0.03	0.85
Predator cues × water level × time	0.98	1	0.80	0.44
Water level × density × time	0.99	2	0.46	0.63
Predator cues × water level × density × time	0.99	2	0.72	0.48

The percentage of total cannibalism (including head, tail and whole body cannibalism) observed from high to low in HD.NP.LW (3.55 ± 5.99 %), LD.NP.DW (2.66 ± 3.37 %), HD.NP.DW (2.48 ± 3.79 %), HD.NP.HW (2.21 ± 2.69 %), HD.P.LW (1.95 ± 3.83 %), LD.NP.LW (1.77 ± 5.32 %), HD.P.DW (1.77 ± 2.17 %), HD.P.HW (1.50 ± 2.51 %), LD.NP.HW (0.88 ± 3.44 %), LD.P.LW (0.88 ± 2.34 %), LD.P.DW (0.44 ± 1.71 %) and LD.P.HW (0.44 ± 1.71 %) treatments, respectively (Fig. 4). The results of MANOVA indicated that predator cues, water level and density independently and interactively had not significant effect on rate of total cannibalism over time (Table 4).

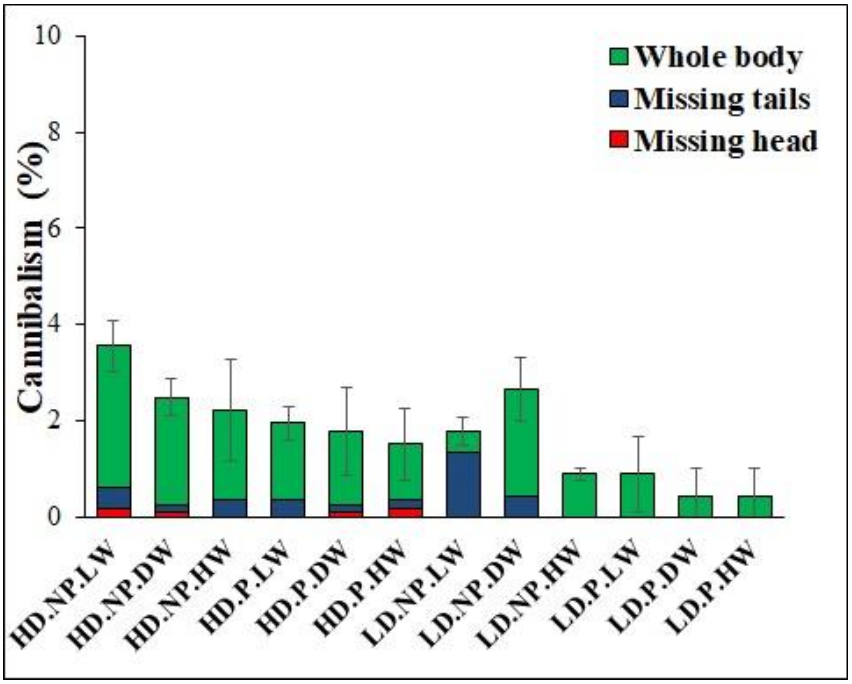

FIGURE 4. The percentage of head, tail and whole-body cannibalism in the different treatments. Abbreviation: presence of predator cues (P), absence of predator's cues (NP), high density (HD), low density (LD), high water level (HW), low water level (LW), decreasing water level (DW).

Head width size at 13th week of experiment measured from high to low in LD.P.LW (8.43 ± 0.6 mm), LD.P.HW (8.38 ± 0.45 mm), LD.P.DW (7.83 ± 1.01 mm), HD.P.LW (6.98 ± 0.21 mm), LD.NP.HW (6.97 ± 0.37 mm), LD.NP.DW (6.85 ± 0.68 mm), HD.P.HW (6.71 ± 0.77 mm), HD.P.DW (6.63 ± 0.06 mm), HD.NP.DW (6.51 ± 0.09 mm), LD.NP.LW (6.41 ± 0.53 mm), HD.NP.HW (6.23 ± 0.33 mm), HD.NP.LW (6.20 ± 0.20 mm) treatments, respectively. Also, the size of head width measured in 29th week of experiment and observed from high to low in LD.NP.HW (9.60 ± 0.39 mm), LD.NP.DW (9.30 ± 0.6 mm), HD.P.LW (9.30 ± 0.36 mm), HD.P.DW (9.27 ± 0.03 mm), LD.NP.LW (9.07 ± 0.41 mm), HD.P.HW (9.01 ± 0.08 mm), HD.NP.DW (8.84 ± 0.08 mm), HD.NP.LW (8.80 ± 0.44 mm) and HD.NP.HW (8.42 ± 0.33 mm) treatments, respectively. LD.P. LW, LD.P. DW and LD.P. HW treatments

TABLE 4. Summary statistics of multivariate analysis of variance (MANOVA) for predator cues, water level and density effects on total cannibalism.

Total cannibalism	**Wilks' lambda**	***df***	***F***	***p-value***
Predator cues	0.99	1	0.01	0.92
Water level	0.97	2	1.76	0.17
Density	0.99	1	0.01	0.91
Predator cues × density	0.99	1	1.33	0.25
Predator cues × water level	0.99	1	0.82	0.44
Water level × density	0.99	2	0.45	0.63
Predator cues × water level × density	0.99	2	0.78	0.45
Predator cues × time	0.96	1	15.01	**0.02**
Water level × time	0.99	2	0.23	0.79
Density × time	0.99	1	0.05	0.82
Predator cues × density × time	0.99	1	0.42	0.51
Predator cues × water level × time	0.99	1	0.43	0.64
Water level × density × time	0.99	2	0.53	0.58
Predator cues × water level × density × time	0.99	2	0.75	0.47

TABLE 5. Summary statistics of multivariate analysis of variance (MANOVA) for effects of predator cues, water level and density on the size of head width.

Head width	**Wilks' lambda**	***df***	***F***	***p-value***
Predator cues	0.90	1	49.91	0.0001
Water level	0.78	2	62.74	0.0001
Density	0.88	1	56.22	0.0001
Predator cues × density	0.93	1	31.43	0.0001
Predator cues × water level	0.96	1	8.06	0.0004
Water level × density	0.98	2	4.38	0.01
Predator cues × water level × density	0.92	2	17.65	0.0001
Predator cues × time	0.86	1	64.24	0.0001
Water level × time	0.95	2	63.71	0.0001
Density × time	0.89	1	53.79	0.0001
Predator cues × density × time	0.82	1	95.88	0.0001
Predator cues × water level × time	0.99	1	1.09	0.33
Water level × density × time	0.98	2	3.03	0.04
Predator cues × water level × density × time	0.96	2	7.61	0.0006

performed metamorphosis on 29th week. The results of MANOVA indicated that predator cues, water level and density independently and interactively had significant effect on head width (Table 5).

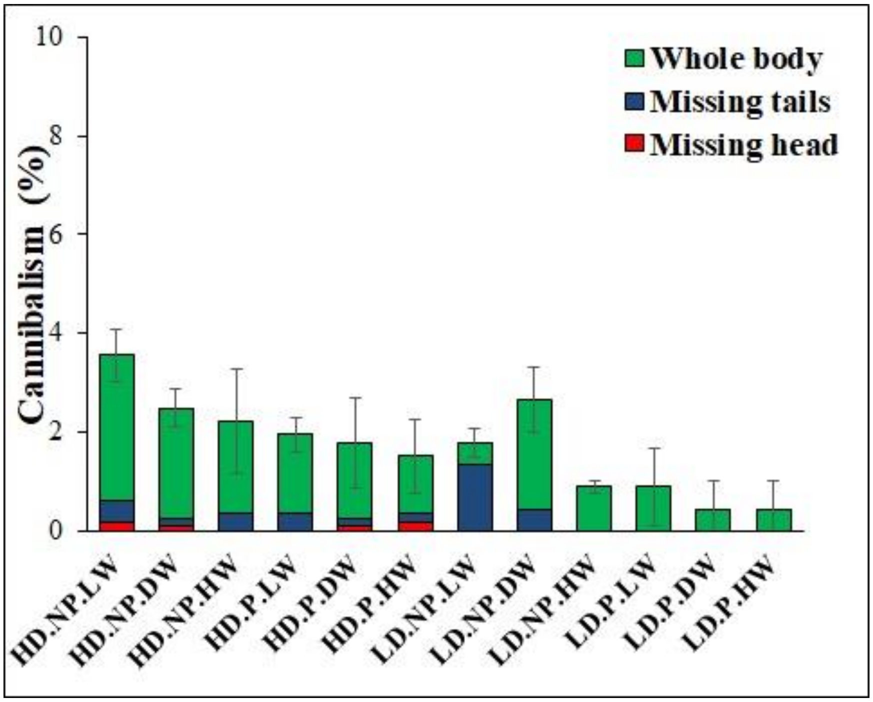

FIGURE 4. The percentage of head, tail and whole-body cannibalism in the different treatments. Abbreviation: presence of predator cues (P), absence of predator's cues (NP), high density (HD), low density (LD), high water level (HW), low water level (LW), decreasing water level (DW).

DISCUSSION

Predator cues play a crucial role in the antipredator behavior of amphibians, particularly in the presence of non-contact predators (Rajchard, 2006). Response to chemical alarm signals has been documented for frog tadpoles and several species of salamander (Marquis et al. 2004). Tadpole activity can be affected by predators, leading to reduce the rate of larval growth (Mogali et al. 2011, 2016). Similarly, to interspecific interactions, antipredator responses can be exhibited in the presence of cannibalistic conspecifics (Kats et al. 1994). Chivers et al. (1997) showed that older larvae of long-toed salamanders exhibited antipredator behavior towards conspecifics possessing the cannibal morphology but not toward conspecifics possessing the typical morphology and observed reduced activity in their presence. In our experiment, significantly the highest rate of total cannibalism documented in presence of predator cues in *P. bedriagae* larvae. The results confirmed a significant increase in head width observed in all treatments where predator cues were present over time.

Previous studies have indicated that cannibalism is often observed among breeding amphibians in temporary water bodies (Wissinger et al. 2010). Such habitats, can dramatically fluctuate in response to changes in environmental conditions (Brooks, 2004). Low precipitation combined with high temperatures can lead to the decreased pond size and rapid pond drying (Brooks, 2004; Jefferson et al. 2014). These restrictions can force larvae into feeding aggregations, increase the degree of intraspecific competition, and lead to aggression and intraspecific predation (Jefferson et al. 2014). Therefore, Cannibalistic behavior of populations is increased to reduce the volume of water and accelerate the growth period because of the decrease in water soluble oxygen and the intraspecific competitions (Mori & Natuhara, 2004). Anderson et al (2013) suggested that predation risk and cannibalism increased in temporary ponds compared to permanent ponds in *Ambystoma talpoideum* larvae. Wildy et al. (1999) indicated cannibalism was not observed in either fast-drying or slow-drying conditions in *Ambystoma macrodactylum*.

Studies suggest that cannibalism is not simply a response to pond drying itself, but is a response to the limited food levels and the increased size variation that can occur when ponds dry up. However, research on the effects of water-level fluctuations on cannibalism rates is scarce. Moradi et al. (2019) reported the highest total cannibalism rate in low water levels in *Bufo variabilis,* but found that water level independently had no significant effect on the total cannibalism rate in *B. variabilis* larvae over time. In our study, we documented the highest total cannibalism rate in *P. bedriagae* observed in the high-water level. The results of MANOVA showed that, except for the significant effect of water level on whole body cannibalism, this factor independently had no significant effect on head, tail and total cannibalism rate over time (Tables 1-4). However, water level independently had significant effect on increase of head width over time (Table 5; Fig. 5).

Habitat desiccation can change a series of environmental factors, including increasing the density of tadpoles (Brady & Griffiths, 2000; Enriquez-Urzelai et al. 2013). Many studies suggest that density is the principal factor affecting aggression (Collins & Cheek, 1983; Nishihara, 1996). Higher densities provide more opportunities for encounters between conspecifics, which can lead to increased aggression. For example, Thibault (1974) observed that increasing density of *Poeciliopsis monacha* negatively affected individuals within the population and stimulated cannibalistic behavior. Collins and Cheek (1983) indicated that high density influenced the production of cannibal morphs in larval Arizona tiger salamanders (*A. t. nebulosum*). In addition, there is a strong interaction between density and food level. Food availability and conspecific density can influence the expression of intraspecific aggression and cannibalistic behavior (Collins & Cheek, 1983; Wildy et al. 2001; Vaissi & Sharifi, 2016). Wildy et al. (2001), showed that the highest incidence of cannibalism was observed in the low food/high density treatment, while the lowest incidence was observed in high food/low density treatments. Maneti et al. (2015) found that aggression among larval European fire salamanders (*Salamandra salamandra*) increased with density and dietary stress, and this increased aggression enhanced the probability of intraspecific attacks and cannibalism. In cane toads, food quality can also conduct populations towards cannibalism and play a major role (Babbitt & Meshaka, 2000). According to Moradi et al. (2019), the highest total cannibalism rate in *Bufo variabilis* was observed at low density. This factor independently and interactively (temperature, water level and density) had a significant effect on cannibalism rate. In our study, the highest total cannibalism rate was observed at high density in *P. bedriagae*, but this effect was not significant (Table 5; Fig. 5).

With growing awareness of the complexity of natural systems, it has become generally accepted that controlled multi-factorial experimental studies are necessary as the combined effect of multiple environmental factors cannot always be predicted from single-factor studies (Alton & Franklin, 2017). Vaissi and Sharifi (2016) examined the effects of food availability and density on agonistic interactions of larval *N. microspilotus* and found that conspecific density and food limitation were associated with increased biting. Pizzatto and Shine (2008) demonstrated that juvenile *Bufo marinus* consumed their smaller conspecifics with the switch from insectivory to cannibalism exhibited during dry seasons and at high densities of small conspecifics. Moradi et al. (2019) indicated that the interactive effects of temperature, water level and density factors had significant effect on cannibalism rate in *B. variabilis* larvae over time. In our study, we found that predator cues, water level and density interactively had no significant effect on head, tail, whole body and total cannibalism rates. However, the interaction of these three factors had a significant effect on the increase of head width over time.

Cannibalistic amphibian larval morphs, which have greater head widths than typical morphs and therefore have larger mouths, provide the basis for the construction of a simple model of cannibalism that describes the growth dynamics of body shape (Wakano et al. 2002). As the relative head sizes of interacting individuals determine the occurrence of cannibalism, the situation is frequency- dependent (Wakano et al. 2002). For example, Pierce et al. (1983) shown that head size does not increase at a faster rate relative to body size in cannibals, but cannibals begin with a larger head early in ontogeny. Nyman et al. (1993) examined body size and head dimensions in relation to cannibalism in autumn-breeding salamander larvae, *Ambystoma annulatum*. A comparison of allometric relationships in cannibals and non-cannibals indicated that cannibals had proportionally broader heads, although the differences were less than those observed between cannibal and non-cannibal morphs of the tiger salamander larvae, *A. tigrinum*. Nishihara-Takahashi (1999) supported the hypothesis that a wide head is an adaptation against frequent cannibalism in larval *Hynobius retardatus*. McLean et al. (2016) suggested environmental factors, such as density inducing the cannibalistic morphism may be more complex than previously thought. They identified a scenario where the presence of an invasive competitor/prey species might have induced the cannibal morph phenotype. In our study, the rate of cannibalism was low in all treatments; as a result, there was no significant relationship between larger head size and higher cannibalism rates.

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RESEARCH ARTICLE **Open Access**

Bats of Guilan, northern Iran: a review and uncovering novel discoveries, with comments on two key cave roosts

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Abstract
Guilan province, located in northern Iran and surrounded by the Caspian Sea to the north and the Hyrcanian forests to the south, boasts the most diverse habitats in the country. However, there is a lack of information on bat fauna in the caves of Guilan province, with all existing records limited to artificial roosts, foraging individuals or echolocation calls. Despite this, available data suggests that 13 species of bats have been reported in the region. In this study, 12 caves throughout the province were observed, resulting in the identification of five bat species, namely *Miniopterus pallidus*, *Pipistrellus pipistrellus*, *Myotis blythii*, *Rhinolophus hipposideros*, and *R. ferrumequinum*. One colony and two individuals in separate localities were also identified at the generic level as *Rhinolophus* sp., while two cave roosts with large populations and considerable number of species were described in detail. Furthermore, this study encompasses data on five new sample records, comprising three from *Pipistrellus kuhlii*, one from *Rhinolophus* sp., and one from *Rhinolophus hipposideros*, from foraging individuals and artificial roosts. Overall, these findings contribute to our understanding of the diversity and distribution of bats in Guilan province, particularly within its caves.

Keywords: *Chiroptera, Fauna, Hyrcanian Forests.*

INTRODUCTION

Caves are large subsurface habitats where peculiar, even ambivalent environmental conditions are experienced by their inhabitants. Compared to other subterranean habitats, caves are more easily accessible, making them a temporary or permanent residence for a diverse range of organisms. These organisms occupy caves for varying reasons and duration (Culver & Pipan, 2019).

Bats are considered as trogloxene mammals which partially depend on the surface environment to fulfill their biological demands (Culver & Pipan, 2019). They benefit from the caves for various reasons including mating, nursery, and hibernation, as well as daily, seasonal and transient roosts. Caves also provide a secure environment for bats to avoid outside temperature fluctuations, competitors and diurnal predators (Altringham, 2011).

The identification of 52 bat species from 9 different families in Iran highlights the country's diverse habitats and suitable climatic conditions for bats to exploit (Yusefi et al. 2019; Khajeh et al. 2020). The study was conducted in the political borders of Guilan province, a coastal province in the

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north of the country that is surrounded by the western Hyrcanian Forests and divided into two main elevated areas: the Talesh mountain ranges in the west and the Alborz Mountain ranges in the east and south. Being surrounded by the Caspian Sea from the north and the mountains from the south, this region contains the most productive landscapes in the country, offering a wide variety of habitats for bats and other wildlife, including caves (Farid-Mojtahedi, 2013).

Compared to the well-studied caves in the Zagros Mountain ranges at the west and southwest of the Iran, the caves of the Talesh and Alborz Mountain ranges have been poorly studied. In recent years, a considerable number of caves in Zagros mountains have been investigated for their bat fauna, while data on chiropteran assemblage of caves in Talesh and Alborz Mountain ranges is scarce (Fathipour et al. 2016; Shahabi et al. 2017; Akmali et al. 2019a, b, 2022). Over a hundred caves and natural rock shelters have been discovered in the western part of the Hyrcanian forests (Dehdar-Dargahi & Farid-Mojtahedi 2014; Jahani, 2017). This study represents the first attempt to collect information about the bat assemblage and their roosts in the caves of this area. Furthermore, all previous records of bats from this area are reviewed.

MATERIAL AND METHODS

Field studies were conducted in 2019-2022, during which a total number of 17 caves were visited on a regular basis all-year-round, and investigated for their bat fauna. Only a few colonies were discovered in certain caves, while only one or two hanging or flying individuals were observed in the most caves. A few individuals were collected by hand, hand-net, or mist-net, photographed, and measured for their distinguishing characteristics, and then released at their roosts. Forearm measurements are provided in Table 3. Bats were identified using identification keys (DeBlase, 1980; Dietz & Von Helversen, 2004). The coordinates of each cave were recorded using a mobile GPS app. All new coordinates, as well as previous records of bats from the studied area, were imported to ArcGIS version 10.2, and a distribution map was prepared. We excluded previous records without coordinates or precise location addresses. Additionally, in six cases, estimated coordinates were assigned for records with accurate addresses using Google Earth Pro. This map illustrates all available record sites, including caves, artificial roosts, foraging individuals, and records of echolocation calls, separately. This study was conducted under the research ethics certificate, with approval ID: IR.RAZI.REC.1399.039.

Study area

Guilan province is a unique area in term of topography, elevations and climatic conditions and diversity of ecosystems. The elevation range varies from -26 meters through the Caspian coastal areas to 3707 meters in Samamoos mountain. This altitude variation is one of the main causes of diverse climatic conditions in the province, where semi-arid climate in the southern plain to highly humid and cold weather at the foothills can be experienced. The main topographic features of the province are the Talesh mountain range in the west and the Alborz range in the south and east. The occurrence of Sefid-rud tectonic valley disconnects the continuity of elevations in the middle of the Guilan province, leading to distinct environmental conditions in the southern lowlands of the Rudbar area where a semi-arid climate is dominant. The Caspian Sea makes the northern margin of the province and has considerable impacts on its topographical and climatical conditions. Estuarine ecosystems are located in the wide and flat plains along the shores of the Caspian Sea. A continuous topographic transition is observed crossing from the lowlands of the coastal plains to high elevations in the mountainous regions of Alborz foothills. The Hyrcanian vegetation belt covers the northern slopes of the mountains throughout the province. The temperature follows a descending pattern southward; then increases at the southern lowlands (Farid-Mojtahedi, 2013; Farid-Mojtahedi et al. 2018).

TABLE 1. summarized information on studied caves.

No.	Name of cave	Elevation (meter)	Length of cave (meter)	Time of visits	Number of visits	Number of bat species
1	Divrash 2	314	30	All-year-round	5	3
2	Divrash 3	339	10	All-year-round	2	1
3	Darband Rashi	720	58	Summer, Autumn, Winter	4	1
4	Shoupar-Chal	137	70	All-year-round	4	2
5	Cheshmeh-Bad	1408	400	Summer	1	1
6	Kaslav	825	10	Summer, Autumn	2	1
7	Liyarud	503	12	Autumn	1	1
8	Shalash	1640	70	Autumn	1	1
9	Eqbal	495	20	Autumn	1	1
10	Diyarjan	1892	52	Summer	2	1
11	Koolak- Avar	293	500	Summer	1	1
12	Espahbodan	1681	24	Autumn	1	1

RESULTS

The review of previous studies

Totally, 18 bat species have been reported from Hyrcanian Forests so far from which 13 species have been observed in the western part of the forests. This number includes 23 site records that refer to echolocation calls, foraging bats, artificial roosts as well as a few records of bat remains in owl pellet. Here, we review the previous records on bats in this region (Benda et al. 2012; Tohidifar et al. 2016).

The sole record of *Rhinolophus ferrumequinum* dates back to 1774; and no recent observation is registered since then. *Pipistrellus nathusii* has been recently reported from Anzali Wetland by Naderi et al. (2017). This is the only record on this species in the country. Although, *Myotis blythii* have a wide distribution range in Iran, there have been no new observations of this species from Guilan Province since 1907. The records on *Rhinolophus hipposideros* from this region are limited to a few records including one museum speciemen from Asalem, an artificial roost in Saghaleksar and foraging individuals in Anzali wetland (Benda et al. 2012; Naderi et al. 2017). Furthurmore, Shahabi et al. (2019a) recorded the echolocation call of this species in Bandar Anzali. The only record on *Eptesicus serotinus* from Guilan province refers to the netting of two individuals in a clearing close to the forest edge in 1968 (Steiner & Gaisler, 1994). Similarly, from a sole record on *Hypsugo savii*, this bat was detected by its echolocation calls in a rocky valley near Rudsar. The observation records of *Pipistrellus pipistrellus* include a large colony in an attic, the remains in owl pellet and two separate foraging individuals. All reports on *P. pygmaeus* are limited to foraging individuals at the Caspian Sea coastal areas. Several records demonstrate the occurrence of *P. kuhli* in the province. These records include the observation of the bats in their roosts, netting foraging individuals, and their remnants in owl pellet. The records of the rare *Nyctalus noctule* from the province have been reviewed by DeBlase (1980). These records refer to two museum collections which were gathered by Thomas (1907) and Farhang Azad (1970) from their roosts. The single record on the *N. lasiopterus* for the whole country belongs to the western part of the Hyrcanian forests where Etemad (1970) detected a migratory individual. *Myotis mystacinus* morpho-group is another bat species which has been identified from Guilan, and has only one observation record.

The results of this study are based on several cave expeditions during 2019 to 2021 in the western parts of Hyrcanian forests. The results of the field trips are summarized in Table 1, and images of some of the specimens are shown in Figure 1. Totally, 12 caves were explored and four bat species were identified. The studied caves include Divrash complex caves (including three separate caves), Darband Rashi, Cheshmeh Bad, Shoupar-Chal, Liyarud, Shalash, Eqbal, Koolak-Avar, Diyarjan, Espahbodan and Keslav. The identified species are listed below with some details on their roosts. One addition colony was identified at generic level and included in the results and analysis of this study. We represent these two taxa as *Rhinolophus* sp. from Divrash 3 cave. The single flying individuals in Diyarjan and Keslav caves weren't identified due to their high flight and immediate disappearance. In Espahbodan cave, only the remnants of a bat skeleton were found, but evidences from previous visitors demonstrate the existence of bats in this cave. Furthermore, two roosts bearing considerable population size were located, monitored all-year-round and depicted. We also present five new records from artificial roosts and foraging individuals. Here, we present the new bat records from the studied area.

Family Miniopteridae Dobson, 1875

Miniopterus pallidus Thomas, 1907

Pale bent-winged bat is a widespread bat in Iran (Akmali et al. 2015). There are some records on the occurrence of this species in the eastern parts of Hyrcanian forests. Shir-Abad cave is the only subterranean habitat in the Hyrcanian forests where this species has been reported. The two other records from the northern part of the country refer to its foraging habitats (Benda et al. 2012).

In this study, *Miniopterus pallidus* was observed in Divrash 2 and Shoupar-Chal caves, where it was observed to share its roost with lesser horseshoe bat. During a summer visit to Divrash 2 cave, four male individuals were collected by a mist-net at the entrance of the cave during their evening emergence. In Shoupar-Chal cave, a large colony of this species was observed, and a few individuals were captured by hand- difficulty net. A detailed description on these two roosts is discussed at the end of this section.

Family Rhinolophidae Gray, 1825

Rhinolophus sp.

Due to of collecting bats in some caves, we were unable to identify flying or hanging individuals at specific level. Although, visual observation and photography confirmed their affiliation to Rhinolophidae family. One colony in Divrash 3 cave, two individuals in Eqbal cave, and one in a manmade underground tunnel were assigned to *Rhinolophus* sp. Eqbal cave is a medium-sized single gallery that is partially separated into two rooms and requires crawling to enter. The bats were hanging separately from the walls of the cave, and no sign of accumulated bat guano was observed on the cave floor.

Rhinolophus ferrumequinum (Schreber, 1774)

The greater horseshoe bat is one of the rhinolophid species whose distribution range and taxonomic status is well-studied from the Northwest to the Southwest and South of the country (Shahabi et al. 2017b, 2019b). Despite being observed in the studied area in the late 18th century, there have been no updated reports on the observation of this species since then. However, in the current study, we collected a dead bat in Shalash cave. In this cave, several hibernating bats were observed on the roof of the main corridor, and a dead individual of this species was found on the cave floor. This cave is a water cave and is known as a sacred place for the local people. The findings of this study provide updated observations on the presence of this species in the studied area and highlight the importance of continuing monitor and study bat populations in these caves.

Rhinolophus hipposideros (Bechstein, 1800)

The lesser horseshoe bat is a relatively common species in Iran, with a continuous distribution in the western half of the country and scattered records from eastern regions and southern coastal margins. However, there are no reports on this species from central deserts. Almost all records of *R. hipposideros* are limited to its roosts, mostly in caves. There are a few records of this species from the western part of Hyrcanian Forests, which refer to artificial shelters (Benda et al. 2012; Naderi et al. 2017). However, the occurrence of the lesser horseshoe bat has not been documented in Guilan caves until this study, which represents the first record of this bat in its subterranean roosts from the western parts of Hyrcanian Forests. In this study, three caves in the studied area were observed to have lesser horseshoe bats.

TABLE 2. Detailed information about new and previous bat records in Guilan province.

No.	Status	description	Altitude (meters)	Coordinates	Identified taxa	Reference
1	Artificial	Lisar, house crevices	0	N37 58 00.0 E48 55 00.0	*Pipistrellus kuhlii*	Benda et al. (2012)
2	Artificial	Rasht, roof of a house	-7	N37 17 00.0 E49 35 00.0	*Pipistrellus kuhlii*	Steiner and Gaisler (1994)
3	Artificial	Saqaleksar, near Rasht, attic of a mosque	50	N37 10 00.0 E49 34 00.0	*Rhinolophus hipposideros*	Benda et al. (2012)
4	Artificial	Saqaleksar, near Rasht, attic of a house	46	N37 09 46.8 E49 31 12.5	*Pipistrellus pipistrellus*	Benda et al. (2012)
5	Artificial	Anzali wetland, rural houses	-29	N37 24 55.0 E49 20 19.0	*Pipistrellus pygmaeus*	Naderi et al. (2017)
6	Artificial	Lisar, under a barn roof	-26	N37 22 51.6 E49 32 32.7	*Rhinolophus hipposideros*	Present study
7	Artificial	Golsarak tunnel, Emamzadeh Hashem	118	N37 01 49.9 E49 37 56.9	*Rhinolophus ferrumequinum*	Present study
8	Artificial	Near Rasht, under a barn roof	17	N37 57 43.1 E48 54 07.6	*Pipistrellus kuhlii*	Present study
9	Cave	Manjil, Cheshmeh-Bad cave	1408	N36 46 02.0 E49 29 10.0	*Myotis blythii*	Present study
10	Cave	Amlash, Liyarud cave	503	N37 00 31.9 E50 02 15.4	*Rhinolophus hipposideros*	Present study
11	Cave	Rezvanshahr, Shalash cave	1640	N37 27 02.2 E48 47 52.7	*Rhinolophus ferrumequinum*	Present study
12	Cave	Lahijan, Shoupar-Chal cave	137	N37 08 38.4 E50 04 56.8	*Miniopterus pallidus, Rhinolophus hipposideros*	Present study
13	Cave	Tutkabon, Darband Rashi cave	720	N36 50 02.0 E49 39 18.7	*Pipistrellus pipidtrellus*	Present study
14	Cave	Tutkabon, Divrash 3 cave	339	N36 54 13.0 E49 35 27.8	*Rhinolophus* sp.	Present study
15	Cave	Tutkabon, Divrash 2 cave	314	N36 54 11.5 E49 35 25.0	*Miniopterus pallidus,* *Rhinolophus hipposideros,* *Myotis blythii*	Present study
16	Cave	Deylaman, Diyarjan cave	1892	N36 53 41.0 E50 01 10.0	Unidentified	Present study
17	Cave	Fuman, Eqbal cave	495	N37 17 21.4 E49 09 46.2	*Rhinolophus ferrumequinum*	Present study
18	Cave	Deylaman, Espahbodan cave	1681	N36 53 51.6 E49 46 21.9	Unidentified	Present study
19	Cave	Shaft, Keslav cave	825	N36 59 54.1 E49 13 39.2	Unidentified	Present study
20	Cave	Siyahkal, Koolak-Avar cave	293	N37 06 39.4 E49 52 59.9	*Myotis blythii*	Present study
21	Echolocation	In a rocky valley at Ziaz near Rudsar	854	N36 51 00.0 E50 14 00.0	*Hypsugo savii*	Benda et al. (2012)
22	Foraging	Above a river in a beech forest near Tutaki	406	N37 02 00.0 E49 54 00.0	*Pipistrellus pygmaeus*	Benda et al. (2012)
23	Foraging	Edge of small clearing in forest near Assalem	1278	N37 39 00.0 E48 49 00.0	*Eptesicus serotinus, Plecotus auritus*	Steiner and Gaisler, 1994
24	Foraging	Rudsar	-23	N37 08 00.0 E50 17 00.0	*Nyctalus lasiopterus*	Etemad (1970)
25	Foraging	Anzali wetland near Khomam	-27	N37 24 36.2 E49 33 52.2	*Rhinolophus hipposideros*	Naderi et al. (2017)
26	Foraging	Sorkhankol protected area, Anzali wetland	-27	N37 23 54.1 E49 27 15.5	*Pipistrellus kuhlii*	Naderi et al. (2017)
27	Foraging	Sorkhankol protected area, Anzali wetland	-28	N37 24 49.7 E49 27 20.0	*Pipistrellus nathusii*	Naderi et al. (2017)
28	Foraging	Anzali wetland	-24	N37 29 06.0 E49 19 27.0	*Pipistrellus pygmaeus*	Naderi et al. (2017)
29	Foraging	Anzali beach	-26	N37 29 40.1 E49 23 38.3	*Pipistrellus kuhlii*	Present study
30	Foraging	Sangachin road, near Anzali	-22	N37 31 08.4 E49 18 26.9	*Pipistrellus kuhlii*	Present study
31	Natural cavities	Tutkabon, near Divrash cave	352	N36 54 15.7 E49 35 35.4	*Rhinolophus hipposideros*	Present study

TABLE 3. Forearm measurements of bat species.

No.	Bat species	Number of measured specimens	Mean forearm length (mm)
1	*Miniopterus pallidus*	7	46.46
2	*Rhinolophus hipposideros*	6	37.1
2	*Rhinolophus ferrumequinum*	1	55.5
4	*Pipistrellus pipistrellus*	2	32.12
5	*Pipistrellus kuhlii*	7	32.62

Miniopterus pallidus from Shoupar-Chal cave

Rhinolophus hipposideros from Liyarud cave

Rhinolophus sp. from Eqbal cave (photo by Pooya Safari)

Rhinolophus hipposideros from Divrash2 cave

Pipistrellus pipistrellus from Darband Rashi cave

Myotis blythii from Cheshmeh-bad cave (photo by Masoud Zare)

Corpse of *Rhinolophus ferrmequinum* from Shalash cave

Pipistrellus kuhlii from Anzali beach

Miniopterus pallidus from Divrash2 cave

FIGURE 1. The images of identified bat species in the present study.

FIGURE. 2. A) The roof of the barn in Lisar, roost of the lesser horseshoe bat; B) Unidentified horseshoe bat in the underground tunnel near Emamzadeh-Hashem; C) the passage of the tunnel.

Divrash 2 cave is a permanent and all-year-round roost for *R. hipposideros*. We observed less than ten immotile individuals hanging separately from the ceiling and walls of the cave near the floor during mid-autumn and early spring visits. During the mid-summer visit, one specimen was collected by a mist-net set up at the cave entrance as it emerged in the evening. Another individual was collected from a small cavity along the valley near Divrash complex caves during the mid-autumn visit. Shoupar-Chal cave, located in a small relict forest in the middle of a tea farm, was observed to have a big mixed colony of about 500 individuals of *Miniopterus pallidus* and *R. hipposideros* during the mid-summer visit. One flying individual of the lesser horseshoe bat was collected by a hand net. During the early spring visit, only a few flying bats were observed. Liyarud cave, located on a steep slope with dense forest coverage, was observed to have two individuals hanging from the low ceiling of the cave. These observations provide valuable insights into the distribution and roosting behavior of the lesser horseshoe bat in the western parts of Hyrcanian Forests, highlighting the importance of monitoring and studying bat populations in this area.

Family Vespertilionidae Gray, 1821

Myotis blythii (Tomes, 1857)

Myotis blythii is one of the most common bat species in Iran, with a widespread distribution from northeast to northwest and southwest. While there are a few records of *M. blythii* from the southeast, no records of this species have been documented in the central deserts and eastern areas of Iran. Most records of *M. blythii* refer to its roosts, especially in caves located in mountainous and humid areas above 1500 meters above sea level. Sheikh-Jabbari (2008) identified *M. blythii* in 12 localities in the closest region to the studied area, Ardabil Province, of which four records refer to caves. There are also several records of this species throughout the Alborz Mountain Ranges. Thomas (1907) collected one male individual from the Alborz Mountains near Rasht, although no further details were given on this record. In the current study, we observed *M. blythii* roosting in the caves. One individual was observed in a torpor state in a small cavity on the ceiling of Divrash 2 cave. This observation was made during the early spring visit when no flying bats were observed in the cave space. Another cave where *M. blythii* was observed in this study is Cheshmeh-Bad. This cave is an aquatic cave with a water flow through the main passage that connects to a pool at the first gallery. A few individuals of *M. blythii* were observed hanging on the walls of the first gallery. Additionally, a flying individual of this species was observed around the entrance of Koolak-Avar cave, during which it unexpectedly landed on the shoulder of a team member. However, due to the cave's impassable passages, the team was unable to explore the cave further, and no bat colony was found. These observations enhanced our understanding of the distribution and roosting behavior of *M.*

blythii in Iran, highlighting the importance of studying and monitoring bat populations in caves located in mountainous and humid areas. Despite being one of the most frequent species in the country, there are still gaps in our knowledge of this species' distribution in certain regions, such as the central deserts and eastern areas, which require further investigation.

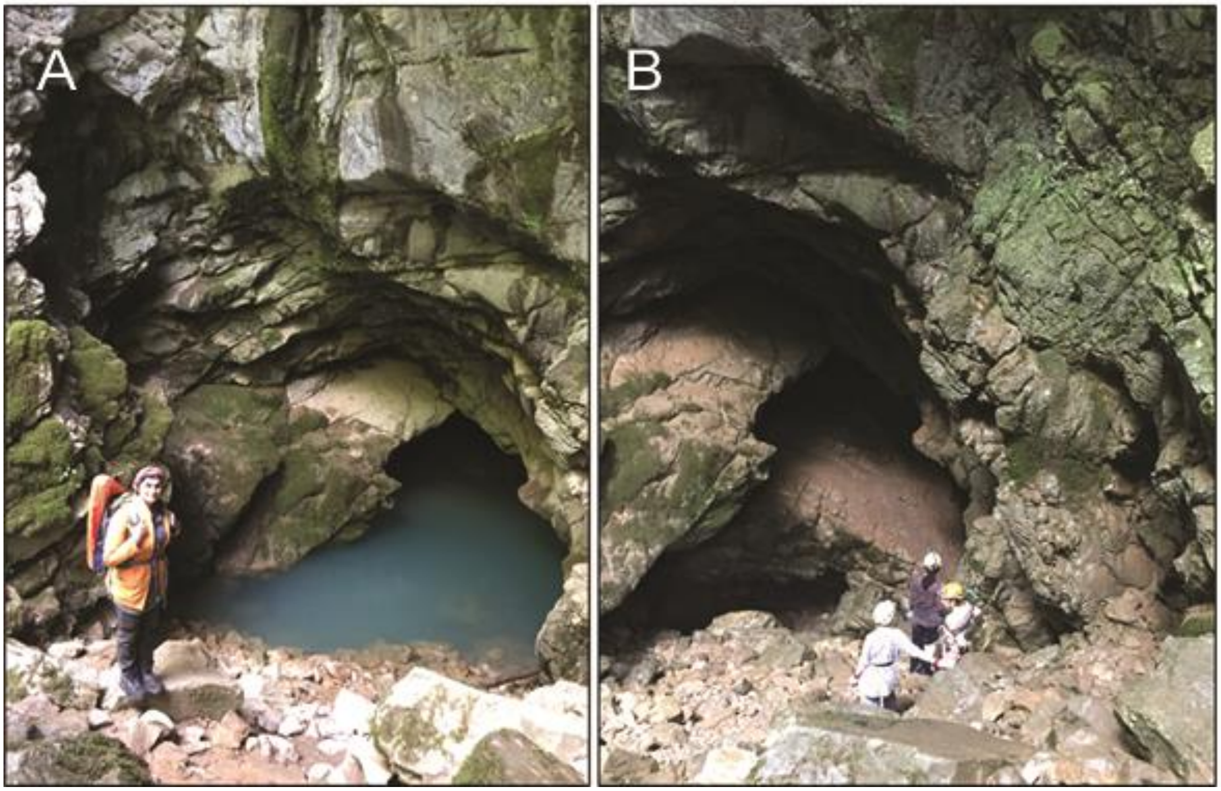

FIGURE 3. A) The level of water in early spring is going down after the flood; B) The water level is back to its normal conditions inside the cave.

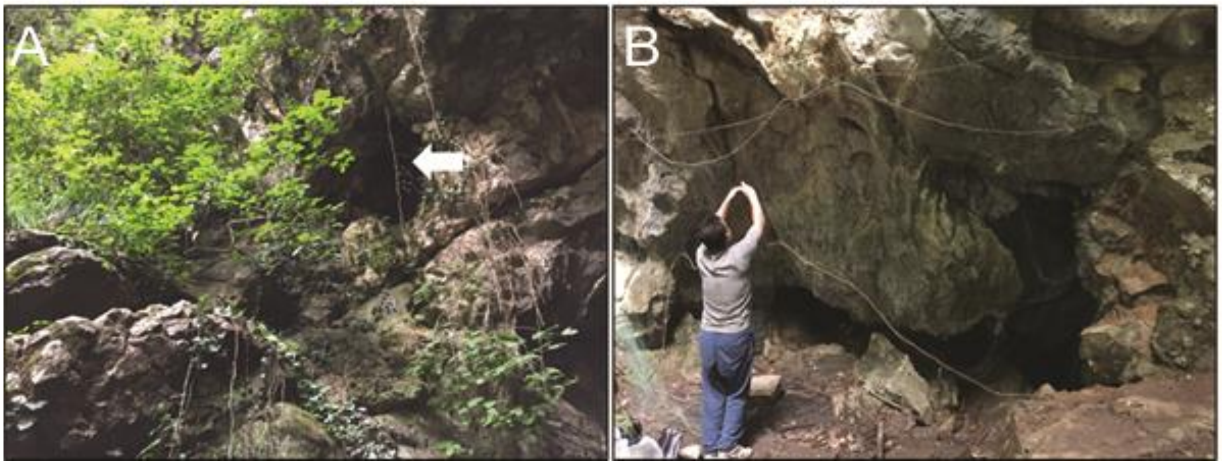

FIGURE 4. A) The entrance of Divrash 3 (white arrow); B) Installing mist-net at the entrance of Divrash 2.

Pipistrellus pipistrellus (Schreber, 1774)

Out of the four *Pipistrellus* species that are known from Iran, only *Pipistrellus pipistrellus* has been observed to roost in caves. *P. pipistrellus* and *P. kuhlii* are both very common species in Iran's bat fauna, while *P. pygmaeous* and *P. nathussi* are rather rare with limited distribution ranges. *P. pipistrellus* has a contiguous distribution in northern regions of the country, while *P. kuhlii* is more frequent in south-west and southern parts (Benda et al. 2012). In the current study, two individuals of this species were netted inside a cave at their emergence time. These two individuals were members of a rather large colony, but due to its inaccessible and high location, the bat assemblage remained unknown. The colony was estimated to comprise around 500 bats hanging from the cave ceiling at a height of 30 meters. During the early winter visit to the cave, no individuals were observed, possibly due to their hiding in invisible and deep crevices during the hibernation interval.

In addition to cave roosts, this study presents five new records of foraging individuals and artificial roosts. Two foraging *Pipistrellus kuhlii* were collected in separate captures, with one individual caught by mist-net at Anzali beach and another accidentally flying into a moving car on Sangachin road. Three more records were attributed to artificial roosts. A group of five *Rhinolophus hipposideros* individuals was observed below the roof of a barn near Lisar (Figure 2-1). Another record refers to the observation of a hibernating *R. ferrumequinum* in a manmade underground tunnel near Emamzadeh-Hashem (Figure 2-2 & 2-3). Furthermore, seven *P. kuhlii* individuals were detected under the roof of a small warehouse in a rural house next to a river bank of Anzali wetland near Rasht. All of these records have been included in the distribution map of bat records in Guilan province.

Divrash complex caves

The Divrash complex caves are located at the end of Divrash valley and comprise three separate caves, each with an independent entrance, although their possible connectivity has yet to be discovered. The caves are referred to as Divrash 1, 2, and 3. To reach the caves, one must begin at the end of Divrash village and continue through the forest along the Khereshk-Rood River. After approximately one hour of navigating and crossing the river, one arrives at the end of the valley. The large entrance of Divrash 1 is visible in front of you, and to enter the cave gallery, you must descend on unstable and loose rocks. The gallery features a permanent underground lake that encompasses the entire area. Discovering the unknown part of the cave requires special equipment and experience in cave diving. As the level of underground water rises during winter, the permanent underground lake in the cave becomes the source of a stream that flows out of the cave through its way at the bottom of the valley to join the Sefidab river.

Divrash 2 is located on the rocky wall of the valley to the right of Divrash 1. After passing through a low entrance passage, a single large gallery is present. There is a platform on the left with a high ceiling, where bats roost. However, due to the accumulation of fresh mud and guano on this platform, it is impossible to ascend it. Additionally, the height of the ceiling makes it difficult to visually identify the bat species. However, there is a colony in a relatively hidden cavity on the ceiling, which can be spotted by observing the movements of bats. Furthermore, bat individuals can be observed singly or in small groups around the ceiling of this area. A few individuals can also be seen hanging from the walls and low ceilings around the large gallery of the cave. These individuals can be easily caught by hand or hand nets and identified as lesser horseshoe bats.

During a mid-summer visit, we set a mist net at the cave entrance and waited outside for their evening emergence. Five individuals were captured, of which four adult pale bent-winged bats were identified, as well as one lesser horseshoe bat. During another visit in late winter, we observed a hibernating individual of a mouse-eared bat in one of the small cavities on the low ceiling at the deepest part of the cave. This was the only record of this species during our visits to this cave. There are some evidences on the connectivity of this cave and Divrash 1. During the wet season, a temporary water stream flows into the cave from inside the crevices in the cave wall at the deepest part of the cave, then goes downward through the crevices on the opposite wall and disappears into the hidden voids. It seems this water flow originates from the same spring that the pond in Divrash 1 emanates from.

FIGURE 5. A) The entrance of Shoupar-Chal from inside the cave; B) The entrance from outside.

The third cave that we assign it as Divrash 3, is located on the outer walls of the Divrash 1, approximately 20 meters above its entrance (Figure 4-1). Two cavities are located above a steep slope next to each other, with the right one being a dead-end. The left cavity is a 10-meter narrow passage that leads to a small room. In this room, a population of 50 horseshoe bats occupied the low ceiling. At the right corner of the room, there is a fairly narrow passage that slopes down sharply to the undiscovered part of the cave that is coated with sticky mud. The connectivity between this passage and Divrash 1 needs to be examined. Several flying bats were also observed in this passage.

We visited this cave several times from 2019 to 2022, covering all seasons. During all visits, we observed a permanent population of bats in Divrash 2 and Divrash 3. Although we did not record any pup or pregnant individuals during our visits, given the continuous presence of bats in these caves, they may serve as maternity roosts as well as hibernacula.

Shoupar-Chal cave

This cave is located in the midst of tea farms within a small relict forest, situated on a karstic outcrop. The entrance of the cave is W-shaped and is quite large, leading to a wide inclined passage that opens up into the main gallery of the cave. Although there is no water source within the cave, the walls in the entrance area are damp, causing water droplets to form. Additionally, a small opening can be observed on the right side of the entrance area in the ceiling. The cave contains a large elevated platform that partially divides the space into two rooms. The highest point of the cave roof reaches 20 meters. Several small cavities can be found within the inner space of the cave.

A deep cavity in the ceiling at the deepest part of the cave was home to a large population of about 500 bats. We applied a visual counting method to estimate the population size as the bats flew out of their roost space. Furthermore, a few lesser horseshoe bats were found hanging from the walls around the cave entrance area. To identify the bat species, we captured a few individuals using a hand-net, and identified *Miniopterus pallidus* and *Rhinolophus hipposideros* in this cave. We observed this population during three visits to the cave in mid and late summer, as well as mid-autumn. The cave is relatively easy to explore, with no special equipment necessary. There are no impassable passages or obstacles in the cave area, and only simple rock climbing is required in certain parts of the cave. The cave is situated on personal property, which provides some protection from human disturbances.

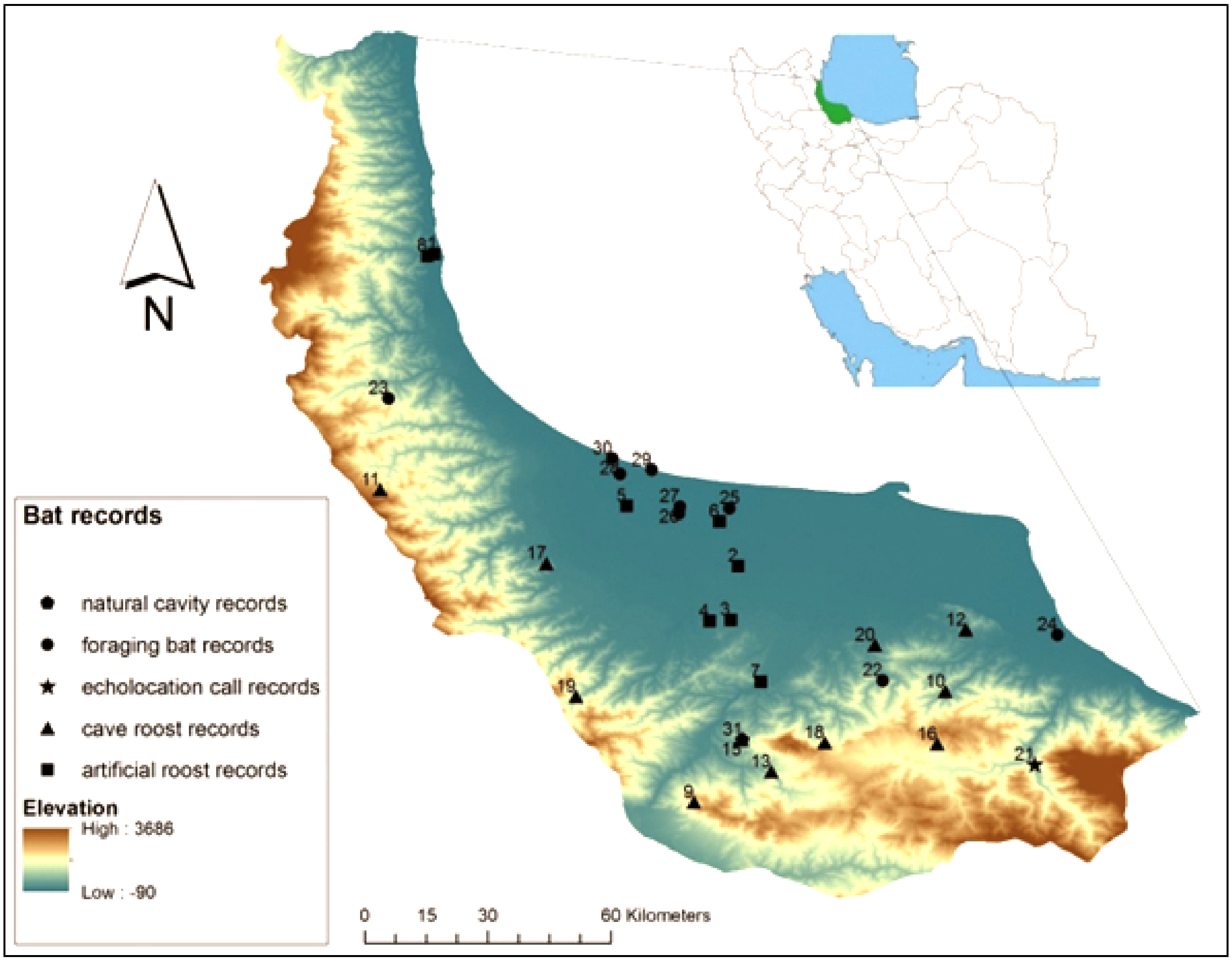

FIGURE 6. Distribution map of all bat records in Guilan province with available coordinates.

DISCUSSION

The caves in Guilan province have not been well studied in terms of their biodiversity, particularly with respect to their bat fauna. In this study, we conducted several exploration efforts to locate bat colonies in the Hyrcanian forests of this area. As a result, we detected two large aggregations of bats in the Divrash complex caves and Shoupar-Chal cave, and monitored them throughout the year. Although we did not directly observe any maternity roosts, the presence of bats in all seasons indicates that these caves are important habitats. The Shoupar-Chal cave is located in the middle of a private tea farm, which has helped to protect it from disturbances by visitors. While we did find evidence of occasional human visitation, such as garbage and waste, our observations demonstrate the persistence presence of bats in this cave.

The conditions in Divrash complex caves are vastly different. This cave is renowned for its picturesque surroundings, and during warm seasons, an average of 1000 visitors from nature-traveler communities and mountaineering clubs visit the cave each week. Their presence inevitably results in disturbance and devastating impacts on the cave's fauna. Unfortunately, this cave is not supervised by any relevant organizations. According to local people, the number of bats in this cave has been declining since the arrival of visitors. The current study has shown that at least three bat species use this cave as their permanent roost, making it imperative to protect the cave.

This study has contributed new records of cave roosts to supplement existing records, which previously only included artificial roosts, foraging individuals, and echolocation calls. We combined our new findings with previous records to gain a comprehensive understanding of the distribution patterns of bats based on the type of record. We used a total of 31 records, including 12 cave roost records, eight artificial roost records, nine foraging individual records, and one record each for echolocation calls and natural cavities, utilizing their available coordinates to create a distribution map. Detailed information on all records can be found in Table 2, which provides a comprehensive review of the data.

The altitudes of each locality were extracted from the Digital Elevation Model (DEM) of Guilan province. The resulting map revealed a regular pattern of distribution for bat records based on their altitudes (Figure 6). All 12 cave roosts, with the exception of Shoupar-Chal, were situated between 300 meters to approximately 2000 meters above sea level, whereas artificial roosts, including barns and attics, were less than 120 meters in height. Around 80 percent of the foraging bats were recorded in the Caspian lowlands, at least 22 meters below sea level. Two foraging records refer to the higher elevations. A few individuals of *Pipistrellus pygmaeus* were netted at an altitude of 406 meters above sea level. The other record was the capture of two individuals of *Eptesicus serotinus* in a wooded area in the Talesh mountains at an altitude of 1278 meters, dating back to 1968.

CONCLUSION

This study represents the first attempt to investigate cave-dwelling bats in the western part of the Hyrcanian forests within the political boundaries of the Guilan province. A total of five species, including *Miniopterus pallidus*, *Myotis blythii*, *Pipistrellus pipistrellus*, *Rhinolophus hipposideros*, and *R. ferrumequinum*, as well as one generic level identification, *Rhinolophus* sp., were recorded from the caves of Guilan. *M. pallidus* was reported for the first time in the studied area, observed in two caves in rather large colonies. Furthermore, *R. hipposideros* was documented for the first time in cave roosts in this region, and the occurrence of *R. ferrumequinum* was confirmed after more than 100 years without any records of observations. Two key cave roosts were monitored throughout the year and described in detail, emphasizing the necessity of continuous monitoring to obtain accurate information about bat colonies. Additionally, *Pipistrellus kuhlii* was reported from non-cave habitats. Although this study has improved the data on caves and their bat fauna in Guilan province, there is still much work to be done. Also, accurate data is crucial for any conservation program, and persistent and regular field studies utilizing the latest technologies and equipment should not be overlooked.

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RESEARCH ARTICLE **Open Access**

Predicting the impacts of climate change on distribution of the genus *Macrovipera* A.F. Reuss, 1927 in Iran (Reptiles: Squamata)

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Abstract
Climate change is an important growing threat to biodiversity and ecosystems. Previous studies have shown that climate change will negatively influence Iran's biodiversity. Reptiles are among the most vulnerable groups to climate change. Until now, 80 terrestrial snakes have been identified in Iran in which 16 are terrestrial venomous. In the present study, we predicted the impacts of climate change on distribution of the genus *Macrovipera* in Iran. We applied an ensemble approach, using five distribution modeling methods (Generalized Linear Models, Generalized Additive Models, Generalized Boosted Models, Maximum Entropy Modelling and Random Forest) to predict the impacts of climate change on distribution pattern of the genus. We also estimated protected areas coverage for the suitable habitat of the genus under current and future climatic conditions. We found that the genus will lose 11% of its suitable habitat under the worst-case scenario (2071-2100SSP585). Results also showed that only around 8% of the genus suitable habitat is covered by protected areas under current and future climatic conditions. Stable suitable but not protected habitats have high priority for conservation of the genus in Iran.

Keywords: *Macrovipera lebetina, Macrovipera razii*, Distribution, Conservation, Iran.

INTRODUCTION

Reptiles are one of the most diverse groups of vertebrates on earth (Uetz *et al.*, 2023). According to the latest surveys, 12021 species of reptiles (7600 species for Sauria, 4061 species for snakes and 360 species for Testudines) have been identified and described that many of which remains ecologically unknown (Uetz *et al.*, 2023). Considering that many new species are added to the list of reptiles every year, the actual number of reptiles is expected to grow. Venomous snakes are more sensitive to human disturbances and expected to be affected negatively by climate change (Maritz *et al.*, 2016; Pintor *et al.*, 2021). Therefore, it is necessary to investigate the impacts of climate change on this group of snakes. Because without considering the effects of climate change, the implementation of conservation programs will face many problems**.**

Formerly similar studies on venomous snakes showed different results (Ahmadi *et al.*, 2019; Nori *et al.*, 2014; Penman *et al.*, 2010; Yousefi *et al.*, 2015). They show that some species such as

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Hoplocephalus bungaroides in Australia will lose more than 80% of their valuable habitats, or some groups such as members of the genus *Montivipera* in Iran and neighboring countries will lose most of the suitable habitats and inevitably have to move to higher areas along the mountainous habitats (Ahmadi *et al.,* 2019; Yousefi *et al.,* 2015). However, the conditions for some other venomous snakes will be completely different, for example, the venomous snakes in Argentina will increase their suitable habitats under the climate change. suitableThus, more people will be exposed to venomous snakes and the risk of snakebite will increase in the future (Nori *et al.*, 2014)**.**

In Iran also snakes are among the most ecological unknown vertebrates and the conservation status of many of which is not clear due to lack of information (Rajabizadeh, 2018). Currently, 80 terrestrial snakes have been identified in Iran (Rajabizadeh, 2018; Yousefi *et al*., 2023). Among reptiles, venomous snakes have a more critical situation due to the negative attitude of humans and the conflicts they have with rural communities due to their bites (Pintor *et al.*, 2021). Every year, the bites of venomous snakes such as *Macrovipera lebetina*, *Echis carinatus*, *Naja oxiana* and *Pseudocerastes persicus* cause the death and permanent disability of hundreds of people in the country (Dehghani *et al.*, 2014; Yousefi *et al.*, 2020a). This factor makes their conservation more challenging than other species**.**

Until 2018, all populations of the genus *Macrovipera* in the country were known under the name of *Macrovipera lebetina* (Rajabizadeh, 2018). In fact, until 2018, this species had one of the widest distributions among venomous snakes in Iran (Rajabizadeh, 2018). But some populations of this species were introduced under a new name, *Macrovipera razii* (Oraie *et al.,* 2018). However, the exact distribution of these two species and their boundaries in the country remain unknown and require a detailed study with extensive non-invasive sampling across distribution range of the the genus (Oraie *et al.,* 2018; Oraie 2020). According to the studies conducted in the country, climate change is one of the most important threats to biodiversity (Yousefi *et al*., 2019), as it is predicted that the suitable habitats of many animal species will be lost. The studies conducted show that out of 37 different species studied in the country, 30 species will be affected by the negative effects of climate change (Yousefi *et al.,* 2019)**.** Like other animal groups, climate change is one of the most important threats to venomous snakes (Ahmadi *et al.,* 2019; Yousefi *et al.,* 2015). Therefore, it is necessary to investigate the impact of future climate changes on venomous snakes of the country**.**

Considering the importance of knowing how species will be affected by future climate changes, the present study was conducted with the aim of measuring the effects of climate changes on the distribution of the genus *Macrovipera* in Iran. Studies conducted worldwide show that species living in mountainous areas will be more sensitive to climate change and will lose most of their suitable habitats (Hannah, 2015). Vipers of the genus *Macrovipera* are living in the mountainous regions of Iran and are present in the Zagros, Alborz, Kopet Dagh and mountains of central Iran (Oraie *et al.,* 2018; Rajabizadeh, 2018; Oraie 2020). Therefore, it is expected that, like other mountain species, *Monivipera* snakes will lose their suitable habitats under climate change (Yousefi *et al.,* 2015). Therefore, the hypothesis of the present study is that it is expected that the genus *Macrovipera* will lose its suitable range under the climate change and is expected shifts its range to higher elevation habitats. Therefore, the genus future range will be smaller and more limited than their present distribution**.**

MATERIAL AND METHODS

Occurrence records

The occurrence records were collected during field surveys of Iranian herpetofauna between 2007 and 2021. It was also checked to obtain the occurrence records of online information sources such as GBIF, VertNet, and HerpNet and 187 occurrence points were obtained from all the avobe mentioned sources. Then, the records with a distance of less than 1 km were removed from the set of species occurrence points for modeling. Determining the distance of 1 km records was determined based on the spatial resolution of the climate layers, which in the present study is 1 km. In this way, the number of occurrence records was reduced to 150 points (Figure 1).

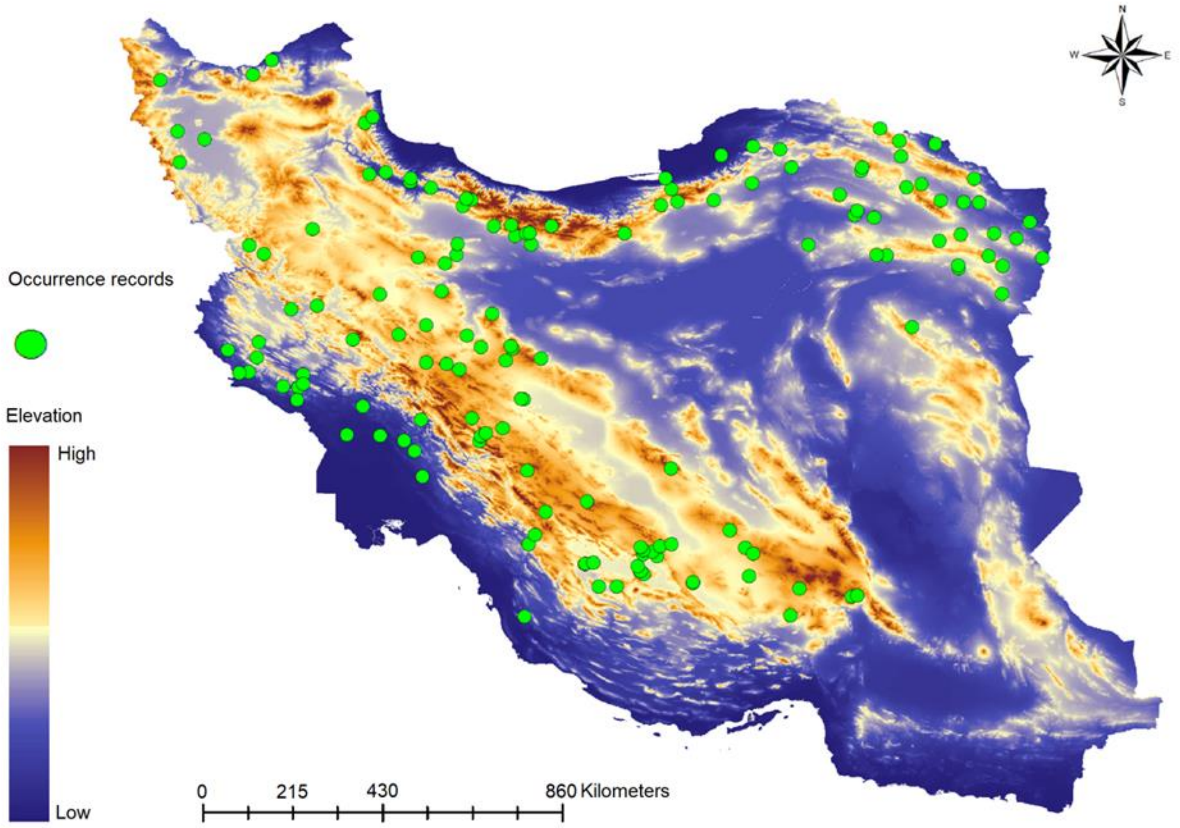

FIGURE 1. Distribution map of the genus *Macrovipera* on the Digital Elevation Model (DEM) of Iran.

Environmental variables.

To predict the impacts of climate change on the distribution of the genus *Macrovipera* in Iran, the climatic variables of the CHELSA database were used (Karger *et al.*, 2017). Current and future climate variables (years 2041-2070 and 2071-2100) from five global circulation models (GFDL-ESM4, IPSL-CM6A-LR, MPI-ESM1-2-HR, MR-ESM2-0, UKESM1-0) -LL) was extracted. The reason for using several climate change models is that each of these models is associated with uncertainties, so by interpolating several models, uncertainties can be reduced to a certain extent and reliable results can be obtained. Two scenarios (SSP126 and SSP585) were considered for each model. These two future climate change scenarios provide optimistic conditions (SSP126) and pessimistic conditions (SSP585). The environmental variables used for habitat suitability modeling include four climatic variables, i.e. cumulative heat units during the growing season above 10 degrees Celsius (GDD), seasonal temperature changes (Bio4), annual precipitation (Bio12), precipitation in the driest season of the year (Bio17) and slope are topographic variables. To consider the topographical conditions in the models, the slope variable was used that was prepared using SRTM elevation digital model (Jarvis *et al.*, 2008). Variance Inflation Factor (VIF) (Quinn & Keough, 2002) was used to ensure the absence of collinearity between climate variables. Based on the results, the mentioned variables had a variance inflation index of less than 10, so they can be used for modeling.

Climate change modelling

Modeling the effects of climate change on the distribution of the genus *Macrovipera* in Iran was done using an ensemble approach of five different algorithms (Araújo & New, 2007; Guisan *et al.*, 2017). The use of an ensemble approach makes it possible to consider the results of different algorithms simultaneously, and it is also possible to reduce the uncertainty resulting from single algorithms (Araújo & New, 2007; Guisan *et al.*, 2017). Ensemble model was created using GLMs (base R-package; R environment), GAMs (gam R-package version 1.20.1), GBMs (gbm R-packge version 2.1.8), RandomForests (randomForest R-package version 2.1.8) and Maxent (dismo R-package version 1.3-5) in the R software environment (R Core Team, 2020). In the present study, to measure the effectiveness of the models, 70% of the points were considered as training data and 30% as tests, and modeling was done with 10 repetitions. The the area under the receiver operating characteristic curve (AUC) was used to measure the performance of the models (Fielding & Bell, 1997). Based on this metric, models with AUC higher than 0.9 have excellent performance, 0.7 to 0.9 have good performance, and less than 0.7 have low performance.

Coverage of protected areas

After developing the distribution models of the genus *Macrovipera*, these models were overlapped with the map of protected areas of Iran in ArcMap 10.3 to determine the coverage of suitable habitats by protected areas of the country (Kafash *et al.*, 2021). For this purpose, in the first step, continuous habitat desirability models were divided into two suitable/unsuitable classes using the maximum sensitivity plus specificity threshold. In the next step, the area of suitable habitats within the protected areas calculated in ArcGIS 10.5.

RESULTS

Based on the AUC metric, the models made for the genus *Macrovipera* have high performance (AUC GLM=0.83, GAM=0.83, GBM=0.84, RF=0.84 and Maxent=0.84) and are therefore reliable (AUC ensemble = 0.85). According to the current distribution model, 614,126 square kilometers of the area of Iran (about 37% of the total area of Iran) are suitable for this species. Alborz, Zagros and Kopet Dagh Mountains have the most suitable habitat for the species of this genus (Figure 2)**.**

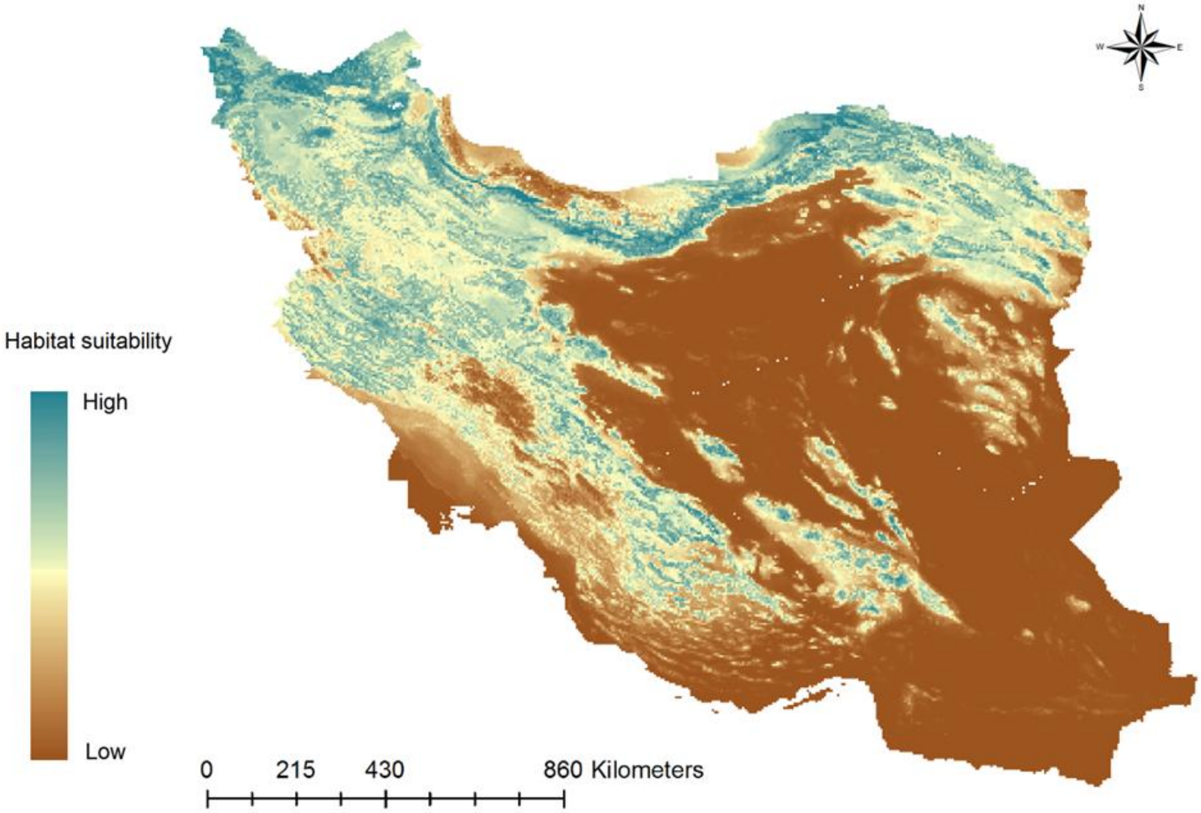

FIGURE 2. *Macrovipera* distribution model in Iran under current climate conditions based on ensemble model.

Future distribution of the genus *Macrovipera*

Based on the future distribution model of the genus *Macrovipera*, suitable habitat of this genus will decrease slightly in the future. Under pessimistic scenarios for the years 2071-2100, about 11% of the suitable habitats will decrease in Iran (Table 1). In other words, the area of suitable habitats will decrease from 614,126 to 548,197 (Figure 3).

TABLE 1. Area and percentage of suitable habitats of the genus *Macrovipera* in Iran under the current and future climate.

Year and Scenario	**Present (2010)**	**2041-2070 ssp126**	**2041-2070 ssp585**	**2071-2100 ssp126**	**2071-2100 ssp585**
Area (km^2)	614126	613888	586128	608993	548197
Percent of area reduction	0	-0.04	-4.56	-0.84	-10.74

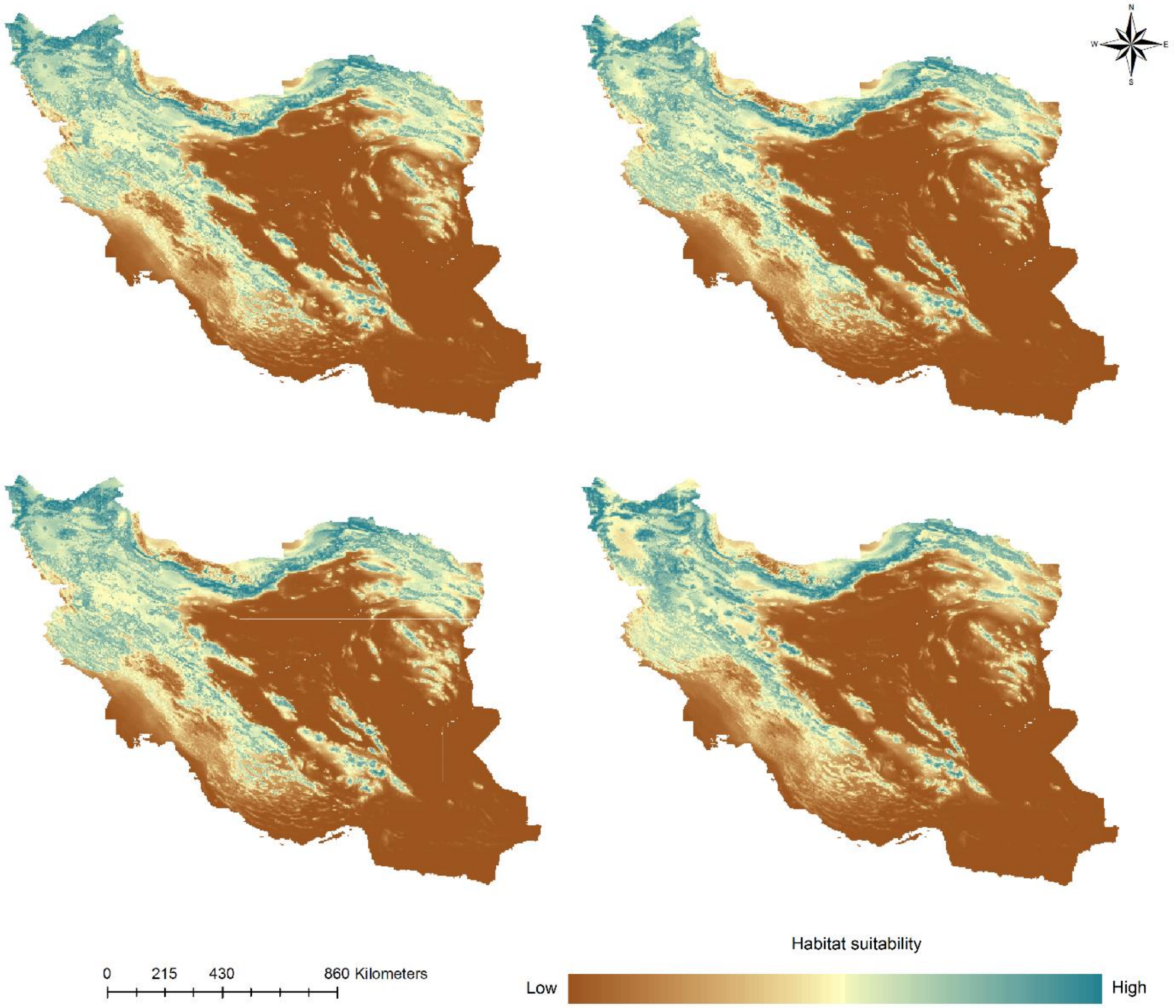

FIGURE 3. Future distribution models of the genus *Macrovipera* in Iran.

Coverage of protected areas

The measurement of the coverage of the protected areas for the suitable habitats of the genus showed that only about eight percent of the suitable habitats of the species are covered by the protected areas (Table 2). Determining the extent of suitable habitats in different categories of protected areas showed that 3,500 square kilometers of protected habitats are located in the national parks, 12,050 square kilometers in the wildlife refuges, and 34,580 square kilometers in the protected areas. In the future, the extent of suitable habitats in protected areas will remain almost the same.

TABLE 2. Area and percentage of protected suitable habitats of the genus *Macrovipera* in Iran.

Year and scenario	**Present (2010)**	**2041-2070 ssp126**	**2041-2070 ssp585**	**2071-2100 ssp126**	**2071-2100 ssp585**
Area of protected habitat (km^2)	50130	51054	47057	49433	44774
Percent of protected habitat	8.16	8.32	8.03	8.12	8.17

DISCUSSION

In the present study, the impact of future climate changes on the genus *Macrovipera* in Iran was investigated. The results show that climate changes will not have a major impact on the distribution of this genus, so that under the pessimistic scenarios for the years 2071-2100, about 11% of the suitable habitats of this genus in Iran will be reduced.

The result of the present study is in line with the studies conducted on other venomous snakes in Iran as well as venomous snakes in other countries, which show that venomous snakes will lose their suitable habitats under climate change (Ahmadi *et al.*, 2019; Penman *et al.*, 2010; Yousefi *et al.,* 2015; Yousefi *et al.,* 2019). The studies carried out in Iran on how the genus *Montivipera* is affected by future climate changes have shown that the suitable habitats for the genus *Montivipera* in the mountainous regions of the country will decrease sharply and the members of this genus will move to higher altitudes to find suitable habitats. (Yousefi *et al.*, 2019). Therefore, it can be concluded that both genus will be negatively affected by climate change, but the severity of climate change will be different on each genus.

Penman *et al.* (2010) predicted the impacts of climate change on a snake in Australia and showed the future distribution of the species was constructed for the years 2030 and 2070. Their results also showed that the range of presence of the species will decrease in the future. According to the pessimistic scenario, only 14% of the currently known populations of the species will survive in the future, and other populations will either adapt to rapidly changing conditions or undergo local extinctions (Penman *et al.*, 2010). Although the rate of loss of suitable habitats of this species is much higher than that of *Macrovipera* in Iran, the status of suitable habitats in both studies is decreasing due to climate change.

Climate changes will change species interactions and cause competition between different species by changing the distribution pattern of species (Hannah, 2015). The distribution of members of the two genera *Macrovipera* and *Montivipera* is separated from each other in terms of elevation (Rajabizadeh, 2018). The genus *Montivipera* lives in high elevation habitats, while the members of the genus *Macrovipera* live at lower elevations (Rajabizadeh, 2018). Based on the results of the present study, the suitable climatic of the genus *Macrovipera* will decrease in low elevation areas and move to higher elevation areas. This pattern of moving species to higher habitats in response to climate change is also predicted for other reptile species (Vaissi, 2022; Yousefi *et al.*, 2015). As a result, it is expected that the populations of the genus *Macrovipera* will move to higher areas than their current habitat. However, as mentioned, in major parts of the Zagros and Alborz mountain ranges, higher elevation habitats are already occupied by members of the genus *Montivipera* (Rajabizadeh, 2018; Yousefi *et al.*, 2019). Therefore, there will be a possibility of competition between the two genera to occupy the suitable habitats. One other possibility is that the genus *Macrovipera* can adapt to the new climatic conditions in low elevation habitats.

Species distribution models are practical tools for measuring the coverage of protected areas for suitable habitats of species (Guisan *et al.,* 2017). In the present study, it was determined that currently eight percent of the suitable habitats of *Macrovipera* vipers are under legal protection. Although the suitable protected habitats will not decrease under the climate change, but considering that the coverage of the protected areas for the suitable habitats of this genus is generally limited, there is a need for protected areas development programs to protected suitable but not unprotected habitats of the genus. Determining the extent of suitable habitats in different categories of protected areas showed that national parks have the lowest percentage of protected habitats of the genus *Macrovipera* and the largest extent of suitable habitats is in the category of protected areas.

Although the results of the present study showed that the genus *Macrovipera* will lose a small part of its suitable habitats and will not be significantly affected by the climate change, but the studies conducted on other reptiles (Hosseinian Yousefkhani *et al.,* 2017; Kafash *et al.,* 2018; Vaissi, 2022), amphibians (Kafash *et al.,* 2018; Vaissi, 2021), freshwater fish (Esmaeili *et al.,* 2018; Yousefi *et al.,* 2020b), birds (Sheykhi Ilanloo *et al.,* 2021) and mammals (Malekian & Sadeghi, 2020; Malakoutikhah *et al.,* 2020; Ebrahimi *et al.,* 2021) show that many species will be influenced by climate changes and they will lose a significant part of their suitable habitats (Yousefi *et al.,* 2019). Therefore, climate change will continue to be a major challenge for biodiversity conservation in Iran.

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REVIEW ARTICLE **Open Access**

A Review on Taxonomy and Distribution of the Genus *Echis* Merrem, 1820 (Serpentes: Viperidae) with Special Reference to the Middle East

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Abstract
Saw scaled vipers of the genus *Echis* belong to the family Viperidae and subfamily Viperinae. These vipers are widely distributed from East Africa to Southwest and Central Asia. *Echis* bite is one of the major causes of mortality in the world. Different populations of these medically important snakes have different venom composition, and the relevant antivenom is highly species-specific. *Echis* has a complex taxonomic history. For many years, only two species were recognized (i.e., *Echis coloratus* and *E. carinatus*). Over the past 50 years, the number of species have been raised to 12, of which six species reside in the Middle East. Phylogenetic studies show that the genus *Echis* fall into four species groups: the *E. carinatus*, *E. coloratus*, *E. ocellatus* and *E. pyramidum* groups. Until recently, only *E. carinatus* in Southwest Asia and India and *E. coloratus* in Arabia were assigned to the Middle East. Several morphological and phylogenetic studies raised the number of *Echis* species in the Middle East from two to six. These Middle Eastern vipers belong to three different species groups. An Asian, an Arabian and an African group. Distribution and basal split of the genus *Echis,* likely have been shaped by vicariance and tectonic events, which have separated or connected the land masses. In this study, a brief review on biogeography, taxonomy and distribution of the genus especially in the Middle East is presented.

Key words: *Echis*, the Middle East, Phylogeny, Saw Scaled Viper, Taxonomy

INTRODUCTION

The carpet vipers of the genus *Echis* Merrem, 1820 belonging to the family Viperidae and subfamily Viperinae, are well adapted to living in arid and semiarid regions. They are distributed extensively across Africa, north of the Equator, Arabian Peninsula, Jordan, Palestine, Israel, United Arab Emirates, Oman, Iraq, Iran and toward north and east to Turkmenistan, Uzbekistan, Afghanistan, Pakistan, India, Sri Lanka and Bangladesh (Leviton et al., 1992; Joger, 1984; Ananjeva et al., 2006; Arnold et al., 2009).

Echis is also known as saw scaled viper as they have scales with serrated keels and when they are threatened, they rub their body parts together and make a sizzling sound instead of hissing. Some believe

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that this respond is an adaptation, preventing loss of water due to hissing, as their habitat is arid and semiarid regions (Mallow et al., 2003).

Echis has a strong virulent venom and the LD 50 is 5.1mg/kg in humans (Daniel, 2002). In many areas such as Africa and especially in West Africa *Echis* bite causes death. About 20,000 *Echis* bite deaths annually has been recorded for the whole Africa (Chippaux, 1998, 2006). In some African countries such as Nigeria the most snake bite mortalities are related to *Echis* bites, about 12% (Warrell, 2013). *Echis* bite is also one of the major causes of mortality in India (Bhat, 1974; Kochar et al., 2007; Arnold et al., 2009; Pook et al., 2009) and in the world as well (Oldfield, 2021). Although hemorrhage, local necrosis, blood coagulation and local blistering are the same clinical symptoms in all the patients bitten by *Echis*, but the venom compositions vary among different populations of *Echis*, remarkably.

Therefore, the antivenom is highly species-specific (Arnold et al., 2009). Since this viper is widely distributed and is highly dangerous, therefore leaving aside the issue of conservation, knowing the exact taxonomic status and distribution range of the *Echis* species can lead specialists to produce more effective antivenom and reduce mortality rate caused by *Echis* bite. The classification scheme of this genus is as follows:

Family Viperidae Oppel, 1811
Subfamily Viperinae Oppel, 1811
Genus *Echis* Merrem.1820

Echis has a long history of taxonomic confusion and controversy (David & Ineich, 1999; Mallow, 2003; Pook et al., 2009). Much debate in taxonomy of *Echis* has been over past 50 years. For many years, only two species were recognized. Klemmer (1963) reported two species: *E. coloratus* (Günther, 1878) in Jordan, Lebanon, Israel, eastern Egypt and Arabia and *E. carinatus* (Schneider, 1801) in most of the distribution range of the genus. Joger (1984, 1987) raised the number of species to three and added *Echis pyramidum* (Geoffroy Saint-Hilaire, 1827) for southwestern Arabia. Cherlin (1990) described three subgenera, 12 species and 20 subspecies but not many researchers accepted all these arrangements (Auffenberg & Rehman, 1991; Schätti & Gasperetti, 1994; Trape & Mané, 2006; Pook et al., 2009; Uetz et al., 2022). David and Ineich (1999) described eight species. Mellow et al. (2003) described 10 species and Pook et al. (2009) clarified some complicated and unresolved taxonomic situations between species by using molecular methods. Therefore, there was no single opinion on phylogeny and taxonomy of the genus *Echis* (Mazuch, 2005).

Until recently much of our understandings of phylogeny and taxonomy of *Echis* were based on morphological studies. The first comprehensive molecular study about the phylogeny of the genus *Echis* was done by Pook et al. (2009). They used four mitochondrial markers in this study and their results showed the populations of *Echis* are divided into four main species groups, the *E. carinatus*, *E. ocellatus*, *E. pyramidum,* and *E. coloratus* groups (Fig. 1). They also resolved interrelationships among these four species groups, although incompletely. Their analyses supported the *E. coloratus* and *E. pyramidum* groups as sister groups, but relationship between the *E. coloratus* and the *E. carinatus-E. ocellatus* groups remained unresolved (Pook et al., 2009).

Barlow et al. (2009), by using the mitochondrial and nuclear genes and only one specimen for each species complex, showed that the *E. carinatus* complex is the sister group of other complexes and the *E. ocellatus* group is the sister group to the *E. pyramidum-coloratus* lineage. In 1990, Cherlin suggested three subgenera for *Echis i.e., Echis, Turanechis* and *Toxicoa,* but phylogenetic study by Pook et al. (2009) showed that these three subgenera were non-monophyletic and they rejected this arrangement. They also showed that *Echis* and *Cerastes* Laurenti, 1768 are monophyletic and sister-group. This sister-group relationship between *Echis* and *Cerastes* was suggested by Joger and Courage (1999) and also by Wüster et al. (2008). According to phylogenetic analysis of mtDNA genes, Arnold et al. (2009) indicated four main clades for *Echis* as well i.e., *E. ocellatus, E. carinatus, E. coloratus* and *E. pyramidum* groups (Fig. 2).

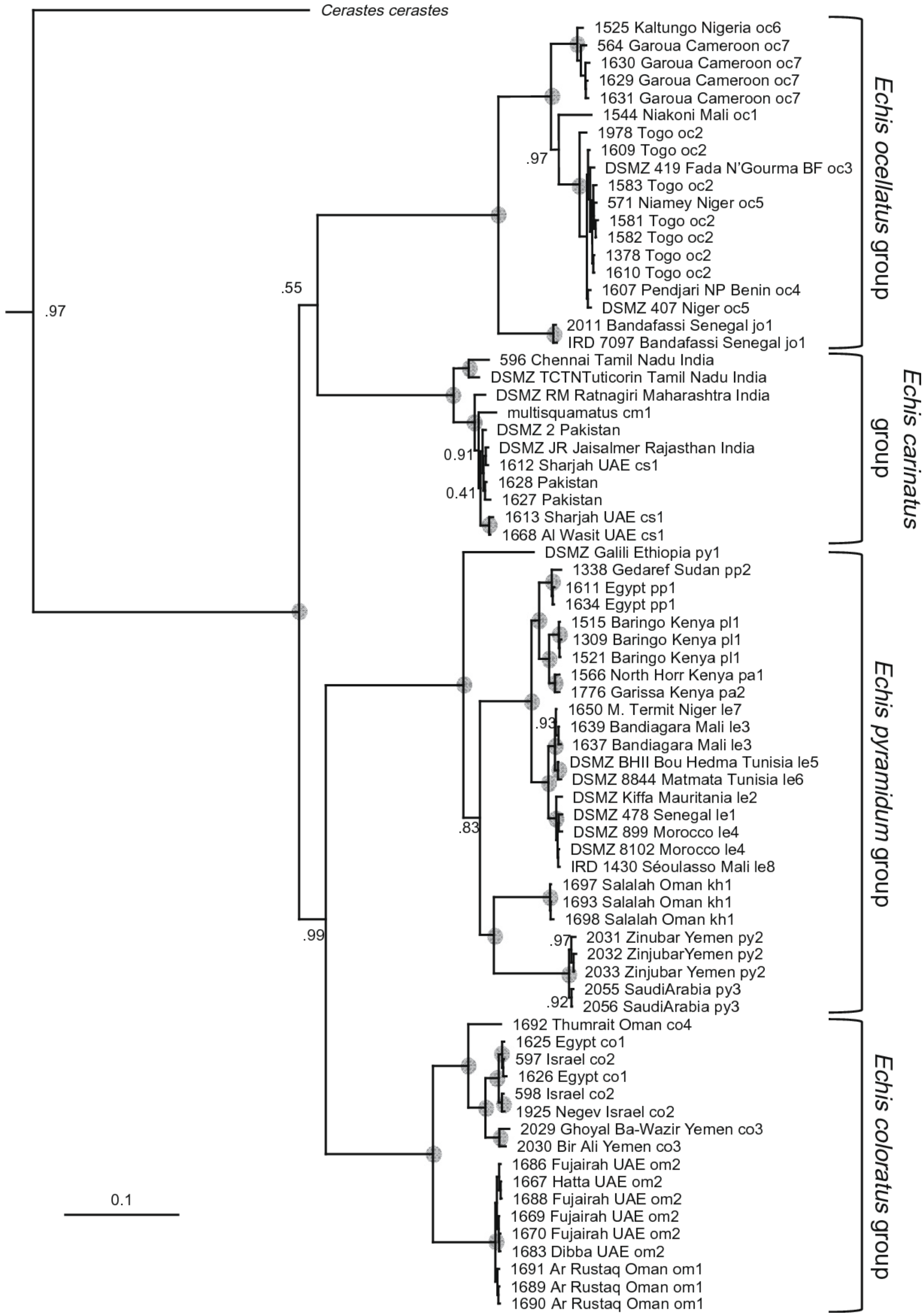

FIGURE 1. Bayesian inference tree of the genus *Echis*. Nodes with gray circles received a Bayesian Posterior probability (bpp) of 1.00 (from Pook et al. 2009).

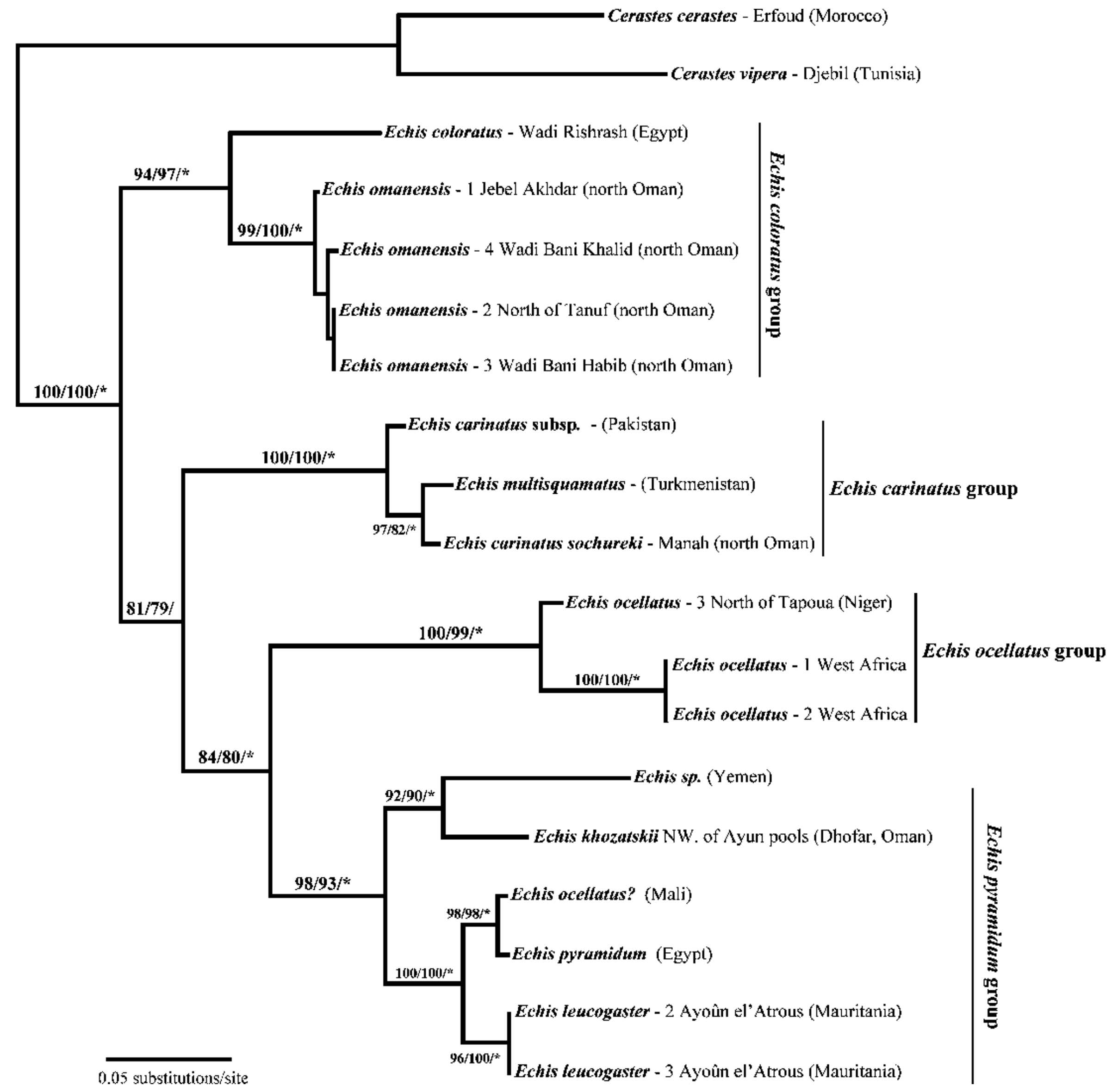

FIGURE 2. Relationships of the genus *Echis* using cytochrome b and 16s rRNA mitochondrial genes based on Arnold et al. (2009). Figures close to nodes are maximum likelihood bootstrap value/maximum parsimony bootstrap value/ Bayesian posterior probability value (only values equal or higher than 0.95 are indicated with an asterisk "*".

Phylogeny of the snakes of the Genus *Echis* Merrem, 1820

Echis Merrem, 1820 Synonyms:

Scythale I. Geoffroy-Saint-Hilaire, 1827 (nomen emendatum), *Toxicoa* Gray, 1849a, *Enchis* Haltom, 1931 (nomen incorrectum), *Ecchis* Rosenfeld, Kelen & Nudel, 1964 (nomen incorrectum), and *Turanechis* Cherlin, 1990 (Wallach, 2014).

Type species: *Pseudoboa carinata* Schneider, 1801.

For the first time Schneider (1801) described *Echis carinatus* under the name of *Pseudoboa carinata* from Madras, eastern India. Geoffroy Saint Hilaire (1827) described *Scytale pyramidium*, now called

E. pyramidum, from Egypt. Gunther (1878) distinguished *E. arenicola* (now called *E. coloratus*) from Arabia. In 1949, Constable referred snakes from northern India to *E. carinatus pyramidum*. Deraniyagala (1951) described *E. carinatus sinhalensis* from Sri Lanka. In 1930, Chernov noted that vipers from central Asia and Iran are different from the vipers of Egypt but he did not have satisfactory samples of these snakes from Africa available to reach authoritative conclusions (Cherlin, 1984). From 1970 onwards, many papers allocated to the taxonomy of these vipers were published. Stemmler and Sochurek described *E. carinatus leakeyi* from Lake Daringo in Kenya (Stemmler & Sochurek, 1969; Cherlin, 1984), at the same time Stemmler (1969) distinguished *E. carinatus sochureki* from central Asia and the adjacent regions. Mertens in 1970, introduced *E. carinatus astole* from the Astole Island in Pakistan. *E. carinatus ocellatus* was described in that time from the Gulf of Guinea by Stemmler (Stemmler, 1970; Cherlin, 1984). In 1972, Roman distinguished *E. carinatus leucogaster* from south of the Sahara (Roman, 1972) and, three years later he declared that this was a new species named *E. leucogaster* (Roman, 1975). Drewes and Sacherer (1974) identified *E. carinatus alliaborri* from Kenya. *E. ocellatus* was described as a separate species by Hughes (1976) from West Africa. It was defined as a subspecies of the *E. carinatus*. In 1981 *E. multisquamatus* was introduced by Cherlin from central Asia and the adjacent regions (Cherlin, 1981). In 1990, Cherlin described 12 species of which only five species are now accepted. *E. varia borkini* (now known as *E. borkini* Cherlin, 1990) from Yemen and Saudi Arabia, *E. hughesi* Cherlin, 1990 from Somalia, *E. jogeri* Cherlin, 1990 from West Africa, Gambia, Mali and Senegal, *E. khosatzkii* Cherlin, 1990 from Yemen and Oman and *E. megalocephalus* Cherlin, 1990 from Eritrea. In 2004, Bobocsay reported a population of *E. coloratus* as *E. omanensis* Babocsay, 2004 from the UAE and Oman. Finally, Trape described *E. romani* trape, 2018 from Cameron, Chad, Niger and Nigeria (Trape, 2018). This newly described species was formerly a population of *E. ocellatus* Stemmler, 1970. Although there is a consensus on 12 species of the Genus *Echis* as of now, studies are still underway to identify and describe new species and subspecies in different regions. The species of the genus *Echis* that are today approved by most researchers are as follows (Uetz et al., 2022):

Echis borkini Cherlin, 1990
E. carinatus (Schneider, 1801)
E. coloratus Günther, 1878
E. hughesi Cherlin, 1990
E. jogeri Cherlin, 1990
E. khosatzkii Cherlin, 1990
E. leucogaster Roman, 1972
E. megalocephalus Cherlin, 1990
E. ocellatus Stemmler, 1970
E. omanensis Babocsay, 2004
E. pyramidum (Geoffroy Saint-Hilaire, 1827)
E. romani Trape, 2018

As explained before the taxonomy of the Genus *Echis* has been in flux especially in Africa. However, in this article, we discuss the taxonomy of the genus *Echis* in the Middle East.

***Echis* in the Middle East**

Until recently, only two species of *Echis* were assigned to the Middle East. *Echis carinatus* in Southwest Asia and India and *E. coloratus* in Arabia. In 1984, and 1987, Joger described *E. pyramidum* from Arabia (southwestern regions) and after that, scientists described new species and subspecies especially in the *E. coloratus* group. After 25 years of research and based on other scientists` results, Stümpel and Joger (2009) raised the number of *Echis* species in the Middle East from two to six. These vipers belong to three different species groups. An Asian, an Arabian and an African group. The Middle Eastern species recognized by Stumpel and Joger (2009) are as follows:

Echis carinatus group (Asian group):
- *E. carinatus* (Oman, UAE, Iran, Central Asia, Afghanistan, Pakistan)

Echis coloratus group (Arabian group):
- *E. coloratus* (Egypt, Arabian Peninsula)
- *E. omanensis* (Oman, UAE)

Echis pyramidum group (one of two African groups):
- *E. pyramidum* (Egypt, Sudan, East Africa)
- *E. khosatzkii* (western Oman, Yemen)
- *E. borkini* (Yemen, SW Saudi Arabia)

***Echis carinatus* (Schneider, 1801)**

Pseudoboa carinata Schneider 1801: 285, *Boa Horatta* Shaw, 1802, *Scytale bizonatus* Daudin, 1803, [*Vipera* (*Echis*)] *carinata* Merrem1820. *E. carinata* Duméril & Bibron 1854: 1448. *E. carinatus* Mith 1943: 487, *E. carinatus sinhaleyus* Deraniyagala 1951: 148. *Echis carinatus* Latifi, 1978. *Echis carinatus* Harding & Welch 1980. *Echis multisquamatus* Cherlin 1981. *Echis carinatus* McDiarmid, Campbell & Touré 1999: 376. *E. carinatus sinhaleyus* David & Ineich 1999, *E. carinatus multisquamatus* David & Ineich 1999. *Echis multisquamatus* Ananjeva et al. 2004. *E. carinatus sochureki* Dobiey & Vogel 2007. *Echis multisquamatus* Rastegar-Pouyani et al. 2008. *E. carinatus sochureki* Afrasiab 2011, *Echis carinatus* Wallach et al. 2014: 255. *E. carinatus astolae* Khan 2017 (Uetz et al., 2022).

Type locality: Yavatmal, Maharashtra State (Arni), West India. No type locality available (fide LOVERIDGE 1936). Type locality (fide SCHMIDT 1939): “Arni” [= Aarni, Yavatmal, Maharashtra, India], (20.073345, 77.954673) (Uetz et al., 2022).

Distribution: Southwest and South Asia. United Arab Emirates, Qatar, Oman, Iraq, Iran, Uzbekistan, Turkmenistan, Tajikistan, Afghanistan, Pakistan, India, Bangladesh and Sri Lanka (O’Shea, 2018).

Echis carinatus is a member of the *Echis carinatus* group (one of the four *Echis* species groups) which is completely an Asian group. *Echis carinatus* has five subspecies as follows:

The nominate subspecies *E. c. carinatus* (Schneider, 1801) in peninsular India, the widely distributed subspecies *E. c. sochureki* Stemmler, 1969 in Pakistan, Iran, Iraq, Bangladesh, Afghanistan and the United Arab Emirates. Population in Astola Island in Pakistan, under the name of *E. c. astolae* Mertens, 1970 and population in Sri Lanka, under the name of *E. c. sinhaleyus* Deraniyagala, 1951 (O’Shea, 2018).

Some Russian herpetologists believe that another species that is called *Echis multisquamatus* cherlin, 1981 occurs in the Central Asia, East of Iran and some parts of western Afghanistan and Pakistan (Ananjeva et al., 2006). Some consider it as a subspecies of *E. carinatus* : *E. c. multisquamatus* Cherlin, 1981 (David & Ineich, 1999; O’Shea, 2018).

***Echis carinatus sochureki* Stemmler, 1969**
Sochurek's saw-scaled viper, Sindh Saw-scale Viper

Diagnosis: Small-sized viper (35-55 cm), head broad and pear- shaped. Ground color brown or gray, white vertebral patches, which may be diagonal. Lateral zigzag with pale arcs, within each arc a single dark spot. Pale cross on head or backwards facing trident on a dark background. Ventral white or gray with dark medial spots. Mid-body scale rows: 25–33, keeled; ventrals: 155–175, wide; anal undivided; subcaudals: 29–48, entire. Circumorbitals: 14–20; scale rows between eye and upper labials: 2–3; upper labials: 10–12 (rarely 13); small imbricate scales on top of head. From see level to 2000 m, ovoviviparous (Egan, 2022).

Echis coloratus Günther, 1878

Palestine Saw-scaled Viper, Burton's carpet viper

Echis froenata Duméril, Bibron & Duméril, 1854: 1449 (nomen oblitum), *Echis frenata* Pfeffer, 1893, *Echis coloratus* Boulenger, 1896, *Echis coloratus coloratus* Cherlin, 1983, *Echis coloratus* Haas, 1957: 83, *Echis coloratus* Gasperetti, 1988, *Echis [(Turanechis)] froenatus* Cherlin, 1990, *Echis froenatus* Cherlin & Broklin, 1990, *Echis coloratus* Welch, 1994: 57. *Echis coloratus* Mcdiarmid, Campbell. Campbel & Tour, 1999: 378, *Echis coloratus* Dobiey & Vogel, 2007, *Echis coloratus* Pook et al. 2009, *Echis coloratus* Wallach et al. 2014:255, *Echis coloratus* Meiri et al. 2019. *Echis coloratus terraesanctae* Babocsay 2003. *Echis coloratus terraesanctae* Bar & Haimovitch 2011: 192 (Uetz et al., 2022).

Type locality: Jebel Sharr, behind El Mewaylah, in Midian, at an elevation of 4500 feet. Holotype: BMNH 1946.1.20.84 (Babocsay, 2003; Uetz et al., 2022).

Distribution: Eastern Egypt, Yemen, Saudi Arabia, Israel, Southern Oman, Jordan.

Diagnosis: ventrals: 175–204, subcaudals: 45–56, nasal almost never fused with the upper prenasal, lower prenasal usually present, 3.5–7.5 scales between the chin-shield and the preventral, conspicuously elongated gulars along the two sides of the midline of the throat. Head dorsally usually uniform gray, mostly with an X-shaped marking, rarely spots. Facial band covers from 17–38 temporal scales, facial band often abruptly ends before the postmandibular blotch, mostly merges with it, usually dark bands below the eye and often fuse with each other or with the facial band, Grows to 716 mm RA, tail relatively long (15.0 in male and 13.0 in female) (Babocsay, 2004).

Remarks: The viper was described for the first time under the name *Echis froenata* Duméril, Bibron et Duméril, 1854. However, since this name had not been used more than 100 years, Stimson (1974) suggested that the International Zoological Commission nomenclature (IZCN) use *E. coloratus* as the valid name. Therefore, *Echis froenatus* should be regarded as a nomen oblitum (Uetz et al., 2022). *E. coloratus* is nested in the *E. coloratus* complex (Arnold et al., 2009; Pook et al., 2009), which was considered as a taxonomically integral species by Cherlin (1990) and Joger (1984). Therefore, studies on taxonomy of this species were abated, and researchers mostly focused on other African and Asian populations of *Echis*. Until Babocsay (2003) realized that, the populations of *E. coloratus* in Levant and Arabia were greatly different. He noticed that there are two variants of *E. coloratus* population in Israel, which differ in color and pattern. The northern variant, in Sinai and toward Arava Valley, with reddish brown or pink ground color, has large dorsal blotches in light pink. The blotches on the sides are shaded as light gray or blue by a dark frame enclosed them. The southern variant, occurring in the central Dead Sea area northwards, with gray or brown-grounded color, has large not framed blotches in light gray or brown. Moreover, several dark dots attached to blotches are recognizable. So, he analyzed these variants and assigned the northern Levantine *E. coloratus* population as a new subspecies (Babocsay, 2003).

Echis coloratus coloratus Günther, 1878

Diagnosis: A medium-sized viper, bumps on the keels of the dorsal scales, unenlarged inframandibular scales, and 3-4 rows of scales between the eye and supralabialia. Drawing bland with large light circular-oval spots on the dorsal surface of the body. The pattern on the head is indistinct or absent. It differs from its closest relative *Echis coloratus terraesanctae* Babocsay, 2003 by more ventral plates, fewer dorsal ones, color and relatively larger eyes (Babocsay, 2003).

Distribution: It occurs in northwestern Arabia (Midian), in the southern Levant and in Egypt, east of the Nile. There is a hybrid zone with *E. c. terraesanctae*. The populations of *E. coloratus* in the Arabian Peninsula are different from the typical *E. coloratus* in coloration form, and need to be taxonomically studied (Babocsay, 2003).

Echis coloratus terraesanctae Babocsay, 2003

Holy land saw scaled viper

Echis colorata Günther 1878:978 (Type locality: Jebel Sharr, Midian; Coll: Sir Richard Burton); Flower 1933:835 (part). *Echis coloratus*, Boulenger 1896:507, pl. 25, fig. 1 (Dead Sea); Anderson 1898:343; Joger 1984:46 (part); Gasperetti 1988:348 (part); Leviton et al. 1992:114 (part); Babocsay 2003 (part). *Echis froenatus* Duméril, Bibron et Duméril 1854; Cherlin 1990: 203 (part, taxonomic review, map; the name was suppressed by Opinion 1176 [ICZN 1981]) (Babocsay, 2003; Uetz et al., 2022).

Type locality: Ma'ale Efrayim, Samaria, Cisjordan; coll: Y. L. Werner et al. on 26.3.1980. Holotype: HUJ R 8926 (Babocsay, 2003).

Distribution: It distributed in the northern and central Dead Sea basin, the Jordan Valley and their western slopes (Babocsay, 2003).

Diagnosis: According to Babocsay (2003), *Echis coloratus terraesanctae* differs from *E. c. coloratus* in the southern Levant in its lower ventral count and higher number of dorsal scale rows on all sections of the body, in colour pattern and in its relatively larger eyes.

Echis omanensis Babocsay, 2004

Oman saw-scaled viper

Echis colorata Gunther: Boulenger, 1887:408 (Muscat; coll: A. S. G. Jayakar). *Echis coloratus* Gunther: Boulenger, 1896:507 (Muscat; coll: A. S. G. Jayakar); Arnold and Gallagher, 1977:69 (Wadi Serin and Wadi Kebir); Joger, 1984:46 (part, locality resume and map); Gasperetti, 1988:348 (part, locality resume and map). *Echis froenatus* Dumeril, Bibron et Dumeril: Cherlin 1990:203 (part, taxonomic review, map; the name was suppressed by Opinion 1176 (ICZN 1981) (Uetz et al., 2022).

Type locality: Wadi as Siji, region of Masafi (25◦18_x0005_ N 56◦10_x0005_ E), United Arab Emirates. Holotype: BMNH 1973.2113 (Babocsay, 2004; Uetz et al., 2022).

Distribution: The species distributes in the United Arab Emirates and north of Oman and ranges from sea level to 1000 m (Babocsay, 2004).

Babocsay (2004) noticed that the species seems to be mostly active in the spring months in the morning and in the afternoon and evening, but less active in autumn. However, In Israel, *E. coloratus* is active in other season as well, particularly in summer. He also found that use of gularis scales is a useful character at the species level, and interestingly the gular area has varied characters in which the variation is correlated. Formerly Cherlin (1983) had used the variation in gular shape and size to divide *Echis* genus into subgenera (Cherlin, 1990; Babocsay, 2004).

By studying a large number of specimens from entire range of the *E. coloratus* complex, Babocsay (2004) described a new species from northern Oman and the United Arab Emirates under the name of *Echis omanensis* Babocsay, 2004. In fact, the new species is separated from the rest of snakes in the *E. coloratus* complex by an extended geographic barrier, the Rhub al Khali Desert in south and southwest part of Arabian Peninsula. As *E. coloratus* is arenicolous, it doesn`t inhabit sandy habitats, so Babocsay (2004) believed that this taxonomic discontinuity and allopatric speciation make sense.

Babocsay (2001), also studied samples from lands around this geographic barrier that were morphologically different. Besides, some authors have shown that snakes in the *E. coloratus* complex had greatly different intraspecific coloration (Gasperetti, 1988; Kochva, 1990; Mendelssohn, 1965). Thereby, different morphological traits and geographic isolation of the populations of the *E. coloratus* complex in its expansion range shows that this complex has a separate evolutionary background (Babocsay, 2004). *Echis omanensis* is different from *E. coloratus* as follows:

Longer tail (16.3) with higher subcaudal counts (49–58); the lower prenasal scale is often missing and the upper prenasal is frequently fused with the nasal; the subnasal is often missing or fused with the nasal. The gular scales between the chin-shield and the preventrals are round or only slightly elongated, not elongated as in *Echis coloratus*, and their number is higher. Ventrals: 184–194, subcaudals: 49–58, Head dorsally usually uniform gray, rarely a dark X-shaped marking or, in young specimens marked by small spots. Ground colour of the body gray with dark edged, conspicuous dorsal blotches; the dark frame may surround the entire blotch or may partly disappear, remaining only laterally and as two transversally positioned spots across the mid-dorsal line, one at the caudal end, one at the cranial end of the blotch. Ventral surface yellowish-white or grayish-white, mostly with a faded line of dots medially and an array of faded dots toward each lateral side of the ventrals or with only one of these components; it may lack any pattern. Grows to 606 mm (rostrum-anus) (Babocsay, 2004).

Echis pyramidum (I. Geoffroy Saint-Hilaire, 1827)
Northeast African Carpet Viper, Egyptian Saw-scaled Viper

Scythale pyramidum Geoffroy Saint-Hilaire 1827: 152. *Echis arenicola* Boie 1827. *Echis pavo* Reuss 1834: 157. *Echis varia* Reuss 1834: 160. *Echis arenicola* Strauch 1868: 292. *Echis carinatus pyramidum* Ionides & Pitman 1965. *Echis carinatus pyramidum* Harding & Welch 1980. *Echis varius darevskii* Cherlin 1990. *Echis (Toxicoa) pyramidum lucidus* Cherlin 1990. *Echis varius* Largen & Rasmussen 1993, *Echis arenicola fide* David & Ineich 1999. *Echis pyramidum* Mcdiarmid, Campbell & Touré 1999: 380. *Echis carinatus pyramidum* Khan 2002 (pers. comm.), *Echis pyramidum* Pook et al. 2009, *Echis arenicola* Gruber 2009, *Echis varius (pyramidum)* Mazuch 2013, *Echis pyramidum* Wallach et al. 2014: 256, *Echis varius* Wallach et al. 2014: 256, *Echis pyramidum* Spawls et al. 2018: 582 (Uetz et al., 2022).

Distribution: North Africa, Egypt, Somalia, Ethiopia, Eritrea, Sudan, Libya, Tunisia, southwest Arabian Peninsula.

Type locality: Cairo, Egypt. Holotype: MNHN-RA 4031, according to Hughes (1976, Rev. Suisse ZooI. 83: 361) (Uetz et al., 2022).

Taxonomy: The species resides in the most complex species group in the genus *Echis*. Both taxonomically and phylogenetically, it has shown a great deal of diversity. And so far, it has gone through different species and subspecies levels (Pook et al., 2009). Cherlin (1990), in an extensive revision study of the genus, splits *E. pyramidum* in many species or subspecies: *E. p. pyramidum*, *E. p. lucidus*, *E. p. leakeyi*, *E. varius*, *E. v. darevskyi*, *E. v. borkini*, *E. v. aliaborri*, *E. hughesi* and *E. megalocephalus* from north and east Africa, *E. varius borkini* and *E. khosatzkii* from southwest Arabia. Schätti and Gasperetti (1994), questioned Cherlin's work, considering *E. varius* a distinct species from *E. pyramidum*. McDiarmid et al. (1999), declared most of these subspecies are synonyms of *E. pyramidum*. But the status of *E. varius* as a separate species or subspecies is in doubt and needs more verification (Pook et al., 2009; Wallach et al., 2014). About *E. borkini* and *E. khosatzkii,* we will discuss their status in the following. Phylogenetic analysis of populations of this complex showed that out of four clades forming the complex, two are Arabic and two are African with significant divergence. *E. borkini* and *E. khosatzkii are* nested in Arabic clades and *E. p.pyramidum,* nested in one of two African clade (Arnold et al., 2009; Pook et al., 2009; Stümpel & Joger, 2009). Currently *E. pyramidum* has two subspecies; *E. p. pyramidum* (Geoffroy Saint-Hilaire 1827) from Egypt, southwest Saudi Arabia, Somalia, Ethiopia, Sudan and *E. c. leakeyi* Stemmler and Sochurek 1969 from Kenya, southern Somalia, and southern Ethiopia.
A brief description of *E. p. pyramidum* is as follows:

***Echis p. pyramidum* (Geoffroy Saint-Hilaire 1827)**

Diagnosis: A medium sized viper (40-50 cm), ground color gray-brown, pale vertebral blotches, large dark spots surrounding with inverted V-shape below dorsal blotches, creating zigzag on flanks. Dark spots near ventral margin. A pale arrow like marking on a dark ground on head. Dark line from eye to posterior labials. White ventral. Dark stripe from eye to posterior labials. Dark line from eye to posterior labials. Mid-body scale rows: 27–31, keeled. Ventrals: 167–186, wide; anal: single; subcaudals: 31–38, undivided. Scale rows between eye and upper labials: 2–3; upper labials: 10–13; lower labials: 9–13; interorbitals: 9–12; circumorbitals: 17–21; medium imbricate scales on top of head (Egan, 2022).
Distribution: Egypt, southwest Saudi Arabia, from below sea level to 500m.

***Echis borkini* Cherlin.1990**
Borkin's Carpet Viper
Echis varius borkini Cherlin 1990, *Echis borkini* Schätti 2001, *Echis borkini* Pook et al. 2009, *Echis borkini* Stümpel & Joger 2009, *Echis borkini* David & Vogel 2010, *Echis borkini* Wallach et al. 2014: 254 (Uetz et al., 2022).

Distribution: Southwest Arabia, western Yemen.

Type locality: Lahej near Aden in Yemen. Holotype: BMNH 99.20.5.18 (Uetz et al., 2022).

Diagnosis: a small to medium sized snake (40-50 cm), ground color light brown, darker dorsally, oval shaped vertebral blotches, as wide as interspaces, to the tip of tail. Vertebral may be divided into paired paravertebral blotches, sometimes has flat zigzag on flanks, small dark spots (usually brown) near ventral edge, an indistinct dark patch with a bright center on head, ventral white. Mid body scale rows: 27-31, keeled, ventrals: 155-181, anal: undivided, subcaudals: 31-44, entire, scales rows between eye and upper labial: 1-2, upper labial: 10-13, lower labial:9-13, interorbitals: 10-12, circumorbitals: 16-20 (Egan, 2022).

Habitat: sandy coastal dunes with vegetation (below 200m). Oviparous (Egan, 2022).
It may be mistaken for *E. coloratus* but *E. coloratus* has dorsally transverse blotches, and when seen from above its head becoming prominent behind eyes. Moreover these two species are not likely sympatric as *E. coloratus* dwells rocky areas and mountains, And in higher altitude (more than 2000 m) (Egan, 2022).

Cherlin (1990) described *Echis varia borkini* from the east Africa, southwest Arabia and western Yemen. Molecular data showed that populations of western Yemen are highly divergent from other species within complex in east Africa and Stumpel and Joger (2009) elevated the subspecies to a full species level as *E. borkini*. Phylogenetic studies by Arnold et al. (2009) and Pook et al. (2009) also showed that populations of western Yemen are genetically distinct from other populations within the *E. pyramidum* complex and considered it as *E. borkini.*

***Echis khosatzkii* Cherlin, 1990**
Khosatzki's Saw-scale Viper
Echis khosatzkii Cherlin 1990 (fide Golay et. al. 1993), *Echis khosatskii* Cherlin 1990 (fide Mcdiarmid et. al. 1999), *Echis khosatzkii* Mallow et al. 2003, *Echis khosatzkii* Pook et al. 2009, *Echis khosatzkii* Arnold et al. 2009, *Echis khosatzkii* Stümpel & Joger 2009, *Echis khosatzkii* David & Vogle 2010. *Echis kozatskyi* Sindaco et al. 2013: 396 (in error), *Echis khosatzkii* Wallach et al. 2014: 255 (Uetz et al., 2022).

Distribution: Eastern Yemen, southern Oman.

Type locality: Arabia, Hadhramaut (eastern Yemen). Holotype: BMNH 97.3.11.117 (Uetz et al., 2022). (From sea level to 800m, oviparous)

Diagnosis: a small to medium sized viper (35-45 cm), ground color pale brown to pinkish, and reddish dorsally, pale vertebral lines or large V-shapes, smaller than or equal to brown interspaces, from neck to tail, pale zigzag on flanks with apex under which there is dark spot, unclear symmetrical head marks and triangular band from eye to posterior labials. Mid-body scale rows: 25–31, keeled, ventrals: 181–189, anal: undivided, subcaudals: 44–47, entire. Scale rows between eye and upper labials: 1–2 (rarely 3), upper labials: 10–13, lower labials: 9–13; circumorbitals: 17–21, scales on head small, ventral pearlish white to pale copper (Egan, 2022).

Cherlin (1990) in an extensive taxonomic study of the snake genus *Echis,* based on morphology recognized *E. khosatzkii* in the *E. pyramidum* complex from Yemen and Oman, which was previously assigned as *E. pyramidum* (Arnold et al., 2009). Arnold (1980) and Schätti and Gasperetti (1994) pointed out the great morphological differences of eastern populations with populations from the western and southwestern regions of Arabia. Phylogenetic study of Pook et al. (2009) confirmed their results, showing that two monophyletic Arabian and African clades in *E. pyramidum* complex are significantly different, and even populations within Arabian clade have diverged so that they represent two endemic Arabian species from south Oman and east Yemen and from west Yemen and southwest Arabia: *Echis khosatzkii* and *E. borkini,* respectively. The same results were obtained from Arnold`s et al. (2009) and Stumpel and Joger`s (2009) molecular studies.

DISCUSSION

In the *Echis carinatus* group, we mentioned five subspecies but usually two of them are considered as valid subspecies; *E. c. carinatus* and *E. c. sochureki*. Most authors place *E. c. sinhaleyus* (in Sri Lanka) in the nominate subspecies. The Astola Island population, in Pakistan, is sometimes recognized as a subspecies (*E. c. astolae*), as is the Central Asian population (*E. c. multisquamatus*) (O'Shea, 2018). Morphological study of Auffenberg and Rehman (1991) showed that all the *Echis carinatus* populations across their expansion range have clinal variation. They assigned these populations as subspecies of *E. carinatus*. Molecular study of Lenk et al. (2001) showed low divergence between *Echis* population from Pakistan (*E. carinatus sochureki*) and Turkmenistan (*E. multisquamatus*). Bagherian and Kami (2008) in a morphological study showed that northern and eastern populations of Iranian *Echis* are different from southern populations. They considered the northern and eastern populations as *E. multisquamatus* and the southern populations as *E. carinatus*. On the other hand, phylogenetic study of Pook et al. (2009) showed that *E. multisquamatus* and *E. carinatus* are conspecific. Morphological study by Todehdehghan et al. (2019) showed that among populations of east, southeast and southwest Iranian *Echis*, southeast and east populations are closely related but they could not resolve the status of species or subspecies of populations. They also provided an identification key for these three populations. Rhadi et al. (2015) in a morphological study of *Echis* population in southern Iraq, near the border of Iran, showed that Iraqi populations are assgined to *Echis carinatus sochureki* but they suggested more ecological, morphological and molecular study in order to achieve the exact taxonomic status of the genus *Echis* in Iraq. Phylogenetic study by Rhadi et al. (2016) also supposed that Iranian *Echis*, which were not sampled in their study, nested between the eastern (India, Pakistan) and western (Iraq) populations and may be closely related to western ones. They also suggested that *E. carinatus* has originated in India and has spread towards west. This finding is in agreement with Pook et al. (2009) which suggested that current distribution pattern of *E. carinatus* is the result of diversification from south India. But Arnold et al. (2009) showed that Oman and southern Iran is center of origin of *E. carinatus* and then it has been diversified to east and west toward India and Iraq, respectively. The initial expansion of the genus *Echis* seems to be at the same time with the collision of Afro-Arabian and Eurasia. Moreover, divergence of the *E. coloratus* and *E. pyramidum* groups took place simultaneously with opening of the Red Sea. Distribution of the *E. pyramidum* groups was later influenced by land connections between Africa, Asia and Arabia especially through the Gomphotherium Landbridge about 18 mya (Rögl, 1999; Pook et al., 2009). Phylogenetic studies by Pook et al. (2009) showed that there is low divergence between populations of north India, central Asia, northeast Arabia and the interstitial range so they concluded that *E. carinatus* diverged recently and rapidly, about 0.9 mya. During the Quaternary glacial maxima, the lowered sea level may have caused a land bridge between Arabian and Asian populations, and this has caused the rapid exchange of Arabian and Asian material. Rhadi et al. (2016) have proposed the same scenario.

Distribution and basal split of the genus *Echis* have been likely shaped by vicariance and tectonic events, which have separated or connected the landmasses (Pook et al., 2009). As Wuster et al. (2008) showed, the basal split within *Echis* dated back to 18.5 Mya, approximately coincide with the Gamphotherium Landbridge between Africa and Eurasia. Therefore, this genus can be considered as a model to study the effects of the collision of African-Eurasian on distribution of other organisms. As mentioned before *Echis* bite is one of the major causes of mortality in India and Africa (Bhat, 1974; Kochar et al., 2007; Arnold et al., 2009; Pook et al., 2009; Warrell, 2013), and the antivenom is highly species-specific (Arnold et al., 2009). Therefore, besides the biogeographical aspects, resolving the problematic taxonomy of this genus leads to higher efficiency of snakebite treatment (Pook et al., 2009). As a conclusion, a comprehensive morphological and phylogenetic study of these snakes is needed to provide a robust background for the genus origin, distribution routes and species delimitation. Undoubtedly, studying more samples from the southern and eastern regions of Iran can help to draw a better conclusion about the distribution of these species in the Middle East. Currently, the senior author of this article is studying samples from different regions of Iran for her PhD thesis, hoping to provide a detailed insight on taxonomic status and pattern of diversification of the genus *Echis* in Iran and subsequently in southwest Asia.

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