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RESEARCH ARTICLE **Open access**

Armada nilotica A. Bang-Haas (Lep., Noctuidae, Acontiinae) new record with first description of male from Iran

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Abstract
Armada nilotica A. Bang-Haas, male specimen is described for the first time and illustration of adult's genitalia are presented. This species is also recorded as new record for the fauna of Iran and diagnosis and description of the newly recorded species are given. Bionomics and distribution of Iranian *Armada* species are presented and an identification key is provided. The tribe Armadini comprises nine genera worldwide, all found in the arid and semiarid zones. The genus *Armada* is represented in Iran by seven species: *A. heliothidia* (Hampson, 1896), *A. dentata* (Staudinger, 1884)*, A. nilotica* A. Bang-Haas, 1912*, A. fletcheri* Wiltshire, 1961*, A. maritima* Brandt, 1939*, A. panaceorum* (Ménétries, 1848) and *A. clio* (Staudinger, 1884). Of these*, A. Panaceorum* and *A. clio* have wider distribution rang in Iran (the former is widespread throughout Iran) while the remaining species are restricted, in the range, to the arid regions of the south and southeast.

Key words: *Acontiinae, Armada, moth, new record, taxonomy.*

INTRODUCTION

The tribe Armadini comprises nine genera worldwide, all found in the arid and semiarid zones (Goater et al., 2003). The systematic position of Armadini has frequently been changed over time. This genus has traditionally been classified in Catocalinae for its quadrified hindwing venation. In order to making the Catocalinae as a monophyletic group, Fibiger and Lafontaine (2005) transferred Armadini to the Acontiinae for the presence of the saccular crest and the paired setose patches on the scaphium. The monompyly of the tribe Armadini is held by the scaphium that has subbasally two laterally setose crests (Goater et al., 2003). In an attempt to stabilize the Noctuidae higher classification, using protein-coding genes, Keegan et al. (2021)inferred a phylogeny and retained the Armadini, based on their achieved results, within Acontiinae.

Having studied the shared character states (almost similar fore-hindwing pattern, palpi and compound eyes shapes, etc.) of *Armada clio* (Staudinger, 1884), *Armada dentata* (Staudinger, 1884), *Armada panaceorum* (Ménétries, 1848) and *Tarachephia hueberi* (Ershov, 1874), Staudinger (1884) established the genus *Armada* according to *Acontia dentata* as the type species. Wiltshire (1961) treated a group of genera (i.e. *Armada*, *Asplenia* Hampson, 1916, *Riadhia* Wiltshire, 1961, *Metoponrhis*

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Staudinger, 1888, *Acrobyla* Rebel, 1903 and *Epharmottomena* John, 1909) based on the characteristics of the male genitalia in the tribe Armadini in Ophiderinae. The old world tibe Armadini was then revised by Wiltshire (1979) and he classified the present taxa into *Metoponrhis* and *Armada* genus-groups. Poole (1989) listed *Armada* in Ophiderinae/Catocalinae too, while Speidel et al. (1996) resurrected Eustrotiinae and provisionally placed *Armada* whitin. Kitching and Rawlins (1998) and Goater et al. (2003) then classified this genus in Catocalinae.

The imagines of the genus *Armada* are characterised by their small-medium body size, well developed haustellum, frons with strong chitinous projection and lamellate male antennae. It is defined, in the male genitalia, by two autapomorphics; first, the scaphium sub-basally with two lateral, setose crest/hills and the second, costally heavily sclerotized valvae bearing small processes (Goater et al., 2003). The genus *Armada* with its nine species (Wiltshire, 1979, Goater et al., 2003) inhabits arid and semi-arid zones of the Middle East and Central Asia. Up to present time, six species of the genus *Armada* have been reported from Iran (Brandt, 1939, Wiltshire, 1961, Wiltshire, 1979). This paper describes the male *Armada nilotica* A. Bang-Haas for the first time. An identification key for the *Armada* species in Iran is provided and bionomics and distribution of the *Armada* species are presented.

MATERIAL AND METHODS

Moth specimens of *A. nilotica* examined in this study were collected using portaple light traps (powered by 12-V batteries and 8-W black light UVB tubes) in Kerman province, south of Iran. The data of other species of the genus *Armada* were obtained from the published literatures or from the specimens deposited in the Collection of Noctuidae, Shahid Bahonar University of Kerman (Kerman, Iran). The genitalia slides of the examined material were prepared using routine method described by Fibiger (1990) with little modifications. Goater et al. (2003) was followed for the terminology used and the systematic order of the taxa. The Photographs of the adult specimens were taken with a Canon digital camera (model: Power Shot A710) and those of the genitalia by an Olympus SZH stereo-microscope with an Omax (18Mp) A35180U3 digital camera.

RESULTS

List of Iranian species

Genus *Armada* Staudinger, 1884

A. heliothidia (Hampson, 1896)
syn. *A. roseifemur* Brandt, 1939
A. dentata (Staudinger, 1884)
syn. *A. afghana* Hampson, 1926
A. nilotica A. Bang-Haas, 1912
A. fletcheri Wiltshire, 1961
A. maritima Brandt, 1939
A. panaceorum (Ménétries, 1848)
syn. *A. distincta* Rothschild, 1915
A. clio (Staudinger, 1884)

Key to Iranian species of *Armada* Staudinger based on the male genitalia (Goater et al., 2003, Wiltshire, 1979)

1. Costa of valvae without process...*A. heliothidia*
- Costa of valvae with a process...2
2. Cucullus weak, costal process small, bare, almost taperred..3
- Cucullus developed, costal process large and wide, hairy...5

3. Vesica without cornutus, left clasper small..*A. fletcheri*
- Vesica with two cornuti, left clasper large and thick 4
4. Costal process taperred, aedeagus with a small cornutus proximally..*A. dentata*
- Costal process broader than long, aedeagus without cornutus.. *A.nilotica*
5. Vesica without cornutus .... *A. panaceorum*
- Cornutus present on vesica 6
6. Vesica with three cornuti .... *A. maritima*
- Vesica with a single cornutus .... *A. clio*

Genus *Armada* Staudinger, 1884
Armada Staudinger, 1884, Romanoff Mémoires sur les Lépidoptères 1: 142. Type species: *Acontia dentata* Staudinger, 1884.

***Armada heliothidia* (Hampson, 1896)**
Melipotis heliothidia Hampson, 1896, Fauna of British India 4: 532. L. t.: India.
syn. *roseifemur* Brandt, 1939
Bionomics: univoltine autumnal species, *A. heliothidia* inhabits the most south-eastern deserts of Iran and the tropical eremic zones of India. The larva was described by Beck (2013), no report on its food plants is available.
General distribution: India, Iran (Wiltshire, 1979).
Distribution in Iran: Sistan-va-Balouchestan and Tehran (Ebert and Hacker, 2002, Brandt, 1939).

***Armada dentata* (Staudinger, 1884)**
Acontia dentata Staudinger, 1884, Romanoff Mémoires sur les Lépidoptères 1: 142. L. t.: Akhal Tekke.
syn. *afghana* Hampson, 1926
Bionomics: univoltine spring flying species, *A. dentata* inhabits semi-desert stony zones of central-south Iran to Afghanistan and Transcaspia. The larval stages and their food plants are unknown as yet.
General distribution: Afghanistan, Turkmenistan, Kazakhstan and Iran (Wiltshire, 1979, Poltavsky et al., 1998, Shovkoon and Trofimova, 2016).
Distribution in Iran: Kerman, Sistan-va-Balouchestan and Khorasan (Wiltshire, 1979, Rabieh, 2018).

***Armada nilotica* A. Bang-Haas, 1912**
Armada dentata v. *nilotica* Bang-Haas, 1912, Deutsche Entomologische Zeitschrift Iris, 26: 160. L. t.: Cairo.
Material examined: 2 ♂♂, Iran, Prov. Kerman, Kerman city, Haftbagh, 1800m, 30°09′50″N 57°09′06″E, 18.04.2011, leg. H. Ramezani., Slide No. ASH558m. 1♀ IRAN, Prov. Kerman, Bardsir, Bidouyeh Protected Area, 1805 m, 30º06′32.2″N 56º55′28.8″E, 26.04.2017, leg. M. Ghaemmaghamian, Slide No. MGH223f.
Diagnosis: *A. nilotica* taxonomically stands between *A. dentata* and *A. fletcheri*, the diagnostic characteristics of adults are given under *A. fletcheri* described by Wiltshire (1961), due to the first description of the male genitalia, the genital characteristics are presented here: compared to both species, valvae of *A. nilotica* are almost paralleled, clasper is longer and stronger, costal process is wider than long and aedeagus bears no sclerotized process distally. Compared to *A. nilotica*, the vesica of *A. fletcheri* lacks any cornutus; the costal process of *A. dentata* is taperred and its aedeagus bears a small thorn-like process.

Description: male (fig. 1A), wingspan 25-28 mm. head, collar, tegulae, thorax and forewing ground colour beige light brown. Antennae filiform, simple, palpi relatively short, the third segment the shortest, compound eyes large, globular. Forewing triangular, basal streak missing, basal line obsolescent, ante-medial line conspicuous, noctuid maculation complete, orbicular and reniform stigmata whitish, outlined by fine beige scales, claviform stigma filled by dark brown, median shade dark brown, post-medial line thinner at costal margin, sub marginal line as blackish wedges, termen fine, fringes white-brown alternately. Hindwing oval, basal two-third white, black discal spot present, longer than wide, marginal band broad, dark-brown, with whitish spot at posterior one third, fringes whitish with brown scales. Underside of forewing grayish white, discal spot present, black, marginal area brownish, underside of hindwing white, discal spot black, ovate, marginal area grayish brown, tornal spot conspicuous. Female (fig. 1B) as male.

Male genitalia: (fig. 2A,B), uncus long, slender, curved, tapering, distal tufts of scaphium present, tegument low; valvae asymmetrical, sclerotized, costa slightly convex, costal process present on the costal extremity, button-liked, sacculus oval-rectangular, saccular extension narrower on right side, both reaching base of cucullus, clasper asymmetrical, thumb-like and thick on the left valva, very short on the right, cucullus and corona weak, juxta longer than wide, pentagonal, wider basally, vinculum V-shaped, saccus large. Aedeagus long, slightly curved, wider distally, vesica short, wide, bent dorsally, two heavily sclerotized thorn-like cornuti present.

Female genitalia: papillae anales weak (fig. 2C), rounded, setose, apophyses slender, posterior one longer, ostium bursae sclerotized, more or less funnel-shaped, ductus bursae short, broad, corpus bursae globular, membranous, appendix bursae elongate.

Bionomics: presumably multivoltine species (Wiltshire, 1979). *A. nilotica* inhabits stony and sandy semi-desert up to 1800 m. altitude vegetated by shrubs and semi-shrubs. It flies, in Iran, from April to May, the larvae have been described by Andres and Seitz (1924), Wiltshire (1948) and Wiltshire (1979) and feed on *Heliotropium luteum* (Wiltshire (1979) and *H. arabense* (Andres and Seitz, 1924).

General distribution: Egypt (Wiltshire, 1979) and Iran.

Distribution in Iran: Kerman (new record from Iran).

FIGURE 1. *Armada nilotica* adults. A. male, Iran, Kerman prov.; B. female, Iran, Kerman prov.

Armada fletcheri Wiltshire, 1961

Armada fletcheri Wiltshire, 1961, Journal of the Bombay Natural History Society 58: 626. L. t.: Khuzestan.

Bionomics: multivoltine species, *A. fletcheri* inhabits desert of Ahwaz city SW Iran and eremic hot deserts of Saudi Arabia. The larvae have not been described and their food plants are still unknown.

General distribution: Saudi Arabia and Iran (Wiltshire, 1979).

Distribution in Iran: Khuzestan (Wiltshire, 1961).

Armada maritima Brandt, 1939

Armada maritima Brandt, 1939, Entomologische Rundschau 56: 294. L. t.: Iran, Sistan va Balouchestan.
Bionomics: multivoltine deserticolous species, *A. maritima* inhabits arid desert of the most south-eastern zone of Iran, Chabahar. The biology, habitat, larval morphology and its food plants are unknown. Kravchenko et al. (2007) collected this species from a stony arid desert with sparce vegetation of semi-shrubs and some *Tamarix* bushes.

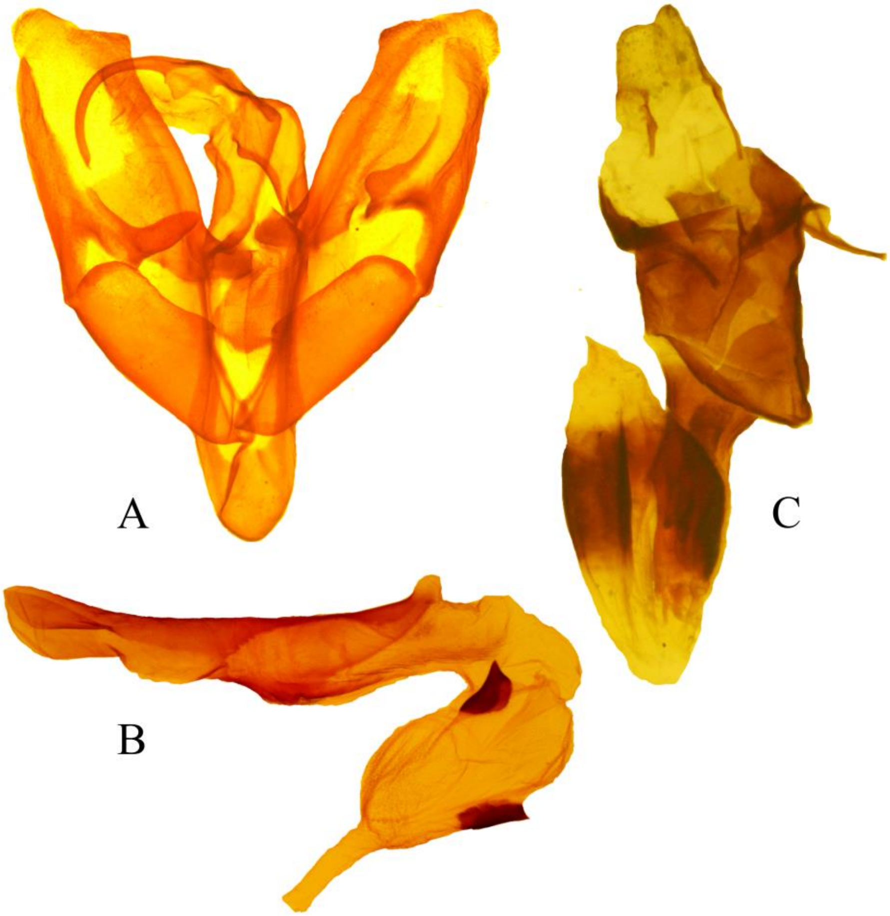

FIGURE 2. *Armada nilotica* genitalia. A. male armature; B. male aedeagus and vesica; C. female genitalia.

General distribution: Iran, Saudi Arabia, Oman, United Arab Emirates, Levant (Brandt, 1939, Wiltshire, 1961, Wiltshire, 1979, Kravchenko et al., 2007).
Distribution in Iran: Sistan va Balouchestan (Brandt, 1939) and Hormozgan (Ebert and Hacker, 2002).

Armada panaceorum (Ménétries, 1848)

Ophiusa panaceorum Ménétries, 1848, Mémoires de l'Académie Impériale des sciences de St. Pétersbourg 6: 292. pl. 6, fig. 6. L. t.: Bokhara.
syn. *distincta* Rothschild, 1915
Material examined: 8♂♂, 2♀♀, Iran, Sistan & Balouchestan, Bazman, 10.03.2008. leg. E. Kazemi. 1♀, Iran, Prov. Hormozgan, 10 km NE of Bandar Abbas, 200 m, 13. 03. 2007, leg. Asghar Shirvani.
Bionomics: univoltine desert and semi-desert species, *A. panaceorum* flies, depend on the inhabited geographical region, from February to June. The reported larval foodplants are *Arnebia decumbens*,

Heterocaryum rigidum and *Lappula ceratophora* (Kravchenko et al., 2007). The early stages are unknown and have not been described.
General distribution: Eurasiatic, a pan-eremic species distributed from North Africa, Canary Islands, Near and Middle East, Kazakhstan, Afghanistan, Tajikistan, Turkmenistan, Uzbekistan, South Russia, Mongolia and Tibet (Wiltshire, 1979, Poltavsky et al., 1998, Goater et al., 2003, Kravchenko et al., 2007, Ivinskis and Miatleuski, 1999, Shovkoon and Trofimova, 2016).
Distribution in Iran: Hormozgan, Tehran, Sistan-va-Balouchestan, Azarbayjan-e-Gharbi, Azarbayjan-e-Sharghi, Khorasan (Ebert and Hacker, 2002, Rabieh, 2018) and Kerman (first record).

Armada clio (Staudinger, 1884)
Acontia clio Staudinger, 1884, In Romanoff Mem. Lep. 1: 145. L. t.: Akhal Tekke.
Bionomics: univoltine early spring flying species. The larvae have not been described and their life history is still unknown.
General distribution: Iran, Uzbekistan, Turkmenistan, Kazakhstan and Tajikistan (Matov et al., 2021, Shovkoon and Trofimova, 2016, Ebert and Hacker, 2002).
Distribution in Iran: Qom (Ebert and Hacker, 2002), Mazandaran and Guilan (Matov et al., 2021).

DISCUSSION

All members of the genus *Armada* are reported from the desert regions of North Africa, Europe, Middle East and Central Asia. Of the *Armada* species, *A. panaceorum,* a pan-eremic species with the widest range of distribution, is extended from the North Africa to Mongolia and Tibet (Goater et al., 2003). This genus is presented in Europe by *A. clio* (Ciscaucasia) and *A. panaceorum* (S Russia). As the arid regions are preferred habitat for this genus, seven species out of nine are now inhabiting Iran territory. Of the Iranian *Armada*, *A. panaceorum* and *A. clio* have wider distribution rang in Iran (the former is widespread throughout Iran) while the remaining species are restricted, in the range, to the arid regions of the south and southeast (Kerman, Sistan-va-Balouchestan, Hormozgan and Khuzestan provinces). Adults of *A. panaceorum*, *A. clio*, *A. dentata* and *A. heliothidia* are flying in a single generation while those of *A. maritima, A. nilotica* and *A. fletcheri* appears in the more generations. Our knowledge on the larval food plant of the genus *Armada* is poor and limited to those reported for host plants of *A. panaceorum*. Similarly, the immature stages of most *Armada* species are undescribed except those of *A. nilotica* and *A. heliothidia.* Since Wiltshire (1979) revised the tribe Armadini, there is no extensive and more complete taxonomic study presenting autapomorphies for the true genera of this taxon.

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RESEARCH ARTICLE **Open access**

Edaphic Mesostigmata mites in central Iran: Twelve new country and local records

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Abstract
Agroecosystems containing edaphic Mesostigmata are considered important predators of nematodes, collembola, and insects, and those living on plants may be effective at controlling pests like spider mites. In this research study we provide new information about Edaphic Mesostigmata mites known from Isfahan in central Iran based on new field collection. In this publication, we give records of thirty-two edaphic species belong to 13 families, and 25 genera collected in Isfahan province (Iran), which four species are new records for the Iran (indicated as a rec. new). Twelve new provincial distribution records viz., *Asca aphidioides* (Linnaeus, 1758), *Blattisocius tarsalis* (Berlese, 1918), *Cosmolaelaps claviger* (Berlese, 1883), *Gaeolaelaps neoaculeifer* (Hrischmann, 1966) a rec. new, *Gymnolaelaps obscuroides* (Costa, 1968), *Hypoaspis quadridentatus* (Allred, 1970) a rec. new, *Hypoaspisella asperatus* (Berlese, 1904) a rec. new, *Hypoaspisella patagoniensis* (Sheals, 1962) a rec. new, *Neoseiulus bicadus* (Wainstein, 1962), *Parasitus mycophilus* (Karg, 1971), *Pogonolaelaps canestrinii* (Berlese, 1903), *Polyaspis berlesei* (Camin, 1954) are presented.

Key words: *Mesostigmata, new records, distribution, Iran, Agroecosystem.*

INTRODUCTION

Soil is one of the most important and diverse ecological habitats (Dustar Sharaf et al., 2016). Soil organisms play an important role in performing soil functions in the environmental system and can be used as indicators to assess soil quality (Schloter et al., 2003). Arthropods are an important component of soil fauna and mites are considered to be the best representatives of arthropods in the soil due to their species diversity, ecological niche and behavior (Bedano et al., 2005). Mites are used as an indicator of soil quality and to inform the soil status of agricultural and forestry environments (Speight et al., 2008).

The number of Iranian Mesostigmata except for the Phytoseiidae family is 348 species belonging to 128 genera from 39 families and 17 superfamilies (Kazemi & Rajaei 2013), while there are more than 12,000 species of Mesostigmata in the world. Comparison of these two numbers shows that the faunistic study of Iranian Mesostigmata mites requires more extensive and more serious studies (Abbaspour et al., 2017). Soil type, moisture, pH, and geographical location cause differences in the density of mites in the soil (Manu, 2013). Management strategies, the vegetation of forests, and human activities can play a significant role in reducing the quantity and quality of Mesostigmata mites in terms of the diversity of species (Sabbatini et al., 2011). The diversity of soil mites in habitats with more vegetation and of course

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pristine is higher compared to habitats with less vegetation and use pesticides and chemical fertilizers (Perez Velazquez et al., 2011).

The study of Mesostigmata mite diversity in green space determines the stability and dynamics of the ecosystem, soil quality and evaluation of management in green space (Maleki et al., 2016). So far, little research has been done in this field in Iran and the world. During the study of soil Mesostigmata mites in parks located in Kerman (Iran), 21 species belonging to 17 genera were identified and only reported one species for the first time from Iran (Kazemi, 2011). In another study, the biodiversity of soil inhabiting Mesostigmata was studied in one of the parks of Tehran and it was found that species diversity is directly related to soil moisture, pH and diversity of vegetation and management applied to the soil. The highest biodiversity was reported in the third quarter of the year and the lowest in the first quarter of the year (Maleki et al., 2016).

This paper contributes to improving baseline biodiversity knowledge of edaphic Mesostigmata mites in central Iran by providing twelve new provincial species records including four new records to Iran. Preparation of a full list of the Iranian edaphic mite species increments our ability to deduce biogeographical patterns and make foresight about their biodiversity. Furthermore, upkeep current records of edaphic mites of Iran can assist us in documenting presented, or anything exclusively relevant to the study of Mesostigmata mites.

MATERIAL AND METHODS

Faunistic study of soil mites in urban green spaces of Isfahan from 24 stations -each of which had different characteristics in terms of vegetation, ecological and geographical aspects - in four seasons (six times per season) was done in 2019 (Table 1). Samples were randomly selected from four points in each station and in a zigzag pattern once every 15 days from a depth of 10–15 cm of soil surface by shovel and in the amount of 500 cm^3 and poured into dark plastic bags and subsequently labeled with information on each the bag separately. The bags containing the samples were then transported to the laboratory. The soil samples of each station were mixed together and a sample of this mixture at a rate of one kilogram was placed in a Berlese funnel for 48 to 72 hours. Collected mites were separated under a stereomicroscope (Olympus, Japan) using special needles and in AG solution (95 parts **A**lcohol 70% + 5 parts **G**lycerin 1–3%). Afterward, the specimens were transferred to small containers containing the lactic acid in order to clear the mites. After clarification, the specimens were mounted using Hoyer fixation solution. The prepared slides were identified according to valid taxonomic sources. A number of samples were also sent to Iranian acarologists for approval. The mite species information is included in table 2.

RESULTS

Following is a list of all the species that were collected and could be identified. A total of thirty-two species belong to 13 families, and 25 genera are treated that contains four new records for Iran (indicated as rec. new). Twelve new provincial distribution records *viz.*, *Asca aphidioides* (Linnaeus, 1758), *Blattisocius tarsalis* (Berlese, 1918), *Cosmolaelaps claviger* (Berlese, 1883), *Gaeolaelaps neoaculeifer* (Hrischmann, 1966) rec. new, *Gymnolaelaps obscuroides* (Costa, 1968), *Hypoaspis quadridentatus* (Allred, 1970) rec. new, *Hypoaspisella asperatus* (Berlese, 1904) rec. new, *Hypoaspisella patagoniensis* (Sheals, 1962) rec. new, *Neoseiulus bicadus* (Wainstein, 1962), *Parasitus mycophilus* (Karg, 1971), *Pogonolaelaps canestrinii* (Berlese, 1903), *Polyaspis berlesei* (Camin, 1954) are presented.

List the Mesostigmata species in the urban green spaces of central Iran (Isfahan province)

Kingdom Animalia Linnaeus, 1758
Phylum Arthropoda von Siebold, 1848
Subphylum Chelicerata Heymons, 1901
Class Arachnida Lamarck, 1801
Superorder Parasitiformes Leach, 1815
Order Mesostigmata G. Canestrini, 1891

FIGURES 1–2. Sampling areas in the Isfahan province, Iran. 1. Area 1 (East); 2. Area 2 (West).

FIGURES 3–4. Sampling areas in the Isfahan province, Iran. 3. Area 3 (North); 4. Area 4 (South).

FIGURE 5. Sampling area 5 (Center) in the Isfahan province, Iran.

Family Ameroseiidae Evans, 1961

Ameroseius lidiae Bregetova, 1997 (Figs 6–13)
Distribution in Iran. Fars, Kerman, Zanjan provinces (Kazemi & Rajaei 2013).

Ameroseius plumosus (Oudemans, 1902) (Figs 14–17)
Distribution in Iran. East Azerbaijan, Isfahan, Khuzestan, Sistan and Baluchestan, West Azerbaijan provinces (Kazemi & Rajaei 2013).

Family Ascidae Voigts & Oudemans, 1905

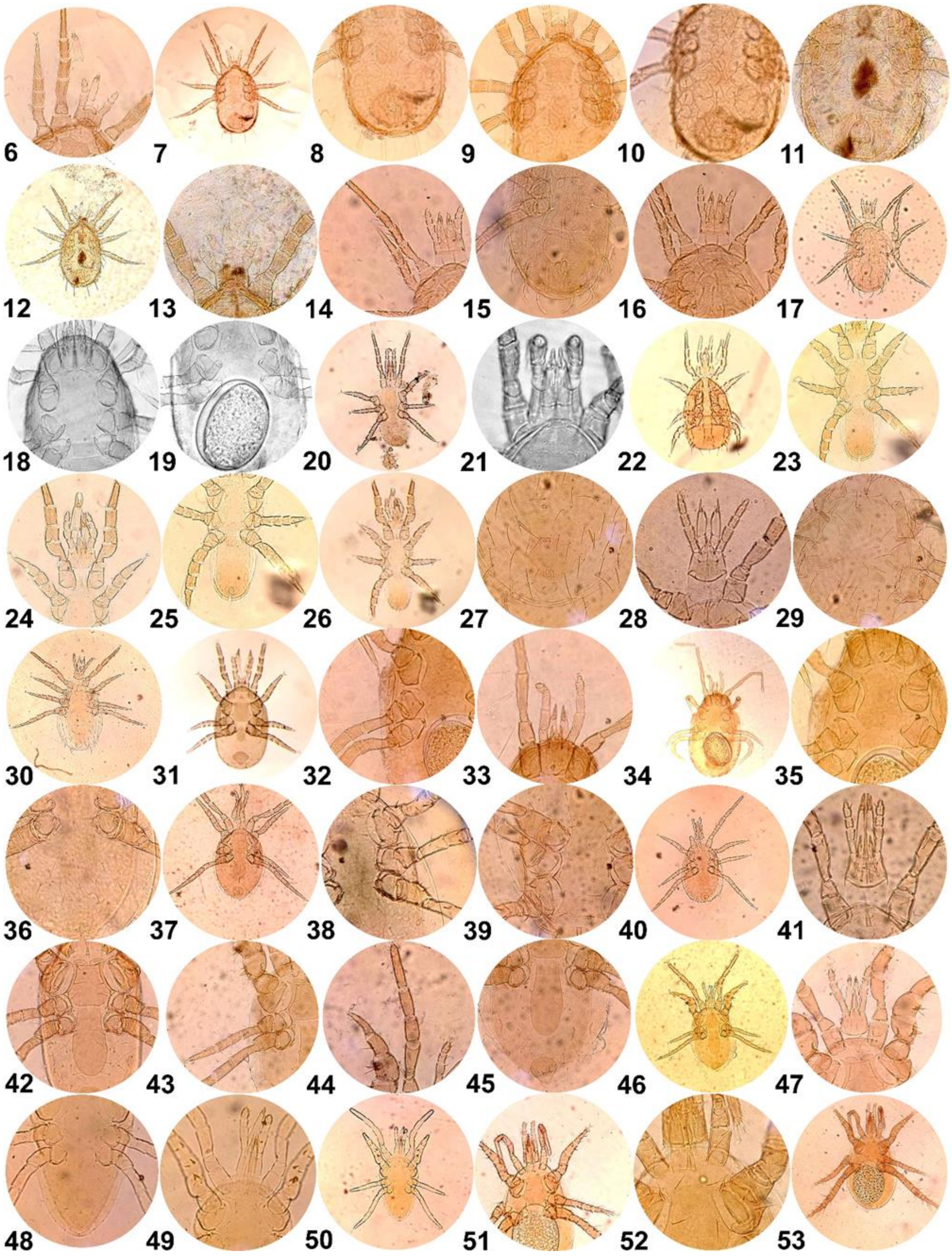
Antenoseius bacatus Athias-Henriot, 1961 (Figs 18–19)
Distribution in Iran. Chaharmahal and Bakhtiari, Fars, Kerman, Kohkiluyeh and Boyer-Ahmad, Khuzestan provinces (Kazemi & Rajaei 2013).

Arctoseius cetratus (Sellnick, 1940) (Fig. 20)
Distribution in Iran. Alborz, Chaharmahal and Bakhtiari, Fars, Golestan, Guilan, Hamedan, Isfahan, Kerman, Khuzestan, Kohkiluyeh and Boyer-Ahmad, Semnan, Tehran, West Azerbaijan, Zanjan provinces (Kazemi & Rajaei 2013).

Arctoseius pristinus Karg, 1962 (Fig. 21)
Distribution in Iran. Isfahan province (Kadkhodae Eliaderani et al. 2013).

Asca aphidioides (Linnaeus, 1758) (Fig. 22)
Distribution in Iran. Golestan, Kohkiluyeh and Boyer-Ahmad, Mazandaran provinces (Kazemi & Rajaei 2013).

Protogamasellus massula (Athias Henriot, 1961) (Figs 23–26)
Distribution in Iran. Fars, Isfahan, Kerman, Semnan, Tehran, West Azerbaijan provinces (Kazemi & Rajaei 2013).



Figures 6–53. 6–13. *Ameroseius lidiae* Bregetova, 1997; 14–17. *Ameroseius plumosus* (Oudemans, 1902); 18–19. *Antenoseius bacatus* Athias-Henriot, 1961; 20. *Arctoseius cetratus* (Sellnick, 1940); 21. *Arctoseius pristinus* Karg, 1962; 22. *Asca aphidioides* (Linnaeus, 1758); 23–26. *Protogamasellus massula* (Athias Henriot, 1961); 27–30. *Blattisocius tarsalis* (Berlese, 1918); 31. *Alliphis halleri* (G. & R.Canestrini, 1881); 32–35. *Cosmolaelaps claviger* (Berlese, 1883); 36–41. *Cosmolaelaps lutegiensis* (Shcherbak, 1971); 42–50. *Euandrolaelaps karawaiewi* (Berlese, 1903); 51–53. *Gaeolaelaps aculeifer* (Canestrini, 1884).

Family Blattisociidae Garman, 1948
Blattisocius tarsalis (Berlese, 1918) (Figs 27–30)
Distribution in Iran. Fars, Golestan, Guilan provinces (Kazemi & Rajaei 2013).

Family Eviphididae Berlese, 1913
Alliphis halleri (G. & R. Canestrini, 1881) (Fig. 31)
Distribution in Iran. East Azerbaijan, Fars, Golestan, Hamedan, Isfahan, Kerman, Markazi, North Khorasan, Tehran, West Azerbaijan provinces (Kazemi & Rajaei 2013).

Family Laelapidae Berlese, 1892
Cosmolaelaps claviger (Berlese, 1883) (Figs 32–35)
Distribution in Iran. Tehran province (Ramroodi et al. 2014).

Cosmolaelaps lutegiensis (Shcherbak, 1971) (Figs 36–41)
Distribution in Iran. Golestan, Guilan, Isfahan, Kerman, Mazandaran, Tehran, West Azerbaijan provinces (Kazemi & Rajaei 2013).

Euandrolaelaps karawaiewi (Berlese, 1903) (Figs 42–50)
Distribution in Iran. Chaharmahal and Bakhtiari, Fars, Guilan, Hamedan, Isfahan, Kerman, Khuzestan, Kohkiluyeh and Boyer-Ahmad, Markazi, Razavi Khorasan, Tehran, West Azerbaijan, Yazd, Zanjan provinces (Kazemi & Rajaei 2013).

Gaeolaelaps aculeifer (Canestrini, 1884) (Figs 51–55)
Distribution in Iran. Chahrmahal and Bakhtiari, East Azerbaijan, Fars, Golestan, Guilan, Hamedan, Isfahan, Kerman, Khuzestan, Markazi, Mazandaran, Razavi Khorasan, Sistan and Baluchestan, Tehran, West Azerbaijan, Yazd, Zanjan provinces (Kazemi & Rajaei 2013).

Gaeolaelaps neoaculeifer (Hrischmann, 1966), rec. new, (Figs 56–62)
Distribution in Iran. Isfahan province (present study).

Gaeolaelaps queenslandica (Womersley, 1956) (Figs 63–68)
Distribution in Iran. Chaharmahal and Bakhtiari, Fars, Guilan, Isfahan, Kerman, Khuzestan, Mazandaran, Razavi Khorasan, Tehran, West Azerbaijan, Yazd provinces (Kazemi & Rajaei 2013).

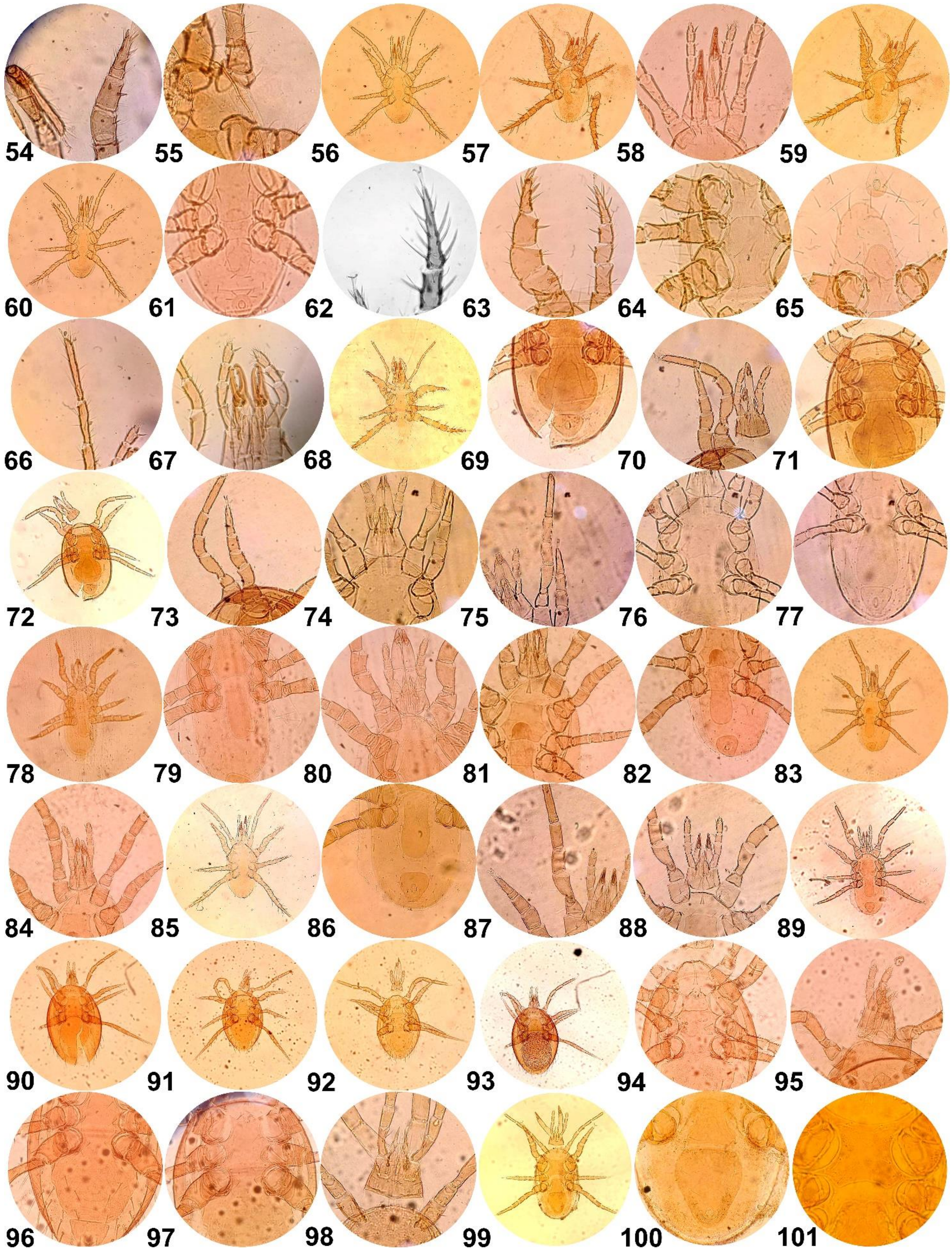
Gymnolaelaps obscuroides (Costa, 1968) (Figs 69–73)
Distribution in Iran. Fars, Isfahan provinces (Kazemi & Rajaei 2013).

Haemolaelaps shealsi (Costa, 1968) (Figs 74–77)
Distribution in Iran. Fars, Razavi Khorasan, Sistan and Baluchestan, Tehran, West Azerbaijan provinces (Kazemi & Rajaei 2013).

Hypoaspis quadridentatus (Allred, 1970), rec. new, (Figs 78–80)
Distribution in Iran. Isfahan province (present study).

Hypoaspisella asperatus (Berlese, 1904), rec. new, (Figs 81–84)
Distribution in Iran. Isfahan province (present study).

Hypoaspisella patagoniensis (Sheals, 1962), rec. new, (Fig. 85)
Distribution in Iran. Isfahan province (present study).



FIGURES 54–101. 54–55. *Gaeolaelaps aculeifer* (Canestrini, 1884); **56–62.** *Gaeolaelaps neoaculeifer* (Hrischmann, 1966); **63–68.** *Gaeolaelaps queenslandica* (Womersley, 1956); **69–73.** *Gymnolaelaps obscuroides* (Costa, 1968); **74–77.** *Haemolaelaps shealsi* (Costa, 1968); **78–80.** *Hypoaspis quadridentatus* (Allred, 1970); **81–84.** *Hypoaspisella asperatus* (Berlese, 1904); **85.** *Hypoaspisella patagoniensis* (Sheals, 1962); **86–89.** *Pneumolaelaps sclerotarsus* costa, 1968; **90–97.** *Pogonolaelaps canestrinii* (Berlese, 1903); **98–101.** *Macrocheles insignitus* (Berlese, 1918).

Pneumolaelaps sclerotarsus costa, 1968 (Figs 86–89)
Distribution in Iran. Chaharmahal and Bakhtiari, East Azerbaijan, Fars, Guilan, Hamedan, Isfahan, Kerman, Khuzestan, Kohkiluyeh and Boyer-Ahmad, Tehran, Zanjan provinces (Kazemi & Rajaei 2013).

Pogonolaelaps canestrinii (Berlese, 1903) (Figs 90–97)
Distribution in Iran. Tehran province (Kazemi & Rajaei 2013).

Family Macrochelidae Vitzthum, 1930

Macrocheles insignitus (Berlese, 1918) (Figs 98–101)
Distribution in Iran. Chaharmahal and Bakhtiari, Golestan, Kerman, Kerman, Mazandaran, North Khorasan, Zanjan provinces (Kazemi & Rajaei 2013).

Family Melicharidae Hirschmann, 1962

Proctolaelaps pygmaeus (Müller, 1859) (Figs 102–105)
Distribution in Iran. Bushehr, Chaharmahal and Bakhtiari, Fars, Guilan, Hamedan, Isfahan, Kerman, Khuzestan, Kordestan, Lorestan, Mazandaran, Semnan, West Azerbaijan, Zanjan provinces (Kazemi & Rajaei 2013).

Family Pachylaelapidae Berlese, 1913

Onchodellus karawaiewi (Berlese, 1920) (Figs 106–107)
Distribution in Iran. Chaharmahal and Bakhtiari, East Azerbaijan, Fars, Golestan, Hamedan, Kerman, Kerman, Kermanshah, Khuzestan, Markazi, Mazandaran, North Khorasan, Razavi Khorasan, Tehran, West Azerbaijan provinces (Kazemi & Rajaei 2013).

Family Parasitidae Oudemans, 1901

Parasitus mycophilus (Karg, 1971) (Figs 108–111)
Distribution in Iran. Fars, Hamedan, Kerman, Razavi Khorasan, Tehran, West Azarbaijan provinces (Kazemi & Rajaei 2013).

Family Phytoseiidae Berlese, 1916

Neoseiulus barkeri Hughes, 1948 (Figs 112–115)
Distribution in Iran. Isafahan, Kurdistan, Lorestan, Zanjan provinces (widely distributed; e.g., Jafari et al. 2011).

Neoseiulus bicaudus (Wainstein, 1962) (Figs 116–120)
Distribution in Iran. Ardebil, Isafahan, Kurdistan provinces (widely distributed; e.g., Rahmani et al. 2010).

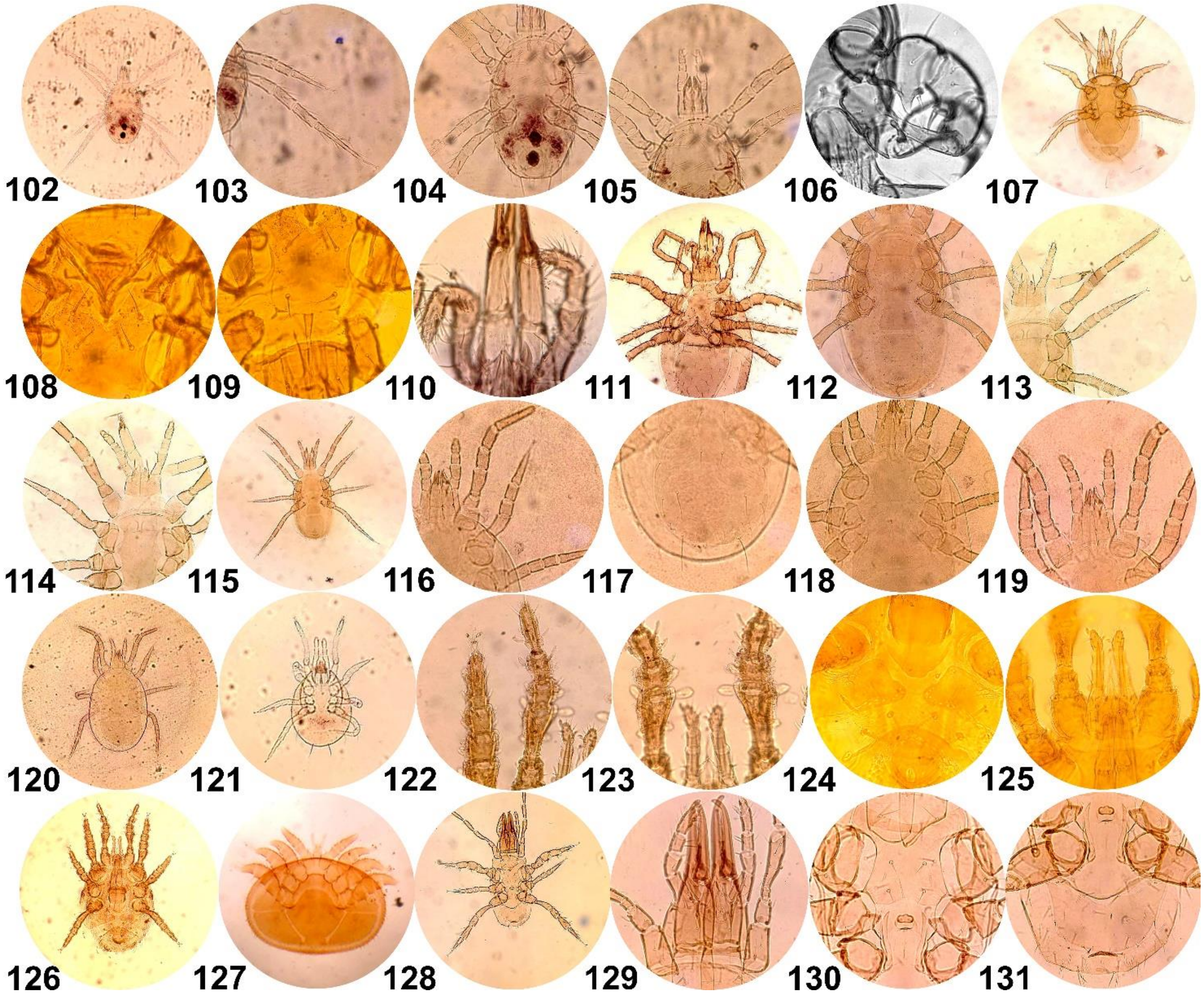
Proprioseiopsis messor (Wainstein, 1960) (Fig. 121)
Distribution in Iran. Fars, Isafahan, Kurdistan, Razavi Khorasan provinces (widely distributed; e.g., Panahi Laeen et al. 2014).

Family Polyaspididae Berlese, 1913

Polyaspis berlesei (Camin, 1954) (Figs 122–126)
Distribution in Iran. Isafahan, Hormozgan provinces (widely distributed; e.g., Nemati et al. 2018).

Family Varroidae Delfinado & Baker, 1974

Varroa destructor Anderson & Trueman, 2000 (Fig. 127)



FIGURES 102–131. 102–105. *Proctolaelaps pygmaeus* (Müller, 1859); 106–107. *Onchodellus karawaiewi* (Berlese, 1920); 108–111. *Parasitus mycophilus* (Karg, 1971); 112–115. *Neoseiulus barkeri* Hughes, 1948; 116–120. *Neoseiulus bicaudus* (Wainstein, 1962); 121. *Proprioseiopsis messor* (Wainstein, 1960); 122–126. *Polyaspis berlesei* (Camin, 1954); 127. *Varroa destructor* Anderson & Trueman, 2000; 128–131. *Veigaia planicola* (Berlese, 1892).

Distribution in Iran. Ardebil, Bushehr, Chaharmahal and Bakhtiari, Fars, Guilan, Isfahan, Kerman, Kermanshah, Khuzestan, Mazandaran, North Khorasan, Qazvin, Razavi Khorasan, Sistan and Baluchestan, South Khorasan, Tehran, Yazd provinces (Kazemi & Rajaei 2013; Hajializadeh et al. 2019).

Family Veigaiidae Oudemans, 1939

Veigaia planicola (Berlese, 1892) (Figs 128–131)

Distribution in Iran. Ardebil, Chaharmahal and Bakhtiari, East Azarbaijan, Isfahan, Kerman, Kermanshah, Tehran, West Azarbaijan provinces (Kazemi & Rajaei 2013; Nemati et al. 2013).

TABLE 1. Five sampling areas in the parks of Isfahan province, Iran.

No. Area	Direction	Coordinates	Vegetation
1	East	32° 38' 32"N, 51°42' 50"E	Conifers and shrubs
2	West	32° 38' 17"N, 51°38' 16"E	Broad-leaved trees and vegetation
3	North	32° 40' 53"N, 51°38' 22"E	Conifers, shrubs, and vegetation
4	South	32° 35' 42"N, 51°39' 11"E	Broad-leaved trees and vegetation
5	Center	32° 38' 26"N, 51°38' 59"E	Conifers, shrubs, and vegetation

TABLE 2. Number of Mesostigmata mites for each sampling area in parks of Isfahan province, Iran.

No.	Family	Species name	Total	Area 1	Area 2	Area 3	Area 4	Area 5
1	Ascidae	*Arctoseius cetratus*	101	17	19	5	31	29
2	Pachylaelapidae	*Onchodellus karawaiewi*	78	13	28	–	23	14
3	Phytoseiidae	*Neoseiulus barkeri*	56	8	16	14	16	2
4	Ameroseiidae	*Ameroseius lidiae*	34	4	10	10	2	8
5	Laelapidae	*Euandrolaelaps karawaiewi*	32	3	3	4	18	4
6	Laelapidae	*Haemolaelaps shealsi*	26	7	–	3	16	–
7	Laelapidae	*Gaeolaelaps queenslandica*	24	–	3	2	15	4
8	Laelapidae	*Cosmolaelaps lutegiensis*	22	5	5	7	–	5
9	Laelapidae	*Pogonolaelaps canestrinii*	16	–	7	7	2	–
10	Phytoseiidae	*Proprioseiopsis messor*	11	–	5	1	–	5
11	Parasitidae	*Parasitus mycophilus*	9	–	5	–	3	1
12	Laelapidae	*Pneumolaelaps sclerotarsus*	7	–	1	2	4	–
13	Laelapidae	*Gymnolaelaps obscuroides*	7	–	6	1	–	–
14	Laelapidae	*Hypoaspisella asperatus*	7	–	–	2	3	2
15	Laelapidae	*Gaeolaelaps aculeifer*	5	3	–	–	2	–
16	Ascidae	*Asca aphidioides*	4	2	2	–	–	–
17	Laelapidae	*Cosmolaelaps claviger*	4	–	–	–	2	2
18	Varroidae	*Varroa destructor*	3	–	2	–	1	–
19	Laelapidae	*Hypoaspis quadridentatus*	3	–	3	–	–	–
20	Ascidae	*Antenoseius bacatus*	3	–	–	–	3	–
21	Ascidae	*Arctoseius pristinus*	3	–	3	–	–	–
22	Ascidae	*Protogamasellus massula*	2	–	–	2	–	–
23	Polyaspididae	*Polyaspis berlesei*	2	–	–	–	2	–
24	Phytoseiidae	*Neoseiulus bicadus*	2	–	–	2	–	–
25	Macrochelidae	*Macrocheles insignitus*	2	–	–	2	–	–
26	Ameroseiidae	*Ameroseius plumosus*	2	–	–	–	–	2
27	Melicharidae	*Proctolaelaps pygmaeus*	2	–	–	–	2	–
28	Laelapidae	*Hypoaspisella patagoniensis*	2	1	–	–	–	1
29	Laelapidae	*Gaeolaelaps neoaculeifer*	2	–	–	–	2	–
30	Eviphididae	*Alliphis halleri*	1	1	–	–	–	–
31	Veigaiidae	*Veigaia planicola*	1	–	1	–	–	–
32	Blattisociidae	*Blattisocius tarsalis*	1	1	–	–	–	–
Total			474	65	119	64	147	79

DISCUSSION

This data shows that the edaphic Mesostigmata mite fauna in Iran is poorly studied. On the basis of previously published data and current fieldwork, we believe that the Iranian edaphic Mesostigmata is exceptionally rich and there will undoubtedly be more species added to the list as more research is conducted. This is because most samplings were restricted to Kerman, and Tehran provinces (Kazemi 2011; Maleki et al., 2016) and are extremely scarce in information, while other provinces i.e., Bushehr, Semnan, Sistan and Baluchestan, South Khorasan remained unexplored.

The present study showed that the areas where broad-leaved trees were densely planted had a richer variety of soil mites than areas with predominantly coniferous vegetation or other ornamental shrubs (Table 1). This is well justified due to the higher shading level of these trees and the retention of more moisture in the soil at the base of the trees. Also, areas that were exposed to direct sunlight and had poor vegetation, such as grasslands and short ornamental shrubs, had less species diversity (Table 1). This may be due to the faster evaporation of soil moisture due to sunlight. Areas one, three and five (north, south and center) had the lowest number of species and areas two and four (west and south) had the highest number of species. Southern parks of Isfahan, such as Soffeh Mountain Park, due to the forest ecosystem and virginity compared to other parks in Isfahan, the increase in the number of species is justified (e.g., Santamaria et al., 2012). However, considering that the central parks of Isfahan are exposed to pollutants, environmental pollution and also human manipulations, the existence of less species diversity in these areas is not far from the mind.

In order to fill this void in Iranian Sciaridae studies, we have commenced data collection for the investigation of the edaphic Mesostigmata mite fauna. The results of this study may be useful for countries in the Middle East as well from the taxonomic point of view. We hope this paper will stimulate local interest in the study of Iranian soil inhabiting Mesostigmata mite and will draw more attention to encourage researchers to study this group.

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RESEARCH ARTICLE **Open access**

Geospatial Analysis of Habitat Suitability for *Capricornis sumatraensis* (Bechstein, 1799) (Mammalia: Herbivora) in Annapurna Conservation Area of Nepal using MaXent Model

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Abstract
Capricornis sumatraensis Bechstein, the only sub-species of Serow found in Nepal, is a threatened species distributed across protected mountainous areas. In this study, we conducted a preliminary systematic survey to record the presence or absence of *C. sumatraensis*, and used satellite imagery, topo-maps, and field data to analyze habitat suitability and vegetation preference using MAXENT and ArcGIS. We also conducted focus group discussions, questionnaire surveys, and key informant surveys to assess the severity of various threats. The results showed that 18.3% of the total area was highly suitable, 16.8% was moderately suitable, and the remaining 64.76% was less suitable habitat. *C. sumatraensis* preferred a *Quercus semecarpifolia* and *Rhododendron arboreum*-dominated forest, where *Drepanostachyum falcatum* and *Girardinia diversifolia* were the dominant shrubs and *Anaphalis busua* and *Tracheophyta* were dominant herbs. The major threats to *C. sumatraensis* were poaching and hunting, open grazing, illegal resource collection, climate change, and development activities. Our findings can inform conservation efforts for this species and benefit conservation area managers, researchers, and academicians.

Key words: *Conservation, Distribution, Habitat preference, IVI, Threats*

INTRODUCTION

The Himalayan serow, *Capricornis sumatraensis* (Bechstein, 1799), commonly found in Bhutan, Northern India and Nepal, known as "Capricornis thar", belongs to the tribe Rupicaprinae under subfamily Caprinae, family Bovidae and order Artiodactyla (Phan et al., 2020). The tribe is also commonly known as goat-antelopes (Mori et al., 2019). *Capricornis sumatraensis* is the only sub-species of Serow that exists in Nepal (Bhattacharya et al., 2012). It has a large head, a thick neck, short limbs, long mule-like ears, and a coarse coat of dark hair. The *C. sumatraensis* looks like a cross between a cow, pig, donkey, and goat. *C. sumatraensis*is considered to be oriental in origin and confined to the forest slopes of the Himalayas (Aryal, 2008).

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The *C. sumatraensis*en joys densely wooded inaccessible river valleys and steep grassy hillsides with a nearby dense cover of oak and rhododendron (Mishra and Mierow, 1976). It prefers damp areas and is less tolerant of dry conditions, favoring an elevation of between 1500 m and 3000 m. Its feeding and habitat preferences also support solitary behavior. It also shows some sort of territorial behavior. *C. sumatraensis*is found to shift towards the lower altitudes in severe winter, but seasonal change in its home ranges in the Himalayas is not noticed. Currently, *C. sumatraensis* is found Bangladesh, Bhutan, China, India, and Nepal (Jnawali et al., 2011). In Nepal, *C. sumatraensis* is reported in Rara National Park (NP), Langtang NP, Sagarmatha NP, Shey-Phoksundo NP, Kanchanjunga NP, Makalu Barun NP, Annapurna Conservation Area (ACA), and Dhorpatan Hunting Reserve (Choudhary, 1998). The *C. sumatraensis* population in ACA is isolated in a small patch of the southern part of the area, with an estimated population density of 1.17 individuals/km and a population sex ratio of 1:1.6 (male: female) (Aryal, 2009). They have also been reported from Kanchanpur, Taplejung, Ramechhap, and Illam districts (Aryal, 2009).

Geographical information systems (GIS) are computer-based systems that are used to store and manipulate geographic information and are ultimately used to produce information needed by users (Aronoff, 1989). The focus of remote sensing in ecology and conservation science is on five broad capabilities: observation of habitat; analysis and management of biological and physical variables; mapping of the condition of a specific area at a specific time; monitoring how features have changed in the past over time and space; and decision support using trend information derived from remotely sensed products (Horning et al., 2010). A niche-based model represents an approximation of a species' ecological niche in the examined environmental dimensions. Species Distribution Models (SDMs), often referred to as Ecology Niche Models (ENM), allow the assessment of the suitability of a given area for one or multiple species and provide important information on ecological factors determining species distributions (Sillero, 2011). The output of SDM is increasingly used for multiple purposes, including the identification of conservation priorities, the prediction of species invasions, and analyses of the impact of environmental changes on biodiversity (Elith and Leathwick, 2009). There are a number of techniques that require presence-only data, such as environmental hype-space inhabited by a species method such as BIOCLIM, surface range envelope (SRE), distance-based methods by a species method such as DOMAIN, and discriminative techniques that require presence-absence data, such as General Linear Model (GLM), General Additive Models (GAM), Multivariate Adaptive Regression Splines (MARS), Classification, and Regression. Furthermore, SDM algorithms can be classified as follows: Regression methods such as GAM, GLM, and MARS; machine-learning methods such as ANN, BRT, MAXTENT, and RF; classification methods such as CTA and FDA; and enveloping methods such as SRE and BIOCLIM (Lazo, 2013). Similarly, in Nepal, several studies have been carried out regarding species habitat suitability using MaxEnt (Bai et al., 2018). The habitat suitability for mammals, birds, vegetation, and even invasive plant species has been previously conducted (Baidar et al., 2017, Thapa *et al*., 2018).

Owing to their population decline, the hunting of *C. sumatraensis* has been prohibited throughout Nepal (Wegge and Oli, 1997). Considering its threatened status, very little is known about the habitat, vegetation preference, and threats of *C. sumatraensis* in the Annapurna Conservation Area. The main objective of the study was to predict the *C. sumatraensis* distribution pattern and to identify the existing threats in Annapurna Conservation Area (ACA) along with the habitat use and to develop a habitat suitability model for the *C. sumatraensis* in ACA using the MaxEnt model.

MATERIAL AND METHODS

Study area

The research was carried out in the Annapurna Conservation Area of Nepal during June and July 2020, which is located in the Annapurna ranges of the Central Himalayas in the Western Region of Nepal (Figure 1). Annapurna Conservation Area (ACA) is the largest protected area in Nepal with high biodiversity and is a treasure house for 1, 226 species of flowering plants, 105 mammals, 518 birds, 40 reptiles, and 23 amphibians (Inskipp, 2003). It has been a refuge for many endangered species, including the snow leopard, since its establishment. Hence, this study adds a new dimension to the conservation and management of the conservation area. ACA was established in 1986 and extended in 1992 and now covers an area of 762900 hectares. The extreme variation in topography and climate has resulted in an exceptionally high diversity of flora and fauna representing subtropical to alpine vegetation types.

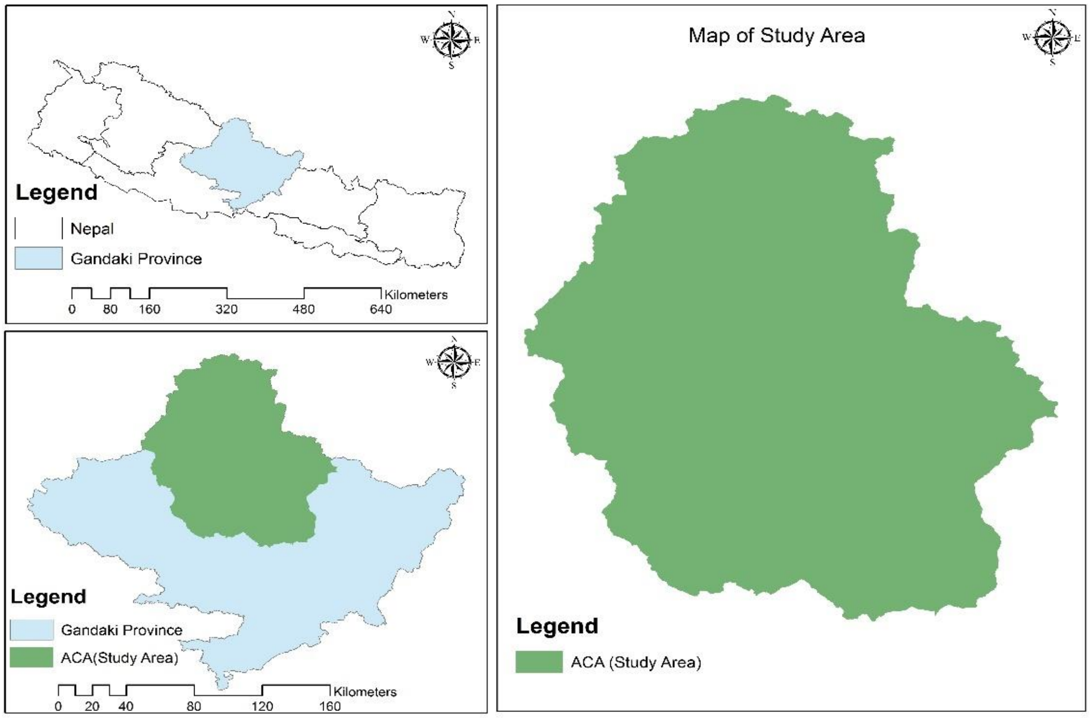

FIGURE 1. Map of the study area.

Sampling design

Important variables were measured in the field in order to estimate Importance Value Index (IVI), and these variables included trees, shrubs, and herbs. Similarly, *C. sumatraensis* sign and a type of disturbance were also observed in the plot. In addition to the record of the presence point in the plot, it was also recorded from outside the plots during a walk from one plot to another. For analysis of food availability, vegetation was estimated by conducting a field survey. Each sample plot had tree concentric circles of different radii for measurement of forest resources as 5.64 m radius for trees (area: 100 m^2), 1.78 m radius for shrubs (area: 10 m^2) and 0.56 m radius for herbs (1 m^2) was established (Figure 2). Similarly, vegetation was recorded in the appropriate plots and classified as trees with a diameter greater than 5 cm above DBH, shrubs with a height greater than 1 m, and herbs with a height less than 1 m.

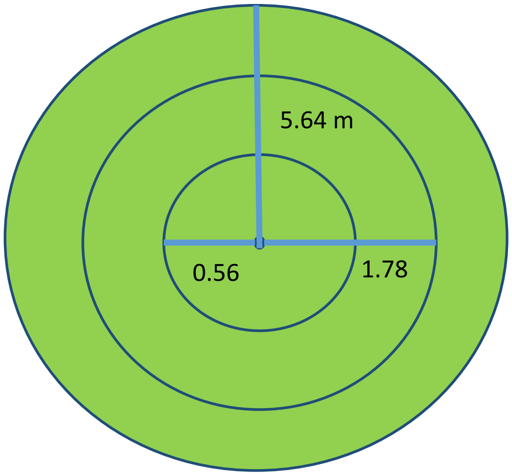

FIGURE 2. Sample plot design.

Data used

In this study, a variety of variables were used, including topographical variables (Digital Elevation Model (DEM), slope, and aspect), bioclimatic variables (worldclim), land use land cover (LULC), water bodies, roads, and riverbeds. These physical variables were taken from different sources: land use land cover (LULC) data was obtained from the International Centre for Integrated Mountain Development (ICIMOD), aspect and slope were taken from Shuttle Radar Topography Mission (SRTM) of the United States Geological Survey (USGS) site (earthexplorer.usgs.gov/), and the drainage and road data were obtained from DIVA-GIS (diva-gis.org). Table 1 gives a brief description of the input variables used for MaxEnt suitability prediction.

TABLE 1. List of input variables before processing.

Layer name	Spatial resolution	Pixel depth	Spatial data type	Projection system	Source
Presence data			Xlsx	UTM 44N	Field visit/NRC 2015
District boundary			polygon/line	WGS 84	ICIMOD
PAs boundary			polygon/line	WGS 85	ICIMOD
Settlement	30	16 bit signed	Point	WGS 86	ICIMOD
Southern part of the Sikles and Parche			polygon/line	WGS 87	PCTMCB
LULC	30	16 bit signed	Polygon	WGS 88	PCTMCB
Water bodies	30	16 bit signed	Polygon	WGS 89	Land use
River Beds	30	16 bit signed	Polygon	WGS 90	Land use
DEM	30	16 bit signed	Grid	WGS 91	ASTER
Slope	30	8 bit signed	Grid	WGS 92	DEM
Aspect	30	16 bit signed	Grid	WGS 93	DEM
Climate	1k	16 bit signed	Grid	WGS 94	Worldclim

Presence data

During a field trip around the study area, 16 presence records were collected. In addition to field data, some previous research and an ACP official's record were also incorporated for the purpose of running the MaxEnt model. For environmental information, the datasets for these 19 bioclimatic variables were derived from globally interpolated datasets for current conditions with a resolution of 1 km (source: http://www.worldclim.org) (Sharma et al., 2022). These variables were used for the modelling study because they are presumed to be the most relevant to animal existence, representing annual trends, seasonality, and extreme or limiting environmental factors (Sodhi et al., 2008). Bioclimatic variables are derived from the monthly temperature and rainfall values in order to generate more biologically meaningful variables for characterizing a species range (Buermann et al., 2008). Several studies have highlighted strong relationships between species abundance and bioclimatic variables (current version 2.0) (Phillips et al., 2006; Jeschke and Strayer, 2008). This scheme follows that of ANUCLIM, except that for temperature seasonality the standard deviation was used because a coefficient of variation does not make sense with temperatures between -1 and 1 (worldclim.org). The various bioclimatic variables, along with other physical variables, are mentioned in Table 2.

Topographical variable

A DEM shows an array of elevations of the land surface at each spatial location (I, j). Terrain visualization using satellite images in association with DEMs has long been explored as a promising

TABLE 2. Bioclimatic variables.

Sr.	BIO	Name	Resolutions
1	BIO1	Annual Mean Temperature	1km
2	BIO2	Mean Diurnal Range (Mean of monthly (max temp - min temp)	1km
3	BIO3	Isothermality (BIO2/BIO7) (* 100)	1km
4	BIO4	Temperature Seasonality (standard deviation *100)	1km
5	BIO5	Max Temperature of Warmest Month	1km
6	BIO6	Min Temperature of Coldest Month	1km
7	BIO7	Temperature Annual Range (BIO5-BIO6)	1km
8	BIO8	Mean Temperature of Wettest Quarter	1km
9	BIO9	Mean Temperature of Driest Quarter	1km
10	BIO10	Mean Temperature of Warmest Quarter	1km
11	BIO11	Mean Temperature of Coldest Quarter	1km
12	BIO12	Annual Precipitation	1km
13	BIO13	Precipitation of Wettest Month	1km
14	BIO14	Precipitation of Driest Month	1km
15	BIO15	Precipitation Seasonality (Coefficient of Variation)	1km
16	BIO16	Precipitation of Wettest Quarter	1km
17	BIO17	Precipitation of Driest Quarter	1km
18	BIO18	Precipitation of Warmest Quarter	1km
19	BIO19	Precipitation of Coldest Quarter	1km

tool in environmental studies (Gugan, 1988). This data layer was downloaded from earthexplorer.usgs.gov/, which was used for the generation of elevation, slope, and aspect.

Slope represents the rate of change of elevation for each DEM cell. It's the first derivative of DEM. The slope was calculated from the ASTERGMT data using ArcGIS desktop's "Spatial Analysis" tool. For the purpose of habitat suitability analysis, the generated slope was reclassified into 9 classes (Figure 3).

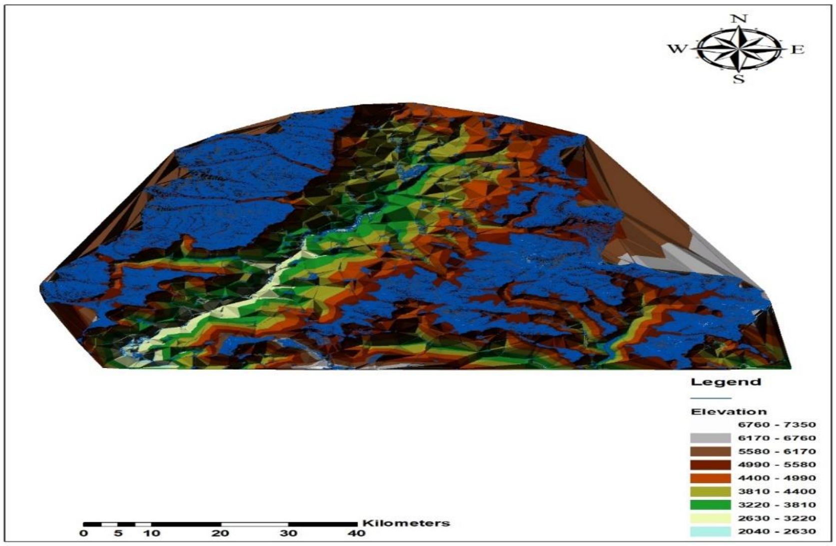

FIGURE 3. Slope map of Landscape.

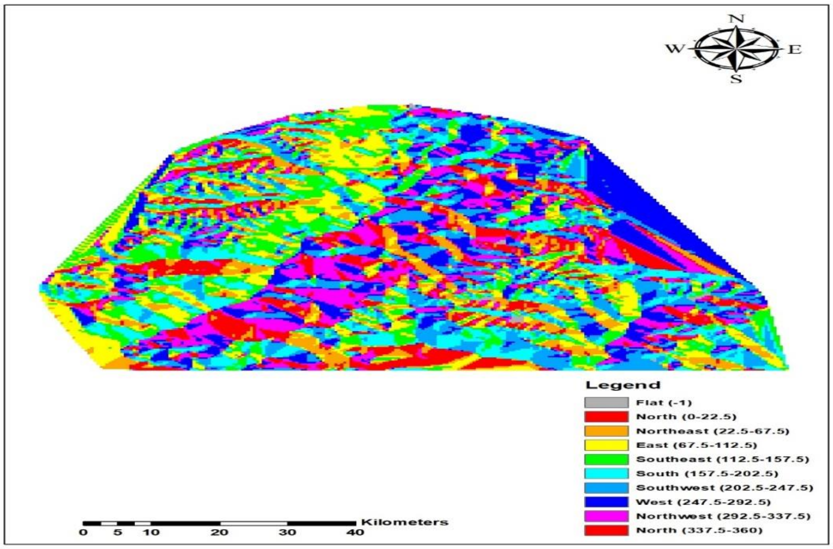

FIGURE 4. Aspect map of the landscape**.**

Data collection

An aspect is the direction towards which a slope is facing. It was derived using the "Spatial Analysis" tool of ArcGIS Desktop and further reclassified into 9 classes (Flat, North, Northeast, East, Southeast, South, Southwest, West, and Northwest) (Figure 4).

The survey was done by purposive sampling with ACP officials, Divisional Forest office staff, local people who have many ideas about *C. sumatraensis*, and other knowledgeable people to obtain information regarding threats. Group discussions were carried out by pursuing the Participatory Method given by Martin (1995), according to which theoretical issues and practical considerations in the conduct and analysis of focus groups were presented and discussed. A focused group discussion segregated by gender was organized to get a better understanding of the availability of the target species and threats to it. The focus group discussion was carried out with women and other people who frequently visited the *C. Sumatraensis* prone areas. A focus group discussion (2 meetings), a questionnaire survey of households with 50 respondents, and a total of three key informant interviews were performed to gather information about the attitude and perception of local people towards *C. sumatraensis*.

Data analysis

Important Value Index (IVI) Calculation

From the data gathered from the field, the IVI for each species was calculated. According to Zobel et al. (1987), the following method was performed for IVI calculation:

i. **Density and Relative density (RD)**

$$\text{Density of species A} = \frac{\text{Total number of individuals of species A}}{\text{Total number of plots sampled} * \text{area of a plot}}$$

$$\text{Relative density of species A} = \frac{\text{Total individuals of species A}}{\text{Total individuals of all species}}$$

ii. **Frequency and Relative frequency (RF)**

$$\text{Frequency of species A} = \frac{\text{No of plots in which species A occurs}}{\text{Total number of plot sampled}} * 100$$

$$\text{Relative Frequency of species A} = \frac{\text{Frequency value of species A}}{\text{Total frequency value of all species}} * 100$$

iii. **Relative dominance (R Dom.)**

$$\text{Relative dominance of species A} = \frac{\text{Total basal area of species A}}{\text{Total basal area of all species}} * 100$$

iv. **Basal area**

The basal area of a species is the sum total of the basal areas of all trees of a species, which was calculated using the following relation:

Basal area= $\pi\left(\frac{d}{2}\right)^2$, where, d=diameter at breast height.

v. **Relative cover (R Cov)**

$$\text{Relative cover of species A} = \frac{\text{Average cover of species A}}{\text{Sum of Average cover of all species}} \times 100$$

vi. **Importance value index (IVI)**

IVI was obtained by the summation of relative density, relative frequency, and relative dominance.

$$\text{IVI of Tree \& Shrub} = \text{Relative density} + \text{Relative frequency} + \text{Relative dominance}$$

Similarly, IVI of Herb $= \text{Relative density} + \text{Relative frequency} + \text{Relative cover}$

Suitability analysis

Software and Tools

The various software and tools, such as ArcGIS 10.2.1, Microsoft Office Suite 2012, Google Earth Pro, and MaxEnt 3.4.1 (prediction mapping), were used for the processing of the data depending on its nature (Table 3 & 4). Input variables were processed with relevant software to make them readable by MaxEnt Model. Presence data was converted to CSV (Comma-separated Value) using MS Excel 2012. Presence data collected in WGS UTM format was plotted and transformed to WGS 1984 (DD) using ArcGIS data management tools. After the transformation, the attributes were exported to MS Excel 2012 and converted to CSV (Comma-separated value) format. All the grid format and shape file format variables were exported to ArcGIS and processed using the ArcGIS 10.2.1 model builder. A data management tool was used for the processing of grid format. Vector (shape file) data was processed using data management and conversion tools in the ArcGIS model builder.

TABLE 3. List of tools for vector data preparation.

Tools	Description
Add layer	Source layer (world bioclimatic)
Project	UTM
Resample	30 m resolution No data = 0
Copy	Pixel depth = 16 bit unregistered
Project	WGS 1984 (degree decimal)
Extract	By smallest layer
Raster to ASCII	Readable by MaxEnt

MaxEnt

All the processed variables are then imported to the MaxEnt model for the prediction of the probability of distribution of *C. sumatraensis* both at the landscape level and localized level. At landscape level distribution prediction, there is no exact record in Nepal, whereas at localized level distribution prediction, 16 sign records collected during a field visit were used. A sample requires a presence point (CSV format) directly, while environmental layers take environmental variables (ASCII format). Create response curves, make pictures of predictions, and the Jackknife test was used to check and measure the importance of variables. Finally, the output format was chosen for logistics. Additional configuration was used during prediction and a total of 15 random partitions of the occurrence localities were made in order to assess the average behaviour of the algorithms (via Wilcoxon signed-rank tests). Each partition was created by randomly selecting 70% of the occurrence localities as training data, with the remaining 30% reserved for testing the resulting models. There is a risk of over-prediction or under-prediction of the relationship by the model if the model doesn't have enough time to converge. To avoid this, the maximum number of iterations is increased to 5000 (where it is 500 by default). The algorithms were run with two sets of habitat sites; first at the landscape level, in which presence points (direct sightings) were extracted from previous data (if available), and second at the localized level, in which presence points (direct sightings and signs) were collected during a field visit by the researcher himself.

Analysis of disruptions and existing threats

The various types of disturbances were recorded from the sample plot inventory. The occurrence of disturbance data was then imported to SPSS version 22 and used descriptive statistics in order to calculate the frequency and percentage of the occurrence. A ranking system based on the Relative Threat Factor Severity Index (RTSFI) by Kiringe and Okello (2007) was used for the assessment of threats. A tally of the threat factors to the *C. sumatraensis* was computed and calculated as indicators of serious threat factors. The following simple formula was used to prioritize the threats to define the (RTFSI).

Table 4. List of input variables after processing.

Layer Name	Spatial resolution (M)	Pixel Depth (radiometric Resolution)	Spatial data type	Projection System	Variable
Presence Data			CSV	WGS 84	
District boundary			Polygon/ Line	WGS 84	
Southern side of Chure hill			Polygon/ Line	WGS 84	
Settlement	30	16 bit unsigned	GeoTIFF	WGS 84	Categorical
Land use	30	16 bit unsigned	GeoTIFF	WGS 84	Categorical
Water bodies	30	16 bit unsigned	GeoTIFF	WGS 84	Categorical
River Beds	30	16 bit unsigned	GeoTIFF	WGS 84	Categorical
DEM	30	16 bit unsigned	GeoTIFF	WGS 84	Continuous
Slope	30	16 bit unsigned	GeoTIFF	WGS 84	Categorical
Aspect	30	16 bit unsigned	GeoTIFF	WGS 84	Categorical
Water bodies	30	16 bit unsigned	GeoTIFF	WGS 84	Categorical
Climate	30	16 bit unsigned	GeoTIFF	WGS 84	Categorical

$$Mean\ score\ of\ each\ threat\ factor = \frac{\text{Sum of all the scores for that particular threat factor}}{\text{The total number of respondents}}$$

$$RTFSI = \frac{\text{The mean score for a particular threat factor}}{\text{The maximum possible score}}$$

The RTFSI was used to categorize threat severity, with severe threats having the highest RTFSI and least threats having the lowest.

RESULTS

Food availability

Vegetation analysis

Identification of habitat preferences of *C. sumatraensis* was done by using IVI of different species of trees found in the study area. The resulting graph shows the preference of the wildlife in habitats containing different tree species; with *Quercus semecarpifolia* (76.22) and *Rhododendron arboreum* (62.31) preferred the most, respectively (Figure 5).

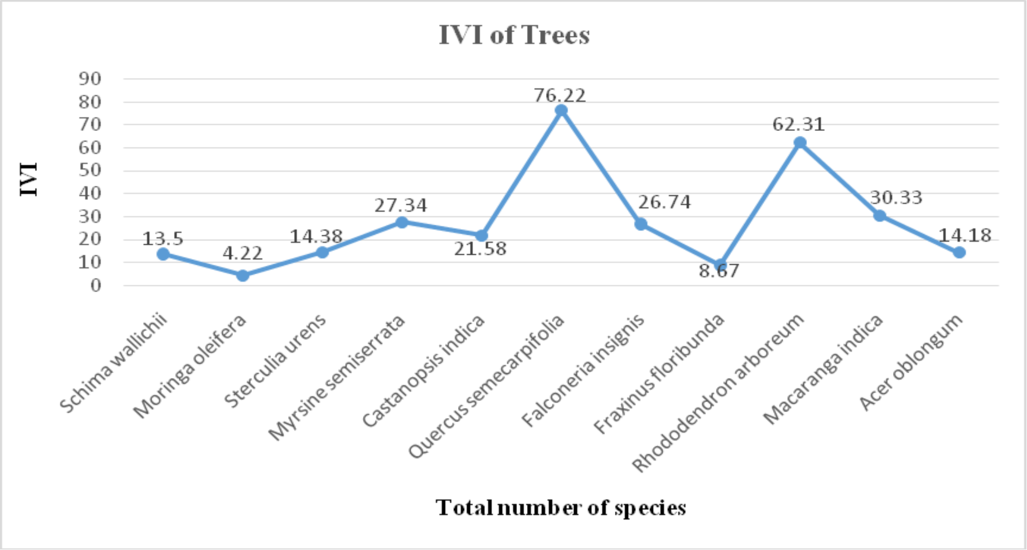

FIGURE 5. IVI of tree species.

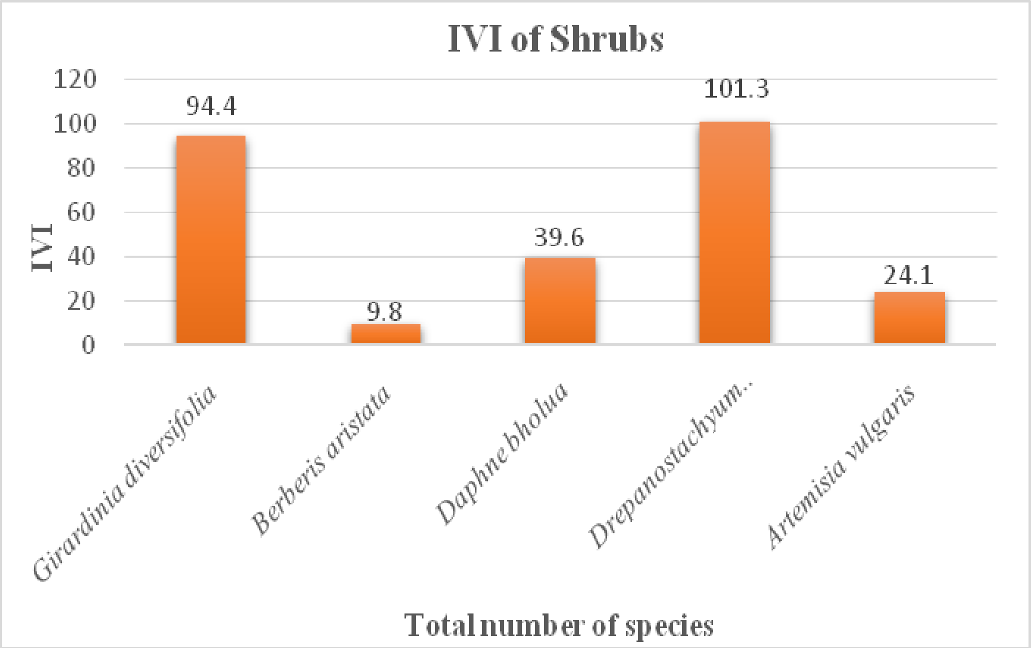

FIGURE 6. IVI of Shrubs.

Identification of habitat preferences of *C. sumatraensis* was done by using IVI of different species of shrubs found in the study area. The resulting graph shows the preference of the wildlife in habitats containing different shrub species; with *Drepanostachyum falcatum* (101.3) and *Girardinia diversifolia* (94.4) preferred the most, respectively (Figure 6).

Similarly, using IVI of different species of herbs found in the study area. The resulting graph shows the preference of the wildlife in habitats containing different herb species, with *Anaphalis busua* (90.3) and *Tracheophyta* (89) preferred the most, respectively (Figure 7).

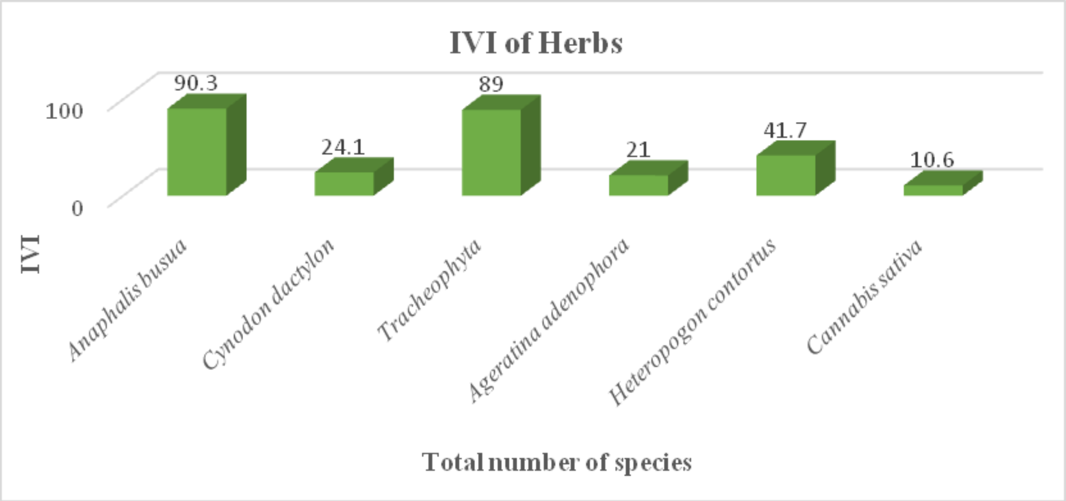

Figure 7. IVI of Herbs.

Habitat suitability and associated factors

Figure 8 shows the probability of occurrence of *C. sumatraensis* generated by the MaxEnt Model. Out of the total area (762900 ha.) of the Annapurna Conservation Area, nearly 76000 ha. of area was found to be suitable, whereas the rest of the area was found to be less suitable. The calculated habitat suitability for *C. sumatraensis* determined that 18.3% of the total area was highly suitable (represented by red colour), 16.8% was moderately suitable (represented by light blue colour) and the remaining 64.76% was less suitable habitat (represented by dark blue colour).

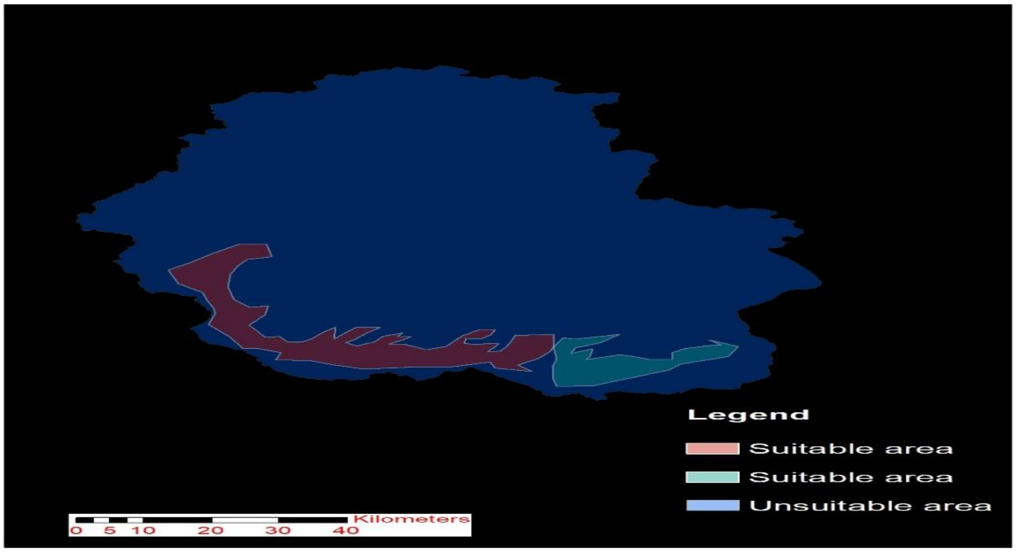

Figure 8. MaxEnt Suitability Map at Landscape level.

Analysis of omission

The test of omission rate and prediction area as a function of the cumulative threshold, averaged over the replicate runs. The omission rate should be close to the predicted omission rate because of the definition of the cumulative threshold.

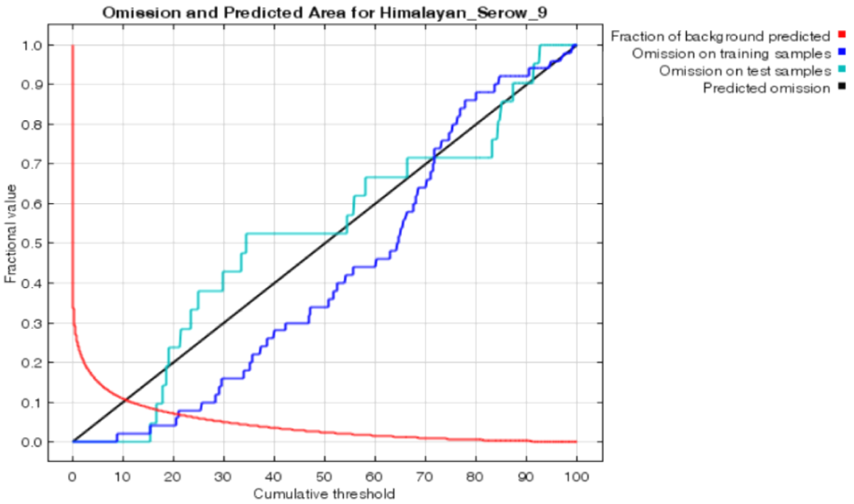

FIGURE 9. Analysis of Omission.

3.2.2. ROC/AUC

Figure 10 shows the receiver operating characteristic (ROC) curve for the same data, again averaged over the replicate runs. Note that the specificity is defined using the predicted area rather than the true commission. The average test AUC for the replicate runs is 0.975 and the standard deviation is 0.003 (Figure 10), which indicated that the model performed well with high accuracy (Swets, 1988).

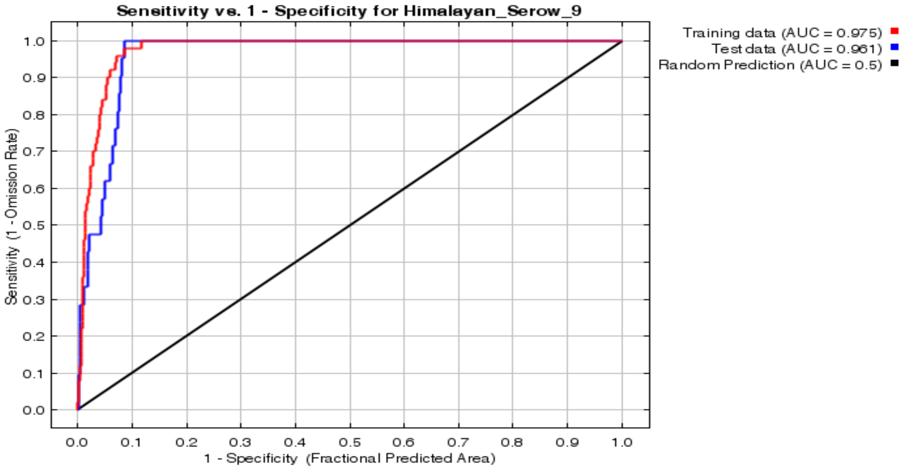

FIGURE 10. AUC curve suitability modeling.

Suitability map

Analysis of variable contributions

The following table gives estimates of the relative contributions of the environmental variables to the Maxent model. To determine the first estimate, in each iteration of the training algorithm, the increase in regularized gain is added to the contribution of the corresponding variable, or subtracted from it if the change in the absolute value of lambda is negative. For the second estimate, for each environmental variable in turn, the values of that variable on training presence and background data are randomly permuted. The model is re-evaluated on the permuted data, and the resulting drop in training AUC is shown in the table, normalized to percentages. As with the variable Jackknife, variable contributions should be interpreted with caution when the predictor variables are correlated. The values displayed are averages of replicated runs.

The following picture shows the results of the Jackknife test of variable importance. The environmental variable with the highest gain when used in isolation is elevation, which therefore appears to have the most useful information by itself. The environmental variable that decreases the gain the most when it is omitted is LULC, which therefore appears to have the most information that isn't present in the other variables. The values displayed are averages of replicated runs.

Attitude and Perception of local people

Local people strongly believed that the population of *C. sumatraensis* had been declining day by day. The majority of people (90%) expressed positive attitudes toward *C. sumatraensis*. About 65% of local people said that the population of *C. sumatraensis* has been declining at a high rate. According to herders and other local people, the main cause of the population's declining is poaching; every year a large number of snares are collected from the study site. Many respondents blamed poaching, killing by predators, and human and livestock disturbance in its habitat as the main causes of population decline. Villagers agreed that poaching activities have been reduced in recent years as compared to the past year.

TABLE 5. Percent contribution of variables to Model at landscape level.

S. N	Variable	Percent contribution	Permutation importance
1	Lulc	36	27.2
2	wb_cl	13.7	0
3	bio_6	12.5	0
4	bio_14	5.1	2.3
5	bio_8	4.3	1.6
6	bio_18	3.8	3.7
7	slop_cl	3.6	0.2
8	bio_5	2.9	3.3
9	bio_13	2.6	6.9
10	bio_3	2.2	0.2
11	Elevate	2	30.9
12	bio_17	1.7	3.4
13	rbed_cl	1.6	0.2
14	bio_4	1.6	1.4
15	bio_15	1.5	2.7
16	bio_16	1.4	0.2
17	bio_7	0.7	0.3
18	bio_9	0.5	0
19	bio_12	0.5	2.7
20	bio_2	0.4	10.2
21	bio_10	0.4	0
22	bio_11	0.3	0.1
23	bio_1	0.2	0.1
24	sett_cl	0.2	0.3
25	asp_cl	0.1	0
26	bio_19	0.1	2

Threats to existing habitats

In the study area, poaching and hunting (0.927), open grazing (0.727), illegal resource collection (0.617), climate change (0.573) and development activities (0.447) were observed as major threats to the *C. sumatraensis* (Figure 12). *C. sumatraensis* poaching is one of the main threats in the study area. Generally, poachers prefer not to hunt *C. sumatraensis*—they only use this species if they cannot find other species. It is said that *C. sumatraensis* meat is not as tasty compared with other ungulates. As a result, hunting is limited and secretive. We found many snares in our study area, especially in the bamboo and deurali-rich sites where there is a high population of *C. sumatraensis*. The development of agriculture areas, the high dependence of local people in *C. sumatraensis* habitat for fuel wood and timber, increasing hotels, trekking routes, increasing settlement areas, and increasing distance from forest has played a critical role in habitat fragmentation, which has brought a critical change in the wildlife profile of the study area in the long run. This habitat fragmentation has a negative impact on the continued survival of the *C. sumatraensis* and other different wildlife species in the area.

The loss of a large population of the *C. sumatraensis* and other ungulates from the study area has created consequent changes in the abundance of predator species like clouded leopards, common leopards, brown bears, and black bears. Generally, such predators attack the local livestock only when their natural prey is either depleted or hard to find, so it is one indicator of the decline of the population of *C. sumatraensis* and other ungulates from the study area. Owing to high seasonality and low primary productivity, the Himalayan region supports a relatively low ungulate and herbivore biomass (Aryal, 2005). It is therefore obvious that with the increase in the biomass of domestic livestock in many areas, wild ungulates such as *C. sumatraensis* have suffered competitive exclusion.

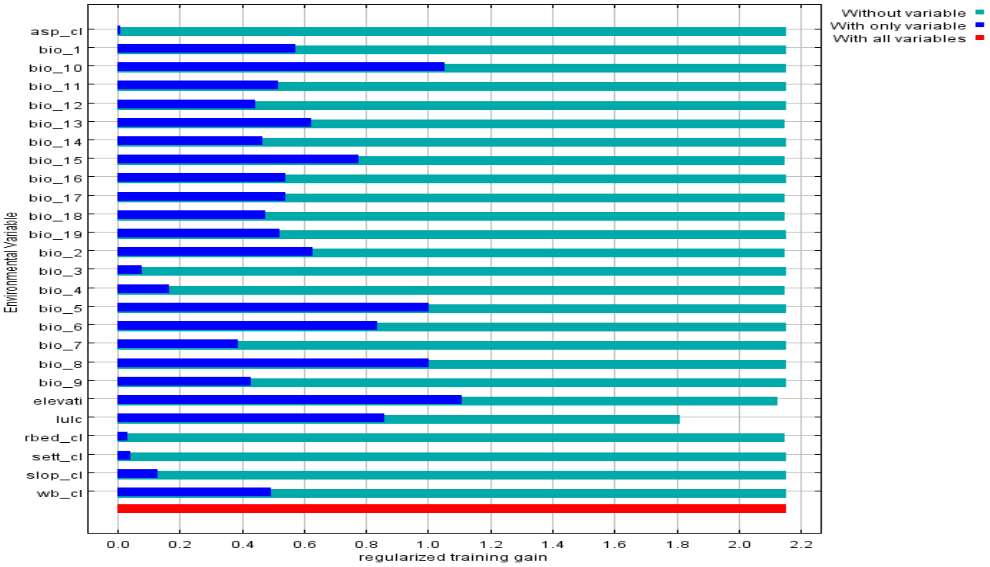

FIGURE 11. Jackknife test of importance of variable in model.

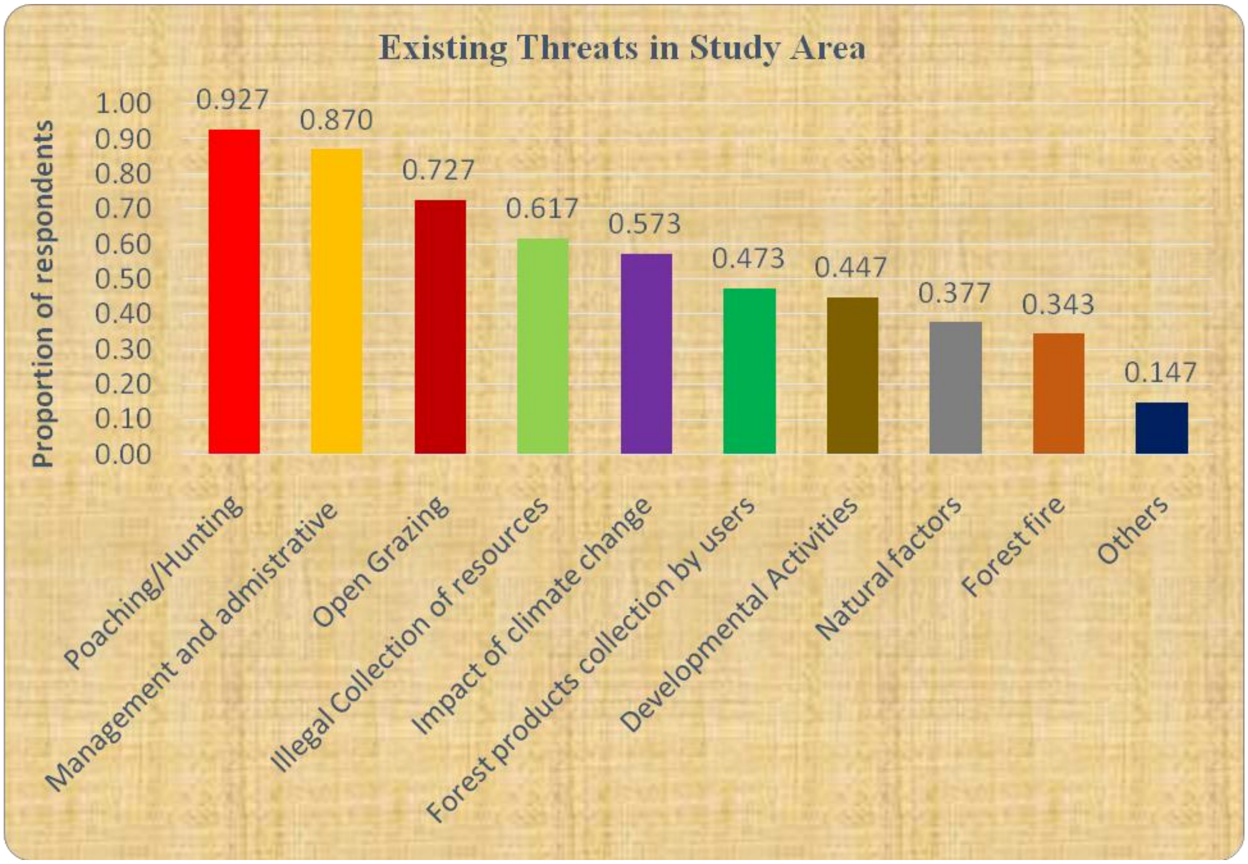

FIGURE 12. Existing threats to serow in Study area.

DISCUSSION

Aryal (2009) reported only 10% of the area as suitable habitat in ACA, which is lower than the present study. It shows that there is improvement in habitat conditions in the ACA region with the implementation of conservation activities. A *Quercus semecarpifolia* and *Rhododendron arboreum* dominated forest was found as the preferred habitat, which was consistent with the previous findings (Aryal, 2009; Giri et al., 2011). Similarly, in preferred habitat, *Drepanostachyum falcatum* and *Girardinia diversifolia* were the dominant shrubs and *Anaphalis busua* and *Tracheophyta* were dominant herbs, which is similar to the results reported by Aryal (2009) and Giri et al. (2011). Poaching and hunting, open grazing, and illegal collection of resources were the major threats to *C. sumatraensis* conservation in ACA. For the threat assessment, the Relative Threat Factor Severity Index was used to rank the threats. Poaching and hunting (0.927), open grazing (0.727), illegal resource collection (0.617), climate change (0.573) and development activities (0.447) were observed as major threats to the *C. sumatraensis*, which is similar to the threats identified by official documents, viz. the ACAP management plan and the National Biodiversity Strategy and Action Plan for biodiversity conservation (GoN, 2015). In this study, a major drawback of the MaxEnt is identified: the model cannot distinguish the nature of the presence point, which supports the work of Phillips and his co-workers (2009). Direct competition with humans is thus clearly a cause for concern in the conservation of species. Additionally, the resulting development of human settlements, human trails, and agricultural lands has led to the fragmentation of *C. sumatraensis* habitat. For example, the main corridor connecting the *C. sumatraensis* populations of Landruk and Ghandruk (Tadapani forest) has been fragmented by settlement and agricultural land. As a consequence, these populations are now isolated. This has had negative impacts on *C. sumatraensis* and other wildlife species in the area (Aryal, 2009). *C. sumatraensis* are a shy species, preferring to live away from human disturbance, but in the study area, human settlement had encroached as close as 400m to the habitat of these animals. The pressures due to direct competition for resources with humans and habitat fragmentation are important concerns for the conservation of *C. sumatraensis* in ACA.

CONCLUSION

The research concluded that *C. sumatraensis* preferred forests dominated by *Quercus semecarpifolia* and *Rhododendron arboreum*. Similarly, in the preferred habitat, *Drepanostachyum falcatum* and *Girardinia diversifolia* were the dominant shrubs, and *Anaphalis busua* and *Tracheophyta* were dominant herbs by IVI analysis. RTFSC analysis showed that poaching and hunting, management, and administrative divisions of the area were major threats to its habitat. These species have more available funds for their conservation and management through national and international sources compared to other species. This situation results in a lack of knowledge about the illegal market value of species such as serow, hispid hare, etc. Therefore, concerned agencies and researchers should just give equal emphasis to in situ conservation of low illegal market value species such as *C. sumatraensis*, which is a favorite prey species of threatened species like leopards. The major problems in *C. sumatraensis* habitats are habitat fragmentation, land use changes, reduction of the *C. sumatraensis* population, conflicts between *C. sumatraensis* and predators and villagers, livestock grazing in *C. sumatraensis* habitats, and poaching. Further research and conservation education are essential to conserve this species. It is not only *Arundinaria* spp. for which *C. sumatraensis* compete with villagers in ACA. The tree species used as food and cover by *C. sumatraensis* are *Rhododendron* spp., *Lyonia* spp., *Acer* spp., etc. They are also important firewood and timber production species in the area and are harvested at high rates for infrastructure development. Further, some of the herbs and shrubs that have been identified in the diet of *C. sumatraensis* are collected for their medicinal value and for other domestic use values. Direct competition with humans is thus clearly a cause for concern in the conservation of *C. sumatraensis*. Human settlements and associated use of land for agriculture are fragmenting *C. sumatraensis* habitat, in some cases, causing populations to become isolated. The pressures due to direct competition for resources with humans and habitat fragmentation are important concerns for the conservation of *C. sumatraensis* in ACA. This study strongly recommended the necessity of effective law enforcement in coordination with local people to reduce the threat of hunting and poaching. Conservation of preferred species is equally important for the enhancement of *C. sumatraensis*. Further research and conservation education are important for the conservation of this species.

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RESEARCH ARTICLE **Open access**

Steinernema feltiae- Xenorhabdus bovienii: more information on this bactohelminthic complex from Iran

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Abstract
Two families of entomopathogenic nematodes (EPNs), Steinernematidae and Heterorhabditidae, symbiotically associated with *Xenorhabdus* and *Photorhabdus* bacteria, are effective biological control agents of insect pests. Native isolates are likely to be better candidates for insect pest control than exotic specimens due to their adaptation to native environmental conditions. In this study, Steinernema feltiae isolate FUM221, was recovered from soil samples collected from the Ardabil Province, Iran. Morphological and morphometric investigations of the first and second-generation adults, infective juveniles, and molecular characterizations were conducted based on ITS and 18S rDNA genes. Molecular analysis based on the 16S rRNA region and phenetic data revealed *Xenorhabdus bovienii* as this isolate symbiont bacterium. The scanning electron microscopy (SEM) verified the identification of this isolate. The molecular characterization using two loci and phylogenetic analyses provided more evidence on the classification of this steinernematid and its distinction from same species from other countries. Moreover, molecular and phenetic characterizations of its symbiotic bacterium indicated minor variations compared to other isolates. Herein, the comprehensive taxonomic data of this steinernematid and its symbiont bacterium, is presented.

Key words: *Characterization; Entomopathogenic nematodes; Steinernema; Survey; Taxon, Xenorhabdus.*

INTRODUCTION

Entomopathogenic nematodes (EPNs) are highly pathogenic to the wide array of insect pests in the foliar environment, cryptic, and especially soil-dwelling habitats (Kaya and Gaugler, 1993; Kaya et al., 2006; Malan and Ferreira, 2017; Askary and Abd-Elgawad, 2017). They mostly belong to the families Steinernematidae and Heterorhabditidae that are symbiotically associated with the entomopathogenic bacteria, *Xenorhabdus* and *Photorhabdus*, respectively (Boemare and Akhurst, 1988; Boemare, 2002; Malan and Ferreira, 2017). Studies show that the bacteria in the *Photorhabdus-Heterorhabditis* association play a principal role in suppressing the immune system and killing the host, causing addiction and sepsis, whereas, in the *Xenorhabdus-Steinernema*, nematodes have a more efficient role in the pathogenicity of the complex (Lewis and Clarke, 2012; Lu et al., 2017; Shapiro-Ilan et al., 2018;

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Koppenhöfer et al., 2020). Nineteen species of *Photorhabdus* and twenty-six species of *Xenorhabdus* have been identified so far (Koppenhöfer et al., 2020).

Efforts to discover new indigenous species/strains of EPNs are necessary because they have adapted climatically as agents that able to regulate native pests (Qiuet et al., 2004; Ehlers, 2005; Stokwe et al., 2011; Torrini et al., 2014; Lulamba and Serepa-Dlamini, 2020). To date, about 21 species of *Heterorhabditis* and 100 species of *Steinernema* have been identified worldwide and the majority of species have been collected from Asia (Lewis and Clarke, 2012; Shapiro-Ilan et al., 2017, 2018; Didiza et al. 2021). The application of EPNs has begun in the 1980s, in the recent two decades, intensive researches were conducted in different fields on EPNs that led to effective results in their taxonomy and commercialization (Koppenhöfer et al., 2020). Currently, they are used commercially as biological control agents on numerous economically important insect pests (Shapiro-Ilan et al., 2002; Azazy et al., 2018; Koppenhöfer et al., 2020). Heretofore leastwise five *Heterorhabditis* species and eight *Steinernema* species have been commercialized (Shapiro-Ilan et al., 2002; Piedra Buena et al., 2015; Azazy et al., 2018; Koppenhöfer et al., 2020; Sivaramakrishnan and Razia, 2021).

In Iran, isolation, identification, and characterization of EPNs have begun since 2000 (Parvizi, 2000; Karimi et al., 2010) and some *Steinernema* and *Heterorhabditis* species have been isolated so far. The collected species of *Steinernema* include *Steinernema feltiae* (Filipjev, 1934) Wouts, Mraček, Gerdin and Bedding, 1982, *Steinernema carpocapsae* (Weiser, 1955) Wouts, Mraček, Gerdin and Bedding, 1982, *Steinernema glaseri* (Steiner, 1929) Wouts, Mraček, Gerdin and Bedding, 1982, *Steinernema bicornutum* Tallosi, Peters and Ehlers, 1995, *Steinernema arasbaranense* Nikdel, Niknam and Ye, 2011, and *Steinernema kraussei* (Steiner, 1923) Travassos, 1927. *Heterorhabditis bacteriophora* is the only species in the *Heterorhabditis* genus that has been identified in Iran until now (Karimi et al., 2010; Nikdel et al., 2010, 2011; Karimi and Salari, 2015; Seddighi et al., 2016; Abdolmaleki et al., 2016; Salari et al., 2019; Ebrahimi et al., 2019; Karimi and Hassani- kakhki, 2021).

This study is the first documented record for the presentation of the 18S tree related to *Steinernema feltiae* to accreditation to identification of this species. The purpose of the current research was the comprehensive characterization of a new native isolate of EPN and its symbiont bacterium by means of morphological and molecular approaches.

MATERIAL AND METHODS

Sampling from soils and entomopathogenic nematodes isolation

The soil samples were gathered from various ecosystems including gardens, pasturelands, strands, parks, and natural undisturbed soils of Ardabil city, Ardabil Province, Iran. The samples were taken randomly from the fertile moisture soils up to a depth of 15-20 cm during late autumn 2018. After transporting to the laboratory, the soils from each site were distributed in a few plastic containers (300 ml) with lids. A routine baiting technique was applied in each container using 10 last instar larvae of the greater wax moth, *Galleria mellonella* Linnaeus, 1758. They were maintained under laboratory conditions (25±2 °C) for 10 days and checked out for dead *Galleria* larvae, considering the noticeable color change (Bedding and Akhurst, 1975). Then the cadavers were retrieved and placed in white traps individually (White, 1927). Finally, to ensure the pathogenicity of emerged nematodes, they were investigated to Koch's postulates (Kaya and Stock, 1997). The collected infective juveniles were stored at 8–10 °C for long-term use.

Light Microscopy

For morphological characterization, all developmental stages of the nematodes were obtained from more than ten *G. mellonella* larvae that were infected by IJ nematodes. *G. mellonella* larvae were placed in a petri dish (10 cm) covered with wet filter papers in its bed at room temperature (25 ºC). Adults of first and second-generation nematodes were harvested by dissecting infected cadavers of *G. mellonella* larvae approx 2-3 and 4-6 days after inoculation of insects, respectively. About 30 specimens of different stages were fixed utilizing hot (80 °C) 4% formaldehyde and transferred to pure glycerin for mounting (Ryss, 2017). Finally, the slides of fixed nematodes were prepared, and morphometric and morphological parameters were performed using an Olympus light microscope CH-2.

Morphological characterization of EPN

Complementary morphological characterization of the infective juveniles and the first generation of adults (both males and females) was conducted by using a scanning electron microscope (SEM) following the procedures in Ye et al., 2010; Nikdel and Niknam, 2015.

1) Each sample was rinsed three times with 0.1 M sodium cacodylate each for 15 min.

2) All nematodes were fixed in 3% glutaraldehyde with 0.1 M sodium cacodylate at pH 7.2 for 24 hours at 4 ºC in the dark condition (wrapped in an aluminum foil).

3) Samples were rinsed three times with 0.1 M sodium cacodylate.

4) The specimens were postfixed with 2% osmium tetroxide solution for 12 hours at 25 ºC (room temperature).

5) They were rinsed with 0.1 M sodium cacodylate three times.

6) Each sample was dehydrated in a graded ethanol series of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, and 100%, each for 20 min, on the dried ice; (dehydrated with 100% ethanol 3 times at room temperature).

7) Samples were mounted on aluminum SEM stubs, and coated with gold. The SEM images were obtained with LEO 1450VP scanning electron microscope (LEO Co. Ltd., Germany).

Molecular characterization of EPN

Extraction of DNA

Genomic DNA contents of nematode was extracted using an individual female in the 5% Chelex®100 solution (SIGMA, Bio-Rad Laboratories, Inc., USA). A single female was picked up by an eyelash, transferred into a 1.5 ml Eppendorf tube, and then was crushed in 50 µl of Chelex and 2 µl of Proteinase K (www.parstous.com) by a micro pestle. The Eppendorf tube was incubated at 60 °C for 3 h, then heating for 10 min at 95 °C utilizing a thermos-block. Finally, the specimen was centrifuged at 13000 rpm for 3 min, then extracted DNA was collected and stored at -20 °C until use.

ITS and 18S genes amplification

A molecular approach was utilized for the characterization of the isolate. For this purpose, the primer sets of TW81 (5′-GTTTCCGTAGGTGAACCTGC-3′) and AB28 (5′-ATATGCTTAAGTTCAGCGGGT-3′) were used for amplification of the internal transcribed spacer (ITS) region (Joyce et al., 1994). Also, in, the other fragment of rDNA containing the 18S gene was amplified using forward primer (5′-AAAGATTAAGCCATGCATG-3′) and reverse primer (5′-CATTCTTGGCAAATGCTTTCG-3′) (Blaxter et al., 1998).

The PCR mixture with a final volume of 25 µl comprising 12.5 µl 2X Taq PreMix, 6.5 µl distilled water, 1 µl of each primer (forward and reverse), and 4 µl genomic DNA, was carried out in a thermo-cycle for amplification of DNA template. The PCR cycling process were started with the initial denaturation at 94 °C for 4 min, then 35 cycles of 94 °C for 1 min (denaturation stage), 55 °C for 1 min (annealing stage), and 72 °C for 2 min (extension stage); in the end, a post-amplification extension at 72 °C for 10 min. Amplified PCR product was loaded into 1% agarose gel. The PCR product was electrophoresed at 80 V for 40 min with 10X TBE buffer 5% and a green-viewer for staining the gel. The ladder was used to determine the PCR product size.

ITS rDNA and 18S rDNA characterization

The PCR products sequencing was employed by Macrogen Co. in Seoul, Korea. Before creating the consensus sequence by the BioEdit software (Hall, 1999), the quality of chromatograms was checked, and the bad peaks from the begining and the end of them (the attachment site of the tag polymerase enzyme) were omitted. Then, the DNA sequence was blasted against the NCBI database and compared with the other presented sequences in the GenBank. Thirty-three sequences of the ITS region and sixteen sequences of the 18S gene of *Steinernema* species, were retrieved from corresponding published gene sequences and aligned using ClustalX.

Isolation of the bacterial strain

The bacteria were extracted from more than 100 infective juvenile nematode (IJs) that newly emerged from the fifth instar larvae of *Galleria mellonella.* After the emerged IJ nematodes were collected in a 1.5 ml micro-tube, they were washed three times with deionized water for 2 min. Then, they were immersed in sodium hypochlorite (NaOCl) 10% (V/V) for 10 min and centrifuged at 8000 speed to precipitate the IJs in the bottom of the tube. To remove remaining sodium hypochlorite, IJ nematodes were washed two times with deionized water and were crushed in 10µl of deionized water. At final step, 100 µl of the target bacterium suspension was streaked onto 9 mm Petri-dishes containing NBTA medium (Nutrient agar, 0.025% bromothymol blue, and 0.004% triphenyltetrazoliumchchloride (TTC)). The plates were incubated at 28 ºC ± 2 for 48 hours in dark condition (Akhurst, 1986). The bacterium was sub-cultured several times to obtain the pure bacterial clones.

DNA extraction, PCR, and characterization

DNA content of bacteria was extracted from a 2-day-old culture using boiling-based PCR cloning (Mcpherson and Møller, 2006). The clone was solved to 20 µl by the sterilized water and boiled for 10 minutes, then it was used as a template for PCR reaction. The universal bacterial primers of the 16S rDNA gene fragment including 27F (Forward primer 5'- AGAGTTTGATCCTGGCTCAG -3') and 1492R (reverse primer 5'-TACGGCTACCTTGTTACGA-3') were used for the amplification process (Heuer et al., 1997). The 25µl reaction mixture consisted of 12.5µl Master Mix, 6.5µl deionized water (dH2O), 1µl of each primer (2 µl), and 4µl of DNA template. The PCR reaction of the 16S rDNA gene was performed as follows: initial denaturation at 95 °C for 10 min, 35 cycles for 1 min at 94 °C, 1min at 56.5 °C, 2 min at 72 °C, followed by the final extension at 72 °C for 8 min. Eventually, the PCR product was electrophoresed at 80 V for 40 min with 10X TBE buffer 5% and a green-viewer for staining the gel. The sequencing of the PCR product was employed by Macrogen Co. in Seoul, Korea. The 16S rRNA gene sequence was edited using the BioEdit program and saved as Fasta format (Hall, 1999). The nucleotide comparison was conducted using the BLAST available on the National Center for Biotechnology Information (NCBI). Thirteen taxa of *Xenorhabdus* species and one species of *Photorhabdus luminescens* subsp. Kleinii strain KMD37 (HM072284) were used to compare with sequences of the studied isolate. The gene sequence was deposited in the Genbank using BankIt software.

Bacteria phenotypic characterization

Some significant phenotypic characterizations were investigated in the present study of the symbiotic bacterium according to Akhurst and Boemare, 1988 and Tailliez et al., 2010. The bacterium was inoculated in different media such as Nutrient Broth (NB), Nutrient Agar (NA), and bromothymol blue and 2, 3, 5- triphenyltetrazolium chloride (NBTA) and then incubated at 28 ± 2 °C for 48h to evaluate some characters such as size, shape, and color. A single clone of the bacterium was cultured on NBTA and NA to investigate the ability of the associated bacterium to absorb dye after 48 hours (Akhurst, 1986). An antibiotic test was carried out to assess the resistance of bacteria through bacteria culture on the NA medium. For this purpose, a disc (0.5 in diameter) of sterilized filter papers was immersed in the suspension of 1% tetracycline. Then it was placed in the center of the bacterial culture and incubated at 28±1°C for 48 h (Kazmierczak et al., 2016). The catalase test was carried out using 5 µl 3% (v/v) drops of H2O2 on a glass slide. Then, a single clone of the bacterium from the pure culture was added to the medium using a sterile plastic loop. The activity of bacterium on the Lecitinase was considered by 2g of NA (2%) and 10 ml of fresh egg yolk mixed with 100 cm3 of sterilized water. Then the bacterium was streaked on the medium culture containing the above mixture. Finally, the petri dish was incubated at 28 ± 1°C for 48 hours. To examine the movement activity of the bacterium, a semi-solid medium containing NA and NB was prepared. Then, a sterilized filter paper disc (0.5 in diameter) was immersed in the formulated bacterial suspension and placed in the center of the medium culture. After incubation at 28±1°C for 48 hours, the bacterial growth around the disc is evidence of motility. To determine the lipase activity, the medium containing 2 grams of agar (2%), 100 µl Tween (0.1 v/v) was prepared in 100 cc of water. The bacterium was cultured on the medium. Then, the plate was incubated at 28±1°C and bacterial activity was recorded after 48 hours. The precipitate around the edge of the bacterial colony was an indication of lipase activity.

Furthermore, the pathogenicity of the bacterium was evaluated against the last instar larvae of *G. mellonella* according to the method of Peel et al. (1999).

Phylogenetic analysis

The authentic and verified sequences were retrieved from peer-reviewed articles and aligned using ClustalX. The alignment file was edited in MEGA 7 manually (Kumar et al., 2016). The number of base differences per site and pairwise distances were computed using Geneious, and MEGA 7.0, respectively (Kumar et al., 2016).

The best fit model was identified under the GTR + I + G (for both genes) and HKY + I + G (for bacterium sequence) model using the MrModeltest 2 (Nylander, 2004). The number of generations was started from two million using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) in the Bayesian analysis. The Markov chain Monte Carlo (MCMC) chains were sampled every 100 generations (Larget and Simon, 1999) and estimated the posterior probabilities (PP) of the phylogenetic trees using the 50% majority rule. At the last step, the burn-in step was set at 25% of the converged runs. The Dendroscope V.3.5.7 (Huson and Scornavacca, 2016) and CorelDRAW version 2020 software were used to visualize the output file of the phylogenetic program and resized the tree, respectively.

RESULTS

The Entomopathogenic nematodes were gathered by baiting with *G. mellonella* larvae from the soil sample taken from the pasture lands in Fandoghloo, Ardabil city, Ardabil Province, Iran with the geographical position 38.3822° N, 48.5550° E. There is no knowledge of natural hosts of this nematode species/ the collected nematodes.

Both classic (morphological and morphometric analysis) and molecular (two loci containing ITS and 18S genes of rDNA, and also phylogenetic analysis) methods were used for identification and characterization of the studied isolate. The infected insects were recognized through change in color to brown and shape. The sequences were blasted on the NCBI database in GenBank. The resulting blast showed that the isolated species belong to the *Steinernema* genus. The partial sequences of this species were deposited in the GenBank by BankIt software. The sequences were submitted under the accession numbers MZ540323 (ITS), MZ540331 (18S), and MZ540333 (16S) for the nematode and its symbiont bacterium, respectively.

Morphological characteristics

Measurement

The measurements of IJ, male and female adults including body length (L), width (W), tail length (T), anal body diameter (ABD), distance from anterior end to excretory pore (EP), distance from anterior end to nerve ring (NR), distance from anterior end to end of esophagus (ES), spicule length (SL), gubernaculum length (GL), vulva (V), spicule width (SW), gubernaculum width (GW), and Standard deviation (Sd) are presented in Table 1. The morphological characterizations were similar to those described by Filipjev, 1934 and then Wouts, Mraček, Gerdin and Bedding, 1982. Briefly:

Infective juvenile

Body slender (Figure 1, A), mouth and anus closed (Figure 1, B). Head offset, labial papilla was not observed. Pharynx long and narrow, isthmus surrounded by nerve ring, basal bulb elongates. Secretory-excretory pore at mid pharynx level (Figure 1, C). Lateral fields distinct and begin with one line and then two additional lines to form two ridges (Figure 1, D). The maximum number of ridges, in the longest part,

Table 1. Morphometric characters of *Steinernema feltiae* FUM221. All measurements are in µm and in the form: mean ± sd (range).

Character	First generation male	female	Second generation male	female	Infective juvenile
n	18	26	12	14	32
L	1128.3 ± 139.5 (870-1330)	3730.6 ± 1204.8 (1170-5550)	1188.3 ± 205.1 (772.5-1480)	4687.5 ± 1035.4 (3150-6825)	562.5 ± 76.0 (417.5-700)
W	109.1 ± 22.1 (64-138)	214.8 ± 52.0 (92-360)	127.4 ± 14.7 (105-145)	267.5 ± 28.7 (212.5-307.5)	38.5 ± 9.4 (24-56)
V	-	1935.4 ± 607.3 (550-3125)	-	2425 ± 534.6 (1650-3375)	-
EP	78.9 ± 9.3 (60-92)	85.0 ± 25.5 (40-129)	84.5 ± 11.4 (61-98)	885.7 ± 317.1 (82.5-1270)	44.4 ± 8.8 (24-56)
NR	89.5 ± 6.4 (76-100)	106.7 ± 10.0 (87-128)	84.6 ± 8.7 (72-98)	103.5 ± 12.0 (84-120)	57.4 ± 10.4 (26-73)
Neck	147 ± 7.4 (127-156)	179.6 ± 14.8 (157-203)	143.7 ± 8.5 (129-155)	179.7 ± 17.0 (160-212)	88.8 ± 12.8 (51-111)
ES	137.9 ± 8.1 118-150	165.3 ± 14.9 (135-194)	136.1 ± 9.3 (123-152)	164.4 ± 15.7 (144-196)	86.9 ± 11.2 59-108
T	31.9 ± 4.8 (23-44)	42.0 ± 12.8 (20-70)	46.4 ± 16.3 (25-75)	42.8 ± 15.7 (24-83)	43.0 ± 8.8 (18-58)
ABD	35.9 ± 3.2 (30-42)	59.7 ± 25.6 (22-111)	45.6 ± 11.4 (25-65)	67 ± 13.3 (40-85)	17.8 ± 3.8 (8-25)
SL	69 ± 5.2 (59-77)	-	73 ± 5.2 (64-81)	-	-
SW	11.9 ± 2.2 (8-18)	-	12.1 ± 2.5 (8-16)	-	-
GL	45.5 ± 8.0 (31-65)	-	45.9 ± 5.9 (36-55)	-	-
GW	7.1 ± 1.1 (6-9)	-	7.1 ± 0.5 (6-8)	-	-
a	10.5 ± 1.6 (8.4-14.8)	17.4 ± 5.5 (9.6-37.0)	9.4 ± 1.6 (7.4-12.6)	17.6 ± 3.8 (11.9-26.1)	15.3 ± 3.7 (10.0-24.1)
b	8.2 ± 0.9 (6.8-9.9)	22.4 ± 7.5 (7.7-38.3)	8.7 ± 1.6 (6.2-11.3)	28.3 ± 4.3 (21.9-35.4)	6.4 ± 0.6 (5.4-8.2)
c	36.3 ± 5.4 (28.8-45.7)	97.7 ± 44.8 (190.0-29.3)	27.8 ± 9.0 (18.1-49.6)	122.0 ± 37.1 (77.4-204.2)	13.2 ± 2.6 (11.3-23.2)
c´	0.9 ± 0.1 (0.8-1.0)	0.8 ± 0.4 (0.5-1.9)	1.0 ± 0.3 (0.7-1.7)	0.7 ± 0.5 (0.4-2.1)	2.4 ± 0.4 (1.7-3.2)
D%	56.8 ± 8.2 (41.1-68.7)	51.5 ± 14.5 (26.3-71.3)	61.8 ± 8.5 (43.6-72.0)	529.3 ± 189.2 (53.2-774.4)	52.5 ± 8.5 (41.7-69.1)
E%	248.9 ± 24.4 (206.8-296.7)	225 ± 118.2 (108.1-645)	200.9 ± 75.4 (130.7-320)	2268.0 ± 1095.8 (250-4233.3)	114.1 ± 30.2 (86.7-180.6)
SW%	193.9 ± 21.8 (163.9-250)	-	169 ± 42.9 (115.4-284)	-	-
GS%	65.5 ± 9.3 (52.5-92.9)	-	63.1 ± 8.0 (48-73.4)	-	-

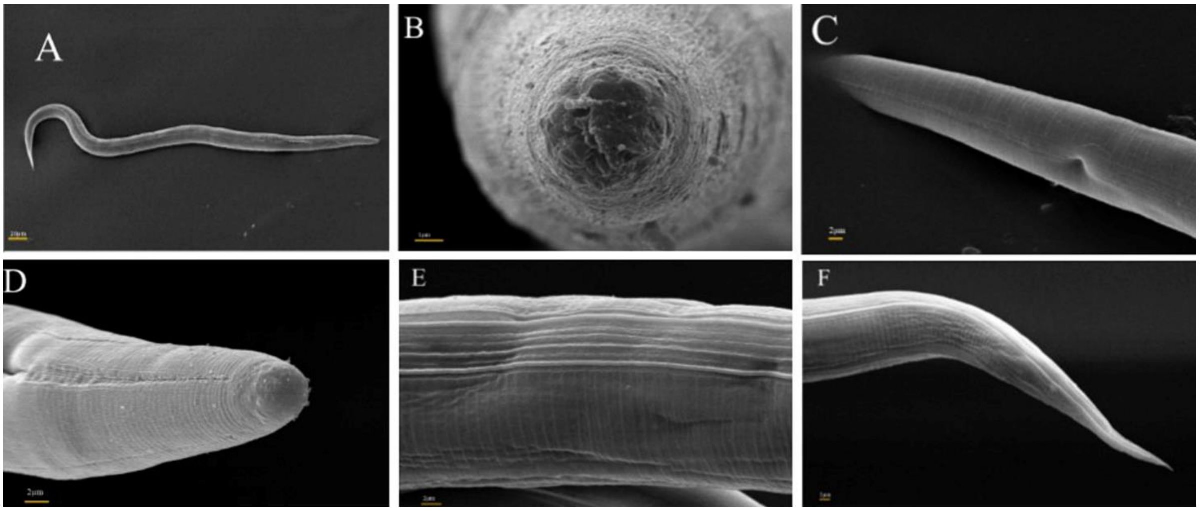

FIGURE 1. Scanning electron microscopy (SEM) photographs of infective juveniles of *Steinernema feltiae* isolate FUM221: A, An overview of larval body; B, head showing closed mouth; C, Secretory-excretory pore; D, lateral fields with one incisure and changes to two ridges; E, lateral field showing eight ridges; F, tail showing reduced number of ridges to four then two at the end of lateral fields. Scale bars: A= 20µm, B= 1 µm, C, D, F= 2 µm.

is eight in the lateral fields. (Figure 1, E). The formula for the arrangement of ridges from head to tail is 2, 8, 6, 4, 2 (Figure 1, F).

Female

Body robust and C-shaped (Figure 2, A). Lateral fields were not observed. Head widely rounded, six labial papillae and four cephalic papillae are visible. (Figure 2, B). Pharynx with cylindrical procorpus, metacorpus slightly swollen, basal bulb pyriform. Secretory-excretory pore at the middle of the pharynx and excretory duct cuticularized (Figure 2, C). Genital system filled with eggs, vulva a median transverse slit (Figure 2, D), protruding from the body, vagina short, oblique with muscular walls. Tail shorter than anal body diameter, with one terminal peg (Figures 2, E) and anus with a wide slit (Figure 2, F). The second generation of female was similar to the first generation.

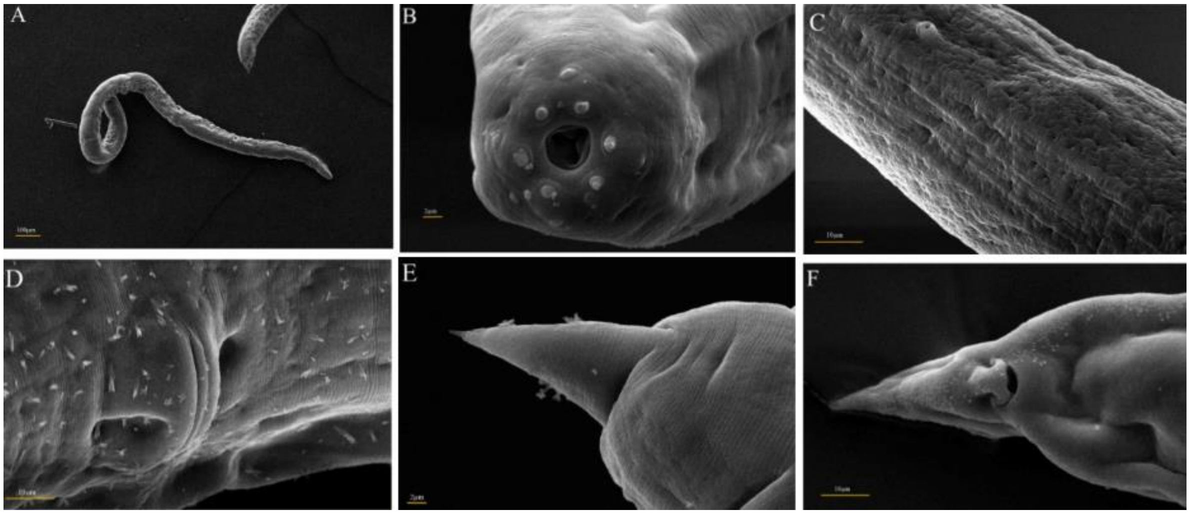

FIGURE 2.. Scanning electron microscopy of first generation females of *Steinernema feltiae* isolate FUM221: A, An overview of female body; B, labial and cephalic papillae; C, Secretory-excretory pore; D, vulva; E, Tail; F, Anus. Scale bars: A= 100 µm, B, E= 2 µm, C , D, F= 10 µm.

Male

Cuticle smooth, Body J-shaped, much smaller and slender than female (Figure 3, A). Head slightly depressed from the body. Lateral fields absent. Anterior region similar to female. Six pointed labial papillae and four cephalic papillae (Figure 3, A). Isthmus distinct, basal bulb pyriform and valvate. Nerve ring in isthmus portion. Deirids not seen. Secretory-excretory pore at the middle of pharynx (Figure 3, B). Testis monarchic and reflexed. Spicules paired, slightly brownish in color, strongly curved, head (manubrium) width is approximately equal to length (Figures 3, C, D), blade arcuate with a straight tip. Gubernaculum approximately three-quarters of spicule length, boat- shaped in lateral view, cuneus short, pointed posteriorly, wing of corpus expanding laterally. There are 11 pairs of papillae (Figure 3, E). Tail conoid with mucron. phasmids imperceptible.

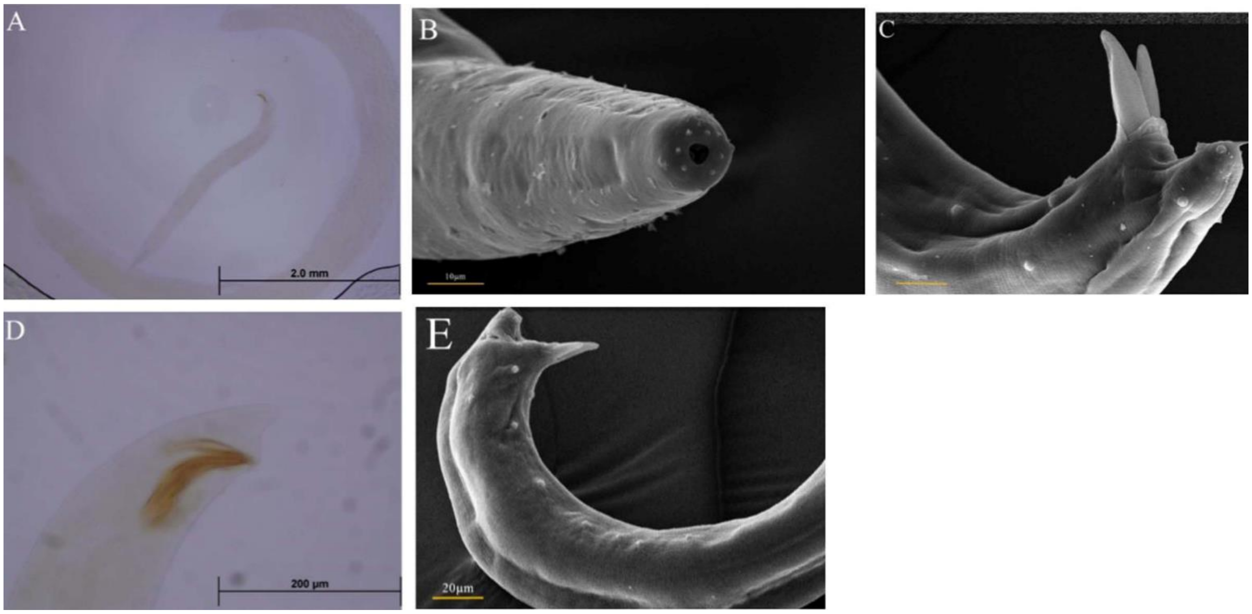

FIGURE 3. Scanning electron microscopy (SEM) (B, C, E) and light microscope (A, D): A, Comparison of male and female body size; B, Labial and cephalic papillae and secretory-excretory pore; C&D, Spicule and gubernaculum; E, Number and distribution of genital papillae in first generation male. Scale bars: A= 2 mm, B, C= 10 µm, D= 200 µm, E= 20 µm.

The ITS sequence analysis of *Steinernema* species

The ITS gene length for the FUM221 isolate was 1012 bp after alignment. The sequence revealed 100% similarities and 99% query coverage with *Steinernama feltiae* from Turkey and Belgium (accession numbers MN861044 and JF728859). The multiple alignments of 1012 bps segment of ITS gene for 33 taxa (the new isolate in this study with 31 taxa of *Steinernema* and a species of *Caenorhabditis elegans* as an outgroup) indicated that 697 sites were variable, 165 sites were conserved, 478 sites were parsimony informative, and 197 sites were singleton. The phylogenetic analysis indicated that the FUM221 isolate forms a monophyletic group with other *Steinernama feltiae* isolates (Figure 4). The overall average distance of ITS sequences was 0.22 ranging from 0.00 to 0.46, calculated by the Kimura 2- parameter model (Table 2).

TABLE 2. The number of bases which are not identical (upper triangle), and pairwise comparison on the number of nucleotide differences (lower triangle) among some of *Steinernema* species and *Steinernema* isolate FUM221 based on ITS rDNA sequences.

	Species	**Acc. no.**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	***S. feltiae***	**MZ540323**	**-**	**1**	**13**	**44**	**66**	**86**	**69**	**61**	**175**	**102**	**133**	**98**
2	***S. feltiae***	AF121050	**0.00**	-	12	45	67	85	70	62	175	103	132	97
3	***S. feltiae***	EU200355	**0.00**	0.00	-	52	73	92	75	68	182	109	139	104
4	***S. ichnusae***	EU421129	**0.03**	0.03	0.03	-	53	76	54	44	170	87	120	90
5	***S. citrae***	EU740970	**0.05**	0.05	0.05	0.04	-	84	26	51	176	90	134	88
6	***S. jollieti***	AY171265	**0.06**	0.06	0.06	0.05	0.06	-	87	79	169	100	129	96
7	***S. nguyeni***	KP325084	**0.04**	0.04	0.04	0.03	0.02	0.05	-	55	182	91	138	93
8	***S. litorale***	AB243441	**0.04**	0.04	0.04	0.03	0.05	0.05	0.04	-	175	98	132	100
9	***S. kushidai***	AB243440	**0.15**	0.15	0.15	0.14	0.14	0.13	0.14	0.15	-	173	164	178
10	***S. texanum***	EF152568	**0.09**	0.09	0.09	0.07	0.09	0.07	0.08	0.09	0.13	-	121	96
11	***S. sangi***	AY355441	**0.10**	0.10	0.10	0.09	0.10	0.09	0.10	0.10	0.13	0.09	-	124
12	***S. sandneri***	MW078536	**0.15**	0.15	0.15	0.15	0.14	0.13	0.14	0.14	0.15	0.14	0.33	-

The 18S sequence analysis of *Steinernema* species

Based on the 18S gene of FUM221 isolate, BLAST analysis revealed 99.51% and 100% similarity and query coverage (respectively) to *Steinernema* sp. (MH084672) from the United Kingdom. The product length of the 18S gene amplified in this study was 840 bps. The multiple alignments of a 1654 bps segment of the18S region for 16 taxa (the new isolate in this study with 14 taxa of *Steinernema* and a species of *Heterorhabditis* as an outgroup) demonstrated that 1366 sites were conserved, 285 sites were variable, 136 sites were singleton, and 149 sites were parsimony informative. The obtained results of the 18S regions were similar to those that resulted from the ITS gene. In the phylogenetic analysis, the FUM221 isolate of *Steinernama feltiae* was placed in the same clade as isolates of *Steinernema* (Figure 5). The overall average was 0.09 (range 0.00–0.14), which were calculated from the 18S gene using the Kimura 2- parameter model (Table 3).

Phylogenetic analysis of symbiont bacteria

The length of the 16S rRNA gene for the bacterium isolate was 1458 bps. The BLAST analysis using the 16S rDNA sequences of the symbiont bacteria of *Xenorhabdus* isolate of this study showed 100% similarity and 98% of query coverage with *X. bovienii* (KJ413078) from Russia. The multiple alignments of the 1413 bps segment of this gene for 14 taxa revealed 110 sites were variable, 1297 sites were conserved, 64 sites were parsimony informative, and 46 sites were singleton. The phylogenetic analysis based on 16S rRNA sequences demonstrated that the isolate of bacterium (FUM221) forms a monophyletic group with other *Xenorhabdus* strains (Figure 6). The overall average distance of 16S rDNA sequences was 0.02 (range 0.00-0.03), calculated by the Kimura 2-parameter model (Table 4).

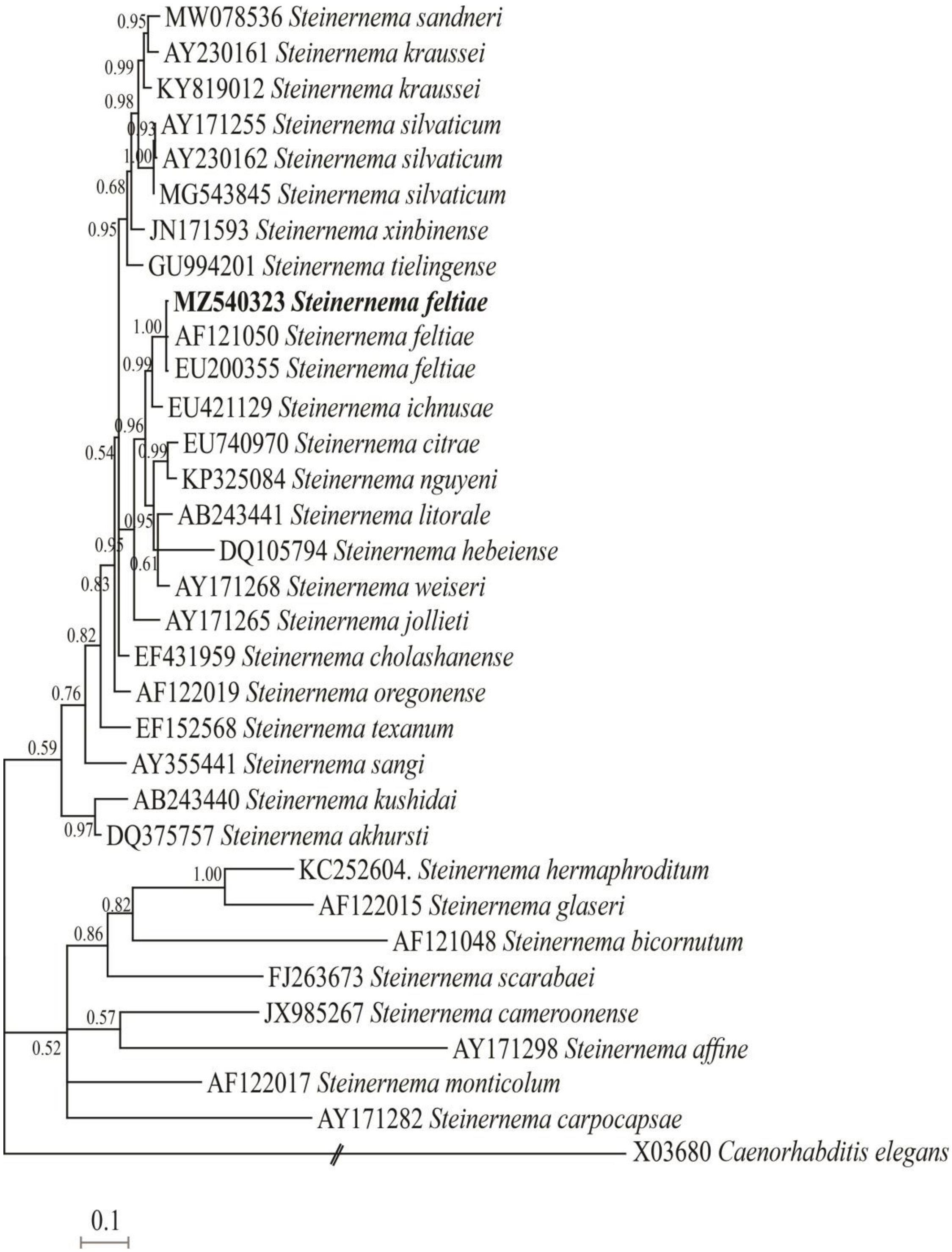

FIGURE I. Phylogenetic relationship the Iranian strain of *Steinernema feltiae* isolate FUM221 with other *Steinernema* species as inferred from Bayesian analysis of sequences of the Internal Transcribed Spacer (ITS rDNA region) under GTR + I + G model. Bayesian posterior probability amounts equal to or more than 0.50 are given for appropriate clades. The scale bar shows the number of substitutions per site.

TABLE 3. The number of bases which are not identical (upper triangle), and pairwise comparison on the number of nucleotide differences (lower triangle) among some of *Steinernema* species and *Steinernema* isolate FUM221 based on 18S rDNA sequences.

	Species	Acc. no.	1	2	3	4	5	6	7	8	9	10	11	12
1	***S. feltiae***	**MZ540331**	**-**	**2**	**2**	**1**	**3**	**2**	**2**	**55**	**17**	**84**	**90**	**107**
2	***S. feltiae***	FJ040418	**0.00**	-	3	3	4	4	3	80	14	84	121	118
3	***S. feltiae***	LN611147	**0.00**	0.00	-	2	3	4	0	76	14	85	106	103
4	***S. feltiae***	FJ040419	**0.00**	0.00	0.00	-	3	4	2	79	13	84	120	117
5	***S. feltiae***	FJ040417	**0.00**	0.00	0.00	0.00	-	5	3	80	15	85	121	118
6	***S. feltiae***	KJ636413	**0.00**	0.00	0.00	0.00	0.00	-	4	81	18	90	120	136
7	***S. feltiae***	LN611148	**0.00**	0.00	0.00	0.00	0.00	0.00	-	76	14	85	106	103
8	***S. scarabaei***	FJ040424	**0.06**	0.06	0.06	0.06	0.06	0.06	0.06	-	54	91	138	135
9	***S. akhursti***	KT878310	**0.01**	0.01	0.01	0.01	0.01	0.01	0.01	0.05	-	92	96	110
10	***S. karii***	AJ417021	**0.09**	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.10	-	121	138
11	***S. carpocapsae***	LN624756	**0.11**	0.11	0.11	0.11	0.11	0.11	0.11	0.12	0.11	0.14	-	4
12	***S. carpocapsae***	AF036604	**0.11**	0.11	0.10	0.11	0.11	0.11	0.10	0.12	0.11	0.13	0.01	-

Phenotypic characterization

The bacterial isolate of the present study could produce pigments, and the isolate was blue to greenish to blue on NBTA media. During the incubation, two phases were recognizable, phase I (round and glossy) and phase II (mucoid). The isolate was gram-negative to the colonies did not show catalase activity on hydrogen peroxide, and they were rod-shaped with wide variable cell length. The colonies could grow and absorbed dye on NBTA and NA medium. The isolate was also motile, and it had a growth inhibition zone with tetracycline. In the end, 24h post-injection by bacterial suspension, the *G. mellonella* larvae were dead compared with the larvae injected with sterilized water. These biochemical tests and others for Iranian bacterial isolate are shown in table 5, and the reaction of the bacterium is presented in figure 7.

DISCUSSION

Iran has a variety of climatic zones, which makes it appropriate for a wide diversity of plants and insects, and could be considered a shelter for several different species and strains of EPNs. As indicated in the present study, the new native isolate of entomopathogenic nematode, *Steinernema feltiae*, which has a mutualistic relationship with *Xenorhabdus bovienii*, was recovered from the Ardabil province of Iran. The northwest of Iran which shares a border with Turkey is a mountainous area with a cool continental climate in which the annual absolute temperature can vary from −38.5 to 44 °C. The out-of-range of 40 °C and 8 °C could be fatal for most EPN populations (Griffin, 1993; Grewal et al., 1994), and studied locations usually experience a temperature below 8 °C which is a limiting factor for EPNs.

Previously, *S. feltiae* was recovered from the coast of the Black Sea and Ankara (Özer et al. 1995; Susurluk et al., 2002; Hazir et al., 2003a), and in some cases, it was the most common species in those

TABLE 4. The number of bases which are not identical (upper triangle), and pairwise comparison on the number of nucleotide differences (lower triangle) among some of *Xenorhabdus* species and *Xenorhabdus* isolate FUM221 based on 16S rRNA sequences.

	Species	**Acc. no.**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	***X. bovienii***	MZ540333	-	2	3	2	2	2	3	3	4	31	32	32
2	***X. bovienii***	KJ413082	0.00	-	2	0	3	0	1	2	4	43	54	43
3	***X. bovienii***	NR119151	0.00	0.00	-	2	5	2	3	4	6	44	55	44
4	***X. bovienii***	HM140697	0.00	0.00	0.00	-	3	0	1	2	4	43	54	43
5	***X. bovienii***	KJ413070	0.00	0.00	0.00	0.00	-	3	2	2	3	45	55	44
6	***X. bovienii***	KJ413065	0.00	0.00	0.00	0.00	0.00	-	1	2	4	43	54	43
7	***X. bovienii***	MG995576	0.00	0.00	0.00	0.00	0.00	0.00	-	1	3	43	55	44
8	***X. bovienii***	KJ413083	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-	3	44	55	45
9	***X. bovienii***	KU312061	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-	43	53	44
10	***X. nematophila***	AY286478	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	-	51	46
11	***X. magdalenensis***	NR109326	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	-	39
12	***X. szentirmaii***	DQ211712	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.02	-

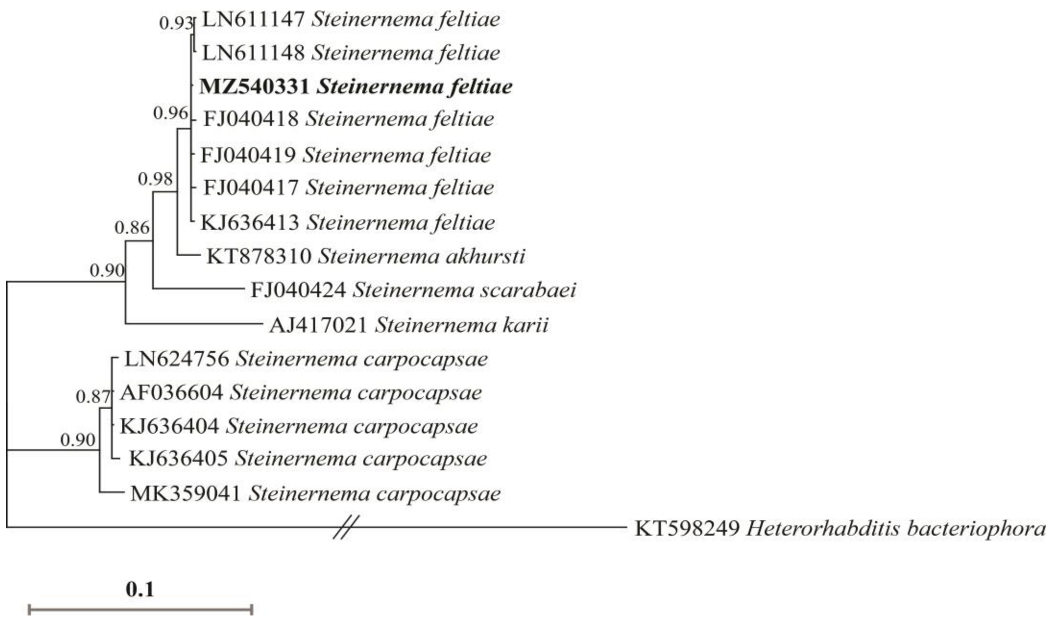

FIGURE 5. Phylogenetic relationship of *Steinernema feltiae* isolate FUM221 with other *Steinernema* species as inferred from Bayesian analysis of sequences of the small subunit (SSU rDNA region) under GTR + I + G model. Bayesian posterior probability amounts equal to or more than 0.50 are given for appropriate clades. The scale bar shows the number of substitutions per site.

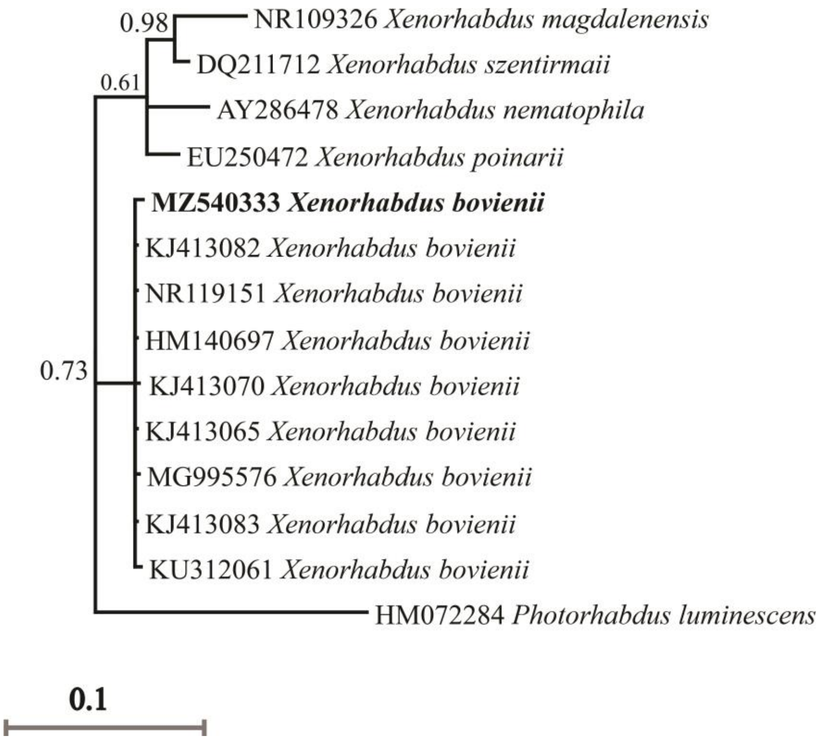

FIGURE 6. Phylogenetic relationship of *Xenorhabdus bovienii* isolate FUM221 with other *Xenorhabdus* species as inferred from Bayesian analysis of sequences of the 16S rRNA region under HKY + I + G model. Bayesian posterior probability amounts equal to or more than 0.50 are given for appropriate clades. The scale bar shows the number of substitutions per site.

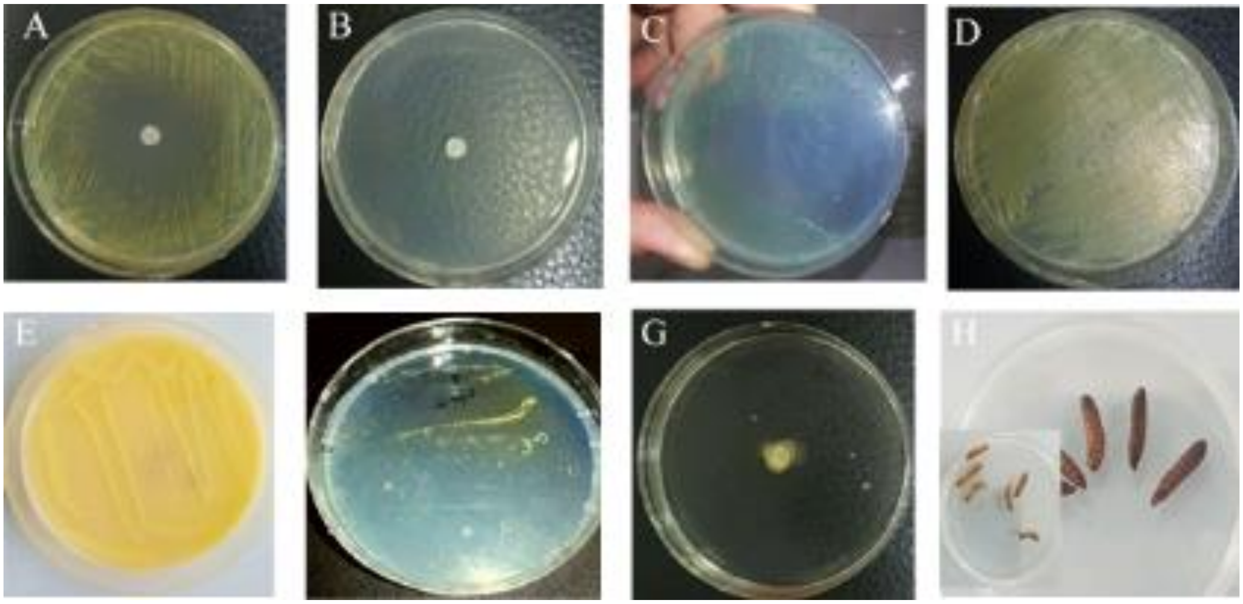

FIGURE 7. Biochemical tests. A and B, Antibiotic and control; C, dye absorption on NBTA; D dye absorption on NA; E, Lesitinase; F, Lipase; G, Motility; H, Mortality, and control.

TABLE 5. General biochemical characteristics of symbiotic bacteria isolated from Steinernema feltiae FUM221.

Biochemical characteristics	***Xenorhabdus bovienii*** **FUM221**
Gram staining	-
Bromothymol blue from NBTA [pigmentation]	Greenish to blue
Pigmentation (nutrient agar)	Yellow
Motility	+
Tetracycline resistance	week
Catalase	_
Lecitinase	+
Lipase	+
Mortality on *G. mellonella*	+

areas (Hazir et al. 2003b; Eivazian Kary et al., 2009; Yuksel and Canhilal, 2019). Similarly, *S. feltiae* was reported in several provinces such as Tehran, Mazandaran, East Azerbaijan, Ardabil, and Kurdistan, Iran (Tanha Ma'afi et al., 2006; Eivazian Kary et al., 2009; Karimi et al., 2009; Nikdel and Niknam, 2015).

In this study, just one species was recovered from the pasture with adequate moisture and herbage, a favorable environment for the growth and establishment of EPNs (Campos-Herrera et al., 2008). This low recovery rate might be due to the use of *Galleria mellonella* as the sole host to trap the insect. As reported previously, *Galleria mellonella* would not be a suitable host for all EPN species/strains (Spiridonov and Moens, 1999). In addition, the room temperature for baiting the soil samples might be another factor for the low recovery rate. However, such a low recovery rate was observed in another investigation performed in different districts of the world and it is not uncommon (Choo et al., 1995; Rosa et al., 2000; Hazir et al., 2003a).

Accurate diagnosis of novel species and isolates of EPNs is necessary to the success of biological control programs due to the adaptability of nematode isolates to native environmental conditions (Stock, 2009). Currently, classical and molecular methods are used for species identification. In classical methods, some structures such as the oral cavity, lips, esophagus, intestine, reproductive system, sensory organs, and tail are measured by light microscopy. This method could be a difficult and sometimes dubious process (Dorris et al., 1999; Abebe et al., 2011). It seems that classical methods are not used optimally in the identification or classification of pathogenic nematodes because of diversity reduction in morphological characteristics (Campos-Herrera et al 2012). In addition, these traits are only suitable for identification but not phylogenetic studies and intraspecific morphometrics variability could be observed within the strains (Yoshida, 2003; Nikdel and Niknam, 2015) and with the original descriptions (Poinar, 1990).

In the present study, some characteristics like the body length of IJs were relatively less than those described *S. feltiae* originally. Some other researchers had the same results (Campos-Herrera et al., 2006; Majić et al., 2018; Flores et al., 2021). The research on morphological characters showed there are up to 14 similarity degrees of body length with a maximum of 28 populations in the same homogenous group (Clausi et al., 2020). On the other hand, it has been suggested that IJs body length is the longest when EPNs are raised at 8 °C, and this character could be limited at higher room temperature conditions (Hazir et al., 2001). In our study, the strain was reared at room temperature could be another reason for shorter morphometric values. The difference in the FUM221 isolate can be due to intraspecific variability (Stock et al., 1999).

Molecular methods are very beneficial and used as a complementary method to unravel the problems like identifying the members of one species and recognizing species with similar morphological traits (Stock and Reid, 2004). These methods are not only substantial for the identification of nematode species but are also beneficial for estimating phylogenetic relationships at different levels of classification (Dorris et al., 1999; Blaxter 2003; Stock, 2009).

The ITS region is a fundamental marker in the separation of species (Adams et al., 1998; Szalanaski et al., 2000; Nguyen et al., 2001). Accordingly, many researchers used this region for the identification of *Steinernema feltiae* species (Nikdel and Niknam, 2015; Tumialis et al., 2016; Majić et al. 2018; Flores et al., 2021). The ITS region is variable between species groups of *Steinernema* and could be observed among individuals of the same species as well. Hence, this region can not be a suitable marker for the distinction of all *Steinernema* species (Stock, 2009). The 18S rDNA has a significant role in the identification of unknown nematode species (Blaxter et al., 1998). Due to its conserved nature, this subunit evolves slowly and uses for classification (Stock and Hunt, 2005). Many researchers have used the ITS region for the recognition of *S. feltiae*, but very little research has been conducted on the 18S rDNA for *S. feltiae*. In the present study, identification of this species was carried out based on ITS and 18S rDNA regions.

Briefly, although molecular analyses are an essential tool for the identification of species, the studies showed that morphological and biological researches on the variability of *S. feltiae* strains are incredibly vital and provide crucial information for species identification. For a comprehensive species characterization, morphological (infective juveniles, spicule and gubernaculum shapes for males), biological (time to achieve adult stage, reproduction, and progeny), and molecular research must be carried out simultaneously (Clausi et al., 2020).

The EPN species have mutual relationships with an exclusive bacterial species. However, few species, particularly *Xenorhabdus* spp. are associated with more than one EPN species. Isolation and identification of the symbiotic bacterium of the EPNs are necessary to prove the exact nematode species. In this study, *Xenorhabdus bovienii* was isolated from *S. feltiae*. The bacterial isolates were characterized by their phenetic characters and 16S ribosomal RNA gene sequences (Agazadeh et al., 2010; Karimi et al., 2011). The isolation of this symbiotic bacterium is inevitable for completing phylogeny and clarifying the ambiguous aspects of its characteristics. Because of the variation in different isolates, the present data for chemical characters, in particular, should be evaluated in the future.

CONCLUSION

As referred before, this study is the first documented record about the presentation of the 18S tree for *Steinernema feltiae* species to accreditation for identification of this species. Also, due to the adaptation of this species to cold climate conditions, it can be suitable to introduce as an option for pest control in cool regions like the west and northwest of Iran. So, this strain can be a candidate for the commercialization of EPNs in these districts. Further studies are suggested to evaluate the potential effectiveness of symbiont bacteria of this EPN species as a new strain with the possibility of having novel metabolites and toxins about its beneficial effects in agriculture and medicine.

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LIST OF ABBREVIATIONS

ABD: Anal body diameter
EP: Distance from anterior end to excretory pore
ES: Distance from anterior end to end of esophagus

EPNs: Entomopathogenic nematodes
GL: Gubernaculum length
GW: Gubernaculum width
ITS: internal transcribed spacer
L: body length
Mega: Molecular evolutionary genetics analysis
n: number of specimens analyzed
NA: Nutrient agar
NBTA: Nutrient Bromothymol blue-triphenyltetrazolium chloride agar
NR: Distance from anterior end to nerve ring
Ph: *Photorhabdus*
PP: posterior probabilities
Sd: Standard deviation
SEM: scanning electron microscopy
SP: Spicule length
SW: Spicule width
T: Tail length
V: Vulva
W: greatest body diam
X: *Xenorahbdus*

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RESEARCH ARTICLE **Open access**

Distribution of long-tailed marmot, *Marmota caudata* in the Badakhshan province of Afghanistan

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Abstract
The current survey was conducted on occurrence and distribution of long-tailed marmot, *Marmota caudata*, in Badakhshan Province, situated in the northeast of Afghanistan. *Marmota caudata* is one of the largest rodents in the cold desert habitats, and an important prey for endangered carnivores. Line transect method was used to collect specimens in the study area. A total of 761 individuals of long-tailed marmot, were observed in this region during 2020 and 2021. The presence of marmots was recorded by direct observation and their symptoms. The results indicate all individuals were occurring in the areas between the snow line and the timberline to near livestock grazing area. The maximum number of observations was in Arghanjkhah with 56 individuals (7.3% of all observations) and the lowest was in Kashim with 5 (0.6%). The highest population density per districts was in Arghanjkhah (11.2±2.5 per Km^2) and the lowest was in Kashim (1±0.2 per Km^2). Marmots were founded in all regions of Badakhshan with more abundance in eastern part of the province. This study was the first study on this species in the region and in Afghanistan.

Key words: *Population density, Abundance, Arghanjkhah, Kashim, High-altitude mammals*

INTRODUCTION

Of the recognized 15 species of marmots, six occur in Asia (Armitage, 2000). The long-tailed marmot, *Marmota caudata* (Geoffroy, 1844) or golden marmot possesses wide distribution in India, Tajikistan, Pakistan and China (Ahmed et al., 2016; Lu et al., 2016; Baseer-ud-Din et al., 2015). The distribution of this species has been assessed by the IUCN red list of Threatened Species in 2017 (Cassola, 2017). *Marmota caudata* has been widely spread in the North and East part of Afghanistan. So far, nothing is known about the distribution and abundance of the *M. caudata*, in the Badakhshan Province (BP) and other part of Afghanistan in details.

Badakhshan Province has relatively cold climate. The winters are characterized by cold temperatures (to –40° C), heavy snow and substantial amounts of precipitation (81–255 millimeter). The summers are warm (28–37.5° C) and dry (precipitation 24–100 millimeter). Despite a good vegetation in BP, continued pressures such as animal grazing, farming, deforestation and uprooting shrubs for fuel,

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most of original natural vegetation region has been diminished (PDMC, 2013). In BP, long-tailed marmot inhabits a region between the upper forest border and snow line in the alpine.

Long-tailed Marmot is a large holarctic rodent adapted to cold climate (Nikol´skii & Ulak, 2007). This species forms an important part of the diet of some globally endangered species in Afghanistan such as the snow leopard, *Panther uncia* (Schreber, 1775), wolf, *Canis lupus* (Linnaeus, 1758), red fox, *Vulpes vulpes* (Linnaeus, 1758), golden eagle, *Aquila chrysaetos* (Linnaeus, 1758) and other associated carnivores in the top of food web (Ahmed et al., 2016).

Marmots live in the colonies in high-altitude mountains and are considered one of the highest altitude mammalians in the world and important component of ecosystem because of their role in web chain (Aryal et al., 2015; Poudel et al., 2016). Long-tailed marmot is classified as a species of "least concern" under IUCN red list category (Sheikh & Molur, 2004). The long-tailed marmot is among the least studied species among marmots in the world and more apparently in the BP.

Habitat utilization in rodents is typically determined by vegetation and soil features (Řičánková et al., 2014). Marmots occupy habitats from small, alpine meadows to the wide range of steppe environments (Ahmed et al., 2016). The relationship between species existence and ecological features is vital for understanding of species distributions. Marmots, in the terms of the benefits associated with obtaining food and risk of predation often display two types of behaviors in response to avoidance of predation, vigilance and group formation. Humans and their livestock like dogs usually are as potential predators, therefore the effects of human distribution are similar to natural predation risks (Poudel et al., 2015). Marmots are commonly baited by domestic dogs for entertainment besides persecute as pests. Furthermore, marmots hunt for a local believe to their extraordinary medicinal properties and their fat (Zaman et al., 2019).

Recently, research on adaptation of marmots to high-altitude and their hibernation have been of great interest. Species of marmots hibernate in family groups during the cold period of the year. Hibernation burrows are usually deep with more than 10 m in some areas. Hibernation in animals is a seasonal state of metabolic suppression and dormancy characterized by a decrease in body temperature, metabolism, heart rate, and oxygen consumption. Marmots are confronted with severe hypoxic and cold stress during winter (Bai et al., 2019). Marmots usually hibernate for up to eight months of the year depending on species. Therefore, they are accessible to the predators for less than half of the year (Tytar et al., 2019).

Regional distribution of long-tailed marmot was not documented in Afghanistan. The aim of this study was to record of the distribution and population density of *M. caudata* in the Badakhshan Province of Afghanistan.

MATERIAL AND METHODS

Study area

The current study was conducted in the Badakhshan Province, situated in the northeast of Afghanistan between 35°26'46"N-69°57'40"E and 38°29' 27"N-74°53'39"E with the area of 43721 per Km^2 (Figure 1). The region is surrounded in the North by Tajikistan, Southern East by Pakistan and Kashmir, in the far end of Wakhan corridor by China and in the West and South by Takhar, Nuristan and Panjshir Provinces. High mountains and steep river valleys are the main geographical features of the province. Nearly 89.9% of the province is mountainous or semi mountainous terrain while 9.7% of the area is made up of flat or semi-flat lands.

METHOD

Line-transect method was used to collect data on distribution of the long-tailed Marmot, *M. caudata* in Badakhshan Province. A total of 56 transects (each district with 2 transects) between 3000–5500 m was taken During April 2020 to September 2021. Each transect was 300 m wide by 2 to 3 Km long (Ahmed, et al., 2016). Length of transects was adjusted according to the land of the regions corresponding with the

TABLE 1. The distribution of long-tailed marmot, *Marmota caudata* in each district of Badakhshan Province. Number of marmots and abundance and density in each district have been observed.

No	**districts**	**Number of marmots observed**	**Abundance* (%)**	**Density (per Km^2) (Mean±SE)**	**Distance travelled (Km)**
1	Arghanjkhan	56	7.3	11.2±2.5	87
2	Sheghnan	54	7	10.8±2.4	147
3	Wakhan	50	6.5	10±2.3	279
4	Kohistan	48	6.3	9.6±2.2	131
5	Raghistan	46	6	9.2±1.2	127
6	Kran & Manjin	44	5.7	8.8±1.5	189
7	Zibak	43	5.6	8.6±1.2	203
8	Darwaz bala	42	5.5	8.4±1.5	371
9	Ishkashim	36	4.7	7.2±1.2	138
10	Tagab	32	4.2	6.4±1.5	147
11	Kuf Ab	28	3.6	5.6±1.2	204
12	Warduj	28	3.6	5.6±1.2	61
13	Yamgan	28	3.6	5.6±1.2	121
14	Yaftalbala	26	3.4	5.2±1.5	29
15	Jurm	26	3.4	5.2±1.4	72
16	Yawan	22	2.8	4.4±1.2	98
17	Shohada	22	2.8	4.4±1.5	78
18	Khwahan	22	2.8	4.4±1.5	279
19	Shaki	18	2.3	3.6±1.3	437
20	Nasi	17	2.2	3.4±1.4	416
21	Maimi	14	1.8	2.8±1.2	360
22	Baharak	12	1.5	2.4±1.5	46
23	Yaftalsafla	12	1.5	2.4±1.3	35
24	Argo	9	1.1	1.8±0.4	32
25	Daraym	8	1	1.6±0.5	54
26	Tishkan	7	0.9	1.4±0.3	73
27	Shahri Buzurg	6	0.78	1.2±0.2	83
28	Kashim	5	0.6	1±0.2	105
Total	28	761	100		4,402

alpine zone (Ahmed et al., 2016; Baseer-ud-Din et al., 2015). Interview was carried out at least with six local residents to be confident of marmots observation (Aryal et al., 2015).

Each district was observed daily, in the morning between 07:00–11:00, and in the afternoon between 14:00–15:00 for two days. During the survey, a few local people helped to collect data and guide authors to the marmot habitats. Each transect was carefully checked for the presence of marmots. In some places, 43 burrows in an area with 200 m2 were recorded. The new burrows were differing from old and inactive ones by the presence of fresh soil and runways. Presence of marmot were recorded by direct observation and their nests and feces (Figure 2).

The human distribution was divided into three types, low, medium, and high density. Human disturbance was considered as low when there was no evidence of human activity or livestock grazing; medium when the plots show only signs of grazing, and high when the plot contained evidence of intensive human activity.

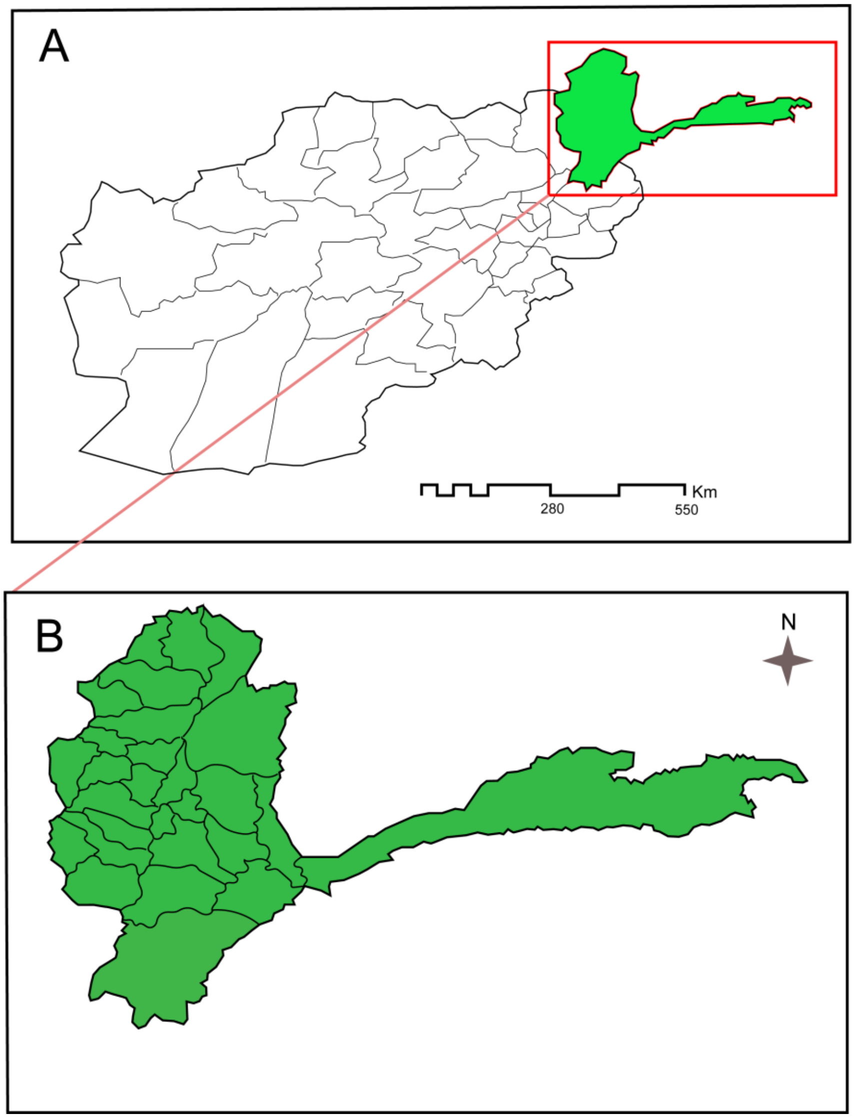

FIGURE 1. A: Map of Afghanistan; B: Badakhshan Province.

Statistical analysis was carried out using IBM SPSS statistics 24. At first, the normality of data was tested using Shapiro-Wilk test, then the significance differences were calculated by Kruskal-Wallis test. The p-value was considered at 0.05.

RESULTS

Data was collected from 28 districts of Badakhshan Province in 2020 and 2021. The Long-tailed marmot, has been distributed throughout the study area. A total of 761 individuals of M. caudata were observed in this region. The distribution of this species was varied in the study area (Table 1; Figure 2B, C). Distribution in each district was recorded separately. The maximum number of observations is related to the Arghanjkhah with 56 individuals (7.3% of all observations) and the lowest observation is in Kashim district with 5 (0.6%).

Mean density of the long-tailed marmot was highest in Arghanjkhah with 11.2±2.5per Km2, follow by Sheghnan, Wakhan and Kohistan (10.8±2.4, 10±2.3 and 9.6±2.2, respectively). lowest density per districts was in Kashim, Shahri Buzurg and Tishkan (1±0.2, 1.2±0.2 and 1.4±0.3, respectively) (Figure 3). Distribution of this species was significantly different between districts ($P< 0.05$). Maximum

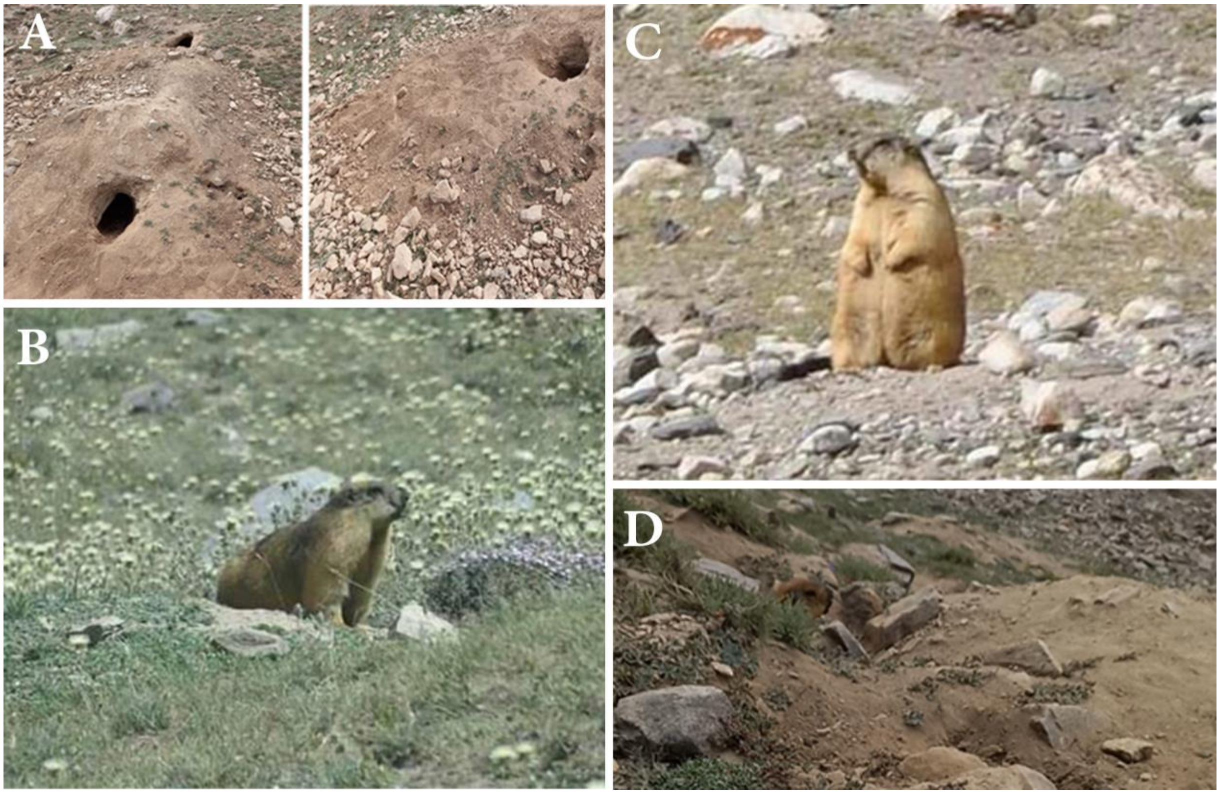

FIGURE 2. A: Burrows of the long-tailed marmot, *Marmota caudata*; B: *M. caudata* in grassland habitat; C: Giving alarm by *M. caudata*; D: A marmot coming out from the burrow.

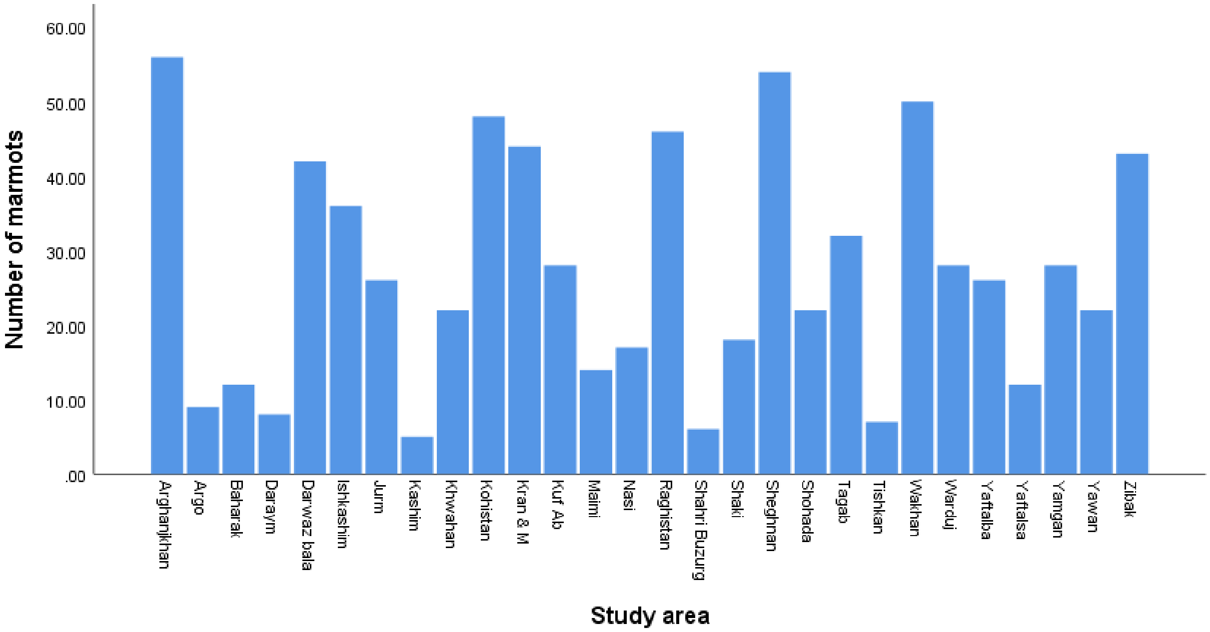

FIGURE 3. The number of the long-tailed marmot, *Marmota caudata* in each district of BP.

number of this marmot was observed between timberline and snowline. In this region, the populations of marmots were observed more in hilly areas followed by valleys and plateaus. The more abundance of marmots in districts like Arghanjkhah, Sheghnan, Wakhan and Kohistan was observed. These districts have vast grassland cover providing adequate food and shelter. grazing activities were observed around the marmot colonies.

DISCUSSION

The current survey was the first time to record distribution and abundance of the long-tailed marmot, *M. caudata* in Badakhshan Province and in all Afghanistan. Our results correspond with Ahmed et al. (2016) from India in which maximum abundance was at herbaceous, meadow, grassland and then in rocky area, respectively (Ahmed et al., 2016). Baseer-ud-Din et al. (2015) reported 551 individuals from Baboon, Bloar and other regions of Pakistan of long-tailed marmot. Chaudhary et al. (2017) recognized that a total of 110 individuals of Marmota himalayana (Hodgson, 1841) were observed in the surveyed stretches of Leh District with a maximum mean of encounter of 2.71. The occurrence and distribution of marmots in other regions in nearby was recorded (Nikol'skii & Ulak, 2006, 2007; Poudyal, 2011).

Distribution of this species was significantly different between districts ($P< 0.05$). In fact, there exist a tendency of decreasing abundance from East to West of Badakhshan. It related to having vast grassland, adequate food, type of suitable soil to excavate the burrow and lower human population in suitable habitat of Eastern part. Maximum number of this marmot was observed between timberline and snowline. Boero (2003) reported that elevation, sun-shine duration, sun exposure, slope, anthropic pressure, food resource, and soil composition impact on the marmot's distribution. According to Nikol’skii & Ulak, (2006), elevation is an important ecological factor for marmots and other mountain species, since most of the other factors depend on it, directly. The maximum number of marmots occurs between the snow line and the timberline with 3000 to 5000 m coinciding with subalpine and alpine mountain belts (Chaudhary et al., 2017). According to Řičánková et al. (2014), the vegetation type explained most of the variability of marmot density. Marmots usually prefer the grasslands as favorable habitat (Figure 2B). Accordingly, the fewer preferential coefficient was also found for the shrubs and clearly avoided the woodlands such as Kashim and Tishkan.

Marmots have often high level of stress during the summer time, due to presence of human and their livestock. They usually approach to the livestock and human disturbances, because they are diurnal and have a small home range, and a high dependency on burrows (Poudel et al., 2016). Nikol’skii and Ulak (2007) documented that burrows are large and deep, the depth of nest borrows in M. himalayana are usually 4–7 m, and they can dig their large burrows only in the regions where the ground is soft and sufficiently be deepened (Figure 2A). In addition, soil conditions and especially the depth of the soft soil layer influence the distribution and abundance of this animal (Barash, 1989).

The geographical distribution of burrows is affected by temperature. The marmots have adapted with lower temperature. Their desired temperature value is between 22–25° C, for normal activity (Lu et al., 2016). A lot of burrows are found near water sources such as streams, rivers, or ponds (Aryal et al., 2015). The same pattern of burrow distribution was observed in the current study.

Generally, we surmised that long-tailed marmot (*M. caudata*) was abundance in suitable areas of Badakhshan province. This is corresponded with the Eastern districts of the province. In addition to human and livestock, four aspects i.e., height, temperature, relief and feeding conditions affect the marmot distribution. The most of the marmot population was observed in Arghanjkhah and lowest in Kashim district. Marmot distributed in all regions and population density are differ in each district of Badakhshan province. Finally, it seems that *M. caudata* is more abundant in Badakhshan province of Afghanistan in comparison with other nearby regions because of tendency of decreasing population from East to West of Province. The same pattern may to be extended to all Afghanistan. Further studies need to reveal the pattern of distribution in another region of Afghanistan.

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RESEARCH ARTICLE Open access

Phenotypic and geographic variation among populations of the *Platyceps rhodorachis-ventromaculatus* species complex (Ophidia, Colubridae) in Iran

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Abstract
Geographical diversity appears in the phenotype of different populations of a given species and specific geographical factors are directly or indirectly involved in this variation. Due to their wide distribution range, snakes of the genus *Platyceps* Blyth, 1860 are wonderful case studies for morphological evaluation. In this study, 49 specimens of the *Platyceps rhodorachis-ventromaculatus* species complex in four geographic areas from Iran were examined using morphological characters. The results of statistical analysis did not show any sexual demorphisem except in the character of Gular scales (*P* value = 0.038), but did show significant variation in the number of scales and in other morphological characters among populations in this group. Generally, four morphological clusters were distinguished with Sistan and Baluchestan Province being the most distinct from each other. Additionally, there was a visible slope of changes in some morphological characters associated with latitude, so that with increasing latitude, the body size of the specimens increases.

Key words: *Platyceps rhodorachis, Platyceps ventromaculatus, Colubridae, Morphology, Phenotypic, Iran*

INTRODUCTION

The genus of *Platyceps* Blyth, 1860 belongs to the Colubridae family and contains 29 species globally, of which 7 have been recorded in Iran (Schaetti *et al.*, 2014; Rajabizadeh, 2018; Uetz, 2020). The genus distribution is quite large and includes the Saharo-Indus biogeographical regions from Africa to East Asia and the Mediterranean region, North Africa, and the Middle East to the Nepalese highlands (Schatti, 2006; Geniez & Gauthier, 2008; Jablonski & Bragin, 2019).

Platyceps rhodorachis (Jan, 1863) is one of the most important species in this genus. This species has a maximum length of 1290 mm. Its body shape is cylindrical and elongated and it is found in brownish-gray, olive, gray, brown colors with a distinct spot under the eyes and dark brindled stains or without dorsal stains (Leviton *et al.*, 1992; Schätti & McCarthy, 2004). The former *Platyceps rhodorachis*

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pecies complex had four subspecies, *P. r. rhodorachis* (Jan in Filippi, 1865), *P. r. subniger* (Boettger, 1893), *P. r. saharacus* (Schätti & Mccarthy 2004), and *P. r. ladacensis* (Anderson, 1871). Currently, two subspecies of this species complex have been elevated to full species status. *Platyceps saharicus* (Schätti & Mccarthy 2004) that cover the western part of the distribution range (North Africa, northwestern Saudi Arabia, Jordan, and Palestine) (SCHÄTTI and McCarthy, 2004; Geniez and Gauthier, 2008). Additionally, a subspecies of *P. r. ladacensis* (Anderson, 1871) from Aden (Yemen) has been elevated to full species status due to differences in ventral scales and lack of overlap of the distribution with *P. rhodorachis* and seems to have a wider distribution. It is also distributed in the northern regions of India (Perry, 1985; Schätti & McCarthy, 2004; Schatti, 2006; Perry, 2012; Шестопал, 2020; Hussain & Tantarpale, 2021).

Currently, *P. rhodorachis* contains two valid subspecies, *P.r.subniger* (Boettger 1893) and *P.r.rhodorachis* (Jan in Filippi 1865), whose distribution in Iran has been confirmed (Latifi & Leviton, 1991; Latifi, 1991,2000; Moradi *et al.*, 2013; Schaetti *et al.*, 2014; Rajabizadeh, 2018). *Platyceps rhodorachis rhodorachis* is distributed through Iran, Afghanistan, India and northeast Iraq (Hussain & Tantarpale, 2021; Malik *et al.*, 2021). *Platyceps rhodorachis subniger* is distributed in Iran, Eritrea and Djibouti to north and central of Somalia and adjacent Ethiopia (Latifi *et al.*, 1991; Khan, 1997; Schaetti *et al.*, 2014). *Platyceps rhodorachis kashmirensis* (Khan, 1997) has been recommended as a synonym of *P. rhodorachis* species by Schätti 2014 (Latifi *et al.*, 1991; Khan, 1997; Schaetti *et al.*, 2014).

Platyceps ventromaculatus (Gray, 1834) is mainly found in Afghanistan, Pakistan, India, and Iran. However, new findings indicate that the distribution of this species is more than has been documented and this species has recently been found in parts of southeastern Antalya, Turkey (Yildiz, 2011; Bhandari *et al.*, 2021). The *Platyceps ventromaculatus* species complex includes three subspecies: *P. v. ventromaculatus* (Gray, 1834), *P. v. bengalensis* (Khan & Khan, 2000), and *P. v. indusai* (Khan & Khan, 2000). Pattern and color differences are evident in the subspecies, but *P. v. bengalensis* and *P. v. indusai* were introduced as subspecies based only on a slight difference in the number of scales (Khan, 1986; Khan & Khan, 2000; Barabanov, 2002). However, because Khan & Khan (2000) didn't specify a neotype for their new subspecies, the current status of these subspecies is unclear.

Platyceps ventromaculatus is morphologically very similar to the *P. rhodorachis* species complex and differs only slightly in the number of dorsal spots and the number of scales. Hence, the situation between these two species has become a species complex and *Platyceps rhodorachis-ventromaculatus* has attracted considerable attention as a polymorphic group**.** Nonetheless, taxonomic status of this group has not yet been resolved and has undergone many changes since its description. Perry (2012) believed that the number of ventral scales is the leading cause of changes in the length of *Platyceps rhodorachis* (Jan, 1865), but did not provide sufficient evidence to support this.

Extensive morphological variations of the number of scales, color patterns, and dorsal stripes have been reported in *P. rhodorachis-ventromaculatus*. However, four significant groups of them can be seen in Iran (Schaetti *et al.*, 2014). Although many attempts have been made to study the morphology of the *P. rhodorachis-ventromaculatus* complex group, most have been limited to a small area and based on initial identifications and this is problematic in that high morphological similarity is observed and individuals are morphologically similar, making them easily misidentified. Another problem is that species descriptions are usually based on a limited number of specimens and due, to their wide distribution range, a comprehensive view of the morphological status of *P. rhodorachis-ventromaculatus* complex group is not available (Khan, 1997; Schatti, 2006; Yildiz, 2011; Schaetti *et al.*, 2014).

We know that a species' geographical range is determined by the complex interaction of species characteristics and environmental factors limiting species distribution (Gaston, 2003); subsequently, a detailed morphological study should be performed in a range commensurate with the range of species distribution.

The range size has been associated with several biogeographical, environmental, and life history factors such as body size (Gaston & Blackburn, 1996), altitude (Stevens, 1989), and latitude (Böhm *et al.*, 2017). According to Bergmann's rule, the body size of animal’s changes in association with latitude changes. In colder regions, the body size of a species is larger than the body size of that species in lower latitudes and warmer regions (Meiri & Dayan, 2003). Additionally, by Rapoport's rule, range size increases with increasing latitude, especially in the northern hemisphere (Böhm *et al.*, 2017). Because Iran has high geographical diversity and natural barriers such as Kopeh Dagh, and the Lut and Kavir Deserts, and Zagros Mountains from northwest to the south, and the Alborz Mountain range that extends from northwest to the northeast (Fu *et al.*, 2007; Edwards *et al.*, 2012; Le Galliard *et al.*, 2012), and based on previous surveys and our results from this research, the spread of *P. rhodorachis-ventromaculatus* complex species is highly probably influenced by these geographical barriers.

In the present study, we tried to compare different populations of *P. rhodorachis-ventromaculatus* complex species in terms of morphological diversity. We also tried to study the effect of geographical barriers in Iran on the morphological variations of *P. rhodorachis-ventromaculatus* complex species.

MATERIAL AND METHODS

A total of 49 adult specimens (25 ♂, 24 ♀) of *P. rhodorachis-ventromaculatus* species complex were examined morphologically. During fieldwork (2002-2010), specimens were collected in western, southern, northeastern, and southeastern parts of Iran by Hakim Sabzevari University and Imam Hossein University researchers (Fig. 1). All specimens were deposited at Sabzevar University Herpetological Collection (SUHC). West Zagros population includes 17 specimens, the southern population includes 9, the southeastern population includes 15, and the northeastern and eastern population contains 8 (Appendix 1 contains the list of specimen collection sites based on OTUs).

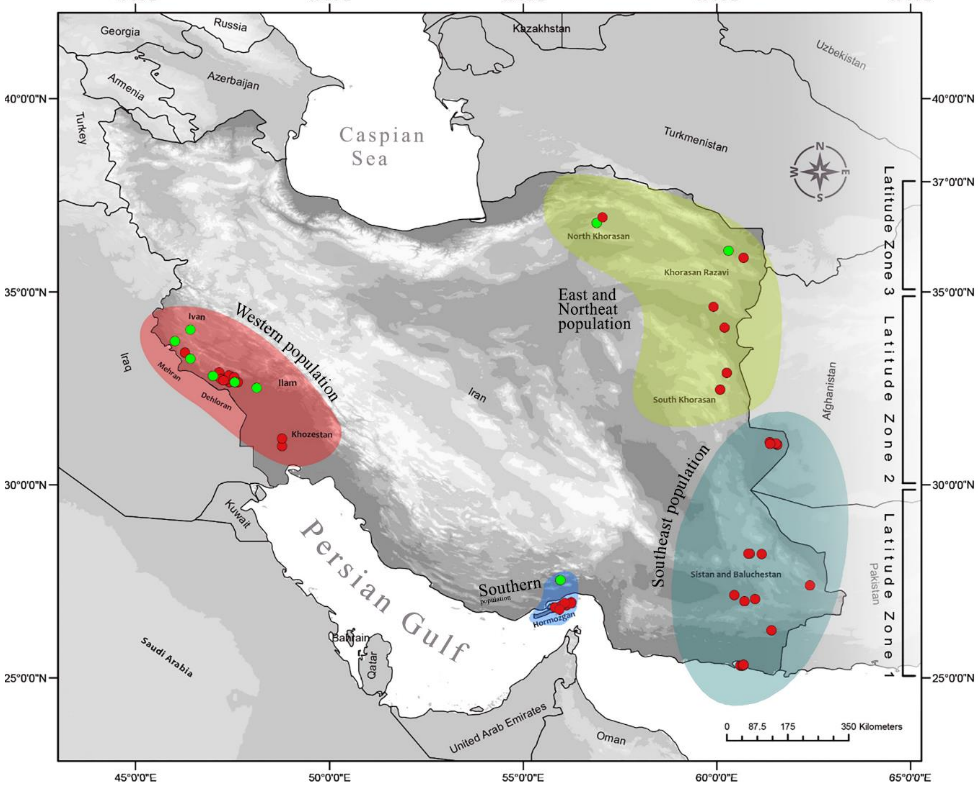

FIGURE 1. Collecting sites of *Platyceps rhodorachis* (red circles) and *Platyceps ventromaculatus* (green circles) in Iran. Western populations belong to Ilam, Mehran, Dehloran, Ivan, and Khuzestan. The populations of the south are from Hormozgan, and the populations of the southwest are from Sistan and Baluchestan Province, and the populations of the northeast and east are collected from Nouth Khorasan, Khorasan Razavi, and South Khorasan.

Characters and measurements

Specimens were identified using the valid identification keys (Habeeb & Pouyani, 2016; Rajabizadeh, 2018), then 21 morphological features were measured to investigate morphological differences in each population and to identify the presence of sexual dimorphism (Yildiz, 2011; Schaetti *et al.*, 2014).

The morphometric and meristic characters were: snout-vent length (SVL), length of tail from the posterior edge of the cloaca to end of the tail (TL), the total length of trunk from the tip of snout to the tip of tail (TOL), length and width of the head (HL, HW), length of the snout from tip of snout to preocular scale (SL), the width of snout from loreal scales area (SW), length of frontal scales (FL), length and width of supraocular scales (SCL, SCW), eye diameter in the largest horizontal position (HED), number of supralabial scales (SUP), number of gular scales (GUL), number of ventral scales (Ventrals), number of dorsal scales rows in mid-body (Dorsals), number of dorsal scales rows at the end of body before tail (DAV), number of one row of tail scales in the direction of ventral (Subcaudals). We also considered four ratio characters as tail length to total body length (TL / TOL), head width to head length (HW / HL), snout width to head length (SW / HL), snout length to head length (SL / HL).

Gender determination in snakes was performed by measuring the depth of the cloaca bag using a sexing probe (McDiarmid *et al.*, 2012). Metric characters were measured using a meter (with an accuracy of ±1.00 mm) and digital caliper (with an accuracy of ±0.01 mm). The meristic characters were counted under the stereomicroscope and, to reduce human error in counting traits, measurements were repeated three times for each specimen (Table 1).

Statistical Analyses

Measured morphological datasets were analyzed using SPSS Statistics V.26. The normality of the data was assessed using descriptive analysis; the normality of metric and ratios characters was determined using the Shapiro-Wilk test and the normality of meristic characters was determined using the Kolmogorov-Smirnov test, The independent samples test (t-test) and 2-independent samples analysis (U-test) were used for normally distributed and non-normally distributed characters, respectively. The dataset is generally divided into four operational taxonomic units (OTUs) based on geographical barriers such as the Zagros Mountains in the west and the Lot Desert or separation of the mainland by sea (Fig. 1). To evaluate the significant level of characters among OTUs, analysis of variance (ANOVA) was performed for normally-distributed characters, and the Kruskal-Wallis test was used for non normally-distributed characters. Principal component analysis (PCA) and Canonical variate analysis (CVA) were performed to determine the most important characters contributing to the variance among populations (Figs 3, 4). The same process was the repeated to determine which characters varied significantly by latitudes changes and a linear regression diagram of the character changes was drawn (Appendix 9), Subsequently, PCA diagrams were drawn for significant characters in three latitudes zone (Fig. 5).

RESULTS

The number of Gular scales showed a significant difference value between male and female specimens, but there was no difference between males and females in the other characters measured. Therefore, this difference may be due to different reasons such as the difference in the number of males and females in OTUs (e.g., in the population of southern Iran) or due to the age difference of the specimens. For this reason, we did not consider sexual dimorphism and examined Gular independently among OUTs (Fig. 2).

TABLE 1. Means±1 SE and ranges for descriptive parameters of 25 morphometric, meristic, and ratio characters measured in two species groups of *P. rhodorachis* and *P. ventromaculatus* collected from four geographic areas in Iran.

	Western Group (*n*=17)		Southern group (*n*=9)		Southeast group (*n*=15)		Eastern group (*n*=8)		
Characters	**Mean ± 1 SE**	**Range**	**Mean ± 1 SE**	**Range**	**Mean ± 1 SE**	**Range**	**Mean ± 1 SE**	**Range**	***P* value**
SVL	593.94±17.49	511-702	679.89±34.48	479-852	390.13±22.23	224-500	710.13±22.09	638-800	**0.000**
TL	254.35±7.59	207-306	212.56±18.04	105-263	159.13±13.8	60-264	251.38±15.74	179-295	0.000
TOL	849.65±24.67	731-1007	895.22±49.99	619-1117	544.07±35.93	286-750	963±30.24	850-1088	**0.000**
HL	16.16±0.47	13.7-19.5	19.31±1.08	14-26	12.44±0.57	8.8-16	19.23±0.77	16-23	**0.000**
HW	566.31±21.23	224-852	13.27±0.85	8-17	8.84±0.71	5-13.8	12.41±0.75	10-16	**0.000**
RL	6.25±0.24	4.8-8	7.7±0.58	5-11	4.42±0.22	3-5.6	7.36±0.5	5-9	**0.000**
RW	6.41±0.3	4.84-8.9	7.79±0.48	5-9.8	4.73±0.22	3-6	7.15±0.39	6-9	**0.000**
TL/TOL	0.3±0	0.28-0.32	0.24±0.01	0.15-0.26	0.29±0.01	0.21-0.38	0.26±0.01	0.2-0.31	**0.000**
HW/HL	0.65±0.02	0.53-0.92	0.69±0.03	0.57-0.84	0.7±0.04	0.55-1.05	0.65±0.03	0.56-0.8	0.426
RW/HL	0.4±0.01	0.33-0.52	0.4±0.01	0.36-0.45	0.38±0.01	0.33-0.45	0.37±0.01	0.33-0.42	0.321
RL/HL	0.39±0.01	0.33-0.44	0.4±0.02	0.29-0.44	0.35±0.01	0.29-0.42	0.38±0.01	0.31-0.42	**0.035**
FL	5.35±0.17	4.4-7	5.73±0.25	5-7.3	4.12±0.19	3-5	5.89±0.2	5.2-7	**0.000**
SCL	4.77±0.2	4-6.8	5.24±0.25	4.3-6.2	3.52±0.16	2-4.3	5.31±0.24	4-6	**0.000**
SCW	2.64±0.14	1.94-4	3±0.17	2-4	1.94±0.07	1-2.2	3.24±0.15	3-4	0.000
HED	3.47±0.14	2.4-4.3	3.64±0.2	3-4.7	2.73±0.13	1.7-3.8	3.83±0.2	3-4.8	**0.000**
SUP	8.94±0.1	8-10	9±0	9-9	8.93±0.15	7-10	8.88±0.13	8-9	0.951
Dorsals	19.31±1.08	14-19	19±0	19-19	18.6±0.27	15-19	19±0	19-19	0.202
DAV	12.88±0.12	12-14	13.33±0.24	13-15	13±0.14	12-14	12.88±0.23	12-14	0.257
GUL	3.76±0.22	2-5	5.11±0.35	4-7	4.53±0.32	3-7	4±0.42	2-6	0.024
Ventrals	222.35±4.05	190-263	211.22±3.13	195-225	204.8±6	154-236	220.25±6.23	196-239	**0.048**
Subcaudals	10.49±0.55	73-121	13.27±0.85	48-100	8.84±0.71	77-140	12.41±0.75	94-137	0.000

According to the analysis of variance (ANOVA), 12 out of 21 characters differed significantly ($P \leq 0.05$) among four groups of *Platyceps rhodorachis-ventromaculatus* species complex in Iran (Bold characters in Table 1), with all normally-distrubted characters showing significance in the one-way ANOVA. We also performed the Kruskal-Wallis test for nine non normally-distributed characters and found TL, SCW, and Subcaudals showed significant values (Table 2).

The lowest means metric values are related to the samples from Sistan and Baluchestan Province in southeastern Iran. In meristic characters, some of the specimens related to the populations from the southeast and west of Zagros showed a significant difference in the number of rows of dorsal scales with other specimens (Table 1). A principal component analysis (PCA) indicated that the first three components explained 86.42% of the cumulative variance and that SVL, TL and Ventral scales are highly diversified characters (Table 3).

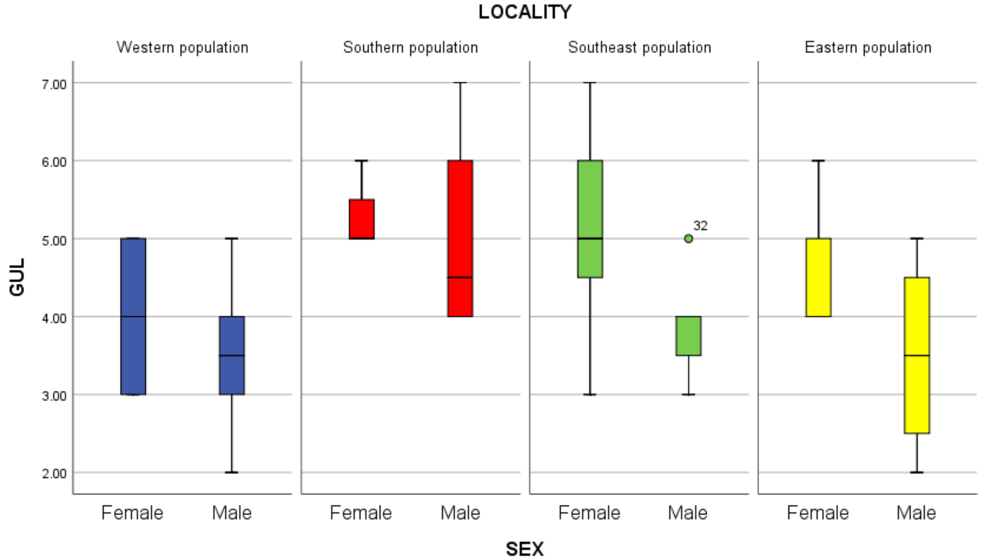

Figure 2. Box diagram showing the difference between the number of Gular scales in male and female *P. rhodorachis-ventromaculatus* specimens collected from 4 regions of Iran.

TABLE 2. Means and ranges for the abnormal character descriptive of 8 morphometric, meristic, and ratio characters measured in adult specimens, collected from four geographic areas across their distribution in Iran. Bold values refer to significant differences in characters among groups.

Characters	Mean	Asymp. Sig.	Kruskal-Wallis H	Range
TL	217.041	**0.000**	21.776	60-306
HW/HL	0.671	0.463	2.570	0.53-1.05
SW/HL	0.390	0.300	3.663	0.33-0.52
SCW	2.591	**0.000**	27.280	1-4
SUP	8.939	0.860	0.756	7-10
Dorsals	18.878	0.069	7.089	15-19
DAV	13.000	0.376	3.106	12-15
Subcaudals	109.875	**0.000**	22.823	48-140

Canonical variate analysis (CVA) showed that the first three functions explained 100% of the variance (Table 4) and, in the scatter plot of the first function relative to the third function, the OTUs are entirely separated from each other (Fig. 4).

Principal component analysis and Canonical variate analysis for 15 significant characters measured from adult specimens of *P. rhodorachis-ventromaculatus* species complex in Iran showed that the four populations studied are morphologically different from each other.

We also performed CVA for metric and relative characters. The results of a Wilks' Lambda test showed that the first three functions comprise 100% of the total variance (Appendix 7) (Appendix 5).

TABLE 3. Factor loadings of the first three Principal Components (PCs) elicited from a correlation matrix of 15 metric, ratio, and meristic significant characters measured in adult specimens.

Characters	PC1	PC2	PC3
SVL	0.953	-0.067	-0.179
TOL	0.848	0.443	-0.065
TL	0.114	0.961	0.147
HL	0.937	-0.074	-0.101
HW	0.886	0.190	0.254
SL	0.945	-0.302	-0.075
SW	0.908	-0.104	-0.134
FL	0.530	0.474	0.346
SCL	0.784	-0.145	0.367
SCW	0.714	0.357	0.227
HED	0.612	0.721	-0.269
Subcaudals	-0.287	0.692	0.515
Ventrals	-0.051	-0.608	0.782
TL/TOL	-0.368	0.899	0.230
SL/HL	0.811	-0.519	0.046
Eigen values	48.038	27.990	10.402
Cumulative variance %	48.04	76.02	86.42

TABLE 4. Function loadings of the first three canonical variate analyses elicited from a correlation matrix of 15 metric, ratio, and meristic significant characters measured in adult specimens.

Characters	CV1	CV2	CV3
SVL	2.840	1.360	-2.459
TL	0.942	3.434	-3.063
TOL	-2.014	-3.480	4.317
HL	1.208	1.443	2.668
HW	-0.819	-0.571	-1.089
SL	-1.997	-2.603	-3.873
SW	0.012	0.337	2.032
TL/TOL	-0.646	-1.593	0.843
SL/HL	0.904	1.243	1.709
FL	-0.074	0.122	0.125
SCL	0.235	0.153	0.312
SCW	0.412	-0.200	-0.964
HED	-0.299	0.238	0.208
Ventrals	-0.201	0.143	0.400
Subcaudals	-0.105	0.740	0.130
Eigen values	4.723	1.953	0.284
Cumulative variance %	67.9	95.9	100

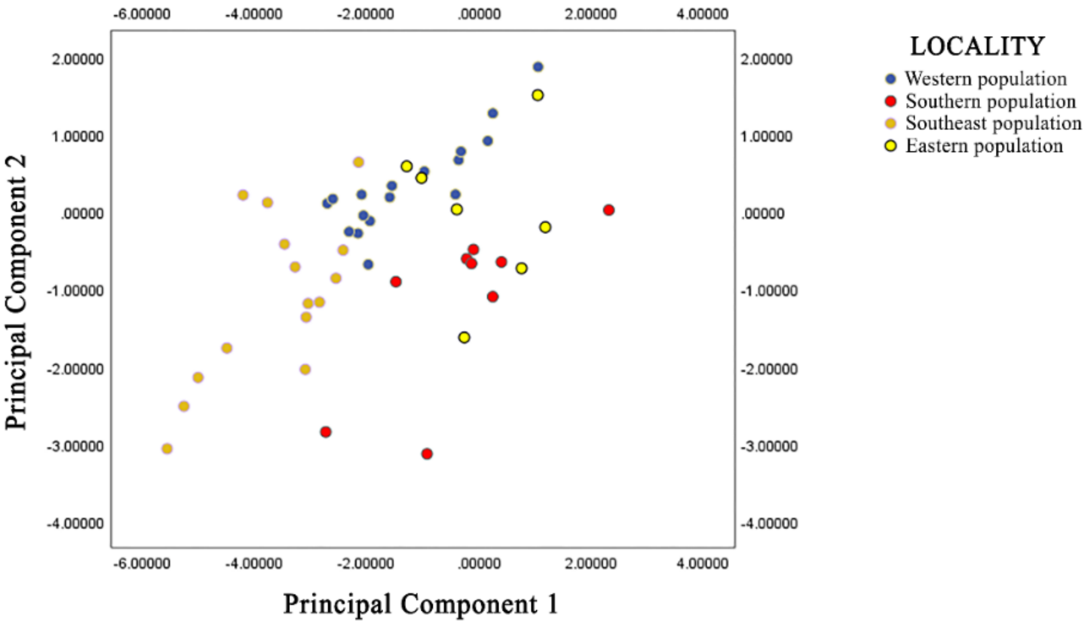

FIGURE 3. The scattered plot of the first and second principal components for 15 metric, meristic, and ratio statistically significant morphological characters in *P. rhodorachis-ventromaculatus* species complex from Iran.

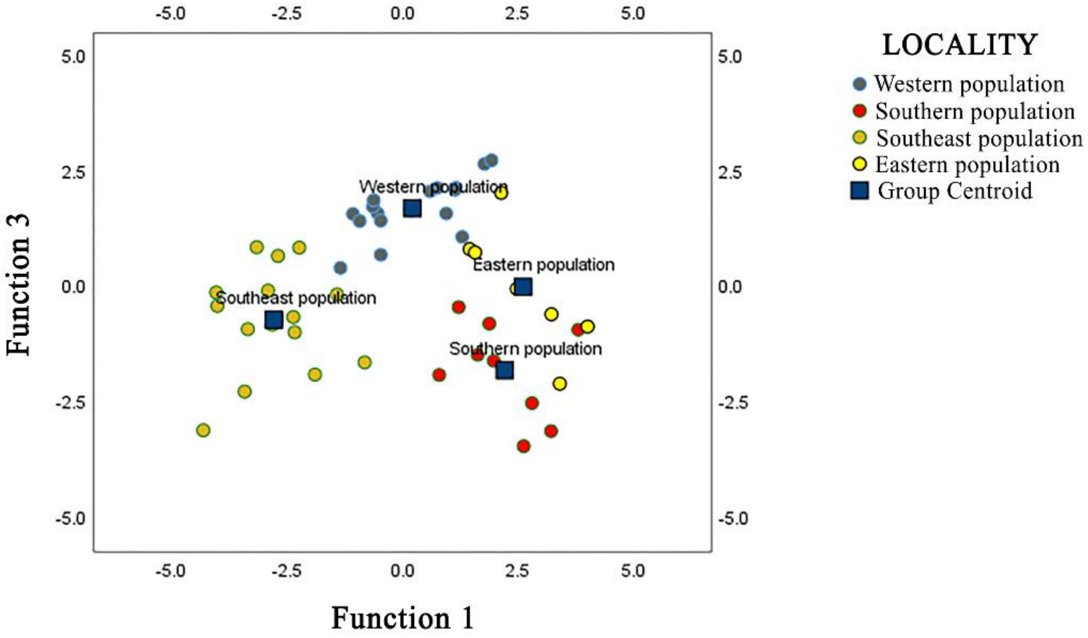

FIGURE 4. The scattered plot of the first and second Canonical components for 15 metric, meristic, and ratio statistically significant morphological characters in *P. rhodorachis-ventromaculatus* species complex from Iran.

Canonical variate analysis (CVA) for meristic characters shows the same results. The number of ventral and Subcaudals scales has the most excellent effect on separating components from each other (Appendix 8). The scatter plot also clearly separates the Group Centroids (Appendix 6).

We also divided the specimens into three different groups based on latitude in Iran and investigated the diversity of morphological patterns of *P. rhodorachis* and *P. ventromaculatus* using metric, ratio, and meristic character analysis (25° N to 29° N, 30° N to 35° N and 36° N to 37° N; Fig. 1).

For this purpose, we used the ANOVA test for the analysis of normally-distributed characters (Appendix 2) and the Kruskal-Wallis test for the analysis of non-normally distributed characters (Appendix 3). Then we performed principal component analysis (PCA) on nine significant characters (Appendix 4; significance level <0.05); the scattered plot of the first and second principal components can be seen in (Fig. 3).

PCA and Linear regression plots obtained for nine significant characters in three latitude zone in Iran (Fig. 5; Appendix 9) show a significant relationship between latitude and character values.

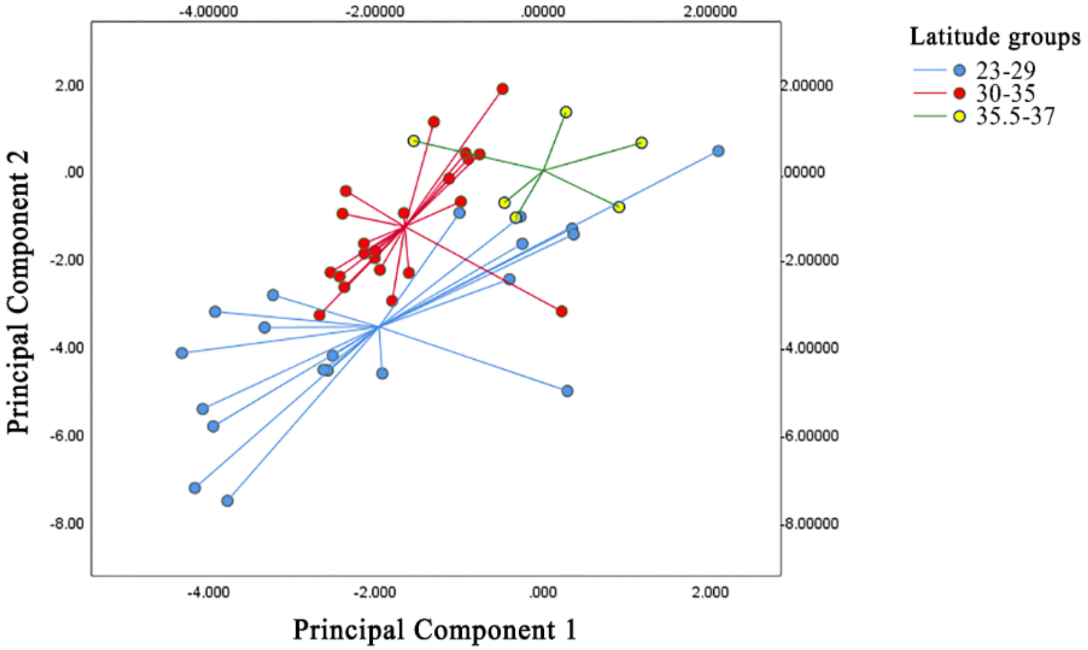

FIGURE 5. Scatter plot of the first and second principal components for 9 significant characters relative *Platyceps rhodorachis-ventromaculatus* grouped in three latitude zone in Iran.

DISCUSSION

Differentiation of four Operational Taxonomic Units (OTUs) based on the distinction between morphological characters in Iran determined that SVL is the leading cause of diversity among populations. SVL in both PC and CV analyses had the highest effect on population separation. SL in PCA and TOL in CVA are determining factors of the degree of separation of populations. The most prominent character with the most impact on morphological variation in snakes (i.e., snout to vent length) shows a severe clinical change (Table 1).

The southeastern population of Sistan and Baluchestan Province showed significant differences with other populations; the differences detected in this morph do not seem irrelevant. For example, the significant difference observed in latitude changes showed high alignment with morphological results and confirms morphological separation to some extent.

Morphological differences between the populations of the West Zagros Mountains and the Southeast with the rest of the populations can be considered due to the existence of geographical barriers in the distribution range of these populations. One of these barriers is the Zagros Mountains and the impact of this barrier on reptile diversity in Iran has been demonstrated many times. Zagros, which belongs to the southern part of the third Mesozoic orogeny in Iran, was created by the opening of the Red Sea with the movement of Arabian plates and now acts as a natural barrier against Iranian reptile species (Falcon, 1974; Kafash *et al.*, 2020). Usually, the western and eastern populations of the Iranian reptile fauna are separated by the Zagros Mountains (Haftlang & Lang, 2003).

The populations of Sistan and Baluchestan Province (southeast population) separated from other populations by the presence of the Dasht-e Lut in the West; the results of PCA and CVA approve this claim to a large extent. The southeastern population shows significant quantitative differences in morphological characters. Also, according to the CVA diagram (Fig. 4), the southeastern population is separated from other populations.

Multivariate analyzes indicate the separation of populations and indicated that the southeastern population of Sistan and Baluchestan Province is more separated from other populations (Fig. 4). As the possibility of establishing a link between the eastern OTU and the southern and western OTUs seems unlikely, meristic characters in the *Platyceps rhodorachis-ventromaculatus* complex are probably conserved and not a good choice for studying the biogeographical effects on the phonetic diversity in Jan’s Cliff Racer snakes. Snout length (SL) and Snout to vent length (SVL) had the most significant effect on OTUs separation (Appendix 7).

If we consider the above assumption, the variations observed in the southern population related to Qeshm Island could be a result of the greater diversity in the ecology and climate of the island compared with the mainland. This is a justification for the overlap of metrics (e.g., TOL, SVL, TL in Appendix 9) among these two populations (Simberloff, 1970; Kreft *et al.*, 2008).

Meristic characters (e.g., number of supralabials and suboculars, longitudinal position of the third reduction) show overlap among all examined populations (Appendix 9), which means the characters may not be affected by origin, gender, age, or macroecological conditions (Schaetti *et al.*, 2014).

Our impression is that the presence of this polymorphism in *P. rhodorachis-ventromaculatus* complex species, along with meristic characters stability, may indicate a phenotypic response to environment selection due to factors such as thermoregulation and response to bioclimatic parameters such as altitude and latitude (Henderson, 1997).

PCA results investigating the effects of latitude change on significant characters separated the groups into three main clusters (Fig. 5); this represents character changes based on latitude differences and largely confirms Bergmann's rule in the *P. rhodorachis-ventromaculatus* complex (Sand *et al.*, 1995).

In Iran, increasing latitudes correspond with decreasing average temperatures (Dastorani & Poormohammadi, 2012). Although there is evidence that snakes and other reptiles do not follow Bergmann's rule in other areas, the results of this study show that the metrics of the *P. rhodorachis-ventromaculatus* complex in Iran increase with increasing latitude (Pincheira-Donoso *et al.*, 2008; Feldman & Meiri, 2014).

The results of linear regression for the significant metric characters show the same results. The metric values increase with increasing latitude in the SVL, TOL, and TL characters (Appendix 9).

Non-observance of this relationship in the Qeshm Island population, which is located in latitude one (Fig. 1), as mentioned earlier, is due to the island ecosystem's variability compared to the mainland (Parker, 1949; Boback, 2003). The pattern is present among the other three populations, and as the latitude increases, so do the metric characters (Appendix 9). In meristic characters, this incremental pattern is less or not present at all; it seems that the number of scales is less affected by environmental factors and latitude.

Therefore, we conclude that the eastern population of Iranian *Platyceps rhodorachis-ventromaculatus* complex species in some characters show an intermediate between the populations of western Zagros and southern Iran. However, the population of Sistan and Baluchestan Province in all cases shows a significant difference from other populations. This discrepancy appears to be due to the separation of Sistan and Baluchestan by Iran's Central Desert Natural barrier. Differences in metric characters in *P. rhodorachis-ventromaculatus* complex species can be seen based on latitudes, but it seems that meristematic characters do not change under the influence of latitude and have a fixed value.

Systematic studies have shown that diversity among specimens representing the same species is influenced by ecological and genetic factors (Sexton *et al.*, 2009). Genetic studies in the *Platyceps rhodorachis-ventromaculatus* complex in Iran are needed to assess whether significant morphological differences are observed in this group affected by cline changes. Further studies in this field will also help assess the extent of morphological plasticity and taxonomic differences between populations with a broader perspective.

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RESEARCH ARTICLE **Open access**

Genetic Diversity of Urial Population in Northeast of Iran

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Abstract
Habitat eradication and loss of animal species have created a new international hazard for wildlife conservation. National parks are considered as suitable places that can serve dual functions of biodiversity conservation and ecotourism. As recommended by the Food and Agriculture Organization (FAO) and ISAG, microsatellites have been used for animal biodiversity assessment. For this reason, Iranian urials population genetic diversity was studied by analyzing of 10 microsatellite markers in 75 skeletal muscle samples that were collected from Tandooreh National Park, Northeastern of Iran. Species of samples validated by sequencing of the control region from mtDNA. Allelic frequencies for each locus in the population and different measurements of within-breed genetic variations were computed by the POPGENE32 software. The number of alleles per locus counted from five to eight, with an average of 6.1. The polymorphism information content was calculated between 0.66-0.74 with the average of 0.7. Observed heterozygosity ranged from 0.223 (MaF214) to 0.776 (OarFCB128) with an average about 0.584 while the average expected heterozygosity for all studied loci was 0.785 ranging from 0.765 (BM8125) to 0.807 (MaF36). High levels of expected heterozygosity can be attributed to some factors such as low level of inbreeding, low selection pressure, and high allele number. However, findings of the present study of the high variability of the Iranian urials showed the presence of a possible 'hot spot' genetic diversity for wild urial population in the Northeast of Iran. In conclusion, values of genetic diversity revealed that the Iranian urial population harbor unique and appreciable reservoirs of diversity.

Key words: *Wild sheep, Urial, Genetic Diversity, Microsatellites, Iran.*

INTRODUCTION

The growing amount of habitat extermination and loss of animal species have aroused a new international perception for habitat and wildlife conservation (Bulte and Rondeau. 2007). Iran, as a large country in the Middle East with diverse ecosystems, is one of the great areas for the conservation of biodiversity (Farshi and Shariati. 2017). As a result of undisciplined hunting and habitat elimination, Iran has already lost two of its most worthy carnivores, the Caspian Tiger and the Persian Lion (NBSAP 2000). Some other

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valuable species are threatened such as Persian crocodile, Persian Bactrian camel, Jebeer, etc (Afsharian et al. 2018; Azghandi et al. 2017; Javadmanesh et al. 2012), which indicate the immediate need for conservation management (NBSAP 2000).

However, one way of conserving biological diversity is the incorporation of environmental concerns in all regional and national development policies (Mace and Baillie 2007). For this goal, the national parks are unique areas of keeping biodiversity in natural ecosystems. Relatively broad natural areas holding specific characteristics and national significance from the geological, biogeographical, ecological, and scenic areas points of view are chosen as national parks with the purpose of keeping the natural and biological conditions, improvement of the populations of animals and vegetation sites. National parks are useful places for research and educational activities as well as ecotourism. In order to protect and maintain the biological diversity, ecological integrity, genetic reserves and scenic areas, residential and consumer utilizations are banned in these areas. (Ramírez and Santana 2019). Outside these areas, the biological diversity conservation is fragile and ecologically sustainable development policies have not been applied effectively (Jowkar et al. 2016).

Tandureh National Park, which is located at Northeast of Iran, has an area level of 37080 hectares. This park is the habitat of a vast variety of plants and wild animals.

Little is known about the actual numbers of Iranian urials. The International Union of the Conservation of Nature (IUCN) lists the *Ovis orientalis* vulnerable to extinction and their numbers are decreasing (Valdez 2008). It has been estimated that about 5000 – 6000 urials are living in Tandureh national park (https://en.wikipedia.org/wiki/Tandooreh_National_Park). Urial’s height is between 80 to 90 cm at the shoulder in males and weighs around 35 to 80 Kg. Females are smaller than males (Valdez 1976; Bon et al. 1993). The gestation period is between 150 – 180 days and single or twin lambs are born in mid-April to early May in Tandureh. Their life span is about 10 – 11 years. Their powerful hearing, sight, and sense of smell are all well-developed. They are extremely wary, based on quick detection of approaching danger and fight for their survival (Ptak et al. 2002).

The microsatellites have become a standard method for estimating genetic diversity (Nassiri et al. 2018; Peter et al. 2007). High levels of variation, merged with the simple analysis of the PCR, makes microsatellites as one of the most extensively used methods for genetic analysis in sheep (Sherrif and Aleymayehu 2018). Microsatellites have been suggested by the Food and Agriculture Organization (FAO) and International Society for Animal Genetics (ISAG) for the identification of animal biodiversity (Baumung et al. 2004; Cortes et al. 2022). Our study was based on the genotyping of ten microsatellite loci in 75 wild sheep samples from Tandureh National Park’s Urials to estimate the genetic diversity.

MATERIAL AND METHODS

Study area

The studied population was distributed over 9250 ha of Tandureh National Park which is located at Northeast highlands of Iran (37°29'E to 37°33'E, 58°33'N to 58°54'N) in Khorasan Razavi province near to Turkmenistan border. From the topography point of view, the area is a mountainous region with an elevation of 900-2586 m. Harirroud, Kashafroud, and Daroungar are the three main rivers around this park. Climatically, Tandureh is a cold rainy area during winters and dry hot summers depend on Siberian and Mediterranean high-pressure circulations. The average annual percipitation is about 370 mm. An annual temperature difference is relatively high as July (the hottest month) has an average temperature of 34.15°C while temperature falls down to 2.7°C in January. From a geological point of view, Tandureh belongs to Koppedaq zone and consists of limestone, sandstone, marn, shale, and conglomerate. The human population density within the park is near zero.

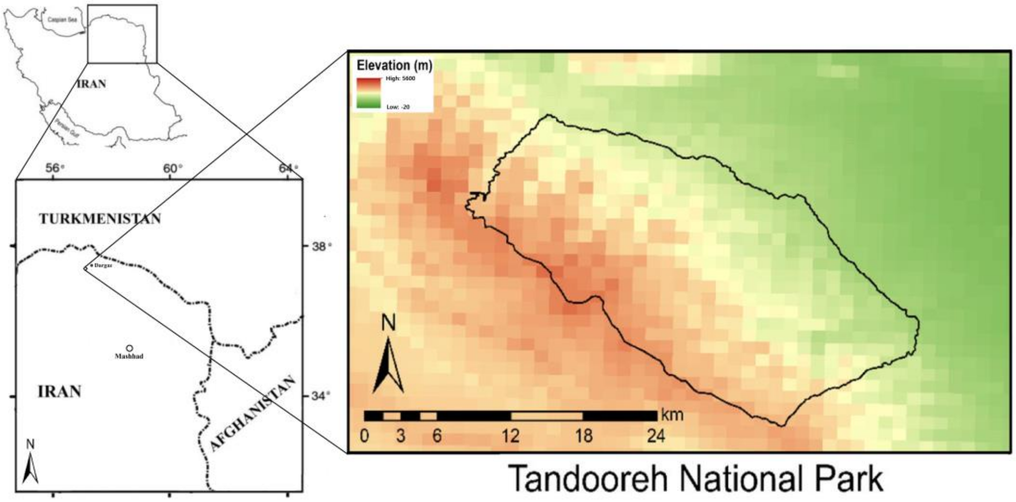

FIGURE 1. The study area.

Sample collection

Meat samples were collected from 75 individuals of legally harvested Iranian urials by hunters from three geographic regions of Tandureh National Park by the permission of Department of Environment, Khorasan-e-Razavi, Iran. Small pieces of skeletal muscle tissues were stored at -20°C as soon as possible.

DNA extraction

Wizard SV Genomic DNA Purification Kit (Promega, USA) was used for DNA extraction according to the manufacturer’s instruction. The quantity and purity of extracted DNA samples were assessed using the NanoDrop ND1000 spectrophotometer (Thermofisher Scientific, USA) and electrophoresis in 0.8% agarose gels. Then DNA samples were diluted to 10 ng/µL concentration using TE buffer (10mM Tris-HCl, pH 8.0, 0.1mM EDTA).

Species validation by mtDNA sequencing

To confirm the species of each sample, a 1075 bp from the control region of mitochondrial DNA was amplified, sequenced, and sequence homology was analyzed by the NCBI BLAST tool (Altschul et al. 1990) based on a method described by Hamadalahmad et al. (2020).

Microsatellite genotyping

Ten microsatellite markers were chosen for this study (Table 1) based on a recommendation by the International Society of Animal Genetics (ISAG) under FAO's MoDAD program for sheep diversity studies (http://dad.fao.org/).

Individual microsatellites were genotyped by polymerase chain reaction (PCR). All primer sets were amplified in separated reactions. Multiplexing information, primer sequences, size ranges, and PCR programs of the microsatellite markers are accessible from the FAO website (http://dad.fao.org/en/home.htm) and shown in Table 1. PCR amplification was accomplished in a total volume of 25 µl containing 2.5 µl of 10X reaction buffer, one unit of Platinum *Taq* DNA Polymerase (Invitrogen, USA), 0.2 mM each of dNTPs, 1.5 mM MgCl2, 10 pmol of each primer and 10 ng of extracted DNA. According to the MoDAD project, thermocycler condition was: primary denaturation at 94°C for 3 min, 30 cycles of 30 s at 94°C, 75 s at the specific annealing temperature (Table 1) and followed by a final extension for 5 min at 72°C. The PCR was performed with no extension step based on the MoDAD project data. Genotyping was performed by electrophoresis on an 8% denaturing polyacrylamide gel at 75 W (Bio-Rad, USA) and visualized by silver nitrate staining method (Bassam and Gresshoff 2007). Allele sizes were approximated using Invitrogen™ M12 and M50 bp DNA Ladders (Invitrogen, USA).

TABLE 1. Microsatellite markers and sequencing primers used in this study.

Locus	Accession no.	Chromosome	T_a	Primer
OarFCB128	L01532	2	64	F: CAGCTGAGCAACTAAGACATACATGCG R: ATTAAAGCATCTTCTCTTTATITCCTCGC
McM527	L34277	5	63	F: GTCCATTGCCTCAAATCAATTC R: AAACCACTEACTACTCCCCAA
OarFCB304	L01535	19	67	F: CCCTAGGAGCTTTCAATAAAGAATCGG R: CGCTGCTGTCAACTGGGTCAGGG
MAF36	M80519	22	65.5	F: TTGCGAAAGTTGGACACAATTGAGC R: CATATACCTGGGAGGAATGCATTACG
MAF65	M67437	15	64	F: AAAGGCCAGAGTATGCAATTAGGAG R: CCACTCCTCCTGAGAATATAACATG
MAF214	M88160	16	69.5	F: GGGTGATCAGGGAGGTTTTGGAGG R: AATGCAGGAGATCTGAGGCGGACG
OarHH47	L12557	18	63	F: TITATTGACAAACTCTCTTCCTAACTCCACC R: GTAGTTATI'TAMAAAATATCATACCTCTTAAGG
CSSM031	U03838	23	56.5	F: CCAAGTITAGTAC'TTGTAAGTAGA R: GACTCTCTAGCACTITATCTGTGT
BM8125	G18475	17	63	F: CTCTATCTGTGGAAAAGGTGGG R: GGGGGTTAGACTTCAACATACG
OarCP34	U15699	3	66	F: GCTGAACAATGTGATATGTTCAGG R: GGGACAATACTGTCTTAGATGCTGC
Control region	NC_001941	mt	58	F: AACTTGCTAAAACTCCCAAACATAC R: GTTGGAGTATGAATTTGAGTATTGAG

Statistical analysis

Allelic frequencies calculated once genotypes were determined. Allele frequencies for each loci were calculated by dividing the number for each allele by the total number of alleles (for every specific loci). Parameters of within breed genetic variations like expected and observed heterozygosity, polymorphic parameters (the number of actual alleles and the number of effective alleles) and Shannon index were calculated by POPGENE32 software (Yeh et al. 1999).

The intra-breed genetic variation was calculated based on observed heterozygosity (Ho) and the mean unbiased estimates of gene diversity (He) (Nei 1978). To assess the population's genetic structure, all F-statistics parameters (Weir and Cockerham 1984) were estimated using POPGENE32 computer program.

RESULTS AND DISCUSSION

Species validation

Sequencing and BLAST results showed that all samples belonged to the Transcaspian urial (*Ovis vignei*); Data not shown (Wilson, 2005). This urial sheep currently inhabitant in Turkmenistan, Uzbekistan, northern Iran, western Kazakhstan, Afghanistan, north Pakistan (Rezaei et al. 2010). The geographic distribution of wild sheep showed that naturally, Iran was the only country that inhabited two species of wild sheep with overlapping habitats. In addition, hybrids of these species, *O. vignei* and *O. orientalis* were reported (Nadler et al. 1973).

It has been shown that the control region of the mitochondria in sheep contributed the highest quantity of variation among other fragments of mtDNA (Meadows et al. 2011). Therefore, it can be concluded that this segment might be the best candidate for species identification. Although, there are other fragments have been used for diversity estimation or species identification, e.g. COXI and CytB in wild sheep as well as other species (Azghandi et al. 2017; Javadmanesh et al. 2017).

TABLE 2. True and effective number of alleles.

Locus	Size Range (bp)	${}^{*}n_a$	${}^{**}n_e$	***PIC	Shannon index (H')
OarFCB128	100-128	6	4.9901	0.72	1.6733
McM527	160-184	6	4.8254	0.74	1.6796
OarFCB304	154-191	5	4.4193	0.67	1.5370
MaF36	84-116	8	5.0314	0.71	1.7231
MaF65	160-184	6	4.4897	0.71	1.6297
MaF214	175-267	6	4.1961	0.67	1.5744
OarHH47	126-146	5	4.1977	0.66	1.5121
CSSM031	136-171	7	4.8033	0.74	1.7056
BM8125	108-120	6	4.1614	0.66	1.5490
OarCP34	105-133	6	4.5072	0.68	1.5779
Mean		6.1	4.5622	0.70	1.6162
SD		0.8756	0.3311	0.03	0.0758

${}^{*}n_a$ = Observed number of alleles
${}^{**}n_e$ = Effective number of alleles
***PIC= Polymorphism information content

Microsatellite loci

All ten microsatellites were amplified in the designed PCR reactions. All studied microsatellite loci were polymorphic and size ranges of amplified alleles were in agreement with the FAO report (2004). Electrophoresis images of all loci are presented in the supplementary material.

The polymorphism information content (PIC) was calculated between 0.66-0.74 with the average of 0.7. The number of alleles per locus counted from five (OarFCB304, OarHH47) to eight (MaF36) for all loci, with an average of 6.1 (Table 2). These results indicated a considerable level of polymorphism among studied loci since all of calculated PICs are above 0.5 (Botstein et al. 1980). We observed a high positive correlation between PIC and both effective allele number and average heterozygosity, 0.84 and 0.81, respectively. It could be inferred that all loci were informative and selected ten loci could be used as an effective panel for estimating genetic diversity.

Allele number is a measure of genetic diversity that has a direct impact on breed development within a species (Buchanan et al. 1994). A total of 61 alleles were detected by the ten microsatellite markers. Also, the highest mean effective number of alleles (n_e) was 5.0314 for MaF36 locus (Table 2). The Chi-square and likelihood ratio tests implemented to evaluate the population for Hardy– Weinberg equilibrium (HWE). Two of independent tests for Hardy-Weinberg equilibrium were rejected at $p < 0.05$. Excess of homozygotes in MaF214 caused its deviation from HWE ($P < 0.05$). On the other hand, a slight excess of homozygotes in OarFCB304 did not affect HW proportions and this locus was still in HWE ($P < 0.05$). In other studied loci, there was not any excess of homozygotes; therefore, no significant departure ($P < 0.05$) from HWE proportions was revealed in these loci. In general, observed HWE deviations were not persistent across all studied loci.

Genetic variation

Observed heterozygosity (H_o) varied from 0.223 (MaF214) to 0.776 (OarFCB128) with an average of about 0.584 while the average expected heterozygosity (H_e, gene diversity) for all loci was 0.785 with variation between 0.765 (BM8125) and 0.807 (MaF36). MaF36 showed the highest level of intrapopulation variation in terms of expected heterozygosity, while BM8125 displayed a lower variability than the other loci. The average means for different genetic diversity parameters, implied that all the studied loci have a high level of genetic variability. All loci were in the relatively similar level of within-breed diversity in terms of H_e ($P < 0.05$).

High levels of expected heterozygosity can be attributed to some factors such a low level of inbreeding, relatively low selection pressure, and high allele number. Since wild sheep breed naturally and no voluntary control made on their reproduction process, consequently, no artificial selection pressure or inbreeding can be imagined. Similar results indicating a high level of diversity was reported on wild sheep population from Northeastern of Iran, however, this study was conducted by the sequencing of the complete length of the control region of mtDNA (Hiendleder et al. 2012).

According to the 95% confidence interval of within-population inbreeding estimates (F_{IS}) were positive in all loci and significantly different from zero ($P < 0.05$). Although these samples did not allow for testing of Mendelian inheritance of the microsatellite alleles, the results revealed that the deficiency of heterozygotes at MaF214 and OarFCB304 microsatellites could be explained by the existence of non-amplifying null alleles. Positive F_{IS} estimates for two studied microsatellites could be assumed as an indication for inbreeding.

Although it is challenging to consider the exact basis of this departure, however, the existence of low-frequency null alleles segregating at these loci may be acknowledged as a possibility (Peter et al. 2007). This deviation could also be connected to reasonably high positive F_{IS} values (Mukesh et al. 2004; Mukesh et al. 2006) observed in the studied population ($P < 0.05$, Table 3). The low number of heterozygotes and high number of homozygotes might be attributed to several aspects such as population heterogeneity, sample relatedness, or null alleles (Peter et al. 2005). However, the main reason for significant F_{IS} values in this population might be the relationship of few individuals under range conditions.

TABLE 3 The observed (H_o), expected (H_e), average heterozygosity and Wright's fixation index (F_{IS}) for different loci.

Locus	**H_o**	**H_e**	**Average Het.**	**F_{IS}**
OarFCB128	0.7763	0.8049	0.7996	0.0291
McM527	0.6579	0.7980	0.7928	0.1701
OarFCB304	0.4605	0.7788	0.7737	0.4048
MaF36	0.5658	0.8066	0.8012	0.2939
MaF65	0.6184	0.7824	0.7773	0.2044
MaF214	0.2237	0.7667	0.7617	0.7063
OarHH47	0.6316	0.7668	0.7618	0.1709
CSSM031	0.6711	0.7971	0.7918	0.1525
BM8125	0.6053	0.7647	0.7597	0.2033
OarCP34	0.6316	0.7833	0.7881	0.1883
Mean	0.5842	0.7849	0.7798	0.2524
SD	0.1497	0.0160	0.0159	0.1865

Heterozygote deficiency analysis showed that one of the studied loci displayed significant deviations from HWE ($P < 0.05$). It may be due to the presence of low-frequency null alleles segregating at these loci (Peter et al. 2005). This lack of deviation could also be connected to a high negative F_{IS} value (Mukesh et al. 2004) observed in this study ($P < 0.05$).

From the demographic structure of this population, it is obvious that rams breed with some of the ewes in the herd, as male and females grazed together and no supervised mating can be imagined. On the other hand, in industrial husbandry systems, generally, few males breed with all the females in the herd. Using individuals with relationship for reproduction may cause high heterozygote deficiency observed in those systems. Generally, a small sample size in this study might be a cause of individual relatedness in the sample and it might be the reason for the heterozygote deficiency in some loci reported in this study.

Major efforts caused by unsuitable management practices to recover individuals and ensure intentional breeding, mating can cause an unwanted damage to the biodiversity (Goyache et al. 2003). Because of limitations of sampling such as collecting samples from one region, and the small size of samples, the hidden genetic structure could not be ruled out.

The present results also displayed the high allelic variation in Iranian urial as represented by mean expected heterozygosity (0.765 to 0.807). The average Shannon index across all loci was calculated 1.62 ± 0.08 (Table 3). Direct comparison of diversity measures from other studies is difficult mainly due to quantity of analyzed individuals and differences in marker sets. However, findings of this study of high diversity of the Iranian urials found in this study is in accordance with other sheep biodiversity studies based on microsatellite markers. Domestic sheep breeds originating from the Near East, and neighboring regions such as the Southeast Europe and Caucasian regions, show elevated levels of genetic diversity because they have retained some diversity from the ancestral wild species, mouflon and urial (*Ovis orientalis*) (Tapio et al. 2006; Peter et al. 2007).

We presented a new report on the genetic diversity of the Iranian urial by analyzing microsatellite loci recommended by ISAG.

In conclusion, estimates of genetic diversity indicated that the Iranian urial population located at the Northeastern of the country; contain a unique and estimable reservoir of biodiversity.

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REVIEW ARTICLE **Open access**

A review on the genus *Trapelus* Cuvier, 1817 (Sauria: Agamidae) in the Iranian Plateau

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Abstract
The genus *Trapelus* Cuvier, 1817, belonging to the subfamily Agaminae, is described with 13 species. There are absolutely four species in Iran: *T. agilis, T. ruderatus, T. sanguinolentus* and *T. persicus*. Within the vast distribution range of *Trapelus*, current and past climate and vicariance events have significantly influenced their diversification patterns and distributions. Recently, the first and the most comprehensive assessment based on molecular genetic analyses within *T. ruderatus* in western Asia including Iran, Turkey, Syria, and Iraq, has shown that Iranian populations have slight divergence whereas are undoubtedly isolated from Turkey's population. Also, this study introduced the populations of southern Iraq as belonging to *T. persicus*. In this review, we have tried to have a complete examination and comparison based on all of the available relevant literature about the taxonomy, phylogeny, evolutionary history, and biogeography of the genus *Trapelus* in the Iranian plateau and neighboring regions, as well as we examined the controversial nomenclature changes and their reasons in some species and subspecies of this genus. We will also give a brief overview of our unpublished dissertation on comparative morphology and dimorphism in the genus *T. ruderatus*. In the end, we present an identification key based on the latest changes in the classification and taxonomy of the genus.

Key words: *species, Iranian plateau, systematics, Zagros Mountain.*

INTRODUCTION

The family Agamidae composed of about 350 species and 50 genera that inhabit the old world (Uetz et al., 2022), including continental Africa, Australia, and Southern Asia, with some extending into the warmer regions of Europe (Pal et al., 2018). The centers of origin of agamid lizards have been hypothesized in The Southeast Asian-Indonesian and Australian-New Guinean tectonic plates by Moody (Ananjeva N. B, 2011a.). Also, new studies on the Asian fauna using molecular methods suggested that taxonomic diversity in agamid lizards, particularly in South and Southeast Asia to be higher than was estimated before (Ananjeva, 2011). One of them is the genus *Trapelus* Cuvier, 1817 belonging to the subfamily Agaminae Gray, 1827 of the family Agamidae. Based on the phylogenetic, morphological, and fossil data, it can be concluded that the ancestors of the Agaminae subfamily occupied Africa through the Arabian Peninsula (Kissling, 2016). Melville et al. (2008) confirmed the Gondwanan origin of the subfamily Agaminae in the Indian subcontinent. They also concluded that the early radiation of Central Asian Agamids is related to the onset of drying during the Late Oligocene and Early Miocene. Moody

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revised the genus *Trapelus* Cuvier, 1817 in the family Agamidae, (Ananjeva, 2011) later for a more inclusive assessment; these studies were followed by Frost and Etheridge (1989). Molecular and phylogenetic analysis can increase the understanding of the composition of genera of agamid lizards, solve the problem of phylogenetic evaluation of morphological characters, and contribute to creating identification keys (Ananjeva et al., 2011). Four species of *Trapelus* occur in Iran: *Trapelus agilis* (Olivier, 1804), *Trapelus ruderatus* (Olivier, 1804), *Trapelus persicus* (Blanford, 1881), *T. megalonyx* (Gunther, 1865), and *Trapelus sanguinolentus* (Pallas, 1814) (Rastegar-Pouyani, 2008, Ananjeva, 2013, Shahamat et al., 2020). In addition, *T. agilis isolepis* has recently been considered as a full species based on molecular investigation by Shahamat et al. (2020). *Trapelus* are unique lizards with short and thick heads, powerful legs, comparatively long tails, a small, deeply sunk tympanum with spiny scales above the ear opening, and a well-formed acrodont dentition for feeding on a wide range of foods. The genus *Trapelus* has not been fully investigated, leading to considerable nomenclature complications (Rastegar-Pouyani, 2000, 2005). Based on extensive morphological work, including work on different populations of *T. agilis* covering almost all of its range, Rastegar-Pouyani (1999) reported four subspecies within this species complex: the nominal subspecies *T. a. agilis*, *T. a. pakistanensis*, *T. a. sanguinolentus* and *T. a. khuzestanensis*. Later *T. a. sanguinolentus* was raised to a full species (Ananjeva et al, 2013). This species complex has recently been investigated in Iran based on morphological and ecological features and molecular phylogeny (Shahamat et al, 2019, 2020). Genetic distances between different populations based on two mitochondrial genes (Cytb and ND2) suggest that some of them are obviously differentiated from the others (e.g *T. khuzestanensis, T. isolepis*).

TAXONOMIC ACCOUNT

Order Squamata Oppel, 1811
Family Agamidae Gray, 1827
Subfamily Agaminae Gray, 1827

Genus *Trapelus* Cuvier, 1816

The genus *Trapelus* is a Saharo-Sindian (Arabian) genus that encompasses about 15 species (Wagner, 2011). The genus occurs in the deserts and steppes of North Africa, the Arabian Peninsula, Southwest and Central Asia (Rastegar-Pouyani, 1999; Melville, 2008). The highest diversity of *Trapelus* occurs in the southern lowlands of Southwest Asia. However, it has one or more species widely distributed in the highlands of Central Asia (Anderson, 1999, Mohammed et al., 2015). Since the closest relative of most African *Trapelus* are found in Pakistan and India the most likely scenario is that their ancestors colonized Africa from Asia via the Arabian Peninsula, including Bab al-Mandab and Sinai (Kissling, 2016). An Agamid lizard of *Trapelus*, which has an Afro-Arabian origin (Macey et al., 2000), is an old genus and has been in Asia since the Miocene. The time of divergence between its species in Africa (*Trapelus savignii*), Arabia (*T. persicus*), the Iranian Plateau (*T. agilis*), and the Caspian-Aral Basin (*Trapelus sanguinolentus*) is estimated to be about 8.3 to 10.7 million years ago (MYBP) (Macey,2004). Agaminae diverged due to Miocene events (Macey, 2006). The subfamily Agaminae, including the genus *Trapelus*, is probably the youngest of the African radiations, with diversification beginning around 23Mya (Kissling2016). The molecular dataset suggests that the *Trapelus* complex originated in the late Oligocene (30 Mya) and then diversified during the early to middle Miocene (22-13 Mya) (Shahamat, 2019). The age of the genus *Trapelus* is estimated to be similar to *Phrynocephalus* (Melville, 2008). It is mainly found in bushes; Leviton (1963) also reports them in thorn bushes (in Iran); however, they have limited climbing ability. Males are often perched on rocks, clay molehills, or other high places (Masroor, 2011). Habitat specifics have been reported to affect the locomotor's performance and thermoregulation (Scott 2005). Habitat preference may differ between males and females of the same species (Eskandarzadeh et al., 2015). In general, the genus Trapelus has created significant difficulties for taxonomists due to the numerous species complexes identified in this genus, just as, Rastegar-Pouyani has had challenges in examining its species (Wagner, 2011).

Description: Tympanum exposed, small with a deep acoustic duct; caudal scales are not arranged as oblique rows; Adults are medium-sized and only males with precloacal scales. Dorsal scalation is homogeneous or heterogeneous (Leviton et al., 1992). These lizards are characterized by short, thick heads (Moody, 1980), in contrast to the species of the genus Agama, and by deeply sunken tympanums with a few spiny scales around the ear opening (Shahamat et al., 2020). The larger head length to head width (HL/HW) ratio in males makes them stronger in biting and more successful in the battle between males and mating. (Rastegar-Pouyani et al., 2013; Eskandarzadeh et al., 2015). This sexual size dimorphism (SSD) is common in lizards, without a genetic basis and result from a wide range of local environmental processes. (Butler 2002). Sexual size dimorphism (SSD) patterns are in *Trapelus*, but it varies in different species. In some species, males are larger than females, and in others, females are larger than males (Eskandarzadeh et al., 2015), which will be discussed.

Trapelus agilis (Olivier, 1807)

Type locality: Iraq, vicinity of Baghdad (Uetz, 2022).

Trapelus agilis commonly known as the Brilliant Ground Agama belongs to central and south Asia, such as the lowlands of southwest Iran (Rastegar-Pouyani, 1999, Khan, 2004; Heidari and Kami, 2009), southeast Pakistan, adjoining northwestern India and the only record in western India in the state of Gujarat since the late 1880s by Murray, was recently confirmed. This species was reported in habitats consisting of sparse xerophytic vegetation, and so far, no record of *T. agilis* has been recorded in the adjoining areas of Gujarat state (Joshi et al., 2017). Also, this challenging and widespread *T. agilis* complex in the Caspian-Aral Basin is divided into two different populations by the Caspian Sea (Macey, 2004; Rastegar-Poyani, 1999, 2005; Shahamat, 2020). In Iran, the most extensive distribution is in the central and eastern regions of the plateau (Yosefabadi et al, 2021). According to Mertens (1969) and based on the comparison of morphological characteristics of samples from Baluchistan (small, pale, long-legged, and large scales on the crown and temporal region), Karachi, and Kandahar, *T. agilis* is a composite of several species. Nilson and Andrén (1981) accepted the subspecies identifications of *Trapelus agilis* in the Iranian plateau. Moody (1980) determined *agilis* and *isolepis* as valid species. Although Anderson 1999 did not believe use of subspecific names for the *T. agilis* complex, some authors considered the three subspecies *T. a. agilis, T. a. isolepis,* and *T. a. sanguinolentus* in this complex (Anderson, 1999). Rastegar-Pouyani, 2005, regrarded *T. a. isolepis* as a synonym of *T a agilis*. Traditionally, four subspecies are recognized within the clade: *T. a. sanguinolentus, T. a. khuzestanensis, T. a. agilis,* and *T. a. pakestanensis* (Rastegar-Pouyani, 2005). *Trapelus a. agilis* from central Iranian Plateau, southern Afghanistan, and southwestern Pakistan; *T. a. sanguinolentus* from northeastern Iran, northern Afghanistan, and the Central Asian Republics of the former USSR as well as Daghestan; *T. a. khuzistanensis* from the lowland southwestern Iran; and *T. a. pakistanensis* from southeastern Pakistan as well as adjoining northwestern India. The analysis was based on significant metric and meristic characters and with a multivariate approach, especially PCA and CVA. The cluster analysis results confirm the existence of several distinct morphotypes that are in agreement with different taxonomic entities (Rastegar-Pouyani, 2005). Rastegar-Pouyani, (1999) believed that there were no accurate records of *T. agilis* from Iraq and introduced the new locality in the city of Isfahan (about 110 km southeast). However, since syntypes still existed, Ananjeva et al. (2013) retained the original type locality as it appears in Olivier's (1804) description. This complex with high genetic diversity diverges into several clades on the Iranian Plateau and Central Asia; the vicariance and dispersal events are the reasons for their species' differentiation from the late Oligocene (Rastegar-Pouyani, 2005; Shahamat et al., 2020). The first population separated from the central continuum as the western clade was *Trapelus ruderatus* (22Mya); *Trapelus persicus* diverged in 18 mya, and finally, *T. sanguinolentus* isolated at approximately 16 mya. (Shahamat et al., 2020).

Based on molecular phylogeny, *Trapelus agilis (senso lato*) can be divided into six genetic lineages in the Iranian plateau. The classification status of the studied populations was investigated using mitochondrial genetic markers. Different genetic markers are suggested for further help (Shahamat et al., 2020). Adaptation to the local conditions and geographic isolation by the uplifting of the Alborz, Zagros, and Kopet Dag mountains are main reasons for the isolation populations in Iran (Rastegar-Pouyani, 1999). The Western Zagros Mountains, northeastern and northwestern parts of the Central Iranian Plateau have the highest number of lizard species (Kafash, 2020). Bhatnagar et al. (2012) suggested that males and females are different in habitat selection, the males choose a shelter under rocks or bushes, and the females dig a burrow to live inside; as a result, the males have a more remarkable ability to change color to avoid a predator. Morphological characters and color patterns are different in both sexes; for instance, males have longer heads and thicker tails than females. In females, the dorsal region of the body has segmented traverse stripes with red or sombrous orange backgrounds. A dark blue color, mainly on the chest, flanks, and gular sac, and a black patch in the shoulder fold has been seen in males (Rastegar-Pouyani, 1998). However, the phylogenetic and taxonomic relationships of this complex are controversial. The key to identifying the subspecies of the *T. agilis* complex is provided bellow.

Description: This specie differs from all described Iranian congeners by its dorsal scalation homogeneous, subequal in size, without larger scales among them, and disposed of in irregular rows, the scales gradually become smaller towards the flanks, 52–103 scales around the middle of the body; gular sac variable, sometimes strongly developed in males to reveal the blue color; males with callous pre anal scales. The dorsal pattern is dark orange or red in gravid females. The female's body size is usually smaller than males and often has further scale counts in different body parts" (Rastegar-Pouyani, 2005).

SUBSPECIES ACCOUNTS

T. a. agilis (Olivier, 1807)

One of the distinct subspecies within the complex is *T. a. agilis*, distributed in the central Iranian Plateau, southern Afghanistan, and southwestern Pakistan, the distribution of these subspecies in the Plateau of Iran is in the southwest, central and northwest regions (Rastegar-Pouyani, 2005). *T. a. agilis* in southwestern and Central Iranian Plateau is the nominative subspecies (as the central core of the complex, which is morphologically most similar to Agama agilis, Olivier's syntypes) (Rastegar-Pouyani, 2007). It has the longest divergence time of the other populations, which is consistent with molecular analysis (Shahamat et al., 2018). Eco-geographical barriers had separated other populations such as *T. a. khuzestanensis*, Qom, and southeastern Iran from the central continuum (Rastegar-Pouyani, 1999). Ecological and morphological approaches also confirmed these differentiated populations (Shahamat et al., 2019). In morphological characters, the principal components include SVL (scales between eyes across the head), HL (length of the head), SAM (scales around the mid-body), and also the number of preanal callose scales and supralabials were significant characteristics. Since beneficial features such as morphological traits need more time to be selected by the natural selection mechanism after ecological niche separation, it can conclude that investigated groups did not have enough time to acquire differentiated morphological traits (Shahamat et al. 2019). The color changes of this species vary depending on the amount of sunlight; for example, it is darker in the cold or shade. Its habitat is a flat, open desert with clay or less sandy soil and scattered bushes or hills covered with vegetation (Minton, 1966). This species is characterized by having nuchal and temporal scales small; ear above with pointed scales; dorsal scales more or less equal in size, keeled; lateral and ventral scales slightly smaller than dorsals, keeled (indistinctly 76–103 keeled and mucronate around the body in Iran (Leviton & Anderson, 1963). tail round, slender, evenly tapering, with oblique scale rows. MASROOR 2012 has observed these characteristics in Pakistan; Males with gular sac and a row of 9-11 callose preanal scales; males and females of nearly equal size, male SVL to 110.2 mm, tail 176.3 mm; female SVL to 119.0 mm, tail 175.0 mm. A medium or short and often compressed tail in males (about 1.3-1.65 body length) and a unique color pattern in many Iranian plain's populations (yellowish cream with distinct or indistinct dorsal cross-stripes) (Rastegar-Pouyani, 1999).

***T. a. isolepis* (Boulenger, 1885)**

Type locality: “between Magas and Bampur, Iran"(Uetz,2022)

The populations of *T. a. isolepis*, distributed in eastern and southeastern Iran, southern Afghanistan, and south and western Pakistan, situations in areas east of the Hub River (in the southeastern range of the range) and in some areas adjacent to the northwestern Indian desert may differentiate it, with the river acting as a barrier to its distribution in the west. However, its geographical isolation does not always guarantee divergence (Rastegar-Pouyani, 2005). This group has been synonymized with *T. a. agilis* by Rastegar-Pouyani (1999). However, this author's multivariate analysis (especially PCA and CVA) in 1999 confirmed *T.a. isolepis* as a new taxon. In the cluster analysis, *T. a. isolepis* is placed as a subgroup of *T.a. pakistanensis*. It presents a three-grouped pattern that is less compatible with the conditions of geographic diversity within the *T. agilis* complex than the four-grouped pattern in the previous analysis. In addition, the clustering method is much more subjective than the ordination techniques (Rastegar-Pouyani, 2005). In a survey of two mitochondrial genes by Shahamat et al., 2020, they found that the highest distance of 15.1% for the ND2 gene exists between *T. isolepis*, which shows that this population is well differentiated from the rest of the *T. agilis* complex, which may be influenced by adaptation to local conditions. Considering the high genetic distance for the southeastern clade, including a local type population of *T. a. isolepis* (Boulenger 1885) in both the Baluchistan regions of Iran and Pakistan, suggested that this clade should become *T. isolepis*. It is currently considered as a subspecies (*T.a. isolepis*) which is separated from the main subspecies (*T.a. agilis*) by different morphological traits including body size medium to large (Max SVL about 105 mm), 60–85 scales around the body; weakly moderately keeled, strongly mucronate, dorsal scales with a rather abrupt change from the larger scales of the median to the smaller lateral (Rastegar-Pouyani, 2000). Molecular genetic analyses are in progress, and the results will be published in forthcoming papers.

***Trapelus agilis khuzestanensis*, Rastegar-Pouyani, 1999**

Khuzistan Ground Agama

Type locality: Iran, Khuzistan Province, 5 km northwest of Haft-Gel on the road to Shushtar. This subspecies has been named based on its distribution area in the lowlands of southwest Iran, Khuzestan province, which is sympatric with *Trapelus persicus* in these areas. However, the exact boundary between the nominal subspecies and this species has not been carefully studied (Smid, 2014). This species presumably also occurs in the bordering areas of Iraq (Rostegar-Pouyani, 1999). One of the most important and prominent factors that play a role in the evolution and divergence of this subspecies is vicariance; after the orogeny of Zagros in the late Miocene, a great variety of ecosystems with significant differences arose due to the complex topography of Zagros and its elevation changes (Mostafavi, 2021). Due to this orogeny event, *T.a. khuzestanensis* in southwestern Iran was isolated from the *T. a. agilis* to adapt to local conditions through natural selection. Rastegar-Pouyani, 1999 for solving the patterns of geographical diversity within and among the four mentioned species, employed uni- and multivariate analysis using 18 valuable morphological characteristics. All populations of this vast range were divided into four distinct groups of subspecies, including *T.a. khuzestanensis*. Given the morphological traits, this subspecies is separated from other populations by having more scales around the body, about 80-97; also, dorsal scales are slightly keeled and smaller as if Leviton and Anderson (1961) introduced the variation in the scale rows of different populations as an important statistical factor, a shorter head, and neck; dorsal scalation almost heterogeneous, small scales in dorsal and ventral; the mean number of subdigital lamellae under the fourth toe significantly lower; keeled or rogues upper head scales; one or two rows of callose preanal scales (absent or slightly developed in females); strongly compressed tail in males, and a particular dorsal coloration (yellowish-grey-cream with weak or without reddish-brown cross bars).

The morphological results of Shahamat et al., 2019 based on 15 morphological characters (the number of preanal callose scales and supralabials were the most important variables), also confirmed the separation of *T.a. khuzestanensis* from other central and northeastern groups. Also, the study of the ecological niche of *T. a. khuzestanensis* announced the separation of this group due to local adaptations and geological history. Ecological niche modeling result covers the habitat suitability of the current distribution range of *T.a. khuzestanensis*. Since after the differentiation of ecological niches, the selection of more valuable characters by natural selection requires more time (Nosil et al. 2005), morphology and ecology cannot confirm each other in the grouping and similarity of groups Shahamat et al. 2019.

Since morphology cannot show a high distinction among all populations of the species, Shahamat et al. 2020 investigated molecular markers based on sequences of two mitochondrial genes (Cytb and ND2) to report phylogenetic relationships, divergence times of the lineages, and intraspecific differentiation within this complex. *Trapelus agilis khuzestanensis* populations with the highest percentage of genetic distance (40%) in the Cytb gene indicated the most significant potential divergence to reach a separate species. Finally, they suggested considering the Persian Gulf's and southeastern Iran's coastal populations as distinct clades. *T.a. khuzestanensis* is the only subspecies in the *Trapelus agilis* complex, it’s morphological, ecological, and molecular results confirm each other to reach the full species level (Shahamat et al., 2019, Shahamat et al., 2020). However, the author suggested different taxonomic methods, such as karyotype analysis, microsatellite analysis, and geometric morphometrics, to improve the taxonomic status of the *T. agilis* complex on the Iranian Plateau. Figure 1b illustrates the distribution of these subspecies on the map.

***Trapelus agilis pakistanensis*, Rastegar-Pouyani, 1999**

Type locality: Gaj-River, Kirthar Range, southeastern Pakistan

The distribution of *T. agilis pakistanensis* is limited to the semi-desert regions of southeastern Pakistan to the border of the Indian Territory, which is parapatric with *T. agilis* in its eastern areas in Baluchistan province. The naming of this subspecies is based on its distribution area, which is mainly limited to Pakistan. The distribution of this taxon is restricted to the west by the Hab River (Rastegar-Pouyani1999). *T.a. pakistanensis* separated from the central chain in southeastern parts in the Pliocene (1.7-5 MYBP) and dispersed eastward to the northwestern Indian desert. Hence, the role of dispersal in the evolution of *T.a. pakistanensis* is prominent. But it is evident that still, much more work needs to be done, depending on many more specimens from critical areas in Pakistan, as well as between these populations to the west and the north, to resolve the problems in this wide-ranging complex (Masror,2011).

Employing multivariate statistical techniques also shows the distinction of these subspecies and reconfirms the previous classification decisions (Rastegar-Pouyani, 1999, 2005). This species is usually distinguished by different key traits from other populations, which include: body and head sometimes compressed (not depressed) in males; males almost always with one row of callose preanal (rarely a second undeveloped row may present); females without callose preanal: dorsal scales relatively flat, subequal to homogeneous, distinctly keeled throughout and mucronate, grading into small dorso-laterals rather abruptly (especially in males), 67-83 around the body; ventral scales also often distinctly keeled in males; body and limbs often strongly slender and head distinctly pointed (in adult males); tail often strongly compressed in adult males, its length more than 1.55 of body length; the mean number of supra-labials and infralabials significantly lower than those of the other subspecies; a rudimentary nuchal crest often present (Rastegar-Pouyani 1999).

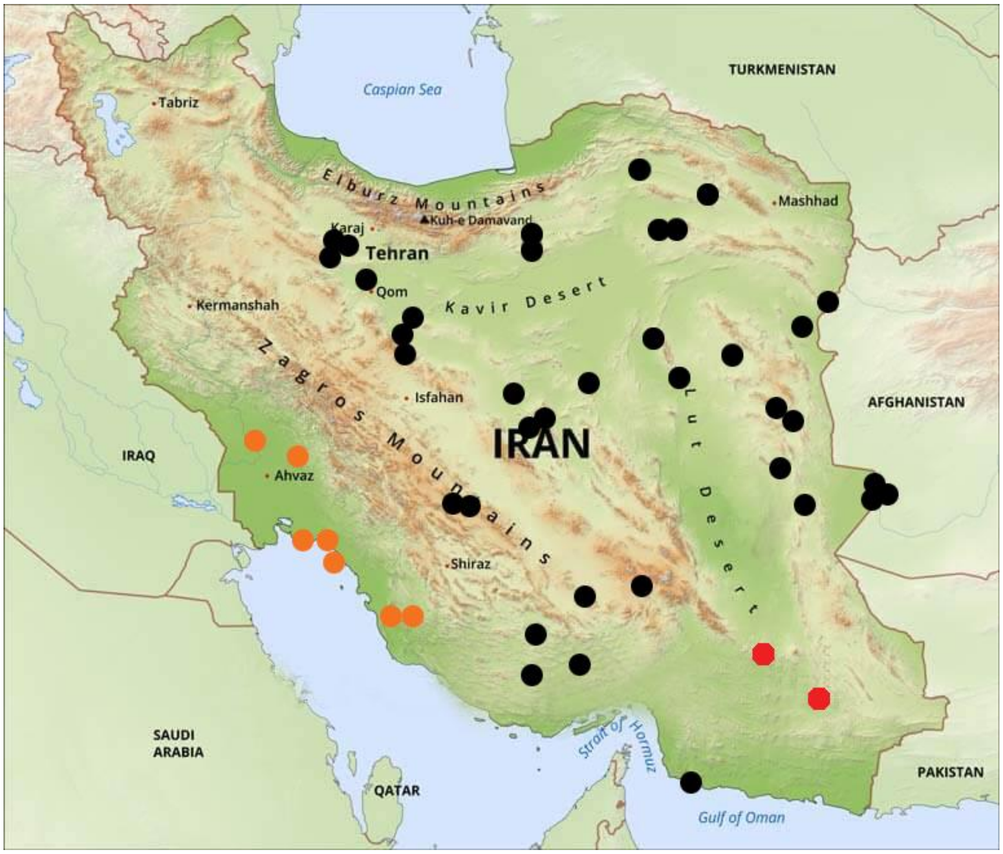

FIGURE 1. Distribution of *Trapelus agilis* complex in Iran: Orange circle: *T.*a. *khuzestanensis*; Black circle: *T.a. agilis*. Red circle: *T.a. isolepis*

***Trapelus sanguinolentus* (Pallas, 1814)**
Type locality: Terek River Valley.

T. sanguinolentus occurred in Russia (Dagestan), Kazakhstan, Kyrgyzstan, Uzbekistan, Turkmenistan, Tajikistan, Kazakhstan, and southward to the north Iranian Plateau (adjoined to Kopet-Dagh valleys), northern Afghanistan, northwestern China and in the west toward southeastern Europe (Ananjeva2011). Like an ecological barrier, the high mountain ranges of the Caucasus and Alborz prevent emigration of the European population from the south, where a continuous land connection does exist to *Trapelus* populations in Iran (Macey, 2004). Central Asian agamids, including the steppe agama (*Trapelus sanguinolentus*), despite their abundance in the region and thus their possible ecological importance, are relatively poorly studied (Novák, 2020). Furthermore, the Caspian-Aral basin clade is in a controversial taxonomic status, either in a separate species called *T. sanguinolentus* or subspecies *T.a. sanguinolentus* (Ananjeva, 2011). Nilsson and Andren (1981) explained that specimens from central (*T. a. agilis*) to northern Iran (*T. a. sanguinolentus*) have a rapid change in the mean number of midbody scales rows and accepted *T. a. sanguinolentus* as a subspecies. Recently, some authors have carried out significant studies with different ideas in the field of historical biogeography, phylogenetic relationships and genetic diversity, intraspecies diversity, morphological and ecological analyses, and taxonomic diversity on this species, which we compare and review. Macey et al. (2000b, 2006), with a phylogenetic analysis (1434 aligned positions) of 72 species and examination of two new sequences from ND1 to COX1, generated parsimonious trees that placed *T. sanguinolentus* as a valid species and its close relationship with *Trapelus savignii* were identified. Likewise, 10.7-13.9% sequence divergence (mitochondrial DNA segment from ND1 to COX1) between *Trapelus* species in Africa (*T. savignii*), Arabia (*T. persicus*), Iranian plateau (*T. agilis*), and Caspian-Aral basin (*T. sanguinolentus*) was calculated (Macey et al., 2000), which estimated the time of divergence among *Trapelus* species to be 8.3 to 10.7 Mya, indicating that this genus has been in Asia since the Miocene. (Macey 2006)

There are also taxonomic disagreements about the status of the isolated European population of *Trapelus*. Ananjeva and Tsaruk, 1987, considered the European populations as distinct subspecies of *T. sanguinolentus* (*T.s. sanguinolentus*) and also named the Central Asian populations *T. s. aralensis*. Bannikov et al., 1977, introduced the European and Asian populations in the Caspian-Aral basin as the full species *T sanguinolentus*, and Wermuth, 1967 considered it as a subspecies. Since the Caspian-Aral basin populations have always been identified as one species or one or two distinct subspecies from the Southwest Asian populations of *T. agilis*, the examination of these populations by evaluating allozyme data shows that these species do not show distinctive forms. Also, its low genetic diversity indicates the recent distribution of this genus throughout the Caspian- Aral Basin. Finally, the results suggest these populations as a single taxon, a distinct species of *T. sanguinolentus*, or a subspecies, *T. a. sanguinolentus* (Macey 2004).

Rastegar-Pouyani, 2005 by analyzing the multivariate approach, examining the significant metric and meristic characters, and considering the ecological and geographical barriers in *T. sanguinolentus*, identified the northeast group of Iran as one of the most extensive groups in the *T. agilis* complex. Despite the wide distribution in all populations of this group, they are very similar and homogeneous in almost all morphological aspects. This is probably due to one or more recent invasions from the southern parts of the range to the northern and northeastern regions. Although this population is geographically separated from the western shores of the Caspian Sea, it has not reached the necessary morphological differentiation. An intermediate form is also distributed in this area as the contact zone between these two groups. These hybrid populations, which indicate the gene flow between this taxon and other subspecies in the introgression regions, do not affirm the taxonomic designation belonging to the nominal subspecies. Melville et al., 2008 within a phylogenetic framework, sequenced a 1200 bp region of mitochondrial DNA and a 1200 bp nuclear gene (RAG-1), including inter- and intraspecific sampling across Central Asian Agamids. The results showed that *T. sanguinolentus* is probably the only species with no apparent genetic structure due to its recent introduction and rapid dispersal in Uzbekistan and Kazakhstan. Therefore, this species was introduced as belonging to the genus *Phrynocephalus*, which has a long and complex history in eastern Kazakhstan. Also, the age of both genera is estimated to be the same. Pyron et al., 2013 presented a new phylogenetic estimate for squamate species with a dataset containing 12,896 bp of 12 genes, including seven nuclear and five mitochondrial. In the case of the genus *Trapelus*, they proposed species limits and estimated that species such as *T. pallidus* may represent populations in other species. Also, the phylogenic tree shows that *T. sanguinolentus* is placed next to *T. mutabilis* and is further away from *T. agilis*. In 2019, Shahamat et al. investigated the ecological and morphological characteristics (three metrics and 12 meristics) of the *Traplelus agilis* complex. Among the 15 morphological characters, the number of pre-anal callose scales and supralabials were the most significant variables. Based on that, the groups of eastern Iran, *sanguinolentus*, Central Khorasan, and Khuzestan were distinguished from other populations. As well as, based on the ecological analysis, one of the clades that are separated from all groups is *T. sanguinolentus*, and the groups of Central Plateau, Eastern Iran, and East-Central Plateau cannot be isolated from each other. They discovered that morphology and ecology disagreed in the grouping and similarity of groups and did not have the same results. They suggested that the lack of sufficient time to complete morphological differentiation and the constant climatic conditions of the Zagros Mountain range did not have enough evolutionary pressure to differentiate populations, and the difference between molecular, morphological, and ecological consequences is raised.

Shahamat et al., 2020, using the nucleotide sequence of two mitochondrial genes (Cytb and ND2) (total length 1322 bp), investigated the phylogenetic relationships and intraspecific differentiation of the *Trapelus agilis* complex. They reported the paraphyly of *T. sanguinolentus*, which had previously been introduced as a species, as its population was in the *T. agilis* species complex, a fact that was declared for the first time. Meanwhile, based on molecular phylogeny, Iranian populations were divided into six genetic lineages. The time of separation of *T. sanguinolentus* from other populations (due to the closing of the Paleo-Tethys and the forming of the Kepeh Dagh Basin) was estimated to be about 12Mya. These lineages are geographically isolated and the genetic distance between them is more than 9.9%, indicating their separation at the species rank. Although, their remarkable similarity in morphology, (Rastegar-Poyani, 1999; Rastegar-Poyani, 2005; Shahamat, 2019) does not properly reflect their genetic differentiation. However, it is possible that genetic differentiation between groups could be reflected in characteristics other than, morphology**.** In a comprehensive study by Novák et al., 2020, basic morphological features, signaling, and agonistic behavior in relation to body condition and lifespan in *T. sanguinolentus* have been investigated. A comparative study of behavior with other subspecies in Central Asia and the Iranian plateau is suggested, which can be useful in deciding the status of this species.

In our opinion, considering all the studies and controversial results about such complexes, it can be practical and useful to consider the following in the final taxonomic decisions. Although taxonomies contribute to a better understanding of geographic variation, species, and taxonomic distinction, since the description of subspecies, there has been disagreement about its necessity (Gippoliti, 2013, Shannon et al 2014). Considering the confusion in the definition of subspecies, some disciplines, such as herpetology, have many authors who reject the classification of subspecies and do not support it to understand evolutionary divergence and conservation (Shannon et al 2014). Amadon, 1949, and Shannon et al. 2014 stated that a population is considered subspecies when 75% of the population's morphological characteristics are outside 99% of the range of other populations. Genetic results regarding the validity of subspecies often conflict with morphological data (Phillimore, 2006). Especially in reptiles, disagreement has been reported (Bryson et al., 2007; Burberink et al., 2008; Daza et al., 2009; Blatti et al., 2011). However, in cases such as the three subspecies of *Trimeresurus flavomaculatus* where genetic data support recognized subspecies, the authors do not recommend taxonomic changes (Slanders et al., 2004). A notable case is a difference in genetic distance values for the height of a subspecies or its collapse, for which there is no universal threshold value and a standard method for species-subspecies boundary detection should be developed (Miralles et al., 2010).

Howlitcheck 2012 proposes the concept of subspecies to unify taxonomy and limit taxonomic inflation. Although many herpetologists oppose the description of new subspecies or even the protection of existing subspecies, most ornithologists use subspecies ranking in conservation and identification studies, the evolutionary divergence of distinct populations, and species without warranted classification. Evidence suggests that there is a level of divergence along the path of speciation where lineages are recognizably distinct but not full species, and this level corresponds to the rank of subspecies. Subspecies differ from other subspecies of the same species but can be mixed with them. However, species are distinct in sympatric and allopatric states (Howlitcheck 2012). Thus, subspecies are "a stage in the process of allopatric speciation". Since species are taxonomic levels, they are considered for analysis, protection aims, and evolutionary studies, as a result, taxonomic inflation within species is a concern (Howlitcheck 2012, Phillimore 2006), and by converting subspecies to the species level, viable units no longer indicate distinct evolving entities. (Howlitcheck 2012). To solve taxonomic problems, increasing the possibility of sequencing whole genomes, instead of sequencing a few hundred base pairs of mitochondrial DNA, allows for better analysis of physical and physiological characteristics (Lippert et al. 2017), especially if descriptions of morphological accuracy, habitats, and high-resolution photos are considered (Uetz, 2019).

FIGURE 2. Distribution of *T. sanguinolentus* in Iran.

***Trapelus ruderatus* (Olivier, 1804)**

Type Locality. Near Esfahan, Esfahan Prov., Iran.

The former type locality, established by Rastegar-Pouyani (2000), between Bandar-e-Ganaveh-Borazjan (50°45'E, 29°35'W), Bushehr Province, SW Iran, (Ananjeva et al. 2013).

Trapelus ruderatus is a widespread, disjunct, with multiple populations, distributed in stony habitats with little vegetation and high-altitude areas. *T. ruderatus* is a species that was recognized by this name after Moody's revision (1980). The *T. ruderatus* complex traditionally has three subspecies: *T.r. ruderatus*, *T.r. baluchianus* and *T.r. megalonyx* (or *T. megalonyx*) Günther, 1864 (Rastegar-Pouyani,1998). The eastern subspecies of this species in Iran, *T. r. baluchianus*, is no longer available and comes under the synonymy of *T. megalonyx* (Rastegar-Pouyani, 2000). *T. megalonyx* has not any zone of intergradation with *T. ruderatus* in Iran due to a distance of more than 500 km and the absence of intermediate populations (Rastegar-Pouyani, 2000). Its distribution after the split of *megalonyx* was limited to Jordan, Syria, and Lebanon through Iraq and S Turkey to W Iran, westwards extending to central Anatolia. In Iran, it has been well recorded in the Zagros Mountain range in most western provinces (Ilam, Kermanshah, and Lorestan)**.** It penetrates the Alborz Mountain range in Tehran province. Toward east, it is distributed in the central parts of the southern plateau of Iran to the south of Shiraz (Anderson, 1999; Rastegar-Pouyani, 2000, Smid et al., 2014). Based on morphological traits, the population of this species in Iran can be easily identified from *T. agilis* and *T. persicus* with key characteristics such as more depressed and smaller body size, shorter limbs and head, more heterogeneous dorsal scalation with nail-like large scales dispersed on the dorsal surface of the body, and the approximate scarcity of gular sac. In central and southcentral regions of the Iranian plateau, it overlaps with *T. agilis*. Thus they are sympatric species

(Rastegar-Pouyani, 2007). There is a 13% percent overlap between the two groups, which shows that the distribution of the two species has little overlap in southern Iran (Hosseinian Yousefkhani, 2016). *T. persicus* and *T. ruderatus* were reported sympatric in the alluvial area of Dehloran city at an altitude of 200 meters, also in Bushehr, Fars, and Esfahan Prov. (Fathinia etal.2009). Since Olivier (1804) described the *T. ruderatus* complex based on a single specimen, taxonomic status and type locality have remained problematic. This uncertainty prompted Rastegar-Pouyani (2000) to examine the holotype of *T. ruderatus* (Olivier, 1804) and compare it with other related specimens of *Trapelus*. In his investigations, he found that the holotype of *T. ruderatus* is identical to the syntype of Blanford's Agama persica. As the name "*ruderatus*" (Olivier, 1804) predates "*persicus*" (Blanford, 1881), all populations of *T. persicus* are preferable to the original name "*ruderatus*." Furthermore, the holotype of De Filippi's *Agama lessonae* was not conspecific with Olivier's *Agama ruderata*, requiring it to be considered a distinct taxonomic entity. Since *T. lessonae* is the oldest name, the population names were changed from *T.r. ruderatus* to *T. lessonae* and designated a lectotype and type locality. He also showed that the western radiation of the complex in Iran is geographically and morphologically distinct from the eastern radiation (*T.r. baluchianus* and *T. megalonyx*). Since *T. megalonyx* Günther, 1864, was described before *T.r. balochianus* (Smith, 1935), all the populations of these two taxa in Afghanistan, Pakistan, and possibly southeast Iran must be named under the nominal and original name of *T. megalonyx* (Rastegar-Pouyani, 2ooo). Ananjeva et al. 2013 provided solutions to avoid nomenclatural problems and prevent further complications in interpreting the taxonomic status of different forms of the genus *Trapelus* in molecular phylogenetic studies. They agreed with Rasteger-Pouyani on the similarity of the *Agama* [*Trapelus*] *ruderata* holotype (MNHN 2610) to *T. persicus*. Still, they considered that the original description of *Agama ruderata* was not based on a single holotype. Still, at least two syntypes, one of them agrees with the diagnosis of the original Atlas. A second syntype completely corresponds to the definition of *Agama ruderata* Olivier, 1804. A point that Rastegar-Pouyani (2000) ignored and did not provide any reference to Olivier's Atlas (1804) (Atlas Livraison: Pl. 29: Fig. 2) and therefore presented a different taxonomic decision. In the latter case, according to previous herpetologists, Boulenger, 1885; Wermuth, 1967,) seems to be the best decision is to designate the holotype of *Agama lessonae* De Filippi 1865 as a neotype *T. ruderatus* Olivier, 1804. Also, the status of *A. lessonae* De Filippi in 1865 as an objective synonym of junior *A. ruderata* Olivier, 1804, is confirmed according to Boulenger (1885:348). Thus, the type locality of *A. ruderata* becomes "Presso Spahan" (De Filippi, 1865) in Isfahan Province, in the center of Iran. Finally, based on the mentioned cases, the nomenclature modifications were not accepted, and the previous names are valid. Older holotypes are often abbreviated and not up to today's standards. They may not accurately represent the current condition of the specimens, significantly when they added later species to the same genus. As a result, the original types often require re-description. Such cases lead to challenges in such research (Uetz, 2019).

In our unpublished study in 2011, we investigated the morphological variation within this species in Iran. We examined 177 individuals of *T. ruderatus* in terms of 40 metric and meristic traits. Three OTUs were determined based on the presence of the Zagros Mountains as an ecological barrier in its east, west, and center. We found that diameter's ear (DE) in the male population of *T. ruderatus* in the center and west of the Zagros Mountains are significantly higher than in the east of it. The Discriminant Functional Analysis (DFA) showed that the specimens of these three populations do not overlap much, and the specimens are placed in separate groups. Finally, Cluster analysis by Ward's method completely separated the populations of the east of Zagros Mountain from the west and the center. Mountain uplifting might also be associated with forming biodiversity gradients, shaping an association between habitat heterogeneity and species richness (Kafash, 2020). Using molecular and morphological data and a comprehensive sampling in Iran and neighboring areas, Yousefabadi et al. 2021 conducted a phylogenetic analysis. Their study revealed that the populations of this species complex are divided into five distinct clades, of which three are in Iran including the populations of northwestern Iran (Azerbaijan provinces), western Iran (Kermanshah province), and Fars province in southern parts of the country. The other two groups were in southeastern Turkey and central-southern Turkey-western Syria. As Arabia collided with the Eurasian plate and as a result of a vicariant event about 8 Mya, the lineages of Northwest and West

Iranians and the lineages of Southeast Anatolia and South Anatolia-West Syria separated. More than 8% of the mean distance in the ND2 gene fragment among the Iranian clades revealed that these clades should be considered as distinct species. The morphological diversity and the high genetic distance between Syria, Turkey, and Iran populations of *T. ruderatus* indicate their high potential to reach a distinct level in species rank.

According to the niche divergence measurements, the habitats' differentiation between the sympatric *T. persicus* and *T. ruderatus* species was confirmed, and the most important environmental variables for the distribution of *T. ruderatus* were precipitation in the wettest and warmest months, temperature seasonality, and slope. The amount of rainfall is necessary for access to water, the growth of plants, and preparing a shelter for them, moreover the accumulation of insects that are an essential source of their nutrition (Hosseinian Yousefkhani, 2016). According to the habitat model and where most records are known, the most suitable habitat for this species is in Southeast Anatolia (Bird, 1936; Schmidt, 1939; Bodenheimer, 1944; Başoğlu and Hellmich, 1970; Baran et al., 1989; Baran and Atatür, 1998; Baran et al., 2012)

The range of distribution of this adaptable species is wide from north to south and east to west, which is compatible with different habitats and altitudes. Anderson, 1999 pointed out that the variable ecological factors can be affected by the morphology of local populations which inhabits heterogeneous habitats. The Zagros Mountains limit the distribution of this species in Iran. The Zagros Mountain range acts as natural barriers and corridors for lizards that have restricted the distribution of this species toward the central plateau of Iran. (Ahmadzadeh et al., 2013; Gül, 2013; Hosseinian Yousefkhani et al., 2016; Kapli et al., 2013; Smidt & Frynta, 2012)

Definition. Body distinctly depressed; limbs relatively short; head roundish; caudal scales obliquely arranged; nostril below canthus rostralis; dorsal scales heterogeneous, back and flanks with scales of varying size intermixed; 66–125 scales around the middle of the body; a patch of enlarged scales on the upper surface of the thigh may be present; callose preanal scales in one, two or three rows; small patch of scales on the neck just posterior to occiput in which direction of imbrication is reversed, or no reverse imbrication; gular sac absent or very weakly developed in males, enlarged scales on the neck (rudimentary nuchal crest) absent, teeth 60. Dorsum sandy gray or grayish-brown, usually with 5 or 6 dark cross bars, interrupted by light vertebral ovoid spots, sometimes marked with very dark brown; sometimes up to three additional series of dark-edged spots on each side of the vertebral line; the tail also has dark bars, interrupted by light vertebral spots; the pattern is sometimes indistinct in males (Rastegar-Pouyani, 2000). In terms of sexual dimorphism in size, in smaller lizards such as *Trapelus ruderatus*, females have a larger size (female-biased), and the adaptation of the female body concerning the production of offspring (eggs) is the main determining factor for the larger size of the female. And in fact, fecundity selection plays a more influential role (Rastegar-Pouyani, 2013). Ahmadzadeh, (2008) reported it in open stony ground and in cropland with low weed vegetation in autumn.

***Trapelus persicus* (Blanford, 1881)**
Persia Agama
Type locality: Deh Bid and Kázrun.

T. persicus complex is known for the lowland regions and desert areas around the Persian Gulf and Mesopotamian Plain. Rastegar-Pouyani described *T. persicus* at an altitude of 300 meters elevation in hot and wet areas with 40 degrees temperature around Borazjan (75 km southwest of Kazerun) of Bushehr province, and he did not have any records in the valleys of the Zagros mountains around Kazerun (the other type locality). According to this author, the populations of *T. persicus* could not penetrate the Zagros Mountains and is restricted to its type locality from Deh Bid in the inner part of the Zagros Mountains to a new one in southern Iran, Fars-Bushehr provinces, the area southwest of Kazerun towards

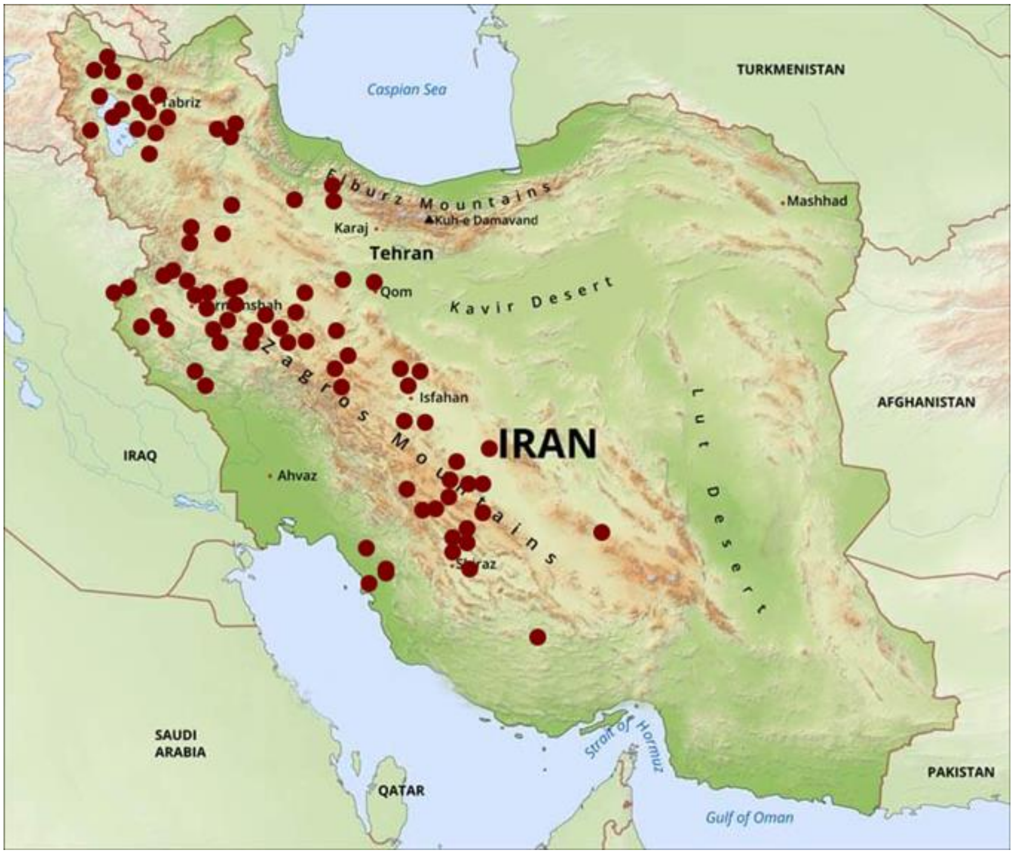

FIGURE 3. Distribution of *Trapelus ruderatus* in Iran.

Borazjan, and considered the syntype collected from this area as the lectotype. Moreover, by re-evaluating the taxonomic status, he made major nomenclatural changes and replacements in the species and renamed all *T. persicus* populations to *T. ruderatus*. Later, this nomenclatural situation was analyzed and resolved some problems of the name-bearing type by Ananjeva et al. (2013), which returned the names to their previous state. In the Iranian plateau, this species belongs to the southern and southwestern regions, a habitat with alluvial plains and different vegetation, including low bushes and cultivated areas, which is entirely different from the habitat of *T. agilis*. In addition, the study and comparison of ecological niches have shown that the central plateau of Iran is not suitable habitat for *T. persicus*. As a result, the Zagros Mountains are a barrier to expanding its territory. The difference niches between the two groups were investigated, and their overlap was about 13% which can be concluded that both species are sympatric in the south of Iran, and *T. persicus* is distributed to a small zone. The results obtained from niche modeling suggest that the environmental conditions for *T. persicus* are restricted. The most significant variable for *T. persicus* is the warmest quarter rainfall since it is associated with water availability in arid regions of southern Iran. One of the special features of this species is that it is the largest species of the genus *Trapelus*. *T. persicus* complex includes two subspecies, *T. p. persicus* and *T. p. fieldii*. These two subspecies differ in the main metric and meristic characteristics of the dorsal pattern, snout-vent length, enlarged scales on the neck, and gular sac. In the southwestern plains of Iran, both subspecies may exist, although their exact distribution range and the degree of intergradation are unknown (Rastegar-Pouyani, 2005). *Trapelus persicus fieldii* (Haas & Werner, 1969) was collected from Abu Kamal (from Syria) near the Syrian-Iraqi borders for the first time. Its distribution range is in Iraq, Kuwait, Jordan, northern Saudi Arabia (Arnold, 1986; Leviton et al., 1992), and probably, southwestern Iran on the lowland coasts of the Persian Gulf. The type locality of *T. p. fieldii* is between Al-Qaysumah and Turaif, Saudi Arabia (Uetz, 2022).

In *T. p. fieldii*, the taxonomic traits such as a larger size, slenderer, and different color patterns of dark and bright longitudinal stripes, as well as a distinct and lighter vertebral pattern, have separated it from the main species, *T. p. persicus*. The subspecies' habitat is hard to wetland with Nitraria retusa shrubs, and the vegetation is mostly Anabasis sp. Males were recorded more than females on the region's highest rock (Zuhair, 2007). The type locality of *T. p. persicus* Deh Bid Kazeron, Fars province, south-central Iran. *T. p. persicus* was distributed in the Mesopotamian Plain in lowland Iraq and southwestern Iran, the northern Arabian Desert, and Jordan and expanded to the Persian Gulf and Saudi Arabia shores. It seems limited to the lowlands and has not penetrated the slopes and mountains of Zagros. *T.p. persicus* in southwest Iran (SVL: 85 mm) is smaller than *T. p. fieldii* (SVL: 135 mm), with faint and weaker lines and enlarged scales present in the midline of the neck less than in *T. p. fieldii*. According to the modeling, there is a possible hybrid zone between the two subspecies. Based on Morphological traits, *Trapulus* has a larger body size, longer tail than body length (smaller SVL/TL), and fewer ventral scales. The molecular genetic results confirmed the morphological differences between *T. persicus, T. ruderatus, and T. agilis*, as well as *T. persicus*, have shown the highest genetic distance from other populations. (Yusef abadi, 2021) According to the molecular study, the divergence time has been estimated as 18.8 mya between *T. persicus* and *T. agilis*. *T. persicus* represents the second clade at the base of the tree (Shahamat et al., 2020).

Definition. Dorsal scales strongly heterogeneous, back and sides with mixed scales of different sizes, 72–97 scales around midbody; ventral body scales distinctly keeled canthus rostralis and supraorbital ridge usually well developed; enlarged scales on the neck more or less grown, almost always, one row of preanal callose scales in males, which in females are weakly developed or missing; create a corrugating gray or light purple stripe on the neck and back as anterior oval vertebral spots linked, connected with darker brown lines stretching onto the dorsal surface of the head, or a noticeable dorsal pattern of dark and light longitudinal stripes, male with extent gular sac. A dark bar on the side of the neck develops across the temporal region to the eye, and a brown line crosses the head at the level of the anterior part of the orbits; almost males have a luminous body and an orange tail. Based on the evidence, it can be concluded that the body color change occurs in defensive and threatening situations, and the dorsal area of the tail turns brick red. In contrast, the sides, gular folds, the lower surfaces of the eyes, and especially the front limbs turn dark blue (Fathinia, 2011).

***Trapelus megalonyx (*Günther, 1864)**

(Ocellate ground Agama: Patta korrh-kirla)
Type locality: vicinity of Ghazni, Afghanistan. (Utez,2022)

Trapelus megalonyx, the Afghan ground agama, distributed in some parts of eastern and southern Afghanistan, north-central Baluchistan, Sindh, and the Cholistan Desert in Punjab, Pakistan, collected up to an elevation of 1800 m. India and Perso-Baluch (Iranian-Pakistani) border (Khan,2003). Rastegar-pouyani 1998 believed that the specimens identified as *T. megalonyx* were synonymous with *Agama ruderata baluchiana*. This species was recorded in the semi-desert and mountainous habitat north and south of Ghazni, Afghanistan. Although there was no reliable report of this species in Iran until then, Werner reported *T. megalonyx* in 1895 in the southeast of Kerman province in Iran, and two records by Annandale from Sistan-Baluchistan Province, on the Iranian-Pakistani border. However, Rastegar-pouyani, 2000 by examining the difference in the heterogeneity of the dorsal scales in both species, believed that *T. r. baluchiana* is not different from the Afghan species, *T. megalonyx*, and considered them synonymous. He noted that *T. r. baluchianus* is a junior synonym of *T. megalonyx*. So, the name *T. megalonyx* includes all the previous populations of *T. r. baluchiana*, and it is no longer valid (Rastegar-pouyani, 2000).

Figure 4. Distribution of *Trapelus persicus* in Iran.

In the description of *T. megalonyx*, Günther (1864) pointed out that the claw nails are much longer, and Boulenger (1885) used this trait in her key to separate it. Still, this character is less common in other males and even less so in females and also juveniles, with the presence of 4 to 12 callose preanal scales in a single row in males, which were less developed in females, and having 67 to 84 scales around the middle of the body. At the same time, Milton considered it 71 to 88 scales in *T. megalonyx*, which was more in *T. r. baluchiana*, about 80–82 scales around the widest middle of the body, but after examining the holotypes of both of them, Anderson did not observe any significant difference.

One of the most recent records of *T. megalonyx* was in the protected area of Sirisha-Goji Torbat Heydarieh in the central region of Khorasan province in 2018 by Assadian Narenji et al, considering that it was not reported in this area before, it was verified as a new record. Considering the reclusive of the species, the author predicts the possibility of its presence in other regions of Khorasan. The key characteristics described were: heterogeneity of dorsal scales and homogeny of ventral scales, 68-88 scales around the widest part of the body, callous preanal scales in a row, and males without gular sac.

Diagnosis: Nostril below canthus rostralis; dorsal scales heterogeneous, back with scales of varying sizes intermixed, enlarged scales not extending onto flanks; 68--88 scales around the widest part of the body; upper surface of thigh usually lacking distinctly enlarged scales, or with an area of large scales not intermixed with small scales; callose preanal scales in a single row; none of the neck scales with reversed imbrication; males without gular sac (Anderson 1999). Even more, Blanford (1875) believed Stoliczka's *Trapelus megalonyx* (from Sindh) is also *T. agilis* (Masroor, 2011).

FIGURE 5. Distribution of *Trapelus megalonyx* in Iran.

Identification keys to the subspecies of *Trapelus agilis* complex (Source: Anderson, 1974, Rastegar-Pouyani, 1998, Nasrabadi, 2017.)

1a. Tail almost always round, two (or more) rows of callose preanal scales…………………….……….. **2**

1b. Tail often compressed, often one or in the case of two row of callose preanal scales (pre anals absent or weakly developed in females) …………………………………………………….………..…...**3**

2a. Body size variable; 65-91 scales around body; dorsal scales subequal, weakly to moderately keeled, often strongly mucronate; ventral scales smooth or weakly keeled; usually 2, sometimes 3 (rarely 4-5) rows of callose preanals; background coloration variable; central Iranian Plateau, central and southern Afghanistan, southwestern Pakistan. ……………..…………………………….….. … ***Trapelus agilis agilis***

2b. Body size medium to large (Max SVL about 105 mm), 60–85 scales around body; weakly moderately keeled, strongly mucronate, dorsal scales with a rather abrupt change from the larger scales of the median to the smaller lateral.; ventral scales weakly keeled; Baluchistan, southeastern Iran, Pakistan , Afghanistan and northwestern India ….…………………………….………..…***Trapelus agilis isolepis***

3a. Body and limbs smaller than those of the other subspecies and sometimes slender not compressed; head and neck distinctly short; body scales smaller than those of the other subspecies; dorsal scales heterogeneous, weakly to moderately keeled, weakly mucronate; 80-97 scales around body; ventral scales slightly keeled; scales of posterior part of head and anterior part of neck reversely imbricated; upper head scales keeled or rugose; 14-19 upper and lower labials; background coloration yellowish grey-cream; lowland southwestern Iran ……………..……………………………...….***Trapelus agilis khuzistanensis***

3b. Body and limbs distinctly slender, sometimes compressed in males; dorsal scales subequal to homogeneous, distinctly keeled and mucronate, usually clearly set off from small dorsolateral; 67-83 scales around body; ventral keeled in adult males; no reversal imbrication of head and neck scales; upper head scales smooth; 12-16 upper and lower labials; background coloration often sandy grey: southeastern Pakistan and northwester India. …………………..…………..……..……. . ***Trapelus agilis pakistanensis***

Key to the Iranian Species of the Genus *Trapelus* (Sources: Rastegar-Pouyani, 1998. Masroor, 2011., Khan.2002,2003, Nasrabadi,2017)

- Dorsal scalation homogeneous, two (or more) rows of callose preanal scales

1a. Dorsal scalation homogeneous, subequal in size, without larger scales among them and disposed of in irregular rows, grading into progressively smaller scales of flanks, 52–103 around the middle of the body; gular sac variable, sometimes strongly developed in males; males with callous preanal scales. …………………………………………………………………………………….…….. ***Trapelus agilis***

1b.Body stout; 52-73 scales around body; all dorsal, ventral, and gular scales larger in size, homogeneous, strongly keeled and mucronate; almost always 2 rows of callose preanals; background coloration of males often dark sandy-grey; northeastern Iran, northern Afghanistan, Central Asian Republics, western coast of the Caspian Sea (Daghestan), western China**………………**………………..……***Trapelus sanguinolentus***

- Dorsal scalation heterogeneous, intermixed with larger scales, ………………………….………..…… 2

2a. Abdominal scales pointed; Largest dorsal scales more than twice the size of smallest; anterior oval vertebral spots linked together on neck and back, bordered by brown stripes extending onto dorsal surface of head, pattern variable in different populations; males with strongly developed gular sac; nuchal crest more or less developed; males with one row of callose preanal scales; canthus rostralis often well developed………………..............……………………………………….…………..... ***Trapelus persicus***

2b. Body distinctly depressed; head roundish; limbs relatively short; nostril below canthus rostralis; abdominal scales smooth or very weakly keeled; largest dorsal scales about three times width of adjacent small scales, usually nail like, 66–125 around body; oval vertebral spots may or may not be distinct, sometimes contained within dark crossbars, not link into longitudinal stripe; enlarged scales on neck absent; gular sac absent or weakly developed in males ……..………..….……………....***Trapelus ruderatus***

2c. Dorsal scales different in size and irregular in arrangement, enlarged dorsal scales pointed; largest dorsal scales about twice the size of the smallest; fewer than 100 scale rows around body; dorsum with reddish or orange ocelli with dark borders.. ***Trapelus megalonyx***

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