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Exploring Balochistan Coast as key Sea Bird Sanctuaries: New Records of Avian Fauna

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Abstract
The study documents two new seabird records for Pakistan, *Anous tenuirostris* (Lesser Noddy) and *Sula leucogaster* (Brown Booby) and confirms additional observations of *Sula dactylatra* (Masked Booby) along the Makran coast of Balochistan. Surveys conducted at Astola Island, Juddi Beach (Pasni), and Gurab Island recorded these species as non-resident visitors primarily using the area for foraging. All three recorded species are currently listed as Least Concern (LC) on the IUCN Red List. However, their sporadic presence in Pakistani waters underscores the need for continued monitoring to assess any potential shifts in distribution patterns, possibly related to climate change, marine productivity, or habitat disturbances in the wider Indian Ocean region. Supported by local ecological knowledge, the findings indicate recent seasonal occurrences and potential range extensions into the northern Arabian Sea. The findings highlight the Makran coast as a potentially important seabird sanctuary and emphasize the need for continued monitoring to better assess avian diversity and inform regional conservation planning.

Keywords: Noddy, Booby, New records, Pasni, Gurrab Island.

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INTRODUCTION

The Makran coast of Balochistan, extending along the northern Arabian Sea and the Gulf of Oman, represents one of the least disturbed and biologically rich coastal regions of Pakistan. Characterized by extensive rocky and sandy shorelines, lagoons, and small islands, the area supports diverse marine life under relatively low anthropogenic pressure. Major fishing and coastal settlements such as Jiwani, Pishukan, Gwadar, Surbandar, Pasni, Ormara, and Damb are the principal centers of marine-based economic activities (Shahzad, 2021; Majeed et al., 2021), while much of the remaining coastline remains sparsely inhabited and ecologically intact. The coastal and offshore waters of Balochistan are strongly influenced by the Arabian Sea monsoon system, with distinct southwest and northeast monsoons and two inter-monsoonal periods (Wilson, 2000). These alternating monsoonal regimes promote nutrient enrichment and enhance marine productivity, supporting a rich food web that sustains fish, shellfish, and seabird populations.

Seabirds are among the most visible and ecologically significant components of coastal ecosystems, acting as indicators of marine productivity and environmental quality. Healthy seabird populations reflect intact habitats, whereas declines often signal ecosystem degradation (Ali et al., 2011). The South Asian biogeographical region comprising Afghanistan, Pakistan, India (including the Andaman

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and Nicobar Islands), Nepal, Bhutan, Bangladesh, Sri Lanka, the Maldives, and the Chagos Archipelago represents only a small portion of the Oriental Region. However, it supports about 1,300 bird species, accounting for roughly 13% of the world's avifauna (Rasmussen and Anderton, 2012). Among these, approximately 143 species (11%) are endemic to South Asia, highlighting the region's significance for avian biodiversity conservation.

Pakistan's avifauna currently includes about 805 species (Lepage, 2025). Within Pakistan, about 30% of avian species are long-distance migrants, 43% are Oriental or Palearctic breeders, and 27% are winter visitors (Roberts, 1991). The country lies along a major migratory route known as the Indus Flyway Zone**,** which provides critical stopover and wintering habitats for cranes, ducks, flamingos, geese, swans, and numerous waders (Ali, 2005).

Balochistan's coastal wetlands, including lagoons, mudflats, and offshore islands, are particularly important for these migratory and resident bird populations. Numerous studies have documented avifaunal diversity along this coast, such as Groombridge, (1989), Scott, (1989), Scott and Poole (1989), Ahmed et al., (1992), Khurshid et al., (1995), Ghalib and Hasnain, (1997, 1999), Khurshid and Jabeen, (2000), Arshad et al., (2002), Ghalib et al., (2004, 2006, 2008, 2009, 2019), Pandrani et al., (2005), Hassan and Khan, (2005, 2009), Khan and Ghalib, (2006), Grimmett et al., (2008), Rasool and Hasnain, (2008, 2009), and Siddiqui et al., (2008), Ali et al., (2011), Begum et al., (2016), Gabol et al., (2018), Kanwal et al., (2018).

From Pakistan, the family Sulidae is currently represented by a single genus, *Sula*, with one confirmed species, *Sula dactylatra* (Masked Booby). In contrast, the family Laridae comprising approximately 11 species including the genus *Anous* of which *Anous stolidus* has been documented (del Hoyo et al., 2014). Despite these efforts, large stretches of the Makran coastline remain poorly surveyed. The present study contributes two new avifaunal records: *Anous tenuirostris* (Lesser Noddy) and *Sula leucogaster* (Brown Booby) with the observation of *Sula dactylatra* (Masked Booby) from the Makran coast of Balochistan, northern Arabian Sea. Notably, *A. tenuirostris* has also been observed at Mubarak Village based on a single photographic record, though no further ecological details are available from that site. Detailed notes on their occurrence, habitat associations, global distribution, and conservation status are provided to enhance knowledge of Pakistan's coastal avifauna and support regional conservation initiatives. These findings demonstrate that new avian records continue to emerge from this underexplored region, emphasizing the need for ongoing monitoring to document avian diversity and identify potential seabird sanctuaries.

MATERIAL AND METHODS

Field observations were conducted along the Makran coast of Balochistan, northern Arabian Sea, during routine visits between 2024 and 2025. Observations were made at Juddi Beach, Pasni (25°15′54″N, 63°28′11″E), Astola Island (25°07′20″N, 63°49′42″E), and Guraab Island (25°06′24″N, 63°51′09″E), which is about 1.5 km south of Astola Island (Fig. 1). Three seabird species were documented: Lesser Noddy, *Anous tenuirostris* (Temminck, 1823) was observed at Juddi Beach and later on Astola Island. The Brown Booby, *Sula leucogaster* (Boddaert, 1783) and Masked Booby, *Sula dactylatra* Lesson, 1831 were observed at Guraab Island. Photographs for species identification and counting were taken from a safe distance to avoid disturbance. Species were identified using standard field guides (Grimmett et al., 2008; Rasmussen & Anderton, 2012). Conservation status was obtained from IUCN Red List (2025) and relevant literature. All observations followed ethical, non-invasive field practices.

RESULTS

In the present study, three seabird species were documented: the Lesser Noddy, *Anous tenuirostris* (Temminck, 1823) and the Brown Booby, *Sula leucogaster* (Boddaert, 1783) are reported for the first time from Pakistan, while the Masked Booby, *Sula dactylatra* Lesson, 1831, previously recorded from the country, was also observed during this study.

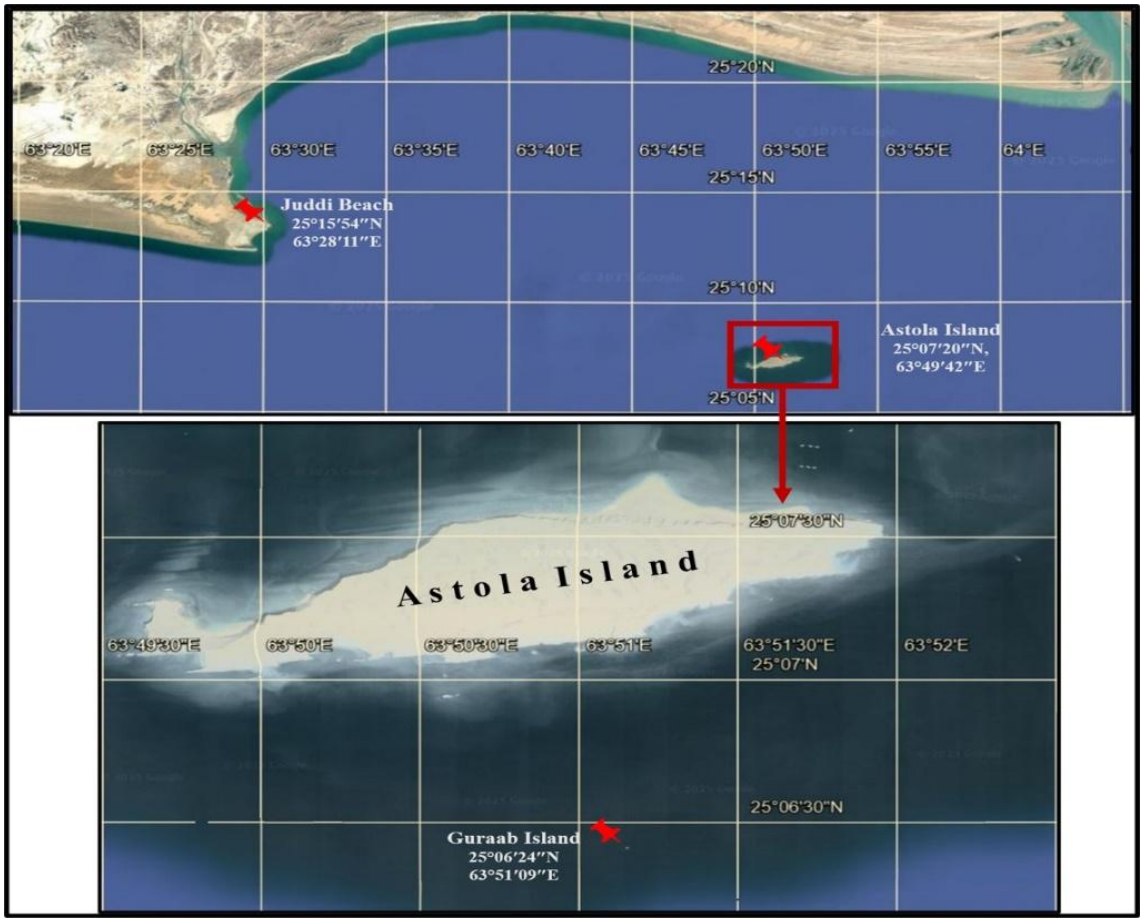

FIGURE 1. Map of study areas, Juddi Beach, Pasni (25°15′54″N, 63°28′11″E), Astola Island (25°07′20″N, 63°49′42″E) and Guraab Island (25°06′24″N, 63°51′09″E).

Family Laridae Vigors, 1825
Genus *Anous* Stephens, 1826
***Anous tenuirostris* (Temminck, 1823)**
Common Name: Lesser Noddy (Fig. 2)

Material examined: Two individuals photographed at Juddi beach, Pasni (25°15′54″N, 63°28′11″E) on 11th August 2024 and Astola island (25°07′20″N, 63°49′42″E) on 16th November 2024.
Description: A small, slender tern-like seabird measuring 28–34 cm in length with a wingspan of 50–61 cm. Plumage dark brown to grayish-black slightly lighter on the tail, and a whitish forehead that merges into a bluish- or lavender-gray crown. Lores softer grayish appearance. Iris brown, bill long, thin, slightly decurved, and black, and the legs and feet brownish-black. Flight is fast, agile, and graceful, often mixing with other terns.
Distribution: Atlantic and Indo-West Pacific: From Belize and north to Oman, south to Madagascar and east to Australia.

Family Sulidae Reichenbach, 1849
Genus *Sula* Brisson, 1760
***Sula leucogaster* (Boddaert, 1783)**
Common Name: Brown Booby (Fig. 3)

FIGURE 2. A, Lesser Noddy on ground. B, side view of Lesser Noddy. C, closer view of *Anous tenuirostris* (Temminck, 1823) with magnification of head recorded at Juddi Beach.

Material examined: Thirty-four individuals photographed at Guraab Island (25°06′24″N, 63°51′09″E), on 16th November 2024, 19th December 2024, 13th January 2025, 26th February 2025, 18th March 2025, 30th August 2025, 10th September 2025.
Description: A medium to large seabird measuring 64–85 cm in length with a wingspan of 135–150 cm. Adults sooty-brown above, including the head, neck, and breast, with a sharply contrasting white belly and undertail coverts. The underwings are mostly white, bordered by dark brown leading and trailing edges and tips. The bill yellow to greenish-gray, often with a pink or bluish tip, and the facial skin ranges from yellow to greenish. Eyes dark brown, and the legs and feet vary from yellowish to greenish-gray.
Distribution: Indo-Pacific and Western Central Atlantic.

Family Sulidae Reichenbach, 1849
Genus *Sula* Brisson, 1760
***Sula dactylatra* Lesson, 1831**
Common Name: Masked Booby (Fig. 3)

Material examined: Seventy-four individuals photographed at Guraab Island (25°06′24″N, 63°51′09″E), on 16th November 2024, 19th December 2024, 13th January 2025, 26th February 2025, 18th March 2025, 30th August 2025, 10th September 2025.
Description: A large white seabird measuring 74–86 cm in length with a wingspan of about 152 cm. Neck long, slender pointed wings, and a tapered wedge-shaped black tail. Adults with pure white plumage, black flight feathers along the wing edges. A distinct dark facial mask surrounds the eyes and base of the stout yellow bill. The eyes yellow, and the legs and feet range from olive to yellowish-gray. Juveniles gray-brown above with pale mottled underparts and a blue-green tinge to the bill. Its dark facial mask, black wings and tail, and large body size distinguish it from other boobies.
Distribution: Indo-Pacific, Atlantic Ocean and the Mediterranean Sea.

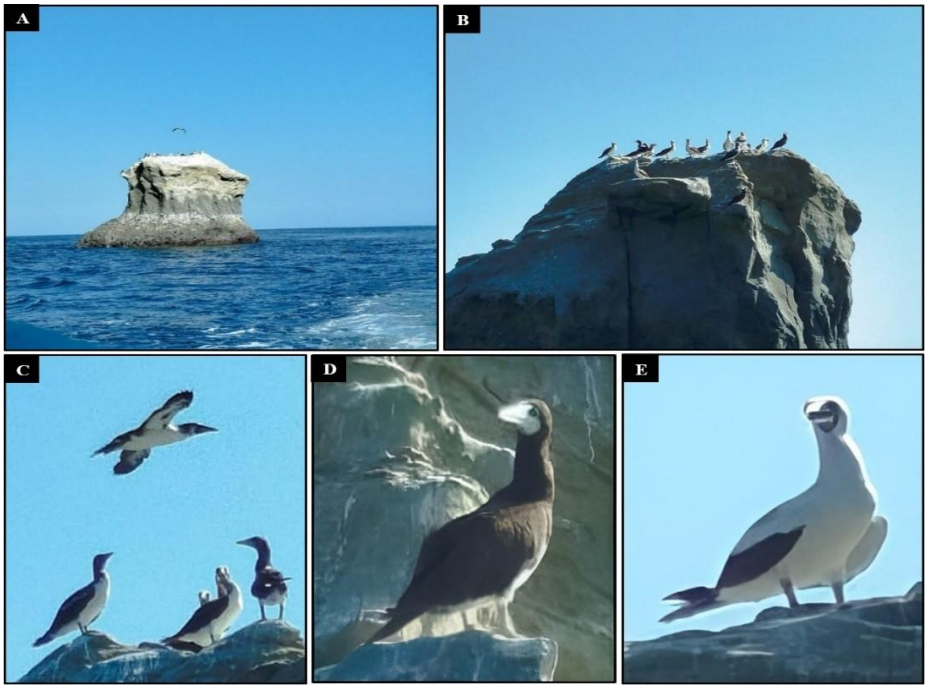

FIGURE 3. A, Guraab Island. B-C, Brown and Masked Boobies. D, Brown Bobby *Sula leucogaster* (Boddaert, 1783). E, Masked Booby, *Sula dactylatra* Lesson, 1831.

DISCUSSION

The present study provides new insights on the occurrence of three seabird species *Anous tenuirostris* (Lesser Noddy), *Sula leucogaster* (Brown Booby), and *Sula dactylatra* (Masked Booby) along the Makran coast of Balochistan, including Astola Island, Juddi Beach (Pasni), and Gurab Island. Field records and local knowledge confirm that these species are non-resident visitors, using these sites mainly for foraging activities rather than for breeding. Among these, *A. tenuirostris* (Lesser Noddy) and *S. leucogaster* (Brown Booby) represent the first confirmed records from Pakistan, while *S. dactylatra* (Masked Booby) has been previously reported from the region. Notably, *A. tenuirostris* has also been observed at Mubarak Village based on a single photographic record, though no further ecological details are available from that site.

From a biogeographical perspective, all three species are widely distributed tropical seabirds. The Lesser Noddy (*Anous tenuirostris*) is known to breed on coral atolls and mangrove islets across the Indian Ocean, including the Seychelles, Chagos Archipelago, and Maldives (Gochfeld et al., 2020; Harrison et al., 2021). Its presence at Juddi Beach and Astola Island, without evidence of nesting, indicates a range extension for feeding activity into the northern Arabian Sea. Similarly, the Brown Booby (*Sula leucogaster*) is distributed across the Atlantic, Indian, and Pacific Oceans, breeding extensively in regions such as the Caribbean, the Red Sea, northern Australia, and several oceanic islands (Nelson, 2005; BirdLife International, 2021). Its detection along the Balochistan coast represents the northernmost edge of its range in the Arabian Sea. The Masked Booby (*Sula dactylatra*), on the other hand, has one of the widest distributions among tropical seabirds, being recorded from nearly every ocean except the eastern Atlantic, northern Indian Ocean, and central-eastern Pacific (del Hoyo et al., 1992).

According to local fishermen, who have lived and fished around Astola Island since 2013, these species were observed for the first time in this region during the 2024 season. According to local reports that these birds visited temporarily for feeding with no evidence of nesting. Local fishermen's long-term familiarity with the area adds credibility to the assertion that these are newly recorded or infrequently sighted seabirds in the coastal waters of Balochistan. This aligns with the lack of previous data or published reports on their occurrence in Pakistan's offshore islands, indicating that the current findings

TABLE 1. Monthly counts of three bird species (*Anous tenuirostris*, *Sula leucogaster*, and *Sula dactylatra*) observed at Juddi Beach, Astola island and Guraab Island of the Makran region between August 2024 and September 2025. Dash (–) indicates no individuals recorded.

S.No.	Species Name	Aug 2024	Nov 2024	Dec 2024	Jan 2025	Feb 2025	Mar 2025	Aug 2025	Sep 2025	Total	IUCN Status
1	*Anous tenuirostris* (Lesser Noddy)	1	1	-	-	-	-	-	-	2	Least Concern (Stable)
2	*Sula leucogaster* (Brown bobby)	-	7	2	3	2	5	9	6	34	Least Concern (Decreasing)
3	*Sula dactylatra* (Masked bobby)	-	14	12	8	8	6	15	11	74	Least Concern (Decreasing)

represent first records of seasonal visitation for these species. Local fishermen further reported that, despite the regular presence of many seabird species around Astola Island and the adjacent areas, only the Sooty Gull is known to breed there. This indicates that while Astola Island serves as a critical foraging ground, it does not function as a major breeding habitat for most seabirds.

The monthly abundance data (Table 1) show that *S. dactylatra* (Masked Booby) was the most abundant species, with 74 individuals observed, followed by *S. leucogaster* (Brown Booby) with 34 individuals, and *A. tenuirostris* (Lesser Noddy) recorded only twice during the study period. The relatively high numbers of Masked Boobies compared to Brown Boobies suggest that Masked Boobies are the dominant visiting species around Gurab island. Behavioral differences were also observed, with Masked Boobies being more active and agile divers than Brown Boobies which indicates that its occurrence is closely linked with abundant fish availability including small pelagic species, cuttlefish, and groupers known to inhabit the reef and offshore areas of Astola Island.

In terms of conservation status, all three recorded species are currently listed as Least Concern (LC) on the IUCN Red List, (2025) However, their sporadic presence in Pakistani waters underscores the need for continued monitoring to assess any potential shifts in distribution patterns, possibly related to climate change, marine productivity, or habitat disturbances in the wider Indian Ocean region.

The integration of local ecological knowledge, particularly from experienced fishermen has proven crucial in documenting these first sightings. Such collaboration bridges scientific and traditional knowledge systems and enhances the accuracy of biodiversity assessments in remote coastal and island habitats.

CONCLUSION

Overall, the findings suggest that Astola Island and the surrounding waters of the Makran coast play an emerging role as important seasonal feeding sites for tropical seabirds extending into the northern Arabian Sea. Future work should focus on systematic seabird surveys, prey abundance assessments, and environmental monitoring to better understand the ecological drivers behind these newly recorded occurrences and to support conservation planning for Pakistan's offshore islands.

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First systematic analysis of marsh frog, *Pelophylax ridibundus* sensu lato (Pallas,1771) (Anura: Ranidae) in central Iran based on morphological, morphometric and histometric data

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Abstract
Although marsh frog (*Pelophylax ridibundus*) is spread all over Iran except desert areas, but there is no systematic information about the populations of central Iran. Totally 35 specimens were collected from Aran-Bidgol and Najaf Abad from March 2021 to April 2022. Morphological results revealed high color polymorphism especially on the dorsal surface of their bodies. 15 morphometric characters were measured in this research. The result obtained from the t-test showed that there is no sexual dimorphism. Aran and Bidgol frogs had a larger body size than Najaf Abad. Results of Anova and discriminant analysis represented populations separation between the two regions**.** Histometric study showed the thickness of the skin on the dorsal surface of Najaf Abad frogs was more than Aran-Bidgol frogs**.** The number of serous and mucous glands in the dorsal and ventral skin of male and female *Pelophylax ridibundus* in Aran-Bidgol was significantly higher than that of Najaf Abad. This issue may be related to the desert climate (hot and dry) of Aran- Bidgol. In summary, systematic analysis of marsh frogs based on morphological, morphometric and histometric data showed significant differences between the marsh frogs of the two regions that can be related to the different geographical conditions.

Keywords: Histometry, Isfahan province, Morphology, Morphometry, *Pelophylax ridibundus***.**

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INTRODUCTION

Order Anura is cosmopolitan except for extreme northern latitudes, Antarctica, and most oceanic islands. There are five family (Dicroglossidae, Pelobatidae, Ranidae, Hylidae and Bufonidae) of Anura in Iran. Ranidae family is cosmopolitan except for southern South America, South Africa, Madagascar, and most of Australia (Frost, 2023). There are two genera of the family in Iran, *Rana* (2 species) and *Pelophylax* (1 species). *Pelophylax ridibundus* (Pallas, 1771), was reported from the northern coast of Caspian Sea and Volga River, for the first time. Genus *Pelophylax* is the most problematic anuran in western Palearctic (Dufresnes et al., 2018) and in fact, there is a species complex (*Pelophylax ridibundus* complex) in Iran

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(Pesarakloo et al., 2017). Marsh frog is spread all over Iran except desert areas. The taxonomic status of the species in Iran is unclear and some populations of marsh frogs are not studied, so far.

Many reports are published about the decline of the amphibian population (Zhao et al., 2022) due to the climate change and global warming (Hinderer, 2024). Skin health is strongly affected in the direct contact with the environment and climate changes. Hence, the study of amphibian skin, which has various biological functions including protection, breathing and defense against external factors, is very important. Isfahan province, which is located at the center of Iran, is a suitable area to study amphibians due to its climatic diversity. Since there is no systematic data about marsh frog populations of central Iran, the main purpose of this research is to investigate the marsh frogs of Najaf Abad and Aran-Bidgol regions of Isfahan province based on morphological, morphometric data and histometric structure of their skin that would be helpful to strengthen future taxonomic revision of the species.

MATERIAL AND METHODS

SAMPLES COLLECTION

From March of 2021 to April of 2022, 35 samples of *Pelophylax ridibundus* were collected (Table1). Aran-Bidgol is the northernmost city of Isfahan province. The elevation of this region is 950 m a.s.l and the climate of this region is hot and dry with hot and scorching summers and cold and dry winters. Najaf Abad is located in a plain with a moderate and relatively dry climate. The elevation of this area is1650 m a.s.l.

MORPHOLOGICAL STUDIES

Photos were taken from the dorsal, ventral and lateral surfaces of all collected specimens. Species identification was done using the identification keys (e.g., Baloutch & Kami, 2006). Presence or absence of dorsolateral folds, tympanic membrane, tongue shape, digital disks, jaw teeth, vomerine teeth, vertebral line, head shape, temporal spot, vocal sacs, eyes shape, color variation, teeth and metatarsal tubercles are important characters in species identification. The sex of samples was recognized by observing the presence or absence of nuptial pads associated with forelimbs.

MORPHOMETRIC STUDIES

In this study, 15 morphological traits were measured (Balouch & Kami,2006). These are: snout-vent length (SVL), head length (HL), thigh length (THL), length of tibia (TL), tarsus length (TAL), , distance between tip of snout and eye (ESD),upper eyelids distance (UED), width of upper eyelid (UEW), horizontal diameter of eye (ED), tympanic diameter (DT), foot length (FL), length of first toe (LFT), length of inner metatarsal tubercle (LMT), distance between nostril and anterior corner of eye (NED), and internarial distance (IND). These characters were measured using a digital caliper with an accuracy of 0.01 mm. Only adult specimens were considered.

TISSUE SAMPLING AND PROCESSING

The samples were scarified and the dorsal and ventral parts of the skin of each frog were peeled off and immersed in Bouin's solution for 48 hours before routine paraffin embedding process. The specimens were dehydrated in alcohol, cleared in xylene, and sections with 5 µm thickness were prepared using rotary microtome (Leica RM2145, Germany) and stained with hematoxylin-eosin. Microscopic images were captured using light microscopy (Olympus BH, Japan) equipped with camera (Olympus DP71, Japan).

HISTOMETRIC ANALYSIS

The thickness of the skin, epidermis and dermis as well as the diameters and number of serous, mucous and granular glands were evaluated by Axiovision 4.5 LE software (*Zeiss*, Oberkochem) on digital images. The skin, epidermis and dermis thickness and diameters of serous, mucous and granular glands were analyzed in 10 microscopic fields in each sample. Mean number of different glands was evaluated in 1mm length of skin in each sample.

TABLE 1. Information of sampling areas of *P.ridibundus* in this study.

No	Sampling areas	N	Coordinates	Elevation
1	Khazagh village, Aran-bidgol, Esfahan Provine	18	34°03'38"N 51°18'52"E	951 m
2	Fooladshahr, Najaf abad, Esfahan Provine	17	32°30'06"N 51°23'44"E	1640 m

TABLE 2. T-test result of *Pelophylax ridibundus.*

Characters	(Sig)	Sig(2tailed)
SVL	0.00	0. 84
HL	0.00	0.12
ESD	0.96	0.36
IND	0.26	0.00
UED	0.10	0. 33
UEW	0.14	0.24
ED	0.00	0.85
DT	0.38	0.08
THL	0.00	0.05
TL	0.00	0.05
TAL	0.00	0.14
FL	0.01	0.13
LFT	0.05	0.12
LMT	0.00	0.13
NED	0.19	0. 84

STATISTICAL ANALYSIS

The data were analyzed using SPSS software, version 16.0 (SPSS Corp., Chicago, IL, USA). An independent sample t-test was used to compare the histometric parameters of the skin between two areas. Statistical differences were considered significant at P-values less than 0.05. For morphometric analysis, univariate and multivariate analyses were used. Univariate analysis includes t test, Anova and descriptive statistics. Discriminant analysis was used to describe functions that maximize the possibility of correct classification of samples to their original population.

RESULTS

MORPHOLOGY AND SPECIES IDENTIFICATION

Samples were carefully examined using the mentioned morphological characteristics and identification keys. Characteristics such as the presence of dorsal folds on the sides, the presence of a bright vertebral stripe on the dorsal surface of most samples and the fact that the digits of hind limbs were not webbed completely so that the last joint of the fourth finger had no web (Fig. 1), indicate that all collected frogs based on the morphological characters belong to *Pelophylax ridibundus* species. The frogs examined in this research show high color polymorphism (Fig. 2), especially on the dorsal surface of their bodies.

TABLE 3. Morphometric measurements (Mean±SD; range) and ANOVA values of *P. ridibundus.*

Characters	Aran - Bidgol (n=16)		Najaf Abad (n=13)		P
	Mean ± SD	Min / Max	Mean ± SD	Min / Max	
SVL	68.84 ± 10.7	52.68-92.26	61.96 ± 4.05	52.32-67.59	0.03
HL	23.25 ± 3.04	19.41-30.79	20.54 ± 1.10	18.60-22.52	0.00
ESD	11.33 ± 2.03	8.33-15.78	8.66 ± 0.61	7.63-9.50	0.000
IND	4.36 ± 0.63	3.23-5.95	3.94 ± 0.56	2.98-5.24	0.07
UED	4.08 ± 0.80	2.51-5.42	3.25 ± 0.59	2.28-4.29	0.00
UEW	4.90 ± 0.66	3.61-6.39	3.77 ± 0.33	3.10-4.19	0.000
ED	8.34 ± 0.98	7.10-10.33	6.49 ± 1.13	3.13-7.78	0.000
DT	4.59 ± 0.61	3.61-5.93	4.39 ± 0.37	3.54-4.91	0.30
THL	33.05 ± 5.1	23.98-44.51	28.03 ± 2.21	22.92-30.80	0.00
TL	34.21 ± 4.5	28.01-44.20	32.03 ± 1.8	28.22-34.94	0.12
TAL	17.53 ± 3.2	14.10-25.51	14.87 ± 1.2	13.01-17.08	0.01
FL	35.10 ± 5.005	28.91-48.07	32.07 ± 2.89	27.18-36.94	0.06
LFT	8.73 ± 1.2	6.92-11.22	8.10 ± 0.87	6.51-10.16	0.14
LMT	3.74 ± 0.59	3.05-5.43	3.24 ± 0.42	2.59-4.04	0.02
NED	4.82 ± 0.59	3.54-5.67	4.21 ± 0.47	3.52-5.09	0.00

TABLE 4. Classification results of discriminate analysis with fifteen morphometric characters.

	Groups	Aran - Bidgol	Najaf Abad	Total
Count	Aran - Bidgol	15	1	16
	Najaf Abad	2	11	13
%	Aran - Bidgol	93.8	6.3	100.0
	Najaf Abad	15.4	84.6	100.0

MORPHOMETRY

The result obtained from the T-Test (Table2) showed that no significant difference (P>0.05) was observed between male and female frogs in all measured characters (except IND). Therefore, there is no sexual dimorphism.

Descriptive statistics revealed that Aran-Bidgol frogs have a larger body size (SVL =68.84 ± 10.7) than Najaf Abad frogs. According to Table 3, there are significant differences in 10 characters, snout-vent length (SVL), head length (HL), distance between tip of snout and eye (ESD), upper eyelids distance (UED), width of upper eyelid (UEW),), horizontal diameter of eye (ED), thigh length (THL), tarsus length (TAL), length of inner metatarsal tubercle (LMT), distance between nostril and anterior

corner of eye (NED) between the populations (Aran-Bidgol and Najaf Abad), (P<0.05). Therefore, separation between populations is observed in many characters.

In discriminant analysis, single function was considered, which represented 100% of the changes and was significant (P<0.05). About 93.8% of the samples from Aran-Bidgol region are in their own group and 6.3% are in the Najaf Abad group. Moreover, 84.6% of the samples from Najaf Abad region are in their own group and 15.4% are in the Aran-Bidgol group. Totally, 89.7% of samples were classified correctly (Table 4).

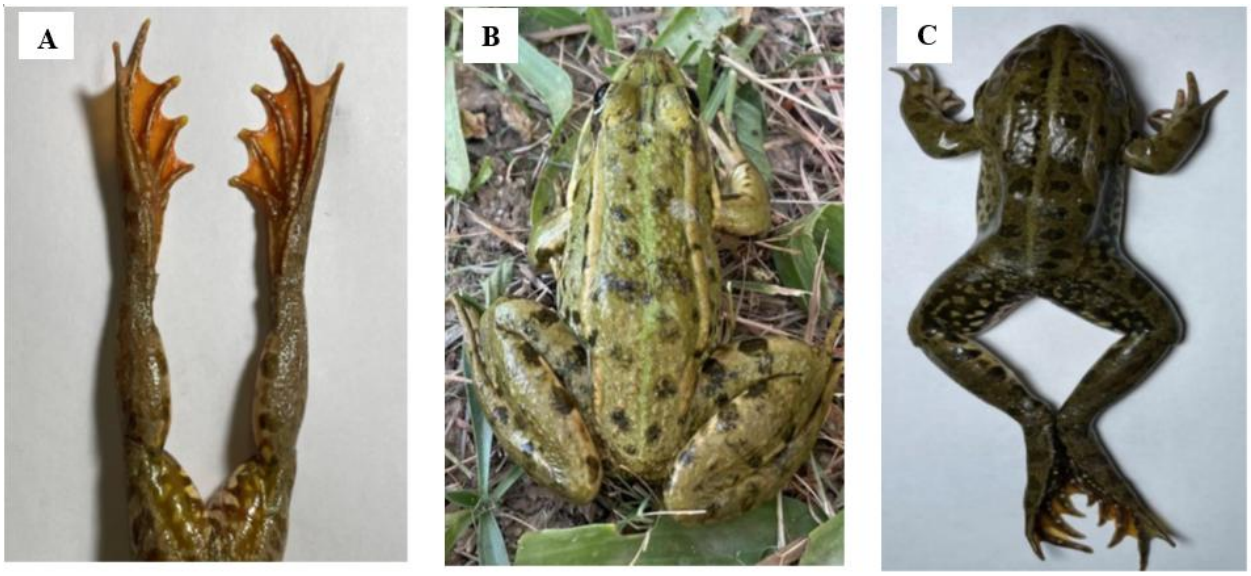

FIGURE 1. Characters used in the identification of *Pelophylaxis ridibundus*. A) web of hindlimbs; B) dorsal folds; C) vertebral strip.

HISTOMETRIC ANALYSIS

The dorsal and ventral skin in male and female of *Pelophylax ridibundus* of Aran-Bidgol and Najaf Abad regions consist of epidermis and dermis. There were two types of small skin glands, including mucous glands and serous glands, and large skin glands, including granular (poisonous) glands, in the spongy layer of the dorsal skin in male and female *Pelophylax ridibundus* of different regions (Fig. 3).

Significant differences were observed in the dorsal dermis ($P<0.001$) and skin ($P<0.001$) thickness of females *Pelophylax ridibundus* between Aran-Bidgol and Najaf Abad (Table 5). There are no significant differences in the ventral skin of male and female *Pelophylax ridibundus* between Aran-Bidgol and Najaf Abad (Table 5). Significant differences ($P<0.001$) were observed in the dorsal skin thickness in the female *Pelophylax ridibundus*, in Najaf Abad and Aran-Bidgol, respectively (Table 5).

There are significant differences ($P<0.001$) in the dorsal dermis and skin thickness ($P<0.001$) between male and female *Pelophylax ridibundus* in Aran-Bidgol (Table 6). The ventral dermis thickness was significantly different ($P<0.001$) between male and female *Pelophylax ridibundus* in Najaf Abad (Table 6). A statistically significant difference ($P<0.001$) was observed in the dorsal skin thickness between male and female *Pelophylax ridibundus* in Aran-Bidgol (Table 6). There is no significant difference in the diameters of serous and mucous glands in the dorsal and ventral skin of male and female *Pelophylax ridibundus* from Aran-Bidgol and Najaf Abad (Table 7). The number of serous and mucous glands in the dorsal and ventral skin of male and female *Pelophylax ridibundus* in Aran-Bidgol was significantly ($P<0.05$) higher than those in Najaf Abad (Table 8).

FIGURE 2. Color polymorphism of *P. ridibundus* in studied areas.

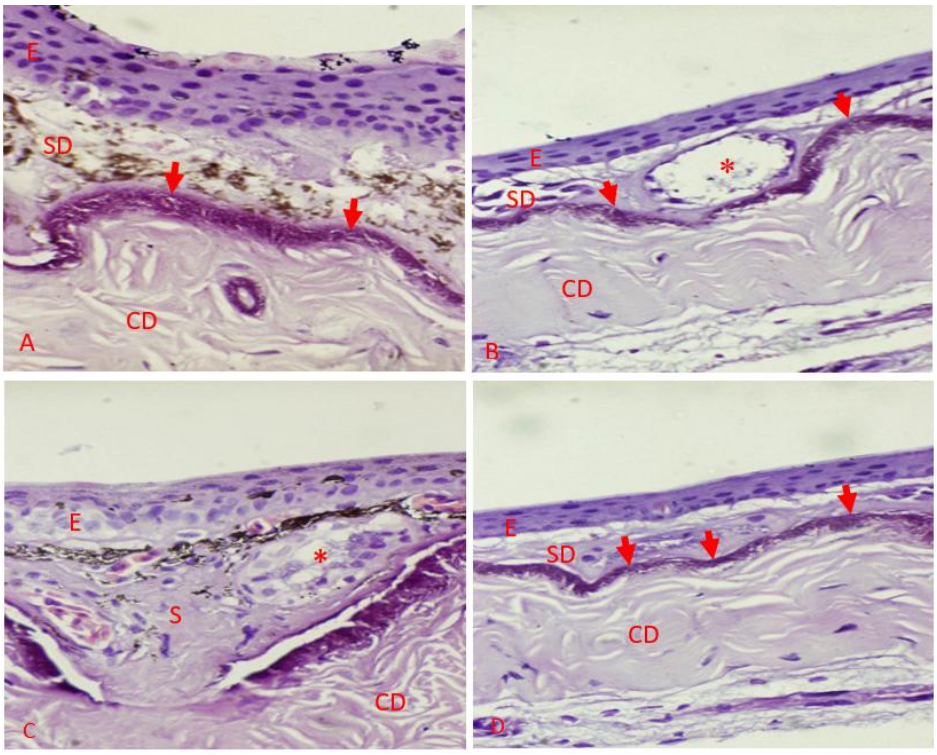

FIGURE 3. Microscopic sections of the skin in *Pelophylax ridibundus* of Aran-Bidgol and Najaf Abad regions (Hematoxylin-Eosin staining, ×40). (A): Dorsal skin in male frog (Najaf Abad), (B): Ventral skin in male frog (Najaf Abad), (C): Dorsal skin in male frog (Aran-Bidgol), (D): Ventral skin in male frog (Aran-Bidgol). Epidermis (E), Spongy dermis (SD), Compact dermis (CD), Mucous gland (Red star), Ebert-Kastchenko layer (Red arrow).

TABLE 5. Comparison of epidermis, dermis and total skin thickness (μm) between male and female *Pelophylax ridibundus* in Najaf Abad and Aran-Bidgol regions.

Parameter	Dorsal surface of body			Ventral surface of body		
	Skin	**Epidermis**	**Dermis**	**Skin**	**Epidermis**	**Dermis**
			Najaf Abad			
Males	41.03±11.611	4.23±2.910	41.10±5.807	32.60±2.143	3.83±0.648	31.10±3.916
Females	45.47±9.333	2.77±0.935	21.47±1.676	28.30±5.240	3.73±0.740	19.00±3.787
***P* value**	0.010	0.001	0.010	0.182	1.000	0.000
			Aran-Bidgol			
Males	40.63±10.601	4.33±1.900	42.20±5.857	3.77±2.046	4.43±0.774	27.77±2.661
Females	32.23±3.390	3.30±0.651	28.43±2.861	27.40±4.940	3.68±0.740	18.91±3.787
***P* value**	0.000	0.001	0.000	0.080	1.000	0.010

TABLE 6. Comparison of epidermis, dermis and total skin thickness (μm) in male and female *Pelophylax ridibundus* between Najaf Abad and Aran-Bidgol regions.

Parameter	Dorsal surface of body			Ventral surface of body		
	Skin	**Epidermal**	**Dermis**	**Skin**	**Epidermis**	**Dermis**
			Males			
Najaf Abad	41.03±11.611	4.23±2.910	41.10±5.807	32.60±2.143	3.83±0.648	31.10±3.916
Aran-Bidgol	40.63±10.601	4.43±1.900	42.20±5.857	32.77±2.046	4.43±0.774	27.77±2.661
***P* value**	0.089	0.234	0.457	1.000	0.236	0.090
			Females			
Najaf Abad	45.47±9.333	2.77±0.935	21.47±1.676	28.30±5.240	3.73±0.740	19.00±3.878
Aran-Bidgol	33.23±3.390	3.30±0.651	28.43±2.861	27.40±4.940	3.68±0.740	18.91±3.787
***P* value**	0.000	0.384	0.000	1.000	0.245	0.412

TABLE 7. Comparison of skin glands diameters (μm) in male and female *Pelophylax ridibundus* between Najaf Abad and Aran-Bidgol regions.

Parameter	Dorsal surface of body		Ventral surface of body	
	Serous glands	**Mucous glands**	**Serous glands**	**Mucous glands**
		Males		
Najaf Abad	182.07±2.07	241.10±3.58	183.55±1.91	237.88±2.85
Aran-Bidgol	187.25±2.35	243.8±3.56	187.68±1.85	241.79±3.06
***P* value**	0.105	0.593	0.127	0.353
		Females		
Najaf Abad	187.57±2.04	240.12±3.41	185.42±2.02	237.88±2.83
Aran-Bidgol	196.04±4.54	238.70±2.85	187.42±2.27	241.79±3.06
***P* value**	0.094	0.750	0.514	0.353

TABLE 8. Comparison of skin glands numbers in male and female *Pelophylax ridibundus* between Najaf Abad and Aran -Bidgol regions.

Parameter	Dorsal surface of body		Ventral surface of body	
	Serous glands	Mucous glands	Serous glands	Mucous glands
		Males		
Najaf Abad	2.86±0.73	2.63±0.13	2.90±0.13	2.90±0.13
Aran-Bidgol	3.26±0.63	3.20±0.12	3.20±0.10	3.13±0.10
***P* value**	0.028	0.002	0.085	0.184
		Females		
Najaf Abad	2.96±0.12	2.90±0.12	2.83±0.13	2.93±0.12
Aran-Bidgol	3.36±0.11	3.33±0.12	3.16±0.15	3.03±0.14
***P* value**	0.019	0.022	0.108	0.609

DISCUSSION

Our morphological results show the collected samples are *P.ridibundus.* This species demonstrates high color polymorphism among sample areas, which is consistent with the results of previous studies of the species in Iran (Poorjafary & Fakharzadeh, 2022; Pesarakloo et al., 2011; Delavar et al., 2017). Marsh frogs of central Iran did not show sexual dimorphism. Although this result is consistent with some of the previous studies (Seydi and Fakharzadeh, 2016; Nemati,1998), most examined populations of the frogs in Iran show sexual dimorphism (e.g. Poorjafary & Fakharzadeh, 2022; Fathinia et al.,2012; Hezaveh et al., 2007). Various reasons have been proposed for the emergence of sexual dimorphism in amphibians. Some researchers believe it is due to sexual selection (Shine, 1979). But some scientists indicate sexual size dimorphism in the Anura can be explained by differences in the age structure between the sexes in breeding populations. If sexual selection has an effect on size dimorphism in anurans, this is probably to be only a secondary one (Monnet and Cherry., 2002). Results of ANOVA and discriminant analysis represent populations separation between the two regions that would be related to different geographical conditions of the studied areas. Such interpopulation variation for this frog from different areas of Iran has been reported in previous studies (e.g. Fakharzadeh et al., 2023; Hezaveh et al., 2007; Seydi and Fakharzadeh, 2016)

According to classical taxonomy, Iranian water frog has been considered as *P. ridibundus.* Recently a molecular study (Pesarakloo et al., 2017) based on mitochondrial gene (Cytb) showed that two major clades of water frog can be recognized in Iran. One of these clades includes samples from the West, the northwest and southwest of Iran called *Pelophylax bedriagae* and the second one (*Pelophylax* sp.) includes North and Northeast populations. Some populations of this frog, including the populations of central Iran (present study), have not been investigated in the mentioned molecular study. Due to lack of sufficient information, definitely cannot be talked about the taxonomic status of this species in Iran. Although the comparison of our results in central Iran with previous papers on marsh frogs in the west of Iran shows some differences in populations, it seems necessary to conduct comprehensive molecular studies on Iranian marsh frogs.

Present study showed that the dorsal and ventral skin of male and female *Pelophylax ridibundus* in Najaf Abad and Aran-Bidgol consist of epidermis and dermis. Our microscopic analysis showed that the dorsal and ventral dermis of the male and female *Pelophylax ridibundus* in the different regions are composed of two distinct layers structurally, which respectively includes the spongy layer, consisting of loose connective tissue containing blood vessels and skin glands, and the dense layer, consisting of dense connective tissue contains thick bundles of collagen fibers that extend parallel to the skin surface. Our histologic findings are in accordance with the results of Felsemburgh (2009), and Saberi et al., (2020). Bingol-Ozakpinar & Murathanoglu (2011) showed that the ventral and dorsal skin in *Triturus karelinii*

have similar morphological structure. However, Azevedo (2006) reported that there is water in the spongy layer in *Rana catesbeiana*, which has turned this layer into a water reservoir.

Furthermore, this study showed that two types of small skin glands, including mucous glands and serous glands, and large skin glands, including granular (poisonous) glands were observed in the spongy layer of the dorsal skin of male and female of *Pelophylax ridibundus* in Najaf Abad and Aran-Bidgol. However, only mucous and serous glands exist in the spongy layer of the ventral skin of male and female frogs in both regions. This result is in accordance with the findings of Haslam (2014), Brunetti (2016) and Papahn et al., (2017).

Histometrical analysis showed that the thickness of the dorsal skin of *Pelophylax ridibundus* in Najaf Abad is greater than Aran-Bidgol. This issue may be related to the desert climate (hot and dry) of Aran-Bidgol, because the skin must be moist to be able to breathe through it. So, the dorsal skin of *Pelophylax ridibundus* in Aran-Bidgol is thinner. The result of a study on the skin of water frogs' populations in Golestan and Mazandaran provinces showed that populations live in higher altitudes have thicker skin, and the authors stated that this observed difference in the dorsal skin between populations in different regions may have an adaptive role (Taziki et al., 2024).

Moreover, our study showed that the number of mucous and serous glands of male and female *Pelophylax ridibundus* in Aran-Bidgol is higher than that in Najaf Abad. Duellman and Trueb (1994) reported that there are intraspecific variations in Ranids in relation to habitat differences.

Sinsch & Lehr (2010) reported that the number of epidermal mucous glands in the *Telmatobius carrillae* is not related to gender and skin thickness. Papahn et al., (2017) found that the number of the epidermal mucous glands of *Pelophylax ridibundus* in cold region is higher than that in warm region.

Mills & Prum (1984) found that the number of granular glands in the dorsal skin of *Pelophylax ridibundus* is more than that of the ventral skin. In addition, Duellman & Trueb (1994) reported that the mucous glands in the dorsal skin of *Pelophylax ridibundus* are more than the ventral skin, and they are more numerous and widespread than granular glands. Thiner skin of dorsal surface in Aran - Bidgol samples comparing to Najafabad samples can be related to their adaptation to hot and dry climate. As we know subcutaneous respiration is a principal phenomenon in amphibia and thinness of the epidermis plays an important role in such respiration (Kardong, 2019). The thickness of the epidermal layer and the distribution of glands differ between the dorsal and ventral areas, implying an underlying strategy of adaptation (Sulaiman et al., 2025). So having thinner skin with more mucous and serous glands for Aran-bidgol marsh frogs that live in such climate conditions seems to be an appropriate strategy for them.

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Molecular Systematics of Zoantharian species (Cnidaria) and their Endosymbionts in the Bostaneh Bay, the Persian Gulf

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Abstract
Zoantharians have an important ecological role in marine ecosystems. Despite their worldwide distribution, studies have been conducted in some areas of the Persian Gulf, these investigations do not cover all regions. For this purpose, 18 samples of zoantharian colonies in three transects of shallow waters of Bostaneh port were collected by snorkeling and scuba diving. 4 extra colonies also were sampled in the intertidal zone of Khark Island for comparison with the sampling areas of BP. All zoantharian specimens were photographed in situ and morphological characteristics of each specimen were recorded, i. e. polyp shape, polyp color, tentacle number and color, external coloration, oral plate color and oral region color. After DNA extraction, mitochondrial 16S ribosomal DNA (16S rDNA) and cytochrome oxidase subunit I (COI) were amplified by polymerase chain reaction (PCR). Based on obtained 16S rDNA and COI gene sequences, three species were identified: *Zoanthus sansibaricus*, *Palythoa* aff. *mutuki* and *Palythoa tuberculosa*. We developed quantitative PCR (qPCR) assays to distinguish each of the three genera (*Symbiodinium*, *Cladocopium* and *Durusdinium*) of dinoflagellate endosymbionts through three clade-specific primer sets aiming at ITS1-5.8S-ITS2 (for *Symbiodinium*), partial ITS-1 (for *Cladocopium*), and LSU (for *Durusdinium*) of the nuclear rDNA of Symbiodiniaceae from which, (genus *Symbiodinium*) commonly found in Zoantharia. In this study, the Zoantharia of Bostaneh Port were identified for the first time.

Keywords: Zoantharian, COI, 16S rDNA, Persian Gulf, Bostaneh, *Zoanthus sansibaricus*, *Palythoa* aff. *mutuki*, *Palythoa tuberculosa*, Symbiodiniaceae, *Symbiodinium*, *Cladocopium*, *Durusdinium*

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INTRODUCTION

The order Zoantharia, which belongs to class Anthozoa, comprises 27 genera allocated to 9 families in 2 suborders: Brachycnemina and Macrocnemina (Belford, 2023; Reimer & Sinniger, 2018). This order is one of the hexacorallian groups that has been categorized by morphological and molecular methods (Bazzaz *et al*., 2024). They are characterized by two rows of tentacles in each polyp and one ventral siphonoglyph, and they form a colony (Haddon & Shackleton, 1891). Despite worldwide distribution in

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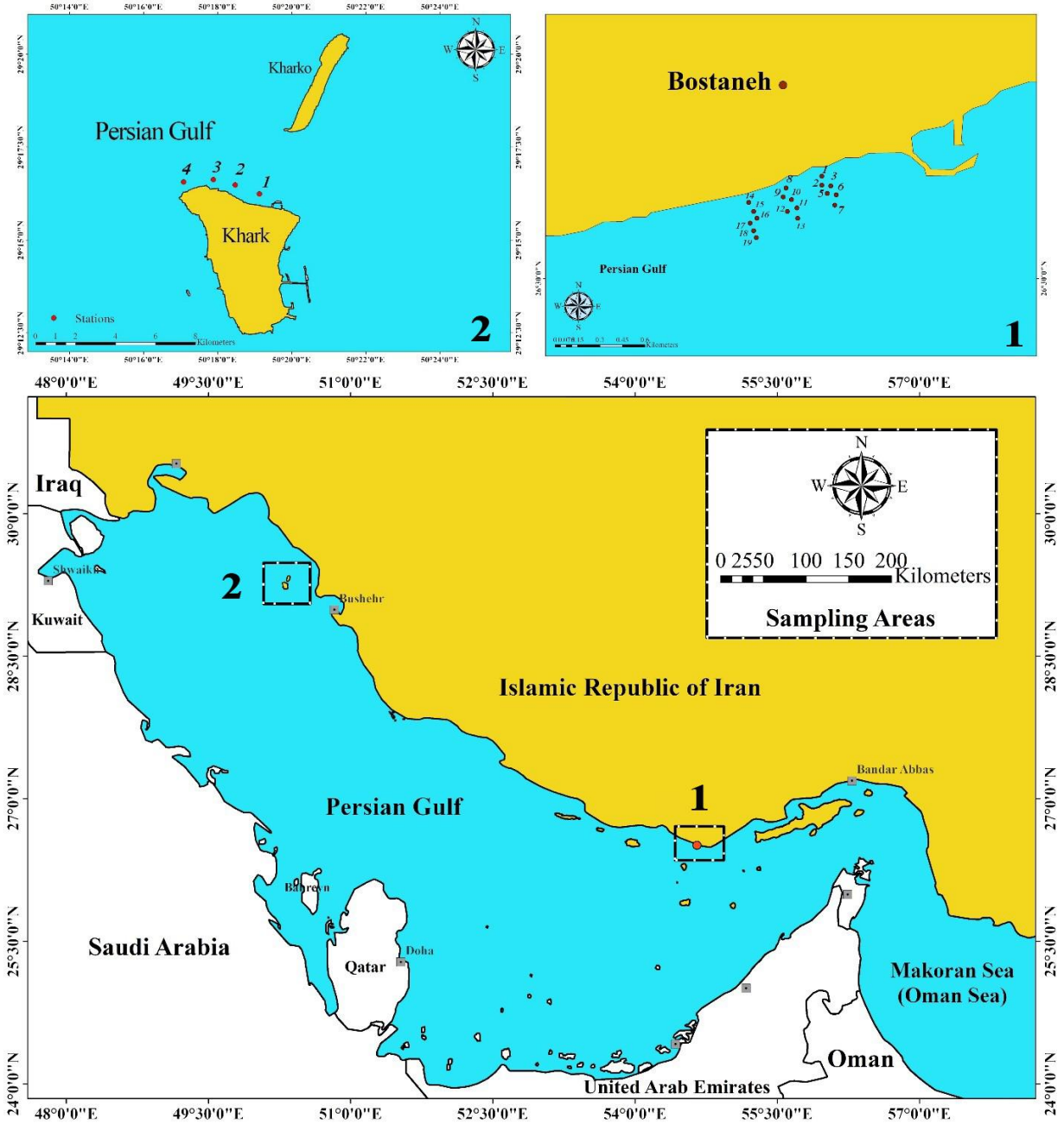

FIGURE 1. Map of the Persian Gulf showing the position of the sampling locations in the (1) Bostaneh Bay and (2) Khark Island.

tropical and subtropical waters (Reimer *et al.*, 2004; Reimer *et al.*, 2012), zoantharians and symbiodiniaceae genera have yet to be identified across all regions of the Persian Gulf.

Zoantharian is the ecologically significant group that is connected to reef-building corals (Reimer *et al.*, 2022). They make mutualistic symbioses with unicellular algae of the Symbiodiniaceae family (Fujiwara *et al.*, 2021). A genus Symbiodinium is a large group of dinoflagellates living as endosymbionts in the endoderm of tropical and subtropical cnidarians, where the products of their photosynthetic processing are exchanged in the host for the inorganic molecules (Baker, 2003; Muscatine & Cernichiari, 1969; Noda *et al.*, 2017). The ability of symbiotic micro-algae not only to carry out

photosynthesis but also to translocate the photosynthetic products to their host species is believed to be the foundation of the success of coral reef-dwelling species such as corals and mollusks (Garcia-Ramos & Banaszak, 2007).

The Persian Gulf is a large, shallow, semi-enclosed marginal sea located in the subtropical northwest of the Indian Ocean. This region is characterized by extreme thermal variability and high salinity, and as a result, many organisms in this area are living near the margins of their physiological limits. Bostaneh port is in the central part of Bandar Langeh city in Hormozgan province, situated on the northern coast of the Persian Gulf. Bostaneh Fishing Port is situated at the geographic coordinates of 26.511°N and 54.653°E and features a seabed characterized by rocky and coral textures. These beds have a diverse range of benthic species due to their distance from urban communities. From the high diversity qualities of marine invertebrate creatures displayed in this ecoregion, the beginning taxonomical endeavors had devoted towards the zoantharian due to their wide substrate cover and taxonomical differing qualities. Comparatively few investigations have been done on the diversity of zoantharian taxonomy in the Persian Gulf: Hormoz, Qeshm, Kish, Hengam, and Larak islands (Koupaei *et al*., 2016a; Koupaei *et al*., 2014; Koupaei *et al*., 2016b; Koupaei *et al*., 2018), Farur, Bani Farur, Hormoz and Chabahar Bay (Panahi Bazzaz *et al.*, 2024). Also, through these investigations, the following species had been recorded: *Zoanthus sansibaricus*, *Z.* sp., *Palythoa* aff. *mutuki*, *Palythoa tuberculosa*, *Palythoa* cf. *mutuki*, and *Neozoanthus* sp.

Due to the lack of existing information on zoantharians in the coastal waters of Bostaneh, we undertook a comprehensive assessment of these organisms using both morphological and molecular approaches. Zoantharian species were identified through mitochondrial markers, including cytochrome oxidase subunit I (COI) and 16S ribosomal DNA (16S rDNA), and the associated Symbiodiniaceae genera were also characterized. This study represents the first documentation of molecular data for these benthic organisms along the northern coast of the Persian Gulf. Furthermore, because no prior research has investigated zoantharians within the islands of Bushehr Province, additional sampling was carried out in the intertidal zone of Khark Island to enable comparison with the sampling sites in Bostaneh Port.

MATERIAL AND METHODS

Specimen collection

In mid-November 2023, 18 samples of zoantharian colonies in three transects of shallow waters of Bandar Bostaneh port (26° 30′ 37″ N 54° 39′ 20″ E), Hormozgan Province, and 4 zoantharian colonies in the intertidal zone of Khark island have been collected by snorkeling in lower intertidal (1-1.5 m depth) and by scuba diving in subtidal zones (2-3.5 m depth) during 3 days (Fig.1 & Table 1). The line Intercept Transect (LIT) method was adopted to estimate intertidal and subtidal zoanthid distribution pattern and abundance. Water temperature and salinity were measured simultaneously using Horiba U10 probe. Specimens were stored in a 20% dimethyl sulfoxide (DMSO) saturated by NaCl buffer and were transferred to the Razi laboratory in Science and Research Branch, IAU for further analysis.

Morphological analyses

All zontharian were photographed in situ and identified following valid identification keys (López *et al*., 2019; Mizuyama *et al*., 2018; Reimer & Sinniger, 2018) based on external morphological features: polyp shape, polyp color, tentacle number and color, external coloration, oral plate color, and oral region color (Fig.2).

DNA extraction, PCR amplification and sequencing

DNA was extracted using the Cetyl Trimethyl Ammonium Bromide (CTAB) method (Baker, 1999). Amplification of mitochondrial cytochrome oxidase c subunit I (COI) has been done using a set of primers (For: 5'-GGTCAACAAATCATAAAGATATTGG-3' and Rev: 5'-TAAACTTCAGGGTGACCA

TABLE 1. Sample sites, depth of sampling, collector, geographical positions and temperature- salinity results

Specimen name	Collection site and depth	Collector	Longitude	Latitude	Temperature (°c)	Salinity (psu)
Bost 1	Bostaneh bay, (1m)	M.Q.Ashrafi	54°39′21.6″	26°30′21.6″	22.71± 0.18	37.20± 0.21
Bost 2	Bostaneh bay, (1.5m)	M.Q.Ashrafi	54°39′20.99″	26°30′20.122″	22.31± 0.11	37.22± 0.18
Bost 3	Bostaneh bay, (1.5m)	M.Q.Ashrafi	54°39′22.931″	26°30′19.973″	22.12± 0.21	37.45± 0.10
Bost 5	Bostaneh bay, (2m)	M.Q.Ashrafi	54°39′22.187″	26°30′18.335″	21.45± 0.20	36.94± 0.11
Bost 6	Bostaneh bay, (2m)	M.Q.Ashrafi	54°39′24.123″	26°30′18.037″	21.32± 0.17	36.55± 0.22
Bost 7	Bostaneh bay, (3.5 m)	M.Q.Ashrafi	54°39′23.825″	26°30′15.804″	21.54± 0.10	36.90± 0.23
Bost 8	Bostaneh bay, (1m)	M.Q.Ashrafi	54°39′13.252″	26°30′19.526″	21.45± 0.12	36.76± 0.32
Bost 9	Bostaneh bay, (1.5m)	M.Q.Ashrafi	54°39′12.656″	26°30′17.59″	22.40 ± 0.12	36.29± 0.31
Bost 10	Bostaneh bay, (2m)	M.Q.Ashrafi	54°39′14.443″	26°30′16.995″	22.40± 0.16	36.47± 0.21
Bost 11	Bostaneh bay, (2m)	M.Q.Ashrafi	54°39′15.635″	26°30′15.208″	22.23± 0.18	37.14± 0.19
Bost 12	Bostaneh bay, (3.5m)	M.Q.Ashrafi	54°39′13.55″	26°30′14.463″	22.15± 0.11	37.54± 0.20
Bost 13	Bostaneh bay, (3.5m)	M.Q.Ashrafi	54°39′15.784″	26°30′12.974″	22.38± 0.19	37.43± 0.33
Bost 14	Bostaneh bay, (1m)	M.Q.Ashrafi	54°39′5.36″	26°30′16.25″	22.41± 0.23	37.45± 0.16
Bost 15	Bostaneh bay, (1.5m)	M.Q.Ashrafi	54°39′6.253″	26°30′14.463″	23.15± 0.27	37.11± 0.27
Bost 16	Bostaneh bay, (1.5m)	M.Q.Ashrafi	54°39′6.998″	26°30′12.974″	23.18± 0.35	37.17± 0.11
Bost 17	Bostaneh bay, (2m)	M.Q.Ashrafi	54°39′5.509″	26°30′11.932″	23.23± 0.15	37.61± 0.35
Bost 18	Bostaneh bay, (3.5m)	M.Q.Ashrafi	54°39′6.253″	26°30′10.294″	21.88± 0.24	37.20± 0.12
Bost 19	Bostaneh bay, (3.5m)	M.Q.Ashrafi	54°39′6.849″	26°30′8.805″	21.46± 0.19	37.30± 0.25
Kharg 1	Kharg island, (3.5m)	M.Q.Ashrafi	50°16′58.8″	29°16′30″	23.18± 0.12	38.15± 0.22
Kharg 2	Kharg island, (3.5m)	M.Q.Ashrafi	50°17′42″	29°16′40.8″	24.11± 0.20	37.84± 0.21
Kharg 3	Kharg island, (3.5m)	M.Q.Ashrafi	50°18′32.4″	29°16′37.2″	24.16± 0.14	38.10 ± 0.10
Kharg 4	Kharg island, (3.5m)	M.Q.Ashrafi	50°19′26.4″	29°16′19.2″	23.97± 0.17	38.19± 0.35

AAAAATCA-3') (Folmer *et al*, 1994). Cytochrome oxidase 1 (COI) gene amplification with a length of 760 base pairs was carried out under the following thermal cycle condition: 30 cycles including 30 seconds at 95°C for denaturation, 30 seconds at 47°C for annealing, and 90 seconds at 72°C for extension. For additional precision, mitochondrial 16S ribosomal DNA (mt 16S rDNA) with a length of 1000 base pairs were amplified using specific primers of zoantharian (For: 5'- GCCATGAGTATAGACGCACA -3'

and Rev: 5'- CGAACAGCCAACCCTTGG -3') provided by Sinniger et al, 2005. PCR amplification was performed under the following thermal condition: 30 cycles, including 30 (s) at 94°C for denaturation, 1 minute at 54°C for annealing, and 90 (s) at 72°C for elongation. Also, three clade-specific primers had been utilized to acquire the replications of Symbiodiniaceae genus genes. These primer sets aiming ITS 1-5.8 S-ITS 2 regions (for *Symbiodinium*, F: 5'-CCTCTTGGACCTTCCACAAC-3' and R: 5'-GCATGCAGCAACACTGCTC-3') (Correa *et al.*, 2009), LSU (for *Cladocopium*, F: AAGGAGAAGTCGTAACAAGGTTTCC and R: AAGCATCCCTCACAGCCAAA) (Ulstrup & Van Oppen, 2003), and domain 2 of the LSU (for *Durusdinium*, F: 5'-GCCGTGTACGGTGCTC GCTCTCAA-3' and R: 5'-GGCCACTCGCAA ATGGACAGC-3') (Correa et al., 2009).

Quantitative PCR

Three clade-specific primer sets aiming at ITS1-5.8S-ITS2 (for *Symbiodinium*) and LSU (for *Cladocopium* and *Durusdinium*) of the nuclear rDNA of *Symbiodinium* were approved in terms of their specificity and efficiency. The thermal condition of qPCR includes 35 cycles of 30 (s) at 94°C for denaturation, 30 (s) at 60°C for annealing, and 30 (s) at 72°C for extension. Reaction voluminosity was achieved using 8 µL ddH_2O, 10 µL SYBR Green Master Mix (2x), 0.5 µL each of forward and reverse (10 µm), and 1 µL template DNA (430 ng/ml). To compare the threshold cycle results for all three genera, equal concentrations of the genomes were used during qPCR (Correa et al., 2009).

Phylogenetic Analysis

The accession numbers that are acquired from this investigation have been submitted to GenBank and have been shown in the table 3. The aligned sequences were analyzed by way of Maximum Likelihood, Maximum Parsimony and Bayesian procedures.

Zoantharia

The genomic sequences obtained from this study, were aligned with COI and 16S-rDNA sequences from NCBI's nucleotide database utilizing the BLAST online software and therefore aligned with earlier reported zoantharian sequences on GenBank, applying the CluscalW algorithm (Thompson *et al.*, 1994). For analyzing the alignment databases, maximum likelihood (ML), maximum parsimony (MP), and Bayesian methods were used.

Maximum likelihood conclusion was accomplished in MEGA 7 software (Kumar *et al.*, 2016) by setting 1000 bootstrap replications. In order to analyze Maximum Parsimony, the PAUP 4.0 beta win version was used (Swofford, 2000). The Bayesian analysis was conducted in MrBayes 2.3 (Ronquist & Huelsenbeck, 2003) and was based on the model selected through MODELTEST 2.3 (Nylander, 2004). For selecting the best model for both ML and Bayesian, the Akaike Information Criterion was used. The Hasegawa–Kishino–Yano model (HKY) was the best fit for both ML and MrBayes of COI data (Hasegawa *et al*., 1985). For the data of 16S-rDNA, the general time-reversible (GTR) model (Rodriguez *et al*., 1990) with invariable sites (GTR + I) gave the best-fit model.

Symbiodiniaceae

The sequences obtained from our study were aligned by the ITS1-5.8S-ITS2 (for *Symbiodinium*), partial ITS-1 (for *Cladocopium*), and LSU (for *Durusdinium*) with the recorded sequences of GenBank. Sequences obtained from this study were deposited in GenBank, and their accession numbers are provided in the table 3. The new nucleotide sequences acquired during the present study were aligned with sequences available from GenBank utilizing the software CLUSTALW (Thompson *et al.*, 1994). Three alignment datasets for the following genus (*Symbiodinium*, *Cladocopium*, and *Durusdinium*) were generated and analyzed through utilizing maximum likelihood (ML), maximum parsimony (MP), and Bayesian methods. The most qualified model selection for ML and Bayesian analyses was carried out using the Akaike Information Criterion (AIC) in MODELTEST 2.3 (Nylander, 2004). The general time-

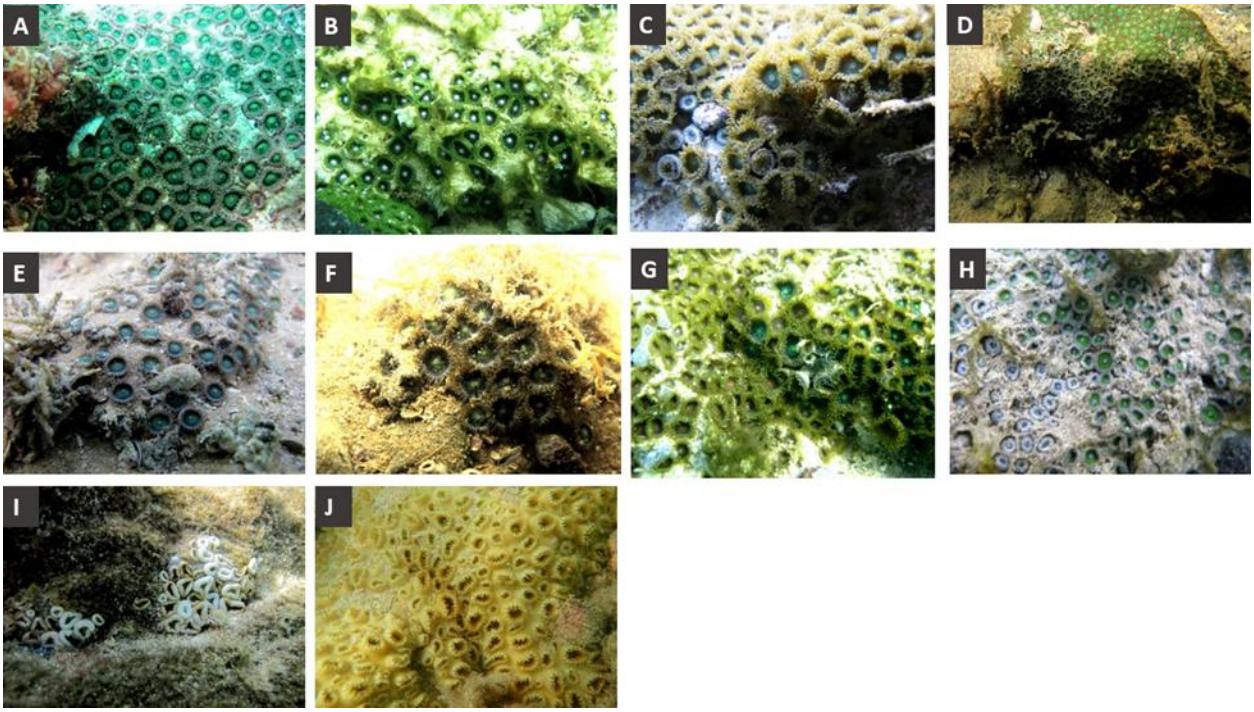

FIGURE 2. In situ images of zoanthid specimens collected in this study. Each image is representative of one morphological group listed in Table1.

reversible model with a gamma parameter (GTR+G+I) presents the best selection for the data (*Symbiodinium*, *Cladocopium*, and *Durusdinium*). Maximum likelihood and maximum parsimony analyses were accomplished by MEGA7 software and PAUP beta version 4.0b10 individually. ML and MP trees were evaluated with 1,000 bootstrap replicates.

RESULTS

In situ external appearance observation

The morphological identification recognized three species: *Zoanthus sansibaricus*, *Palythoa* aff. *mutuki*, and *Palythoa tuberculosa*, which belong to two families, Zoanthidae and Sphenopidae. Morphological features are placed in a table based on verified identification keys (Koupaei *et al.*, 2016; Koupaei *et al.*, 2018; López *et al*., 2019; Mizuyama *et al*., 2018; Reimer & Sinniger, 2018). Also, the images that have been taken from specimens in situ have been shown in Figure 2.

DNA sequence and phylogenetic analysis

COI

COI gene fragment sequences were obtained from 18 samples of Bostaneh port and 4 samples of Kharg island. The outgroup sequence for both the COI and 16S-rDNA trees was *Parazoanthus gracilis*. The results from the COI alignment tree presented that the sequences of these specimens (Bost: 15/16/9/2/17/14/11/10/8/3/6/13/7/1/12 and Kharg: 1/2/3) were identical to *Zoanthus sansibaricus*. with high support (ML=96%, MP=99%, PP=1). The *Zoanthus kuroshio* and *Zoanthus gigantus* were sister to the *Zoanthus sansibaricus* cluster with great support (ML=99%, MP=100%, PP=1), apparently distinct from *Palythoa* spp. The *Palythoa* genus was supported moderately (ML=96%, MP=74%, PP=0.9). *Palythoa tuberculosa* species create a moderately supported cluster (ML=61%, MP=64%, PP=0.99). Two sequences, specimens Kharg4 and Bost5, had one or two base pair differences from *Palythoa mutuki* and formed a distinct moderate cluster (ML=63%, MP=65%, PP=0.95). Consequently, we define them as *Palythoa* aff. *mutuki* (Fig. 3).

TABLE 2. Summary of morphological characteristics of collected zoantharians and final molecular identification**.**

Morphological group	Group name	Sample name	Polyp form, colour	Oral colour	Oral zone colour	Oral disc colour	Tentacle number, colour	Final molecular identification
Zoanthus sansibaricus.	A	Kharg 1- Kharg 2	Liberae, Light Brown	Fluorescent green	Dark grey with fluorescent green circle around it	Dark green	Light brown, approx. 56-61	*Zoanthus sansibaricus*
	B	Bost 10- Bost 7- Bost 9	Liberae, Brown	White	White, with gray circle and red circle around it	Purple	Fluorescent green, approx. 49-58	*Zoanthus sansibaricus*
	C	Bost 3- Bost 2	Liberae, Light Brown	White	Brown with fluorescent green circle around it	Purple with white lines	Light brown, approx. 48	*Zoanthus sansibaricus*
	D	Bost 1- Kharg 3	Liberae, Light Brown	White	Dark green with white lines	Light green	Light brown, approx. 60	*Zoanthus sansibaricus*
	E	Bost 12- Bost 14- Bost 16	Liberae, Light Brown	Light grey	Light grey with fluorescent green circle	Purple with white lines	Light brown, approx. 58-65	*Zoanthus sansibaricus*
	F	Bost 6- Bost 15- Bost 17	Liberae, Light Brown	Light grey	Dark grey with light green circle	Dark green with white lines	Light brown, approx. 54-70	*Zoanthus sansibaricus*
	G	Bost 13- Bost 11	Liberae, Brown	Some light green, Some white	Some red, Some green	Purple with white lines	Light brown, approx. 52	*Zoanthus sansibaricus*
	H	Bost 8	Liberae, Light cream	White	Purple	Fluorescent green	Cream, approx. 50	*Zoanthus sansibaricus*
Palythoa aff. *mutuki*	I	Kharg 4- Bost 5	Liberae, cream	White	Cream	Green to brown with white lines	Brown, approx. 62	*Palythoa* aff. *mutuki*
Palythoa tuberculosa	J	Bost 19- Bost 18	Immersae, Brown	Cream	Cream	Brown	Brown, approx. 30	*Palythoa tuberculosa*

Mt 16s- rDNA

The sequences of the mt 16S-rDNA gene fragment were obtained from 18 samples collected in the Bostaneh area and 4 samples collected from Kharg Island. In the phylogenetic trees obtained by matching mt 16S-rDNA sequences, the genera *Zoanthus* and *Palythoa* each formed a large clade. Bootstrap values and posterior probability for *Zoanthus* and *Palythoa* clades are as follows, respectively: *Zoanthus* (ML=96%, MP=100%, and PP=0.1) and *Palythoa* (ML=99%, MP=91%, and PP=0.57) (Fig 4).

SYMBIODINIACEAE FAMILY

The results of phylogenetic trees showed that there are three genera of the Symbiodiniaceae family from Bostaneh port: *Symbiodinium*, *Cladocopium*, and *Durusdinium*. Sequences belonging to *Symbiodinium* (ITS1-5.8S-ITS2) were identified with high support (ML=100%) from *Palythoa* aff. *mutuki*, *Zoanthus sansibaricus*, and *Palythoa tuberculosa*; *Cladocopium* was well supported (ML=92%) from *P.* aff. *mutuki*, Z. sansibaricus, and *P. tuberculosa*; and *Durusdinium* was well supported (ML=99%) from *P.* aff. *mutuki*, *Z. sansibaricus*, and *P. tuberculosa*. Figs. 4, 5, and 6 show the ML tree for the aligned sequences. Because of the similarity in the tree topologies in all of the analyses, the bootstrap values of Mr. Bayes and MP are shown on the ML tree.

REAL-TIME PCR

Detection of *Symbiodinium*, *Cladocopium* and *Durusdinium* DNA

The average threshold cycle (Ct) was obtained from quantitative PCR of each of the studied groups. Examining the Ct of the samples showed that the Ct obtained from the PCR related to the genus *Symbiodinium* was lower compared to other genera, which means that this genus was more abundant in the studied zoantharian samples. Also, the information obtained from quantitative PCR showed that the Ct related to the *Durusdinium* genus was higher than other genera, which means less frequency of this

genus in the studied samples. The results of the quantitative comparison of three species of symbiotic micro algae in the hosts of *Zoanthus sansibaricus*, *Palythoa* aff. *mutuki*, and *Palythoa tuberculosa* are given in Table 2. The ratio of species to each other was obtained from the following formula, where Ct was the threshold cycle associated with each extracted DNA sample (Correa *et al*., 2009). *Clade x (DNA) / Clade y (DNA)= 2* $^{(Ct\ Clade\ y-\ Ct\ Clade\ x)}$

DISCUSSION

The aim of this investigation was identifying the zoantharian species of Bostaneh bay through morphological features and the mitochondrial molecular markers include COI and 16s-rDNA, which were applied successfully to distinguish the most species of them (Kise *et al*., 2021; Koupaei *et al*., 2014; Koupaei *et al*., 2018; López *et al*., 2019; Bazzaz *et al*., 2024).

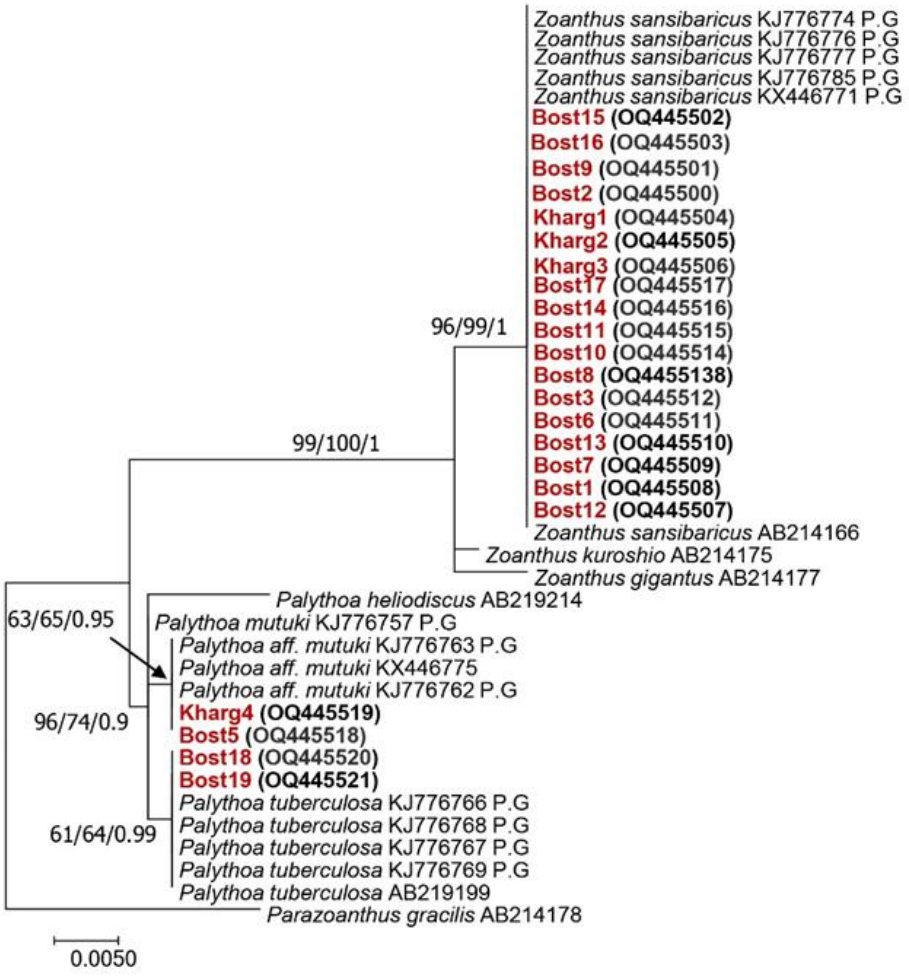

FIGURE 3. Maximum likelihood tree of COI sequences for zoantharian specimens. Values at branches represent maximum likelihood bootstrap percentages from 1000 trees/maximum parsimony bootstrap percentages from 1000 trees/Bayesian posterior probabilities.

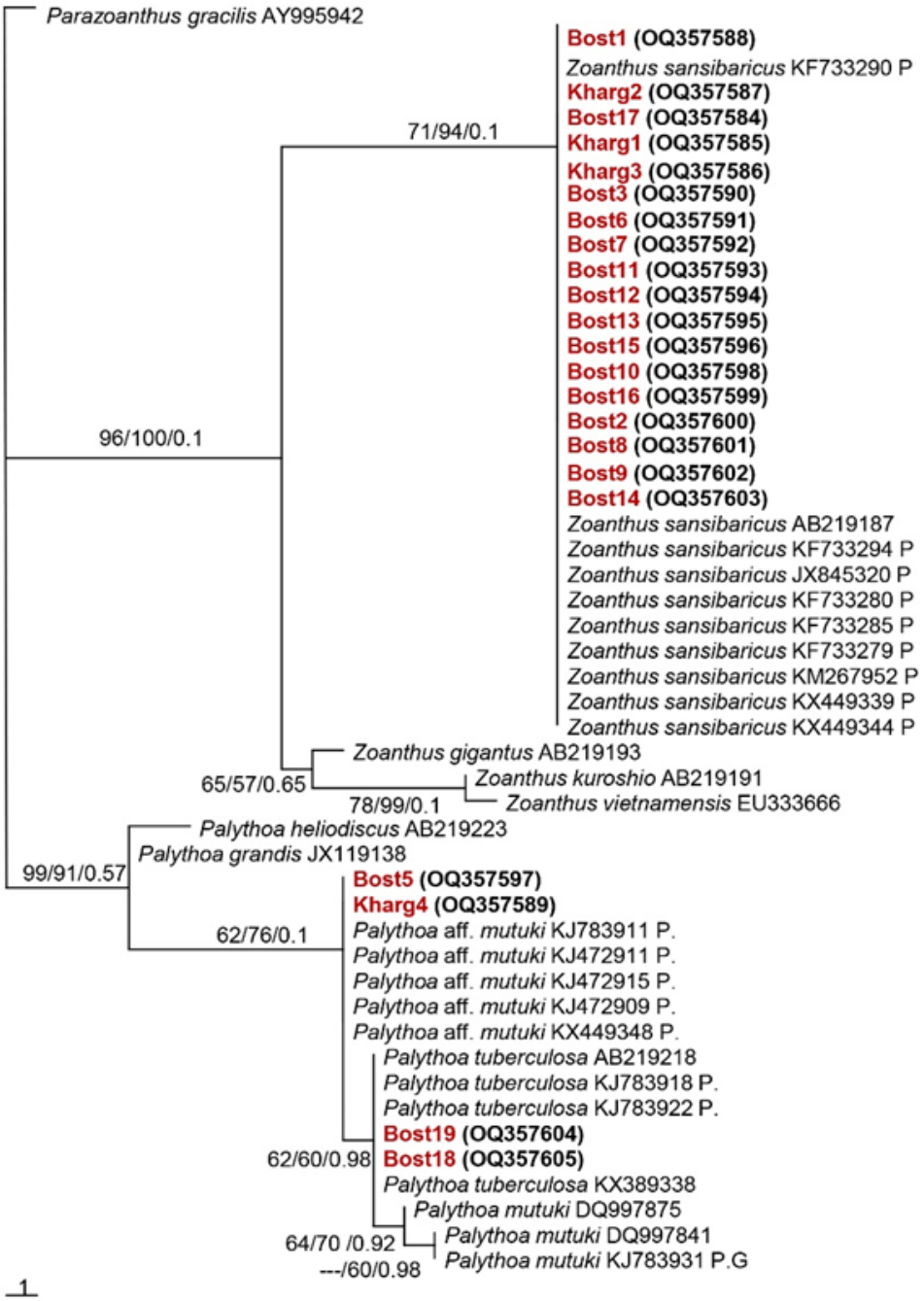

FIGURE 4. Maximum likelihood tree of 16s-rDNA sequences for zoantharian specimens. Values at branches represent maximum likelihood bootstrap percentages from 1000 trees/maximum parsimony bootstrap percentages from 1000 trees/Bayesian posterior probabilities.

TABLE 3. accession numbers and final molecular identification of each specimen**.**

Specimen name	Mt 16srDNA accession numbers	COI accession numbers	Molecular identification
Bost 1	OQ445508	OQ357588	*Zoanthus sansibaricus*
Bost 2	OQ445500	OQ357600	*Zoanthus sansibaricus*
Bost 3	OQ445512	OQ357590	*Zoanthus sansibaricus*
Bost 5	OQ445518	OQ357597	*Palythoa aff. mutuki*
Bost 6	OQ445511	OQ357591	*Zoanthus sansibaricus*
Bost 7	OQ445509	OQ357592	*Zoanthus sansibaricus*
Bost 8	OQ445513	OQ357601	*Zoanthus sansibaricus*
Bost 9	OQ445501	OQ357602	*Zoanthus sansibaricus*
Bost 10	OQ445514	OQ357598	*Zoanthus sansibaricus*
Bost 11	OQ445515	OQ357593	*Zoanthus sansibaricus*
Bost 12	OQ445507	OQ357594	*Zoanthus sansibaricus*
Bost 13	OQ445510	OQ357595	*Zoanthus sansibaricus*
Bost 14	OQ445516	OQ357603	*Zoanthus sansibaricus*
Bost 15	OQ445502	OQ357596	*Zoanthus sansibaricus*
Bost 16	OQ445503	OQ357599	*Zoanthus sansibaricus*
Bost 17	OQ445517	OQ357584	*Zoanthus sansibaricus*
Bost 18	OQ445520	OQ357605	*Palythoa tuberculosa*
Bost 19	OQ445521	OQ357604	*Palythoa tuberculosa*
Kharg 1	OQ445504	OQ357585	*Zoanthus sansibaricus*
Kharg 2	OQ445505	OQ357587	*Zoanthus sansibaricus*
Kharg 3	OQ445506	OQ357586	*Zoanthus sansibaricus*
Kharg 4	OQ445519	OQ357589	*Palythoa* aff. *mutuki*

Formerly, five species of zoantharian were reported from the Persian Gulf through the mitochondrial markers (COI and 16S-rDNA) (i.e., Z.*sansibaricus*, *Palythoa* aff. *mutuki*, *Palythoa tuberculosa*, *Palythoa* mutuki, *Neozoanthus* sp.) which were collected from Kish, Hormoz, Lark, Hengam, Qeshm, Farur, BaniFarur islands and Chabahar Bay (Koupaei *et al.*, 2014; Koupaei *et al.*, 2016a,b ; Koupaei *et al.*, 2018; Bazzaz *et al.*, 2024). The present investigation vividly demonstrated the presence of three species groups in Bostaneh port, which included *Zoanthus sansibaricus*, *Palythoa* aff. *mutuki*, and *Palythoa tuberculosa*, also reported in Koupaei et al. (2014), Koupaei et al. (2016a,b), Koupaei et al. (2018) and Bazzaz et al. (2024). Mitochondrial marker sequencing revealed that all Zoanthus morphotypes, despite their wide variation in coloration, belonged to the same species. In particular, every specimen analyzed was identified as *Zoanthus sansibaricus*, a species known for its extensive range of color forms.As can be seen in Fig 3, all eighteen *Zoanthus* specimens of our investigation formed a well-supported sub-cladal monophyly (ML=96%, MP=99%, PP=1) with *Zoanthus sansibaricus* reported from the Persian Gulf, Hengam (KJ776774, KJ776776, KJ776777), Larak (KJ776785), Kish (KX446771), and Pacific Ocean, Japan (AB214166). Within the *Palythoa* genus, sequences Bost18 and Bost19 were identical to each other and also to *Palythoa tuberculosa* sequences from the Persian Gulf, Hengam (KJ776766, KJ776768, KJ776767, KJ776769), and Japan (AB219199) (ML=61%, MP=64%, PP=0.99). Nevertheless, Kharg4 and Bost5, being somewhat distant from *Palythoa mutuki*, resolved with formerly reported *Palythoa* aff. *mutuki* from the Persian Gulf, Hengam (KJ776763, KJ776762), and Kish (KX446775) (ML=63%, MP=65%, PP=0.95)(Koupaei *et al*, 2016; Koupaei *et al.*, 2018). Although the anthozoans' mitochondrial markers are really conservative (Shearer *et al*, 2002), a low level of variation displays species-level differences in *Palythoa* species. The position of these specimens in the phylogeny trees was comparable to that of the *Palythoa* sp. collected from Sakurajima and Ogasawara Island, which was introduced as *Palythoa* aff.*mutuki* in a study by (Koupaei *et al.*, 2016a).

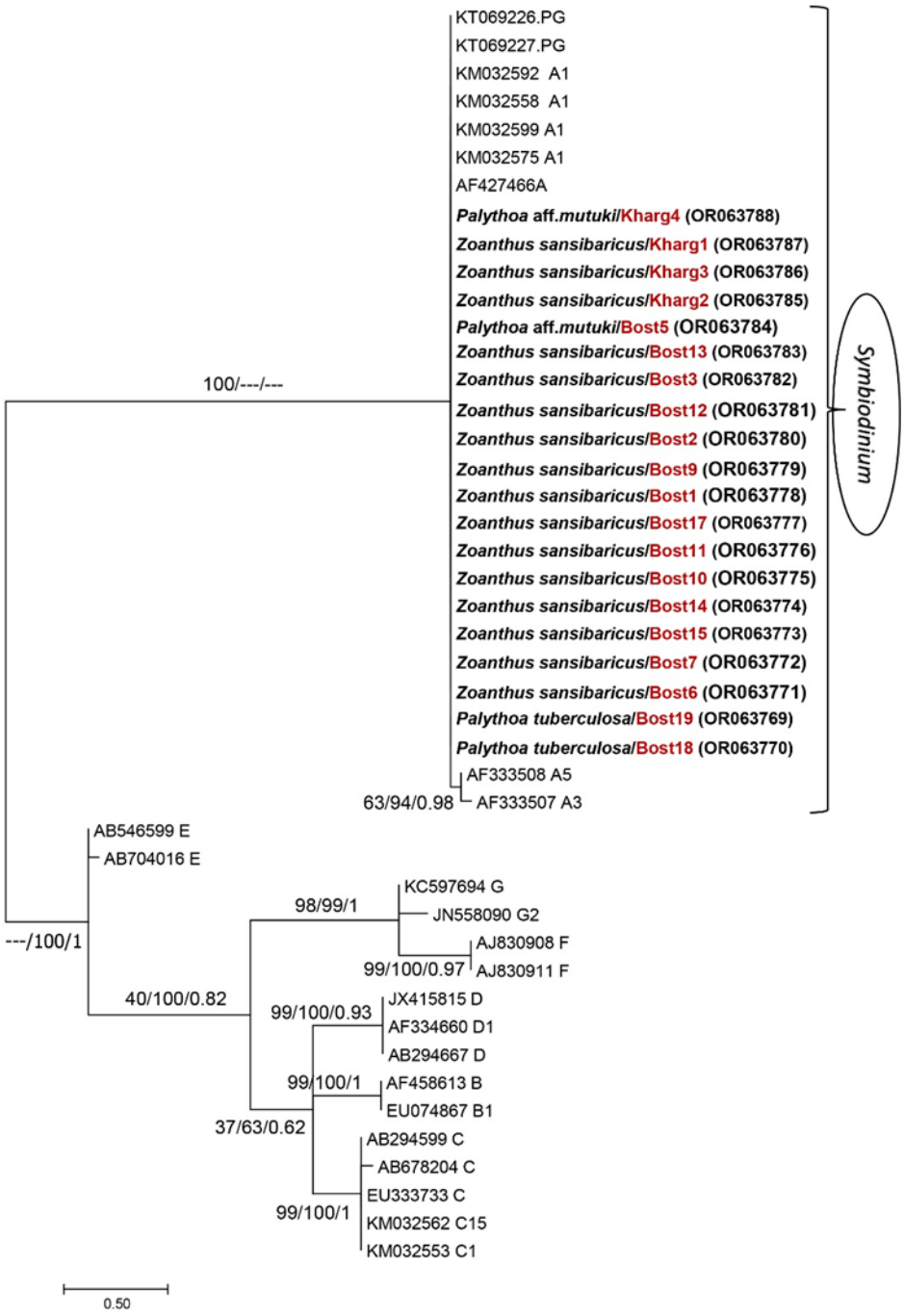

FIGURE 5. Maximum likelihood tree of ITS1-5.8S-ITS2 sequences for *Symbiodinium*. Values at branches represent maximum likelihood bootstrap percentages from 1000 trees/ maximum parsimony bootstrap percentages from 1000 trees /Bayesian posterior probabilities.

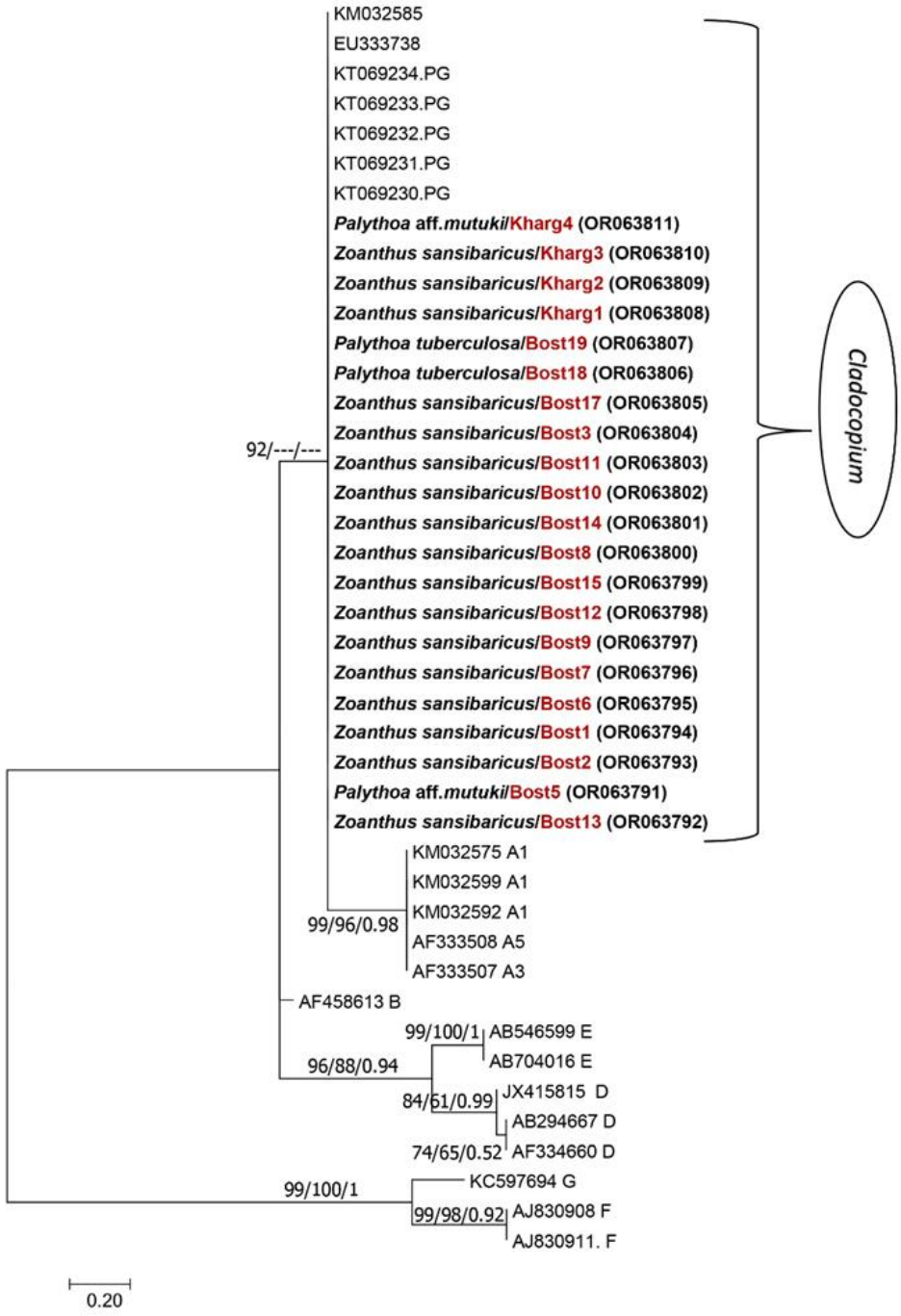

FIGURE 6. Maximum likelihood tree of partial ITS 1 for *Cladocopium*. Values at branches represent maximum likelihood bootstrap percentages from 1000 trees/ maximum parsimony bootstrap percentages from 1000 trees /Bayesian posterior probabilities.

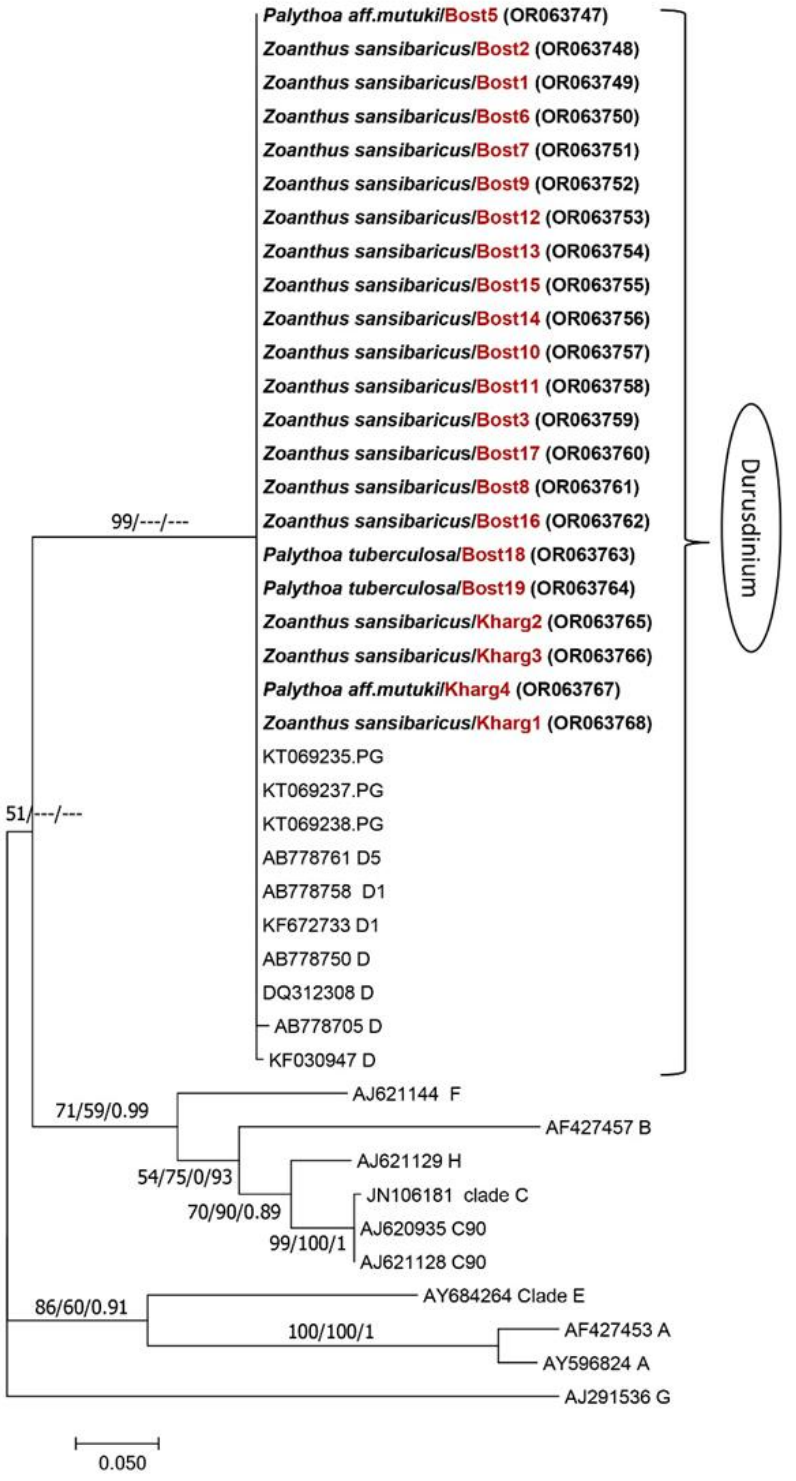

FIGURE 7. Maximum likelihood tree of LSU for *Durusdinium*. Values at branches represent maximum likelihood bootstrap percentages from 1000 trees/ maximum parsimony bootstrap percentages from 1000 trees /Bayesian posterior probabilities

TABLE 4. Zooxanthellae species abundance ratio based on quantitative comparison

Host Zoantharian		*Symbiodinium*/*Cladocopium*	*Cladocopium*/*Durusdinium*	*Durusdinium*/*Symbiodinium*
Zoanthus sansibaricus	Bost 1	75.58	107.63	8135.41
	Bost 2	427.57	4.35	1858.60
	Bost 3	1234.75	7.46	9216.48
	Bost 4	1209.34	2.58	3125.78
	Bost 6	9089.59	0.40	3615.55
	Bost 7	537.45	82.71	44453.21
	Bost 8	*Cladocopium* only	0.81	*Durusdinium* only
	Bost 9	0.19	0.37	0.07
	Bost 10	14562.80	0.52	7590.61
	Bost 11	6746.86	0.62	4153.18
	Bost 12	8135.41	1.59	12944.04
	Bost 13	0.01	30.70	0.38
	Bost 14	16728.26	1.02	17079.76
	Bost 15	7.62	5.86	44.63
	Bost 16	*Durusdinium* only	*Durusdinium* only	*Durusdinium* only
	Bost 17	891.44	14.12	12590.08
	Kharg 1	942.27	4.06	3821.70
	Kharg 2	3848.29	1.54	5914.33
	Kharg 3	1200.98	5.94	7131.55
Palythoa aff. *mutuki*	Bost 5	0.00	230.72	0.20
	Kharg 4	1.27	237.21	302.33
	Bost 19	278.20	6.36	1770.57

The present study results were compared with historical data in the literature and recent results from the Indian ocean, the Red Sea and the Persian Gulf. Results show a relatively limited number of zoantharian species documented, despite being an early area of study within the Indo-Pacific (Reimer et al., 2017). The largest number of zoantharian species identified in the Red Sea belongs to the study by Reimer et al (2017) with 13 species in 2017. Kumari et al, (2015) and Risi and Macdonald (2016) each identified seven species of zoantharian in the Indian Ocean and Kise (2019) identified three new species from the Indopacific Ocean. This is while three species have been identified in the present study, which is similar to studies conducted in Qeshm, Hormuz and Kish Islands by Koupaei et al., (2014, 2015, 2018) with three species, Darvishi et al., (2017) on the coast of Bushehr with four species and Koupaei et al., (2016a,b) in Larak Island with five species.

Comparing the obtained sequences by mt 16s-rDNA with the previously reported sequences showed that all samples of the *Zoanthus* morphological group are consistent with the recorded sequences of the *Zoanthus sansibaricus* species from the Persian Gulf, Larak (KF733290, KF733294), Qeshm (JX845320, KF733280, KF733285, KF733279), Hormuz (KM267952), Kish (KX449339, KX449344) (Koupaei et al. 2014, 2016), and Japan (Reimer *et al*. 2006a) (AB219187) with high support (ML=71%, MP=94%, and PP=0.1). The sequences of the morphological group (Bost18 and Bost19) of *Palythoa tuberculosa* also completely matched the recorded sequences of this species from the Persian Gulf, Hengam (KJ783918, KJ783922), and southwestern Japan (AB219218), which is supported moderately (ML=62%, MP=60%, and PP=0.98) (Koupaei et al. 2014, 2016; Reimer *et al*. 2006b). However, the sequences obtained from the samples of the morphological group of *Palythoa mutuki* (Bost5 and Kharg4),

having two nucleotide differences with the samples from Japan and Persian Gulf (DQ997841, DQ997875) and Larak (KJ783931) and supported moderately (ML=62%, MP=76%, and PP=0.1) with the recorded sequences of *Palythoa* aff. *mutuki*, which are introduced from the Persian Gulf, Larak (KJ783911), Qeshm (KJ472911, KJ472915, KJ472909), and Kish (KX449348) (Koupaei et al. 2014, 2016) (Fig 4).

Symbiotic Micro Algae in Zoantharia

The phylogenetic tree of *Symbiudinium* (ITS1-5.8S-ITS2 sequence) from three species strongly clustered (ML=100%) with subclade A1 that is hosted by *Dipsastrea pallida* (KT069226), *Leptastrea transversa* (KT069227) of the Persian Gulf, Larak Island (Dehghani *et al*. 2018), *Zoanthus sansibaricus* (KM032592), *Zoanthus natalensis* (KM032558, KM032599, KM032575) of South Africa (Risi, 2014), and *Zoanthus sociatus* (AF427466) of New York (Santos *et al*., 2002) (Fig 5). The sequences of *Cladocopium* (partial ITS-1) from three zoantharian species with previously reported sequences, including some sequences from *Zoanthus durbanensis* (KM032585) of South Africa (Risi, 2014), *Zoanthus sansibaricus* (EU333738) of Singapore (Reimer and Todd, 2009), *Dipsastrea pallida*, *Leptastrea transversa*, *Psammocora contigua*, *Stylophora pistillata*, and *Pocillopora damicornis* (KT069230-KT069234) of the Larak island (Persian Gulf) (Dehghani et al. 2018), supported highly with *Cladocopium* radiation (ML=92%). According to the phylogenetic tree of the *Durusdinium* genus, sequences belonging to LSU showed more similarity with the sequences of *Durusdinium* includes KT069235, KT069237, and KT069238 (Dehghani *et al*., 2018) from hard corals (the Persian Gulf), AB778761, AB778705, AB778750, and AB778758 from nudibranchs (Yorifuji *et al*., 2015), and, DQ312308 (Ghavam Mostafavi *et al*., 2007), and KF030947 (Fard Yazdani *et al*., 2014) from hard corals.

qPCR

Since several types of symbionts can be present on the zoanthid at the same time, the qPCR method can show us the amount of these symbionts. The qPCR investigation and the primers have been used for detecting the presence of Symbiodiniaceae genuses, establishing a very sensitive and well-optimized method. In the present study, *Symbiodinium* was the dominant symbiont with zoantharians in Bostaneh bay, the previous studies in the Persian Gulf by Koupaei et al 2016 also showed that the most dominant symbionts in zoantarians are genus *Symbiodinium*. This type of symbiotic micro algae appears to be adapted to stressful environmental conditions by producing substantial amounts of mycosporine-like amino acids (MAAs) (Banaszak *et al*., 2000), compounds which provided strong protection against UV radiation (Neale *et al*., 1998).

CONCLUSION

This study demonstrated the presence of two families, two genera and three species of zoanthids including *Zoanthus sansibaricus*, *Palythoa* aff. *mutuki* and *Palythoa tuberculosa* and their endosymbionts namely *Symbiodinium*, *Cladocopium* and *Durusdinium* in the Bostaneh bay- Hormozgan province in the Persian Gulf. Zoantharians are valuable genetic and biological resources that have been neglected in the Persian Gulf until now. Identifying these species is the first step towards protecting these genetic resources in the region. Based on the phylogenetic results obtained in the present study, the markers COI and 16S ribosomal mitochondrial DNA are considered appropriate for similar genetic studies in the Persian Gulf.

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RESEARCH ARTICLE Open access

Contributions to the spider fauna (Arachnida: Araneae) of the South-Central Iran: new species and new records from Iran and Fars Province

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Abstract

The spider fauna of Iran is remarkably diverse but remains incompletely documented, particularly in the south-central area. We present here the result of a faunistic survey conducted in Darab County, Fars Province. A total of 28 species representing 23 genera and 11 families were collected and identified. All species represent new records for Darab County, among them a new record for Iran fauna (*Langona pallida*, Prószyński, 1993), 13 species are newly recorded for the Fars Province spider fauna, including *Berlandina artaxerxes* Zamani et al. 2021, *Cryptodrassus helvolus* (O. Pickard-Cambridge, 1872), *C. iranicus* Zamani et al., 2021, *Cyrba ocellata* (Kroneberg, 1875), *Filistata maguirei* Marusik & Zamani, 2015, *Langona pallida* Prószyński, 1993, *L. tartarica* (Charitonov, 1946), *Minosiella intermedia* Denis, 1958, *Zimiris doriae* Simon, 1882, *Plexippus minor* Wesołowska & van Harten, 2010, *P. paykulli* (Audouin, 1826), *Steatoda trianguloides* Levy, 1991, and *Wadicosa fidelis* (O. Pickard-Cambridge, 1872). Additionally, we discovered three species belonging to the families Dysderidae, Gnaphosidae, and Salticidae as possibly new species, highlighting the region's unexplored diversity. One of these species belongs to the genus *Dysdera* (♂, Dysderidae) from the *ninni*–group, diagnosed by the extremely short stylus and distinctive triangular projection on the anterior side of the posterior apophysis. Another species, *Berinda* sp. (Gnaphosidae), differs from its congener in the longer and thinner tibial apophysis, shallowly bifurcated retrolateral tibial apophysis, and pitchfork-shaped median apophysis in lateral view. Moreover, *Evarcha* sp. (♂, Salticidae) is distinguished by a short and thin retrolateral tibial apophysis and a short triangular apophysis in the lateroventral view.

This species list is composed of widespread Palearctic elements and Iranian endemics, confirming Fars Province as a biogeographic confluence of diversity and highlighting the need for continuing research. This work establishes a foundational dataset and demonstrates that targeted surveys in poorly explored regions of Iran yield significant faunistic discoveries.

Keywords: New records, Spider, *Dysdera*, *Berinda*, *Evarcha*, Biodiversity, Faunistic, Araneae.

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INTRODUCTION

The spider fauna of Iran is remarkably diverse, comprising over 1,000 species in 328 genera and 57 families, and is among the most diverse in the Western Palearctic (Zamani et al. 2024). Since the first national checklist (Mozaffarian & Marusik, 2001), numerous studies have expanded the known diversity (Hosseinpour et al., 2019; Mirshamsi et al., 2015; Zamani et al., 2014, 2015a, 2015b, 2016, 2017a, 2017b,

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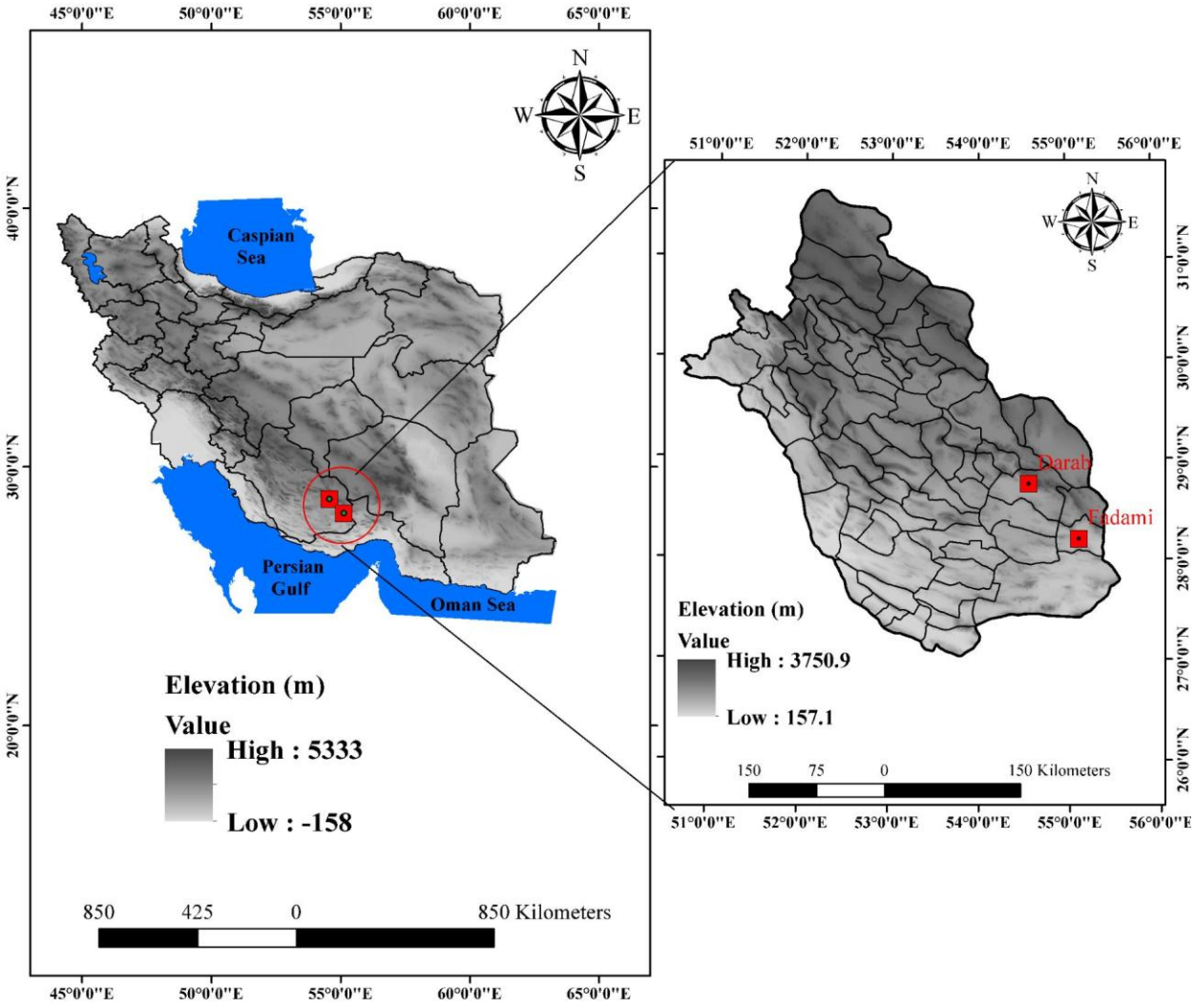

FIGURE 1. Collecting localities in Darab County.

2022, 2024a, 2024b, 2026), and significant geographical biases persist.

Fars Province, characterized by the Zagros Mountains and arid plains, contains diverse microclimates (Khadivi, 2010). Existing records are incidental, derived from scattered reports lacking systematic methodology (Logunov, 2023; Tahami et al., 2017). Consequently, there is no reliable baseline for assessing species richness, endemism, or ecological patterns in the region, a major impediment to biogeographic synthesis and conservation planning. The spider fauna of Fars Province has not been studied as a project for the region; only some publications dealing with recording of new species and introducing of some species in the checklists of various studies (Azarkina and Zamani, 2019; Hosseinpour et al. 2019; 2022; Kiany et al. 2016; Logunov, 2023; Logunov et al. 2007; Malek Hosseini et al. 2015; Malek-Hosseini and Zamani, 2017; Tahami et al. 2017; Zamani 2015; Zamani et al. 2014; 2021; Zamani & Marusik, 2023) have been noted. As a result, there remains a significant gap in our understanding of the diversity and ecology of spiders in this area, highlighting the need for comprehensive research efforts to better document and analyze these essential arachnid populations.

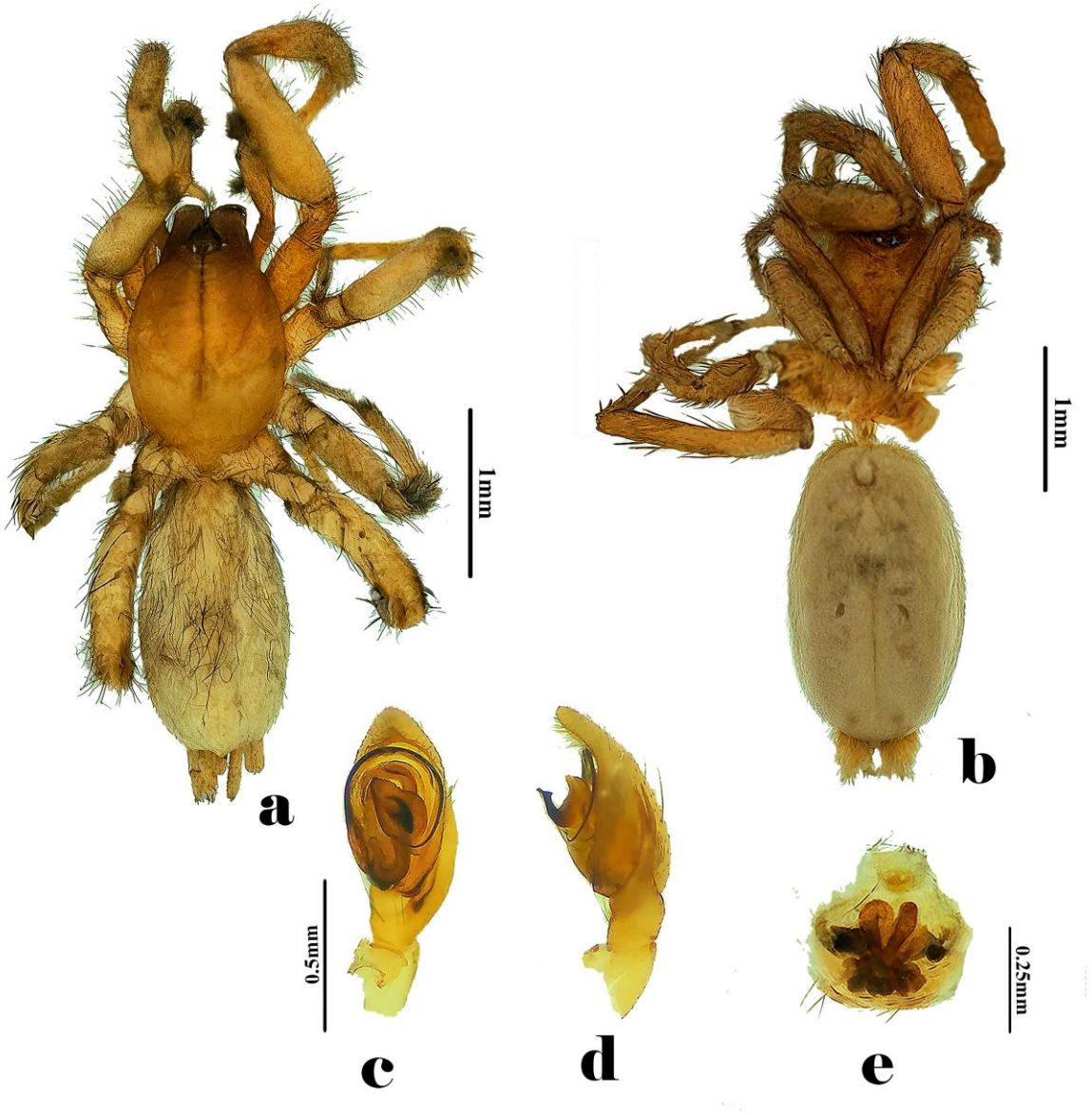

FIGURE 2. *Berinda* sp.: Male: a, habitus, dorsal, c, palp, ventral, d, palp, posterior; female: b, habitus, dorsal; e, epygine.

This contribution addresses this deficiency by providing the first rigorously documented spider inventory for Darab County in eastern Fras. We provide collection records to establish a verifiable benchmark for future research.

MATERIAL AND METHODS

Sampling was conducted in 2022 at two localities in Darab County, Fars Province, south-central Iran (Figure 1): Darab (54°34'40.725"N, 28°45'38.779"E) and Fadami (55°7'54.317"N, 28°13'10.058"E). We collected 51 specimens by hand and pitfall trapping across gardens, agricultural lands, and plains. Specimens were preserved in 70% ethanol.

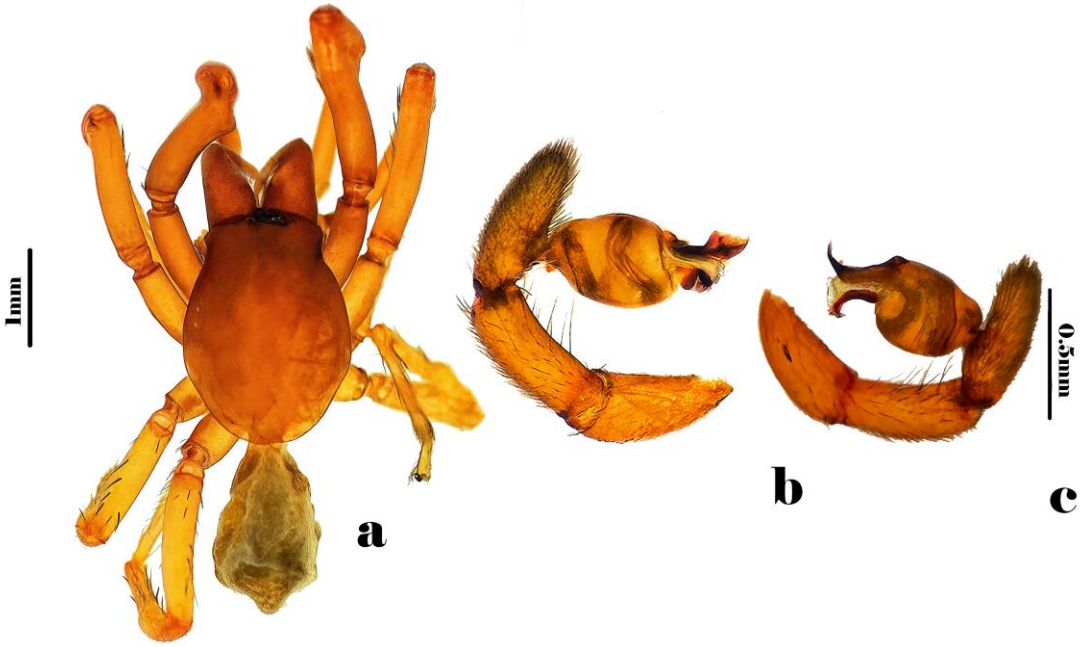

FIGURE 3. *Dysdera* sp.: Male: a, habitus, dorsal, b, palp, anterior, c, palp, posterior.

In the laboratory, specimens were photographed using an Olympus SZ40 stereomicroscope equipped with an Olympus 58MP FHD Camera V8 at the University of Jiroft, Jiroft, Kerman Province. For critical identification, female epigynes were cleared in 10% KOH, and male palps were excised and examined. Dr. Alireza Zamani confirmed the final identification.

RESULTS

We examined 51 adult spiders, identifying 28 species across 23 genera and 11 families. Three species, *Dysdera* sp., *Berinda* sp., and *Evarcha* sp. (families Dysderidae, Gnaphosidae, and Salticidae, respectively), represent putative new species. These species are not fully described here, as we had either only one male specimen (*Dysdera* sp. and *Evarcha* sp.) or a few samples (one female and two males for *Berinda* sp.) in our collection. Below, the list of species is presented with the diagnostic characters of the three putative species documented.

***Berinda* sp.**

Material examined: 1♂2♀ Fars Province, Fadami 55°7'54.317"N, 28°13'10.058"E, 1247m a. s. l. 04 July 2022 A. Mashayekh leg.

Female (Figure 2b): total body length 5mm, including carapace (2mm) and abdomen (3mm). Epigyne 0.4 mm. Carapace, chelicera, and fangs yellow to orange. Legs and spinnerets yellow. Abdomen cream-colored and unpatented with two pairs of sigilla. With a posterior conical hood shaped like a tornado.

Male (Figure 2a): total body length 4.5 mm, including carapace (2mm), abdomen (2.5mm). Palp length 1mm. Carapace, chelicera and fangs yellow to orange. Legs and spinnerets yellow. Abdomen cream-colored, unpatroned and without sigilla. A sclerotized hook-like structure that acts as a median apophysis and conducts the embolus. The embolus is narrow and long, originating from the left side of the pulp. Scutum almost triangular. The retrolateral apophysis of the tibia very long and slender, bifurcating from anterior to medial.

Remarks: Collected materials were found in the Yard without vegetation. This species is similar to *B. bifurcate*, but it differs in a longer, thinner tibial apophysis and in the shape of the median apophysis in a retrolateral view. The retrolateral tibial apophysis is shallow, bifurcated, clung to palp, very long, and transparent.

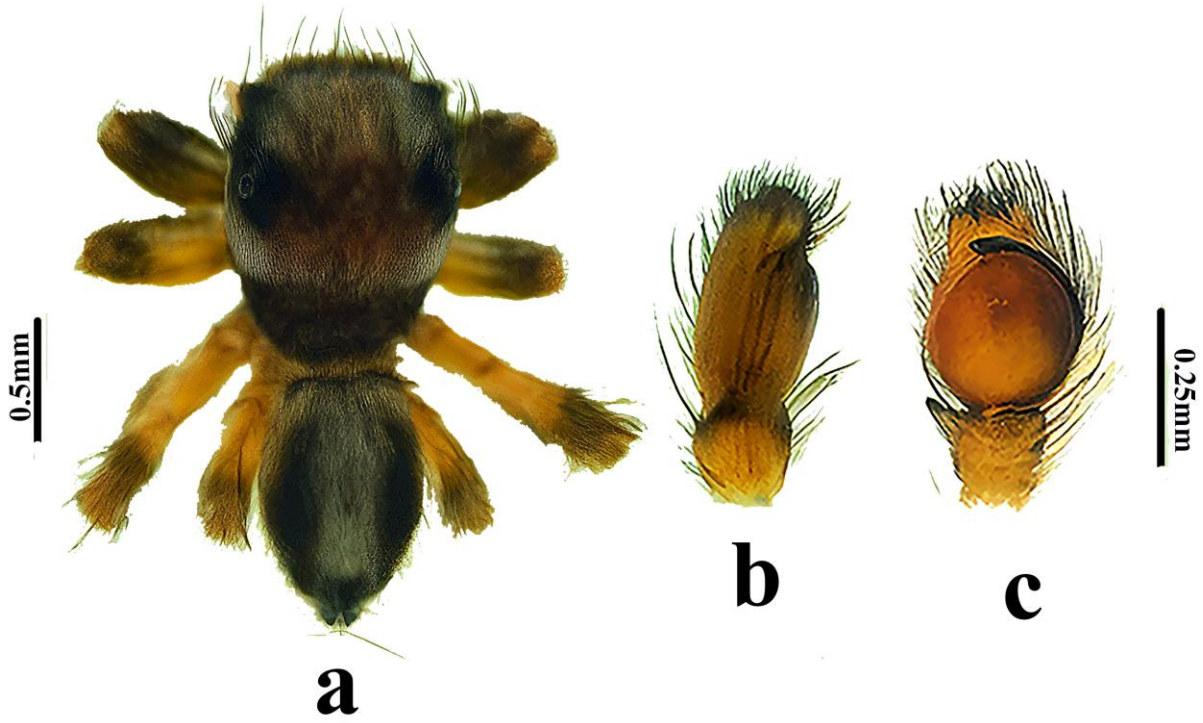

FIGURE 4. *Evarcha* sp.: Male: a, habitus, dorsal, b, palp, anterior, c, palp, ventral**.**

***Dysdera* sp.**

Material examined: 1♂ Fars Province, Darab 54°34'40.725"N, 28°45'38.779"E, 750 m a. s. l. 15 January 2022, A. Mashayekh leg.

Male (Figure 3)**:** total body length 7 mm, including carapace (4.5 mm), and abdomen (2.5 mm). Palp length 1mm. Carapace, fang, and chelicerae orange. Palp and legs orange to yellowish. Abdomen and spinnerets light. Bulb small. Tegulum almost large, its length greater than its width, and bell shaped. Embolus shorter than tegulum. Median crest and apex triangular. Posterior apophysis, claw-shaped, with triangular growth on the anterior side. The stylus slightly bent and very short. This species differs from other species by having short stylusis.

Remarks: Specimen was found in a mountainous area. The male was captured, and the female is unknown. The bulb in palp is small. Tegulum is almost large, its length is greater than its width, and bell shaped. Pembolus is shorter than the tegulum. Median crest and apex are triangular. Posterior apophysis is claw-shaped with triangular growth on the anterior side. Stylusis bent, short, and weak.

***Evarcha* sp.**

Material examined: 1♂2♀ Fars Province, Fadami 55°7'54.317"N, 28°13'10.058"E, 1247m a. s. l. 15 Septemebr 2021 A. Mashayekh leg.

Male (Figure 4)**:** total body length 3 mm, including carapace (2 mm), and abdomen (1 mm). Palp length almost 0.5 mm. Eyes brown with black eye-ring. Legs and spinnerets brown to light brown. Palps, chelicera and fangs light brown. Anterior eyes on a brown band between two white bands. Anterior part of embolus short triangle and visible in lateral view, sloping towards the abdomen.

Remarks: Male specimens were collected on the grass in the yard. The female is unknown. The male has differenced with other congeners in that the retrolateral tibial apophysis is short and thinner; the apophysis is a short triangle in lateroventral view.

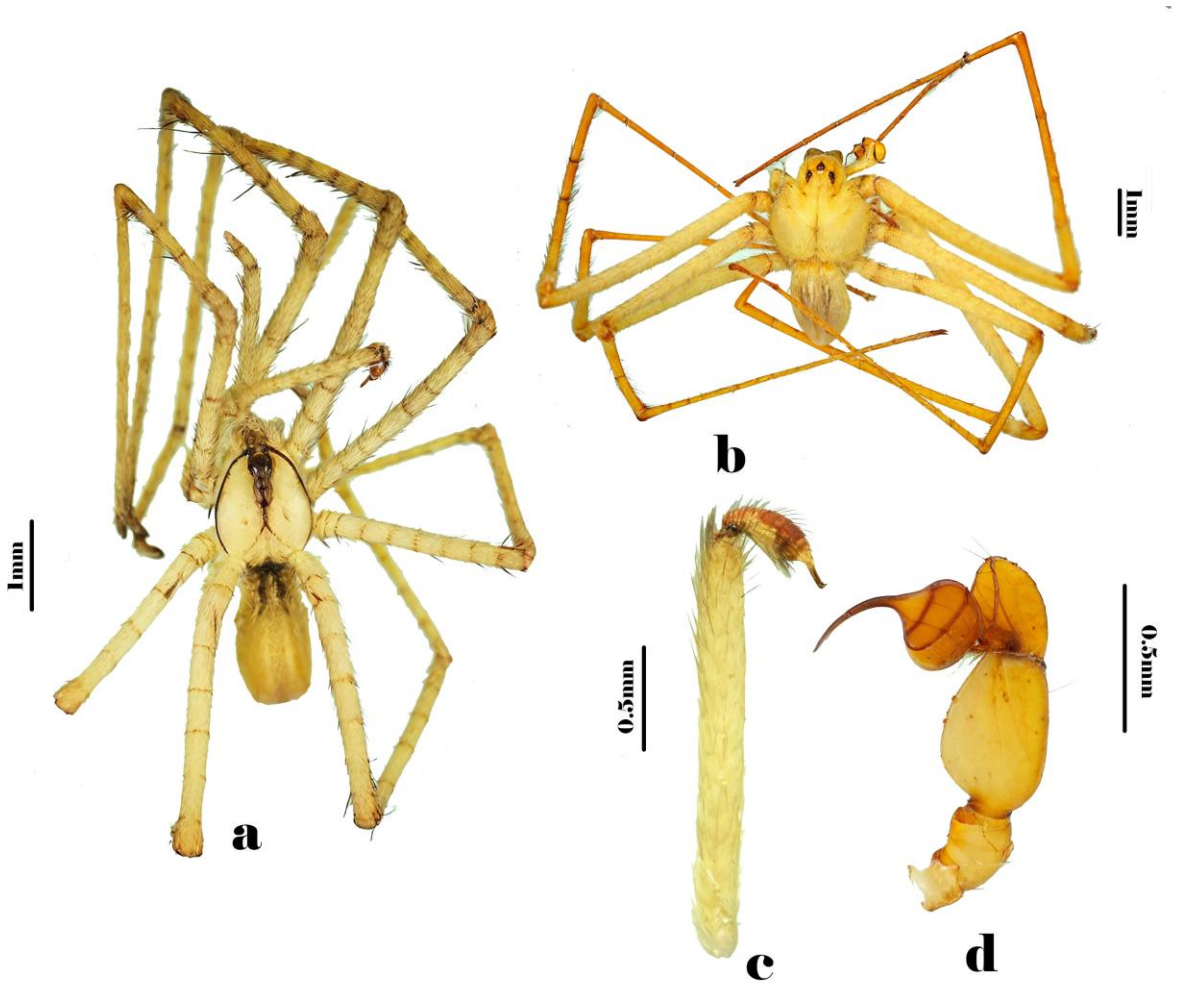

FIGURE 5. *Filistata maguirei*: male: a, habitus, dorsal; c, palp; *Loxosceles persica*: male: b, habitus, dorsal; d, palp.

Family Filistatidae Ausserer, 1867
Filistata maguirei Marusik & Zamani, 2015
(Figure 5A, C)
Material examined: 1♂ Fars Province, Fadami 55°7'54.317"N, 28°13'10.058"E, 1247m a. s. l. 03 January 2022 A. Mashayekh leg.
Global distribution: Iran: Hormozgan Province (Marusik and Zamani, 2015)
Remarks: This species was found and described in a dried mountainous habitat from Bandar Abbas (Hormozgan Province). New materials collected from the house yard in Fadami city (Fars Province). This is the first provincial record in Fars.

Family Gnaphosidae Banks, 1892
Anagraphis pallens Simon, 1893
(Figure 6)
Material examined: 1♀ Fars Province, Darab 54°34'40.725"N, 28°45'38.779"E, 750 m a. s. l.7 January 2022, A. Mashayekh leg.

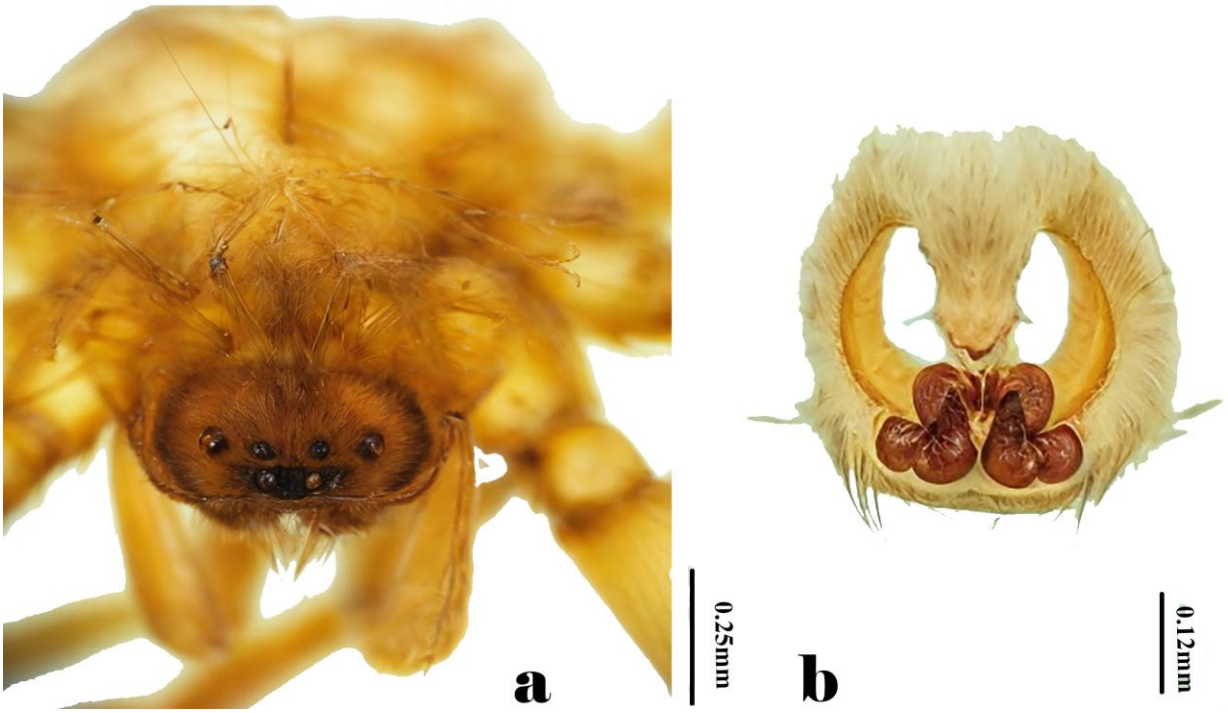

FIGURE 6. *Anagraphis pallens*: female: a, habitus, dorsal; b, epigyne.

Global distribution: Iran: East Azerbaijan; Fars; Golestan; Ilam; Khuzestan; Kohgiluyeh & Boyer-Ahmad Province; Lorestan; Khorasan-e-Razavi Province; Sistan & Baluchistan; Tehran. Libya, Malta, Greece, Turkey, Israel, Syria, Russia (Europe, Caucasus), Georgia, Azerbaijan, Kazakhstan, Central Asia (Zamani et al., 2014; Zamani et al., 2025).
Remarks: Collected material was found in the mountain area in Darab City. This species has been recorded from East Azerbaijan, Fars, Golestan, Ilam, Khuzestan, Kohgiluyeh & Boyer-Ahmad, Lorestan, Razavi Khorasan, Sistan & Baluchistan, and Tehran. Globally, recorded from Libya and Malta to Tajikistan (Zamani et al., 2025).

Berlandina artaxerxes Zamani et al. 2021
(Figure 7A, C)
Material examined: 2♂ Fars Province, Fadami 55°7'54.317"N, 28°13'10.058"E, 1247m a. s. l. 01 November 2021 A. Mashayekh leg.
Remarks: The species was found in a desert area in Yazd Province. This is the second time reported from Iran. Materials were captured from house yards in Fadami City. It's new to the fauna of Fars Province.

Berlandina plumalis (O. Pickard-Cambridge, 1872)
(Figure 7B, D)
Material examined: 1♀Fars Province, Darab 54°34'40.725"N, 28°45'38.779"E, 750 m a. s. l. 05 March 2022, A. Mashayekh leg.
Global distribution: Iran: Khorasan-e-Razavi Province, Fars ProvinceMediterranean to Central Asia, Afghanistan, Myanmar, and the United Arab Emirates (Zamani et al., 2026).
Remarks: The species was hitherto known from the Mediterranean to Central Asia, Afghanistan, Myanmar, and the United Arab Emirates (Zamani et al., 2026). The Iranian records are from Khorasan-e Razavi Province and Fars Province. The species was reported from the Maharloo Lake area in Fars Province. The new material was collected from the mountains in Darab City. This species is the first record for Darab County.

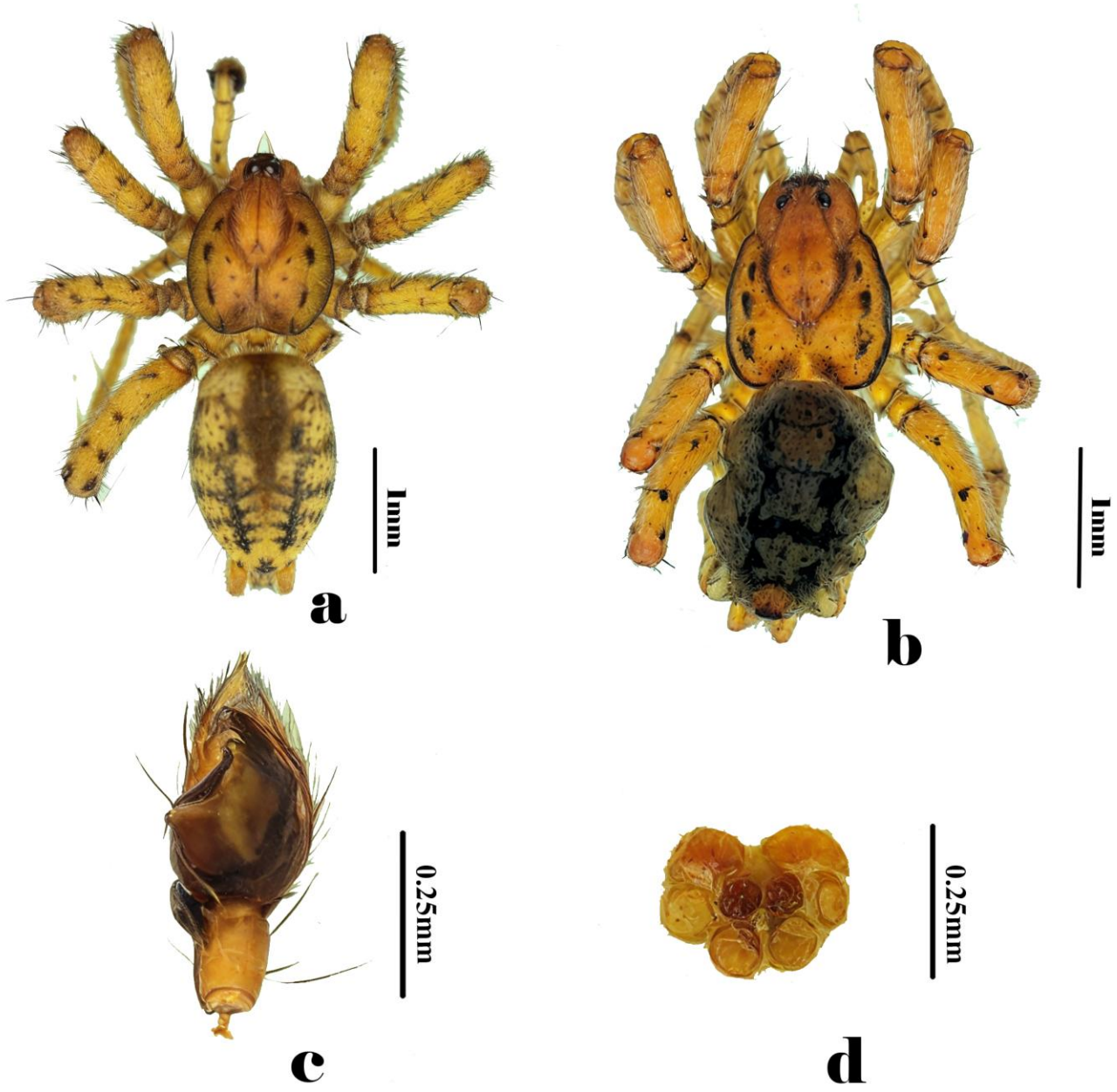

FIGURE 7. *Berlandina artaxerxes*: male: a, habitus, dorsal; c, palp; *Berlandina plumalis*: female: b, habitus, dorsal; d, epigyne.

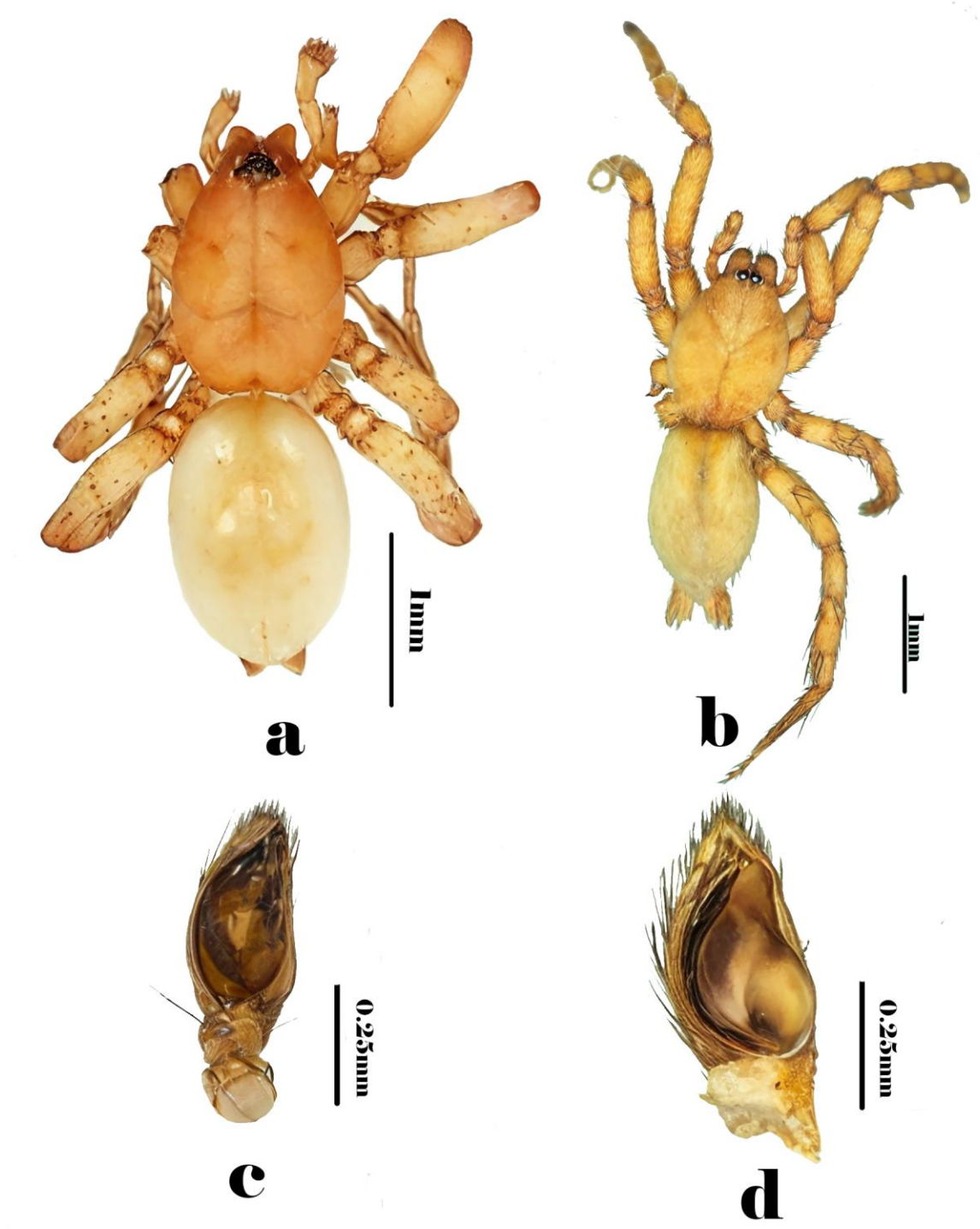

FIGURE 8. *Cryptodrassus iranicus*: male: a, habitus, dorsal; c, palp; *Cryptodrassus helvolus*: male: b, habitus, dorsal; d, palp.

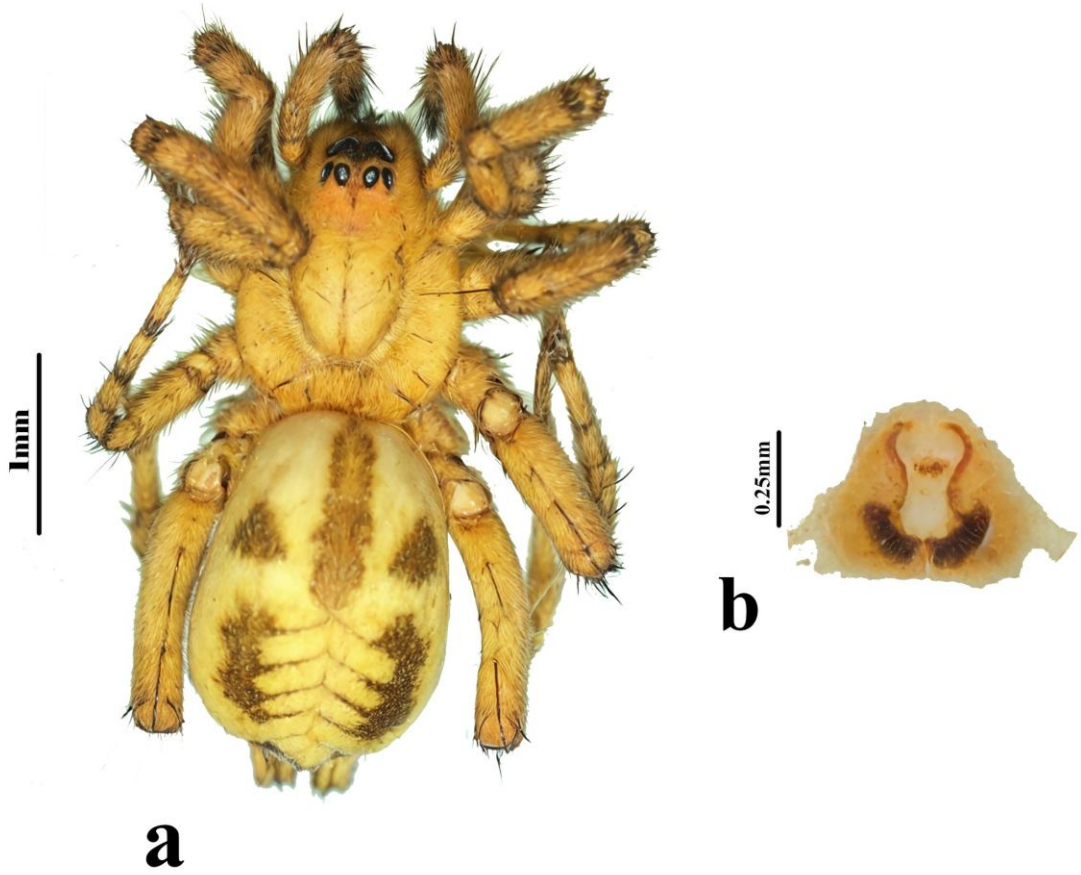

FIGURE 9. *Minosiella intermedia*: female: a, habitus, dorsal; c, epigyne; *Steatoda trianguloides*: female: b, habitus, dorsal; d, epigyne.

Cryptodrassus helvolus (O. Pickard-Cambridge, 1872)
(Figure 8B, D)
Melanophora helvola O. Pickard-Cambridge, 1872: 243, pl. 16, f. 23 (Dm) Original description.
Material examined: 2♂ Fars Province, Fadami 55°7'54.317"N, 28°13'10.058"E, 1247m a. s. l. 31 October 2022 A. Mashayekh leg.
Global distribution: Iran: Kohgiluyeh & Boyer-Ahmad Province; Sistan & Baluchistan Province. Russia (Europe), Turkey, Cyprus, Israel, Kazakhstan, and Morocco (Chatzaki & Russell-Smith, 2017; Zamani et al., 2026).
Remarks: This species was recorded from Morocco to the Caucasus and Kazakhstan (Chatzaki & Russell-Smith, 2017; Zamani et al., 2026). It was known in Iran from Kohgiluyeh & Boyer-Ahmad and Sistan & Baluchistan (Hosseinpour et al., 2019; Zamani et al., 2026). This is the first record of the species in Fars Province. Materials were collected in the house yard, devoid of vegetation.

Cryptodrassus iranicus Zamani et al. 2021
(Figure 8A, C)
Material examined: 1♂ Fars Province, Fadami 55°7'54.317"N, 28°13'10.058"E, 1247m a. s. l. 01 November 2022 A. Mashayekh leg.

Remarks: This species was known only from a single male specimen from Kermanshah Province (Zamani et al., 2021). Newly collected material is considered a new record for Fars Province and the southernmost distributional limit of the species globally.

Minosiella intermedia Denis, 1958
(Figure 9A, B)
Material examined: 1♀ Fars Province, Fadami 55°7'54.317"N, 28°13'10.058"E, 1247m a. s. l. 06 February 2023 A. Mashayekh leg.
Global distribution: Iran: Khorasan-e-Razavi Province. Central Asia, Afghanistan (Denis, 1958; Platnick, 2014), Egypt (El-Gendy, 2022), Iraq (Al-Khazali, 2024), and the United Arab Emirates (Zamani et al., 2026).
Remarks: This species has been reported from Afghanistan, Central Asia, Egypt, Iran (Khorasan-e-Razavi Province), Iraq, and the United Arab Emirates (Denis, 1958; Platnick, 2014; Zamani et al., 2014; El-Gendy, 2022; Al-Khazali, 2024; Zamani et al., 2026). Our records were collected under a grass bush and represented a new to Fars Province.

Nomisia conigera (Spassky, 1941)
(Figure 10)
Material examined: 1♀ 2♂ Fars Province, Darab 54°34'40.725"N, 28°45'38.779"E, 750 m a. s. l. 19 February 2022, A. Mashayekh leg.
Global distribution: Iran: Fars Province, Isfahan Province, Khuzestan Province, Kohgiluyeh & Boyer-Ahmad, North Khorasan Province, Sistan & Baluchistan Province, Tehran Province. Turkey, Tadzhikistan, Central Asia republics and Azerbaijan (World Spider Catalog, 2026).
Remarks: This species is globally recorded from Turkey to Central Asia and Iran, the southernmost extent of its distribution. It has been widely distributed in Iran, and recorded from Ardabil, East or West Azarbaijan, Kermanshah, Kohgiluyeh & Boyer-Ahmad, Kurdistan, Tehran, Zanjan, Fars, Golestan, Isfahan, Khuzestan, Lorestan, North Khorasan, and Sistan & Baluchistan (Hosseinpour et al., 2017; Zamani et al., 2020). This is the second record from Fars Province and is new to Darab County.

Pterotricha strandi Spassky, 1936
(Figure 11A, B)
Material examined: 2♂ Fars Province, Darab 54°34'40.725"N, 28°45'38.779"E, 750 m a. s. l. 22 February 2022, A. Mashayekh leg.
Global distribution: Iran: Alborz Province; Fars Province, Isfahan Province, Hormozgan Province, Kerman Province, Kohgiluyeh & Boyer-Ahmad Province, Yazd Province. Afghanistan (Sajadi et al., 2024), India (Gajbe, 1983), Iraq (Zamani et al., 2024b), and Turkmenistan (Zamani et al, 2018).
Remarks: This species is widespread on the Iranian plateau. It has been recorded from Afghanistan (Sajadi et al., 2024), India (Gajbe, 1983), Iraq (Zamani et al., 2024b), and Turkmenistan (Zamani et al, 2018). Records in Iran are from Alborz, Bushehr, Fars, Hormozgan, Isfahan, Kerman, Kohgiluyeh & Boyer-Ahmad, Tehran, and Yazd. It was previously known from Shiraz in Fars Province. It is herewith the second provincial record and the time from Darab County.

Family Lycosidae Sundevall, 1833
Hippasa deserticola Simon, 1889
(Figure 12A, B)
Material examined: IRAN: 3♀ Fars Province, Fadami,55°7'54.317"N, 28°13'10.058"E, 1247m a. s. l, 24 September 2021, A. Mashayekh.

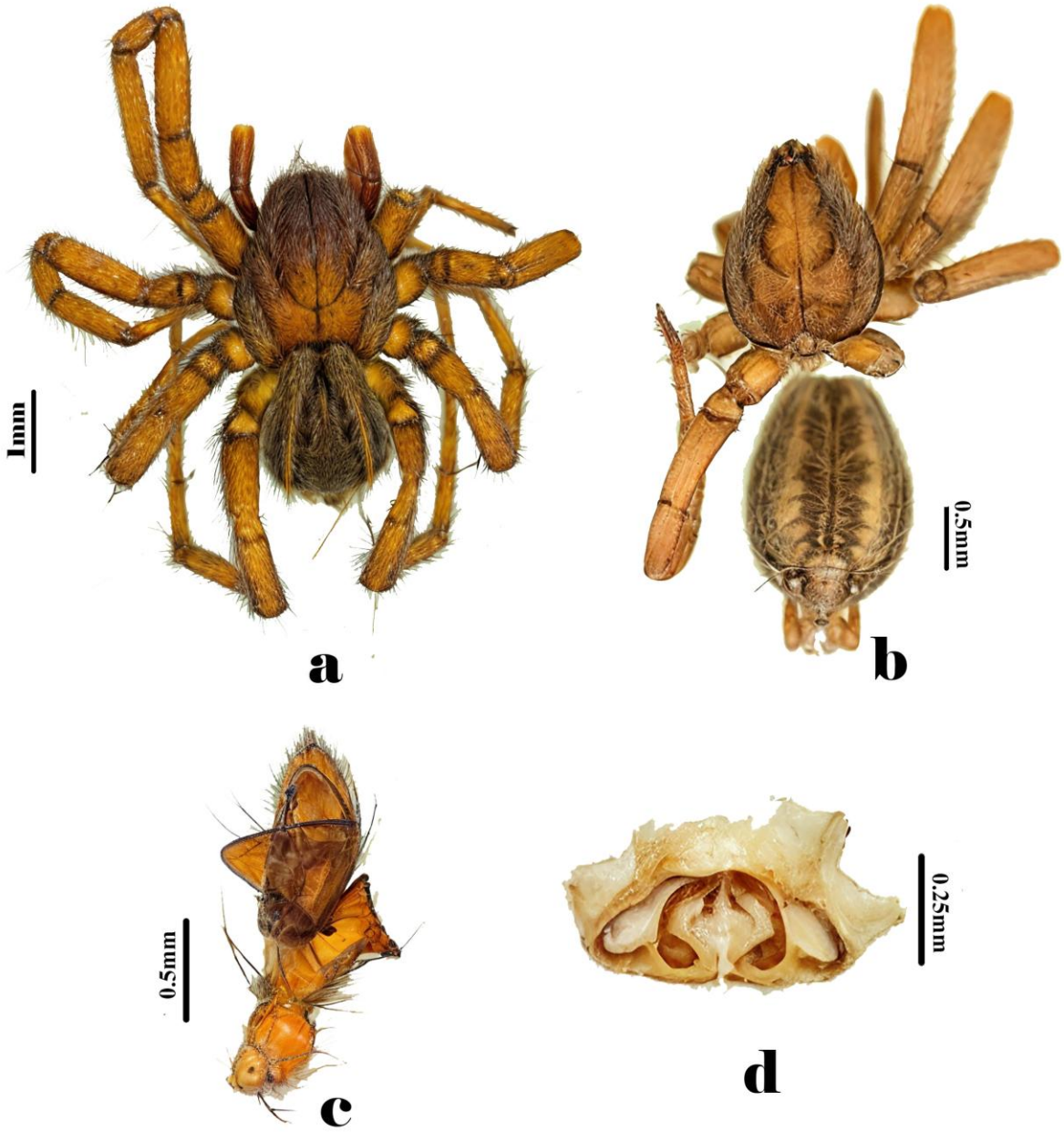

FIGURE 10. *Nomisia conigera*: male: a, habitus, dorsal; c, plap; female: a, habitus, dorsal, d, epigyne.

Global distribution: Iran: Fars Province, Hormozgan Province, Lorestan Province, Sistan & Baluchistan Province. Egypt, Saudi Arabia, Iraq, Uzbekistan, Turkmenistan, Tajikistan, Afghanistan, Pakistan, India, and Bangladesh (Zamani et al., 2022; Sankaran & Caleb, 2023).
Remarks: This species has been recorded from Iran (Fars, Hormozgan, Lorestan, and Sistan & Baluchistan), Egypt, Saudi Arabia, Iraq, Uzbekistan, Turkmenistan, Tajikistan, Afghanistan, Pakistan, India, Bangladesh (Zamani et al., 2022; Sankaran & Caleb, 2023). Freshly collected materials were collected in the palm grove in Fadami City.

Hogna effera (O. Pickard-Cambridge, 1872)
(Figure 13A, B)

Material examined: 1♂ Fars Province, Fadami 55°7'54.317"N, 28°13'10.058"E, 1247m a. s. l, 12 September 2021, A. Mashayekh leg.
Global distribution: Iran: East Azerbaijan Province, Golestan Province, Kerman Province, Kohgiluyeh & Boyer-Ahmad Province, Sistan & Baluchistan Province, South Khorasan Province. Cyprus, Turkey, Egypt, Israel, Lebanon, Syria, Yemen (Socotra), Saudi Arabia, United Arab Emirates, Iraq (Zamani et al., 2025).
Remarks: The species has been recorded from Cyprus, Turkey, Egypt, Israel, Lebanon, Syria, Yemen (Socotra), Saudi Arabia, United Arab Emirates, Iraq, Iran (Alborz, East Azerbaijan, Fars, Golestan, Hormozgan, Isfahan, Kerman, Kohgiluyeh & Boyer-Ahmad, Kurdistan, Mazandaran, Semnan, South Khorasan, Tehran, and Sistan & Baluchistan) (Zamani et al., 2025).

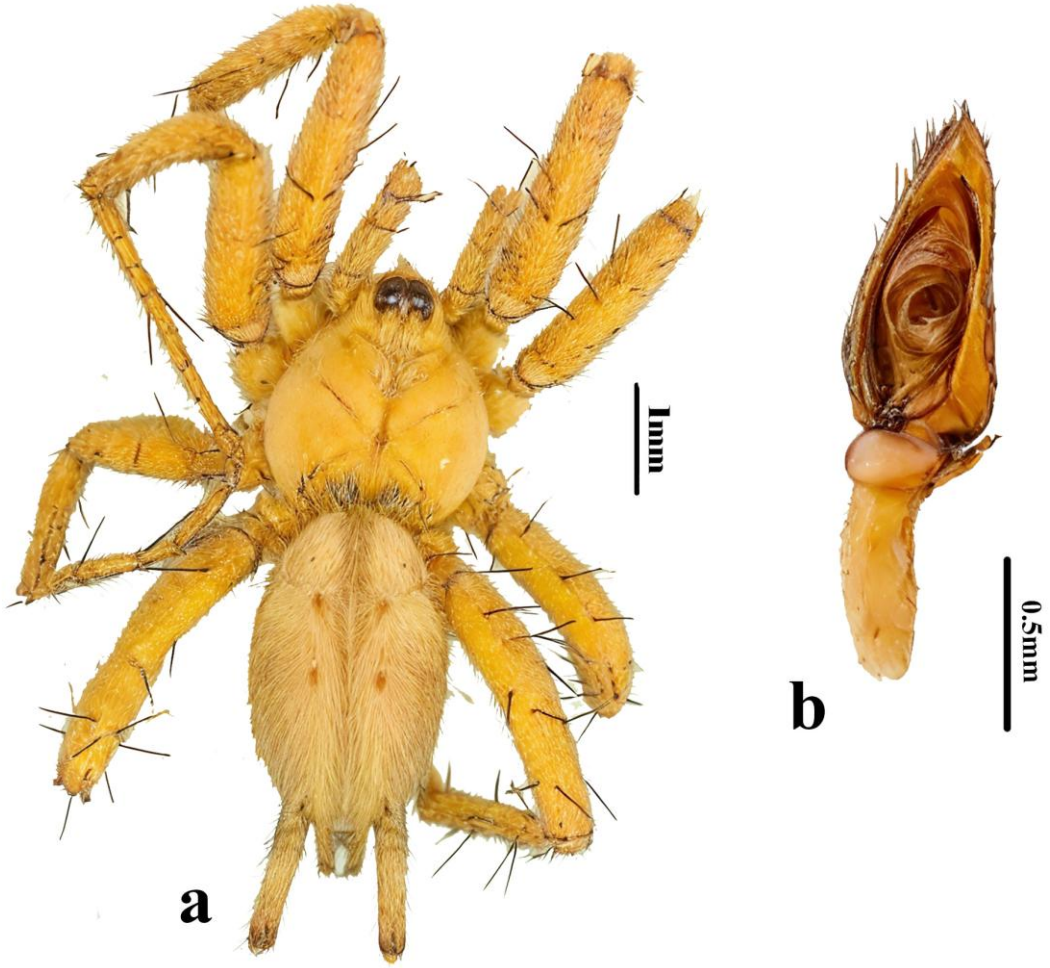

FIGURE 11. *Pterotricha strandi*: male: a, habitus, dorsal; b, palp.

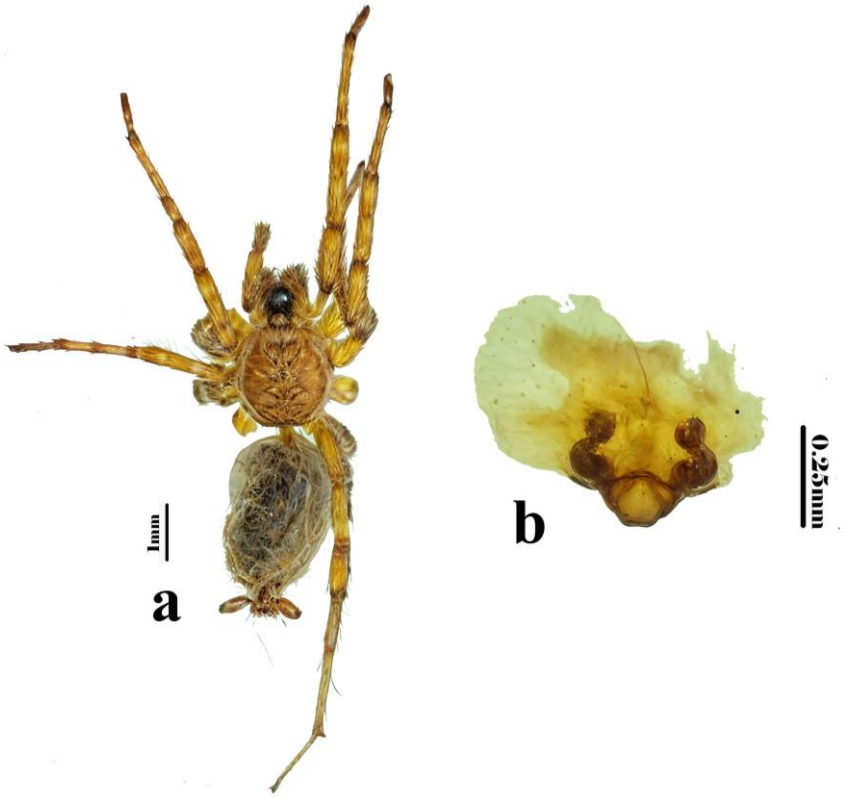

FIGURE 12. *Hippasa deserticola*: female: a, habitus, dorsal; b, epigyne.

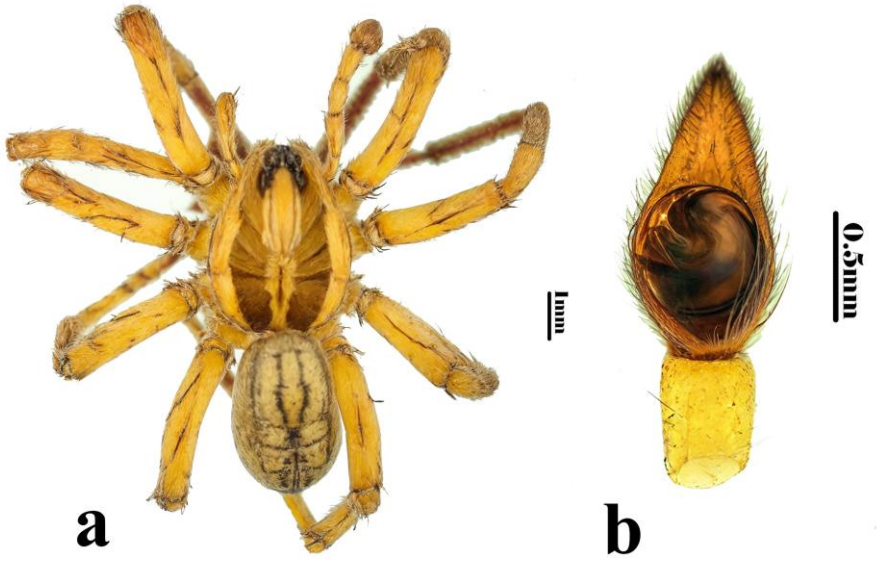

FIGURE 13. ; *Hogna effera*: male: a, habitus, dorsal; b, palp.

Wadicosa fidelis (O. Pickard-Cambridge, 1872)
(Figures 14)
Material examined: IRAN: 2♂ 1♀ Fars Province, Fadami 55°7'54.317"N, 28°13'10.058"E, 1247m a. s. l, 12 September 2021/2 September 2022 A. Mashayekh leg.
Global distribution: Iran: Sistan & Baluchistan Province, Hormozgan Province. Macaronesia, North Africa, Southern Europe, Caucasus, , Central Asia, China, Japan, Pakistan, Indonesia (Sumatra), India, Bhutan, Bangladesh, Philippines, and the United Arab Emirates (Hosseinpour et al., 2022; Zamani et al., 2026).

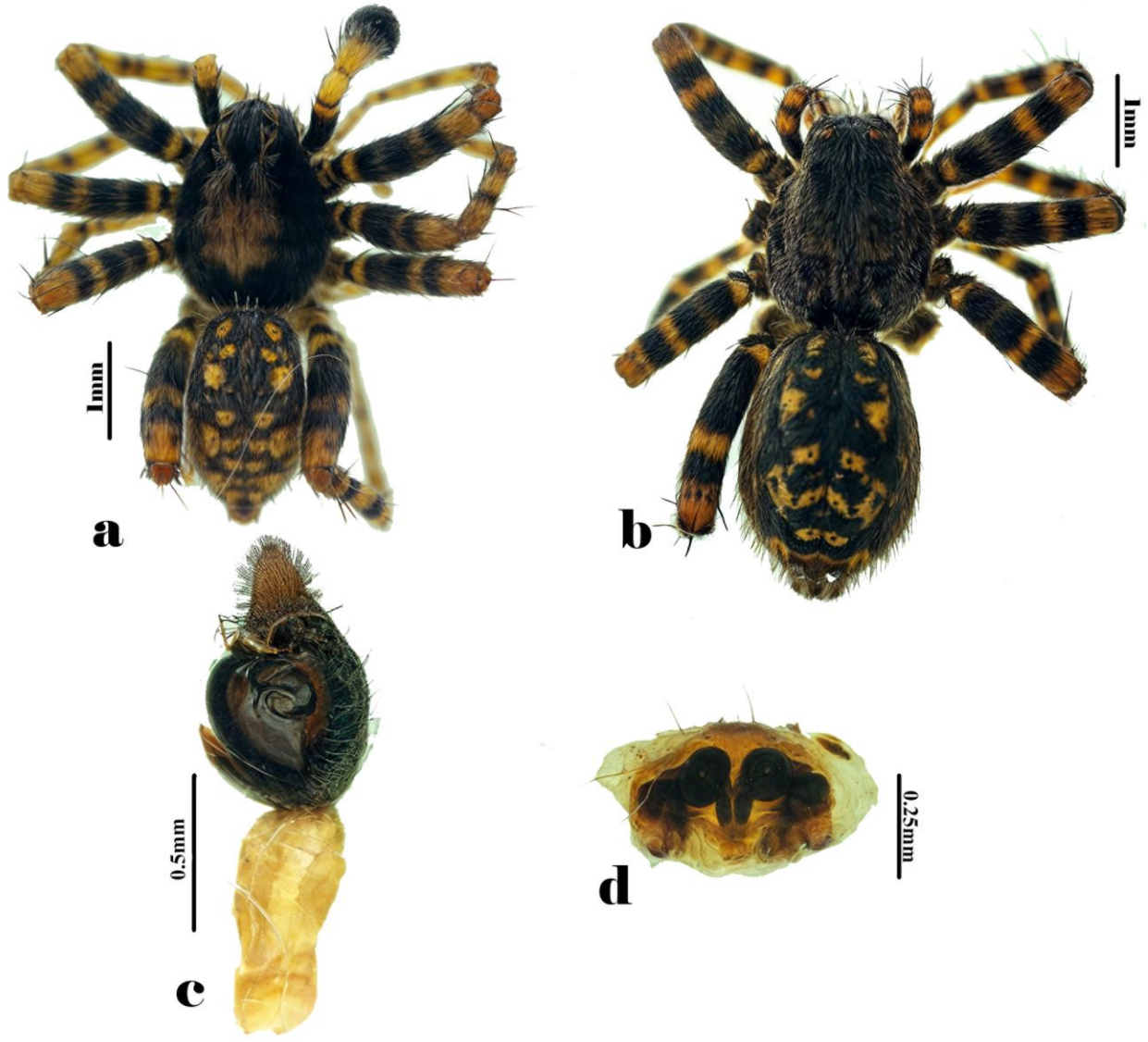

FIGURE 14. *Wadicosa fidelis*: male: a, habitus, dorsal; c, palp; female: b, habitus, dorsal; d, epigyne.

Remarks: The species has been recorded from Macaronesia, North Africa, Southern Europe, Caucasus, Middle East, Central Asia, China, Japan, Pakistan, Indonesia (Sumatra), India, Bhutan, Bangladesh, Philippines, Indonesia (Sumatra), and the United Arab Emirates. This species was recorded from several provinces in Iran -Golestan, Hormozgan, Ilam, Kermanshah, Khuzestan, Kohgiluyeh and Boyer-Ahmad,

Kurdistan, and Razavi Khorasan (Hosseinpour et al., 2022; Zamani et al., 2026). New materials were collected on a palm tree and vegetation in the garden from Fadami City (Fars Province). Recorded here for the first time from Fars Province.

Family Oecobiidae Blackwall, 1862
Oecobius pasargadae Zamani & Marusik, 2023
(Figure 15A, B)
Material examined: 1♂ Fars Province, Fadami 55°7'54.317"N, 28°13'10.058"E, 1247m a. s. l, 06 February 2023, A. Mashayekh leg.

FIGURE 15. *Oecobius pasargadae*: male: a, habitus, dorsal; b, plap.

Global distribution: Iran: Fars Province: ♂ (MMUE) Shiraz, 29.6°N, 52.516667°E, Y.M. Marusik (Zamani & Marusik, 2023).
Remarks: This species was recorded from Fars Province (Shiraz). This is the first record from Darab County (Fars Province). Fresh material was found on the wall in the car garage.

Family Philodromidae Thorell, 1869
Thanatus vulgaris Simon, 1870
(Figure 16A, B)
Material examined: 2♂ Fars Province, Darab 54°34'40.725"N, 28°45'38.779"E, 750 m a. s. l, 07 January 2022, A. Mashayekh leg.
Global distribution: Iran: Isfahan Province, Ilam Province, Sistan & Baluchistan Province.
Remarks: Collected from the mountainous area and in-house next to the drainage (Darab). This species is probably the most widespread philodromid species, and is mostly transported by humans to new areas (Jäger, 2002). It is widely distributed in Iran (Alborz, Fars, Golestan, Hormozgan, Ilam, Isfahan, Kermanshah, Khuzestan, Kohgiluyeh &
Boyer-Ahmad, Mazandaran, Qazvin, Tehran, Zanjan, Sistan & Baluchistan, Europe, North Africa, Turkey, Caucasus, Russia (from Europe to the Far East), Middle East, Iran, Kazakhstan, Central Asia, China, Korea, Japan. Introduced to North America, South Africa, and Australia (Zamani et al., 2025).

Family Prodidomidae Simon, 1882
Zimiris doriae Simon, 1882
(Figure 17A, B)
Material examined: 2♀ Fars Province, Fadami 55°7'54.317"N, 28°13'10.058"E, 1247m a. s. l, 12 September 2021/2 September 2022 A. Mashayekh leg.
Global distribution: Iran: Hormozgan Province. Ivory Coast, Sudan, Eritrea, Yemen, Iran, Pakistan, India. Introduced to Mexico, the Caribbean, French Guiana, Brazil, Ascension, Germany, Indonesia (Java), and Malaysia (World Spider Catalog, 2026).

FIGURE 16. *Thanatus vulgaris*: male: a, habitus, dorsal, b, palp.

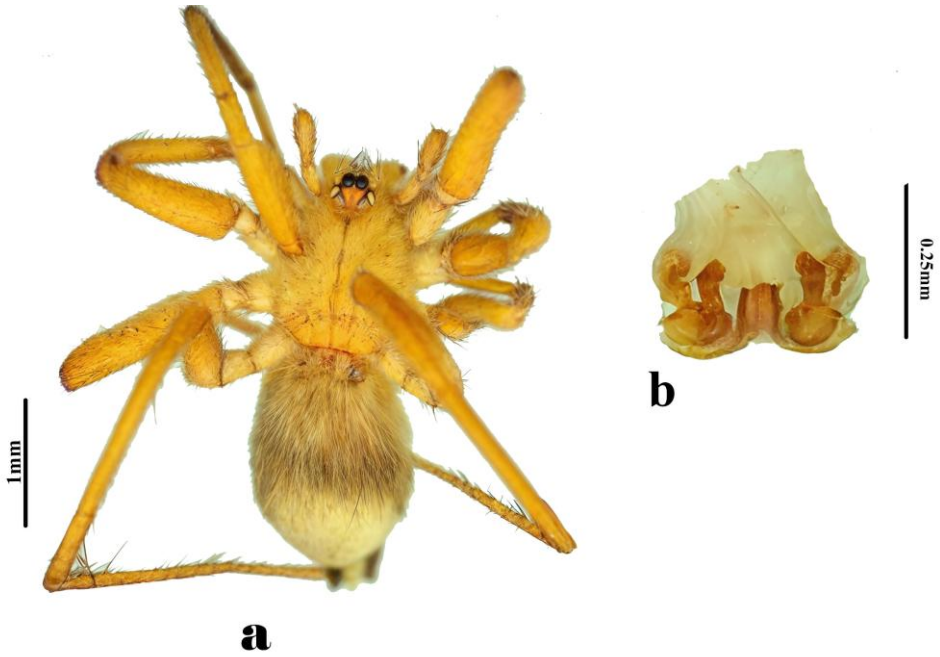

FIGURE 17. *Zimiris doriae*: female: a, habitus, dorsal.

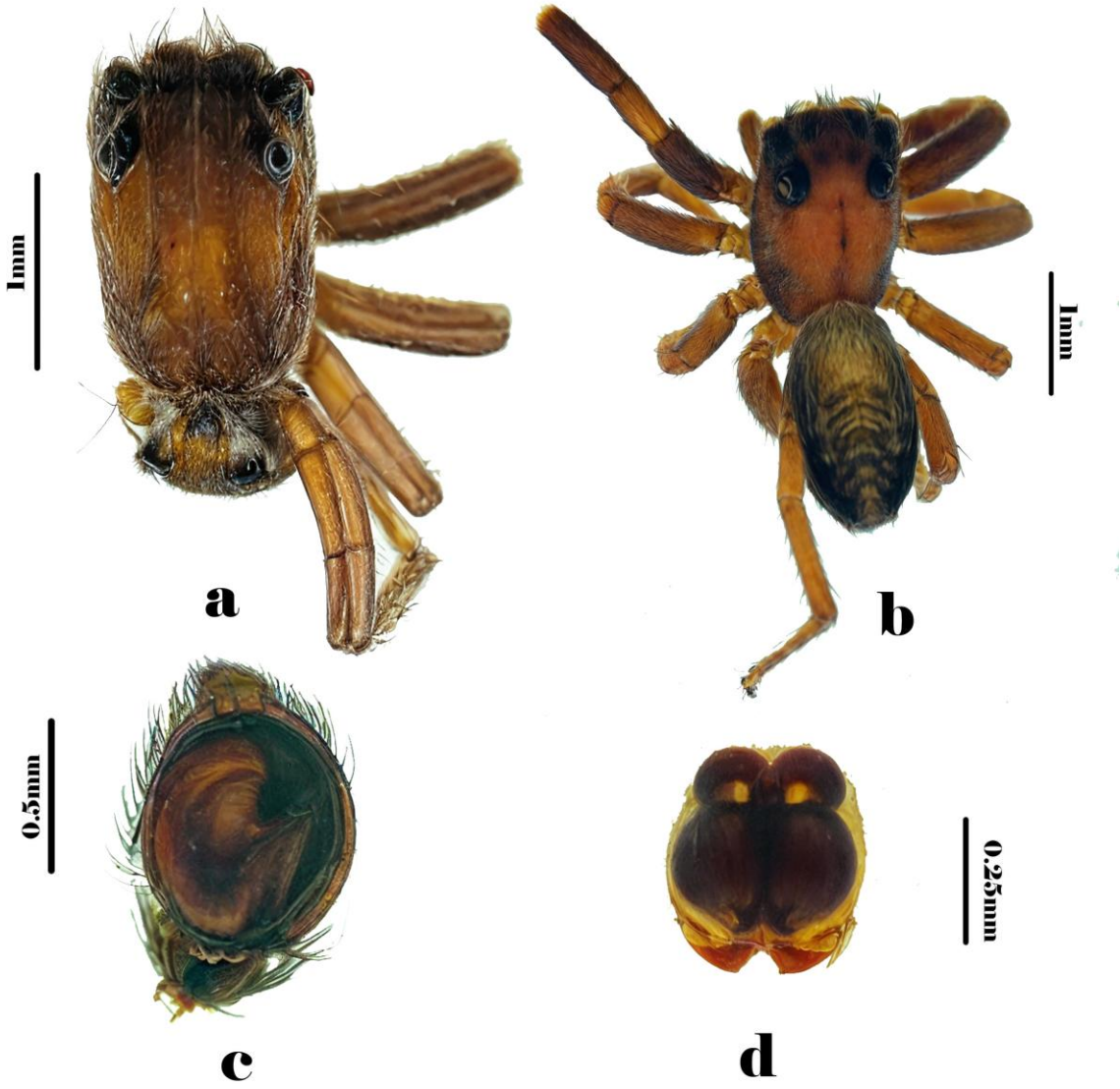

FIGURE 18. *Cyrba ocellata*: male: a, habitus, dorsal; c, plap; female: habitus, dorsal; d, epigyne.

Remarks: This species is distributed in tropical areas from Mexico to Malaysia (Zamani et al., 2015; World Spider Catalog, 2026). Specimens were found in the garden under the grass bushes. This is the first record in Fars Province.

Family Salticidae Blackwall, 1841
Cyrba ocellata (Kroneberg, 1875)
(Figure 18)
Material examined: 1♂ 1♀ Fars Province, Darab 54°34'40.725"N, 28°45'38.779"E, 750 m a. s. l, 13 October 2022, A. Mashayekh leg.

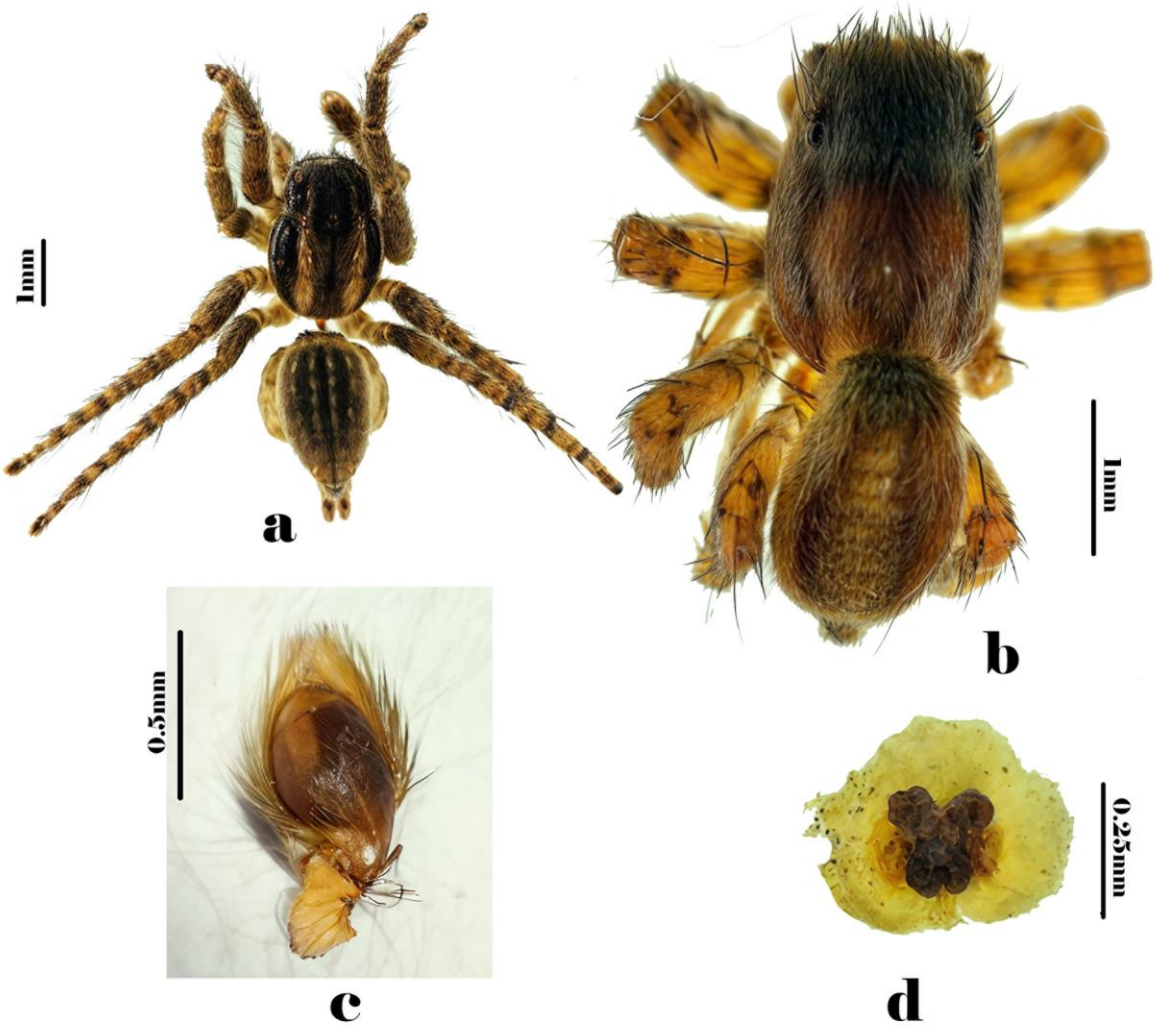

FIGURE 19. *Langona pallida*: male: a, habitus, dorsal; c, palp; *Langona tartarica*: female: b, habitus, dorsal; d, epigyne.

Global distribution: Iran: Sistan & Baluchistan Province, Mazandaran Province, Razavi Khorasan Province, Tehran Province. Eastern Africa to India and Indonesia, Caucasus to Central Asia and China, and the United Arab Emirates are recorded as the distributional area of the species. to Australia (Zamani et al., 2026).
Remarks: This species is globally recorded from Eastern Africa to India and Indonesia, the Caucasus to Central Asia and China, the United Arab Emirates, and introduced to Australia. It has previously been reported from Golestan, Kerman, Mazandaran, Razavi Khorasan, Tehran, and Sistan & Baluchestan in Iran (Mirshamsi et al., 2015; Zamani et al., 2026). The specimens were collected under a grass bush. Herewith recorded for the first time from the spider fauna of Fars Province.

Langona pallida Prószyński, 1993
(Figure 19A, C)
Material examined: 2♂ Fars Province, Fadami 55°7'54.317"N, 28°13'10.058"E, 1247m a. s. l, 22 November 2022 A. Mashayekh leg.
Global distribution: Iran: Turkmenistan, Saudi Arabia, United Arab Emirates, Afghanistan (Sajadi et al., 2024; Zamani et al., 2025).

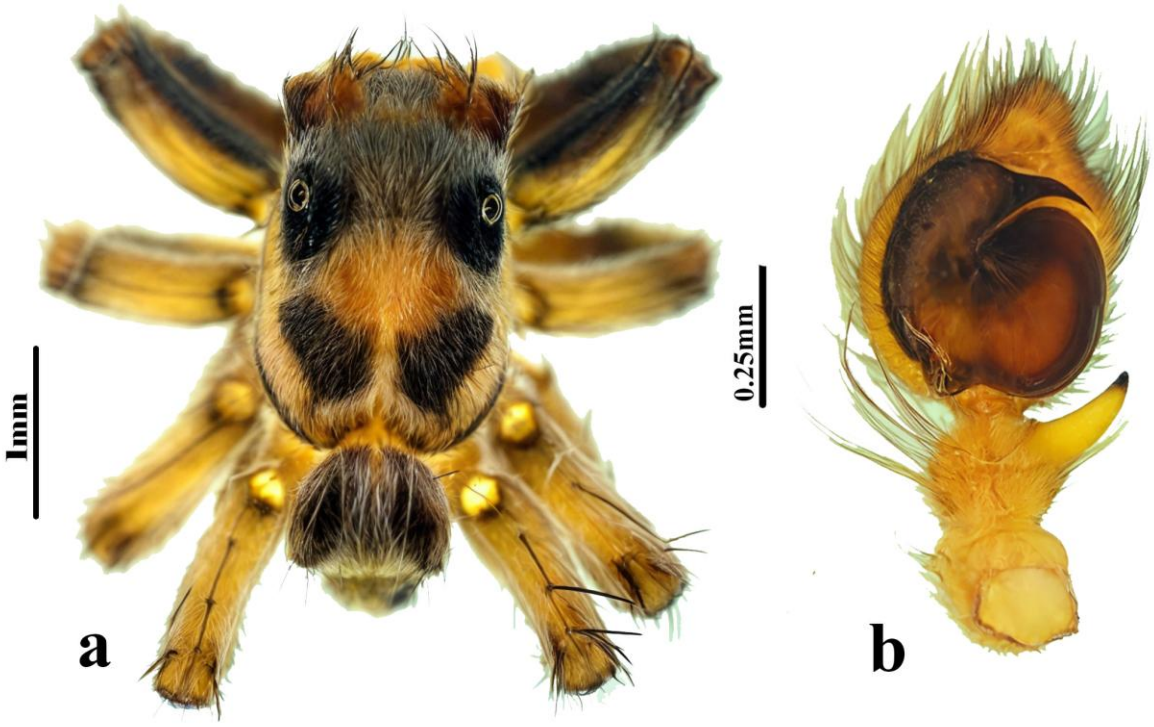

FIGURE 20. *Plexippus minor*: male: a, habitus, dorsal; b, palp.

Remarks: This species is recorded from Afghanistan to Saudi Arabia to the United Arab Emirates. According to Logunov et al. (2023), *L. pallindula* Logunov & Rakov, (1998) has been recorded in Iran (Khorasan-e-Razavi), as reported by Mirshamsi et al. (2015) as *L. pallida*. Collected materials from Fars Province belong to *L. pallida*. Therefore, this is the first record of the species in Iran.

Langona tartarica (Charitonov, 1946)
(Figure 19B, D)
Material examined: 1♀ Fars Province, Fadami 55°7'54.317"N, 28°13'10.058"E, 1247m a. s. l, 5 December 2022 A. Mashayekh leg.
Global distribution: Iran: Hormozgan Province, Sistan & Baluchistan Province. Turkmenistan, Uzbekistan, Tajikistan, Afghanistan, Pakistan, China, Yemen? (Mirshamsi et al., 2015; World Spider Catalog, 2026).
Remarks: Material was collected under a grass bush. This is the first record of Fars Province. Other records from Iran are in Hormozgan and Sistan & Baluchistan Province.

Plexippus minor Wesołowska and van Harten, 2010
(Figure 20A, B)
Material examined: 2♂Fars Province, Fadami 55°7'54.317"N, 28°13'10.058"E, 1247m a. s. l, 6 December 2022 A. Mashayekh leg.
Global distribution: Iran: Hormozgan Province. United Arab Emirates, India (World Spider Catalog, 2026).

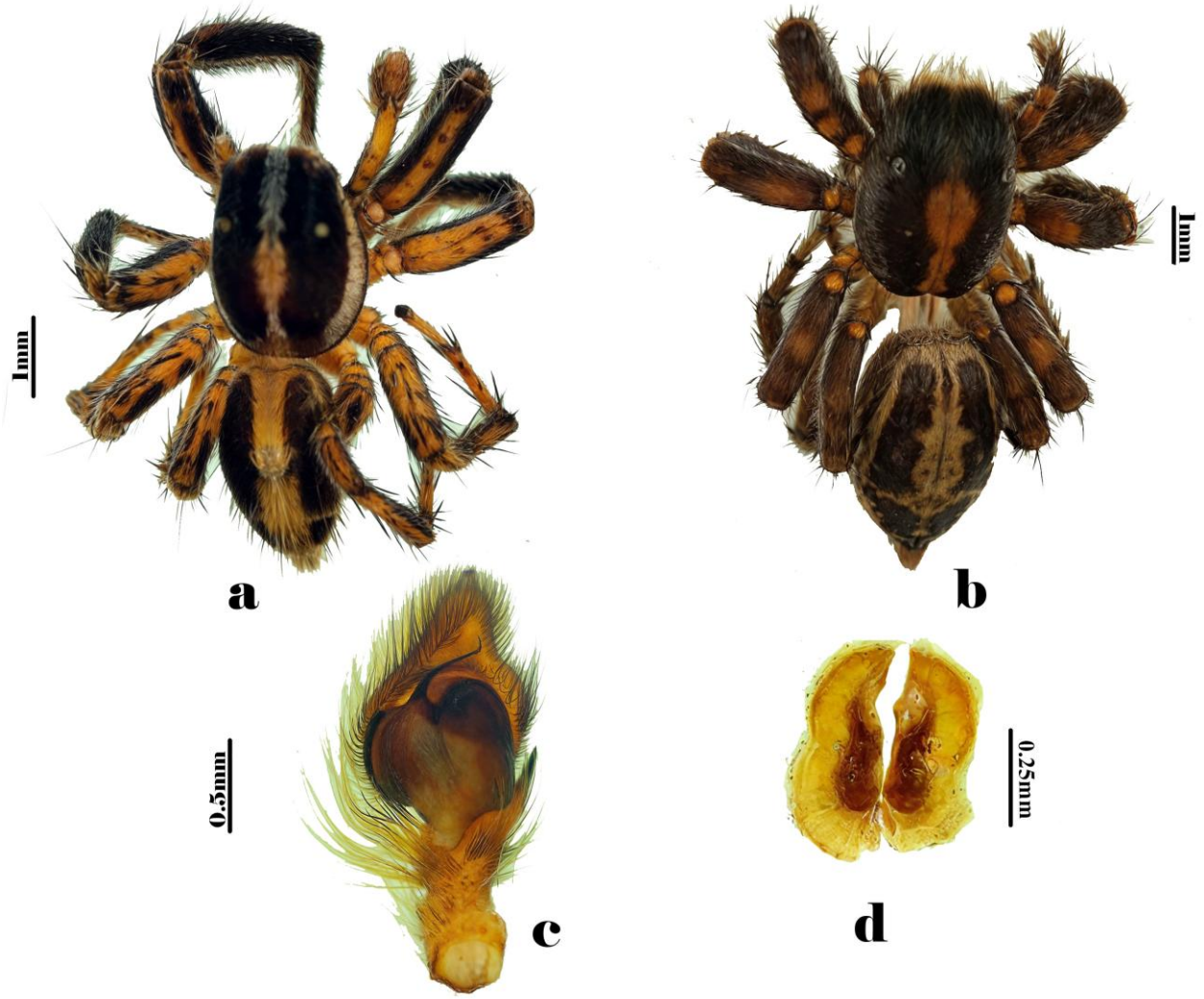

FIGURE 21. *Plexippus paykulli*: male: a, habitus, dorsal; c, palp; female: b, habitus, dorsal; d, epigyne.

Remarks: This species has been recorded from the United Arab Emirates, India, and Iran. This is the second record of the species in Iran and the first in Fars Province. Collected materials were found under a grass bush.

Plexippus paykulli (Audouin, 1826)
(Figure 21)
Material examined: 2♂ 1♀ Fars Province, Fadami 55°7'54.317"N, 28°13'10.058"E, 1247m a. s. l, 10 January 2023 A. Mashayekh leg.
Global distribution: Iran: Golestan Province, Hormozgan Province, Sistan & Baluchistan Province, Isfahan Province, Kerman Province, Khuzestan Province, Mazandaran Province, Tehran Province. Africa. Introduced to both Americas, Europe, West Asia, Nepal, South Asia, Australia, Pacific Islands (Zamani et al., 2025).
Remarks: This species is cosmopolitan, distributed, and introduced to all continents. This is the first record of the species in Fars Province. Materials were observed in vegetation.

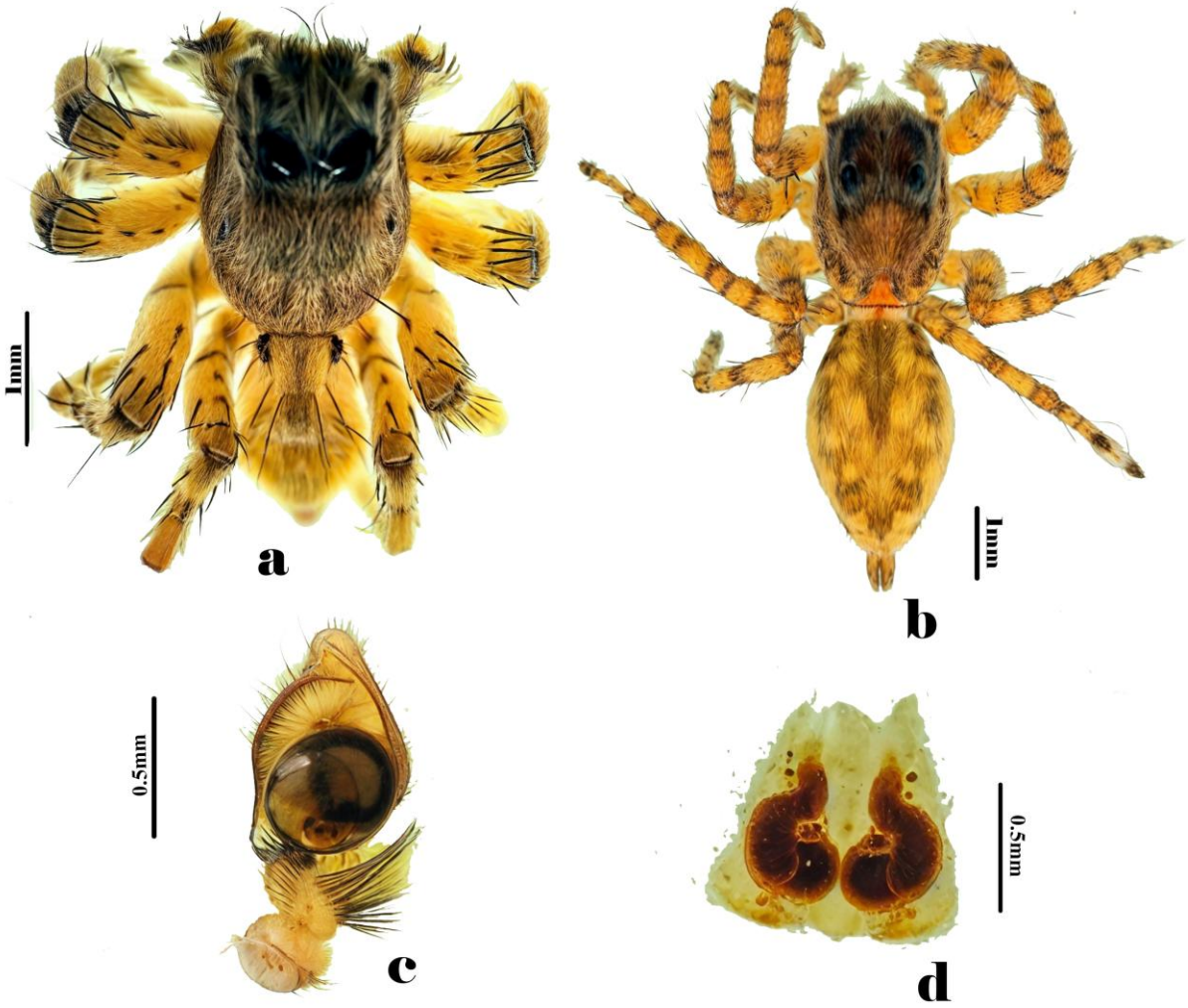

FIGURE 22. *Plexippoides flavescens:* male: a, habitus, dorsal, c, palp; female: b, habitus, dorsal; d, epygine.

Plexippoides flavescens (O.Pickard-Cambridge, 1872)
(Figure 22)
Material examined: 1♂ 2♀Fars Province, Fadami 55°7'54.317"N, 28°13'10.058"E, 1247m a. s. l, 18, 30 January 2023 A. Mashayekh leg.
Global distribution: Iran: Fars Province, Kerman Province, Khuzestan Province, Tehran Province, Chahar Mahal & Bakhtiari Province, Isfahan Province, Kerman Province, Kohgiluyeh & Buyer-Ahamd Province, Lorestan Province, Mazandaran Province, Khorasan-e-Razavi Province (Mirshamsi et al., 2015). Egypt, Sudan, West Asia, Iran, Kyrgyzstan, Turkmenistan, and Afghanistan. Introduced to Ukraine and the USA (World Sprider Catalog, 2026).
Remarks: This species has been found in vegetation. It is mostly distributed in Asia and Africa and has been introduced to Europe and America. This is the first record in Darab County.

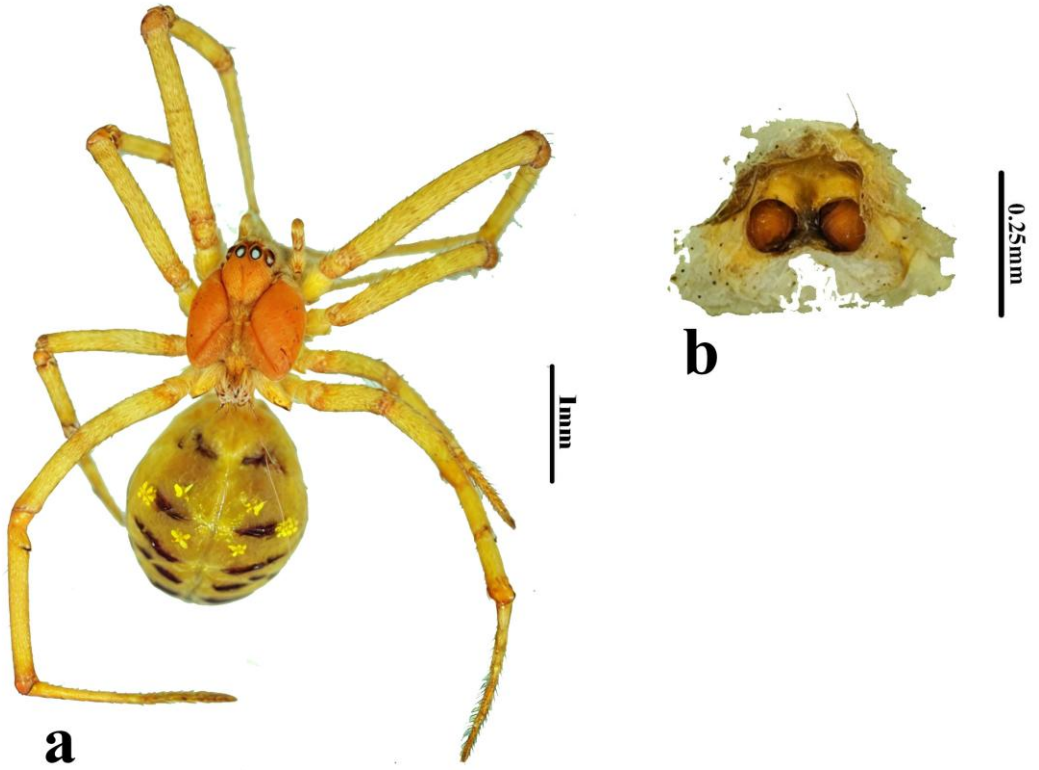

FIGURE 23. *Steatoda trianguloides*: female: a, habitus, dorsal; b, epigyne.

FIGURE 24. *Bassaniodes loeffleri*: male: a, habitus, dorsal; b, plap.

Family Sicariidae Keyserling, 1880
Loxosceles persica Ribera & Zamani, 2017
(Figure 5B, D)
Material examined: 1♂ Fars Province, Darab 54°34'40.725"N, 28°45'38.779"E, 750 m a. s. l, 28 December 2021, A. Mashayekh leg.
Global distribution: Iran: Khuzestan Province, Fars Province, Yazd Province,

Remarks: The species is endemic to Iran. It has been recorded from Fars, Khuzestan, and Ilam (Zamani et al., 2021). The new material was found in the Montanus area in Darab County.

Family Theridiidae Sundevall, 1833
Steatoda trianguloides Levy, 1991
(Figure 23A, B)
Material examined: 1♀ Fars Province, Darab 54°34'40.725"N, 28°45'38.779"E, 750 m a. s. l, 21 January 2022, A. Mashayekh leg.
Global distribution: Iran: Khuzestan Province. Portugal, Spain, France (Corsica), Greece, Turkey, Cyprus, Israel (Zamani et al., 2022; World Spider Catalog, 2026).
Remarks: This is the second record of the species in the Iranian fauna. Herewith is the first provincial report of Fars. It has been collected in a mountain area of Darab City.

Family Thomisidae Sundvall, 1833
Bassaniodes loeffleri (Roewer, 1955)
(Figure 24A, B)
Material examined: 2♂ Fars Province, Darab 54°34'40.725"N, 28°45'38.779"E, 750 m a. s. l, 25 January 2022, A. Mashayekh leg.
Global distribution: Iran: Alborz Province, Fars Province, Gilan Province, Kohgiluyeh & Boyer-Ahmad Province, Isfahan Province, West Azerbaijan Province, Zanjan Province. Greece, Turkey, Caucasus, Kazakhstan, Central Asia (World Spider Catalog, 2026).
Remarks: This species has been recorded from Greece to Central Asia. It was found in Fars, Gilan, Isfahan, Kohgiluyeh & Boyer-Ahmad, West Azerbaijan, and Zanjan in Iran. New materials were captured in the mountains of Darab City.

DISCUSSION

The richness of spider species recorded from Iran showcases the country's rich biodiversity. Researchers continue to explore various habitats, contributing to our understanding of these fascinating arachnids. In our study of the spider fauna of Darab County in Fars Province, 28 species were collected and identified. *Langona palida* is being recorded for the first time for the spider fauna in Iran. Logunov et al (2013) have recorded *L. pallidula* from Khorasan-e-Razavi. All species were recorded for the first time from Darab. Moreover, 13 species, including *Berlandina artaxerxes*, *Cryptodrassus helvolus*, *C. iranicus*, *Cyrba ocellata*, *Filistata maguirei*, *Langona pallida*, *L. tartarica*, *Minosiella intermedia*, *Pardosa parathompsoni*, *Plexippus minor*, *P. paykulli*, *Steatoda trianguloides*, *Wadicosa fidelis*, and *Zimiris doriae*, are newly recorded for Fars Province, significantly expanding known distributions in Iran. The detection of three putative new species further suggests that the region's arachnid diversity is understudied. To verify these records, we compared our findings with the most comprehensive recent catalogues of Iranian spiders and all available regional publications (Zamani et al., 2021, 2022, 2023; World Spider Catalogue, 2026). Dr. Alireza Zamani verified the final identification.

In our collection, only males of *Dysdera* sp. and *Evarcha* sp. were found. Both male and female of *Berinda* sp. were collected, but we could only catch one female, so we need more female specimens to compare with other congeners for a definite decision. Future research will focus on locating the missing sexes to enable more accurate descriptions and to enhance our knowledge of their ecology and distribution.

Family Dysderidae C.L. Koch, 1837 is distributed in the West Palearctic. 15 species of the genus *Dysdera* Latreille, 1804 were recorded from Iran (Zamani et al., 2023). *Dysdera* Latreille, 1804 has 10 species groups (Zamani et al., 2023). The species recorded here belongs to the *ninnii*–species group. The *ninnii*–species group is distinguished from other groups by chelicerae shorter than the width of the carapace, relatively short with anteriorly converging lateral margins, a bulb with a simple crest, a simple apex bearing a long subapical tooth, and a crescent-shaped lateral projection (Deeleman-Reinhold and

Deeleman, 1988). *Dysdera* sp., differs from other species by an extremely short stylus and a distinctive triangular projection on the anterior side of the posterior apophysis.

Evarcha Simon, 1902, a genus of jumping spiders, with a relatively large body, often brownish and not colorful (World Spider Catalog, 2026), found mainly on grass, trees, branches, and in the forest. The retrolateral tibial apophysis of *Evarcha* sp. is similar to *E. nenilini*, except it is slightly thicker and shorter in the new species. The anterior part of the embolus is inclined towards the abdomen, which is in the shape of a short triangle and is visible in lateral view.

The discovery of three putative new species within a limited sampling area is particularly compelling. It strongly suggests that the spider fauna of Fras Province is not only rich, but also contains a notable proportion of undocumented taxa. Fars Province occupies a transitional zone between the Zagros Mountains and the Persian Gulf lowland, also a marginal desert, encompassing diverse habitats from oak forests to arid steppes. This environmental heterogeneity likely underlies the region's high spider diversity. The presence of species with both Mediterranean/Afrotropical affinities (*Plexippus*, Cyrba) and Irano-Turanian elements (*Berlandoina*, *Cryptodrassus*) reflects this transitional position.

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SHORT COMMUNICATIONS Open access

Notes on the Heyden's gecko (*Hemidactylus robustus*) (Squamata: Gekkonidae) from Tunb-e Bozorg and Faror Islands, Persian Gulf

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Abstract
The Heyden's gecko (*Hemidactylus robustus*) is a rare taxon in the Iranian herpetofauna, and its distribution range in Iran has remained largely unclear. In this study, we report on relatively recently collected three specimens from Tunb-e Bozorg and Faror Islands, Persian Gulf, and compare them with published data. The specimens examined show morphological congruence in the main taxonomic features and colour patterns with those previously described in the existing literatures. This study provides for the first time molecular evidence for the specimens of *Hemidactylus robustus* on the southern coast of Iran. The results show that the prevailing hypothesis of a recent, possibly anthropogenically favored dispersal of the species in its range is not sufficiently supported.

Keywords: Iranian Islands, Persian Gulf, Gekkonidae, mtDNA, Haplotype, taxonomy.

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Hemidactylus robustus Heyden, 1827 is a gecko belonging to the diverse genus that includes 199 recognized species, making it one of the most species-rich genera of reptiles worldwide, seven of which have been recorded in Iran (Uetz et al., 2025). This species is widely distributed and inhabits the coastal regions of the Red Sea, Arabian Sea and Persian Gulf, but also extends its range to the interior regions of the Horn of Africa and Arabia (Sindaco and Jeremcenko, 2008; Uetz et al., 2025).

For an extended period, *Hemidactylus robustus* has been regarded as a junior synonym of *H. turcicus* due to morphological similarities and the absence of comprehensive taxonomic studies. Nevertheless, it has been recognized as a distinct species by several researchers (Baha El Din, 2003, 2005, 2006; Lanza, 1990; Moravec & Böhme, 1997). Genetic analyses have corroborated its status as a distinct taxon (Carranza & Arnold, 2006). Further investigations have reinforced this taxonomic classification (Carranza & Arnold, 2012; Moravec et al., 2011; Šmíd et al., 2013, 2015).

Although *Hemidactylus robustus* is recognized as a component of the Iranian herpetofauna (Bauer et al., 2006; Hosseinzadeh et al., 2014), molecular phylogenetic analyzes of specimens from the Iranian coast of the Persian Gulf have not yet been performed. In this study, a segment of the mitochondrial gene encoding the fourth subunit of NADH dehydrogenase (ND4) is used to determine the phylogenetic position of three specimens originating from Tunb-e Bozorg and Faror islands. In addition, a table comparing the morphological characters between the specimens and the existing scientific literature is provided.

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TABLE 1. Morphological comparison of *Hemidactylus robustus* from Tunb-e Bozorg and the Faror Islands in the Persian Gulf with the literature. Values are given as follows: sample size, mean ± standard deviation above, minimum and maximum values below. (It should be noted that males and females were not measured separately for metric characters in the analysis of Hosseinzadeh et al., 2014).

Species / Character	*H. robustus* (Šmid et al., 2013)		*H. robustus* (Hosseinzadeh et al., 2014)		*H. robustus* (Carranza& Arnold, 2012.)		*H. robustus* (Faror)	*H. robustus* (Tunb-e Bozorg)	
							HAC 1381	HAC 1377	HAC 1377
Upper labials	27	9.4 ± 0.7 8–11	7	9.34±0.16 8-10	45	9±0.08 7-11	9/9	9/9	9/9
Lower labials	27	7.7 ± 0.6 6–9	7	7.62±0.11 7-8	45	7.6±0.07 7-9	8/9	8/9	9/9
Nasals in contact (%)	27	22.2					Separated	Separated	Separated
1st postmental in contact with 2nd lower labial (%)	27	70.3					Contacted	Contacted	Contacted
Rows of dorsal tubercles	27	14.8 ± 1.2 13–18			45	14.8 ± 0.12 14-17	13	13	14
Number of preanal Pores	9	6.1 ± 0.8 5–8			22	6 ± 0.09 5–7	7	6	-
Lamellae under 1st toe	27	6.1 ± 0.5 5–8	7	5.95±0.13 5-7	22 (M) 23 (F)	6±0.11 (M) 6.2± 0.12 (F) 6-7 (M) 5-7 (F)	7	7	8
Lamellae under 4th toe	27	10.1 ± 0.7 8–12	7	10.14±0.23 9-14	22 (M) 23 (F)	9.6 ±0.13 (M) 10 ± 0.09 (F) 9-11(M) 9-11 (F)	8	8	8
Snout-Vent Length (Males)	8	41.8 ± 2.3 37.0–43.7	7	36.30±1.84 18.59-51.98	22	43±0.93 33.4-54.6	42.73	37.22	
Snout-Vent Length (Females)	16	43.6 ± 4.7 32.7–50.1		- -	23	43.3±1.11 31.2-51.3			43.90

During the field survey conducted on the Tunb-e Bozorg and Faror Islands during May 6-9, 2024, aimed at examining the reptile's fauna, three specimens of *Hemidactylus* were collected (Figure 1). The collected specimens (HAC 1381, HAC 1370, HAC 1376) were preserved in 80% Ethanol in the Shahrekord University Herpetological Collection (HAC), Iran. The specimens were studied for one metric and eight meristic characters following Šmid et al., (2013) (Table 1).

Total genomic DNA was extracted from the tissue samples following standard extraction protocols (Green & Sambrook, 2012). Mitochondrial gene encoding the fourth subunit of NADH dehydrogenase (plus downstream Serine, Histidine, and Leucine tRNAs; hereafter collectively referred to as ND4 was amplified using standard PCR procedures with the following primers, ND4F, 5'-CACCTATGACTACCAAAAGCTCATGTAGAAGC-3' (Thaung et al., 2009) and Leu R, 5'-CATTACTTTTACTTGGATTTGCACCA-3'(Arevalo et al., 1994). PCR reactions performed in 20µl with the following conditions: Initial denaturation stage of 95° C (05:00) followed by the 36 cycles with denaturation at 95°C (00:40), annealing at 50° C (00:40) and extension at 72° C (01:40) then single extension cycle at 72° C (05:00). The amplification products were sequenced on an automated sequencer ABI 3730XL (Shahrekord University of Medical Sciences, Shahrekord, Iran) according to standard protocols.

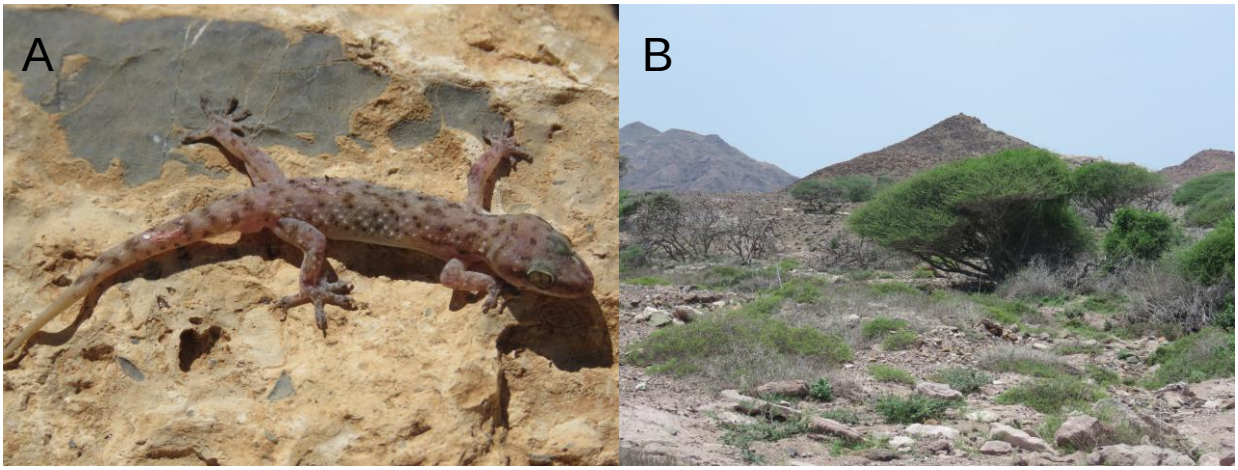

FIGURE 1. Live specimens of *Hemidactylus robustus* caught on the island of Faror, Persian Gulf (A); Natural habitats of *Hemidactylus robustus* on the island of Faror, Persian Gulf (B).

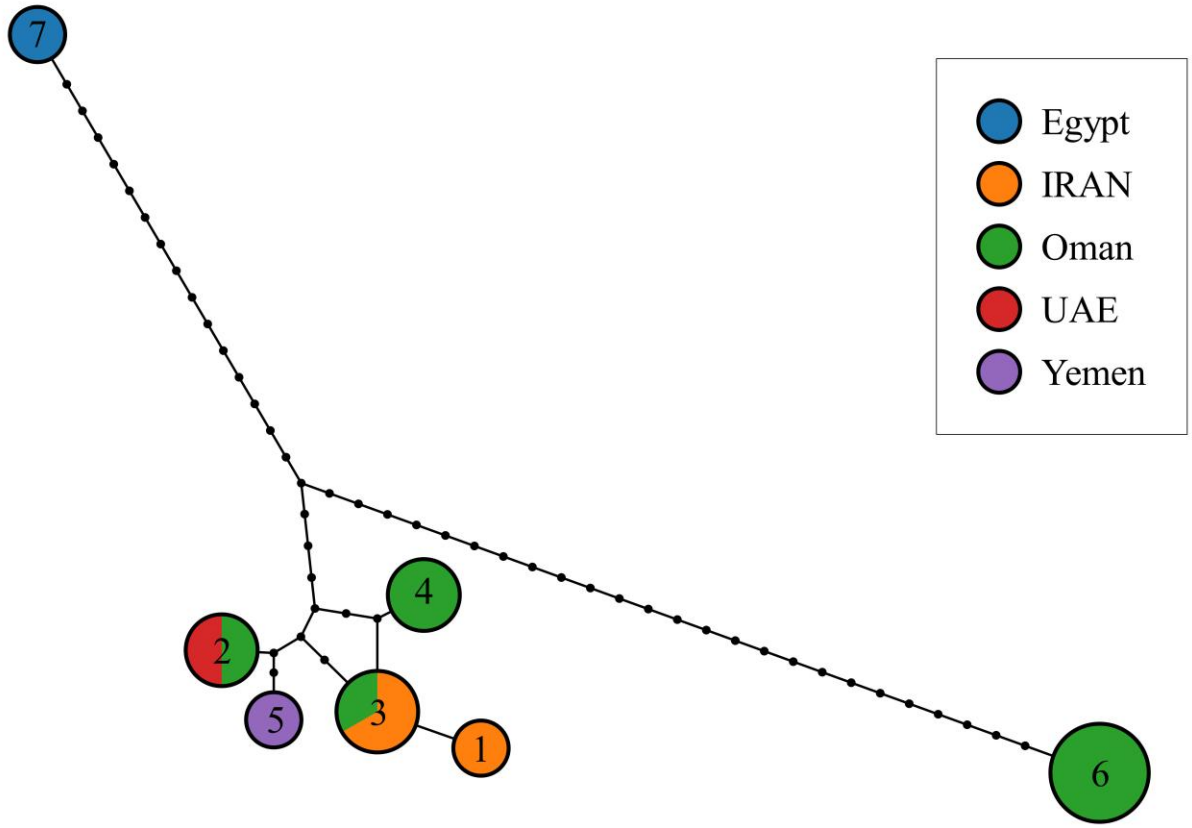

FIGURE 2. Phylogenetic network based on haplotypes for the *ND4* fragment (491bp) in *Hemidactylus robustus*. Circles correspond to haplotypes, with size proportional to the number of individuals per haplotype. Vertical lines correspond to mutational steps. Colors represent geographic origins of haplotypes according to Table S1.

On the islands of Tunb-e Bozorg and Faror, *Hemidactylus robustus* was easily found in most of the natural habitats of these islands (Figure 1). They were active at night and could be found hiding under rocks during the day. The examined specimens show morphological congruence in the main taxonomic features and color patterns with those previously described in the existing literature (Table 1). A segment comprising 585 base pairs of the mitochondrial DNA sequences pertaining to the ND4 gene was sequenced in the present investigation and subsequently archived in the National Center for Biotechnology Information (NCBI) (PV863223 (HAC 1376); PV863222 (HAC 1370); PV863221 (HAC 1381)). The sequences underwent editing utilizing Bioedit version 7.0.0 (Hall 1999) and were aligned employing the CLUSTAL W algorithm (Thompson et al. 1994). Furthermore, existing sequences of ND4 for *H. robustus* were retrieved from GenBank (Table S1). The obtained sequences were subjected to trimming, culminating in the creation of a dataset characterized by a final sequence length of 491 nucleotides. An unrooted phylogenetic network delineating the ND4 haplotypes of *Hemidactylus robustus* was established through the application of the TCS network algorithm (Clement et al. 2002), incorporated within Hapsolutely 0.2.2 (Vences et al. 2021), utilizing the default parameters. Comparison of the DNA sequences obtained showed that our samples belonged to two mitochondrial haplotypes. One of them is a unique haplotype and the other two belong to a haplotype that is also found in Oman (Al Zaiba) (Figure 2). The highest haplotype diversity is found in samples from Oman (Figure 2), although this result could be due to a sampling gap in the distribution ranges of this species. The pattern of haplotype diversity discovered in this study clearly contradicts the hypothesis of a recent, potentially anthropogenically facilitated, dispersal of the species in this region (Šmíd et al., 2015). It seems that we need further studies with more samples to better understand the distribution pattern of this species.

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TABLE S1. Data pertaining to all *Hemidactylus robustus* sequences incorporated in this study, together with their geographical distributions and corresponding NCBI accession numbers.

CODE	Haplotype no.	Geographic Origin	NCBI Accession Number
H_robustus_AO3_Oman	3	Oman	JQ957388
H_robustus_AO164b_Oman	4	Oman	JQ957387
H_robustus_AO165_Oman	4	Oman	JQ957387
H_robustus_S1688_Oman	2	Oman	JQ957390
H_robustus_R1415_Yemen	5	Yemen	JQ957392
H_robustus_SPM001859_Egypt	7	Egypt	JQ957394
H_robustus_SPM001503_UAE	2	UAE	JQ957393
H_robustus_S1677_Oman	6	Oman	JQ957389
H_robustus_S2150_Oman	6	Oman	JQ957391
H_robustus_S2151_Oman	6	Oman	JQ957389
H_robustus_S1905_Oman	6	Oman	JQ957389
H_robustus_S1962_Oman	6	Oman	JQ957389
H_robustus_S1778_Oman	6	Oman	JQ957389
HAC_1381_Faror	3	IRAN, Island of Faror	PV863221
HAC_1370_Tunb-e Bozorg	3	IRAN, Island of Tunb-e Bozorg	PV863222
HAC_1376_Tunb-e Bozorg	1	IRAN, Island of Tunb-e Bozorg	PV863223

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RESEARCH ARTICLE Open access

A preliminary study of the effects of long-term potato monoculture on earthworm community

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Abstract
Earthworms are widely recognized as ecosystem engineers because their activities significantly modify soil properties and ecosystem functioning. Earthworm community composition is primarily determined by soil characteristics, land use, and climate. In agro-ecosystems, earthworm populations are strongly affected by management practices, particularly tillage intensity, crop rotation schemes, and the application of various agrochemicals. The aim of the present study was to investigate the effects of intensive potato (*Solanum tuberosum*) cultivation on earthworm community structure and diversity. Earthworm samples were collected by hand-sorting from long-term potato monoculture farms subjected to ploughing and the application of pesticides, fungicides, herbicides, and chemical fertilizers, as well as semi-natural, non-agricultural habitats used as reference sites. A total of eight anecic, epigeic, and endogeic earthworm species belonging to the family Lumbricidae were identified in the semi-natural habitats, namely *Aporrectodea caliginosa*, *A. longa*, *A. trapezoides*, *Dendrobaena hortensis*, *D. pentheri*, *D. semitica*, *Eisenia andrei*, and *Perelia kaznakovi*. In contrast, only a single anecic species (*A. longa*) was recorded in potato fields. These results indicate that intensive agricultural activities have adversely affected the earthworm community in potato fields, leading to species loss by eliminating epigeic and endogeic earthworms.

Keywords: Fauna, *Androctonus*, taxonomy, morphology, Azarbaijan, Iran.

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INTRODUCTION

Earthworms are recognized as ecosystem engineers due to their significant impact on soil biota, structure, and chemical properties. Consequently, earthworm abundance, biomass, and diversity have strong impacts on overall soil quality (Singh et al., 2021; Edwards & Arancon, 2022). Earthworms play a key role in improving soil structure and function through their burrowing and casting activities, which loosen the soil and enhance aeration. They also contribute to carbon and nutrient cycling through litter decomposition, support the maintenance of soil microbial community, and promote both the chemical and biological fertility of the soil (Edwards & Arancon, 2022; Vidal et al., 2023).

Agricultural monoculture has become a defining feature of modern intensive farming, often leading to soil degradation and loss of biodiversity (Foley et al., 2011; Lal, 2015). Potato monoculture, in particular, is widely practiced due to the crop's economic value and global demand, yet long-term cultivation of a single crop can substantially alter soil structure, nutrient dynamics, and microbial activity (Larkin, 2015). Such changes directly affect soil-dwelling organisms, especially earthworms, which play

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key roles in nutrient cycling, soil aggregation, and overall soil health (Edwards & Arancon, 2022). Because earthworm communities are highly sensitive to management practices and shifts in soil conditions (Bogužas et al., 2022), understanding the ecological consequences of prolonged potato monoculture is critical. This preliminary study therefore examines how long-term potato monoculture influences earthworm community structure and abundance.

The composition of earthworm communities is strongly shaped by the availability of soil nutrients and prevailing climatic conditions, both of which determine habitat suitability and resource accessibility. In agro-ecosystems, a variety of factors can influence their population dynamics, including tillage, cropping sequence, geographical location, fertilizer overuse, and climate (Edwards & Arancon, 2022). Regarding farming practices, the majority of the studies have shown that organic farming improves soil organic matter and increases earthworm abundance, biomass, and species diversity compared to conventional farming (Hole et al., 2005; Flohre et al., 2011; Crittenden et al., 2014). Incorporating organic matter during ploughing may enhance food availability for endogeic earthworm species (Ernst & Emmerling, 2009; van Capelle et al., 2012). However, tillage remains a major cause of earthworm mortality, particularly for epigeic and anecic species (Briones & Schmidt, 2017). In addition, soil compaction resulting from certain farming practices can adversely affect earthworm communities (Capowiez et al., 2009).

Inorganic fertilizers can exert adverse effects on the environment and pose a significant threat to the long-term sustainability of agricultural systems (Zhou et al., 2013). Soil pH is reduced by the use of inorganic fertilizers (Zhou et al., 2013; Ngosong et al., 2020), which results in detrimental effects on earthworm populations (Ngosong et al., 2020). In contrast, organic farming systems promote biodiversity (Suthar, 2009), and earthworm abundance, biomass, and diversity increase in organic farmlands (Crittenden et al., 2014). Jin et al. (2022) further demonstrated that organic fertilizers may positively affect the microbial diet of certain species, especially endogeic and anecic earthworms.

Pesticide application has been shown to reduce earthworm abundance (Bart et al., 2018). The sensitivity of earthworms to pesticides varies among species and is closely related to the efficiency of their xenobiotic detoxification systems (Lu et al., 2017). Anecic and endogeic earthworms are generally less tolerant to pesticide exposure than epigeic species (e.g. *Eisenia* spp.) (Robinson et al., 2021).

In the present study, the effect of long-term potato (*Solanum tuberosum* L., 1753) monoculture on the earthworm communities in agricultural lands was investigated. Because monoculture has been practiced for a long time in the study area, it is likely that the composition of the earthworm community has been significantly altered, leading to reduced diversity and biomass in potato monoculture fields compared to semi-natural habitats.

MATERIAL AND METHODS

Study area

The experimental design included 15 potato agricultural lands and 15 semi-natural fields in Hamedan Province, Iran, in May 2023 (Fig. 1). For more than 20 years, the potato fields have been used exclusively for the cultivation of potatoes without any crop rotation. The soil type of the farmlands is silty loam. The fields are ploughed three times a year using heavy machinery. The potato-growing areas have been irrigated by rain for the past 10 years; prior to that, flooding was used for irrigation. Chemical and organic fertilizers, pesticides, fungicides, and herbicides have been used on these farmlands (Table 1).

The semi-natural habitats examined in this study were located in the vicinity of potato fields. The vegetation in these areas comprises grassy and bushy plants, including *Avena fatua*, *Chenopodium album*, *Cynodon dactylon*, *Descurainia sophia*, *Digitaria sanguinalis*, *Echinochloa crus-galli*, *Fumaria officinalis*, *Hordeum murinum*, *Papaver rhoeas*, and *Portulaca oleracea*. These habitats have been impacted by human activities such as soil compaction, vegetation cutting, vegetation burning, and grazing by sheep.

The climate of the study area is temperate. The average annual precipitation and air temperature in the area are about 325 mm and 11.2 °C, respectively (Mosammam et al., 2016; Talebmorad et al., 2021).

TABLE 1. Application of agrochemicals in potato farmlands (based on interviews with farmers).

Agrochemical inputs	Application rate (per hectare)	Applications/year (time)	Application time
Fertilizers			
Arcta NPK fertilizer	3-5 L	1-2	Spring
Phosphate (granular)	100-150 Kg	1	Fall
Poultry manure	6-10 t	1	Fall
Sulo potash (granular)	5-10 Kg	2-4	Spring
Urea (granular)	50-100 Kg	2-3	Spring
Pesticides			
Carbaryl	1-2 Kg	2-3	Spring
Chlorpyrifos	1-2 L	1-3	Spring
Diazinon	1-1.5 L	1-2	Spring
Imidacloprid	1-1.5 L	1	Early Spring
Metam-sodium	100-200 L	1	Fall
Thiacloprid	0.1-0.2 L	1-2	Spring
Fungicides			
Chlorothalonil	1.5-2 Kg	2-3	Spring
Mancozeb	2-2.5 Kg	3-5	
Nordox	2.5-3 Kg	1-2	Spring
Prochloraz	0.5-0.75 L	2-3	Spring
Signum	0.75-1 Kg	2-3	Spring
Thiabendazole	0.75-1 Kg	1-2	Spring
Herbicides			
Metribuzin	0.5-1 Kg	1	Early Spring
Paraquat	1-2 L	1-2	Early Spring
Pendimethalin	1-1.5 L	1	Early Spring
Rimsulfuron	20-40 gr	1	Mid-Spring

Earthworm sampling

Earthworms were hand-sorted in the field from three randomly selected replicates using quadrats (50 × 50 × 25 cm deep). Quadrats were arranged at 10-meter intervals along transects in each sampling site, with the starting point randomly selected. To determine the starting point, each station was divided into 16 sections, and one of them was selected using a random number table. In the potato fields, transects began at least 20 meters from the field margins, while in the semi-natural habitat quadrats were positioned at least 5 meters from the agricultural lands (Bartz et al., 2024; Mittmannsgruber et al., 2025). Sampling was conducted on sunny days between 9 a.m. and 12 p.m., with air temperatures ranging from 17 to 23 °C. The earthworm specimens were transported back to the laboratory in cool boxes, and their fresh weight was immediately measured. Thereafter, three adult specimens of each species were fixed in 80% ethanol for identification, and the rest of the specimens were returned to the sampling sites. Species identification was done based on Latif et al. (2021). The ecological categories of the earthworms were determined according to Latif et al. (2016), Reynolds and Mýsýrlýoðlu (2018), and Bottinelli et al. (2020).

Statistical analyses

The density of earthworms was expressed as the number of individuals per square meter (ind. m^{-2}). Biomass was defined as the fresh weight of worms per square meter (g. m^{-2}). For each species, the rate of occurrence was calculated as the percentage of stations in which the species was present. Relative abundance was calculated as the proportion of individuals of a given species relative to the total number of individuals observed at each station. These two metrics were treated separately to capture different aspects of species distribution: presence/absence patterns and the distribution of individual numbers across stations.

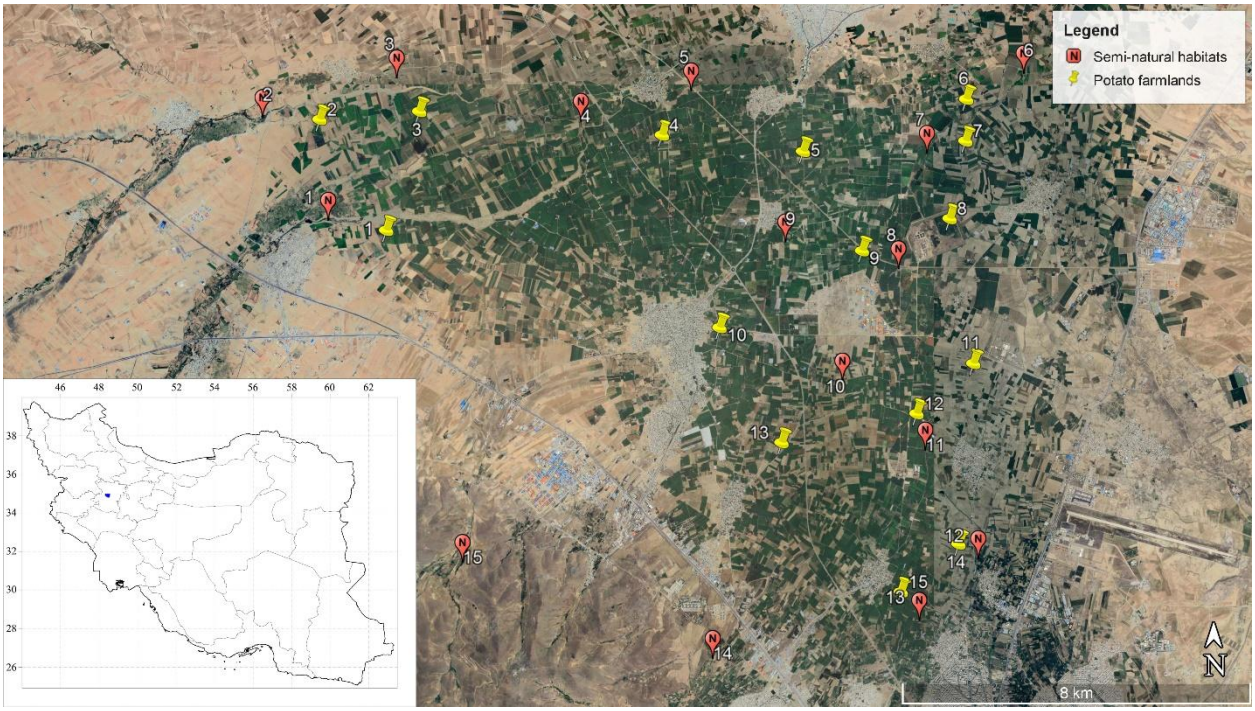

FIGURE. 1. Locations of sampling sites. Yellow markers indicate potato farms, and red markers indicate semi-natural habitats.

The Menhinick diversity index (D_{Mn}), Shannon-Wiener diversity index (H′), Simpson index (D), and Jaccard similarity index were used for biodiversity assessment (Singh et al., 2020).

Normality of variables was examined using the Kolmogorov-Smirnov test and the homogeneity of variances assessed using Levene's test. Independent-Samples t-test was used to compare earthworm abundance, weight, biomass, and diversity between agricultural and non-agricultural habitats (Student's t-test was applied when variances were equal, and Welch's t-test was used when variances were unequal). Kruskal-Wallis H test was conducted to detect differences among relative abundance and biomass, and Mann–Whitney U test was used for pairwise comparisons. Chi-square test (χ^2) was used to compare the distribution of species among stations. Data are presented as mean ± standard error (SE). Statistical analyses were performed using SPSS version 22 at a significance level of 0.05.

RESULTS

A total of eight earthworm species belonging to the family Lumbricidae were found at the semi-natural habitats, namely *Aporrectodea caliginosa* (Savigny, 1826), *A. longa* (Ude, 1885), *A. trapezoides* (Dugés, 1828), *Dendrobaena hortensis* (Michaelsen, 1890), *D. pentheri* (Rosa, 1905), *D. semitica* (Rosa 1893), *Eisenia andrei* Bouché, 1972 and *Perelia kaznakovi* (Michaelsen, 1910). In contrast, at the potato farmlands, only one species was observed: *A. longa*.

The total abundance of earthworms was very similar between the two land uses —agricultural and semi-natural habitats ($t = 1.513$, $df = 28$, $p = 0.141$), although it was slightly higher in the farmlands (19.20 ± 1.522 ind. m^{-2}) than in the non-agricultural habitats (16.27 ± 1.201 ind. m^{-2}). The abundance of different earthworm species was significantly different between treatments ($\chi^2 = 62.534$, $df = 8$, $p < 0.0005$). Specifically, the density of *A. longa* in agricultural lands was about six times higher than in semi-natural habitats, and this difference was statistically significant (pairwise Mann-Whitney U, $p < 0.0005$). Moreover, its density was also significantly higher than that of all other species (pairwise Mann-Whitney U, $p < 0.0005$). No statistically significant differences were found between densities per species in semi-natural habitats (Fig. 2 and Table 2).

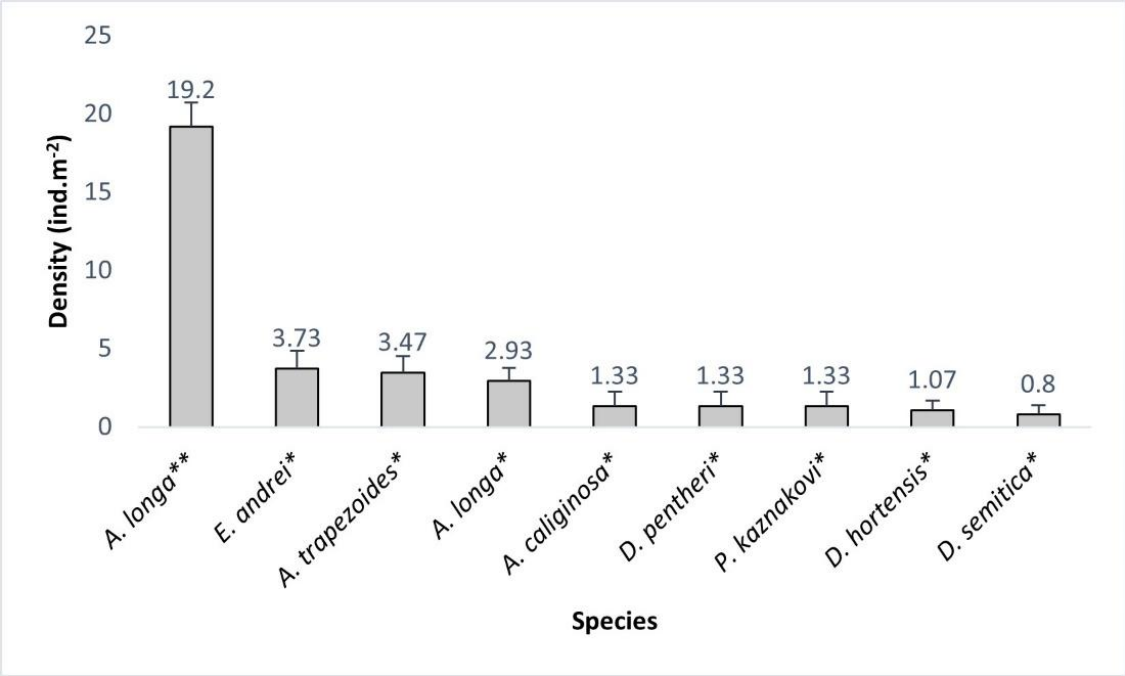

FIGURE. 2. Earthworm density per species in potato farmlands (**) and semi-natural (*) habitats. Data are presented as mean ± standard error (SE).

TABLE 2. Comparison of earthworm species densities among different stations.

Species	χ^2 (H)	df	p-value	η^2	Cohen's d	Effect Size Interpretation
A. longa	12.80	14	0.543	0.059	0.48	small-medium
A. caliginosa	18.00	14	0.210	0.084	0.42	small-medium
A. trapezoides	16.40	14	0.290	0.076	0.64	small-medium
D. hortensis	14.00	14	0.450	0.065	0.46	Small
D. pentheri	22.00	14	0.078	0.102	0.42	medium
D. semitica	20.00	14	0.130	0.093	0.38	small-medium
E. andrei	15.20	14	0.367	0.071	0.64	small-medium
P. kaznakovi	19.10	14	0.280	0.112	0.42	small-medium

In the semi-natural habitats, species composition (both the type and number of species, as well as the number of individuals of each species) varied among different stations. *A. longa*, *A. trapezoides*, and *E. andrei* occurred more frequently than other species at these atations (Fig. 3). The differences in the rate of species occurrence among stations were statistically significant ($\chi^2 = 91.551$, df = 7, $p < 0.0005$). These same three species also showed higher relative abundance in non-agricultural habitats compared to other species, although the pattern of abundance differed among stations (Fig. 4). The differences in relative abundance of species in non-agricultural habitats across stations were statistically significant ($\chi^2 = 30.535$, df = 7, $p < 0.0005$).

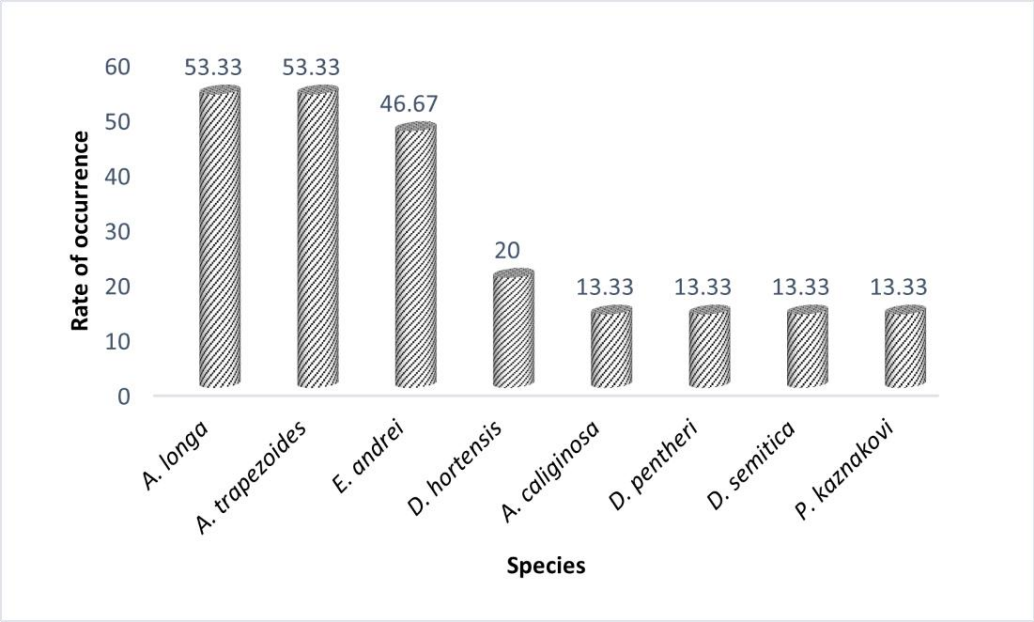

FIGURE 3. Rate of species occurrence in semi-natural stations.

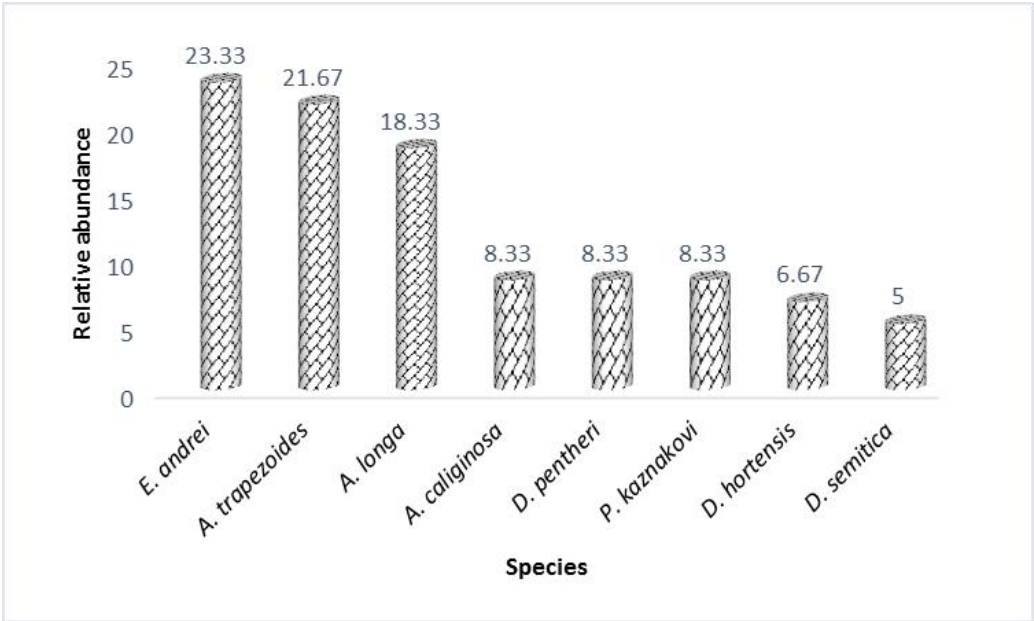

FIGURE 4. Relative abundance of species in semi-natural habitats.

The total biomass of earthworms in agricultural lands (82.15 ± 6.46 g. m^{-2}) was significantly higher than that of semi-natural habitats (28.16 ± 1.86 g. m^{-2}) ($t = 8.034$, $df = 16.294$, $p < 0.0005$). There was also a significant difference in relative biomass among the semi-natural habitats ($\chi^2 = 22.124$, $df = 7$, $p = 0.002$), with *A. longa* having the highest value, followed by *A. trapezoides* and *E. andrei*, respectively (Fig. 5). These three species differed significantly from each other and from other species. A significant difference ($t = 6.016$, $df = 14.504$, $P < 0.0005$) was also found in the mean fresh weight of *A. longa* between farmlands (4.28 ± 0.06 g) and non-agricultural habitats (3.43 ± 0.13 g).

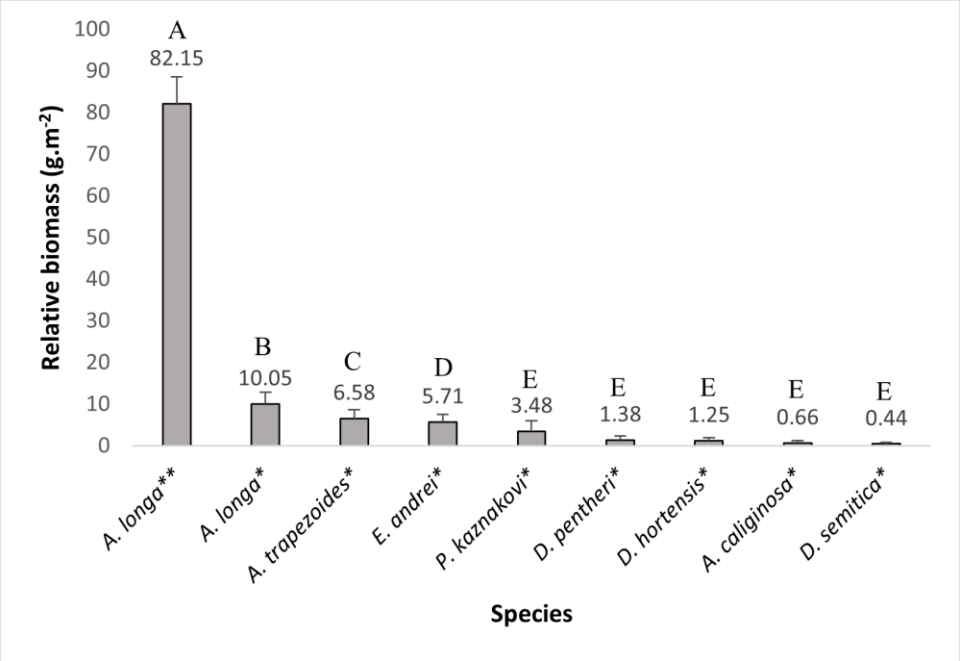

FIGURE 5. Species relative biomass in potato farmlands (**) and semi-natural (*) habitats. Data are presented as mean ± standard error (SE). Different letters above the bars indicate statistically significant difference at $p < 0.05$.

As shown in Table 3, biodiversity in potato fields is considerably lower than in semi-natural habitats, as indicated by Menhinick, Shannon-Wiener, and Simpson indices (0.24 vs 0.56, 0 vs 2.11, and 1 vs 0.48, respectively). Consequently, similarity between the two ecosystems is very low, as reflected by the Jaccard similarity index (0.11).

TABLE 3. Ecological indices for comparing biodiversity in semi-natural and agricultural habitats.

Ecological index	Mean ± SE		t/Z	df	p
	Potato fields	Semi-natural habitats			
Menhinick diversity index (D_{Mn})	0.24 ± 0.01	0.56 ± 0.04	-8.307	16.633	< 0.0005
Shannon-Wiener diversity index (H′)	0 ± 0	2.11 ± 0.34	-4.474	---	< 0.0005
Simpson diversity index (D)	1 ± 0	0.48 ± 0.06	-4.474	---	< 0.0005

DISCUSSION

In this study, four ecological categories of earthworms with a relatively even distribution were identified in non-agricultural environments: anecic (*A. longa*), endogeic (*P. kaznakovi*, *A. caliginosa*), epi-endogeic (*A. trapezoides*), and epigeic (*D. hortensis*, *D. pentheri*, *D. semitica*, *E. andrei*). In contrast, only the anecic species was found in the potato fields.

The single-species occurrence of *A. longa*, with higher density and biomass than in semi-natural habitats, observed in potato fields in this study is partly consistent with findings from other agricultural systems, where *A. longa* often dominates the anecic component of earthworm communities under intensive land use (Baker, 2002; Sutri et al., 2025). Several mechanisms may explain this pattern. As a deep-burrowing anecic species, *A. longa* can be partially buffered from short-term surface disturbances, including surface-applied agrochemicals and microclimatic extremes, reducing mortality relative to epigeic and endogeic species (Baker, 2002). Additionally, the species exhibits tolerance to various management regimes, including tillage and reduced organic-matter inputs, which often favor only a few resilient species in intensively managed soils (Sutri et al., 2025). The absence of other species in the

potato fields has likely contributed to the increased abundance and biomass of *A. longa*, possibly due to reduced interspecific competition (Eriksen-Hamel & Whalen, 2007; Eijsackers, 2011). Because pesticide residues were not measured and species-specific toxicity tests were not conducted in this study, the relative contributions of these mechanisms cannot be determined. Future studies integrating residue analysis, laboratory toxicity assays, and comparative sampling across different management practices are therefore recommended to clarify whether pesticide tolerance, habitat suitability, or specific management practices drive the single-species occurrence of *A. longa* in this region.

In this study, natural habitats sustained a more diverse earthworm community —both in terms of species richness and ecological groupings —than intensively cultivated soils, as reflected in the diversity indices ($H' = 2.11 \pm 0.34$ vs. 0; $D_{Mn} = 0.56 \pm 0.04$ vs. 0.24 ± 0.01; $D = 0.48 \pm 0.06$ vs. 1.00, respectively). These values indicate that natural habitats support higher species richness and more even community structure, whereas cultivated soils exhibit extremely low diversity and complete dominance by a single species, highlighting the strong simplifying effect of intensive agricultural practices on earthworm communities.

A similar negative effect of potato cultivation systems on earthworms was observed by Curry et al. (2002) in silty loam to silty clay loam in Ireland. In their study, earthworm populations were almost eliminated from these agricultural lands within two years: five of the nine species disappeared during the first year after potato harvest, and three more were lost in the second year, leaving only a single species remaining, *Murchieona minuscula* (Rosa, 1906). The density of the remaining species in the second year after potato harvest was, however, higher than in the first year.

Important differences exist between the two studies. First, while Curry et al. (2002) monitored earthworm responses over a two-year period following the introduction of intensive potato cultivation, the potato fields in the present study have been subjected to more than 20 years of continuous monoculture without rotation, representing a much longer and more persistent disturbance regime. Second, soil management intensity at the study sites is substantially higher: fields are ploughed three times annually using heavy machinery and receive repeated inputs of fertilizers, pesticides, fungicides, and herbicides, whereas the Irish fields experienced intensive tillage primarily during potato establishment and mechanical harvesting. Finally, the climatic context differs markedly: Hamedan Province has a semi-arid temperate climate with an average annual precipitation of ~325 mm, whereas the Irish site has a temperate oceanic climate with 650–750 mm rainfall. These contrasts in long-term land use, management intensity, and climate likely contribute to the even stronger and more persistent reduction in earthworm diversity and abundance observed in the studied fields.

Although Curry et al. (2002) reported a slight rebound in the density of the single remaining species during the second year after potato harvest, the sampling design used here does not allow detection of comparable short-term fluctuations. Because earthworm sampling in the present study was conducted only once, in May, transient seasonal changes—whether increases or declines—may have occurred outside this sampling window. This temporal limitation is therefore acknowledged, and multi-season or multi-year sampling would be necessary to determine whether similar short-term recovery patterns occur in long-term potato monoculture systems. Future studies should incorporate repeated sampling across different seasons to capture potential temporal dynamics more accurately.

Furthermore, Buckerfield and Wiseman (1997) cropped pasture paddocks once and returned them to pasture. They applied sulphate of potash, lime, NPK fertilizer (Nitrogen, Phosphorus, and Potassium), urea or ammonium nitrate, liquid nitrogen, fungicide, herbicide, insecticide, and trace elements S, Cu, Zn, and Mo to the cropping fields. In the paddocks cultivated with potatoes, the population of earthworms was halved compared to the pasture in the year immediately following potato cropping. However, in the second year, the earthworm community was similar in the cultivated and non-cultivated paddocks. According to the researchers, the increase in earthworm density two years after potato harvest may be attributed to fertilizer use (increasing soil organic matter) and lime application (raising soil pH).

Unlike the transient rebound observed by Buckerfield and Wiseman (1997) following short-term potato cultivation, earthworm populations in the long-term monoculture fields studied showed no signs of

recovery. This persistent decline is likely driven by repeated tillage, continuous application of agrochemicals, and prolonged habitat simplification. Analyses of station-level heterogeneity confirmed that this pattern was consistent across all fields, with no significant deviations, highlighting the long-term negative effects of intensive management on earthworm communities.

Physicochemical factors (e.g., soil temperature, moisture, pH, inorganic salts, aeration, and texture) and food availability (herbage, litter composition, dung, consolidated organic matter) are known to influence earthworm community composition (Shakir & Dindal, 1997; Edwards & Arancon, 2022). However, these variables were not measured in the present study, and thus direct inference of their effects on the observed patterns is not possible. Future studies should include measurements of soil pH, organic matter, total nitrogen, available phosphorus and potassium, electrical conductivity, texture, and bulk density or penetration resistance to better understand their influence on earthworm communities.

The loss of diversity in the potato farmlands could be related to the findings of Lagerlöf et al. (2002) and Singh et al. (2016). They reported that declines in epigeic species diversity in farmland result from physical soil disturbances during ploughing and intensive pesticide use, as these species do not enter diapause under adverse conditions, leading to higher mortality during warm and dry periods. These authors also stated that the diversity of endogeic species in agricultural fields is reduced by heavy soil cultivation, which destroys their burrows. In the potato fields studied here, heavy machinery is used for harvesting and soil preparation, which can lead to soil compaction. This may contribute to restrictions on earthworm burrowing activity (Marinissen, 1992; Capowiez et al., 2012).

Soil nutrients are known to influence earthworm diversity (Edwards & Arancon, 2022); however, nutrient levels were not measured in this study, and future work should include such measurements to better interpret community differences. In potato farmlands, herbicides and fungicides are applied to control weeds and fungi, which may limit the diversity of plant litter inputs. By contrast, semi-natural habitats receive organic material from a wider variety of plants, particularly grasses. The observed differences between earthworm populations in these habitats may therefore be related to the quantity and quality of available food, depending on litter composition (Shakir & Dindal, 1997), although this remains a hypothesis that requires direct measurement of litter inputs.

Steroidal glycoalkaloids, which serve as chemical protectants against plant pathogens and herbivores, are commonly found in the Solanaceae plant family. In *Solanum tuberosum*, the main glycoalkaloids are α-solanine and α-chaconine, present in all plant parts at varying concentrations, with the highest levels in sprouts, followed by flowers, leaves, berries, and tubers. These compounds are released into the soil during plant senescence and through the decomposition of residues and leftover tubers after harvest. Although glycoalkaloid concentrations were not measured in the present study, their presence could potentially influence soil biota, including earthworms. Reported topsoil concentrations in potato fields suggest that glycoalkaloids are largely bound to organic matter. While data on annelid-specific toxicity remain limited, future studies should investigate whether the observed levels approach toxicity thresholds for earthworms (Jensen et al., 2009a; Jensen et al., 2009b; Milner et al., 2011).

Both α-solanine and α-chaconine are toxic to a wide range of organisms, including mollusks, amphibians, and mammals, particularly embryos and juveniles (Milner et al., 2011; Akiyama et al., 2021). The potential effects of these toxins on earthworm populations in potato fields remain untested, but they may represent one of several factors influencing community composition, alongside tillage, soil compaction, pesticide use, and soil moisture conditions. Even deep-dwelling anecic species such as *A. longa*, which feed at the soil surface, may be exposed to these compounds.

Another factor influencing earthworm survival is soil moisture (Edwards & Arancon, 2022). Moreover, ploughing at the appropriate time of year is crucial, as early-spring ploughing negatively affects earthworm populations, while autumn ploughing has comparatively little impact (Chan, 2001). In the potato fields examined here, ploughing occurs during harvest, and in many cases the fields are ploughed twice in summer to prepare the land for the following season. Although soil moisture was not measured, management records indicate that irrigation ceases after harvest, which may accelerate topsoil drying. Such conditions are known to be detrimental to earthworms (Marinissen, 1992), potentially placing epigeic and endogeic species at risk when humidity becomes insufficient. By contrast, anecic

species such as *A. longa* can burrow down to 50 cm, enabling them to enter aestivation or quiescence during adverse periods (Lagerlöf et al., 2002; Jiménez & Decaëns, 2004; Singh et al., 2016).

Soil pH is a key determinant of earthworm community structure, as earthworms are highly sensitive to acidic conditions and are unable to persist below species-specific pH thresholds (Edwards & Arancon, 2022). Acidic soils—often associated with elevated concentrations of soluble metal ions—limit earthworm survival, feeding, and burrowing activity (Springett & Syers, 1984; Lagerlöf et al., 2002). Most species reach their highest abundances in near-neutral soils (Gerard & Hay, 1979; Nair et al., 2005; Stoscheck et al., 2012; Singh et al., 2016).

Within this general framework, species-specific tolerance thresholds become important for interpreting habitat-level patterns. The anecic species *A. longa* has an acid-tolerance threshold around pH 4.4–4.6, below which it is unable to burrow (Laverack, 1960; Baker & Whitby, 2003). Although *A. longa* generally prefers near-neutral soils (Bouché, 1972, as cited in Baker & Whitby, 2003), laboratory observations indicate relatively weak discrimination between soils of pH 4.5 and 7.0, with only slight avoidance of soils above pH 7.0 (Baker & Whitby, 2003). Its deep-burrowing lifestyle may further reduce exposure to acidity in surface layers compared to epigeic and endogeic species.

These physiological and ecological differences help contextualize the management practices in the potato fields examined in this study. Poultry manure and NPK fertilizers are commonly applied, together with fungicides, herbicides, and insecticides. Poultry manure—neutral to moderately alkaline (pH 6.9–8.0; Ravindran et al., 2017) and nutrient-rich (Burton et al., 2004)—is generally considered beneficial for earthworm populations (Ngosong et al., 2020). Nitrogen-rich amendments can enhance earthworm abundance, biomass, and cocoon production, and NPK fertilizers may increase biomass through elevated soil N and P availability (Shipitalo et al., 1988; Edwards & Arancon, 2022). However, these potentially positive nutrient-driven effects coexist with the well-documented acidifying influence of long-term inorganic nitrogen application, which can disadvantage epigeic and endogeic species (Haynes & Naidu, 1998; Timmerman et al., 2006; Ngosong et al., 2020). Accordingly, organic farming systems—where acidification pressures are lower—consistently show higher earthworm abundance, biomass, and diversity than conventional systems (Crittenden et al., 2014).

The earthworm patterns observed in the studied potato fields may therefore reflect the interplay of multiple, potentially opposing processes: nutrient enrichment from poultry manure and NPK, long-term soil acidification, species-specific pH tolerance, and functional-group differences in exposure pathways. While *A. longa* may be comparatively less affected by surface acidification and may benefit from nutrient inputs and deep organic matter placement, epigeic and endogeic species are likely to be more vulnerable. However, soil pH was not measured in this study; therefore, all pH-related interpretations should be considered provisional until field-specific measurements become available.

Chemical pesticides can disrupt enzyme activities, reduce fecundity and growth, alter behaviors such as feeding rate, and ultimately decrease the biomass and density of earthworm communities. Insecticides and fungicides, in particular, pose high toxicity risks by impairing survival and reproduction (Pelosi et al., 2013; Pelosi et al., 2014). Singh et al. (2016) reported high earthworm diversity in fields receiving low levels of inorganic pesticides and noted that burrowing earthworms are generally less directly affected by these chemicals. In the potato fields examined here, epigeic and endogeic species are likely more intensively exposed to pesticides due to their surface activity, whereas *A. longa*, as a deep-burrowing anecic species, may be less exposed, although surface feeding still brings them into contact with treated residues. Exposure pathways include contact with soil-active compounds and plant residues containing pesticides. According to our pesticide inventory (Table 1), the active ingredients commonly applied in these fields include carbaryl, chlorpyrifos, diazinon, imidacloprid, metam-sodium, and thiacloprid, which could differentially affect earthworm functional groups.

The findings of this study on the abundance and biomass of the large-bodied anecic species *A. longa* contradict previous reports on anecic earthworm populations in farmlands (e.g., Gerard & Hay, 1979; Edwards & Lofty, 1982; Capowiez et al., 2009; Ernst & Emmerling, 2009; De Oliveira et al., 2012; Crittenden et al., 2014; Briones & Schmidt, 2017). Wyss and Glasstetter (1992) reported that in

minimally tilled cornfields, *A. longa* abundance exceeded that of endogeic species, whereas in conventionally tilled fields, endogeic species were abundant. In contrast, in the potato fields examined here, both the abundance and biomass of *A. longa* were higher than in adjacent semi-natural habitats (mean biomass difference: 53.99 g. m^{-2}, 95% CI: 40.23-67.76; mean fresh weight difference: 0.85 g, CI: 0.55-1.16; Welch's t-test was used for comparisons due to unequal variances). This conflict may be explained by several hypothesized mechanisms, including (i) the contribution of poultry manure, (ii) the absence of competing species, and (iii) the increased availability of food resulting from the burial of organic matter into deeper soil layers during ploughing. These hypotheses require targeted testing, such as measuring soil OM, N, P, and pH; quantifying bulk density, compaction, and the vertical distribution of organic matter; conducting microcosm experiments manipulating manure inputs; and applying community-level analyses (e.g., PERMANOVA, NMDS) to relate species composition to measured soil properties. An additional possibility is that the collapse of earthworm diversity may have reduced interspecific competition, allowing *A. longa* to attain higher biomass and body size even without substantial increases in absolute resource availability.

CONCLUSIONS

The study revealed a significant reduction in earthworm diversity in long-term monoculture potato fields compared to semi-natural habitats. This decline is likely attributable to intensive agricultural practices, including deep ploughing with heavy machinery during periods of earthworm vulnerability, as well as the application of chemical fertilizers, herbicides, fungicides, and pesticides.

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