

UNIVERSITY OF LJUBLJANA

BIOTECHNICAL FACULTY

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**DISCOVERY, DIVERSITY, AND THE ORIGINS
OF TROGLOBIOTIC FAUNAS IN IRAN**

DOCTORAL DISSERTATION

Ljubljana, 2022

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**ODKRIVANJE, PESTROST IN IZVOR TROGLOBIOTSKIH
FAVN V IRANU**

DOKTORSKA DISERTACIJA

Ljubljana, 2022

Based on the Statute of the University of Ljubljana and the decision of the Biotechnical Faculty senate, as well as the decision of the Commission for Doctoral Studies of the University of Ljubljana adopted on October 22, 2018, it has been confirmed that the candidate meets the requirements for pursuing a PhD in the interdisciplinary doctoral programme in Biosciences, Scientific Field Biology. Assoc. Prof. Dr. Matjaž Kuntner is appointed as supervisor.

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 AB We investigated the troglobiont biodiversity of Iran, with a focus on Zagros karstic areas. This study followed three main goals. First, a discovery component involved faunistic investigations into the troglobiont organisms in Zagros Mountains of Iran, as well as taxonomic descriptions of new taxa. Three new troglobiont species were described: a carabid beetle, an asellid isopod and a truncatelloid gastropod. Additionally, we report on the discovery of six species under description (Oligochaeta, Decapoda, Isopoda) and over 10 species that are awaiting description. DNA extraction and PCR of selected markers from most of these taxa have been done, enabling species delimitation and phylogenetic analyses. We recorded new habitats for previously described Iranian cave fishes. Our second aim was to examine patterns of troglobiont biodiversity and endemism in the Zagros Mountains of Iran. We published a checklist of Iranian subterranean arthropods in 2017. In an additional chapter, we listed all Iranian troglobiont animals (previously published and our new discoveries, including yet undescribed species). The species richness and distribution patterns of Iranian troglobiont diversity were presented, including one currently recognized biodiversity hotspot. As the third goal, we aimed to analyze Iranian troglobiont animals phylogenetically to understand their relationships and to identify their origin and estimate a timeframe for their colonization into subterranean habitats. The new troglobiont species and some of sampled surface relatives were used for DNA isolation and molecular analysis. Related sequences were mined from GenBank. Given the poor knowledge of Iranian subterranean fauna, this speleobiological dissertation enriched this knowledge by reporting new findings and describing new species. The discovery component of this thesis enabled us to establish Iran as an important part of the world map of subterranean biodiversity. We now understand more about the origins of Iranian troglobionts and the time of their adaptation to subterranean environment. This fauna is likely to show high endemism and is important from the conservation perspective.

KLJUČNA DOKUMENTACIJSKA INFORMACIJA

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AI	<p>Raziskali smo biotsko raznovrstnost troglobiontov v Iranu s poudarkom na kraških območjih gorovja Zagros. Disertacija je sledila trem glavnim ciljem. Prvi cilj, komponenta odkrivanja, je vključevala favnistične raziskave troglobiotičnih organizmov v gorovju Zagros v Iranu ter taksonomske opise novih taksonov. Opisane so bile tri nove troglobiotične vrste: karabidni hrošč, asellidni izopod in truncatelloidni gastropod. Poleg tega poročamo o odkritju šestih vrst v postopku opisovanja (Oligochaeta, Decapoda, Isopoda) in več kot 10 vrst, ki čakajo na opis. Iz večine teh taksonov sta bili opravljeni ekstrakcija DNK in PCR izbranih markerjev, kar omogoča razmejitve vrst in filogenetske analize. Za prej opisane iranske jamske ribe smo zabeležili nove habitate. Naš drugi cilj je bil preučiti vzorce biotske raznovrstnosti in endemizma troglobiontov v gorovju Zagros v Iranu. Leta 2017 smo objavili vrstni seznam iranskih podzemeljskih členonožcev. V dodatnem poglavju smo navedli vse iranske troglobionte (prej objavljene in naša nova odkritja, vključno s še neopisanimi vrstami). Predstavili smo vrstno bogastvo in vzorce razširjenosti iranske troglobiotične pestrosti, vključno z eno trenutno priznano vročo točko biotske raznovrstnosti. Tretji cilj je bil filogenetska analiza iranskih troglobiontov, da bi razumeli njihove medsebojne odnose ter ugotovili njihov izvor in ocenili časovni okvir njihove kolonizacije podzemnih habitatov. Nove vrste troglobiontov in nekatere vzorčene površinske sorodnike smo uporabili za izolacijo DNK in molekularno analizo. Relevantna zaporedja DNK so bila dodatno pridobljena iz baze GenBank. Glede na slabo poznavanje iranske podzemeljske favne je ta biospeleološka disertacija obogatila to znanje s poročanjem o novih najdbah in opisom novih vrst. Del disertacije posvečen odkrivanju nam je omogočil uveljavitev Irana kot pomembnega dela svetovnega zemljevida podzemeljske biotske raznovrstnosti. Zdaj bolje razumemo izvor iranskih troglobiontov in čas njihovega prilagajanja podzemnemu okolju. Ta favna verjetno izkazuje visok endemizem in je pomembna z vidika ohranjanja narave.</p>

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Malek-Hosseini M. J., Jugovic J., Fatemi Y, Kuntner M, Kostanjšek R, Douady C. J., Malard F. 2022. A new obligate groundwater species of *Asellus* (Isopoda: Asellidae) from Iran. Subterranean Biology, 42: 97-124

Malek-Hosseini M. J., Zamani A. 2017. A checklist of subterranean arthropods of Iran. Subterranean Biology, 21: 19-46.

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ANNEX B: Permission from publisher for the publication of article Fatemi et al. (2019) in the printed and electronic versions of the doctoral dissertation

ANNEX C: Permission from publisher for the publication of article Malek-Hosseini et al. (2022) in the printed and electronic versions of the doctoral dissertation

ANNEX D: Permission from publisher for the publication of article Malek-Hosseini and Zamani (2017) in the printed and electronic versions of the doctoral dissertation

ABBREVIATIONS AND SYMBOLS

Mya	million years ago
COI	cytochrome c oxidase subunit I
PCR	polymerase chain reaction
H3	Histone H3
UTM	universal transverse mercator

1 INTRODUCTION

1.1 SUBTERRANEAN ENVIRONMENT

1.1.1 A brief history of Speleology and Speleobiology

Subterranean environments, mostly caves, have been fascinating subjects for many scientists in several fields such as biology, geology, and archaeology. Speleology is a cross-disciplinary field that combines the knowledge of chemistry, biology, geology, physics, meteorology, and cartography to develop portraits of caves as complex, evolving systems. Speleology has a recent origin as a field of science. Cave study traces back to 1680s when John Beaumont studied caves in the Mendip Hills of England and presented his conclusions to the Royal Society in 1676. During that decade, Janez Vajkard Valvasor from Slovenia, wrote about caves and karst hidrology, also presenting to the Royal Society. Although, several sporadic speleological studies had been carried out by 1900, Édouard-Alfred Martel (1859–1938), the 'father of modern speleology', who through his extensive and well-publicised cave explorations introduced in France the concept of speleology as a distinct area of study. In 1895 Martel founded the Société de Spéléologie, the first organization devoted to cave science in the world. The French entomologist René Jeannel and the Romanian biologist Emil Gheorghe Racoviță studied more than 1400 caves in southern Europe and North Africa and published descriptions of the caves and their fauna. Emil Racoviță" Institute of Speleology, the first one in the world dealing with cave research, was founded by Racoviță in Cluj, Romania. The first obligate cave-dwelling animal to be studied scientifically and described was the cave salamander *Proteus anguinus* Laurenti, 1768 from Slovenian caves. However, first time, it was described from springs and wrongly as living in Lake Cerknica. The first troglobiont invertebrate was the cave beetle *Leptodirus hochenwarti* Schmidt, 1832 from Slovenia (Camacho, 1992; White and Culver, 2012).

During recent 100 years, lots of studies have been carried out on subterranean biology in different parts of the world and many books and articles have been published. Also, several journals are specially focused on publications of different disciplines of speleobiology.

1.1.2 Characteristics of the subterranean environment

Subterranean environment represents an interesting and unique ecosystem. Several kinds of subterranean environments exist that harbor troglobiont animals such as caves, karstic

springs, wells, interstitial waters, and lava tubes. Regarding the biodiversity and evolutionary points of view, caves are the most important subterranean environment. Several kinds of caves exist but our main interest in this study is natural solution caves in limestone. A series of general features (such as stability, predictability, being simple and isolated, nutrient poor) have been emphasized as the main characteristics of the subterranean environments (Camacho, 1992; Culver and Pipan, 2009) which are both terrestrial and aquatic.

Light is the origin of most life, and primary production is the source of biodiversity; nevertheless, life also exists in darkness. Based on the amount of light and the interaction of the subterranean environments with the surface, three main zones are considered for caves: a) entrance (where the light reaches), b) twilight zone (transition zone), c) dark zone (deep zone or total darkness) (Vandel, 1965; Camacho, 1992). The most obvious characteristic of caves (except at the entrance zone) is darkness. Subterranean environments are characterized not only by continuous darkness but also by a reduced variability in the number of specific abiotic conditions such as moisture, temperature, and water chemistry, in some cases low oxygen, as well as by isolation and restriction in space. Additionally, hypogean systems are relatively energy-limited (restricted food supply) compared to photosynthetically based epigean systems (Vandel, 1965; White and Culver, 2012).

1.1.3 Energy resources in caves

Due to the lack of sunlight, there is no photosynthesis in subterranean environments. So, the green plants are not the primary producer. The utilization of the energy of chemical bonds and chemoautotrophy by bacteria are the basic source of energy. Some truly chemoautotrophic-based cave ecosystems such as Frasassi in Italy and Movile in Romania are the two best studied examples to date. Fatemi et al. (2019) mentioned Tashan cave in Iran likely represents a chemoautotrophically based cave ecosystem. This cave is a subterranean habitat with sulfide-rich waters. The sulfide caves host interesting ecosystems with unusually rich and diverse invertebrate communities (Brad et al., 2021) such as crustaceans (Peterson et al., 2013; Por, 2014; Brad et al., 2015), insects (Tobler et al., 2013) gastropods (Falniowski and Sarbu, 2015; Fatemi et al., 2019) and occasionally fishes (Reisch et al., 2010; Roach et al., 2011; Mousavi-Sabet et al., 2016). External energy sources enter subterranean habitats in a variety of ways. Flowing water, especially streams entering caves, carries with it not only dissolved organic material, but also particulate organic material. Percolating water provides input of dissolved organic matter. Wind and gravity bring nutrients into caves when organic material comes into an entrance. Examples include falling leaves as well as the body of dead animals. Guano is one of the most important food sources in many caves. (Culver and Pipan, 2009; Fatemi et al., 2019). Culver et al. (2003) hypothesized a connection between surface productivity and the amount of food available in

caves: in nearly all caves, there is no significant chemoautotrophy so that all available food results from the surface productivity.

1.1.4 Cave animals

Subterranean organisms can be classified into three main groups, according to their affinity to life in this habitat (ecologically, in relation to their association with subterranean/cave environments):

a) **Troglobiont:** any of the organisms found in caves (subterranean habitats) that display the typical, convergent phenotypes (morphological, physiological, and behavioral) such as loss of eyes and pigmentation, and elongation of appendages. However, the degree of troglomorphy could be different in different troglobiont animals. Sket (2008) mentioned that we have to accept that the degree of troglomorphy does not necessarily correspond to the strength of the “troglobiosis”. Troglobionts are obligatory cave species (troglos: cave and bios: life). The term stygobiont is used for the aquatic obligate troglobionts (Sket, 2008; Culver and Pipan, 2009).

b) **Troglophile:** an organism that can complete its life cycle in caves but may also do so outside of caves. This group could be essentially epigean species able to maintain a permanent subterranean population (which may become troglobiont). Sket, 2008 considered two unnecessary categories for troglophiles: a) eutroglophile is an essentially epigean species, but able to maintain a permanent subterranean population; b) subtroglophile is inclined perpetually or temporarily to inhabit a subterranean habitat but is bound to the surface for some biological functions (e.g., feeding) (Sket, 2008; Romero, 2009).

c) **Trogloxene:** species only occurring sporadically in a hypogean habitat and unable to establish a subterranean population. An organism that habitually enters caves but must return periodically to the outside for certain of its living requirements, usually food. Bats and cave birds are good examples. This term was introduced by Racovitza (1907) to replace accidental or ‘occasional guest’ (Camacho, 1992; Culver and White, 2005; Sket, 2008; Culver and Pipan, 2009; Romero, 2009).

1.1.5 Troglomorphic traits

These adaptations are the response of animal to survive in the lightless, food-poor cave environment. There are some regressive and convergent characters such as varying degrees of eye loss and depigmentation. Some predictive adaptations are increase in tactile and chemical sensitivities (elongation of appendages and increase in numbers of sensory hairs, setae and other receptors), and increase in metabolic economy (reduction of the routine metabolic rate, aggressive and territory behavior and fright reactions). Some adaptations in terrestrial troglobionts are to the moisture. Development of paedomorphic forms is another predictive adaptation in obligate cave-dwellings. There are some adaptations regarding the life cycles such as longer duration of phases of life cycle. They show some developments of certain reproductive strategies such as low fecundity, increase in egg size and decrease in the number of eggs (Camacho, 1992; Christiansen, 1992).

Many abovementioned adaptations could be toward saving energy. The ability of saving more fat in body has been improved in these animals. They have an increase in food-finding ability. Although an increase in food-finding ability in cave animals often seems to go along with an increase in food-searching activity, changed motion patterns result in a reduction of energy expenditure, sometimes to exaggerated extension. For example, in the most cave-adapted species of the amblyopsid fish in North America, over 90% of the total energy savings by adaptations are based on the reduced activity (Poulson, 1985; Camacho, 1992; Culver and white, 2005; Culver and Pipan, 2009).

1.1.6 Global biodiversity of troglobiotic animals

Although relatively poorly studied in comparison with the epigeal ecosystems, subterranean ecosystems show a rather high biodiversity at the global scale. However, subterranean biodiversity is much lower than epigeal biodiversity, but it is not as poor as it seems to be. Extensive biological studies have been carried out in the main karst areas around the world, namely in the eastern United States of America and in the region from Pyrenees to Slovenia and the Dinaric Karst (Culver et al., 2000; Culver and Pipan, 2009). Globally, over 7000 aquatic subterranean dwellers have been catalogued (Botosaneanu, 1986), but unfortunately, such data are not available for the terrestrial subterranean fauna. It is likely that subterranean habitats harbor even larger numbers of terrestrial species, e.g., Coleoptera alone includes more than 1927 underground-limited species (Juberthie, 1998; Gibert and Deharveng, 2002). Culver and Holsinger (1992) proposed a potential biodiversity of about 50,000 to 100,000 obligate terrestrial subterranean species on the basis of nested regional estimates. Sket et al. (2004) reported more than 975 terrestrial and 650 aquatic obligate subterranean species for the Balkan Peninsula. More than 1,138 obligate cave-dwelling

species and subspecies from 112 families and 239 genera have been described in the United States (Hobbs, 2012). The subterranean aquatic fauna in most karst areas in Europe has been fairly well studied, with around 1800 stygobiont species known to date (Stoch and Galassi, 2010).

Two groups of Vertebrata have troglomorphic representatives: salamanders (Amphibia Gray, 1825; Caudata Scopoli, 1777; Urodela Duméril, 1806) (13 species) and fishes (Actinopterygii Klein, 1885) (more than 280 species) (White and Culver, 2012).

There are troglomorphic forms of many groups of invertebrates such as Porifera, different groups of worms such as Platyhelminthes (flatworms), Nematoda (roundworms), Annelida (segmented worms), Mollusca (mollusks) especially Gastropoda, many groups of Arthropoda such as Crustacea, Arachnida, Myriapoda (millipedes and centipedes) and Insecta (insects) (Camacho, 1992; Romero, 2009).

1.1.7 Hotspots of troglomorphic biodiversity

The syntagm “biodiversity hotspot”, one of the most important global tools for nature conservation, has been used in different ways, but with the same goal: identifying areas around the world that have high biological diversity (Myers, 1988; Reid, 1998; Hughes et al. 2002; Brooks et al., 2006). Biodiversity hotspots were defined by Myers et al. (2000) as the richest places in terms of biodiversity, with high numbers of species found nowhere else and that have already lost 70% of their original vegetation. Hotspots were most commonly referred to areas with high biodiversity that also are with high productivity (Briscoe et al., 2016). Culver and Sket (2000) used the term hotspots of subterranean biodiversity to define subterranean habitats with an arbitrary cutoff of twenty or more obligate subterranean species. Understanding patterns of subterranean biodiversity requires an understanding of regional patterns. In general, the number of species found in any one cave or subsurface site is small relative to the number of species in the region. Culver and Pipan (2009) have set the value to 25 stygobionts or 25 troglomorphic species per site. Whether an area is a hotspot or not is thus arbitrarily defined, but can have value within a comparative framework. A particularly vexing problem in the analysis of species numbers in subterranean sites is the nearly universal problem of undescribed species. The difficulty is that not all records reported as new species turn out to be new species (Culver et al., 2012, 2021).

Culver and Sket 2000, published a paper listing 20 caves and karst wells with 20 or more known species. In 2021, there was a Special Issue of the journal of Diversity: “Hotspots of Subterranean Biodiversity” expanded and enriched previous hotspot lists (Gibert and Deharveng, 2002; Culver and Pipan, 2009, 2013; Deharveng and Bedos, 2019). These hotspots may not capture all regions of high subterranean species richness; all these hotspot caves are worthy of study and protection in their own right, and any protection strategy should include these exceptional sites as well as regional areas of species richness (Culver et al., 2021). It is worthy reiterating that, compared to surface habitats, the underground diversity is low (Sket, 1999a) and that most diversity in subterranean habitats is expressed regionally rather than locally. The fragmented nature of the cave habitat and restricted opportunities for dispersal keep local diversity much lower than regional diversity (Culver and Sket, 2000). To improve our knowledge of the diversity of a region, it is essential to have large inventories of well-identified species and georeferenced localities for them in order to conduct standardized geospatial analyses that can be compared among regions and at different spatial scales (Culver and Pipan, 2013; Zagnajster et al., 2018). In a study by Trajano et al., 2016, they determined some spots of high diversity of troglobionts in Brazil. They described five Brazilian areas and a cave with their unique troglobiotic faunas, which were considered as spots of high diversity of troglobionts based on the following criteria: (1) high number of troglobionts, when compared to other studied cave areas, (2) presence of higher taxa (families, order) so far not recorded in subterranean habitats elsewhere in Brazil, (3) presence of highly troglomorphic taxa, and (4) presence of phylogenetic relicts (taxa which have no close living relatives, and distributional relicts, whose relatives survived somewhere else sensu Holsinger, 1988) (Trajano et al., 2016). Their criteria seem to be logical, especially for areas that have been poorly studied in regards of troglobiotic faunas. These criteria with modifications and rationalization, considering the alpha and beta species diversity, phylogenetic and functional diversity and habitat diversity in Iran, could be considered for Iran, as well as the entire Middle-East.

Prior to this study, the cave with the highest diversity in Iran was Loven cave in Zagros that harbors three species of Iranian cave fishes (Vatandoust et al., 2019). There has been no hotspot of troglobiotic biodiversity or spots of high diversity of troglobionts detected in Iran. Based on some studies on Iranian caves conducted on some regions the presence of some hotspots is expected. However, some parts of Iran are completely arid and as deserts do not support any subterranean hotspots.

1.1.8 Origin of troglomorphs

From evolutionary and biogeographic points of view and for the study of speciation and adaptation, subterranean environment and its fauna is a natural laboratory. Reduced aboveground dispersal of cave organisms produces phylogenetic patterns of area distribution that largely match the geological history of mountain ranges and cave habitats. Most current hypotheses assume that subterranean lineages arose recently from surface dwelling, dispersive close relatives, but for terrestrial organisms there is scant phylogenetic evidence to support this view. Understanding the origin of subterranean fauna and adaptations have been an interesting topic of numerous evolutionary studies (Culver, 1982; Culver and Pipan, 2009; Ribera et al., 2010).

There are two general hypotheses regarding the origin of the subterranean fauna: the climatic relict and the habitat shift hypotheses. The climatic relict hypothesis (Peck and Foinston, 1993) claims that during special climatic conditions, the populations of epigean species are forced into a subterranean environment, so it acts as a refuge for them. These populations become isolated from their surface relatives, and then develop the morphological and physiological adaptations to this new habitat (Peck and Finston, 1993). The adaptive shift hypothesis (Howart, 1980) posits that the opportunity to exploit new resources leads to the colonization of the subterranean environment by epigean populations. These populations are not forced below ground by changing conditions, and there may be limited gene flow between the two environments for some time (Howarth 1980; 1987). Both scenarios assume that subterranean lineages have originated from closely related epigean relatives, with recurrent colonization of the subterranean medium (Ribera et al., 2010).

1.2 SPECIFICATION OF THE RESEARCH PROBLEM

Comprising a land area of 1,648,195 km², Iran is the second-largest country in the Middle East and the 17th-largest in the world. Iran is geologically a part of the Alpine-Himalayan orogenic belt. It lies between latitudes 24° and 40° N, and longitudes 44° and 64° E. Five major structural zones, different in structural history and tectonic style, can be distinguished in Iran (Stöcklin, 1968): (a) The Zagros Range, (b) The Sanandaj-Sirjan Range, (c) Central Iran, (d) East and South-East Iran, and (e) The Alborz and Kopet-Dagh Ranges. The Zagros and Alborz Mountains are the two main mountain chains in western and northern Iran, which

comprise nearly a third of the Iranian land area (Figure 1) (Raeisi et al., 2012; Malek-Hosseini and Zamani, 2017).

More than 10% of the earth's surface is composed of karst. Karstic carbonate formations cover about 11 % of Iran's land area (Figure 2). The total area of the karstified carbonate rocks in Iran is about 185.000 km², with 55,2 % in the Zagros, 24,3 % in Central Iran, 15,2 % in Alborz, 4,7 % in East and South Iran, and less than 0,5 % in the Sanandaj-Sirjan Range (Raeisi, 2004; Raeisi et al., 2012). Most of the outcrops of carbonate rocks are of the Cretaceous and Tertiary Age. Alavi (2004) recently revised the Zagros stratigraphic column to consist of four age-groups of rocks from Cambrian to Post-Miocene. The age of some main karstic formations in Iran is listed in Table 1. Orogeny and then speleogenesis occurred after the mentioned times in Table 1 and referred to Paleocene- Eocene- Oligocene and after that (Cucchi and Zini, 2003; Popov et al., 2004; Agard et al., 2011; Raeisi et al., 2012). As Iran has arid areas, deserts, low rainfall in many parts of the country, the importance of karstic areas as a resource for groundwater is significant. Groundwater represents about 30% of global freshwater resources (Shiklomanov, 2000). This part of the aquatic ecosystems hosts numerous species that cannot be found in surface freshwaters (Culver et al., 2009; Trontelj et al., 2009; Esmaili-Rineh et al., 2018).

Most of the cave studies in Iran concentrate merely on visiting, photography, and/or mapping the explored caves. Sometimes the complete information about a cave has not been published or it cannot easily be found. The speleological committee of Iran has started to collect cave data, but this has not yet been published. Marefat (1994) reported 258 caves, but the report did not cover all the caves in Iran, and had only partial information about location, altitude, survey, and length of some of the caves; neither geological settings nor hydrologic features were reported. There are many highland karst aquifers without any cave systems yet known, while big springs emerge at their bases of erosion. Several levels of caves are expected in the karst mountains of Iran, as a result of the rapid rates of uplifting and local valley incision. There are two main reasons for the small number of known caves. First, most of the karst areas are high mountains with steep slopes, so that many cave entrances have been filled in by talus or transported sediments or have been blocked by entrance breakdown in the high-risk earthquake regions of Iran. Second, many springs with high discharges are of the vaclusian type, with no explorable dry cave systems. Conduit systems of these springs, which have developed at higher levels in the past, cannot presently be seen on the surface. The deepest cave in Iran is Ghar Parau, 751 m deep (Raeisi and Kowsar, 1997; Gunn, 2003). Also, some other deep caves have been discovered in Iran.

Vast amount of faunistic data from Iran has been collected during the last decade but its subterranean fauna remains inadequately known and most reported species from the caves

are in fact troglloxenes (Malek-Hosseini and Zamani, 2017; Tahami et al., 2017; Esmaeili-Rineh, 2018; Fatemi et al., 2019). Nevertheless, 46 obligate subterranean organisms have been reported from Iran, of which as many as 31 have been described since 2013. 43 species of these troglobionts are restricted to Iran (Löffler, 1959; 1961; Pesce, 1979, 1980 1981; Pesce and Maggi, 1982; Malek-Hosseini and Zamani, 2017; Mamaghani-Shishvan and Esmaeili-Rineh, 2019; Bargrizaneh et al., 2021). The only known troglobiont vertebrates are four fish species. Troglobiont invertebrates include a spider, a diplopod, a beetle and 38 crustaceans. More than 20 troglobionts are waiting to be reported or described. The most species-rich group in subterranean environment is Amphipoda with the genus *Niphargus* Schiødte, 1849. All Iranian troglobionts were described from the central and northern parts of Zagros and northern slopes of Alborz (Mousavi-Sabet et al., 2016; Malek-Hosseini and Zamani, 2017). This small knowledge of Iranian caves fauna is due to a lack of comprehensive studies of all taxa. So, according to Malek-Hosseini and Zamani (2017), the seemingly low species richness of Iranian troglofauna is not realistic. The number of described caves of Iran is more than 2000 (Raeisi et al., 2012), with many more to be added. There are potential subterranean hotspots in the karst regions of western Caucasus in Georgia and possibly the Tien Shan Mountains in Kyrgyzstan. Although biologically poorly-studied, based on their positive relationship to the productivity maps and the presence of major cave regions they seem to harbor potential subterranean hotspots (Gvozdetski et al., 1994, Klimchouk 2004a, b). These regions are the closest to Iran which somehow have been studied in term of caves fauna. There are a lot of similarities between them and the north and western parts of Iran. Based on the studies conducted on these regions as well as some sporadic studies on Iranian caves in the mentioned areas, we could consider that there are some hotspots there (Malek-Hosseini and Zamani, 2017). From east of Kyrgyzstan to the eastern coast of the Asian continent, the climate is probably too dry to support any subterranean hotspots (Culver et al., 2006).

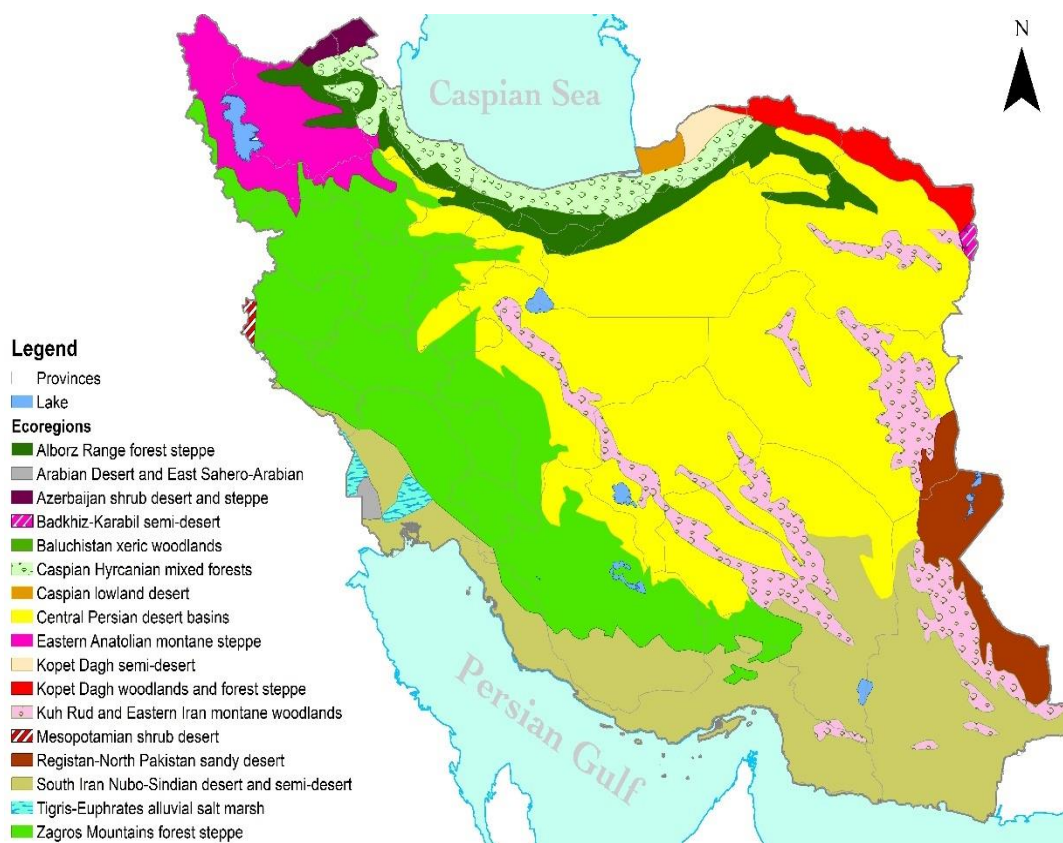


Figure 1: Map of terrestrial ecoregions in Iran (Map derived from WWF (Olson et al. 2001) and Safaei-Mahroo et al. (2015).

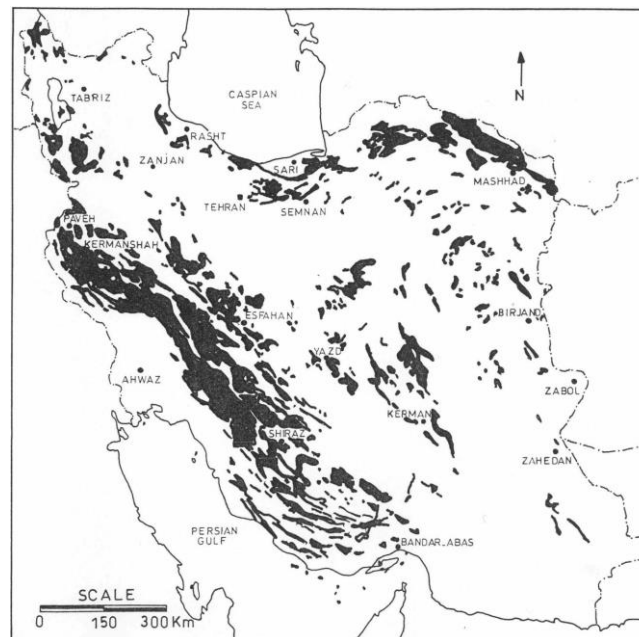


Figure 2: Carbonate location map of Iran (according to Raeisi and Kowsar 1997).

Table 1: Age of some karst formations in Iran (Raeisi et al., 2012)

Zone	Formation	Age
Zagros	Surmeh	Early to Late Jurassic (20.3-145.4 Mya)
	Fahliyan	Lower Cretaceous (145-100.5 Mya)
	Bistoon	Upper Triassic / Upper Cretaceous (237-66.72 Mya)
	Sarvak	Upper Cretaceous (100.5-66.72 Mya)
	Ilam	Upper Cretaceous (100.5-66.72 Mya)
	Jahrum	Tertiary (65-2.58 Mya)
	Asmari	Tertiary (65-2.58 Mya)
	Guri	Tertiary (65-2.58 Mya)
Central Iran	Jamal	Paleozoic (541-251.9 Mya)
	Orbitolina	Cretaceous (145-66 Mya)
Alborz	Lar	Upper Jurassic (163.5-145.4 Mya)
	Tizkuh	Lower Cretaceous (145-100.5 Mya)
Kopet Dag	Mozduran	Upper Jurassic (163.5-145.4 Mya)
	Tirgan	Lower Cretaceous (145-100.5 Mya)

The origin of Iranian troglobionts is mostly unknown. However, some taxonomical studies on Iranian cave fishes and the genus *Niphargus* from amphipods just mentioned some time period for their divergence, but they did not focus on their origin and time of the colonisation of subterranean environments by these troglobiont animals (Esmacili-Rineh et al., 2015b; Hashemzadeh Segherloo et al., 2018). Speleobiological studies are required to address many questions. What was the time of cave colonization by these organisms? While such questions have been addressed in most of other known karst biodiversity hotspots such as Balkan Peninsula, such knowledge is currently not available in the Middle-East, the Caucasus and in Central Asia. In order to address this problem, the first step is to survey these troglofaunas. Although faunistic studies in Iran have produced preliminary data, these are largely unavailable for numerous neighboring countries, e.g., Iraq, Afghanistan, Tajikistan, and Armenia. This means that there is a gap of knowledge that extends from the Dinaric karst in the west, through Turkey into Iran, and further east to oriental faunas. Most diverse groups

of troglobionts in subterranean environments of Middle East are aquatic crustaceans and fish. Some scattered research has been done on biogeography, phylogeography and molecular phylogeny of Middle Eastern subterranean organisms (Bargrizaneh et al., 2021; Kirchner et al., 2021).

Iranian subterranean biology must be included into the story. The question is what is the origin of this fauna? Is this the same for all parts of Iranian subterranean environments or different for each structural zone? Speleobiological studies should address this problem for Zagros karst. With taking a look at lithological-paleogeographic maps of Paratethys, we will face with these questions: where did the Iranian troglofauna originate from? From which successors of Tethys or Paratethys?

1.3 REVIEW OF LITERATURE

At present, information on the taxonomic composition of the Iranian troglobiotic fauna is far from complete. The discovery goes slowly but it has shown that the numbers of discovered organisms is increasing. Most of studies have been faunistic. Some sporadic studies have been carried out on the phylogeny of these troglobiont animals.

First study on Iranian troglofauna was done on Iranian cave fish. Bruun and Kaiser (1944) described a cave fish from a subterranean habitat (Loven cave) accidentally in the place where the railway was on constructions. This species had been collected in 1937. They named the new genus and species as *Iranocypris typhlops* (Bruun & Kaiser, 1944). Recent studies showed that this species belongs to the genus *Garra* (Farashi et al., 2014). Results of a study by Mousavi-Sabet and Eagderi, 2016 showed that *Garra* fishes in Loven cave belong to two different species: *Garra typhlops* (Bruun & Kaiser, 1944) and they also described *Garra lorestanensis* Mousavi-Sabet & Eagderi, 2016 (Greenwood, 1976).

In 1950, Anthony Smith, who led a group of Oxford University students, visited Iran in search of a reputedly blind white cave fish (Smith, 1953). Their quest failed. Twenty-six years later, Anthony Smith returned to Iran and, using information supplied by Bruun and Kaiser in their paper, visited (not without considerable difficulty) the type locality of *Garra typhlops*. There, exactly as described by Bruun and Kaiser, he found a small group of fishes swimming in the well-like outlet of a subterranean water-body (Greenwood, 1976). This species was described by Greenwood, 1976 as *Noemacheilus smithi*. When Nalbant and

Bianco (1998) reviewed the loaches of Iran, they transferred *N. smithi* to *Paracobitis* without giving reasons. Hashemzadeh Segherloo et al., 2016 studied this species molecularly as well. They named *Eidinemacheilus*, as a new generic name for *Noemacheilus smithi* Greenwood, 1976. Another species of this genus is living in Iraqi Kurdistan: *Eidinemacheilus proudlovei* Freyhof, Abdullah, Ararat, Ibrahim & Geiger, 2016 (Freyhof et al., 2016).

Mahjoorazad and Coad (2009) and Vatandoust et al., 2019 reported *G. typhlops* and *G. lorestanensis* from two different localities that first one is 130 km far from Loven cave and the second one is 30 km to the south of Loven cave. These reports may reveal the potential presence of a large freshwater aquifer in the Zagros Mountains.

Mousavi-Sabet et al., 2016 described *Garra tashanensis* Mousavi-Sabet, Vatandoust, Fatemi & Eagderi, 2016 from Tashan Cave in southeastern part of Iran in Zagros Mountains.

Karaman (1998), Esmaili-Rineh and Sari (2010, 2013), Esmaili-Rineh et al. 2015a, 2016, 2017, Mamaghani-Shishvan and Esmaili-Rineh, 2019, Zamanpoore et al., 2019, Bargrizaneh et al., 2021 published several new species of the genus *Niphargus* in Iran. They have done some molecular analysis on genus *Niphargus* of Middle-East. According to Esmaili-Rineh et al., 2015 b, the origin of one of the identified clades from Iran and Lebanon corresponds to marine transgression between the Black Sea and Mediterranean approximately 12 Mya.

Three species of troglobiont Isopoda have been discovered from Zagros karstic areas in Iran. *Microcharon raffaellae* Pesce, 1979 was reported from a well in Shahr-e Kord city (Pesce, 1979). Gakal cave in south-western Iran in Zagros harbors *Protracheoniscus gakalicus* Kashani, Malek-Hosseini & Sadeghi, 2013, a member of Oniscidea that shows high level of troglomorphy (Kashani et al., 2013). *Stenasellus tashanicus* Khalaji-Pirbalouty, Fatemi, Malek-Hosseini & Kuntner, 2018 from Tashan cave was the first representative of Stenasellids from the country (Khalaji-Pirbalouty et al., 2018).

Trilacuna qarzi Malek-Hosseini & Grismado, 2015 as the first troglobiont spider from Iran, was discovered from Gakal Cave (Malek Hosseini et al., 2015a, b).

Chiraziulus troglopersicus Reboleira, Malek-Hosseini, Sadeghi & Enghoff, 2015 as the first troglobiont species of Diplopoda from Iran was reported from Neyneh cave in south-western part of the country in Zagros (Reboleira et al., 2015).

About 12 species of Copepoda have been reported from subterranean waters of different parts of Iran as troglobionts during 1937-1981 by several authors. Unfortunately, the precise localities for these species have not been determined in literature (Löffler, 1959; 1961; Pesce, 1980; 1981; Pesce and Maggi, 1982).

Erpobdella borisi Cichocka, Bielecki, Kur, Piłkuła, Kilikowska & Biernacka, 2015 from Erpobdellidae family of Hirudinida was discovered from Sahoolan Cave in north-western Iran as the first troglobiont Hirudinea from the country (Cichocka et al., 2015).

Foranotum perforatum Nabozhenko and Sadeghi, 2017 (Coleoptera: Tenebrionidae) as the first troglobiont beetle from Iran was reported from a cave in Southern Zagros Mountains (Nabozhenko and Sadeghi, 2017).

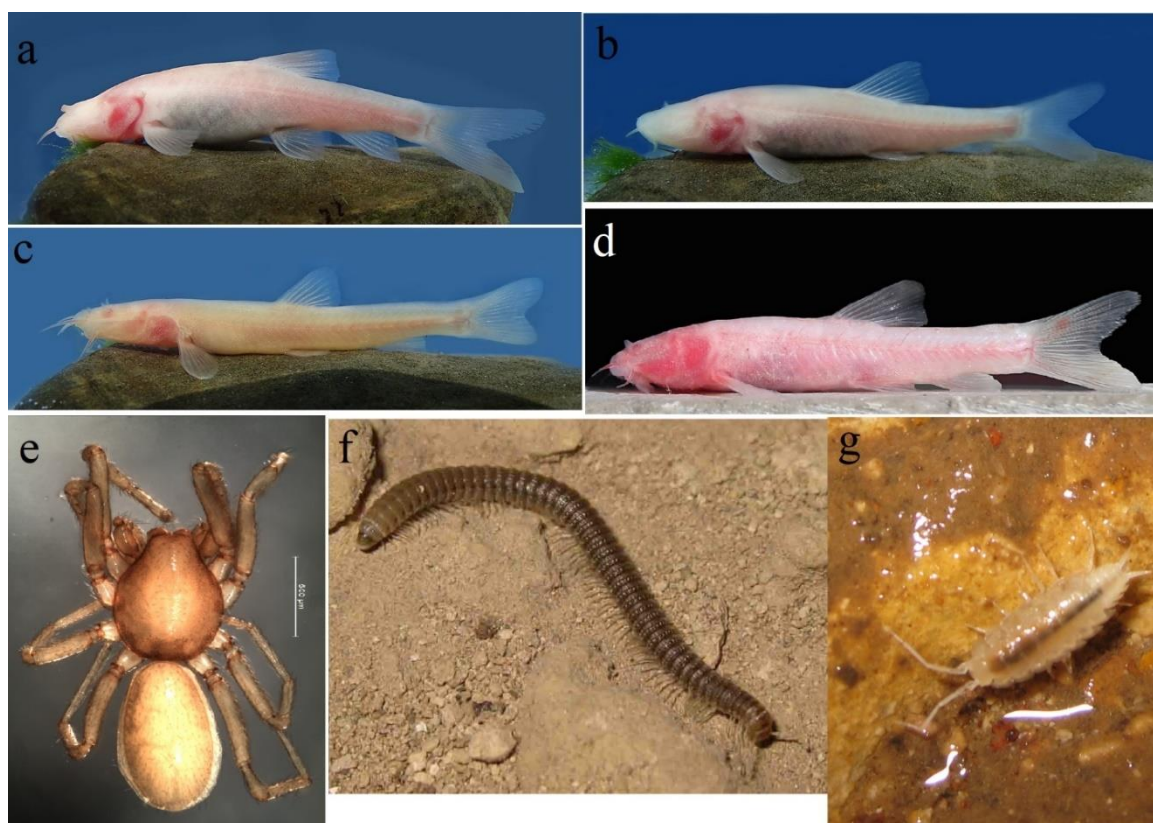


Figure 3: Some of Iranian obligate subterranean dwelling animals: a: *Garra lorestanensis*; b: *Garra typhlops*; c: *Eidinemacheilus smithi*; d: *Garra tashanensis*; e: *Trilacuna qarzi*; f: *Chiraziulus troglopersicus*; g: *Protrachaeoniscus gakalicus* (photos: Kashani et al., 2013; Malek-Hosseini et al., 2015a; Reboleira et al., 2015; Esmaeili et al., 2016; Mousavi-Sabet et al., 2016)

1.4 CONSERVATION

The identification and protection of priority areas are common goals in managing and conserving biodiversity (Margules et al., 2002). However, our knowledge of subterranean biodiversity is inconsistent and often deficient in many areas. With few exceptions, cave ecosystems and habitats are poorly sampled when compared with surface ecosystems. Caves have an important role in the cycle of nature. Caves are valuable in many perspectives such as ecological (environmental), scientific, cultural heritage, tourism and economic (Romero, 2009). Considering the fact that the life cycles of animals in subterranean environments and especially caves depend on all elements of these environments from abiotic and inanimate parts such as the habitat itself and to all types of cave animals (troglobiont, troglophile, troglaxene), and regarding the vulnerability and high sensitivity of these habitats, they must be protected by applying especial management principles. Conserving an ecosystem implies the protection of habitats and species. Causes of deterioration in subterranean environments are mostly related to human activities. Organic pollutions, problem of heavy metals, petroleum products, excessive visiting by cavers and tourists even scientists, destructions and diggings or vandalism are kinds of these causes. These negative factors that have human origin, threaten the animal population in subterranean environments. Cave species are extremely limited in numbers and also many of them have extremely limited climatic or other environmental requirements (Camacho, 1992). The entry of surface contaminants by such as pesticides and chemical fertilizers, also water extraction from underground aquifers threatens the life in subterranean habitats.

Scientific depredation is one of the main causes of some decline even extinction of troglobiont species. Numerous samplings of Iranian cave fishes by different research groups for unnecessary studies have been done as for a single study 44 specimens were collected (Abbasi and Gharezi, 2008; Sargeran et al., 2008). Collecting cave fishes from the same locality and for several times per year and even using invasive methods such as electroshock by researchers is scientific depredation. Reboleira et al., 2015 mentioned some destruction in Neyneh cave in Iran due to the activities of treasure hunters. Fatemi et al., 2019 mentioned that due to the negligence of authorities, cavers, locals, and even the research groups, a lot of habitat destruction has occurred in the recently-discovered Tashan cave that harbors some troglobiont species such as fish, gastropod and isopod.

Troglobionts are unique and seem to be restricted to a single cave or karstic system. Many cave species are extremely limited in number. Subterranean environments and specially

caves, karstic springs and Qanats¹ are the most important habitats for troglofauna in Iran. Despite the shortage of water, drought, excessive water extraction from underground, many pollutions from surface to the groundwaters, the Karstology and Speleobiology and thus, conservation of these resources, have been neglected in Iran.

Only Iranian cave fishes from Loven cave have the status of Vulnerable (VU) in IUCN list (Coad 1996; Coad et al., 2009; IUCN Red List of Threatened Species, 2018; World Conservation Monitoring Centre, 2018 a, b).

In general, biodiversity in Iran is under serious threat; modern infrastructures such as road systems cutting across natural or semi-natural habitat have damaging effects on biodiversity via wildlife-vehicle collision (Farashi and Shariati, 2017).

1.5 THESIS AIMS AND OUTLINE

This dissertation follows three main goals to improve the knowledge about Iranian troglobiont animals in several aspects such as faunistic and biodiversity, evolutionary and the origin.

First, a discovery component involves faunistic investigations into the troglobiont organisms in Zagros Mountains of Iran, as well as taxonomic descriptions of new taxa. We aim to substantially increase the taxonomic and faunistic knowledge about Iranian subterranean fauna. The putative presence of representatives of all major animal lineages in subterranean environments of Iran that are known as troglobionts from other karst areas is tested.

The second aim is to establish a georeferenced database of Iranian subterranean fauna and conduct an analysis of troglobiotic diversity patterns. The presence of one, or several troglobiotic diversity hotspots in Iran is tested.

¹ A qanat or kariz, is a system for transporting water from an aquifer or water well to the surface, through an underground aqueduct.

The third aim is to propose a temporal and phylogenetic frame for the origin of the subterranean fauna of the Zagros Mountains. Considering the age of some karstic areas in Iran and also some preliminary studies on Iranian troglobiont animals, the colonization of subterranean environments by these animals and also some divergence in surface relatives could have occurred in Late Miocene and Pliocene or even as recent as Pleistocene.

In the first part of the thesis, we present the description of a troglobiont carabid beetle genus *Duvalius* Delarouze, 1859 and one of its surface-dwelling congeners as a step towards the first aim of this study. This chapter also includes a part of the third aim. A species level phylogeny of *Duvalius* and closely related genera, and a dated phylogeny that enables understanding of the timing of cave colonization by this troglobiont beetle are presented.

The description of a new obligate cave-dwelling genus and species of gastropods is presented in this thesis. Using our original sequences and sequences from GenBank, we reconstruct the phylogenetic trees of the related taxa with our new genus.

We present the description of a new subterranean-dwelling species of the genus *Asellus* from Iran. Both morphological and molecular studies confirm that this is a new species. Species phylogenetic relationships using original and datamined mitochondrial and nuclear rDNA, and estimation of molecular divergences with other *Asellus* species, suggest that *A. ismailsezarai* sp. nov. is sister to a larger clade that also contains the European *A. aquaticus* species complex. This indicates that this species does not belong to the European *A. aquaticus* species complex.

In this thesis, we present the checklist of subterranean arthropods of Iran as a published article. In an addition to this article, we represent an overview of troglobiotic biodiversity in Iran. In this part, we represent all Iranian troglobiotic animals (previously published and our new discoveries, including yet undescribed species). The species richness and distribution patterns of Iranian troglobiotic diversity are presented, including the currently recognized hotspot.

Given the poor knowledge of Iranian subterranean fauna, this speleobiological dissertation adds critical knowledge by reporting new findings and describing new species. The discovery component of this thesis enables us to establish Iran as an important part of the world map of subterranean biodiversity by discovery of some new species that previously

had not been reported from the country even to the order or family level. This dissertation attempts to detect the origins of Iranian troglobionts and the time of their adaptation to subterranean environment. This fauna shows high endemism. This small discovered species and what will be discovered is very important from conservation perspective. Such studies about cave fauna should lead to decisions about conservation approaches.

2 SCIENTIFIC WORKS

2.1 PUBLISHED SCIENTIFIC WORKS

2.1.1 First insights into the origin of Iranian cave beetle diversity with description of two new species of the genus *Duvalius* (Carabidae)

Malek-Hosseini M. J., Muilwijk J., Gregorič, M., Kuntner M., Čandek, K. 2021. First insights into the origin of Iranian cave beetle diversity with description of two new species of the genus *Duvalius* (Carabidae).

Journal of Zoological Systematics and Evolutionary Research, 59, 7:1453-1469, doi: 10.1111/jzs.12537

Naslov: **Prvi vpogled v izvor raznolikosti iranskih jamskih hroščev z opisom dveh novih vrst rodu *Duvalius* (Carabidae)**

Izvleček:

Podzemna okolja Irana so zelo slabo raziskana. V tem prispevku želimo izboljšati znanje o iranski jamski biotski raznovrstnosti, pri čemer sledimo trem ciljem: (i) raziskati iranske jame za troglobiotske hrošče; (ii) razumeti filogenetske odnose znotraj rodu *Duvalius* Delarouze, 1859 in oceniti časovno obdobje kolonizacije iranskih jam; in (iii) komentirati trenutno poznavanje iranske troglobiotske favne za lažje prihodnje raziskave. S terenskim delom in morfološkim pregledom smo opisali dve novi vrsti rodu *Duvalius* iz jam v gorovju Zagros v Iranu: troglobiotski *Duvalius nezelensis* sp. nov. in netroglobiotski *Duvalius achaemenius* sp. nov. Za filogenetske analize smo pridobili izvorna zaporedja dveh mitohondrijskih (COI, 16S) in dveh jedrnih (18S, 28S) genov za tri vrste rodu *Duvalius* iz gorovja Zagros in jih združili z objavljenimi molekularnimi podatki drugih vrst in rodov hroščev. Z uporabo Bayesovega sklepanja in največje verjetnosti smo rekonstruirali filogenijo rodu *Duvalius* in tesno sorodnih rodov, nato pa s programom BEAST izračunali čas razhajanja glavnih linij. Naše filogenije so dobro podprle klad "Zagros", katerega ločitev od drugih sorodnikov je ocenjena na 9,7 milijona let. V kladu Zagros je razcep *D. nezelensis* in njegove sestrške skupine ocenjen na 7,8 milijona let, medtem ko je razcep *D. achaemenius* in *D. kileri* ocenjen na nedavnih 0,78 milijona let. V študiji smo prispevali nekaj razrešitve pri razumevanju vrstnega bogastva jamskih hroščev v Iranu in časa njihove podzemne kolonizacije. Vendar naše filogenije potrjujejo taksonomske težave, saj je več rodov ugnезdenih globoko v "rodu" *Duvalius*.

Ključne besede: *Duvalius*, molekularna filogenija, Trechini, troglobiont, Zagros

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First insights into the origin of Iranian cave beetle diversity with description of two new species of the genus *Duvalius* (Carabidae)

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Abstract

Subterranean environments of Iran are severely understudied. Here, we advance the knowledge of Iranian cave biodiversity by following three goals: (i) to investigate Iranian caves for troglomorphic beetles; (ii) to understand the phylogenetic relationships and estimate the timing of Iranian cave colonization by *Duvalius* Delarouze, 1859; and (iii) to comment on the current knowledge of the Iranian troglomorphic fauna to facilitate future research. Through field efforts and morphological examination, we describe two new *Duvalius* species from caves of Zagros Mts., Iran: the troglomorphic *Duvalius nezelenis* sp. nov. and the non-troglomorphic *Duvalius achaemenis* sp. nov. For phylogenetic analyses, we provide original sequences of two mitochondrial (COI, 16S) and two nuclear (18S, 28S) genes for three *Duvalius* species from the Zagros Mts., and combine them with published molecular datasets using other *Duvalius* species with relevant outgroup genera. Using Bayesian inference and maximum likelihood, we reconstruct a species-level phylogeny of *Duvalius* and closely related genera, then use BEAST to explore divergence times of major lineages. Our phylogenies recover a well-supported "Zagros clade," with its split from other congeners estimated at 9.7 Ma. Within the Zagros clade, the split of *D. nezelenis* and its sister group is estimated at 7.8 Ma, while the split of *D. achaemenis* and *D. kileri* is estimated to a recent 0.78 Ma. We provide some resolution in understanding the species richness of cave beetles in Iran and the timing of their subterranean colonization. However, our phylogenies confirm taxonomic problems as several genera are nested deep within the *Duvalius* tree.

KEYWORDS

Duvalius, molecular phylogeny, Trechini, troglomorphic, Zagros

Résumé

Les environnements souterrains de l'Iran sont très peu étudiés. Dans le présent article nous améliorons les connaissances sur la biodiversité des grottes iraniennes en suivant trois objectifs : (i) étudier les grottes iraniennes à la recherche de coléoptères

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troglomorphes ; (ii) comprendre les relations phylogénétiques et estimer la date de colonisation des grottes iraniennes par le genre *Duvalius* Delarouze, 1859 ; et (iii) commenter les connaissances actuelles de la faune troglomorphe iranienne afin de faciliter les recherches futures. Par du travail de terrain et un examen morphologique, nous décrivons deux nouvelles espèces de *Duvalius* provenant de grottes des monts Zagros, en Iran : la troglomorphie *Duvalius nezelenis* sp. nov. et la non-troglomorphie *Duvalius achaemenius* sp. nov. Pour les analyses phylogénétiques, nous fournissons les séquences originales de deux gènes mitochondriaux (*COI*, 16S) et de deux gènes nucléaires (18S, 28S) pour trois espèces de *Duvalius* des monts Zagros, et nous les combinons avec des données moléculaires publiées sur d'autres espèces de *Duvalius* et sur des groupes externes pertinents. En utilisant l'inférence bayésienne et le maximum de vraisemblance, nous reconstruisons une phylogénie au niveau des espèces de *Duvalius* et des genres qui leur sont étroitement apparentés, puis nous utilisons BEAST pour explorer les temps de divergence des principales lignées. Nos phylogénies révèlent un "clade Zagros" bien étayé, dont la séparation avec ses congénères est estimée à 9,7 Ma. Au sein du clade Zagros, la séparation de *D. nezelenis* et de son groupe frère est estimée à 7,8 Ma, tandis que la séparation de *D. achaemenius* et *D. kileri* est estimée à une date récente de 0,78 Ma. Nous apportons une certaine résolution dans la compréhension de la richesse en espèces des coléoptères des cavernes en Iran et de la chronologie de leur colonisation du milieu souterrain. Cependant, nos phylogénies confirment des problèmes taxonomiques, car plusieurs genres sont imbriqués profondément dans l'arbre du genre *Duvalius*.

1 | INTRODUCTION

Globally, subterranean environments have been colonized by several groups of animals, for example, fishes, amphibians, molluscs, and many arthropod groups (Vandel, 1964). Iran has some of the largest cave systems in the world (Raeisi et al., 2012; Vatandoust et al., 2019), yet most of them are poorly investigated. Troglomorphic representatives of fishes, millipedes, spiders, crustaceans, and snails are already recorded from Iranian caves (Bargrizaneh et al., 2021; Fatemi et al., 2019; Malek-Hosseini & Zamani, 2017). However, even though beetles are among the arthropod groups with globally species-richest terrestrial troglomorphic representatives (Zagmajster et al., 2008), only a single true cave-dwelling beetle, *Foranotum perforatum* Nabozhenko & Sadeghi, 2017 (Tenebrionidae), is known from Iran (Nabozhenko & Sadeghi, 2017). Troglomorphic and troglomorph representatives of several beetle families occur in Iran, including the genera *Laemostenus* and *Duvalius* (Carabidae) from caves in the Zagros and Central zone (Tahami et al., 2017). All Iranian *Duvalius* are recorded almost exclusively from the Zagros Mountains (Malek-Hosseini et al., 2016), an extensive, 1500 km mountain range from north-west to south Iran. These mountains have a sedimentary origin being composed primarily of limestone. Geologically, Zagros is part of the Alpine-Himalayan orogenic belt (Agard et al., 2011; Cucchi & Zini, 2003).

With around 300 species, the genus *Duvalius* Delarouze, 1859 is one of the most species-rich genera of the family Carabidae. Most *Duvalius* species are West Palearctic elements, but some

are found in West China, Kyrgyzstan, Caucasus, Armenia, Pakistan, Afghanistan, Turkmenistan, and most species have restricted distributions (Belousov, 2017). *Duvalius* belongs to the tribe Trechini, which is one of the main insect groups that have colonized subterranean environments (Faille et al., 2011). Comprising numerous true cavernicolous species, trechine ground beetles of West Palearctic are well studied morphologically, yet the evolutionary origins of some groups of these beetles are enigmatic due to incomplete sampling (Faille et al., 2013; Maddison et al., 2019). Trechini includes over 2500 species distributed worldwide (Bousquet, 2012), originated in Eocene to early Oligocene, and comprise numerous lineages that have colonized the subterranean world (Maddison et al., 2019). *Duvalius* is one of the Trechini lineages ("isotopic" Trechini), which has probably rapidly diversified during late Miocene (Faille et al., 2013). However, the genus level taxonomy is unresolved: While the *Duvalius* lineage is reported to include no fewer than eight genera (*Anophthalmus*, *Arctaphaenops*, *Agostinia*, *Duvalius* sensu stricto, *Luraphaenops*, *Trichaphaenops*, *Aphaenopidius*, and *Petrarchaenops*), the relationships among these genera, and even their monophyly, remain ambiguous (Delić et al., 2020; Faille et al., 2013).

Species described as *Duvalius* s.s. are found in different ecological environments, and the cavernicolous ones show different degrees of troglomorphy. These species show slight or complete depigmentation, and reduced or absent eyes and wings, while other species are pigmented and even winged (albeit exceptionally; Deuve, 2000; Faille et al., 2013). To date, four species of *Duvalius* have been

known from Iran, of which three are endemic to Zagros Mts., and *D. hetschkoi* (Reitter, 1911) with a wide distribution area. The apterous nominate form of *D. hetschkoi* (Reitter, 1911) is described from Turkmenistan and also occurs in Afghanistan, and *D. hetschkoi orbayi* Hernandez, 1999 is known from Pakistan (Belousov, 2017; Deuve, 2000). The Iranian subspecies *D. hetschkoi matilei* Deuve, 2000 is the only known *Duvalius* with the exceptional feature of having functional wings. *D. hetschkoi*, including its subspecies, has an exceptionally large and disjointed distribution range (Belousov, 2017; Deuve, 2000). In the prominent monograph by Jeannel (1928), *D. hetschkoi* is placed together with *D. bodoanus* (Reitter, 1913) and *D. dromioides* (Reitter, 1897) in the "*bodoanus*" species group. Later Kryzhanovskij et al. (1995) assumed that *D. dromioides* (Reitter, 1897) should be placed in the genus *Oroblemites* (Ueno & Pawlowski, 1981). Jeannel based the similarity of the species of the "*bodoanus*" species group on the big eyes and stated that these species possess the same type of bifid copulatory piece in the median lobe as species from central Caucasus, Greece, and Italy.

The other Iranian species are restricted to the Central and Southern parts of the Zagros Mts.: *Duvalius koeni* Muilwijk & Felix, 2008, *Duvalius mohammadzadehi* Muilwijk & Felix, 2008 (Muilwijk & Felix, 2008), and *Duvalius kileri* Muilwijk & Malek Hosseini 2016 (Malek-Hosseini et al., 2016). These species are similar to *D. hetschkoi* and form the *hetschkoi* species group. Other *Duvalius* species occur in the neighboring countries of Iran.

D. antoniae (Reitter, 1892) has been recorded from the border area between Armenia and Azerbaijan (Mount Ilandag, North of Ordubad). This species is very close to *D. stepanavanensis* labllokhoff-Khnzorian 1963. Also, two species from East Turkey are close to this

group *D. martinae* Jeanne 1996 and *D. armeniacus* Casale, 1979. This Armenian species group is recognizable by its tibiae without external sulcus, its chaetotaxy, lack of eyes, and morphology of the copulatory apparatus (Casale, 1979) and therefore differs from the *hetschkoi* species group (Jeannel, 1928).

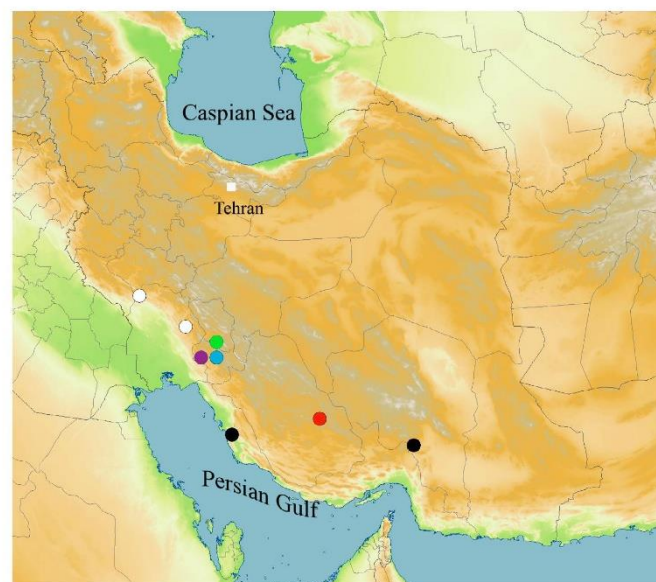
Here, we investigate the subterranean beetle fauna of Iranian caves of the Zagros Mts. We diagnose and describe two newly discovered species of *Duvalius* Delarouze, 1859 from two caves in Zagros. The troglomorphic *Duvalius nezelenis* sp. nov. occurs in the Nezel cave, while the troglomorphic *Duvalius achaemenius* sp. nov. occurs in the small Suq cave. We then use molecular phylogenetic analyses to include the new species in a phylogenetic framework. Specifically, we aim to obtain a dated phylogeny that will enable understanding of the timing of cave colonization by beetles in the region. Finally, we use the newly presented data to provide preliminary interpretations of the troglomorphic Iranian fauna species richness.

2 | METHODS

2.1 | Taxon sampling

We obtained original, four marker sequences (see below) of three *Duvalius* species from the Zagros Mts., Iran: *D. achaemenius* sp. nov., *D. kileri*, and *D. nezelenis* sp. nov. For morphological examination and measurements, we used five specimens of *D. achaemenius*, seven specimens of *D. kileri* (one specimen was previously published in Malek-Hosseini et al., 2016), and eight specimens of *D. nezelenis*, of which we used one specimen per species for DNA isolation. The

FIGURE 1 Location of investigated caves in Iran and distribution of Iranian *Duvalius*. Purple circle: *Duvalius* (*D.*) *achaemenius* sp. nov. Black circles: *Duvalius* (*D.*) *hetschkoi matilei* Deuve, 2000. Green circle: *Duvalius* (*D.*) *kileri* Muilwijk & Malek Hosseini, 2016. White circles: *Duvalius* (*D.*) *koeni* Muilwijk & Felix, 2008. Orange circle: *Duvalius* (*D.*) *mohammadzadehi* Muilwijk & Felix, 2008. Blue circle: *Duvalius* (*Duvalius*) *nezelenis* sp. nov.



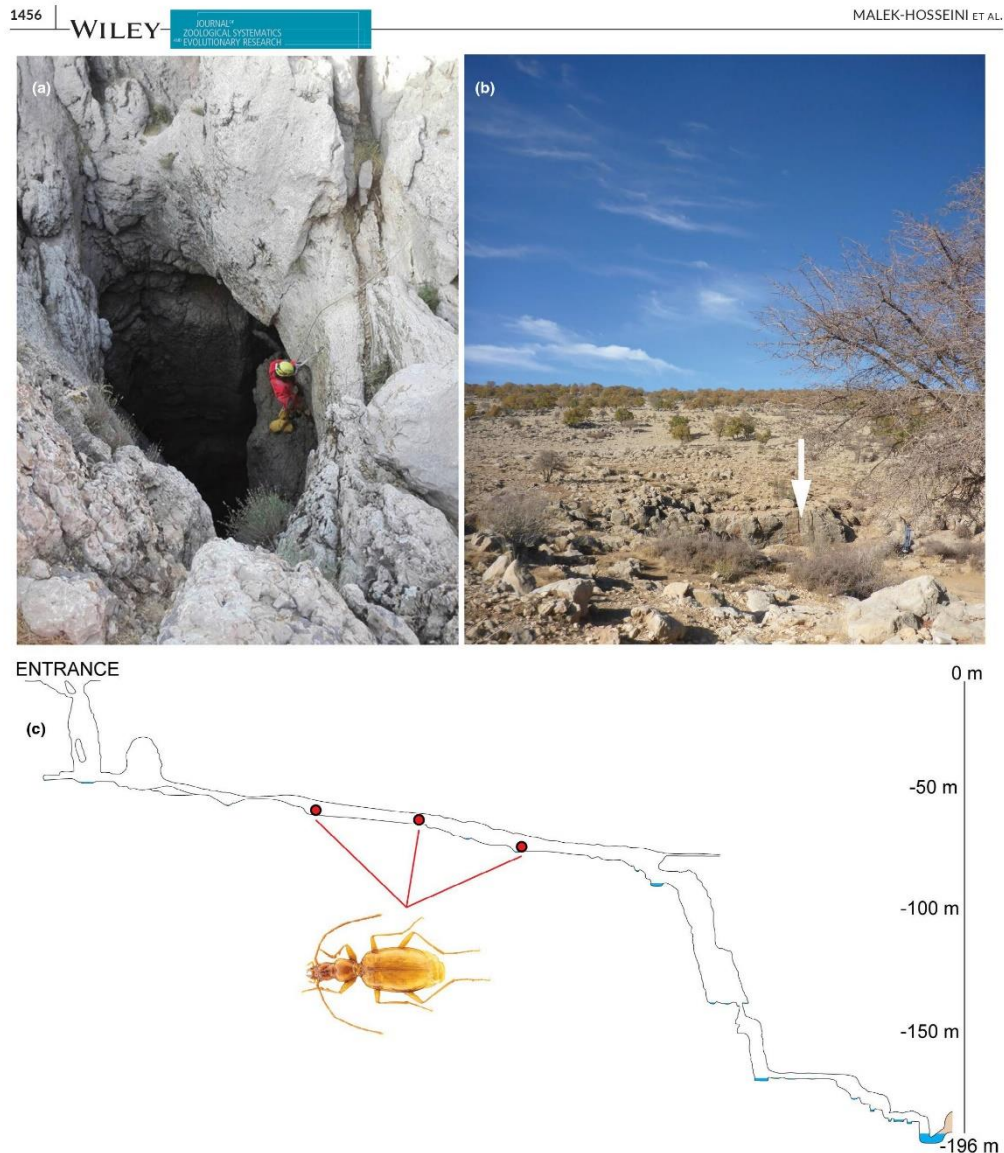


FIGURE 2 Nezel cave: (a) The entrance of the cave that is a 50-m pit. (b) The same entrance of the cave as figure 2a from another view that is located in a flat plain (white arrow showing the entrance). (c) Sampling localities within the Nezel cave (red circles showing the places where specimens of *Duvalius nezelensis* sp. nov. were found)

Iranian *Duvalius* species *D. koeni* (two specimens, previously published in Muilwijk & Felix, 2008), *D. mohammadzadehi* (two specimens, previously published in Muilwijk & Felix, 2008), and *D. hetschkoi matilei* (one specimen, previously published in Deuve, 2000) were used for morphological comparison with the above three species (Appendix 1). From GenBank, we additionally mined the same four

marker sequences for nine genera that are hypothesized to be nested within the *Duvalius* clade (Delić et al., 2020; Faillie et al., 2013). In total, we selected sequences of 43 species of *Duvalius*, 11 species of *Anophthalmus*, and for representatives of *Agostina* (1), *Aphaenopidius* (2), *Arctaphaenops* (1), *Luraphaenops* (1), *Petrarchaenops* (1), and *Trichaphaenops* (1) (Table S3). *Pheggomisetes globiceps* Buresch, 1925,

Geotrechus discontignyi (Fairmaire, 1867), *Aphaenops cereberus* (Dieck, 1869), and *A. leschenaulti* Bonvouloir, 1862 were used as outgroups.

2.2 | Field work and cave details

During several expeditions to Nezel and Suq caves, both in the Kohgiluyeh Va Boyer Ahmad Province in the Central part of the Zagros Mountains, South-Western Iran (Figure 1) between 2015 and 2019, the first and second authors collected the material by hand.

The Nezel cave is a natural cave in Southwest Iran near Jookhooneh Village, Kohgiluyeh Va Boyer Ahmad Province (30°47'28.62" N, 50°56'53.83" E, altitude 2307 m above sea level (a.s.l.), depth of the cave: ~196 m, length of discovered part is 546 m). The entrance of the cave is a 50-meter pit, located in a flat plain (Figure 2). The cave consists of several pits of different depths, the last pits are siphons, and the total depth is 196 m ending in a siphon. The cave contains two big halls. During heavy rain, streams of water flow through the first hall. The second hall includes a big water pool with almost no mud. Environmental conditions at the collection site were as follows: water temperature: 8.6°C; pH: 7.8–8.3; air temperature: 10–11°C; CO₂: 388–1493 ppm; and relative humidity: 86–95%.

The Zagros Mts. chain represents the southern, Asian branch of the Alpine geosynclines. The Nezel cave is located on the Sarvak Formation in the Zagros Mountains. The age of this formation is determined as the Cenomanian (100–93 Ma) in most parts (Jamalpour et al., 2017; Parnian et al., 2019; Razin et al., 2010; Setudehnia, 1973). Orogeny and then speleogenesis could have occurred during Miocene (Popov et al., 2004). Nezel is still an active cave.

The Suq cave is a small natural cave in Southwest Iran, Kohgiluyeh Va Boyer Ahmad Province near Suq and Lendeh (30°49'25.9" N, 50°25'37.9" E, altitude 812 m a.s.l.). In the summer, the region is hot and dry, with temperatures exceeding 40°C. The cave is located in a gorge where a small brook flows through, with the opening at the level of the brook. The passable part of the cave is approximately 100 m long, with water flowing between small rocks. There is hardly any debris or mud between the stones. At the far end of the cave streams a larger brook.

2.3 | Morphological examination and imaging

Specimens were examined with a Leica MZ 20.5 C stereomicroscope. Genitalia were extracted after soaking of the specimen for at least 24 h in a commercial protein enzyme solution. Male genitalia were immersed in a water/alcohol mixture in several stages and preserved in Euparal. Measurements were mostly conducted from photographs. For measurements of the median lobe, a 10x Nikon microscope objective was used. Measurement of the length of median lobe was obtained with a microscope objective micrometer (0.01 mm). The median lobe length was measured from basal bulb to apex of apical lamella at the widest distance. Microsculpture was studied at 120x magnification. The elytral chaetotaxy is seen as black spots on the left elytron (Figures 3 and 4).

Photographs of beetles were taken with a Canon 5Ds digital camera and Canon MP-E 65 mm macro lens or a Nikon 10x objective, and subsequently stacked using Helicon Focus 7. The label text of type specimens or of historical specimens is cited as originally given, a forward slash (/) indicates the end of each line. A double forward slash (//) separates labels. All measurements are reported in millimeters.

The following morphological acronyms are used:

TL: total length measured from the anterior margin of labrum to the apex of elytra;
EL: elytral length measured along suture from the basal bead of the elytron to the apex;
EW: maximum width of the elytra;
PL: pronotal length measured along the middle line;
PW: maximum width of the pronotum.

The following museum and collection acronyms are used:

CMU: Working collection of Jan Mulder, Leiden, Netherlands;
HMIM: Hayk Mirzayan's Insect Museum, Tehran, Iran;
MNP: Národní Muzeum Praha, Czech Republic;
NBCL: Naturalis Biodiversity Centre, Leiden, Netherlands;
ZM-CBSU: Zoological Museum and Biological Collection of Shiraz University, Shiraz, Iran.

2.4 | Molecular procedures

One leg of a specimen from each species was taken for DNA extraction. The protocol included Robotic DNA extraction using Mag MAX™ Express magnetic particle processor Type 700 with DNA Multisample kit (Thermo Fisher Scientific kit) and modified protocols following Videgar et al. (2014). We amplified fragments of four genes, two mitochondrial and two nuclear. Mitochondrial markers: 3' end of cytochrome c oxidase subunit 1 gene (CO1); a section including the 3' end of the 16S rRNA gene, the tRNA-Leu gene, and the 5' end of the NADH dehydrogenase 1 gene (16S-Leu-nad1); nuclear markers: the 5' end of the 18S rRNA gene (18S); and an internal fragment of the 28S rRNA gene (28S). Partial gene sequences were amplified by PCR using primers listed in Table S1 (Ober, 2002; Ribera et al., 2010; Shull et al., 2001; Simon et al., 1994), and following PCR amplification protocols listed in Table S2. PCRs were made in a 35 µl volume using EH2O: 18.8 µl, Buffer: 7.1 µl, dNTPS (2 mM): 3.5 µl, MgCl₂ (25 mM): 3.2 µl, Primer (20 mM): 1 µl forward and 1 µl reverse, polymerase 0.2 µl, and BSA 0.2 µl. For some samples with multiplied DNA bands, we ran PCR with 75 µl volume. Gel electrophoresis was done using special agarose (low EEO, for electrophoresis, Acros Organics), and then, separated bands were cut from gel and DNA was purified using the Thermo Scientific DNA purification kit. PCR products were sent to Macrogen Europe (Amsterdam, the Netherlands) for sequencing. Each fragment was sequenced in both directions using PCR amplification primers. The lengths of the PCR products (Amplicon length) generated with primers are listed in Table S1.

2.5 | Phylogeny inference and molecular dating

Geneious v. 5.6.7 (Kearse et al., 2012) and ChromasPro 2.1.3 (Technelysium, Tewantin, Australia) were applied to assemble sequences, as well as for editing and proofreading. Alignments were done using MEGA (Kumar et al., 2018). We concatenated all marker matrices in Mesquite version 3.6 (Maddison & Maddison, 2018). The aligned concatenated matrix contained 3540 characters (ch) including 739 ch for 16S, 622 ch for 18S, 984 ch for 28S, and 1195 ch for COI. Mined sequences varied in length and starting positions within a gene; therefore, some characters are represented by gaps, for example, the longest sequence of the COI alignment was 857 ch. MEGA (Kumar et al., 2018) was used to find the best nucleotide substitution model and the optimal partitioning scheme for each marker. To reconstruct the phylogeny of *Duvalius* and related genera, we conducted Bayesian inference using MrBayes v. 3.2.7a (Huelsenbeck, & Ronquist, 2001). The optimal settings for our data involved four partitions, each using an independent nucleotide substitution model (16S: HKY+I+G; 18S: JC+G; 28S: K2+G; COI: GTR+I+G) (Alignment S1–S5). We ran two independent Bayesian runs, each with four MCMC chains, for 15 million generations with a sampling frequency of 3000. A relative burn-in was set to 25% and checked for the MCMC chain convergence in Tracer 1.7 (Rambaut et al., 2018). To check whether any marker creates unexpected noise in analyses, and to compare node supports, we performed Bayesian analyses for each individual marker and for combinations of two and three markers, in addition to concatenated matrices.

We ran maximum likelihood analyses (hereafter ML) with a separate substitution model for each of the four partitions (16S: HKY+I+G; 18S: JC+G; 28S: K2+G; COI: GTR+I+G) and ultrafast bootstrapping in IQ-tree 1.6.7 (Nguyen et al., 2014). We obtained node support values with 1000 bootstrap replicates (Hoang et al., 2018).

We employed BEAST2 (Bouckaert et al., 2019) to reconstruct time-calibrated phylogenies (chronograms). In BEAUti (Bouckaert et al., 2019), the bModelTest (Bouckaert & Drummond, 2017) was selected as the substitution model for each of the four gene partitions. bModelTest, a package implemented in BEAUti, uses a reversible jump MCMC that integrates over all substitution models available in the software (Bouckaert & Drummond, 2017). To time calibrate our phylogeny, we used molecular clock rates and models optimized for the beetle family Carabidae and regularly used for Trechini (Andújar et al., 2012). The COI partition used a strict clock with normal prior distribution (mean 0.0134, sigma 0.0015), the 16S partition used a strict clock with normal prior distribution (mean 0.0016, sigma 3e-4), while the 18S and 28S partitions ran under the same relaxed log-normal clock model with normal prior distribution (mean 0.0029, sigma 5e-4). The birth-death tree prior was used. BEAST analyses ran for 20 million MCMC generations with a sampling frequency of 2000.

Time-calibrated analyses can be sensitive to the inclusion or exclusion of outgroup taxa (Andújar et al., 2012). Therefore, to avoid any time-calibration biases introduced by the outgroup, we reconstructed another chronogram with settings as above, but this time using in-group taxa only.

After examining the log files with Tracer 1.7 (Rambaut et al., 2018), we discarded 20% of the trees as burn-in and summarized the remainder of trees with TreeAnnotator (Bouckaert et al., 2019). The target tree was set as the Maximum clade credibility tree, and the node heights were set as median heights. All phylogenetic and molecular dating of BEAST and Bayesian analyses were performed on CIPRES (Miller et al., 2010), while maximum likelihood was run on IQ-TREE webserver (Trifinopoulos et al., 2016).

3 | RESULTS

3.1 | Taxonomy

Duvalius (Duvalius) nezelenis Muilwijk & Malek Hosseini sp. nov.

(Figure 3a–e).

<http://zoobank.org/urn:lsid:zoobank.org:act:53D2B6C8-8496-4B36-B2D3-A518FC4EE287>

Type material

Holotype male labeled: "Iran Kohgiluyeh Va Boyer Ahmad, Zagros / Nezel cave, 12.vi.2018 / M. Porebrahim, S. Azizi, Z. Rozbehi, S. Rahideh, & J. Muilwijk leg." // "Holotype / *Duvalius (Duvalius) nezelenis* sp. nov. / Muilwijk & Malek Hosseini des. 2021" (black print on red label). Paratypes, one male labeled: "Iran, Kohgiluyeh va Boyer Ahmad, Zagros / Nezel cave, 15.xi.2016 / Mohammad Javad Malek Hosseini leg.";—one female labeled: "Iran, Kohgiluyeh va Boyer Ahmad, Zagros / Nezel cave 9.x.2017 / Tahami leg.";—three males and two females labeled: "Iran Kohgiluyeh Va Boyer Ahmad, Zagros / Nezel cave, 12.vi.2018 / M. Porebrahim, S. Azizi, Z. Rozbehi, S. Rahideh, & J. Muilwijk leg." // "Paratype / *Duvalius (Duvalius) nezelenis* sp. nov. / Muilwijk & Malek Hosseini des. 2021" (black print on red label). Holotype and female paratype deposited at HMIM, one female paratype at ZM-CBSU, one male paratype at NBCL, three males and one female paratypes at CMU.

Additional examined material

Duvalius (D.) hetschkoi matilei Deuve, 2000

Iran: Kerman Jiroft, Narab, 900 m, 16.xi.1999, Badii, Bar. Mof leg.; HMIM. 1 male.

Duvalius (D.) kileri Muilwijk & Malek Hosseini, 2016.

Iran: Kohgiluyeh Va Boyer Ahmad, Kiler cave, 150 km North West of Yasuj, 27.x.2011, N 31°17'96", E 50°35'13", 1610 m, Mohammad Javad Malek Hosseini leg., ZM-CBSU. Male holotype; —Kiler cave, 14.vi.2018, S. Rahideh & J. Muilwijk leg., HMIM, CMU. Three males and three females.

Duvalius (D.) koeni Muilwijk & Felix, 2008

Iran: Khuzestan, Pole-e Tang, 60 km NW Andimeshk, Loc. No. 284, 10–11.iv.1977, Exped. Nat. Mus. Praha. MNP. male holotype; —Lake Izeh, 01.iv.2007, Muilwijk leg., CMU. Female paratype.

Duvalius (D.) mohammadzadehi Muilwijk & Felix, 2008

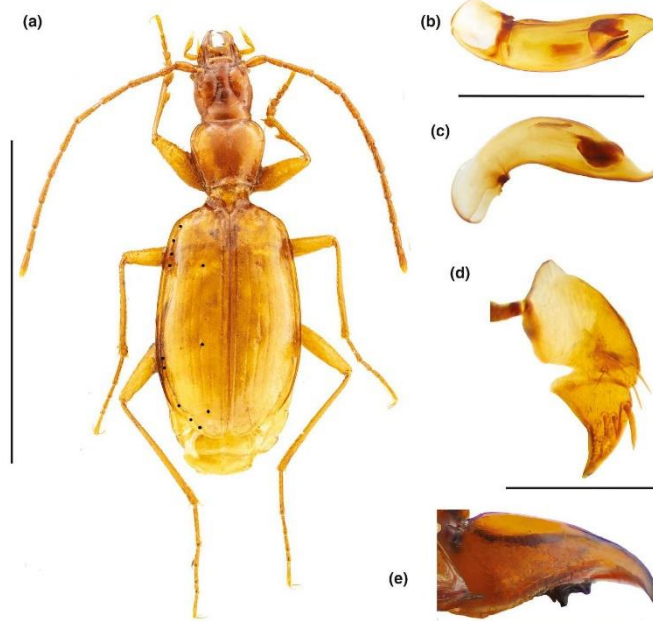


FIGURE 3 *Duvalius nezelenis*: (a) HT, habitus, scale: 4 mm, chaetotaxy indicated with black spots on elytra. (b) HT, median lobe median lobe, dorsal. (c) PT, median lobe median lobe, left lateral. scale: 1 mm. (d) PT, Gonocoxite and laterotergite IX, ventral, scale: 0.2 mm. (e) PT, right mandible ventral, scale: 0.5 mm

Iran: Fars, Estahban, 25.iv.2006, 2380 m, Muilwijk leg., MNP. Male holotype, one female paratype, CMU.

Description

(Figure 3), a large-sized anophthalmic, wingless *Duvalius* with upper-side of head testaceous, pronotum, and elytra yellow. Appendages long. Fine hairs on genae, pronotum, lateral sides of elytra, and lateral sides of ventrites. Total length 5.48–6.15 mm, males: 5.53–5.89 mm (holotype 5.84 mm, average 5.73 mm, $n = 5$), females: 5.48–6.15 mm (average 5.71 mm, $n = 3$).

Head elongated, as long as wide, with slightly swollen genae narrowed to the neck; frontal furrows half-circular, impressed; eyes absent; very small ocular scars without pigment and covered by integument (in some individuals barely recognizable); genae with fine hairs; two pairs of long supraorbital setae, anterior pair just behind the middle and posterior pair in the basal part of frontal furrows; clypeus with two pairs of setae, labrum with three pairs of setae; right mandible with three teeth at the base, the first two teeth are along the ventral side, the third small flat tooth is located dorsally of the large one (as shown in Figure 3e), left mandible with a ridge of three very small teeth; apical border of ligula with eight setae, concave; antennae long and slender, reaching over the middle of elytra, covered with dense decumbent hair; antennomeres 3–7 nearly equally long.

Pronotum rounded anteriorly, then almost straight, slightly concave to hind angles (ratio PL/PW: 0.87–0.93 (HT: 0.91), wider than head; lateral sides rounded, slightly sinuate toward the acute upwards pointed hind angles, front angles rounded, obtuse; base with a slight indentation; lateral margin with two pairs of setae, anterolateral setae situated in the anterior fifth of the lateral margin, basolateral pair before hind angles; basal fovea short and moderate deep; lateral sides with fine hairs.

Elytra elongate (ratio EL/EW: 1.47–1.65; HT: 1.56), widest at the apical third, much wider than pronotum; basal line of the elytra clearly oblique; lateral furrows wide; all striae superficially and provided with superficial punctures; first stria of elytra curved back to the fifth stria. Scutellar stria not deepened. Sides with fine hairs, denser to the lateral sides. Elytra with a scutellar setiferous pore, three setiferous discal punctures (two discal in stria 3, one pre-apical in interstria 3), umbilicate series consist of eight setiferous pores, divided over three groups shown as black spots in Figure 3a. The humeral group consists of four pores at equal distance from each other. The second group of two pores is situated just behind the middle. The apical group consists also of two pores; angulo-apical punctures present.

Legs long, slender; femora and tibia with many tiny bristles, tarsi with dense decumbent hairs; protibiae with a longitudinal furrow dilated with fine apical hairs; protarsi in the male with two basal tarsomeres dilated, first tarsomere slightly denticulate inwards, second

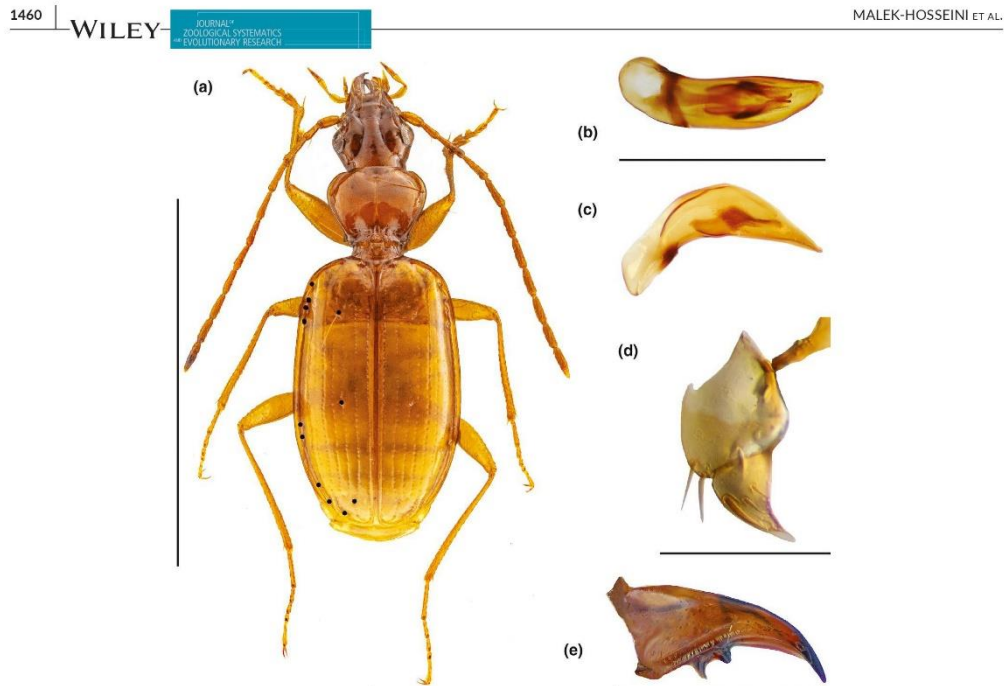


FIGURE 4 *Duvalius achaemenius*: (a) HT, habitus, scale: 4 mm, chaetotaxy indicated with black spots. (b) HT, median lobe, dorsal, scale: 1 mm (c) PT, median lobe, left lateral, scale: 1 mm. (d) PT, Gonocoxite and laterotergite IX, ventral, scale: 0.2 mm. (e) PT, right mandible ventral, scale: 0.5 mm

tarsomere with three very short, blunt dents along apical margin. Tarsal claws of front leg of normal size.

Ventral side: mentum with two short, blunt teeth; proepisternum and ventrites with fine hairs; ventrites 4–6 with a pair of paramedial setae. Along the apical border of the anal ventrite in both sexes also two setae.

Microsculpture: polygonal meshes on the head and pronotal disk, transversal meshes on lateral sides of pronotum, and dense transversal meshes on elytra.

Male genitalia as in Figure 3b,c: median lobe 1.25 mm long, in lateral view regularly curved and upwards sinuated at the apex; in dorsal aspect slightly sinuate in basic fifth, middle part parallel-sided, apex asymmetrical, triangular, tip rounded. Copulatory piece isotopic, consisting of two long sclerotized lateral parts, a horseshoe-shaped sclerotized median part and a sclerotized rectangular basal part embedded in a hyaline structure; in dorsal view, the symmetrical lateral lamellae enclose at the base the rectangular basal lamella and consist apical of dense sclerous scales; the horseshoe-shaped part ends apical in a tip, the hyaline structure is provided with scattered scales; in lateral view the lateral lamellae form apically a semicircle of dense sclerous scales, the horseshoe-shaped lamella is enclosed by the lateral lamellae and ends apically near the orifice in a hooked tip,

the basal part forms a dorsal plate in the middle of the median lobe. Length of parameres about half of the length of median lobe, each paramere with four apical setae.

Female gonocoxites and laterotergites as in Figure 3d: few hairs on the gonocoxite, laterally with a big spine.

Differential diagnosis

D. nezelenis sp. nov. differs from all known Iranian *Duvalius* species by the absence of eyes, small elongated head, clearly longer appendages, more and longer pubescence on legs and antennae, the short hairs on the elytra and sides of the head, and the copulatory piece with an accessory horseshoe-shaped lamella (Figures 3–5).

Distribution and habitat

Known only from the type locality; the new species was collected on wet clay in the first hall of the Nezel cave, Zagros Mts., South-western Iran.

Etymology

Topotypic, the species epithet refers to the cave where the species was collected.

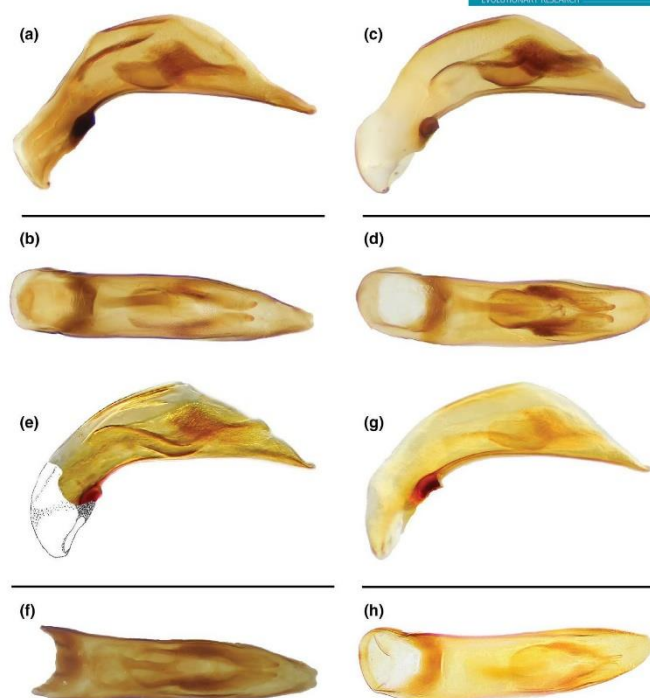


FIGURE 5 Median lobe of Iranian *Duvalius*. (a) *D. hetschkoi matilei*, median lobe, left lateral. (b) *D. hetschkoi matilei*, median lobe, dorsal. (c) *Duvalius* (*D.*) *kileri* median lobe, left lateral. (d) *Duvalius* (*D.*) *kileri* median lobe, dorsal. (e) *Duvalius* (*D.*) *koeni*, median lobe, left lateral. (f) *Duvalius* (*D.*) *koeni*, median lobe PT, dorsal. (g) *Duvalius* (*D.*) *mohammadzadehi* HT median lobe, left lateral. (h) *Duvalius* (*D.*) *mohammadzadehi* HT median lobe, dorsal. Scale: 1 mm

***Duvalius* (*Duvalius*) *achaemenius* Muilwijk & Malek Hosseini sp. nov.**
 (Figure 4a–e).

<http://zoobank.org/urn:lsid:zoobank.org:act:1F57B27C-43F8-40EA-B4D9-082E7F334BDF>

Type material

Holotype male labeled: "Iran, Kohgiluyeh Va Boyer Ahmad / Suq cave near Suq / 16.vi.2018 / S. Rahideh & J. Muilwijk leg." // "Holotype / *Duvalius* (*Duvalius*) *achaemenius* sp. nov. Muilwijk & Malek Hosseini des. 2021" (black print on red label). Paratypes, male labeled: "Iran, Kohgiluyeh Va Boyer Ahmad / Suq cave near Suq / 31.iv.2017 / Yaser Fatemi leg.". —two males and one female labeled: "Iran, Kohgiluyeh Va Boyer Ahmad / Suq cave near Suq / 16.vi.2018, S. Rahideh & J. Muilwijk leg." // "Paratype / *Duvalius* (*Duvalius*) *achaemenius* sp. nov. Muilwijk & Malek Hosseini des. 2021" (black print on red label). Holotype deposited at HMIM, one male paratype at NBCL, two males and one female paratypes at CMU.

Additional examined material

The same material was used for this species as in the description of the previous species.

Description

Figure 4a: A medium-sized *Duvalius* with testaceous upper side and yellow appendices yellow. Pronotum shiny, head and elytra dull. Vestigial wings present. Total length 4.99–5.45 mm, males: 4.99–5.45 mm (holotype 5.43 mm, average 5.24 mm, $n = 3$), female: 5.13 mm).

Head wider than long; frontal furrows half-circular, impressed; eyes well developed, much larger than genae (1.32x); two pairs of long supraorbital setae, anterior pair above the eye and posterior pair in the basal part of frontal furrows; clypeus with two pairs of setae, labrum with three pairs of setae; right mandible with two large teeth at the base (as shown in Figure 4e), left mandible with a ridge of three very small teeth; apical border of ligula with eight setae, slightly convex; antennae long and slender, exceeding somewhat over middle of elytra, antennomeres 3–7 nearly equally long; antennomere 1 with a few setae, second half of antennomere with scattered setae, from antennomere three onwards more dense setae.

Pronotum cordiform (ratio PL/PW: 0.77–0.81 (HT: 0.77); wider than head; lateral sides rounded, slightly sinuate toward the acute upwards pointed hind angles, front angles rounded, obtuse; lateral margin with two pairs of setae, anterolateral setae situated in the

anterior third of the lateral margin, basolateral pair before hind angles; basal fovea short and deep, median line well marked.

Elytra subparallel (ratio EL/EW: 1.55–1.64; HT: 1.64), widest at apical third, humeri rounded; lateral furrows wide; striae 1–5 impressed, striae 6–7 superficially and striae 1–7 clearly punctured, first stria of elytra curved back to fifth stria, second stria reaching the apical puncture, stria 3–7 reaching to pre-apical setiferous discal puncture.

Elytra with a scutellar setiferous pore, three setiferous discal punctures (two discal in stria 3, one pre-apical in interstria 3), umbilicate series consist of eight setiferous pores, divided over three groups as shown as black spots in Figure 4a). The humeral group consists of four pores at equal distance from each other. The second group of two pores is situated just behind the middle. The apical group consists also of two pores; angulo-apical punctures present.

Microsculpture: head with reticulate and elytra with fine transverse meshes.

Legs: femora and tibia with some tiny bristles, tarsi with some decumbent hairs, protibiae with a longitudinal furrow on their dorsal side, slightly dilated with a fine apical hair; protarsi in the male with two basal tarsomeres dilated.

Ventral side: mentum tooth bifid; ventrites 4–6 with a pair of paramedial setae. Along the apical border of the anal ventrite in both sexes also two setae.

Male genitalia as in Figure 4b,c. Median lobe 0.98 mm long, median lobe in lateral view dorsal side strongly convex, with maximum width around the middle, tip rounded, in dorsal view basal part curved, apex asymmetrical, tip rounded. The copulatory piece consists of two sclerotized lateral parts and a sclerotized elongated basal part embedded in a hyaline structure; in dorsal view the symmetrical undulating lateral lamellae enclose partly the elongated basal lamella, and consist in the middle of dense sclerous scales and end apically in a tip, the elongated basal lamella is narrow in the middle and widened to the base and apex, the apex is more or less triangular; the hyaline structure is provided with scattered scales; in lateral view, the lateral lamellae are in the middle provided with dense sclerous scales, apically ending in a bended tip near the orifice, the undulating elongated basal lamella medio-dorsally situated.

Female gonocoxites and laterotergites as in Figure 4d. Gonocoxites with two visible hairs and a spine laterally.

Differential diagnosis

D. achaemenius sp. nov. (Figure 4) differs from *D. nezelsensis* (Figure 3) by the presence of eyes; from all other Iranian *Duvalius* species by the form of the median lobe and copulatory piece (Figure 5a–h), from *Duvalius* (*D.*) *koeni* by more rounded humeri and less impressed striae and from *Duvalius* (*D.*) *koeni* and *Duvalius* (*D.*) *hetschkoi matilei* by reduced wings; from *Duvalius* (*D.*) *kileri* and *Duvalius* (*D.*) *mohammadzadehi* by more developed eyes.

Distribution and habitat

Known only from the type locality, the new species was found under wet stones about 50 m. from the entrance of the Suq cave, Zagros Mts., Southwestern Iran.

Etymology

The species epithet refers to the Achaemenid dynasty, a royal house of the Old Persian Empire.

3.2 | Key to the Iranian species of *Duvalius*

1- Eyes absent, pronotum slightly cordiform, habitus as in Figure 3a; copulatory piece with a horseshoe-shaped sclerotized median part (Figure 3b, c);

..... *D. nezelsensis* sp. nov.

- Eyes present, pronotum strongly cordiform; copulatory piece without a horseshoe-shaped sclerotized median part.

..... 2

2- Species with developed wings..... 3

- Species without reduced or without wings.

..... 4

3- Eyes more prominent, clearly protruding; pronotum with acute upwards pointed hind angles; sides of elytra more or less parallel; median lobe as in Figure 5a, b.

..... *D. hetschkoi matilei* Deuve, 2000

- Eyes less prominent; hind angles pronotum less acute; sides of elytra more oval; median lobe as in Figure 5e, f.

..... *D. koeni* Muilwijk & Felix, 2008

4- Eyes not reduced; humeri regularly bent; striae moderately impressed; habitus as in Figure 4; apex of median lobe curved as in Figure 4b.

..... *D. achaemenius* sp. nov.

- Eyes reduced.

..... 5

5- Striae on elytra superficial; species from Fars; median lobe as in Figure 5f, g.

..... *D. mohammadzadehi* Muilwijk & Felix, 2008

- Striae on elytra impressed; species from Kohgiluyeh va Boyer Ahmad; median lobe as in Figure 5c, d.

..... *D. kileri* Muilwijk & Malek Hosseini, 2016.

3.3 | Annotated catalogue of *Duvalius* from Iran with a distribution map of the species (Figure 1)

Duvalius (*D.*) *achaemenius* sp. nov.; Kohgiluyeh Va Boyer Ahmad, Suq cave, 30°49'25.9" N, 50°25'37.9" E, altitude 812 m a.s.l.

Duvalius (*D.*) *hetschkoi matilei* Deuve, 2000; Bushehr, Ahrām Omar, near the Persian Gulf, altitude 100 m a.s.l.; Kerman Jiroft, Narab, altitude 900 m a.s.l. 16.xi.1999 Badii, Bar. Mof.

Duvalius (*D.*) *kileri* Muilwijk & Malek Hosseini, 2016; Kohgiluyeh Va Boyer Ahmad, Kiler cave 150 km North West of Yasuj, 31°17'96" N, 50°35'13" E, altitude 1610 m a.s.l.

Duvalius (*D.*) *koeni* Muilwijk & Felix, 2008; Khuzestan, Pole-e Tang, 60 km NW Andimeshk; Khuzestan, Izeh.

Duvalius (*D.*) *mohammadzadehi* Muilwijk & Felix, 2008; Fars, Estahban, altitude 2380 m. a.s.l.

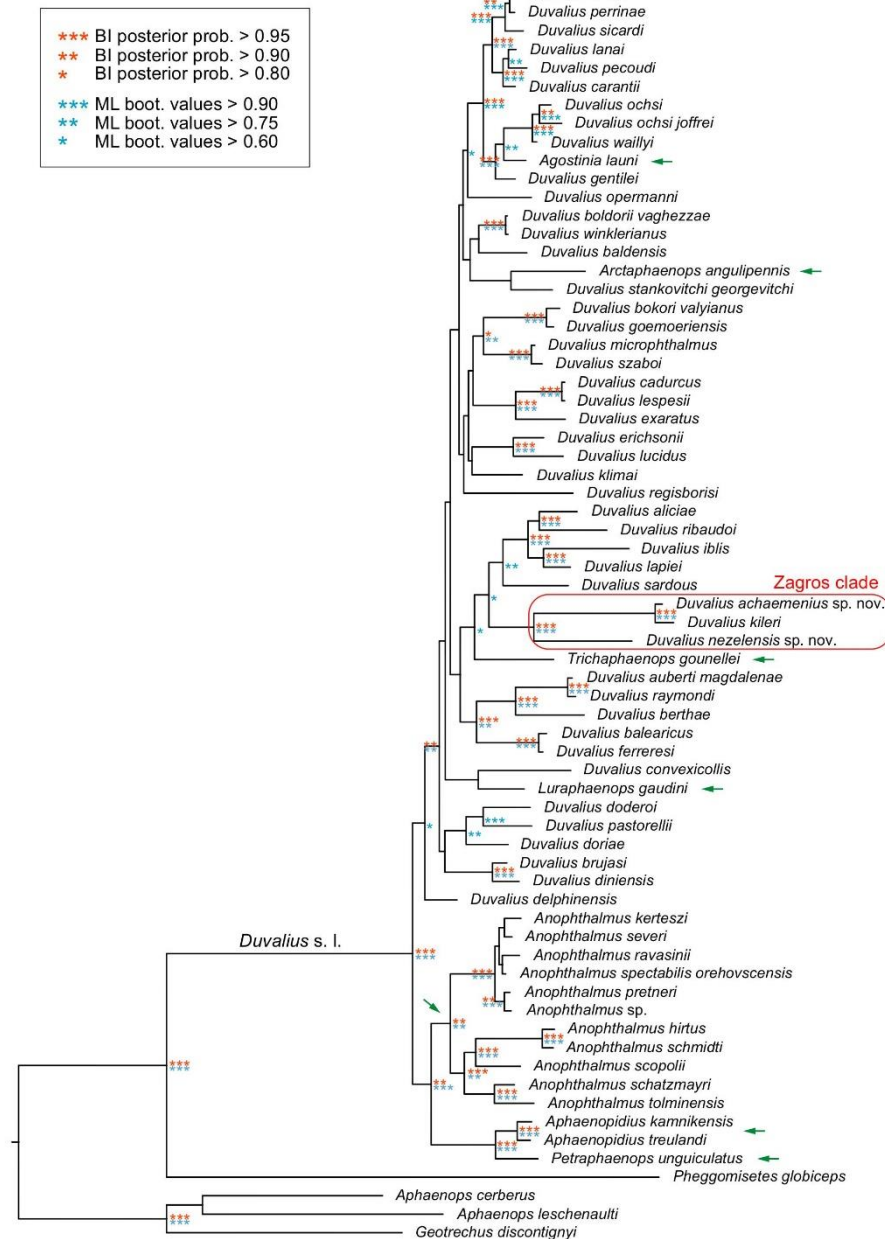


FIGURE 6 Phylogeny of the *Duvalius* lineage group of the Trechini beetles. The topology is from the Bayesian inference analysis, with all compatible groups. Colored asterisks mark both Bayesian inference (BI; orange) and maximum-likelihood (ML; blue) node supports. The red ellipse marks the highly supported Zagros clade. The relationships within Trechini are poorly resolved. Green arrows mark genera other than *Duvalius*

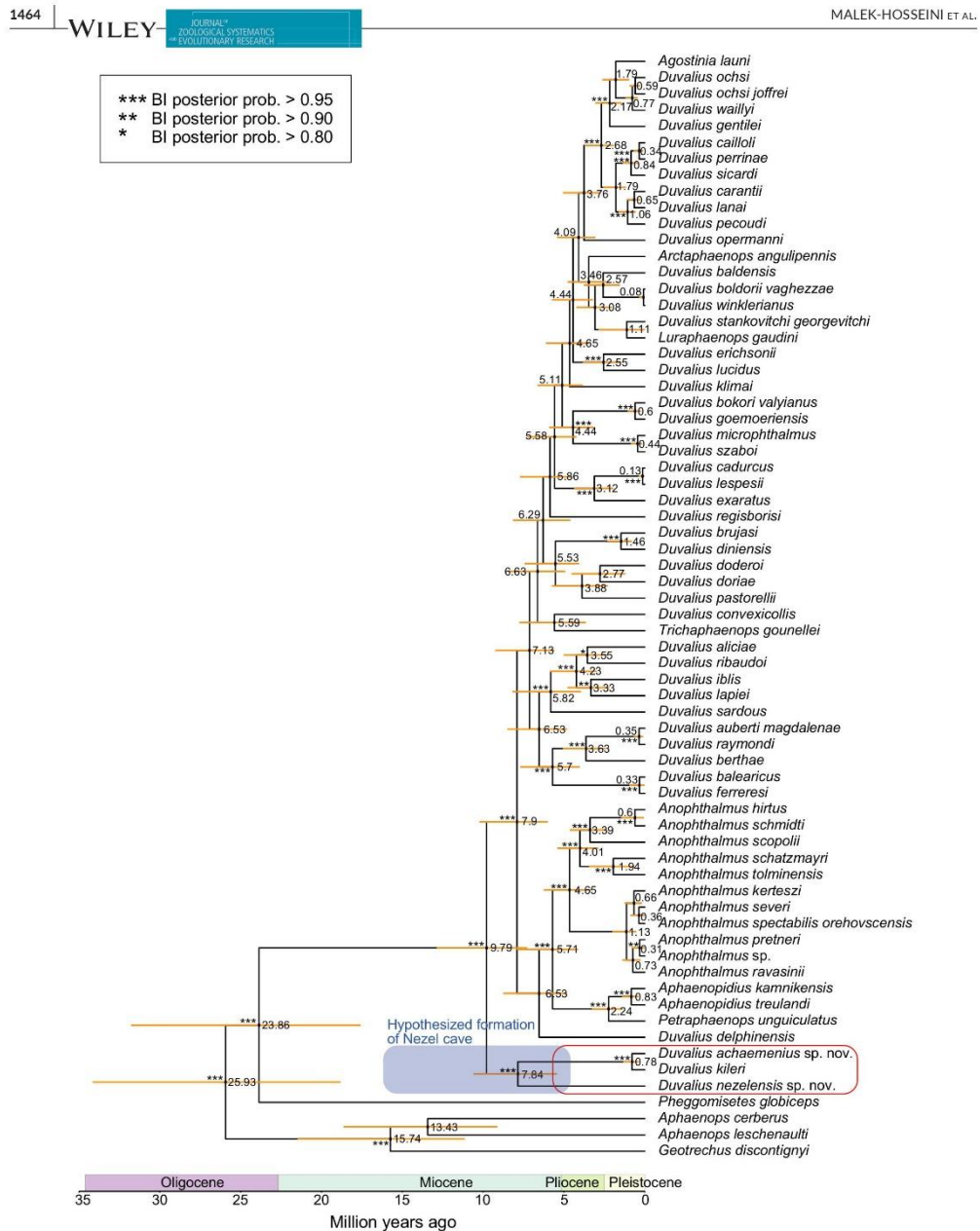


FIGURE 7 BEAST chronogram of the *Duvalius* lineage of the Trechini beetles, based on molecular clock rates for four markers. Orange bars on the nodes indicate 95% highest posterior probability density for node age. Asterisks indicate nodal support from BEAST. This chronogram suggests that Zagros clade split from the rest of *Duvalius* between 7.3 and 12.9 Ma (mean 9.7 Ma) in Late Miocene and soon thereafter diversified. *D. nezelenis* sp. nov. probably colonized the Nezel cave and developed its troglomorphic features between 5.4 and 10.6 Ma (mean 7.8 Ma) in Late Miocene and present time. The figure shows estimated time of the Nezel cave formation after Popov et al. (2004)

Duvalius (*Duvalius*) *nezelenis* sp. nov.; Kohgiluyeh va Boyer-Ahmad, Nezel cave, 30°47'28.62" N, 50°56'53.83" E, altitude 2307 m a.s.l.

3.4 | Phylogeny

All analyses strongly support the monophyly of the *Duvalius* species group from the Zagros Mts. labeled and discussed as the "Zagros clade", consisting of *D. kileri*, *D. achaemenius*, and *D. nezelenis*. They recover a sister relation of *D. nezelenis* with *D. achaemenius* + *D. kileri* with high node support (Figures 6, 7 and S1–S3). However, the relationships within the *Duvalius* lineage of Trechini are poorly resolved in all Bayesian and ML analyses, and thus, the position of the Zagros clade cannot yet be resolved. This may be due to the lack of molecular data for most of the potentially sister candidates from the neighboring countries of Iran. Although poorly supported, both Bayesian and ML analyses consistently recover the Zagros clade as sister to a clade containing Algerian (*D. iblis* (Peyerimhoff, 1910), *D. lapiei* (Peyerimhoff, 1908)), Sicilian (*D. aliciae* Magrin, Baviera & Petrioli, 2007, *D. ribaudi* Magrin, Petrioli & Degiovanni, 2010), and Sardinian (*D. sardous* (Doderò, 1917)) representatives (Figures 6, S1 and S2). This larger clade is sister to a troglotrophic species from France, *Trichaphaenops gounellei* (Bedel, 1879). However, the support for this relationship is relatively weak. One further inclusive node in this phylogeny is also weakly supported, but contains a clade sister to the above with a well-structured, and supported clade of Mediterranean, troglotrophic *Duvalius* species, including the type of the genus, *D. raymondi* Delarouze, 1859.

The BEAST chronogram shows a phylogenetic proximity of the Zagros clade quite different from the above phylogenies. This analysis recovers the Zagros *Duvalius* clade as sister to the entire *Duvalius* lineage. However, we only use this BEAST analysis to test the timing of the major splits rather than as an alternative topological hypothesis of the relationship of the Zagros clade with other *Duvalius*. BEAST estimates the split of the Zagros clade to have occurred between 7.3 and 12.9 Ma (mean 9.7 Ma) in Late Miocene, which is congruent with the estimated time by Faille et al. (2013). The diversification of the Zagros clade is estimated to have occurred between 5.4 and 10.6 Ma (mean 7.8 Ma) in Late Miocene for the split of *D. nezelenis* from its sister group, and more recently in Middle Pleistocene between 0.4 and 1.3 Ma (mean 0.78 Ma) for the split of *D. achaemenius* and *D. kileri* (Figure 7). According to our BEAST estimation, the colonization of the subterranean environment by *D. nezelenis* must have occurred at any recent time since the Late Miocene (7.8 Ma) (Figure 7). Multiple, independent cases of eye loss are already well known in the *Duvalius* clade (Faille et al., 2013, 2018). The alternative explanation, that the ancestor of Zagros clade was already a cave-dweller, is unlikely because the implied ancestor to all *Duvalius* species would have to be spread in caves over a vast area of the Old World, which are clearly not interconnected. Our chronogram inferred the separation of the *Duvalius* group from the basal Trechini to have occurred between 18 and 32 Ma (mean 24 Ma) in late Oligocene (Figure 7), congruent with Faille et al. (2013) who estimated this split at 30 Ma. The alternative BEAST analysis with ingroups only produced comparable time estimates (Figure S3).

4 | DISCUSSION

In this study, we investigate cave-dwelling beetles of Zagros Mts in south-western Iran with the goals to make a three-pronged advancement in our understanding of the regional biodiversity: i) to aid in new species discovery of troglotrophic beetles; ii) to obtain a dated phylogeny of selected beetle taxa that would enable estimating the timing of cave colonization by beetles; and iii) to comment on the current knowledge of troglotrophic Iranian fauna more broadly.

4.1 | Discovery of troglotrophic beetles

Although Coleoptera and especially carabids have many representatives in subterranean environments, no troglotrophic carabid beetle has been discovered from Iran prior to this study and the only known troglotrophic coleopteran from Iran was the tenebrionid *Foranotum perforatum* (Nabozhenko & Sadeghi, 2017). This seemingly low species richness of cave-dwelling beetles in Iran is likely an artifact stemming from lack of focused research on this fauna. Most caves of the country that might potentially harbor troglotrophic beetles are not easy to reach, and there are no particular surveys on Iranian cave beetles. Moreover, given the vastness of the country and extension of karstic areas from north to south, an extensive study would require tremendous effort. After our discovery of two new species, a total of six *Duvalius* species are known in Iran. Of these, only *D. nezelenis* sp. nov. is a troglotrophic. No doubt, we expect the presence of other new species in Iran, particularly from subterranean environments. However, our discovery of one new non-troglotrophic species, *D. achaemenius* sp. nov., and its closely related troglotrophic, *D. nezelenis* sp. nov., is the first step toward a better understanding of the phylogeny and the origin of Iranian *Duvalius*.

Beside the absence of eyes and long appendages, the most striking features of *D. nezelenis* sp. nov. is the deviant shape of the copulatory piece in the median lobe. However, the chaetotaxy does not differ from other *Duvalius* species. The four previously known species (*D. hetschkoi matilei*, *D. kileri*, *D. koeni*, and *D. mohammadzadehi*) and the newly described *D. achaemenius* species are morphologically alike and can be described as the *hetschkoi* species group. The setiferous punctures of the series umbilicata and copulatory piece in the median lobe of the Iranian specimens match the description of the genus *Duvalius*, and differ from other genera of the *Duvalius* lineage (Casale & Laneyrie, 1982).

4.2 | Phylogeny and evolutionary history of the Zagros clade

Our study adds to phylogenetic resolution of Trechini by adding three *Duvalius* species from Iran. However, incomplete sampling of *Duvalius* lineage species from all around the Palearctic from Western Europe to Caucasus and from Iran to China (no molecular data exist for over 80% of described species) does not allow us to resolve the

phylogeny of this vast lineage. The genus *Duvalius* contains over 300 described species and is among the most species-rich genera of the Trechini (Belousov, 2017). Faille et al. (2011; 2013; 2018) hypothesized that the *Duvalius* lineage, consisting of eight "genera," was monophyletic. Our phylogenies are consistent with a large *Duvalius* clade (Delić et al., 2020; Faille et al., 2013, 2018) and confirm non-monophyly of the genera nested within as found in previous analyses (Delić et al., 2020; Faille et al., 2013, 2018). In other words, *Duvalius* is paraphyletic with respect to these other genera. Therefore, a genus level taxonomy of *Duvalius* will require multiple redefinitions. Rather than fixing the *Duvalius* taxonomy, we here focus our discussion on the evolutionary implications of the Zagros clade relationships.

Our molecular analyses agree on the monophyly of the Zagros clade that unites both newly discovered species with the troglomorphic *D. kileri*. We hypothesize that this clade will also include the three species of *Duvalius* from Iran (*D. hetschkoi matilei*, *D. koeni*, and *D. mohammadzadehi*) that were not included in our phylogeny. This expectation seems warranted by morphological resemblance, and geographic proximity of the included and omitted Iranian *Duvalius*.

Due to the lack of species from the neighboring countries, our analyses cannot unequivocally pinpoint the immediate phylogenetic relative of the Zagros clade. Although ML and Bayesian topologies both suggest this clade to consist of five species from Algeria, Sicily, and Sardinia, the node supports are weak. In addition, BEAST analyses consistently place the Zagros clade as sister to a vast assemblage of *Duvalius*. While we place more confidence in the ML and Bayesian topologies for sister group relationships, and resort to BEAST more for estimated time of major splits, this inconsistency in topologies precludes a more resolute conclusion as to which of the West Palearctic clades of *Duvalius* is the closest relative to the Zagros clade.

According to our chronogram, the three species from Zagros shared the most recent common ancestor between 7.3 and 12.9 Ma (mean 9.7 Ma) in the Late Miocene. This implies that *D. nezelenis* developed its troglomorphic features between the Late Miocene and today. In fact, considering that the estimated split overlaps with the potential time frame of the Nezel cave formation (Figure 7; Popov et al., 2004), the cave colonization by *D. nezelenis* is likely to have taken place around the time of this hypothesized split, and this must have been followed by accrual of troglomorphies as described in the contemporary *D. nezelenis* population. The basal diversification of Zagros clade is estimated to have occurred between 5.4 and 10.6 Ma (mean of 7.8 Ma) in the Late Miocene, and the recent split occurred between 0.4 and 1.3 Ma (mean 0.78 Ma) in the Late Pleistocene. Given the estimated 7 million years between these two splits, the inclusion of other Iranian *Duvalius* species as well as those from neighboring countries could illuminate future studies about the evolutionary events of this period.

A more recent cave colonization of species of the *hetschkoi* species group in Pleistocene is implied by *D. achaemenius* sp. nov. and *D. kileri*. This agrees with Hernando (1999) who hypothesized a recent colonization of the subterranean environment by *D. hetschkoi* in Afghanistan. The speciation of the *hetschkoi* species group in Iran

is limited to a small area in the Zagros Mountains. *D. kileri* has several adaptations such as reduced wings, small eyes, long antennae (Malek Hosseini et al., 2016) that implies this species has had a life in darkness but not long enough to completely obtain troglomorphic features. On the other hand, *D. achaemenius* sp. nov. shows no troglomorphies, and this species may have had recent or no continuous history in hypogean environments. This conclusion agrees with the qualities of Suq cave, the collection locality of *D. achaemenius* sp. nov. being a shallow cavern without absolute darkness.

All evidence combined point to our conclusion that the Zagros clade represents an independent evolutionary origin of subterranean colonization and troglomorphic evolution. As pointed out by Faille et al. (2013), multiple colonizations of the subterranean environment by *Duvalius* have occurred elsewhere. We see the alternative explanation that all *Duvalius* may have shared a subterranean ancestor, unlikely. First, such scenario would imply an unusually broad area of occupancy of this hypothetical ancestor. Second, this vast area would be spread over karsts of different ages and origins that are clearly not interconnected. Third, such scenario would imply numerous cases of re-emergence from subterranean environments. For these reasons, we find the alternative explanation as highly unlikely. Rather, the *Duvalius* lineage contains beetles predisposed for living underground. The development of karstic systems then triggers numerous and repeated cases of cave colonization.

4.3 | Iranian troglomorphic fauna species richness

Karstic carbonate formations cover about 11% of Iran's land where over 2000 caves have been registered (Raeisi et al., 2012). Nonetheless, Iran's subterranean fauna remains poorly known with only about 45 reported troglomorphic species. Of these, most are crustaceans (38), while the other represent fishes, gastropods, arachnids, and myriapods (Bargrizaneh et al., 2021; Fatemi et al., 2019; Malek Hosseini & Zamani, 2017). Most of these species have been discovered from Zagros karst. Clearly, the currently reported diversity of Iranian troglomorpha is a vast underestimation. Compared to some karstic areas such as the Dinaric or the Balkan Peninsula (Skeet et al., 2004), the areas studied, the number of studies and discovered troglomorphs in Iran are very low. Considering ours and similar ongoing biospeleological research efforts, the detection of additional undescribed troglomorphic diversity, perhaps even troglomorphic hotspots in Iran, are possible. Such research effort is important for the understanding of the regional troglomorpha including the colonization of subterranean environments in different parts of the Iranian karst for both terrestrial and aquatic fauna, as well as for inclusion of Iranian subterranean biology within a context of general processes that shape cave colonization and present-day patterns of subterranean biodiversity.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Table S1. Primer details with targeted gene, primer names (F: forward; R: reverse) and sequences, amplicon lengths, and reference information.

Table S2. PCR amplification protocols.

Table S3. Taxonomic and gene information (GenBank accession numbers) data and the proportion of missing data for the 68 taxa used in our analyses.

Figure S1. Phylogeny of the *Duvalius* sensu lato group of the Trechini beetles, obtained by Bayesian inference, with all compatible groups and posterior probability supports for all nodes.

Figure S2. Phylogeny of the *Duvalius* sensu lato group of the Trechini beetles, obtained by IQ-tree, with bootstrap supports for all nodes.

Figure S3. BEAST chronogram of the *Duvalius* lineage of the Trechini beetles for ingroup taxa only.

Alignment S1. Nexus matrix of concatenated 16S, 18S, 28S and COI sequences.

Alignment S2. Fasta file of 16S sequences used in our analyses.

Alignment S3. Fasta file of 18S sequences used in our analyses.

Alignment S4. Fasta file of 28S sequences used in our analyses.

Alignment S5. Fasta file of COI sequences used in our analyses.

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APPENDIX 1

Specimen list of examined individuals, with collection and locality info, museum collection information, and genbank accession numbers

Duvalius species	Collection details	COI	16S	18S	28S
<i>D. achaemenius</i> sp. nov.	1 male, holotype, Iran, Kohgiluyeh Va Boyer Ahmad, Suq, Suq cave, 30°49'25.9" N, 50°25'37.9" E, 812 m, 16.vi.2018, HMIM, Rahideh, S., & Muilwijk, J.	—	—	—	—
<i>D. achaemenius</i> sp. nov.	1 male, paratype, Iran, Kohgiluyeh Va Boyer Ahmad, Suq, Suq cave, 30°49'25.9" N, 50°25'37.9" E, 812 m, 31.iv.2017 / CMU, Fatemi, Y.	MZ033000	MZ042533	MZ042980	MZ043751
<i>D. achaemenius</i> sp. nov.	1 male, paratype, Iran, Kohgiluyeh Va Boyer Ahmad, Suq, Suq cave, 30°49'25.9" N, 50°25'37.9" E, 812 m, 16.vi.2018, NBCL, Rahideh, S., & Muilwijk, J.	—	—	—	—
<i>D. achaemenius</i> sp. nov.	1 male, 1 female, paratypes, Iran, Kohgiluyeh Va Boyer Ahmad, Suq, Suq cave, 30°49'25.9" N, 50°25'37.9" E, 812 m, 16.vi.2018, CMU, Rahideh, S., & Muilwijk, J.	—	—	—	—
<i>D. hetschkoi matilei</i>	1 male, Iran, Kerman Jiroft, Narab, 900 m, 16.xi.1999, HMIM, Badii, Bar. Mof.	—	—	—	—
<i>D. kileri</i>	1 male, holotype, Iran, Kohgiluyeh Va Boyer Ahmad, Kiler cave, 31°17'96", E 50°35'13", 1610 m, 27.x.2011, N 31°17'96", E 50°35'13", 1610 m, ZM-CBSU. Malek-Hosseini, M.J.	—	—	—	—
<i>D. kileri</i>	1 males and 2 females, Iran, Kohgiluyeh Va Boyer Ahmad, Kiler cave, 31°17'96", E 50°35'13", 1610 m, 14.vi.2018, CMU, Rahideh, S., & Muilwijk, J.	—	—	—	—
<i>D. kileri</i>	1 male, 1 female, Iran: Kohgiluyeh Va Boyer Ahmad, Kiler cave, 31°17'96", E 50°35'13", 1610 m, 14.vi.2018, HMIM, Rahideh, S., & Muilwijk, J.	—	—	—	—
<i>D. kileri</i>	1 male, Iran, Kohgiluyeh Va Boyer Ahmad, Kiler cave, 31°17'96", E 50°35'13", 1610 m, 14.vi.2018, CMU, Rahideh, S., & Muilwijk, J.	MZ033183	MZ042532	MZ043110	MZ044285
<i>D. koeni</i>	1 male, Iran, Khuzestan, Pole-e Tang, 60 km NW Andimeshk, Loc. No. 284, 10–11.iv.1977, MNP, Muilwijk, J.	—	—	—	—
<i>D. koeni</i>	1 female, paratype, Iran, Khuzestan, Lake Izeh, 01.iv.2007, CMU. Muilwijk, J.	—	—	—	—
<i>D. mohammadzadehi</i>	1 male, holotype, Iran, Fars, Estahban, 25.iv.2006, 2380 m, MNP, Muilwijk, J.	—	—	—	—
<i>D. mohammadzadehi</i>	1 female, paratype, Iran, Fars, Estahban, 2380 m, 25.iv.2006, CMU. Muilwijk, J.	—	—	—	—
<i>D. nezelensis</i> sp. nov.	1 male, holotype, 1 female, paratype, Iran, Kohgiluyeh Va Boyer Ahmad, Nezel cave, 30°47'28.62" N, 50°56'53.83" E, 2307 m, 12.vi.2018, HMIM, Porebrahim, M., Azizi, S., Rozbehi, Z., Rahideh, S., & Muilwijk, J.	—	—	—	—
<i>D. nezelensis</i> sp. nov.	1 male, paratypes, Iran, Kohgiluyeh va Boyer Ahmad, Nezel cave, 30°47'28.62" N, 50°56'53.83" E, 2307 m, 15.xi.2016 / CMU, Malek-Hosseini, M. J.	MZ033192	MZ042534	MZ043721	MZ043751
<i>D. nezelensis</i> sp. nov.	2 males and 1 female, paratypes, Iran, Kohgiluyeh Va Boyer Ahmad, Nezel cave, 30°47'28.62" N, 50°56'53.83" E, 2307 m, 12.vi.2018, CMU, Porebrahim, M., Azizi, S., Rozbehi, Z., Rahideh, S., & Muilwijk, J.	—	—	—	—
<i>D. nezelensis</i> sp. nov.	1 female, paratype, Iran, Kohgiluyeh va Boyer Ahmad, Nezel cave, 30°47'28.62" N, 50°56'53.83" E, 2307 m, 9.x.2017, ZM-CBSU, Tahami, M. S.	—	—	—	—

2.1.2 Description of a new genus and species as the first gastropod species from caves in Iran.

Fatemi Y., Malek-Hosseini M. J., Falniowski A., Hofman S., Kuntner M., Grego J. 2019. Description of a new genus and species as the first gastropod species from caves in Iran. *Journal of Cave and Karst Studies*, v. 81, no. 4, p. 233-243, https://caves.org/pub/journal/PDF/v81/81_4_233.pdf

Naslov: **Opis novega rodu in prve vrste polžev iz jam v Iranu**

Izvleček:

Poročamo o novem stigobiontskem trunkateloidnem polžu iz sulfidnih jezer v jami Tašan v gorovju Zagros v jugozahodnem Iranu. Habitat bogat z vodikovim sulfidom je podoben doslej znanim habitatom polžev iz s sulfidi bogatih jam v Romuniji, Italiji in Grčiji. Novo opisani rod *Trogloiranica* n. gen. z novo opisano vrsto *T. tashanica* n. sp. predstavljata prve prave stigobionske polže, najdene v Iranu. Na podlagi filogenije, pridobljene iz mitohondrijskega (citokrom oksidaza podenota I) in jedrnega (histon 3) genoma ter anatomskih znakov, je *T. tashanica* uvrščena v družino Moitessieriidae Bourguignat, 1863. Novi rod lahko predstavlja evolucijski relikv iz obdobja po miocenu, preden sta se razcepili družini Moitessieriidae in Cochliopidae Tryon, 1866.

Yaser Fatemi, Mohammad Javad Malek-Hosseini, Andrzej Falniowski, Sebastian Hofman, Matjaž Kuntner, and Jozef Grego. Description of a new genus and species as the first gastropod species from caves in Iran. *Journal of Cave and Karst Studies*, v. 81, no. 4, p. 233-243. DOI:10.4311/2019LSC0105

DESCRIPTION OF A NEW GENUS AND SPECIES AS THE FIRST GASTROPOD SPECIES FROM CAVES IN IRAN

Yaser Fatemi¹, Mohammad Javad Malek-Hosseini^{2,3,4}, Andrzej Falniowski⁵, Sebastian Hofman⁶, Matjaž Kuntner^{2,3}, Jozef Grego^{7,c}

ABSTRACT

We report on a new stygobiont truncatelloid gastropod from the sulfidic ponds of Tashan Cave in the Zagros Mountains of Southwest Iran. The hydrogen sulfide habitat resembles those hitherto known for gastropods from sulfide-rich caves in Romania, Italy and Greece. The newly described genus *Trogloiranica* n. gen. with a newly described *T. tashanica* n. sp. represent the first true stygobiont gastropods found in Iran. The phylogeny inferred from the mitochondrial (cytochrome oxidase subunit I) and the nuclear (histone 3) genomes, as well as anatomical evidence, place *T. tashanica* in the family Moitessieriidae Bourguignat, 1863. The new genus may represent an evolutionary relict from the post Miocene before the split of the families Moitessieriidae and Cochliopidae Tryon, 1866.

INTRODUCTION

The freshwater Mollusca of Iran have been a focus of research during past two decades (Mansoorian, 2001; Glöer and Pešić, 2009, 2012; Moghadam and Chegini, 2009; Mowlavi et al., 2009; Shahabuddin et al., 2012; Ektefa et al., 2013; Nouroozi, 2014), and the taxonomic position of the local Ponto-Caspian Hydrobiidae has been clarified only recently (Dellicado et al., 2016). Iran is situated at the interface of three zoo-geographic realms, Palaearctic, Oriental (Indo-Malayan), and Afrotropical (Ethiopian). The karstifiable carbonate formations cover around 11% of Iran's surface area with more than half (55.2%) being located at the Zagros Mountains in western Iran (Raeisi and Laumanns, 2012). Thus, it is not surprising that more than 90% of known Iranian troglotrophic taxa are found in the Zagros karst formations (Malek-Hosseini and Zamani, 2017).

Tashan Cave, located near the Sarjooshar Village, harbors a unique ecosystem. The recent discovery of a blind fish, *Garra tashanensis* Mousavi-Sabet, Vatandoust, Fatemi and Eagderi, 2016, and of the isopod *Stenasellus tashanicus* Khalaji-Pirbalouty, Fatemi, Malek-Hosseini, Kuntner, 2018, has been followed by other discoveries including the new gastropod genus and species reported here. The Tashan Cave represents a specific hypogean habitat with several cave ponds containing groundwater rich in hydrogen sulfide. Earlier studies performed in sulfide-rich caves by Hose et al. (2000); Engel (2012); and Jones et al. (2014) have shown that chemoautotrophic sulfur-oxidizing microorganisms thrive in such environments using the redox interface between the sulfide in the water and the oxygen in the atmosphere to produce food *in situ*, independent of the external light energy. Sulfuric acid is a product of the bacterial sulfur oxidation and accelerates the dissolution of the limestone bedrock in the process called *sulfuric acid speleogenesis* (SAS), which results in the formation of *sulfide caves* (Engel et al., 2004; Porter et al., 2009). It is estimated that hydrogen sulfide-rich phreatic waters and microbially-generated sulfuric acid were involved in the speleogenesis of around 10% of worldwide known caves (Palmer, 2007). However, only a few of these caves remain active ducts containing hydrogen sulfide-rich groundwater. The sulfide caves host interesting ecosystems with unusually rich and diverse invertebrate communities dominated by crustaceans (Peterson et al., 2013; Por, 2014), insects (Tobler et al., 2013) and occasionally fishes (Reisch et al., 2010; Roach et al., 2011; Mousavi-Sabet et al., 2016). While sulfide-based ecosystems are usually well-recognized inside deep sea trenches (Deming and Baross, 1993), the groundwater sulfide ecosystems remain poorly studied (Engel, 2007). The sulfide cave ecosystems received attention with the studies of Sarbu et al. (1996). The first studied cave containing a sulfide ecosystem was Movile Cave in Romania (Sarbu and Popa, 1992; Sarbu et al., 1996; Sarbu, 2000; Engel, 2012), followed by the Frasassi caves and Grotto Azzurra in Italy (Macalady et al., 2007; Peterson et al., 2013), Tito Bustillo and Maltravieso caves in Spain (Schabereiter-Gurtner, 2002; Arzo et al., 1997), Fiume Coperto Cave in Italy (Latella et al., 1999), Melissotrypa Cave in Greece (Falniowski and Sarbu, 2015), El Hamma in Tunisia (Por, 1963), Ayalon Cave and the Tabgha Spring in Israel (Por, 1963; 2007; 2011), Kugitangtoug caves in Turkmenistan (Maltsev and Korshunov, 1998), Cueva de Villa Luz in Mexico (Hose et al., 2000; Engel, 2007), Bungonia and Nullarbor caves with Bunder Sinkhole in Australia (Holmes et al., 2001; Jaume et al., 2001), Lower Kane

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Cave in Wyoming, USA (Porter et al., 2002), Cesspool Cave in Virginia, USA (Engel et al., 2001), and the Mammoth and Parker Caves in North America (Hutchins et al., 2016).

Hydrogen sulfide is toxic for most organisms (Kelley et al., 2006), and only five of the around twenty so far known sulfide cave ecosystem sites host stygobiont gastropod species, as likely outcomes of extreme sulfide adaptation: *Heleobia dobrogica* (Grossu and Negrea, 1989) from Movile Cave (Falniowski et al., 2008), *Islamia sulfurea* Bodon and Cianfanelli, 2012 from the Frasassi caves (Bodon et al., 2009; Bodon and Cianfanelli, 2012), *Physella spelunca* Turner and Clench, 1974 from Lower Kane Cave in Wyoming (Porter, 2002; Wethington and Guralnick, 2004) as well as *Iglica hellenica* and *Daphioli magdalenae* (Falniowski and Sarbu, 2015) from Melissotrypa Cave in Greece. The discovery of a sixth sulfidic stygobiont gastropod species in the Tashan Cave of Iran, reported here, is thus of general ecological importance.

MATERIAL AND METHODS

Snails were collected from pools in Tashan Cave, located under a limestone plateau close to Sarjooshar Village in the south-eastern part of the Zagros Mountains, Tashan district, Behbahan County, Khuzestan Province, southwest Iran (Fig. 1 and 2). Specimens were collected by using dropper pipettes and forceps and preserved in 96 % ethanol. A few specimens were transferred into a small rimstone basin at the cave pool shelf for photographing and subsequently returned to the pool.

The sampled snails were fixed in 80% ethanol. The dissections were done under a NIKON SMZ18 microscope with dark field, and the structures were photographed with a CANON EOS 50D digital camera. Frontal, ventral, and lateral images of the holotype were made using a Nikon SMZ25 microscope with a Nikon D200 camera and an AF-S Micro NIKKOR 60 mm lens at the Vienna Natural History Museum (NHMW), Austria. Morphological terms follow Hershler and Ponder (1998).

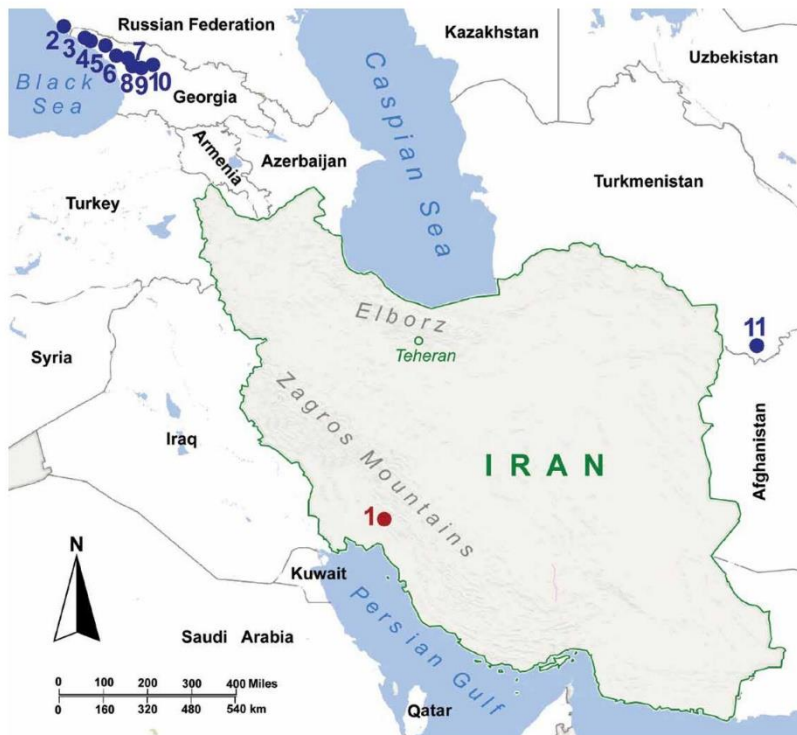


Figure 1. Map of Iran with the type locality of *Troglairanica tashanica* n. sp.: Khuzestan Province, Behbahan County, Tashan district, Sarjooshar Village, Tashan Cave (1 - Red ring) and geographically closest records of stygobiont gastropod species from SW Caucasus (2-10 - Blue dots) and from Turkmenistan, Kushka, Shar-Arab spring (11 - Blue dot).

DNA was extracted from foot tissue, hydrated in Tris-EDTA (TE) buffer, using a Sherlock extraction kit (A&A Biotechnology), and dissolved in 20 mL of TE buffer. The extracted DNA was stored at -80°C at the Department of Malacology of Institute of Zoology and Biomedical Research of the Jagiellonian University in Kraków. Our study targeted nucleotide data from the mitochondrial (cytochrome oxidase subunit I - COI) and the nuclear (histone 3 - H3) genomes, to produce phylogenetic inference from individual and combined gene datasets. Details of PCR conditions, primers used, and sequencing methods follow Szarowska et al. (2016). Sequences were aligned by MUSCLE (Edgar, 2004) implemented in MEGA 6

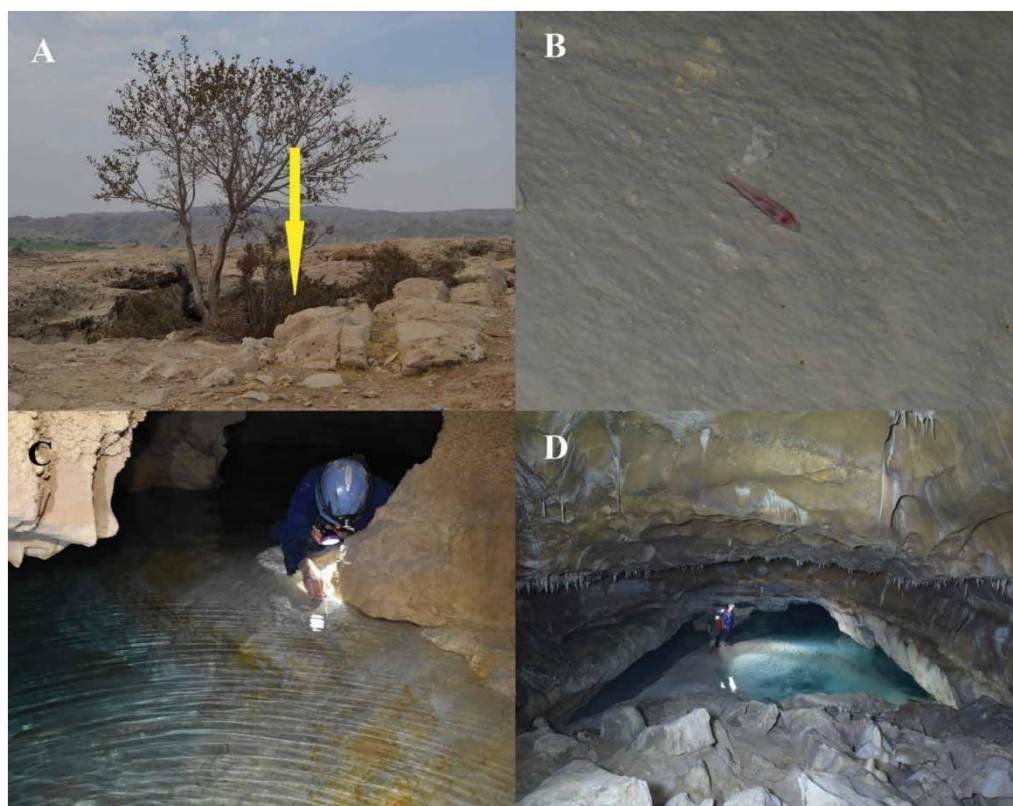


Figure 2. Photos of the type locality of *Troglorhina tashanica* n. sp.: A: Tashan Cave entrance on the semi-desert limestone plateau; B hydrogen sulfide-rich pond inside the Tashan cave showing *Garra tashanensis*; C and D: sampling at the type locality (photo: Mohammad Javad Malek-Hosseini and Yaser Fatemi).

(Tamura et al., 2013) and in Bioedit 7.1.3.0 (Hall, 1999). The saturation test (Xia, 2000, Xia et al., 2003) was performed using DAMBE (Xia, 2013). Additional sequences from GenBank were used in the phylogenetic analysis (Table S1). The phylogenies were inferred using Bayesian inference (BI) and maximum likelihood (ML).

The Bayesian analyses were run using MrBayes v. 3.2.3 (Ronquist et al., 2012) with the default priors. Two simultaneous analyses were performed, each of which lasted 10,000,000 generations, with one cold chain and three heated chains, starting from random trees and sampling the trees every 1,000 generations. The first 25 % of trees were discarded as burn-in. The analyses were summarized as a 50 % majority-rule tree. The ML approach was applied with RAxML v. 8.0.24 (Stamatakis, 2014). We applied the GTR model, whose parameters were estimated by the RAxML (Stamatakis, 2014). One thousand searches were initiated with starting trees obtained through the randomized step-wise addition maximum parsimony method. The tree with the highest likelihood score was considered as the best representation of the phylogeny. Bootstrap support was calculated with 1,000 replicates and summarized on the best ML tree. RAxML analyses were done in CIPRES Science Gateway (Miller et al., 2010).

Abbreviations

NMBE	Naturhistorisches Museum, Bern, Switzerland
NHMK	Natural History Museum, London, UK
NHMW	Natural History Museum, Vienna, Austria
ZMUH	Zoological Museum, University of Hormozgan, Iran

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SMF	Senckenberg Museum, Frankfurt, Germany
H	Shell height
W	Shell width
BH	Height of the body whorl
BW	Width of the body whorl
AH	Aperture height
AW	Aperture width
LT	Type locality

RESULTS

The molecular and anatomical investigation of the live gastropods from the type locality in Tashan Cave revealed it to be a new species belonging to a new genus in Moitessieriidae Bourguignat, 1863.

Superfamily Truncatelloidea Gray, 1840

Family Moitessieriidae Bourguignat, 1863

Genus *Trogloiranica* n. gen.

Diagnosis

The diagnostic features of the genus are the same as those of the type species, *Trogloiranica tashanica* n. sp. The oval shell shape with low spire is unique within the Moitessieriidae. Nevertheless, similar shell shapes seem to have convergently evolved in other truncatelloid families such as Hydrobiidae Stimpson, 1865, Cochliopidae Tryon, 1866, Pomatiopsidae Stimpson, 1865, and Tateidae Sacco, 1896.

Etymology

The name derives from a prefix troglo- referring to the cave habitat of the new taxon and suffix -iranica referring to the country of origin.

Trogloiranica tashanica sp. n.

Type locality

Iran, Khuzestan Province, Behbahan County, Tashan district, Sarjooshar Village, sulfidic freshwater ponds inside Tashan Cave, 30°51'54"N; 50°10'29"E (altitude 559 m a.s.l.).

Type material

Holotype: type locality: Yaser Fatemi and Mohammad Javad Malek-Hosseini leg. August 23, 2017 (holotype NMBE 558282).

Paratypes: type locality (same data) one paratype NMBE 558283; one paratype in each lot of ZMUH 123, 124 and 125; SMF 358125 one specimen, four specimens in coll. Grego; type locality Yaser Fatemi and Mohammad Javad Malek-Hosseini leg. August 27, 2017, two specimens in coll. Malek-Hosseini; type locality (same data); Yaser Fatemi and Mohammad Javad Malek-Hosseini leg. 17. March 2018 coll. 10 specimens in coll. Malek-Hosseini, two in coll. Jagiellonian University, Krakow.

Measurements

Holotype: H 2.39 mm; W 1.69 mm; BH 1.73 mm; NW 1.14; AH 1.69, AW 0.86 (holotype). Figure 3, A1-A6.

Diagnosis

The low spire with oval shell shape of the new species is rather unusual within the family Moitessieriidae, but a similar shape with more elevated spire is present in *Bythiospeum lamperti* (Geyer, 1907). Similar shell morphology can be found in representatives of other truncatelloid families such as: *Pseudamnicola hauffeni* Delicado and Ramos, 2012, Hydrobiidae from Spain; *Aroapyrgus pasionensis* Goodrich & Van der Schalie, 1937, Cochliopidae from Guatemala; *Tricula spelaea* Grego, 2018, Pomatiopsidae from Laos; *Pseudotricula auriforma* Ponder, Clark, Eberhard and Studer, 2005, Tateidae from Tasmania, and in the hydrobiid *Turkmenamnicola lindholmi* (Zhadin, 1952) from Turkmenistan. The cochliopid *Heleobia dobrogica* (Grossu & Negrea, 1989) found in similar sulfidic habitats in Movile Cave, Romania, has much more elongate shell shape. However, the molecular and anatomical data distinguish the new species from all other morphologically convergent species.

Description

Oval shell with conical spire with four rounded slightly convex whorls with a semi-deep suture and a blunt apex. Shell surface smooth with faint transverse growth lines covered by horny yellowish periostracum and partly by precipitated reddish-brown mineral crystals especially at the suture and marginal part of the body whorl. Aperture elongate-oval,

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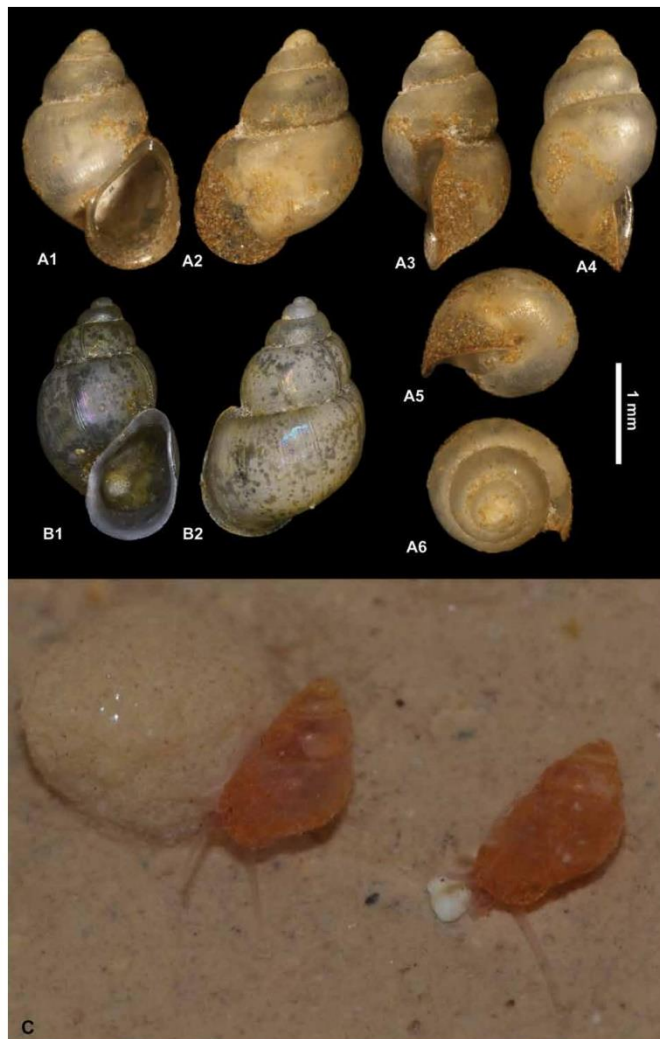


Figure 3. *Trogloiranica tashanica* n. sp., Iran, Khuzestan Province, Behbahan County, Tashan District, Sarjooshar Village, Tashan Cave. A1-A6: Holotype NMBE 558282; B1-B2: Paratype ZMUH 123; C: Live specimens of *Trogloiranica tashanica* n. sp., in their natural habitat within sulfide ponds of Tashan Cave (Specimens were transferred into a small stet basin at the pool shelf for photography) (photo: Jozef Grego, Yaser Fatemi and Mohammad Javad Malek-Hosseini).

clear H3 (Fig. 7) clearly demonstrated that *T. tashanica* belongs to the Moitessieriidae (Bootstrap support 86%). Likewise, the concatenated analysis supported this (Fig. 8).

Etymology

The species is named after the type locality, the Tashan Cave.

pear-shaped and slightly depressed form labral side, adapically separated from the body whorl by a weak furrow. Peristome margin expanded especially at its lower side and blunt along its outline. The labral lip characteristically sinuous in lateral view, columellar lip straight. Umbilicus closed and obscure.

Anatomy

Animal without eyes and pigment. Female reproductive organs (Fig. 4) typical of the Moitessieriidae: with rather big bursa copulatrix and one small receptaculum seminis (distal one (i.e., in the position of r_s after Radoman 1983)). Penis (Fig. 5) simple, without any outgrowths. Loops of rectum (Fig. 5) with the faecal pellets arranged characteristically for the Moitessieriidae (Boeters & Gittenberger 1990), like a bandolier, not a chain.

Molecular phylogenetic relationships

We present two original COI (cytochrome oxidase subunit I) (552 bp, GenBank Accession numbers MK906039-MK906040) and two histone 3 sequences (283 bp, GenBank Accession numbers MK906041-MK906042). In both of these protein-coding loci the tests of Xia et al. (2003) revealed no saturation. Topology of the trees obtained with BI and ML analyses were identical. The obtained topologies using BI and ML were congruent. The phylogeny inferred with COI (Fig. 6) suggested a close relationship with the Moitessieriidae, but did not confirm the monophyly of the latter. Between the clade consisting of the *Bythiospeum* and the other grouping *Iglica* and *Paladilhiopsis* there were representatives of the family Cochliopidae, and *T. tashanica* between the Cochliopidae and *Iglica*/*Paladilhiopsis* clade. However, the low bootstrap supports are typical of deep nodes inferred with COI. On the other hand, the phylogeny inferred with nu-

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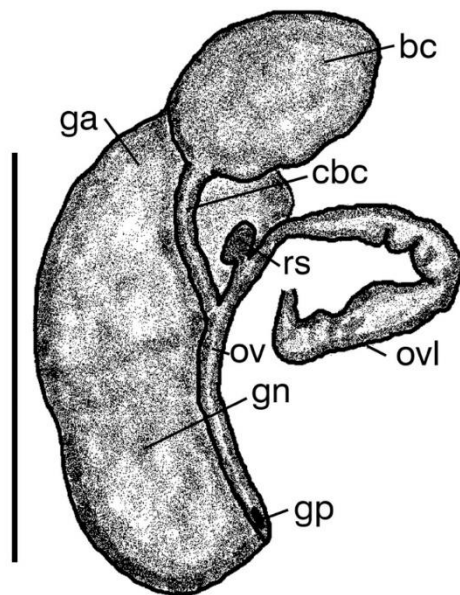


Figure 4. Renal and pallial part of the female reproductive organs of *Troglairanica tashanica* n. sp. ((bc—bursa copulatrix, cbc—duct of bursa copulatrix, ga—albumen gland, gn—capsule gland, gp—gonopore, ov—oviduct, ovl—loop of oviduct, rs—seminal receptacle); bar equals 0.5 mm.

Distribution

Known only from the type locality.

Ecology

Tashan Cave developed in Miocene limestones of Upper Red—Fars formation in the southeast part of the Zagros Mountain chain. The cave is situated under a semi-arid steppe plateau near the Sarjooshar village. The mostly horizontal cave with numerous passages and domes is several hundred meters long (detailed cave mapping is currently in progress). The morphology of the cave suggests a strong influence of sulfide-rich hydrothermal waters during the process of speleogenesis. Numerous pools and lakes inside the dark zone of the cave are rich in hydrogen sulfide, which is the main energy substrate for chemoautotrophic bacteria that utilize CO_2 and methane as a carbon source (Palmer, 2007). Preliminary stable isotope data (Sarbu, personal communication) suggest that the microbial biofilms that thrive in the cave pools represent the main source of food for the rich cave fauna including the *Troglairanica tashanica* n. sp., the blind fish *Garra tashanensis* Mousavi-Sabet, Vatandoust, Fatemi and Eagderi, 2016, and the isopod *Stenasellus tashanicus* Khalaji-Pirbalouty, Fatemi, Malek-Hosseini, Kuntner, 2018 that were recently described from this cave. Specimens of *T. tashanica* n. sp. were found on the limestone walls, on the bottom rocks and on the mud covered by microbial biofilm in the cave pools. The water temperature was 25.4 °C and the pH was 7.8. The atmospheric conditions in the cave are almost constant throughout the year at 25.2 °C – 25.4 °C, 530 ppm – 755 ppm CO_2 saturation and 99.9 % relative humidity.

DISCUSSION

Despite their ecological sensitivity and importance, subterranean ecosystems are very poorly studied worldwide and, except the areas of southern Europe, Balkans, southwestern Caucasus, eastern United States with north-east Mexico, Japan, Southeast Asia, New Zealand, and Tasmania, stygobiont gastropod records are extremely scarce (Bole and Velkovrh, 1986; Kabat and Hershler, 1993; Culver, 2012). This distribution represents only our present knowledge, while, most likely, the stygobiont Gastropoda inhabit all suitable habitats worldwide. The subterranean fauna of Iran is poorly known. Some cave-adapted groups of animals such as fishes (4 species), Crustacea (amphipods and isopods, more than 20 species), diplopods, and insects have been reported from Iran (Malek-Hosseini and Zamani, 2017). We suggest the putative presence of several groups of animals in hypogean environments of Iran, but a lot of field work is needed. *Troglairanica tashanica* n. sp. is the first obligate cave-dwelling snail from Iran. The closest stygobiont gastropod records to this Iranian species can be found in the south-eastern part of Greater Caucasus in Georgia, in the vicinity of Sochi

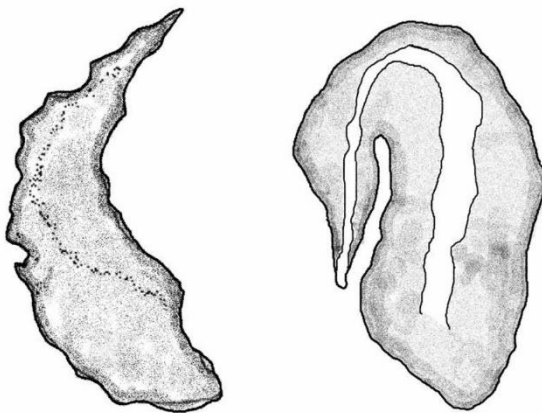


Figure 5. *Troglairanica tashanica* n. sp., A. penis: at the left dorsally, in situ, in natural position, at the right ventrally, somewhat flattened, under the cover sleep. B. loops of the rectum, with the faecal pellets arranged characteristically for the Moitessieriidae, like a bandolier, not a chain.

Troglairanica tashanica n. sp. is the first obligate cave-dwelling snail from Iran. The closest stygobiont gastropod records to this Iranian species can be found in the south-eastern part of Greater Caucasus in Georgia, in the vicinity of Sochi

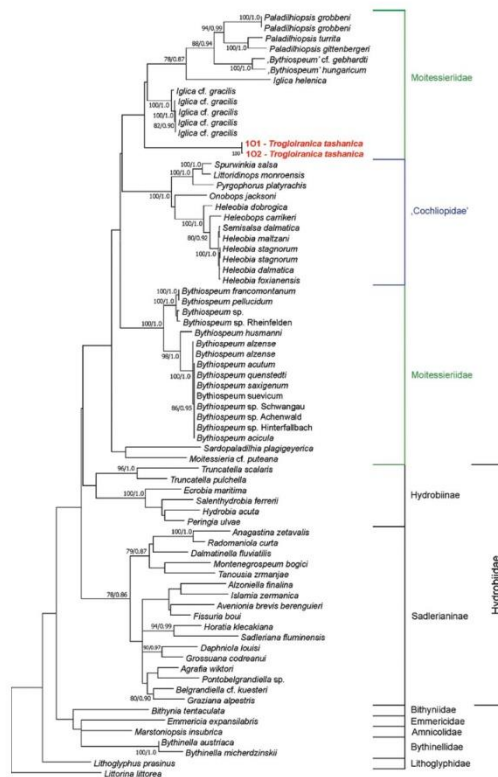


Figure 6. ML phylogeny based on COI. Bootstrap supports if > 70 % and Bayesian probabilities given. New sequences of *Troglairanica tashanica* are indicated in bold red; for the other sequences, see Table S1.

in Russia (Starobogatov, 1962; Vinarsky et al., 2014), and southern Turkmenistan (Zhadin, 1952), where we could expect the closest relations. The records from the Balkans (Glöer and Grego, 2015; Grego et al., 2017) in west and southeast Asia (Grego, 2018) towards the East, are likely not at all closely related to the Ponto-Caspian stygobionts, as it is proven for the related freshwater crenobionts of the family Hydrobiidae (Delicado et al., 2016). Of the five other known sulfide stygobiont gastropods, *Heleobia dobrogica* from Movile Cave represents the closest known lineage to *T. tashanica* n. sp. not only geographically, but also ecologically and by its three million-year old separation from its surface relatives (Falniowski et al., 2008). It appears that the other four known sulfidic stygobionts from the Frasassi caves in Italy, Lower Kane Cave in Wyoming, and Melissotrypa Cave in Greece could represent species with much younger adaptation history (Wethington and Guralnick, 2004). The communication of *Islamia sulfurea* with their surface relatives in the Frasassi caves (Bodon et al., 2009) is indicated by the distribution of the same species in other habitats and by the presence of the recent invader *Potamopyrgus antipodarum* Gray, 1843 at the same locality. This invasive species was found for the first time in Europe in 1889, and in Italy in 1961. Considering the special and rich biodiversity encountered in sulfidic caves and the estimation that 10% of the world's caves were formed by sulfidic speleogenesis (in some regions such as Greece these estimates reach up to 20 % of the accessible caves (Vaxevanopoulos, 2009), we hypothesize that the sulfide-induced high subterranean diversity persisted in most of these ex-sulfidic caves after the input of hydrogen sulfide faded out. According to our experience, the ex-sulfidic cave Tahm Nam Dôn in Khammouane, Laos, hosted significantly higher stygobiontic gastropod diversity than any other neighboring cave without traces of the sulfide corrosion in its vicinity (Grego, 2018). If so, the importance

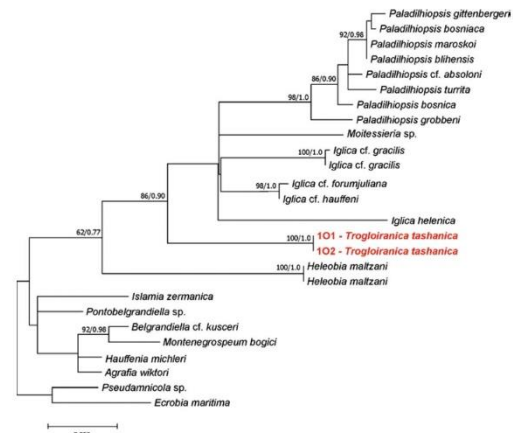


Figure 7. ML phylogeny based on H3. Bootstrap supports if > 60 % and Bayesian probabilities are given. New sequences of *Troglairanica tashanica* given in bold red for the other sequences see Table S1.

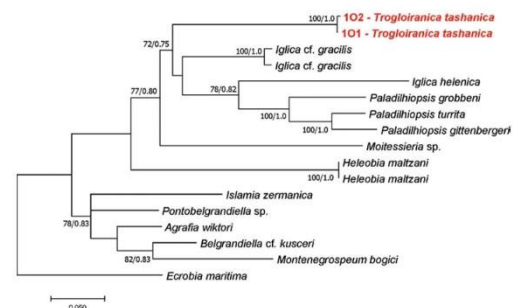


Figure 8. ML phylogeny based on concatenated data from COI and H3. Bootstrap supports if > 60 % and Bayesian probabilities are given. New sequences of *Troglairanica tashanica* given in bold red.

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of the sulfidic processes in the formation of the recent stygobiont diversity would be significantly strengthened, and the high subterranean diversity of many isolated subterranean environment could be better explained.

CONCLUSIONS

Tashan Cave likely represents another chemoautotrophically based cave ecosystem. The new species record is the sixth worldwide known stygobiont gastropod inhabiting subterranean sulfide-rich waters and the first records of stygobiont gastropod from Iran, indicating the large potential of the country for the presence of additional subterranean life forms. Although the whole ecosystem has not been well understood yet, at least we know that the water contains sulfide. The long natural history leads the subterranean ecosystems towards narrow, sometimes extreme sulfide specialization by ecological adaptation to the stable underground environment. Such a narrow specialization and long-term stable conditions reduced the adaptability of all the single ecosystem components and made it extremely sensitive against all, even very small changes, in the environmental conditions. The resulting very fragile web could be to some extent regarded as a multisymbiotic system. The disappearance of one component can lead to the collapse of the entire ecosystem. That is the main reason why the protection and conservation of such unique habitats as the Tashan Cave ecosystem have worldwide importance. Most of the caves worldwide hosting such rich sulfide ecosystems are heavily protected with restricted access, reduced to a small number of researchers per year. We believe the new locality also deserves such strict protection and that with the help of local environmentalists and cavers it can be soon achieved. Unfortunately, due to the negligence of authorities, cavers, locals, and even the research groups, a lot of habitat destruction has already occurred in this recently-discovered cave.

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Table 2 (S1): Our original sequences and additional sequences from GenBank were used in the phylogenetic analysis.

Species name	COI	H3
<i>Agrafia wiktoria</i>	JF906762	MG543158
<i>Alzoniella finalina</i>	AF367650	
<i>Anagastina zetavalis</i>	EF070616	
<i>Avenionia brevis berengueri</i>	AF367638	
<i>Belgrandiella cf. kusceri</i>	KT218511	MG551366
<i>Belgrandiella cf. kuesteri</i>	MG551327	
<i>Bithynia tentaculata</i>	AF367643	
<i>Bythinella austriaca</i>	JQ639858	
<i>Bythinella micherdzinskii</i>	JQ639854/-	
<i>Bythiospeum acicula</i>	KU341350	
<i>Bythiospeum acutum</i>	HM107120	
<i>Bythiospeum alzense</i>	KU341354	
<i>Bythiospeum francomontanum</i>	HM107128/-	
<i>Bythiospeum cf. gebhardti</i>	KP296922	
<i>Bythiospeum hungaricum</i>	KP296923	
<i>Bythiospeum husmanni</i>	HM107134	
<i>Bythiospeum pellucidum</i>	HM107124	
<i>Bythiospeum quenstedti</i>	HM107113	
<i>Bythiospeum saxigenum</i>	HM107125	
<i>Bythiospeum suevicum</i>	HM107118	
<i>Bythiospeum</i> sp.	AF367634/-	
<i>Bythiospeum</i> sp. <i>Achenwald</i>	KU341341	
<i>Bythiospeum</i> sp. <i>Hinterfallbach</i>	KU341347	
Continuation		

Continuation of Table 2 (S1)

<i>Bythiospeum</i> sp. <i>Rheinfelden</i>	KU341360	
<i>Bythiospeum</i> sp. <i>Schwangau</i>	KU341338	
<i>Dalmanella fluvialis</i>	KC344541	
<i>Daphniola louisi</i>	KM887915	
<i>Ecrobia maritima</i>	KJ406200	MG551322
<i>Emmericia expansilabris</i>	KC810060	
<i>Fissuria boui</i>	AF367654	
<i>Graziana alpestris</i>	AF367641	
<i>Grossuana codreanui</i>	EF061919	
<i>Hauffenia michleri</i>	-	KY087878
<i>Heleobia dalmatica</i>	JQ973022	
<i>Heleobia dobrogica</i>	EU938131	
<i>Heleobia foxianensis</i>	JQ973023	
<i>Heleobia maltzani</i>	KM213723	
<i>Heleobia stagnorum</i>	JQ973024	
<i>Heleobops carrikeri</i>	AF213347	
<i>Horatia klecakiana</i>	KJ159128	
<i>Hydrobia acuta</i>	AF278808	
<i>Iglica</i> cf. <i>gracilis</i>	MH720985-MH720989	MH721003- MH721004
<i>Iglica</i> cf. <i>hauffeni</i>		MH720995
<i>Iglica</i> cf. <i>forumjuliana</i>		MH721006
<i>Iglica hellenica</i>	KT825581	MH721007
<i>Islamia zermanica</i>	KU662362	MG551320
<i>Lithoglyphus prasinus</i>	JX073651	
<i>Littoridinops monroensis</i>	AF367644	

Continuation

Continuation of Table 2 (S1)

<i>Littorina littorea</i>	KF644330	
<i>Marstoniopsis insubrica</i>	AF322408	
<i>Moitessieria</i> cf. <i>puteana</i>	AF367635	MH721012
<i>Montenegrospeum bogici</i> ()	KM875510	MG880218
<i>Onobops jacksoni</i>	AF367645	
<i>Paladilhiopsis</i> cf. <i>absoloni</i>		MH721021
<i>Paladilhiopsis blihensis</i>		MH721015
<i>Paladilhiopsis bosniaca</i>		MH721020
<i>Paladilhiopsis bosnica</i>		MH721021
<i>Paladilhiopsis grobbeni</i>	MH720990-MH720991	MH721013
<i>Paladilhiopsis maroskoi</i>		MH721017
<i>Paladilhiopsis turrita</i>	MH720992	MH721015
<i>Paladilhiopsis gittenbergeri</i>	MH720993	MH721025
<i>Peringia ulvae</i>	AF118302	
<i>Pontobelgrandiella</i> sp.	KU497024	MG551321
<i>Pseudamnicola</i> sp.		KT710579
<i>Pyrgophorus platyrachis</i>	AF367632	
<i>Radomaniola curta</i>	KC011814	
<i>Sadleriana fluminensis</i>	KF193067	
<i>Salenthidrobia ferrerii</i>	AF449213	
<i>Sardopaladilhia plagigeyrica</i>	HQ623176	
<i>Semisalsa dalmatica</i>	AF367631	
<i>Spurwinkia salsa</i>	AF367633	
<i>Tanousia zrmanjae</i>	KU041812	
<i>Trogloiranica tashanica</i>	MK906040-MK906039	MK906042-MK906041

Continuation

Continuation of Table 2 (S1)

<i>Truncatella pulchella</i>	AF253085
<i>Truncatella scalaris</i>	JX970621

2.1.3 A new obligate groundwater species of *Asellus* (Isopoda: Asellidae) from Iran

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Naslov: Nova obligatna podzemeljska vrsta iz rodu *Asellus* (Isopoda, Asellidae) iz Irana

Izvleček:

Z le 43 opisanimi stigobionti in le dvema vrstama enakonožnih rakov je obligatna podzemeljska favna Irana, obsežne države z več kot 10 % površine apnenca, nezadostno poznana. Tu poročamo o odkritju vrste *Asellus ismailsezarii* sp. nov. iz gorovja Zagros, prvega brezokega in depigmentiranega asellidnega enakonožca iz Irana. Nova vrsta je morfološko podobna vrsti *Asellus monticola* Birstein, 1932, vendar je brez oči in popolnoma depigmentirana, ima rahlo ukrivljen pereopod IV in nima sete na proksimalnih robovih eksopodita pleopodov IV in V. Filogenetska razmerja med vrstami z uporabo izvirne in podatkovno rudarjene mitohondrijske DNK in jedrne rDNK ter ocena molekularnih razlik z drugimi vrstami rodu *Asellus* kažejo, da je *A. ismailsezarii* sp. nov. sestrška vrsta večjega klada, ki vsebuje tudi evropski kompleks vrst *A. aquaticus*. Površinske populacije vrst iz rodu *Asellus* so ob različnih priložnostih in v več krajih v Evropi in Aziji kolonizirale podtalnico, pri čemer so nastale vrste in podvrste, ki so razvile troglomorfizme, kot sta depigmentacija in izguba oči. Od 37 uradno opisanih vrst in podvrst rodu *Asellus* jih je 15 iz podzemne vode, vključno z *A. ismailsezarii* sp. nov. Predvidevamo, da bo v Aziji odkritih še veliko vrst rodu *Asellus*, ki obligatno poseljujejo podzemne vode.

Ključne besede

Azija, Crustacea, podzemeljske vode, molekularna filogenija, taksonomija, troglomorfija

A new obligate groundwater species of *Asellus* (Isopoda, Asellidae) from Iran

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Abstract

With only 43 described stygobionts and only two isopod species the obligate groundwater fauna of Iran, a vast country with over 10% of limestone surface, is inadequately known. Here, we report the discovery of *Asellus ismailsezarai* sp. nov. from Zagros mountains, the first eyeless and depigmented asellid isopod from Iran. The new species is morphologically similar to *Asellus monticola* Birstein, 1932, but it is eyeless and fully depigmented, has a slightly curved pereopod IV and does not bear any setae on proximal margins of exopodite of pleopods IV and V. Species phylogenetic relationships using original and datamined mitochondrial DNA and nuclear rDNA, and estimation of molecular divergences with other *Asellus* species, suggest that *A. ismailsezarai* sp. nov. is sister to a larger clade that also contains the European *A. aquaticus* species complex. Surface populations of *Asellus* have colonized groundwater at multiple occasions and localities, both in Europe and Asia, giving rise to species and subspecies that have evolved troglomorphisms, such as depigmentation and loss of eyes. Of the 37 formally described species and subspecies of *Asellus*, 15 are from groundwater, including *A. ismailsezarai* sp. nov. We predict that many more obligate groundwater *Asellus* taxa are yet to be discovered in Asia.

Keywords

Asia, Crustacea, groundwater, molecular phylogeny, taxonomy, troglomorphy

Introduction

Groundwater harbors a high diversity of metazoans that represents an important, yet underestimated, component of the Earth's freshwater biodiversity (Culver and Holsinger 1992; Dole-Olivier et al. 2009). Knowledge of groundwater biodiversity is uneven across the globe: species inventories are far more advanced in Europe, North America, and Australia, for example, than in South America, Africa and Asia (Zagmajster et al. 2018). We know of only 43 obligate groundwater species from Iran, despite the fact that 10.5% of the country (compared with approximately 1.7% of Europe) is underlain by carbonate rock formations. The obligate groundwater fauna includes four fish species, 23 amphipods of the genus *Niphargus* Schiødt, 1847, 12 copepods, one oligochaete, one gastropod and two isopods (Cichocka et al. 2015; Mousavi-Sabet et al. 2016; Malek-Hosseini and Zamani 2017; Vatandoust et al. 2019; Bargrizaneh et al. 2021; Malek-Hosseini et al. 2021). The only two obligate groundwater isopods known from Iran are *Microcharon raffaellae* Pesce, 1979 (Lepidocharontidae) collected from a well in Pliocenic sandstone and clay at Shahr-e-Kord city, Chaharmahal and Bakhtiari Province, and *Stenasellus tashanicus* Khalaji-Pirbalouty, Fatemi, Malek-Hosseini & Kuntner, 2018 (Stenasellidae) collected from Tashan karstic cave in Khuzestan province (Pesce 1979; Khalaji-Pirbalouty et al. 2018). In the present article, we describe the third obligate groundwater isopod from Iran belonging to the family Asellidae (Pancrustacea, Isopoda, Asellota).

The Asellidae is one of the few families of metazoans containing a large number of both surface and subterranean aquatic species. To date, the family contains 23 genera and 428 species and subspecies (Malard et al. in press). Of these 428 species and subspecies, 279 are eyeless and depigmented obligate groundwater species, 18 are mostly confined to groundwater, often showing reduced eyes and pigmentation, and 131 are oculated and pigmented surface water species. The family is widely distributed in the northern hemisphere with species in northern part of North America, Europe, northern Africa and Asia. The systematics of asellids is not yet fully established but morphological and molecular evidence support the existence of three major clades whose species occur in America, Europe and Northern Africa, and Asia, respectively (Morvan et al. 2013). One of these major clades to which Henry and Magniez (1995) referred to as the “*Asellus* pattern” contains 59 species and subspecies among which 26 inhabit surface waters and 33 groundwaters. They belong to the genera *Asellus* Geoffroy, 1762, *Calasellus* Bowman, 1981, *Columbasellus* Lewis, Martin & Wetzer, 2003, *Limnoasellus* (*Nomen nudum* in Hidding et al. 2003), *Mesoasellus* Birstein, 1939, *Nipponasellus* Matsumoto, 1962, *Phreatoasellus* Matsumoto, 1962, *Sibinasellus* Henry & Magniez, 1993 and *Uenasellus* Matsumoto, 1962.

With the exception of the genus *Asellus*, all genera within the “*Asellus* pattern” have relatively narrow distribution ranges either in Lake Baikal, Far East Russia, South Korea, Japan, or the Pacific Northwest coast of North America (Matsumoto 1963, 1966;

Bowman 1981; Henry and Magniez 1991, 1993, 1995; Hidding et al. 2003; Lewis et al. 2003; Sidorov and Prevorčnik 2016). The genus *Asellus* shows a wide geographic range, being present in Alaska (1 species, Bowman and Holmquist 1975), Asia (15 species, Henry and Magniez 1995; Sidorov and Prevorčnik 2016) and Europe (2 species). The occurrences of *Asellus* in Europe were all attributed to the *Asellus aquaticus sensu lato* species complex (Verovnik et al. 2005), with the exception of occurrences from two nearby surface water sites in eastern Ukraine, which were attributed to *Asellus* sp. (River Kharkiv and Pionersky pond; see Verovnik et al. 2005). The taxonomic status of *Asellus* populations within the *A. aquaticus* complex is not yet fully resolved. That complex actually contains two species, *A. aquaticus* Linnaeus, 1758 and *A. kosswigi* Verovnik et al., 2009, and eleven formally described subspecies (Sket 1965; Turk-Prevorčnik and Blejec 1998). COI-based species delimitation methods indicate that there may be many more species (Sworobowicz et al. 2015, 2020). Also, several *Asellus* populations belonging to that complex have colonized caves in Europe and have evolved traits such as loss of pigmentation and eye reduction (Balázs et al. 2021).

The eastern geographic boundaries of the *A. aquaticus* complex are not clearly established. The global biodiversity information facility does not report any records of *A. aquaticus* Linnaeus, 1758 in Iran (consultation date: 16/11/2021). However, the presence of that species was reported by Rémy (1941) at a pond located 6 km from Chahi, Mazandaran province, and more recently by Henry and Magniez (1995) at a surface freshwater site in the Gorgan province contiguous to Turkmenistan. Birstein (1945) described *Asellus aquaticus messerianus* from Turkmenistan Lakes Delili, Yashka and Karatogelek located near the northern border of Iran. Another asellid species, *Asellus monticola*, originally described by Birstein (1932) from freshwater surface sites in Armenia and Georgia was collected in Northern Iran at Bora Laan Spring, a locality located near the Turkish border (Henry and Magniez 1996).

Here, we report on the discovery of *Asellus ismailsezarii* sp. nov. (Asellidae, Asellota, Isopoda, Pancrustacea), the first eyeless and depigmented asellid isopod described from Iran. We also provide morphological comparisons with *A. monticola*, the species that morphologically resembles *Asellus ismailsezarii* sp. nov. as well as comparisons with European cave-species and subspecies belonging to the *A. aquaticus* complex. We then use molecular data to corroborate the species status of *A. ismailsezarii* sp. nov. and to document its phylogenetic relationship to the *A. aquaticus* complex.

Material and methods

Sampling

On several occasions from December 2018 to June 2019, we collected by hand four males, four females and 17 juveniles of *Asellus* at Ganow (Gandab) spring, Iran. This karstic spring is seasonal, flowing during winter and spring. The spring is located close to Tuveh village, Andimeshk, Khuzestan Province (geographic coordinates: 32°48'31"N, 48°43'32"E; altitude: 470 m above sea level) (Fig. 1).

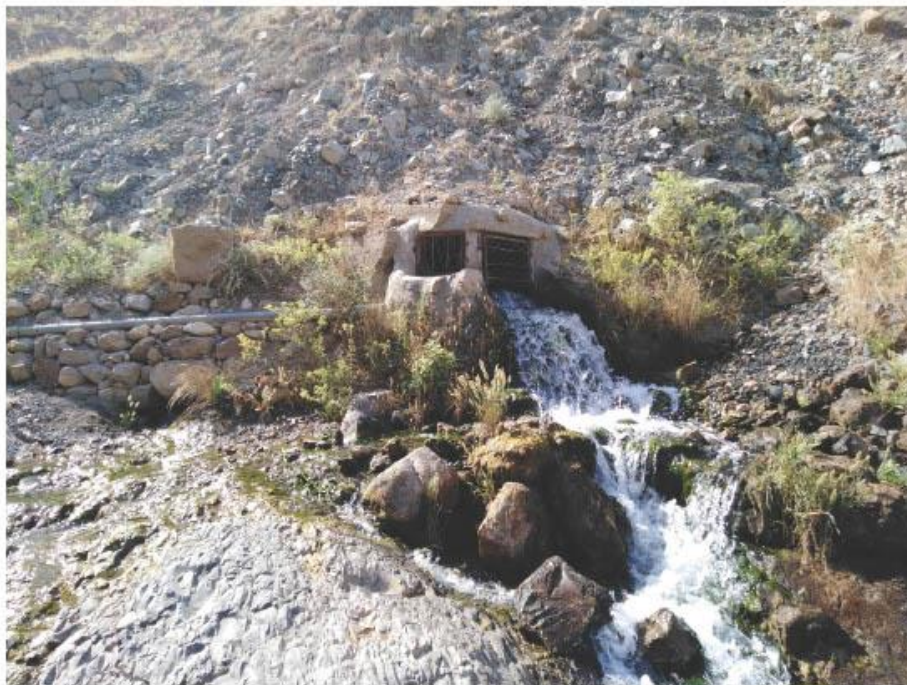


Figure 1. Photo of Ganow spring, the type locality of *Asellus ismailsezarai* sp. nov., Tuveh village, Andimeshk, Khuzestan Province, Iran. Stream width is about one meter.

Morphological description

When necessary, pereopods I, IV and VII from one side of the body were dissected and heat-treated in a KOH solution, dyed with Trypan Blue for better visibility of spinulation and then temporary mounted on slides in glycerine. After dissection, also antennae I, II, pleopods, uropods and pleotelson, were mounted on slides in glycerine. Specimens and their body parts were photographed and measured using a Sony DXC390P digital camera mounted on a stereomicroscope or microscope (depending on the size of the structure), and measured with Leica Application suite – LAS EZ. The remains of the dissected specimens were then transferred to 70% ethanol for storage. In one of the specimens from the type locality, all appendages were dissected and prepared for drawing. All pereopods, as well as the trunk were heat-treated and dyed as described above, and then temporarily mounted in glycerine, alongside the rest of dissected specimen (mouth appendages, antennae, pleopods, uropods). Vector drawings were made from microphotographs using a graphics tablet (Wacom, Cintiq 13HD Creative Pen Display) and the free software KRITA 4.1.1 (<https://krita.org/>). In males, we measured 90 morphometric characters (cf. Prevorčnik et al. 2004) for describing body proportions (trunk, appendages) and characterizing cuticular and sensory structures (number and length of spines, setae, aesthetascs). In antenna II, only the last two articles of the

antennal basis (fourth and fifth) were included in the total antennal length together with the length of the flagellum. In pereopods IV and VII, length of the coxa was not included in the total pereopod length. We did not report full length of antenna II and pereopods because in most cases the basal articles of both appendages remained attached to the body during dissection. Throughout the ensuing description, we provide a single measurement value for the holotype, together with a range of values (in parentheses) referring to the males (♂♂) and females (♀♀) from the type material.

For observation with scanning electron microscopy (SEM), Plp I and Plp II of the male holotype and paratype stored in 70% ethanol were air-dried and mounted on sample stubs using conductive double sided carbon tape. Mounted samples were sputter-coated with platinum and observed with a JEOL JSM-7500F field emission scanning electron microscope (Jeol. Japan) at the Department of Biology, Biotechnical Faculty, University of Ljubljana.

Molecular taxonomy

DNA extraction, amplification and gene sequencing

We used the last three pereopods of three specimens (specimens AS1, AS2 and AS3, see Material examined) of *A. ismailsezarii* sp. nov. to obtain sequences of the mitochondrial cytochrome oxidase subunit I (COI) gene and 28S nuclear rDNA gene. We performed molecular analyses at the Evolutionary Zoology Laboratory, Jovan Hadži Institute of Biology ZRC SAZU, Ljubljana (EZ LAB, Slovenia) and LEHNA laboratory, Villeurbanne (LEHNA, France). We crosschecked DNA extraction protocols for the COI gene using specimen AS1 and DNA extraction and PCR protocols for the 28S using specimens AS1 and AS2. Full detail of the molecular protocol for each gene and specimen is provided in Suppl. material 1: Table S1 and Suppl. material 2: Table S2.

We performed robotic DNA extraction at EZ LAB on the three specimens using Mag MAX™ Express magnetic particle processor Type 700 with DNA Multisample kit (Thermo Fisher Scientific kit) and modified protocols following Vidergar et al. (2014). We also extracted DNA from specimen AS1 at LEHNA using a modified Chelex protocol from Casquet et al. (2012). We incubated pereopods in a solution of 150 µl of 7% chelex and 10 µl of proteinase K at 15 mg / ml for 90 minutes at 56 °C, then 15 minutes at 90 °C.

We performed polymerase chain reactions (PCRs) for COI fragments at LEHNA using a previously optimized protocol (Calvignac et al. 2011). However, we used a Taq polymerase (Eurobiotaq) amount of 0.05 U instead of 0.15 U and a PCR volume of 25 µl instead of 35 µl. To detect amplification of nuclear paralogs of mtDNA, we used a mix of four half-nested PCR strategies applied on a dilution range of initial DNA template (pure DNA solution of 2 µl to 1/2500 dilution, primers in Suppl. material 1: Table S1 and Suppl. material 2: Table S2). We ran the first round PCRs with one step of 3 min at 95 °C; 37 cycles of 20 s at 95 °C, 30 s at 51 °C, 45 s at 72 °C; and one step of 5 min at 72 °C. The second round PCRs were run on 1 µl of the first round PCR product using the same parameters but 35 cycles. Service providers (BIOFIDAL, Vaulx-en-Velin, France) performed Sanger sequencing using PCR amplification primers.

We performed PCRs for 28S fragments at EZ LAB on the three specimens and at LEHNA on specimens AS1 and AS2. At EZ LAB we performed PCRs in 25 µl final volume using H₂O: 18.3 µl, 10X Buffer: 2.5 µl, dNTPS (20 mM): 0.5 µl, Primer (10 µM): 0.6 µl forward and 0.6 µl reverse, polymerase (Eurobiotaq 5U/µl): 0.25 µl, and BSA (10 mg/ml): 0.25 µl (primers in Suppl. material 1: Table S1 and Suppl. material 2: Table S2). We applied the following PCR settings: one step of 3 min at 94 °C; 36 cycles of 45 s at 94 °C, 1 min at 47 °C, 1 min at 72 °C; and one step of 3 min at 72 °C. PCR products were sent to Macrogen Europe (Amsterdam, the Netherlands) for sequencing. At LEHNA, we amplified 28S fragments with 2 independent pairs of primers (Suppl. material 1: Table S1 and Suppl. material 2: Table S2) using the following settings: one step of 3 min at 95 °C; 37 cycles of 30 s at 95 °C, 30 s at 62 °C, 30 s at 72 °C; and one step of 5 min at 72 °C. BIOFIDAL performed Sanger sequencing. We visualized all chromatograms using FinchTV (Geospiza, Seattle, WA, USA). Sequences were submitted to GenBank (accession numbers [OM640761–OM640764](#); [OM501360–OM501366](#), Suppl. material 3: Table S3).

Molecular species delimitation

Species and higher taxa and their associated names represent hypotheses of independently evolving lineages that should ideally be supported by different lines of evidence (de Queiroz 2007; Padial et al. 2010; Fišer et al. 2018). In addition to morphological evidence, we used a COI-based molecular species delimitation approach to corroborate whether *A. ismailsezarii* sp. nov. was indeed a new species. In effect, we follow the diagnosable interpretation of the phylogenetic species concept (de Queiroz 2007).

COI sequences of *A. ismailsezarii* sp. nov. produced in this study were analysed with all COI sequences of taxa belonging to the “*Asellus* pattern” (*sensu* Henry and Magniez 1995) available in NCBI (Sequence extraction date: 25 August 2021). In addition to sequences of *A. ismailsezarii* sp. nov., we retrieved 581 sequences belonging to the *A. aquaticus sensu lato* species complex (including *A. kosswigi*, Verovnik et al. 2005, 2009; Sworobowicz et al. 2015, 2020; Lafuente et al. 2021), *Asellus* sp. from Ukraine, *Asellus hilgendorffii* from Japan, and two species from Lake Baikal, Russia – *Mesoasellus dybowskii* and *Limnoasellus poberezhnii* (*Nomen nudum* in Hidding et al. 2003) (Suppl. material 3: Table S3). We aligned the sequences with ClustalO in Seaview (Gouy et al. 2010) (Suppl. material 4: Table S4). We checked visually for the presence of anomalies, including stop codons and frameshifts, and removed ambiguous sites with the embedded Gblocks (with options for less stringed selection enforced) (Castresana 2000). COI maximum-likelihood phylogenies were reconstructed using PhyML (Guindon et al. 2010) under the GTR + G + I model of substitution with 4 substitution rate categories and a gamma shape parameter ($\alpha = 0.519$) as well as a proportion of invariant sites (0.392) estimated by maximum likelihood in PhyML. This model of evolution was previously used in several studies for delimiting species and reconstructing phylogenetic relationships among asellids (Lefébure et al. 2006; Morvan et al. 2013; Eme et al. 2018). We used MEGA (Kumar et al. 2018) as well, to find the best nucleotide substitution model and the optimal partitioning scheme for each marker. Molecular operational taxonomic units

(MOTUs) were delimited using the fixed threshold method implemented by Lefébure et al. (2006) for crustaceans. This method is based on the observation made from 1500 COI sequences belonging to 276 species of crustaceans that two clades diverging by more than 0.16 substitution per site, as measured by patristic distances, have a strong probability (ca. 0.99%) of belonging to different species. That method is conservative insofar as it identifies both fewer MOTUs and MOTUs that are more divergent than other delimitation methods, thereby limiting the risk of considering two populations of the same species as belonging to two distinct MOTUs. Several studies (Morvan et al. 2013; Sworobowicz et al. 2015; Erme et al. 2018) showed that evolutionary units of Asellids as delimited by distance-based methods, including that implemented by Lefébure et al. (2006), were further subdivided by tree-based methods such as the General Mixed Yule-Coalescent method (Pons et al. 2006) and the Poisson tree processes (Zhang et al. 2013). Our conservative estimate avoids potential oversplitting.

We used the R package “ape” (Paradis et al. 2004) to compute patristic distances and “cluster” to delimit MOTUs (Maechler et al. 2012).

To further assess the genetic differentiation of *A. ismailsezarii* sp. nov., we reconstructed the most likely phylogenetic relationships among taxa belonging to the “*Asellus* pattern” using all 28S sequences available in NCBI. Non-overlapping sequences (accession number KX467625) and short sequences (less than 300 bp, accession numbers AY739195 and HG322482) were excluded from the analyses. In addition to sequences of *A. ismailsezarii* sp. nov., we obtained 23 sequences belonging to the *A. aquaticus sensu lato* species complex (including *A. kossigii*), *Asellus* sp. from Ukraine and *A. hilgendorffii* from Japan (Suppl. material 3: Table S3). As part of this study, we produced 28S sequences for *M. dybowskii* and *L. poberezhnii* using protocols described above for *A. ismailsezarii* sp. nov. We aligned sequences in Seaview with MAFFT (Katoh and Standley 2013) under the e-insi setting and removed ambiguous sites with the embedded Gblocks (with default options) (Suppl. material 5: Table S5). We reconstructed most likely relationships in the same way as for the partial COI gene.

Results

Taxonomy

Asellidae Rafinesque, 1815

***Asellus* E. L. Geoffroy, 1762**

***Asellus ismailsezarii* Malek-Hosseini, Jugovic, Fatemi & Douady, sp. nov.**

<http://zoobank.org/5AD48986-0231-4775-9D8F-6F0D30169382>

Figs 2–7

Material examined. Holotype: mature male, body length (BL) 8.2 mm (TU-SP1), Ganow spring, Tuveh village, Andimeshk, Khuzestan Province, Iran, (32°48'31"N, 48°43'32"E, altitude 470 m above sea level). coll. Yaser Fatemi; 01. June.2019.

Paratypes: 2 mature males, BL 8.2 mm (TU-SP2), BL 6.5 mm (TU-SP5), 1 juvenile male, BL 7.3 mm (TU-SP7); 3 mature females, BL 8.1 mm (TU-SP3), BL 8.1 mm (TU-SP4), BL 6.7 mm (TU-SP6); data same as holotype. **Other material:** 17 juvenile specimens (including specimens AS2 and AS3), data same as holotype. 1 mature female, BL 8.6 mm (specimen AS1), 01. December.2018, Same locality as holotype. coll. Mohammad Javad Malek-Hosseini and Yaser Fatemi. All material was preserved in 70% ethanol and deposited at UCBLZ (University Claude Bernard Lyon-Zoology, Lyon, <http://cerese.univ-lyon1.fr/>), under deposit numbers 2012.11.23.91–2012.11.23.99 and 2012.11.24.1–2012.11.24.47. We also deposited tubes containing stubs onto which pleopods I and II of holotype and paratype were mounted for producing SEM images.

Diagnosis of male. Species of *A. ismailsezar* sp. nov. with depigmented body and complete loss of eyes (Fig. 2). Body elongated oval, more than three times (3.2–3.5) as long as wide. Head up to 1.4 times as wide as long; frontal margin bisinuate, medially concave, lateral margins rounded, each with posterolateral prominence with few weak setae. Pereonites I–III with slightly convex lateral margins, and pereonites IV–VII with straight lateral margins, with only few small simple short setae of about the same length. Pleomere I–II width about 60% of pereonite VII width. Pleotelson subrectangular, lateral margins densely fringed with simple short setae of about the same length, terminal edge rounded, with only weak median prominence. Antennae I and II, lengths about 15% and 70% of body length and with 8 and 55 flagellar articles, respectively. Propodus I slender ovoid, with weak apophysis on palmar margin. Carpus and propodus IV only slightly curved, superior margin and submarginal surface of propodus IV with few (6) short and weak simple setae. Length of longest spiniform



Figure 2. Habitus of *Asellus ismailsezar* sp. nov. (Female AS1). The photo shows a specimen exposed to air after the stone it was under had been turned over. Body length is 8.6 mm.

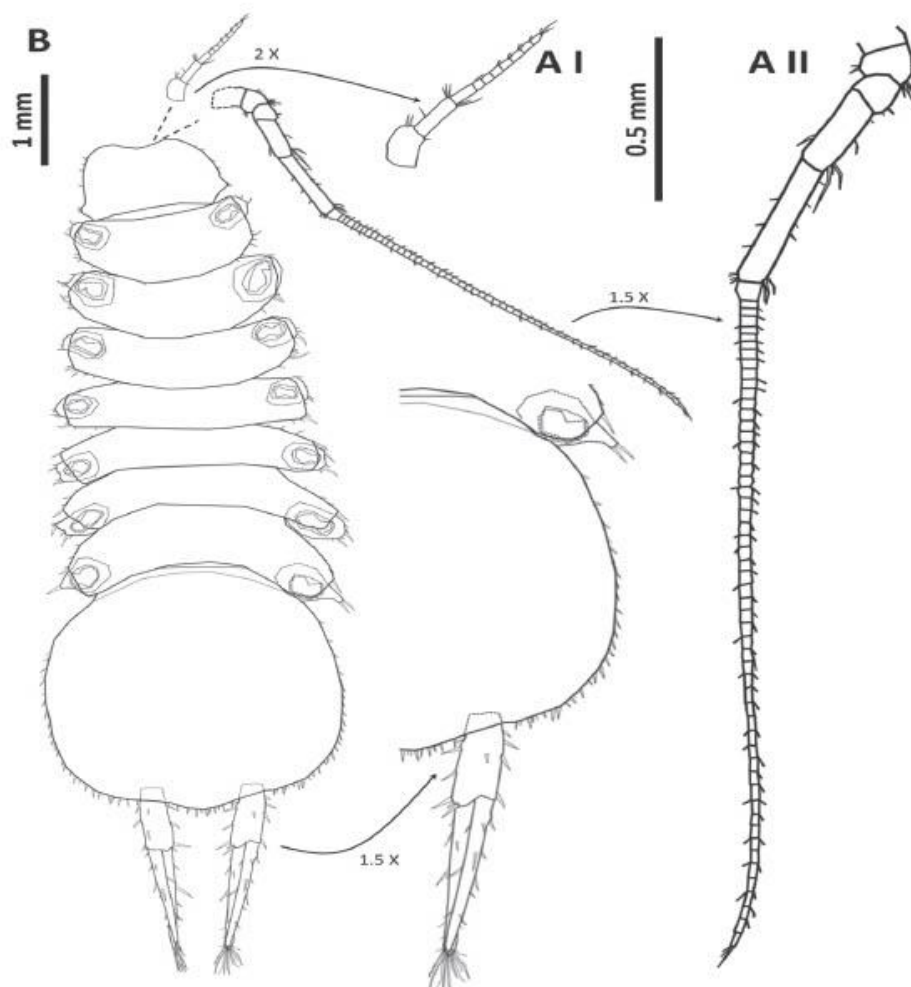


Figure 3. *Asellus ismailbezarai* sp. nov., Ganow spring, Western Iran. Holotype male, 8.2 mm: **A I, II** antennae I and II **B** body dorsal view.

robust seta on propodus VII inferior margin only about 3% pereopod VII length. Pleopod I exopodite without symmetric concavity on lateral margin, lateral margin being rather straight to slightly convex. Pleopod II architecture following typical “*Asellus* pattern” sensu Henry and Magniez (1995). Its endopodite with short (around 25% of protopodite length) horn-shaped basal spur (*processus calcariformis*). Pleopod IV and V with small respiratory areae, linea areae beginning and ending at the distal exopodite margin. Uropod short, up to about a quarter of body length.

Description of holotype and male type material (values in parenthesis). Body (Fig. 3B) 3.4 (♂♂: 3.3–3.5 ♀♀: 3.3–3.8) times as long as wide, elongated oval,

without pigmentation. Head (Fig. 3B) width 1.3 ($\sigma\sigma$: 1.1–1.4; $\varphi\varphi$: 1.3–1.4) times of its length. Frontal margin bisinuate, medially concave, without rostral process, lateral margins rounded, each with small posterolateral protuberance, with few small, short stiff setae. No eye structures visible.

Pereonites (Fig. 3B) I–III with straight to slightly convex lateral margins and weakly rounded antero- and posterolateral angles, slightly protruding forwards. Last three pereonites with antero- and posterolateral angles slightly protruding backwards. Pereonite VI widest. Anterolateral, lateral and posterolateral margins of pereonites almost bare, with only sparse short simple setae. Coxopods well developed, margins of all epimerae dorsally visible, last three the most prominent.

Pleomere I–II short but wide (Fig. 3B), their width about 62% of pereonite VII width, forming a stalk largely covered by posterior margin of pereonite VII. Pleotelson (Fig. 3B) subrectangular, its width 1.02 ($\sigma\sigma$: 1.02–1.13; $\varphi\varphi$: 0.96–1.01) times of its length, terminal edge rounded, with only weak median prominence between uropods. Lateral margins in their anterior quarters with few short setae, other three quarters with 30 ($\sigma\sigma$: 26–30; $\varphi\varphi$: 26–36) (left side of pleotelson) and 32 ($\sigma\sigma$: 25–32; $\varphi\varphi$: 29–35) (right side of pleotelson) dense but minute spiniform setae of about the same length. Terminal edge with a series of 12 ($\sigma\sigma$: 11–15; $\varphi\varphi$: 11–19) short setae between the uropods. Dorsal surface almost bare, without setation.

Antenna I length (Fig. 3AI) 15% ($\sigma\sigma$: 15–16%; $\varphi\varphi$: 16–17%) of body length, with 3 peduncular segments. First segment robust, with curved superior (longer) and inferior (shorter) margin, other two segments cylindrical. Second segment 1.3 ($\sigma\sigma$: 1.26–1.33; $\varphi\varphi$: 0.98–1.30) times as long as first and 1.9 ($\sigma\sigma$: 1.28–1.88; $\varphi\varphi$: 1.43–2.08) times as long as third. Longest setae on segment 1 approximately as long as width of second article, and length of longest setae on segment 2 about as long as article 3. Flagellum of 8 segments ($\sigma\sigma$: 6–8; $\varphi\varphi$: 7–8), with 5 ($\sigma\sigma$: 3–5; $\varphi\varphi$: 1–4) aesthetascs on distal segments. Aesthetascs shorter as their parallel segments.

Antenna II length (Fig. 3AII) 62% ($\sigma\sigma$: 50–62%; $\varphi\varphi$: 80–81%) of body length, with 6 peduncular and 55 ($\sigma\sigma$: 32–55; $\varphi\varphi$: 47–55) flagellar segments. Sixth peduncular segment 1.6 ($\sigma\sigma$: 1.39–1.61; $\varphi\varphi$: 1.39–1.43) times as long as fifth, both with only short setae, long setae present only on superior distal angles. Flagellum length 77% ($\sigma\sigma$: 66–77%; $\varphi\varphi$: 76–77%) of antenna II length.

Mandibulae (Fig. 4MdbL) robust: *Pars molaris* (molar process) U-shaped, with toothed margin. *Pars incisiva* (incisor) formed by few blunt cusps arranged in semi-circle. Left *lacinia mobilis* with few cups and spine row of about 15 biserrate setae, the distal ones being longest. Palp of three segments. First palpal segment widest, with few simple setae distally. Second palpal segment 1.6 times longer than first, without setation along external margin, with two simple setae distalo-mesially, and few simple setae along internal margin. Third palpal segment around half as long as second, with row of about 20 robust biserrate setae along external margin. Maxilla I (maxillule; Fig. 4MxI) lateral lobe with few smooth (the outer spines cone-shaped) and few weakly serrate robust spines. Distal part of outer margin with 1 long slender seta. Mesial lobe with 4 robust long plumose setae. Maxilla II (Fig. 4MxII) lateral and middle lobe with 19 slender and simple, and 13 curved pectinate robust setae, respectively, mesial lobe

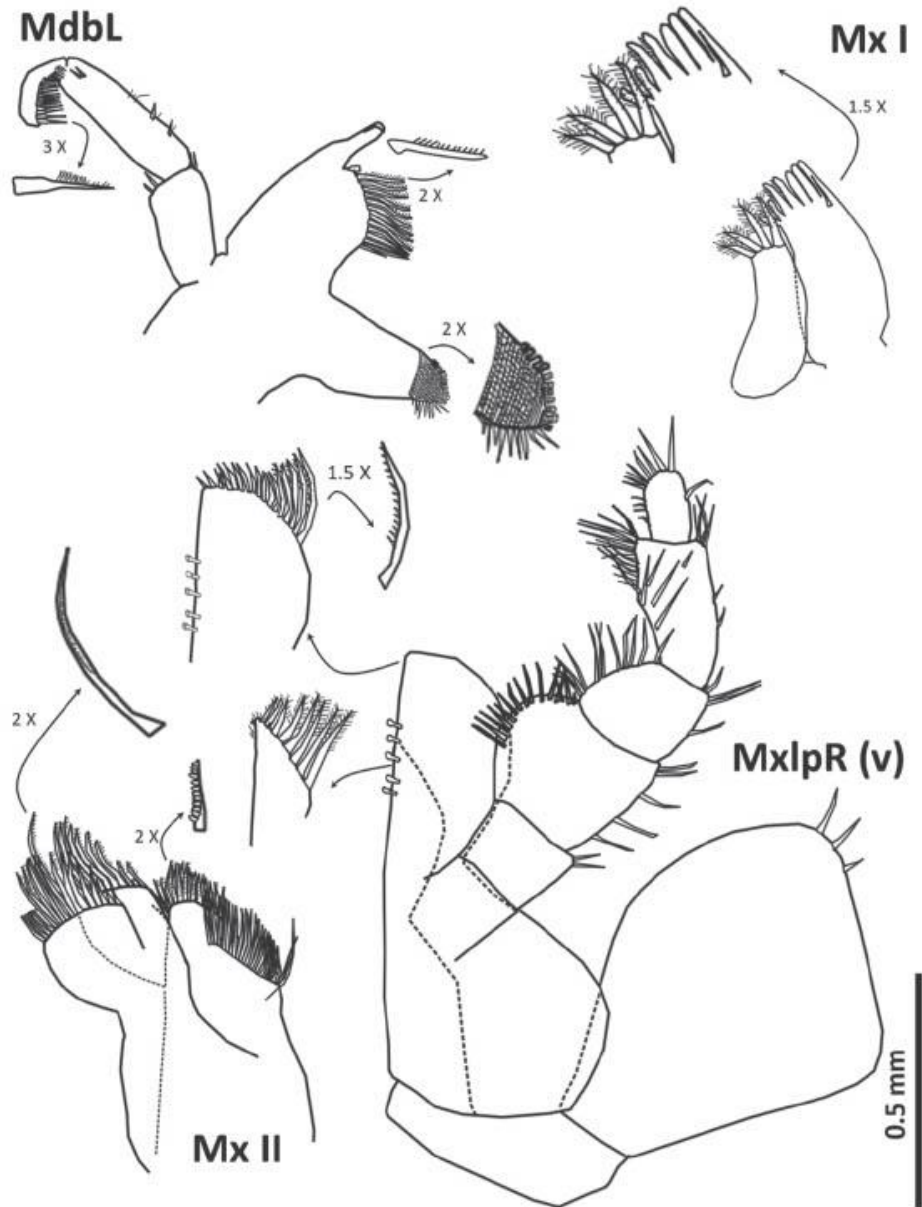


Figure 4. *Asellus ismailsezarrii* sp. nov., Ganow spring, Western Iran. Paratype male, 8.1 mm (TU-SP. 2), mouthparts: MdL, left mandible; Mx I, maxilla I; Mx II, maxilla II, MxlpR (v), right maxilliped (ventrally).

with about 10 biserrate setae and parallel row of about 13 long simple setae along inner margin. Maxilliped (Fig. 4MxlpR) endite distal margin with about 11 biserrate robust setae, subapically with several rows of short simple setae. Mesial margin curved dorsally, with row of about 8 long biserrate setae, distomesial margin with setulose

fringe and around 5 coupling hooks. Palp of five articles. First article with 2 short setae apically on outer margin; second about 2.5 times as long as first, subtrapezoidal, with 5 long stiff setae on outer margin and row of about 15 longer medially directed simple setae on inner margin. Third article a bit shorter as second, less broad, with 4 long stiff setae on outer margin and row of about 11 slender simple setae on inner margin. Fourth article approximately twice as long as third, slender, distally wider, with a row of 6 and about 20 long slender setae along outer and inner margin, respectively. Fifth article as long as first, ovoid, fringed with around 10 long slender setae and 2 longest stiff simple apical setae. Epipodite subrectangular, lateral margin almost bare, with only few (3) short simple setae.

With the exception of the first and the fourth pair, seven pairs of pereopods similar in construction and ambulatory, increasing in length towards posterior pairs. Pereopod I (Fig. 5PpI) grasping, subchelate. Propodus I (article 6) slender ovoid, 2.2 (♂♂: 2.2–2.5; ♀♀: 2.6–2.8) times as long as wide, with weakly expressed proximal apophysis with few stronger spiniform setae (♂♂: 3–4; ♀♀: 2–4) and other sparsely set row of shorter simple setae (about 10 in total). Dactylus I (article 7) length about 70% (♂♂: 70–82%; ♀♀: 70–78%) of propodus length, with 5 (♂♂: 4–6; ♀♀: 5) sparsely placed slender stiff robust setae along inferior margin (their length increasing towards unguis). Pereopod I length 31% (♂♂: 31–38%; ♀♀: 31–40%) of body length, length relations of articles from ischium (article 2) to dactylus (article 7): 1: 0.9 (♂♂: 0.7–0.9; ♀♀: 0.7–1.0); 0.5 (♂♂: 0.3–0.5; ♀♀: 0.4–0.5); 0.2 (♂♂: 0.15–0.20; ♀♀: 0.20–0.24); 0.9 (♂♂: 0.7–0.9; ♀♀: 0.8–1.0); 0.7 (♂♂: 0.7–0.8; ♀♀: 0.7–0.8), unguis length 18% (♂♂: 18–25%; ♀♀: 19–24%) of dactylus length.

Pereopod IV (Fig. 5PpIV) grasping, with parallel, but only slightly curved superior and inferior margins of propodus. Pereopod IV length 36% (♂♂: 36–47%; ♀♀: 42–47%) of body length, length relations of articles from ischium (article 2) to dactylus (article 7): 1: 0.6 (♂♂: 0.6–0.8; ♀♀: 0.6–0.7); 0.4 (♂♂: 0.4–0.5; ♀♀: 0.4–0.5); 0.7 (♂♂: 0.7–0.9; ♀♀: 0.7–0.8); 0.8 (♂♂: 0.8–0.9; ♀♀: 0.8–1.0); 0.4 (♂♂: 0.3–0.4; ♀♀: 0.3–0.4), unguis length 32% (♂♂: 32–39%; ♀♀: 29–41%) of dactylus length. Carpus IV superiodistal angle with 5 (♂♂: 5–6; ♀♀: 5–6) spiniform setae, longest one 26% (♂♂: 18–26%; ♀♀: 17–26%) of carpus length. Propodus IV inferior margin and mesial surface with 6 (♂♂: 3–6; ♀♀: 3–4) acute stiff robust setae, longest robust seta 7% (♂♂: 7–10%; ♀♀: 8–11%) of propodus length, inferodistal surface with 3 (♂♂: 2–4; ♀♀: 0–5) short simple and penicillate setae, superior margin and submarginal surface with 6 (♂♂: 6–13; ♀♀: 12–13) short simple and penicillate setae, superior distal angle with 4 (♂♂: 4–6; ♀♀: 5–6) long simple setae and 1 penicillate seta. Dactylus IV inferior margin with 2 (♂♂: 2; ♀♀: 1–2) robust stiff setae, superior margin distally with 2–5 simple setae.

Pereopod VII (Fig. 5PpVII) with long slender articles, its length 70% (♂♂: 67–81%; ♀♀: 65–72%) of body length, length relations of articles along pereopod VII (given as in pereopod IV): 1: 0.9 (♂♂: 0.9–1.0; ♀♀: 0.8–0.9); 0.7 (♂♂: 0.6–0.7; ♀♀: 0.6–0.7); 1 (♂♂: 1.0–1.1; ♀♀: 0.9–1.1); 1.4 (♂♂: 1.2–1.4; ♀♀: 1.1–1.4); 0.3 (♂♂: 0.3–0.4; ♀♀: 0.3), unguis length 30% (♂♂: 30–39%; ♀♀: 33–43%) of dactylus length. Ischium VII with 8 (♂♂: 6–8; ♀♀: 6–6) spiniform setae along its margins,

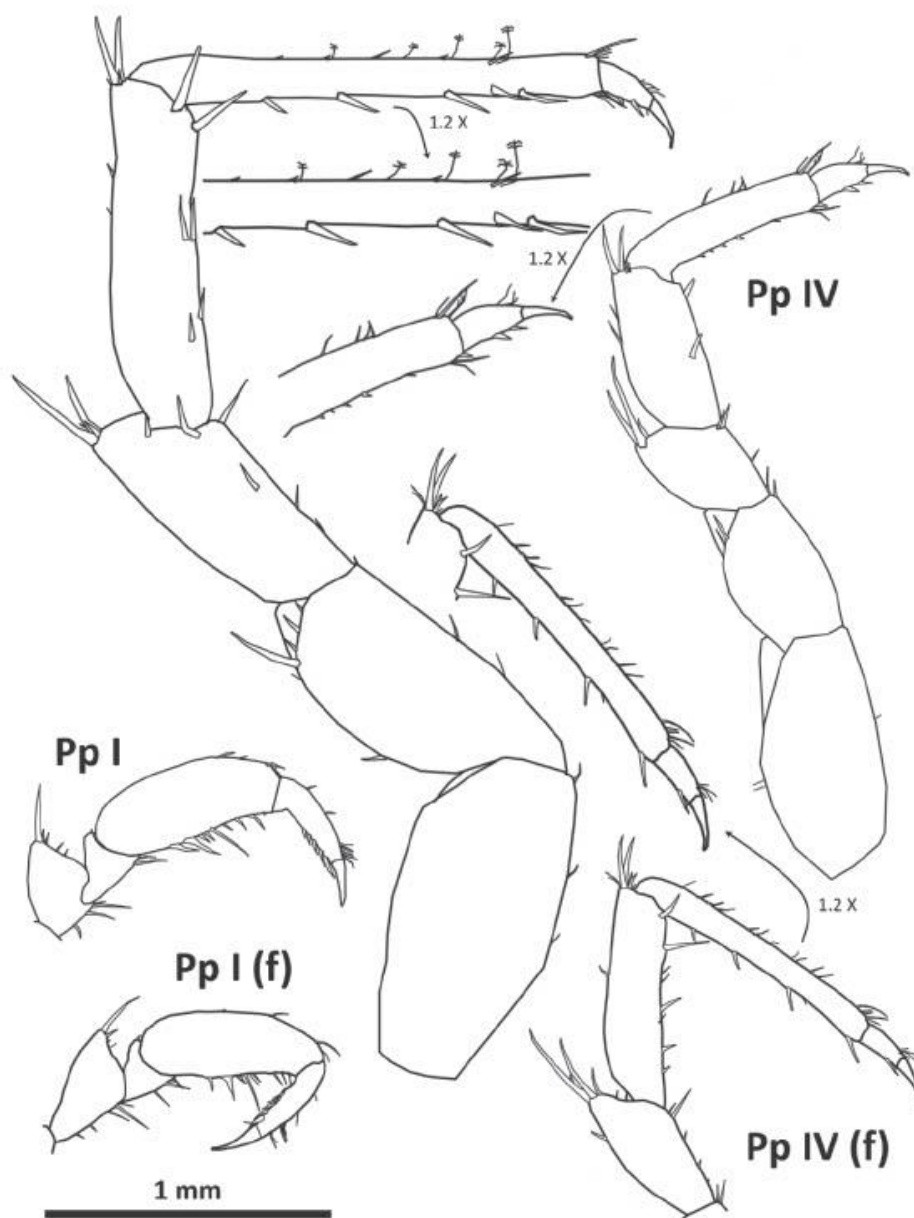


Figure 5. *Asellus ismailsezarai* sp. nov., Ganow spring, Western Iran. Holotype male, 8.2 mm: Pp I, IV, VII – pereopods I (distal articles), IV, VII. Female, 8.1 mm: Pp I, IV (f) – distal articles of pereopods I, IV.

with around 7 spiniform setae along inferodistal margins of merus, and a group of around 4 spiniform setae on superiodistal angle of merus VII, longest one 42% (♂♂: 42–58%; ♀♀: 44–53%) of merus length. Carpus VII with around 6 strong spiniform setae along inferior and inferodistal margins, few (around 2) weaker spiniform setae

along superior margin, and a group of (around two strong and two weak) spiniform setae at superior-distal angle. Propodus VII inferior margin with row of 6 (♂♂: 6; ♀♀: 6) acute stiff robust setae, longest robust seta 10% (♂♂: 9–11%; ♀♀: 11–15%) of propodus length, mesial surface and inferodistal angle with 6 (♂♂: 2–6; ♀♀: 3–5) submarginal simple setae, with few weak simple setae at inferodistal angle. Superior margin and submarginal surface with 10 (♂♂: 7–10; ♀♀: 3–12) short simple and penicillate setae, superior distal angle with 1 (♂♂: 1–2; ♀♀: 1–2) simple setae and around 5 penicillate seta. Dactylus VII inferior margin with 2 (♂♂: 2; ♀♀: 1–2) robust stiff setae, longest 30% (♂♂: 30–39%; ♀♀: 33–43%) of dactylus length, superior margin distally with around 5 (♂♂: 4–6; ♀♀: 4–5) simple setae.

Male pleopod I (Fig. 6PlpI, Fig. 7) protopodite 0.92 (0.88–0.96) times as wide as long, retinacle on medial margin of 5 (4–5, exceptionally less) hooks. Exopodite elongated ovoid, its width 77% (57–77%) of its length, with 19 (6–23) simple setae along lateral and distal margins, and 7 (7) plumose terminal marginal setae. Without concavity on lateral margin, lateral margin being rather straight to slightly convex. In juvenile males (Fig. 6PlpI (juv)), pleopod I exopodite external margin proximally elongated in a finger-like projection, with one plumose seta on it, and around 8 plumose setae along lateral and distal margins.

Male pleopod II (gonopod; Fig. 6PlpII, Fig. 7) protopodite subtrapezoidal, with rounded angles, its width 77% (76–83%) of its length. Lateral and medial margins without setae. Exopodite oval, almost twice (1.76–1.96) as long as wide, its proximal segment with around 2 simple setae on its lateral margin; lateral and medial margins of distal segment fringed with 11 (6–11) simple and 6 (0–6) long plumose setae, respectively. A well-developed and rounded catch lobe on the dorsal side and medial margin of the distal segment of exopodite. This catch lobe partly overlaps the proximal segment of exopodite. Endopodite elongated ovoid, its length 60% (60–71%) of protopodite length, with a short horn-shaped basal spur (*processus calcariformis*): 43% (37–55%) of endopodite length. Dorsal side of endopodite entirely smooth. Ventral side with a finger-like labial spur (*processus cylindriciformis*), an anterior lobe densely covered with serrated scales, and a spoon-shaped *Capitulum*. Surface of *Capitulum* is smooth and without spines. A short but large-diameter *Cannula* is clearly visible with scanning electron microscopy between the anterior lobe and *Capitulum*. In juvenile males, pleopod II (Fig. 6PlpII (juv)) without endopodite, exopodite almost without setation.

Pleopod III (Fig. 6PlpIII) exopodite rounded triangular, about 1.6 times as long as wide, with almost straight medial margin. Medio-distal, terminal and latero-distal margins with around 8 long plumose setae. No setation along medial and lateral margins. Endopodite length about 0.7 of exopodite length.

Pleopod IV (Fig. 6PlpIV) exopodite broadly ovoid, about 1.6 times as long as wide, its area equally shaped as in pleopod V. Without setation along margins. Endopodite subrectangular, its length about 0.8 of exopodite length.

Pleopod V (Fig. 6PlpV) exopodite ovoid, 1.6 (♂♂: 1.6–1.7; ♀♀: 1.5–1.6) times as long as wide, its margins without setation. Respiratory area small, its surface 21% (♂♂: 21–28%; ♀♀: 22–28%) of exopodite surface, lineae areae beginning and ending

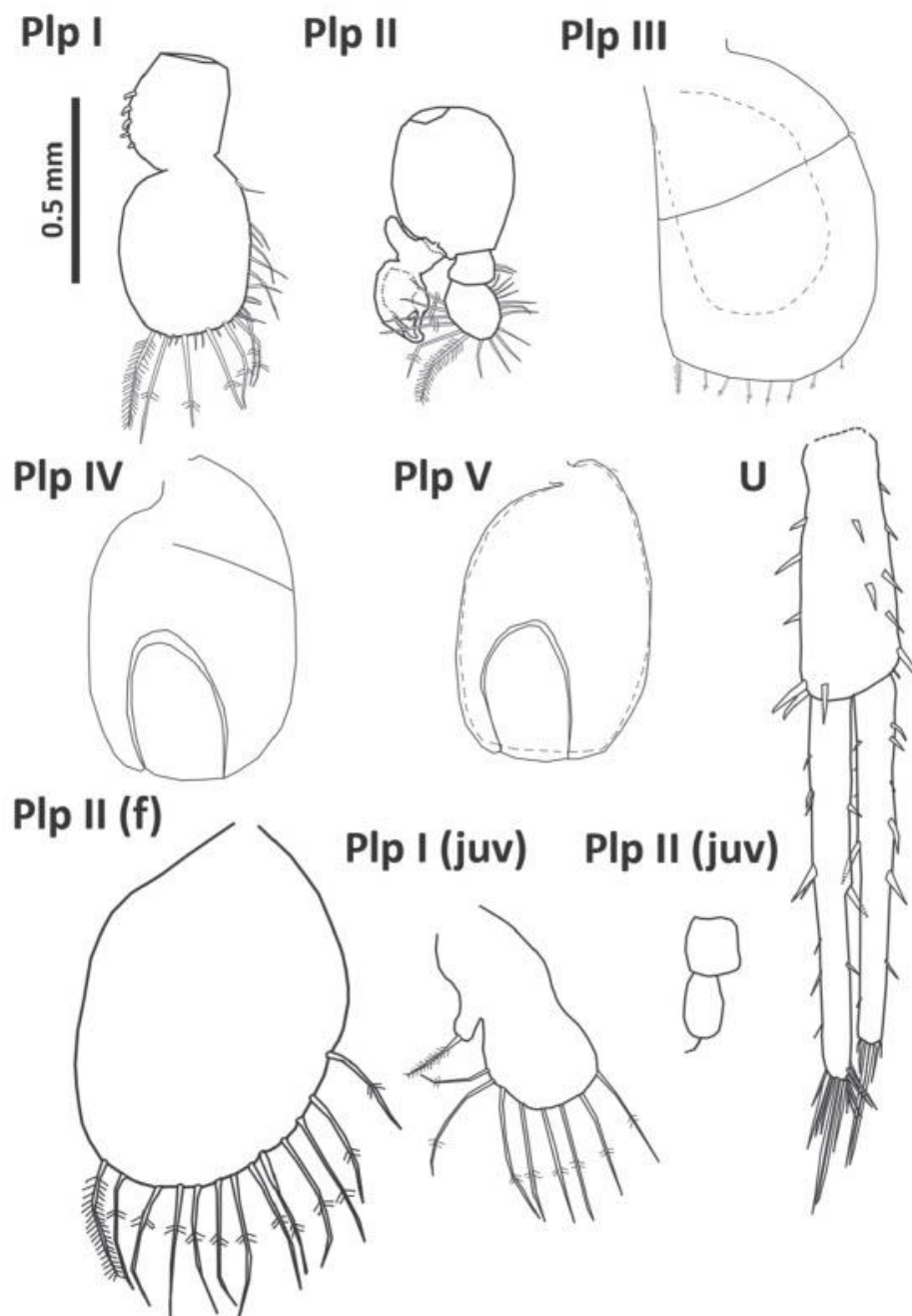


Figure 6. *Asellus ismailsezarai* sp. nov., Ganow spring, Western Iran. Holotype male, 8.2 mm: Plp I, II; II, IV, V – pleopods I, II, III, IV, IV; U – uropod (pleopod VI). Female, 8.1 mm: Plp II (f) – pleopod II. Juvenile male, 7.3 mm: Plp I, II (juv) – pleopods I, II.

on the distal exopodite margin. Endopodite suboval, its length almost as long (holotype: 97%) as exopodite.

Uropod (Fig. 6U) length 24% (♂♂: 24–25%; ♀♀: 17–24%) of body length. Proto-, endo- and exopodite length relations: 1:1.52 (♂♂: 1.40–1.52; ♀♀: 1.53–1.77):1.49 (♂♂: 1.25–1.49; ♀♀: 1.30–1.56). Endopodite with 9 (♂♂: 9; ♀♀: 8–9) spiniform simple setae and 7 (♂♂: 7–9; ♀♀: 8–9) penicillate setae, longest simple seta length 14% (♂♂: 14–15%; ♀♀: 10–13%) of endopodite length.

Female type material. Body length 6.7–8.1 mm (3.3–3.8 times of body width), almost identical to male except, antenna II seems longer than in males, around $\frac{4}{5}$ of body length, but with similar number of flagellar segments (47–55). Pereopod I (Fig. 5PpI(F)) propodus with less expressed proximal apophysis, slender and longer propodus, 2.6–2.8 times as long as wide (♂♂: 2.2–2.5). Pereopod IV (Fig. 5PpIV(f)) not for grasping, ambulatory, little longer than the preceding pairs. Without pleopod I. Pleopod II (Fig. 6PlpII(f)) suboval, about 1.3 (1.3–1.4) times as long as wide, with 12–13 long marginal plumose setae. Uropods seem a bit shorter than in males (♂♂: 24–25%; ♀♀: 17–24%), with relatively longer endopodite compared to protopodite (♂♂: 1.40–1.52 versus ♀♀: 1.53–1.77 times as long as protopodite).

Morphological comparison with other *Asellus* species. Among the 18 species of *Asellus* presently described, *A. ismailsezarii* sp. nov. resembles morphologically *A. monticola* Birstein, 1932 (Suppl. material 6: Table S6). Pleopods I and II appear to be almost identical between the two species, although Henry and Magniez (1996) did not provide SEM photos of these pleopods for *A. monticola*. In both species, the endopodite of pleopod II has a spoon-shaped *Capitulum* with no spines. However, *A. ismailsezarii* sp. nov. differs from *A. monticola* in that it is eyeless and fully depigmented, has a slightly curved pereopod IV and bears no setae on proximal margins of exopodite of pleopods IV and V. These morphological differences are sufficient to consider the studied specimens as belonging to a distinct species. We also provided in Suppl. material 6: Table S6, a morphological comparison with *A. kosswigi*, *A. aquaticus aquaticus*, *A. aquaticus infernus* and *A. aquaticus cavernicolus* showing that *A. ismailsezarii* sp. nov. shows many morphological differences with both surface and subterranean aquatic species and subspecies of the *A. aquaticus* complex. Contrary to *A. ismailsezarii* sp. nov., all members of the *A. aquaticus* complex show a helical *Capitulum*, suggesting the new species does not belong to that complex.

Molecular species delimitation. We found eleven MOTUs within the “*Asellus* pattern”, including one MOTU corresponding to *A. ismailsezarii* sp. nov. (Fig. 8A). Maximal patristic distances among COI sequences within the same MOTU (i.e., within-MOTU distances) ranged from 0 to 0.19 (0.01 for *A. ismailsezarii* sp. nov.) (Suppl. material 7: Table S7). Minimal patristic distances among sequences belonging to *A. ismailsezarii* sp. nov. and sequences belonging to the other MOTUs (i.e., between-MOTU distances) ranged from 0.76 to 1.00 (Suppl. material 7: Table S7). The considerable gap between within- and between-MOTU patristic distances supports our taxonomic assessment that *A. ismailsezarii* sp. nov. is a distinct species.

Phylogenetic relationships as inferred from the COI gene indicated that *A. ismailsezarii* sp. nov. does not belong to the *A. aquaticus* complex, which itself contained

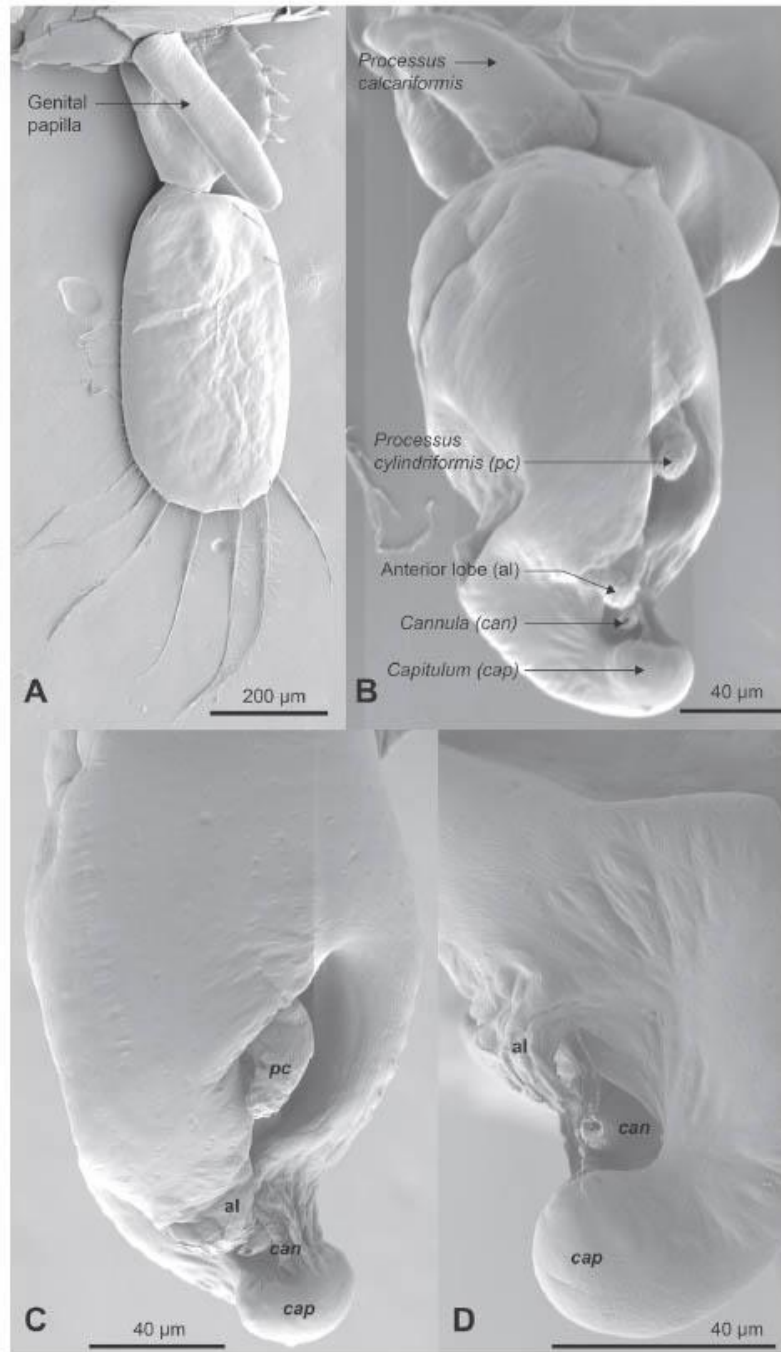


Figure 7. *Asellus ismailsezarit* sp. nov., male; SEM micrographs of (A) pleopod I (paratype male BL 8.2 mm: TU-SP.2). Photo also shows one genital papilla inserted on ventral side of pereionite VIII. B, C, D pleopod II endopod (holotype male BL 8.2 mm: TU-SP.1) B, C ventral view and D lateral view.

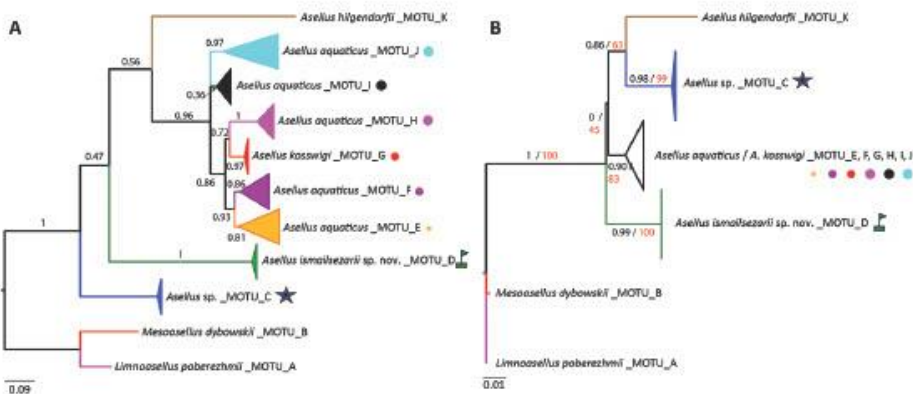


Figure 8. Phylogenetic relationships among taxa belonging to the *Asellus* pattern derived from Maximum likelihood analysis of (A) 585 COI mtDNA sequences, (B) thirty 28S rDNA sequences. The clade comprised of *M. dybowskii* and *L. poberezhnyi* was used as outgroup. In A, branches to molecular operational taxonomic units (MOTU) as delimited with the fixed threshold method implemented by Lefebvre et al. (2006) were collapsed. In B, branches to the main clades were collapsed. Colors and symbols as in Fig. 9. Branch supports are approximate likelihood ratio test (aLRT) values (black) and bootstrap values (red).

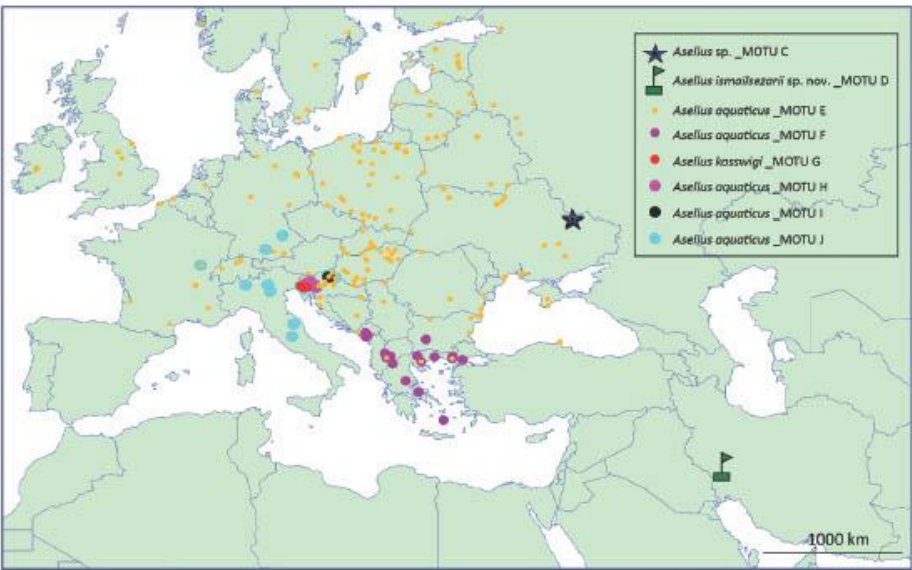


Figure 9. Map showing the distribution of molecular operational taxonomic units (MOTU) within the “*Asellus* pattern”. Site locations for *Asellus hilgendorffii* (Japan) and *Mesoasellus dybowskii* and *Limnoasellus poberezhnyi* (Lake Baikal, Russia) are not shown. Colors and symbols as in Fig. 8.

six MOTUs, including *A. kosswigi* (Fig. 8A). Within that complex, only MOTU E corresponding to *A. aquaticus* Linnaeus, 1758 *sensu stricto* was widely distributed across Europe (Fig. 9). The resulting tree of the nuclear 28S rDNA sequences agreed well with the COI phylogeny, again indicating that *A. ismailsezarai* sp. nov. was a sister species

of the *A. aquaticus* complex (Fig. 8B). Patristic distances derived from the 28S loci also corroborated COI results by revealing a strong divergence between *A. ismailsezarii* sp. nov. and the other MOTUs.

Etymology. The name of the new species is a patronym for “Mohammad Ismail Sezari”, the legendary guard of the train tracks from the area of the type locality. In year 1986, he sacrificed his life to save 750 others that were on a collision course train.

Discussion

The present study provides morphological and molecular evidence supporting the species status of *Asellus ismailsezarii* sp. nov. This newly discovered species shows diagnostic morphological features of the *Asellus* genus and *Asellus* subgenus (Magniez and Henry 1970; Henry and Magniez 1993, 1996). In addition to being eyeless and fully depigmented, *A. ismailsezarii* sp. nov. differs from the surface aquatic species *A. monticola* to which it is supposedly most closely related, by the shape of its pereopod IV and the lack of setation on pleopods IV and V. Most but not all groundwater species lack a grasping pereopod IV, suggesting adult males no longer engage in precopulatory mate guarding (Henry 1976). The occurrence of a still curved pereopod IV among some groundwater asellids could indicate that groundwater colonization has occurred recently. However, this hypothesis deserves to be tested using a large number of population and/or species (see for example Balázs et al. 2021). We also present morphological evidence that *Asellus ismailsezarii* sp. nov. is distantly related to species belonging to the *A. aquaticus* complex. All species of that complex are characterized by a helical *Capitulum*, whereas both *Asellus ismailsezarii* sp. nov. and *A. monticola* have a spoon-shaped *Capitulum*.

In addition to morphological evidence, we show that patristic distances for the COI gene between *A. ismailsezarii* sp. nov. and any other MOTUs delimited within the “*Asellus* pattern” largely exceed the 0.16 threshold value above which two clades can be considered to belong to distinct species (Lefébure et al. 2006). Phylogenetic relationships as inferred from the COI and 28S gene confirm that *A. ismailsezarii* sp. nov. does not belong to the *A. aquaticus* complex.

Our findings together with previous asellid reports from Iran and neighboring countries (Turkmenistan, Georgia, Armenia) suggest that the Caspian Sea region is a contact zone between species of the European *A. aquaticus* complex, including *A. aquaticus* Linnaeus, 1758 and *A. aquaticus messerianus* and Asian species, including *A. monticola* and *A. ismailsezarii* sp. nov. Although the number of MOTUs within the *A. aquaticus* complex varies according to the species delimitation method – Sworobowicz et al. (2015) recognized 13 MOTUs using the automatic barcode gap discovery – one MOTU corresponding to the neotype population of *A. aquaticus* Linnaeus, 1758 (see Verovnik et al. 2009) systematically shows a large distribution throughout Europe. According to records by Rémy (1941) and Henry and Magniez (1995), *A. aquaticus* Linnaeus, 1758 is also present in Iran. Further sampling and sequencing of asellids in the Caspian Sea region is necessary to ascertain the existence of that contact zone between species of the European *A. aquaticus* complex and Asian species.

The molecular systematics of the *Asellus* and more largely of the “*Asellus* pattern” is yet to be established since most of its species have not yet been sequenced. Hence, except from the fact that *A. ismailsezarii* sp. nov. does not belong to the *A. aquaticus* complex, its phylogenetic position within the *Asellus* remains to be more precisely determined. We expect that *A. ismailsezarii* sp. nov. is phylogenetically closely related to *A. monticola* because the two species share many morphological characters. This hypothesis can be tested when fresh material of *A. monticola* becomes available for DNA sequencing. Ancestral populations of *A. monticola* might have colonized groundwater on multiple occasions and localities. If so, many more obligate groundwater *Asellus* taxa could be discovered in Iran.

We have several reasons to expect many more groundwater species discoveries in Iran as sampling effort increases. First, the number of 43 obligate groundwater species presently known from Iran is exceedingly low considering the extent and diversity of groundwater habitats (Vardanjani et al. 2017). Of the 2000 registered caves and many more wells and springs in Iran, a tiny proportion has actually been sampled. Second, about 70% of the species were described in the past eight years when sampling and taxonomic efforts intensified. Third, the number of species is very unevenly distributed among taxa, reflecting a strong taxonomic bias in description effort. Of the 43 obligate groundwater species, 23 belong to the genus *Niphargus* Schiödte, 1847, due to a recent increase in species description activity among Iranian experts (Malek-Hosseini and Zamani 2017; Zamanpoore et al. 2020; Bargrizaneh et al. 2021). Yet, recent species descriptions of Stenasellidae, aquatic Isopoda (Khalaji-Pirbalouty et al. 2018), Moitessieriidae, aquatic snails (Fatemi et al. 2019), Agnariidae, terrestrial Isopoda (Kashani et al. 2013) and Carabidae, terrestrial Coleoptera (Malek-Hosseini et al. 2021) indicate that many animal groups are likely to have presently unknown subterranean representatives in Iran. Local but repeated sampling is also revealing the presence of potentially species-rich aquifers with high conservation value. The karst aquifer feeding the Ganow spring, the type locality of *A. ismailsezarii* sp. nov., also harbors three described species of cave fish (Mousavi-Sabet et al. 2016; Vatandoust et al. 2019) as well as yet-undescribed species of gastropods (in first author collection). This paper contributes a piece in the puzzle of the Iranian cave biodiversity.

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Supplementary material 1

Table S1. DNA extraction and PCR protocols

Authors: Mohammad Javad Malek-Hosseini, Jure Jugovic, Yaser Fatemi, Matjaž Kuntner, Rok Kostanjšek, Christophe J. Douady, Florian Malard

Data type: pdf. file

Explanation note: Molecular protocols indicating for each gene (COI and 28S) and specimen of *Asellus ismailsezarii* sp. nov. (AS1, AS2 and AS3) the DNA extraction protocol (see text), PCR protocol (see text), primer names, sequencing institutions and sequence codes.

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Link: <https://doi.org/10.3897/subtbiol.42.79447.suppl1>

Supplementary material 2

Table S2. List of primers

Authors: Mohammad Javad Malek-Hosseini, Jure Jugovic, Yaser Fatemi, Matjaž Kuntner, Rok Kostanjšek, Christophe J. Douady, Florian Malard

Data type: pdf. file

Explanation note: List of primers used in this study.

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Link: <https://doi.org/10.3897/subtbiol.42.79447.suppl2>

Supplementary material 3

Table S3. COI and 28S sequence data set with sampling localities

Authors: Mohammad Javad Malek-Hosseini, Jure Jugovic, Yaser Fatemi, Matjaž Kunter, Rok Kostanjšek, Christophe J. Douady, Florian Malard

Data type: GenBank codes (excel file)

Explanation note: COI and 28S sequence data set used in the present study. TH: Molecular operational taxonomic units (MOTU) as delimited with the 16% COI divergence threshold; Long.: longitude of the locality, in decimal degrees; Lat.: latitude of the locality, in decimal degrees, There may be more than one locality per sequence in case haplotype sequences were deposited to GenBank.

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Link: <https://doi.org/10.3897/subtbiol.42.79447.suppl3>

Supplementary material 4

Table S4. COI alignment

Authors: Mohammad Javad Malek-Hosseini, Jure Jugovic, Yaser Fatemi, Matjaž Kunter, Rok Kostanjšek, Christophe J. Douady, Florian Malard

Data type: mase. file

Explanation note: COI alignment data.

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Link: <https://doi.org/10.3897/subtbiol.42.79447.suppl4>

Supplementary material 5

Table S5. 28S alignment

Authors: Mohammad Javad Malek-Hosseini, Jure Jugovic, Yaser Fatemi, Matjaž Kunter, Rok Kostanjšek, Christophe J. Douady, Florian Malard

Data type: mase. file

Explanation note: 28S alignment data.

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Link: <https://doi.org/10.3897/subtbiol.42.79447.suppl5>

Supplementary material 6

Table S6. Morphological comparison with other *Asellus* species

Authors: Mohammad Javad Malek-Hosseini, Jure Jugovic, Yaser Fatemi, Matjaž Kunter, Rok Kostanjšek, Christophe J. Douady, Florian Malard

Data type: pdf. file

Explanation note: Comparison of *Asellus ismailsezarii* sp. nov. with *A. monticola*, *A. kossuigi*, and *Asellus aquaticus* and its known hypogean subspecies from Europe (*A. a. infernus*, *A. a. cavernicolous*). Abbreviations: A II – antenna II, Prp IV, VII – pereopods IV and VII, Plp IV, V – pleopods IV and V. Most discriminative features between *A. ismailsezarii* and any other species analysed here are presented in bold (note value overlapping).

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Link: <https://doi.org/10.3897/subtbiol.42.79447.suppl6>

Supplementary material 7

Table S7. Genetic distances among and within MOTUs

Authors: Mohammad Javad Malek-Hosseini, Jure Jugovic, Yaser Fatemi, Matjaž Kunter, Rok Kostanjšek, Christophe J. Douady, Florian Malard

Data type: excel file

Explanation note: Genetic distances among MOTUs. Letters correspond to MOTUs as shown in Figs 8 and 9. Below diagonal: Minimum patristic distances among MOTUs. Above diagonal: minimum uncorrected distances (p-distance) among MOTUs. Diagonal: maximum patristic distances within MOTUs / maximum uncorrected distances (p-distance) within MOTUs.

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Link: <https://doi.org/10.3897/subtbiol.42.79447.suppl7>

2.1.4 A checklist of subterranean arthropods of Iran

Malek-Hosseini M. J., Zamani A. 2017. A checklist of subterranean arthropods of Iran. *Subterranean Biology*, 21: 19–46, doi: 10.3897/subtbiol.21.10573

Naslov: **Vrstni seznam podzemnih členonožcev Irana**

Izvleček:

Razumevanje podzemeljske biotske raznovrstnosti je pomembno, vendar so obsežna območja sveta še vedno slabo raziskana. V tem prispevku predstavljamo prvi korak k popisu podzemeljskih členonožcev Irana. Po pregledu in analizi dostopne literature ter pregledu vzorcev, ki smo jih zbrali v iranskih jamah, smo našli 89 jamskih vrst (iz 42 jam in 5 kraških izvirov), ki pripadajo štirim poddeblom členonožcev: Chelicerata (1 razred, 4 redovi, 36 vrst), Crustacea (2 razreda, 3 redovi, 15 vrst), Hexapoda (2 razreda, 5 redov, 34 vrst) in Myriapoda (2 razreda, 3 redovi, 4 vrste).

Ključne besede

Jame, favna, Srednji vzhod, Zagros, troglorafna

A checklist of subterranean arthropods of Iran

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Abstract

Understanding subterranean biodiversity is important, yet vast regions of the world remain poorly explored. Here, we provide the first step towards cataloguing the subterranean arthropods of Iran. After review and analysis of the available literature and the examination of samples collected by us from Iranian caves, we listed 89 cavernicolous species (from 42 caves and 5 karstic springs) belonging to four arthropod subphyla: Chelicerata (1 class, 4 orders, 36 species), Crustacea (2 classes, 3 orders, 15 species), Hexapoda (2 classes, 5 orders, 34 species) and Myriapoda (2 classes, 3 orders, 4 species).

Keywords

Cave, fauna, Middle East, Zagros, troglotauna

Introduction

Although relatively poorly studied in comparison with the epigeal ecosystems, subterranean ecosystems show a rather high biodiversity at the global scale. Globally, over 7000 aquatic subterranean dwellers have been catalogued (Botosaneanu 1986), but unfortunately, such data are not available for the terrestrial subterranean fauna. It is likely that subterranean habitats harbor even larger numbers of terrestrial species, e.g. Coleoptera alone includes more than 1927 underground-limited species (Gibert and Deharveng 2002, Juberthie and Decu 1998). Culver and Holsinger (1992) proposed a potential biodiversity of about 50,000 to 100,000 obligate terrestrial subterranean species on the basis of nested regional estimates.

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In spite of more than a century of taxonomic and biogeographic studies, large-scale patterns of diversity of the obligate cave-dwelling fauna have remained obscure. The two major apparent determinants of biodiversity for the subterranean terrestrial fauna, i.e. productivity and habitat availability, are the same as for many other faunas (Hawkins et al. 2003, Turner and Hawkins 2004). Culver et al. (2003) hypothesized a connection between surface productivity and the amount of food available in caves: in nearly all caves, there is no significant chemoautotrophy so that all available food results from the surface productivity. Hotspots are most commonly referred to as areas of high biodiversity, followed by areas of high productivity (Briscoe et al. 2016). There are potential subterranean hotspots in the karst regions of western Caucasus in Georgia and possibly the Tien Shan Mountains in Kyrgyzstan. Although biologically poorly-studied, based on their positive relationship to the productivity maps and the presence of major cave regions they seem to be promising research subjects (Gvozdetzki et al. 1994, Klimchouk 2004a, b). These regions are the closest to Iran which somehow have been studied in term of caves fauna. There are a lot of similarities between them and the north and western parts of Iran. Based on the studies conducted on these regions as well as some sporadic studies on Iranian caves in the mentioned areas, we could consider that there are some hotspots there. From east of Kyrgyzstan to the eastern coast of the Asian continent, the climate is probably too dry to support any subterranean hotspots (Culver et al. 2006).

Covering 164.8 million hectares in western Asia at the interface between three zoogeographic realms, Palearctic, Oriental (Indo-Malayan) and Afrotropical (Ethiopian), Iran is situated at the conjunction of three climatic zones: the Mediterranean, the arid West Asian, and the temperate humid/semi-humid Caspian zone. Nevertheless, it lies predominantly in an arid environmental zone. The Zagros and Alborz Mountains are the two main mountain chains in western and northern Iran, which comprise nearly a third of the Iranian land area. Geologically, Iran is a part of the Alpine-Himalayan orogenic belt, divided into five major structural zones: a) Zagros Range, b) Sanandaj-Sirjan Range, c) Central Iran, d) East and South-East Iran and e) Alborz and Kopet-Dagh Ranges. However, more detailed divisions exist. The number of described caves is more than 2000 (Raeisi et al. 2012), with many more to be added.

During the last decade, several faunistic studies have been carried out in Iran, resulting in new records of already known species, as well as the descriptions of many new ones. However, despite the vast amount of collected faunistic data, the Iranian subterranean fauna remains inadequately known, and most reported species from the caves are in fact troglonemes (Esmacili-Rineh and Sari 2013). Nevertheless, 16 obligate cave-dwellers are reported from Iran, of which as many as 13 have been described since 2013. Fifteen species of these troglonemes are restricted to Iran. The only known troglotic vertebrates are three fish species: *Garra typhlops* (Bruun & Kaiser, 1944), *G. lorestanensis* Mousavi-Sabet & Eagderi, 2016 and *Paracobitis smithi* Smith, 1976 (Cypriniformes) (Farashi et al. 2014, Mousavi-Sabet and Eagderi 2016). Troglotic invertebrates include a spider, a diplopod and 11 crustaceans. In this paper, we aim to provide the first checklist of Iranian subterranean arthropods, based on the available literature and our unpublished material.

Material and methods

Unique adaptations of troglofauna have evolved in relations to the significant characteristics of subterranean environments such as caves, e.g. darkness, constant climatic conditions and restricted food supply. Based on their life style and adaptations to the conditions of the unique habitat, cave organisms (troglofauna) are divided into three categories: 1) troglobionts (organisms that display characteristics of troglomorphy, such as depigmentation and loss of eyes, and are considered “obligatory” residents of the underground environment), 2) troglaphiles (organisms that can live and complete their life cycle either in the cave or in the surface; some biospeleologists subdivide this group into the eutroglaphiles and subtroglaphiles) (Gavish-Regev et al. 2016, see Sket 2008); 3) troglaxenes (organisms only occurring sporadically in hypogean habitat and unable to establish a subterranean population that habitually enter caves but must return periodically to the outside for certain of their living requirements, usually food) (Sket 2008, Romero 2009, Culver and Pipan 2009). We classified cave organisms into the mentioned three groups, based on the classification used in the available literature or on our own observations. Troglomorphic and non-troglomorphic species that were found in all cave zones throughout the year are classified as troglaphiles. Troglaxenes were found either around cave entrances or within the entrance zone of smaller caves. Ectoparasites of bats were not categorized, however, they could be considered as troglaphiles or troglaxenes. Major structural zones of Iran and the distribution of all troglobionts have been shown in Figure 1. Specimens from caves were collected by means of diverse sampling items (e.g. the plankton net, small hand net and aspirator), and placed directly into 96% ethanol. The collected specimens were brought to the laboratory and identified using identification keys and also with the assistance of specialists. Records of undetermined species are not referenced in this catalog. Material is deposited in the Zoological Museum of University of Tehran (ZUTC) and in the Collection of Biology Department, Zoological Museum of Shiraz University, Shiraz, Iran (ZM CBSU).

List of species

Phylum Arthropoda von Siebold, 1848

Subphylum Chelicerata Heymons, 1901

Class Arachnida Lamarck, 1801

Order Araneae Clerck, 1757

Based on the World Spider Catalog (2016), 45,987 species of spiders, grouped in 3,985 genera and 114 families have been described so far. To date, more than 600 spider species of 243 genera and 48 families have been recorded from Iran (Zamani et al. 2016). Only a few recent publications deal exclusively with the cave spider fauna of Iran (Marusik et al. 2014, Malek Hosseini et al. 2015a, b), while in previous reports only scattered

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records are provided (e.g. Brignoli 1970, Senglet 2008, Moradmand and Jäger 2011). Here, we have listed 29 species from 15 families and 21 genera, most of which have been classified as edaphic troglaphiles and troglaxenes, and one troglabiont.

Family Agelenidae C. L. Koch, 1837

***Tegenaria lenkoranica* (Guseinov, Marusik & Koponen, 2005)**

Status: **troglaxene**.

Records: Roodafshan Cave (Tehran Province) (Zamani et al. 2014), Shirabad Cave (Golestan Province) (present study).

***Tegenaria zamanii* Marusik & Omelko, 2014**

Status: **troglaphile**.

Records: Shirabad Cave (Golestan Province) (Marusik et al. 2014, Marusik and Zamani 2015).

Family Eutichuridae Lehtinen, 1967

***Cheiracanthium mildei* L. Koch, 1864**

Status: **troglaxene**.

Records: Nevel Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek Hosseini et al. 2015b).

Family Gnaphosidae Pocock, 1898

***Anagraphis pallens* Simon, 1893**

Status: **troglaphile**.

Records: Gakal Cave, Neyneh Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek Hosseini et al. 2015b).

Family Liocranidae Simon, 1897

***Mesiotelus scopensis* Drensky, 1935**

Status: **troglaxene**.

Records: Nevel Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek Hosseini et al. 2015b).

Family Linyphiidae Blackwall, 1859

***Lepthyphantes iranicus* Saaristo & Tanasevitch, 1996**

Status: **troglophile**.

Records: Shirabad Cave (Golestan Province) (Marusik et al. 2014).

***Megalepthyphantes camelus* (Tanasevitch, 1990)**

Status: **trogloxene**.

Records: Unnamed cave (Alborz Province) (present study).

***Palliduphantes sbordonii* (Brignoli, 1970)**

Status: **troglophile**.

Records: Ab Ask Cave (Mazandaran Province) (Brignoli 1970).

***Sengletus latus* Tanasevitch, 2009**

Status: **troglophile**.

Records: Deh Sheikh (Pataveh) Cave, Nevel Cave, Chek Cave, Tikow (Tang-e Tikab) Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek Hosseini et al. 2015b).

Family Oonopidae Simon, 1890

***Trilacuna qarzi* Malek Hosseini & Grismado, 2015**

Status: **troglobiont**.

Records: Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek Hosseini et al. 2015a, b); Fig. 1, locality 2.

Family Oxyopidae Thorell, 1870

***Oxyopes lineatus* Latreille, 1806**

Status: **trogloxene**.

Records: Nevel Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek Hosseini et al. 2015b).

Family Pholcidae C. L. Koch, 1850

***Artema doriai* (Thorell, 1881)**

Status: **trogloxene /troglophile.**

Records: Behesht Makan Cave, Palangan cave (Fars Province) (Malek Hosseini et al. 2015b).

***Pholcus armeniacus* Senglet, 1974**

Status: **troglophile.**

Records: Unidentified caves (Western Azarbayjan Province) (Senglet 2008).

***Pholcus arsacius* Senglet, 2008**

Status: **troglophile.**

Records: Unidentified caves (Southern Khorasan Province) (Senglet 2008).

***Pholcus elymaeus* Senglet, 2008**

Status: **troglophile.**

Records: Unidentified cave (Kordestan Province) (Senglet 2008).

***Pholcus medicus* Senglet, 1974**

Status: **troglophile.**

Records: Unidentified caves (Eastern Azarbayjan and Mazandaran Provinces) (Senglet 2008).

***Pholcus parthicus* Senglet, 2008**

Status: **troglophile.**

Records: Unidentified caves (Northern Khorasan Province) (Senglet 2008).

***Spermophora persica* Senglet, 2008**

Status: **troglophile.**

Records: Unidentified cave (Kordestan Province) (Senglet 2008).

***Spermophora senoculatoides* Senglet, 2008**

Status: **troglophile.**

Records: Unidentified caves (Kordestan Province) (Senglet 2008).

Family Salticidae Blackwall, 1841

***Plexippoides flavescens* (O. P.-Cambridge, 1872)**

Status: **trogloxene**.

Records: Unnamed cave (Alborz Province) (present study).

Family Scytodidae Blackwall, 1864

***Scytodes thoracica* (Latreille, 1802)**

Status: **trogloxene**.

Records: Tikow (Tang-e Tikab) Cave (Fars Province) (Malek Hosseini et al. 2015b).

Family Segestriidae Simon, 1893

***Segestria mirshamsii* Marusik & Omelko, 2014**

Status: **trogloxene**.

Records: Shirabad Cave (Golestan Province) (Marusik et al. 2014).

Family Sicariidae Keyserling, 1880

***Loxosceles rufescens* (Dufour, 1820)**

Status: **troglophile**.

Records: Charkhab Cave (Fars Province) (Sadeghi et al. in press).

Family Sparassidae Bertkau, 1872

***Spariolenus iranomaximus* Moradmand & Jäger, 2011**

Status: **troglophile**.

Records: Khofash Cave (Ilam Province) (Moradmand and Jäger 2011).

***Spariolenus zagros* Moradmand & Jäger, 2011**

Status: **troglophile**.

Records: Kenesht Cave (Kermanshah Province) (Moradmand and Jäger 2011),
Dalkhoon Cave (Fars Province) (Malek Hosseini et al. 2015b).

Family Tetragnathidae Menge, 1866

***Metellina merianae* (Scopoli, 1763)**

Status: **troglophile**.

Records: Shirabad Cave (Golestan Province) (Marusik et al. 2014).

***Metellina orientalis* (Spassky, 1932)**

Status: **troglophile** (this species was observed the whole year in deep parts of Gakal Cave)

Status: **trogloxene** (in other caves it just observed near entrances)

Records: Nevel Cave, Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province), Chek Cave, Tikow (Tang-e Tikab) Cave (Fars Province) (Malek Hosseini et al. 2015b).

Family Theridiosomatidae Simon, 1881

***Theridiosoma gemmosum* (C. L. Koch, 1877)**

Status: **troglophile**.

Records: Shirabad Cave (Golestan Province) (Marusik et al. 2014), Nevel Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek Hosseini et al. 2015b).

Family Uloboridae Thorell, 1869

***Octonoba yesoensis* (Saito, 1934)**

Status: **trogloxene**.

Records: Shirabad Cave (Golestan Province) (Marusik et al. 2014).

Order Pseudoscorpiones Haeckel, 1866

According to our own unpublished data, 56 species and subspecies of pseudoscorpions, from 12 families are known from Iran, of which, only one troglophile species is listed here.

Family Chernetidae Menge, 1855

***Megachernes pavlovskyi* Redikorzev, 1949**

Status: **troglophile**.

Records: Deh Sheikh (Pataveh) Cave (Kohgiluyeh and Boyer-Ahmad Province) (Christophoryová et al. 2013), Karafto Cave (Kordestan Province), Kila Sefid Cave (Kermanshah Province) (Mirmoayedi et al. 2000).

Subclass Acari Leach, 1817

This subclass is with probably over 45,000 described species and has very complex systematics. There are more than 1,000 described species reported from caves with some being troglomorphic or troglophiles to some degree, generally showing reduction or loss of eyes and depigmentation, as well as elongation of appendages and well-developed sensory setae (Romero 2009). No detailed study of Acari in caves (e.g., as ectoparasites or guanobionts) has been done in Iran so far, and only five species of bat's ectoparasites are listed in here. We have collected several undetermined species that some of them are guanophilic and some other are associated with beetles.

Order Ixodida Leach, 1815

Family Ixodidae Koch, 1844

***Ixodes simplex* Neumann, 1906**

Status: **bat ectoparasite**.

Records: Kiler Cave (Kohgiluyeh and Boyer-Ahmad Province), Tadovan Cave (Fars Province) (Malek-Hosseini et al. 2016b).

***Ixodes vespertilionis* Koch, 1844**

Status: **bat ectoparasite**. This species shows certain degree of troglomorphy, such as extremely elongated appendages and well-developed sensory setae.

Records: Mahi-Dasth Limy Cave (Kermanshah Province) (Vatandoost et al. 2010), Deh Sheikh (Pataveh) Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek-Hosseini et al. 2016b).

Order Mesostigmata Canestrini, 1891

Family Spinturnicidae Oudemans, 1902

***Meristaspis lateralis* (Kolenati, 1856)**

Status: **bat ectoparasite**.

Records: Bishapur, cave at the Sasan spring (Fars Province) (Benda et al. 2012).

***Spinturnix myoti* (Kolenati, 1856)**

Status: **bat ectoparasite.**

Records: Tadovan Cave (Fars Province) (Benda et al. 2012), Kiler Cave (Kohgiluyeh and Boyer-Ahmad Province), Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek-Hosseini et al. 2016b).

***Spinturnix psi* (Kolenati, 1856)**

Status: **bat ectoparasite.**

Records: Tadovan Cave (Fars Province), Bishapur Cave (Fars Province), Dashkahun Cave (Ardabil Province) (Benda et al. 2012), Kiler Cave (Kohgiluyeh and Boyer-Ahmad Province), Gakal cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek-Hosseini et al. 2016b).

Subphylum Myriapoda Latreille, 1802

Class Chilopoda Latreille, 1817

Approximately 3,000 species of centipedes are found in the class Chilopoda (Vazirianzadeh et al. 2007). About 60 species have been found in caves (Romero 2009). No checklist is available regarding the number of species in Iran; here we list two troglomorphic species. Specimens were identified by Dr. Marzio Zapparoli (Tuscia University, Viterbo, Italy).

Order Geophilomorpha Pocock, 1896

Family Mecistocephalidae Bollman, 1893

***Mecistocephalus evansi* Brolemann, 1922**

Status: **troglophile.**

Records: Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (present study).

Order Lithobiomorpha Pocock, 1895

Family Lithobiidae Newport, 1844

***Lithobius erythrocephalus cronebergii* Sseliwanoff, 1881**

Status: **troglophile.**

Records: Deh Sheikh (Pataveh) Cave, Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (present study).

Class Diplopoda De Blainville in Gervais, 1844

About 10,000 species of millipedes have been described, of which, about 200 species in five orders have been reported from caves (Romero 2009). Enghoff and Moravvej (2005) listed 50 species of Iranian millipedes, of which only one species was reported from a cave. Here, we list two species, including one troglomorphic.

Order Spirostreptida Brandt, 1833

Family Cambalidae Hoffman, 1979

***Chiraziulus kaiseri* Mauries, 1983**

Status: **troglomorphic**.

Records: Sarab Cave (Hamadan Province) (Enghoff and Moravvej 2005).

***Chiraziulus troglopersicus* Reboleira, Malek Hosseini, Sadeghi & Enghoff, 2015**

Status: **troglomorphic**.

Records: Neyneh Cave (Kohgiluyeh and Boyer-Ahmad Province) (Reboleira et al. 2015); Fig. 1, locality 3.

Subphylum Crustacea Brünnich, 1772

Class Malacostraca Latreille, 1802

Order Amphipoda Latreille, 1816

This subphylum is constituted of about 52,000 known living marine, freshwater and terrestrial species with worldwide distribution (Martin and Davis 2006). It is by far the richest and the most diverse group in subterranean waters. A literature search yielded more than 4,800 species of cave, phreatic, and anchialine crustaceans from all over the world, including marine, freshwater, and terrestrial ones (Romero 2009). By far the most species-rich crustacean groups are Amphipoda and Copepoda, although Decapoda (due to their size) are probably the most obvious (Holsinger 1993, White and Culver 2012). With 319 species, niphargoids are the most diverse Palearctic hypogean amphipods, which include also a few epigean taxa. They are distributed through central and particularly southeastern Europe, where they exhibit high levels of endemism in karst systems. More than 305 species of *Niphargus* have been described so far, and this is currently the largest freshwater amphipod genus (Fišer et al. 2005, Väinölä et al. 2008). Esmaeili-Rineh and Sari (2010) listed several localities for *Niphargus* in Iran: Dimeh spring in Chaharmahal and Bakhtiari, Brolan spring in West Azarbayjan, Sasan River in Fars, Ghor-Ghale cave in Kermanshah, Razbashi spring in Lorestan, Ghaemshahr and Danial Cave in Mazandaran and Cheshmeh-Siah in Koh-

giluyeh and Boyer-Ahmad Provinces. However, the identification data haven't been published for all of them so far.

Freshwater amphipods from Iran include the family Gammaridae with 18 species (Zamanpoore et al. 2011, Semsar-Kazerooni et al. 2016) and Niphargidae with 10 species of the single genus *Niphargus*. All Iranian *Niphargus* have been recorded from caves and karstic springs.

Family Niphargidae Bousfield, 1977

***Niphargus alisadri* Esmaili-Rineh & Sari, 2013**

Status: **troglobiont**.

Records: Alisadr Cave (Hamedan Province) (Esmaili-Rineh and Sari 2013). Fig. 1, locality 5.

***Niphargus bisitunicus* Esmaili-Rineh, Sari & Fišer, 2015**

Status: **troglobiont**.

Records: Sarab-e-Bisitun (Kermanshah Province) (Esmaili-Rineh et al. 2015). Fig. 1, locality 10.

***Niphargus borisi* Esmaili-Rineh, Sari & Fišer, 2015**

Status: **troglobiont**.

Records: Belqis Spring (Kohgiluyeh and Boyer-Ahmad Province) (Esmaili-Rineh et al. 2015). Fig. 1, locality 4.

***Niphargus daniali* Esmaili-Rineh & Sari, 2013**

Status: **troglobiont**.

Records: Danial Cave (Mazandaran Province) (Esmaili-Rineh and Sari 2013). Fig. 1, locality 6.

***Niphargus darvishi* Esmaili-Rineh, Sari & Fišer, 2015**

Status: **troglobiont**.

Records: Dimeh Spring (Chaharmahal and Bakhtiari Province) (Esmaili-Rineh et al. 2015). Fig. 1, locality 12.

***Niphargus kermanshahi* Esmaili-Rineh, Heidari, Fišer & Akmal, 2016**

Status: **troglobiont**.

Records: Kangarshah Spring close to Sahneh City (Kermanshah Province) (Esmacili-Rineh et al. 2016). Fig. 1, locality 13.

***Niphargus kbayyami* Hekmatara, Zakšek, Heidari Baladehi & Fišer, 2013**

Status: **troglobiont**.

Records: Ghoori-Ghaleh Cave (Kermanshah Province) (Hekmatara et al. 2013). Fig. 1, locality 8.

***Niphargus khwarizmi* Hekmatara, Zakšek, Heidari Baladehi & Fišer, 2013**

Status: **troglobiont**.

Records: Cheshmeh Kahriz (Qanat) (East Azarbayjan Province) (Hekmatara et al. 2013). Fig. 1, locality 7.

***Niphargus sharifi* Esmacili-Rineh, Sari & Fišer, 2015**

Status: **troglobiont**.

Records: Sarab-e-Robat (Lorestan Province) (Esmacili-Rineh et al. 2015). Fig. 1, locality 11.

***Niphargus valachicus* Dobreanu & Manolache, 1933**

Status: **troglobiont**.

Records: unidentified cave near Ghaem Shahr (Mazandaran Province) (Karaman 1998). Fig. 1, locality 9.

Order Isopoda Latreille, 1817

Suborder Oniscidea Latreille, 1802

The order Isopoda contains over 10,000 species worldwide. Around 4,500 marine species are found mostly on the seabed; 500 species live in fresh water, while another 5,000 species from suborder Oniscidea are terrestrial (Schotte et al. 2016). The checklist of Oniscidea of Iran has not been published yet, but there are more than 40 recorded species from the country. Four species of this order have been listed here, including one troglobiont.

Family Agnaridae Schmidt, 2002

***Protracheoniscus faramarzi* Kashani, Dashan & Sadeghi, 2016 (in press)**

Status: **troglophile**.

Records: Shafagh Cave (Fars Province) (Kashani et al. 2016. In press).

***Protracheoniscus gakalicus* Kashani, Malek Hosseini & Sadeghi, 2013**

Status: **troglobiont**.

Records: Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (Kashani et al. 2013). Fig. 1, locality 2.

Family Porcellionidae Brandt, 1831

***Agabiformius lentus* (Budde-Lund, 1855)**

Status: **trogloxene**.

Records: Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (Kashani et al. 2013).

***Porcellionides pruinosus* (Brandt, 1833)**

Status: **trogloxene**.

Records: Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (Kashani et al. 2013).

Class Maxillopoda Dahl, 1956

Order Cyclopoida Burmeister, 1834

Family Cyclopidae Rafinesque, 1815

***Diacyclops* cf. *crassicaudis* (Sars, 1863)**

Status: **troglophile**.

Records: Kiler Cave (Kohgiluyeh and Boyer-Ahmad Province) (present study).
Species was identified by Dr. Frank Fiers from the Royal Belgian Institute of Natural Sciences.

Subphylum Hexapoda Latreille, 1825

Class Entognatha Stummer-Traunfels, 1891

Order Entomobryomorpha Börner 1913

There are more than 8,600 described species of collembola worldwide (Bellinger et al. 2016), of which, more than 400 are hypogean (Romero 2009). The collembolan fauna of Iran is poorly known, it consists of 112 species, belonging to 18 families and 57 genera (Shayanmehr et al. 2013). There has been no detailed study

of cavernicolous collembola in Iran. Three species which have been listed below had been previously reported from soil and leaf litter (Shayanmehr et al. 2013). Specimens were identified according to Fjellberg (2007), by Dr. Masoumeh Shayan Mehr (Sari University of Agricultural Sciences and Natural Resources, Sari, Mazandaran, Iran).

Family Cyphoderidae Börner, 1913

***Cyphoderus albinus* (Nicolet, 1842)**

Status: **troglophile**.

Records: Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (present study).

Family Entomobryidae Schäffer, 1896

***Heteromurus nitidus* (Templeton, 1835)**

Status: **troglophile**.

Records: Deh Sheikh (Pataveh) Cave, Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (present study).

***Pseudosinella octopunctata* (Boerner, 1901)**

Status: **troglophile**.

Records: Kiler Cave (Kohgiluyeh and Boyer-Ahmad Province) (present study).

Class Insecta Linnaeus, 1758

Order Blattodea Brunner von Wattenwyl, 1882

To date, over 4,500 cockroach (order Blattodea or Blattaria) species have been reported from different parts of the world. The cockroach fauna of Iran includes three families, 14 genera, and 26 species (Hashemi-Aghdam and Oshaghi 2015). Many geographical regions of the country have not been studied yet, hence a systematic research is required to reveal the real cockroach biodiversity of the country. They were observed as guanophilic animals in most of Iranian caves and in some cases with a high density of populations. However, these cavernicolous cockroaches haven't been studied so far. Adaptations to life in darkness such as very long antennae and long legs with numerous sensory hairs and setae, have been observed in some populations.

Family Blattidae Latreille, 1810

***Shelfordella* cf. *monochroma* (Walker, 1871)**

Status: **troglophile**.

Records: Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (Tahami and Sadeghi 2015).

Family Polyphagidae Saussure, 1864

***Polyphaga aegyptica* (Linnaeus, 1758)**

Status: **troglophile**.

Records: Rood Fargh Cave (Kerman Province) (Tahami and Sadeghi 2015).

Order Coleoptera Linnaeus, 1758

There are over 330,000 species of beetles in the world; 1,927 terrestrial and 32 aquatic troglobionts have been described so far (Gibert and Deharveng 2002). The checklists of most families in Iran are not available.

Family Carabidae Latreille, 1802

Azadbakhsh & Nozari (2015) listed 955 species and subspecies in 155 genera, belonging to 26 subfamilies of Carabidae from Iran. Here we have listed 8 species from caves.

***Abacetus zarudnyi* Tschitschérine, 1901**

Status: **troglophile**.

Records: Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek Hosseini et al. 2016a).

***Bembidion* (*Ocyturanus*) *parsorum* Netolitzky, 1934**

Status: **trogloxene**.

Records: Nevel Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek Hosseini et al. 2016a).

***Duvalius kileri* Muilwijk & Malek Hosseini, 2016**

Status: **troglophile**.

Records: Kiler Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek Hosseini et al. 2016a).

***Laemostenus (Antispodrus) aequalis* Muilwijk & Malek Hosseini, 2016**

Status: **troglophile**.

Records: Kiler Cave, Deh Sheikh (Pataveh) Cave, Nevel Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek Hosseini et al. 2016a).

***Laemostenus (Antispodrus) bulirschii* Dvořák, 1995**

Status: **troglophile**.

Records: unidentified cave in Elburz, Demavend (Lohajr and Mlejnek 2007).

***Laemostenus (Antispodrus) deconincki* Casale & Quéinnec, 2001**

Status: **troglophile**.

Records: Gamasiab Cave (Hamadan Province) (Casale and Quéinnec 2001), unidentified cave (Lorestan Province) (Lohajr and Mlejnek 2007).

***Laemostenus (Antispodrus) glasunowi* Semenov, 1895**

Status: **troglophile**.

Records: unidentified cave in Elburz, Demavend (Mazandaran Province) (Lohajr and Mlejnek 2007).

***Paratachys fulvicollis* (Dejean, 1831)**

Status: **troglophile**.

Records: Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek Hosseini et al. 2016a).

Family Leiodidae Fleming, 1821

This family has not been studied in Iran so far. Less than 5 species are known from the country. Here we have listed one species from a cave.

***Eocatops beroni* Szymczakowski, 1976**

Status: **troglophile**.

Records: Shapur Cave (Fars Province) (Szymczakowski, 1976).

Family Ptinidae Latreille, 1802

Currently, about 220 genera and 2,200 species of spider beetles are known in the world. Several ptinids have been previously reported from caves, mostly in Europe (Arango and Young 2012). However, little is known about the spider beetle fauna of Iran.

***Niptus hololeucus* (Faldermann, 1835)**

Status: **troglophile**.

Records: Kangohar Cave (Fars Province) (Dashan et al. 2014a).

***Gibbium aequinoctiale* Boieldieu, 1854**

Status: **troglophile**.

Records: Kangohar Cave, Shafagh Cave, Tadovan Cave, Sahlak Cave (Fars Province) (Dashan et al. 2014b).

Family Salpingidae Leach, 1815

About 45 genera and 300 species are recognized worldwide, of which, a single troglophile species has been listed here (Sadeghi et al. 2014).

***Aglenus brunneus* (Gyllenhal, 1813)**

Status: **troglophile**.

Records: Tadovan Cave (Fars Province) (Sadeghi et al. 2014).

Family Staphylinidae Lameere, 1900

The family Staphylinidae, or rove beetles, with 56,768 described species in 3,624 genera and 33 subfamilies, is one of the most diverse families of animals. Staphylinidae of Iran contains 594 species and subspecies in 150 genera belonging to 13 subfamilies. Amongst those, 104 species and subspecies (17,5 %) are known only from Iran (Anlas and Newton 2010).

***Quedius iranicus* Coiffait, 1976**

Status: **troglophile**.

Records: Bishapur, Shapur Cave (Fars Province) (Coiffait 1976, 1978, Bordoni and Oromi 1998, Herman 2001, Smetana 2004).

***Stenus guttula* Muller, 1821**

Status: **trogloxene**.

Records: Behesht Makan Cave (Fars Province) (Dashan et al. 2014b).

***Stenus limicola* Korge, 1967**

Status: **trogloxene**.

Records: Behesht Makan Cave (Fars Province) (Dashan et al. 2014b).

Family Tenebrionidae Latreille, 1802

Darkling beetles, with more than 15,000 known species are one of the most common families of the order Coleoptera. The fauna of Iranian Tenebrionidae is considered poorly studied (Ghahari and Bunalski 2011). The most common genus in Iranian caves is *Blaps* Fabricius, 1775. In some caves this beetle was observed in high numbers and in all zones.

***Blaps variolaris* Allard, 1880**

Status: **troglophile**.

Records: Behesht Makan Cave, Dalkhoon Cave, Sahlak Cave, Tadovan Cave, Kangohar Cave (Fars Province) (Dashan et al. 2014b), Kiler Cave, Gakal Cave, Deh Sheikh (Pataveh) Cave (Kohgiluyeh and Boyer-Ahmad Province) (present study).

***Trachyderma christophi* Faust, 1875**

Status: **trogloxene**.

Records: Neyneh Cave (Kohgiluyeh and Boyer-Ahmad Province) (present study). The species was identified by Dr. Wolfgang Schawaller from the State Museum of Natural History, Stuttgart, Germany.

Order Diptera Linnaeus, 1758

Here, we have only mentioned ectoparasite Diptera that were collected from bats. The most conspicuous bat ectoparasites are the bloodsucking nycteribiid and streblid flies that are obligatory ectoparasites of bats (Marshall 1982, Theodor 1967). Till now only very limited studies have been done on the ectoparasites of bats in Iran (Benda et al. 2012). Here, 10 species from 6 genera and 2 families of Diptera have been listed.

Family Nycteribiidae Samouelle, 1819

***Eucampsipoda hyrtlii* Kolenati, 1856**

Status: **bat ectoparasite.**

Records: Shafagh Cave (Fars Province) (Malek-Hosseini et al. 2016b).

***Nycteribia pedicularia* Latreille, 1805**

Status: **bat ectoparasite.**

Records: Neyneh Cave (Kohgiluyeh and Boyer-Ahmad Province), Shafagh Cave (Fars Province) (Malek-Hosseini et al. 2016b).

***Nycteribia schmidlii* Schiner, 1853**

Status: **bat ectoparasite.**

Records: Mozduran Cave (Razavi Khorasan Province) (Kock 1983), Tadovan Cave (Fars Province) (Benda et al. 2012), Gakal Cave, Neyneh Cave and Kiler cave (Kohgiluyeh and Boyer-Ahmad Province), Shafagh Cave (Fars Province) (Malek-Hosseini et al. 2016b).

***Nycteribia vexata* Westwood, 1835**

Status: **bat ectoparasite.**

Records: Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (Malek-Hosseini et al. 2016b).

***Penicillidia conspicua* Speiser, 1901**

Status: **bat ectoparasite.**

Records: Tadovan Cave (Fars Province), Dashkahul Cave (Ardabil Province) (Benda et al. 2012), Kiler Cave (Kohgiluyeh and Boyer-Ahmad Province), Tadovan Cave (Fars Province) (Malek-Hosseini et al. 2016b).

***Penicillidia dufourii* (Westwood, 1835)**

Status: **bat ectoparasite.**

Records: Tadovan Cave (Fars Province), Dashkahul Cave (Ardabil Province) (Benda et al. 2012), Kiler Cave, Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province), Shafagh Cave (Fars Province) (Malek-Hosseini et al. 2016b).

***Phthiridium biarticulatum* Hermann, 1804**

Status: **bat ectoparasite.**

Records: Mozduran Cave (Razavi Khorasan Province) (Kock 1983), Dashkahul Cave (Ardabil Province) (Benda et al. 2012), Tadovan Cave, Charkhab Cave (Fars Province) (Malek-Hosseini et al. 2016b).

***Stylidia biarticulata* Hermann, 1804**

Status: **bat ectoparasite.**

Records: Mozduran Cave (Razavi Khorasan Province) (Kock 1983).

Family Streblidae Kolenati, 1863

***Brachytarsina alluaudi minor* Falcoz, 1923**

Status: **bat ectoparasite.**

Records: Charkhab Cave (Fars Province) (Malek-Hosseini et al. 2016b).

***Brachytarsina flavipennis* Macquart, 1851**

Status: **bat ectoparasite.**

Records: Mozduran Cave (Razavi Khorasan Province) (Kock 1983), Tadovan Cave (Fars Province) (Benda et al. 2012), Tadovan Cave (Fars Province) (Malek-Hosseini et al. 2016b).

Order Hemiptera Linnaeus, 1758

Family Cimicidae Latreille, 1802

***Stricticimex namru* Usinger, 1960**

Status: **bat ectoparasite.**

Records: Dehloran Cave (Ilam Province) (Benda et al. 2012).

Order Siphonaptera Latreille, 1825

Family Ischnopsyllidae Wahlgren, 1907

***Ischnopsyllus dolosus* Dampf, 1912**

Status: **bat ectoparasite.**

Records: Dashkasan Cave, Dashkahul Cave (Ardabil Province) (Benda et al. 2012).

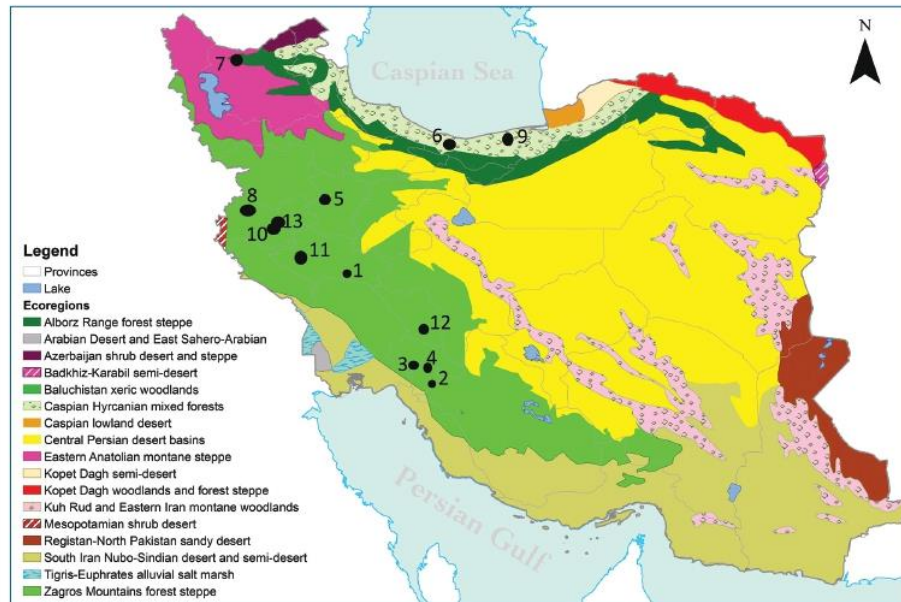


Figure 1. Map of terrestrial ecoregions in Iran, showing the distribution of troglobiotic organisms (16 species). Map derived from WWF (Olson et al. 2001) and Safaei-Mahroo et al. (2015). **1** Loven Cave (Lorestan Province) (33°04'N, 48°35'E) *Garra typhlops*; *G. lorestanensis*; *Paracobitis smithi* **2** Gakal Cave (Kohgiluyeh and Boyer-Ahmad Province) (30°18'N, 51°09'E) *Protracheoniscus gakalicus*; *Trilacuna qarzi* **3** Neyneh Cave (Kohgiluyeh and Boyer-Ahmad Province) (30°40'N, 50°2'E) *Chiraziulus troglopersicus* **4** Belqais Spring (Kohgiluyeh and Boyer-Ahmad Province) (30°45'N, 50°44'E) *Niphargus borisi* **5** Alisadr Cave (Hamedan Province) (35°16'N, 48°17'E) *Niphargus alisadri* **6** Danial Cave (Mazandaran Province) (36°41'N, 50°53'E) *Niphargus daniali* **7** Cheshmeh Kahriz (Qanat) (East Azarbayjan Province) (38°41'N, 46°10'E) *Niphargus khwarizmi* **8** Ghoori-Ghaleh Cave (Kermanshah Province) (34°53'N, 46°30'E) *Niphargus khayyami* **9** unidentified cave near Ghaem Shahr (Mazandaran Province) *Niphargus valachicus* **10** Sarab-e- Bisitun (Kermanshah Province) (34°25'N, 47°28'E) *Niphargus bisitunicus* **11** Sarab-e-Robat (Lorestan Province) (33°35'N, 48°18'E) *Niphargus sharifi* **12** Dimeh Spring (Chaharmahal and Bakhtiari Province) (32°30'N, 50°13'E) *Niphargus darvisi* **13** Kangarshah Spring close to Sahneh City (Kermanshah Province) (34°36'N, 47°39'E) *Niphargus kermanshahi*

Discussion

In our checklist, we compiled faunal lists of subterranean arthropods for Iranian caves based on scattered published literature together with unpublished data from our own collections. There are some ongoing projects addressing issues of biodiversity that will improve our knowledge of the cave fauna in this vast country, e.g. studies of cavernicolous pseudoscorpions, myrmecophilid crickets, oniscid isopods and niphargid amphipods by students of the University of Shiraz and Razi University of Kermanshah are being carried out. Moreover, there are other groups of cavernicolous arthropods in our collections that we did not list here because of incomplete identification, e.g. some species of Collembola, Coleoptera, Orthoptera (Grylloidea), Crustacea, Myriapoda, Araneae, Pseudoscorpiones and Acari. Ac-

According to our investigations, caves of Alborz and Zagros chains are rich in fauna. All Iranian troglomorphic species were described from the central and northern parts of Zagros and northern slopes of Alborz, which are mostly forested. About 10 species of troglomorphic crustaceans from Zagros caves and karstic springs are waiting to be described. The Zagros region exemplifies the continental variant of the Mediterranean climate pattern, while the northern slopes of Alborz are distinctly humid. So, these areas have a high level of productivity. The low species richness of Iranian caves is not realistic and it is due to a lack of comprehensive studies of all taxa (as a comparison, Sket et al. (2004) reported more than 975 terrestrial and 650 aquatic obligate subterranean species for the Balkan Peninsula).

An important problem is related to the identification keys for cavernicolous animals. To date, all studies have been sporadic. There is an ongoing comprehensive study on *Niphargus* of Iranian caves by Dr. Cene Fišer from University of Ljubljana, Slovenia with Iranian collaborators. The complete keys are needed to help future researchers.

From all 16 identified troglomorphic species of Iran (3 terrestrial, 13 aquatic), 13 are arthropods, and out of these, 11 are crustaceans and 10 belong to the genus *Niphargus* (Niphargidae). From all 89 species of this checklist (from 42 caves and 5 karstic springs), Araneae with 29 species and Coleoptera with 17 species are dominant groups. A problem with some literature records are ambiguous names and localities of some caves that need to be clarified in further studies. Our studies about cave fauna should lead to decisions about conservation approaches. Unfortunately, based on our exploration and some other reports from caving groups, several caves have already been destroyed by human activities, which could cause drastic changes in food chains in these habitats, or even species extinction.

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2.2 REMAINING LINKING SCIENTIFIC WORK

2.2.1 Overview of troglobiont biodiversity in Iran

This part of dissertation represents updated information to the checklist we published in 2017. In that checklist 2017 (Malek-Hosseini and Zamani, 2017), 16 troglobiont species were listed for Iran. However, already at the time of this publication, some new troglobionts were being described from Iran. The list also missed 12 species of copepods from older literature (mostly in French and German languages) and one oligochaete. Including these missing sources, 29 species had been reported as troglobionts from Iran in 2017. Here, we represent all Iranian troglobiont animals (previously published and our new discoveries, including yet undescribed species). The species richness and distribution patterns of Iranian troglobiotic diversity are presented, including the currently recognized hotspots. So, the aims of this chapter are:

- to provide the updated checklist of troglobiont species of Iran
- to map the distribution of troglobionts in Iran
- to detect any hotspots of troglobiont biodiversity in Iran

2.2.1.1 Introduction

Iran is 1.648 million km² large country (the second-largest country in the Middle East after Saudi Arabia), situated in western Asia. Most of the land lies in an arid environmental zone. The area coverage of different types of climates in Iran is 35.5% hyper-arid, 29.2% arid, 20.1% semi-arid, 5% Mediterranean and 10% wet (of the cold mountainous type). Thus, more than 82% of Iran's territory is located in the arid and semi-arid zone of the world. The average rainfall in Iran is about 250 mm, which is less than 1/3 of the average rainfall in the world (860 mm) (Amiri and Eslamian, 2010). In addition, this sparse precipitation is also unfavorable with respect to time and location. Another important climatic element is extreme temperature changes that at some places range from -20 to +50°C. Severe drought is also recognized as a feature of Iran's climate (Amiri and Eslamian, 2010). The rainfall is different from more than 1800 mm in northern parts of Iran on the Caspian seashore and in some parts of the Zagros about 800 mm, but average is about 400 mm in the sloping region of Alborz and Zagros mountains, and in arid parts and central Iran less than 100 mm (Raziei et al., 2005; Soltani et al., 2012). During this decade, the country has suffered severe desiccation

and this lack of rainfall has resulted in extensive losses of water resources, vegetation, and productivity. It is one of the world's most mountainous countries, its landscape dominated by rugged mountain ranges that separate various basins or plateaux from one another. The Zagros and Alborz Mountains are two main mountain chains in western and northern Iran, which comprise nearly a third of the Iranian surface. Geologically, Iran is a part of the Alpine-Himalayan orogenic belt, divided into five major structural zones: a) Zagros Range, b) Sanandaj- Sirjan Range, c) Central Iran, d) East and South-East Iran and e) Alborz and Kopet- Dagh Ranges (Raeisi et al. 2012). The proportion of carbonate karst surface in Iran land area is more than 11% (Raeisi et al. 2012), with more than 2000 discovered caves up to now (Raeisi et al. 2012), and many new expected. This value of carbonate karst surface increases to about 23% in the south-central region of Iran (Raeisi and Kowsar, 1997).

During the last decade, several faunistic studies have been carried out in Iran, resulting in new records of already known species, as well as the descriptions of many new ones. However, despite the vast amount of collected faunistic data, the Iranian subterranean fauna remains inadequately known, and most reported species from the caves are in fact troglaphiles and troglloxenes (Malek-Hosseini et al., 2015a, b; Malek-Hosseini and Zamani, 2017; Malek-Hosseini et al., 2021).

During this decade, more discoveries have been carried out in underground environments of Iran and mostly in Zagros Mountains. Another new species of cave fishes, new habitats and new troglbiont arthropods have been discovered in Iran. 23 species of the genus *Niphargus* Schiødte, 1849 from amphipods have been recorded from Iran from 28 localities. So, the genus *Niphargus* is with highest species richness of troglbionts in Iran (Mousavi-Sabet et al., 2016; Bargrizaneh et al., 2021). Other troglbionts include a spider, a millipede, a terrestrial oniscid isopod, few species of copepods and a coleopteran beetle. Most of these troglbionts have been described during last 15 years (Kashani et al., 2013; Malek-Hosseini et al., 2015a; Reboleira et al., 2015; Malek-Hosseini et al., 2021).

Endemism and species richness are highly relevant to the prioritization of conservation efforts in which Iranian subterranean environments have remained relatively neglected. The discovery component and preparation of a checklist of Iranian troglbionts with the map of distribution, will enable us to establish Iran as an important part of the world map of subterranean biodiversity. This will therefore facilitate conservation decisions.

2.2.1.2 Material and methods

We used all published literature in any language and supplemented it with our own data. Some new taxa were described recently, but there are many more new species that were identified via DNA analysis and await morphological descriptions. Records of yet undescribed species are referenced in the separate section of this list.

In this chapter, we provide the updated checklist of Iranian troglobiont animals based on the available literature, our collection and published and unpublished material. We mentioned the species that have been reported as troglobiont in literature. For our own observations of collected species in this study, we used the term of troglobiont that is determined in the introduction of this thesis. Up to date, more than 100 caves, wells and springs have been surveyed for subterranean animals (arthropods, mollusks, oligochaetes and fishes). The caves that have been reported as habitats for bats only, are not included. However, many of these caves out of these 100 habitats, harbor bats. Most of reported animals are indeed troglaphiles and troglonexes (Malek-Hosseini and Zamani., 2017; Tahami et al., 2017). Prior to this study, 46 species had been reported as troglobiont from Iran. Some taxa that were reported for the first time from Iran as troglobiont, were discovered by a group that the author of this thesis (Mohammad Javad Malek-Hosseini) was involved in (e.g. Malek-Hosseini et al., 2015a; Khalaji-Pirbalouti et al., 2019; Zamanpoore et al., 2020), and so, most of discoveries are recent (2010-2021) (e.g. Hekmatara et al., 2013; Mousavi-Sabet et al., 2016; Esmaeili-Rineh, et al., 2018; Bargrizaneh et al., 2021).

When mapping species richness and sampled localities distribution, the problem of finding suitable scale for analyses needs to be addressed because different scales shows that the pattern depends on the scale size we use (Stoms, 1994). If the basic sample cells are too large, the regional patterns and details are lost, but if the cells are too small, the data are over-scattered (Fortin and Dale, 2005).

Regarding this fact that not many subterranean habitats have been studied in Iran and thus considering the vastness of the country and number of known caves and karstic springs from Iran, the density of caves and springs in each area, we decided to consider 100×100 km scale for analyses. We generated a troglobiont species-richness map by calculating the number of species present in each 100×100 -km UTM grid cell.

Our research area covers the whole Iran but as showed in the maps, most species have been reported from west and north of the country. Considering the low number of species, we mentioned all troglobiont species in this study.

Localities were identified according to the descriptions in the literature sources, and we also used our own data. For some old literatures without the exact locality, we could not determine exact position, so we put the coordinates to the central parts of the closest town or village; this was done for five localities, harboring 14 species.

In the first part of the analysis, we prepared maps with pointed localities by numbers. The details are shown in table 2. Number of species are determined for each subterranean habitat. Richness per locality is shown on map by the size of circles and points. We used QGIS to prepare the distribution maps of species.

2.2.1.3 Updated checklist of troglobiont species of Iran

From all 49 identified and described troglobiont species of Iran (five terrestrial, 44 aquatic), 43 species are arthropods, and of these, 39 species are crustaceans, and 23 belong to the genus *Niphargus* (Niphargidae).

In this study, we discovered 26 new species of troglobiont animals. We have described three of them. We described also a troglophile species of beetles, sister to the new discovered troglobiont one (Malek-Hosseini et al., 2021; Fatemi et al., 2019). In this thesis, we only presented the three published articles including the descriptions of two of our new troglobiont species (*Duvalius nezelensis* Muilwijk and Malek-Hosseini, 2021 of carabid beetles; *Trogloiranica tashanica* Fatemi, Malek-Hosseini, Falniowski, Hofman, Kuntner & Grego 2019 of gastropods and *Asellus ismailsezarii* Malek-Hosseini, Jugovic, Fatemi & Douady, 2022 from Isopoda) (Malek-Hosseini et al., 2022) and a troglophile *Duvalius achaemenius* Muilwijk and Malek-Hosseini, 2021.

The description of another species of obligate cave-dwelling species of crustacea (a *Caridina* atyid shrimp) is under preparation.

List of species

Phylum Chordata

Class Actinopterygii Klein, 1885

Order Cypriniformes Bleeker, 1859

Family Cyprinidae Linnaeus, 1758

***Garra lorestanensis* Mousavi-Sabet & Eagderi, 2016**

Synonym: *Iranocypris typhlops*, Bruun, E. W. Kaiser, 1944

Records: Tang-e-Haft- Darreh Bagh - Sirom Village, Pol Dokhtar City, Lorestan Province, Loven Cave, N33°4'39" E48°35'33" (Mousavi-Sabet et al., 2016), Figure 9, locality 20.

Records: Tuveh Village, Andimeshk City, Khuzestan Province, Tuveh Spring, N32°48'48.8" E48°43'7", (Vatandoust et al., 2019), Figure 9, locality 19.

Records: Talkhab Hendmini Village, Darrehshah City, Ilam Province, Drainage, the Simarreh River basin, intake tunnel for a dam, N33°16'56" E47°12'16", (Mahjoorazad and Coad, 2009), Figure 9, locality 21.

***Garra typhlops* (Bruun & Kaiser, 1944)**

Synonym: *Iranocypris typhlops*, Bruun & Kaiser, 1944

Records: Tang-e-Haft- Darreh Bagh - Sirom Village, Pol Dokhtar City, Lorestan Province, Loven Cave, N33°4'39" E48°35'33" (Bruun & Kaiser, 1944; Mousavi-Sabet et al., 2016), Figure 9, locality 20.

Records: Tuveh Village, Andimeshk City, Khuzestan Province, Tuveh Spring, N32°48'48.8" E48°43'7", (Vatandoust et al., 2019), Figure 9, locality 19.

***Garra tashanensis* Mousavi-Sabet, Vatandoust, Fatemi & Eagderi, 2016**

Records: Sarjoosher Village, Tashan district, Behbahan County, Khuzestan Province, Tashan Cave, N30°51'54" E50°10'29", (Mousavi-Sabet et al., 2016), Figure 9, locality 13.

Family Nemacheilidae Regan, 1911

Eidinemacheilus smithi (Greenwood, 1976)

Synonym: *Paracobitis smithi* (Greenwood, 1976)

Synonym: *Noemacheilus smithi* Greenwood, 1976

Records: Tang-e-Haft- Darreh Bagh - Sirom Village, Pol Dokhtar City, Lorestan Province, Loven Cave, N33°4'39" E48°35'33" (Greenwood, 1976; Hashemzadeh Segherloo et al., 2016), Figure 9, locality 20.

Phylum Annelida Lamarck, 1809

Class Clitellata Michaelsen, 1919

Subclass Oligochaeta Grube, 1850

Subclass Hirudinea Lamarck, 1818

Family Erpobdellidae Blanchard, 1894

Erpobdella borisi Cichocka, Bielecki, 2015

Records: Sahoolan-Eisa Kand Village, Mahabad City, West Azerbaijan Province, Sahoolan Cave N36°39'16.9" E45°57'10.8", (Cichocka et al., 2015), Figure 9, locality 37.

Class Gastropoda Cuvier, 1795

Subclass Caenogastropoda Cox, 1960

Order Littorinomorpha Golikov & Starobogatov, 1975

Moitessieriidae Bourguignat, 1863

****²Trogloiranica tashanica* Fatemi, Malek-Hosseini, Falniowski, Hofman, Kuntner & Grego 2019***

Records: Sarjoosher Village, Tashan district, Behbahan County, Khuzestan Province, Tashan Cave, N30°51'54" E50°10'29" (Fatemi et al., 2019), Figure 9, locality 13.

Phylum Arthropoda von Siebold, 1848

Subphylum Chelicerata Heymons, 1901

Class Arachnida Lamarck, 1801

Order Araneae Clerck, 1757

Family Oonopidae Simon, 1890

***Trilacuna qarzi* Malek Hosseini & Grismado, 2015**

Records: Basht City, Kohgiluyeh and Boyer-Ahmad Province, Gakal Cave, N30°18' E51°9' (Malek-Hosseini et al., 2015a, b), Figure 9, locality 5.

Subphylum Myriapoda Latreille, 1802

Class Diplopoda De Blainville in Gervais, 1844

Order Spirostreptida Brandt, 1833

² The asterisk symbol (*) in the beginning and end of the species names means that this species has been discovered and described in part of this thesis

Family Cambalidae Hoffman, 1979

Chiraziulus troglopersicus Reboleira, Malek Hosseini, Sadeghi & Enghoff, 2015

Records: Khaeez Vilage, Kohgiluyeh and Boyer-Ahmad Province, Neyneh Cave, N30°40'18" E50°21'71", (Reboleira et al., 2015), Figure 9, locality 12.

Subphylum Crustacea Brünnich, 1772

Class Malacostraca Latreille, 1802

Order Amphipoda Latreille, 1816

Family Niphargidae Bousfield, 1977

Niphargus alisadri Esmaeili-Rineh & Sari, 2013

Records: Hamedan Province, Alisadr Cave, N35°16' E48°17' (Esmaeili-Rineh and Sari, 2013), Figure 9, locality 32.

Niphargus bisitunicus Esmaeili-Rineh, Sari & Fišer, 2015

Records: Kermanshah Province, Sarab-e-Bisitun, N34°25' E47°28', (Esmaeili-Rineh et al., 2015), Figure 9, locality 28.

Niphargus borisi Esmaeili-Rineh, Sari & Fišer, 2015

Records: Choram City, Kohgiluyeh and Boyer-Ahmad Province, Belqis Spring, N30°45' E50°44', (Esmaeili-Rineh et al., 2015), Figure 9, locality 10.

Niphargus daniali Esmaeili-Rineh & Sari, 2013

Records: Lireh Sar Village, Salmanshahr City - Abbasabad district, Mazandaran Province, Danial Cave, N36°41' E50°53', (Esmaeili-Rineh and Sari, 2013), Figure 9, locality 40.

***Niphargus darvishi* Esmaeili-Rineh, Sari & Fišer, 2015**

Records: Kohrang-Chelgerd Village, Kohrang City, Chaharmahal and Bakhtiari Province, Dimeh Spring, N32°30' E50°13', (Esmaeili-Rineh et al., 2015), Figure 9, locality 17.

***Niphargus fiseri* Mamaghani-Shishvan & Esmaeili-Rineh, 2019**

Records: Badin Abad Village, Piranshahr City, West Azerbaijan Province, Badin Abad Spring, N36°34'55" E45°10'34" (Mamaghani-Shishvan and Esmaeili-Rineh, 2019), Figure 9, locality 36.

***Niphargus hakani* Esmaeili-Rineh, Mirghaffari & Sharifi, 2017**

Records: Razan City, Hamedan Province, Kheder- Goli Spring, N35°27' E49°07', (Esmaeili-Rineh et al., 2017a), Figure 9, locality 33.

***Niphargus hosseiniei* Esmaeili-Rineh, Sari, Fišer & Bargrizaneh, 2017**

Records: Sahneh City, Kermanshah Province, Nojivaran Spring, N34°29' E47°29', (Esmaeili-Rineh et al., 2017b), Figure 9, locality 29.

Records: Bazargan City, West Azerbaijan Province, Brolan Spring, N39°47' E44°35', (Esmaeili-Rineh et al., 2017b), Figure 9, locality 45.

Records: Ravansar City, Kermanshah Province, Ghori-Ghale Cave, N34°53' E46°34', (Esmaeili-Rineh et al., 2017b), Figure 9, locality 31.

***Niphargus ilamensis* Esmaeili-Rineh, Sari, Fišer & Bargrizaneh, 2017**

Records: Darreh Shahr City, Ilam Province, Sarab-e-Moord, N33°14' E46°54', (Esmaili-Rineh et al., 2017b), Figure 9, locality 23.

Records: Darreh Shahr City, Ilam Province, Sarab-e-Kanipahn, N33°20' E46°46', (Esmaili-Rineh et al., 2017b), Figure 9, locality 24.

***Niphargus keeleri* Zamanpoore & Bakhshi, 2019 (Figure 8b)**

Records: Roodrish Village, Dishmook County, Kohgiluyeh va Boyer-Ahmad Province, Keeler Cave, N31°18' E50°35' (Zamanpoore et al., 2019), Figure 9, locality 11.

***Niphargus kermanshahi* Esmaili-Rineh, Heidari, Fišer & Akmali, 2016**

Records: Sahneh City, Kermanshah Province, Kangarshah Spring, N34°36'53.7" E47°39'44.2", (Esmaili-Rineh et al., 2016), Figure 9, locality 30.

***Niphargus khayyami* Hekmatara, Zakšek, Heidari Baladehi & Fišer, 2013**

Records: Ravansar City, Kermanshah Province, Ghoori-Ghaleh Cave, N34°53' E46°34', (Hekmatara et al., 2013), Figure 9, locality 31.

***Niphargus khwarizmi* Hekmatara, Zakšek, Heidari Baladehi & Fišer, 2013**

Records: Kharvana village, Tabriz City, East Azarbayjan Province, Cheshmeh Kahriz (Qanat), N38°41' E46°10', (Hekmatara et al., 2013), Figure 9, locality 44.

***Niphargus kurdistanensis* Mamaghani-Shishvan, Esmaili-Rineh & Fišer, 2017**

Records: Baneh City, Kurdistan Province, Shoei Cave, N36°0'18" E45°53'45", (Mamaghani-Shishvan et al., 2017), Figure 9, locality 35.

Records: Marivan City, Kurdistan Province, Darvish-Olya Cave, N35°37'14" E46°37'59" (Mamaghani-Shishvan et al., 2017), Figure 9, locality 34.

***Niphargus lorestanensis* Esmaili-Rineh, 2018**

Records: Aligudarz City, Lorestan Province, Shol-Abad Spring, N33°09' E49°5' (Esmaili-Rineh, 2018), Figure 9, locality 22.

***Niphargus nasrullahi* Bargrizaneh, Esmaili-Rineh & Fišer, 2021**

Records: Kakan Village, Yasuj City, Kohgiluyeh va Boyer-Ahmad Province, Nahr Spring, N30°40' E51°43' (Bargrizaneh et al., 2021), Figure 9 locality 6.

***Niphargus persicus* Esmaili-Rineh, Sari, Fišer & Bargrizaneh, 2017**

Records: Tir-e-Bagh Village, Marvdasht City, Fars Province, Tir-e-Bagh Spring, N30°14' E52°40' (Esmaili-Rineh et al., 2017b), Figure 9, locality 4.

***Niphargus sari* Esmaili-Rineh, Niyakan & Akmal, 2018**

Records: Arkavaz Village, Chardavol City Ilam Province, Jo-Khanem Spring, N33°19' E46°40' (Esmaili-Rineh, et al., 2018), Figure 9, locality 25.

***Niphargus sharifi* Esmaili-Rineh, Sari & Fišer, 2015**

Records: Khoram-Abad City, Lorestan Province, Sarab-e-Robat, N33°35' E48°18', (Esmaili-Rineh et al., 2015), Figure 9, locality 26.

***Niphargus sohrevardensis* Esmaili-Rineh, Sari, Fišer & Bargrizaneh, 2017**

Records: Zanjan City, Zanjan Province, Sohrevard Spring, N36°40' E48°29', (Esmaili-Rineh et al., 2017b), Figure 9, locality 39.

Records: Khoram-Abad City, Lorestan Province, Razbashi Spring, N33°38' E48°35'
(Esmaili-Rineh et al., 2017b), Figure 9, locality 27.

***Niphargus urmiensis* Mamaghani-Shishvan & Esmaili-Rineh, 2019**

Records: Oshnavieh City, West Azerbaijan Province, Randole Spring, N37°3'25" E45°2'48"
(Mamaghani-Shishvan & Esmaili-Rineh, 2019), Figure 9, locality 38.

***Niphargus valachicus* Dobrea & Manolache, 1933**

Records: Ghaem Shahr, Mazandaran Province, unidentified cave (Karaman, 1998),
Approximate point: Figure 9, locality 43.

***Niphargus yasujensis* Bargrizaneh, Esmaili-Rineh & Fišer, 2021**

Biareh Village Sisakht City, Kohgiluyeh va Boyer-Ahmad Province, Biareh Spring,
N30°51' E51°27' (Bargrizaneh et al., 2021), Figure 9, locality 8.

Order Isopoda Latreille, 1817

Suborder Oniscidea Latreille, 1802

***Protracheoniscus gahalicus* Kashani, Malek Hosseini & Sadeghi, 2013 (Figure 8c)**

Records: Basht City, Kohgiluyeh and Boyer-Ahmad Province, Gahal Cave, N30°18' E51°9'
(Kashani et al., 2013), Figure 9, locality 5.

Suborder Asellota Latreille, 1802

Family Lepidocharontidae Galassi & Bruce, 2016

***Microcharon raffaellae* Pesce, 1979**

Records: Shahr-e-Kord City, Chaharmahal va Bakhtiari Province, undetermined well in literature (Pesce, 1979), Approximate point: Figure 9, locality 16.

Family Stenasellidae Dudich, 1924

Stenasellus tashanicus Khalaji-Pirbalouty, Fatemi, Malek-Hosseini, Kuntner, 2018 (Figure 8f)

Records: Sarjoosher Village, Tashan district, Behbahan County, Khuzestan Province, Tashan Cave, N30°51'54" E50°10'29" (Khalaji-Pirbalouty et al., 2018), Figure 9, locality 13.

Family Asellidae Rafinesque, 1815

**Asellus ismailsezarii* Malek-Hosseini, Jugovic, Fatemi & Douady, 2022* (Figure 8a)

Records: Tuveh village, Andimeshk, Khuzestan Province, Ganow spring, N32°48'31" E48°43'32", (Malek-Hosseini et al., 2022), Figure 9, locality 18.

Class Maxillopoda Dahl, 1956

Order Cyclopoida Burmeister, 1834

Family Cyclopidae Rafinesque, 1815

Megacyclops dusssarti orientalis Pesce, Pace & Maggi, 1984

Synonymy: *Acanthocyclops dussarti orientalis* Pesce, Pace & Maggi, 1984

Records: Cialestore Village, Shahr-E-Kord City, Chaharmahal va Bakhtiari Province, undetermined point in literature. (Pesce et al., 1984), Approximate point: Figure 9, locality 16.

Diacyclops zschokkei (Graeter, 1910)

Synonymy: *Cyclops zschokkei* Graeter, 1910; *Diacyclops languidoides zschokkei* (Juberthie, 2001).

Records: Kalarabad City, Mazandaran Province, undetermined point in literature. (Pesce et al., 1984), Approximate point: Figure 9, locality 41.

Records: Cialestore Village, Shahr-E-Kord City, Chaharmahal va Bakhtiari Province, undetermined point in literature. (Pesce et al., 1984), Approximate point: Figure 9, locality 16.

***Diacyclops iranicus* Pesce & Maggi, 1982**

Records: Kalarabad City, Mazandaran Province, Approximate point: Figure 9, locality 41.

Records: Shahr-e-Kord City, Chaharmahal va Bakhtiari Province, undetermined point in literature (Pesce and Maggi, 1982), Approximate point: Figure 9, locality 16.

***Haplocyclops neuter* Kiefer, 1955**

Synonymy: *Bryocyclops neuter* (Kiefer, 1955)

Records: Shahr-E-Kord City, Chaharmahal va Bakhtiari Province, undetermined point in literature (Kiefer, 1955), Approximate point: Figure 9, locality 16.

Order Harpacticoida G. O. Sars, 1903

Family Ameiridae Boeck, 1865

***Parapseudoleptomesochra iranica* (Löffler, 1959)**

Synonymy: *Nitocrella iranica* Löffler, 1959

Records: Neyriz City, Fars Province, Qualeh Kirmiz, bord du lac Niriz, undetermined point in literature (Löffler, 1959), Approximate point: Figure 9, locality 3.

***Nitocrella kirgizica* Borutsky, 1972**

Records: Neyriz City, Fars Province, Qualeh Kirmiz, bord du lac Niriz, undetermined point in literature, (Borutzky, 1972), Approximate point: Figure 9, locality 3.

***Nitocrella mara* Löffler, 1959**

Records: Neyriz City, Fars Province, Qualeh Kirmiz, bord du lac Niriz, undetermined point in literature, (Löffler, 1959), Approximate point: Figure 9, locality 3.

***Nitocrella paceae* Pesce, 1980**

Records: Shahr-e-Kord, Chaharmahal va Bakhtiari Province (Pesce, 1980), Approximate point: Figure 9, locality 16.

Records: Farokhshahr, Chaharmahal va Bakhtiari Province, (Pesce, 1980), Approximate point: Figure 9, locality 15.

***Nitocrella petkovskii* Pesce, 1980**

Records: Shahr-e-Kord, Chaharmahal va Bakhtiari Province (Pesce, 1980), Approximate point: Figure 9, locality 16.

Records: Farokhshahr, Chaharmahal va Bakhtiari Province, (Pesce, 1980), Approximate point: Figure 9, locality 15.

***Nitocrella stentinai* Sterba, 1973**

Records: Saidabad, no more data, (Sterba, 1973; Juberthie, 2001)

***Nitokra fallaciosa fallaciosa* Klie, 1937**

Records: Shahr-e-Kord, Chaharmahal va Bakhtiari Province (Juberthie, 1994; 1998; 2001),
Approximate point: Figure 9, locality 16.

Family Leptastacidae

***Paraleptastacus spinicaudus trisetatus* Noodt, 1954**

Synonymy: *Paraleptastacus spinicauda trisetosa* Serban, Lang, 1957

Record: Mahmoodabad City, Mazandaran Province, (Juberthie, 1994; 1998; 2001),
Approximate point: Figure 9, locality 42.

Class Insecta Linnaeus, 1758

Order Coleoptera Linnaeus, 1758

Family Tenebrionidae Latreille, 1802

***Foranotum perforatum* Nabozhenko & Sadeghi, 2017**

Record: Ashna village, Khonj City, Fars Province, Khan Cave, N27°44'41" E53°20'15"

(Nabozhenko and Sadeghi, 2017), Figure 9, locality 1.

Family Carabidae Latreille, 1802

Duvalius nezelensis* Muilwijk & Malek-Hosseini, 2021

Records: Jookhooneh Village, Sarparyab Town, Kohgiluyeh va Boyer-Ahmad Province,
Nezel Cave, N30°47'28.6" E50°56'53.8" (Malek-Hosseini et al., 2021), Figure 9, locality 9.

2.2.1.4 Unpublished or undescribed new troglomorphic species

Rather than discovered (described and undescribed) species in this study, there is another new species of the genus *Asellus* from an Iranian caves in literature (Beko, 2016) that has not been described yet.

Some animals have been identified to the family even order level. For three species of Oligochaeta, we may be dealing with new family for science. Regarding the molecular studies, it shows that this group is not included in any of other families. For morphology the same, but we need fresh and well-preserved specimens.

Despite this fact that in many cases, undescribed species are problematic and counting them as new species are doubtful, but in this study, the collected specimens from different groups of animals that we considered them as new species, first: are geographically far from other congeners; second: some have been discovered from the country for the first time even in order level; third: as we mentioned, initial DNA analysis showed that they are indeed new species and even far from the congeners from other parts of the world and also placed in distinct clade; and forth: the initial morphological check, dissection and comparisons, revealed that they are new species and different from described taxa.

List

Phylum Annelida Lamarck, 1809

Class Clitellata Michaelsen, 1919

Subclass Oligochaeta Grube, 1850

Order Opisthopora Michaelsen, 1930

Lumbricidae Rafinesque-Schmaltz, 1815

***Helodrilus* sp.**

Records: Dangezloo village, Padana County, Isfahan Province, Dangezloo Cave, N30°51' E51°38', Figure 9, locality 7.

Order Haplotaxida Brinkhurst, 1971

Oligochaeta sp. Not identified

Records: Torang Village, Baft City, Kerman Province, Torang Cave, N28°45' E56°49', Figure 9, locality 2.

Oligochaeta sp. Not identified

Records: Sarjoosher Village, Tashan district, Behbahan County, Khuzestan Province, Tashan Cave, N30°51'54" E50°10'29", Figure 9, locality 13.

Oligochaeta sp. Not identified

Records: Sarjoosher Village, Tashan district, Behbahan County, Khuzestan Province, Chah-Kabootari Cave, N30°52'10" E50°10'1", Figure 9, locality 14.

Order Cyclopoida Burmeister, 1834

Family Cyclopidae Rafinesque, 1815

***Mesocyclops* sp.**

Records: Sarjoosher Village, Tashan district, Behbahan County, Khuzestan Province, Tashan Cave, N30°51'54" E50°10'29", Figure 9, locality 13.

***Tropocyclops* sp. A** (Figure 8h)

Records: Sarjoosher Village, Tashan district, Behbahan County, Khuzestan Province, Tashan Cave, N30°51'54" E50°10'29", Figure 9, locality 13.

***Tropocyclops* sp. B**

Records: Sarjoosher Village, Tashan district, Behbahan County, Khuzestan Province, Tashan Cave, N30°51'54" E50°10'29", Figure 9, locality 13.

***Tropocyclops* sp. C**

Records: Sarjoosher Village, Tashan district, Behbahan County, Khuzestan Province, Tashan Cave, N30°51'54" E50°10'29", Figure 9, locality 13.

Records: Sarjoosher Village, Tashan district, Behbahan County, Khuzestan Province, Chah-Kabootari Cave, N30°52'10" E50°10'1", Figure 9, locality 14.

Order Harpacticoida G. O. Sars, 1903

Family Ameiridae Boeck, 1865

***Nitocrella* sp. A (Figure 8g)**

Records: Sarjoosher Village, Tashan district, Behbahan County, Khuzestan Province, Tashan Cave, N30°51'54" E50°10'29", Figure 9, locality 13.

Records: Sarjoosher Village, Tashan district, Behbahan County, Khuzestan Province, Chah-Kabootari Cave, N30°52'10" E50°10'1", Figure 9, locality 14.

Order Thermosbaenacea Monod, 1927

Family Monodellidae Taramelli, 1954

***Tethysbaena* sp. (Figure 8e)**

Records: Sarjoosher Village, Tashan district, Behbahan County, Khuzestan Province, Tashan Cave, N30°51'54" E50°10'29", Figure 9, locality 13.

Order Decapoda Latreille, 1802

Family Atyidae De Haan, 1849

***Caridina* sp. (Under description) (Figure 8d)**

Records: Sarjoosher Village, Tashan district, Behbahan County, Khuzestan Province, Tashan Cave, N30°51'54" E50°10'29", Figure 9, locality 13.

Order Amphipoda Latreille, 1816

Family Melitidae Bousfield, 1973

Records: Sarjoosher Village, Tashan district, Behbahan County, Khuzestan Province, Chah-Kabootari Cave, N30°52'10" E50°10'1", Figure 9, locality 14.

Family: Stegocephalidae Dana, 1852

Records: Sarjoosher Village, Tashan district, Behbahan County, Khuzestan Province, Tashan Cave, N30°51'54" E50°10'29", Figure 9, locality 13.

Family Niphargidae ³Bousfield, 1977

Records: Torang Village, Baft City, Kerman Province, Torang Cave, N28°45' E56°49', Figure 9, locality 2.

Order Isopoda Latreille, 1817

Suborder Asellota Latreille, 1802

³ The specimens are in the collection of Shiraz University, Iran.

Family Stenasellidae Dudich, 1924

***Stenasellus* sp.**

Records: Sarjoosher Village, Tashan district, Behbahan County, Khuzestan Province, Chah-Kabootari Cave, N30°52'10" E50°10'1", Figure 9, locality 14.

Asellidae Rafinesque, 1815

⁴*Asellus* sp.

Records: Sahoolan-Eisa Kand Village, Mahabad City, West Azerbaijan Province, Sahoolan Cave, N36°39'16.9" E45°57'10.8", (Bevk, 2016), Figure 9, locality 36.

Order Araneae Clerck, 1757

Family Oonopidae Simon, 1890

Records: Sarjoosher Village, Tashan district, Behbahan County, Khuzestan Province, Tashan Cave, N30°51'54" E50°10'29", Figure 9, locality 13.

***Araneae* sp.**

Records: Sarjoosher Village, Tashan district, Behbahan County, Khuzestan Province, Chah-Kabootari Cave, N30°52'10" E50°10'1", Figure 9, locality 14.

Subclass Acari Leach, 1817

Mesostigmata G. Canestrini, 1891

⁴ This species is not from our collection. It is from Bevk, 2016.

Records: Sarjoosher Village, Tashan district, Behbahan County, Khuzestan Province, Tashan Cave, N30°51'54" E50°10'29", Figure 9, locality 13

Class Diplopoda De Blainville in Gervais, 1844

Order Polydesmida Leach, 1815

Records: Sarjoosher Village, Tashan district, Behbahan County, Khuzestan Province, Tashan Cave, N30°51'54" E50°10'29", Figure 9, locality 13.

Class Insecta Linnaeus, 1758

Order Zygentoma Börner, 1904

Family Nicoletiidae Escherich, 1905

Records: Sarjoosher Village, Tashan district, Behbahan County, Khuzestan Province, Tashan Cave, N30°51'54" E50°10'29", Figure 9, locality 13.

Subclass Collembola Lubbock, 1871

Records: Sarjoosher Village, Tashan district, Behbahan County, Khuzestan Province, Tashan Cave, N30°51'54" E50°10'29", Figure 9, locality 13.

Class Gastropoda Cuvier, 1795

Subclass Caenogastropoda Cox, 1960

Order Littorinimorpha Golikov & Starobogatov, 1975

Hydrobiidae Stimpson, 1865

Records: Tuveh Village, Andimeshk City, Khuzestan Province, Tuveh Spring, N32°48'48.8" E48°43'7", (Vatandoust et al., 2019), Figure 9, locality 19.

2.2.1.5 New records of cave fish

During field works, we discovered some new localities for described Iranian cave fishes.

Chah-Kabootari cave (Figures 4-5), a limestone cave with sulfur water, a kilometer to west of the Tashan cave harbors *Garra tashanensis*. Several pools were discovered in Tashan cave as well. Although, the difference in morphology, DNA analysis determined that the isolated populations of Tashan cave belong to a single species. Tashan cave and Chah-Kabootari populations belong to the single species of *G. tashanensis*.

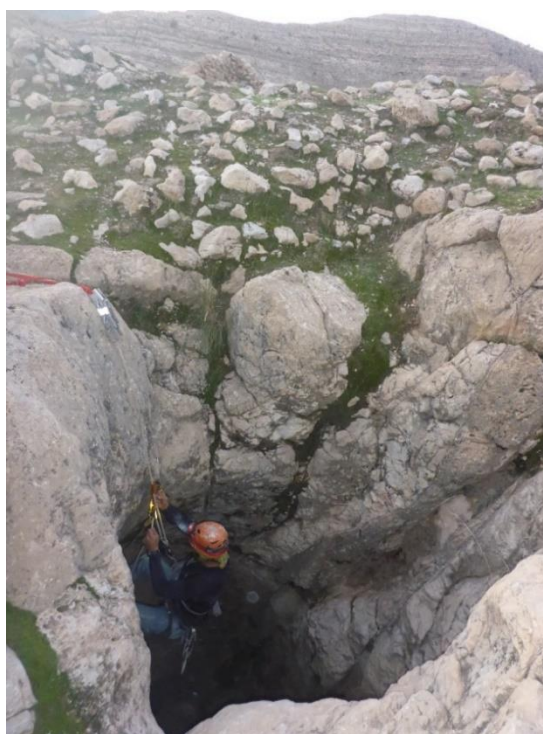


Figure 4: Entrance of Chah-Kabootari cave (Photo: Malek-Hosseini M. J., 2018).



Figure 5: Population of *Gara tashanensis* in Chah-Kabootari cave (Photo: Malek-Hosseini M. J., 2018).

Tuveh spring, is another locality was discovered as habitat for *Eidinemacheilus smithi* (Figure 6). Tuveh spring (Figure 7) that was reported in 2019 as a new locality for the Iranian cave barbs *Garra lorestanensis* and *G. typhlops*, harbors the cave loach fish as well. Although Vatandoust et al., 2019 mentioned that *E. smithi* potentially could exist in this habitat, they did not collect this species there. These three species were reported previously from Loven cave, about 30 km to the north of Tuveh Spring.



Figure 6: *Eidinemacheilus* cf. *smithi* from Tuveh spring (Photo: Fatemi Y., 2018).

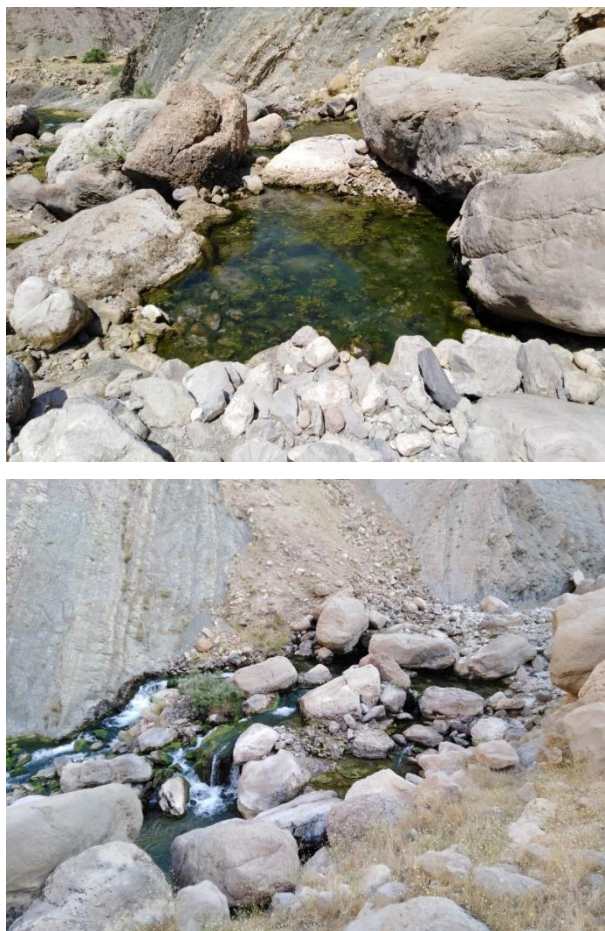


Figure 7: Tuveh Spring, habitat locality of Iranian cave fish (Photos: Malek-Hosseini M. J., and Fatemi Y., 2018).

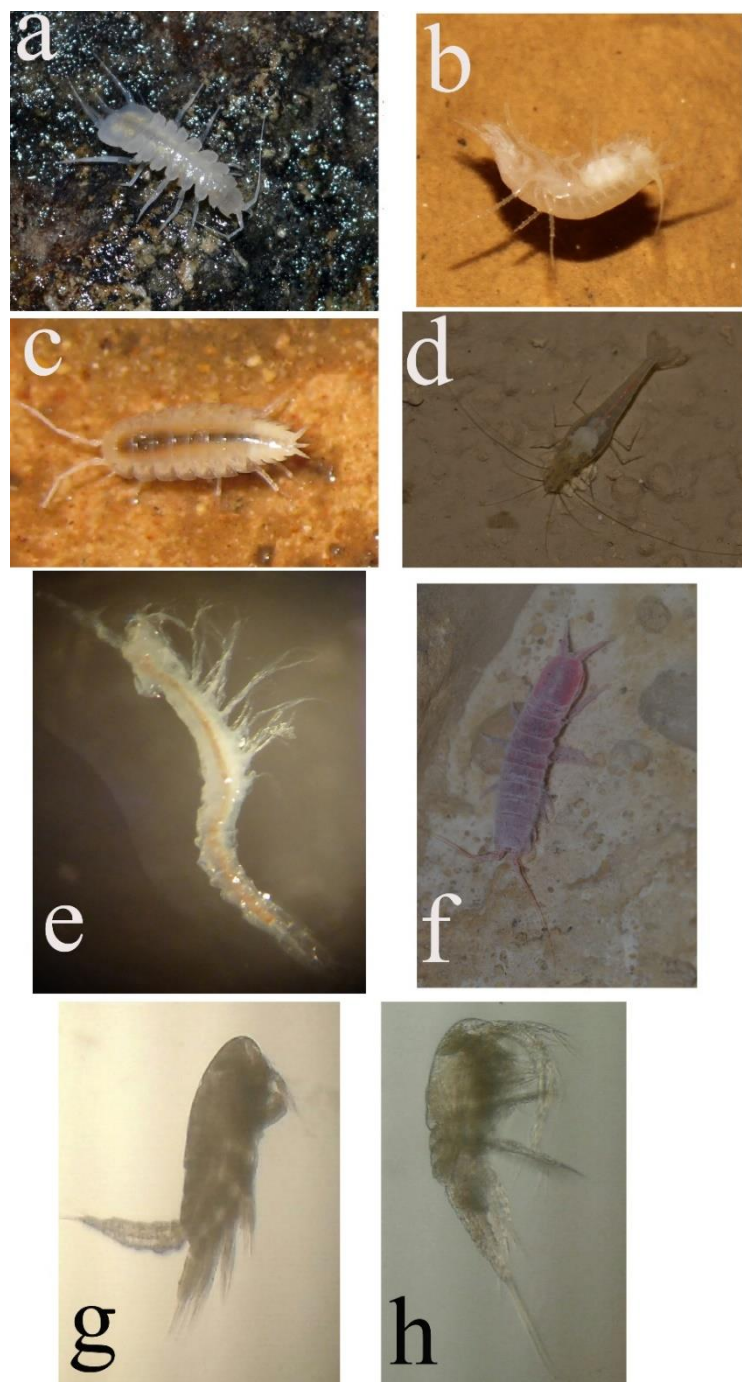


Figure 8: Some of Iranian troglomorphic Crustacea a) *Asellus ismailsezari*. b) *Niphargus keeleri* c) *Protrachaeoniscus gakalicus*; d) *Caridina* sp.; e) *Tethysbaena* sp.; f) *Stenasellus tashanicus*; g) *Nitocrella* sp.; h) *Tropocyclops* sp. (Photos: Malek-Hosseini M. J., and Fatemi Y., 2015-2019).

Table 3: All Iranian troglomorphic animals

Group of animals	Number of Species	Genus-Genera	Family	Terrestrial	Aquatic	Described, known from literature	Described and published recently in this study	Undescribed
Amphipoda	26	4	3	0	26	23	0	3
Copepoda	17	9	3	0	17	12	0	5
Decapoda	1	1	1	0	1	0	0	1
Isopoda	6	4	4	1	5	3	1	2
Thermosbaenacea	1	1	1	0	1	0	0	1
Fishes	4	2	2	0	4	4	0	0
Oligochaeta	5	5	3	0	5	1	0	4
Araneae	3	3	2	3	0	1	0	2
Diplopoda	2	2	2	2	0	1	0	1
Coleoptera	2	2	2	2	0	1	1	0
Gastropoda	2	2	2	0	2	0	1	1

Continuation

Continuation of Table 3

Zygentoma	1	1	1	1	0	0	0	1
Collembola	1	1	1	1	0	0	0	1
Acarina	1	1	1	0	1	0	0	1
Total	72	38	28	10	62	46	3	23

2.2.1.6 Biodiversity patterns

Despite only modest research on subterranean fauna in Iran in comparison with some well-studied parts of the world such as Balkan Peninsula, (Sket, 2008), the number of species at some sites or areas are increasing with more studies. Thus, even if preliminary, preparing the overview of current knowledge is informative both from the taxonomic as well as the conservation point of view. Recent discoveries have enabled the updated distributions for all troglobiont species from Iran. 45 localities are shown harboring 72 obligate subterranean-dwelling species from Iran (caves, wells, and karstic springs). 36 localities are located in Zagros karstic areas. As discussed in this thesis, this is due to more substantial research in these areas. The localities of Iranian troglobiont animals are shown in Figure 9. Details are found in Table 4.

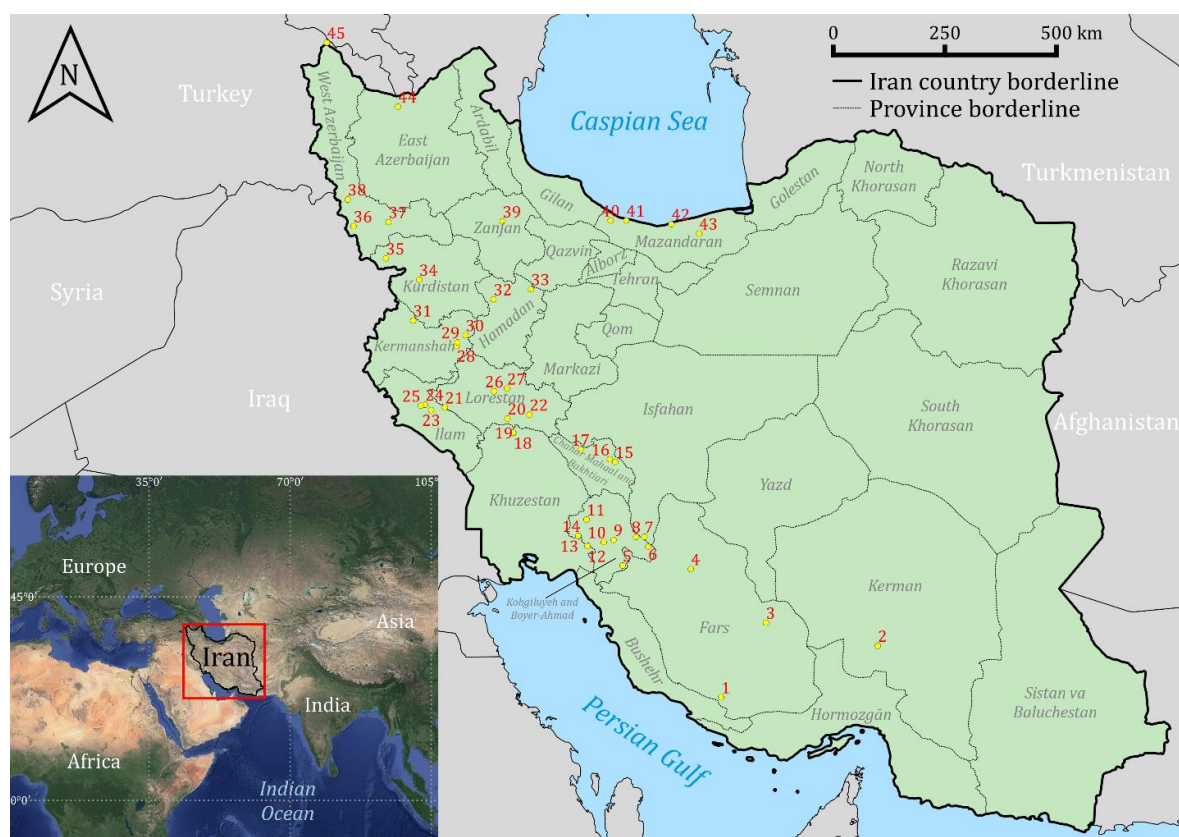


Figure 9: Map of Iran, showing the distribution of troglobiont animals. For the localities details see Table 2. Species names are found in the checklists.

2.2.1.7 Overview of species richness per cave and richness of taxa

In the published sources, prior to this study, Loven cave with three species of cave fishes was the richest cave in number of troglobionts (Vatandoust et al., 2019). Some caves and springs with two troglobionts are listed as well (Malek-Hosseini et al., 2015a; Esmaeili-Rineh et al., 2017b).

In our study, we identified two caves (Table 4) as having over five troglobionts. There is Tashan-Chah-Kabootari cave system with 21 troglobiont species (Figures 8, 9). Three species have been described, and more are under description. 18 species have been analyzed molecularly, which revealed they are new species for science. These two caves that are connected by their groundwater, have been visited several times. In any new visits, we

discovered new paths, pools, streams, and kinds of microhabitats. So, with more surveys, we collected more species. This means that more fieldwork is needed to understand this cave system. Some species are found in both caves. According to our DNA and morphological analyses, two species including a fish and a copepod are living in both caves. Crustacea with 11 representatives, is the richest group in this cave system. There are some springs and wells in Tashan area around Tashan and Chah-Kabootari caves just in four to six square kilometers that all have sulfur water as same as these two caves. Obviously, they have formed a big aquifer. These caves and aquifer potentially harbor more terrestrial and aquatic troglotic species

Tuveh spring with four species is the second richest habitat after Tashan-Chah-Kabootari caves system. Tuveh spring is part of a big aquifer including Loven cave as well. Loven-Tuveh aquifer harbors three species of Iranian cave fishes that have been found in both sites. A new species of troglotic gastropods have been collected from Tuveh spring. Also, we discovered the new species *Asellus ismailsezarii* from Ganow spring about 1 km to the east of Tuveh spring from the same aquifer.

The most diverse group of troglotons in subterranean environments of Iran is Crustacea with 51 species, of which 39 species are described and 12 have not been officially described yet (Table 3). Amphipoda with 26 species is the most diverse group and within Amphipoda, the genus *Niphargus* Schiödt, 1849 including 23 species from 28 localities shows the highest species richness of troglotons in Iran. *Niphargus* is the largest freshwater amphipod genus in the world, with species living in subterranean waters of the Western Palearctic (Fišer et al. 2008).

Copepoda with 17 species is the second most diverse group in Iranian groundwaters. The family Ameiridae with seven described species from three genera is species richest Copepoda in groundwaters of Iran, with one more species not described yet (Table 3).

Cave fishes with four representatives from two families and two genera from five localities are the next diverse group of troglotons in Iran after copepods.

Distribution of richness of troglobiont animals is shown in Figures 10, 11, 12. As showed in figure 10, cells with highest species numbers are situated in western Iran and especially Southwestern in the border of Khuzestan and Kohgiluyeh and Boyerahmad Provinces. These two cells include 27 troglobiont species. Together with the eastern cell, they have 30 troglobiont species, which is considered as the richest part of the country in regard of troglobiotic fauna.

Table 4: Locality details and species number of Iranian troglobiont animals (localities with asterisk symbol are not the exact points)

No	Locality name	Number of species	Terrestrial	Aquatic	Described	Undescribed	Locality number on the map
1	Tashan Cave	17	4	13	3	14	13
2	Chah-Kabootari Cave	7	1	6	1	6	14
3	Tuveh Spring	4	—	4	3	1	19
4	Loven Cave	3	—	3	3	—	20
5	Gakal Cave	2	2	0	2	0	5
6	Torang Cave	2	—	2	0	2	2
7	Ghoori-Ghaleh Cave	2	—	2	2	—	30
8	Sahoolan Cave	2	—	2	1	1	36
9	Alisadr Cave system	1	—	1	1	—	31
10	Khan Cave	1	1	0	1	—	1

Continuation

Continuation of Table 4

11	Tir-e-Bagh Spring	1	–	1	1	–	4
12	Nahr Spring	1	–	1	1	–	6
13	Dangezloo Cave	1	–	1	0	–	7
14	Biareh Spring	1	–	1	1	–	8
15	Nezel Cave	1	1	0	1	–	9
16	Cheshmeh-Belqeis (Spring)	1	0	1	1	–	10
17	Keeler Cave	1	0	1	1	–	11
18	Neyneh Cave	1	1	0	1	–	12
19	Dimeh Spring	1	–	0	1	–	17
20	Ganow Spring	1	–	1	0	–	18
21	Simarreh River basin	1	–	1	1	–	21
22	Shol-Abad Spring	1	–	1	1	–	22
23	Sarab-e-Moord	1	–	1	1	–	23
24	Sarab-e-Kanipahn	1	–	1	1	–	24
25	Jo-Khanem Spring	1	–	1	1	–	25
26	Sarab-e-Robat	1	–	1	1	–	26

Continuation

Continuation of Table 4

27	Razbashi Spring	1	—	1	1	—	27
28	Sarab-e- Bisitun	1	—	1	1	—	28
29	Nojivaran Spring	1	—	1	1	—	29
30	Kangarshah Spring	1	—	1	1	—	30
31	Kheder- Goli Spring	1	—	1	1	—	33
32	Darvish-Olya Cave	1	—	1	1	—	34
33	Shoei Cave	1	—	1	1	—	35
34	Badin Abad Spring	1	—	1	1	—	36
35	Randole Spring	1	—	1	1	—	38
36	Sohrevard Spring	1	—	1	1	—	39
37	Danial Cave	1	—	1	1	—	40
38	Cheshmeh Kahriz - Qanat	1	—	1	1	—	44
39	Brolan Spring	1	—	1	1	—	45
40	* ⁵ Qualeh Kirmiz, bord du lac Niriz	3	—	3	3	—	3

Continuation

⁵ * Symbol: These localities with this symbol are not the exact point

Continuation of Table 4

41	*Farokhshahr	2	—	2	2	—	15
42	*Shahr-E-Kord City	6	—	6	6	—	16
43	*Kelarabad City	2	—	2	2	—	41
44	*Mahmoodabad	1	—	1	1	—	42
45	*Ghaem Shahr	1	—	1	1	—	43

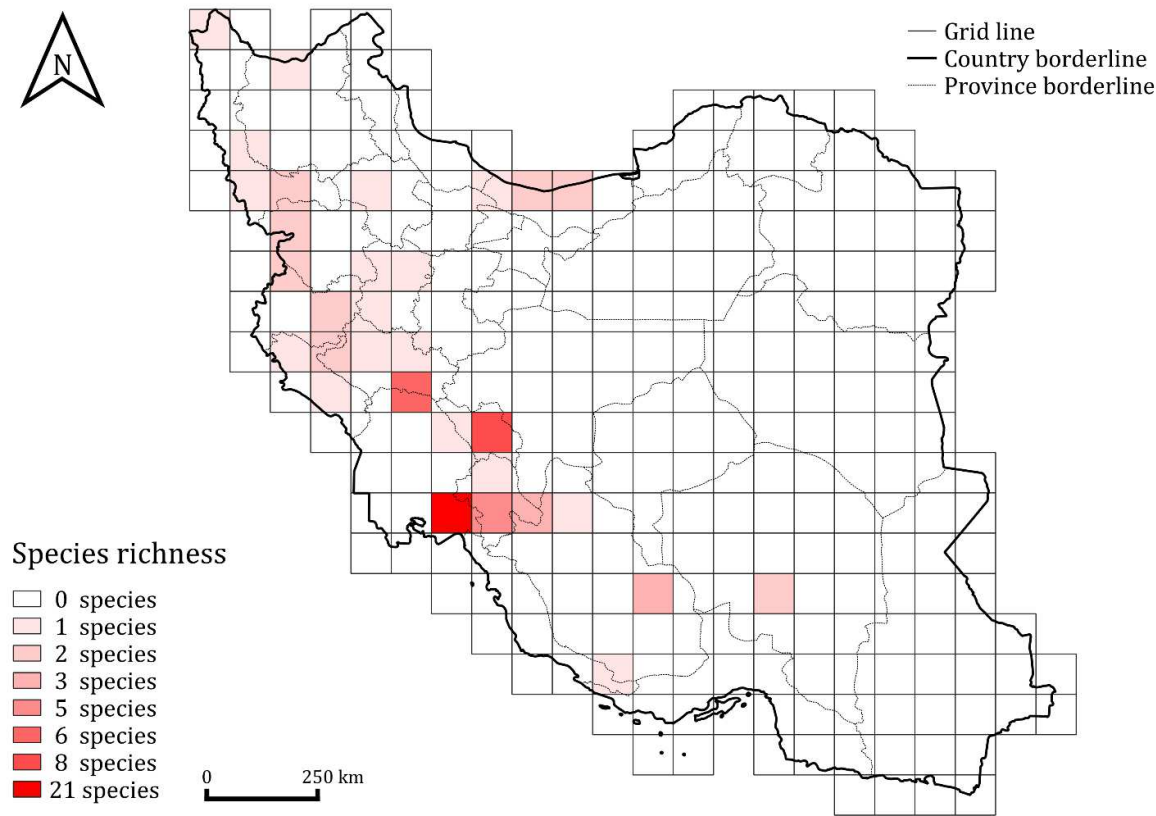


Figure 10: Distribution of troglobiont species number in Iran at grid cell size 100 × 100 km.

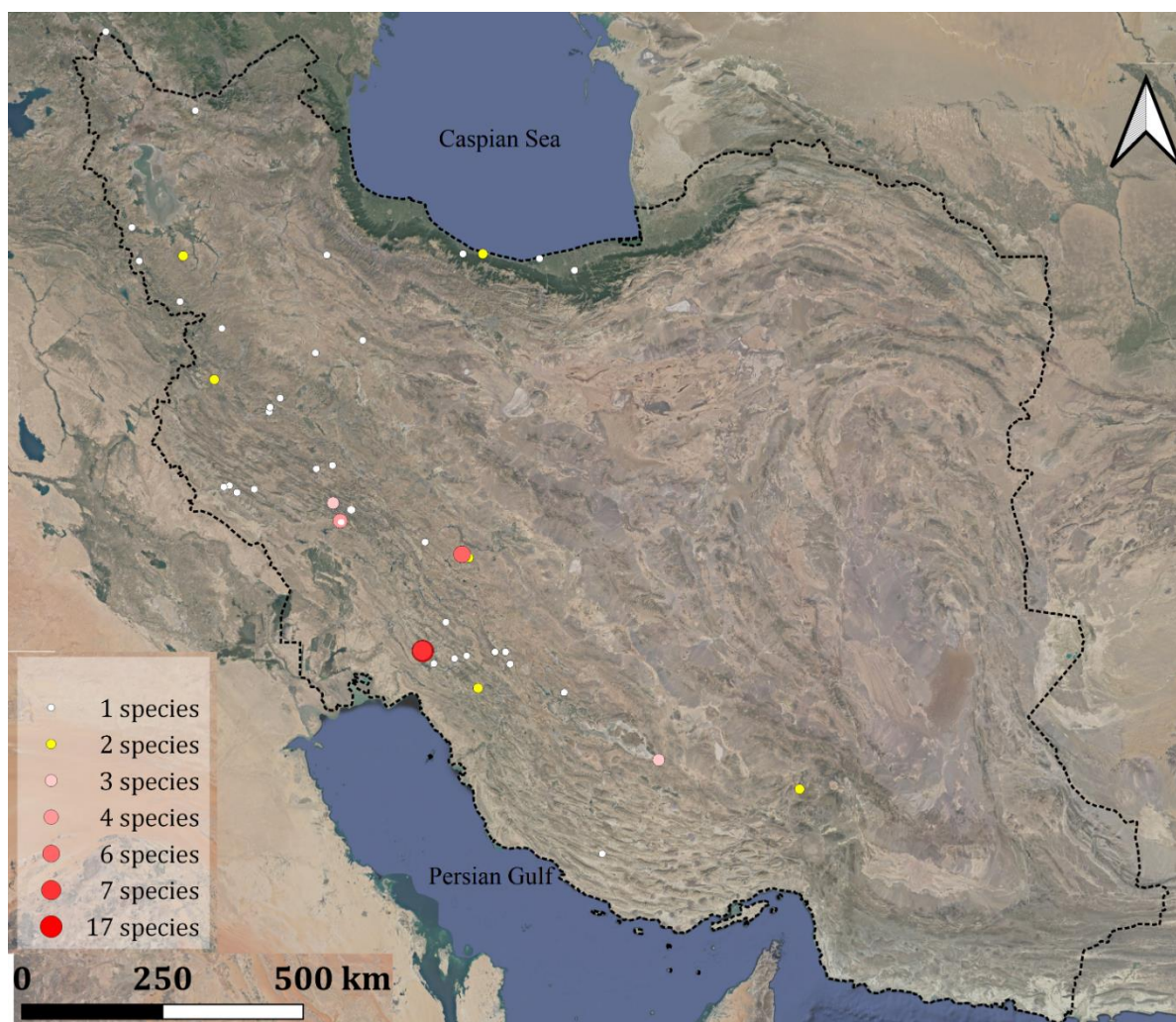


Figure 11: Map of Iran showing species richness per localities (caves, wells, springs).

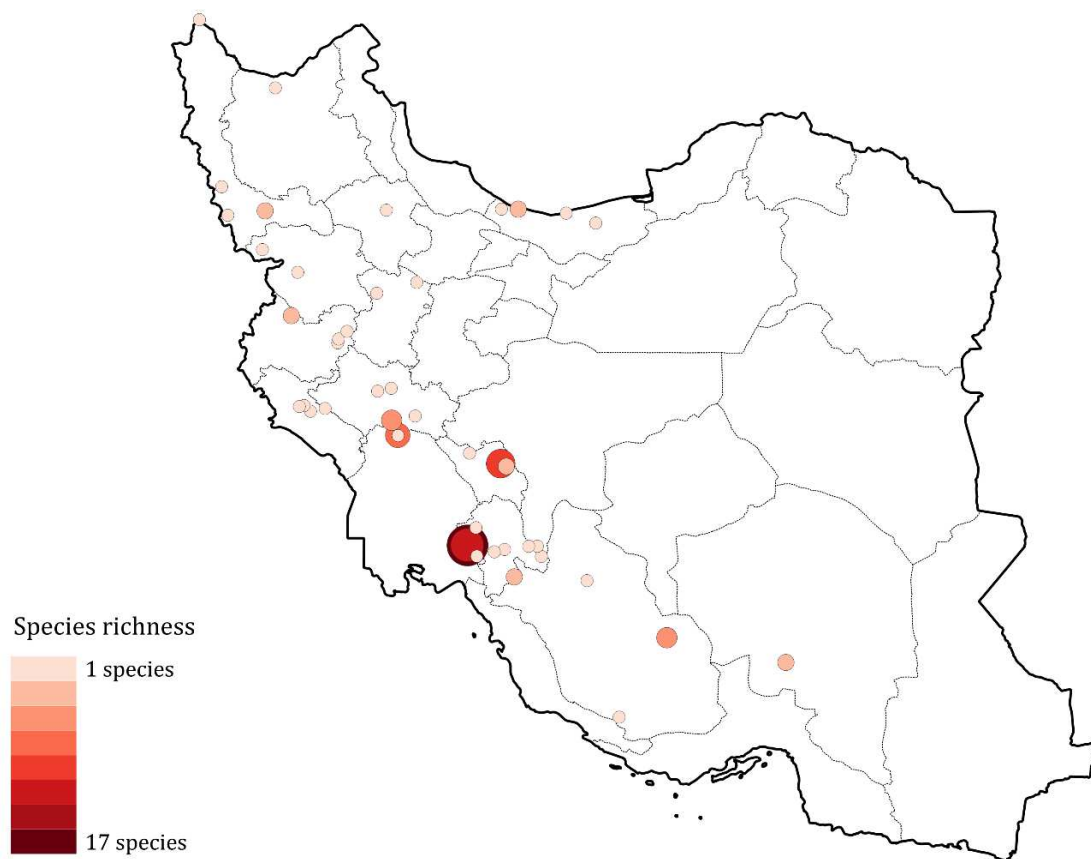


Figure 12: Map of Iran showing species richness per localities (caves, wells, springs).

2.2.1.8 Endemism

The degree of endemism is very high in Iranian troglobionts. However, we should consider this fact that that not many caves, wells and karstic springs in Iran have been faunistically studied, and not many troglobiont species have been reported from the country. Most of the known species are restricted to a single cave, a spring or an aquifer. In general, subterranean communities are with high endemism but low local diversity relative to regional diversity (Gibert and Deharveng, 2002).

Regarding grid cell map, 93% of Iranian troglobiont animals are restricted to a single cell and only 7% including five species have been reported for more than an area as a single grid cell. So, this is congruent with the worldwide characteristics (from Gibert and Deharveng,

2002) of subterranean faunas. We have to consider that low sampling efforts could artifactually concluded in short-range endemism.

Garra lorestanensis has been reported from three localities: 19, 20 and 21.

Niphargus sohrevardensis has been reported from two far localities: 27 and 39.

Niphargus valachicus Doboreanu & Manolache, 1933 was reported by Karaman (1998) from undetermined site from northern Iran. It has wide distribution from Europe to Iran. This species was reported first time from Romania (Doboreanu and Manolache, 1933). However, the molecular data is not available for this species from Iran and potentially, it could be a separate species. Iran represents the eastern boundary of *Niphargus* range.

Two species of copepods, *Diacyclops zschokkei* and *D. iranicus* have been reported from two far localities 16 and 41.

This degree of endemism implies the importance of priorities for the protection of the habitats of these troglobiont species in Iran.

2.2.1.9 Conclusion

In this chapter, we compiled faunal lists of troglobiont animals of Iran based on scattered published literature together with published and unpublished data from our own collections. As showed in the maps (Figures 9, 10, 11, 12), most of Iranian troglobionts have been discovered in west and northern parts of the country from Alborz and Zagros. These parts of the country are mostly mountainous with limestone karsts that harbor many caves and karstic springs. The other reason for being more studied is that some research groups in the universities in these providences are studying troglofauna of these areas. Our original field works focused on Zagros. From all 72 species (both described and undescribed), more than 60 have been collected from Zagros karstic areas. Zagros, as host many karstic areas and thus many caves and springs, as well variety of vegetations, in some parts with 800 mm rainfall (Raziei et al., 2005; Soltani et al., 2012), many rivers, also rich biodiversity, it harbors most of Iranian troglobiont animals. However, this region has been better studied.

The number of troglobionts is rising in Iran due to ongoing researches. However, the relatively low species richness of Iranian caves is not realistic, and it is due to a lack of

comprehensive studies of all taxa. Eastern parts of the country have been neglected in the aspect of subterranean biology. Despite this fact that most part of Eastern-Iran is located in very arid zones, there are some caves and karstic spring that would be very interesting. Northeast of the country is also mountainous with many caves that potentially could harbor troglobiont animals. As for many other taxa for Zagros, the putative presence of troglobiont representatives of many taxa is predicted

Central Zagros and especially west of Kohgiluyeh and Boyer-Ahmad and East of Khuzestan Provinces harbor about 30 troglobiont species and many more to be discovered.

Speleobiological studies are needed in Iran. Compared to some karstic areas such as Dinaric or Balkan Peninsula, the number of studies and discovered troglobionts are low. Most diverse groups are aquatic crustaceans and fish (Mousavi-Sabet et al., 2016; Bargrizaneh et al., 2021). Considering the vastness of Iran and big karstic areas harboring more than 2000 caves and thousands of karstic springs, more field work studies are needed to understand the subterranean environments of the country.

For the above reasons, it is too preliminary to discuss the troglobiotic fauna of Iran within a global context. With over 2000 records of specialized subterranean species from both Europe and the United States, we could say that these parts of the world have been the most studied parts in regards of subterranean fauna. Several datasets on the georeferenced occurrence of these obligate subterranean species have been presented till now (Deharveng et al., 2009; Zgmajster et al., 2014; Bregović and Zgmajster, 2016; Christman, et al., 2016; Pipan et al., 2020; Culver et al., 2021).

Until 1999, more than 45000 caves were known from the USA and many more have been added up to date (Culver et al., 2009). More than 2000 caves and approximately 10000 karstic springs and wells are known for Iran. Of course, not all caves and also karstic springs and other groundwaters need to be sampled to obtain a good estimate of species richness for a region, but a rather large number do (Pipan et al., 2020). Since 2010 and including this study, about 56 troglobiont species have been discovered (including non-described ones) from Iran that is 77 % of discovered troglobiont species from Iran. 33 species have been described since 2010.

Nonetheless, this dissertation is a start of a more methodical study of the cave biotas in Iran and wider. We developed a georeferenced database of troglobionts (cave-obligate species) in Iran to examine spatial patterns of species richness and endemism, including >88 records

for 48 described and 24 under-description or undescribed species. Although, we have discovered more troglobiont taxa from Iran, due to obvious time constraints of a single work, all initial results could not be included in this thesis.

3 DISCUSSION AND CONCLUSIONS

3.1 DISCUSSION

3.1.1 Goal 1: Discovery of troglobiotic fauna in Iran (Zagros karsts)

Several taxa that prior to this study had no troglobiont representatives in Iran, are now included in the troglobiotic list of fauna of the country. Although we discovered several new troglobiont species from subterranean environments of Iran that represent a significant number for the troglobiotic fauna of the country, this still is a relatively small amount of data in comparison with the epigeal fauna of the country or the subterranean fauna of some well-studied parts of the world such as Balkan Peninsula. This seemingly low species richness of obligate subterranean animals in Iran is likely an artefact stemming from lack of focused research on this fauna. Most caves, wells and karstic springs of the country that might potentially harbor troglobiont species are not easy to reach and there are not many particular surveys on Iranian troglobiont organisms. Moreover, given the vastness of the country and extension of the karstic areas from north to south, an extensive study would require tremendous effort. Some cave-adapted groups of animals such as fishes (4 species), Crustacea (amphipods and isopods, copepods, more than 30 species), diplopods, and insects have been reported from Iran (Malek-Hosseini and Zamani, 2017; Fatemi et al., 2019; Bargrizaneh et al., 2021). We suggest the putative presence of several groups of animals in hypogean environments of Iran, but to test this presumption, much more intensive field work is needed.

Although Coleoptera and especially carabids have many representatives in subterranean environments, no troglobiont carabid beetle has been discovered from Iran prior to this study and the only known troglobiont coleopteran from Iran was the tenebrionid *Foranotum perforatum* (Nabozhenko and Sadeghi, 2017). In this study, we investigated the subterranean beetle fauna of Iranian caves of the Zagros Mts. We diagnosed and described two newly discovered species of *Duvalius* Delarouzée, 1859 from two caves in Zagros. The troglobiont *Duvalius nezelensis* Muilwijk and Malek-Hosseini, 2021 that occurs in the Nezel cave, while the troglophile *Duvalius achaemenius* Muilwijk and Malek-Hosseini, 2021 occurs in the small Suq cave (Malek-Hosseini et al., 2021).

Troglairanica tashanica Fatemi, Malek-Hosseini, Falniowski, Hofman, Kuntner & Grego, 2019 was the first obligate cave-dwelling snail from Iran. We reported the discovery of the

sixth sulfidic stygobiont gastropod species of the world in the Tashan Cave of Iran with the general ecological importance of the habitat.

Asellus ismaeilsezarai Malek-Hosseini, Jugovic, Fatemi & Douady, 2022, is a new obligate groundwater asellid isopod from Ganow spring close to Tuveh spring and 30 km to the Loven Cave, both localities harboring Iranian cave fishes. Ganow spring is a part of big aquifer that harbors three species of cave fishes and a new trogllobiont species of gastropods. We described this *Asellus* species based on morphology and molecular data.

Several trogllobiont species that have been discovered in this study are under-description. A trogllobiont species of atyid shrimps: *Caridina* sp. from the sulfidic water of Tashan cave as the first obligate cave-dwelling species of shrimps from Iran that is also first trogllobiont one from Atyidae from sulfidic hypogean waters in the world, has been described and awaiting publication. This family has several trogllobiont representatives worldwide.

Another trogllobiont isopod is under description. *Stenasellus* sp. From Chah-Kabootari that genetically is sister to *Stenasellus tashanicus* from Tashan cave.

Molecular analyses of copepods from Tashan-Chah-Kabootari cave system showed that we have five different species from three genera of two families. Two new species of Amphipoda have been collected in Tashan and Chah-Kabootari caves. They are waiting to be described. A new species from Thermosbaenacea that is the first representative of this order from Iran was collected in Tashan cave. Molecular and morphological analyses both showed it belongs to the genus *Thetysbaena* Wagner, 1994.

We discovered four new species of Oligochaeta. A new trogllobiont species of the genus *Helodrilus* (family Lumbricidae) from water of Dangezloo cave, and two interesting species from sulfidic waters of Tashan and Chah-Kabootari caves. These oligochaetes should be sampled again for more appropriate specimens for morphology. DNA analyses have been done and very interesting results have been obtained.

3.1.2 Goal 2: Iranian trogllobiotic fauna species richness and hotspot detection

Iran covers 164.8 million km² of area and about 11% of the country's land are karstic carbonate formations. The country located in western Asia at the interface between three zoogeographic realms, Palaearctic, Oriental (Indo-Malayan) and Afrotropical (Ethiopian).

Iran is situated at the conjunction of three climatic zones: the Mediterranean, the arid West Asian, and the temperate humid/semi-humid Caspian zone (Raeisi et al., 2012; Amiri and Eslamian, 2010). Variety of climate, vegetation, rainfall, temperature and then very different productivity exist in Iran from south to the north and from east to west. Although many parts of the country, especially central parts, are desert, a significant difference could be seen in western and northern parts of the country.

The Zagros region exemplifies the continental variant of the Mediterranean climate pattern, while the northern slopes of Alborz are distinctly humid. So, these areas have a high level of productivity.

Determining the global patterns of subterranean species richness has remained elusive (Pipan et al., 2020). There are several poorly studied subterranean faunas in the world especially in Central Asia and Middle Eastern countries such as Iran. To understand the subterranean fauna of Iran, considering the karstic areas of the country, at least 600 sites from all around the country that potentially could harbor troglobiont animals need to be studied. As up to date, more than 100 caves, wells and springs have been surveyed for subterranean animals (arthropods, mollusks, oligochaetes and fishes) and only 49 troglobiont species have been reported from them, and considering this fact that the subterranean environments of huge areas of the country have not been studied yet, we suggest 600 sites to obtain a realistic estimation of the troglofaunal of the country. For Iran, to determine spots of high subterranean biodiversity, we should consider some criteria that used by Trajano et al. (2016) for Brazil with consideration to the difference of climate in Iran with Brazil. First, we should consider the number of troglobionts, when compared to other studied cave areas in the region. Presence of higher taxa (families, order) so far not recorded in subterranean habitats elsewhere in Iran even in the region must be considered. Regarding the country and the region, with a comparative point of view, any site with more than 15 troglobiont species could be considered as hotspot of troglobiont diversity for this region.

Undescribed species are problematic in the analysis of species numbers in subterranean sites because sometimes some of them are not new species. According to our analyses (morphological and molecular), this has not been true regarding Iranian troglobiont diversity as all discovered species are new for science.

There is a remarkable concentration of subterranean sites in the Dinaric karst of Slovenia, Croatia, and Bosnia-Herzegovina, which is also the richest known region of subterranean biodiversity in the world (Sket, 1999b). This concentration ~~and connection~~ of subterranean sites in Iran may be just referred for Loven-Tuveh aquifer and for the sulfidic water system that we detected in Tashan and Chah-Kabootari caves. This system harbors a unique

ecosystem and represents a specific hypogean habitat with several cave ponds containing groundwater rich in hydrogen sulfide. The first studied cave containing a sulfide ecosystem was Movile Cave in Romania (Sarbu and Popa, 1992). According to the local people and our observations, there are more wells and springs with sulfur water in that area close to Tashan. With more field works, we could understand the fauna of this big unique system. As we are preparing the species level testing to corroborate the species status of several taxa, we saw some different species from the same genera in Tashan and Chah-Kabootari caves that are sisters genetically, and some groups of animals that only exist in one of these caves and even in a single isolated pool, we could say that with detection of more places in these two caves, new taxa would be discovered that are restricted to a microhabitat. Despite numerous samplings, covering the different microhabitats (chambers, passages, guano, stream, ponds, lakes), large sections of Tashan cave remain unexplored, from both a topological (e.g., permanently flooded sections, dangerous streams down to the pools with loose, unstable, and falling walls) and biological perspectives. The inability to sample the entirety of the karst system implies an underestimation of its species richness. During several visits and samplings from Tashan-Chah-Kabootari system, we collected new taxa that we were not able to collect them in previous field works. So, here, we considered Tashan-Chah-Kabootari cave system with 21 obligate cave-dwelling species as a hotspot for troglomorphic diversity in Iran. Regarding the topography of this cave system with sulfidic water, new paths in these caves, and unused techniques of sampling in both aquatic and terrestrial parts of these caves, undiscovered species are expected. The number of Iranian troglomorphic species is likely to increase in the future since new records are occurring regularly.

The most diverse group of troglomorphs in Iran was Crustacea. This is congruent with global pattern of subterranean diversity. Crustaceans constitute a major animal group in groundwater (Culver and Pipan, 2013) with around 3400 species known worldwide, 1200 of which are known to occur in Europe (Camacho and Puch, 2021; Stoch and Galassi, 2010). This is more than 65% of the total groundwater species currently known from Europe (Iannella et al. 2020; Iepure et al., 2021).

The new, developing Iranian database on subterranean biodiversity can be build on a clearer premises than other databases in the world. All other databases needed to clean masses of data and find the coordinates for localities, here the database can be constructed in a modern way from the scratch.. This enables not only more exact mapping, but also spatial modeling, including spatial autocorrelation (Zagmajster et al., 2008), conditional autoregression (Christman and Culver, 2001), and kriging (Christman and Zagmajster, 2012).

3.1.3 Goal 3: Phylogeny and evolutionary history of Iranian troglobionts

With this study, the discovery of several new taxa adds to phylogenetic resolution of several groups such as Trechinae beetles, Isopods, and Gastropoda family Moitessieriidae. Also, some time frames for their origins and colonization of subterranean environments were estimated. The phylogenetic relationships were reconstructed in big frames of taxa from Palearctic and Africa. For some groups, even species from Oriental were included to see a better results. Here we discuss about the abovementioned groups separately and then compare the estimated time of colonization of Iranian subterranean environments by different terrestrial and aquatic taxa. Due to incomplete sampling of some taxa and as there is no close relatives in surface (epigean relatives), for some Iranian troglobiont taxa, our analyses cannot unequivocally pinpoint the immediate phylogenetic relative these troglobiont taxa. So due to that, providing a chronogram (datation) was totally irrelevant at the moment. The problem is not to make a chronogram but first to get enough sequences of different genes from more taxa within the “Iranian pattern” clades and second to have a reliable calibration point for time estimation (datation) within those clades. For some taxa, at the moment, we have neither the first nor the second.

The monophyly of some taxa of Iranian troglobionts have been shown in our analysis. *Duvalius* species group of beetles from the Zagros Mts. made the “Zagros clade”. The split of the Zagros clade to have occurred between 7.3 and 12.9 Mya (mean 9.7 Mya) in Late Miocene. Two diversifications of the Zagros clade that estimated to have occurred between 5.4 and 10.6 Mya (mean 7.8 Mya) in Late Miocene for the split of *D. nezelensis* from its sister group, and more recently in Middle Pleistocene between 0.4 and 1.3 Ma (mean 0.78 Mya) for the split of *D. achaemenius* and *D. kileri*. The colonisation of subterranean environments by *D. nezelensis* could have occurred in any time from 10.6 Mya.

Molecular analysis of newly discovered gastropod from Tashan cave confirmed that it belonged to the family Moitessieriidae. This new genus and species, *Trogloiranica tashanica* may represent an evolutionary relict from the post Miocene before the split of the families Moitessieriidae and Cochliopidae. The separation of this species from its surface relatives was estimated on 3 Mya.

The molecular analyses we did on the first troglobiont *Asellus* from Iran that was discovered and described in this study, showed that this species did not belong to the *A. aquaticus* complex. Reconstructed trees showed that *A. ismailsezarii* is sister to a larger clade that also contains the European *A. aquaticus* species complex. In this case, lack of data from Iran and

neighboring countries did not allow us to understand the phylogenetic relationship of this Iranian species and also estimate a timeframe for its divergence from its congeners. *A. monticola* have been reported from the Caspian Sea region. This region could be considered as a contact zone between species of the European *A. aquaticus* complex and Asian species, including *A. ismailsezaraii* and *A. monticola*. Regarding the similarities in morphological characters of *A. ismailsezaraii* and *A. monticola*, we expect that these two species are phylogenetically close as well.

3.1.4 Conservation

Iran harbors many caves with historical values. Several caves with troglobiont species are located in historical sites. Unfortunately, there is no protection of valuable and unique subterranean habitats of the country. Some caves such as Tashan and Chah-Kabootari should be in priority for special protection. Up to date, much of destruction has been caused by cavers, treasure hunters and local people in these caves. Scientific depredation is one of the main reasons for Iranian cave fishes to be in danger. Considering available literature and our studies, most of Iranian troglobionts are restricted to a single cave and any changes in the environment, could end up in extinction. Although subterranean fishes of Iran have been reported from more than one locality, all threats and risk of extinction still exist. The importance of preparation of the databases on subterranean biodiversity of Iran is obvious. So, the management and strategies for protection of Iranian subterranean environments could be organized better. Identification of troglobiont diversity hotspots should results in protection of these unique habitats. Tashan-Chah-Kabootari system, Loven-Tuveh aquifer and Gakal cave are the most important habitats in terms of subterranean fauna in Iran. Unfortunately, there is no protection of these habitats. Tashan-Chah-Kabootari system likely represents another chemoautotrophically based cave ecosystem harboring 21 obligate cave dwelling species that some of them in order level, are recorded from the country for the first time. Although, most of species are waiting to be described morphologically, molecular studies on these animals from this system showed that they are new species and also some of them as distinct clades sister to the rest of congeners. From evolutionary view, the estimated time for this undescribed species showed that study of this cave system is very important and will reveal worthy stories about the origins of Iranian troglobiotic faunas. This cave system has not been completely studied yet and much evidence may be lost and destroyed as there is no protection at all. Regarding the threats for this cave system, especially scientific depredation and uncontrolled visits, the priority should be in direction of protection of this valuable and unique hotspot of troglobiont biodiversity. Regarding our visits and also some geologist and archaeologist visits of Tashan Cave, lot of valuable historical clues and objects from ancient times have been completely manipulated and

plundered by treasure hunters during last 7 years. Their destructive activities affect cave life of animals as well.

Four fish species are known as the only true subterranean vertebrates from Iran: *Garra typhlops* (VU on IUCN), *G. lorestanensis*, *G. tashanensis* and *Eidinemacheilus smithi* (VU) (Cypriniformes). Three species have been described from Loven cave and Tuveh spring and *G. tashanensis* is known only from Tashan cave and Chah-Kabootari cave, Khuzestan Province.

First threat for Iranian cave fish is scientific depredation. Fish are sampled by several research teams. Just in a study by well-known professors in 2008, 44 specimens were killed. Although Loven cave fish are as national natural heritage of Lorestan province, they are considered as ornamental fish and are easily caught. Loven cave is close to road and railroad. Dam and road construction activities are underway. Soil texture at the cave opening is sandy and alluvial. Wall rocks are sloping and loose. Flooding has been problematic and could clogged the entrance with soil. Extreme earthquakes are potentially risky and fallen rocks could completely fill the opening pool of Loven Cave.

In addition to scientific depredation and treasure hunters, water extraction is a big threat for Tashan Cave. Water well is very close to this cave and directly extract water from the cave. The rainfall in this area has decreased significantly. This cave is in middle of three villages. Pesticides and human wastes directly flow into the system.

So, without exaggeration and overstatement, these Iranian cave fishes are headed towards extinction. All effort should be made to avoid this. Considering all habitats and species as national heritages, education to research groups and local communities, installing appropriate doors, impose heavy fines and control all visits must be applied. Also fencing loose walls at the opening of Loven Cave is necessary.

3.2 CONCLUSIONS

In this thesis, we followed three main goals related to the Iranian troglotrophic fauna. We studied several caves and karstic springs in Zagros Mountains. We tried to discover more localities that harbor troglotrophic animals. Considering the promises in the dissertation proposal, we tried to describe some new species and new habitats for Iranian troglotrophic

animals. However, there remain several new species to be described. Regarding our original data and available data on GenBank, we tried to reconstruct phylogeny of Iranian troglobionts from different groups of animals. We focused on a dated phylogeny to estimate a timeframe for their colonization to the subterranean environment. Then, considering the available faunistic data and our discoveries, we did a species richness analysis and looked for any hotspot for troglobiotic diversity in Iran.

We can draw the following conclusions, coming from our work:

1. The presence of several taxa in obligate cave-dwelling forms is confirmed by this study.

For the discovery and description chapter, we described three new troglobiont species including:

Coleoptera: Carabidae: *Duvalius nezelensis* Muilwijk and Malek-Hosseini, 2021 (Malek-Hosseini et al., 2021).

Gastropoda: Hydrobiidae: *Trogloiranica tashanica* Fatemi, Malek-Hosseini, Falniowski, Hofman, Kuntner and Grego, 2019 (Fatemi et al., 2019).

Isopoda: Asellid isopods *Asellus ismailsezarii* Malek-Hosseini, Jugovic, Fatemi and Douady, 2022 (Malek-Hosseini et al., 2022)

Several new species are under-description such as a new troglobiont atyid shrimp from the genus *Caridina* Milne-Edwards, 1837.

Although diverse, Iranian's subterranean fauna remains poorly studied and many additional species await discovery and description. We identified several under-sampled regions and outlined conservation and management priorities to improve our knowledge and aid in protection of the subterranean biodiversity in Iran and specially Zagros karstic areas.

We envisage that the exploration of Iranian troglobiont animals has just begun and that many more species still await formal description. Although the available data clearly demonstrate that the subterranean biodiversity in the Zagros karstic areas of Iran is relatively high (at least in some parts), especially compared with that of other caves in the world and when you

consider the climate, amount of rainfall and productivity (Deharveng and Bedos, 2000; Pipan et al., 2020).

2. 72 obligate cave-dwelling species (49 described and 23 undescribed and under-description) have been discovered from Iran. Out of this, more than 60 species have been collected from Zagros karstic areas. Zagros, as hosts many karstic areas and thus many caves, wells and springs and the karstic parts of the Zagros have been more understudy.

3. Clearly, the currently reported diversity of Iranian troglofauna is a vast underestimation and it remains poorly known. The low species richness of Iranian subterranean environments is not realistic, and it is due to a lack of comprehensive studies of all taxa.

4. Central Zagros and especially west of Kohgiluyeh and Boyer-Ahmad and East of Khuzestan Provinces harbor about 30 troglobiont species and many more to be discovered. Tashan-Chah-Kabootari cave system harbors 21 troglobiont species (five terrestrial and 16 aquatic). Considering the discoveries of new paths in this cave system, more troglobiont species are expected with more field work and sampling.

5. Phylogenetic relationships of Iranian troglobiont animals with their epigean congeners were studied and in some cases monophyly was reconstructed. Lack of complete samplings from many taxa made the understanding of these relationships difficult. This problem is global for some taxa.

6. Different timeframes were estimated for the colonisations of the subterranean environments of Iran by different taxa. In addition to finding the closely relatives of Iranian troglobiotic animals, phylogenetic analyses were done to estimate a timeframe for their colonisation to the subterranean environment.

For some taxa, it was not, and it is not easy to find their close relatives. In some cases, the order is first record from the country. Also, no surface-dwelling species have been collected from the same genera.

Three million-year was estimated for the separation of the obligate cave-dwelling gastropod: *Troglairanica tashanica* from its surface relatives.

Time frames estimated for the colonisation of subterranean environments by animals are affected by the rates of evolution used in analyses. The colonisations of subterranean environments of Iran by different groups of animals could have occurred in different times and as independent populations colonising.

Our study adds to phylogenetic resolution of Trechinae by adding three *Duvalius* species from Iran. The split of the troglomorphic carabid beetle *D. nezelensis* from its sister group occurred between 5.4 and 10.6 Ma (mean 7.8 Mya) in Late Miocene. So, the colonisation of the subterranean environment by *D. nezelensis* must have occurred at any recent time since the Late Miocene (from 10.6 Mya). This implies that *D. nezelensis* developed its troglomorphic features between the Late Miocene and today. estimated split overlaps with the potential time frame of the Nezel cave formation.

7. Most of the habitats for Iranian troglomorphic animals, especially Tashan-Chah-kabootari cave system and Loven-Tuveh aquifer are not protected. The evidence suggests protection of both is the main conservation priority.

4 SUMMARY

4.1 SUMMARY

One of the interesting environments for biological studies such as taxonomy, evolution, ethology, etc., are subterranean habitats, both aquatic and terrestrial. The main feature of such environments is continuous darkness. Some abiotic conditions such as moisture, temperature, water chemistry and oxygen are almost constant during the year. Additionally, hypogean systems are relatively energy-limited compared to photosynthetically based epigean systems. These habitats harbor three main categories of animals based on degree of their dependence on subterranean environments. The focus of this study was on troglobionts, a term that refers to organisms found only in caves and that display cave-specific phenotypes (morphological, physiological, and behavioral) such as loss of eyes and pigmentation, and elongation of appendages. Troglobionts are obligatory cave-dwelling species.

Although relatively poorly studied in comparison with the epigean ecosystems, subterranean ecosystems show a rather high biodiversity at the global scale. However, in comparison with epigean environments, this subterranean biodiversity is low, but not as low as it seems to be. Over 7000 aquatic subterranean dwellers have been globally catalogued but such data are not available for the terrestrial faunas. About 50,000 to 100,000 obligate terrestrial subterranean species are estimated to exist worldwide.

The syntagm “biodiversity hotspot” is one of the most important global tools for nature conservation. Several hotspots of subterranean biodiversity have been detected mostly in Europe and United States of America, and an additional few are known from Australia and Asia. This is according to the literature that used an arbitrary cutoff of 20-25 obligate subterranean species for a site to be considered as a hotspot for troglobiotic biodiversity. This cutoff depends on comparative value to others. Prior to this study, the cave with the highest diversity in Iran was Loven cave in Zagros that harbors three species of Iranian cave fishes. There has been no hotspot of troglobiont biodiversity or spots of high diversity of troglobionts detected in Iran.

Iran with 1,648,195 km² of land area, is the second-largest country in the Middle East and the 17th-largest in the world. This country is geologically a part of the Alpine-Himalayan orogenic belt.

The total area of the karstified carbonate rocks in Iran is about 185.000 km², with 55,2 % in the Zagros, 24,3 % in Central Iran, 15,2 % in Alborz, 4,7 % in East and South Iran. More than 2000 caves, 10000 springs and wells have been reported from Iran and mostly from Zagros and Alborz. The subterranean fauna of Iran remains inadequately known not only faunistically, but also from the evolutionary view, especially when considering species origins.

In this study, we followed three aims. The first aim was discovery of troglobiont organisms in Zagros Mountains of Iran, and taxonomic descriptions of new taxa. Three new troglobiont species were described: a carabid beetle, an asellid isopod and a truncatelloid gastropod. Six species (Oligochaeta, Decapoda, Isopoda) are under description and more than 10 species are awaiting description. We also recorded new habitats for previously described Iranian cave fishes.

The second aim was to prepare a checklist of all Iranian troglobionts to examine patterns of troglobiont biodiversity and endemism in the Zagros Mountains of Iran. In this part of the thesis, we detected and discussed the first hotspot for troglobiont biodiversity in Iran.

The third aim was to study the phylogenetic relationships among selected troglobiont groups, and investigate the origins of this troglofauna. DNA extraction and PCR for several markers was performed for numerous new troglobiont species we discovered as well as for their relevant surface relatives, and we have published a subset of these original data in scientific publications. DNA isolations and PCRs were done at Evolutionary Zoology Laboratory, Biological Institute Jovan Hadzi, ZRC SAZU, Slovenia. Related sequences were mined from GenBank for meaningful reconstructions of phylogenetic relationships of studied taxa, for tests of their species status, and for estimations of the timeframe of their colonization of subterranean environments.

In a scientific paper within this dissertation, we described two new species of cave beetles of the genus *Duvalius* from caves of Zagros Mts. in Iran. *Duvalius nezelensis* was the first troglobiont carabid beetle from the country. The main troglomorphic features of *D. nezelensis* were absence of eyes and long appendages. We also described *D. achaemenius* as a troglophile species. Although Coleoptera and especially carabids have many representatives in subterranean environments, no troglobiont carabid beetle had been discovered from Iran prior to this study and the only known troglobiont coleopteran from Iran was *Foranotum perforatum* from Tenebrionidae. Now, a total of six *Duvalius* species are known from Iran.

For phylogenetic analyses, we obtained original sequences of two mitochondrial (COI, 16S) and two nuclear (18S, 28S) genes for three *Duvalius* species from the Zagros Mts. and combined them with published molecular datasets using other *Duvalius* species. We applied Bayesian inference and maximum likelihood for reconstruction of a species level phylogeny of *Duvalius* and closely related genera. To explore divergence times of the major lineages of *Duvalius*, we used BEAST. Our phylogenies recovered a well-supported “Zagros clade” with the three examined Iranian species. About 9.7 Mya (Late Miocene) was estimated for the isolation time of this clade from other congeners. Within the Zagros clade, the split of *D. nezelensis* and its sister group was estimated at 7.8 Mya (Late Miocene). We concluded that the colonization of subterranean habitats by *D. nezelensis* could have occurred at any time since 7.8 Mya.

In the second publication, we described a new gastropod species from the sulfidic ponds of Tashan Cave in the Zagros Mts. of Southwest Iran. *Trogloiranica tashanica* represented the first troglomorphic/stygobiont gastropods found in Iran and sixth stygobiont gastropod species of the world from sulfide rich water. We used two markers (COI and H3) to infer the phylogeny of this species and relatives, and studied their anatomical evidence for the placement of *T. tashanica* in the family Moitessieriidae. The phylogeny inferred with COI suggested a close relationship with the Moitessieriidae but did not confirm the monophyly of the family. The phylogeny inferred with nuclear H3 clearly demonstrated that *T. tashanica* belongs to the Moitessieriidae. Likewise, the concatenated analysis of COI and H3 supported this relationship.

We commented on the unique hydrogen sulfide habitat of the newly discovered gastropod. Tashan Cave harbors a unique ecosystem. The recent discovery of a blind fish, *Garra tashanensis*, and the isopod *Stenasellus tashanicus*, have been followed by other discoveries including the new gastropod genus and species reported in this study. Hydrogen sulfide is toxic for most organisms and only five of the around twenty so far known sulfide cave ecosystem sites worldwide, host stygobiont gastropod species.

Another troglomorphic species we described in this study and published the finding within a paper was *Asellus ismailsezarai* from Asellidae family, order Isopoda, from Ganow spring close to Tuveh Village, Andimeshk City, Khuzestan Province, Zagros Mts, Western Iran.

Asellus ismailsezarai was the first obligate groundwater *Asellus* reported from the country. This species showed troglomorphies such as depigmentation and loss of eyes. We provided morphological and molecular evidence that supported the species status of *A. ismailsezarai*. COI and 28S genes of *A. ismailsezarai* were analyzed with available sequences of the genus

Asellus on GenBank. 11 MOTUs within the *Asellus* pattern were found, including one MOTU corresponding to *A. ismailsezaraii*. The resulting tree of the nuclear 28S rDNA sequences was congruent with the COI phylogeny. These analyses showed that this species was not nested within the European *A. aquaticus* clade. This new species of *Asellus* from Ganow spring showed that this aquifer, which includes important cave of Loven, Tuveh and Ganow springs, harbors more troglobiont animals. Previously reported Iranian cave fishes *Garra lorestanensis*, *G. typhlops* and *Eidinemacheilus smithi* from this aquifer from three sites, as well as some other unpublished gastropods confirm this hypothesis. With the new discovery, up to date, two *Asellus* species have been reported from Iran. *Asellus monticola* was reported from a spring in the extreme Northwest of Iran. With the new discovery, the number of obligate subterranean-dwelling isopod species from Iran raised up to four (three aquatics and one terrestrial). We suggested the putative presence of many more obligate groundwater Isopoda and especially *Asellus* taxa in Iran.

The main problem with the study of phylogenies and finding the origin of Iranian troglobionts was the lack of data for close relatives of studied taxa in this dissertation. There was not enough data available on GenBank. Also, as some genera were new for the country and science, there were no epigean/hypogean close relatives for those taxa, neither genetically nor geographically.

Regarding our results and the available literature, most of colonization of Iranian subterranean habitats by animals, have occurred during Middle Miocene to Early Pleistocene (15 Mya – 2 Mya). However, few data were available in this regard and most of Iranian troglobionts had not been tested yet for understanding their origins. Several groups of troglobionts in different karstic areas must be examined. Also, the estimated times could be compared in both aquatic and terrestrial troglobionts.

To examine the species distribution patterns of troglobiont diversity in Iran, we used available literature and our own data. Complementing the checklist of subterranean arthropods of Iran which we published in 2017, we represented all Iranian troglobiont animals (previously published and our new discoveries, including yet undescribed species) in this dissertation to update the checklist. One of the main problems for diversity analyses is whether or not to include yet undescribed species that had not been taxonomically evaluated. In our case, we believe this not to be the problem, because we examined most of the sampled taxa morphologically and molecularly, and, considering the geographical distribution of the taxa in question, we eliminated reasonable doubt as to their taxonomic status.

From all 49 identified and described troglobiont species from Iran (six terrestrial, 43 aquatic), 42 species were arthropods, and out of these, 39 species were crustaceans, and 23 species of them belonged to the genus *Niphargus* (Niphargidae). Terrestrial troglobionts included two species of beetles, a spider, a diplopod, and an isopod. In this study, we discovered 26 new species of troglobiont animals. To date, we described three of them. During field works, we discovered some new localities for previously described Iranian cave fishes. Chah-Kabootari cave, a limestone cave with sulfur water, a kilometer to west of the Tashan cave harbors *Garra tashanensis*. Tuveh spring is another locality, which was discovered to harbor *Eidinemacheilus smithi*.

In total 45 localities we listed 72 obligate subterranean-dwelling species from Iran (caves, wells, and karstic springs). Most (36) of localities were in Zagros Mts, potentially indicating sampling bias.

The most diverse group of troglobionts in subterranean environments of Iran was Crustacea with 51 species, of which 12 await taxonomic description. With 26 species Amphipoda was the most diverse group. Within Amphipoda, the genus *Niphargus* counted 23 species (28 localities), making it the genus with the highest number of troglobionts in Iran. The second most-diverse group in Iranian groundwaters was Copepoda with 17 species. Cavefishes with four representatives from two families and two genera from five localities were the next most diverse groups of Iranian troglobionts.

We prepared maps to show the distribution and richness of troglobiont species per site. We showed the number of species present in each 100 × 100-km UTM grid cell prepared using QGIS. Cells with the highest species numbers were situated in western Iran and especially Southwest. There were two cells, which included 27 troglobiont species. Together with the eastern cell, they showed to have 30 troglobiont species, which was considered as the richest part of the country regarding troglobiont diversity.

The richest cave system that we detected consisted of Tashan and Chah-Kabootari caves that harbor 21 troglobiont species, of which five species were terrestrial, and 16 species were aquatic. In addition to these cave systems, other springs and wells in that area within six square kilometers all have sulfidic water. Obviously, they have formed a big aquifer. These caves and aquifer could harbor more terrestrial and aquatic troglobiont species. To understand this, more fieldwork, and sampling are needed. More springs with sulfidic water in that area have been observed by locals, which mostly are seasonal, flowing during winter and spring, and they harbor cave-fish as reported by local people as “red-pink fish”. Potentially, Tashan district is an important part for Iranian subterranean biodiversity as its

sulfidic aquifer makes it unique. This cave system was the first hotspot for troglobiotic biodiversity in Iran that needs high priority for protection. Considering the checklist we prepared, Tashan-Chah Kabootari cave system, Loven-Tuveh aquifer and Gakal cave, as important habitats for troglobionts, must be protected. Subterranean habitats are neglected parts of Iranian environments in protection point of view.

The degree of endemism in Iranian troglobiont animals was high and most of species were restricted to one cave, spring, or aquifer. This implies the importance of priorities for the protection of the habitats of these troglobiont species in Iran.

Caves and springs have been to a large extent neglected from the conservation point of view. According to our observations and some reports from locals, cavers and scientific publication, lots of destruction has occurred in these habitats. On the top of it, scientific depredation endangers Iranian cave-fishes. Excessive samplings using rather invasive sampling as electroshock can be reconstructed from the publications.

Although, most of the Iran land lies in an arid environmental zone, and considering the low rainfall in many parts of the country, there are many karstic areas in western and northern Iran. So, the currently reported Iranian subterranean biodiversity, even after this thesis, is still an underestimation. Speleobiology is in its first stage in Iran and regarding the discoveries of the recent decade, forthcoming decades will reveal more facts about this biodiversity and its evolutionary history.

Given the poor knowledge of Iranian subterranean fauna and its evolutionary history, this biospeleological dissertation enriched our knowledge by reporting new findings, describing new species, reporting on troglobiont biodiversity patterns, hotspot recognition, performing phylogenetic analyses to test species validity, as well as to understand the relationships and the origin of examined taxa. Our discoveries will therefore facilitate conservation decisions.

4.2 POVZETEK

Eno najzanimivejših okolij za biološke študije, kot so taksonomija, evolucija, etologija itd., so podzemni habitati, tako vodni kot kopenski. Glavna značilnost takšnih okolij je stalna tema. Nekateri abiotski pogoji, kot so vlaga, temperatura, kemija vode in kisik, so skorajda konstantni vse leto. Poleg tega so hipogeni sistemi v primerjavi s fotosintetičnimi epigeni relativno energetsko omejeni. V teh habitatih živijo tri glavne kategorije živali glede na vrsto uporabe podzemnih okolij. V tej študiji smo se osredotočili na troglobionte, izraz, ki se nanaša na organizme, ki jih najdemo samo v jamah in ki kažejo konvergentne fenotipe (morfološke, fiziološke in vedenjske), kot so izguba oči in pigmentacije ter podaljšanje okončin. Troglobionti so torej vrste, ki obligatno živijo v jamah (Vandel, 1965; Camacho, 1992; Culver in White, 2005; Sket, 2008; Culver in Pipan, 2009; Romero, 2009; White in Culver, 2012).

Čeprav so podzemni ekosistemi v primerjavi z epigeičnimi ekosistemi razmeroma slabo raziskani, kažejo precej visoko biotsko raznovrstnost na svetovni ravni. Na svetu je bilo popisanih več kot 7000 vodnih podzemeljskih vrst živali (Botosaneanu, 1986), za kopenske favne pa takšnih podatkov ni na voljo. Ocenjuje se, da na svetu obstaja približno 50.000 do 100.000 obligatnih kopenskih podzemeljskih vrst (Culver and Holsinger, 1992; Culver in sod., 2000; Culver and Pipan, 2009).

Sintagma "vroča točka biotske raznovrstnosti" je eno najpomembnejših globalnih orodij za ohranjanje narave (Myers, 1988; Reid, 1998; Hughes in sod., 2002; Brooks in sod., 2006). Več vročih točk podzemeljske biotske raznovrstnosti je bilo odkritih predvsem v Evropi in Združenih državah Amerike, nekaj pa jih je znanih tudi iz Avstralije in Azije (Culver in Sket, 2000; Culver in sod., 2021). To je v skladu z literaturo, ki je uporabila arbitrarno mejo 20-25 obligatnih podzemeljskih vrst, da se območje šteje za vročo točko troglobionske biotske raznovrstnosti (Culver in Sket, 2000; Souza Silva in Ferreira, 2016; Pipan in sod., 2020). Ta meja je odvisna od primerjalne vrednosti z drugimi (Culver in sod., 2021). Pred to študijo je bila jama z največjo raznolikostjo v Iranu jama Loven v Zagrosu, v kateri živijo tri vrste iranskih jamskih ribic (Vatandoust in sod., 2019). V Iranu doslej ni bila znana vroča točka troglobionske biotske raznovrstnosti.

Iran je z 1 648 195 km² površine druga največja država na Bližnjem vzhodu in 17. največja na svetu. Večina ozemlja leži na sušnem okoljskem območju. Različne vrste podnebja v Iranu pokrivajo 35,5 % površine, 29,2 % je sušnega, 20,1 % polsušnega, 5 % sredozemskega in 10 % vlažnega podnebja (hladnega gorskega tipa). Tako se več kot 82 % iranskega

ozemlja nahaja na sušnem in polsušnem območju sveta. Povprečna količina padavin v Iranu znaša približno 250 mm, kar je manj kot tretjina povprečne količine padavin na svetu (860 mm) (Amiri in Eslamian, 2010). Padavine se razlikujejo od več kot 1800 mm v severnih delih Irana na obali Kaspijskega morja in v nekaterih delih gorovja Zagros do približno 800 mm, vendar je povprečje približno 400 mm na nagnjenem območju gorovja Alborz in Zagros, v sušnih delih in osrednjem Iranu pa manj kot 100 mm (Raziei et al., 2005; Soltani et al., 2012). Iran je geološko del alpsko-himalajskega orogenega pasu. Skupna površina kraških karbonatnih kamnin v Iranu je približno 185.000 km², od tega 55,2 % v Zagrosu, 24,3 % v osrednjem Iranu, 15,2 % v Alborzu ter 4,7 % v vzhodnem in južnem Iranu. Zagros in Alborz sta dve glavni gorski verigi v zahodnem in severnem Iranu, ki obsegata skoraj tretjino iranske površine. V Iranu je bilo popisanih več kot 2000 jam, 10000 izvirov in vodnjakov, večinoma v Zagrosu in Alborzu (Raeisi, 2004; Raeisi in sod., 2012). Podzemna favna Irana je še vedno nezadostno poznana, ne le s favnističnega, temveč tudi z evolucijskega vidika, zlasti pri obravnavi izvora vrst.

V tej disertaciji smo sledili trem ciljem. Prvi cilj je bilo odkrivanje troglobiontskih organizmov v gorovju Zagros v Iranu, skupaj s taksonomskimi opisi novih taksonov. Opisane so bile tri nove troglobiontske vrste: karabidni hrošč, asellidni izopod in truncatelloidni gastropod. Šest vrst (Oligochaeta, Decapoda, Isopoda) je opisanih, več kot 10 vrst pa čaka na opis. Zabeležili smo tudi nove habitate že opisanih iranskih jamskih rib.

Drugi cilj disertacije je bil pripraviti seznam vrst vseh iranskih troglobiontov, da bi preučili vzorce biotske raznovrstnosti in endemizma troglobiontov v gorovju Zagros v Iranu. V tem delu disertacije smo odkrili in obravnavali prvo vročo točko biotske raznovrstnosti troglobiontov v Iranu.

Tretji cilj disertacije je bil preučiti filogenetske odnose med izbranimi skupinami troglobiontov in raziskati izvor te troglofavne. Za številne nove vrste troglobiontov, ki smo jih odkrili, ter za njihove ustrezne površinske sorodnike smo izvedli ekstrakcijo DNK in PCR za več markerjev, del teh izvirnih podatkov pa smo objavili v znanstvenih publikacijah. DNA izolacije ter reakcije PCR so bile narejene v Laboratoriju za Evolucijsko Zoologijo na Biološkem inštitutu Jovana Hadžija, ZRC SAZU. Sorodna zaporedja smo pridobili iz baze GenBank za smiselne rekonstrukcije filogenetskih odnosov proučevanih taksonov, za preizkuse njihovega vrstnega statusa in za ocene časovnega okvira njihove kolonizacije podzemnih okolij.

V znanstvenem članku v okviru te disertacije smo opisali dve novi vrsti jamskih hroščev iz rodu *Duvalius* iz jam v gorovju Zagros v Iranu. *Duvalius nezelensis* je bil prvi troglobiontski

karabidni hrošč iz te države. Glavne troglomorfne značilnosti vrste *D. nezelensis* so bile odsotnost oči in podaljšane okončine. Opisali smo tudi vrsto *D. achaemenius* kot troglofilno vrsto. Čeprav imajo Coleoptera in zlasti karabidi številne predstavnike v podzemnih okoljih, pred to študijo iz Irana ni bil odkrit noben troglobiontski karabid, edini znani troglobiontski hrošč iz Irana pa je bil *Foranotum perforatum* iz družine Tenebrionidae. Zdaj je iz Irana znanih šest vrst rodu *Duvalius*.

Za filogenetske analize smo pridobili izvirna zaporedja dveh mitohondrijskih (COI, 16S) in dveh jedrnih (18S, 28S) genov za tri vrste *Duvalius* iz gorovja Zagros in jih združili z objavljenimi zbirkami molekularnih podatkov o drugih vrstah *Duvalius*. Za rekonstrukcijo filogenije rodu *Duvalius* in tesno sorodnih rodov smo uporabili Bayesovo sklepanje in največjo verjetnost. Za raziskovanje divergenčnih časov glavnih linij rodu *Duvalius* smo uporabili BEAST. Naše filogenije so pokazale dobro podprt klad "Zagros" s tremi preučevanimi iranskimi vrstami. Za čas izolacije tega rodu od drugih sorodnikov smo ocenili približno 9,7 milijonov let (pozni miocen). Znotraj klada Zagros je bila ločitev *D. nezelensis* in njene sestrške skupine ocenjena na 7,8 milijonov let (pozni miocen). Sklepali smo, da bi se lahko kolonizacija podzemeljskih habitatov z *D. nezelensis* zgodila kadar koli po 7,8 milijoni let.

Ta znanstveni prispevek je bil objavljen z naslovom »Prvi vpogled v izvor raznolikosti iranskih jamskih hroščev z opisom dveh novih vrst rodu *Duvalius* (Carabidae)« leta 2021 v reviji *Journal of Zoological Systematics and Evolutionary Research*. Izvleček: Podzemna okolja Irana so zelo slabo raziskana. V tem prispevku želimo izboljšati znanje o iranski jamski biotski raznovrstnosti, pri čemer sledimo trem ciljem: (i) raziskati iranske jame za troglobiontske hrošče; (ii) razumeti filogenetske odnose znotraj rodu *Duvalius* Delarouze, 1859 in oceniti časovno obdobje kolonizacije iranskih jam; in (iii) komentirati trenutno poznavanje iranske troglobiontske favne za lažje prihodnje raziskave. S terenskim delom in morfološkim pregledom smo opisali dve novi vrsti rodu *Duvalius* iz jam v gorovju Zagros v Iranu: troglobiotski *Duvalius nezelensis* sp. nov. in netroglobiotski *Duvalius achaemenius* sp. nov. Za filogenetske analize smo pridobili izvirna zaporedja dveh mitohondrijskih (COI, 16S) in dveh jedrnih (18S, 28S) genov za tri vrste rodu *Duvalius* iz gorovja Zagros in jih združili z objavljenimi molekularnimi podatki drugih vrst in rodov hroščev. Z uporabo Bayesovega sklepanja in največje verjetnosti smo rekonstruirali filogenijo rodu *Duvalius* in tesno sorodnih rodov, nato pa s programom BEAST izračunali čas razhajanja glavnih linij. Naše filogenije so dobro podprle klad "Zagros", katerega ločitev od drugih sorodnikov je ocenjena na 9,7 milijona let. V kladu Zagros je razcep *D. nezelensis* in njegove sestrške skupine ocenjen na 7,8 milijona let, medtem ko je razcep *D. achaemenius* in *D. kileri* ocenjen na nedavnih 0,78 milijona let. V študiji smo prispevali nekaj razrešitve pri razumevanju vrstnega bogastva jamskih hroščev v Iranu in časa njihove podzemne kolonizacije. Vendar

naše filogenije potrjujejo taksonomske težave, saj je več rodov ugnезdenih globoko v “rodu” *Duvalius*.

V drugi objavi smo opisali novo vrsto polža iz sulfidnih ribnikov v jami Tašan v gorovju Zagros v jugozahodnem Iranu. *Trogloiranica tashanica* je prvi troglobiontni/stigobiontni gastropod, najden v Iranu, in šesta vrsta sulfidnega stygobiontnega gastropoda na svetu. Z dvema markerjema (COI in H3) smo sklepali o filogeniji te vrste in sorodnikov ter preučili njihove anatomske dokaze za uvrstitev *T. tashanica* v družino Moitessieriidae. Filogenija, izpeljana s COI, je nakazovala tesno sorodstvo z družino Moitessieriidae, vendar ni potrdila monofilije družine. Filogenija, izpeljana z jedrnim H3, je jasno pokazala, da *T. tashanica* spada v družino Moitessieriidae. Podobno je to potrdila tudi združena analiza COI in H3.

Razpravljali smo o edinstvenem habitatu novo odkritega polža, ki je poln vodikovega sulfida. V jami Tašan se nahaja edinstven ekosistem. Nedavnemu odkritju slepe ribe *Garra tashanensis* in izopoda *Stenasellus tashanicus* so sledila druga odkritja, vključno z novim rodом in vrsto gastropoda, o katerih poroča ta študija. Vodikov sulfid je strupen za večino organizmov in le pet od približno dvajsetih doslej znanih nahajališč sulfidnih jamskih ekosistemov po vsem svetu gosti stigobionske vrste gastropodov.

Ta znanstveni prispevek je bil objavljen z naslovom »Opis novega rodu in prve vrste polžev iz jam v Iranu« leta 2019 v reviji *Journal of Cave and Karst Studies*. Izvleček: Poročamo o novem stigobionskem truncatelloidnem gastropodu iz sulfidnih ribnikov v jami Tašan v gorovju Zagros v jugozahodnem Iranu. Habitat vodikovega sulfida je podoben doslej znanim habitatom polžev iz s sulfidi bogatih jam v Romuniji, Italiji in Grčiji. Novo opisani rod *Trogloiranica* n. gen. z novo opisano vrsto *T. tashanica* n. sp. predstavljata prve prave stigobionske polže, najdene v Iranu. Na podlagi filogenije, pridobljene iz mitohondrijskega (citokrom oksidaza podenota I) in jedrnega (histon 3) genoma ter anatomskega znakov, je *T. tashanica* uvrščena v družino Moitessieriidae Bourguignat, 1863. Novi rod lahko predstavlja evlucijski relikv iz obdobja po miocenu, preden sta se razcepili družini Moitessieriidae in Cochliopidae Tryon, 1866.

Druga vrsta troglobiontov, ki smo jo opisali v disertaciji in objavili v članku, je *Asellus ismailsezarii* iz družine Asellidae, red Isopoda, iz izvira Ganow blizu vasi Tuveh, mesto Andimeshk, provinca Khuzestan, Zagros Mts, zahodni Iran. To je bil prvi oblikatni stigobionski *Asellus* iz Irana. Družina Asellidae je ena redkih družin metazojev, ki vsebuje veliko število površinskih in podzemnih vodnih vrst. Do danes družina vsebuje 23 rodov ter 428 vrst in podvrst. Od teh 428 vrst in podvrst je 279 vrst brez oči in depigmentiranih vrst, ki so značilne za podzemne organizme, 18 vrst je večinoma omejenih na podzemne vode,

pogosto imajo zmanjšane oči in pigmentacijo, 131 vrst pa je takih, ki imajo oči in pigment ter poseljujejo površinske vode. Družina je splošno razširjena na severni polobli z vrstami v severnem delu Severne Amerike, Evropi, severni Afriki in Aziji.

Pri vrsti *Asellus ismailsezarii* so bile ugotovljene troglomorfije, kot sta depigmentacija in izguba oči. Navedli smo morfološke in molekularne dokaze, ki potrjujejo status vrste *A. ismailsezarii*. Gena COI in 28S vrste *A. ismailsezarii* smo analizirali z zaporedji vrst rodu *Asellus*, ki so na voljo v GenBank. Najdenih je bilo 11 MOTU znotraj rodu *Asellus*, vključno z enim MOTU, ki ustreza vrsti *A. ismailsezarii*. Nastalo drevo jedrnih zaporedij 28S rDNK se je ujemalo s filogenijo COI. Te analize so pokazale, da ta vrsta ni v evropskem kladu *A. aquaticus*. Ta nova vrsta rodu iz izvira Ganow je pokazala, da ta vodonosnik, ki vključuje pomembne jame Loven, Tuveh in izvire Ganow, gosti več troglobiontskih živali. Prej opisane iranske jamske ribe *Garra lorestanensis*, *G. typhlops* in *Eidinemacheilus smithi* iz tega vodonosnika s treh najdišč ter nekateri drugi neobjavljeni polži potrjujejo to hipotezo. Z novim odkritjem sta bili doslej iz Irana najdeni dve vrsti rodu *Asellus*. *Asellus monticola* je bil najden na izviru na skrajnem severozahodu Irana. Z novim odkritjem se je število obligatnih podzemeljsko živečih izopodov iz Irana povečalo na štiri vrste (tri vodne in ena kopenska). Predlagali smo domnevno prisotnost še več obligatnih podzemeljskih izopodov in zlasti taksonov rodu *Asellus* v Iranu. Naše ugotovitve skupaj s prejšnjimi poročili o asellidih iz Irana in sosednjih držav (Turkmenistan, Gruzija, Armenija) kažejo, da je območje Kaspijskega morja kontaktno območje med vrstami evropskega kompleksa *A. aquaticus*, vključno z *A. aquaticus* Linnaeus, 1758 in *A. aquaticus messerianus* ter azijskih vrst, vključno z *A. monticola* in *A. ismailsezarii*.

Ta znanstveni prispevek je bil objavljen z naslovom »Nova obligatna podzemeljska vrsta iz rodu *Asellus* (Isopoda, Asellidae) iz Irana« leta 2022 v reviji *Subterranean Biology*. Izvleček: Z le 43 opisanimi stigobionti in le dvema vrstama izopodov je obligatna podzemeljska favna Irana, obsežne države z več kot 10 % površine apnenca, nezadostno poznana. Tu poročamo o odkritju vrste *Asellus ismailsezarii* sp. nov. iz gorovja Zagros, prvega brezokega in depigmentiranega asellidnega izopoda iz Irana. Nova vrsta je morfološko podobna vrsti *Asellus monticola* Birstein, 1932, vendar je brez oči in popolnoma depigmentirana, ima rahlo ukrivljen pereopod IV in nima sete na proksimalnih robovih eksopodita pleopodov IV in V. Filogenetska razmerja med vrstami z uporabo izvirne in podatkovno rudarjene mitohondrijske DNK in jedrne rDNK ter ocena molekularnih razlik z drugimi vrstami rodu *Asellus* kažejo, da je *A. ismailsezarii* sp. nov. sestrška vrsta večjega klada, ki vsebuje tudi evropski kompleks vrst *A. aquaticus*. Površinske populacije vrst iz rodu *Asellus* so ob različnih priložnostih in v več krajih v Evropi in Aziji kolonizirale podtalnico, pri čemer so nastale vrste in podvrste, ki so razvile troglomorfizme, kot sta depigmentacija in izguba oči. Od 37 uradno opisanih vrst in podvrst rodu *Asellus* jih je 15 iz

podzemne vode, vključno z *A. ismailsezarii* sp. nov. Predvidevamo, da bo v Aziji odkritih še veliko vrst rodu *Asellus*, ki obligatno poseljujejo podzemne vode.

Glavna težava pri preučevanju filogenij in iskanju izvora iranskih troglobiontov je bilo pomanjkanje bližnjih sorodnikov za preučevane taksone v tej disertaciji. V GenBank ni bilo na voljo dovolj podatkov. Ker so bili nekateri rodovi novi za državo in znanost, za te taksone ni bilo bližnjih sorodnikov iz epigejskega/hipogejskega območja, niti genetsko niti geografsko.

Glede na naše rezultate in dostopno literaturo se je večina kolonizacij iranskih podzemnih habitatov z živalmi zgodila v srednjem miocenu do zgodnjega pleistocena (15 mio - 2 mio). Vendar je bilo v zvezi s tem na voljo malo podatkov, saj večina iranskih troglobiontov še ni bila testirana, da bi bolje razumeli njihov izvor. V prihodnosti bo potrebno preučiti še več skupin troglobiontov na različnih kraških območjih. Prav tako bi lahko primerjali ocenjene čase pri vodnih in kopenskih troglobiontih.

Za preučitev vzorcev razporeditve vrstne pestrosti troglobiontov v Iranu smo uporabili razpoložljivo literaturo in lastne podatke. Kot dopolnitev vrstnega seznama podzemeljskih členonožcev Irana, ki smo ga objavili leta 2017, smo v tej disertaciji predstavili vse iranske troglobionte (prej objavljene in naša nova odkritja, vključno s še neopisanimi vrstami) in tako posodobili seznam. V letu 2017 smo objavili seznam podzemeljskih členonožcev Irana z naslovom »Vrstni seznam podzemnih členonožcev Irana« v reviji *Subterranean Biology*. Izvleček: Razumevanje podzemeljske biotske raznovrstnosti je pomembno, vendar so obsežna območja sveta še vedno slabo raziskana. V tem prispevku predstavljamo prvi korak k popisu podzemeljskih členonožcev Irana. Po pregledu in analizi dostopne literature ter pregledu vzorcev, ki smo jih zbrali v iranskih jamah, smo našli 89 jamskih vrst (iz 42 jam in 5 kraških izvirov), ki pripadajo štirim subfilumom členonožcev: Chelicerata (1 razred, 4 redovi, 36 vrst), Crustacea (2 razreda, 3 redovi, 15 vrst), Hexapoda (2 razreda, 5 redov, 34 vrst) in Myriapoda (2 razreda, 3 redovi, 4 vrste).

Eden glavnih problemov pri analizah raznolikosti je, ali vključiti še neopisane vrste, ki še niso bile temeljito taksonomsko preverjene. Menimo, da v našem primeru to ne predstavlja težave, saj smo večino vzorčenih taksonov pregledali morfološko in molekularno ter, glede na geografsko razširjenost obravnavanih taksonov, odpravili dvom o njihovem taksonomskem statusu.

Od vseh 49 ugotovljenih in opisanih vrst troglobiontov iz Irana (šest kopenskih, 43 vodnih) je bilo 42 vrst členonožcev, od teh je bilo 39 vrst rakov, 23 vrst pa jih je pripadalo rodu *Niphargus* (Niphargidae). Med kopenskimi troglobionti sta bili dve vrsti hroščev, pajek, diplopod in izopod. V tej študiji smo odkrili 26 novih vrst troglobiontov. Do danes nam je

uspelo opisati in objaviti tri od njih. Med terenskim delom smo odkrili nekaj novih nahajališč že prej opisanih iranskih jamskih rib. V jami Chah-Kabootari, apnenčasti jami z žvepleno vodo, kilometer zahodno od jame Tashan, živi *Garra tashanensis*. Izvir Tuveh je še eno najdišče, kjer je bil odkrit *Eidinemacheilus smithi*.

Glede na literaturo in naše podatke je bilo ugotovljeno, da je na 45 nahajališčih v Iranu (jame, vodnjaki in kraški izviri) prisotnih 72 vrst obligatnih podzemljskih vrst. Kar 36 nahajališč je bilo na kraških območjih Zagrosa. Kot je bilo obravnavano v tej disertaciji, je to posledica večjega števila raziskav na teh območjih. Vendar pa je gorovje Zagros večinoma kraško in vsebuje največ jam in izvirov v državi. Tako majhno število raziskanih nahajališč podzemeljske biotske raznovrstnosti ni primerljivo z ZDA in Balkanskim polotokom ter njihovimi odkritimi troglobiontskimi vrstami. Do leta 1999 je bilo v ZDA znanih več kot 45.000 jam, do danes pa so jih dodali še veliko več (Culver et al., 2009). Sket in sodelavci (2004) so za Balkanski polotok navedli več kot 975 kopenskih in 650 vodnih obligatnih podzemljskih vrst. V Združenih državah Amerike je bilo opisanih več kot 1138 obligatnih jamskih vrst in podvrst iz 112 družin in 239 rodov (Hobbs, 2012). Podzemna vodna favna na večini kraških območij v Evropi je dokaj dobro raziskana, saj je doslej znanih okoli 1800 vrst stigobiontov (Stoch in Galassi, 2010). V Iranu je znanih več kot 2000 jam ter približno 10000 kraških izvirov in vodnjakov. Čeprav ni treba vzorčiti vseh jam in tudi kraških izvirov ter drugih podzemnih voda, da bi dobili dobro oceno vrstnega bogastva za določeno regijo, pa bi jih bilo potrebno povzorčiti znatno število (Pipan in sod., 2020).

Najbolj raznolika skupina troglobiontov v podzemnih okoljih Irana so bili raki (Crustacea) z 51 vrstami, od katerih je bilo 39 vrst opisanih, 12 pa jih še ni bilo uradno opisanih. Najbolj raznolika skupina je Amphipoda s 26 vrstami, v okviru Amphipoda pa je največje vrstno bogastvo troglobiontov v Iranu pokazal rod *Niphargus*, ki vključuje 23 vrst iz 28 lokalitet. Druga najbolj raznolika skupina v iranskih podzemnih vodah je Copepoda s 17 vrstami. Jamske ribe s štirimi predstavniki iz dveh družin in dveh rodov iz petih lokalitet so bile na naslednjem mestu raznolikih skupin troglobiontov v Iranu.

Pripravili smo zemljevide, ki prikazujejo razširjenost in bogastvo troglobiontskih vrst na posameznem območju. Prikazali smo število vrst, prisotnih v vsaki 100 × 100-kilometrski celici mreže UTM, ki smo jo pripravili s programom QGIS. Celice z največjim številom vrst so se nahajale v zahodnem Iranu in zlasti na jugozahodu. V dveh celicah je bilo 27 vrst troglobiontov. Skupaj z vzhodno celico sta imeli 30 troglobiontskih vrst, kar je veljalo za najbogatejši del države glede raznolikosti troglobiontov.

Najbogatejši jamski sistem, ki smo ga odkrili, sta sestavljali jami Tashan in Chah-Kabootari, v katerih se je nahajalo 21 vrst troglobiontov, od katerih je bilo pet vrst kopenskih, 16 vrst pa vodnih. Poleg teh jamskih sistemov imajo sulfidno vodo tudi drugi izviri in vodnjaki na tem območju v območju 6 kvadratnih kilometrov. Očitno je, da so ustvarili velik vodonosnik. V teh jamah in vodonosniku bi lahko živelo še več kopenskih in vodnih vrst troglobiontov. Da bi to razumeli, je potrebno več terenskega dela in vzorčenja. Domačini so na tem območju opazili več izvirov z žvepleno vodo, ki so večinoma sezonskega značaja, tečejo pozimi in spomladi, v njih pa se zadržujejo jamske ribe, ki jih domačini imenujejo "rdeče-rožnate ribe". Okrožje Tashan je potencialno pomemben del iranske podzemne biotske raznovrstnosti, saj je zaradi sulfidnega vodonosnika edinstveno.

V sulfidnih jamah so zanimivi ekosistemi z nenavadno bogatimi in raznolikimi nevretenčarji, med katerimi prevladujejo raki in žuželke, in občasnimi ribami. Ekosisteme sulfidnih jam so bili preučevali Sarbu s sodelavci (1996). Prva raziskana jama s sulfidnim ekosistemom je bila jama Movile v Romuniji, sledile so jama Frassassi in Grotto Azzurra v Italiji, jama Melissotrypa v Grčiji ter jama Ayalon in izvir Tabgha v Izraelu, zdaj pa še študija vodonosnika Tashan-Chah Kabootari v Iranu. V teh zgoraj omenjenih sulfidnih jamah živijo zanimive troglobiontske živali in njihove favne bi lahko primerjali, da bi ugotovili morebitne podobnosti. V prihodnjih študijah želimo preveriti, ali so sulfidne jame Tashan-Chah Kabootari resnično kemoavotrofne ali ne. Tudi z evolucijskega vidika je še vedno vprašanje, kdaj so jamski sistem Tashan-Chah Kabootari naselili organizmi in ali je bila ta kolonizacija enkratni dogodek ali zaporedje neodvisnih dogodkov.

Jamski sistem Tashan in Chah-Kabootari je tako prva vroča točka troglobiontske biotske raznovrstnosti v Iranu, ki jo je treba prednostno zaščititi. Glede na seznam vrst, ki smo ga pripravili, je treba zaščititi jamski sistem Tashan-Chah Kabootari, vodonosnik Loven-Tuveh in jamo Gakal kot pomembne habitate troglobiontov. Podzemni habitati so z vidika varstva zanemarjeni deli iranskega okolja.

Stopnja endemizma pri iranskih troglobiontih je bila visoka in večina vrst je bila omejena na eno jamo, izvir ali vodonosnik. To pomeni, da je treba prednostno varovati habitate teh troglobiontskih vrst v Iranu.

Jame in izviri, ki so pomemben del iranskega okolja, so bili z vidika ohranjanja v veliki meri zanemarjeni. Po naših opazanjih in nekaterih poročilih domačinov, jamarjev in znanstvenih publikacij, je v teh habitatih prišlo do številnih uničenj. Žal je ena glavnih groženj za iranske jamske ribe znanstvena plenitev. Domačini so poročali o številnih vzorčenjih ihtiologov, celo o invazivnih metodah, kot je elektrošok, kar smo zlahka razbrali iz njihovih publikacij.

Čeprav večina iranskega ozemlja leži na sušnem okoljskem območju in glede na majhno količino padavin v številnih delih države, je v zahodnem in severnem Iranu veliko kraških območij. Zato je trenutno poznana podzemna biotska raznovrstnost v Iranu tudi po tej disertaciji še vedno podcenjena. Speleobiologija je v Iranu v prvi fazi in glede na odkritja zadnjega desetletja bodo prihodnja desetletja razkrila več dejstev o tej biotski raznovrstnosti in njeni evlucijski zgodovini.

Glede na slabo poznavanje iranske podzemeljske favne in njene evlucijske zgodovine je ta biospeleološka disertacija obogatila naše znanje s poročanjem o novih najdbah, opisom novih vrst, poročanjem o vzorcih biotske raznovrstnosti troglobiontov, prepoznavanjem vročih točk, izvedbo filogenetskih analiz za preverjanje veljavnosti vrst ter za razumevanje odnosov in izvora preučevanih taksonov. Naša odkritja bodo tako olajšala odločitve o ohranjanju.

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