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Parsimony Analysis of Endemicity (PAE) in Iranian freshwater basins based on subterranean amphipods genus *Niphargus* (Crustacea, Malacostraca)

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Abstract

The order Amphipoda is one of the most diverse taxa that globally distributed in all environments and virtually habitats. The members of the genus *Niphargus* represent an important part of the Western Palearctic subterranean amphipod fauna. Accurate data on the occurrence and distribution of amphipods are only available for some regions. In this study, we were applied Parsimony analysis of endemicity (PAE) to analyze the distribution of amphipod freshwater genus *Niphargus* occurring strictly at the basin or sub-basin level along of Elburz and Zagros Mountains in Iran. The analysis was carried out using the computer program PAUP* 4.0a166, based on a data matrix built with 27 populations from 21 taxa and 13 areas for genus *Niphargus*. The rooting was made on a hypothetical all-zero out-group. Applying the exact algorithm heuristic search, the consensus tree was obtained with 30 steps, a consistency index of 0.9, and a retention index of 0.1. The procedure based on distributions of the 21 species of *Niphargus* delimited five areas of endemism in the study area with a minimum of two species. The high levels of endemism observed in West boundary rivers1 sub-basin with six species and then in Urmia and West boundary rivers2 sub-basins with three species.

Key words: *Niphargid species, Freshwater amphipods, Hydrological basins, Areas of endemism, Parsimony, Iran.*

INTRODUCTION

Amphipods are an important and diverse group of macroinvertebrates (Altermatt *et al.*, 2014). The members of this group inhabit freshwater environments including epi-benthic, benthic and subterranean habitats (Väinölä *et al.*, 2008). So far, more than 1500 species of freshwater amphipods are known worldwide, which about 70% of these species found in the Palaeartic (Väinölä *et al.*, 2008).

With over 330 species, genus *Niphargus* is the largest group of groundwater amphipods distributed across the Western Palearctic (Väinölä *et al.*, 2008). The most niphargid members inhabit in subterranean waters such as caves and springs (Fišer, 2019). So far, 19 species have been described from Iran along with Elburz and Zagros Mountains (Karaman, 1998; Hekmatara *et al.*, 2013; Esmaeili-Rineh *et al.*, 2015; Mamaghani-Shishvan & Esmaeili-Rineh, 2019). The most of known species comprises a narrow range with high endemism.

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Iran lies in an extremely complex area with extreme distributions in terms of altitude, climate and temperature (Frey & Probst, 1986). The high biodiversity of the Iranian fauna is also the result of its location and influences of four ecozones (Palaeartic, Nearctic, Afrotropical and Oriental) (Madjnoonian *et al.*, 2005). According to the Iranian National Geographic Organization, there are six main freshwater basins and 30 sub-basins in Iran (Fig. 1) including Caspian Sea basin with seven sub-basins (Aras, Talesh, Sefid-Rud, between Haraz and Sefid-Rud, Haraz, Gorgan-Rud and Atrek sub-basins), the Persian Gulf and Oman sea basin with nine sub-basins (Karkheh, Karun, Jarahi, West boundary rivers, Helleh, Mehran, Baluchistan, Mand and Between Bandar-Abbas and Sedih), Urmia Lake basin in the north-west with one sub-basin, the Central Plateau basin in the middle of the country with nine sub-basins (Namak lagoon, Gaavkhoni, Maharloo, Abarghoo, Hamoun, Lut desert, Central desert, Siah-koh desert and Saghand desert), East Border basin with three sub-basins (Khaf, Hirmand and Hamoon Mashkil) and Kara-Kum basin in the north-east (with one sub-basin). Caspian Sea, Persian Gulf and Oman Sea, Urmia Lake and the Central Plateau basins were visited within the Elburz and Zagros Mountains from north-west to south-east Iran (Afshin, 1994).

Parsimony analysis of endemism (PAE) is a biogeographical method first proposed by Rosen (Rosen, 1998), which uses a parsimony algorithm to obtain area cladograms based on the geographical distributions of specified taxa (Morrone & Crisci, 1995). PAE can be used to infer relationships among different biogeographical units (e.g., localities, quadrats, continents, islands) (Morrone & Escalante, 2002). Although there is an ongoing debate about the value of PAE, it has also proven to be a useful and important tool for identifying areas of endemism (AOEs) (Morrone, 1994; Morrone & Escalante, 2002; Hubert & Renno, 2006; Huang *et al.*, 2008). PAE is a cladistics method that groups areas by their shared taxa based on the most parsimonious solution (Morrone & Crisci 1995). This methodology allows an interpretation of the occupation of an area by taxa, to clarify the relationships among the studied areas, and to identify putative areas of endemism using a matrix built with taxa versus areas (or localities) (Nihei, 2006).

The present study surveyed the relationships of Iranian freshwater basins, using a PAE based on the distributional patterns of subterranean amphipod genus *Niphargus*.

MATERIAL AND METHODS

In this study, the samples were derived from various collections. Data were comprised of the database published in Esmaeili-Rineh *et al.* (2015, 2020). We analyzed a matrix composed of the presence (1) or absence (0) of 21 species of genus *Niphargus* distributed along the Zagros and Elburz Mountains to find the most parsimonious area cladogram. The analysis is based on a data matrix (Table 1) built with taxa (columns) versus areas (rows). The areas are treated as taxa and the taxa as characters (Rosen, 1988); character states are the presence/absence of taxa in the "terminal areas". A hypothetical area to root the tree with all the taxa absence was added to the data matrix. The data were analyzed using PAUP*4.0a166 (Swofford, 2018), applying the bootstrap method with a heuristic search to perform a search of the most parsimonious of all possible tree topologies.

TABLE 1. Data matrix of taxa (columns) x sub-basins (rows) of the species of *Niphargus* from Iran used in Parsimony Analysis of Endemicity (PAE).

Basin/ Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
	<i>N. khwarizmi</i>	<i>N. khayyami</i>	<i>N. daniali</i>	<i>N. darvishi</i>	<i>N. alisadri</i>	<i>N. borisi</i>	<i>N. bistunicus</i>	<i>N. shariifi</i>	<i>N. kurdistanensis</i>	<i>N. kermanshahi</i>	<i>N. hakani</i>	<i>N. loristanensis</i>	<i>N. ilamensis</i>	<i>N. valachicus</i>	<i>N. sohrevardensis</i>	<i>N. hosseiniei</i>	<i>N. sarii</i>	<i>N. persicus</i>	<i>N. fiseri</i>	<i>Niphargus</i> sp1. (Mergeh Mir)	<i>Niphargus</i> sp2. (Jandaran)	<i>Niphargus</i> sp3. (Kani Rasoul)	<i>Niphargus</i> sp4. (Vordekan)	<i>N. urmiensis</i>	<i>Niphargus</i> sp5. (Shinkeh 1)	<i>Niphargus</i> sp6. (Shinkeh 2)	<i>Niphargus</i> sp7. (Sahoolan)	
Haraz Sub-basin	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Namak Lagoon Sub-basin	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aras Sub-basin	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Sefid-Rud Sub-basin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
West boundary rivers1 Sub-basin	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0	1	1	0	
Between Haraz and Sefid-Rud Sub-basin	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
West boundary rivers2 Sub-basin	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
Maharloo Sub-basin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Karun Sub-basin	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jarahi Sub-basin	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Karkkeh Sub-basin	1	0	0	0	0	0	1	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Gaavkhoni Sub-basin	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urmia Basin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1



FIGURE 1. Distribution map of the species of *Niphargus* Schiödte, 1849 in freshwater sub-basins of Iran.

RESULTS

Parsimony analysis of endemism (PAE) for the genus *Niphargus* produced a single most parsimonious tree (Tree length = 30, Consistency index (CI) = 0.9000, Homoplasy index (HI) = 0.1000, Retention index (RI) = 0.6250) (Fig. 2) after 2000 independent replicates. Twenty-one species of the genus *Niphargus* were included in this analysis (Table1). Tree indicated a basal polytomy with four single clade "Gaavkhoni, Maharloo, Namak Lagoon and Urmia sub-basins", and two groups named "Haraz + Between Haraz and Sefid-Rud sub-basins" and "Jarahi + Karun sub-basins" and large clade (Sefid-Rud + Karkheh and Aras sub-basins, West boundary rivers1 and West boundary rivers2 sub-basins) that some ones are resolved. The clade "Haraz + Between Haraz and Sefid-Rud sub-basins" appeared in 62 percent of trees, the Jarahi + Karun sub-basins were present in 60 percent of them. Internally, in two clades of large clade had more than 45% support by bootstrap/jackknife values including "Karkheh and Aras sub-basins (54%) and West boundary rivers1 and West boundary rivers2 sub-basins (45%)" (Fig. 3).

Most of the clades were well supported by apomorphies (Fig. 2) as characters (species; Table 1), such as ch. 4 (*N. darvishi*) for Gaavkhoni sub-basin, ch. 18 (*N. persicus*) for Maharloo sub-basin, ch.3 (*N. daniali*) and ch.14 (*N. valachicus*) for Between Haraz and Sefid-Rud and Haraz sub-basins, respectively; ch.5 (*N. alisadri*) and ch.11 (*N. hakani*) for Namak Lagoon sub-basin; ch. 1 (*N. khwarizmi*) for both Aras and Karkheh sub-basins, ch.9 (*N. kurdistanensis*) for West boundary rivers1 and West boundary rivers2 sub-basins, ch.6 (*N. borisi*) for Karun and Jarahi sub-basins and characters 20/24/27 (*Niphargus* sp1./ *N. urmiensis*/ *Niphargus* sp7.) for Urmia Basin (Fig. 2).

Areas of endemism comprise regions in which more than one taxon is found with congruent distributional patterns (Morrone, 1994). Five areas of endemism with more than two species identified from this analysis. The first area is composed of the Urmia basin, is defined by three species (20/24/27). The second area is constituted from West Boundary Rivers 2 sub-basin which is defined by three endemic species (2/13/17). The third area is composed of West Boundary Rivers 1 sub-basin, is defined by six species (19/21/22/23/25/26). The fourth area of endemism is composed of Karkheh sub-basin that is defined by two species (7/10). The fifth area of endemism is composed of Namak Lagoon sub-basin that is defined by two species (5/11).

DISCUSSION

The most obtained parsimonious tree represents hypothetical relationships between different hydrological basins in Iran. Relationships on the area cladogram are based on a database from Esmaeili-Rineh *et al.*, 2015, 2020. The database for the freshwater amphipod genus *Niphargus* is incomplete because each hydrological system has only been partially explored. Consequently, no information exists on the occurrence of this taxon in some basins, meaning that these results can be modified or supported by new data in the future; also, there may be a considerable number of freshwater amphipod species not detected in one area because sampling effort was not sufficient, then some of the zero values in the presence/absence matrix may not be true absences. Despite these limitations, the database used in this study is the most complete reference of freshwater amphipod genus *Niphargus* records in each hydrological basin, so far.

The general topology based on distributions of the 27 populations from 21 species of *Niphargus* revealed seven main branches (Fig. 2). The analysis indicated that there are five distinct areas of endemism of *Niphargus* in the study area. The high levels of endemism observed for this taxon in West Boundary Rivers 1 sub-basin with six species and one species share with West Boundary Rivers 2 sub-basin and then in Urmia and West Boundary Rivers 2 sub-basins with three species.

The first area of endemism is composed of Urmia basin with three species (*Niphargus* sp1. (Mergeh Mir)/ *N. urmiensis*/*Niphargus* sp7. (Sahoolan)), the second area is composed of West Boundary Rivers 2 sub-basin also with three endemic species (*N. khayyami*/ *N. ilamensis*/ *N. sarii*), the third area is composed of West Boundary Rivers1 sub-basin with six species (*N. fiseri*/*Niphargus* sp2. (Jandaran)/ *Niphargus* sp3. (Kani Rasoul)/ *Niphargus* sp4. (Vordekan)/*N. urmiensis*/*Niphargus* sp5. (Shinkeh 1)/ *Niphargus* sp6. (Shinkeh 2)), the fourth area of endemism is composed of Karkheh sub-basin with two species (*N. bisitunicus*/ *N. kermanshahi*) and the fifth area of endemism is composed of Namak Lagoon sub-basin with two species (*N. alisadri*/ *N. hakani*). Urmia, West Boundary Rivers 2 and West Boundary Rivers 1 sub-basins are located in the west of Iran. The highest number of endemic species of *Niphargus* (hotspot for endemic species) occurs in these sub-basins. According to White (2007), the underlying geology, annual precipitation, and the quality of water resources especially in the karstic areas are affecting the frequency of niphargid populations. Therefore, the west and northwest of Iran is suitable climatically and hydrologically (Raisi, 2004) for the *Niphargus* members (Mamaghani-Shishvan & Esmaeili-Rineh, 2019). Also, Zagros Mountains are among the most important ecosystems in Iran (Mamaghani-Shishvan *et al.*, 2017) and the species richness is high in this area especially in northern parts of Iran due to locate at mid-latitudes belt (Culver *et al.*, 2009; Eme *et al.*, 2015, 2017).

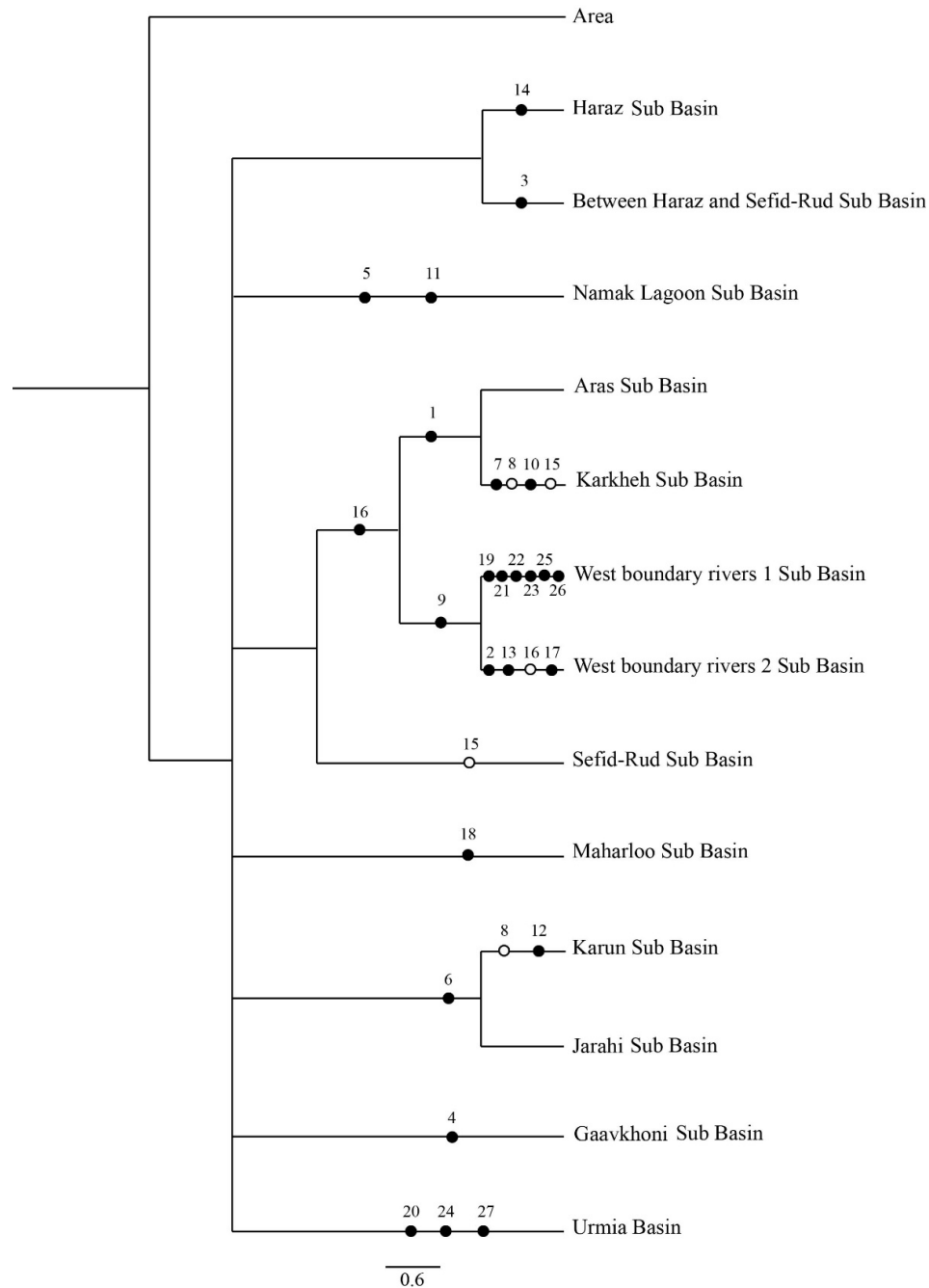


FIGURE 2. The majority rule consensus tree cladogram of genus *Niphargus* (Length=30 steps) obtained from parsimony analysis of the data matrix of table 1. The number showing the characters that were an apomorphy (black circles) or a homoplasy (white circles).

Karkheh sub-basin has two endemic species (*N. bisitunicus*/*N. kermanshahi*), although, this sub-basin also, share two species (*N. khwarizmi* and *N. hosseiniei*) with Aras sub-basin. Karkheh sub-basin in Persian Gulf and Oman sea basin and Aras sub-basin in Caspian Sea basin constitute a monophyletic group based on *N. khwarizmi*. The synapomorphy between two basins is related to the occurrence of two populations of *N. khwarizmi*. Two populations locate 570 apart (Esmaili-Rineh *et al.*, 2015) which demonstrate the connectivity of groundwater aquifers in the karstic area of the Zagros Mountains.

The Fifth area of endemism (Namak Lagoon sub-basin) has two endemic species (*N. alisadri*/*N. hakani*). This sub-basin is located in the three Iranian geological zones of Sanandaj-Sirjan, Central Iran and Elburz. Also, Namak Lagoon sub-basin is bounded by the Southern Elburz Mountains to the north and by the north-northeastern Zagros Mountains to the south. Mean annual precipitation ranges from less than 200 mm in the southeast to more than 800 mm in northern parts (Feiznia *et al.*, 2007).

The Karun basin area is defined by one endemic species (*N. loristanensis*) and one species share with Jarahi sub-basin (*N. borisi*). The Karun River has one of the largest river basins in Iran and located in the southwestern semi-arid mountainous area, has huge water resources potential due to the relatively abundant precipitation in the Zagros Mountain ranges and topography suitable for storage of water by dam construction (Hishinuma *et al.*, 2014).

Haraz, Sefid-Rud, and Between Haraz and Sefid-Rud sub-basins are located in the Caspian Sea basin. The present results indicate that these basins do not form a monophyletic group. Elburz is a branch of the Alpine-Himalayan orogenic system and its climate, geology and topography are very variable in the northern and southern slopes as well as in the eastern and western slopes (Stocklin, 1974). This dissimilarity can be related to the very variable topology of these mountains. More taxonomic studies will be needed in all sub-basins of the Caspian Sea basin to evaluate their relationships.

The two sub-basins Maharloo (*N. persicus*) and Gaavkhoni (*N. darvishi*) have each one endemic species. Maharloo sub-basin located in the south of Iran has been facing water shortage and declining in groundwater level during the last decade. In the center, south, east and southeast of the sub-basin, the groundwater does not have suitable quality because existence of the salt formations. But, the groundwater has a suitable quality in the north and northwest part of sub-basin (Agard *et al.*, 2005). *N. persicus* population was sampled from the northern part of Maharloo sub-basin and the lack of *Niphargus* population in other parts of sub-basin may be related to the geological conditions of the area.

The data assembled here is especially useful in proposing a general hypothesis of the relationships between several Iranian hydrological basins. These data formed into the nested sets in the area cladogram, indicating hypothetical relationships between 13 sub-basins included in the study. These should be treated as preliminary results because they are based on a relatively small number of *Niphargus* species. A more accurate biogeographical analysis should be undertaken in the future by considering a larger data set, and incorporating more freshwater amphipod taxa, and other hydrological systems. The hypothetical relationships between Iranian basins proposed here can be tested through a comparison of taxon–area cladograms of their taxa through an analysis of cladistic biogeography.

In summary, PAE was applied to identify the areas of endemism of freshwater amphipods genus *Niphargus* (Crustacea, Malacostraca) at the level of hydrological basins. Five areas of endemism (AOEs) *i.e.*, Urmia, West Boundary Rivers1, West Boundary Rivers 2, Karkheh and Namak Lagoon sub-basins, were identified for *Niphargus* genus. PAE are also found to be useful in freshwater amphipods conservation priority in terms of biodiversity, biogeography and evolution through identifying AOEs. Areas of endemism constitute the basic units of evolutionary biogeographic studies and they would be very useful in biodiversity conservation. The Endemic taxa are an important part of the natural heritage of a country. Areas with significant numbers of endemics and/or systematically significant endemics are prime candidates for conservation. In the recent years, amphipods (eg. *Niphargus* and *Gammarus*) are generally better known than many other, smaller, aquatic organisms, particularly in large and geographically diverse areas without an extensive history of systematic research like Iran. On this basis, it can be useful indicators of areas in concern to management decisions about biodiversity conservation priorities.

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