Original Article

Molecular Characterization of *Paederus* Spp (Coleoptera: Staphylinidae, Paederinae) the Agent of Human Linear Dermatitis in the Caspian Sea Coast, North of Iran

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Abstract

Background: A combined morphological and molecular survey was performed to determine the agent of human linear dermatitis *Paederus* Fabricius, 1775 (Coleoptera: Staphylinidae, Paederinae) species composition in Mazandaran Province in the Caspian Sea coast in northern Iran, where most of linear dermatitis cases of the country occurred.

Methods: Altogether, 397 *Paederus* specimens were collected from May to August 2021 and classified using morphological characters and ITS2-rDNA sequence analysis.

Results: Morphological investigation revealed that all the specimens were *Paederus fuscipes.* ITS2 polymerase chain reaction (PCR) direct-sequences and the profiles of restriction fragment length polymorphism (RFLP) derived from digestion of PCR products by *Hinf*I, *Hpa*II, and *Sal*I enzymes were identical confirming the morphological results, implying that all specimens belonged to a single taxon.

Conclusion: *Paederus fuscipes* (Fabricius, 1775) is considered the dominant taxon and responsible for linear dermatitis in Mazandaran Province. To our knowledge, we have provided the first molecular typing of *Paederus* beetles at the species level, suggesting that ITS2-rDNA characterization is an alternative tool for species discrimination of *Paederus* spp.

Keywords: *Paederus fuscipes*; Rove beetle; Linear dermatitis; ITS2-rDNA; Iran

Introduction

Rove beetle (Staphylinidae) is the prime family of Coleoptera order with more than 6.000 species (1, 2). *Paederus* Fabricius, 1775, is categorized into the tribe Paederini and subfamily Paederinae, presently encompasses around 490 species (3). According to Herman (2001), Paederinae subfamily is the third largest subfamily within the Staphylinidae family, and it is represented about 6.000 species (4). In the Palaearctic region, the genus *Paederus* is included 85 species and subspecies. Some Rove beetle are scavenger (5) or predator and act as biological agents in control of pests (6).

Paederus beetles are nocturnal and attracted by incandescent and fluorescent lights. As *Paederus* species turn towards particularly fluorescent light, they encounter with people. These insects that can enter homes at nights from an open window or underneath of door, excrete toxic substance in their hemolymph when they are disturbed or rushed.

The hemolymph of some species within the *Paederus* has long been recognized to be an irritation as, once released, it leads to human linear dermatitis and conjunctivitis (7). The signs are as a result of a toxic amide substance, named

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Pederin (C25 H45 O9 N, LD_{50}: 0.14mg/kg rat i.p.) (8) and makes up roughly 0.025% of an adult *P. fuscipes* weight. After pederin touches the skin, this substance to be proportional to its amount may cause important results from a simple rash to large wounds. At least 20 species of *Paederus* beetles have been associated with linear dermatitis (9). Along with causing rigorous skin lesions, their ordinary substructure accounts for similar antitumor and antiviral activities, cytotoxicity and interruption of DNA metabolism which are primarily based on obstruction of eukaryotic protein biosynthesis (10–13). At the same time owing to have the ability of inhibiting protein and DNA synthesis and being a very strong toxic substance, pederin has been habited in tumor studies in current years. The secretion or production of pederin depends on the activities of a *Paederus* bacterial endosymbiont (*Pseudomonas* sp.) (14–18). The production of pederin is mainly limited to adult female beetles which protects the beetles against predators (14, 15, 19). Males and larvae only accumulate pederin gained maternally (i.e., through eggs) or by ingestion (13).

Systematics of *Paederus* species is rather problematic and is relied on the male main and secondary sexual morphological characters (20). This has ended a very complex history for *Paederus* taxonomy and has reformed it intensely (21) and some species are regarded as synonyms of each other and or lowered to a single subspecies/species (22 and references herein). Recent advances in molecular methods have provided an extensive range of molecular markers for taxonomy and systematics of insects (23–30). The ribosomal DNA (rDNA) of insect genome has many advantages comprising known PCR primers, easy to use, a high interspecies mutation rate, and low intraspecies mutation rate due to homogenization has acknowledged particular attention for taxonomic and systematic studies of insects (24, 31–39).

Earlier studies on the ecology, geographical distribution, medical importance, and fauna of *Paederus* beetles in Iran showed presence of fourteen *Paederus* subspecies or species in the country, in which six species *P. littoralis ilsae*, *P. fuscipes*, *P. balachowskyi*, *P. balcanicus*, *P. riparius*, and *P. duplex* are reported in Caspian Sea coast (Mazandaran, Guilan, and Golestan Provinces) (22). However, due to problems in *Paederus* species identification, various lists of species have been reported for a same region. In north of country, for example, three species of *P. pietschmanni* (synonym of *P. mesopotamicus*), *P. fuscipes*, and *P. spectabilis* have been reported by Janbaksh and Ardalan (1977) in Mazandaran Province at the Caspian Sea shore (40). Later, Majidi-Shad et al. (1989) registered three species of *P. riparius*, *P. littoralis* and *P. fuscipes*, from the same region (41). Afterwards, *P. balcanicus*, *P. fuscipes*, and *P. kalalovae* were reported from the same region by Nikbakhtzadeh and Tirgari (2008) (42). Ultimately, three species of *P. balachowskyi* (synonym of *P. mesopotamicus*), *P. balcanicus*, and *P. fuscipes*, was reported from the same region by Nikbakhtzadeh et al. (2012) (22). This discrepancy and inconsistency stimulated us to test genetic variation of ITS2-rDNA, as a powerful molecular marker, of *Paederus* specimens collected from the province to unravel systematic status of the *Paederus* beetles in the area.

Materials and Methods

Study area and Paederus collection

Mazandaran Province (MP) is 23,833km^2, situated on the southern coast of the Caspian Sea; clockwise it is bounded by the, Tehran, Alborz, Golestan Semnan, Qazvin, and Guilan Ps. City of Sari is the capital and the largest city of the province. The province is geographically partitioned into two parts: the mountainous areas, and the coastal plains. The Alborz Mountain Range borders the coastal belt and plains of the Caspian Sea. There is often snowstorm throughout most of the seasons in the Alborz regions, which run correspondence to the Caspian Sea's southern coast, partitioning the province into

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many remote valleys. The province includes 15 counties including Sari, Babol, Amol, Behshahr, Babolsar, Chaloos, Tonekabon, Ramsar, Juybar, Qaem Shahr, Savad Kooh, Neka,Mahmood Abad, Noshahr, and Noor (Fig. 1).

Adult *Paederus* samples were collected by mouth aspirator on various vegetation (particularly rice) in early morning or afternoon and under soils at warm hours of daytime. In some areas, an ultraviolet black light was used for specimen collection from dusk to midnight at night as previously described by Nikbakhtzadeh and Tirgari (2008) (42). The collections were done mostly in rice farms in 33 sites belong to the 15 MP's counties (Fig. 1, Table 1). The rice fields were selected randomly, and sampling was conducted throughout the growth time from May to August 2011. The captured specimens were kept in 70% ethanol till morphological and molecular identification.

Morphological identification

The pictorial keys of Coiffait (1982) were used to determine the specimens to genus level (20) and then we send them to the specialist (Dr Sinan Anlaş laboratory) in Turkey for species identification. The specimens were identified on the base of habitus and sound structure of male basal and secondary sexual characters (Fig. 2).

DNA extraction

A subset (n=66) of *Paederus* specimens' representative of the populations and counties, were chosen for DNA analysis. Total body of individual samples was used for genomic DNA extraction using DNeasy® Blood and Tissue Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Preceding to DNA extraction, the specimens were frozen and then pounded in the Kit supplied buffer and extraction followed based on the manufacturer's directions.

DNA amplification

The primer pair ITS3f (5′-gcatcgatgaagaacgcagc-3') and ITS4r (5′- tcctccgcttattgatatgc-3') was used to amplify ITS2 (Internal Transcribed Spacer number 2) region of rDNA gene of the specimens. The primer pair has already been proposed by White et al. (1990) and utilized for some Coleopteran (43). PCR amplification cocktail included 5µl 10× PCR-Buffer, 120µM of each dNTPs, 50pmol of each primer, 2µl (around 100ng) of template DNA, and 2.5U of *Taq* polymerase (Sinaclon, Iran) in a 25µl reaction volume. PCR amplification was accomplished with an Eppendorf thermal cycler (Germany). The cycling program was: 3min denaturation at 94 °C followed by 35 cycles of 1min at 94 °C, 1min at 52 °C, and 2min at 72 °C, and a final 7min chain elongation. Well-characterized DNA samples and double distilled water (ddH2O) were used as positive and negative controls.

Sequencing and PCR-RFLP

A subset (n=17) of the specimen PCR products were refined from gels by using a gel purification kit and used for sequencing. An ABI 3730 sequencer machine by Bioneer (South Korea) was used for sequencing the samples. The ambiguities of resultant sequences were verified and their homologies with the accessible sequence data in GenBank was blasted by using basic local alignment search tool (BLAST) analysis software (www.ncbi.nlm.nih.gov/BLAST).

To thrifty in time and cost of sequencing, the rest of specimens were tested by restriction enzyme fragment polymorphism (RFLP) assay. To do this, the ITS2 sequences attained in present study was tested to prepare its physical map and to choose restriction/digestion enzymes by using the Nebcutter program (44) (Fig. 3). Restriction/digestion enzymes were selected according to their sites on the PCR product, costs, profiles, and obtainability in the marketplace. Digestion or cutting of PCR products was accomplished in 25µL of a solution containing 15µL of PCR product associated with 2.5µL of enzyme buffers and 5 units of individual restriction enzymes (*Hinf*I, *Hpa*II, and *Sal*I) overlaid with two droplets of mineral oil. The blend was nursed at the temperature suggested by the

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enzyme supplier. An aliquot part (14µL) of the restricted product was blended with 6µL of loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol), loaded on to a 2% agarose gel, and electrophoresed. Gels were contaminated with ethidium bromide (2mg/mL) and the RFLP outlines were envisioned under ultraviolet light.

Results

Species identification and PCR-RFLP

During the study period, a total of 397 adult *Paederus* specimens were gathered by hands or black-light trap. Except one specimen of *Philonthous* cf. sp which belongs to Staphylininae subfamily, (tribe Staphylinini, subtribe Philonthinina), all the specimens were classified morphologically as *Paederus fuscipes* (Fig. 2). This species was the predominant taxon representing 99.75% (396) of the total number of collected rove beetles. A subsection of the morphologically distinguished *P. fuscipes* were laid open to ITS2-rDNA PCR amplification. A 495bp fragment was amplified for all tested specimens. The ITS2 PCR products of a total of 17 morphologically identified *P. fuscipes* were selected based on their geographical origin for sequence characterization. The specimens were sequenced for both strands and the consensus data were submitted to GenBank database with GenBank identification numbers (IDs): KC414867- KC414883 (Table 1). The amplified fragment comprises 126bp of 3' end of 5.8S ribosomal DNA gene, 312bp internal transcribed spacer number 2 (ITS2), and 57bp of 5' end of 28S ribosomal DNA gene. The sequences were relatively high (58.4%) GC content. Among the sequences, there was no substitution or indel throughout the 495bp of the fragment indicating identical sequences. Afterward, a BLAST search on the sequences showed that there were some matching ITS2 sequences for *P. fuscipes* species in GenBank including a specimen from south of Iran (GenBank ID: KM086334) with 98.75% identity), and a specimen from China (GenBank ID: MZ172410) with 96.34% identity. Also, there were some homologous sequences with 89.45% maximum identity belonged to *P. mesopotamicus* from Turkey (GenBank ID: KC414866) and with 85.18% maximum identity belonged to *P. littoralis* from south of Iran (GenBank ID: KM098054).

Sequence analysis of *P. fuscipes* ITS2 fragment revealed appropriate restriction sites for *Hpa*II (CC↓GG) at 146, *Sal*I (G↓TCGAC) at 221, and *Hin*fI (G↓ANTC) at 310 nucleotide positions (Fig. 3). Restriction digestion of the ITS2 PCR products gave two fragments of 310/185, 146/349, and 221/274bp for *Hinf*I, *Hpa*II, and *Sal*I respectively for all the 396 morphologically identified *P. fuscipes*.

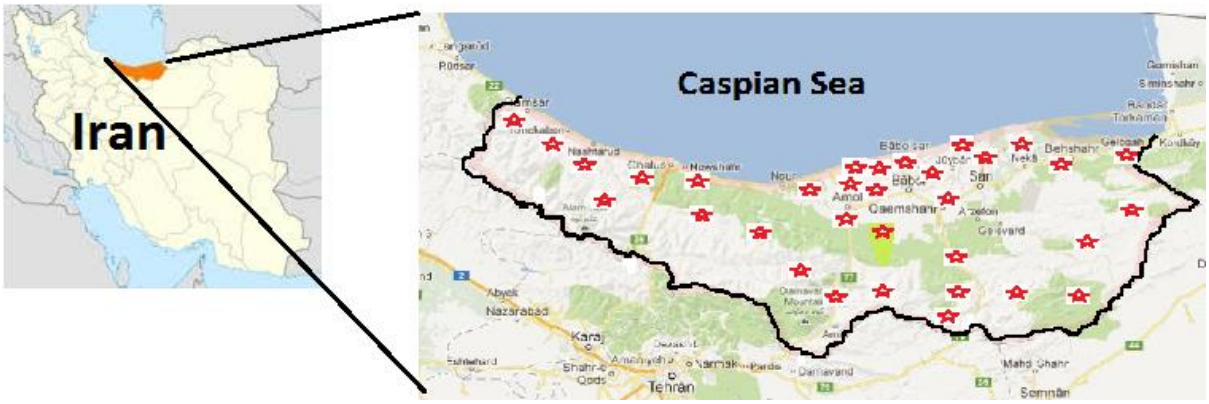

Fig. 1. Map of the area study (Mazandaran Province) and the collection sites (stars) of *Paederus* specimens

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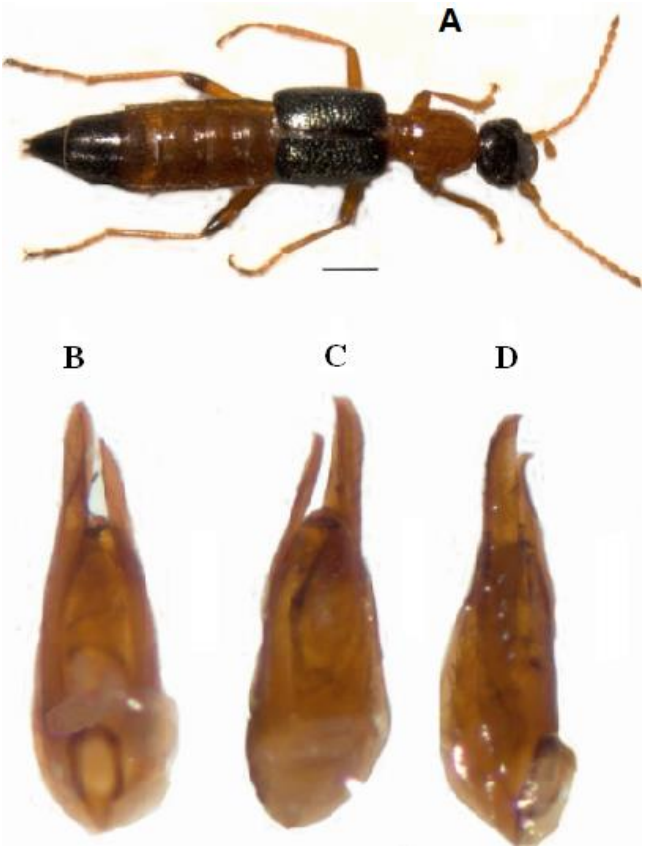

Fig. 2. Details of *Paederus fuscipes* (A)- habitus; (B)- aedeagus in ventral view (C)- aedeagus in dorsal view (D)- aedeagus in lateral view. Scale bars: 1.0mm (A); 0.2 mm (Figs. B-D)

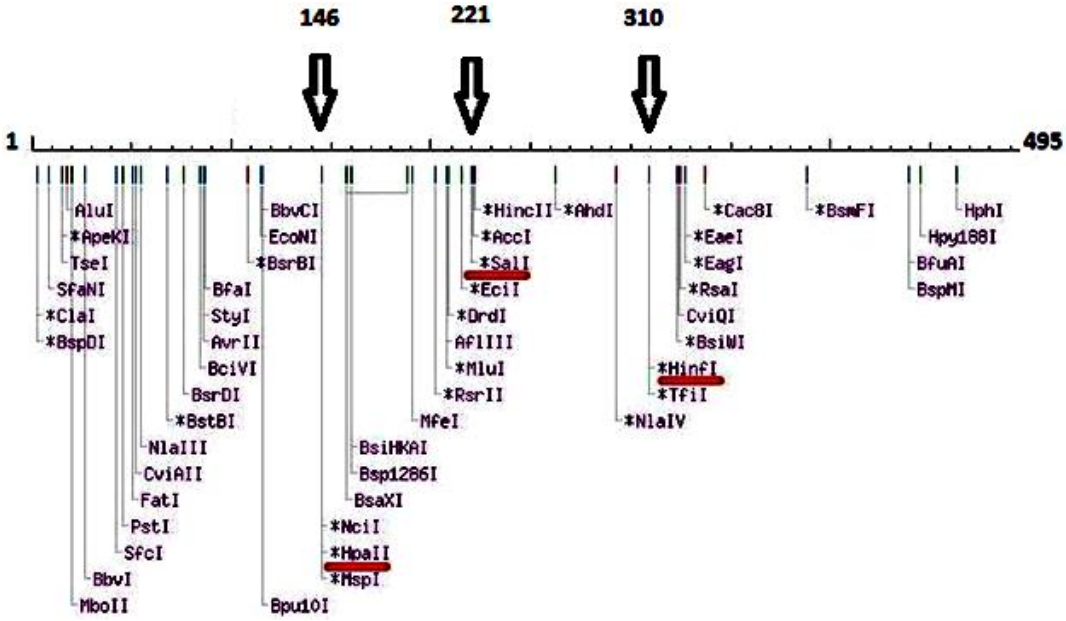

Fig. 3. Physical map of 495bp fragment of ITS2-rDNA region of *Paederus fuscipes*. The *Hpa*II, *Sal*I, and *Hinf*I restriction sites at 146, 221, and 310 nucleotid positions are shown by arrows

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Table 1. Details of morphologically identified *Paederus fuscipes* specimens collected in this study

Location (township)	Date 2011	Site	No.	Further Identification Method	GenBank Accession Number
Mahmood-Abad	May	Zardab, Hezarpey-Shomali	11	Sequencing	KC414874
		Abolhassan-Abad	12	PCR-RFLP	
	June	Ahlmarestaghe-Shomali	11	PCR-RFLP	KC414875
	May	Ahlmarestaghe- Jonobi	12	PCR-RFLP	
	July	Mirdeh-Olya, Daboye-Shomali	27	PCR-RFLP	NA
Amol	May	Bisheh-Mahaleh	13	Sequencing	KC414873
	June	Roodbar	12	PCR-RFLP	NA
	June	Aghozin, Linkooh	10	PCR-RFLP	NA
	July	Osko-Mahaleh, Mirza KochakKhan Jungle, Linkooh	13	PCR-RFLP	NA
	April	Marzango	21	Sequencing	KC414878
Ghaem-Shahr	June	Haji-Kola, Balanjan	9	PCR-RFLP	NA
	June	Ahangar-Kola, Noukandeh	14	Sequencing	KC414872
Noor	June	Banafsheh, Mianrood	12	Sequencing	KC414877
Babol	June	Markazi Kati Tayebi, Babolkenar	11	Sequencing	KC414871
	July	Mansour-Kandeh, Feyziyeh	8	PCR-RFLP	NA
Babolsar	June	Sorkh-Dasht, Bahmanmir	17	PCR-RFLP	NA
Location (township)	**Date 2011**	**Site**	**No.**	**Further Identification Method**	**GenBank Accession Number**
Sari	June	Jafar-Abad, Banaft	15	PCR-RFLP	NA
	July	Taher-Abad, Roodpeye-Shomali	16	Sequencing	KC414879
Fereydoon-Kenar	June	Shahrae-Daryasar, Barikrood	10	Sequencing	KC414880
Juybar	June	Seraj-Mahaleh, Siahrood	11	PCR-RFLP	NA
Neka	June	Ghaleh-Sare-Olya, Peyrajeh	12	PCR-RFLP	NA
Behshahr	June	Abbas-Abad, Kouhestan	11	Sequencing	KC414876
Gelogah	June	Mosayeb Mahaleh, Tooska, Spring	16	Sequencing	KC414870
Shirgah	June	Kati-Lateh	14	PCR-RFLP	NA
Pool-Sefid	July	Pool-Sefid	10	Sequencing	KC414881
Zirab	July	Zirab	12	PCR-RFLP	NA
Alasht	July	Larzaneh	11	Sequencing	KC414882
Noushahr	July	Kajour, Khachak	9	PCR-RFLP	NA
Chalous	July	Namak Abrood, Kalarestake-Gharbi	8	Sequencing	KC414869
Tonekaboun	July	Kelarabad, Yalbandan,	10	Sequencing	KC414868
Ramsar	July	Janate-Roodbar, Grasma-Sar	8	Sequencing	KC414883
Abbas-Abad	July	Kelar-Abad	11	PCR-RFLP	NA
Royan	Aug.	Noor	10	Sequencing	KC414867

Discussion

To our knowledge, this is the pioneer molecular analysis at population level aiming at Identifying *Paederus* beetles in literature. Based on the results of present study, the *Paederus* spp population in Mazandaran Province in the Caspian Sea shore almost exclusively consists of *P. fuscipes*. In the other hand, previous studies (22, 41, 42) reported the presence of five more species including *P. kalalovae*, *P. littoralis*, *P. balcanicus*, *P. riparius*, and *P. balachowskyi* (synonym of *P. mesopotamicus*) in the province. This discrepancy may be due to the collection method we used in this study. The validity of our findings requires a thorough process from design to using various sample collection methods. However, the present study

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failed to consolidate presence of other five species in the province. Thus, *P. fuscipes* can be regarded as the main local causative agent of linear dermatitis. It is worth mentioning that *P. kalalovae* (one of the five species mentioned above) and *P. fuscipes* is treated as synonym species in the modern systematics of Staphylinidae (45, 46). *Paederus fuscipes* has been found in north, centre and south of Iran (22, 40, 47–54). It has also an extensive geographical dispersal globally in the old-world countries (20, 55, 56).

Species classification of the rove beetles is mostly dependent on the examination of the genitalia and demands a distinguished level of proficiency particularly for doubtful species. This results in struggle to identify and confines their examination, management, and control. In current study the ITS2 of rDNA region was used for species identification of the rove beetle. This locus is a recognized powerful molecular indicator for species diagnostic assay. It is proven that inter-specific variation in this moderately fast evolving region is higher than intraspecific sequence variation (38, 57–60).

Further studies are now necessary to clarify the *Paederus* species composition of in the country using type specimens or morphologically distinguished species. Nikbakhtzadeh et al. (2012) reported 14 subspecies and species of the genus *Paederus*, belonged to five subgenera in the country (22). It is also suggested to examine sequence variation of ITS2 region or other genes such as mitochondrial DNA cytochrome oxidase subunit one (mtDNA COI), *28S*, *Topoisomerase I*, and *wingless*, in *Paederus* spp to provide a molecular key for distinguishing of the Iranian *Paederus* species. There is a substantial report on application of COI in species identification, systematics, and population genetics of beetles in the literature (32, 36, 57, 61, 62). There are a scarce existing COI sequence data of *P. ruficollis* (JX416589), *P. littoralis* (ANs: DQ155980; JX416591), *P. fuscipes* (DQ156010), and *P. riparius* (JX 416588) in Genbank database. Species identification can be performed by using RFLP outlines on PCR amplicons or developing species-specific primers to supply species-specific band/s. Bazrafkan et al. (2016) demonstrated that molecular typing using mtDNA COI gene followed by RFLP was useful to differentiate between two species *P. littoralis* (=syn: *P. lenkoranus*, *P. ilsae*) and *P. fuscipes*, and recommended PCR-RFLP of mtDNA COI for distinguishing of other *Paederus* species, which morphologically are identical or very challenging to be distinguished (63).

Conclusion

The composition of the local *Paederus* population in Mazandaran Province in north of Iran consist typically of *P. fuscipes* and seems to be the main source of linear dermatitis in the region. According to our estimation, this dermatitis is probably seen in almost every place of MP at Caspian Sea coast and in adjacent regions during hot and humid summer months, it is confused with various dermatoses thus detailed research must be done. Due to difficulties and doubtful morphological identification of *Paederus* species, more detailed morphological and molecular systematic and faunal investigations need to be done in the future to determine the *Paederus* fauna of MP and Iran more accurately, and the number of species confirmed.

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Ethical considerations

This study was performed based on Tehran University of Medical Sciences (TUMS) Ethical Committee Guideline.

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Conflict of interest statement

The authors declare there is no conflict of interests.

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