Gyrodactylus (Monogenea, Gyrodactylidae) parasite fauna of fishes in some rivers of the southern Caspian Sea basin in Mazandaran provin ce

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Abstract:

BACKGROUND: Members of the genus Gyrodactylus species are one of the extensive groups of monogenean ectoparasites which parasitize marine, brackish and freshwater fishes. **OBJECTIVES:** The main objective of the present study is the identification and diagnosis of the Gyrodactylus species on the fishes in some rivers of the southern Caspian Sea basin in Mazandaran province. **METHODS:** The field investigations were carried out from March 2015 to June 2016 and approximately, 1240 fish specimens belonging to two families, nine genera and nine species from Babolrud, Tajan, Shirud, Tonekabon, Nekarud, Siahrud, Talar and Haraz rivers were examined. **RESULTS:** At least eleven Gyrodactylus species were isolated from skin and gills of the examined fishes. Among them, *Gyrodactylus mutabilitas*, *G. sprostonae* and *G. prostae* have been previously reported to be found in Iran. The rest, including *Gyrodactylus ctenopharyngodonis*, *G. gobioninum*, *G. katharineri*, *G. nemachili*, *G. proterorhini*, *G. ophiocephali*, *G. varicorhini* and *G. vimbi* are reported for the first time from Iranian fishes in the present study. **CONCLUSIONS:** The host spectrum of Gyrodactylus species in Iran is actually wider, and further research may demonstrate that classification of individual species in terms of their host specificity will have to be changed and additional species of Gyrodactylus species would be found.

Introduction

Members of the genus Gyrodactylus species are one of the extensive groups of monogenean ectoparasites with an elongated body which attaches to the host by means of a small organ called opisthaptor with sixteen marginal hooks and one pair of anchors connected by one dorsal bar and one ventral bar (Vanhove et., 2014). These parasites are hyperviviparous, which means that the embryos develop within each other inside the mother's uterus and asexual reproduction alternates with sexual reproduction (Cable et al., 2002). It means that they experience only one adaptive barrier in their life. The direct life-cycle leads to auto-infection of the host. Despite detachment usually being fatal for the parasite (Bakke et al., 2007), gyrodactylid monogeneans which are detached from their host may form an important source of infection in fish communities. This is particularly significant for these ectoparasites, which have no specific free-living stage (Cable et al., 2002). Their hyperviviparous reproduction and monoxenous life cycle can lead to a sudden increase in worm numbers per host, particularly when the host densities are relatively high. Gyrodactylid monogeneans are frequently found on gills, skin and the fins of fishes (Shinn et al., 2010). They feed on the mucus and epithelial cells and can move freely on the host in almost a "caterpillar-like" fashion by alternatively attaching to the host's epidermis with their posterior opisthaptor and anterior attachment glands. Many gyrodactylids exhibit distinct site preferences on the host but may display characteristic migrations on the host during their life cycle (Shinn et al., 2010). They parasitize marine, brackish and freshwater fishes and the lower aquatic invertebrates and usually have a narrow host range and are restricted to a single species, genus or family (Rhode, 1982; Noga, 2010).

In 50 years of research on the monogenean parasitic fauna of the fishes in Iran, only 14 species including *Gyrodactylus cichlidarum* Paperna, 1968 from Astronotus ocellatus; *G. chinensis* (Ling, 1962) from *Carassius auratus*; *G. cyprini* (Diarova, 1969) from *Cyprinus carpio*; *G. derjavini* (Mikhailov, 1975) from *Salmo caspius* and *Oncorhynchus mykiss*; *G. elegans* (Nordmann, 1832) from *Abramis brama*, *Cyprinus carpio* and *Alburnus filippi*; *G. fossilis* (Ergens, 1973) from *Heteropneustes fossilis*; *G. gurleyi* (Price, 1937) from *Carassius auratus*; *G. kobayashii* (Hukuda, 1940) from *Carassius auratus* and *C. gibelio*; *G. mutabilitas* (Bychowsky, 1957) from *Capoeta gracilis*; *G. prostae* (Ergens, 1963) from *Rutilus kutum*; *G. shulmani* (Ling, 1962) from *Cyprinus carpio*; *G. sprostonae* (Ling, 1992) from *Arabibarbus grypus*, *Cyprinus carpio*, *Hypophthalmichthys molitrix* and *Hypophthalmichthys nobilis*; *G. stankovici* (Ergens, 1970) from *Cyprinus carpio* and *G. jalalii* (Vanhove, Boger, Bukinga, Volckaerte Huyse et Pariselle 2012) from the only Iranian cichlid, Iranocichla hormuzensis have been described on the species level. Gyrodactylus jalalii (Mokhayer, 1981; Jalali et al., 1995; Papahn et al., 2004; Jalali and Barzegar, 2006; Jalali et al., 2007, Ebrahimzadeh Mousavi et al., 2009; Vanhove et al., 2012; Omidzahir et al., 2012; Ebrahimzade Mousavi et al., 2013; Omidzahir et al., 2015).

Gyrodactylus species in low numbers may appear to have little effect on their hosts, but their mechanical and chemical stimulation do have an impact on the respiratory function of the gills and the skin, and also on the host's ability to regulate its ion balance due to the puncture wounds inflicted by the attachment and the feeding of the parasites (Ferguson, 1989). Also, monogenean Gyrodactylids like the other parasites have proven useful in host identification, biogeography and evolutionary history, both at the interspecific (Pariselle et al., 2011; Gillardin et al. 2012; Muterezi Bukinga et al. 2012; Vanhove et al. 2013) and intraspecific (Vanhove 2014) level. The main objective of the present study is the identification and diagnosis of Gyrodactylus species from the native and exotic fishes in some rivers of the southern Caspian Sea basin in Mazandaran province.

Material and Methods

Study area and fish collection: Field investigations on the parasites of the fish-

Table 1. Native and exotic fish species studied in some rivers of the southern Caspian Sea basin in Mazandaran province in the present study.

Family	Scientific name	Weight (g)	Length (cm)	Number of examined specimens	Estimated prevalence (%)
Cyprinidae	*Alburnoides bipunctatus* (Bloch, 1782)	6.5 ± 1.4	8.7 ± 0.6	144	27%
	Alburnus chalcoides (Güldenstaedt, 1772)	7.7 ± 0.9	7.56 ± .67	130	25%
	Alburnus hohenackeri (Kessler, 1877)	4 ± 1.3	7 ± 0.7	137	35%
	Barbus lacerta (Heckel, 1843)	3.4 ± 0.4	7 ± 0.4	135	39%
	Carassius gibelio (Bloch, 1782)	38 ± 7.1	14.6 ± 1.3	135	35%
	Capoeta Capoeta gracilis (Keyserling, 1861)	33 ± 2.6	16.5 ± 2.4	138	24%
	Squalius cephalus (Linnaeus, 1758)	6.2 ± 3.6	8.2 ± 1.9	148	44%
	Vimba vimba (Linnaeus, 1758)	24.4 ± 10	14 ± 1.2	140	24%
Gobiidae	*Neogobius pallasi* (Berg, 1916)	6.3 ± 2.1	8.3 ± 1	133	19%

es in some rivers of the southern Caspian Sea basin, in the Mazandaran province were carried out from March 2015 to June 2016. The fish samples were caught using a bag net, hook or electrofishing by local fishermen from different stations in Babolrud, Tajan, Shirud, Tonekabon, Nekarud, Siahrud, Haraz and Talar rivers. The fish samples were immediately transported alive in oxygen-filled plastic bags and then, sent to the parasitology laboratory, and wet mounts were prepared. Fish identification was performed by using keys of Berg (1965), Goad (2016) and Keivany et al (2017).

Parasitological study: Only fresh or already killed fish samples were subjected to the parasitological investigation. First, the fish samples were sedated by a drop of clove oil and then wet mount of fins, skin and gills were prepared and studied under a light microscope at 40X to 100x magnification. Vigorously moving Gyrodactylus worms were separated from the smears and subsequently fixed with ammonium picrate-glycerine (Malmberg, 1970) and mounted on a slide under a cover slip.

For the morphological study, the images of the opisthaptoral hard parts and the male copulatory organ (MCO) were captured at magnifications of 400X and 1000X oil immersion, using a digital microscope camera (Sony, SSC-DC80P), and the diagnostic variables were measured by Axiovision software (Carl Zeiss Vision AxioVision LE Rel. 4.5) on the captured images. The drawings of the taxonomic features were made from the captured images. A total of 18 morphological features were analyzed in this paper including body length (BL), body width (BW), diameter of haptor spherical (DHS), hamulus length (HL), hamulus shaft length (HSL), hamulus point length (HPL), hamulus process (HP), hamulus angle (HA), length of ventral bar (LVB), width of ventral bar (WVB), length of ventral bar membrane (LVBM), anterior bilateral processes of ventral bar (ABPVB), length of dorsal bar (LDB), width of dorsal bar (WDB), total length of marginal hook (TLMH), length of marginal hook sickle (LMHS), diameter of the male copulatory organ (DMCO) and greatest diameter of pharynx (GDP). All data are given in micrometers (μm) and are presented as a range and number of measurements (both in parentheses) preceded by the Mean ± Standard deviation. The identification and a comparison with the known species were based on Bychovskaya-Pavlovskaya et al., (1962); Yamaguti (1961); Gussev (1983) and Ergens (1983).

Table 2. The Gyrodactylus parasite isolated from fishes in some rivers of the southern Caspian Sea basin in Mazandaran province in the present study.

Parasite	Type host	Infection site	Type locality	Other host(s)
Gyrodactylus ctenopharyngodonis Ling, 1962	*Barbus lacerta*	Gills	Telar	*Ctenopharyngodon idella*
Gyrodactylus gobioninum Gussev, 1955	*Alburnus hohenackeri* *Capoeta capoeta*	Skin, Gills	Telar and Babolrud	*Abbotina rivularis, Gobio gobio, Gobio kessleri, Hemibarbus maculatus, Microphysogobio amurensis, Pseudorasbora parva, Romanogobio tenuicorpus, Sarcocheilichthys czerskii, Sarcocheilichthys sinensis*
Gyrodactylus katharineri Malmberg, 1964	*Vimba vimba* *Capoeta capoeta*	Gills and skin	Tonekabon and Telar	*Alburnus alburnus, Aristichthys nobilis, Barbus barbus, Barbus meridionalis, Barbus peloponnesius, Capoeta capoeta, Carassius auratus, Carassius carassius, Cyprinus carpio, Gobio gobio, Hypophthalmichthys molitrix, Scardinius eryophthalmus*
Gyrodactylus mutabilitas Bychowskii, 1957	*Vimba vimba* *Alburnus hohenackeri*	Skin	Telar and Shirud Rivers	*Capoeta capoeta*
Gyrodactylus nemachili Bykhovskii, 1936	*Capoeta capoeta*	Gills and skin	Babolrud	*Barbatula barbatula, Triplophysa dorsalis, Triplophysa stoliczkae, Triplophysa strauchi*
Gyrodactylus ophiocephali Gussev, 1955	*Squalius cephalus*	Gill, Skin	Tajan	*Cyprinus carpio, Cyprinus carpio haematopterus*
Gyrodactylus prostae Ergens, 1963	*Alburnoides bipunctatus* *Alburnus chalcoides*	Gills and skin	Tajan and Telar	*Abramis ballerus, Abramis bjoerkna, Abramis brama, Leucaspius delineatus, Leuciscus cephalus, Leuciscus idus, Leuciscus leuciscus, Phoxinus phoxinus, Rutilus rutilus, Vimba vimba.*
Gyrodactylus proterorhini Ergens, 1967	*Neogobius pallasi*	gills, skin	Tajan	*Gobius cobitis, Gobius niger, Neogobius melanostomus, Proterorhinus marmoratus, Zosterissesor ophiocephalus*
Gyrodactylus sprostonae Ling, 1962	*Carassius gibelio* *Capoeta capoeta*	gills, skin	Babolrud, Telar and Tajan	*Carassius carassius, Carassius gibelio, Cyprinus carpio, Hypophthalmichthys molitrix Aristichthys hybrids, Pseudaspius leptocephalus*
Gyrodactylus varicorhini Ergens et Ibragimov, 1976	*Alburnoides bipunctatus*	Skin	Telar	*Capoeta capoeta*
Gyrodactylus vimbi Schulman, 1953	*Vimba vimba*	skin and gills	Tonekabon	*Abramis bjoerkna, Abramis brama, Abramis sapa, Alburnus alburnus, Barbus meridionalis, Carassius carassius, Cyprinus carpio, Gobio gobio, Leuciscus cephalus, Leuciscus leuciscus, Rutilus pigus. Rutilus rutilus. Scardinius erythrophthalmus, Scardinius scardafa, Vimba vimba*

Taxon and author names in this study were checked according to Coad (2016) and Eschmeyer (2017) for the hosts and Harris et al. (2008) and Shinn et al. (2010) for the gyrodactylids.

Results

Approximately, 1240 fish specimens belonging to two families, nine genera and nine species were examined between March 2015 and June 2016. The list of fish species used for the parasitological study is given in Table 1.

Eleven Gyrodactylus species including *Gyrodactylus mutabilitas*, *G. sprostonae*, *G. prostae*, G. ctenopharyngodonis, *G. gobioninum*, *G. katharineri*, *G. nemachili*, *G. proterorhini*, *G. ophiocephali*, *G. varicorhini* and *G. vimbi* (Fig. 1) are isolated from skin (head, flank, operculum) and gills of the examined fishes from Babolrud, Tajan, Shirud, Tonekabon, Nekarud, Siahrud and Talar rivers belonging to the southern Caspian Sea basin. Parasites isolated from the studied fishes, the hosts and the infected organs are shown in Table 2. Gyrodactylus specimens have been described particularly in terms of size and morphological characters of the hamulus, the opisthaptor, the dorsal and ventral bars and other diagnostic variables. All point-to-point measurements are presented in Table 3.

Table 3. Morphometric data of body: haptoral hard parts and male copulatory organ (MCO) of the described Gyrodactylus species, in µm (distances) or ° (angles), depicted as average ± standard deviation, with range and number of measurements in parentheses

Discussion

Malmberg (1970) sub-divided the genus Gyrodactylus, based on the structure of the excretory systems, into six subgenera: G. (Gyrodactylus), G. (Mesonephrotus), G. (Paranephrotus), G. (Metanephrotus), G. (Neonephrotus) and G. (Limnonephrotus). Within each of these subgenera, Gyrodactylus species were classified based on their marginal hook morphology, host identity and site of infection. In 50 years of research on the monogenean parasitic fauna of the Iranian fishes, only 14 Gyrodactylus species were found, as the gyrodactylid parasites are notoriously difficult to identify owing to the heavy dependence on hook morphology, mainly morphometrics, but also made more difficult owing to a shape component (García-Vásquez et al., 2012). Further confounding factors are the considerable intra-species variation (Harris, 1998) and marginal hook variation that can arise due to abiotic factors such as water temperature and seasonality (Bakke et al., 2007). Furthermore, regarding the low outbreak of parasites in wild fishes (Kennedy, 2009), the extraction of sufficient DNA and subsequently molecular identification of the parasite would be difficult.

In the present investigation, 11 Gyrodactylus species were described, particularly in terms of size and the morphological characters of the hamulus, opistohaptor, the dorsal bar and other diagnostic variables from the different fishes in some rivers of the Caspian Sea basin. Among these 11 Gyrodactylus species, *Gyrodactylus mutabilitas*, *G. sprostonae* and *G. prostae* have been described previously in the context of Iranian freshwater fishes and the rest, including *Gyrodactylus ctenopharyngodonis*, *G. gobioninum*, *G. katharineri*, *G. nemachili*, *G. proterorhini*, *G. ophiocephali*, *G. varicorhini* and *G. vimbi* are reported for the first time in Iran. According to the Gyrodactylus database (Harris et al., 2008; Shinn et al., 2010), some fishes are introduced as new hosts for these species (Table 2).

The Gyrodactylus species are remarkably host-specific and their specific host species and also the precise microhabitats on them

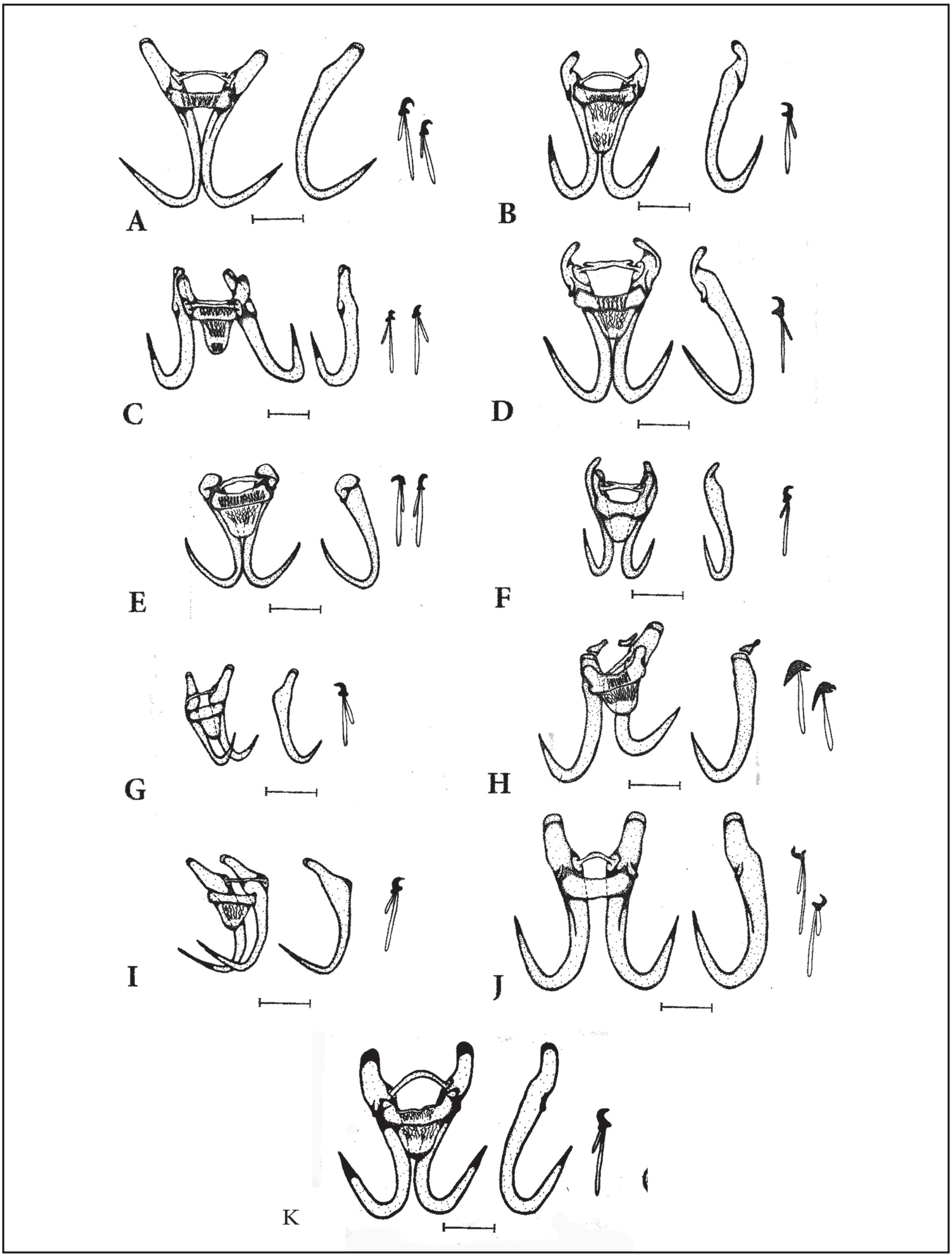

Figure 1. Morphological variability of anchors, bars and marginal hooks of A) *Gyrodactylus ctenopharyngodontis*, B) *Gyrodactylus gobioninum*, C) *Gyrodactylus katharineri*, D) *Gyrodactylus mutabilitas*, E) *Gyrodactylus nemachili*, F) *Gyrodactylus ophicephali*, G) *Gyrodactylus prostae*, H) *Gyrodactylus protrohini*, I) *Gyrodactylus sprostonae*, J) *Gyrodactylus varicorhini*, K) *Gyrodactylus vimbi*; Scale bars represents: A, B, D, E, F, G, H, I, J and K = 10 µm, C = 20 µm.

are defined structurally, biochemically and physiologically such that they are almost identical in each specimen of a particular host species (Noga 2010; Sukhdeo and Bansemir, 1996). The ones with the widest host range included *Gyrodactylus prostae* and *G. sprostonae*. *Gyrodactylus prostae* has already been found on *Carassius auratus* and Rutilus frisii kutum (Jalali, 1998) and the present investigation added to the list three new host species including: *Alburnoides bipunctatus*, *Alburnus chalcoides* and *Carassius gibelio*. *G. sprostonae* was also found for the first time by Jalali (1998) in *Cyprinus carpio*, *Hypophthalmichthys molitrix* and *Hypophthalmichthys nobilis* in almost all fish farms in Iran and it seems this species is translocated by the introduction of the common carp and Chinese carp to Iran and now it is linked to the parasite fauna of the native fishes (Jalali, 1998). In this paper, *Capoeta gracilis* and *Carassius gibelio* are introduced as new hosts for *G. sprostonae* in Iran. *Gyrodactylus mutabilitas* was formerly isolated from the gills of *Capoeta capoeta* (Jalali et al. 2007). In the present study, it was found on the gills and skin of two fish species from two different genus, *Vimba vimba* and *Alburnus hohenackeri* which shows its low host specificity.

Specific differences in the host fish epithelium and specific differences in the monogenean anterior adhesive, which contains mannose-rich glycoproteins, are implicated in stimulating the alternate complement pathway in the host and may all contribute to parasite-host specificity among the monogenean parasites (Buchmann, 1998b).

It is noteworthy that *Gyrodactylus ctenopharyngodonis* and *Gyrodactylus varicorhini*, both with two host species, have the narrowest host ranges.

The monogeneans live on the host's epidermis; they live in its products (e.g. mucus), and feed on it. Some of the host products are "attractants" and may be inhospitable surfaces because of the immunological activity and therefore, tissue-specific microenvironment can be seen among Gyrodactylus species (Whittington et al., 2000). In the present study, *Gyrodactylus proterorhini* was isolated from the gills, but mostly from the surface of the skin of *Neogobius pallasi* with the prevalence and mean intensity of 34% and 1.2, respectively. Moreover, so far the parasites were only isolated from the members of Gobiidae family including *Gobius cobitis*, *Gobius niger*, *Neogobius melanostomus*, and *Proterorhinus marmoratus.* The gobies are a family found worldwide, mostly in warmer sea waters, although some species enter the brackish water of estuaries and coastal areas. The *Neogobius pallasi* were caught from the *Mirud estuary* (Talar river's entrance to the Caspian Sea) with the salinity being around 12.5 ppt, which indicates that *G. proterorhini* is mainly specific to the brackish waters of coastal areas and estuaries. Here, *Neogobius pallasi* is considered as a new host for *G. proterorhini*.

The existing knowledge of the diversity, the host spectrum and the geographical distribution of the gyrodactylid monogenean parasites among the Iranian fishes is incomplete. Further studies should be carried out in order to identify and determine the species composition of the parasites in the different basins, their ecology and other biological specifications of the Gyrodactylus species in Iran. Also, an appropriate fixation and the processing of these monogenean parasites, as well as their molecular characterization, seem to be necessary requirements for providing new and reliable information. It is supposed that further research may demonstrate the host spectrum of Gyrodactylus species in Iran is actually wider and the classification of individual species in terms of their host specificity will have to be changed and additional species of Gyrodactylus species would be found.

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