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Non-lethal detection of mtDNA from the monogenean *Gyrodactylus mutabilitas* which recorded for the first time from the gill mucus of *Cyprinus carpio*, Iraq

Detecção não letal de mtDNA do monogenético Gyrodactylus mutabilitas que registrou pela primeira vez no muco branquial de Cyprinus carpio, Iraque

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ABSTRACT

The monogenean species of the genus *Gyrodactylus* are harmful to the common carp *Cyprinus carpio*. In this study fish sampling were collected from al-Tarmiyahm fish farm, Baghdad Iraq. The presence of the monogenean *Gyrodactylus mutabilitas* is reported in the present study for the first time on the gills of the common carp. Morphological characterization and measurements of this parasite are provided. The distribution of this monogenean on the gill arches of the hosts revealed a specific right-side preference, with a notable affinity for the second gill arches. Typically, accurate diagnosis of the monogenean relies on traditional methods by their morphology and it can only be achieved by fish post-mortem dissection. To support common carp conservation and management in the fish farm, a non-lethal method for pathogen detection is urgently needed. In this study, gill swabbing proved to be a promising technique for obtaining *Gyrodactylus mutabilitas* DNA samples.Fisheries managers may find this method be a useful tool to assess infection status which may prevent the spreading of any possible infection.

KEYWORDS: *Gyrodactylus mutabilitas;* common carp; non-lethal sampling; site preference; Iraq.

RESUMO

As espécies monogenéticas do gênero *Gyrodactylus* são nocivas à carpa comum *Cyprinus carpio*. Neste estudo, amostras de peixes foram coletadas na fazenda de peixes al-Tarmiyahm, em Bagdá, Iraque. A ocorrência do monogenético *Gyrodactylus mutabilitas* é relatada no presente estudo pela primeira vez em brânquias da carpa comum. São fornecidas caracterização morfológica e medidas deste parasita. A distribuição deste monogenético sobre os arcos branquiais dos hospedeiros mostrou uma preferência específica pelo lado direito, havia preferências óbvias para os segundos arcos branquiais registrados por este monogenético. Normalmente, o diagnóstico preciso do monogenético baseia-se em métodos tradicionais pela sua morfologia e só pode ser alcançado por dissecação post-mortem do peixe. Para apoiar a conservação e gestão da carpa comum na piscicultura, é urgentemente necessário um método não letal para a detecção de agentes patogénicos. Neste estudo, o esfregaço branquial parece fornecer um método útil para amostras de DNA de *Gyrodactylus mutabilitas*. Os gestores das pescas podem considerar este método uma ferramenta útil para avaliar o estado da infecção, o que pode impedir a propagação de qualquer possível infecção.

PALAVRAS-CHAVE: *Gyrodactylus mutabilitas;* carpa comum; amostragem não letal; preferência de site; Iraque

INTRODUCTION

The common carp is an economically important teleost fish in the aquaculture industry, with over 20 million metric tons of fish produced worldwide, approximately a total of 70% of global freshwater aquaculture production (XU et al. 2014). On the other hand, this considerable growth in aquaculture has led to some serious parasitic diseases affecting the health and productivity of carp and other fish. species (BONDAD-REANTASO et al. 2005). *Several studies have reported that common carp are commonly infected with the monogenetic ectoparasite Gyrodactyl.* As a result of their viviparous reproduction, these monogenetic have been described as one of the most invasive fish parasites, especially attacking fish gills and skin through their 16 marginal hooks and a pair of medium hooks and feeding on dermal mucus, blood, and the remains of host cells (BAKER et al. 1998). Although most *Gyrodactylus* species are considered to be almost less harmful to their hosts, intense parasitic infection with Gyrodactylus sp. leads to increased mucus secretion with serious damage to the skin and gill blades, especially in juvenile fish, which often have a high mortality rate (BUCHMANN 2012). *For example, Gyrodactylus salaris* was recognized as a fatal injury to a young Atlantic salmon population, causing enormous economic losses in Norway (BAKKE et al. 2007).

The first *Gyrodactylus* species described in Iraq was G. elegans from the gills of C. carpio and Planiliza abu (which was reported as Liza abu) (ALI & SHAABAN 1984). Since then, 58 species of gyrodactylus have been reported in various Iraqi fish.

The absence of a notable clinical sign of illness in some fish can lead to easy misidentification of Gyrodactyl infection, especially in the early stage of infection (PALADINI et al. 2014). This is precisely noted by a study by (PALADINI et al. 2009), in which an old sample of scraped skin from an infected rainbow trout, dated 2000 and preserved in formalin, was examined to indicate that the *G. salaris infection had been found in Italy at least nine* years before its first discovery.

Currently, the detection of monogenetic is performed with post-mortem examination of gills, which requires lethal sampling of fish; therefore, morphological characterizations are fundamentally used to identify genera or species of parasites. In the case of Gyrodactyl, the hard parts of the haptor, with the marginal hook and reproductive organs, are often used to discriminate between very close species (MALMBERG 1970). Serological tests are normally used to identify parasites. The serum of tilapia *Oreochromis niloticus* infected with the monogenetic Cichlidogyrus spp. was used to evaluate the humoral immune response using an immunoenzyme assay (ELISA). (SANDOVAL-GÍO et al. 2008). However, both morphological and serological tests are lethal to the host, both of which require a high level of technical expertise, and the presumptive diagnosis of parasites in a fish farm has an additional financial cost for treatment and management. (EK-HUCHIM et al. 2012).

Non-lethal sampling is ideal for endangered species or for fish that are frequently resampled. The widespread use of animals in research projects shed light on regulations and ethical implications (FERREIRA et al. 2019, HENDERSON et al. 2016). Recently, several studies evaluated the non-lethal DNA detection of various fish pathogens using different methods to examine virally infected fish (DRENNAN et al. 2007); bacterial (TAVARES et al. 2016) and parasitic (DA CUNHA et al. 2020, DE NOIA et al. 2022, DUVAL et al. 2021, KRKOŠEK et al. 2005, NORRIS et al. 2020).

It is clear that, based on previous studies, there has been no published work on *G.* mutabilitas in Iraq. Therefore, this article aimed to investigate the gyrodactylus and successfully register this parasite for the first time in Iraq. The second objective is to investigate whether it is possible to detect *Gyrodactylus infection* in the branchial mucus of common carp and how noninvasive tests can be used to examine low levels of parasitic infection.

MATERIALS AND METHODS

Fish sampling and parasite testing

Between September and December 2020, 35 common carp were collected from a fish farm in the al-Tarmiyah region, Baghdad province, using a throwing net with the assistance of fish farm owners and workers. The fish were transported alive to the laboratory and immediately examined for parasites. The gills were examined separately under a stereomicroscope to detect any metazoan infection. *Skin swabs, flippers, and gills were prepared to investigate infection by Gyrodactylus sp.* To examine whether *G.* mutabilitas exhibits a preference for distribution between the right and left gills or other parts of the body examined, the number of parasites in each branchial arch or body part was counted. To identify the species, we prepared the parasites following the standard CRIBB & BRAY protocol (2010). Freshly prepared parasites were photographed and measured using a specific digital camera attached to the microscope. The drawings were created using a lucid

camera. The parasite was identified according to the keys of BYKHOVSKAYA-PAVLOVSKAYA et al. (1962) and GUSSEV (1985).

Isolation of mucus from common carp gills and DNA extraction

In total, 25 common carp that were known to be infected with *G.* mutabilitas were collected, and the gills were subsequently cleaned to collect mucus. Following the procedure of EK-HUCHIM et al. (2012), the fish were then dissected and had their gills removed to determine the worm load. The infected gills were removed and individually placed in Petri dishes containing 0.7% saline. For DNA extraction, the parasites were counted and kept individually in Eppendorf tubes containing 70% ethanol. For negative controls, mucus was collected from 10 uninfected fish following the same protocol as that used for a positive sample.

DNA extraction from gill swab samples and PCR assays

Briefly, the fish were placed in a clean glass tray with the help of a technician who held them firmly with his hand and then cleaned them with a sterile swab. To avoid serious tissue damage, the swab was carefully placed inside the operculum and rotated for five seconds on the outer surface of the left and right sides of the fish's gills. Thereafter, the swab was carefully removed from the gills, returned to the sterile container, and immediately submitted for DNA extraction. DNA extraction was performed using the Geneaid, GSync ™ DNA Extraction Kit according to the manufacturer's protocol. After swab collection, the fish were dissected to assess the worm load and to collect infected tissue samples from the gills for comparison of the parasite's genomic DNA. Mucus was obtained from 10 uninfected fish and used for DNA extraction from negative control samples. The DNA concert was measured using a Nanodrop, which was used to assess the purity and quantity of the DNA, which was then stored at -20 º C for future use. Like a DNA barcode, mtDNA cytochrome c oxidase (COI) subunit I was amplified using the polymerase chain reaction (PCR) method. PCR amplification was attempted using primer pairs (Bioneer/Korea), the direct primer

(LCO1490) 5´- GGTCAACAAATCATAAAGATATTGG-3´ and the reverse primer (HC02198) were 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' by FOLMER et al. (1994).

The polymerase chain reaction (PCR) mixture for amplification of the *COI* gene was optimized as follows: primers were performed in a total volume of 25 µl with 5 µL of the mixture (Bioneer\ Korea) PCR mix, 5 µL of DNA (30-50 ng/µl) 1 µL of each primer, 13 µL of twice distilled water. The PCR mixture mentioned above was placed in a thermocycler (Bioneer/Korea). The PCR program used in this amplification was optimized as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles of 40 s at 95 °C, 1 min of annealing at 45 °C; extension step of 45 s at 72 °C, and a final extension of 7 min at 72 °C.

PCR products were verified using a standard 1.5% agarose gel dissolved in 1X TBE (Bioneer, Korea), 0.5 µl of ethidium bromide was added and poured into the gel former, after which 2 µl of 100 bp DNA ladder (Bioneer, Korea) were added. The PCR products were run at 75 V for 2 h. The agarose gel was prepared using a UV-documentation system.

Statistical analysis

The spatial distribution of the monogenean *G.* mutabilitas between the left and right sides of the fish gills, including the branchial arches numbered 1–4, from the first branchial arch below the operculum to the posterior one, was used in the mixed-effects model ANOVA to test the correlation between the average abundance of infection in the right and left gill arches. All statistical analyses were performed using the Minitab18 statistical software.

RESULTS

Gyrodactylus mutabilitas were isolated from common carp gills. All measurements were based on 11 parasite specimens that were particularly described in terms of size and morphological characteristics of the hamulus, opisthaptor, dorsal and ventral bars, and other diagnostic variables. Four parasite specimens were used to obtain the measurements.

As illustrated in Figure 1, a brief description and measurements (in mm) of this parasite are presented: The worms are small in size 0.37-0.45 (0.40), width 0.14-0.18 (0.16), opisthaptor length 0.1-0.15 (0.12), opisthaptor width 0.1-0.15 (0.12), dorsal bar 0.019-0.025 (0.021) x 0.0017- 0.0023-0.0028 (0.0026), hamulus length 0.06-0.075 (0.064), humulus tip length 0.02-0.026 (0.023), hamulus axis length 0.021-0.026 (0.023), 0.038-0.043 (0.04), ventral bar 0.017-0.02 (0.018) x0.0038-0.0042 (0.004), length of the membranoid extension 0.017-0.021 (0.019), length of the marginal hooks 0.030-0.040 (0.035) and finally it was 0.009-0.014 (0.011).

Site selection of *G.* Mutabilites in the gills of C. carpio

Data analysis showed significant differences in the average intensity of infection over the months of the study between the left and right sets of branchial arches when significantly higher numbers of *G.* mutabilitas were reported on the right side of the host. gill ($P < 0.0001$; Table 1; Figure 2). In addition, the number of parasites in the branchial arches infected with G. mutabilitas varied significantly; the analysis revealed that there were fewer parasites in the first, third, and fourth branchial arches than in the second branchial arch ($P < 0.002$, Figure 3).

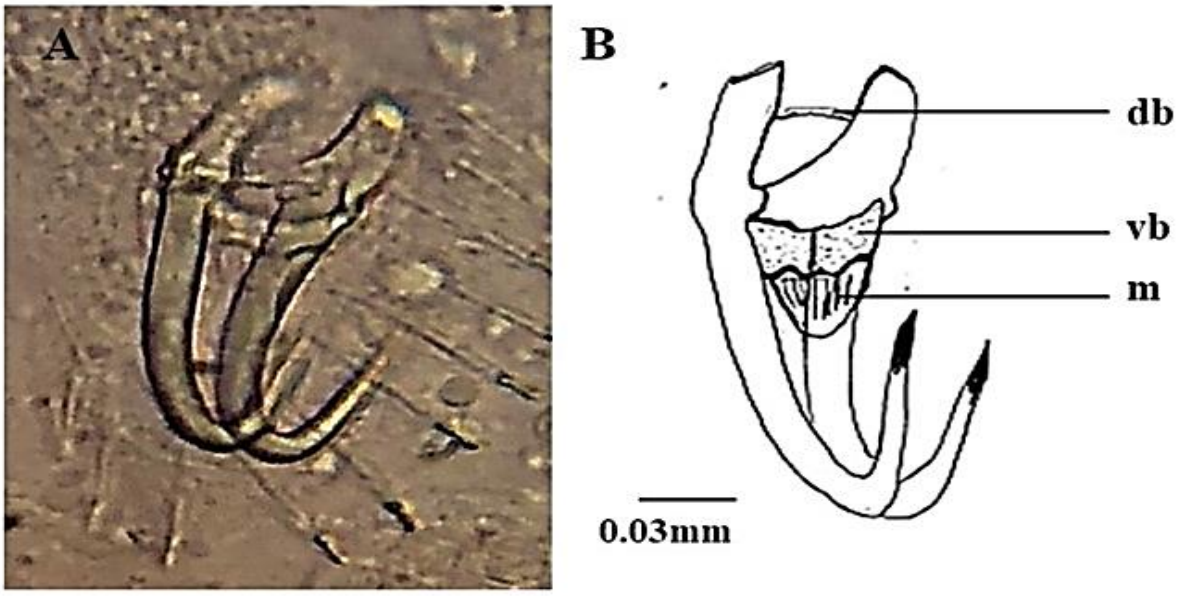

Figure 1. *Gyrodactylus mutabilitas* from the common carp. A- Light micrograph of the haptoral part. B- Design of the haptor's lucid camera. db = dorsal bar; vb = ventral bar; m = membranoid extension.

Table 1. *G.* mutabilitas distribution (mean and SD) in the right and left sets of common carp gills. The branchial arches were numbered from 1 to 4, from the first arch below the operculum to the posterior arch.

Side	Right gill	Left gill
Gill arches		
1	1.76 ±1.12	072 ±0.79
2	3.32 ±1.52	1.72 ±1.10
3	0.36 ±0.57	0.16 ±0.37
4	0.16 ±0.79	0.08 ±0.28

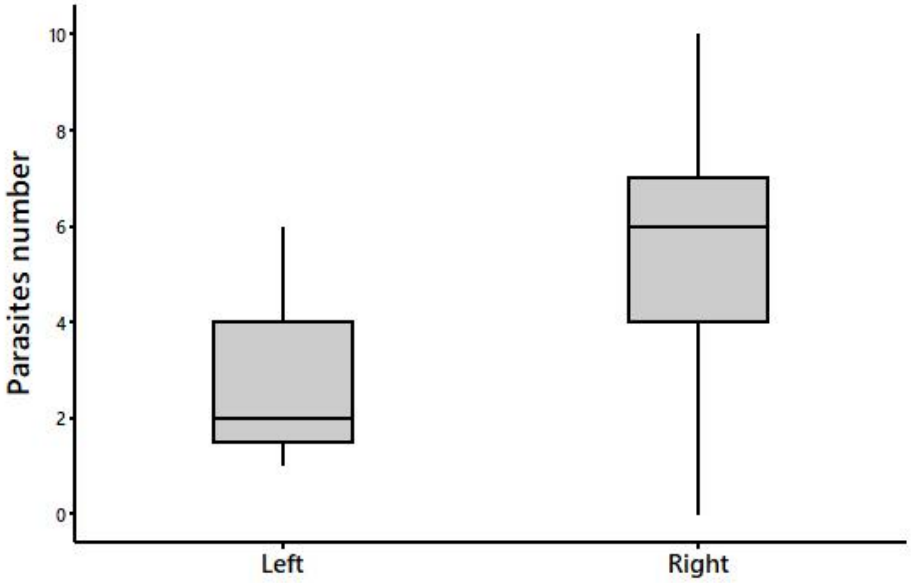

Figure 2. The number of *G.* mutabilitas was isolated from C. carpio gills present on the side of the infection.

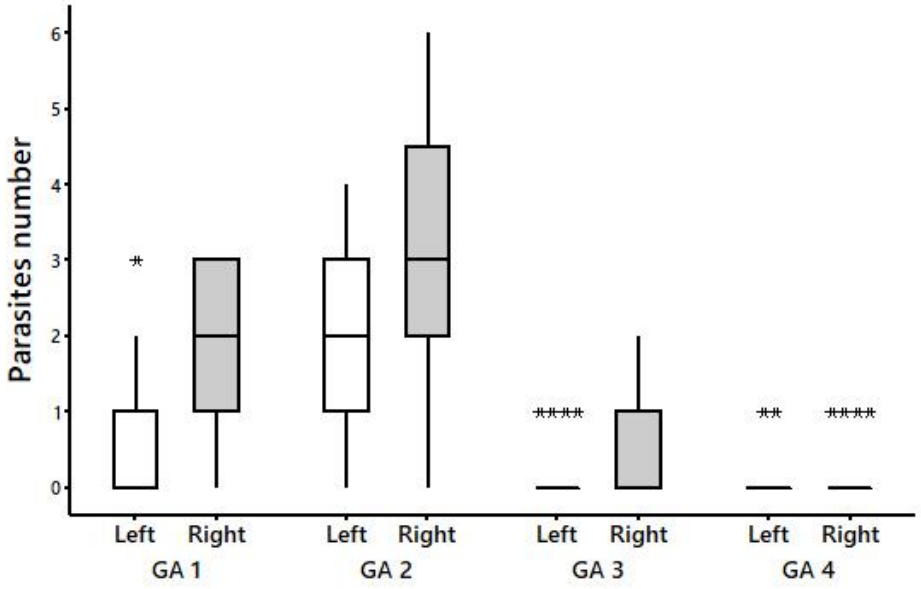

Figure 3. Boxplot showing the distribution of *G.* mutabilitas in the GA 1-GA 4 gill arches between the right and left gills.

Parasite*: MtDNA COI gene amplification and PCR*

The mtDNA *COI* parasite gene was detected using PCR with the primers LCO1490 and HC02198. To examine the sensitivity of the test for subsequent use in common carp mucus, DNA from collected parasites was used for PCR standardization. The positive PCR reaction in the presence of parasite DNA was achieved when the primers successfully amplified two areas of mtDNA in the form of two 709-bp fragments.

An objective of this study was to test whether the mucosal DNA approach is a reliable method for detecting parasitic infection in fish. The DNA concentrations in the mucus samples and the DNA from the collected parasite samples were strongly and positively correlated (Figure 4), indicating that both approaches produced very similar estimates of parasite DNA concentrations. DNA amplification was not observed in the mucus of 10 uninfected *C.* carpio. PCR tests amplified DNA fragments of the same size as G. *mutabilitas* from 25 common infected carp.

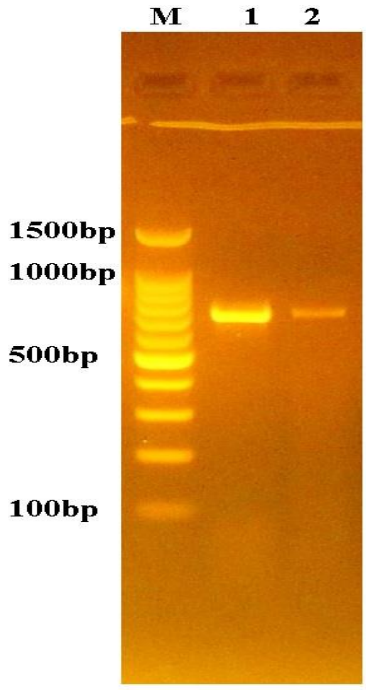

Figure 4. *The 1.5% electrophoresis agarose gel amplified the CO1 target gene.* (1) sample of parasites and (2) gill mucus from infected *Cyprinus* carpio.

DISCUSSION

In this study, the monogenetic *species G.* mutabilitas was reported for the first time in Iraq. According to the Iraqi fish parasites and disease agents index catalog [Mhaisen, 2022 Iraq fish parasites and disease agents index catalog (Unpublished: mhaisenft@yahoo.co.uk)], no previous record of this parasite in Iraq fish has been documented. Data from MHAISEN showed that 58 *Gyrodactylus species have been recorded in different Iraqi fish; specifically, 34 of* them have been described mainly from C. carpio.

Transportation, establishment, and demographic growth are just some of the variables that can affect a species becoming invasive in a new area (TORCHIN & MITCHELL 2004).

Gyrodactylus mutabilitas was previously described in several Iranian freshwater fish species (BARZEGAR et al. 2018, MASHALY et al. 2019, MIRGHAED et al. 2018). Iran is a neighboring country to Iraq, which may explain why this monogenetic was introduced into a new habitat area.

Monogenetics is a recurring concern for common carp for many years. Current detection methods, which are widely used, require the fatal sampling of fish from farms; this method is sometimes ineffective and increases costs for local farms. In this study, the non-lethal method was used for the detection of ectoparasite infection; the PCR-based test was used to detect infection by *G.* mutabilitas using DNA isolated directly from parasites collected recently, as well as by the parasite's DNA in the mucus of C. carpio.

Because they are highly conserved and present in numerous copies, mitochondrial *COI* genes are the main target genes in PCR-based detection techniques (PAOLETTI et al. 2018). Mitochondrial genes are useful markers for the diagnostic and taxonomy of population studies. In *Gyrodactylus* populations, the COI gene is often used as a universal barcode for species identification. For example, many strains of G. salaris and G. thymalli have been reported in Norway (HANSEN et al. 2003).

Gyrodactylus is highly variable, as demonstrated by the COI mtDNA sequences (HANSEN et al. 2007). To identify the presence of *Gyrodactylus* parasites in commercial and wild fish species, the mitochondrial gene COI has been considerably used, for example (BUENO-SILVA & BOEGER 2014, GILMORE et al. 2010, KUUSELA et al. 2008, ONDRAČKOVÁ et al. 2020, PINACHO-PINACHO et al. 2021, PLAISANCE et al. 2007).

The test in this study was based on simple PCR, not qPCR. However, real-time PCR was highly efficient and accurate for detecting a parasite within its host. However, in relation to their use in the detection of diseases in fish, the costs of these methods can be high; on the other hand, standard PCR uses inexpensive reagents and equipment.

Generally, monogenous present a selective pattern of spatial distribution in the host's body, when most species are found only in fish gills and may have a preference for specific gill arches or even specific sectors of those arches (BAGGE & VALTONEN 1999). The selective distribution of monogenous in the body of their hosts is associated with various environmental and biological parameters (PIE et al. 2006). Several studies have investigated the distribution of monogenetic microhabitats in the gills of their hosts (IANNACONE &

ALVARIÑO 2012, KADLEC et al. 2003, KUMAR et al. 2017, RUBIO-GODOY 2008, SIMKOVÁ et al. 2000).

There was a significant difference in the average intensity of infection between the left and right gills when the right side had the highest number of parasites reported. This also agrees with previous observations of two monogenetic, which found that *Dactylogyrus amphibothrium has a* right-sided preference over the ruffle gill apparatus (WOOTTEN 1974). Microcotyle mugilis also has a definite preference on the left in the gills of striped mullets (EL HAFIDI et al. 1998).

This result is contrary to that reported by MASHALY and others. (2019), who found no preference observed by *Gyrodactylus rysavyi* between the right and left sets of gills of the Nile catfish Clarias gariepinus. The authors' findings suggest that host fish are symmetrical and receive equal amounts of water on both sides of their bodies.

According to CHAPMAN et al. (2000), the monogenetic gill *Neodiplozoon polycotyleus from the African cyprinid fish Barbus neumayeri can use* the second branchial arch to position itself in a region with greater laminar flow in the gill. This may indicate that some monogenetic species are rheophilic, meaning that they prefer lotic streams to lentic streams. The distribution of *G.* mutability over the branchial arches of the hosts revealed a distinct preference for the right side, as well as a clear predilection for the second branchial arches, according to this monogenean. This finding is consistent with that of MASHALY et al. (2019), who reported that the second branchial arch was the most favorable attachment site for *G.* rysavyi. Differences in water circulation between different sections of the gill surface are believed to play a role in the distribution of parasites in the gills (WOOTTEN 1974). The most powerful water flow runs through the middle of the branchial arches, providing ideal conditions for establishing the parasite. The amount of water that passes through the gills may alter the aerobic conditions in some sections of the gills, thereby facilitating the attachment of the parasite and reflecting the larger surface area accessible for attachment of the parasite to these gills (WOOTTEN 1974).

The main factors that promote monogenetic invasion of the gills are morphology, particularly the degree of differentiation of the gill apparatus, as well as the fine-scale histological structure of the gills (IZJUMOVA 1956).

In the present study, gill mucus DNA was used as a non-lethal method for detecting specific common carp parasites. This unique method is not invasive to the host and can identify parasitic DNA in infected gill mucus, which may facilitate the use of tests to avoid financial losses on carp farms. To assess *C.* carpio stock in fish farming, low-cost tests and sensitive and rapid non-lethal techniques are urgently needed. In addition, frequent and rapid screening of fish farms can prevent the spread of pathogens, thus preventing economic losses.

CONCLUSION

The monogenean *Gyrodactylus mutabilitas* was first recorded in common carp gills in Iraq. DNA from gill mucus can be used as a non-lethal method to detect parasitic infections in fish. This non-lethal technique is urgently needed because it represents a frequent and rapid screening of fish farms and can help prevent the spread of pathogens, thus avoiding economic losses.

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REFERENCES

ALI M & SHAABAN F. 1984 Some species of parasites of freshwater fish raised in ponds and in Tigris-Al-Tharthar canal region. Seventh Sci. Paper presented at the Conf. Iraqi Vet. Med. Assoc., Mosul.

BAGGE A & VALTONEN E. 1999. Development of monogenean communities on the gills of roach fry (*Rutilus rutilus*). Parasitology 118: 479-487.

BAKER JR et al. 1998. Advances in parasitology: Academic Press.

BAKKE TA et al. 2007. The biology of gyrodactylid monogeneans: the "Russian-doll killers". Advances in parasitology, 64: 161-460.

BARZEGAR M et al. 2018. *Gyrodactylus* (Monogenea, Gyrodactylidae) parasite fauna of fishes in some rivers of the southern Caspian Sea basin in Mazandaran province. Iran J Vet Med, 12: 35-44.

BONDAD-REANTASO MG et al. 2005. Disease and health management in Asian aquaculture. Veterinary parasitology 132: 249-272.

BUCHMANN K. 2012. *Gyrodactylus salaris* and *Gyrodactylus derjavinoides*. In Fish parasites: pathobiology and protection. Wallingford: CABI. p. 193-208.

BUENO-SILVA M & BOEGER WA. 2014. Neotropical Monogenoidea. 58. Three new species of *Gyrodactylus* (Gyrodactylidae) from *Scleromystax* spp. (Callichthyidae) and the proposal of COII gene as an additional fragment for barcoding gyrodactylids. Folia Parasitol 61: 213-222.

BYKHOVSKAYA-PAVLOVSKAYA I et al. 1962. Key to parasites of freshwater fish of the USSR Akad. Nauk, SSSR, Moscow: 727pp.

CHAPMAN LJ et al. 2000. Ecology of a diplozoon parasite on the gills of the African cyprinid *Barbus neumayeri*. African Journal of Ecology 38: 312-320.

CRIBB TH & BRAY RA. 2010. Gut wash, body soak, blender and heat-fixation: approaches to the effective collection, fixation and preservation of trematodes of fishes. Systematic parasitology 76: 1-7.

DA CUNHA FP et al. 2020. Non-lethal molecular diagnostic for acanthocephalosis in *Colossoma macropomum*. Aquaculture 519: 734860.

DE NOIA M et al. 2022. Towards an in situ non-lethal rapid test to accurately detect the presence of the nematode parasite, *Anguillicoloides crassus*, in European eel, *Anguilla anguilla*. Parasitology 149: 605-611.

DRENNAN J et al. 2007. Evaluation of lethal and non‐lethal sampling methods for the detection of white sturgeon iridovirus infection in white sturgeon, *Acipenser transmontanus* (Richardson). Journal of fish diseases 30: 367-379.

DUVAL E et al. 2021. Urine DNA (uDNA) as a non‐lethal method for endoparasite biomonitoring: Development and validation. Environmental DNA 3: 1035-1045.

EK-HUCHIM JP et al. 2012. Non-lethal detection of DNA from *Cichlidogyrus* spp. (Monogenea, Ancyrocephalinae) in gill mucus of the Nile tilapia *Oreochromis niloticus*. Diseases of aquatic organisms 98: 155-162.

EL HAFIDI F et al. 1998. Microhabitat distribution and coexistence of Microcotylidae (Monogenea) on the gills of the striped mullet *Mugil cephalus*: chance or competition? Parasitology Research 84: 315-320.

FERREIRA IA et al. 2019. Detection of Betanodavirus in experimentally infected European seabass (*Dicentrarchus labrax*, Linnaeus 1758) using non‐lethal sampling methods. Journal of fish diseases 42: 1097-1105.

FOLMER O et al. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294-299.

GILMORE S et al. 2010. The placement of *Gyrodactylus salmonis* (Yin & Sproston) in the molecular phylogeny of studied members of the *Gyrodactylus wageneri*‐group parasitizing salmonids. Journal of fish diseases 33: 461-467.

GUSSEV A. 1985. Parasitic metazoans: class Monogenea. In: BAUER ON. (Ed.) Key to the parasites of freshwater fish fauna of the USSR. Leningrad: Nauka.

HANSEN H et al. 2003. Mitochondrial DNA variation of *Gyrodactylus* spp. (Monogenea, Gyrodactylidae) populations infecting Atlantic salmon, grayling, and rainbow trout in Norway and Sweden. International Journal for Parasitology 33: 1471-1478.

HANSEN H et al. 2007. DNA taxonomy and barcoding of monogenean parasites: lessons from *Gyrodactylus*. Trends in parasitology 23: 363-367.

HENDERSON C et al. 2016. Assessing the suitability of a non-lethal biopsy punch for sampling fish muscle tissue. Fish physiology and biochemistry 42: 1521-1526.

IANNACONE J & ALVARIÑO L. 2012. Microecology of the monogenean *Mexicana* sp. on the gills of *Anisotremus scapularis* (Tschudi, 1846) (Osteichthyes, Haemulidae) of the marine coast of Lima, Peru. Neotropical Helminthology 6: 277-285.

IZJUMOVA N. 1956. Materials on the biology of *Dactylogyrus extensus*. Nybelin, Parazitologiya 16: 229-243.

KADLEC D et al. 2003. The microhabitat distribution of two *Dactylogyrus* species parasitizing the gills of the barbel, *Barbus barbus*. Journal of Helminthology 77: 317-325.

KRKOŠEK M et al. 2005. Nonlethal assessment of juvenile pink and chum salmon for parasitic sea lice infections and fish health. Transactions of the American Fisheries Society 134: 711-716.

KUMAR R et al. 2017. Spatial distribution of ectoparasites on the gills of the mullet, *Liza macrolepis*: the effects of pollution. Journal of Parasitic Diseases 41: 40-47.

KUUSELA J et al. 2008. Description of three new European cryptic species of *Gyrodactylus* Nordmann, 1832 supported by nuclear and mitochondrial phylogenetic characterization. Acta Parasitologica 53: 120-126.

MALMBERG G. 1970. The excretory systems and the marginal hooks as a basis for the systematics of *Gyrodactylus* (Trematoda, Monogenea). Arkiv for Zoologi 23: 1-235.

MASHALY MI et al. 2019. Microhabitat Selection of Ectoparasitic Monogenean Populations of the Nile Catfish, *Clarias gariepinus*. Jordan Journal of Biological Sciences 12: 573-580.

MIRGHAED AT et al. 2018. Parasite fauna of Chub, *Squalius turcicus* De Filippi, 1865 (Teleostei: Cyprinidae) from some rivers of the Southern Caspian Sea basin in Iran. Iranian Journal of Ichthyology 5: 109-117.

NORRIS L et al. 2020. Detection of the parasitic nematode, *Pseudocapillaria tomentosa*, in zebrafish tissues and environmental DNA in research aquaria. Journal of fish diseases 43: 1087-1095.

ONDRAČKOVÁ M et al. 2020. Morphometric and genetic evidence for cryptic diversity in *Gyrodactylus* (Monogenea) infecting non-native European populations of *Ameiurus nebulosus* and *A. melas*. Parasitology 147: 1700-1711.

PALADINI G et al. 2009. The first report of *Gyrodactylus salaris* Malmberg, 1957 (Platyhelminthes, Monogenea) on Italian cultured stocks of rainbow trout (*Oncorhynchus mykiss* Walbaum). Veterinary parasitology 165: 290-297.

PALADINI G et al. 2014. Reservoir hosts for *Gyrodactylus salaris* may play a more significant role in epidemics than previously thought. Parasites & vectors 7: 1-13.

PAOLETTI M et al. 2018. Species-specific Real Time-PCR primers/probe systems to identify fish parasites of the genera Anisakis, *Pseudoterranova* and *Hysterothylacium* (Nematoda: Ascaridoidea). Fisheries Research 202: 38-48.

PIE MR et al. 2006. Density-dependent topographical specialization in *Gyrodactylus anisopharynx* (Monogenoidea, Gyrodactylidae): Boosting transmission or evading competition? Journal of Parasitology 92: 459-463.

PINACHO-PINACHO CD et al. 2021. Species delimitation of *Gyrodactylus* (Monogenea: Gyrodactylidae) infecting the southernmost cyprinids (Actinopterygii: Cyprinidae) in the New World. Parasitology Research 120: 831-848.

PLAISANCE L et al. 2007. The complete mitochondrial DNA sequence of the monogenean *Gyrodactylus thymalli* (Platyhelminthes: Monogenea), a parasite of grayling (*Thymallus thymallus*). Molecular and Biochemical Parasitology, 154: 190-194.

RUBIO-GODOY M. 2008. Microhabitat selection of *Discocotyle sagittata* (Monogenea: Polyopisthocotylea) in farmed rainbow trout. Folia Parasitologica 55: 270-276.

SANDOVAL-GÍO JJ et al. 2008. Humoral antibody response of the tilapia *Oreochromis niloticus* against *Cichlidogyrus* spp. (Monogenea). Journal of Parasitology 94: 404-409.

SIMKOVÁ A et al. 2000. Co-existence of nine gill ectoparasites (Dactylogyrus: Monogenea) parasitising the roach (*Rutilus rutilus* L.): history and present ecology. International Journal for Parasitology 30: 1077-1088.

TAVARES GC et al. 2016. Nonlethal sampling methods for diagnosis of *Streptococcus agalactiae* infection in Nile tilapia, *Oreochromis niloticus* (L.). Aquaculture 454: 237-242.

TORCHIN ME & MITCHELL CE. 2004. Parasites, pathogens, and invasions by plants and animals. Frontiers in Ecology and the Environment 2: 183-190.

WOOTTEN R. 1974. The spatial distribution of *Dactylogyrus amphibothrium* on the gills of ruffe *Gymnocephalus cernua* and its relation to the relative amounts of water passing over the parts of the gills. Journal of Helminthology 48: 167-174.

XU P et al. 2014. Genome sequence and genetic diversity of the common carp, *Cyprinus carpio*. Nature genetics 46: 1212-1219.