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An Expansion of the Invasive Species *Gussevia asota* Kritsky, et al., 1989 (Monogenea: Dactylogyridae) In Western Uttar Pradesh, India

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Research Article

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Abstract

Non-native fish species and their parasites are a threat to aquatic ecosystems, posing a risk to inherited communities by diminishing biodiversity and causing severe commercial and public health impacts. Along with their fish hosts, monogenean parasites are often co-introduced into new areas. In the western part of the state of Uttar Pradesh, India, a range expansion for the non-native monogenean parasite was recorded. This study confirmed the co-introduction of the dactylogyrid, *Gussevia asota* Kritsky et al. (1989), into India with their fish host, the invasive Oscar, *Astronotus ocellatus* Agassiz, 1831. The present species were also distinguished based on molecular analysis of their 18S rDNA sequence and the haptoral parts and male copulatory organ. Phylogenetic analysis of *G. asota* showed that it clustered with other dactylogyrid species, supporting the contention that it co-introduced parasites, increasing the number of monogeneans acquired in Indian water.

Keywords: Non-Native Species; Fish; Molecular; India; Meerut

Introduction

Non-native host species introduce their non-native parasites into a new range of geographical locations that can help settle them into new habitats [1]. Several non-native parasite species are co-introduced into new regions with their original host species. These non-native parasites frequently persist as a part of the host's fauna and can extend their range as the host increases their range [2,3]. Sometimes a few co-introduced parasite species can switch to a new host species that is native to that particular region, though it spreads in the new environment [4-8]. The majority of non-indigenous fish hosts in India were imported for aquaculture and as ornamental fish, which can cause disease transmission and the co-introduction of new parasite species that might further increase the threats of unintended introduced non-native species for local fauna [1,9]. As important fish parasites, Monogeneans are common parasites that are very host-specific. Reports on monogenean parasites co-introduced into India include various monogenean

species, and most of them are host-specific and generally do not switch to other fish host species [10-17]. The genus *Gussevia* was proposed as an ancryocephaline species by Kohn, et al. [18]. *Gussevia* Kohn, et al. [18] comprises three monogenean species *Gussevia asota, Gussevia astronoti*, and *Gussevia rogersi* that were described by Kritsky, et al. [19] from *Astronotus ocellatus* collected from countries Brazil and the United States. In all of the above mentioned species, particularly *G. asota* was found an important pathogen that may well kill their host [19,20].

The main aim of the present study was to identify monogenean species *Gussevia asota* Kritsky, et al. [19] infecting invasive Oscar, *Astronotus ocellatus* Agassiz, 1831, in the Meerut region, a district of Uttar Pradesh, India, using ribosomal 18S DNA data and morphological analysis that make available to understand the phylogenetic relationships with other monogenean species.

Materials and Methods

Host-Parasite Collection and Morphological Analysis

Non-native Oscar *Astronotus ocellatus* was collected in the Meerut region, a district of Uttar Pradesh, India. In total, 38 Oscars were collected and transported alive in aerated boxes to the molecular taxonomy laboratory, department of Zoology, Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India, and kept in aerated aquariums. Prior to the collection of helminth parasites, the fish specimens were subjected to euthanasia utilizing clove oil within three days of capture. They were further dissected for monogenean parasites, but before dissection, every fish host was measured for standard length (SL).

Collected dactylogyrid monogenean parasites from gill filaments were mounted in glycerine-ammonium-picrate [21] for semi-permanent slide preparation for morphological and morphometric analyses, while for permanent slides, the protocol of Verma, et al. [22] was followed. Monogeneans mounted in Canada balsam are deposited in the Museum of the Department of Zoology, Chaudhary Charan Singh University, Uttar Pradesh, India, and the Museum d'Histoire naturelle, Geneva, Switzerland. Morphological observations were made using an Olympus CH30 (Shinjuku, Tokyo, Japan) fitted with the image analysis software Axio Cam ERc5s. Drawings of haptoral hard parts were made with the aid of a drawing attachment. The morphological identification was based on the shape and measurements of the sclerotized parts of the haptor and reproductive organs, according to Kritsky, et al. [19]. All the measurements were taken in micrometers as the mean ± standard deviation, followed by the range in parentheses unless otherwise stated. A subsample of the collected parasites was preserved in 95% ethanol for further molecular analysis. Prevalence (in %) was calculated according to Bush, et al. [23].

DNA Extraction, PCR Amplification and Sequencing

Parasites collected in the present study, placed in 95% ethanol for genomic DNA extraction, were used to extract the DNA using a DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany), following the method. The region of the 18S ribosomal DNA (rDNA) was amplified using the primers WormA+1270R and 930F+WormB [24]. The PCR reaction was performed at a final volume of 25 µL, according to Shinad, et al. [25], and the cycling conditions were as follows: initial denaturation for 2 min at 95 °C, 35 cycles of 55 s at 95 °C, 50 s at 55 °C, 1 min at 72 °C, and a final extension for 7 min at 72 °C. Obtained PCR products were electrophoresed on 1.5% agarose gels and then purified using the Purelink™ Quick Gel Extraction and PCR Purification Combo Kit (Invitrogen, Löhne, Germany), following the manufacturers protocol. The purified PCR products were then sequenced directly in both directions using the same primers as mentioned above using a Big Dye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Thermo Fisher Scientific, Foster City, California, USA). Generated DNA sequences were assembled and edited using the Geneious Pro 5.4 platform [26], and the newly generated monogenean sequences were deposited in GenBank for accession numbers.

Molecular phylogeny was analyzed using maximum likelihood (ML) and Bayesian inference (BI) methods. Sequences of other monogenean species for phylogenetic reconstruction were retrieved from GenBank. For the alignment of 18S gene sequences, MUSCLE algorithm implemented in Molecular Evolutionary Genetics Analysis (MEGA) version 11 [27] was used with default parameters. Model Finder [28] was used to infer the ideal evolutionary model for the 18S gene using the Bayesian information criterion. The GTR + I + G evolutionary model were selected in the monogenean phylogeny of the current species. To conduct ML analysis, the program MEGA version 11 was used with nodal support assessed by 1,000 bootstrapping replicates. The BI phylogenetic analysis was performed with TOPALi version 2.5 software [29], using four simultaneous chains of the Markov Chain Monte Carlo (MCMC) algorithm run twice for 1,000,000 generations. For every 100 generations, the tree topologies were sampled, whereby the first 25% of trees from each run were discarded as burn-in. Finally, the genetic divergence was calculated using uncorrected p-distances for the 18S gene in MEGA version 11. The species of *Scutogyrus* and *Cichlidogyrus* was used as outgroup.

Results

Family Dactylogyridae Bychowski, 1933
Genus *Gussevia* Kohn and Paperna, 1964
***Gussevia asota* Kritsky, Thatcher and Boeger, 1989**

Description (n= 27) (Figure 1: *Gussevia asota* microphotograph). Body-elongated, fusiform, moderate-sized worms with a narrow cephalic region; maximum width attained at the level of gonads. Cephalic lobes poorly developed, having two terminals and two bilateral. Two pairs of eyes, equidistant; the posterior pair larger than anterior pair. Ovate shaped pharynx present. Well-developed haptor present with anterior and posterior lobes having small glandular patches. Dorsal anchor, variable in length with superficial root, shaft curved with elongate point. Dorsal bar rod-shaped have slightly expanded ends. Ventral anchor comprises poorly differentiated roots, short anterior projection arising from tip of superficial root, shaft short with acute point. Filamentous ventral bar has ends curling around ventral anchor base. Hook pairs 1, 2, 3, 4, 6, and 7 similar with a slender shank; hook pair 5 modified, usually elongate. Eggs spherical in shape and smooth outline. Cirrus comprises a base from which a double-walled, coiled tube arises, having a coil of about 1.5 rings. Accessory piece basally articulated with cirrus having terminal flabellate piece possessing sclerotized ring. Vagina not observed. Vitellaria dense, dispersed along through intestinal caeca.

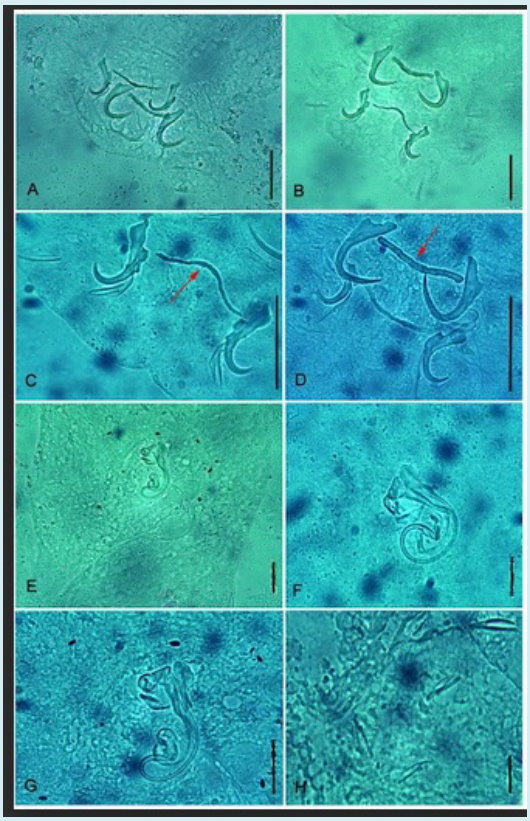

Figure 1: Micrographs of *Gussevia asota* Kritsky et al. (1989) from *Astronotus ocellatus*. (A) and (B) Haptoral armature; (C) An enlarged view of the ventral anchors and ventral bar (VB), VB indicated by red arrow; (D) An enlarged view of the dorsal anchors and dorsal bar (DB), DB indicated by red arrow; (E) Male copulatory organ; (F) and (G) Two different enlarged views of male copulatory organ; (H) Marginal hooks. Scale bars: 35 µm (A, D), 32 µm (B), 30 µm (C) 23 µm (E) 12 µm (F) 17 µm (G) 11 µm (H).

Taxonomic Summary

Host: Oscar, *Astronotus ocellatus* Agassiz, 1831 (Cichliformes: Cichlidae).
Locality: Meerut (29˚01′N and 77˚45′E), Uttar Pradesh, India.
Site of infection: Gill filaments.
Prevalence: 71.05% (27 host infected out of 38); a range of >50 to 200 parasites per fish.
Material deposited: Voucher specimens were submitted to the collection of the Museum, Department of Zoology, Chaudhary Charan Singh University, Meerut (U.P.), India

(HS/mon/2022/13) and to the Museum d'Histoire Naturelle, Geneva, Switzerland (MHNG-PLAT-0144330).
Molecular sequence data: For 18S gene, we have generated sequences from two isolates of *Gussevia asota*: PP352272 (1787 bp), PP352273 (1695 bp).

Remarks: This is the second record of *G. asota* from India based on morphological and molecular data. It is characterized primarily by having a dextral vagina, cirrus with a long spiral tube, and an accessory piece attached to the distal end of the cirrus. *Gussevia asota* was originally described from the gill filaments of *Astronotus ocellatus* from Brazil. This parasite was also reported from various geographical regions: East Asia [30]; Europe [20]; North America [19]; South America [19,31-33]. From the Indian region, this parasite was reported by Tripathi, et al. [17] and exhibited some more features, like the presence of a vaginal pore that is present in the dextro-marginal, funnel-shaped, and large median seminal receptacle (Table 1).

Study of the present specimen showed that this is conspecific to previous descriptions of *G. asota* [17,19, 31]. In our study, this parasite was morphologically compared with isolates of other geographical regions. Among them, it shows maximum similarity (in respect of body length) with isolates from Peru [33] (Table 1). The present species comprises features that are a little bit different from the original description in having: large worms, well-developed anterior and posterior lobes in the haptoral region, and small glandular patches, as also shown in numerous studies [17,20,31] (Table 1).

Body parts	*G. asota* Kritsky et al. (1989) Brazil	*G. asota* Mendoza-Franco et al. (2010) Peru	*G. asota* Smigh et al. (2016) Slovakia	*G. asota* Tripathi and Matey (2023) India	*G. asota* Present study India
Body:					
Body length	394(380-462)	630	544(540-564)	-	634.46±19.93 (606.67-666.67)
Body width	88(73-111)	103	92(81-121)	-	146.18±14.74 (126.47-166.62)
Pharynx diameter	25(21-30)	23-29	-	-	30.59±2.45 (26.67-33.33)
Haptor length	42(34-54)	-	90-97	-	123.73±7.25 (113.75-130.76)
Haptor width	66(61-78)	160	105(102-111)	-	145.65±3.90 (140.33-150)
Dorsal anchor:					
Length	27(24-30)	26(25-27)	27(24-28)	27 (27-28)	28.27±2.23 (25-31.33)
Base width	12(10-13)	12(10-13)	7(6-8)	-	6.86±0.88 (5.33-8)
Dorsal bar:					
Length	31(23-36)	33(31-38)	23-36	32 (31-36)	32.5±1.57 (31-35)
Width	-	-	-	-	2.99±0.62 (2.33-4)
Ventral anchor:					
Length	26(25-28)	25(24-27)	26(24-29)	24 (23-26)	26.16±1.30 (24-27.33)
Base width	14(12-16)	18(17-19)	7(5-8)	-	5.64±0.99 (4.33-7)
Ventral bar					
Length	31(24-35)	32(30-33)	26-35	31 (26-36)	33.66±3.31(29-36.33)
Width	-	-	-	-	1.55±0.41 (1-2)
Female reproductive organs:					
Ovary length					
Ovary width	109(85-132)	-	-	-	142.30±6.90 (135.89-153.84)
Egg length	45(36-57)	-	-	-	43.07±4.41 (35.89-48.71)

Egg width	-	-	-	-	70.91±2.08 (68.75-74.16)
	-	-	-	-	63.24±1.90 (60.83-66.25)
Male reproductive organs:					
Testis length	90(71-108)	-	-	-	101.11 ±11.38 (82.5-115.38)
Testis width	40(33-51)	-	-	-	41.66±4.92 (35.89-48.71)
Cirrus length	61-62	30(28-35)	54-73	57 (56-62)	56.91±5.05 (52.67-65.33)
Proximal ring diameter	14(10-16)	-	-	-	13.67±1.44 (11-15.33)
Accessory piece length	27(23-29)	29(26-31)	-	22 (24-40)	31.58±3.70 (27-36.67)
Hooks length:					
1,2,3,4,6,7th pair	11(10-12)	11(10-12)	-	11 (11-12)	11.55±0.98 (10.16-13)
5th pair	14(13-15)	15(14-15)	-	14 (13-15)	14.38±0.47 (13.83-15)

Table 1: A comparison of the measurements of *Gussevia asota* Kritsky, et al. (1989) parasitizing *Astronotus ocellatus*.

Phylogenetic Analysis

The final alignment of the 18S rRNA gene was used for phylogenetic reconstruction, and the species of the genera *Scutogyrus* and *Cichlidogyrus* were used as an-outgroup. Identical sequences of 18S rDNA were obtained from two specimens in this study. For alignment of the 18S dataset, selected monogenean parasite data from GenBank was retrieved. The topologies generated using the ML and BI methods were similar; therefore, ML reconstruction with bootstrap support and posterior probabilities outcomes from both analyses were shown along their nodes (Figure 2). The 18S rDNA phylogeny using both ML and BI demonstrated that the sequences generated in the present study and sequences for other isolates of the same species from the same host, *A. ocellatus*, Peru, form a well-supported group (Figure 2). A sister clade to the second grouping, comprised of *G. astronoti* from the same host *A. ocellatus*, Peru was also shown. The intraspecific range for isolates of *G. asota* was 0.07%, while the interspecific range of isolates of *G. asota* with *G. astronoti* was 0.30%.

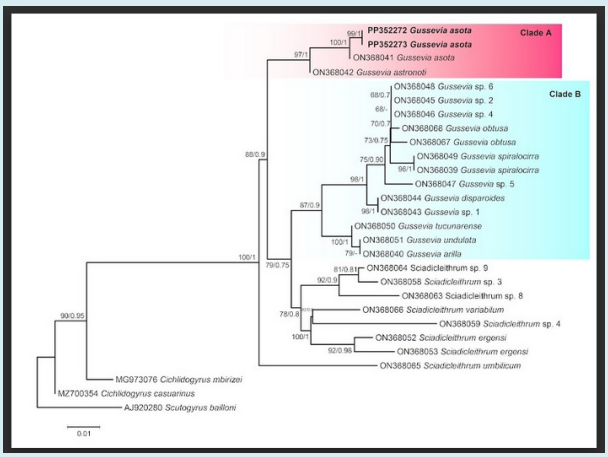

Figure 2: Maximum likelihood phylogenetic tree of *Gussevia asota* parasitizing cichlid fish based on 18S rRNA sequences. Values alongside branches designate bootstrap values and posterior probabilities resulting from maximum likelihood (ML) and Bayesian inference (BI) analyses. A and B letters symbolized the well-supported clades. Values below 0.70 (BI) are shown as dashes. The GenBank accession numbers of the species used in the phylogenetic analysis are also shown. The branch-length scale bar indicates the number of substitutions per site.

The phylogenetic reconstruction for *Gussevia* species in the present study revealed two well-supported clades parasitizing Neotropic cichlids. *Gussevia asota* and *G. astronoti* parasitizing cichlid *A. ocellatus* from Peru belongs to South America, formed clade A, while *Gussevia* species that also parasitizing cichlids of South American countries, Peru and Venezeula, were nested together in clade B (Figure 2). Representatives of the genus *Gussevia* show a polyphyletic assemblage in the present analysis, whereas *G. asota* and *G. astronoti,* with Indian isolates of *G. asota* (clade A) and other *Gussevia* species of South American cichlids (clade B), formed a well-supported monophyletic group.

Discussion

The present study reports the presence of an neotropical cichlid, *Astronotus ocellatus,* in Meerut, western Uttar Pradesh, India*.* This cichlid, *A. ocellatus was* originally found in the Amazon basin, in the countries of Peru, Colombia, and Brazil [34,35]. This species has growing economic interest as an ornamental fish and is abundantly consumed for its delicious meat [35-37]. Insufficient controls of fish species imports and exports at quarantine places enable the spreading of novel and undocumented pathogen or parasite species [30].

In spite of the great economic value of *A. ocellatus*, studies appraising monogenean infection in Indian water for non-native fish parasites are still scarce. This is the first report of high infection by *G. asota* in *A. ocellatus* (found >50 to 200 parasites per fish) in western Uttar Pradesh, India, and it establishes that this fish pathogen is spreading in the global ornamental fish trade. In the present study, for the first time, we have amplified the 18S ribosomal RNA gene for molecular characterization and to supplement the morphology of the parasite. The occurrence of *G. asota* was confirmed in this study, i.e., in the western UP district of Meerut, and in a study by Tripathi, et al. [17] in the northern UP district Lucknow, in New Delhi, and in the eastern part, i.e., Kolkata, India. These reports show the occurrence of this parasite species, which agrees with our results, indicate high prevalence in both studies, and show the potential and probability of this parasite species co-introduction into new areas. According to a study on the Neotropical cichlid monogeneans by Seidlová, et al. [38], we also have similar observations. Our phylogenetic analysis indicated the inclusion of *G. asota* Indian isolates clustered with Peru isolates of the same species and another species, *G. astronoti* parasitizing cichlid host (Figure 2). Seidlová, et al. [38] have suggested a monophyletic origin for the monogenean parasites of Neotropical cichlids which is also consistent with our results. Furthermore, Seidlová, et al. [38] proposed *Gussevia* as monophyletic group with which the current results shows not in agreement and indicating the requirement for the taxonomic revision of *Gussevia*.

India has obtained less responsiveness for investigations regarding parasite co-introduction by exotic fishes as compare to other geographical regions [10-17]. Although the National Fisheries Development Board (NFDB), Ministry of Fisheries, the Government of India implemented guidelines to import ornamental fishes in India and to control the entry of exotic ornamental fish along with associated parasites [39]. As per the conventions of the National Fisheries Development Board, Government of India [39], Oscar is one of the 92 commercially significant freshwater ornamental fish that are authorized to be imported in India. The Oscars have the capability to survive and reproduce in non-native environmental tolerances. For example, hypoxia, the ability to colonize interrupted habitats, generalist feeding behaviors, and reproductive potential in association with fast growth [40-43]. Moreover, to reduce the risk of the introduction of non-native parasites and their associated diseases, quarantining is one of the essential biosecurity measures. In the course of this period, imported fish are detained in a quarantine facility in which they can be monitored, made a diagnosis, and treated successfully beforehand before being discharged to a farm [44]. According to the International Union for Conservation of Nature (IUCN), *A. ocellatus* is listed as a species of "least concern." It is widely distributed across South America, including the western Amazon and Orinoco basins, but has been translocations throughout elsewhere under the ornamental fish trade [45], and as a result, it is now present in the various geographical localities, which also shows the tight host specificity of *G. asota*.

Conclusion

In conclusion, our study confirmed the presence of the monogenean fauna of Neotropical cichlid *A. ocellatus* in India. Morphology, morphometry, and molecular data generated subsidize the existing literature about the invasive monogenean parasite *G. asota*. Additionally, the generated taxonomic evidence for this parasite may be employed in future studies to track the cause and range of its invasive hosts in the Indian freshwater system and worldwide.

Conflicts of Interest

The authors declare no competing interests.

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