addition, the increase in soil temperature will be faster than air temperature with the degradation of *L. chinensis*. This needs further attention, as it may change the adaptive strategies of grassland plants due to the asymmetric warming between aboveground and underground.

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ACKNOWLEDGEMENT. This research was supported by the National Natural Science Foundation of China (grant no. 41330640).

Received 26 February 2015; accepted 24 March 2015

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Monogenoidea on exotic Indian freshwater fish. 3. Are Indian guidelines for importation of exotic aquarium fish useful and can they be implemented; The case of Neotropical *Gussevia spiralocirra* Kohn and Paperna, 1964

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Gussevia spiralocirra, a Neotropical parasitic monogenoid (Platyhelminthes), has been recorded from the type host, freshwater angelfish *Pterophyllum scalare* (Cichlidae), collected from the post-quarantine populations in local aquarium markets of Lucknow and Itanagar, India. The finding establishes India as a geographical distribution record for *G. spiralocirra*, and more importantly, reveals a potentially serious breach of quarantine regulations of the country. The present communication provides a summary assessment of existing Indian guidelines for importation of exotic aquarium fish and highlights some of its major shortcomings.

**Keywords:** *Gussevia spiralocirra*, exotic aquarium fish, *Pterophyllum scalare*, quarantine regulations.

AQUARIUM trade is a potential pathway for the global translocation of exotic aquarium fish<sup>1,2</sup> and their parasites<sup>3,4</sup>, especially the monogenoids (Platyhelminthes)<sup>5</sup>. Should these fish escape from the culture facilities and establish self-sustaining populations in the wild waters of an importing country<sup>6,7</sup>, they can pose a serious threat to the native aquatic biodiversity and economy<sup>8,9</sup>. Not surprisingly, the aquarium species, dominated by freshwater fish, comprise one-third of the world's 100 worst aquatic invasive species<sup>10</sup>.

The freshwater angelfish *Pterophyllum scalare* (Schultze, 1823), which originates from the river basins of tropical South America, is one of the most treasured of all the aquarium fish. Kohn and Paperna<sup>11</sup> established the monogenoidean genus *Gussevia* and designated *G. spiralocirra* from the gills of *P. scalare* 'raised in aquariums in various places in Israel' as its type species. Kritsky *et al.*<sup>12</sup> emended the generic diagnosis of *Gussevia* and restricted the genus to member species parasitizing the gills of Neotropical cichlid fish. Further, these authors redescribed *G. spiralocirra* based on specimens collected from the type host, but in a new locality in Peru, South America. The species has not been recorded since then.

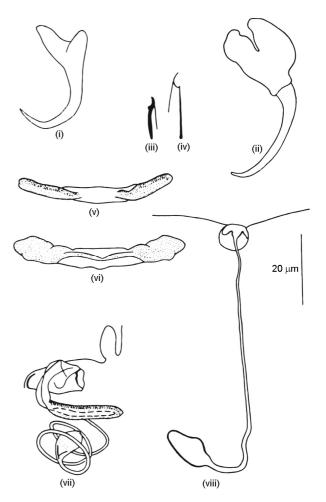
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This article, third in a series to document the parasitic monogenoids on exotic Indian freshwater fish<sup>13,14</sup>, aimed to establish if the angelfish carried exotic *G. spiralocirra* into India, and if so, to study the major shortcomings of existing guidelines and practice for importation of exotic aquarium fish into India.

During 2008–2013, a total of 55 live specimens of angelfish were randomly collected from their postquarantine populations available in aquarium markets of Lucknow in northern India (lat.  $26^{\circ}50'$ N; long.  $80^{\circ}56'$ E) and Itanagar in North East India (lat.  $27^{\circ}6'$ N; long.  $93^{\circ}37'$ E). Fish were killed by brain spiking soon after collection and their gills were preserved in 4% mild hot formalin solution until parasitological examination. The worms isolated were stained, mounted and illustrated as described by Kritsky *et al.*<sup>12</sup>. Voucher specimens were deposited in the US National Parasite Collection in Belts-ville, Maryland (100507.00). The host name follows that provided by FishBase<sup>15</sup>.

The morphology of present specimens was consistent with the diagnostic features of Gussevia characterized by overlapping gonads, haptor with anterior and posterior lobes, modified ventral anchors, modified hook pair 5, copulatory tube with clockwise rings and a distally ornate accessory piece (Figure 1). Comparison of the present specimens with the voucher specimens of G. spiralocirra, studied by Kritsky et al.<sup>12</sup>, and borrowed from the US National Parasite Collection (USNPC78778; four slides), further confirmed their conspecificity and the occurrence of G. spiralocirra in India. Based on the comparative morphology of copulatory complex and dorsal and ventral bars, G. spiralocirra is found to be similar to and can be confused with the native species Chandacleidus recurvatus (Jain, 1961), Agrawal et al. 2006 from Chanda nama (Hamilton, 1822). These species are easily distinguished, however, by the number of rings in the couplatory tube (3-4 rings in G. spiralocirra; one ring in C. recurvatus), the position of the vagina (sinistral in G. spiralocirra; dextral in C. recurvatus), and the presence of modified hook pair 5 in G. spiralocirra (absent in C. recurvatus). Though very little is known about the pathological implications of G. spiralocirra, many congeneric species are known to have caused mortality, for example, of the aquarium fish in Pocatello, USA<sup>16</sup> and cultured fish in the Peruvian Amazon<sup>17</sup>. Indeed, all monogenoids are potential pathogens to their hosts reared in captivity, such as fish farms, aquaria or ponds<sup>18</sup>, which means G. spiralocirra is of concern and interest to numerous Indian stakeholders. The present finding, after 28 years, of G. spiralocirra constitutes India as a geographical distribution record for the species and also indicates a serious breach of quarantine regulations of the country. Since the ease with which a parasite species can establish and colonize a new locality is inversely related to the complexity of its life cycle<sup>19</sup>, G. spiralocirra, with its direct life cycle (i.e. intermediate hosts are absent) is more likely to become established in India.

Since its independence in 1947, India has accorded a relatively low national priority to its aquatic animal health. Indeed, the fact that the country enacted limited number of overt legislations on fisheries and aquaculture emphasizes this point. Mention may be made, for example, of the Indian Fisheries Act (1897), Environment (Protection) Act (1986), Water (Prevention and Control of Pollution) Act (1974), and the Wild Life Protection Act (1972). And, all of them, except a century-old Indian Fisheries Act (1897) have been the umbrella Acts containing certain provisions for fishery-related issues. In particular, there has been no legislation to regulate the translocation of invasive aquatic species and concomitant ingress of diseases. India has thus long suffered from outbreaks of exotic aquatic diseases. A good example is the exotic white spot syndrome virus (WSSV), which had been introduced into India in the 1990s, probably with the importation of broodstock of Penaeus japonicas (Bate, 1888) and P. chinensis (Osbeck, 1765) from China. The virus cost the Indian shrimp industry millions of dollars



**Figure 1.** Sclerotised haptoral and copulatory structures of *Gussevia* spiralocirra Kohn and Paperna, 1964 from *Pterophyllum scalare*. (i) Dorsal anchor, (ii) Ventral anchor, (iii) Hook pairs 1–4, 6 and 7, (iv) Hook pair 5, (v) Dorsal bar, (vi) Ventral bar, (vii) Copulatory complex (dorsal view) and (viii) Vagina.

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in just one decade<sup>20</sup>. Similarly, the epizootic ulcerative syndrome (EUS), which is caused by *Aphanomyces invadans* David and Kirk, 1997, an exotic fungus of freshwater and estuarine fish, escaped into India in the 1990s from neighbouring countries, possibly Bangladesh, through the common river system, and caused an estimated loss of US\$ 42.5 million during 1992–1995 (ref. 21).

India is a contracting member of a number of important international conventions and organizations, such as the World Organization for Animal Health (OIE), Convention on Biological Diversity (CBD), and Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES), which resolve to prevent the introduction of invasive alien species to conserve biological diversity. In pursuit of its international agreements, the Ministry of Agriculture, Government of India (GoI) enacted the 'Guidelines for the import of ornamental fishes into India' for effective control and management of ornamental fish and associated disease introduction into the country<sup>22</sup>. The guidelines basically adopt a twopronged policy: (1) formulating an 'indicative list' of 97 individual species agreed for import, and (2) imposing import procedures and requirements (pre-quarantine, quarantine and post-quarantine). An 'indicative list' here is the same as 'permitted list' or 'white list' approach, which bans the import of all species unless they are on a permitted list<sup>23</sup>. Under the pre-quarantine actions, the import of ornamental fish is not allowed unless accompanied by a valid import permit issued by GoI. The guidelines also demand a pre-quarantine certificate from the competent authorities of exporting countries of the consignments. Under the quarantine actions, the imported species of fish are subjected to a mandatory quarantine protocol for 15 days (21 days for goldfish) in a guarantine facility. Under the post-quarantine follow-up, the guidelines make it an offence to release, or to allow escaping fish into the wild; in addition, the guidelines prohibit the direct sale of imported brood stocks in the domestic market, but only the F1 and F2 progeny.

A closer look at the guidelines makes it clear that the only restriction, which any of the species included in the 'indicative list' needs to face, is the quarantine protocol. However, the effectiveness of even this sole restriction is questionable, since, the angelfish, which is a vector of G. spiralocirra reported in this communication, is included in the 'indicative list' and as such must have passed the quarantine protocols. An important moot point involved here is whether the invasion of G. spiralocirra is due to poor framing or poor enforcement of the guidelines. While a detailed examination of this question is beyond the extent of this communication, it will be sufficient here to mention that no details of risk analysis and quarantine are given in the guidelines. For instance, it is not known whether or not the guidelines have a separate risk analysis for fish as pets and for the parasite which they

may have carried. Similarly, it is not clear whether or not the quarantine facility mentioned in the guidelines is a highly specialized diagnostic laboratory employing a wide range of fish health professionals, including particularly the taxonomists. The recommendations of guidelines regarding the undertaking of risk analysis and quarantine are also confusing and even contradictory. Thus, the guidelines endorse, 'every species of ornamental fish imported into the country shall have to be subjected to the quarantine procedures'. However, risk analysis shall be carried out 'if the request for import of a particular species is sought for the first time'. These two statements when read together promote the idea that the quarantine is uniform, and risk analysis is selective, when, in fact, the opposite is the case. The objective of risk analysis is to identify the potential risks associated with the trade and to recommend the required regulatory measures, including quarantine. It is important to realize that the quarantine without risk analysis may be useful in reducing risk for certain diseases, but useless for others. If it is assumed that no risk analysis was undertaken for P. sca*lare*, as it appears to be the case, it is quite possible that G. spiralocirra succeeded in invading India because it was never identified as a potential risk and therefore, did not receive applicable quarantine treatment.

Further, the guidelines do not focus on exotic species at the ecosystem level, but at the geopolitical level, and therefore, do not consider risk analysis for the domestic movement, i.e. movement of ornamental fish within the country. In the strict ecological sense, it does not cause a change, whether a species is moved from one river basin to another within the country (transplanted) or across national borders (introduced/translocated), because both could generate similar ecologically disastrous outcomes<sup>24</sup>. This is even more true for a country like India, which has as many as 20 geographically distinct drainage basins<sup>25</sup>. The 'indicative list' should therefore include not only the species exotic to India, but also the native species which are introduced outside their natural range within India. This is important because the Indian domestic market comprises both exotic and native species of ornamental fish, though it favours the exotic species more than the native ones $^{26}$ . It is worth pointing out here that some countries, including England and Wales, France and Spain have already enacted legislations to control domestic transfer of fish. In England and Wales, for example, Section 30 of the Salmon and Freshwater Fisheries Act 1975 (SAFFA) makes it illegal to transfer both native and non-native fish within their political boundaries without a written consent.

More complications transpire when one investigates the criteria behind the preparation of the 'indicative list'. Strangely, the list includes a large number of taxa that are either invasive or potentially invasive. Thus, as many as 6 of 97 species on the list have already established their wild populations in Indian waters<sup>5</sup>. In addition, at least 23 species on the list have established themselves in many other parts of the world<sup>15</sup>, suggesting a potential to also become invasive in Indian waters. Inclusion of fishes, for instance, the goldfish Carassius auratus (Linnaeus, 1758), koi carp Cyprinus carpio (Linnaeus, 1758) and even freshwater angelfish, which are recognized pests and/or hosts of pathogens not present in India, in the 'indicative list' is another unusual consideration. While the goldfish is a potential pest and has caused adverse ecological impacts in several countries following its introduction<sup>15</sup>, the koi carp is included in 100 of the world's worst invasive alien species database managed by the IUCN<sup>27</sup>. Furthermore, goldfish, koi carp, and angelfish are known to harbour a diversity of viruses, bacteria, and protozoan and metazoan parasites<sup>28-31</sup>. Their inclusion in the 'indicative list', thus, repudiates the very basis of risk analysis, which allows only those exotic species that have been proven safe or at least of low risk. It can always be argued, obviously, that there are little points in maintaining a quarantine barrier to a species of fish (and its parasite fauna) which is already endemic to the country. Nevertheless, while minimizing the further spread of these pests/diseases is important, it is also necessary to minimize additional entry of these agents. Note that the 'permitted list' of ornamental fish of New Zealand, for instance, strictly excludes the importations of both goldfish and koi carp<sup>28</sup>.

The absence of an effective surveillance and reporting system, and/or a systematic impact mitigation strategy is another downside of the Indian guidelines. That this communication, since the implementation of the guidelines, is apparently the only report of disease diagnosis from a post-quarantine population of ornamental fish in India is a good indication of the lack of surveillance and reporting of ornamental fish pathogens entering India. While the guidelines make it an offence to release, or to allow escaping exotic ornamental fish into the wild, there is neither an explicit requirement for their control nor a contingency plan for actions to be taken. The only documented impact mitigation effort reads, 'In case, the consignment does not pass quarantine, the entire consignment shall be destroyed at importers cost as per the prescribed protocols'. The guidelines also do not make any specific recommendation(s) in relation to the movement and sale of those species of exotic ornamental fish which have already established their wild populations in India, or those which were introduced into India before the implementation of the current guidelines. According to Ghosh et al.<sup>32</sup>, for example, India has more than 200 domestically bred exotic species of ornamental fish, which is clearly far more than those included in the 'indicative list'.

Last but not the least, the guidelines set little restrictions in the practices, allowing the continued illegal introduction of exotic species of aquarium fish and their parasites into India. For example, the striped catfish Pangasianodon hypophthalmus (Sauvage, 1878) is not included in the 'indicative list' and yet is freely available in the aquarium markets of the country along with two potentially pathogenic exotic parasitic monogenoids<sup>14</sup> – *Thaparocleidus caecus* (Mizelle & Kritsky, 1969) Lim, 1996 and *T. siamensis* (Lim, 1990) Lim, 1996. What is even worse is that such illegally imported fish are easily available for purchase over the Internet from the domestic breeders and dealers, which will only further facilitate the inter-state transfer of exotic fish species and their parasites (see above).

In conclusion, the occurrence of G. spiralocirra from the post-quarantine populations of freshwater angelfish in India demonstrates considerable inadequacies in the structure and enforcement of the current Indian guidelines for import of exotic aquarium fish and their parasites, thus providing a case for revision. India is particularly vulnerable to additional invasions of imported ornamental fish and their parasites<sup>5</sup> and, therefore, the country needs to adopt a conservative, if not 'zero risk', policy to help protect its aquacultural industries and environment. An important step to realize this goal should be, of course, besides strengthening and implementing the standing guidelines effectively, establishing a dedicated 'Central Institute of Invasive Species Management' as a multidisciplinary research, teaching and extension unit.

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ACKNOWLEDGEMENTS. I thank Eric P. Hoberg and Patricia Pilitt (US National Parasite Collection, Beltsville, MD20705, USA) for loaning the voucher specimens; University Grant Commission, [39-603/2010 (SR)] and the Department of Science and Technology, Government of India [SR/SO/AS-56/2011] for providing financial assistance, and the two anonymous reviewers for their useful comments that helped improve the manuscript.

Received 19 May 2014; revised accepted 13 March 2015