Bioinvasion: a paradigm shift from marine to inland ecosystems

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Received: 13 September 2013 / Accepted: 5 August 2014 / Published online: 24 August 2014
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Abstract Anisakidosis is one of the most fearsome zoonotic food borne disease in aquaculture. The natural infections by anisakidoids or related variety in freshwater fish are not known, though sporadic experimental reports are available abroad (Butcher and Shamsi 2011). Invasive severity of anisakidoids in fish from Gangetic riverine ecosystems, i.e., in river Ganges at Fatehpur and Allahabad, as well as in river Yamuna at Allahabad, and molecular heterogeneity among these worms have been extensively investigated. The pathways of transmission of non-native alien species due to long distance migratory habits of *Rita rita*, man-made alterations including dredging in long stretches of the river bed of Ganges to facilitate ballast water transfer mechanism owing to the commercial ship movements between Haldia and Allahabad; and sudden water chemistry (salinity, hardness, alkalinity) alteration (due particularly to rainy period) oriented microfauna interchange are identified, and remedial measures suggested.

Keywords Bioinvasion · Invasive species · Anisakid · *Rostellascaris spinicaudatum* (Malhotra and Anas 2001) · *Polyonchobothrium armatii* · *Polyonchobothrium allahabadense* · Biogeographic

Abbreviations

CP	Sunflower like caudal papillae
EP	Excretory pore
FL	Frontal lid
HS	Single spines on head
L	Lip
PP	Pre-anal papillae
SP, FP	Spicule
T	Mucron
W	Worm in situ in liver

Introduction

The instances of anisakidosis in human beings are frequent (Butcher and Shamsi 2011). This disease is one of the interesting examples of food-borne infections, about which little is known from Indian Ocean and adjoining coastal areas. The worms of Anisakidae are commonly reported from Indian marine fish (Malhotra et al. 2012a, b). These worms (Figs. 1a, b, c, d) were never reported in literature from freshwater fish before 21st century. But the investigators recorded their occurrence (Malhotra and Anas 2001) in the catfish, *Rita rita* since 2001 (Fig. 1d), whose migration from estuarine waters in West Bengal to the Gangetic plains at Allahabad is not a confirmed event yet (Chauhan and Malhotra Sandeep 1984), although these anisakids were most frequent in sharks and catfish in Arabian Sea. The question, therefore, could be on the entry of parasitic organisms of saline environment of marine

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ecosystems into non-salinity zones, their passage through the low-salinity zones of estuaries, and survival and establishment in the non-native zero saline environment of fresh water rivers, particularly upstream Ganges, in India. The reason could be assigned to:

- i. An opportunity of transport of the larval and other developmental stages of the parasites by the movement of ballast water under the bottom of the larger commercial ships.
- ii. Migration of catfish which have thus far been found to be mostly infected by members of family Anisakidae that are of immense zoonotic significance.
- iii. Long distance migration of birds from marine/estuarine areas to the Gangetic riverine ecosystems, and sudden variations in water chemistry parameters and siltation patterns due to sudden flush of water in river Ganges from Tehri Dam during October to February, while the volume of water flow in this river remained severely restricted during most of the months of the every year.

The author's contribution to GenBank Accession Nos. FJ172979 and EU 741046 form *mitcoi* gene of *Rostellascaris* (Malhotra and Anas 2001) and GQ265673 for its 18S rDNA gene of *Rostellascaris*; GQ265670, GQ265671,

Q265674, GQ265675 for 18S rDNA gene of *Iheringascaris* (Malhotra et al. 2012a, b); KF609490–KF609492 for ITS1, ITS2 and 18S rDNA gene, respectively of anisakid-gnathostomatoid type larvae from fish of river Ganges; KF609487 for ITS1 gene of their mature worms; KF609493 and KF609495 for ITS2 and 18S rDNA gene, respectively of mature female worms and KF609496 for 18S rDNA gene of their adult worms; and BankIt Nos. 1,659,188 and 1,659,192 for ITS1 gene of Anisakidoid larva, 1,659,187 for ITS1 gene of *Contracaecum* larva; 1,659,181 for ITS2 gene of Anisakidoid larva, 1659176 for ITS2 gene of *Contracaecum* larva have provided evidence of genetic heterogeneity among marine and freshwater anisakidoids.

The present investigations were aimed at identifying the range of distribution of species of zoonotic significance, and their distribution patterns within interactive zones of climatic change *vis-à-vis* oceans and freshwater bodies, that displayed congruence with respective definitive hosts.

India is a vast country with tremendous magnitude of diversity of fauna which is up against environmental barriers, extending from hill-stream fish fauna and the helminth parasites harboured by them, down to their confluence within Himalayas to flow down to the Gangetic plains, where the domain of fresh water fish virtually doesn't allow fish from Himalayas to survive, and vice versa. This follows an entirely different parasitic helminth fauna in marine fish of coastal areas of Indian Ocean and Arabian Sea. The present study encompasses two significant pathways for entry of bioinvasive alien species—(i) From marine environment of Arabian Sea, via Indian Ocean, to the fish of river Ganges at Allahabad and Fatehpur in Uttar Pradesh, and (ii) From coastal zones abroad to Arabian Sea and Indian Ocean, around India.

The investigations conducted in this study have continued since 1988, during which the focus was on collection and identification of marine and freshwater riverine zones at Ganga and Ganga and Yamuna rivers. A total of 8 780 fish were examined for collection of trematodes, cestodes and nematodes, which were killed in lukewarm water and processing (Malhotra et al. 2012a, b; Chauhan and Malhotra Sandeep 1984) done after fixation by specific methods for each parasite. The analysis of hydrobiological parameters to assess marine and freshwater environmental variations (Malhotra et al. 2012a, b; Chauhan and Malhotra Sandeep 1984) was done by standard methods.

From marine environment of Arabian Sea to fish of river Ganges

Mechanism of transmission through salinity barrier

The critical issue related to the intrinsic hydrological environment to trigger an event under which certain

Fig. 1 Scanning electron micrograph of Anisakid larva from *Rita rita*: **a–c** at Fatehpur, U.P. **a** anterior end, **b** showing excretory pore on head, **c** sunflower like preanal caudal papillae, and **d** at Allahabad, U.P.—Anterior end of worm. Scale bars **a** 100 μm, **b,c** 10 μm, 50 μm

physiological adaptations within the organisms are remodeled need specific attention.

The monitoring of salinity changes over a period of time at the Mandovi estuary has drawn attention to focus on this aspect. The maximum anisakid (Figs. 2a, b) outbreak including larval dominance was noticed in catfish, *Arius maculatus* at Mandovi estuary during the period of sudden drop in salinity content of water during rainy period with maximum rainfall in the area. It was at the level of 37–40 ppt during end summer period in May, while it varied from 29–34 ppt after rainfall in July and up to winter period. On a similar note, to emphasize the record of sudden variations in salinity at Hooghly estuary, it was emphasized (Chauhan and Malhotra Sandeep 1984) that in the estuarine zone of Rupnarayan near Bay of Bengal (i.e., zone IV), salinity decline occurred after construction of Farakka Barrage during 1975 up to 1977, because fresh-water being flushed into estuarine water. But later, in the zones II and III during 1988, 1992 and 1994–1998, the salinity values escalated, and these fluctuations resulted into change in fauna as well as vegetation. The heavier dredging operations to facilitate the activities of National Waterways Authority since 1986, could easily become instrument to have changed copepod and other micro-fauna due to major shifting of sand from bottom to the surface of riverine stretch from Haldia (West Bengal) upstream river Ganges up to Saraswati Ghat at Allahabad (Uttar Pradesh). Thus, the dynamics and outreach of the changed micro-fauna under altered hydrological conditions beyond the estuarine zone in the areas upstream river Ganges could have facilitated the introduction of exotic parasitic organisms into the non-native zones up to Allahabad. This possibly facilitated sudden survival and establishment of a newer anisakid species that possessed the characteristics of invader anisakid genera from Arabian Sea. Such parasitic adaptations were corroborated by the conclusions of an earlier study to assert that the temperature-salinity profiles (Reed et al. 2012; Ayyappan and Gopalakrishnan, 2008) and their association with specific masses of water determined the distribution patterns of marine parasites. It has also emphasized in the Indian context that the sudden salinity changes were significant enough to trigger changes in the faunal populations of zooplanktons (Ayyappan and Gopalakrishnan 2008), that, in turn, were the part of life cycle of a variety of helminthes.

Simultaneously, the hydrographic changes in the Gangetic river bed would have added to the biology and dynamics of the intermediate host as well. This is because such changes have occurred over the years because of severely restricted riverine flow, as the majority of available quantity of water is held back at the reservoir of Tehri Dam, but for the 4–5 months every year, during October to February, when the water is flushed into the river from this

dam, besides the floods in river Ganges during July–August.

Ecology of alien species

The findings on distribution pattern of infection prevalence during July through winter period (Table 1) was corroborated in both the marine fish studied with the results being—(i) *Rhincodon typus*: peak infection prevalence of male nematodes in male fish, 62.5–71.4 % and peak infection prevalence of female nematodes in female fish, 57.14–66.66 % as well as peak mean intensity of male nematodes in male fish, 3.4, and peak mean intensity of female nematodes in female fish, 4; ii.) *Arius maculatus*: peak female nematodes in female fish, 62.7–85.4 % and peak mean intensity of male nematodes in male fish, 4. Therefore, a stronger supportive influence of same sex of fish hosts on the similar sex of nematodes, *Rostellascaris spinicaudatum* was predominantly apparent under the overall influence of hydrological conditions of the water body in sharks as well as *A. maculatus* in the central west coast of India at Goa. However, a couple of aberrations to this pattern i.e., peak prevalence of female nematodes in male fish, 66.66 %, and peak mean intensity of male as well as female nematodes in female and male fish, respectively occurred in *A. maculatus*. This could be the result of interactive influence of a variety of hydrological factors in the marine ecosystems, other than temperature-salinity effect, which would require further detailed investigations.

A closer look on infection prevalence by *R. spinicaudatum* in *Mystus tengra* in the freshwater riverine ecosystems provided evidence of peak infection prevalence, 66.66 % by female nematodes in female fish during late winter and spring periods as well as of peak mean intensity (18) by female nematodes in female fish during winter period. The findings have projected strikingly similar pattern of distribution of *R. spinicaudatum* in the fish of freshwater as well as marine habitats, thereby necessitating further investigations to analyse regulatory influence of a combination of water chemistry parameters in coordination with the effect of hormonal interference to support peak prevalence and mean intensity of either sex of nematodes of genus *Rostellascaris* in the same sex of fish. Though the authors have collected long term data on the monthly and seasonal oscillations in the water chemistry parameters in freshwater riverine ecosystems in northern India, the conclusive analyses at length, on above lines would be beyond the scope of the present paper which has focused mainly on the inventory and biology of transfer of alien anisakid species from marine to inland freshwater ecosystems in India. The detailed ecology to deal with the aspects of bioinvasion of the helminth parasites of Indian fish shall be

Fig. 2 **a–b** Mature *Rostellascaris* from *Rita rita* at Allahabad **a** sunflower caudal papilla, **b** tail with spicules, **c** Gnathostome larva from shark, *Rhynchodon typus*. **d–h** Anisakid from *Xenentodon cancila* at Allahabad **d** In situ in liver, **e–h** scanning electron micrograph

of: larva (whole body), **e** head of mature worm, **f** tail with caudal papillae of male **g** and Sunflower like preanal caudal papillae **h**. Scale bars 50 μ m, 200 μ m, **e** 200 μ m, 20 μ m, **g** 100 μ m, **h** 3 μ m

included in the forthcoming publications which the authors intend to contribute in future.

Human concerns of alien species

The nematodes of Anisakidae were never encountered in freshwater fishes in river Ganges between Bay of Bengal and Ganges at Allahabad since past century (Baylis 1930, 1936; Freze 1969; Yamaguti 1961). These worms are of serious cause of concern because of their zoonotic

significance. The peculiar overlapping morphological characteristics between subfamily Goeziinae and Anisakinae, like sunflower-shaped caudal papillae in the posterior 1/3rd of the body of larva, that had an unspined body (Figs. 2a, g, h) with three lips on head (Fig. 2f) that were devoid of interlabia, and an excretory pore opening at the base of the ventral interlabia on head were noticeable. The sunflower caudal papillae have been reported in the recorded anisakid nematode so far from Bangladesh i.e., *Goezia bangladeshi*, but this worm had an entirely different

Table 1 Monthwise distribution of *Rostellascaris goai* n.sp. and *R. spinicaudatum* in *M.tengra*, *R. typus* and *Arius maculatus*

Year/month	Infection prevalence(%) in male fish by		Infection prevalence (%) in female fish by		Mean intensity in male fish by		Mean intensity in female fish by	
	Mn*	Fn**	Mn*	Fn**	Mn*	Fn**	Mn*	Fn**
<i>R. spinicaudatum</i> in <i>Mystus tengra</i>								
1997								
November	0	0	28.57	14.28	0	0	1	7.5
December	0	0	0	5.88	0	0	0	18
1988								
January	20	20	0	0	2	9	0	0
February	10	0	22.22	66.66	1	0	3.5	4.5
March	0	0	33.33	66.66	0	0	7	11.5
April	28.57	28.57	33.33	33.33	1	3.5	6	6
May	40	40	0	0	1	5	0	0
June	20	20	14.28	14.28	1	2	3	3
October	0	0	14.28	14.28	0	0	5	14
<i>R. spinicaudatum</i> in <i>R. typus</i>								
2004								
January	71.4	42.8	12.5	50	3.4	3.3	3	4
February	30	50	40	30	2.6	1	1	1.3
March	26.6	20	23	30.7	1	2.3	1.66	1.75
April	33.3	50	14.28	42.8	1.5	2.3	1	1
May	0	0	25	25	0	0	2	2
June	34.7	47.8	22.2	33.3	1.5	2	2	2
July	20	22.2	33.33	66.67	2	1.5	1	1
August	12.5	12.5	18.18	27.2	7	2	2	1.3
September	0	14.2	16.67	33.33	0	2	1	1.5
October	35.7	57.1	27.2	50	1	1.8	1	1.09
November	62.5	37.5	28.57	57.14	1.3	1.8	1	1
December	44.4	38.8	28.57	42.8	1.5	1.8	1.1	1.3
2005								
January	25	50	42.8	38.2	6	1.8	1.1	1
<i>R. goai</i> in <i>Arius maculatus</i>								
2006								
September	11.11	11.11	9.09	18.18	3	4	4	2
October	8.33	8.33	11.11	11.11	2	3	2	2
November	14.28	7.14	9.09	22.22	3	8	4	2
December	20	20	0	0	3	2	0	0
2007								
January	13.33	13.33	10.58	26.31	1	1	1.5	1.4
February	25	37.5	20	50	4	1.33	3	1.6
March	30	40	33.33	50	2	1.25	1.5	1.5
April	33.33	41.66	25	50	1	1.2	1.5	1
May	14.28	21.4	31.25	62.5	3	2	1.6	1.5
June	20	20	25	25	2	2	3	2
July	9.09	18.18	14.28	21.4	4	1.5	3	1.67
August	33.33	66.67	28.57	85.7	3	2	3.5	1

heavily spined body on cuticular annulations, contrary to larvae from Indian fish. The question, therefore, was not

only the appearance of hitherto unreported marine fauna of roundworms in the freshwater Gangetic riverine ecosystem

at Allahabad, but their peculiar morphological features as well. This has thus attracted our attention to the interesting phenomenon that, not only that the outbreak of anisakid worms has frequented in freshwater riverine zone after about more than a century of non-reporting of such infections, but the possibility of spontaneous mutations in roundworms in adult as well as larval forms of anisakids are on record (Gederaas et al. 2012). More than a decade ago adult worms of *R. spinicaudatum* (Malhotra and Anas 2001) with three lips and distinct interlabia, unspined body but intense cuticular annulations and excretory pore at a short distance behind head, similar to anisakid roundworms of genus *Hysterothylacium* and *Iheringascaris* reported extensively from marine sharks, catfishes and a host of other fish in Arabian Sea, began appearing in the catfish *Mystus vittatus* and *Rita rita* at Allahabad, having been reported in 2001 for the first time (Malhotra and Anas 2001).

The genetic composition of anisakid worms analysed during investigations provided evidence of molecular differentiation between nucleotide sequences of GenBank Accession Nos. FJ172978 and FJ172979 for *mitcoi* and EU 741046, GQ265670, GQ265671, GQ265674, GQ265675, GQ265673 (total six genes) for 18S rRNA. The significance of this event was because the appearance of these nematodes was noticed after dredging in the river bed of Ganges was conducted between 1986 and 2001 in the long stretches from Saraswati Ghat at Allahabad (Uttar Pradesh) to Haldia in West Bengal. Their transport from Arabian Sea to Indian Ocean [by sharks (*R. typus*) and spotted catfish (*A. maculatus*)] would not be very difficult to presume because of long distance migratory habits of the definitive host fish, *R. rita* from estuarine areas to upstream river Ganges at Allahabad. But the likelihood of a change in invertebrate fauna in the area of Hooghly estuary, after traversing salinity barrier, might have supported propagation of these marine species to undergo transformation, and develop adaptability in a newer variety of intermediate host in estuarine waters, and later in areas, upstream River Ganges, up to Allahabad, between 1986 and 2001, as explained by Gederaas et al. (2012). The characteristic affinity to a particular group of hosts, multifactorial, interactive and opportunistic, with a striking influence of historical contingency were some of the pertinent factors that functioned as drivers of invasion (Hoberg 2010). It was also emphasized that the potential for introduction of a helminth species was determined under the strong influence of its life history pattern that interacted with invasion pathways. The role of human-mediated alterations that regulated global expansion of pathogens linked to broad transport networks and environmental perturbation associated with climate change was specifically highlighted (Hoberg 2010), along with other drivers, which enhance the

pervasive influence on the distribution of helminthes. Therefore, another possible mechanism of transmission of the larval and other developmental stages of the alien parasitic species and their intermediate hosts could be through ballast water (Ruiz et al. 1997) at the bottom of huge commercial ships of Shipping Corporation of India, that operated between Haldia (near Hooghly estuary), via river Bhagirathi, near Farakka Barrage, and river Ganges, Saraswati Ghat at Allahabad from 2001 onwards. The regular finding of cyclopoid copepods from coastal fish infesting eggs of invader parasitic species has supported the author's findings further (Geetanjali et al. 2002, Geetanjali 2002, Unpubl. D.Phil. Thesis).

The recent finding of another anisakid larva of genus *Contracaecum* in the catfish, *R. rita* from Allahabad, which were identified to be *Type 1* larvae reported in anisakid worms (Shamsi et al. 2011), possessing tooth on head that is typical to the genus, a distinctly medium-sized intestinal ceca, with typical anisakid ventriculus, and a smaller ventricular appendix, strengthened the viewpoint on bio-invasion that, hitherto unknown anisakids infesting freshwater fish, have now frequently occurred in freshwater habitats near Allahabad as was noticed periodically during continuous long term infestations since 2001 through 2013.

The zoonotic potential of anisakids and fish spirurines, collected from the bottom dweller, *Xenentodon cancila* in River Ganges at Allahabad, was an interesting common factor between the two groups of worms, the former ones of which showed wider host specificity and habitat preference from marine to fresh water regions, in geographically different parts of the country, as these worms were encountered in a variety of catfish, *M. vittatus* and *R. rita* in river Ganges and *A. maculatus* as well as sharks, *R. typus* in Arabian Sea. But, the latter roundworms exhibited restricted host specificity, in riverine freshwater, as these were reported only from *X. cancila*, along with tapeworms of genus *Monobothrioides*, that occurred only in sharks in marine environment but *Clarias batrachus* in river Ganges.

The larvae of gnathostomes (Fig. 2c) recovered from *A. maculatus* this year by the investigators, after the continuous collections for over a decade, in the central west coast of India at Goa, have affiliation to the worms encountered in another bottom dwelling freshwater fish in River Ganges at Allahabad, *X. cancila* (Fig. 2d). The two pairs of cervical glands in both the worms from marine as well as freshwater environments was the unique common feature besides single spines on outer body surface. However, the site specificity of adult worms infesting liver of freshwater fish was unique and differentiating from fish spirurines, particularly Gnathostomatoidea from *X. cancila*, than the closest genus *Goezia* and other anisakid worms, that commonly infected gut of definitive host, though sporadic reports on the occurrence of larvae of *Gnathostoma*

spinigerum in liver of fish are on record (Rojekittikhun et al. 2002).

In the sharp contrast from the characteristic features of gnathostomatoids in nematodes recovered from fishes in Arabian Sea at Goa, the specialized features developed by the worms collected from *X. cancila* in fresh water indicated striking adaptations leading to the completion of direct life cycle without an intermediate host, after three moults only (Fig. 2e), after which the non-spined second stage larvae with the boring ‘larval tooth’ (de Freitas and JK, Lent H 1946; Cabrera et al. 2009) migrated through the muscles and other organs to reach at the final site of infection i.e., liver. The developing forms like, AdvL₃ and adult worms co-occurred in liver. The oesophagus comprised of an anterior muscular and a posterior glandular part; eggs with polar plug and filament at one end; mature eggs, that were uncleaved when laid, later contained fully formed first-stage larvae, which undergo moults within egg, to differentiate these worms in freshwater host from gnathostomatoids, and three cephalic lips; ventriculus like formation between oesophagus and the intestine (Moravec and Nagasava 2000; Moravec 2007) and the ‘sunflower’ like tail, as observed in genus *Goezia* (Akther et al. 2004), clearly distinguished these worms away from fish spirurines. In addition, a post-labial, elevated double-ridged collarette at the base of cephalic bulb in the worms of the new genus, was the typical character of Physalopteridae present in the invasive helminths, while frontal sclerotized lid-plate to cover triangular oral cavity apically in the latter worms (Fig. 2f), was a distinctly unique feature that differentiated these from spirurines, anisakids as well as the worms of Physalopteridae.

From coastal zones of the different parts of the world to Arabian Sea and Indian Ocean, around India

Marine to marine transfers and archaic interrelationships of alien helminths

The phenomenon of marine to marine transfer of a variety of parasitic helminths like the first report of genus *Wenyonia*, *W. rhinchodonti* (Malhotra et al. 2012a, b) in sharks, *R. typus* of Arabian Sea among tapeworms and anisakids in a variety of marine fish of Arabian Sea, among roundworms, are significant points that have attracted attention of Indian Parasitologists. The tapeworms of genus *Wenyonia* were never reported from coastal areas of Arabian Sea or adjoining areas for the past several decades. It is common knowledge that the developmental stages of parasites with zooplanktons and phytoplankton and small fish etc. are brought in ballast water of large commercial ships which upload minerals, grains, consumables and other articles of commercial use at the coast of a country

abroad, and then offload these articles at any of the coasts of India in Arabian Sea (Origin—100 M.Y.A.) or Indian Ocean (Origin—200 M.Y.A.). Despite several remedial measures to permit such off loadings at an appropriate distance from Indian coast, and other such measures, no significant control on spread of exotics through these activities could succeed, and due to this the inputs for introduction of species like, *W. rhinchodonti*, were made possible in Indian marine fish by man-made alterations.

The biogeographic changes followed by the historical continental drift illustrated a distinct connectivity between Atlantic and Indian Oceans via the Tethys Ocean 65 M.Y.A. during the late Cretaceous period (Fig. 3); (Strahler and Strahler 1989). A recent study (Reed et al. 2012), emphasized upon the potential biological tag status of fish parasites in two different stocks of sardines in South Africa, the climate change between warm water from the western Indian Ocean (Agulhas Current) and cold water from South Atlantic Ocean (Benguela Current) ascertained specific difference in parasite species that were identified as potential bioindicators. Therefore, contrary to vicariance, the event of biological dispersal is a greater likelihood.

It is possible that the fish of Cretaceous period became susceptible to infections by *Monobothrioides*, *Wenyonia* and *Polyonchobothrium*, under the latitudinal variations in water temperature of Atlantic Ocean (Overall: 0.5–30 °C; Atlantic South: 2.2–30 °C, Atlantic North: 0.5–22.2 °C, North Central: 9.4–20 °C) (NOAA (National Oceanic and Atmospheric Administration) 2013), prior to the emergence of Gondwana region. Later, during the continental drift 200 M.Y.A. from which Atlantic and Indian Oceans evolved, these tapeworms could not succeed to establish in fish of those times, at 15–35 °C water temperature of Arabian Sea (this study). However, there is a likelihood that over the period of time, after 100 M.Y.A., when

Fig. 3 Biogeographic connectivity of subcontinents (schematic)

Arabian Sea emerged, the overlapping range of water temperature might have facilitated adaptations in the species of *Monobothrioides*, *Wenyonia* in sharks and *Polyonchobothrium* in the walking catfish (African and Asian catfish), *C. batrachus*, that occurred in inland waters throughout much of the Old World, and spread emerging infections (helminth mentioned in parentheses), by natural selection, to the modern day fish, *R. typus* (*Wenyonia*) in Arabian Sea (Malhotra et al. 2012a, b) and *C. batrachus* (*Monobothrioides* and *Polyonchobothrium*), as discussed in Gangetic riverine ecosystems (Malhotra et al. 2013). On the question of transfer of species from marine fish to freshwater ones, like *C. batrachus*, the intriguing upwelling of saline water resources intermittently at different places in Uttar Pradesh (Watanbe et al. 2012), could well be due to the partial affinity of groundwater resources to the inherent water quality characteristics of the reminiscent of Tethys Ocean from which these might have emanated, after the former receded after continental drift, into Arabian Sea giving way to emergence of Gangetic plains, and that became now exposed due to deep dredging under National Waterways Authority of India between Haldia (W.B.) to Allahabad in U.P. This exactly is the course of riverine pathway from where anisakids, and cestodes, *Monobothrioides* and *Polyonchobothrium* are being reported.

A closer study conducted recently (Knoff and Fernandes 2010) recounted occurrence of a digenean *Tormopsolus* (Poche) in *Genypterus brasiliensis* (Regan) (Ophidiidae), that also occurred in the subtropical waters of the Southwest Atlantic Ocean, in depths that range 60–200 m (Froese and Pauly 2013) from the North Atlantic and Indian Oceans as well as Arabian Sea simultaneously.

It is obvious, therefore, that the marine water comprising high salinity environment facilitated the survival and establishment of these worms in Arabian Sea at Goa. The comparative hydrochemical features of the Gangetic riverine ecosystem at Allahabad and estuarine as well as typical marine water chemistry data summed up to 2001 were corroborated with the role of parasites as bioindicators in earlier conclusions by the authors (Geetanjali et al. 2002). Long term investigations further revealed extended information on enhanced hardness levels up to 29,700–31,050 mg/l in Arabian Sea at Dona Paula, Goa during May, 2005, while salinity level dipped down to 32 ppt in September from 40 ppt in May. Coincidentally, alkalinity augmented up to 410 mg/l in May in river Yamuna from where a variety of anisakid outbreak is a regular feature now. The most striking observation though, was the peak of hardness in river Yamuna near Sangam, the point of confluence of the two rivers, Ganga and Yamuna, that varied from 800 to 1,000 mg/l during August–September each year. These findings summarily highlighted the sudden changes to influence the inherent water quality

characteristics with predominant role of salinity, hardness and alkalinity in riverine freshwater at Allahabad. Hence the inference the interference of sudden alterations in water quality that resulted into conducive environment for survival and establishment of non-native intermediate hosts, essential for completion of life cycle of helminth parasites. While calcium and magnesium levels—8.8 and 0.74 mg/l, respectively, were considered safe by WHO (1985), the frequent presence of 9.62–30.5 mg/l (calcium) 3.89–8.77 mg/l (magnesium), respectively in the water of river Yamuna supposedly contributed to the raised levels of hardness in Yamuna at Allahabad. Thus the environment could largely have been a barrier for long, to restrict host specific distribution of these tapeworms only in sharks in the central west coast of India, and over the years, the passage for the transport of developmental stages of tapeworms with possible small fish intermediate hosts, was made possible by human activities. The question now really was the support from environmental attributes. In that light the existence of saline water resources in remote areas of district Allahabad and certain other parts of the country (Watanbe et al. 2012) is significant.

In addition, the affinity of environmental attributes, as well as similarity of distribution of fauna, after the historic continental drift, has resulted into identical taxa having been distributed in African and Indian coasts which, at one time belonged to the same continent before continental drift. The ancient divergence of fauna was well supported by earlier published reports (Reed et al. 2004) in literature. The change in morphological features of such organisms that inhabited different biogeographical zones that were hitherto separated by the barriers of temperature and salinity (Reed et al. 2012) were peculiarly influenced by the genetic changes that were reflected in the barcode structure of each (GenBank Accession Nos., *Polyonchobothrium* spp.–*Mitcoi*, EU912552, EU912553, EU912554, FJ172982–FJ172993; 18S rRNA, GQ265685–GQ265699). Most of the time the environment in aquatic ecosystems was suitable for growth and development of exotic species, and the inputs for introduction of species were provided by man-made alterations, like transport of developmental stages or small fish etc. from foreign lands to Indian coastal areas.

Simultaneously, the entry of additional zooplankton species, as potential non-native intermediate hosts, that never had access to the riverine areas of Uttar Pradesh, created conducive conditions for newer parasitic helminth species to thrive at the cost of natives. From this standpoint, the transmittance of eggs or other developmental stages through the migratory birds, like *Larus ridibundus* from coastal areas around Goa to the Gangetic plains around Sangam (Allahabad) at the banks of river Ganges is also a distinct possibility. Thus, an aerial route of

introduction through faecal matter of migratory birds could facilitate inputs to varied infection prevalence that was encountered in fish as a regular feature. According to the evidence available in literature (Sousa 1990), there is a strong support to the significant role of autumn arrival of migratory birds at the mudflats and the introduction of a new snail cohort. Thus an opportunity was provided to these biological invaders which succeeded to establish by following the Rule of Ten (Bright 1998) and have flourished over the years to record 10–15 % prevalence in catfish, *R. rita* presently in river Ganges.

The reports of infections by *Polyonchobothrium allahabadense* (Gairola and Malhotra Sandeep 1986), *Polyonchobothrium armatii* (Malhotra Sandeep 1985; Chauhan and Malhotra Sandeep 1986; Malhotra Sandeep and Chauhan 1986) in Garhwal Himalayan fish, *Mastacembelus armatus*; *Polyonchobothrium humidii* (Geetanjali and Malhotra Sandeep 2003) from *Mystus vittatus* in the Gangetic riverine ecosystems are fairly common. Genetic characterization of bothriocephalid worms to establish valid species of genera *Senga* and *Polyonchobothrium* has been discussed recently (Malhotra et al. 2013). In light of marine connectivity of Oceans and water bodies in India with Atlantic and Pacific Oceans via Tethys Ocean, that was well illustrated (Strahler and Strahler 1989), and was further substantiated in context of parasitic helminths (Reed et al. 2012), the likelihood of similarity of fauna at genera level between African and Indian subcontinents cannot be ruled out. It was established in a recent study in the flood plain of the Yangtze River, China (Li et al. 2009) that the historical connectivity of habitats could well be attributed the reason for similarity in the helminth communities, as observed in the infections by genus *Polyonchobothrium* in the old world catfish, *Clarias* in the aquatic bodies of Egypt and Sudan (Freze 1969), as have been reported in the freshwater fish in India (Malhotra Sandeep and Chauhan 1986). Therefore, the claim that '*Polyonchobothrium* is only found in Africa' (Scholz, T., 2013, *pers. comm.*) is apparently a scientifically unsubstantiated statement, particularly because the inputs pertaining to invertebrate copepod fauna that acts as intermediate host to worms of *Polyonchobothrium* in hillstream fishes of Garhwal Himalayas, would not have the identical dynamics under environmental conditions of water bodies in the Gangetic plains or in African subcontinent. However, Chuck Blend (*pers. comm.*) from Texas, USA while illustrating differences in newer digenean worms of genus *Neolebouria*, collected from the same area of study in Arabian Sea, by the senior author, commented, "Already suspecting you have sp. based on deep water vs. tropical/sub-tropical host spp. and differing geographic localities (i.e., different spp. of intermediate hosts, etc.)". The assertion by earlier investigators (Freze 1969) on the role

of bioinvasive species as bioindicators of cestodes infesting fish, has further upheld the validity of biogeographic separation of helminth species.

Remedial measures

Therefore, the answer to the riddle of putting restraints on invasive alien helminth species, that have entered into riverine ecosystems of the Gangetic plains, lied on developing a strategy in future, to deescalate salinity enhancement spurts in concentration of salinity in estuarine zones, particularly during the period when the flushing of water occurred, i.e., either (i) due to rains (as recorded at Mandovi estuary in central west coast of India) or (ii) due to man-made alterations, that resulted into flushing of water into areas associated with dams, like Farakka Dam, where salinity was monitored (Jhingran 1991) to be high before flushing commenced. The thoughtful strategy need be developed before implementing decision to extend shipping pathways up to Fatehpur (U.P.).

Secondly, the sudden fluctuations in hydrochemical parameters that alter the inherent water quality at or around Sangam area need strict monitoring, particularly during the period of sudden temperature change, i.e., autumn and late spring periods.

Thirdly, this is the period when the quantum of flow of riverine water is severely restricted due to blockade at Tehri Dam each year. Resultantly islands of dried land interrupt riverine pathway, that would contribute to the enhanced concentration of ions in water. And in turn, the emerging soil component in the river bed would alter the faunal composition of invertebrates, like copepods, annelids, molluscs and a host of zooplanktons and phytoplanktons, that invariably play the role of intermediate hosts in the life cycle of a variety of helminthes. Therefore, it is essential to monitor change of species of biotic fauna, in the zone of influence of water scarcity, particularly in the Gangetic plains, during the months when barriers to water flow are imposed at Tehri Dam after February each year. The implications of detailed analysis on the collected long term data of hydrobiological attributes (Malhotra Sandeep 1994) and their correlation with parasitic variations in the area of study have been discussed in the fore-going text.

The possible changes in siltation patterns, as well as critical water chemistry attributes, need be critically analysed, at the point of release of water in excessive volumes from Tehri Dam area (Garhwal Himalayas in the State of Uttarakhand), and simultaneously at the point of flash floods in the Gangetic plains at Allahabad generated by this release, every year, and because of pilgrimage activities of Magh mela every year, Ardh Kumbh mela after a gap of every 6 years, and Maha Kumbh (similar to the one that was celebrated in January, 2013) at Prayag, the zone of confluence of twin rivers, Ganges and Yamuna.

Thus, the siltation patterns under influence of severe flooding, as experienced this year due to the devastation influenced by glaciers in Kedarnath valley in the upper reaches of Himalayas this year, climate change, in the hydrological environment of river bodies in Northern India, alterations in microfauna in the river stretch from Himalayas to Haldia in West Bengal, are indeed the critical factors that contribute to create conducive environment for the survival and establishment of non-native helminth species in the environment that is exotic to these species.

Acknowledgments NJ is grateful to the University Grants Commission, New Delhi for a Post-Doctoral Fellowship. SKM is thankful to the University Grants Commission for a major research project no.F. 41-17/2012(SR).

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