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## **Diversity of tapeworms (Cestoda) in freshwater fish of India**

Ph.D. Thesis

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### **Annotation**

The Indian fauna of cestodes of freshwater fish has been one of the long standing and frequently discussed issues in the field of helminthology. Due to incomplete descriptions and lack of adequate supportive documentation, the validity of these cestode taxa remained questionable. The present study has contributed, to some extent to a better understanding of the systematics of cestodes of freshwater fish in India. Critical evaluation of newly collected material using morphological and molecular approaches made it possible to clarify the species composition, host specificity and phylogenetic relationships of selected groups (Caryophyllidea and Proteocephalidea).

### **Declaration (in Czech)**

Prohlašuji, že svoji rigorózní práci jsem vypracoval/a samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

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## List of papers and author's contribution

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Paper 1. **Ash A.**, Scholz T., Oros M., Kar P. K. 2011. Tapeworms (Cestoda: Caryophyllidea), Parasites of *Clarias batrachus* (Pisces: Siluriformes) in the Indomalayan Region. **Journal of Parasitology** 97: 435–59. (IF = 1.208)

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Paper 3. Oros M., **Ash A.**, Brabec J., Kar P. K., Scholz T. 2012. A new monozoic tapeworm, *Lobulovarium longiovatum* n. g., n. sp. (Cestoda: Caryophyllidea), from barbs *Puntius* spp. (Teleostei: Cyprinidae) in the Indomalayan region. **Systematic Parasitology**, in press. (IF = 1.056)

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# 1. INTRODUCTION

## 1.1. Biodiversity and parasites

Biodiversity represents a continuum across a variety of scales, ranging from genetic to population, species, community, habitat, ecosystem, and landscape diversity (Brooks and Hoberg, 2000). Species diversity, however, plays a pivotal role in the study and perception of biodiversity. After the agreement to conserve biodiversity in the Rio Convention (1992), the exploration of biodiversity became more imperative than ever since we cannot defend or manage something if we do not know it.

Parasites, constituting more than half of all biodiversity (Toft, 1986), are the integrative core of biodiversity survey and inventory, conservation and environmental integrity and ecosystem function. In the realm of conservation biology parasites have dual and conflicting significance (Brooks and Hoberg, 2006), because they may regulate host populations, playing a central role in maintenance of genetic diversity and structuring host communities and, at the same time, they represent threats to human health, agriculture, natural systems, conservation practices, and the global economy (see Horwitz and Wilcox, 2005). At a higher level than the communities of parasites themselves, they can track broadly and predictably through ecosystems. Within the ecological-trophic context, according to Brooks and Hoberg (2000), parasites can tell us about (1) trophic positions of hosts in food webs; (2) time spent by hosts in different microhabitats; (3) whether hosts are accumulating parasites via host switching, and if so, which hosts might be in potential competition; (4) whether any host harbours disease parasites; (5) whether the host changes its diet during its life time; and (6) if the hosts are residents or colonizers in the community. Thus parasites can be sensitive indicators of subtle changes within ecosystems. This is specifically true for parasites with heteroxenous life cycles such as helminths, many of which use one or two, exceptionally three intermediate hosts.

The word 'helminths' was first used by Aristotle (384–322 B.C.) for some of the worms found parasitic in animals (Hugot et al., 2001). Helminths, as parasites in general, do not represent a monophyletic assemblage since under that term members of phylogenetically not related phyla are included, i.e., Platyhelminthes ("flatworms") comprising cestodes, monogeneans and digeneans; Nematoda ("roundworms"), previously placed in the phylum Nemathelminthes (or Aschelminthes); and Acanthocephala ("thorny-headed worms").

Parasitic disease is the single most important factor threatening the fishery industry worldwide, particularly in the tropics (Williams and Jones, 1994; Schmidt and Roberts, 2000). Among the parasites that infect teleostean



fishes, helminths represent the largest and important group. No other group of vertebrates has such a diversity of helminth species and some of the helminth groups like monogeneans are unique to fish. It is estimated that there are more than 30000 helminth species parasitizing marine and freshwater fish (Williams and Jones, 1994) and some of them are known to be the agents of serious fish diseases or may represent an important public health problem.

Among the helminths, monogeneans are mostly ectoparasites of fish with relatively high host specificity. Buchmann and Bresciani (2006) assumed that many fish hosts including freshwater ones could harbour at least one unique monogenean species. Apart from being hosts to less harmful adult digeneans, fish may also be infected with metacercarial larval stages, which are the main agents of fish diseases (Paperna and Dzikowski, 2006). Most of the cestode orders (except Cyclophyllidea, Diphyllbothriidea, “Mesocestoidea” and Tetrabothriidea) have members that can infect fish (both Chondrichthyes and Osteichthyes) as adults. The number of species of nematodes infecting fish is relatively low compared with their terrestrial counterparts, but is still quite high (Molnár et al., 2006). A large number of nematodes of piscivorous birds, mammals or reptiles infect fish during their larval stages. Among approximately 1100 species of acanthocephalans (Golvan, 1994), nearly one-half parasitize as adult in the intestine of bony fish (Teleostei), especially in Cypriniformes (Nickol, 2006).

Fish helminths with their mostly complex life cycles may also represent excellent models for the solution of a number of theoretical questions, including host-parasite relationships including host manipulation, biology, ecology, zoogeography and phylogeny of these parasites and their hosts (Williams and Jones, 1994).

## **1.2. Tapeworms (Cestoda)**

Cestoda is the name given to a monophyletic assemblage, commonly called tapeworms, of exclusively parasitic platyhelminths (the Neodermata). The adult body of most cestodes consists of an anterior end called scolex (plural scoleces), which is often substantially modified to serve for attachment to the intestine of the vertebrate host; a proliferative zone termed “neck”; and the remaining part of the body, strobila, in which the reproductive organs are located. The scoleces of cestodes are typically categorized as either bothriate: characterized by the presence of two, or rarely four (Trypanonyncha), longitudinally arranged, shallow depressions called bothria (singular bothrium) (see Noever et al., 2010); or acetabulate: characterized by the presence of one to five muscular cups (suckers or bothridia) sunk into the equatorial surface of the scolex (Caira et al., 1999). In polyzoic cestodes, the strobila usually consists of a chain of segments,

each generally housing one set, but occasionally two or more sets (with a maximum of 300 sets in *Baylisia supergonoporis* Yurakhno, 1989) (Yurakhno, 1992) of male and female reproductive organs. However, in the relatively few monozoic cestodes (Gyrocotylidea, Amphilinidea and Caryophyllidea), the body is undivided and houses a single set of reproductive organs. Cestodes entirely lack a digestive system and instead most of the time, absorb nutrients through tegument (neodermis) (exception *Sanguilevator yearsleyi* Caira, Mega, and Ruhnke, 2005; see Caira et al., 2005) which is covered with microtriches. Microtriches are unique to cestodes and present in different forms (see Chervy, 2009).

Cestodes are known to humankind for a long time. Tyson, Andry, Frisch, Linnaeus and Pallas in the 16<sup>th</sup>–17<sup>th</sup> Centuries were the pioneers of cestode taxonomy (see Wardle et al., 1974). However, it was the Belgian researcher van Beneden, who made a closer approach to a scientific arrangement of cestodes in 1849 (van Beneden, 1849). Later Carus (1863) made the foundation of the modern classification scheme by modifying Beneden's ordinal terms in Latinized form. He adopted the term "Platyhelminthes" under which he placed Turbellaria, Trematoda and Cestoda. Under Cestoda he created five families, namely, Caryophyllidea, Tetrphyllidea, Diphyllidea, Pseudophyllidea and Taeniadea, to accommodate all cestode taxa known at that time (see Wardle and McLeod, 1952). Thereafter, numerous authors, such as Monticelli, Braun, Ariola, Lühe, Southwell, Poche, Pintner, Fuhrmann, Yamaguti, Wardle, McLeod, Freze, Protasova, Schmidt, Khalil, Jones, Bray and others have enriched our knowledge of cestode systematics (see Wardle and McLeod, 1952; Freze, 1965; Wardle et al., 1974; Schmidt, 1986; Scholz, 2001).

Monogeneans are believed to be the closest relatives of cestodes (Hoberg et al., 2001; Olson et al., 2001), but phylogenetic relationships and the classification of cestodes are still a matter of discussion. However, a wide consensus is achieved in several points mostly as a result of extensive phylogenetic studies based on morphology (including ultrastructure) and molecular data (Olson et al., 2001; Waeschenbach et al., 2007, 2012).

Two subclasses have been recognized within the monophyletic class Cestoda: Cestodaria, including the monozoic orders Gyrocotylidea and Amphilinidea; and Eucestoda, comprising 15 (mostly polyzoic) orders (see Olson et al., 2001; Waeschenbach et al., 2012). One of the major morphological characters supporting their monophyly is the lack of intestine in all stages of their development. Structural peculiarities of osmoregulatory canals and the presence of microtriches in all groups including gyrocotylideans (see Poddubnaya et al., 2009) also confirm their origin from a common ancestor (Xylander, 2001).

There are no fossil records of cestodes, but phylogenetic studies and analyses of the evolutionary associations with hosts suggest a long period of the cestode-vertebrate coevolution, perhaps since Devonian, i.e. 350–420 million years ago (Hoberg et al., 1999). Existing evidence also suggests that extant cestode groups evolved as parasites of fish and radiated to parasitize all major vertebrate groups (Hoberg et al., 1999). Within the Cestoda, Gyrocotylidea has a basal position to the branch containing the remaining taxa (Amphilinidea plus orders of the Eucestoda). Among the Eucestoda, the monozoic order Caryophyllidea is considered basal to the remaining orders (Waeschenbach et al., 2012), though this hypothesis is not always supported by molecules (see Olson and Caira, 1999; Kodedová et al., 2000; Olson et al., 2008). Among the polyzoic orders, cestodes having acetabulate scolex (previously referred to as tetrafossate) are considered more derived than those having bothriate scolex (previously referred to as difossate). Recent results also suggest that strobilization may have been a stepwise process evolving from non-proglottized, non-segmented forms (Caryophyllidea) to proglottized, non-segmented cestodes (Spathebothriidea), to the proglottized, segmented condition (higher Eucestoda) (Waeschenbach et al., 2012).

Members of different orders of cestodes live in the digestive tract of vertebrates as adults and, depending on the cestode group, during their larval stages, in the body cavity, musculature, or occasionally in a diversity of other sites in one or more invertebrate and/or vertebrate hosts. Over 5000 species and 740 genera of cestodes have been described, but known diversity seems to be just a small fraction of the true number due to their hidden existence (Waeschenbach et al., 2012).

The life cycle of most cestode species includes at least two hosts, final or definitive and intermediate. The final host is that harbouring adult worms (reproducing sexually) and the intermediate host is that in which larvae (also known as metacestodes) develop. The two hosts are in close associations, facilitating the transmission of the parasite. The transmission of the cestodes from the intermediate hosts to the final hosts is along the food chains only (transmission *via* food ingestion or trophic transmission), thus intermediate host is a common component of the diet of the final host (Schmidt and Roberts, 2000).

The general scheme of a life cycle of most aquatic cestodes, including fish cestodes, is as follows: cestode eggs in the uterus, which may contain embryos, named oncospheres (lycophora in Cestodaria), pass with host's faeces into the environment. Eggs (except those taxa have coracidium) are eaten by the intermediate hosts (crustaceans). Larvae hatch in the gut of the intermediate hosts (with some exceptions), and using their hooks and glands, penetrate through the intestinal wall and locate in the body cavities or other

internal organs where they metamorphose into infective larval stages (metacestodes).

Chervy (2002) identified six basic types of metacestodes. Three of them can be found in the life cycles of freshwater fish cestodes: (i) proceroid, an alacunate form which cannot develop further until ingested by a second intermediate host (e.g. Diphyllbothriidea, some Bothriocephalidea); (ii) plerocercoid, an alacunate form with an everted scolex (e.g. Caryophyllidea, some Proteocephalidea); and (iii) merocercoid, an alacunate form with an invaginated scolex. The final host is infected by eating intermediate host that harbours metacestodes. The scolex of the metacestode attaches to the intestinal wall of the final host and the neck of the cestode starts to produce proglottides and thus the strobila is formed.

Cestodes of six orders can be found in freshwater fish (Teleostei) as adult, namely Amphilinidea, Caryophyllidea, Spathobothriidea, Bothriocephalidea, Proteocephalidea and Nippotaeniidea. Their life cycles are briefly described here. Life cycles of Amphilinidea of freshwater teleosts are poorly known, but two hosts are involved. Decacanth (lycophora) larvae hatch from non-operculated eggs, then develop as a juvenile stage in intermediate crustacean hosts such as amphipods, crayfish and freshwater prawns (Rohde and Georgi, 1983), and finally find their piscine definitive hosts like aba (*Gymnarchus niloticus*).

The basal orders of the Eucestoda, i.e. Caryophyllidea and Spathobothriidea, have mostly two-host life cycles. The eggs are operculate and the larvae hatch in the gut of the intermediate host. Caryophyllideans use aquatic oligochaetes as intermediate hosts, which is not common in helminths (Mackiewicz, 1972, 1981a, 1982). Plerocercoid stage develops in coelom or seminal vesicle of tubificids. From this stage, three types of development are possible (Kulakovskaya, 1962; Mackiewicz, 1972, 1982): (i) egg-producing progenetic stage e.g. *Archigetes limnodrili*; (ii) long-time span and advanced development of reproductive organs in intermediate host, but no egg production e.g. *Glaridacris confusa*; and (iii) short-time span in intermediate host and normal cycle, i.e. development of genitalia occurs only in the definitive host (most species). Definitive hosts of caryophyllideans are freshwater fish of the orders Cypriniformes (Cyprinidae and Catostomidae) and Siluriformes (Bagridae, Clariidae, Heteropneustidae, Mochokidae, Plotosidae and Schilbeidae) (Mackiewicz, 1981a). Spathobothriideans use amphipod crustaceans as intermediate hosts (Amin, 1978), progenetic development is also seen in this group. Freshwater teleosts, especially salmonids (trout, grayling) serve as typical definitive hosts, whereas pike and perch act as accidental ones (Gibson, 1994).

Bothriocephalideans have two or three hosts in their life cycle. Eggs are with or without operculum and usually develop in the water (*Bothriocephalus*, *Triaenophorus*), but also in the uterus (*Eubothrium*). Some taxa (usually when eggs are not embryonated in the uterus) liberate ciliated, free swimming coracidium in the water, which is eaten by crustacean intermediate host, generally a copepod. The hexacanth penetrates into the haemocoel where it develops into next larval stage. In the case of three-hosts life cycles (*Senga*, *Triaenophorus*) (Williams and Jones, 1994), the proceroid develops in the first intermediate host and plerocercoid develops in the musculature or body cavity of the second intermediate host, which is a teleost (Whitfield and Hegg, 1977). One third of the valid taxa of this order are found in freshwater fish, mostly in the Perciformes (Kuchta and Scholz, 2007).

A typical life cycle of the proteocephalidean cestodes includes an intermediate and definitive hosts (Wagner, 1954). Freze (1965) suggested three types of development in proteocephalideans, among them two types, namely, 'proteocephalinoidean' and 'corallobothriinoidean' can be found in freshwater fish. Current knowledge suggests that most species parasitizing fish (except for species of the Corallobothriinae) use only one intermediate host, mostly copepods of the order Cyclopida and rarely Calanoida (Scholz, 1999), in which plerocercoid develops (Scholz and de Chambrier, 2003). In the case of poorly known life cycles of Corallobothriinae (*Megathylacoides*), metacestode stages like proceroid or merocercoid can be present in the first intermediate host (copepod) and plerocercoids can be found in reservoir or paratenic hosts (Scholz and de Chambrier, 2003). Definitive hosts include several phylogenetically unrelated groups of teleosts. The richest fauna of proteocephalideans exists in the Neotropical region, mainly in pimelodid catfishes (Rego, 1994, 2004).

The life cycle of nipotaeniids, a very small group of cestodes typical in the possession of an apically situated sucker (Bray, 1994) includes a single intermediate host, usually a copepod. Definitive hosts are perciform (*Perccottus*) and osmeriform (*Galaxias*) fish (Bray, 1994).

Cestodes have received considerable attention of systematists, not only because they are ubiquitously distributed, having radiated with their hosts into all habitats (Khalil et al., 1994), but because of their importance as pathogens of humans (e.g. *Diphyllobothrium* and *Taenia*) and livestock (*Moniezia*, *Taenia* and others). They exhibit a range of morphological, physiological, biochemical and ecological adaptations, which make them suitable models for studies of various biological phenomena, including host-parasite relationships and evolution of parasitism (Williams and Jones, 1994; Kern 1998). For example, cestodes which exhibit narrow host specificity

such as many caryophyllideans (Mackiewicz, 1982) and proteocephalideans, especially those parasitic in catfishes in South America (de Chambrier and Vaucher, 1997, 1999; Zehnder et al., 2000), can be a potentially important model for studies on host-parasite co-evolution (Škeříková et al., 2001). A high diversity of scolex morphology also makes cestodes a suitable model for studies on morphological adaptations (Rego, 1999; Scholz and de Chambrier, 2003).

The aim of modern taxonomy is not only to describe, identify and arrange organisms in convenient systematic categories, but also to understand their evolutionary histories and mechanisms (Boero, 2010). Earlier approaches were mainly based on observed morphological characters without considering interspecific differences and without any knowledge on population variability and genetic characteristic, which resulted in inflation of descriptions of conspecific taxa. Thus several approaches have recently been taken to more rigorously circumscribe species for producing accurate inventories and biodiversity surveys.

Several tools for studying cestode micromorphology such as scanning and transmission electron microscopes have been used to provide accurate and, most importantly, more stable morphological characters (Scholz et al., 1998; Levron et al., 2010; Oros et al., 2010). Phylogenetic classification systems have been shown to be the most effective framework for prediction of relationship of organisms and their place in the biosphere (Brooks and McLennan, 1993). Phylogenetic analyses of parasitic flatworms (Platyhelminthes – Neodermata) began more than 25 years ago (Brooks, 1985) and cestodes represented one of the helminth groups in which cladistics was first applied (Brooks et al., 1991; Brooks, 1995). As Boero (2010) correctly stated – “it is much more ‘scientific’ to identify specimens with machines than doing it by simply looking at them”, molecular approaches are now integrated with morphological ones to provide much reliable results (Hoberg et al., 2001; Olson et al., 2001; Kuchta et al., 2008).

Dayrat (2005) suggested seven guidelines to prevent the over-abundance of specific names on the basis of his experience with systematics of free living animals: (i) No species names should be created in a given group unless a recent taxonomic revision has dealt with the totality of the names available for the group; (ii) No new species names should be created if the infra- and interspecific character variation has not been thoroughly addressed; (iii) No new species names should be created based on fewer than a certain number of specimens (a number which specialists of each group could agree upon), and never with a single specimen; (iv) A set of specimens differing in some regard from existing species can be described with the abbreviation ‘sp.’ (for species) and not with a real species name regulated by the codes of

nomenclature; (v) Ideally, names should only be created for species that are supported by broad biological evidence (morphology, genealogical concordance etc.); (vi) No new species names should be created if type specimens deposited in a museum collection are preserved in a way that prevents any further molecular study; and (vii) All neotypes designated from now on should be preserved in a way that allows DNA extractions and sequencing. Although Dayrat (2005) suggested this approach for free living organisms, this approach is perfectly applicable for helminth parasites and it was followed in this study (see below).

### **1.3. Cestodes of freshwater fish in India**

India is among the 17 megadiversity countries (Mittermeier et al., 1997) and hosts as many as 55 families of freshwater fish (Teleostei) (Froese & Pauly, 2012). For the last few decades, fish (both Chondrichthyes and Osteichthyes) have been extensively used as a protein rich diet for human consumption in the Indian subcontinent and thus contribute substantially to its economy. It is estimated that about 10 million tons of fish are required annually to meet the present-day demand of fish proteins in India compared to an actual annual production of only 3.5 million tons (Shukla and Upadhyay, 1998).

Catfishes are an important part of the fish fauna in wetlands and many of them are economically important as a food source of high nutritive value. In India, there have been described about 160 species of inland catfishes from 50 genera distributed in 13 families, namely Akysidae, Amblycipitidae, Ariidae, Bagridae, Chacidae, Clariidae, Heteropneustidae, Olyridae, Pangasiidae, Plotosidae, Schilbeidae, Siluridae and Sisoridae (Talwar and Jhingran, 1991). Five of them, namely Bagridae, Clariidae, Heteropneustidae, Schilbeidae and Siluridae, have been reported as definitive hosts of cestodes (Hafeezullah, 1993; Jadhav et al., 2010).

Species of three orders of the Eucestoda, namely Caryophyllidea, Bothriocephalidea and Proteocephalidea, are found in the Indian freshwater fish. The British researchers Southwell (1913a,b) and Woodland (1924) provided the first data on fish cestodes from India, followed by the Indian helminthologists Moghe (1925) and Verma (1926). Since then, freshwater fish cestode diversity has been documented from different parts of the country by a number of Indian helminthologists. Most of these studies comprised descriptions of new taxa and as a result, high numbers of species (more than 250) of cestodes from freshwater fish, have been described from the Indian subcontinent (see Mackiewicz, 1981a; Agarwal, 1985; Chakravarty and Tandon, 1989b; Hafeezullah, 1993; Jadhav et al., 2010; Ash

et al. 2011a,b). Fewer publications dealt with the ecology of these parasites (Ahmed and Sanaullah, 1977; Niyogi et al, 1982; Power et al., 2011), their biology and life cycles (Ramadevi, 1976; Niyogi and Agarwal, 1983; Lyngdoh and Tandon, 1998), pathology (Satpute and Agarwal, 1974; Chakravarty and Tandon, 1989a; Irshadullah and Mustafa, 2010) and, most recently, genetic structure (Jyrwa et al., 2009; Valappil et al., 2009). The majority of the new taxa were described from Maharashtra (as many as 59 taxa, of Caryophyllidea and Proteocephalidea only), Uttar Pradesh (35 taxa), Assam, Jammu and Kashmir, West Bengal, etc. (see Fig. 1).

Three orders of cestodes (Eucestoda), the species of which occur in freshwater fish in India, are briefly characterized below.

**Caryophyllidea:** This is a small group of monozoic, non-segmented cestodes parasitizing freshwater teleost fishes. Caryophyllidea seems to be closely related to the Spathebothriidea and morphological data indicate they represent the most basal group of the Eucestoda. However, molecular analyses have not unequivocally supported this placement (Olson et al., 2008; Waeschenbach et al., 2012). A total of 41 genera and about 150 species of caryophyllideans distributed worldwide (except for the Neotropical Region) were recognized by Mackiewicz (1994). Of these 14 genera and 90 species belonging to three families have been described from the Indomalayan region from catfishes (Siluriformes: Bagridae, Clariidae, Heteropneustidae, Schilbeidae and Siluridae), cyprinid and cobitid fishes (see valid taxa in Table 3). In India, the first species, *Caryophyllaeus indicus* (now syn. of *Lytocestus indicus*), was described from the walking catfish *Clarias batrachus* (Linnaeus) in Nagpur by Moghe (1925).

**Bothriocephalidea:** In the case of the cosmopolitan Bothriocephalidea, which previously formed part of the order “Pseudophylliidea” (see Kuchta et al., 2008), 125 nominal species of 41 genera distributed worldwide are considered valid (Kuchta and Scholz, 2007). Bothriocephalidea are divided into four families, but they do not reflect natural groupings of phylogenetically related taxa, especially members of the largest and most diverse family Triaenophoridae (Kuchta et al., 2008). Sister groups are probably Trypanorhyncha, Diphyllidea and rest of acetabulate groups (Waeschenbach et al., 2012). Woodland (1924) described *Bothriocephalus pycnomerus* (= *Senga pycnomerus*) from snakehead *Channa marulius* (Hamilton), which was the first bothriocephalidean cestode described from the Indian subcontinent. A total of 108 nominal species of eight genera of this order were described mainly from perciform and synbranchiform fish from the Indian subcontinent, but Kuchta and Scholz (2007) considered valid only 17 species of three genera, namely *Bothriocephalus* Rudolphi, 1808; *Ptychobothrium*, Lünberg, 1889 (though Kuchta and Scholz, 2007 casted



doubts upon taxa reported as species of *Ptychobothrium* from Indian freshwater fish); and *Senga* Dollfus, 1934. Just within the last five years (2007–2012) as many as 20 new species of *Senga* and *Circumoncobothrium* Shinde, 1968 have been described from India.

**Proteocephalidea:** This cosmopolitan cestode group comprises mostly parasites of freshwater fish, less frequently reptiles and amphibians, with one species found in a mammal; most genera are limited to South America (42 of 54 genera) (Rego, 1994). About 400 species in 54 genera were recognized as valid by Schmidt (1986). Of these, more than 50 species of four genera (*Gangesia* Woodland, 1924; *Proteocephalus* Weinland, 1858; *Silurotaenia* Nybelin, 1942; and *Vermaia* Nybelin, 1942) have been described from the siluriform and cypriniform fish in the Indian subcontinent (see Table 4). Southwell (1913a,b) described *Ophryocotyle bengalensis* from the intestine of snakehead *Channa striata* (Bloch), rohu *Labeo rohita* (Hamilton) and wallago *Wallago attu* (Bloch and Schneider). Later, Woodland (1924) proposed a new genus *Gangesia* to accommodate two new species and Verma (1928) transferred *O. bengalensis* to this genus. This order is almost certainly monophyletic but relationships of individual groups (subfamilies and genera) are not clear. Molecular analyses strongly support the validity of basal groups (Acanthotaeniinae and Gangesiinae) as well as the monophyly of the Palaearctic species of the nominotypical genus *Proteocephalus* (see de Chambrier et al., 2004; Hypša et al., 2005). However, the relationships of numerous genera parasitic in reptiles, amphibians and Neotropical catfish are still unresolved.

The Indian fauna of the three cestode groups mentioned above was studied only superficially (excepting a few) and the lack of proper and adequate supportive documentation have raised a number of questions, not only with respect to the validity of several species, but also of the genera described from Indian freshwater fish. The main problems regarding the systematics of the cestodes parasitic in freshwater fish of India can be summarised as follows:

(i) Descriptions of most of the species were based on decomposed or deformed specimens. If helminths, including cestodes, are not fixed properly, they can substantially change their shape and size so that their morphology is impossible to describe (Cribb and Bray, 2010; Oros et al., 2010; Justine et al., 2012). The presence of artifacts caused by inappropriate fixation may produce misleading information on their morphology (Mackiewicz, 1981b; Hafeezullah, 1993).

(ii) Type-specimens are almost always unavailable, which impedes any comparative study and confirmation of the published data. In most cases type-specimens are either lost or damaged or have not been deposited at all in the designated place (Thapar, 1979).

(iii) Both intraspecific- and interspecific variability has never been studied in detail and new taxa were described on the basis of negligible, often doubtful or incorrect differences in morphology of inappropriate material (see point (i)). As a result, excessive numbers of cestode species have been described from the same fish host species. For example, as many as 59 species of 15 caryophyllidean genera and 3 families were described from *Clarias batrachus*, a very common catfish in the Indian subcontinent. Similarly as many as 17 species of the proteocephalidean genus *Gangesia* were described from *Wallago attu*, another common catfish in the subcontinent. Considering the relatively strict host specificity of most caryophyllideans and proteocephalideans from other well-studied geographical regions (Mackiewicz, 1972, 1981a; Dick et al., 2006; Scholz et al., 2007, 2011), such a high number of taxa from one fish host is surprising and requires confirmation.

(iv) Most data were published in regional journals, without peer review, which has resulted in publication of papers violating basic rules of modern systematics or even the International Code of Zoological Nomenclature (homonyms, unavailability of original description, *nomina nuda*, etc.).

(v) Scanning electron microscopy, which provides valuable information on scolex morphology and surface ultrastructure (Scholz et al., 1998; Chervy, 2009; Oros et al., 2010, etc.) has almost never been used.

(vi) Very few molecular data are available to support taxonomic conclusions inferred from morphological data.

To clarify this unsatisfactory situation, a multidisciplinary approach has been applied in the present study, which had the following objectives.

## **2. OBJECTIVES**

### **Principal objective**

The main objective of this study was to critically assess the species composition of fish cestodes of selected commercially important groups of freshwater fish (which were reported with huge number of taxa) in India and their phylogenetic relationships using morphological, ultrastructural (scanning electron microscopy) and molecular data.

### **Particular objectives**

- Critical re-evaluation of the original descriptions of Indian species and examination of available museum material.
- Evaluation of newly obtained material of fish cestodes using morphological, ultrastructural and molecular methods.
- Assessment of the validity of the nominal species and redescriptions of the taxa considered to be valid.
- Clarification of the host spectrum of these groups of fish cestodes.
- Unravelling phylogenetic relationships of the cestodes studied.

### 3. METHODOLOGY

#### 3.1. Sampling expeditions

This study was based on evaluation of newly collected material of cestodes from economically important freshwater fish (Teleostei) in India and from mostly two principal river basins. To successfully realize this plan, considerable effort, time and financial support were required, especially when no reliable data on seasonality and the actual host spectrum of freshwater fish cestodes of the region were available. Luckily we had the opportunity to conduct research expeditions within the framework of the Indian National Science Academy (INSA) – Academy of Sciences of the Czech Republic (ASCR) Bilateral Exchange Programme. Three sampling expeditions in India were realized during last four years (2008–2011); they were financially and logistically supported by the Jhargram Raj College in Jhargram, Paschim Medinipur, West Bengal under the management of Dr. Pradip Kumar Kar. Additionally, sampling was carried out in Bangladesh.

(i) First expedition (2009). During the first expedition, which was realized by A. Ash, P.K. Kar and T. Scholz during February and March, a vast area of West Bengal (Ganges River basin) that included, Kolkata (including Rishra and Howrah), Jhargram, Mukutmanipur, Malda, Balurghat, Siliguri and Bijanbari, was covered (Fig. 1). A small area of south Sikkim (Jorethang) which belongs to the Brahmaputra River basin was also covered. In this pilot study the main focus was on sampling the fish hosts which have been previously reported to harbour cestode parasites. A total of 409 freshwater fish of 27 species from different water bodies (rivers, dam lakes, fishponds) were dissected and at least 13 of them (precise identification of some fish hosts was not possible) namely, *Bagarius bagarius* (Hamilton) (Siluriformes: Bagridae); *Barilius* sp. (Cypriniformes: Cyprinidae); *Channa punctata* (Bloch) (Perciformes: Channidae); *Clarias batrachus* (Siluriformes: Clariidae); *Clupisoma garua* (Hamilton) (Siluriformes: Schilbeidae); *Heteropneustes fossilis* (Bloch) (Siluriformes: Heteropneustidae); *Mastacembelus armatus* (Lacepède) (Synbranchiformes: Mastacembelidae); *Mystus* cf. *tengara* (Hamilton) (Siluriformes: Bagridae); *Ompok* sp. (Siluriformes: Siluridae); *Puntius* spp. (Cypriniformes: Cyprinidae); *Rita rita* (Hamilton) (Siluriformes: Bagridae); and *Wallago attu* (Siluriformes: Siluridae), were infected with as many as 19 species of cestodes of three orders (Bothriocephalidea, Caryophyllidea and Proteocephalidea) (see Table 1).

(ii) Second expedition (2010). This short expedition realized by A. Ash and P.K. Kar in January included sampling in Malda and Siliguri (West Bengal). A total of 150 fish of seven species were examined but just 11 fish

of two taxa, namely *Clarias batrachus* (4 specimens) and *Puntius sophore* (Hamilton) (7 specimens) were infected with caryophyllidean cestodes (see Table 1).

(iii) Third expedition (2011). During this expedition of A. Ash, P.K. Kar, M. Oros and T. Scholz in March almost the same areas of West Bengal of the 2008 sampling expedition were visited. In addition sampling was carried out in Assam along the Brahmaputra River, i.e. in Dhuburi, Guwahati, Tejpur, Kaziranga and Jorhat (Fig. 1). The main focus was to collect cestodes from uncommon hosts such as *Bagarius*, *Mystus*, *Ompok*, *Rita* etc., which were found rarely infected during the trip in 2009. More than 350 fish of 24 taxa were dissected and 10 species of cestodes (belonging to Bothriocephalidea, Caryophyllidea and Proteocephalidae) were collected from the following fish hosts: *Barilius* sp., *Channa* spp., *Clarias batrachus*, *Heteropneustes fossilis*, *Mastacembelus armatus*, *Monopterusuchia* (Hamilton) (Synbranchiformes: Synbranchidae), *Mystus* spp., *Puntius* spp., *Rita rita*, and *Wallago attu* (see Table 1).

(iv) Bangladesh expedition (2011). Based on the logistic support provided by Dr Mostafa A. R. Hossain and his co-workers from the Faculty of Fisheries, Bangladesh Agricultural University in Mymensingh, Bangladesh, and Dr Andrew P. Shinn (Institute of Aquaculture, University of Stirling, UK), a collecting trip to northern Bangladesh (Mymensingh and Durgapur – Brahmaputra river basin) was realized by A. Ash, M. Oros and T. Scholz in March 2011. This trip enabled us to obtain fish cestodes from the lower Brahmaputra basin and to compare them with those collected in the middle Brahmaputra (Assam) and Ganges. Among 243 fish of 29 taxa, fishes of seven species (*Clarias batrachus*, *Clupisoma garua*, *Heteropneustes fossilis*, *Mastacembelus armatus*, *Puntius sophore*, *Sperata seenghala* and *Wallago attu*) were found infected with nine species of cestodes (belonging to Bothriocephalidea, Caryophyllidea and Proteocephalidae) (see Table 2).

### **3.2. Materials studied**

#### **New materials**

This study was mainly based on the fresh material of a total of 18 species of cestodes (Caryophyllidea and Proteocephalidea), which were collected from dissection of around 1,150 freshwater fish from different water bodies (rivers, dam lakes, fish ponds), during sampling expeditions to Bangladesh (Mymensingh and Durgapur) and India (Assam, Sikkim and West Bengal).

In addition, cestodes collected by other researchers from freshwater fish from different parts of the Indomalayan region (Cambodia, India – Maharashtra, and Indonesia) were also included in this study.

(i) Cambodia: Drs. A. de Chambrier, R. Kuchta and T. Scholz, in 2010, collected proteocephalidean cestodes from *Wallago attu*.

(ii) India (Maharashtra – Godavari River basin): Drs. M. Oros (2008) and S.P. Chavan (2010–2012) collected proteocephalidean cestodes from *Wallago attu* and *Sperata seenghala* (Sykes).

(iii) Indonesia: Drs. R. Kuchta and M. Říha, in 2008, collected caryophyllidean cestodes from *Clarias gariepinus*, a catfish of African origin, recently subject to aquaculture in some countries of South East Asia.

### **Type-specimens and museum vouchers**

As mentioned above, most of the types or vouchers of cestodes from Indomalayan freshwater fish were unavailable upon request (almost all written requests to the authors of the original species descriptions and/or to the heads of the departments where specimens should have been deposited to obtain types or voucher specimens remained unanswered). Types or voucher specimens of only the following caryophyllidean and proteocephalidean species were available for this study:

(i) The Natural History Museum, Geneva, Switzerland (courtesy of Drs. A. de Chambrier and J. Mariaux):

- *Caryophyllaeus javanicus* Bovien, 1926. Holotype (MHNG 60963) from *Clarias batrachus* (L.), Java, Indonesia;
- *Caryophyllaeus serialis* Bovien, 1926. Holotype (MHNG 60964) from *C. batrachus*, Java, Indonesia;
- *Djombangia penetrans* Bovien, 1926. Syntypes (MHNG 36035) from *C. batrachus*, Java, Indonesia.

(ii) The Natural History Museum, London, UK (courtesy of Mrs. E. Harris and Dr. D.T.J. Littlewood):

- *Gangesia macrones* Woodland, 1924. Syntypes (BMNH 1927.8.10.3 and 1964.12.15.246–255) from *Sperata seenghala*, India;
  - *Gangesia wallago* Woodland, 1924. Syntypes (BMNH 1927.8.10.1–2 and 1964.12.15.256–280) from *Wallago attu* (Bloch & Schneider), India;
  - *Gangesia sindensis* Rehana and Bilquees, 1971. Not designated explicitly as types but in fact representing syntypes (BMNH 1982.5.13.27) from *W. attu*, Gharo, Pakistan;
  - *Lytocestus birmanicus* Lynsdale, 1956. Holotype (BMNH 1998.10.22.35–36) from *C. batrachus*, Rangoon, Myanmar.
- (iii) U. S. National Parasite Collection, Beltsville, USA (courtesy of Drs. P. Pilitt and E. Hoberg):

- *Crescentovitus biloculus* Murhar, 1963. Holotype (USNPC 70469) from *Heteropneustes fossilis* (Bloch), Nagpur, Maharashtra, India;
- *Lytocestus longicollis* Rama Devi, 1973. Holotype and paratype (USNPC 72796 and 72797) from *C. batrachus*, Visakhapatnam District, Andhra Pradesh, India.
- (iv) School of Studies in Life Sciences, Pt. Ravishankar Shukla University, Raipur, India (courtesy of Dr. A. Niyogi Poddar):
- *Djombangia indica* Satpute and Agarwal, 1974. Holotype from *C. batrachus*, Raipur, India;
- *Introvertus raipurensis* Satpute and Agarwal, 1980. Holotype from *C. batrachus*, Raipur, India;
- *Lucknowia indica* Niyogi, Gupta and Agarwal, 1982. Two specimens (probably syntypes) from *C. batrachus*, Raipur, India.

### **Other comparative materials**

Materials kindly borrowed by the following researchers, from their personal collection were also studied:

**Dr. John S. Mackiewicz** (State University of New York at Albany, New York, USA): “*Bovienia serialis*” vouchers from *C. batrachus*, Nagpur, India (JSM X24.6 and XII.2); 12 specimens of “*B. serialis*” from *C. batrachus* collected by B. M. Murhar in India, including six specimens from Nagpur, Maharashtra (now deposited as HWML 49518 and 49519, ICAS C-353, JSM – not numbered, and USNPC 104233–104235); 6 adult specimens of “*Crescentovitus biloculus*” from *H. fossilis* from India (Nagpur and Selu, Maharashtra, India), collected by B. M. Murhar (now deposited as HWML 49520, IPCAS C-578, JSM – not numbered, and USNPC 104240 and 104241); “*Introvertus raipurensis*” from *C. batrachus*, probably Howrah, West Bengal, India (now deposited as USNPC 104236); two specimens from *C. batrachus*, India, collected by B. M. Murhar and identified as *Clariocestus indicus* n. gen. n. sp. (now deposited as IPCAS C-569 and USNPC 104239); four specimens from *C. batrachus*, India, collected by B. M. Murhar and identified as “*Lytocestus birmanicus*” (now deposited as HWML 49517, IPCAS C-538, and USNPC 104244); eight specimens of “*Lytocestus indicus*” from *C. batrachus*, India, collected by B. M. Murhar (including one from Nagpur, Maharashtra) (now deposited as HWML 49512 and 49513, IPCAS C-541, and USNPC 104237 and 104238); and eight specimens collected by B. M. Murhar from *C. batrachus*, India, identified as “*Lytocestus moghei* n. sp.” (including one from Nagpur, Maharashtra); and four specimens collected by Rama Devi and identified as “*Lytocestus longicollis*” (all from J. S. Mackiewicz’s collection, now deposited as HWML 49514–49516, IPCAS

C-541 and USNPC 104242 and 104243).

**Dr. Anindo Choudhury** (St. Norbert College, De Pere, Wisconsin, USA): one adult specimen of “*Crescentovitus biloculus*” from *H. fossilis*, West Bengal (local ponds around Calcutta), India (now deposited as IPCAS C-578).

**Dr. Ajit Kalse** (North Maharashtra University, Chalisgaon, India): Two specimens of “*Lytocestus indicus*” from *C. batrachus*, Maharashtra, India.

### 3.3. Methods

#### Morphology and histology

Tapeworms were almost always alive when fixed because they were obtained by dissection of fresh, usually purchased live fish at fish markets or provided by local fishermen. Cestodes were gently isolated from the host intestine to avoid loss or damage of the scolices. Specimens used for morphological studies, including observations with scanning electron microscopy (SEM) and histology, were rinsed in saline solution (physiological solution 0.9% NaCl), placed in a small amount of saline solution in a beaker or large vial, and hot, almost boiling 4% formaldehyde solution (10% formalin) was immediately added to keep worms straight and stretched, not contracted or deformed (see Oros et al., 2010 for more data on this fixation procedure). After 2–3 weeks, formalin was replaced by 70% ethanol for storage before further processing of specimens (staining, sectioning and/or preparation for SEM study).

For light microscopy, specimens were stained with Mayer’s hydrochlorid carmine, destained in 70% acid ethanol, i.e., ethanol with several drops of HCl, dehydrated through a graded ethanol series, clarified in clove oil (eugenol), and mounted in Canada balsam as permanent preparations (Scholz and Hanzelová, 1998). Pieces of the strobila and some scoleces were embedded in paraffin wax, sectioned at 12–15  $\mu\text{m}$  (cross sections of the strobila and longitudinal sections of scoleces), stained with Weigert’s haematoxylin and counterstained with 1% acidic eosin B solution (de Chambrier, 2001).

Illustrations were made using a drawing attachment for an Olympus BX51 microscope (Olympus Corporation, Tokyo, Japan) with the use of Nomarski differential interference contrast. Measurements were taken with the aid of analySIS B v.5.0 software (Soft Imaging System – Olympus). Eggs dissected from the uterus were measured and photographed in wet mounts in tap water.



## **Surface ultrastructure**

For SEM studies, specimens were either dehydrated through a graded ethanol series followed by a graded amylacetate series, dried by a critical-point method, sputter-coated with 20–25 nm of gold, or dehydrated through a graded ethanol series, transferred to hexamethyldisilazane (HMDS, see Kuchta and Caira, 2010), dried on air and sputtered with gold. All specimens were examined with a Jeol JSEM 7401F microscope (JEOL Ltd., Tokyo, Japan).

## **Molecular taxonomy and phylogenetics**

In the case of caryophyllidean specimens, the middle parts of selected worms containing only testes and vitelline follicles were fixed with pure ethanol (95–99%) for DNA sequencing before fixing the remaining parts of the body (scolex and anterior part and posterior part containing the ovary, uterus and gonopore(s)) with hot formalin. In the case of bothriocephalidean and proteocephalidean specimens, a small piece, usually a few posteriormost proglottides, was cut off and placed in pure ethanol (95–99%) for DNA sequencing, before fixing the remaining parts of the worm with hot formalin. The latter served as morphological vouchers.

Genomic DNA was extracted using a standard phenol-chloroform protocol (Sambrook & Russell 2001) from 96% ethanol preserved samples. The D1–D3 large subunit nuclear ribosomal RNA gene (18S rDNA) or (28S rDNA) region was amplified by PCR using the primers and conditions described in Brabec et al. (2012). All products were verified on a 1% agarose gel and purified using exonuclease I and shrimp alkaline phosphatase enzymes (Werle et al., 1994). BigDye® Terminator v3.1 cycle sequencing kit and PRISM 3130xl automatic sequencer (Applied Biosystems) were used for bidirectional sequencing of the PCR products using the set of PCR and internal sequencing primers (see Brabec et al., 2012). Sequences were assembled and inspected for errors in Geneious Pro 5.3.6 (Drummond et al., 2010), aligned using the E-INS-i algorithm of the program MAFFT (Kato et al., 2005) and the ambiguously aligned positions were manually excluded from resulting alignments in MacClade 4.08 (Maddison and Maddison, 2005). The phylogenetic relationships were evaluated under the maximum likelihood (ML) criteria in the program RAxML ver. 7.2.8-ALPHA (Stamatakis, 2006; Stamatakis et al., 2008), employing the GTR+ $\Gamma$  substitution model. All model parameters and bootstrap nodal support values (1000 repetitions) were estimated using RAxML.

To verify the correct identification of the definitive hosts, small piece of the muscle of fish infected with cestodes, was fixed with pure EtOH (95–

99%) for DNA sequencing. In the case of barbs (*Puntius* spp.), which were infected with a new caryophyllidean species, genomic DNA was isolated from muscles of these fish from different localities and a 581 bp long fragment of the large mitochondrial ribosomal subunit (*rrnL*) gene was amplified using the primers and the protocol of Lakra and Goswami (2011). PCR products were purified and sequenced as described above. BLAST was used to search GenBank for the most closely matching *rrnL* sequences of *Puntius* spp. and aligned with those characterized within this study. ML analysis was run as described above to search for closely related sequences (see Oros et al., in press).



Figure 1: Map outlining the Indian states, with numbers of described species of cestodes (Caryophyllidea and Proteocephalidea only) from freshwater fish. Inset: maps of Assam and West Bengal with sampling areas of the present study.

**Table 1. List of dissected freshwater fish from India during field expeditions (2009–2011).**

Host	1 <sup>st</sup> exp 2009	2 <sup>nd</sup> exp 2010	3 <sup>rd</sup> exp 2011	Cestode order
<b>Bagridae</b>				
<i>Mystus cf. tengara</i>	2/38	20	3/87	<b>Caryo + Proteo</b>
<i>Mystus cf. cavasius</i>			1/24	<b>Proteo</b>
<i>Rita rita</i>	4/18	9	3/30	<b>Proteo</b>
<b>Channidae</b>				
<i>Channa morilius</i>			1/1	<b>Bothrio</b>
<i>Channa punctata</i>	5/8		4	
<i>Channa striata</i>	9		3	
<i>Channa stewartii</i>			2	
<b>Clariidae</b>				
<i>Clarias batrachus</i>	22/22	4/6	15/18	<b>Caryo</b>
<i>Clarias gariepinus</i>	4			
<b>Cobitidae</b>				
<i>Botia</i> sp.	4		1	
<i>Lepidocephalus guntea</i>	3		12	
<i>Lepidocephalus</i> sp.	1/1			<b>Bothrio</b>
<b>Cyprinidae</b>				
<i>Barilius</i> sp.				
<i>Cirrhinus cirrhosa</i>	2			
<i>Garra</i> sp.	4			
<i>Labio calbasu</i>	1		1	
<i>Osteobrama</i> sp.	2		2	
<i>Puntius sophore</i>	2/34	7/34	4/59	<b>Caryo</b>
<i>Puntius</i> sp.	2/18	27	17	
<i>Puntius ticto</i>	68	40		
<i>Schizothorax</i> sp.	36			
<b>Heteropneustidae</b>				
<i>Heteropneustes fossilis</i>	4/44	10	13/45	<b>Caryo</b>
<b>Mastacembelidae</b>				
<i>Mastacembelus armatus</i>	1/3		2/4	<b>Bothrio</b>
<b>Notopteridae</b>				
<i>Notopterus notopterus</i>	1		1	
<b>Schilbeidae</b>				
<i>Clupisoma garua</i>	5/12		8	
<i>Eutropiichthys vacha</i>	39	4	5	
<b>Siluridae</b>				
<i>Ompok</i> sp.	1/12		33	<b>Proteo</b>
<i>Sperata aor</i>	16		7	
<i>Sperata seenghala</i>	1		8	
<i>Wallago attu</i>	5/8		4/7	<b>Proteo</b>
<b>Sisoridae</b>				
<i>Bagarius</i> sp.	1/1		4	<b>Proteo</b>
<b>Synbranchidae</b>				
<i>Monopterusuchia</i>			1/7	<b>Bothrio</b>
<b>Total</b>	<b>409</b>	<b>150</b>	<b>390</b>	

Note: Fish found with cestodes in bold; in case of infection number is expressed by - infected fish / total fish examined; Caryo – Caryophyllidea; Bothrio – Bothriocephalidea and Proteo – Proteocephalidea.

Table 2. List of dissected freshwater fish from Bangladesh during field expedition (2011).

Host	Numbers	Cestode order
<b>Bagridae</b>		
<i>Mystus cf. cavasius</i>	7	
<i>Rita rita</i>	2	
<b>Channidae</b>		
<i>Channa marulius</i>	1	
<i>Channa punctata</i>	13	
<i>Channa striata</i>	3	
<i>Channa gachua</i>	3	
<b>Clariidae</b>		
<b><i>Clarias batrachus</i></b>	<b>7/7</b>	<b>Caryophyllidea</b>
<i>Clarias gariepinus</i>	4	
<b>Cobitidae</b>		
<i>Botia dario</i>	2	
<i>Lepidocephalus guntea</i>	4	
<b>Cyprinidae</b>		
<i>Amblypharyngodon mola</i>	2	
<i>Cirrhinus cirrhosa</i>	2	
<i>Labio calbasu</i>	1	
<i>Osteobrama</i> sp.	2	
<b><i>Puntius sophore</i></b>	<b>11/95</b>	<b>Caryophyllidea</b>
<i>Puntius conchoniuis</i>	16	
<i>Puntius ticto</i>	17	
<b>Gobiidae</b>		
<i>Glossogobius giuris</i>	5	
<b>Heteropneustidae</b>		
<b><i>Heteropneustes fossilis</i></b>	<b>1/17</b>	<b>Caryophyllidea</b>
<b>Mastacembelidae</b>		
<b><i>Mastacembelus armatus</i></b>	<b>1/7</b>	<b>Bothriocephalidea</b>
<b>Nandidae</b>		
<i>Nandus nandus</i>	5	
<b>Notopteridae</b>		
<i>Notopterus notopterus</i>	2	
<b>Schilbeidae</b>		
<b><i>Clupisoma garua</i></b>	<b>1/1</b>	<b>Proteocephalidea</b>
<i>Eutropiichthys vacha</i>	12	
<b>Siluridae</b>		
<i>Ompok</i> sp.	2	
<i>Sperata aor</i>	2	
<b><i>Sperata seenghala</i></b>	<b>1/3</b>	<b>Proteocephalidea</b>
<b><i>Wallago attu</i></b>	<b>1/4</b>	<b>Proteocephalidea</b>
<b>Sisoridae</b>		
<i>Bagarius</i> sp.	2	
<b>Total</b>	<b>243</b>	

Note: Fish found with cestodes in bold; in case of infection number is expressed by - infected fish / total fish examined.

## 4. RESULTS

### 4.1. Critical re-evaluation of species original descriptions and examination of museum material

Type-materials of most of the taxa described from Indian subcontinent have not been available and it is not known to exist. Consequently, the validity of these species had to be assessed solely by comparison of the data from the original descriptions, which, however, suffered from many flaws and deficiencies, which are listed here:

(i) Most, if not all, taxa were described on the basis of inadequately fixed, deformed, or decomposed specimens (e.g. Fig. 2).

(ii) Most species descriptions were less than satisfactory, usually incomplete, and did not include information from cross-sections, which is crucial for assignment to a superfamily, family, or subfamily (Freze, 1965; Mackiewicz, 1994; Rego, 1994).

(iii) Some measurements appear apparently erroneous, as obvious from discrepancies between the text and figures, and good-quality, realistic illustrations (line drawings) were almost always missing (see e.g. Fig. 2).

(iv) The morphology of the tapeworms has been frequently misinterpreted, e.g., ovarian follicles have been confused with postovarian vitelline follicles; swollen vas deferens filled with sperm has been identified as an external seminal vesicle; subtegumental cells have been misinterpreted as vitelline follicles, etc.

(v) Species have been differentiated on the basis of questionable taxonomic characters, such as negligible differences in the number of testes, unspecified shape of the cirrus-sac, etc.; frequently these differential characteristics overlapped between differentiated taxa.

(vi) The authors generally ignored previously published data, including those from the same region e.g., *Lytocestus clariasae* Jadhav and Gavahne, 1991 and *L. clariasae* Pawar and Shinde, 2002 were described under the same name, the latter thus becoming a homonym of the former.

(vii) Several new species were proposed in abstracts from national conferences or in PhD theses, thus failing to comply with the requirements of the International Code of Zoological Nomenclature and makes these names invalid as *nomina nuda* (see **Papers 1, 2 and 6** for details).

As stated earlier, most of the types and voucher specimens could not be obtained (if they have been allegedly deposited, they are never borrowed upon request), but those few that were available (see a list of type specimens and museum vouchers in the materials studied) helped us considerably in reaching taxonomic conclusions and clarifying taxonomic status of some species, especially in *Bovienia* Fuhrmann, 1931 (see **Papers 1, 2 and 6**).

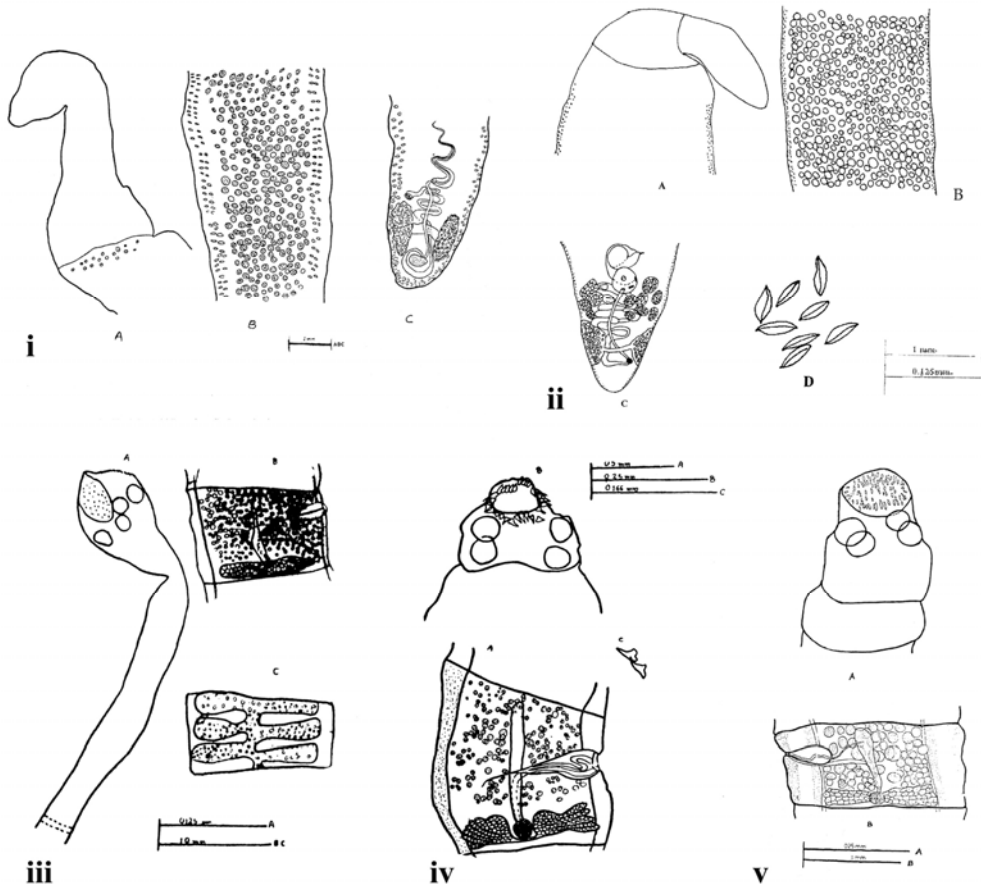


Figure 2: Original line drawings of few caryophyllidean and proteocephalidean cestodes described from Indian freshwater fish. i. *Lytocestus govindae* Patil and Jadhav, 2002; ii. *Lytocestus murhari* Kaul, Kalse and Suryawanshi, 2010; iii. *Silurotaenia behairvnathi* Deshmukh and Shinde, 1989; iv. *Silurotaenia tictoi* Shinde, Kadam and Jadhav, 1984; v. *Silurotaenia macroni* Shinde, Kadam and Jadhav, 1984.

## 4.2. Evaluation of the newly obtained materials of fish cestodes using morphological, ultrastructural and molecular methods

Successful sampling of extensive material of fish cestodes enabled us to obtain reliable data on their morphology including surface ultrastructure and DNA sequences in some groups. This made it possible to provide new, robust data on several taxa whose morphology was not sufficiently known. Molecular data (DNA sequences) justified some of the taxonomic conclusions.

Detailed data on surface morphology (distribution and types of microtriches; shape of the scolex) of most species redescribed (see 4.3) were provided for the first time, with the aid of scanning electron microscopy (SEM) (**Papers 1–6**). Use of SEM also enabled us to reliably describe the surface structure of the eggs, including the presence of an operculum in caryophyllidean cestodes, which may be easily overlooked in eggs observed in utero using only light microscopy (**Papers 1–3**).

The present study has demonstrated the suitability of several morphological characteristics for species and generic differentiation, based on properly fixed material. It was found that in caryophyllidean cestodes from walking and stinging catfish, the shape of the body and scolex, the length of the neck, the position and shape of the ovary, and the extent of vitelline follicles represent suitable characteristics to distinguish genera and species (**Papers 1 and 2**).

Application of a multidisciplinary approach was also crucial for the revision of proteocephalideans parasitizing catfish, especially *Wallago attu* and *Sperata seenghala* (**Paper 6**). It was found that the following characteristics are of taxonomic importance: (i) number of rows of rostellar hooks and their size; (ii) number of rows of hooklets on the suckers; (iii) ratio of the width of the rostellum-like organ and diameter of the suckers; (iv) relative length of the ovary, i.e. ratio of its length to the length of proglottis; (v) width of the scolex; (vi) diameter of the suckers; (vii) relative width of ventral osmoregulatory canals, i.e. ratio of their width to the width of the proglottis; and (viii) types of microtriches on suckers. A table with differential characters of all species from the Indomalayan Region is also provided, together with a key to identification of the genera of the subfamily *Gangesiinae* Mola, 1929 (**Paper 6**).



### 4.3. Assessment of the validity of the nominal species described from India and redescriptions of the taxa considered valid

After analysing caryophyllideans from the walking catfish *Clarias batrachus* in the Indomalayan Region using morphological methods, this study revealed that only eight taxa of the family Lytocestidae are valid, instead of 59 nominal taxa of 15 genera from three caryophyllidean families. All other are nothing but synonyms of the following taxa (see Table 3): *Bovienia indica* (Niyogi, Gupta and Agarwal, 1982) n. comb.; *B. serialis* (Bovien, 1926); *B. raipurensis* (Satpute and Agarwal, 1980) n. comb.; *Djombangia penetrans* (Bovien, 1926); *Lucknowia microcephala* (Bovien, 1926) n. comb.; *Lytocestus indicus* (Moghe, 1925); *Pseudocaryophyllaeus ritai* Gupta and Singh, 1984 (resurrected); and *P. tenuicollis* (Bovien, 1926) n. comb. All valid taxa were redescribed and an identification key was provided (**Paper 1**).

Nine caryophyllidean species of seven genera (*Capingentoides* Gupta, 1961; *Lucknowia* Gupta, 1961; *Lytocestus* Cohn, 1908; *Pseudoadenoscolex* Mathur and Srivastav, 1994; *Pseudocaryophyllaeus* Gupta, 1961; *Pseudoheteroinverta* Srivastav and Khare, 2005; and *Sukhpatatae* Srivastav, Khare and sahu, 2007) parasitising the stinging catfish *Heteropneustes fossilis* (Siluriformes: Heteropneustidae) were critically reviewed and newly synonymised with *Lucknowia fossilisi* Gupta, 1961. Previous synonymies of other nine species proposed by Hafeezullah (1993) were confirmed and the generic diagnosis of *Lucknowia* Gupta, 1961 was amended. In addition, one species of *Pseudobatrachus* Castelnau, 1875 and two species of the monotypic genera *Pseudoneckinverta* Srivastav, Narayan and Singh, 2011 and *Sudhaena* Srivastav, Khare, Sahu and Yadav, 2011 were invalidated as *nomina nuda* (**Paper 2**) (see also Table 3).

Hyperapolytic cestodes found in the catfish *Clupisoma garua* (Siluriformes: Schilbeidae) were identified as *Vermaia pseudotropii* (Verma, 1928). This poorly known species of phylogenetic importance (i.e. the only hyperapolytic proteocephalidean, which possesses a rostellum-like organ with large hooks, thus closely resembling taeniids; see Freeman, 1973), was redescribed (**Paper 4**).

*Gangesia* was one of the most species-rich genera of proteocephalidean cestodes with as many as 53 nominal taxa. However, a complex study based on molecular and morphological data has demonstrated that the number of valid species in India is in fact considerably lower because as many as 40 species were invalidated. Based on the present revision, the genus consists only of *G. agraisensis* Verma, 1928; *G. bengalensis* (Southwell, 1918); *G. macrones* Woodland, 1924; *G. margolisi* Shimazu, 1994; *G. oligonchis*

Roitman and Freze, 1964; *G. parasiluri* Yamaguti 1934; *G. polyonchis* Roitman and Freze, 1964; *G. pseudobagrae* Chen, 1962; and *G. vachai* (Gupta and Parmar, 1988) n. comb. The present study has also revealed that all Indian species of *Silurotaenia* are invalid, having been synonymised with some of the four species of *Gangesia* (**Paper 6**) (see also Table 4).

#### **4.4. Clarification of the host spectrum of the selected model groups of fish cestodes**

Literary data indicated that some fish harboured extraordinarily rich fauna of cestodes, but the present study did not confirm these data and clarified host specificity of several taxa.

A critical revision of caryophyllidean cestodes of the walking catfish (locally known as Magur), *Clarias batrachus* (Siluriformes: Clariidae), has revealed that the number of the valid species is much lower (eight species of five genera) than the number of nominal species (as many as 59 species of 15 genera were reported in the literature). Among these eight valid species, three *Bovienia* spp. seem to be specific to the *C. batrachus*. The remaining five taxa have also been found in other catfishes, especially in *Heteropneustes fossilis* (Siluriformes: Heteropneustidae), but *C. batrachus* is always the most-heavily parasitized host, as indicated by a higher prevalence than in other catfish. Only one species, *Lucknowia fossilisi* Gupta, 1961 (Lytocestidae), instead of 19 taxa reported by Indian authors, is a specific parasite of *H. fossilis* (**Papers 1 and 2**).

The present study has also shown the newly described taxon, *Lobulovarium longiovatum* (Cestoda: Caryophyllidea), from the pool barb, *Puntius sophore* (Cypriniformes: Cyprinidae), can infect phylogenetically closely related barbs, but not the much more abundant occurring in sympatry unrelated barbs (**Paper 3**). Photos of all hosts were uploaded on the web of the Global Cestode Database (<http://teleosts.tapewormdb.uconn.edu>) and *rnl* sequences (two new) were submitted to GeneBank for future reference.

In the silurid catfish *Wallago attu*, only two species of *Gangesia* Woodland, 1924, namely *G. bengalensis* (Southwell, 1913) and *G. agraensis* Verma, 1928, occur commonly, which was first noticed by Verma (1928). Another economically important catfish, *Sperata seenghala*, has also been reported to host a number of proteocephalidean species, but it seems that the only valid species that is specific to *S. seenghala* is *Gangesia macrones* described by Woodland (1924). Another species, *Gangesia vachai* (Gupta and Parmar, 1988) has been found in catfish of as many as three families, but more data are necessary to clarify the host specificity and distribution of this apparently uncommon species. *Gangesia* tapeworms have also been recorded

by Indian authors from non-siluriform fish, such as cyprinids (*Cirrhina*, *Labeo*, *Puntius*) and the mastacembelid, *Mastacembelus armatus*. However, all these findings are doubtful because no vouchers of any of these remarkable, but suspicious findings have been deposited. Misidentification of fish hosts (cyprinids and zig-zag eel versus siluriforms) is impossible but mislabelling of samples cannot be excluded (**Paper 6**).

#### 4.5. Unravelling phylogenetic relationships of the cestodes studied

Due to time limitation, molecular markers (DNA sequences) were used for the assessment of the validity and phylogenetic relationships of fish cestodes in two studies.

Twenty-one 1556–1644 bp long lsrDNA sequences of *Gangesia* were characterized in a revision of the *Gangesia* cestodes, within the scope of this study, including samples of two *Gangesia* spp. from other than the Indomalayan geographical region (*G. parasiluri* from *Silurus asotus*, Japan; *Gangesia* cf. *polyonchis* from *Tachysurus fulvidraco*, Russia). In addition, all remaining genera of the subfamily Gangesiinae (*Electrotaenia* Nybelin, 1942 from Africa; *Postgangesia* Akhmerov, 1969 from the Palaearctic region; *Ritacestus* de Chambrier, Scholz, Ash and Kar, 2011; and *Vermaia* Nybelin, 1942, both from India) were sequenced and their sequences were combined with those available in GenBank. A phylogenetic analysis has shown that three Indomalayan species, namely *G. bengalensis*, *G. macrones* and *G. vachai*, form a monophyletic group within *Gangesia*, whereas the fourth species from the Indomalayan Region, *G. agraensis*, forms a clade with the Palaearctic species of the genus, even though this species and *G. bengalensis* parasitize the same fish host, *Wallago attu*. **Paper 6**.

Molecular data (partial lsrDNA sequences) indicate that the new taxon, *Lobulovarium longiovatum* (Cestoda: Caryophyllidea) from barbs (Cypriniformes), belongs to the most basal caryophyllidean cestodes, being unrelated to any species from siluriform catfish in the Indomalayan Region. Its distinct phylogenetic position among caryophyllideans well corresponds to the possession of some unique morphological traits, such as an irregularly-shaped, lobular ovary and long, narrow eggs (**Paper 3**).

**Table 3. Updated list of species of the Caryophyllidea described from freshwater fish (Teleostei) from the Indomalayan Region.**

Valid taxon	Synonym	Typical host	Reference
Family Caryophyllaeidae			
<i>Paracaryophyllaeus leptocephali</i> (Kundu, 1985)	<i>Lytocestoides leptocephali</i> Kundu, 1985	<i>Lepidocephalus gunita</i>	Hafeezullah, 1993
Family Capingentidae			
<i>Adenoscolex oreini</i> Fotedar, 1958		<i>Schizothorax plagiotomus</i>	Hafeezullah, 1993
<i>Breviscolex aurangabadensis</i> (Shinde, 1970)	<i>Lytocestoides aurangabadensis</i> Shinde, 1970	<i>Hypselobarbus kolus</i> and <i>Labeo calbasu</i>	Hafeezullah, 1993
<i>Breviscolex naldurgensis</i> (Shinde, Moheker, Jadhav and Hafeezullah, 1987)	<i>Lytocestoides naldurgensis</i> Shinde, Moheker, Jadhav and Hafeezullah, 1987 <i>Lytocestoides mackiewiczzi</i> Shinde, Moheker, Jadhav and Hafeezullah, 1987	<i>Cirrhinus cirrhosus</i>	Hafeezullah, 1993
Family Lytocestidae			
<b><i>Bovienia indica</i></b> (Niyogi, Gupta and Agarwal, 1982)	<i>Lucknowia indica</i> Niyogi, Gupta and Agarwal, 1982 <i>Heeradevina barnasagarensis</i> Srivastav and Khare, 2005 <i>Lytocestus attenuatus</i> Tandon, Chakravarty and Das, 2005 <i>Lytocestus clariae</i> Tandon, Chakravarty and Das, 2005	<i>Clarias batrachus</i>	Ash et al. 2011a Ash et al. 2011a Ash et al. 2011a Ash et al. 2011a
<b><i>Bovienia raipurensis</i></b> (Satpute and Agarwal, 1980)	<i>Bovienia nagpurensis</i> Murhar, 1972 ( <i>nomen nudum</i> ) <i>Introvertus raipurensis</i> Satpute and Agarwal, 1980 <i>Lucknowia raipurensis</i> (Satpute and Agarwal, 1980) Schmidt, 1986 <i>Bovienia ilishai</i> Zaidi and Khan, 1976 <i>Capingentoides faizabadensis</i> Pande, Dubey and Mittal, 2007	<i>Clarias batrachus</i>	Ash et al. 2011a Ash et al. 2011a Ash et al. 2011a Ash et al. 2011a Ash et al. 2011a Fuhmann, 1931
<b><i>Bovienia serialis</i></b> (Bovien, 1926)		<i>Clarias batrachus</i>	





**Table 3 – continued**

<b>Valid taxon</b>	<b>Synonym</b>	<b>Typical host</b>	<b>Reference</b>
<i>Pseudocaryophyllaeus ritai</i> Gupta and Singh, 1983	<i>Pseudocaryophyllaeus lucknowensis</i> Gupta and Sinha, 1984 <i>Lytocestus marathwadaensis</i> Shinde and Phad, 1986 <i>Pseudobatrachus moolchandrai</i> Srivastav, Sahu and Khare, 2006	<i>Clarias batrachus</i>	Ash et al. 2011a Ash et al. 2011a Ash et al. 2011a
<i>Pseudocaryophyllaeus tenuicollis</i> (Bovien, 1926)	<i>Caryophyllaeus tenuicollis</i> Bovien, 1926 <i>Lytocestus birmanicus</i> Lynsdale, 1956 <i>Pseudocaryophyllaeus indica</i> Gupta, 1961 <i>Capingentoides batrachii</i> Gupta, 1961 <i>Lytocestus parvulus</i> Furtado 1963 <i>Lytocestus moghei</i> Murhar, 1972 ( <i>nomen nudum</i> ) <i>Lytocestus longicollis</i> Rama Devi, 1973 <i>Pseudocaryophyllaeus mackiewiczii</i> Gupta and Parmar, 1984 <i>Pseudobatrachus chandrai</i> Pathak and Srivastav, 2005 <i>Lytocestus bokaronensis</i> Poonam, 2007 <i>Lytocestus majumdari</i> Poonam, 2007 <i>Pseudobatrachus chhatrasalli</i> Sahu, Srivastav and Bagel, 2009 <i>Pseudocaryophyllaeus chandurensis</i> Jaysingpure, 2009	<i>Clarias batrachus</i>	Ash et al. 2011a Ash et al. 2011a Ash et al. 2011a Ash et al. 2011a Ash et al. 2011a Ash et al. 2011a Ash et al. 2011a Ash et al. 2011a Ash et al. 2011a Ash et al. 2011a Ash et al. 2011a Ash et al. 2011a Ash et al. 2011a Ash et al. 2011a Ash et al. 2011a

Note: Taxa not in bold were considered as valid by Hafeezullah (1993); they were not available in this study to critically review their validity. \* – validity of these taxa are doubtful (see also Hafeezullah, 1993).





**Table 4 – continued**

<b>Valid taxa</b>	<b>Synonyms</b>	<b>Typical host</b>	<b>Reference</b>
	<i>Gangesia rohita</i> Shinde, Mahajan and Begum, 1999 <i>Gangesia seenghali</i> Hiware, 1999 <i>Gangesia rohita</i> Pawar, Lakhe, Shinde and Patil, 2004 (homonym) <i>Gangesia ramkaei</i> Pawar and Hiware, 2008 <i>Gangesia bendsurensis</i> Reddy, Wankhede, Dhole and Anand, 2011 <i>Silurotaenia tictoi</i> Shinde, Kadam and Jadhav, 1984.		§ present study § present study § present study § present study § present study
<b><i>Gangesia macrones</i> Woodland, 1924</b>	<i>Silurotaenia paithanensis</i> Shinde, Majid and Solunke, 1983 <i>Silurotaenia barbubus</i> Shinde, Kadam and Jadhav, 1984 <i>Silurotaenia macroni</i> Shinde, Kadam and Jadhav, 1984 <i>Silurotaenia singhali</i> Shinde, Kadam and Jadhav, 1984 <i>Silurotaenia tictoi</i> Shinde, Kadam and Jadhav, 1984 <i>Silurotaenia behairvathi</i> Deshmukh and Shinde, 1989 <i>Silurotaenia shastri</i> Gavhane and Jadhav, 1991 <i>Silurotaenia raoii</i> Bhure, Pathan, Nanware and Dhondge, 2010	<i>Sperata seenghala</i>	§ present study § present study § present study § present study § present study § present study § present study
<b><i>Gangesia vachai</i> (Gupta and Parmar, 1988)</b>	<i>Silurotaenia vachai</i> Gupta and Parmar, 1988	cat fish	§ present study
<b><i>Ritacestus ritaii</i> (Verma, 1926)</b>	<i>Proteocephalus ritaii</i> Verma, 1926	<i>Rita rita</i>	de Chambrier et al., 2011
<b><i>Vermaia pseudotropii</i> (Verma, 1928)</b>	<i>Gangesia pseudotropii</i> Verma, 1928 <i>Gangesia sumani</i> Shinde & Wankhede, 1990	<i>Clupisoma garua</i>	Nybelin, 1942 Ash et al., 2010
<i>Vermaia sornakowahi</i> Zaidi and Khan, 1976		<sup>y</sup> <i>Scoliodon laticaudus</i>	Ash et al., 2010
Subfamily Proteocephalinae		<i>Bagarius yarrelli</i>	de Chambrier et al., 2011
* <i>Proteocephalus vitellaris</i> Verma, 1928			

Note: Validity of taxa in bold was evaluated in this study; § – see Paper 6 (Ash et al., in preparation); <sup>y</sup> – doubtful host (see Ash et al., 2010, i.e. Paper 4); \* – Probably belongs to *Ritacestus* (see de Chambrier et al., 2011, i.e. Paper 5).

## 5. GENERAL DISCUSSION

As mentioned in the beginning, Dayrat's (2005) view was implemented in the context of the present study of Indian freshwater fish cestodes. Even though a large number of specimens were made available and examined in the course of the present study, only one new species, for which a new genus was proposed, was described. In contrast, almost all cestodes could be easily accommodated to already known taxa, most of them having been described by Bovien (1926) from Indonesia (Caryophyllidea) and Southwell (1913) and Verma (1928) from India (Proteocephalidea). Surprisingly, the oldest papers, such as those by Woodland (1924), Moghe (1925, 1931), Bovien (1926), Verma (1926, 1928), Lynsdale (1956), and Gupta (1961), provided much better and more complete morphological descriptions than the taxonomic accounts published during the last four decades. The present study has also shown that, remarkably, a large number of species described from India are not valid (e.g. as many as 86% of the nominal species of caryophyllidean cestodes described from *Clarias batrachus* are invalid). The most recent species considered to be valid, *Gangesia vachai*, was described almost 24 years ago. Inappropriate handling of specimens has led to significant misinterpretations of individual structures and has provided unreliable and often incorrect data regarding the morphology of the worms. The present study also confirms that properly fixed material is a key factor and prerequisite for reliable taxonomic research..

The present study has contributed, to some extent, to a better understanding of the systematics of the Caryophyllidea and Proteocephalidea described from freshwater fish in India (see Tables 1 and 2). However, considerable gaps still exist in our knowledge of the diversity and species composition of fish cestodes in the Indian subcontinent. In addition, there are still vast areas, where ichthyoparasitological surveys oriented at the fish parasites have not been carried out.

For example, some cestodes, one caryophyllid and one proteocephalid precisely, from catfish *Mystus* sp. and *Ompok* sp., respectively were collected during the field expeditions in India. Preliminary results were indicated that they may belonged to new taxa, but the insufficient material impeded us to reach taxonomic decision.

Molecular study of caryophyllidean taxa has been partially done in the scope of this study. But detailed molecular study, including phylogenetic analyses, of all valid taxa from walking and stinging catfish will be carried out in the near future.

Bothriocephalidea is another species-rich order from the Indian subcontinent. According to Kuchta and Scholz (2007) the species complexes

of *Bothriocephalus* and *Senga* described from the Indomalayan region require a critical revision. During this project several cestodes belonging to order Bothriocephalidea were collected from freshwater fish and preliminary examination has indicated that the number of valid species is much lower than that of the nominal taxa, which fully corresponds to the situation in the two other cestode orders, i.e. Caryophyllidea and Proteocephalidea. Due to shortage of time it was not possible to evaluate these specimens, but the materials are ready for future complex studies.

This project was probably the first of this kind in India, at least in the case of cestodes, thus had been confined to a comparatively smaller scale (in the context of the geographical area of India). The Northern part, especially Kashmir region (report of a valid member of family Capingentidae, rare in the subcontinent) and whole Southern part (among the world's ten "Hottest biodiversity hotspots"), which hosts some endemic catfish, were not explored in the current project. The future plan envisages enlarging our construal by continuing the pending studies along with the panned exploration of the unexplored regions in the same way. Final aim will be not to confine this research just into cestodes but to spread it in all helminth groups.

## 6. CONCLUSIONS & PERSPECTIVES

The present study has shown that overwhelming majority of literary data from India needs to be handled with caution, because morphological descriptions are poor or erroneous, overlook basic rules of taxonomy and systematics or even violate the International Code of Zoological Nomenclatures (ICZN). It has also revealed that the number of valid species parasitic in freshwater fishes in India is significantly lower than that of the nominal taxa (see Table 3 and 4). In contrast, the cestode fauna of Indomalayan freshwater fish is still poorly known, which is demonstrated by the finding of a new caryophyllidean tapeworm from barbs (*Puntius* spp.), for which a new genus is proposed. In the future, more attention should be paid to less studied fish hosts, instead of describing “new” species or even “new” genera of caryophyllideans from *Clarias batrachus* and *Heteropneustes fossilis*.

Interesting patterns in host specificity, distribution and phylogenetic relationships of some studied taxa were observed, which indicates that the Indomalayan fauna of cestodes represents an important part of cestode diversity in the globe (see <http://tapewormdb.uconn.edu>).

For future research, it is strongly recommended that any taxonomic study on cestodes should be based on the evaluation of well-fixed material (see Cribb and Bray, 2010; Oros et al., 2010; Justine et al., 2012). Detailed morphological examination should be carried out using good quality optics, histological sections should always be provided and morphological variability should be assessed in order to reach justified descriptions of the new species. Recommendations of Dayrat (2005) should be followed whenever possible, especially the first five points (see Introduction). Type- and voucher specimens must be deposited in internationally recognized collections, from which material can be readily borrowed.

Future projects should be aimed at conducting fieldwork with the focus on unexplored areas and less studied hosts. Modern molecular methods should be applied in systematic studies to bring the knowledge of the India’s helminth fauna to the level corresponding to the current global knowledge. Simultaneously, young people should be trained in proper application of various modern methods of taxonomy, systematics, and phylogenetics using suitable model organisms. The main emphasis should be given to building a network of specialists all over India. Furthermore, reliable data on various hosts and their helminth parasites shall be incorporated into publicly available on-line database resources, which will ensure their availability for anybody dealing with parasites and their impact on hosts and ecosystems, including farmed animals and aquaculture.

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## 8. PUBLISHED PAPERS & MANUSCRIPT IN PREPARATION

### Caryophyllidea

#### 8.1. Paper 1.

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**Tapeworms (Cestoda: Caryophyllidea), Parasites of *Clarias batrachus* (Pisces: Siluriformes) in the Indomalayan Region**

**Ash A., Scholz T., Oros M., Kar P. K.**

*Journal of Parasitology* (2011) 97: 435–59

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## 8.2. Paper 2.

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**Cestodes (Caryophyllidea) of the stinging catfish  
*Heteropneustes fossilis* (Siluriformes: Heteropneustidae)  
from Asia**

**Ash A., Scholz T., Oros M., Levron C., Kar P. K.**

*Journal of Parasitology* (2011) 97: 899–907

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### 8.3. Paper 3.

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**A new monozoic tapeworm, *Lobulovarium longiovatum* n. g.,  
n. sp. (Cestoda: Caryophyllidea), from barbs *Puntius* spp.  
(Teleostei: Cyprinidae) in the Indomalayan region**

Oros M., Ash A., Brabec J., Kar P. K., Scholz T.

*Systematic Parasitology* (2012) in press

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## Proteocephalidea

### 8.4. Paper 4.

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**Redescription of *Vermaia pseudotropii*, a hyperapolytic freshwater tapeworm, and composition of *Vermaia* Nybelin, 1942  
(Cestoda: Proteocephalidea)**

**Ash A.**, de Chambrier A., Scholz T., Kar P. K.

*Revue Suisse de Zoologie* (2010) 117, 665–677

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## 8.5. Paper 5.

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***Ritacestus* n. gen. (Cestoda: Proteocephalidea) and  
redescription of *R. ritaii* n. comb., a parasite of *Rita rita*  
(Siluriformes) in India**

de Chambrier A., Scholz T., **Ash A.**, Kar P.K.

*Folia Parasitologica* (2011) 58, 279–288

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## 8.6. Paper 6.

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### **Revision of *Gangesia* (Cestoda: Proteocephalidea) in the Indomalayan Region: morphology, molecules and surface ultrastructure**

**Ash A.**, Scholz T., de Chambrier A., Brabec J., Oros M.,  
Kar P. K., Chavan S. P., Mariaux J.

Manuscript in advanced preparation; target journal:  
*Plos one*

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