Chapter 3

MATERIALS AND METHODS

3.1. Brief Description of Families and Species Selected for the Study

The order Perciformes is the largest and most diversified of all fish orders. The classification of this order is unsettled and will certainly be subject to changes that will better reflect the evolutionary history of the higher percomorphs. Only the families to which the species under investigation belong have been discussed under this section.

Fishes of family Ambassidae exhibit greater diversity in which freshwater forms are morphologically and biologically more diverse than marine ones. Many species with semitransparent body and large scales, but Chanda nama have very fine scales (125 in lateral series). About 21 of the approximately 46 species are confined to freshwater (Talwar and jhingran, 1991; Roberts, 1989 and 1994; Kottelat, 1995 and 2003; Anderson and Heemstra, 2003; Nelson, 2006). Only three genera with six species are included in this study, viz., Ambassis commersoni, Chanda nama, Parambassis thomassi, Parambassis dayi, Parambassis ranga and Parambassis lala.

Sillaginidae is a small family of fishes of sandy shores and estuarine waters, confined to the Indo-West Pacific region. It comprises of only three genera and thirty one species (McKay, 1992), of which many are with dubious identity. They are elongate, slightly compressed fish, often light brown to silver in colour with a variety of markings and patterns on their upper body. For my study I have taken Genus Sillago with two species S. sihama and S. vincenti, which are reported from freshwater also.

There are eight genera with about 44 species recorded in family Gerreidae (Iwatsuki et al., 2002; Ruiz-Carus and Uribe-Alcocer, 2003; Gilmore and Greenfield, 2003). The fishes of this family have a protrusible mouth and are
easily recognised by visible scales, which also cover the head. One genus *Gerres* with two species *G. filamentosus* and *G. abbreviatus* have been added to the study, which are basically brackishwater species, but enter rivers and caught even from upstreams.

Family *Nandidae* has been split in various ways by past authors and remain a poorly-diagnosed group of freshwater percomorphs (Britz, 1997), with membership recently restricted to a single Asian genus, *Nandus* (Kullander and Britz, 2002). The nandids exhibit extreme morphological specializations correlated with exceptionally well-developed predatory habits. This family comprises a fascinating group of relatively small fishes. The species included in the study are *Nandus nandus*, *Nandus marmoratus*, *Badis badis*, *Badis assamensis* and *Dario dario*.

The fishes of family *Pristolepidae* inhabit a small area of peninsular India and have been recognized with one genus, *Pristolepis*, with about three species. Two species *Pristolepis rubripinnis* (Britz *et al*., 2012) and *Pristolepis marginata* have been included for the study.

Terapons belonging to the family *Terapontidae* inhabit Indo-Pacific inshore marine and brackishwaters, some also enters the freshwaters. One genus with three species has been taken into account for the study, viz., *Terapon jarbua*, *Terapon puta* and *Terapon theraps*.

Family *Cichlidae* is the second largest and one of the most speciose and ecological diverse groups of freshwater fishes inhabiting the tropical zone among the Perciforms. The South Asian cichlids, endemic to southern India and Sri Lanka comprise the only genus *Etroplus*, with three valid species. *Etroplus* with three species have been used for this study, viz., *E. maculatus*, *E. suratensis*, *E. canarensis*.

Species of family *Scatophagidae* occasionally enter freshwater. The family gets its name from their habit of including human faeces in the diet. Two genera with four species are recognized (Parenti, 2004). *Scatophagus argus* has been investigated in the present study.

Fishes of the family *Anabantidae* inhabit freshwaters of the Indo-west Pacific. Four genera with 33 species (Norris and Douglas, 1992) are recognized. Most of the species occur in Africa. A single genus *Anabas* occurs in Asia. Fishes of genus *Anabas* from three regions have been included for the study.
Family *Belontidae* (Talwar and Jhingran, 1991; Jayaram, 1999) is the largest and most diverse family of anabantoids, with 3 subfamilies, 11 genera and more than 30 species. The fishes of this family are known for their beauty and many of them have been kept as pets in the aquaria. Two species of genus *Pseudosphromenus* viz., *P. cupanus* and *P. dayi*, three species from genus *Colisa* viz., *C. sota*, *C. lalia* and *C. fasciatus* have been studied in this thesis.

The family *Channidae* consists of only two genera, Asian *Channa* and African *Parachanna*. The Asian genus *Channa*, which presently contains 26 valid species, is widely distributed in the Indian subcontinent (Musikasinthorn, 2000, 2003; Berra, 2001; Courtenay and Williams, 2004). Fishes of this family can be recognised by the shape of the head which resembles that of a snake. The sneakheads inhabit mainly permanent shallow, lentic waters (ponds and lakes) and rivers. Three species of *Channa* viz., *C. marulius*, *C. striata* and *C. diplogramma* have been used for the study.

The species *Mugil cephalus* from the order Mugiliformes was choosen as outgroup to represent divergence at higher systematic levels.

### 3.1.1. Family: *Ambassidae*

**Ambassis commersoni** Cuvier, 1828  
**Synonym:** *Ambassis commersonii* Cuvier 1828  
**Common Name:** Commerson’s glassy perchlet  
**Diagnosis:** Body is elongate and fairly broad with massive head and a prominent chin. Supraorbital ridge is smooth terminating with a spine. Preorbital ridge, suborbital and interoperculum margins are entire, not serrated. Lateral line complete with 27-30 scales. Cheek is with 2 transverse rows of scales.  
**Geographical Distribution:** Indo-west Pacific. Benthopelagic. It inhabits the sea and estuaries, ascending rivers and brackishwater lakes. Common in Maharashtra, Kerala and Tamilnadu.  
**Habitat:** Freshwater, brackish, marine.  
**Economic Importance:** These fishes are caught in fairly large numbers during June and September along the coasts, river mouths and estuaries, particularly in Kerala. They are used as food as they are cheap and tasty.
and also as bait for capturing other economically important fishes. These fishes play a significant role as a source of abundant cheap protein food to the poorer classes.

*Chanda nama* Hamilton, 1822  
**Synonym:** *Ambassis nama* Day, 1875  
**Common Name:** Elongate glass-perchlet  
**Diagnosis:** Body is ovate and strongly compressed with minute and often irregularly arranged scales. Lateral line is with 100 to 107 scales. Mouth is large with thin lips, very prominent lower jaw with strong projection and three canines on either side of lower jaw. Fins are bright orange; upper half of first dorsal fin is deep black; caudal fin is dusky and orange, with pale outer border.  
**Geographical Distribution:** The only species so far referred to this genus. Inhabits both in standing and running waters of Ganga, Krishna, Mahanadi basins of India. Abundant in clear streams, canals, beels, ponds, and inundated paddy fields during rainy season. Asia: Pakistan, India, Nepal and Bangladesh.  
**Habitat:** Freshwater, brackish.  
**Economic Importance:** The species attains a length of 11 cm and contributes to the artisanal fish catches in Uttar Pradesh, Bihar and West Bengal. These small, bony, fleshy fishes are sold in heaps along with other small fishes in the fish markets. As low-priced fishes they are preferred by many. This species could effectively be used in the control of guinea worms and also for malarial control. Popular for aquarium purposes.

*Parambassis thomassi* (Day, 1870)  
**Synonym:** *Ambassis thomassi* Day, 1870; *Parambassis thomassi* Guha and Talwar, 1985  
**Common name:** Western Ghat glassy perchlet  
**Diagnosis:** Body rather stout, deep and compressed. Head fairly large; snout rather elongate, with a pronounced elevated tip. Mouth large; teeth on
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jaws villiform, differentiated in size, outer row slightly enlarged particularly anterioly. Scales large; lateral line with 35 to 43 scales; predorsal scales 15 to 19; cheek with 4 transverse scale-rows. Humeral spot absent. Fins hyaline; second dorsal spine slightly dusky on its anterior side. Dorsal and anal fins are short with only 9 to 11 soft rays. Suborbital ridge with about 12 very small serrae. Preopercular ridge with seven small serrae. Vertical limb non-serrate. Gill rakers on lower limb of first arch 12 to 18.

**Biology:** Greenish with silver colouration. Young fishes entirely pale yellow, transparent, with a pronounced silvery sheen on flanks. Maximum size obtained is 12cm. Omnivorous, but prefers live feed, do not accept pelleted feed. Sexual dimorphism and breeding is not known, not so far bred in captivity.

**Distribution:** India: Inhabits stream and lakes Western Ghats of Kerala and Karnataka.

**Habitat:** Demersal. Freshwater.

**Economic Importance:** This species contributes to the artisanal catches in the Vembanad Lake, Kerala. They have already secured positions in the national and international markets as ornamental fishes. Survives well in clean, aerated tanks. Good for single species aquarium.

**Parambassis dayi** (Bleeker, 1874)

**Synonym:** *Ambassis nalua* (Day, 1865); *Ambassis dayi* Bleeker, 1874

**Common name:** Day’s glassy perchlet

**Diagnosis:** Silvery coloured fish, glossed with purple; with a broad lateral burnished band. Fins hyaline; membrane between 2\textsuperscript{nd} and 3\textsuperscript{rd} dorsal spine dusky; soft dorsal fin; caudal and anal fins with dusky edges. Body elongate, rather slender and compressed. Head fairly elongate, with a moderately pointed snout. Lateral line with about 30 scales; predorsal scale 15; cheek with 6 transverse scale rows. Humeral spot absent. Dorsal and anal fins are short with only 9 to 11 soft rays. Suborbital ridge with about ten small weak serrae. Preopercular ridge with 18 strong large serrae on ventral limb.
Geographical Distribution: Inhabits freshwaters and estuarine lakes of Western Ghats of Kerala, India. This species is caught from the Vembanad Lake and other lakes of Kerala, usually by gill-nets. It is very common in Kuttanad throughout the year; and also in the hilly regions of north and central Tranvancore where these fishes are adapted for life in swift currents. The species is probably endemic to Kerala.

Habitat: Freshwater, Brackish.

Economic Importance: Important as ornamental fish in international market.

Parambassis lala (Hamilton, 1822)

Synonym: Pseudambassis lala (Hamilton, 1822)

Common name: Highfin glassy perchlet

Diagnosis: Translucent brilliantly coloured (orangish-yellow) with three longitudinal dusky bands along sides extending dorsoventrally. Adult males with two broad vertical dark bands. Body small and almost rounded with minute scales. Lateral line with about 90 scales. Mouth oblique. Operculum with dark stripes. Humeral spot present. Dorsal and anal fins are long with 12 to 17 soft rays. Gill rakers on lower limb of first arch 12 to 18. Fins reddish orange with blackish outer margin; Second spine of dorsal fin very elongate. Some workers consider this fish as the young of Parambassis ranga, but the species is undoubtedly distinct. It can be distinguished by its small (1½") adult size and the presence of three vertical bars behind its eye.


Habitat: Freshwater, occasionally enters brackish waters.

Economic Importance: This translucent little fish is prized high as an aquarium fish. It is a fascinating fish that resembles a bit of crystal moving about in the aquarium. The species is also harvested by lower income groups as a subsistence food.

Ambiguity: Many authors consider this species as a juvenile of Parambassis ranga.
Parambassis ranga (Hamilton, 1822)

Synonym: Chanda ranga Hamilton-Buchanan, 1822; Pseudambassis ranga (Hamilton-Buchanan, 1822)

Common name: Indian glassy fish

Diagnosis: Transparent with a greenish-yellow tinge and a silvery gloss on dorsum; a silvery broad lateral stripe on side of body. Body stout, deep and compressed. Preopercular hind edge smooth, at most with one or two serrations at angle. Body depth 2.3 to 2.4, caudal peduncle depth 8.3 to 9.1, diameter of eyes 11.3 to 11.8 times in standard length. Scales small; lateral line with 47 to 63 scales; cheek with 7 transverse scale-rows. Gill rakers on lower limb of first arch 21 to 25. Humeral spot present. Dorsal and anal fins are long with 12 to 17 soft rays. Fins hyaline; dorsal and caudal fins with blackish edges. It has a dark area behind the eye.


Distribution: Inhabits waters of Gangetic provinces of India, Bangladesh, Malaysia, Myanmar, Pakistan, Burma, Thailand. This is an exceeding common freshwater fish in the Indian region. It breeds everywhere during the rains. It remains appreciably smaller in captivity. Found in sluggish and standing water. A common species proliferating in impoundments

Habitat: Freshwater, brackish.

Economic Importance: Rare in markets. But it does fairly well in aquarium trade. This is a delightful aquarium novelite.

3.1.2. Family: Sillaginidae

Sillago sihama (Forsskål, 1775)

Synonym: Atherina sihama, Forsskål 1775

Common name: silver whiting and sand smelt

body depth. Snout and head not depressed. Air bladder with two very distinct postcoelomic extensions. The species has a low lateral line with about 70 scales.

**Distribution**: Indo-west Pacific. Inhabits shallow sandy bottoms of shores and bays, also estuaries. This is a nearshore species that frequently penetrates estuaries for considerable distances. The species is a permanent inhabitant of the Vembanad Lake (Kerala) and Chilka Lake (Orissa) and goes out to the sea or the mouth of the lake for breeding which takes place by about February. Juveniles are available in the lake from close of winter till rainy season.

**Biology**: It is a carnivore, taking a variety of polychaetes and crustaceans.

**Habitat**: Brackish; mainly reef associated, amphidromous. Common along beaches, sandbars, mangrove creeks, estuaries and freshwater.

**Economic Importance**: The species is of major economic importance throughout the Indo-Pacific and marketed fresh. Mostly used as food fish.

**Ambiguity**: Among the five individuals morphologically identified as *Sillago sihama* used for the study, two different unknown species of *Sillago* were observed and named as *Sillago sp1* (Si si sp1?) and *Sillago sp2* (Si si sp2?).

*Sillago vincenti* McKay, 1980

**Common name**: Estuarine whiting

**Diagnosis**: Very similar in external morphology to *Sillago sihama*. Preopercle serrated. Dorsal spine: 12; Dorsal soft rays: 21-23; Anal spines: 2; Anal soft rays: 22-24. Second dorsal spine not exceeding body depth. Snout and head not depressed. Air bladder with a single postcoelomic extension. No tubular extensions anteriorly. Body color is uniform pale tan, with the second dorsal fin spotted

**Distribution**: India: Karanad near Neendakara, north of Quilon, Kerala. Inhabits shallow bottoms of estuaries and freshwaters. This species is fairly common in the estuarine waters of southwest India. May be endemic to Kerala.

**Biology**: Biology is relatively unknown. The egg bearing females of 25 to 28 cm in length were present in January and February, suggesting this to be at least part of the spawning period.
**Habitat:** Brackish, Marine, Occasionally enters to freshwater.

**Economic Importance:** The species is recognized as having considerable potential for aquaculture in impoundments and tidal ponds, with a reported rapid growth rate. A very good foodfish of excellent flavour; marketed fresh.

### 3.1.3. Family: Gerreidae

**Gerres filamentosus** Cuvier, 1829

**Common name:** Whipfin mojarra, Whiptail silver-biddy

**Diagnosis:** Silvery with 7 to 10 vertical series of ovoid bluish spots on upper portion of sides. Pectoral, pelvic, caudal and anal fins are dusky; dorsal fin hyaline, except for end of filamentous spine which is black. Body is deep and compressed, its depth 2 to 2.5 times in standard length. Dorsal spines: 9; Dorsal soft rays: 10-11; Anal spines: 2-3; Anal soft rays: 7-8. Second dorsal spine long and laterally compressed, produced into a filament whose tip extends past level of first anal spine. Scales moderate; lateral line with 44 to 47 scales.

**Geographical Distribution:** Red Sea, Indo-west pacific: East Africa, Madagascar and western Mascarenes east to Fiji, north to southern Japan, south to Western Australia, Queensland (Australia) and New Caledonia.

**Habitat:** Demersal; amphidromous Inhabits coastal waters, enters lakes and lower freshwater reaches of rivers and brackishwaters.

**Economic Importance:** This species which attains a length of 20cm standard length, contributes to the artisanal fish catches in the Vembanad Lake (Kerala), Nethravathi Estuary (Mangalore), Chilka Lake (Orissa). Salted or made into fish sauce.

**Gerres abbreviatus** Bleeker, 1850

**Synonym:** *Gerres erythrourus* (Bloch 1791)

**Common name:** Deepbody mojarra

**Diagnosis:** Body deep, its depth 1.9 to 2.3 times in standard length. Dorsal spines: 9; Dorsal soft rays: 10; Anal spines: 3; Anal soft rays: 7. Scale-rows
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3.5 to 6 between 5th dorsal spine and lateral line. Indistinct dark stripes along scale rows above and 4-6 rows immediately below lateral line. Second dorsal fin spine longer than the 3rd. Ventral and anal fins yellow with white tips.

**Distribution:** Indo-West Pacific: Madagascar to Australia.

**Habitat:** Marine; brackish; reef-associated; oceanodromous; enters freshwater.

**Economic Importance:** Marketed fresh or made into fish balls.

3.1.4. Family: *Nandidae*

**Subfamily:** Nandinae

*Nandus nandus* (Hamilton, 1822)

**Synonym:** *Coius nandus* Hamilton, 1822

**Common name:** Mottled nandus, Gangetic leaffish.

**Diagnosis:** Greenish-brown with brassy reflections; vertically marbled with three broad patchy blotches; a dusky blotch on caudal fin base. Fins greenish; yellowish narrow bands of spots across soft portions of dorsal, anal and caudal fins. Body oblong and fairly deep, compressed. Head large and compressed. Mouth very large, protrusible; teeth villiform on jaws. Eyes large, the diameter 5 to 6 times in head length. Dorsal spines rather strong; anal spines moderately strong, second spine longest. Caudal fin slightly rounded. Scales ctenoid; lateral line interrupted at about 36th scale; 46 to 57 scales in longitudinal series.

**Distribution:** Inhabits fresh and brackishwaters of Pakistan: Indus plain; India; Nepal; Bangladesh; Burma; and Thailand. This species is common in ditches and inundated fields and attains a length of 20cm. It is very popular in West Bengal, particularly when freshly caught. It is common in summer months when it is collected from dried-up beds of tanks, bheels, bheries etc. This is a piscivorous species preying upon small carps in paddy fields, ditches. It is very tenacious of life.

**Biology:** Attains a maximum size of 20 cm. Carnivorous. Attacks smaller fishes. So far not bred in captivity. Hides in between leaves of aquatic vegetation, feeding the fish is a problem as it does not accept artificial feed. Being piscivorous, smaller fishes cannot be kept along with this.

**Habitat:** Benthopelagic. Freshwater, brackish.
Economic Importance: It is a high-prized fish inspite of its spinous fins and ugly black bands and blotches all over the body, fetches good price as ornamental fish.

Ambiguity: 10 individuals (Five from Periyar river, Kerala and five from drainages of river Ganges, Kolkata) were examined. Distinct variation in DNA samples was observed between the collections from the two regions and Nandus nandus from Kerala (Na na Ker) can be Nandus marmoratus Valenciennes as reported in Day, 1875 (Fishes of India: 129, Pl. 32, fig. 1) which needs confirmation.

Subfamily: Badinae

**Badis assamensis** Ahl, 1937

**Synonym:** *Badis badis assamensis* Ahl, 1937

**Common name:** Assambadis

**Diagnosis:** Dorsal spine: 16-17; Dorsal soft rays: 9-11; Anal soft rays: 7-8. Possess a prominent dark blotch posterodorsally on opercle and two rows of irregular blackish blotches along side. Differs from *B. blosyrus* by shorter jaw, upper jaw 9.7-10.9 SL vs. 12.0-13.6; lower jaw 12.7-14.6 SL vs. 16.3-18.5, having more scales in lateral row (28-29 vs. 27-28), fewer gill rakers (7-9 vs. 10-13), and more vertebrae (29-30 vs. 27-29)

**Distribution:** Upper Assamese portion of the Brahmaputra River drainage, Dibrugarh, India.

**Habitat:** Benthopelagic. Freshwater.

**Economic Importance:** Important as ornamental fish.

**Badis badis** (Hamilton-Buchanan, 1822)

**Synonym:** *Badis labrus* Hamilton-Buchanan, 1822

**Common Name:** Badis, Dwarf chameleon fish
Diagnosis: Body is moderately elongate and slightly compressed with ctenoid scales. Conspicuous dark blotch covering superficial part of cleithrum above pectoral fin base. Eyes large, Mouth is small with villiform teeth on jaws. Dorsal spines: 16-18; Dorsal soft rays: 7-10; Anal spines: 3, soft rays: 6-8; Pectoral soft rays: 12, Ventral spine: 1, soft rays: 5. Dorsal spine is slender and anal spines are short. Caudal fin is rounded. Lacks a dark caudal peduncle blotch. Lateral line is interrupted, often absent. 26 to 30 scales in longitudinal series. In life colour is highly variable. Fins yellowish green, bluish or dark blue; a row of dark spots along base of dorsal fin.

Geographical Distribution: Asia: Ganges River drainage in India, Bangladesh, and Nepal, from Himachal Pradesh (Yamuna River) to the estuary; the Mahanadi River drainage; Assam lowlands close to Brahmaputra (Kaziranga, Guwahati, and Dibru River).

Habitat: Benthopelagic. Inhabits freshwaters; in rivers, ponds, ditches and swamps.

Economic Importance: This is one of the most interesting colourful aquarium fish because of its range of pigmentation. In the aquarium it has a habit of remaining at the bottom and nibbling at vegetable detritus. It is shy with highly changeable colouration and markings. It attains a maximum length of 8cm. It is a column feeder and feeds on mosquito larvae when a chance permits.

_Dario dario_ (Hamilton 1822)

*Synonym:* _Dario labrus_ Hamilton 1822

Diagnosis: Dorsal spines: 12-14; Dorsal soft rays: 6-8; Anal soft rays: 6-7. No palatine dentition. Scales in transverse row 8.5. Pre-, post, and supraorbital stripes present. Small badid fish with adult size below 25mm distinguished from *Badis* by absence of bony tubed lateral line scales, absence of dentary and infraorbital pores. Males with 7 prominent dark vertical bars across sides; females uniform or with indistinct vertical bars. Pelvic fin is prolonged reaching to beginning or end of anal fin base in males.

Biology: Males show a different reproductive behaviour lacking an elaborate parental care as in *Badis* species but rather defending any territory around spawning site.
Geographical Distribution: India: Brahmaputra river system.

Habitat: Freshwater.

Economic Importance: Fetches good price as ornamental fish.

3.1.5. Family: Pristolepidae

*Pristolepis rubripinnis* (Bleeker 1851)

Diagnosis: Differs from all other congeners by its colour pattern. It shows orange red soft dorsal, soft anal and caudal fins and a yellow to orange pelvic fin. It differs further from its south Indian congener *P. marginata* in having 4-5 scales above the lateral line (vs. 3) and 10 scales below (vs. 9) and from *P. fasciata* in the absence of prominent bars on the body. Strongly compressed thick body; Head large, Eye inserted in anterior half of head. Mouth comparatively small. Opercle with two spines posteriorly, lower longer and stronger. Background colour olive green, darker on the back, lighter towards belly. Yellowish green marks present on head. Six or seven irregular zigzagging and interrupted yellowish greenish bars along the body up to caudal peduncle.

Distribution: Currently known from Pamba and Chalakkudy Rivers in Kerala, southwestern India.

Habitat: Potamodromous. Found in slow or standing waters, among bushes of shore vegetation. Occurs in medium to large-sized rivers and flooded fields, lakes, ponds and swamps.

Economic Importance: Used for aquarium purpose.

*Pristolepis marginata* Jerdon 1849

**Synonym:** *Pristolepis marginatus* Jerdon 1848

**Common name:** Malabar leaffish

**Diagnosis:** Dorsal spines 14-16, soft rays 11-14; Anal spines 3 (rarely 4), soft rays 8; Pectoral soft rays 14-15; Ventral spine 1, soft rays 5. Body oblong and compressed. Mouth
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moderate; teeth on vomer and jaws is villiform. Dorsal spine stout; second anal spine strongest but as long as third spine. Lateral line interrupted opposite fourth dorsal finray on 21st scale, with 25-27 scales. This species attains a length of 15cm SL.

**Distribution:** Western Ghats of Kerala, Karnataka, Tamil Nadu.

**Habitat:** Benthopelagic. Inhabits clear and rapid streams of Mananthavady River, Wayanad, Kerala.

**Economic Importance:** Commercially important as aquarium fish.

3.1.6. Family: Terapontidae

**Terapon jarbua** (Forsskål, 1775)

**Synonym:** *Sciaena jarbua* Forsskål, 1775

**Common name:** Jarbua terapon

**Diagnosis:** Dorsal spines: 11-12; Dorsal soft rays: 9-11; Anal spines: 3; Anal soft rays: 7-10. Lower opercular spine extending well beyond the opercular flap. Post temporal bone exposed posteriorly and serrate. Body color is fawn above, cream below, nape dark; head, body and fins with and iridescent sheen. Three or four downwardly curved longitudinal stripes along body, the lowermost continuing across the middle of the caudal fin.

**Distribution:** Indo-west Pacific.

**Biology:** Attains a length of 25cm. Spawn in the sea and juveniles migrate to freshwater. Eggs are guarded and fanned by the male parent. Produce sound. Omnivorous. But prefers animal food.

**Habitat:** Catadromous. Inhabits inshore waters and moves considerable distances upstream in the vicinity of river mouths; Enter estuaries and rivers.

**Economic Importance:** This is a very robust and hardy fish for brackish aquaria. As food fish, they are marketed fresh, dried or salted.

**Terapon puta** Cuvier, 1829

**Synonym:** *Therapon puta* cuvier, 1829

**Common name:** Small scale terapon, Squeaking perch
Diagnosis: Dorsal spines: 11-12; Dorsal soft rays: 9-11; Anal spines: 3; Anal soft rays: 8-9. Three or four downwardly straight longitudinal stripes along side of body.

Distribution: Indo-west Pacific. This species is found throughout the year. Mostly found in Chilka Lake, Orissa.

Biology: Feed on fishes and invertebrates. Eggs are guarded and fanned by the male parent.

Habitat: Benthopelagic; amphidromous. Inhabits coastal waters, enters freshwater, brackish water, estuaries and mangrove areas.

Economic Importance: Minor commercial importance.

*Terapon theraps* Cuvier, 1829

Synonym: *Therapon theraps* Cuvier, 1829

Common name: Banded grunter, Large scaled terapon

Diagnosis: Dorsal spines: 11-12; Dorsal soft rays: 9-11; Anal spines: 3; Anal soft rays: 7-9; Pectoral 14-15; Ventral spine 1; ventral soft rays: 5. Body oblong, compressed, robust. Mouth slightly oblique. 6 to 8 rows of scales above lateral line and 14-16 rows below it. Lower opercular spine extending well beyond the opercular flap. Post-temporal bone exposed posteriorly and serrate. Color is dusky green above, silvery below; body, head and fins with an iridescent sheen. Four longitudinal brown stripes extend on the upper side from the head. A large prominent black blotch is on the distal end of the spinous portion of the dorsal fin. Similar brown stripes run across the caudal fin. Caudal lobes with two bars, upper lobe with black tip.

Distribution: Reef-associated; Indo-West Pacific: East Africa, Madagascar, Seychelles, Red Sea, Arabian Peninsula, Persian Gulf to India and Andaman Islands; and southeast Asia. Reaches south to the Arafura Sea and northern Australia; enters freshwater.

Biology: Juveniles with floating weeds, often far offshore. Eggs are guarded and fanned by the male parent.
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Habitat: Marine; freshwater; brackish.

Economic importance: Marketed fresh; ideal for brackishwater aquariums.

3.1.7. Family: Cichlidae

*Etroplus canarensis* Day, 1877

Common name: Canara pearlspot

Diagnosis: Yellowish buff coloured fish with about eight vertical blackish bands; each side darkest at its base and some with a light central yellow spot. Dorsal spines: 21-22, soft rays: 8; Anal spines: 14-16, soft rays 6-7; Pectoral soft rays: 16; Ventral spine: 1, soft rays: 5. Dorsal and caudal fins dirty greenish; pectoral fins yellow, with a black base; pelvic and anal fins are dusky. Body is deep and compressed. Eyes large, its diameter 3 to 3.5 times in head length. Mouth small; teeth villiform, in 2 or 3 rows on jaws. Caudal fin emarginated. Scales weakly ctenoid; Lateral line interrupted after 15 to 25 scales; 30 or 31 scales in longitudinal series.

Geographical Distribution: Inhabits freshwater of South Karnataka (Dakshina Kannada), India. Rediscovered by Menon et al., 1993 from Kumaradhara river near Pullikayam, Uppinangudi, Dakshina Kannada

Habitat: Benthopelagic; amphidromous. Freshwater.

Economic Importance: This species attains a length of 11.5 cm and has great demand as aquarium fish.

*Etroplus maculatus* (Bloch, 1785)

Synonym: *Chaetodon maculates* Bloch, 1785

Common name: Spotted etroplus, Orange chromide

Diagnosis: Yellowish, back greenish, with numerous horizontal lines of deep golden spots; three large, round black blotches on flanks, middle blotch largest and darkest. Dorsal spines: 17-20, soft rays: 8-10, Anal spines 12-15, soft rays: 8-9; Pectoral spine: 1, soft rays 15-16; Ventral spine: 1, soft...
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rays: 5. Spinous dorsal fin with several brown and yellow spots; pelvic fins deep black; anal and caudal fins yellowish, the former with a deep black border while the latter fin with a reddish edge. Body is disc-shaped, very deep and strongly compressed. Eyes large, its diameter three times in head length. Mouth small; teeth villiform, in 2 or 3 rows on jaws. Caudal fin lunate. Scales weakly ctenoid; Lateral line interrupted with about 35 scales in longitudinal series.

**Biology:** Omnivorous, readily accepts pelleted feed. It also consumes aquatic vegetation. Breeding in tank is successful. Exhibits nest building and parental care.

**Geographical Distribution:** Inhabits fresh and brackishwaters along coastal areas of India: Tamil Nadu, Kerala and South Karnataka; and Sri Lanka. It has been introduced into Andhra and Maharashtra waters.

**Habitat:** Benthopelagic amphidromous. Occurs in lagoons and small streams. Enters estuaries

**Economic Importance:** A very individual cichlid and a rather touchy one in the aquarium. This small species attains a length of 8cm and is not very useful as a food fish. Males are more colourful.

**Etroplus suratensis** (Bloch, 1785)

**Synonym:** Chaetodon suratensis Bloch, 1785

**Common name:** Banded pearlspot, Striped chromide, Pearlspot

**Diagnosis:** Body is light green in colour with six to eight transverse bands. Most of the scales above lateral line with a central white pearly spot; some irregular black spots on abdomen. Dorsal spines: 18-19, soft rays 14-15; Anal spines: 12-13, soft rays: 11-12; Pectoral spine:1, soft rays:16; Ventral spine: 1, soft rays: 5. Dorsal, caudal, pelvic and anal fins are bluish or dirty green; pectoral fins yellow, with a black blotch at its base. Body is very deep, short, oval and strongly compressed. Eyes large, its diameter 3 to 4 times in head length. Mouth small; teeth villiform, in a single row anteriorly but in 1 or 2 rows posteriorly on both jaws. Caudal fin is slightly emarginated. Scales weakly
Materials and Methods

cntenoid; Lateral line interrupted at 16\textsuperscript{th} or 18\textsuperscript{th} scale; 35 to 40 scales in longitudinal series.

**Biology:** Attains a maximum size of 50cm. Omnivorous. But prefers vegetable food. Males and females can be identified by examining the genital papilla. Captive breeding is successful. Exhibits parental care.

**Distribution:** Kerala and Sri Lanka, introduced to other states.

**Habitat:** Benthopelagic. Inhabits brackish waters and mouth of rivers; This is a brackishwater fish but known to tolerate freshwater.

**Economic Importance:** It is an excellent delicious fish, especially when large. This species is extensively cultivated in ponds and tanks in Kerala where they breed naturally. Juveniles fetch good price as ornamental fish. It is the state fish of Kerala.

3.1.8. **Family: Scatophagidae**

*Scatophagus argus* (Linnaeus, 1766)

**Synonym:** *Chaetodon argus* Linnaeus, 1766

**Common name:** Spotted scat

**Diagnosis:** Ground colour greenish. Juveniles with a few large roundish blotches, about size of eye, or with about 5 or 6 broad, dark, vertical bars. In large adults, spots may be faint and restricted to dorsal part of flanks. Body quadrangular, short, elevated, much compressed with round abdomen. Anal fin with six spines and 14 or 15 rays, anal spines are all of same length, each alternate one being the strongest. Two dorsal fins, united at their bases, inserted above tip of pectoral fins, first dorsal with ten or eleven spines and another single recumbent forwardly directed spine, second with one spine and 16 to 18 rays and covered with scales.

**Distribution:** Indo-west Pacific. India and Sri Lanka and north to southern Japan.

**Biology:** The species attains 30cm in length. Feeds on worms, crustaceans, insects and plant matter.

**Habitat:** Reef-associated; amphidromous. Inhabit brackish, marine and the lower reaches of freshwater streams, frequently occurring among mangroves.
Economic Importance: The dorsal, anal and pelvic spines are believed by Philippine fishers to be venomous and capable of inflicting wounds. Used in Chinese medicine. Marketed as fresh for consumption. While young, it makes a handsome aquarium fish; old fish become dull and less attractive.

3.1.9. Family: Anabantidae

*Anabas testudineus* (Bloch, 1795)

*Synonym:* *Anthias testudineus* Bloch, 1795

*Common Name:* Climbing perch, Koi

*Diagnosis:* Dorsal spines: 16-18, soft rays: 8-10; Anal spines 8-11, soft rays: 9-11; Pectoral spine: 1, soft rays: 13-14; Ventral spine: 1, soft rays: 5. Body is elongated and 3.0 to 3.5 times deeper in standard length, Mouth fairly large with villiform teeth on jaws. Scales are large with 21 to 29 in lateral series. Dorsal fin is with eight to ten rays. Dark spot at base of the caudal fading with age.

*Geographical Distribution:* West Pacific: native to southeastern Asia from India to Sri Lanka to Indonesia, Philippines and China; introduced widely elsewhere. Throughout India. These fishes inhabit all kinds of freshwater, including large streams but flourish most in canals, ditches, lakes, ponds and swamps. It is caught mainly from low lying swamps and marshy tracts as well as derelict pits, pools and puddles which remain in the process of drying up during summer months. By means of its supplementary breathing apparatus, it can thrive in water deficient in oxygen.

*Habitat:* Freshwater, brackish.

*Economic Importance:* This is a very hardy fish and is of considerable fisheries interest. Despite its moderate size, fish is regarded as a highly esteemed fish for its fine flavour, restorative values and prolonged freshness out of water. The fish is suitable for cultivation in ponds, reservoirs and rice fields as monoculture or with carps, *Clarias batrachus* and *Heteropneustes fossilis*.

*Ambiguity:* Individuals of Kerala collection showed distinct genetic variation from collections of Andhra and Orissa indicating the presence of a second species in the Indian fauna, the *Anabas oligolepis* Bleeker, 1855, though the latter is considered as the synonym of *A. testudineus* by many authors.
Anabas oligolepis Bleeker, 1855

Diagnosis: *Anabas oligolepis* can be distinguished from *A. testudineus* by wide body, smaller pectorals, longer snout, without dark spot at base of caudal. Dorsal fin with nine or ten rays.

Geographical Distribution: India: Bhimavaram, West Godavari district, Andhra Pradesh, also Orissa and West Bengal.

3.1.10. Family: Belontidae

Sub Family: Macropodinae

*Pseudosphromenus cupanus* (Cuvier, 1831)

Synonym: *Polyacanthus cupanus* Valenciennes, 1831; *Macropodus cupanus* (Valenciennes, 1831)

Common name: Day’s paradise fish


Distribution: Inhabits freshwater ponds and ditches of Kerala, Maharashtra: Mutha-Mula river, Pune, Corommandel coasts in India; Sri Lanka; Bangladesh; Pakistan; Western Burma; Malay Peninsula and Sumatra. Also, reported from Karnataka and Tamilnadu.

Biology: A hardy species and is rather more of a jumper than most bubblenest builders. It is also a fairly good larvicidal fish; attains a length of 7.5cm.

Habitat: Benthopelagic. Freshwater; brackish; Often found in ditches, paddy fields and shallow water not far from tidal influence.

Economic Importance: Mostly used in the aquarium.
**Pseudosphromenus dayi** (Köhler 1908)

**Synonym:** Macropodus cupanus dayi (Köhler 1908)

**Diagnosis:** Body oblong and compressed. Mouth small; teeth small, conical and fixed on jaws. One soft ray of pelvic fin is produced into a single moderately long filament. Caudal fin pointed with median rays elongated. Scales large; lateral line rudimentary; 29 to 32 scales in longitudinal series. Dark olive to green, darker on back; often with a brown stripe from eye to corner of opercle and brown spots on head; a dusky blotch on caudal peduncle. Elongated pelvic fin rays scarlet; fins otherwise pale green; soft dorsal and caudal fins with rows of black spots; edges of fins peculiar white that might be called phosphorescent.

**Biology:** Eggs shrink to the bottom and are then collected by one or both parents and are embedded among the foam bubbles in the nest. Attains a maximum size of 6cm. Omnivorous, accepts artificial feed. Males are brilliantly coloured with fluorescent margin in tail, captive breeding is successful. Males prepare bubble nest as in the case of Siamese fighter and take care of the eggs and young ones. Fairly good larvicidal fish. They can survive in less oxygenated water also, being air breathing. It hides among aquatic vegetation, moves in the upper halves of the water column.

**Distribution:** Kerala, India. Occurs in lentic environment. Found in ditches and swamps.

**Habitat:** Pelagic; Freshwater; brackish.

**Economic Importance:** An aquarium fish owing to its beautiful colouration.

**Ambiguity:** Considered as a synonym of *Pseudosphromenus cupanus* by Talwar and Jhingran (1991).

**Sub Family:** Trichogasterinae

**Colisa fasciatus** (Schneider, 1801)

**Synonym:** Trichogaster fasciatus Schneider, 1801

**Common Name:** Striped Gourami, Giant Gourami
Diagnosis: Body egg-shaped and strongly compressed with 14 or more Bands. Color greenish with oblique orange or bluish bars descending downwards and backwards from the back to the anal fin. Vertical fins with alternating dark and pale spots or bars; the anal fin often with a red margin. Dorsal spines: 15-17, soft rays: 9-14; Anal spines: 15-18, soft rays: 14-19; Pectoral soft rays: 9-10. Mouth is small and slightly protrusile with thick upper lip. Dorsal and anal fins long-based, Pelvic fins are thread-like, caudal fin is slightly notched or cut square (truncate). Scales large, 29 to 31 in longitudinal series, anal fin is scaly at base only. Anal fin is often with a red margin.

Geographical Distribution: Inhabits large rivers and estuaries, also tanks, ditches and ponds of Pakistan, India, Nepal, Bangladesh and Upper Burma. In India, it is distributed upto Krishna river in the south. It is fairly common and abundant in the Hooghly estuary (West Bengal) during the rainy season.

Habitat: Benthopelagic. Freshwater.

Economic Importance: A peaceful and beautiful aquarium fish and is traditionally liked for its good taste. It adapts well to life in community aquaria.

*Colisa lalia* (Hamilton, 1822)

**Synonym:** *Trichopodus lalius* Hamilton-Buchanan, 1822; *Trichogaster lalius* Day, 1876

**Common name:** Dwarf Gourami

Diagnosis: Body egg-shaped and strongly compressed. Dorsal spines: 15-17, soft rays: 7-10; Anal spines: 17-18, soft rays: 13-17; Pectoral soft rays: 10. Mouth is small and strongly protrusible with normal lips. Preorbital denticulate. Soft part of dorsal and anal fins are not produced, but rounded. Caudal fin is rounded to truncate. Anal fin is densely scaled. Body is with oblique scarlet and light blue vertical bands.

Geographical Distribution: Distributed in the Ganga, Yamuna river systems of India; Pakistan and Bangladesh. It inhabits stagnant puddles.

Habitat: Freshwater; benthopelagic.
Economic Importance: It is the smallest of the gouramis and one of the highly satisfactory and most beautiful little fish. It is apt to be timid and hide away in the foliage and well known in the aquarium world.

**Colisa sota** (Hamilton, 1822)

**Synonym:** *Trichopodus sota* Hamilton-Buchanan, 1822; *Trichogaster chuna* (Hamilton-Buchanan, 1822)

**Common name:** Sunset gourami

**Diagnosis:** Dull, greenish, lighter along belly, often with dark stripes along series of scales from back to belly. Body is oblong and compressed with large scales, 27 to 29 in longitudinal series. Dorsal spines: 17-18, soft rays: 6-9; Anal spines: 18-22, soft rays: 11-13; Pectoral soft rays: 9. Mouth is small and strongly protrusible. Preorbital serrated. Dorsal spine increasing in length to last, anal fins with scales at base. Caudal fin is slightly emarginated and often with a black spot at base.

**Geographical Distribution:** Distributed in the Ganges provinces and Assam of India; Pakistan; Bangladesh.

**Habitat:** Freshwater; benthopelagic.

**Economic Importance:** This species is a good ornamental fish and thrives well in aquarium.

### 3.1.11. Family: Channidae

**Channa marulius** (Hamilton, 1822)

**Synonym:** Ophiocephalus marulius Hamilton-Buchanan, 1822

**Common name:** Bullseye Snakehead; Giant Sneakhead

**Diagnosis:** Dorsal soft rays: 45-55; Anal soft rays: 28-36; Pectoral: 16-18; Ventral: 6. Predorsal scales: 15-16. Body elongate and fairly rounded in cross-section. A large black ocellus on caudal fin base (fades with growth). Numerous white spots on body and fins. Pectoral fins about half head length; pelvic fin about 75% of pectoral fin length. Scales on
Materials and Methods

summit of head of moderate-size; rosette of head-scales lies between orbits; two scales between rosette and basal head-scale; predorsal scales 16; scales 60 to 70 in lateral series.

**Distribution:** Pakistan, India, Sri Lanka, Bangladesh, Nepal, Burma, Thailand and China.

**Biology:** Carnivorous and subsist on fish, frogs, snakes, insects, earthworms and tadpoles. Males are territorial. May bite when caught.

**Habitat:** Freshwater; benthopelagic; potamodromous. Inhabit waters with submerged aquatic vegetation. Adults occur in sluggish or standing water with sandy or rocky bottom in canals, rivers, lakes, and swamps.

**Economic Importance:** Used as a favourite gamefish; in public aquaria; esteemed food.

*Channa diplogramma* (Day, 1865)

**Synonym:** *Channa micropeltes* (Cuvier, 1831)

**Common name:** Malabar Snakehead

**Diagnosis:** *Channa diplogramma* differs from all other species in the genus by its high number of lateral line scales (103–105 vs. 36–91); except *C. bankanensis, C. lucius, C. diplogramma* and *C. pleurophthalma* by the presence of gular scales, a patch of scales between the anterior tips of the lower jaws, visible in ventral view. *C. diplogramma* differs from its sister species *C. micropeltes* by a combination of characters viz., number of caudal fin rays, lateral line scales, scales below lateral line; total vertebrae, pre-anal length and body depth. Physically it has relatively long broad body with a small amount of lateral compression with a more extended pointed head.

**Distribution:** A valid endemic species to the southern Western Ghats of peninsular India, Cochin, Kerala; Malabar coast.

**Biology:** *Channa diplogramma* reported to be largest sneakhead in India and shows multiple color phases during its life history. Feed on smaller sneakheads, shrimps and other open water invertebrates.

**Habitat:** Being a tropical snakehead that lives in a variety of environments. Freshwater; brackish.
**Economic Importance:** As a game fish, has quite a reputation among anglers. In India juveniles are in demand as aquarium fish.

*Channa striata* (Bloch, 1793)

**Synonym:** *Ophicephalus striatus*  
Bloch, 1793; *Channa striatus*  
(Bloch, 1793)

**Common name:** Striped Snakehead

**Diagnosis:** Dorsal spines: 0; Dorsal soft rays: 38-43; Anal spines: 0; Anal soft rays: 23-27. Body sub-cylindrical; head depressed; caudal fin rounded. The dorsal surface and sides is dark and mottled with a combination of black and ochre, and white on the belly; a large head reminiscent of a snake’s head; deeply-gaping, fully toothed mouth; very large scales.

**Distribution:** Southern Asia: Native range Pakistan to China, Thailand, Malaysia and Indonesia; introduced elsewhere.

**Biology:** Feed on fish, frogs, snakes, insects, earthworms, tadpoles and crustaceans.

**Habitat:** Freshwater; brackish; benthopelagic. Potamodromous. Adults inhabit ponds, streams and rivers, preferring stagnant and muddy water of plains. Found mainly in swamps, but also occurs in the lowland rivers. More common in relatively deep (1-2m), still water. Survives dry season by burrowing in bottom mud of lakes, canals and swamps as long as skin and air-breathing apparatus remain moist and subsists on the stored fat.

**Economic Importance:** Very economically important in both culture and capture throughout southern and southeastern Asia. Firm white flesh almost bone-free, heavy dark skin is good for soup and usually sold separately. Also common in public aquaria. It is the state fish of Andhra Pradesh.

### 3.2. Sample Collection

Altogether 32 perciform species from 11 families as described above were collected for the study from different agro-climatic zones in India. Majority of the species occur in freshwater, while some are migratory ones, spending part of their life cycle in freshwater habitats. The details of species are given in Table 3.1. As the classification and phylogeny of this group remain extremely
Table 3.1. Details of the fish specimens used for the current study

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Species</th>
<th>Family</th>
<th>Code used for Species</th>
<th>Reference</th>
<th>State</th>
<th>Place/River Basin</th>
<th>Number of individuals (n)</th>
<th>Latitude (N)</th>
<th>Longitude (E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Ambassis commersoni</em> Cuvier, 1828</td>
<td>Ambassidae</td>
<td>Am co</td>
<td>Talwar and Jhingran, 1991</td>
<td>Kerala</td>
<td>Vembanad Lake</td>
<td>5</td>
<td>9°37'48&quot;</td>
<td>76°22'45&quot;</td>
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<tr>
<td>2</td>
<td><em>Chanda nama</em> Hamilton-Buchanan, 1822</td>
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<td>Ch na</td>
<td></td>
<td>Orissa</td>
<td>Daya River</td>
<td>5</td>
<td>20°12'32&quot;</td>
<td>85°51'15&quot;</td>
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<tr>
<td>3</td>
<td><em>Parambassis thomassi</em> (Day, 1870)</td>
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<td>Pa th</td>
<td></td>
<td>Kerala</td>
<td>Chalakkudy River</td>
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<td>10°17'29&quot;</td>
<td>76°28'41&quot;</td>
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<td>4</td>
<td><em>Parambassis dayi</em> (Bleeker, 1874)</td>
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<td>Pa da</td>
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<td>Kerala</td>
<td>Chalakkudy River</td>
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<td>5</td>
<td><em>Parambassis lata</em> (Hamilton, 1822)</td>
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<td>West Bengal</td>
<td>Kolkata</td>
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<td>22°33'13&quot;</td>
<td>88°27'18&quot;</td>
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<td>6</td>
<td><em>Parambassis ranga</em> (Hamilton, 1822)</td>
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<td>Kerala</td>
<td>Chalakkudy River</td>
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<td>10°17'29&quot;</td>
<td>76°28'41&quot;</td>
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<tr>
<td>7</td>
<td><em>Sillago sihama</em> (Forsskål, 1775)</td>
<td>Sillaginidae</td>
<td>Si si</td>
<td>Talwar and Jhingran, 1991</td>
<td>Kerala</td>
<td>Back water, Kochi</td>
<td>5</td>
<td>9°55'67&quot;</td>
<td>76°18'26&quot;</td>
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<td>8</td>
<td><em>Sillago vincenti</em> McKay, 1980</td>
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<td>Si vi</td>
<td></td>
<td>Kerala</td>
<td>Back water, Kochi</td>
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<td>9°55'67&quot;</td>
<td>76°18'26&quot;</td>
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<tr>
<td>9</td>
<td><em>Gerres flamentosus</em> Cuvier, 1829</td>
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<td>Kerala</td>
<td>Back water, Kochi</td>
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<td><em>Gerres abbreviatus</em> Bleeker, 1850</td>
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<td>Kerala</td>
<td>Back water, Kochi</td>
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<td>11</td>
<td><em>Nandus nandus</em> (Hamilton, 1822)</td>
<td>Nandidae (Subfamily-Nandinae)</td>
<td>Na na Kol</td>
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<td>West Bengal</td>
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<td><em>Nandus nandus</em> (Hamilton, 1822)</td>
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<td>Periyar River</td>
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<td>10°4'22&quot;</td>
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<td><em>Dario dario</em> (Hamilton, 1822)</td>
<td>Nandidae (Subfamily- Badinae)</td>
<td>Da da</td>
<td>Kullander and Britz, 2002</td>
<td>Assam</td>
<td>Brahmaputra River drainage</td>
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<td>26°27′20″</td>
<td>89°49′7″</td>
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<td>14</td>
<td><em>Badis badis</em> (Hamilton-Buchanan, 1822)</td>
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<td>Ba ba</td>
<td>Talwar and Jhingran, 1991</td>
<td>West Bengal</td>
<td>Kolkata</td>
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<td>22°33′13″</td>
<td>88°27′18″</td>
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<td>15</td>
<td><em>Badis assamensis</em> Ahl, 1937</td>
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<td>Assam</td>
<td>Brahmaputra River drainage</td>
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<td><em>Pristolepis marginata</em> Jordan, 1849</td>
<td>Pristoepidae</td>
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<td>Britz et al., 2012</td>
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<td><em>Terapon jarbua</em> (Forsskål, 1775)</td>
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<td><em>Terapon puta</em> Cuvier, 1829</td>
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<td><em>Etroplus suratensis</em> (Bloch)</td>
<td>Cichlidae</td>
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<td><em>Etroplus canarensis</em> Day, 1877</td>
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<td>Scatophagidae</td>
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<td>Anabantidae</td>
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<td>Talwar and</td>
<td>Andhra Prades</td>
<td>Kolleru Lake</td>
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<td>An te Bhu</td>
<td>Jhingran, 1991</td>
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<tr>
<td>26</td>
<td><em>Anabas testudineus</em> (Bloch)</td>
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<td>An te Ker</td>
<td></td>
<td>Kerala</td>
<td>Periyar River</td>
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<td></td>
</tr>
<tr>
<td>27</td>
<td><em>Colisa fasciatus</em> (Schneider, 1801)</td>
<td>Belontidae</td>
<td>Co fa</td>
<td></td>
<td>Andhra Prades</td>
<td>Krishna River</td>
<td>5</td>
<td>16°26′50″</td>
<td>80°40′43″</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td><em>Colisa lalia</em> (Hamilton-Buchanan, 1822)</td>
<td></td>
<td>Co la</td>
<td></td>
<td>West Bengal</td>
<td>Kolkata</td>
<td>5</td>
<td>22°33′13″</td>
<td>88°27′18″</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td><em>Colisa sota</em> (Hamilton-Buchanan, 1822)</td>
<td></td>
<td>Co so</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td><em>Pseudosphromenus cupanus</em> (Cuvier 1831)</td>
<td></td>
<td>Ps cu</td>
<td></td>
<td>Kerala</td>
<td>Periyar River</td>
<td>5</td>
<td>10°4′22″</td>
<td>76°16′55″</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td><em>Pseudosphromenus dayi</em> (Köehler 1908)</td>
<td></td>
<td>Ps da</td>
<td></td>
<td>Kerala</td>
<td>Periyar River</td>
<td>5</td>
<td>10°4′22″</td>
<td>76°16′55″</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td><em>Channa marulius</em> (Hamilton-Buchanan, 1822)</td>
<td>Channidae</td>
<td>Ch ma</td>
<td>Talwar and</td>
<td>Kerala</td>
<td>Meenachil River</td>
<td>5</td>
<td>9°34′53″</td>
<td>76°31′31″</td>
<td>Sequence information was obtained from NCBI database</td>
</tr>
<tr>
<td>33</td>
<td><em>Channa diplogramma</em> (Day, 1865)</td>
<td></td>
<td>Ch di</td>
<td>Eschmeyer,</td>
<td>Kerala</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td><em>Channa striata</em> (Bloch, 1793)</td>
<td></td>
<td>Ch st</td>
<td>Jayaram, 2010</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sequence information was obtained from NCBI database</td>
</tr>
</tbody>
</table>
confused (Nelson, 2006), field identification and nomenclature of all the species were followed based on the morphological, meristic and anatomical characters described by Talwar and Jhingran (1991) and Jayaram (1999). Fresh fin clips and muscle tissue samples (10mm, approximately) were collected from the live fish using non-invasive methods immediately after capture and preserved with 95% ethanol (EtOH) in properly labeled sterile Eppendorf tubes and sealed with parafilm and kept at 20°C until further analysis.

3.3. DNA Marker Selection

Three DNA fragments were targeted for species identification at molecular level and phylogenetic analysis in order to provide a range of mtDNA markers representing faster and slower evolving regions of the genome. The large subunit 16SrRNA gene represents a slowly evolving conserved region of mtDNA that typically exhibits levels of variation useful for answering phylogenetic questions among distantly related taxa (Meyer, 1994b; Simon et al., 1994). The 16SrRNA is a non-coding region and mutations in this region can include insertion/deletion events (indels) that can change the sequence lengths, as well as substitution mutational events. Cyt b is a moderately fast evolving region of mtDNA containing both slowly and rapidly evolving codon positions, as well as more conservative and more variable regions or domains overall and is widely used in systematic studies to resolve divergences at many taxonomic levels (Farias et al., 2001; Johns and Avise, 1998; Zardoya and Meyer, 1996c). Species-level diagnoses can routinely be obtained through COI analysis (Hebert et al., 2003a, b). The universal primers for this gene are very robust, enabling the recovery of its 5' end from most animal species. Furthermore, COI has a greater taxonomic signal range than Cyt b. However, COI provides better resolution of deeper taxonomic affinities because its amino acid sequence changes more slowly than that of Cyt b.

3.4. Total DNA Extraction, Amplification and Sequencing of PCR Products

Genomic DNA was isolated from muscle/fin tissue fixed in 95% EtOH following standard salt extraction protocol (Miller et al., 1988) (See Appendix I for details on extraction method). The quality of DNA isolated was checked through 0.8% agarose gel (See Appendix II for full details). Three DNA fragments were amplified, representing three partial gene regions such as 16SrRNA, COI
Table 3.2. List of primer pairs used to amplify mtDNA regions evaluated for the study

<table>
<thead>
<tr>
<th>mtDNA Genes</th>
<th>Primers (Forward/Reverse)</th>
<th>Primer Sequence (5’-3’)</th>
<th>Melting Temp. (T_m)</th>
<th>Annealing Temp. (T_a)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>16SrRNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16Sar-5’ rRNA</td>
<td>L 2510 (F)</td>
<td>CGC CTG TTT ATC AAA AAC AT</td>
<td>63°C</td>
<td>58°C</td>
<td>Palumbi et al., 1991</td>
</tr>
<tr>
<td></td>
<td>H3080 (R)</td>
<td>CCG GTC TGA ACT CAG AT ACG T</td>
<td>63°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16Sbr-3’ rRNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COI 1-5’</td>
<td>WARD-1(F1)</td>
<td>TCA ACC AAC CAC AAA GAC ATT GGC AC</td>
<td>58°C</td>
<td>50°C</td>
<td>Ward et al., 2005</td>
</tr>
<tr>
<td></td>
<td>WARD-1(R1)</td>
<td>TAGACTTCTGGGGCCAAAGAAATCA</td>
<td>58°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WARD-2(F1)</td>
<td>TCGACTAATCATAAAGATATGCGC</td>
<td>58°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WARD-2(R1)</td>
<td>ACTTCAGGGTGACCGAAGAATCAGA</td>
<td>58°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COI 1-3’</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COI 2-5’</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COI 2-3’</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyt b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyt b -5’</td>
<td>Glu Fish-F (F1)</td>
<td>AACCACCGTTGGTTATCACTACAA</td>
<td>57.7°C</td>
<td>54°C</td>
<td>Rafael et al., 2007</td>
</tr>
<tr>
<td>Cyt b -3’</td>
<td>THR-Fish-R (R1)</td>
<td>ACCCTGGATCTCGGAATCAAGAC</td>
<td>64.4°C</td>
<td>56°C</td>
<td></td>
</tr>
<tr>
<td>Cyt b -5’</td>
<td>Glu Fish-F (F2)</td>
<td>AACCACCGTTGGTTATCACTACAA</td>
<td>57.7°C</td>
<td>54°C</td>
<td>Rafael et al., 2007</td>
</tr>
<tr>
<td>Cyt b -3’</td>
<td>TruccytB-R (R2)</td>
<td>CCGACTTCCGGATTACAAGAC</td>
<td>64.6°C</td>
<td>56°C</td>
<td></td>
</tr>
<tr>
<td>Cyt b -5’</td>
<td>FishCyt B-F (F3)</td>
<td>ACCCTGGATCTCTCGGAATCAAGAC</td>
<td>60.7°C</td>
<td>59°C</td>
<td></td>
</tr>
<tr>
<td>Cyt b -3’</td>
<td>THR-Fish2-R (R3)</td>
<td>AACCTGGATCTCTCGGAATCAAGAC</td>
<td>72.1°C</td>
<td>59°C</td>
<td></td>
</tr>
<tr>
<td>Cyt b -5’</td>
<td>L 14724 (F4)</td>
<td>GACTTGAACACACCCTTTG</td>
<td>50°C</td>
<td>50°C</td>
<td>Xiao et al., 2001</td>
</tr>
<tr>
<td>Cyt b -3’</td>
<td>H 15915(R4)</td>
<td>CTCCGATCTCCTCGGAATCAAGAC</td>
<td>57°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
and Cyt b by employing specific universal primers. For COI and Cyt b more than one set of primers (varied primers) were used depending on the compatibility. Annealing temperatures ($T_a$) were adjusted depending on the melting temperature ($T_m$) of the respective primer used. The details of primer sequences used are listed in Table 3.2. PCR conditions for each fragment are detailed in Table 3.3.

Each PCR procedure included a negative control (no DNA template). Verification of successful amplification was assessed by 1.8% agarose gel electrophoresis. A molecular weight standard ladder (100 bp DNA ladder ranging from 100 to 1000 bp for 16SrRNA and COI; pBR322 DNA/Hinf I Digest for Cyt b) was also included in one of the wells on each agarose gel. After successful PCR amplification of the target fragments, amplified products were purified before the template was sequenced in both directions. The cleaned

### Table 3.3. PCR conditions used to amplify target DNA fragments

<table>
<thead>
<tr>
<th>DNA Region</th>
<th>Reaction Mix (25 μl)</th>
<th>Thermal Cycler Profile</th>
<th>Product Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>16SrRNA</td>
<td>20 ng of template DNA, 1x assay buffer (100 mM Tris, 500 mM KCl, 0.1% gelatin, pH 9.0), 1.5 mM MgCl₂ (Genei, Bangalore, India), 5 pmoles of each primer, 200 mM of each dNTP (Genei, Bangalore, India), 1.5 U Taq DNA polymerase</td>
<td>95°C - 5 min 29 X 94°C - 45 sec 58°C - 30 sec 72°C - 45 sec 72°C - 5 min</td>
<td>520-570 bp</td>
</tr>
<tr>
<td>COI</td>
<td>20 ng of template DNA, 1x assay buffer (100 mM Tris, 500 mM KCl, 0.1% gelatin, pH 9.0), 1.5 mM MgCl₂ (Genei, Bangalore, India), 5 pmoles of each primer, 200 mM of each dNTP (Genei, Bangalore, India), 1.5 U Taq DNA polymerase</td>
<td>95°C - 5 min 29 X 94°C - 45 sec 50°C - 30 sec 72°C - 45 sec 72°C - 5 min</td>
<td>630-678 bp</td>
</tr>
<tr>
<td>Cyt b</td>
<td>20 ng of template DNA, 1x assay buffer (100 mM Tris, 500 mM KCl, 0.1% gelatin, pH 9.0), 1.5 mM MgCl₂ (Genei, Bangalore, India), 5 pmoles of each primer, 200 mM of each dNTP (Genei, Bangalore, India), 1.5 U Taq DNA polymerase</td>
<td>95°C - 5 min 30 X 95°C - 30 sec 50-59°C - 30 sec 72°C - 45-75 sec 72°C - 10 min</td>
<td>800-1142 bp</td>
</tr>
</tbody>
</table>
up PCR products were used as the template for sequencing PCR to increase the amount of product linearly with the number of cycles (See Appendix III). Nucleotide sequencing was performed by the dideoxy chain-termination method (Sanger et al., 1977) using ABI Prism Big Dye Terminator v3.1 Cycle Sequencing kit, (Applied Biosystems, USA). Terminators are dideoxynucleotides labelled with different coloured fluorescent dyes that will present different emission spectra on an electrophoresis gel illuminated by laser. Each PCR product was sequenced using both forward and reverse amplification primers. The resulting Cycle sequencing DNA fragments were cleaned before sending to the Sequencing facility (See Appendix III).

**3.5. Electrophoresis and Visualization of Sequences**

The cleaned up products were sent for sequencing to the sequencing facility. The dried products were dissolved in 10% formamide and electrophoresed in a polyacrylamide gel and visualized using an AB 3730 XL capillary sequencer (Applied Biosystems). The products were visualized by laser detection of fluorescence emitted by different emission spectra of fluorescent labelled terminators. The raw DNA sequence information was generated as electropherograms read using DNA Sequencing Analysis Software Version 3.3 ABI (Applied Biosystems).

**3.6. Sequence Analysis**

A total of 495 partial sequences of mitochondrial 16S rRNA (520-570 bp), COI (630-678 bp) and Cyt b (800-1142 bp) genes were generated of specimens (n = 5 each species) collected from different drainages in India in the present study. The sequence information thus generated were examined and good quality sequences (468 numbers) having no background noise (by examining the peaks in the electropherogram) were selected and blasted using the Basic Local Alignment Search Tool (BLAST) available on the NCBI website (http://www.ncbi.nih.gov/BLAST/) with that of other synonyms/subgenera/species from other parts of the world (as provided in NCBI/BoLD/EMBL databases) to resolve the taxonomic ambiguity. The raw DNA sequences were aligned, truncated and edited manually using BIOEDIT sequence alignment editor version 7.0.5.2 software (Hall, 1999). Multiple alignments were performed with CLUSTAL W (Thompson et al. 1994) alignment editor (Bootstraps 1000) and checked manually for obvious misalignments as implemented in BioEdit. Since
Materials and Methods

COI and Cyt b are protein-coding genes, the COI and Cyt b sequences were translated and the polypeptides were checked for erroneous stop codons. The sequences (different haplotypes only) after their confirmation were submitted to GenBank using a standalone multiplatform submission programme called “Sequin” (www.ncbi.nlm.nih.gov/Sequin/index.html) for public access (List of NCBI accessions generated was given in Appendix IV).

3.7. Data Analysis

By using the three genes, the independent evolutionary markers, I attempted to infer a robust evolutionary tree addressing both the recent and older evolutionary events. Phylogenetic and molecular evolutionary analyses (Exploratory data analysis of sequences) were performed using MEGA Version 5 (Tamura et al., 2011). A K2P, Pairwise sequence divergence within families were generated using Kimura’s two-parameter method (Kimura, 1980). The K2P model provides a suitable metric model when genetic distances are low (Nei and Kumar, 2000) as anticipated with many of the species here.

A cluster analysis within and between families was done with the Neighbour-Joining algorithm (NJ) as implemented in MEGA. Gaps were considered as missing data on the phylogenetic reconstructions. 1000 bootstraps were performed to assign confidence levels to the nodes in the branches of trees (Felsenstein, 1985). The simple NJ algorithm was considered at this juncture to be an appropriate starting point for the analyses, given that specimen identification is based entirely on sequence similarity, rather than on strictly phylogenetic relationships, and the speed of analysis that is necessary for biosecurity diagnostic purposes. The Conserved (C), Variable/Polymorphic (V), Parsimony Informative (Pi) and Singleton (S) sites were recorded. Average nucleotide composition was analysed to examine the relationships between the families. The average number and rate of transitions (si) / transversions (sv) within families were also calculated using the program MEGA. The numbers of haplotypes were calculated using the program DnaSP version 4.10 (Rozas et al., 2003). The sequences that are used from NCBI GenBank for analysis such as outgroup are given in Table 3.4.
Table 3.4. Details of GENBANKTM sequences used for the current study

<table>
<thead>
<tr>
<th>Species</th>
<th>DNA Region</th>
<th>GENBANK Accession Number</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ch ma</td>
<td>16SrRNA</td>
<td>AY763725</td>
<td>Ruber et al., 2006</td>
</tr>
<tr>
<td>Ch ma</td>
<td>COI</td>
<td>HM117195</td>
<td>Lakra et al., 2010</td>
</tr>
<tr>
<td>Ch ma</td>
<td>Cyt b</td>
<td>AY763771</td>
<td>Ruber et al., 2006</td>
</tr>
<tr>
<td>Ch st</td>
<td>16SrRNA</td>
<td>HM117250</td>
<td>Lakra et al., 2010</td>
</tr>
<tr>
<td>Ch st</td>
<td>COI</td>
<td>HM117203</td>
<td></td>
</tr>
<tr>
<td>Ch st</td>
<td>Cyt b</td>
<td>GU288571</td>
<td>Adamson et al., 2010</td>
</tr>
<tr>
<td>Ch di</td>
<td>COI</td>
<td>EU342210</td>
<td>Benziger et al., 2011</td>
</tr>
<tr>
<td>Te pu</td>
<td>Cyt b</td>
<td>JQ769417</td>
<td>Davis et al., 2012</td>
</tr>
<tr>
<td>Te th</td>
<td>Cyt b</td>
<td>JQ769418</td>
<td></td>
</tr>
<tr>
<td>Co fa</td>
<td>Cyt b</td>
<td>AY763745</td>
<td>Ruber et al., 2006</td>
</tr>
<tr>
<td>Mu ce</td>
<td>16SrRNA</td>
<td>NC_003182</td>
<td></td>
</tr>
<tr>
<td>Mu ce</td>
<td>COI</td>
<td>NC_003182</td>
<td>Miya et al., 2001</td>
</tr>
<tr>
<td>Mu ce</td>
<td>Cyt b</td>
<td>NC_003182</td>
<td></td>
</tr>
</tbody>
</table>