A Review of Fish Taxonomy Conventions and Species Identification Techniques

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Abstract

Taxonomists, ecologists, geneticists or researchers from other biological fields who wish to adopt fish as a constituent of their studies often become discouraged when they find that ichthyology is a complex subject. In fish-based studies, the failure to recognize fishes as distinct biological units can lead to wrong diagnosis. Hence, this review paper attempts to clarify and discuss the latest schools of thought pertaining to fish taxonomy and the techniques for species identification. It is hoped that the contents and illustrations in this paper will assist researchers in laying a good foundation to inform their studies.

Keywords: Fish, Morphology, Molecular, Taxonomy, Species identification

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Introduction

The term "fish" is usually a convenient description for a group of poikilothermic (cold-blooded) aquatic vertebrates under the Chordata phylum that breathe with gills (Nelson, 2006). Scientifically, the collective term of "fish" primarily refers to Agnatha (jawless fishes), Chondrichthyes (sharks and rays), Sarcopterygii (lobe-finned fishes), and Actinopterygii (ray-finned fishes). Actinopterygians, the bony or ray-finned fishes, are without a doubt the majority of fishes found in freshwaters. Actinopterygians have lepidotrichia which are characterized by fins of membranous webs held together by bony spines, or rays. This niche character differentiates Actinopterygians from Sarcopterygians which possess lepidotrichia that are fleshy.

Although in early 20th century, the ichthyologist Regan (1910) defined a fish species as a product of interrelated communities with common morphological features (today this is termed as "morphospecies"), it should be noted that the species classification concept differs among scientists. While authorities such as Nelson (2006) and Mayr (1942) accept the "biological species" concept, others like Simpson (1951) promotes the "evolutionary species" concept. Then there is Cracraft (1983) who prefers to adopt the "phylogenetic" or "cladistic" species concept.

The oldest and most widely practised "biological species" concept postulates that species are part of a group composition that breed or can potentially interbreed in natural conditions. In the "evolutionary species" concept, a species is a representative of a lineage having its own evolutionary affinity and historical destiny. As for the "phylogenetic species" concept, species is viewed as a monophyletic set of organism with common ancestors. In practice, each of these major concepts is prone to some level of subjectivity. Regardless of concept, wildlife scientists, especially ichthyologists, typically identify and name fishes by either by their consistency in morphological, and more recently, molecular characteristics.

While most researchers are concerned with fishes as a food source and their work involves enriching the body of aquaculture knowledge, there are some who are interested in their diversity, distribution patterns, ecology and functional physiology. Recently, there has also been an overwhelming interest in the molecular constitution of fishes (Wong *et al.*, 2011; Pereira *et al.,* 2013; Rakshit *et al.*, 2015 Quraishia *et al.*, 2015) and their function as biological indicators to monitor waterbody pollution (Fonge *et al.,* 2011; Khodadoust *et al.,* 2013; Authman *et al.,* 2015). Correspondingly, the interest in fish has expanded exponentially, and the ichthyology discipline is often sought to contribute too many other fields of studies (Padilla and Williams, 2004; Lauder *et al.,* 2007; Feist and Longshaw, 2008; Rudkowska *et al.*, 2010). Generally, species is the basic unit in these studies and sound taxonomy is a prerequisite to prevent confusion and misinterpretation.

However, researchers who wish to adopt fish as a major or minor component of their studies will be discouraged when they discover that ichthyology is not an easy subject, more so fish taxonomy. Also, it is a common knowledge that there is already an acute shortage of fish taxonomists and finding a fish taxonomist to assist in a study is often difficult. Such is the same case with many other taxa. Since fish diversity can be high, especially in tropical countries, a taxonomist is typically overwhelmed with constant scientific name revisions, field collection expeditions and managing a museum. Moreover, taxonomy cannot be commercialized and it sees very little funding in many countries. Thus, the discipline rarely attracts students or can sustain career taxonomists in the universities. Such a problem has caused taxonomic errors in published papers and the condition is now widely known as the "taxonomy impediment" (Wheeler *et al.,* 2004; de Carvalho *et al.,* 2007). It is often said that the discipline of taxonomy would be extinct earlier than most endangered species.

On hindsight, the advancement of molecular, computerized and statistical techniques have led many to believe that "species" can be easily characterized by nucleotide sequences, software and mathematical calculations, and the knowledge in taxonomy is no longer needed. Ebach and Holdrege (2005) warn us that there are a growing number of researchers who have never wet their feet in the rivers to observe species or build competency in applying nomenclature rules, and yet set out to conduct fish-based studies and publish papers. Since they lack field experiences, many are unaware of distinctive fish characteristics such as phenotypic polymorphisms, sexual dimorphism and behavioural divergence due to the regional speciation process (Waugh, 2007). Failing to recognize such exceptions can lead to wrong species diagnosis. Some researchers restrict themselves to laboratories, ornamental fish and aquaculture farms, and their specimens may look very different from the wild type which the scientific name was derived from. Ideally, it is advisable to treat a selective bred variety raised in artificial conditions differently. Otherwise, publication with nomenclature errors will be perpetuated through citation and cause a chaotic situation.

In lieu of concerns highlighted, this review attempts to provide a concise introduction to this complex but important field. To understand fish as a biological unit, the readers should also have some background in zoology. Our objective is not to polarize techniques for fish identification because we are convinced that each technique has its merits. Their shortcomings will, however, be discussed to assist researchers in making sound judgments and decisions.

This paper reviews and discusses taxonomic concerns of freshwater species that belong to the Actinopterygii class, and we assume that readers have some familiarity with ray-finned fish anatomy and the common species. Given that a picture is worth a thousand words, we

believe that taxonomy can be easier to master if the reader is provided with detailed scientific illustrations and colour images. This review is by no means comprehensive but we hope it will set a good foundation for those who wish to make a competent start in species-level research.

Why are there do many species?

Teleost species form the largest category among vertebrate animals and their numbers have reached more than 32,500 valid marine and freshwater species under 515 families (Nelson, 2006). In the global context, there are roughly 11,952 of freshwater species (Helfman *et al.*, 2009). Ricklefs (2004) suggests that competition and mutualism among species have an effect on species abundance while Wright *et al.* (2003) speculate that species diversity is the result of productivity from available "energy" in a particular region. If a region lacks energy (e.g., low food availability in the desert), species richness is typically low. However, in the case of fish, MacArthur's (1969) hypothesis seems to be the most probable as he suggests that species diversity is accelerated if there are more areas to host suitable habitats. The hypothesis corresponds with the reality today as large freshwater regions seem to demonstrate more genera and species, for example the Neotropical region (705 genera, 4,035 species) compared to the Australian region (94 genera, 261 species) (Leveque *et al.*, 2008) (Fig. 1).

Early taxonomists, namely Linnaeus and Darwin, had started cataloguing and clustering species that looked similar because they believed that these species share a common biological lineage. In the mid-18th century, the evolution hypothesis was a new and strange concept. Eventually this was proven to be factual in the advent of modern molecular and genetic assessment technologies. In general, today, there is a consensus among scientists that all species on Earth are interlinked with a hierarchical and evolutionary tree with millions of branches. Each branch itself is a representation of the natural history of a particular species and its pedigree. But why did the branches have to extend and multiply in the first place? What causes the divergence?

When gene flow among populations is interrupted by natural (e.g., geographic barriers created by earthquakes) or artificial means (e.g., man-made barriers in aquaculture farms and dams), two types of speciation will occur, namely allopatric speciation and sympatric speciation (Butlin *et al.,* 2016) (Fig. 2). Allopatric speciation occurs when a population is separated by a barrier and such isolation prevents the two or more sub-populations from mating. Given time, the lack of gene flow among the sub-populations will cause biological incompatibility and divergence would be triggered (Mayr, 1959; Turelli *et al.*, 2001; Singh 2012).

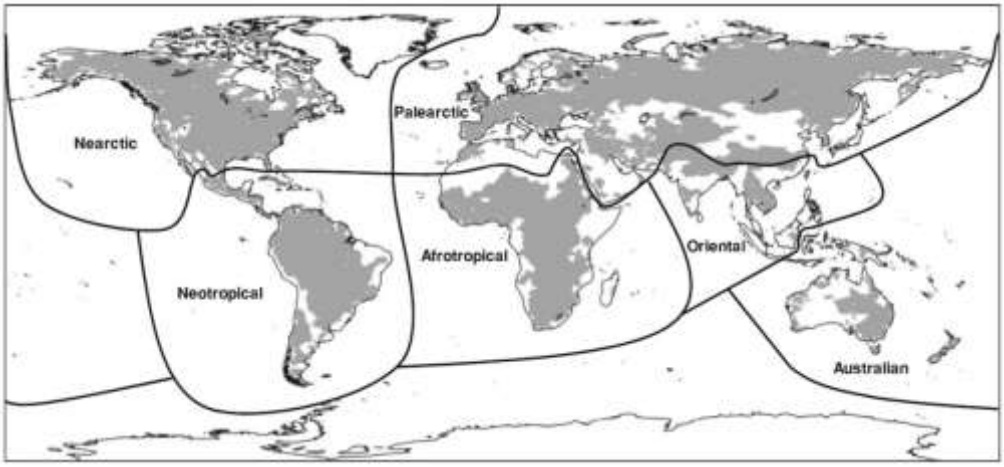

Figure 1: Major freshwater fish Eco regions encompassing 1,054 rivers as classified by the Fish-SPRICH database (Source: Brosse *et al.*, 2012).

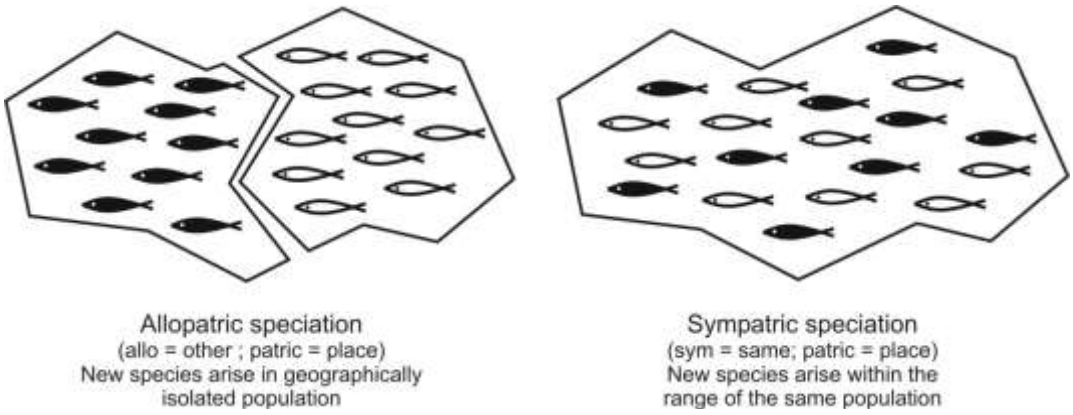

Figure 2: Modes of speciation.

In sympatric speciation, biologists highlight that ecological shift and resource competition are the key drivers (Mayr, 1947; Dieckmann and Doebeli, 1999; Bolnick and Fitzpatrick, 2007; Mallet *et al.*, 2009). They reasoned that new species may arise within a population from biological reproductive barriers between mutants that are better adapted and parent populations. Nonetheless, despite further speciation in both space and time, and regardless of allopatric or sympatric speciation, species within a branch would still maintain a certain degree of morphological similarity (Butlin *et al.*, 2016).

Taxonomy and systematics

The word taxonomy originated for Greek word *taxis*, meaning arrangement, and *nomos*, meaning law. The science of biological taxonomy is responsible for discovering, describing, classifying, naming and treating each species as the basic unit. Species are given names in accordance to the protocol set by

Linnaeus' binomial nomenclature system (Enghoff, 2009). Systematics on the other hand is the science of distinguishing orderliness and classification of a taxon into hierarchical series to emphasize their interrelationships (Mayr, 1942; Guerra-Gracia *et al.*, 2008). The word systematics stems from Greek word *systema*. Nelson (2006) tells us that a systematist seeks the broadest outlook to resolve family, relative, order or grouping orderliness. Taxonomy and systematics are not entirely different schools of thought, but rather overlapping fields (Wilson, 1985; Lincoln *et al.*, 1998). Kapoor (1998) and Wägele (2005) also highlight that the term "systematics" is often used synonymously with taxonomy. It must be clarified that this review adopts Nelson's (2006) argument that biological classification is based on systematic studies and taxonomy is part of systematics.

A proper taxonomy is a first-hand and exhaustive undertaking. Whether one adopts the "biological species" concept (Mayr, 1942; Nelson, 2006), "evolutionary species" concept (Simpson, 1951) or the "phylogenetic species" concept (Cracraft, 1983) as explained earlier, all identification processes start which examining morphotypes at the earliest stage of discovery. Specimens are physically scrutinized from small microscopic configuration of scales to large membrane patterns of the caudal fin. Each physical variance, no matter how small, on a fish body is useful information. Naturally, all fish-based studies require considerable identification skills, experiences and familiarity with local species. Nonetheless, this may not be as complex as it sounds because each species is normally distinctive in appearance and has a certain "look" (Fig. 3).

At a higher level and broader perspective, systematists are experts who examine historical discrepancies, ambiguities, errors and variant names of species, genus and family. For example, there is the problem with regards to whether Eleotridae or Eleotrididae should be used for the members of sleeper fishes (Robins, 1991). Thankfully, species classification and naming consistency is slowly being resolved and currently governed by the International Commission on Zoological Nomenclature (ICZN). However, fish taxonomic problems cannot be resolved quickly as desired because ICZN also has to address issues affecting other taxa. For the fish taxon, appreciatively, there are dedicated ichthyologists who take it upon themselves to diligently keep track of the latest development and progressively publish the most updated information.

Every species belongs to a genus (plural: genera), every genus to a family, every family to an order, every order to a class, every class to a phylum (plural: phyla) and finally all phyla are placed under an overarching kingdom (Fig. 4). Each phylum is regarded as a representation of a large grouping of species that shares a common ancestor in evolution.

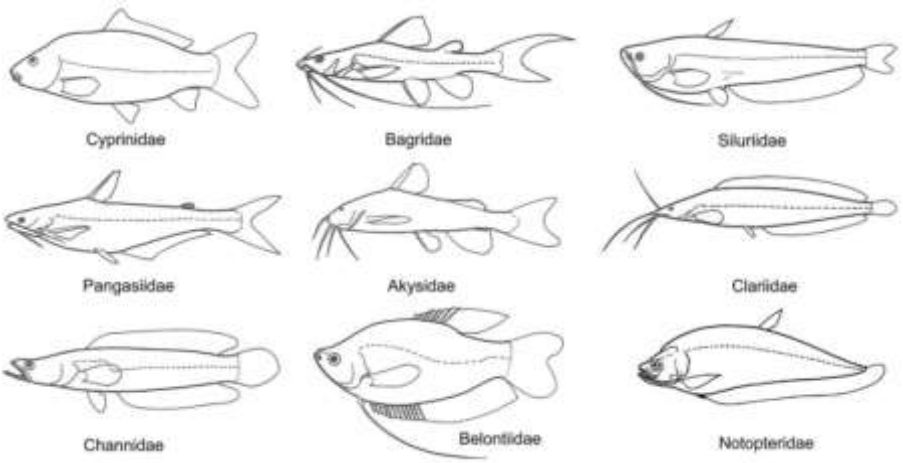

Figure 3: Species from each family have a similar morphological profile to provide identification clues (Source: Adapted from Rainboth, 1996).

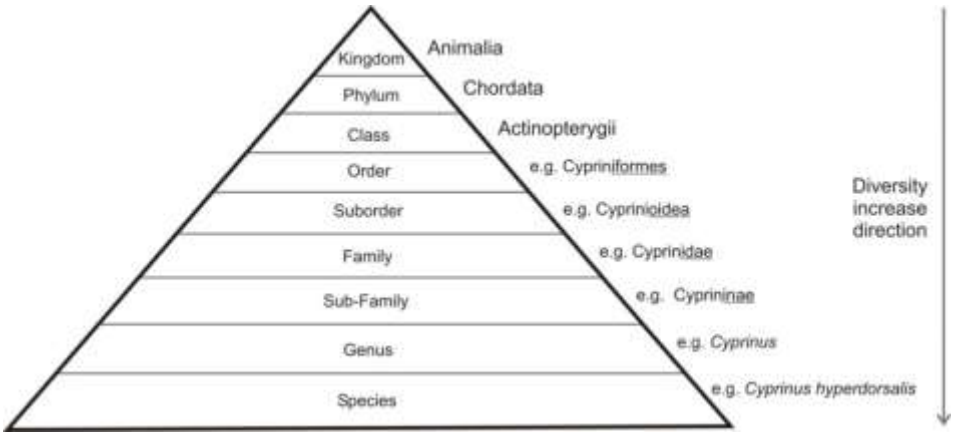

Figure 4: Taxonomic ranking and naming convention.

Ray-finned fish which is reviewed in this paper belongs to the Actinopterygii class under the Chordata phylum in the Animalia kingdom. Such is the ranking and principle of taxonomy practice used to organize all biological units. At time of writing, some experts have arrived at some agreement and the "Family-group Names of Recent Fishes" published by Van der Laan *et al.* (2014) seems to be the most updated for family naming.

In fish taxonomy, there are established conventions for expressing taxonomic ranking. According to ICZN regulation, the family-group name must always end with the "idae" suffix (e.g., Cyprinidae) and the subfamily-group name must end with the "inae" suffix (e.g., Cyprininae). At a higher level, the order-group name should end with the "iformes" suffix (e.g., Cypriniformes) and the suborder-group name must end with the "oidea" suffix (e.g., Cyprinioidea). The family names are always capitalized but never italicized.

How species are named and why

In Latin, species means "a kind, appearance and quality". Depending on locality, people have various vernacular and common names for a fish species. For example, the common names Pearl Gourami, Diamond Gourami and Mosaic Gourami all refer to the same species. Therefore, the use of common names can be confusing and misleading. However, its scientific name *Trichogaster leeri* is a unique name and there is no chance that the name may be mistaken with other species. Ideally, a scientific name may even tell something about the species' key characteristics (Fig. 5), its habit, discoverer and perhaps the location where it was first found.

Figure 5: The species name *Leptobarbus rubripinna* is derived from Greek word "leptós" which means thin or slender, and the Latin word "barbus" meaning barbel. *Rubripinna* originates from the Latin words "ruber" and "pinna" which mean red and fin, respectively.

Another interesting example is the fighting fish species, *Betta persephone*, which is named after Persephone the daughter of Zeus in Greek mythology. Also known as the princess of darkness because she is said to rule the underworld, Persephone was the perfect epithet for *B. persephone* which typically inhabits the black water in peat-swamp habitats of Southeast Asia. Another fighting fish species the *Betta gladiator* need no further explanation as to why the epithet was given.

The *Kottelatia* genus (with only one recognized cyprinid species, *Kottelatia brittani*) was named after a prominent ichthyologist Maurice Kottelat, and a species that is named *Anguilla borneensis* tells us that it was first described from specimens found in Borneo island. Those familiar with Latin or Greek, the traditional language used in scientific names, would also be able to tell that the catfish *Clarias leiacanthus* should have pectoral spines with smooth anterior edge; in Greek, "leios" means smooth and "akathos" means thorn. The *Bihunichthys monopteroides* was named after a popular food in Southeast Asia; "bihun" is a local name for rice noodle and "ichtys" means

fish in Greek. Sure enough, it is a very small and thin spineless eel that resembles rice noodles. From examples mentioned, a scientific name can be communicative and gives species stable and universal designations for easy retrieval.

The scientific naming that we practice today would not be possible without the foundation laid by Carl Linnaeus (1707-1778). Often confused by the many dialectal names during his specimen collection work in various countries, Linnaeus was convinced that species names must be standardized. In 1735, he published a small pamphlet titled Systema Naturae (The System of Nature) to introduce his new system of giving and organizing species names. Linnaeus also decided that species names should be given in Latin or Greek in two parts. Thus the binomial nomenclature system was conceived. Although the Systema Naturae was meant for naming plants, it soon gained popularity due to its practicality and it was quickly adopted by taxonomists of various taxa.

The rule of binomial nomenclature dictates that the first part of species name comprises of its genus (noun) and it is always capitalized (e.g., *Cyprinus*). The second part is used to describe the species' attribute or epithet (adjective) and it is never capitalized (e.g., *Cyprinus hyperdorsalis;* "hyper" meaning "over" in Greek and "dorsalis" meaning the back part of the body in Latin). The first and second part is always italicized. However, if the neighbouring texts are italicized, then the first and second name would be non-italicized (e.g., *in mesohabitat that hosts an isolated* Channa gachua *population* …). This is to ensure that the species name is outstanding and can be easily singled out during reading. When handwritten, species name should be underlined for the same reason. The first part may be used alone but the second part is never used by itself.

A species name must be written in full the first time is it expressed in a manuscript (e.g., *Cyprinus hyperdorsalis*) and thereafter the first part, or genus name, can be abbreviated with initial capital letter (e.g., *C. hyperdorsalis*) on the condition that there is no misconstruing with other genera (i.e., bearing in mind "C. can also mean *Channa* or *Clarias* if these genera appear on the same paper). In cases where an abbreviated initial capital letter may cause confusion, two letters may be used (e.g., *Ch.* for *Channa* or *Cl.* for *Clarias*). There is no absolute rule and the objective is to avoid misinterpretation.

Additionally, "sp." (Singular) or "spp." (Plural) may be used to represent "species" to indicate partially identified species with the genus known. For example, when a specimen is recorded as *Cyprinus* sp. it denotes that a specimen of the *Cyprinus* genus cannot be identified to species level, possibly due to it being a small juvenile which makes positive identification difficult. For species with problematic identification, it may also be recorded with the term "cf." added between the scientific names. It is simply a short term for "confer" or "compare with". For example, when a species is referred as *Rasbora* cf. *elegans*, it implies

the species it most likely belongs to but the designation is still marred by unresolved taxonomic issues or more work is needed to be completely sure. Alternatively, the term "aff." is sometimes added between the scientific name when a specimen cannot be matched to any species known to science, or it is a new species that is yet to be named. The term is a short form of "affinis" in Latin which means "akin to". The insertion of "aff." is to associate the possibility of a new species with the closest species (e.g., *Rasbora* aff. *elegans*).

It would be advisable to describe the genus, species, author and year in full when a species name is expressed as part of a manuscript title, or the first time it is written in the manuscript. The author(s)' name(s) who first coined the binomial name and year of publication should be included (e.g., *Cyprinus hyperdorsalis* Nguyen, 1991) because it is a good practice to ensure the manuscript author is explicitly referring to a species described by a particular taxonomist in a particular year. The author's name is typically enclosed in parentheses when the genus is no longer the original one used. Alternatively, the author's name is enclosed within parenthesis when a species has been transferred from the original genus in which it was first described; e.g. *Cyprinus melanes* (Mai, 1978). This puts the author and the reader in a safer position because literature that adopts fish as a subject can be flawed by synonyms and obsolete species' names. It should be noted that the author citation is treated differently in various taxa. Also, when submitting a manuscript for peer review, it would be wise to countercheck the journal's submission instructions on how to quote the target species.

Species identification at the morphological level

Since humans learnt how to hunt fish, species were identified based on some simple anatomical features. Observation of specimen anatomy and differentiating fish species based on their morphological features is the most practical, rapid and low cost method. Besides experienced local fisherman and fish mongers, people who live by the river or wetland would learn to identify fishes at a young age. This is due to knowledge and memory acquired from long-term observation or through oral tradition maintained by elders. Such traditional knowledge has been interweaved into modern ichthyology by many researchers (Calamia, 1999; Drew, 2005; Stacey *et al.,* 2008; MacLean *et al.,* 2009; Ferreira *et al.,* 2014), and the term for it is "traditional ecological knowledge" (TEK) (Berkes *et al*., 2000). For those keen in taxonomy, however, there is a need to adopt a more precise and sound approach.

Fish identification is the most important component in any fish-based study and acquiring reasonable competency always begins with the study of fish anatomy. Fundamentally, fish species have to "go with the flow". It is apparent that the anatomy of fish is shaped by the physical properties of water, the essential liquefied medium in which the fish adopts as habitat. Water is

dense but holds relatively small amounts of oxygen which affects fish respiratory function. Water also absorbs light in higher intensity than air which affects fish visual capacity. Water can flow fast or not at all, and this affects how fishes manoeuvre in water. Such a complex and fluid environment calls for special adaptations to live in and fishes have evolved precisely to fit in.

In general, most biological adaptations in fish occur in the body, mouth, fins, skin coloration and reproductive traits. The body of a bony ray-finned fish comprises 3 sections; the head, trunk and caudal or tail. The head is a region from the mouth tip to the posterior edge of the gill cover. The trunk contains the abdominal cavity and it forms the main body that lies between the head and caudal (Fig. 6). In most species, the trunk is narrowed down at an area called the caudal peduncle where it is connected to the caudal fin, which is a prominent feature on the body of a fish.

Body flexure attained by contractions of the myomeres and thrust from fins is responsible for fish propulsion. Fin shapes and sizes vary tremendously and they are used for stabilizing, reversing, stopping, descending, ascending and manoeuvring. In morphometric (Figs. 7 and 8) and meristic identification of fishes, the positions of fins, numbers and types of ray or spine composition are useful (Figs. 9, 10 and 11).

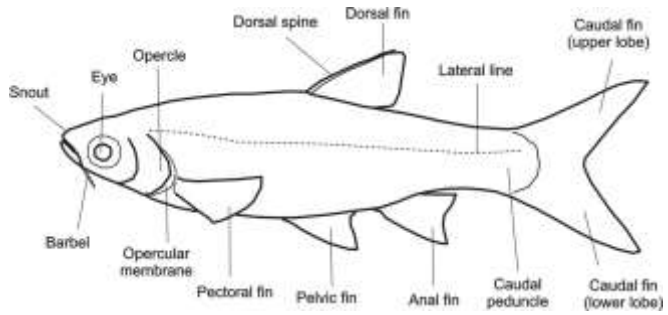

Figure 6: An example of key morphological features of a species from the Cyprinidae family (Source: Adapted from Rainboth, 1996).

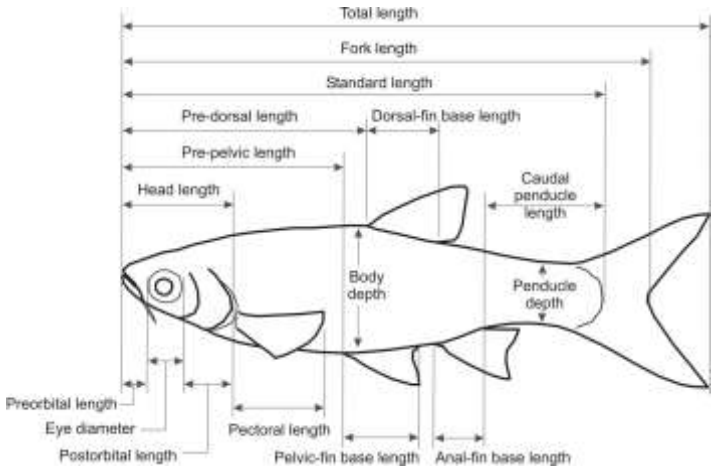

Figure 7: Common morphometric data collected for fish identification (Source: Adapted from Rainboth, 1996).

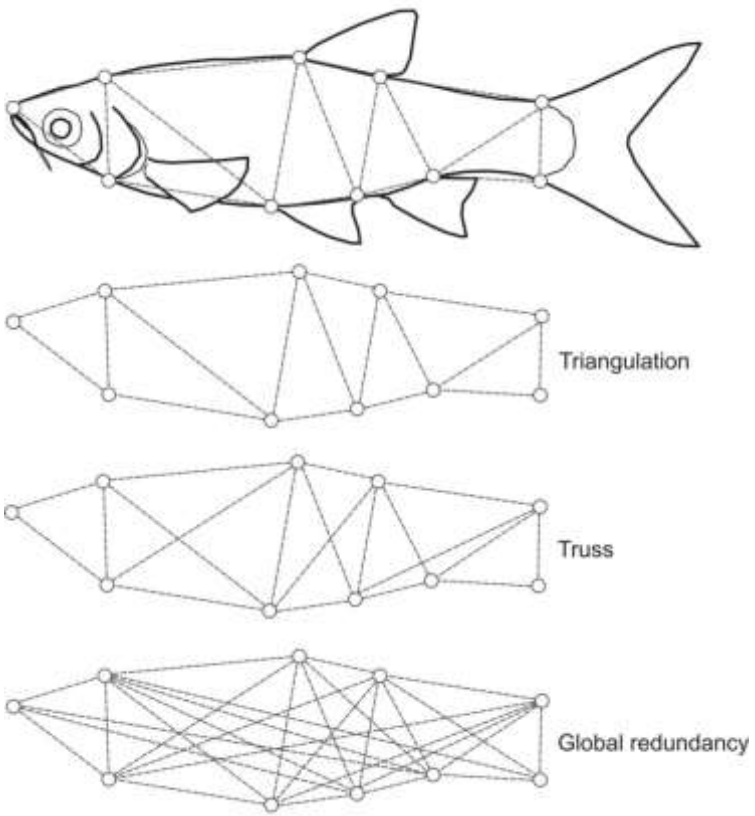

Figure 8: Common measurements between key points to construct patterns that quantify morphometric variance between species (Source: Adapted from Strauss and Bookstein, 1982).

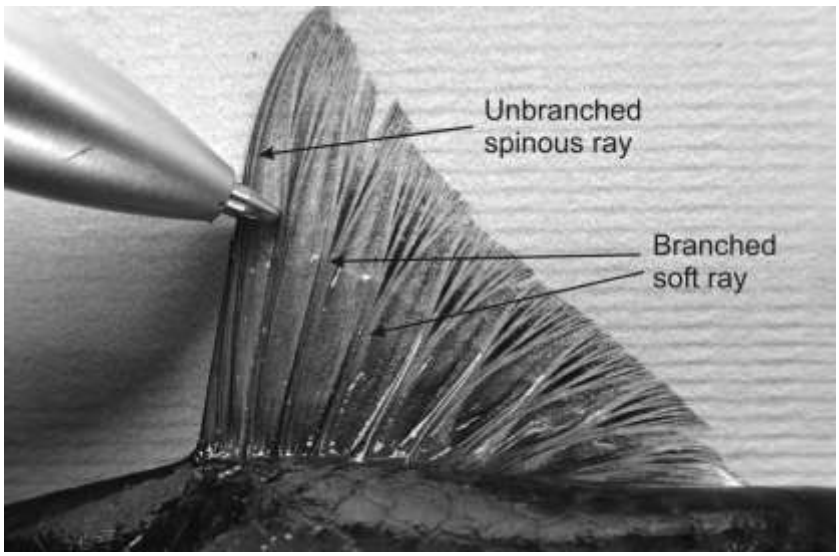

Figure 9: A close-up view of fin rays which epitomises fish under the Actinopterygii class.

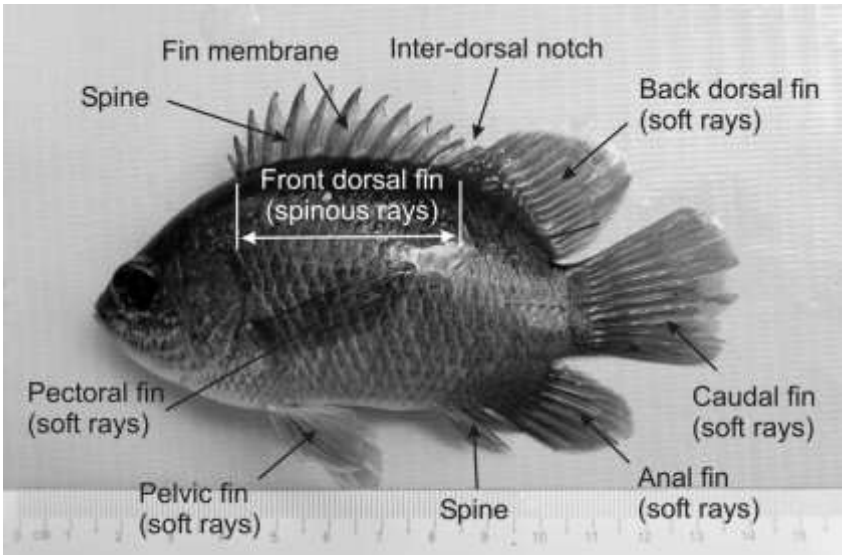

Figure 10: An example of fin positions and ray structures of a fish with a contiguous dorsal fin.

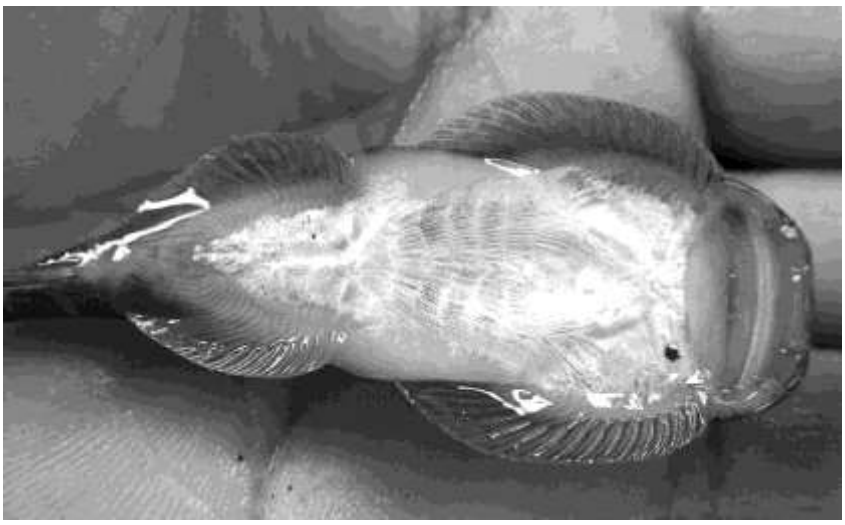

Figure 11: A specimen from the *Gastromyzon* genus with specialized suctorial pectoral fins and fused pelvic fins for adhering to substratum rocks in riffles.

There are two fundamental types of fin rays, namely the true spinous rays and soft rays that form the framework for fins. Spinous rays are stiff and typically unbranched, and they are located in a single fin's anterior part while soft rays consist of longitudinal supports and are typically branched. Even so, there are exceptions. Some species like the *Silurichthys indragiriensis* and *Wallago attu* propel themselves forward or backward by wavelike flexure of long anal fins. Ichthyologists call these fins "ribbon-fins" (Curet *et al.*, 2011), and what makes them so special is that the entire stretch of the anal fin is actuated by muscles along the body length. In eels, a ribbon-fin may be present but the caudal fin is almost absent and they rely mostly on snake-like rectilinear locomotion for swimming.

Most bony fishes have homocercal caudal fins that can be forked, rounded, truncated, and other symmetrical and non-symmetrical forms (Fig. 12). Generally, fishes with tapered body and fins are

proficient in high-speed propulsion in fast flowing waters while fish with rounded body and fins are associated with low-speed movement in slow waters.

Mouth form and position also vary greatly among fishes (Fig. 13). In most fishes, the mouth is located terminally at anterior tip of the head (e.g., *Pristolepis* spp. and *Oreochromis* spp.), but in some others it may be inferior (beneath the snout, e.g., *Pangio* spp.) or superior (upturned, e.g., *Belodontichthys dinema*) depending on their feeding habits. Generally, terminal mouths belong to species that prefer to bite or seize their prey while those with inferior mouths are bottom feeders. Insectivorous fishes such as the archer fish (*Toxotes* spp.) typically have superior mouths and they feed on insects that fall on the water surface. Some freshwater species like the river pipefish of the genus *Doryichthys* have a tubular mouth to suck food from crevices.

Most fishes have protective scales which vary greatly in size, structure, squamation (scale coverage), arrangement, sequence and colouration. Scientifically, scales of teleost fishes can be classified into the placoid, cosmoid, elasmoid and ganoid categories (Sudo *et al.*, 2002). Teleost scales possess outstanding hydrodynamic properties and provide a resistant layer to protect fishes from injury (Bruet *et al.*, 2008). Scaled skin is also known to play a critical role in supporting fish locomotion by synchronizing wave propagation (Long *et al.*, 1996), and by accumulating potential energy like tendons (Hebrank and Hebrank, 1986) for swimming efficiency. As expected, scale counts are crucial for fish identification (Fig. 14). Some genera such as *Clarias*, *Mystus* and *Ompok* are without scales, but they are no less vulnerable. The skin of scaled and scale-less fishes also secretes mucus to function as a sealant or protection against infection, and to reduce hydraulic friction while swimming.

Evidently, some of the most interesting features of fishes are their pigmentation patterns (Fig. 15) which can be used as critical references for taxonomic identification purposes. Body colouration is indeed an interesting subject in the context of fish as a biological indicator. Like all animals, fishes cannot synthesize pigments. They have to ingest colourant pigment like carotenoids from the food they consume within their habitats, and these can include fruits, insects and phytoplankton (Grether, 2000). This can reflect their foraging tendencies, habitat health and especially the presence of insect diversity as a rich source of pigments. Many studies have shown that fish mating behaviour is affected by pigment availability from feeding habitats and this, in turn, affects mate choice and population abundance (Endler, 1980; Basolo, 1990; Houde, 1997; Blount *et al.*, 2003).

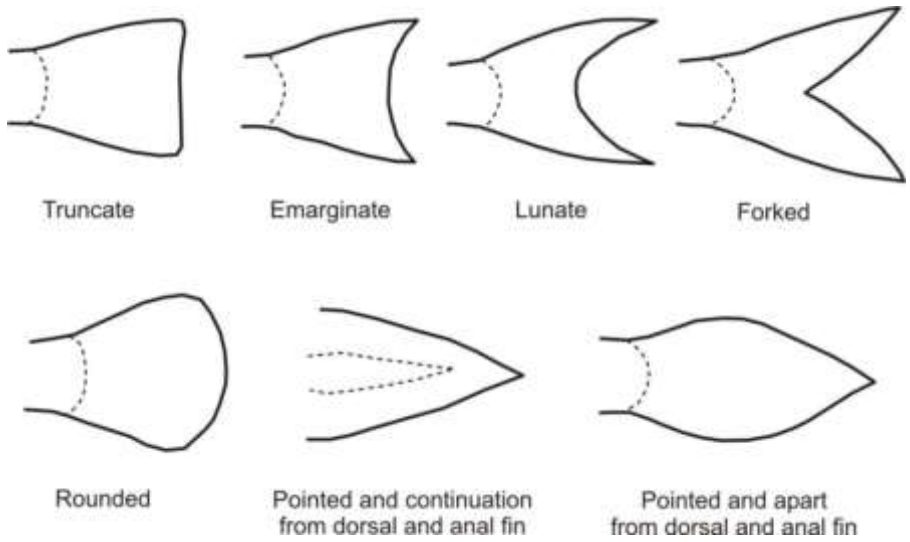

Figure 12: Common caudal fin shapes (Source: Adapted from Rainboth, 1996).

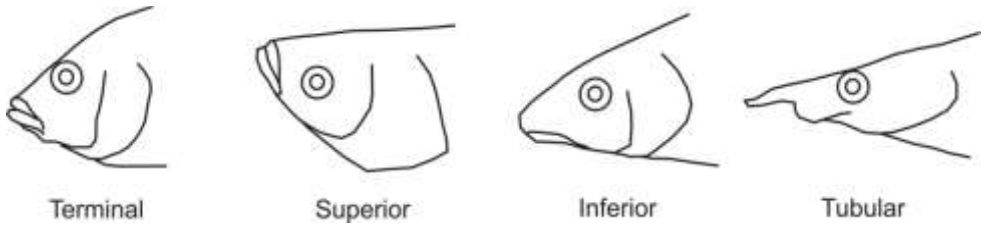

Figure 13: Common mouth types.

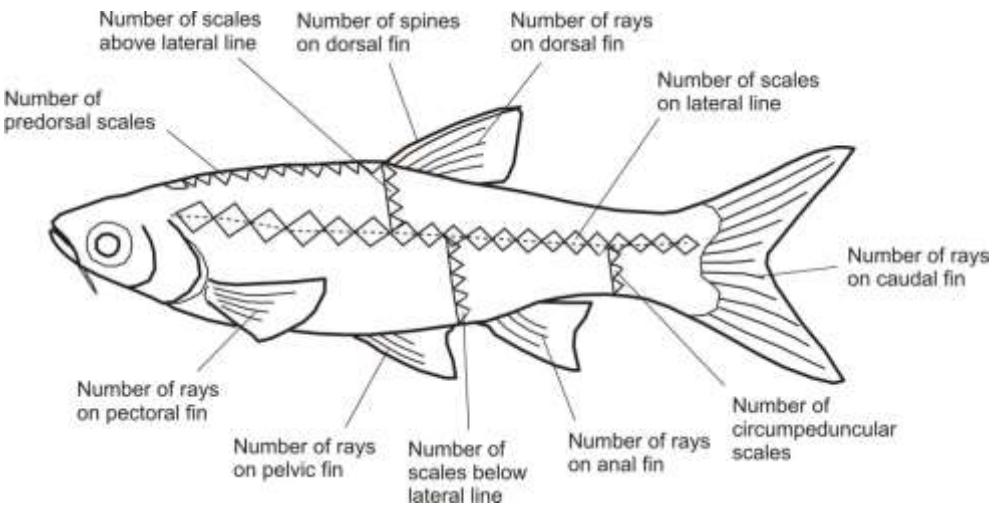

Figure 14: Common meristic data collected by taxonomists (Source: Adapted from Rainboth, 1996 and Ambak, 2012).

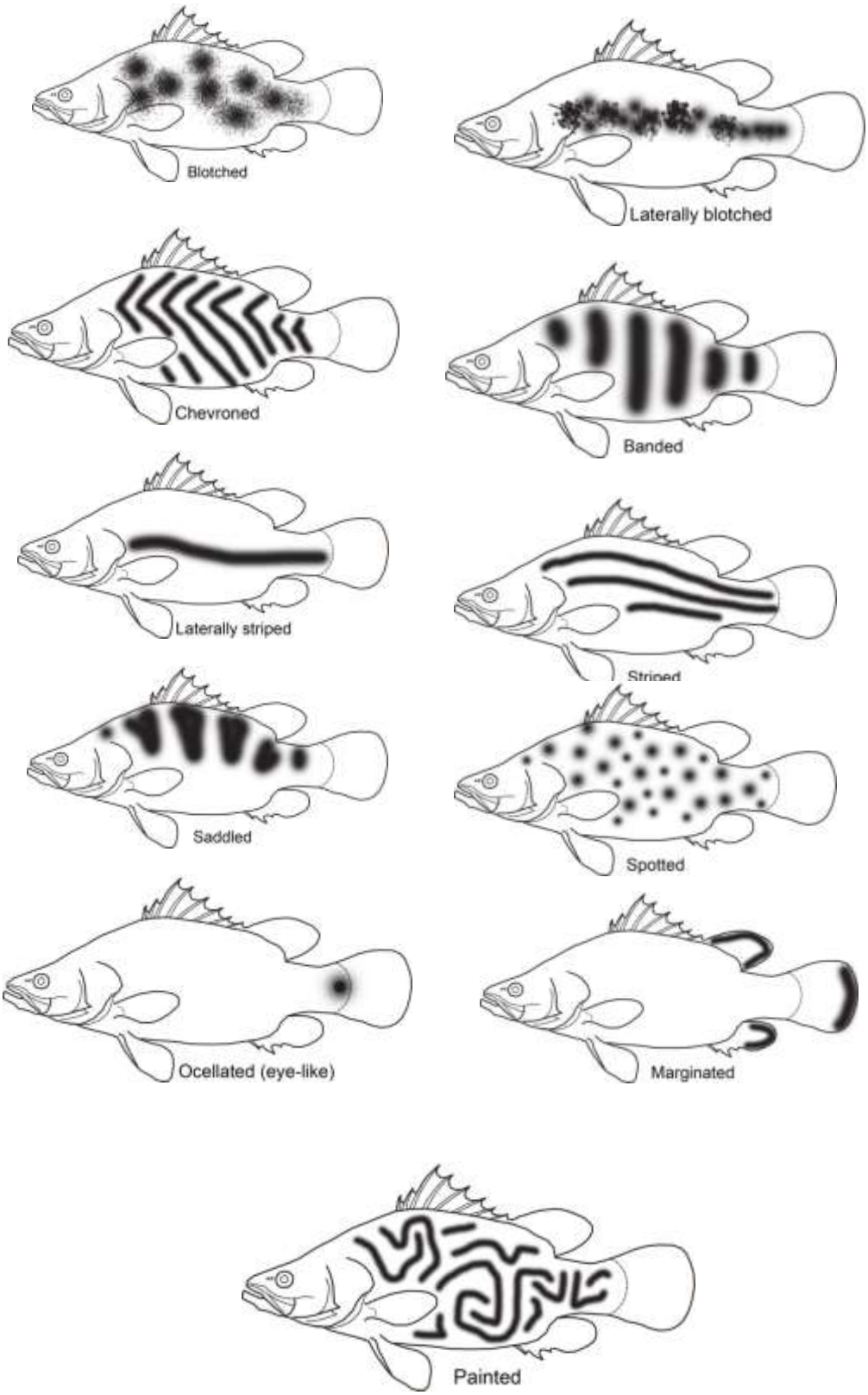

Figure 15: Common terms used to describe body markings and patterns on a representative teleost.

The fundamental colour constituent in fishes is the dermal chromatophore unit (pigment cell) which consists of the: 1) melanophore which contains melanin (browns, blacks, and greys), 2) xanthophore and erythrophore which harbour carotenoids (yellows, orange and red), and 3) iridophore, or sometimes termed as iridocyte, which naturally reflects external light source and provides fishes their iridescence (Hawkes, 1974; Fujii *et al.,* 1989; Metz *et al.,* 2006). Chromatophores can naturally contract or expand to induce or change colours to blend into the aquatic environment for camouflaging purposes. In some cases, a combination of chromatophore units can produce interesting colours. For example, in the Siamese fighting fish (*Betta splendens*), a wide variety of bodily colours can be exhibited by the permutation of iridophores, melanophores, erythrophores and xanthophores (Khoo *et al.,* 2012; Khoo *et al.*, 2014).

It must be cautioned that identifying species by their body colouration and patterns alone is not entirely robust. In certain cases, individuals in a natural population may be affected by genetically inherited conditions that cause them to exhibit albinism, leucistic, melanistic and xanthic abnormalities. Albino individuals such as *Silurus glanis, Astyanax mexicanus, Hydrolagus colliei* and *Genidens planifrons* have been reported (Dingerkus *et al.,* 1991; Jeffery, 2006; Reum *et al.*, 2008; Leal *et al,* 2013) and these individuals are unable to synthesize tyrosine and melatonin hormones partially or fully (Slavík *et al.,* 2016). Individuals affected by albinism are typically pale, light pink, white or yellow (Lechner and Ladich, 2010). Their eyes are generally pink because blood is seen through the colourless retina (Van Grouw, 2006).

Conversely, leucism is a condition characterized by the reduction or absence of most, if not all, of the pigment cell types. Leucoptic individuals are generally pale, white or yellow but they exhibit normal retinal pigmentation (Quigley and Wallace, 2013). Melanism is the exact opposite of albinism where excessive development of dark-coloured pigment melanin occurs and the affected individual is typically dark brown or black (Regan, 1961). It is a form of polymorphism that is widespread in fishes, and it may be genetically determined and/or influenced by the environment (Price *et al.,* 2006). For example, melanin polymorphism has been report in *Gambusia holbrooki*, *Siphateles bicolor mohavensis* and *Amphilophus labiatus* (Horth, 2002; Henkanaththegedara and Stockwell, 2011; Sowersby *et al.,* 2014). When an individual exhibits abnormal deep yellow, orange or reddish pigmentation on the body, this is attributed to excessive development of the xanthophores and erythrophores (Khoo *et al.,* 2012). Xanthic variety of the *Cyprinus carpio* and *Carasszus auratus* with red, orange and yellow pigmentation were first reported and domesticated in China and Japan as ornamental species (Kajishima, 1977; Balon, 1995). Wild individuals from species such as *Cyprinodon bifasciatus* have also been found to be

xanthic (Carson 2011). On the other hand, an axanthic individual that lacks xanthophores may be black, white, and/or blue (Lewand *et al.*, 2013). For example, Parichy (2006) reported that axanthic individuals have been found in species from the *Danio* genus.

Being fully aquatic, fishes have vastly different sensory systems compared to those of terrestrial vertebrates. In fact, they are endowed with more sensitive and complex sensory organs to detect vibration and sound. Fishes possess the cephalic-lateralis system (Nelson, 1972) that comprises a series of neuromast sensory cells that run across the outer layer of the head and body (Fig. 16).

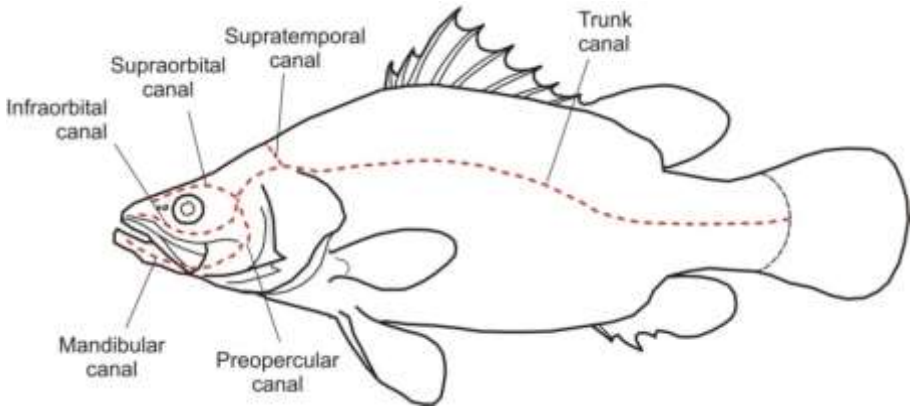

Figure 16: An illustration of the fish cephalic-lateralis system consisting of neuromast sensors that run across canals on the head and body (Source: Adapted from Nelson, 1972 and Iwata and Jeon, 1995).

The neuromast contains fine hair cells oriented in a manner to detect the direction of vibration. Vibration signals are then transmitted through special canals and pits containing endolymphic fluid to amplify the signals and thereafter pass on to the brain. The location of the lateralis system in fish can reflect their feeding habits. Bottom feeders tend to have the lateralis system on top of the body to detect vibration from predators that are lurking above. Those which feed on the water surface normally have the system along the ventral margin of the body to track the presence of predators below. Studies have also shown that the lateralis system is responsible for obstacle avoidance, triggering startle response and schooling synchronization (Partridge and Pitcher, 1979). The patterns and configuration of cephalic-lateralis system on fish bodies are sometimes used for fish identification such as those from the *Kryptoglanis*, *Pseudorasbora* and *Caecieleotri* genus (Moncey, 2012; Kawase and Hosoya, 2015; Walsh and Chakrabarty, 2015).

In the case of cryptic species when the species are too difficult to be differentiated because they show small external anatomical and morphological deviations between species in the same genus, ichthyologists have relied on the internal organs to characterise them (McCune, 1981). Where necessary, gill rakers from the first gill arch on the left

side of the body are also counted for meristic characterization such as species from the *Coregonus, Garra* and *Labeo* genus (Amundsen *et al.*, 2004; Ayoade *et al.*, 2004; Krupp and Budd, 2009). Correspondingly, in some species, the pharyngeal teeth found on the fifth gill arch are counted from left to right to assist in meristic identification of species such as those from the *Moxostoma, Cycleptus, Danio* and *Epalzeorhynchos* genus (Eastman 1977; Pasco-Viel *et al.*, 2010).

All teleost fishes have an inner ear as an auditory system. Instead of bony ossicles, fishes have three pairs of calcareous "ear stones", or scientifically termed as otoliths (Greek for "ear stones"). The lapillus ("little stone" in Latin) functions in maintaining body balance and orientation, and the other two, namely asteriscus ("little star" in Latin) and sagittal ("arrow" in Latin), peruse acoustic reception. Otoliths are enclosed in a membranous sac together with sensory hair cells or ciliary bundles. Detection of mechanical signals will occur when there are dynamic interactions between otoliths and cilia (Assis, 2003; Campana, 2005). Since Koken (1884) reported that fish species can be characterised by having otoliths of different shapes and sizes, otolith morphometry is fast becoming a trend to identify fishes and some species such as *Netuma bilineata, Nuchequula nuchalis, Coilia dussumieri* and *Garra rufa* (Chen *et al.*, 2011; Thuy *et al.*, 2015; Salimi *et al.*, 2016; Yedier *et al.*, 2016). In 2006, the AFORO online database was launched (http://www.cmima.csic.es/aforo/) to archive and share Fourier spectrum (FFT), wavelet analysis (WT) and curvature scale space analysis (CSS) data of otoliths (Lombarte *et al*., 2006). At time of this writing, the AFORO archive contains 4,672 high resolution images of 1,441 species from 221 families for reference.

Species Identification at the Molecular Level

Morphological characterization is not entirely robust. As mentioned earlier, the concept of "species" is still subjected to debate and not all taxonomists assign meaningful categories to organisms by morphotype. In many cases when certainty cannot be attained, researchers use terms like "subspecies", "strain" or "variant". These are highly subjective and confusing. In fish especially, morphological plasticity between individuals of the same species is inherent. For example, body colour tones and polymorphism exhibited by individuals of the same species may vary considerably depending on the diet regime, habitat and season such as species from the *Betta*, *Poecilia* and *Danio* genus (Khoo *et al.*, 1997; Price *et al.*, 2008). And this is where molecular analysis has become a viable alternative.

Each organism is characterized by a unique set of biological attributes that enhance its fitness to survive in a niche environment. Correspondingly, to adjust to any changes in the environment, an organism is naturally subjected to genetic drift, mutation or variation (polymorphism) as a mechanism to adapt. Such natural phenomenon provides

markers at molecular level to detect individual or species uniqueness (Sanger *et al.,* 1977). All molecular methods depend on DNA marker or protein sequence analysis and comparison to determine molecular divergence over evolutionary time based on the null hypothesis of molecular evolution, or better known as the "neutral theory" (Kimura, 1968). Essentially, all methods assume that individuals from the same species have specific DNA (or protein) sequences that vary, to a certain extent, from individuals that belong to other species. Nonetheless, the variance, or "signature" of speciation (Sbordoni, 2010), is dependent on time and space and subjected to biological productivity of individuals, dispersal pattern and natural genetic drift. Therefore, genetic variability also occurs among individuals of the same species. This means establishing a credible locus, or the position of a gene (Khoo *et al.,* 2011) from a phenotype is a prerequisite as master reference for comparison purposes.

DNA markers can be classified in two different types, namely type I which are markers associated with genes with known function and type II which are markers are with anonymous genomic segments (Chauhan and Rajiv, 2010). For example, allozyme markers are classified as type I protein markers and microsatellites and other neutral markers are considered type II (Hinsinger *et al.,* 2015). The DNA in all organisms is a composition of four chemical bases – adenine (A), guanine (G), cystosine (C), and thymine (T) (Avise, 1994). Their order or sequence in each gene is unique to every species. Since Razin and Rottem (1967) have successfully employed protein analysis for characterising microorganism species, modern advances have adapted the concept of analysing A-G-C-T bases in various DNA genetic markers. In fish identification, the common DNA analyses applied are length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), microsatellites or simple sequence repeat (SSR), single nucleotide polymorphism (SNP) and expressed sequence tag (EST) markers (O'Reilly and Wright, 1995; Khoo *et al.,* 2003; Sampaio *et al.,* 2003; Teletchea, 2009; Chauhan and Rajiv, 2010; Khoo *et al.,* 2011; Kress *et al.,* 2015).

Fish genome typically contains roughly a billion nucleotide pairs (Stepien and Kocher, 1997), and analysing all of them would be too daunting and the results would cause an information overload. Since Herbert *et al.* (2003) discovered a technique to amplify the mitochondrial *cytochrome c* oxidase subunit 1 (COI) gene. There is now a consensus to analyse a 648-base pair (bp) region of COI to rapidly identify a fish species (Cawthorn *et al.,* 2012). Such an approach is now known as DNA barcoding and it has gained widespread acceptance as a fast, cost effective and standardised technique. So far, the Fish Barcode of Life Initiative has barcoded 7,882 fish species, which is only approximately 25% of the estimated

31,220 species present globally (Jinbo *et al.*, 2011).

It should be stressed that identified species through DNA barcode is only as good as the voucher specimen's DNA barcode made available and archived in platform such as GenBank. Hence, if a DNA barcode is inaccurate or compromised due to issues such as carryover DNA contamination, incomplete genotyping of loci throughout the genome, low/wrong quality DNA material, human errors, biases and some other critical risks (Bridge *et al.*, 2003; Forster, 2003; Moritz and Cicero, 2004; Smith and Burgoyne, 2004; Ebach and Holdrege, 2005; Meyer and Paulay, 2005; Will *et al.*, 2005), the identification process would be erroneous. Ross *et al.* (2008) proposed that reference sequences of at least five or more voucher specimens from various geographical sites should be acquired. Unfortunately, this also means that DNA analysis is not a one minute procedure. To conduct it properly, the process of acquiring exemplar genotypes is tedious and costly because some species are found in large regions and various countries.

In the recent years, DNA sequencing techniques have made substantial progress and they are able to analyse 30 to 1,500 nucleotides (nt) for hundreds of thousands to millions of DNA molecules in a single process within a complex or degraded DNA source (Davey and Blaxter, 2011; Mehinto *et al.*, 2012). These are classified as next-generation sequencing (NGS) technologies (Tillmar *et al.*, 2013) and sometimes termed as "DNA metabarcoding" to refer to their ability to automatically identify multiple species from a single bulk sample (Taberlet *et al.*, 2012). Because NGS technologies can extract massive numbers of reads, the results increase the chances of finding and annotating matches (Hemmer-Hansen *et al.*, 2014). So far, technological platforms that are able to produce and analyse gigabases of DNA sequence include Illumina, Roche, AB SOLiD, 454 GS FLX, Ion Torrent, HeliScope, Starlight and PacBio (Rothberg and Leamon, 2008; Pandey *et al.*, 2008; Davey and Blaxter, 2011; Hemmer-Hansen *et al.*, 2014). They differ from each other based on amount of sequence information generation, chemistry protocol for sequencing and the length of sequence read (Mehinto *et al.*, 2012).

Reference Type and Traceability

The hallmark of the taxonomy discipline is its persistency in collecting, preserving and managing specimens as essential physical references for species-level research. Although ecologists, conservationists, aquaculturists or researchers who adopt fish as a part of their studies need not master the specifics in specimen management, it is useful to briefly understand the ICZN zoological code and terms as a precaution against negligence when tracing the correct binomial nomenclature and description of fish.

Fundamentally, in taxonomy, a “type” is a specimen of a distinct species which is used as master reference (Krell and Wheeler, 2014). Each species

or subspecies that is scientifically named by the original author (discoverer) is traceable to a name-bearing specimen which was first found and kept in a particular museum. A new species cannot be described and registered formally without depositing a single physical whole fish known as a "holotype" in the museum (Clemann *et al.*, 2014; Kumar and Hassan, 2015). As sexual dimorphism occurs in many fish species, it is good practice to deposit an opposite sex specimen of the holotype and it is assigned as an "allotype" (Jorge *et al.*, 2014). Also, polymorphisms may occur in certain fishes and a holotype is not expected to be a typical representative (Hulsey, 2005), although in an ideal case it should be. To mitigate this, morphotypes that show morphological variants of a species can be established, and molecular analysis is usually carried out to determine whether the variations are due to polymorphism or if it is a new species (Simonov, 2008).

A researcher may also designate a duplicate specimen of the holotype and such a specimen is termed as an "isotype". When a better specimen is eventually deposited, the holotype is not superseded. According to the ICZN code, such a specimen which provides better clarity is known as an "epitype" and it may be deposited when the holotype is evidently imprecise. In the case when a holotype is lost or damaged, a "neotype" specimen may also be deposited as replacement. While there can only be one holotype, taxonomists can continue to deposit specimens in any museums around the world for the type series (a range of specimens showing variation in the species). These comparable specimens are termed as "paratypes" and they have no name-bearing role.

In situations when the researcher fails to establish a holotype, the existing two or more specimens collected and deposited in the museums can be used to describe and name a species. These deposited specimens are known as "syntypes" although such practice is now rarely used in contemporary taxonomy. A master reference specimen selected from syntypes is termed as "lectotype" and when a lectotype is finally established, all other syntypes shall be, by default, reassigned as "paralectotypes". Correspondingly, any duplicate specimen of the lectotype is called an "isolectotype". Finally, specimens that have been erroneously described, named or labelled are annotated as "non-types". All terminologies pertaining to types mentioned in this section are elaborated in detail by ICZN at the website http://www.iczn.org/iczn/index.jsp.

The responsibility rests on the taxonomist to maintain the specimens and make them accessible for current and future studies (Krell and Wheeler, 2014; Rocha *et al.*, 2014). To consolidate a large quantity of voucher specimen database in a museum, the taxonomist is also expected to occasionally publish monographs that contain summarised information of all species in a group to update the scientific community (Grinnell, 1910; Pyke *et al.*, 2010; Kottelat, 2013).

In the advent of technological advancement, non-destructive methods such as high-resolution photography, computerised tomography (CT) scan and radiography methods that can record the physical characteristics of specimens, the work of a taxonomist has expanded over the years. There are already attempts to digitalize specimens using these technologies. For example, Berquist *et al.* (2012) have progressively scanned specimens with the magnetic resonance imaging (MRI) technology and created an online digital archive called Digital Fish Library (DFL, http://www.digitalfishlibrary.org) to share high-resolution and high-contrast visual data. The Biovisualization Center at the University of Washington has also just initiated a program that applies the CT scanning technology and the captured 3D morphology data of fish specimens is shared through the Open Science Framework website (https://osf.io/ecmz4/wiki/Fishes/). Such innovations have the potential to replace pale and degraded voucher specimens that have been preserved for a long period of time in alcohol. Morphological and meristic database compilation can be instantly peer-reviewed and shared online with any amateurs or experts located anywhere around the world. This is an opportunity for scientists to engage more with the society and garner support from the public and policymakers for fish conservation.

Specimen Collection and Preservation

In principle, a scientific finding must be reproducible. When an author declares and publishes the description of a new species, a specimen (i.e., haplotype) must be deposited permanently in the museum so that the author's claim can be critically appraised and reappraised by others (Rocha *et al.*, 2014). The specimen can be refuted or disputed by other ichthyologists to allow for revisions. Similarly, when a researcher conducts a fish inventory investigation that results in a scientific or journal report, it is a good practice (although not compulsory) to collect voucher specimens for future verification and to promote transparency. This approach also provides physical records that allow researchers to scrutinize the inter- and intraspecific variation of species collected at different periods of time and localities.

Specimens are typically acquired by various capturing methods as discussed widely elsewhere by literature such as by handnets, traps, seine nets, electrofishing and even by buying directly from fishermen or the local fresh markets. Once obtained, they must be immediately labelled with 1) serial number, 2) species name, 3) name of collector, 4) location, 5) date of collection, 6) GPS coordinates, and 7) some description of the specimens' colour and body markings in fresh should be recorded (Fischer, 2013; Motomura and Ishikawa, 2013). This may be supported by high resolution photographs of the whole fish and specific parts with distinctive characters (Figs. 17, 18 and 19).

Figure 17: Fresh specimen is photographed facing left together with a ruler to indicate scale.

Figure 18: A live specimen is best photographed with high-resolution photography to record subtle details and colouration.

Figure 19: Noticeable variations of the same species should be photographed for species-level studies. For example, in the case of *Tor tambra* (above), individuals in the same population may display differing forms of mental lobe and barbels (Roberts, 1999).

Specimens should be cleaned and can either be frozen or immediately fixed in formalin or alcohol before being transferred to depositories.

In fish taxonomy, the left side of the fish body is examined. Therefore if muscle tissue is required for molecular analysis, it should be dissected from the right side of the body. In small fishes where muscle tissues are difficult to excise, the right pelvic fin may be collected as an alternative (Shiozawa *et al.,* 1992). Typically, 1.0 cm^3 tissue from the fresh muscle contains enough genomic material for molecular study and it should be stored in 95% alcohol and then refrigerated at -20°C (Motomura and Ishikawa, 2013). Preferably, it should be free from fat and blood which may hamper the DNA purification process (Wong *et al.,* 2012). Chakraborty *et al.* (2006) suggest that in the absence of refrigeration during field work, specimens fixed in 10% buffered formalin or 95% alcohol should be analyzed with DNA processes within one week to produce the best results.

In the museums and depositories, taxonomists are cautious when fixing and storing specimens (Fig. 20) since any flaw in the process will devalue their academic significance. Specimens are usually fixed in 10% formalin solution and large sized specimens are incised on the right side to enable better absorption of formalin (Schander and Halanych, 2003; Garrigos *et al.,* 2013). For the same purpose, formalin may also be injected into the abdomen by using a syringe (Motomura and Ishikawa, 2013).

Figure 20: Fixed specimens are usually stored in glass jars with heads pointing downwards. Note that specimens become pale and lose their colouration quality, which is why photography of live specimens is crucial as part of the data collection process.

Taxonomic Key

After fixing, labelling and cataloguing a specimen, a competent taxonomist would carry out the standard morphometrics, meristics and sometimes molecular analyses as mentioned earlier. As a minimum, the taxonomist would subsequently generate a report with the following information (Fischer, 2013) for each species;

1. Species name, author and year.
2. Material examined – description of type material and voucher specimen examined.
3. Diagnosis – description of the specimen's key markers or morphological features that differentiates it from nearest congeners from the same watershed and other watersheds. Diagrams may be included for clarity.
4. Description – description of major morphological and meristic data.
5. Pigmentation in life – description of colour, marking and patterns on the body and fins of a fresh specimen.
6. Colour in formalin or alcohol – description of colour, markings and patterns on the body and fins of the fixed specimen.
7. Distribution – description of location where the specimen and the species can be typically found according to published literature.
8. Etymology – description of the Greek/Latin word or rationale behind the scientific name (binomial nomenclature) assigned to the species.
9. Field notes – description of sympatric and syntonic species found in the same habitat when the specimen is collected.
10. Remarks or comments – description of precautions as a measure against misidentification and any other useful information for effective identification. If a potential new species is encountered, the specimen and full-colour photographs are to be sent to an authority of the genus for further investigation. This shall be recorded in this section.

A competent taxonomist would update the field "taxonomic key" for the corresponding genus once a new species has been identified. It is a conventional tool meant for quick and practical identification based on the major morphological characters (Fig. 21) of a species (Fischer, 2013). A typical taxonomic key is organized in a series diagnostic characteristic of a species that lead the user to the correct name of a given specimen. A taxonomic key is called a dichotomous key (in Latin "dichotomous" means in two parts) because only two marker options are offered in each step (Fig. 22). The markers provided may be quantitative (e.g., scale count) or qualitative (e.g., body colour). Once created, the key can be continuously improved by taxonomists based on feedback from users.

Figure 21: The key characteristics of a genus are typically described at the start of the taxonomic key. For example, the *Glyptothorax* genus's key characteristic is the presence of a unique thoracic adhesive apparatus (arrow) that can only be observed in the ventral view.

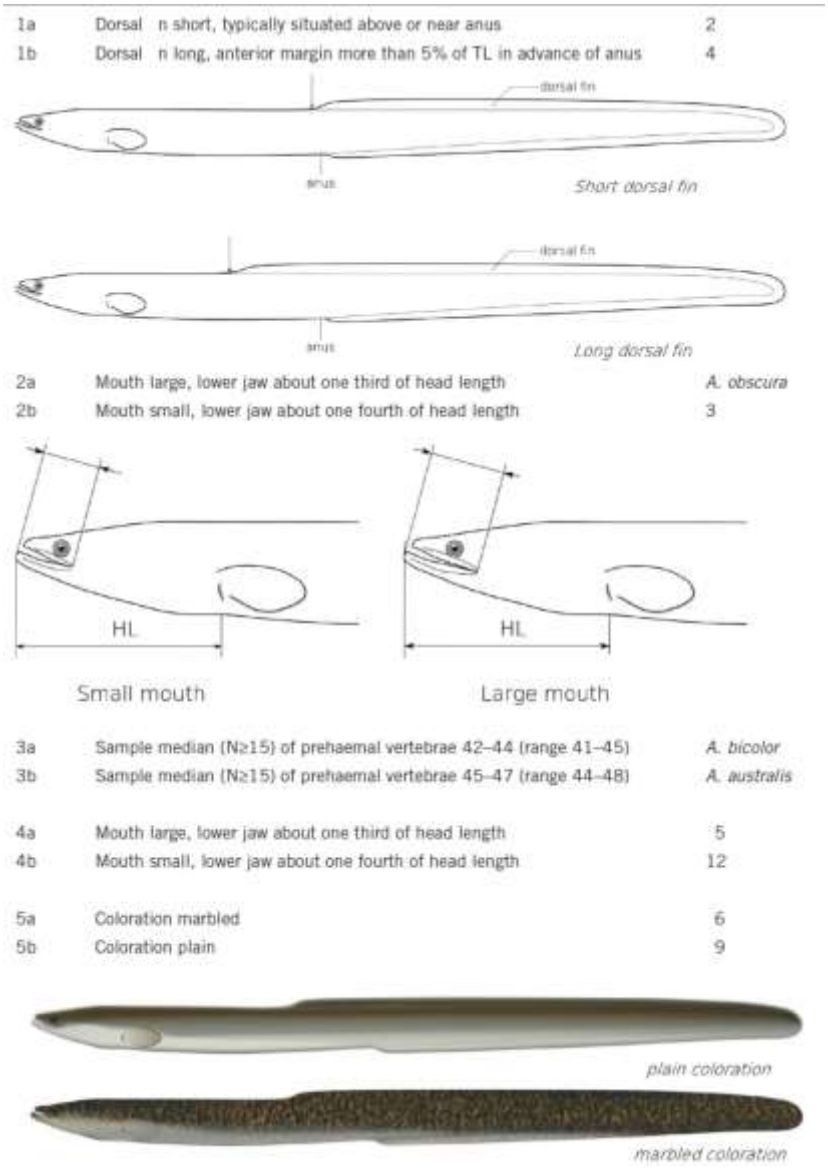

Figure 22: An example of a dichotomous key produced by Silfvergrip (2009, p.25) for the identification of freshwater eels from the Anguillidae family.

Integrative taxonomy

There are species within the same genus that possess small variations that are not easy to discriminate. This is compounded by the unstable and convoluted taxonomic history of some species which can experience numerous revisions of species names because taxonomists may not share the same assumptions and approaches. Correspondingly, it is fair to expect that the science of taxonomy and systematics is constantly in flux and revisions may be frequent.

A classic example is the case of *Neolissochilus* spp. and *Tor* spp. found in Southeast Asia. These genera are typically prone to trophic polymorphism and display conspicuous oral morphology variation (Roberts and Khaironizam, 2008) that have confused even the most experienced ichthyologists. For example, in early 20th Century, members of the *Neolissochilus* genus were placed in the *Barbus* genus (Boulenger, 1893; Duncker, 1904). Later they were reassigned respectively to *Labeobarbus*, *Crossochilus, Puntius* and *Acrossocheilus* (Weber and de Beaufort 1916; Ahl 1933; Fowler 1934; Herre and Myers 1937; Smith 1945) before being finalized as *Neolissochilus*, a new genus created by Rainboth (1996).

In cases when the taxonomy of a particular genus has stabilized, misidentification can still be common because small variations can be difficult to be distinguished. For example, in the past decades, there were numerous reports that highlighted misidentification of the freshwater eels from the Anguillidae family due to species and subspecies from the family have sympatric distribution and the morphological characters among them are hard to distinguish (Castle and Williamson, 1974; Aoyama *et al.,* 2000; Arai and Wong 2016). For example, Sugeha and Suharti (2009) found difficulty in distinguishing *Anguilla bicolor bicolor* and *Anguilla bicolor pacifica* based on morphological attributes alone. However, they were able to make a distinction between the species by their adult sizes. Generally, *A. bicolor bicolor* is longer and heavier than *A. bicolor pacifica*. They also found that *A. bicolor bicolor* tend to occur in Sumatra and Java region and *A. bicolor pacifica* is found in Sulawasi and New Guinea region of Indonesia. As such, they have proposed that the two subspecies can be distinguished by the geographic approach. Subsequently, Teng *et al*. (2009) resolved the issue by conducting a phylogenic study and validated the notion that *Anguilla* species and subspecies can cluster regionally (Fig. 23).

The case of *Anguilla* genus is a classic example how the combination of morphological and molecular approaches, generally termed as integrative taxonomy (Goulding and Dayrat, 2016), are crucial in gaining and expanding the understanding of taxonomy and diversity in fish. This demonstrates that the broadest range of methods should be utilized to answer and solve taxonomic concerns.

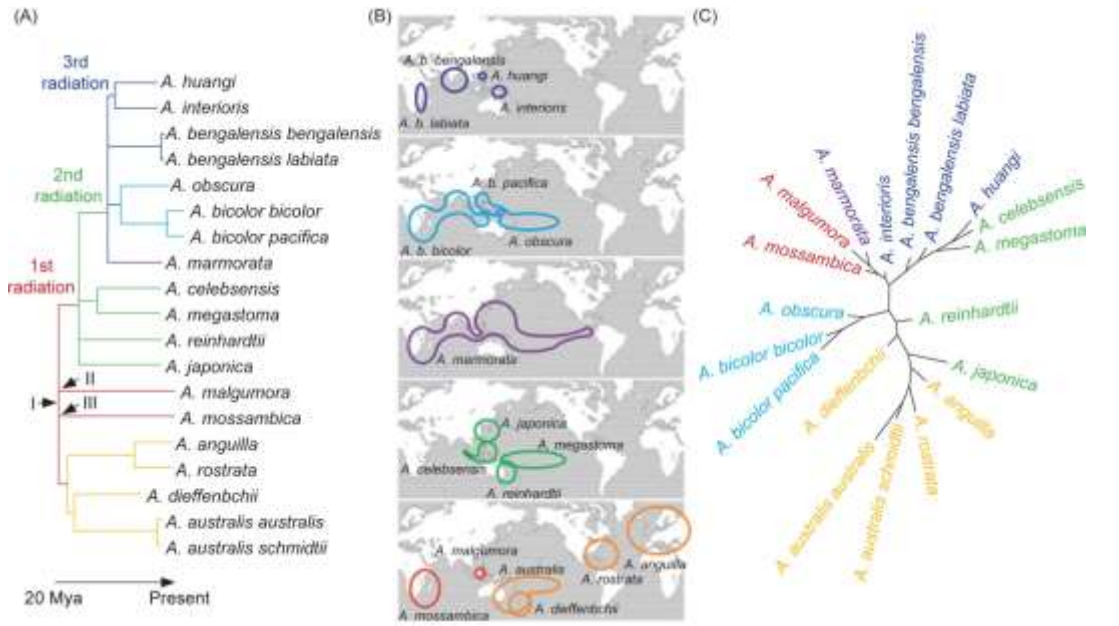

Figure 23: The morphological phylogenetic tree of the genus *Anguilla* showing correlation species occurrence in various regions (Source: Teng *et al.*, 2009).

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