

Chapter 6

Morphological Descriptions and Taxonomy

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6.1 TOPIC BACKGROUND

Morphology, the study of biological form, involves examination of structure, shape, color, and all other physical attributes of organisms. It can be used to describe the features within an individual organism but also provides useful information about variation among populations, species, families, and higher taxonomic levels. The ability to evaluate morphological variation, either descriptively or quantitatively, is fundamental to many fields of biology, including physiology, biomechanics, developmental biology, and systematics.

The morphology of fishes varies enormously, and virtually any feature can be used as a **diagnostic character**, helping us to distinguish one taxon (species, genus, or any other taxonomic group) from another. However, in order to reliably identify and propose diagnostic characters, it is crucial to differentiate taxonomically meaningful morphological differences from other forms of variation. Grande (2004) and Hilton and Bemis (2012) characterize four main types of morphological variation: ontogenetic, variation that occurs over development within an individual; sexual dimorphism, differences between males and females within the same species; individual, variation between representatives of a single species (i.e., at the population level); and last, phylogenetic (or taxonomic), which consists of morphological variation characterizing differences among species (or other taxonomic levels of interest). Ontogenetic, dimorphic, and individual variation in morphology all occur within the same species and therefore need to be carefully considered when seeking characters that provide distinguishing features at the species level or higher.

Traditionally, morphology has played an important role in taxonomy, the science of classifying living organisms. The main focus of taxonomy is to identify differences among related taxa and to assign characters that facilitate their recognition. As suggested above, this process is based on evolutionary relationships among organisms, and it is the task of systematics to unravel these relationships. Clearly, taxonomy and phylogenetic systematics are tightly linked and overlap broadly in scope. Early on, taxonomic characters were based mostly on morphology, but the incorporation of molecular data has driven much of the progress in systematics in recent years (Betancur-R. et al. 2013, 2017; Near et al. 2013; Arcila and Ortí 2021, Chapter 5, this volume). The limitations of morphology for systematics arise in cases like **cryptic species**, which are difficult to distinguish based solely on morphological characters. Molecular

approaches have proven very useful for identification in instances like these (Tornabene et al. 2016). Nonetheless, morphology maintains an important role in taxonomy, and as you will see in this chapter, also provides vital context to investigate the ecological and lifestyle diversity that we see in fishes.

This chapter is divided into three main topics. The next several sections (6.2 to 6.6) walk through a series of morphological systems in fishes commonly used in fish systematics, both external and internal, detailing the components that constitute them and describing how they may vary across fishes. Section 6.7 provides an overview of the primary methods used in morphological studies of taxonomy and systematics of fishes. Section 6.8 details the main taxonomic and nomenclatural acts and procedures. Section 6.9 covers **geometric morphometrics**, a common quantitative approach for evaluating morphological variation in fishes and other organisms. The methods described in this last section can be used to complement traditional morphological descriptions in taxonomic studies but are also useful for general exploration of morphological diversity in fishes. Generally, we have focused this chapter on descriptions of bony fishes and elasmobranchs. The morphology of jawless fishes are not covered here. For more information on the morphology and systematics of hagfishes (Myxini-formes) and lampreys (Petromyzontiformes), see Hubbs and Potter (1971), Ota and Kuratani (2008), Mincaroni and Fernholm (2010), and Song and Kim (2020).

6.2 DATA AND OUTCOMES

In the following sections, we will lead you through detailed descriptions of various morphological systems that are commonly used in taxonomic studies of elasmobranchs and bony fishes. However, it is first important to discuss the key concept of morphological characters. Here we use a definition from Sereno (2007: 570), describing a character as a “heritable, organismal feature (i.e., an observable condition) expressed as an independent variable.” Under this convention, characters are composed of two parts, a locator that is simply the morphological structure of interest (e.g., the caudal fin) and a variable, which is a feature of the locator that varies across taxa (e.g., shape). The same character might have distinct conditions or variants among different taxa, which are called character states. For example, caudal-fin shape may be forked in one taxon and emarginate in another. Here, “forked” and “emarginate” represent alternate states of the character “caudal-fin shape.” One important property of character states is that they must be mutually exclusive conditions. The character, and all of its character states, make up the character statement. An example of a character statement is shown here:

- Caudal fin, shape: forked (0); emarginate (1).

Here, the caudal fin is given the state “0” when it is forked in shape and “1” when emarginate. A common mistake when constructing character statements is considering the absence of a locator simultaneously with other forms of variation in physical state. For example, the following is an example of an incorrectly written character statement:

- Caudal fin, shape: forked (0); emarginate (1); absent (2).

In this case, the absence of the structure is not a feature of its shape and it is not an exclusive condition comparable to the character states of caudal-fin shape (i.e., forked and

emarginate). Instead, “absent” is an observation about the locator under investigation (i.e., the caudal fin of the fish). The only character state that can be mutually exclusive with respect to the state “absent” is the alternate state, “present.” Therefore, the incorrect character statement above could be rewritten as two different character statements:

- Caudal fin: present (0), absent (1).
- Caudal fin, shape: forked (0), emarginate (1).

The incorporation of these character concepts and methodologies in the diagnosis of a taxon allows more precise comparisons and clearly defined distinctions among taxa.

6.2.1 External Morphology

The general body plan of fishes consists of three primary regions, including (from anterior to posterior) the head, trunk, and tail. These regions can be distinguished by a combination of internal and external features and are illustrated for a representative bony and cartilaginous fish (Figure 6.1).

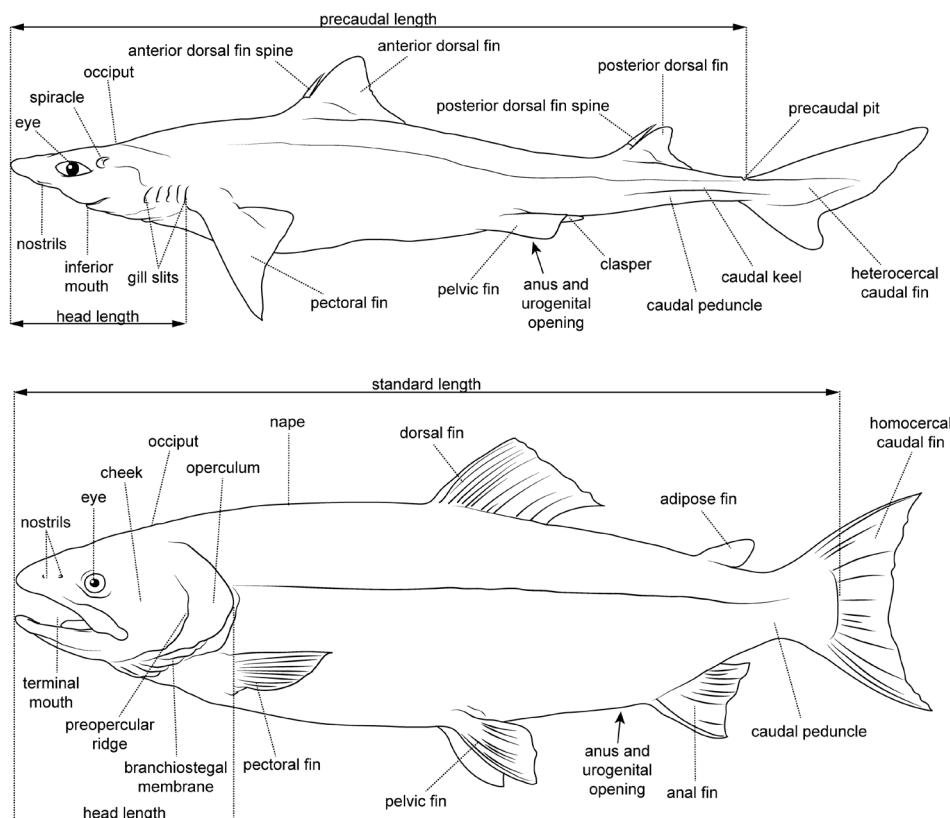


Figure 6.1. General external morphological features and primary measurements for cartilaginous (top) and bony fishes (bottom).

In bony fishes, the head region occurs between the anterior tip of the snout and the posterior margin of the operculum (a thick membrane supported by a series of bony plates that forms the gill cover). The ventral margin of the head is defined by the ventral and posterior boundaries of the branchiostegal membrane. Dorsoposteriorly, the head region extends to the occiput, a region where axial muscles in the trunk attach to the back of the neurocranium. Located directly posterior to the head, the nape (or nuchal region) is the space between the occiput and the origin of the dorsal fin. In contrast to bony fishes, elasmobranchs (a group of cartilaginous fishes including sharks, skates, and rays) lack an operculum. For sharks, the posterior-most gill slit marks the posterior extent of the head region. In skates and rays, greatly expanded pectoral fins are fused to the head, making the distinction of a discrete head region much more difficult to identify externally.

The trunk, or abdominal region of the body, extends from the back of the head to the anus, near the anterior origin of the anal fin. In several fishes, such as pirate perches (*Aphredoderidae*), swampfishes (*Amblyopsidae*), and some species of sculpins (*Cottidae*), the anus is shifted anteriorly from its typical position (Jenkins and Burkhead 1994; Mecklenburg et al. 2002). The terminal body region, the tail, comprises the area from the origin of the anal fin to the posterior end of the caudal fin. The caudal peduncle is one of the primary features of the tail and occurs between the posterior insertion of the anal fin and the anterior origin of the caudal fin.

Conventional measurements taken on the fish body are summarized in Figures 6.1 and 6.2. In bony fishes, ichthyologists generally represent body measurements as proportions of standard length, the linear distance from snout to the posterior tip of the caudal peduncle (often at the origin of the caudal fin or “flexure”). Given the fragile nature of fin rays, especially in museum-preserved specimens, standard length is generally preferred over the total length.

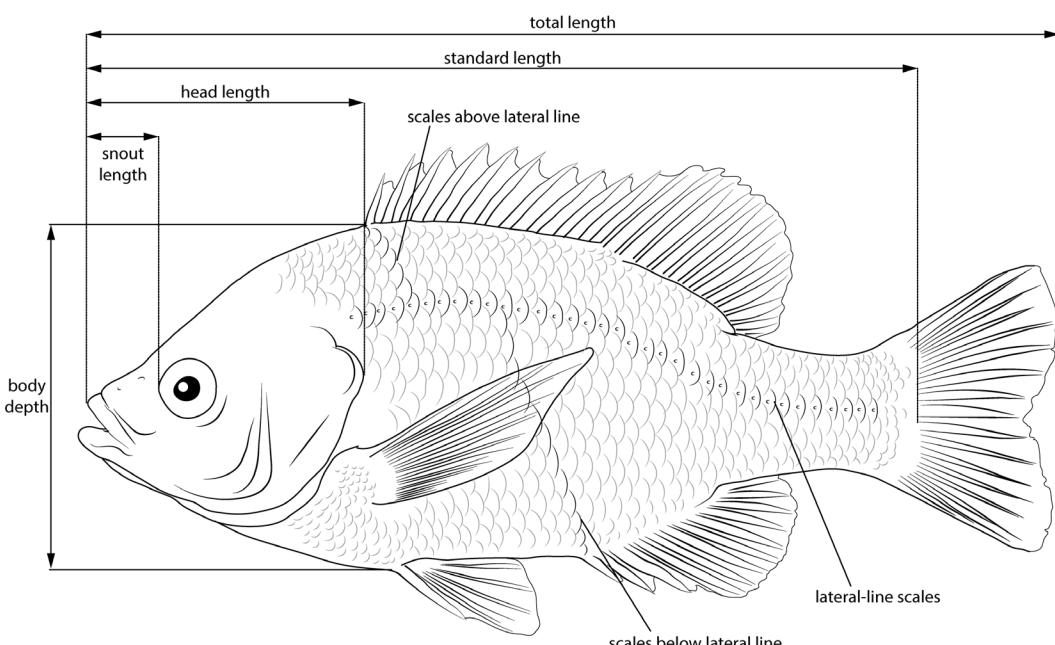


Figure 6.2. Common measurements and scale counts used in morphological studies of bony fishes.

of the fish (i.e., distance from the snout to the end of the tail), as it reduces unwanted variability (i.e., noise) due to damaged or broken caudal fins. In sharks, body size is often measured as precaudal length, which is taken from the tip of the snout to the upper origin of the caudal fin (Figure 6.1). In rays and skates, body width (often called disc width) is commonly used as a size reference, given their dorso-ventrally flattened body shapes.

6.2.2 Head

6.2.2.1 Cranial Features

The head of fishes is complex and, for simplicity, can be divided into several discrete regions. It is important to keep in mind that however convenient these regional groupings are, the head, like other parts of the body, is an integrated feature, and many of its components are highly correlated and function cooperatively. The snout is the anterior-most part of the head and is positioned directly anterior to the eye (orbit). The interorbital region is the dorsal surface of the head between the orbits, and its width is commonly measured as the shortest distance between the outer margins of the eyes. The cheek region is located ventral and posterior to the eyes when viewed in lateral profile. Posterior to the cheek is the opercular region, which comprises several bones that make up the operculum, or gill cover. An ossified bony ridge along the preopercular bone usually marks the boundary between the cheek and opercular regions (see Figure 6.1).

The position of the mouth varies widely across fishes. Terminal mouths are forward facing and occur at the anterior-most point of the body, while subterminal mouths are displaced slightly posterior to the snout. A mouth is considered superior when it opens dorsally and inferior when its position is well posterior to the tip of the snout and opens downward. Mouth placement is generally indicative of feeding behavior. For example, the subterminal mouth of drums (Sciaenidae) reflects their diet consisting of benthic invertebrates. Monkfishes (Lophiidae) and stargazers (Uranoscopidae) can bury themselves in the sand and use their upward-facing, superior mouths to prey on organisms directly above them.

The upper jaw of acanthomorph fishes is usually composed of two bones, the maxilla and premaxilla (see section 6.4.1.2), and varies widely in degree of protrusion across fishes. Several groups, such as suckers and darters, have nonprotruding upper jaws that are attached to the snout by a fleshy connection, the frenum. The lower jaws of bony fishes are composed of multiple bones but often function as single unit, referred to as the mandible. The anterior-most tip of the lower jaw is the mentum. The gular region is the area between the mentum and branchiostegal rays. Teleosts have an external membrane, the branchiostegal membrane, that occurs over the gular region and covers the isthmus, a slender anterior extension of the body that connects to the head. The branchiostegal membrane is located ventrally on the head, continuous to either side of the operculum, and is supported by rod-like bones called branchiostegal rays. The number and shape of branchiostegal rays have been used as diagnostic characters for some taxa (e.g., Percidae; Jenkins and Burkhead 1994).

Elasmobranchs have between five and seven gill slits located laterally, toward the posterior of the head. Chimaeras (Chimaeriformes), in contrast, have a dermic membrane covering the gills that is similar in appearance to an operculum. Most chondrichthyans have inferior mouths (except for angel sharks and whale sharks, which have terminal mouths). Additionally, many chondrichthyans, as well as some non-teleost actinopterygians, such as bichirs

(Polypteriformes), sturgeons, and paddlefishes (Acipenseriformes), have a pair of spiracles located posterior to each eye. Spiracles are hypothesized to be a remnant of the gill slit of the first visceral arch in the ancestors of jawed vertebrates (Liem et al. 2001). Skates, rays, and some species of sharks (e.g., angel sharks) have robust and well-developed spiracular muscles that can be used to pump water into the oral cavity. Bichirs use their spiracles to breathe air into their paired ventral lungs (Graham et al. 2013).

6.2.2.2 Cephalic Sensory Structures

Nostrils are external openings of the nasal cavity. **Chemosensory** organs within the nasal cavity are responsible for the sense of smell. Nostrils are located dorsolaterally on the snout in bony fishes but are ventrally oriented in chondrichthyans (Figure 6.1). Nostrils come in one of two general morphological forms: single, with one opening and a median flap between anterior and posterior sections, as seen in most elasmobranchs, or double with distinct anterior and posterior openings (found in bony fishes). The shapes of nostrils and distances between anterior and posterior apertures are extremely variable (Strauss and Bond 1990). Commonly in bony fishes, nostrils are simple, paired openings directly on the head. However, in some fishes, nostrils are present as tube-like projections (e.g., bichirs [Polypteridae]; bowfin [Amidae]) or fringes (e.g., toadfishes [Batrachoidiformes]).

Eyes are **photosensory** organs that are generally located dorso-laterally on the head. The size and position of eyes vary both ontogenetically and across taxa. Species occurring in clear, shallow waters usually have well-developed eyes, while those that live in low light environments, such as caves and turbid waters may have reduced eyes or lack them entirely (Kröger 2011; Trajano et al. 2017). In flatfishes (Pleuronectiformes), one of the eyes undergoes migration during early ontogeny, such that adults have both eyes on the same side of the head.

Cephalic lateral line canals are cranial components of the larger laterosensory or lateral line system (Northcutt 1989; Webb 2014). These canals bear **mechanosensory** organs, called neuromasts, that sense stimuli produced by the flow of water around a fish's body. The arrangement, position, and number of pores and canals are sometimes used as diagnostic characters for distinguishing fish species (Rizzato and Bichuette 2017). Canals may be complete in some taxa, interrupted, or altogether absent in others (Nelson 1972; Webb 1989; Pastana et al. 2019). Nomenclature of cephalic sensory canals is based on the innervation of the canal neuromasts and relative position on the head (Figure 6.3). The supraorbital canal runs dorsomedial to the orbit and nostrils, usually extending to the tip of the snout. The infraorbital canal surrounds the orbit posteriorly and ventrally, often extending onto the snout, laterally. The supraorbital and infraorbital canals may connect to each other and also to the otic canal, which runs dorsolaterally along the head, behind the orbit. The preoperculomandibular canal runs laterally on the lower jaw and posteriorly on the cheek, usually connecting dorsally with the otic and postotic canals. The postotic canal is continuous with the otic canal and connects posteriorly with the supratemporal canal, which runs transversely on the nape and longitudinally over the scapular region. The supratemporal canal eventually merges posteriorly with the trunk canal, which then continues along the midline of the body. Pores along the lateral line canals are open to the external environment and may appear as single or multiple apertures. In addition to the lateral line canals, superficial neuromasts may be distributed across the head and body, either, scattered or forming distinct lines, notably in gobies (Gobiidae) and swampfishes (Amblyopsidae; Nickles et al. 2020).

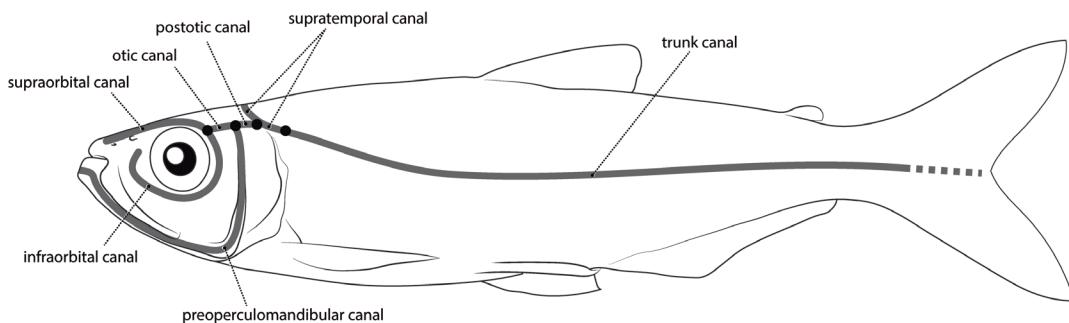


Figure 6.3. General distribution of lateral line canals in bony fishes. Black dots represent connections between canals.

Sensory barbels can serve both chemosensory and mechanosensory functions and are commonly found on fish heads near the nostrils and mouth. Barbel morphology is highly variable and the topology, shape, and number can be used as distinctive characters. Catfishes (Siluriformes) have up to four pairs of barbels, which can be quite elongate. In contrast, barbels on minnows (Cyprinidae) and croakers (Sciaenidae) are usually short and thin (Ovalle and Shin 1977; Chao 2002; LeClair and Topczewski 2010; Page and Burr 2011).

6.2.2.3 Other Cephalic Structures

Cirri (singular: cirrus) are dermic projections sometimes present on the head and body of fishes and which vary widely in their morphology. Shapes vary from slender and unbranched (similar in appearance to a barbel; e.g., Silverspotted Sculpin *Blepsias cirrhosus*, Hemipteridae) to stout, branched, and fringed, like those around the mouth of the Oyster Toadfish *Opsanus tau* (Batrachoididae). Cirri are usually located around the eyes, nostrils, and mouth but can also be found across the entire body, as seen in midshipmen fishes (Batrachoididae), which have a pair of cirri flanking each pore of the lateral line. Cirri are generally not thought to possess a specific sensory function (Strauss and Bond 1990). However, there is some evidence that certain species of blennies (Blennidae) and greenlings (Hexagrammidae) possess cirri that have a **gustatory** function (von Bartheld and Meyer 1985).

Some fishes possess spines or other ossified projections that protrude from their heads. Bones of the opercular series sometimes bear spines, like the preopercle spines of sculpins (Cottiformes; Buser et al. 2019) or opercular and subopercular spines of toadfishes (Batrachoidiformes). Scorpionfishes (Scorpaeniformes) and some species of sculpin (e.g., Spinyhead Sculpin *Dasyscottus setiger*; Psychrolutidae) also have parietal spines, which are ossified projections arising directly from the skull. Additionally, some stonefishes (Synanceiidae) have a lachrymal bone that has been modified into a mobile saber-like structure that can rotate laterally and lock into position (Smith et al. 2018).

6.2.3 Fins

Fins are a primary source of taxonomically important morphological variation across fishes. As such, they provide several commonly used characters for distinguishing species and

higher taxonomic groups, such as the number, position, composition of fin elements, and even the presence or absence of fins. Most fins are composed primarily of soft rays that are formed from two segmented and rod-like structures called hemitrichia. Soft rays are usually branched at their distal extremities. Spiny-rayed fishes (Acanthomorphata) possess true fin spines, which comprised a single unsegmented and unbranched bone. Spines always precede soft rays anteriorly within a given fin. Most catfishes (Siluriformes) and some carps and minnows (Cypriniformes) may also have robust spines in their fins, but they are not considered true spines. These spinous soft rays, as they are called, are not homologous with acanthomorph spines, as they are formed by fusion of the hemitrachia of soft fin rays (Kubicek et al. 2019).

The fins of elasmobranchs are not composed of soft rays. Instead, fin support is provided by ceratotrichia, a flexible protein filament that attaches to cartilaginous basal radials. Methods for fin notation and enumeration of elements are discussed later in section 6.3, Meristic Characters.

6.2.3.1 Median Unpaired Fins

Dorsal fins may be single or divided into two parts. In bony fishes, when two dorsal fins are present, the anterior (or first) fin is formed by spines, and the posterior (or second) is composed of soft rays. Notably, some species of cod (Gadidae) possess three dorsal fins. Despite external divisions of the dorsal fin, the skeletal support for soft rays and spines is continuous (see section 6.4.2).

The shape and size of dorsal fins, as well as their position on the body and the relative spacing between fin segments, may be used to distinguish species. Some snailfishes (Liparidae) and toadfishes (e.g., *Thalassophryne amazonica*, Batrachoididae) have dorsal fins that form a confluent structure with the caudal fin. Gunnels (Pholidae) are distinct in possessing a dorsal fin that is composed entirely of fin spines.

Another fin that may be positioned along the dorsal midline of a fish is the adipose fin, which is mostly formed of connective tissue and lacks skeletal support. Adipose fins are present in non-acanthomorph fishes, such as minnows (Cypriniformes), characins (Characiformes), catfishes (Siluriformes), bristlemouths (Gonostomatidae), and salmon (Salmonidae). However, even within these groups, the adipose fin may be absent in several taxa (e.g., *Notropis*, Cyprinidae).

The anal fin is generally shorter than the dorsal fin and is usually single (with few exceptions in cod species, having two). The origin of the anal fin is located posterior to the anus, but the position of the anal-fin insertion, as well as the fin's length is highly variable. The anal fin is particularly elongate in groups such as Neotropical knifefishes (Gymnotiformes) and gunnels (Pholidae). Anal-fin spines are common in acanthomorph fishes, most with one to three spines, such as sunfishes (Centrarchidae) and basses (Moronidae). Higher numbers of anal-fin spines can be found in families such as Gerreidae (Johnson 1984).

Finlets are small, nonretractable fins that often occur in series and are isolated from dorsal and anal fins (Figure 6.4D). They are positioned posterior to the dorsal and anal fins and play a functional role in swimming behaviors of pelagic species such as tuna and mackerel (Scombridae; Nauen and Lauder 2000).

The caudal fin is a distinct structure in most fishes, but it may be reduced in groups with elongate body plans, such as eels (Anguilliformes). Ocean sunfishes (Molidae) are unique in lacking a caudal fin (Johnson and Britz 2005), although they have pseudocaudal fins, which

are supported by modified rays from the dorsal and anal fins. In other fishes, the overall shape of the caudal fin is highly variable. Most elasmobranchs and non-teleost actinopterygians have heterocercal caudal fins, which are asymmetrical and have an elongate dorsal lobe and short ventral lobe. A unique feature of teleosts is a homocercal tail, in which dorsal and ventral caudal-fin lobes are externally symmetrical, despite asymmetry of internal skeletal support. Within fishes with homocercal tails, the shape can be truncate, with a nearly vertical posterior margin; emarginate, with a shallow indentation along the midline; or forked, with a deep indentation. Other variants include lunate, rounded, and pointed (Figure 6.4).

6.2.3.2 Paired Fins

Paired fins, as the name implies, consist of two matching fins on opposite sides of the body. Relative positions of paired fins on the body are highly variable across fishes. In some groups, including rays (Batoidea) and wrasses (Labridae), species have become specialized in using paired fins (specifically, pectoral fins) as their primary means of locomotion.

Pectoral fins are located posterior to the gill openings in sharks and operculum in ray-finned fishes (Figure 6.1). In non-teleost actinopterygians and early radiations of teleosts, pectoral fins

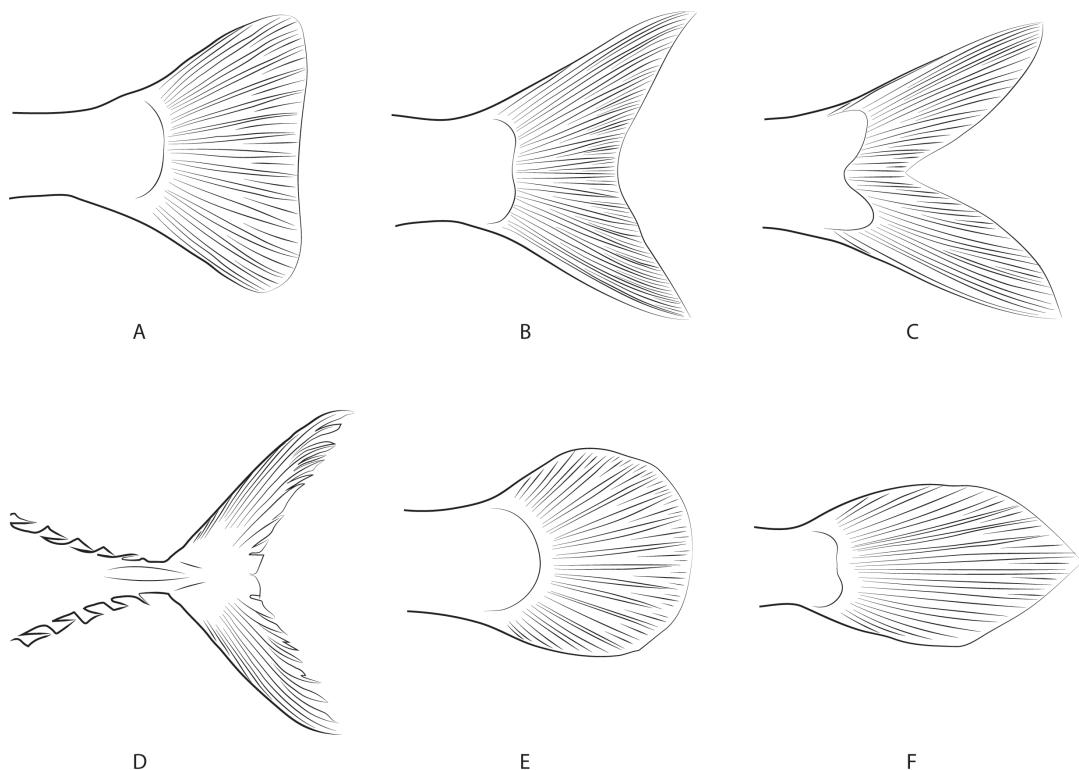


Figure 6.4. Types of homocercal caudal fins in representative teleosts. A = truncate: Blackside Darter *Percina maculata*, Percidae. B = emarginate: Striped Mullet *Mugil cephalus*, Mugilidae. C = forked: Blueback Herring *Alosa aestivalis*, Clupeidae. D = lunate: Pacific Bluefin Tuna *Thunnus orientalis*, Scombridae. E = rounded: Alaska Blackfish *Dallia pectoralis*, Umbriidae. F = pointed: Freshwater Goby *Ctenogobius shufeldti*, Gobiidae.

are positioned ventrally on the body, with a horizontally angled base. In acanthomorphs, by comparison, pectoral fins are located laterally and have more obliquely angled bases. A great number of pectoral fin modifications can be observed in fishes. For example, eels have highly reduced fins (Anguilliformes; Silva and Johnson 2018), contrasting with skates and rays (Batoidea) in which the pectoral fin is the most prominent feature of the overall body plan (Martinez et al. 2016). Specializations in pectoral fins of flying fishes (Exocetidae) give them the ability to glide over the surface of the water, while frogfishes (Antennariidae) and mudskippers (Oxudercidae) have modifications for benthic walking. Furthermore, several species within the order Batrachoidiformes have pit-organs in the axil of the pectoral fins that are thought to have a secretory function (Vernick and Chapman 1968; Hamlett and Schwartz 1979; Maina et al. 1998). Despite the unclear function of this organ, vertical position of these pits in the pectoral axil have been used as taxonomic characters (Collette 1966; Greenfield et al. 2008).

Pelvic fins are found along the ventral margin of the body, anterior to the anus. Like pectoral fins, they also serve as a rich source of morphological variation in fishes. The highly derived pelvic fins of gobies (Gobiiformes), clingfishes (Gobiesocidae), and snailfishes (Liparidae) independently modified into suction disks used for adhesion. In other groups of fishes, pelvic fins are absent altogether (e.g., pufferfishes, Tetraodontidae). In hakes (Merlucciidae), the pelvic fins are reduced to a filament that is modified for a sensory function (**proprioceptive**; Bardach and Case 1965; Williams et al. 2013).

Fishes from early diverging lineages of Actinopterygii, such as sturgeons (Acipenseridae), paddlefishes (Polyodontidae), and herring (Clupeidae), have pelvic fins positioned further back along the body, in an “abdominal” position. In certain non-acanthomorph fishes, such as beardfishes (Polymixiiformes), pelvic fins are located mid-way along the ventral margin of the abdominal region. Several groups within Acanthomorphata have pelvic fins anteriorly located, just below the pectoral fin, in a “thoracic” position. In some spiny-rayed fishes, pelvic fins have migrated even further anteriorly and are located anterior to the pectoral fins, a condition called “jugular” (e.g., anglerfishes [Lophiiformes] and cusk-eels [Ophidiiformes]). Despite the external location of the pelvic fin in these fishes, the internal support (the pelvic girdle) remains posterior to the pectoral girdle.

6.2.4 Lateral Line

The term lateral line typically refers to the trunk canal of the lateral line system (Figure 6.3). This structure runs laterally along the body and is associated with a row of perforated scales, when scales are present (Figure 6.2). These pores allow surrounding water to enter the canal and stimulate the lateral line mechanosensory organs, the neuromasts. The trunk canal originates anteriorly, connecting to the cephalic lateral line canals (section 6.2.2.2), and extends into the tail in most fishes. In fishes, such as drums (Sciaenidae), the trunk canal continues posteriorly onto the caudal-fin membrane. Several species within the family Percidae have lateral line canals that end short of the origin of the caudal fin (Jenkins and Burkhead 1994). Additionally, most species in the family Cichlidae have a discontinuous trunk canal, creating distinct upper and lower segments. Pricklebacks (Stichaeidae), flatfishes (Pleuronectiformes), and some toadfishes (Batrachoidiformes) have multiple lateral line trunk canals. A small number of groups, including herrings (Clupeidae), do not possess lateral line canals (Strauss and Bond 1990).

6.2.5 Scales and Dermal Structures

Fish scales are originally derived from three tissue layers: the epidermis, dermis, and a lower layer of bony tissue. Ganoid scales have an outer surface made of a mineralized and layered tissue called ganoine, which is followed by dentine (of dermal origin), and underlain by a bony layer. These scales are found in some extinct fishes and early-diverging actinopterygians, such as bichirs (Polypteriformes) and gars (Lepisosteiformes). Cosmoid scales contain an outer vitrodentine layer over a cosmine layer and are present only in extinct coelacanths and lungfishes (the early radiations of Sarcopterygii) (for illustrations on ganoid and cosmoid scales, see Liem et al. 2001 and Schultze 2016).

Placoid scales are tooth-like and occur in elasmobranchs and other chondrichthyan fishes. Like cosmoid scales, they have an outer layer of vitrodentine, but it is underlain by a dentine layer that surrounds a vascularized pulp cavity (Helfman et al. 2009).

Scales occurring in teleosts evolved from ganoid scales but have lost the ganoine and dentine layers, leaving a thin bony layer. Teleosts have four types of scales (Figure 6.5; following Roberts 1993): cycloid or circular scales have a smooth, flattened surface and a rounded posterior margin; crenate scales have simple indentations and projections along the posterior margin; spinoid scales have spines that are continuous with the surface of the scale; and ctenoid scales have a distinct posterior region bearing spines. Ctenoid scales are classified into three configurations: peripheral cteni, characteristic for having a single row of distinct spines on the posterior margin of the scale; transforming cteni, which have two or three rows of distinct spines along the posterior margin (spines anterior to the margin are present, but not fully developed; see Roberts 1993); and whole cteni, which have whole spines both marginally and submarginally (see Figure 6.5).

Many fishes possess scale structures that are modified for defense, such as the scutes of sturgeons (Acipenseridae) and sticklebacks (Gasterosteidae), and enlarged bony plates in armored catfishes (Callichthyidae and Loricariidae). Similarly, scale modifications create a bulky carapace around boxfishes (Ostraciidae), spines covering porcupine fishes (Diodontidae), and bony rings that surround the bodies of seahorses (Syngnathidae) (Sire 1993; Helfman et al. 2009; Lujan et al. 2015; Matsuura 2016).

6.2.6 Sexual Dimorphism

Fishes exhibit various forms of sexual dimorphism, morphological differences between males and females within a species, which can sometimes be useful for taxonomic purposes. Some closely related elasmobranch species can be distinguished by detailed morphological examination of paired male intromittent organs, the claspers (Moreira et al. 2017; Moreira and Carvalho 2018; Vaz and Carvalho 2018). Several species of darters (Percidae) and shiners (Cyprinidae) display sexual dichromatism, in which one of the sexes (usually males) has a distinctive nuptial coloration (Page and Burr 2011). Williams et al. (2013) showed that female Splendid Darters (*Etheostoma barranense*) prefer males with orange coloration, over those with red. Sexual selection can also result in extreme morphologies, such as the classic example in which males in some species of swordtails (Poeciliidae) evolve exaggerated caudal fin ornamentation to attract females (Basolo 1990).

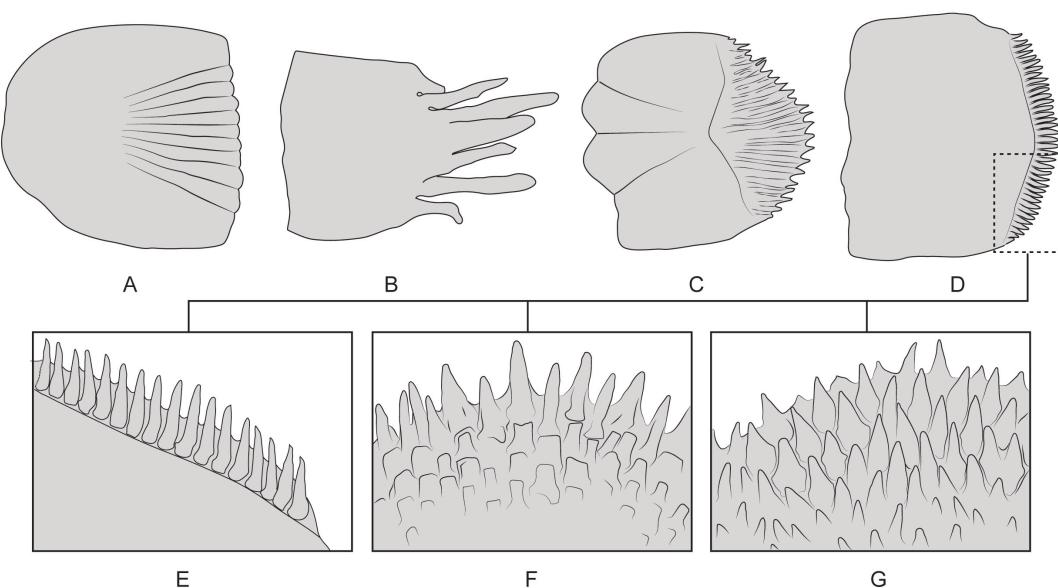


Figure 6.5. Types of scales in Teleostei. A = cycloid: Barred Livebearer *Poeciliopsis turubensis*, Poeciliidae. B = crenate: *Bathypterois quadrifilis*, Ignopidae. C = spinoid: *Ctenolucius beani*, Ctenoluciidae. D = ctenoid: *Eugnathichthys eetveldti*, Distichodontidae. E = peripheral cteni: Pacific Sleeper *Gobiomorus maculatus*, Eleotridae. F = transforming cteni: *Perca flavescens*, Percidae. G = whole cteni: Striped Mullet *Mugil cephalus*, Mugilidae. Figures based on Roberts (1993).

6.2.7 Light Organs

Light-producing organs vary from small, circular **photophores** to complex structures, like the large organs below the eyes of flashlight fishes (Anomalopidae). Photophores serve different functions across fishes possessing them, from predatory behaviors to intraspecific communication. Researchers have recently demonstrated that bioluminescence functions to maintain schooling cohesion in flashlight fishes (Gruber et al. 2019). The morphologies of light organs, and their distribution across a fish's body, can also be used as diagnostic characteristics. Circumesophageal light organs have been important in systematic studies of ponyfishes (Leiognathidae) and are hypothesized to serve a role in intraspecific mate recognition for some species (Sparks et al. 2005; Chakrabarty et al. 2011). Additional studies have shown that the arrangement of photophores is species specific in lanternfishes and midshipmen (Bathracoididae) (Walker and Rosenblatt 1988; Richards 2006).

6.2.8 Pigmentation and Coloration

Coloration is a fundamental characteristic of fish skin and it is among the most prominent features of their overall physical appearance. Patterning of colors is commonly produced by **chromatophores** embedded in the integument. Coloration can be pigmentary, in which cells absorb specific wavelengths of light via pigments contained in chromatophores, structural, whereby light is instead reflected, or a combination of the two.

Patterns of coloration and pigmentation may be useful in taxonomic studies. Variables such as presence, shape, and number, can be used to describe character locators such as spots, blotches or stripes, serving as potential diagnostic features at the species level. For example, Walker and Rosenblatt (1988) used the number and relative size of black blotches along the body for differentiating species of midshipmen (*Porichthys*). Similarly, species of South American angel sharks (Squatinidae) can be distinguished by differences in the relative size and density of white blotches on the dorsal surface of the body (Vaz and Carvalho 2013, 2018). Additionally, closely related species of freshwater stingrays can be identified by the shape of ocelli (pigmentation resembling eye spots) and coloration pattern (Silva and Carvalho 2011, 2015a, 2015b). A promising area of future work, researchers have also begun leveraging the use of machine learning to characterize ecological correlates of fish coloration patterns at larger phylogenetic scales (Alfaro et al. 2019).

Specimen preservation greatly impacts coloration in fishes, as common fixatives, such as formalin, cause chromatophores to collapse (Mattox et al. 2013). While it can be valuable to document the color of specimens in preservation, descriptions of natural coloration should be made from live or fresh individuals in order to ensure accuracy (Sidlauskas and Konstantinidis 2021, Chapter 19, this volume).

Many fish species have the ability to change color for intra- and interspecific communication, for camouflage, in response to changes in environment, or in response to stressors (Burton 2011). Under stress, the Oyster Toadfish can increase the intensity of its spots and stripes (D. Vaz, personal observation). Maturity and seasonality can also affect species-specific coloration (Page and Burr 2011). Additionally, juvenile coloration often undergoes drastic transformation as the fish matures, as seen in marine angelfishes (Pomacanthidae; Taquet and Diringer 2012; Bacchet et al. 2017).

6.3 MERISTIC CHARACTERS

Meristic characters are features that can be counted, such as the number of spines and rays in the dorsal fin or the number of pharyngeal teeth. These traits are still widely used in identifying and describing species of fishes. General characters and methods are described below and listed in Table 6.1. Given the extensive morphological diversity of fishes, modified approaches may be necessary for particular groups of interest.

6.3.1 Vertebral Counts

Individual vertebrae form the vertebral column of fishes and may be separated into three types: abdominal, caudal, and ural. The abdominal region extends from the first vertebrae articulating with the neurocranium to the last vertebrae without complete haemal arches (see section 6.4.2 for further description). Caudal vertebrae have complete haemal arches, with the last caudal vertebra bearing the parhypural. Ural vertebrae articulate with the hypural plates, which are modified, closed haemal arches.

In elasmobranchs, it is difficult to distinguish haemal arches in radiographs (the most common method for obtaining vertebral counts). As such, Springer and Garrick (1964) established two main regions: the precaudal region, which are all vertebrae anterior to the origin of the upper lobe of the caudal fin, and the caudal region, including vertebrae located posterior

Table 6.1. Examples of morphometric characters in the snailfish *Careproctus staufferi* (Liparidae; modified from Orr 2016).

Meristic character	n	Range
Dorsal-fin rays	16	40–42
Anal-fin rays	16	33–37
Pectoral-fin rays	16	36–44
Rays of lower lobe of pectoral fin	15	6–11
Caudal-fin rays	13	11–13
Precaudal vertebrae	16	10–10
Caudal vertebrae	16	34–36
Total vertebrae	16	44–46
Tooth rows	11	9–13
Tooth count per row	14	7–10
Gill rakers	16	6–10
Pyloric caeca	6	10–19

to the origin of the upper caudal-fin lobe. Chondrichthyans also have two types of vertebrae, which are counted separately. Monospondylyous vertebrae are located anteriorly and are associated with one myomere, and diplospondylyous vertebrae are smaller, located posteriorly within the body and usually arranged as two vertebrae associated with a single myomere. Usually near the origin of the caudal region, there is a marked transition from monospondylyous to diplospondylyous vertebrae (Compagno 1988).

6.3.2 Fin Counts

Counts of fin rays and spines are useful diagnostic features for differentiating fish species and, as such, are common taxonomic characters. Conventional fin annotation is as follows: dorsal, D; anal, A; caudal, C; pectoral, P; and pelvic, V (for ventral fin) or P2. Fin spines are denoted by roman numerals and soft fin rays by Arabic numbers. Authors usually do not use Roman numerals for the spinous rays of catfishes and carps (for definition of spinous soft rays, see section 6.2.3). The fin formula varies depending on whether spiny and soft parts are separated or continuous in the same fin. If separated, counts include a hyphen (e.g., D X-12), whereas counts of fins with confluent spiny and soft parts are separated by a comma (e.g., D X, 12). In both of these examples, the fish has 10 fin spines and 12 rays. Soft fin rays are usually branched distally; it is therefore crucial when counting them to observe the base of the structure for accuracy. This is less problematic for fin spines, which are unbranched and unsegmented. In species such as sunfishes (Centrarchidae), fin spines are substantially more robust than rays, making the two relatively easy to distinguish. However, in other fishes, such as some sculpins (e.g., Great Sculpin *Myoxocephalus polyacanthocephalus*; Cottidae), fin spines are nearly as slender and flexible as fin rays and must be examined under a well-lit microscope to distinguish the two.

The caudal fin in actinopterygians contains only soft fin rays and is usually divided into two series: principal and procurrent. The principal series is formed by all branched rays plus the first adjacent nonbranched ray, both dorsally and ventrally. All others form the dorsal and ventral series of unbranched procurrent fin rays. The number of procurrent fin rays is extremely

variable across fishes and they are sometimes even absent (e.g., monkfishes). In teleosts, there is often a gap in the middle of the caudal fin, the **diastema**, separating the dorsal and ventral rays. In taxonomic studies, the caudal fin formula is usually written with the following sequence: the single dorsal unbranched principal ray in Roman numeral, dorsal branched principal rays in Arabic numbers, ventral branched principal rays in Arabic, and the ventral unbranched principal ray as a Roman numeral. For example, a caudal fin with the formula (I, 7, 8, I) has seventeen total principal fin rays, including eight that are dorsal (seven branched and one non-branched) and nine that are ventral (eight branched and one non-branched).

Elasmobranchs do not have fin rays, but it is common practice to count the number of distal radials of pectoral and pelvic fins (i.e., elements articulating with the basal radials: the propterygium, mesopterygium, and metapterygium).

6.3.3 Scale Counts

A common character for distinguishing species is the number of scales associated with the lateral line (Figure 6.2). The lateral line can also be used as a reference for other **meristic traits**, such as the number of scale rows that occur above or below the lateral line. Counts of circumferential scales, or the vertical scale columns around the body, are also often used. Circumferential counts involve the location (i.e., left, right, dorsal, ventral) and number of scale columns.

6.3.4 Gill Rakers, Pharyngeal Teeth, and Other Features

The number of gill rakers on the first gill arch can be used to diagnose species. The gill arch in lateral view is a V-shaped structure. Counts of the lower limb (ceratobranchial and hypobranchial bones) and upper limb (epibranchial) are usually taken separately, unless otherwise noted. The operculum is fragile and may become damaged in the counting process. As a result, it is standard practice to make counts on the right side of the specimen to keep the left side intact for future imaging and examination (Sidlauskas and Konstantinidis 2021, Chapter 19, this volume).

Branchiostegal rays, located below the operculum, are also a common meristic character and are particularly useful for distinguishing species of darters (Jenkins and Burkhead 1994). Counts of branchiostegal rays include all elements associated with the thin membrane connecting them.

Carps and minnows (Cyprinidae) lack oral teeth but have highly developed and toothbearing **pharyngeal jaws** on their fifth ceratobranchial element (see section 6.4.1.3 for a detailed description of gill arches). These pharyngeal teeth are usually organized into rows and can be helpful for distinguishing species. The tooth formula is given for both ceratobranchials, from left to right. Tooth row counts taken from the same element are separated by a comma, whereas counts of the two ceratobranchials are separated by a hyphen (e.g., 3,5 – 5,3). Visualization of pharyngeal teeth requires removal of the fifth ceratobranchial by manual dissection. The teeth are constantly being replaced and those that have yet to be replaced may be present as tooth germs or grooves (for example of tooth replacement, see figure 3 in Conway et al. 2017a).

Another feature used to generate meristic characters is the number of pores on cephalic sensory canals. For example, some species of freshwater sculpin can be distinguished by the

number of pores associated with the preoperculomandibular canal (Jenkins and Burkhead 1994). However, these characteristics can be highly variable. For example, Rizzato and Bi-chuette (2017) demonstrated high intraspecific variation in the presence and number of cephalic pores in species of **troglomorphic** catfishes (Trichomycteridae).

6.4 SKELETAL FEATURES

Morphological features of the skeleton have historically been a primary source of evidence for the classification of fishes (Greenwood 1966; Rosen and Patterson 1969; Johnson and Patterson 1993; Stiassny et al. 1996; Arratia 1999, 2010, 2013, 2017; Wiley and Johnson 2010). Despite the rise of genetic data in the modern era, the study of the skeletal characters remains a valuable resource for phylogenetic studies, especially those incorporating fossil data in reconstructions of evolutionary relationships. Skeletal information also serves as an incredibly important source of morphological variation at the population level and is commonly used to distinguish between closely related species (Hutchins 1974, 1976; Vaz and Carvalho 2013).

Bones of the skeleton can be broadly categorized into one of three groups based on their embryonic origin. Endochondral bones (E) are those that ossify from cartilaginous precursors during ontogeny, dermal bones (D) ossify by contacting the dermis, and membrane bones (M) ossify within ligaments or other connective tissues. In the following sections, we include the above abbreviations in the descriptions of different bones. While other approaches exist for the classification and study of the skeletal system, we follow Hilton (2011), who divides the skeleton into topographic regions and functional units (e.g., the axial versus appendicular skeleton). This section is based on a generalized skeletal structure of a teleost fish and does not discuss or illustrate the wide variation that exists across fishes.

6.4.1 Cranial Axial Skeleton—The Skull

The skull is the anterior-most part of the axial skeleton of a fish (Figure 6.6), broadly including the cranium, jaws and opercular series. However, we note that owing to the integrated nature of the skull, in which different regions often interact with each other, functional units are not always clearly defined.

6.4.1.1 Neurocranium

The neurocranium encompasses the bones and cartilages that form the braincase, dorsal roof, and infraorbital series (Hilton 2011). While the primary function of the neurocranium is to protect the brain, its shape is highly diverse and can reflect ecological diversity (Evans et al. 2017).

The braincase (Figure 6.7) consists of four primary regions: the ethmoid, orbital, otic, and occipital. Bones of the ethmoid region surround the olfactory bulbs. This region also articulates with bones of the suspensorium (described below). The mesethmoid (E) is a non-paired bone positioned medially between the nasal capsules. It contacts the lateral ethmoid (E), which is paired. The fenestra for the passage of the olfactory tract can be used as an indicator for identifying the lateral ethmoid. Ventrally, the ethmoid region has a single dermal bone, the vomer (D), which sometimes bears teeth.

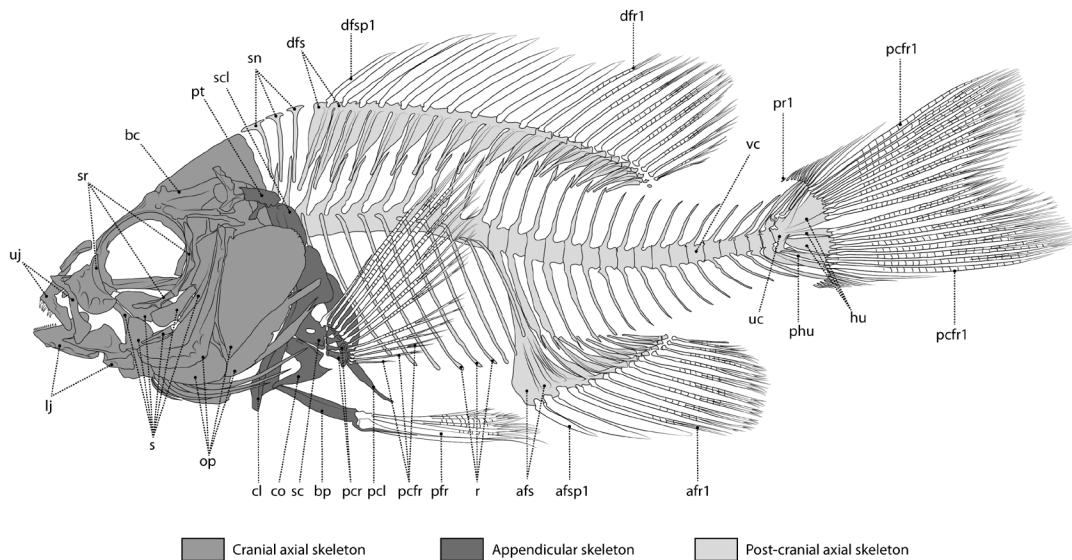


Figure 6.6. Skeletal anatomy of a perciform fish (genus *Lepomis*), showing the main bones and skeletal regions. afr1 = first anal-fin ray; afs = anal-fin support; afsp1 = first anal-fin spine; bc = braincase; bp = basipterygium; cl = cleithrum; co = coracoid; dfr1 = first dorsal-fin soft ray; dfs = dorsal-fin support; dfsp1 = first dorsal-fin spine; hu = hypurals; lj = lower jaw; op = opercular bones; pcfr = pectoral-fin rays; pcfr1 = first (upper and lower) principal caudal-fin ray; pcl = postcleithra; pcr = pectoral-fin radials; pfr = pelvic-fin rays; phu = parhypural; pr1 = first procurrent ray; pt = posttemporal; r = ribs; s = suspensorium; sc = scapula; scl = supracleithrum; sn = supraneurals; sr = sclerotic ring; vc = vertebral column.

The orbital or sphenoid region is formed mostly by endochondral bones that are associated with the orbit. The orbitosphenoid (E) is positioned dorsoanteriorly within this region. This bone is absent in several groups of Teleostei, especially within Acanthomorphata. The pterosphenoid (E) is located dorsoposteriorly in the orbital region, adjacent to the otic region and the basisphenoid (E) located ventrally.

The otic region is composed mostly of endochondral bones that ossify from the embryonic cartilaginous auditory capsule that surrounds the semicircular canals and ampullae of the inner ear. The sphenotic is positioned dorsoanteriorly and often has a compound structure, with both endochondral and dermal components (the autosphenotic and dermosphenotic, respectively). These components generally fuse during ontogeny, but in non-teleostean actinopterygians, such as bowfins (Amiiformes), the endochondral and dermal parts of the sphenotic remain distinct. The sphenotic is easily identifiable by its articulation with the anterior limb of the hyomandibula. Posterior to the sphenotic, the pterotic articulates with the posterior limb of the hyomandibula. The pterotic, too, is often a compound bone formed by the autopterotic (E) and dermopterotic (D). The ventral and largest bone of the otic series is the prootic (E). This bone encloses the trigeminal and fascialis cranial nerves (forming the trigeminusfascialis chamber) and possesses one or more **foramina** for these nerves to transmit sensory information.

The occipital region interfaces with postcranial anatomy, including musculature and bones of the axial skeleton. Medioventrally, the basioccipital (E) ossifies around the anterior

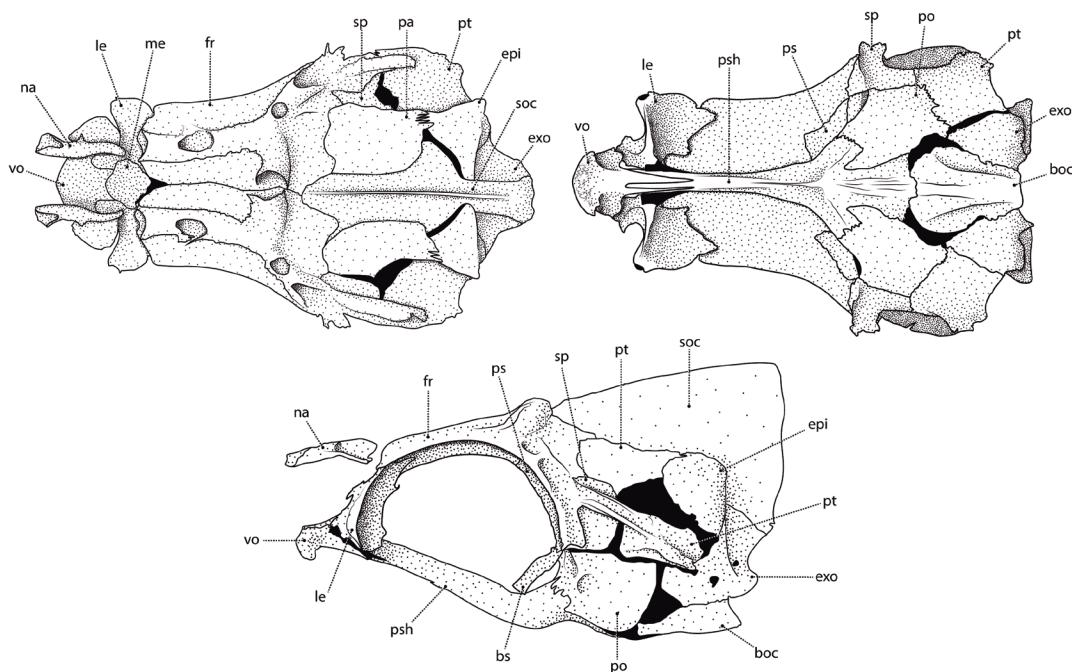


Figure 6.7. Braincase of a perciform fish (genus *Lepomis*) in dorsal (top left), ventral (top right), and lateral (bottom) views. boc = basioccipital; bs = basisphenoid; epi = epiotic; exo = exoccipital; fr = frontal; le = lateral ethmoid; me = mesethmoid; na = nasal; pa = parietal; po = prootic; ps = pterosphenoid; psh = parasphenoid; pt = posttemporal; soc = supraoccipital; sp = sphenotic; vo = vomer.

tip of the notochord and articulates with the vertebral column. Dorsal to the basioccipital, a pair of bones, the exoccipitals (E), forms the foramen magnum, the opening where the spinal cord exits the neurocranium. The exoccipitals can be identified by the foramen that allows for passage of the vagal nerve. The supraoccipital (E), present only in teleosts, is an unpaired bone that is positioned dorsal to the foramen magnum, along the dorsal midline of the skull. Dorsolaterally to the exoccipitals are two paired bones, the epioccipital (E) and intercalar (M). The epioccipital is positioned more dorsally and can be identified by its articulation with the dorsal limb of the posttemporal (D), the dorsal-most bone of the pectoral girdle. The intercalar is ventral to the epioccipital and contacts the ventral limb of the posttemporal.

The parasphenoid bone (D) extends ventrally through both orbital and otic regions, with its anterior margin contacting the vomer and its posterior limit contacting the basioccipital. Three to four pairs of dermal bones form the dorsal roof of the neurocranium. The nasal bones (D) are positioned dorsoanteriorly to the lateral ethmoid. In most teleosts, nasal bones are small tubular bones surrounding lateral line canals, but in early actinopterygians, nasal bones can be well developed (see Hilton 2011). Posterior to the nasals, are the frontal bones (D), which usually form the main part of the anterior roof of the skull. Parietal bones (D) are located posterior to the frontals, usually medial to the supraoccipital. Extrascapular bones are greatly developed in non-teleost actinopterygians, but similar to nasal bones, are usually reduced to tubular structures surrounding lateral line canals in teleosts.

Infraorbital bones (D) are associated with the dorsal roof and encapsulate the infraorbital branch of the cephalic lateralis system (Figure 6.8A). Infraorbitals define the orbit and are enumerated antero-posteriorly. The first, anteriormost infraorbital bone is the lachrymal and the posteriormost, the dermosphenotic.

6.4.1.2 Suspensorium, Oral Jaws, Hyoid Arch, and Opercular Series

Combined, the suspensorium, upper and lower jaws, and branchial arches (Figure 6.8) form the splanchnocranum, or visceral skeleton. Although these skeletal components form an integrated functional unit, they are consistently classified as distinct parts of the cranial axial skeleton.

A convenient place to start for identifying bones of the suspensorium is the articulation between the quadrate (E) and articular (E) bones, which serves as the point of rotation for the mandible and delimits the upper and lower jaws (Figure 6.8B). The quadrate has a process that articulates with a groove in the articular bone of the lower jaw. In Teleostei, the quadrate also has a posterior process that forms a deep indent in the main body of the bone. Dorsal to the quadrate, is the metapterygoid (E) and anterior to the quadrate are two dermal bones: the endopterygoid (D) and ectopterygoid (D). These bones flank the cartilage that connects the quadrate to the palatine bone (the embryonic palatoquadrate cartilage), with the endopterygoid positioned medially and the ectopterygoid positioned laterally. At the anterior end of the palatoquadrate cartilage is the palatine bone. The palatine has distinct endochondral and dermal parts: the autopalatine (E) and the dermopalatine (D). The autopalatine is continuous with the palatoquadrate cartilage and articulates with the lateral ethmoid, while the dermopalatine usually has a rougher texture and is tooth bearing. In some fishes, a distinction between the auto- and dermopalatines is not present, and the palatine is referenced without distinction of individual components. Anterior to the palatine, there are two dermal bones. The anterior tip of the maxilla (D) is positioned anterior to the palatine, but it projects ventroposteriorly, contacting the lower jaw via the supralabial membrane and ligament (Datovo and Vari 2013). The premaxilla (D) is positioned just anterior to the maxilla. The premaxilla in Teleostei usually has a distinct ascending process, and in Acanthomorphata the ascending process sits in the rostral cartilage. The ascending process of the premaxilla allows for protrusion and increased mobility of the jaw, a feature that has contributed to the widespread success of spiny-rayed fishes (Bellwood et al. 2015).

To identify the bones in the lower jaw, we again orient ourselves to the joint between the quadrate and articular. Ventral to the articular, usually surrounding it, is the angular (D). In several fishes (e.g., Batrachoidiformes), the articular and angular bones are fused. From the medial surface of the angular or angulo-articular bone, a piece of cartilage extends anteriorly along the lower jaw. This cartilage is what remains of the embryonic Meckel's cartilage, which forms the primary structure of the lower jaw in early stages of development. At the posterior tip of the angular (or angulo-articular), there is a small bone, the retroarticular (E). The retroarticular can be identified by its attachment to the interopercular-retroarticular ligament, which connects the lower jaw to the interopercle (opercular series). Anterior to the angular, the dentary (D) is the tooth-bearing bone of the lower jaw. Despite variability in the shape of the dentary across taxa, the posterior margin is generally indented, forming a V-shape where the articular inserts. Several groups of fishes have independently evolved mobility between the dentary and angulo-articular through an intramandibular joint (Gibb et al. 2015), including fishes as distantly related as *Distichodus* (Characiformes) and *Escenius* (Blenniiformes).

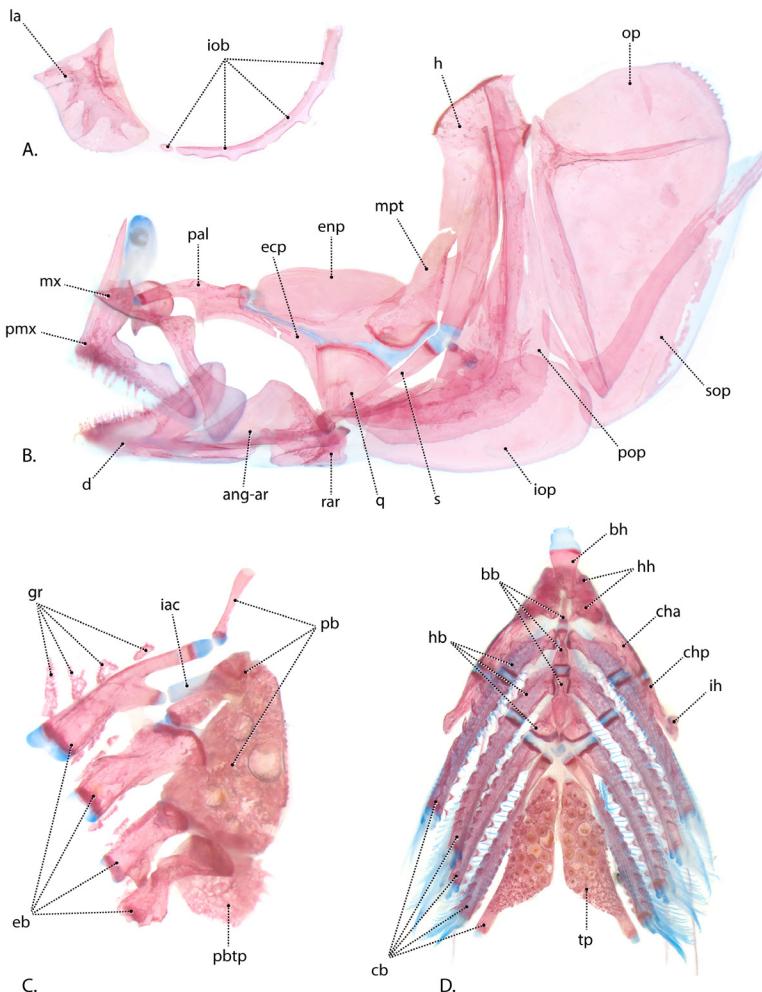


Figure 6.8. Skeletal elements of a cleared and double-stained specimen of a perciform fish (genus *Lepomis*), with bones stained in red and cartilage in blue. (A) Left infraorbital bones in lateral view; (B) left upper and lower jaws and suspensorium in lateral view; (C) right dorsal branchial-arch elements in dorsal view; (D) ventral branchial-arch elements in dorsal view. ang-ar = angulo-articular; bb = basibranchial; bh = basihyal; cb = ceratobranchial; cha = anterior ceratohyal; chp = posterior ceratohyal; d = dentary; eb = epibranchials; ecp = ectopterygoid; enp = endopterygoid; gr = gill rakers; h = hyomandibula; hb = hypobranchial; hh = hypohyal; iac = interarcual cartilage; ih = interhyal; iob = infraorbital bones; iop = interopercle; la = lacrimal; mpt = metapterygoid; mx = maxilla; op = opercle; pal = palatine; pb = pharyngobranchials; pbt = pharyngobranchial tooth plate; pmx = premaxilla; pop = preopercle; q = quadrate; rar = retroarticular; s = symplectic; sop = subopercle; tp = tooth plates.

The hyoid arch has dorsal elements that interact with the suspensorium and ventral elements that articulate with the branchial arches. The dorsal-most element is the hyomandibula (E), which is positioned posteriorly to the metapterygoid. The hyomandibula is a uniquely shaped bone with three points of articulation. Along its dorsal margin, there are two heads; the anterior head articulates with the sphenotic and the posterior with the pterotic. Along its posterior margin, the hyomandibula has another articular head that inserts in the socket of the opercle (D). The symplectic (E), generally a rod-shaped bone, is positioned ventral to the hyomandibula, usually inserting in the indent formed between the main body and posterior process of the quadrate. Medial to the dorsal margin of the symplectic, there is another rod-shaped bone, the interhyal (E). This bone connects the dorsal elements of the hyoid arch to the ventral series.

The ventral series of the hyoid arch (Figure 6.8D) is formed by a pair of ceratohyal bones (E): the posterior ceratohyal has a small socket where the interhyal bone inserts. Ceratohyals support the branchiostegal rays (D). The anterior ceratohyal projects anteromedially, articulating with a pair of hypohyal bones (E), the dorsal and ventral hypohyal. Hypohyals usually surround the medially oriented basihyal (E). Finally, the urohyal (M) is located ventral to the basihyal.

The opercular series is formed by four bones, all of dermal origin (Fig. 6.8B). The opercle can be identified by the socket in the anterior margin that articulates with the hyomandibula. The subopercle is positioned ventral to the opercle. Anterior to the opercle and posterolaterally to the hyomandibula, is the preopercle. The preopercle is distinct in having a large groove that surrounds the preopercular-mandibular branch of the cephalic lateralis system. The interopercle bone is positioned ventral to the preopercle and anterior to the subopercle. It can be identified by its attachment to the interopercle-retroarticular ligament that inserts onto the retroarticular.

6.4.1.3 Branchial Arches

Branchial arches (Figure 6.8C, D) provide the primary skeletal support for the respiratory apparatus of fishes. In Actinopterygii, several elements of the gill arches have been co-opted as pharyngeal jaws, which function to process food. For the most part, branchial arches are connected to other elements of the cranial skeleton by muscles and ligaments. Jawed fishes (Gnathostomata) have five branchial arches (with the exception of a few elasmobranch species), and the generalized gill arch has five elements, from dorsal to ventral: pharyngobranchial (paired), epibranchial (paired), ceratobranchial (paired), hypobranchial (paired), and basibranchial (unpaired). Across fishes, several elements of each arch may be reduced or lost. A good point of reference for identifying gill elements is to find the articulation between epibranchials and ceratobranchials, which form a V-like structure when the operculum is open. From there, dorsal elements are projected dorsoanteriorly and the ventral elements are projected ventroanteriorly.

Dorsal gill arches include the pharyngobranchials (E) and epibranchials (E). Species in Gnathostomata generally have three to four pharyngobranchials. In Actinopterygii, the first pharyngobranchial is usually a rod-like element that contacts the neurocranium. All other pharyngobranchials may support tooth plates (dorsal plates of the pharyngeal jaw). The posterior tips of pharyngobranchials articulate with the anterior margins of the epibranchials. Actinopterygians have four epibranchials, and elasmobranchs have a fifth epibranchial, which

is fused to both the fourth epibranchial and pharyngobranchial. Dorsally, all epibranchials except the fourth support gill filaments. Ventrally, all support gill rakers (D), which are small dermal projections that are often used for protection of gill filaments (although co-opted for filter feeding in anchovies and other Clupeiformes).

Ventral gill arches include the ceratobranchials (E), hypobranchials (E), and basibranchials (E). Ceratobranchials are the only branchial elements that are still present in all five arches. The first three ceratobranchials support gill filaments ventrally, and ceratobranchials one to four bear gill rakers dorsally. The fifth ceratobranchial is highly modified for supporting the tooth plate of the ventral part of the pharyngeal jaw. The anterior margin of the first three ceratobranchials articulate with the first three hypobranchials. Whereas actinopterygians usually have three hypobranchials, elasmobranchs usually have four. Basibranchials articulate laterally with the anterior tips of the first three hypobranchials and the fourth ceratobranchial. The number of basibranchials is highly variable across taxa, with some (usually the posterior most) composed of cartilage. *Hiodon* and other bony-tongued fishes (Osteoglossomorpha) have tooth plates covering the basibranchials (Hilton 2001, 2011).

6.4.2 Post-cranial Axial Skeleton

The post-cranial axial skeleton (Figure 6.6) comprises all unpaired elements posterior to the skull, including squamation (section 6.2.5). In this section, descriptions are focused on the caudal skeleton of Teleostei (for variation in the caudal skeleton of non-teleost actinopterygians and elasmobranchs, see Grande and Bemis 1998; Moreira et al. 2019). The vertebral column is composed of many individual vertebrae, which are themselves formed by a number of elements. The centrum is the ossification of the notochord. Dorsal to the centrum are the paired neural arches (E), which fuse during development. Neural arches enclose and protect the spinal cord exiting from the neurocranium. Neural spines (E) are unpaired and positioned dorsal to the neural arches. Abdominal vertebrae have paired parapophyses (E) ventral to the centrum. The caudal region of the vertebral column is composed of vertebrae with haemal arches (E), located ventrally, which enclose and protect blood vessels extending caudally and are developmentally homologous with parapophyses. Ventral to the haemal arches is an unpaired haemal spine (E).

The caudal skeleton is formed from modified vertebrae. The ural region (i.e., the region enclosing the caudal skeleton) begins where there are no longer haemal arches, such that blood vessels run laterally to the bones. The parhypural (E) is the name given to the haemal spine associated with the haemal arch of the last pre-ural vertebra (Schultze and Arratia 2013; Vaz and Hilton 2020).

Additionally, the notochord displays a strong dorsal flexion, resulting in an asymmetrical caudal skeleton. In the caudal region, the hypural bones (E) are homologous with haemal arches. Lineages that diverged early within Teleostei, such as anchovies (Clupeiformes) and tarpon (Elopiformes) have seven hypural plates. The two anterior hypurals are associated with the first centrum. The third to the seventh hypural are associated with the second ural centrum. Teleosts generally have two ural centra, and species within Acanthomorphata sometimes have a single urostylar centrum, reflecting the evolutionary trend toward reduction and fusion of hypurals across acanthomorphs. Dorsal to the centrum, there are usually one or two uroneurals (E), which are homologous to neural arches, and three epurals (E), which are homologous to neural spines.

Caudal-fin rays may extend anteriorly beyond the ural region. Vertebrae that have either or both neural and haemal spines supporting caudal-fin rays are called pre-ural vertebra. Enumeration of pre-ural vertebra runs caudorostrally.

The generic name of the skeletal complex that supports fin rays and spines is the pterygiophore (E). In general, a pterygiophore is formed from three radial elements: proximal, middle, and distal. The proximal radial is usually the longest bone of the pterygiophore complex. In several species, the proximal and middle radials are fused into a proximal-middle element. The distal radial usually remains cartilaginous and is surrounded by the base of fin rays.

Fin rays are formed by two halves of dermal bone, the lepidotrichia (D). The fin spine (D) of Acanthomorphata is formed by the fusion of the halves of lepidotrichia into a single, non-segmented element. The true spine present in acanthomorphs is characterized by its chain-like linkage of the spine to the corresponding pterygiophore. The developmentally distinct spines present in catfishes and some species of cyprinids have a spine–pterygiophore articulation. In acanthomorphs, each pair of lepidotrichia unite early in development and form an unsegmented, hollow element (Reed 1924). In catfishes, each pair of lepidotrichia segments fuses together, forming a single, solid element (Kubicek et al. 2019).

6.4.3 Appendicular Skeleton

The appendicular skeleton (Figure 6.6) is paired and homologous to the limbs of tetrapods. The pectoral girdle is the set of skeletal elements that support pectoral-fin rays, while the pelvic girdle supports the pelvic-fin rays. For more information on the appendicular skeleton of elasmobranchs, see da Silva and de Carvalho (2015a, 2015b), and da Silva et al. (2015).

The easiest pectoral-fin element to identify is the L-shaped cleithrum (D). This is generally the longest bone in the complex and serves as the insertion point for much of the axial musculature. In the posterior margin of the cleithrum, there are two or three endochondral bones, the scapula, coracoid, and mesocoracoid. The scapula supports the pectoral radials and usually has a large fenestra when the mesocoracoid is absent. The scapula supports four pectoral basal radials (E), and each of these radials support several distal radials surrounded by lepidotrichia, similar to those described for dorsal and anal fins. The mesocoracoid is usually positioned medial to the scapula, whereas the coracoid is located ventral to the scapula.

Dorsal to the cleithrum is the supracleithrum (D). The posttemporal bone (D) is positioned dorsoanteriorly to the supracleithrum and attaches to the neurocranium. The posttemporal has somewhat of a Y shape, with the dorsal limb contacting the epioccipital and the ventral limb inserting on the intercalar bone. The attachment of the pectoral girdle to the neurocranium is unique to Actinopterygii and often serves as a point of rotation of the head during prey capture.

The pelvic girdle is relatively simple. Its largest bone is the pelvic bone (or basipterygium; E). Pelvic rays either articulate on small pelvic radials or directly onto the posterior margin of the pelvic bone. In most teleosts, the pelvic bones contact each other medially.

6.5 SOFT ANATOMY

Anatomical accounts of nonhuman vertebrates date back to 1550s (Belon 1553, 1555). Detailed descriptions of muscles, brains, and abdominal organs in fishes date from the 1800s (Owen 1846; Clapp 1898; Herrick 1899; Allis 1903, 1909; Garman 1913; see Hilton et al.

2015 for historical perspective). These early anatomical descriptions pioneered the study of soft anatomy in fishes and, while limited in taxonomic scope, they were vital resources for large comparative studies in the twentieth and twenty-first centuries (Monod 1960; Winterbottom 1973; Northcutt 1977, 1978, 1989; Yabe 1985; Springer and Johnson 2004; Datovo and Vari 2013; Datovo et al. 2014; Pupo and Britto 2018). Traditionally, descriptions of species utilize skeletal information for diagnoses, but very few taxonomic manuscripts include accounts of soft anatomy (e.g., Vaz and Carvalho 2013; Petean and Carvalho 2018). For example, Datovo et al. (2014) showed that 72% of all synapomorphies of Teleostei described by Wiley and Johnson (2010) were based on characters from the skeleton.

6.5.1 Musculature

One of the primary references for actinopterygian **myology** is the 1973 monograph by Winterbottom, which offers a standardized nomenclature for all striated muscles. Realizing that the configuration of nerves is more conserved in vertebrates than the shape of muscles, he proposed the use of muscle innervation as the basis for identifying homologues across fish diversity. More recently, however, Datovo and Vari (2013) demonstrated that the sole use of innervation for tracing homologies across the diversity of Teleostei can be problematic, showing that the motor branch of the trigeminal nerve can vary in its position while the topology of adductor muscles remains the same. Datovo and Vari (2013) and Datovo and Rizzato (2018) proposed a new nomenclature system for the facial muscles of ray-finned fishes, which is followed here (Figure 6.9). Additional studies by Springer and Johnson (2004) and Datovo et al. (2014) described muscles attached to both dorsal and ventral gill arches for a large proportion of Teleostei, identifying an important, and previously unknown, component of myological diversity.

6.5.2 Central Nervous System

The central nervous system includes the brain and spinal cord. The brain is divided into five regions, the anterior most being the telencephalon, which processes information from the olfactory bulbs. Posterior to this region is the diencephalon, which is involved in regulating cyclical behaviors and processing information related to homeostasis (Helfman et al. 2009). This is followed by the mesencephalon, which receives information from the optic lobes and relays it to regions responsible for motor responses. Next, the metencephalon (or cerebellum) maintains body position in the water and regulates muscle activity. Elephantfishes (Mormyridae) are unique for having an extremely hypertrophied cerebellum that covers all other regions of brain (Lauder and Liem 1983; Butler 2011). The posterior most region of the brain, the myelencephalon, receives and transmits sensory information, triggers motor responses, and regulates respiration.

Variation in brain divisions has implications for, and is associated with, ecology and behavior, but can also reflect phylogenetic relationships (Northcutt 1989; Pupo and Britto 2018). Two seminal books describe the nervous systems of fishes (Northcutt 1978; Northcutt and Davis 1983), but neuroanatomy is seldom used in systematic studies. Some exceptions exist for Chondrichthyes (Northcutt [1977, 1989] and Fontanelle and Carvalho [2016]) and recent research on actinopterygian fishes has begun to incorporate central nervous system morphology in systematic analyses. Examples can be found in Lauder and Liem (1983) for Osteoglos-

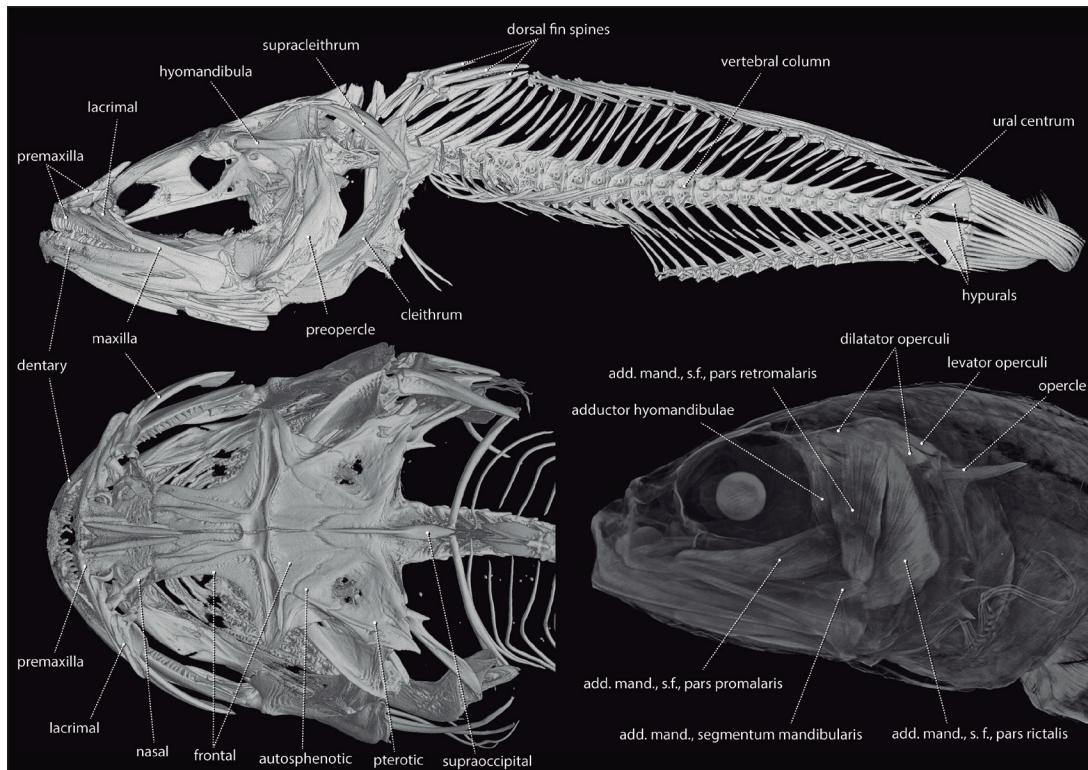


Figure 6.9. Tridimensional models generated by CT-scan of ethanol-preserved specimens. Two preparation types are shown: plain scanning for analysis of skeletal features (top and bottom left, opercle, sub-opercle, interopercle, and pectoral-fin rays removed) and specimen staining with phosphomolibdic acid (bottom right) to enhance soft tissue contrast for examination of facial muscles. Skeleton: Somalian Toadfish *Barchatus indicus* (Batrachoidiformes), whole skeleton in left lateral view (top) and cranial skeleton in dorsal view (bottom left). Musculature: specimen of Digitate Cusk Eel *Dicrolene introniger* (Ophidiiformes), head in left lateral view. add. mand. = adductor mandibulae. s.f. = segmentum facialis.

somorpha (especially Mormyridae); Eastman and Lannoo (2003a, 2003b, 2011) and Lannoo and Eastman (1995, 2000) for Notothenioidei; and Albert et al. (1998), Abrahão et al. (2018), and Pupo and Britto (2018) for several groups of Ostariophysi (knifefishes, characins, and catfishes, respectively).

6.5.3 Abdominal Organs

Major abdominal organs include the stomach, intestines, reproductive tract, kidneys, spleen, liver, heart, and swim bladder. Similar to the musculature and nervous systems, the use of abdominal organs in systematic and taxonomic studies is limited. An exception, however, is the reproductive tract of chondrichthyan fishes. Musick and Ellis (2005) discussed the phylogenetic distribution of reproductive modes and found that a uterus with well-developed **trophonemata** for delivering **histotroph** to growing embryos is a synapomorphy for Myliobatoidea (stingrays and eagle rays). Additionally, Vooren and da Silva (1991), Vooren and

Klippel (2005), and Vaz and Carvalho (2013, 2018) used the number of functional ovaries to distinguish southwestern Atlantic species of angelsharks (Squatiniformes). The intestinal morphologies of sharks and rays have also received attention in systematic studies. Compagno (1988) described several types of spiral intestinal valves in sharks, proposing that a scroll intestinal valve, present in both hammerheads (Sphyrinidae) and some requiem sharks (Carcharhinidae), indicates a close relationship between the families. Last, Yano and Tanaka (1983) used the number of spiral folds of the intestinal valve to differentiate species of deep-water dogfishes (*Centroscymnus*).

In Teleostei, swim bladder morphology has been the subject of taxonomic investigation. Collette and Russo (1981) used morphological differences in swim bladders to distinguish between species of toadfishes in the genus *Batrachoides*. In addition, Birindelli et al. (2009) thoroughly investigated swim bladder morphology in thorny catfishes (Doradidae), and discovered a high degree of morphological variation not previously described for catfishes. This study also showed that swim bladder shape can be used for distinguishing closely related, sympatric species.

6.6 LARVAL FISH MORPHOLOGY

Larval fishes are extremely morphologically diverse across species but also commonly exhibit drastic transformations in appearance throughout development within a species. In fact, the use of morphology to connect early life stages to juveniles and adults has proven to be difficult and, in some instances, the use of molecular tools is necessary (Baldwin and Johnson 2014; Leis 2015). Owing to the large degree of ontogenetic variation in morphology, distinguishing characters are usually stage and size specific. The most commonly used characters include pigmentation patterns and vertebral, fin, and myomere counts. In fishes that have photophores (e.g., lanternfishes, Myctophidae), distributional patterns of light organs can serve as reliable distinguishing characters. Important sources for the taxonomy of larval fishes include Leis and Rennis (1983), Moser et al. (1984), Leis and Trnski (1989), Matarese et al. (1989), Richards (2006), and Fahay (2007). Leis (2015) provides a list of studies that have produced significant advances in larval taxonomy over recent years.

6.6.1 Larval Stages

Definitions of larval stages follow Richards (2006). The egg stage is defined by a period in which the embryo and yolk are housed within a shell, called the chorion, and are separated from it by a perivitelline space. In most species of fishes, the shell is spherical, but shells can also be elliptical, spindled, or urn shaped (e.g., gobioids; Richards 2006). In skates and some sharks, eggshells sometimes possess unique features, such as elongate projections (skates) or an overall helicoid shape (bullhead sharks, Heterodontidae; Compagno et al. 2005). Richards (2006) lists a few groups of teleost fishes, such as flatfishes, in which the perivitelline space can be helpful for taxonomic purposes despite being generally inconspicuous. Several groups of non-acanthomorph Teleostei have segmented yolk masses (e.g., salmons, Salmonidae and anchovies, Engraulidae), whereas most acanthomorphs have homogeneous yolks. Distinguishing characters at this stage include the number of myomeres and pigmentation patterns, similar to those used for early stages of free-swimming larvae.

The yolk-sac larval stage is defined by free-swimming larvae that still possess at least some portion of their yolk sac. This stage is common in fishes with a nondispersing larval stage, such as toadfishes (Batrachoididae), in which individuals spend their entire larval stage within nests.

Finally, the larval stage proceeds through three substages: preflexion, flexion, and postflexion, defined by the degree of dorsal deflection of the posterior end of the notochord. In preflexion, the notochord is straight throughout. During flexion, the tip of the notochord starts curving dorsally to approximately 45 degrees, and hypural plates, caudal vertebrae, and fin rays begin to ossify (Richards 2006). Last, postflexion is achieved when the notochord is fully flexed, leading into the juvenile stage.

6.7 TECHNIQUES/METHODS

6.7.1 Methods for Obtaining Skeletal Information

6.7.1.1 Dry Skeletal Preparations

Traditionally, skeletal information was obtained almost exclusively through manual dissection of bony parts and subsequent drying for long-term storage. However, when working with large specimens, the removal of connective tissue can be incredibly laborious and time consuming. Moreover, it is nearly impossible to visualize all skeletal foramina with manual dissection. Bemis et al. (2004) provides a recent review of methods for obtaining clear and partially articulated skeletons. This method involves a partial dissection of the body before placing the specimen in a colony of dermestid beetles that will consume the soft tissues. For elasmobranchs with low calcification of skeletal structures, Enault et al. (2016) describes a method that involves soaking a specimen in warm water, manually dissecting its skeleton, then placing it in a solution of ethanol and hydrogen peroxide for further removal of the **perichondrium**.

6.7.1.2 Clearing and Staining

A common method for obtaining skeletal information from small specimens (up to 150 mm) is enzyme clearing with double staining (Figure 6.8). Two advantages of this method are that skeletal preparations can remain entirely articulated and the staining process results in a clear distinction between bony and cartilaginous structures. Specimens are initially dehydrated in a graded ethanol series. After dehydration, cartilage is stained blue by immersing the specimen in a solution of ethanol, glacial acetic acid, and Alcian Blue 8 GX. Specimens are then rendered transparent by placing them into distilled water with trypsin, which digests muscles but leaves the collagen and connective tissues intact. Bones are stained red by placing the fish in a solution with Alizarin Red S powder, diluted in potassium hydroxide. Finally, specimens are stored long term in glycerin (Sidlauskas and Konstantinidis 2021, Chapter 19, this volume). Full protocols for clearing and staining can be found in Dingerkus and Uhler (1977) and Taylor and Van Dyke (1985). Smith et al. (2018) describe a method for visualizing cleared and stained specimens with fluorescent microscopy that gives images greater depth and dimensionality and also facilitates identification of otherwise hard-to-see margins and sutures between bones.

6.7.1.3 Radiography and Computed Tomography (CT-scans)

The use of radiographs for viewing skeletal anatomy dates to the discovery of X-rays in 1895 (Sansare et al. 2011), and they have been a widely used resource for taxonomic research since the 1960s (Springer and Garrick 1964; Collette 1966). However, the use of radiographs in this field has been largely restricted to meristic data, such as counts of vertebrae, fin rays, and teeth (Vaz and Carvalho 2013, 2018). One of the primary limitations of using radiographs for evaluation of skeletal morphology is the intrinsic loss of dimensionality, which can produce artifacts with the slightest change in specimen orientation.

X-ray computed tomography (CT) is the oldest method for topographic imaging (Metscher 2009). The use of CT scans for studying fish morphology started in the early 1990s, with an investigation of coelacanth anatomy by Schultze and Cloutier (1991). A CT scan gathers data from a specimen that is placed in the path of an X-ray beam while being rotated at various angles (Metscher 2009). The anatomical information resulting from CT scans can be reconstructed as a stack resembling histological cross sections. Keklikoglou et al. (2019) provide a comprehensive overview of the steps involved in CT scanning biological specimens, including detailed protocols and uses for different types of specimen-based data. While the newest CT scanners produce images with increasing resolution, computing power for processing these images can be a limiting factor that researchers should consider, ideally with consultation from a CT specialist.

Examination of an image stack from a CT-scanned specimen can be helpful for determining boundaries of bones and articulations between structures. Specialized software is needed to generate and manipulate three-dimensional volumes and models (Figure 6.9). Software, such as 3D-Slicer, ImageJ, and its extension Fiji, are free for download and can be used across computing platforms. Horos® is another option for working with three-dimensional models, but is currently available only for Mac OS.

Computed tomography is a key tool for modern studies on fish skeletal systems. One of the greatest benefits of using CT scans for fish taxonomy is the ability to obtain non-destructive skeletal data from rare and type specimens. Figure 6.9 shows the type specimen of Somalian Toadfish *Barchatus indicus*, a species of toadfish known only from three individuals. Without CT scanning capabilities, we would know little about the species' skeletal anatomy beyond the number of vertebrae. Instead, access to volumetric data enabled a detailed examination of the skeleton, allowing a digital dissection of the specimen. Another benefit of the non-destructive nature of CT is the ability to sample large numbers of museum specimens, where permission would not likely be given for invasive procedures on many individuals. This is particularly helpful for researchers studying intraspecific variation in skeletal morphology (e.g., Witten and Hall 2015). Finally, CT-scans can provide new or otherwise difficult-to-obtain types of morphological data. For example, bone densities can easily be sampled to obtain information on material properties of skeletal structures (Silvent et al. 2017).

6.7.2 Methods for Examining Soft Anatomy

Abdominal organs are relatively easy to access with a simple dissection, requiring little more than a longitudinal cut along the ventral margin of the fish's body. Dissections for examining muscles and the nervous system, however, require training and can be time-consuming.

Unfortunately, the invasive nature of manual dissection often prevents investigations of rare taxa. Datovo and Bockmann (2010) describe an effective method to observe the origin and insertion of muscles precisely by removing the trypsin step from the clearing and staining protocol (see section 6.7.1.2).

Alternative and less invasive methods have been developed for investigating soft anatomy with CT (Figure 6.9). Metscher (2009) offers a list of contrast-enhancing techniques for soft tissues by means of different staining solutions, such as iodine and phosphotungstic acid. The effectiveness of each solution depends on the specimen's permeability, which can be limited by specimen size. For example, in Plainfin Midshipmen *Porichthys notatus*, larvae and small juveniles up to 28 mm in total length stained fully, while trunk, abdominal, and caudal musculatures stained only peripherally in larger specimens (D. Vaz, personal observation).

Several methods exist for staining nerves in cleared specimens. Filipski and Wilson (1984, 1986) and Song and Parenti (1995) developed protocols for triple staining specimens; in addition to staining cartilage and bone (as described above), cleared individuals are treated with Sudan Black B to stain nerves just prior to transferring the specimen to glycerin for long-term storage.

6.7.3 Microscopy: Histology and Scanning Electron Microscopy

Histology, the study of microscopic cells and tissues, is a technique that allows for examination of both soft and hard anatomical structures. Histological preparations of organisms have been implemented since the nineteenth century and remain important to a number of biological fields today (Hilton et al. 2015; Konstantinidis et al. 2015; Cohen and Hernandez 2018). There are various staining and preparation techniques available, which depend on the cellular structure of interest. One of the more common preparations is haematoxylin and eosin, which stains acidic and basic components of the cell pink-red and purple, respectively (Clardy et al. 2015). While histology allows for examination of multiple systems (e.g., skeletal, muscular, nervous) in the same, integrated sample, the preparations are size limited, as well as invasive, requiring at least partial destruction of a specimen.

Scanning electron microscopy (SEM) is another method that can be used to examine extremely small anatomical structures. It is versatile and can be used to visualize three-dimensional microstructure of solid objects with resolution on the order of 10 nanometers. Details of methods and protocols vary widely, including textbooks that cover the physical and technical aspects of SEM (e.g., Goldstein 1975). The uses of SEM in ichthyology are widespread, including investigations of fine structure of scales (Roberts 1993), skeletal elements (Britz and Johnson 2002), egg surfaces (Britz and Toledo-Piza 2012), and dentition (Conway et al. 2015).

6.8 TAXONOMIC PROCEDURES

Taxonomy is the science of classification. An important aspect of this field is binomial nomenclature, which is the standardized system used by taxonomists to name extant and extinct organisms (i.e., taxa; singular, taxon). The International Code of Zoological Nomenclature (ICZN) that regulates this system can be found online and contains definitions and recommendations for various taxonomic issues. See Box 6.1 and 6.2 for examples.

Box 6.1 Case Study: Taxonomy of a New Species

The following sections present a few examples compiled directly from scientific articles, with comments on their particularities and functions. The first example is a description of a new species of angel shark from Vaz and Carvalho (2018):

SYSTEMATIC ACCOUNT

Squatinaidae Bonaparte, 1838

Squatina Duméril, 1806

Squatina varii, new species

urn:lsid:zoobank.org:act:960E616F-C7B7-438F-B8AAE8466FA044F3

Figures 1–8, Tables 1, 2

Squatina dumeril: Nunan and Senna, 2007:174, pl. III (list of species).

Squatina occulta: Vaz and Carvalho, 2013:18, 79, table 2 (in part; misidentification of specimen MNRJ 30191).

Squatina sp.: Vaz and Carvalho, 2013:45–50, figs. 32–36 (taxonomic review; cited as a possible new species).

“Systematic Account” is equivalent to the results section of a typical scientific manuscript. Although not required, it is desirable to state the family and genus (along with the original describing author) in which the new species will be allocated, preventing confusion caused by **homonyms**. The ICZN requires that the status of a new species be clearly stated, which is why the term “new species” is placed immediately after the name. The register number of the nomenclatural act is placed below the name of the new species.

The second paragraph is the synonymous list. The function of this list is to describe potential previous citations (often under an incorrect name), clarify misidentifications, and more importantly, propose synonymy between two or more species. In the given example, the new species was previously cited under incorrect names (*Squatina dumeril* and *S. occulta*) and as an undescribed species (*Squatina* sp.).

The following synonymous list, extracted from Vaz and Carvalho (2013) exemplifies the proposal of a synonym between two species.

Squatina guggenheim Marini, 1936

(figs. 12–24; table 3–4)

Squatina guggenheim Marini, 1936:19–30, fig. 2 (original description, Southwestern Atlantic Ocean, Necochea, Province of Buenos Aires, Argentina)

Squatina punctata Marini, 1936: 19–30, fig. 4 (original description).

(box continues)

Box 6.1 Continued.

In this article, Vaz and Carvalho (2013) redescribed the species *Squatina guggenheim* Marini, 1936. In its synonymous list, they include the two names and nomenclatural acts (i.e., original descriptions of *S. guggenheim* and *S. punctata*) that are associated with the name *Squatina guggenheim*. Therefore, the authors proposed that *Squatina punctata* is not a valid species, but a junior synonym of *S. guggenheim*. Note that when proposing synonyms, the colon is placed after the author name and year, whereas when correcting misidentifications, the colon is placed immediately after the species name (as listed above from Vaz and Carvalho 2018).

Continuing with the example of *Squatina varii* (Vaz and Carvalho 2018), after the synonymous list, authors list the type series (definition by the International Code of Zoological Nomenclature (ICZN): all specimens on which the author established a nominal species-group taxon).

Holotype.—MNRJ 43106, adult male, 1110 mm TL, continental slope of Espírito Santo state, Brazil, Southwestern Atlantic Ocean, 19°42'54"S, 39°25'57"W, 195 m depth, bottom trawl, vessel N/O Thalassa, 30 June 2000.

Paratypes.—(13 specimens) MNRJ 30190, juvenile female, 690 mm TL, continental slope of Bahia state, Brazil, Southwestern Atlantic, 15°42'40.5"S, 38°37'58.8"W, 251 m depth, bottom trawl, vessel N/O Thalassa (remaining paratypes not listed).

The holotype is the name-bearing type specimen (MNRJ 43106) that is designated in the original publication. It is the only specimen of the type series immutably tied to the name. The paratype includes each specimen of the type series other than the holotype. These two type specimens are the most common, but there are other types for addressing particular situations (e.g., syntypes, lectotypes) discussed within the ICZN code.

The next section provides an example of a diagnosis, which is a section where morphological features are used to recognize a new (or re-described) taxon. It is common to list all combining characters in the diagnosis, but also to provide comparisons of each diagnostic feature for the new species with other related species in a later section. In the present example from Vaz and Carvalho (2018), the authors use a comparative diagnosis, which includes a comparison with related species. The advantage of this approach is that it emphasizes the differences among species upfront, clearly distinguishing the character combination that is unique to the new species.

Diagnosis.—*Squatina varii* is distinguished from all western Atlantic congeners (with exception of *S. david*) by a higher number of vertebral centra, from 138 to 150 (vs. 132–136 in *S. argentina*; 128–136 in *S. guggenheim*; 127–136 in *S. occulta*; 130–135 in *S. dumeril*; 121 in *Squatina* sp. NW1). *Squatina varii* differs from all Southwestern Atlantic species and *S. david* in their dorsal coloration, which has very few (<20) or lacks white circular spots (vs. dorsal surface of the skin having hundreds of white spots in *S. argentina*, *S. occulta*, *S. david*, and *S. guggenheim*).

(box continues)

Box 6.1 Continued.

Squatina varii is further distinguished from *S. argentina*, *S. guggenheim*, and *S. occulta* by having a wide and semielliptical lobe projecting from the proximal third of the lateral dermal fold of the head (vs. lateral dermal head folds entirely straight or slightly convex, without any emargination in *S. argentina*, *S. guggenheim*, and *S. occulta*). From *S. dumeril* and *S. david*, *S. varii* differs by lacking black blotches on the dorsal surface of the pectoral origin (vs. pectoral origin with rounded black blotches in *S. dumeril* and *S. david*).

Squatina varii is further distinguished from *S. guggenheim* by the slit shape of the pseudopera (vs. drop-shaped aperture in *S. guggenheim*), and by males reaching maturity at 980 mm TL (in contrast to male maturity ranging from 730–800 mm TL in *S. guggenheim*). The accessory dorsal marginal cartilage of the clasper of *S. varii* is trapezoidal in shape (vs. rectangular in *S. guggenheim*) and is positioned in the clasper shaft, laterally in relation to the dorsal marginal cartilage (vs. dorsal marginal cartilage positioned in the clasper glans, posterior to the dorsal marginal cartilage in *S. guggenheim*).

After the diagnosis, authors provide an overall description of the new or re-described species, along with the explanation for the chosen name. The ICZN code provides instructions for correctly creating a name. The example below is also from Vaz and Carvalho (2018).

Etymology.—This new species is named in honor of the late ichthyologist Dr. Richard Vari, who passed away in January 2016, in recognition of his outstanding contributions to Neotropical ichthyology.

Box 6.2 Case Study: Paleontological Taxonomy

Taxonomic studies of fossilized animals follow many of the same practices used for extant organisms, including nomenclature. The main difference lies in the methods for obtaining fossilized material and the completeness of specimens used in descriptions (oftentimes consisting of incomplete or fragmented elements only). The ICZN does accept parts or fragments of an organism as a type specimen.

The example presented below contains a fossil-based description of a new genus and species of extinct sturgeon (Acipenseriformes) from Grande and Hilton (2006). As you read this example, compare it with the description of the extant species in Box 6.1.

SYSTEMATIC PALEONTOLOGY

Order ACIPENSERIFORMES Berg, 1940

Family ACIPENSERIDAE Bonaparte, 1831

Subfamily †PSAMMORHYNCHINAE new subfamily

Genus †*Psammorhynchus* new genus

Type species. †*Psammorhynchus longipinnis* n. gen. and sp., by monotypy.

Diagnosis.—As for species (monotypic).

Etymology.—*Psammos*, sand; *rhynchos*, snout (from the Greek), referring to the sandy matrix that this specimen was found in and the large snout typical of sturgeons.

†*Psammorhynchus longipinnis* new species

Figures 1-29, Tables 2 and 3, and Appendix 1

Diagnosis.—This species differs from all other known acipenserids in having an extremely long dorsal fin (>140 rays, extending along most of the trunk length) with a highly falcate anterior margin. Trunk is almost completely covered with thick scales. Median extrascapular absent; two pairs of extrascapulars present: an inner pair and an outer pair. Dorsal scutes more numerous than in any other sturgeon; more posterior dorsal scutes chevron-shaped and grade smoothly into anterior fin rays of dorsal fin. Dermopterotics with an elongate posteromedially directed process extending beneath first few dorsal scutes.

In paleontological systematics, the use of skeletal data in the diagnosis is more common and prominent than in extant fishes. The reason for this is merely practical; preservation of external characteristics and soft morphological features is rare, making hard tissues (e.g., teeth, scales, skeleton) the primary source of information in these studies.

6.9 GEOMETRIC MORPHOMETRICS IN FISH BIOLOGY

In earlier sections of this chapter, you were introduced to the extraordinary morphological diversity that fishes possess and the structural complexity that is contained within their body plans. Morphometrics—the quantification and analysis of form—allows researchers to study observed patterns of morphological variation within a statistical framework. This has proven vital for addressing fundamental questions and hypotheses surrounding the diversity, evolution, and ecology of fishes.

Methods for quantitative evaluation of morphologies have long been employed, with geometric morphometrics (GM) representing a relatively recent approach but one that has greatly influenced the way in which biologists compare and visualize variation in organismal form. Geometric morphometrics relies on **landmarks**, homologous anatomical coordinates, to capture and evaluate morphology (Figure 6.10). A key distinction of GM, in comparison to traditional methods based on linear measurements, is that landmark coordinates inherently retain spatial information (i.e., shape) of the morphologies in question, and after an alignment procedure, the resulting shape data are comparable across individuals. Geometric morphometrics methods have been used to study macroevolutionary trends of fish body plans (Sallan and Friedman 2012; Friedman et al. 2019; Price et al. 2019), patterns of growth and development (Loy et al. 1998; Powder et al. 2015), morphological differentiation of putative taxonomic groups for systematics and stock identification (Ibañez et al. 2007; Martinez et al. 2015; Geiger et al. 2016), as well as relationships between morphology and resource partitioning (Clabaut et al. 2007; Antonucci et al. 2009; Muschick et al. 2012).

This section provides an introduction to geometric morphometric research as it relates to fish biology. Topics covered include best practices for data collection, the process of shape alignment, exploratory methods and data visualization, statistical analyses and data treatments, and emerging ideas and directions of GM research. In order to maximize the coverage of a broad range of topics, some details and concepts could not be included (e.g., statistical shape theory, partial warps, among others). For more detailed treatments of various subjects, we provide suggestions and references for further reading throughout the section.

6.9.1 Data Collection and Exploratory Methods for Geometric Morphometrics

6.9.1.1 Image Acquisition

The first step of any morphometrics study is to image specimens. While photographs, X-rays, CT or magnetic resonance imaging (MRI) scans, video, or any other image can be used for GM studies, we focus here on the best practices for specimen photography, which is the most common image acquisition method in two-dimensional morphometrics. For the purposes of GM, images do not need to be aesthetically pleasing, but it is essential that photographs clearly show the landmark locations and that specimens are consistently oriented relative to the camera. In particular, specimens (or the anatomical region of interest) should be centered and perpendicular to the camera to prevent spurious shape variation due to inconsistent positioning or the parallax effect (Mullin and Taylor 2002). Importantly, each photograph must include a scale bar. During analysis, this scale bar will allow for conversion of the distances between landmarks to a unit of length. Once every specimen has been photographed, the next step will be to place landmarks on the images in order to capture shape.

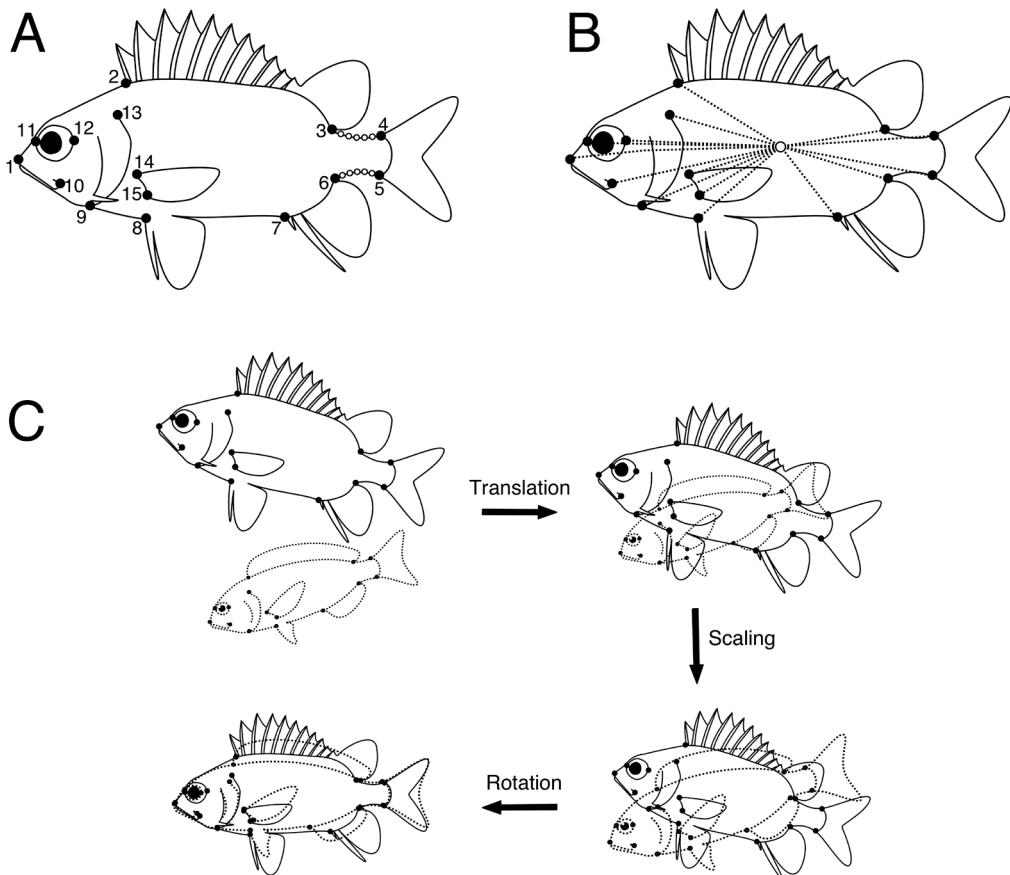


Figure 6.10. Graphical depiction outlining steps in the digitizing process and alignment of shape data. (A) Sample landmark configuration to capture external body shape variation in fishes, including typical external locations, such as fin insertion points and articulations between bones. Filled dots represent landmarks and unfilled circles are semilandmarks used to capture the curvature of the caudal peduncle region. (B) Illustration of the centroid, or average position, of the landmark configuration. Dashed lines represent the distance from each landmark to the centroid (unfilled circle). (C) Generalized Procrustes analysis is used to remove nonshape variation from two species, including position, size, and orientation. Figure design inspired by Klingenberg (2010).

6.9.1.2 Study Design

Landmarks are discrete anatomical points that, combined in a landmark configuration—the complete set of landmarks that represent an object—summarize the form of a single specimen. Through the **digitization** process, landmarks are placed on images of specimens and are recorded as two- or three-dimensional Cartesian coordinate data. As such, landmark data are inherently multidimensional and serve as points of correspondence between each specimen. After a shape alignment procedure (described below), landmark configurations can be mathematically compared, and the shape variation quantified across populations, between species,

or among other groups of interest. As all subsequent GM analyses use landmark data, it is critically important to consider the study design and quality of landmarks carefully from the start.

When designing a GM study, a preliminary question that may arise is how many landmarks are sufficient to capture anatomical variation across the study system. Sampling too few landmarks will obscure true structural variation across the data set and potentially lead to bias. On the other hand, sampling too many landmarks is labor intensive and time-consuming and can reduce statistical power of some downstream analyses (Gunz and Mitteroecker 2013; Collyer et al. 2015). One recent approach evaluates the number of landmarks that significantly contribute to describing patterns of shape variation across a data set. Implemented in the R statistical language (R Core Team 2018), “Landmark Sampling Evaluation Curve” determines the point at which landmarks can be removed from the configuration without surrendering shape information (Watanabe 2018). Keep in mind that landmarks only provide information at the point location, and shape variation between landmarks cannot be directly detected. Thus, it is important to ensure adequate sampling of the study region. Ultimately, however, the ideal number of landmarks is highly dependent on the study and anatomical region of interest.

Another essential aspect of GM study design is deciding where to place landmarks (e.g., Figure 6.10A). Landmarks should provide significant coverage of the morphology of interest such that features of biological variation can be quantified. Ideally, landmarks should be (1) homologous, (2) found reliably and consistently across all specimens, and (3) adequate to cover the structure of interest (Zelditch et al. 2004; Webster and Sheets 2010). Homology between landmarks can be anatomical or functional, but they must represent corresponding locations on specimens for both mathematical and biological reasons. For example, while the wing tips of a bird and an insect are not anatomically homologous, they represent corresponding, and thus comparable, aerodynamic locations. Landmark loci that can be identified to a single point on a structure, also known as a type 1 landmark, are desirable as they lack ambiguity (Roth 1993; Bookstein 1997). For instance, we can easily point to the precise location that the anterior spine of the dorsal-fin inserts on a fish’s body. In contrast, a landmark at the posterior-most point along that same fish’s operculum would be difficult to place in precisely the same location in every specimen (given the gentle curvature of that bone). Additionally, landmarks for which the structure is absent or broken in some specimens should be avoided. In these instances, either missing landmarks should be estimated (e.g., Gunz et al. 2009) or the landmark/specimen in question will have to be omitted from the data set.

When curves, outlines, or surfaces of structures are of interest to a specific research question, **sliding semilandmarks** can be used (Bookstein 1997; Mitteroecker and Gunz 2009). As the name suggests, these landmarks are constrained to slide along a two-dimensional curve (or three-dimensional surface) during shape alignment and, as such, do not identify discrete, anatomical loci like fixed landmarks. For example, semilandmarks may be useful to characterize the contour of a fish’s caudal peduncle region in order to gain information about relative swimming performance (Figure 6.10A). Methods and considerations for the alignment of semilandmark coordinates are beyond the scope of this chapter, but there are many excellent resources for further information (Zelditch et al. 2004; Mitteroecker and Gunz 2009; Gunz and Mitteroecker 2013). Freely available programs commonly used to digitize landmarks are tpsDig2 (Rohlf 2006) and Geomorph (Adams et al. 2021), a package implemented in R. Regardless of the software used, homology is denoted by the order in which landmarks are digitized.

6.9.1.3 Data Preparation

Once the landmarks for all specimens have been digitized, shapes are aligned using **generalized Procrustes analysis**, or Procrustes superimposition. In morphometrics, **shape** is defined as the features of a landmark configuration aside from its location, size, and orientation. Procrustes superimposition is used to isolate shape information by removing all of these non-shape attributes. This iterative least-squares procedure takes the raw landmark data through three sequential steps: (1) translation of all specimens to the origin, (2) scaling each to unit **centroid size**, and (3) rotation of specimens to minimize the square root of the sum of squared distances between corresponding landmarks, or their **Procrustes distance** (Figure 6.10C). The scale factor, centroid size, is a measure of the amount of dispersion in landmarks around the centroid, or the center of the landmark configuration (Figure 6.10B). It is calculated as the square root of the sum of squared distances from each landmark to the centroid (Bookstein 1997). Centroid size, hereafter referred to simply as size, is typically log-transformed in morphometrics studies to linearize relationships between variables. It should be emphasized that, while Procrustes superimposition does isolate size from shape, it does not account for shape change that is due to size (allometry). All subsequent analyses should be conducted on the multivariate Procrustes-aligned landmark coordinates (shape) and univariate log-transformed centroid size data (size) for each specimen or observation.

6.9.1.4 Data Exploration and Visualization

After preparation of landmark data, it is advisable to first use exploratory methods and visualization procedures. The most widely used technique for exploring patterns of variation in multidimensional data is principal component analysis (PCA). Principal component analysis is a rigid rotation of data that aligns it along new axes, called principal components (PCs), which are linear combinations of the original traits (landmark coordinates). The first principal component (PC 1) captures the greatest shape variation across the data set, with each subsequent PC orthogonal (uncorrelated) to all others and successively describing the maximum portion of remaining variance. With it, one can determine which aspects of shape change strongly contribute to variation across the data set, identify outliers and digitizing errors, as well as visualize the grouping and distribution of specimens by shape (Figure 6.11). While PCA is commonly used as a dimension reduction tool, the practice of analyzing and interpreting individual PC axes in isolation is not suitable for landmark-based GM data (Du 2018); since PCs are mathematical constructs with no inherent biological meaning, it is not advisable to use individual axes as a substitute for shape data in its full dimensionality.

When interpreting results from a PCA, it is helpful to visualize how shape changes across a PC plot. For this and other instances in which visualization is desired, there are a number of ways to graphically display shape change. Here, we focus on deformation grids (Figure 6.11), which are conceptually based on the theory of shape transformations popularized by D'Arcy Thompson (1942). With this approach, shape change between landmark coordinates is interpolated by a thin-plate spline model, and comparisons between forms are displayed as localized expansion, contraction, and warping of otherwise square grid cells (Thompson 1942; Bookstein 1989; Klingenberg 2013). Deformation grids can be used to easily identify regions of shape change that strongly differ between landmark configurations.

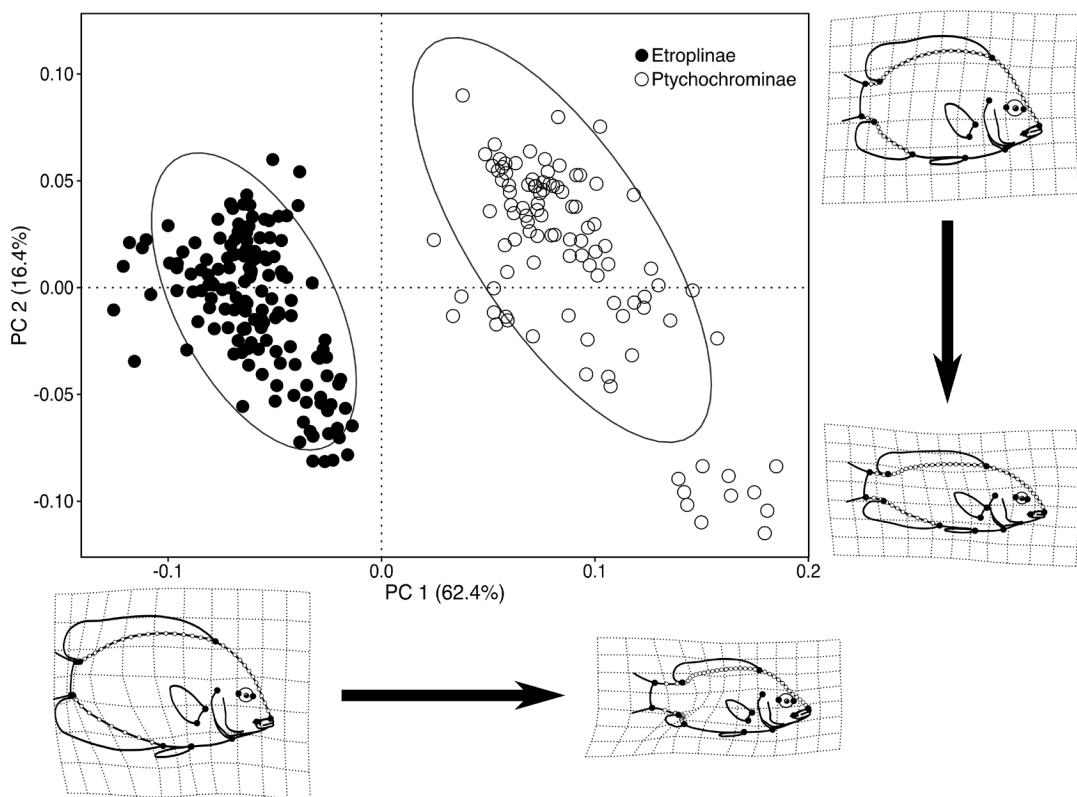


Figure 6.11. Major axes of body shape variation (PC1 and PC2) in Malagasy and South Asian cichlids, containing 78.8% of overall shape variation. Points represent shapes of individual specimens across 33 species. Subfamilies are shown as open versus closed circles, with 95% data ellipses displaying similar directions of shape change (from deep to shallow bodied) in each. Visualization of shapes along PC axes, relative to mean shape, are shown as deformation grids with landmarks in black and sliding semilandmarks in white. Data from Martinez and Sparks (2017).

6.9.2 Morphometric Data and Statistical Analyses for GM

Data exploration, such as PCA, can help to identify unwanted sources of variation that confound results and make interpretation difficult. Fortunately, methodological approaches have been developed to address some of these issues, such as artifactual variation due to bending in preserved specimens (Valentin et al. 2008) or arbitrary rotation of articulated structures, such as fish jaws, that makes standardization difficult (Adams 1999). One of the most commonly encountered sources of variation, however, involves shape variation due to size, allometry. As mentioned above, the shape alignment procedure does not remove the effects of size on shape. When shape allometry is not the focus of a study, researchers may choose to remove the effects of size so that it is not confounded with other factors that potentially influence morphological diversity. This is done by performing a multivariate regression of shape on the logarithm of centroid size and then using the residuals as allometrically adjusted shape data (e.g., Evans et al. 2017; Hipsley and Müller 2017).

After spurious variation has been removed from shape data, it is time to proceed with statistical analyses. Though the precise data analysis pipeline will depend on the study, Table 6.2 contains a list of research questions, and associated analyses, commonly asked by investigators using GM (all applicable to both two- and three-dimensional shape data). Note, however, that the table is intended as a starting point for shape analyses and is by no means an exhaustive list nor does it include all of the ways that any one question can be evaluated. Additionally, the table includes three research questions that incorporate a phylogeny, but several of the other methods have been extended for use in comparative studies.

Section 6.9 was designed as practical workflow for applying landmark morphometric techniques to questions in fish biology. In addition to the references provided throughout this section, readers interested in a more comprehensive overview of GM can refer to books by Bookstein (1997) and Zelditch et al. (2004).

Table 6.2. Common research questions and associated analyses for geometric morphometric shape data.

Research Question	Analysis/Approach
What are the primary axes of shape variation in my data?	Principal component analysis (PCA)
In which ways does shape vary between groups?	Discriminant function analysis (DFA) or canonical variate analysis (CVA)
How do I visualize shape differences?	Thin-plate spline/deformation grids
Do shapes differ when grouped by a discrete factor?	Multivariate analysis of variance (MANOVA)
Does one group have greater shape diversity than another?	Comparison of Procrustes variances
Does shape covary with a continuous factor?	Multivariate regression
Does shape covary with a multivariate-continuous factor?	Two-block partial least-squares (2B-PLS)
Is there a significant effect of size on shape (allometry)?	Multivariate regression of shape on size
Is there modularity between landmark subsets?	Covariance ratio (CR) test
Is there covariation (integration) between landmark subsets?	Two-block partial least-squares (2B-PLS)
How do shape transition rates vary across a phylogeny?	Phylogenetically independent contrasts (PIC)
Does the rate of shape evolution differ by group?	Multivariate rate (σ^2_{mult}) test
Does shape covary with a continuous factor, given patterns of shared ancestry?	Phylogenetic generalized least-squares regression (PGLS)

6.10 EMERGING IDEAS/FUTURE DIRECTIONS

Taxonomic procedures, as described in Section 6.8, have been consistent for the past century and are unlikely to change in the near future. However, technology has revolutionized the ways in which scientists can investigate taxonomic or systematic questions. Perhaps the most dramatic shift in the study of fish systematics was brought by the invention of molecular methods (Arcila and Ortí 2021, Chapter 5, this volume). Genetic and genomic tools can be used to provide strong support for phylogenetic relationships across morphologically dissimilar species. For example, Pelagiaria (composed of cutlassfishes, snake mackerels, mackerels, tunas, and others) and Syngnatharia (pipefishes, seahorses, dragonets, cornetfishes, and others; see Betancur-R. et al. 2017) are two groups that have only relatively recently been recognized and accepted by ichthyologists. Conversely, molecular analyses have also resulted in the splitting of groups with poor morphological support; toadfishes (Batrachoidiformes) and cusk-eels (Ophidiiformes), long considered to be part of Paracanthopterygii (Rosen and Patterson 1969; Patterson and Rosen 1989) have been reclassified as Percomorphacea (Miya et al. 2005; Betancur-R. et al. 2013, 2017; Near et al. 2013) as a result of genetic evidence. At the species level, genetic information has been used to identify previously unrecognized cryptic species diversity in coral reef dwarf gobies (Tornabene et al. 2016). Sequencing data has also been invoked to piece together the distinct ontogenetic stages of Yellow-spotted Golden Bass *Liopromus olneyi* (Serranidae; Baldwin and Johnson 2014). As technology improves, the next logical frontier of the field is genomics. With the power and data to provide unprecedented biological insights, there is potential to completely resolve the Fish Tree of Life in the not-so-distant future (Alter 2021, Chapter 3, this volume).

The field of morphometrics, too, has quickly advanced in recent decades with the rapid growth of technological and analytical methods. One such area is the use of three-dimensional shape data. While the ability to analyze three-dimensional images with GM is not new, there has been a recent explosion in the accessibility of imaging systems such as CT and MRI scanners. Just as we describe the recent impacts of these technologies for traditional descriptive morphology earlier in the chapter, the ease at which we can now capture shape variation in its full dimensionality is rapidly expanding the breadth of research questions that we can address with GM. Although the focus of this chapter has been primarily on two-dimensional geometric morphometrics, it is sometimes necessary to evaluate morphologies in three dimensions. Structures like the skull are inherently curved, and the shapes of wide-bodied and/or dorsoventrally depressed fishes can contain important morphological variation in multiple planes (Buser et al. 2018). Although the analytics are generally the same for both two- and three-dimensional landmark data, the digitizing procedure differs significantly. For further information on three-dimensional geometric morphometrics there is a wealth of resources from the primary literature (e.g., Mitteroecker and Gunz 2009; Parr et al. 2012, Bardua et al. 2019) as well as a book by Zelditch et al. (2004) that touches on the subject.

A currently underutilized area of GM research, especially relating to fishes, is its application to organismal function, biomechanics, and kinematics (Cooke and Terhune 2015). A number of studies in nonfish systems cast morphological variation in an explicitly functional light, sometimes pairing GM with finite element analysis to study the mechanical performance of skeletal structures (O'Higgins et al. 2011; Polly et al. 2016). Recent work on the evolution of fish feeding systems uses GM to map form–function relationships for a biomechanical model of jaw function (Martinez and Sparks 2017; Martinez and Wainwright 2019). In addition to

functional simulation, GM may be used to evaluate kinematics in living organisms. As an organism or biomechanical system completes a motion, it will trace out a trajectory through shape space (or a loop, if the start and end shapes are the same; Adams and Collyer 2009). Features of these trajectories provide information about the nature of motions. For example, a study on feeding motions in African cichlids showed that the degree of relative nonlinearity and the length of a species' shape trajectory were associated with prey evasiveness (Martinez et al. 2018). Given the potential to expand these methods to other systems, GM applications to kinematic studies will undoubtedly lead to greater knowledge of evolution and diversity in functional systems.

In the pursuit of an evolutionary perspective, many studies have also begun to couple morphometric data with phylogenetic comparative methods to address hypotheses of shape change through time (Monteiro 2013; Davis and Betancur-R. 2017; Borstein et al. 2018). Though methods exist to infer tempo and mode of morphological evolution on a macroevolutionary scale, statistics to analyze multivariate and multidimensional shape data in a phylogenetic context are unfortunately limited (but see Monteiro 2013; Adams and Collyer 2018). With methodological advancements, especially as it relates to phylogenetics, geometric morphometrics will continue to be a powerful tool, not only to compare biological forms, but to study the evolution of morphological diversity.

One of the primary challenges in ichthyology is to understand how ecological and evolutionary processes lead to shape differences both within and across taxa. Geometric morphometrics serves as a bridge between descriptions of organismal form and a framework to quantify and analyze that morphological variation. From differentiating between fish stocks, to species identification and characterizing morphological diversity, advancements in the fields of systematics and morphometrics will continue to make valuable contributions and improvements to the study of fish biology.

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6.11 TERMS

Definitions of GM terms are drawn from Zelditch et al. (2004) and Mitteroecker and Gunz (2009). Definition of homonyms extracted from the ICZN (1999).

Centroid size: a measure of geometric scale, capturing the dispersion of landmarks around the center of a landmark configuration; it is calculated as the square root of the sum of squared distances from each landmark to the centroid

Chemosensory: relating to chemosensation, the process of sensing chemical stimuli; the sense of smell is an example

Chromatophore: pigment-containing cells that are embedded in the integument of fishes and that give them coloration

Cryptic species: species of any organism that are morphologically similar, but are genetically distinct; cryptic species may result from recent divergences, where incipient sister species have not been isolated from each other long enough to accumulate readily observable morphological differences

Diagnostic character: one or a combination of characters that are used to recognize and distinguish a species; also known as distinguishable features, these characters are often presented in the diagnosis section of a species description (see Box 6.1 for an example)

Digitization: the process of converting information, in this case geometric landmark coordinates, to a digital format

Foramina: plural form of foramen, a hole or opening in a skeletal structure, usually for the passage of nerves and blood vessels

Generalized Procrustes analysis: also known as Procrustes superimposition, this is the process whereby shape is isolated from other nonshape attributes: scale, position, and orientation; this method minimizes the Procrustes distance (see definition, below) between landmark configurations in a data set

Geometric morphometrics: a suite of methods that use landmarks to describe, quantify, and analyze shape

Gustatory: relating to the sense of taste

Histotroph: nutritive “milk” secreted by the trophonemata in the uterus of stingrays (Myliobatiformes)

Homonyms: available names that have the same spelling, but refer to distinct nominal taxa (species, genus, or higher); it is still considered homonymy if only the nominal suffix differs

Landmark: a discrete, anatomical locus that represents a single homologous point of correspondence between specimens

Mechanosensory: relating to mechanosensation, the process of sensing mechanical stimuli, such as touch or hearing; fishes have the ability to detect changes in water flow around the body based on its physical or mechanical interaction with small sensory structures (neuro-masts) in the lateral line system

Meristic traits: a countable quantitative trait (e.g. fin rays, scales, etc.) that can be used for taxonomy and distinction of species

Myology: the study of muscles, including their structure and function

Perichondrium: layer of connective tissue that surrounds cartilage

Pharyngeal jaws: a secondary set of jaws located in the posterior region of the oral cavity, anterior to the opening of the esophagus; it is formed by dorsal tooth plates, fused to pharygo-branchials and by ventral tooth plates, which are attached to the ceratobranchial

Photophore: the light-producing organ of fishes, which commonly functions in intraspecific communication; the pattern of photophores on many fishes are species specific and can be used as a diagnostic tool

Photosensory: relating to photoreception, the process of perceiving different intensities and wavelengths of light

Principal component analysis: a multivariate statistical tool used to assess patterns of variation and covariation within a data set. This method involves a rigid rotation of data to align it along new axes, called principal components (PCs), composed of linear combinations of the original variables. The first principal component captures the greatest shape variation across the data set, with each subsequent PC orthogonal to all others and successively describing the maximum portion of remaining variance.

Procrustes distance: the distance between landmark configurations in shape space, calculated as the square root of the sum of squared distances between corresponding landmarks after Procrustes superimposition

Proprioceptive: the sense of self-awareness of the body position and its movements

Shape: the geometric information contained within a landmark configuration after removing the effects of position, scale, and orientation

Sliding semilandmarks: a class of landmarks that occur along an outline, curvature, or surface and are used to capture a contour; in contrast to fixed landmarks, semilandmarks are constrained to vary along their defined contour

Troglobomorphic: animals that are adapted to cave-dwelling; most species converge on a suite of morphological characteristics including reduction or loss of pigments and eyes

Trophonemata: uterine appendages of rays that secrete a nutritious substance rich in proteins and lipids for developing embryos

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