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# Systematics of myliobatoid elasmobranchs: with emphasis on the phylogeny and historical biogeography of neotropical freshwater stingrays (Potamotrygonidae: Rajiformes)

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The neotropical freshwater family Potamotrygonidae appears to be the only stingray group that has radiated in a non-marine environment. To assess the affinities of potamotrygonids to other rays, a phylogenetic analysis was undertaken using 39 morphological characters from 18 stingray groups. The single tree produced (CI = 0.80, RI = 0.88) suggests that neotropical freshwater rays are a monophyletic group, and that within Potamotrygonidae, *Paratrygon* is basal to a clade composed of *Plesiotrygon* and *Potamotrygon*. The sister group to potamotrygonids was determined to be amphi-American *Himantura* — these taxa share synapomorphies of the ventral mandibular musculature and the hyomandibular/mandibular articulation. The topology suggests that potamotrygonids are derived from a freshwater-invading ancestor that was distributed along the northern coast of South America (Pacific and Caribbean) prior to the emergence of the isthmus of Panama. This hypothesis conflicts with parasite-based biogeographic scenarios of a strictly Pacific origin for potamotrygonids. General systematic results concerning urolophids, dasyatids, and pelagic myliobatoid stingrays are also discussed.

ADDITIONAL KEY WORDS:—South America – Amazon – fishes – paleogeography – parasites – marine – *Himantura* – Dasyatidae – Urolophidae.

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## INTRODUCTION

The family Potamotrygonidae consists of exclusively freshwater stingrays distributed throughout most of the major river systems of South America. Although marine sharks and stingrays invade the large rivers of most continents (e.g. Compagno & Roberts, 1982, 1984; Roberts & Karnasuta, 1987; Snelson, Williams-Hooper & Schmid, 1988; Thorson, 1983), fully freshwater rays appear to have only diversified in the Neotropics. The potamotrygonid family comprises some 20 species divided into three genera (two of which are monotypic), and demonstrates a considerable array of morphological variability (Rosa, 1985, 1990; Rosa, Castello & Thorson, 1987). All potamotrygonids possess a unique (among elasmobranchs) suite of characteristics related to their obligate freshwater lifestyle. These include: the inability to retain high blood concentrations of urea to counter water loss caused by high salinity (Thorson, 1970; Thorson, Cowan & Watson, 1967); the degeneracy of the rectal gland, a salt excretory organ (Thorson, Wooton & Georgi, 1978); and modifications of the electroreceptive Ampullae of Lorenzini for freshwater operation (Raschi & Mackanos, 1989).

The distribution of stingrays in freshwaters of South American is paralleled by several other families of predominantly marine fishes, including anchovies (Engraulidae), herrings (Clupeidae), needlefishes (Belonidae), flatfishes (Achiridae or Soleidae), croakers (Sciaenidae), etc. (Géry, 1969; Fink & Fink, 1979). Roberts (1972) has tabulated some 14 families of mainly marine fishes with freshwater neotropical representatives. In contrast, the Congo basin has only four. This difference in diversity has been attributed to the accessibility of the two river basins — while the Congo is largely above sea level and defended by rapids, the Amazon is low-lying and relatively open to marine invaders (Roberts, 1972). Thus, marine taxa (including stingrays) are thought to have independently invaded South America through the mouth of the Amazon. Brooks, Thorson & Mayes (1981), however, provide an alternative perspective. Based on studies of parasite systematics, they suggest that the South American stingray parasites and hosts originated from the Pacific, before the uplift of the Andes. This hypothesis, although derived from stingray parasites, has not been tested using stingray phylogeny, largely because the affinities of potamotrygonids to marine rays are poorly known.

Potamotrygonids have been considered closely related to members of two marine stingray families: Dasyatidae and Urolophidae. Together, these taxa make up a group of predominantly benthic rays that swim by the undulation of their pectoral fins, as distinct from the more active, pelagic marine species that swim by 'flapping' their pectorals (e.g. Myliobatidae, Rhinopteridae, Mobulidae). Brooks *et al.*'s (1981) systematic treatment of parasites suggested that potamotrygonids are most closely related to Pacific coast members of the genus *Urolophus* (hereafter referred to as *Urobatis*, a name originally suggested by Garman [1913], and more recently by Miyake [1988] and McEachran [in press], to designate amphi-American stingrays of the genus *Urolophus*). Rosa (1985) and Rosa *et al.* (1987) considered urolophids to be the sister group to the neotropical freshwater rays, and used them as the outgroup to assess intra-potamotrygonid relationships. Other authors, however, have suggested that few differences, besides habitat, distinguish potamotrygonids from dasyatids (Garman, 1913; Bigelow & Schroeder, 1953), and some have grouped freshwater stingray genera within the family Dasyatidae (e.g. Arambourg & Bertin, 1958). Miyake (1988) and Miyake, McEachran & Hall (1992a) mentioned that there is little evidence to support a close relationship between urolophids and potamotrygonids, and instead posit that *Taeniura lymma* (Forskal), an Indo-West Pacific reef dasyatid, may be more closely related to neotropical freshwater rays. Nishida (1990) presented a phylogeny for stingrays in which dasyatid and potamotrygonids were grouped together. However, only a single character supported this particular grouping, and Nishida did not include two of the three freshwater genera (*Paratrygon* and *Plesiotrygon*) in his analysis.

Two hypotheses are therefore available concerning the marine sister group to Potamotrygonidae. Parasitological evidence (Brooks *et al.*, 1981) suggests that potamotrygonids are most closely related to urolophids, or some subset thereof. On the other hand, the anatomical work of Miyake and McEachran, and the phylogenetic analysis of Nishida (1990) suggests that potamotrygonids may be nested within dasyatid rays. The latter hypothesis also derives support from physiological/ ecological data — while urolophids are obligate marine fishes, many dasyatid species are euryhaline and enter freshwaters for extended periods of time. An intuitively pleasing ecological scenario would have potamotrygonids evolving from a freshwater-invading dasyatid ancestor.

The purpose of the present investigation was to evaluate these hypotheses in the context of a phylogenetic systematic analysis of stingray genera. The resultant cladogram is used to explore the origins and biogeographic history of neotropical freshwater potamotrygonids.

## METHODS

Specimens were collected in the field, purchased from aquarium dealers (*Paratrygon*), and borrowed from a variety of institutions; the complete list of stingrays examined is provided in Appendix 1.

Lateral line canals were exposed by dissecting away the skin and superficial tissue using a binocular dissecting microscope with illumination from a fibre-optic light source. The canals of some specimens were injected under water with black india ink using a hypodermic syringe to improve visibility. Drawings were then made by placing the specimen under a flat plate of glass and tracing the canal patterns from directly above.

Most anatomical characters were examined by radiography and gross morphological dissections, rather than clearing and staining. Small specimens (under 20 cm disc width) were placed on Kodak Industrex SR film (size: 20.3 × 25.4 cm) and exposed to x-rays in a Hewlett Packard 43805N Faxitron series at 35 KVP, 35 mA, for between 1.5 to 3.5 minutes (9- 14 inches to specimen). Larger specimens were placed on Kodak Industrex AA film (size: 35.6 × 43.2 cm) and exposed to x-rays from a General Electric rotating anode MBN 1.0-2.0 beryllium x-ray tube at 35 to 45 KVP, 100s mA, for between 4/5ths to 1 and 1/2 seconds. Radiographs were viewed under a dissecting scope and traced and examined on a light table. Clearing and staining followed the methods outlined by Dingerkus & Uhler (1972).

Dissections of alcohol-preserved and cleared and stained specimens were also made under the dissecting scope, with muscles occasionally stained using a potassium iodide solution. Drawings were made using a camera lucida. Figures were produced using Desk Scan 2.04 for the HP ScanJet Plus (Hewlett-Packard) and Canvas 3.0 (Deneba Software).

Other data were derived from the anatomical literature, primarily Rosa (1985), Miyake (1988), Miyake & McEachran (1991), Miyake *et al*. (1992a), and Nishida (1990). Whenever possible, these data were verified by examining additional specimens.

Nishida's (1990) phylogeny was used to delimit ingroups and outgroups: the stingrays *Plesiobatis daviesi* (Wallace) and *Hexatrygon* were together considered the primary outgroup to an ingroup consisting of all other stingray taxa. Stingrays to the exclusion of *Plesiobatis* and *Hexatrygon* are monophyletic based on the characters: ventrolaterally expanded nasal capsules (however, see below), and nasal curtain completely united and reaching mouth (Nishida, 1990). More distant secondary outgroups included *Raja, Psammobatis, Rhinoraja, Bathyraja, Platyrhina*, and other skate and guitarfish taxa. Stingrays, to the exclusion of these groups are supported as a monophyletic taxon based on six characters (discussed by Nishida [1990]).

As the purpose of the study was to determine the sister group to neotropical freshwater stingrays, emphasis was placed on the examination of benthic amphi-American marine taxa. The biogeographically conservative assumption that the sister group would be distributed along the Pacific and/or the Atlantic Coast of South America was based on previously considered vicariant and dispersalist scenarios (see Brooks *et al*. [1981]). The fact that potamotrygonids are not known from any other continents (particularly Africa) suggests a post-Gondwanan origin for the group. Nevertheless, representative stingrays from more distant geographical locales were considered in the analysis. The pelagic stingray groups were also included; however, they were not closely scrutinized for characters — data were taken from the literature, and not all possible genera (or autapomorphies) were used in the analysis. This de-emphasis on pelagic rays was based on preliminary morphological work, which suggested that potamotrygonids would be nested somewhere within the other benthic ray groups.

Characters were analysed using cladistic parsimony methods, as originally proposed by Hennig (1966), and elaborated by authors such as Farris (1983), Nelson & Platnick (1981) and Wiley (1981). Outgroup methods were used to root trees and polarize characters (Watrous & Wheeler, 1981; Nixon & Carpenter, 1993). Multistate characters were ordered whenever possible with consideration to morphocline analysis (see Mickevich & Weller, 1990), the method of intermediates (Wilkinson, 1992), and similarity (Lipscomb, 1992). These techniques essentially involve ordering by minimizing morphological change or distance between states. Inapplicable, unknown, and polymorphic character states were analysed with respect to the warnings of Maddison (1993) and Nixon and Davis (1991) about potential unexpected and undesired effects; in general, however, these types of states were not problematic because of their rarity in the data.

A matrix was constructed for the taxa under consideration and analyzed using the branch and bound search algorithm of Swofford's (1993) PAUP 3.1, and by hand, using Hennigian argumentation. Ordered multistate characters were also run unordered. Character evolution was examined using the ACCTRAN and DELTRAN optimization options of PAUP 3.1, by hand, and by using MacClade 3.0 (Maddison & Maddison, 1992).

## CHARACTER SURVEY

Characters were derived from (1) the lateral line canal system, and (2) other morphological systems (skeletal, myological, physiological, and embryological); claspers were not investigated. Stingray lateral line canals have rarely been examined from a comparative perspective. Garman (1888) reported lateral line canal patterns from a wide variety of elasmobranchs, deriving a key for batoids based on his illustrations. Ewart (1892) and Ewart & Mitchell (1892) later organized and named four primary lateral line canal systems, thus simplifying Garman's (1888) terminology. Chu & Wen (1979) next used a hybrid nomenclature of Garman (1888) and Ewart & Mitchell (1892) to construct a classification and evolutionary scenario for Chinese elasmobranchs. Lateral line canal patterns have, therefore, infrequently been used to infer evolutionary relationships among stingrays, and have never been applied in a cladistic context.

Morphological characters (to the exclusion of lateral line canal patterns) have been more commonly exploited as a source of phylogenetic information. Most recently, Nishida (1990), revising an earlier study (Nishida, 1985), performed a cladistic analysis of stingray relationships, using a broad spectrum of anatomical features. Miyake (1988) provided a comparative anatomical study of batoids, with an emphasis on Urolophidae, and Rosa (1985) provided a cladistic analysis of stingrays, with an emphasis on Potamotrygonidae. Compagno (1977) gave a broad overview of the skeletal anatomy of sharks, skates, and rays. Daniel (1934) discussed comparative anatomy of myliobatoids (among other cartilaginous fishes), and Garman (1913) presented invaluable anatomical illustrations for representative species of several stingray genera.

More taxonomically specific studies on stingrays include the comprehensive morphological investigation of the Indo-West Pacific stingray, *Himantura imbricata* (Schneider), by Chandy (1957), and detailed work by Hamdy, Khalil & Hassan (1974a, b), and El-Toubi & Hamdy (1969a, b) on the neurocranium and visceral arches of *Hypolophus sephen* (Forskal) and *Himantura uarnak* (Forskal) (see Miyake [1988] for a complete list of references). The vast majority of stingray species descriptions are limited to meristics (including counts of fin rays, vertebrae, etc.) and external characters. However, Heemstra & Smith (1980), and Compagno & Heemstra (1984) describe internal anatomical characters of, respectively, *Hexatrygon bickelli* Heemstra & Smith and *Himantura draco* Compagno & Heemstra; Compagna & Roberts (1982) provide descriptions of the neurocranium, pectoral and pelvic girdle, and claspers of

*Himantura signifer* Compagno & Roberts, a freshwater species from Southeast Asia; and Rosa *et al*. (1987) illustrate various skeletal features of *Plesiotrygon iwamae* Rosa, Castello & Thorson.

Other sources of morphological data include comparative anatomical studies, which focus on a particular organ system or skeletal component, and include one or more stingray taxa for comparison with other batoids or elasmobranchs. De Beer (1932) considered the hyoid arch in skates and rays. Holmgren (1940, 1941, 1943) examined the ontogeny of the neurocranium and visceral arches in *Urobatis halleri* (Cooper), building on the work of Balfour (1878) and others, on elasmobranchs in general. Miyake *et al*. (1992b) examined the rostral cartilage in batoids, and Miyake & McEachran (1991) discussed the ventral gill arch skeleton. Bell (1993) investigated the nasal structure in several batoid groups, including stingrays. Cranial and ventral gill arch muscles were considered by, among others, Marion (1905), Edgeworth (1935), Kesteven (1942), De Andrés, García Guzmán & Muñoz-Chápuli (1987), and Miyake *et al*. (1992a).

The strength of this legacy of batoid investigations is primarily descriptive rather than phylogenetic (until recently). Nevertheless, it provides a sound basis for the quest for apomorphies undertaken here. Characters are discussed below under headings corresponding to convenient structural or functional units. Previously suggested characters are designated by a citation following the derived state; those that resolve relationships within pelagic myliobatoid stingrays (including *Myliobatis*, *Aetomylaeus*, *Aetobatus*, *Rhinoptera*, *Mobula* and *Manta*) are discussed only briefly. Characters used for analysis are enumerated; character consistency indices are included in brackets following the character number. All characters are represented in matrix format (Table 1).

### *Dorsal lateral line canals*

Preliminary examination of the dorsal lateral line canal patterns suggested that they would hold little phylogenetically informative variation; they were not closely examined for this study.

*1.* $^{(CI\,=\,1.0)}$ *Dorsal tubules of the pleural loop* extend towards the borders of the disc in a radius from the hyomandibular canal. At their tips, they branch dichotomously one or more times and open to the surface of the skin. In *Gymnura*, the pleural tubules are consistently more branched and cover the outer edges of the disc in a ‘web’ of overlapping tubules (Garman, 1888, plates 43–45; Chu & Wen, 1979, figs 57–59). This is considered the derived state of character 1.

### *Ventral lateral line canals*

All canal terminology follows Ewart & Mitchell (1892); components of canals are named following Garman (1888). Only three of the four primary canals occur on the ventral surface of stingrays (Fig. 1A). The hyomandibular canal penetrates to the ventral surface of the disc near the anterior border of the nasal capsule. It runs anteriorly for a short distance, then curves posterolaterally at a variable distance from the edge of the disc to form the outer part of the subpleural loop. The medial part of the subpleural loop passes along the gill slits to connect with the infra-orbital canal. The infra-orbital canal descends posteriorly from the tip of the disc to between the nostrils, turns laterally along the nasal capsule, passes its connection to the hyomandibular and supra-orbital, and loops (sometimes with modifications) anteriorly to eventually penetrate to the dorsal surface of the disc. The supra-orbital, from its connection to the infra-orbital and/or hyomandibular canal moves medially along a curving path to eventually extend for a variable distance towards the anterior

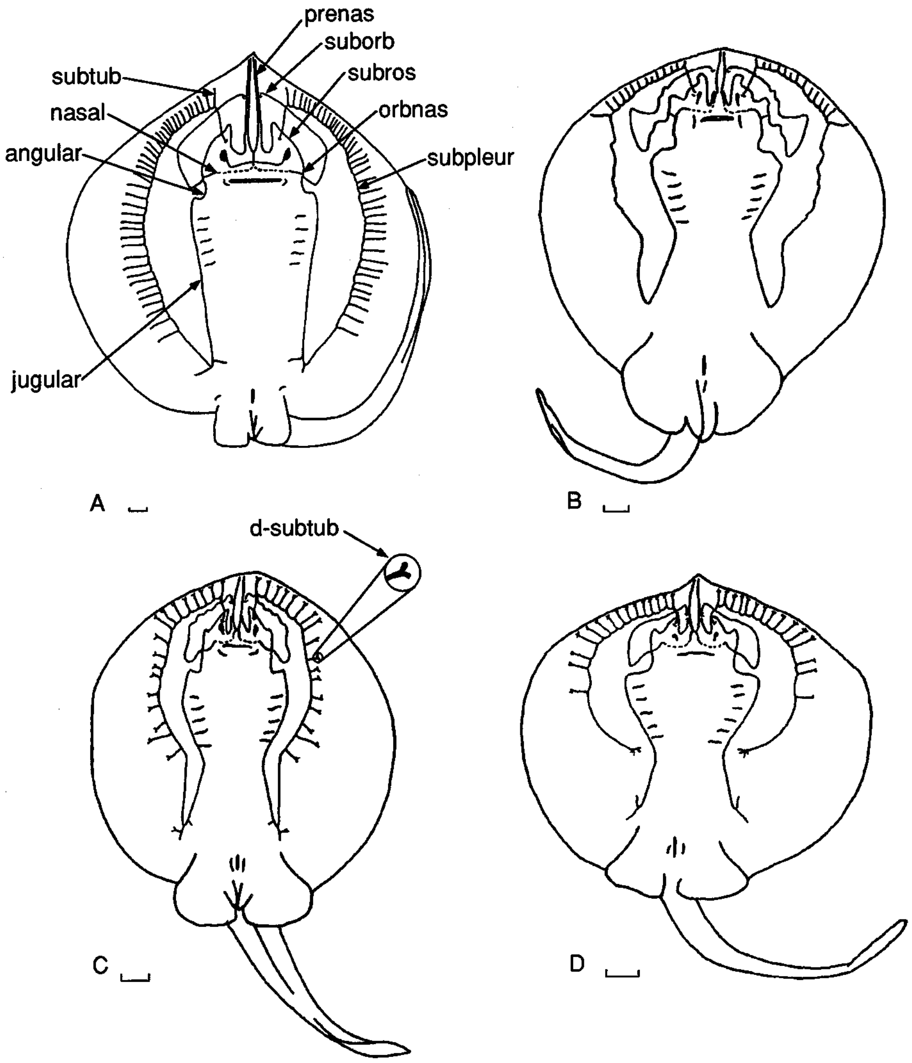

Figure 1. Ventral lateral line canals of stingrays. A: *Plesiobatis daviesi* (BPBM 24578); B: *Urolophus cruciatus* (LACM CSUF); C: *Urobatis jamaicensis* (Cuvier) (ROM 28276); D: *Urotrygon microphthalmum* Delsman (USNM 222693). C shows magnified subpleural tubules. Scale bar = 10 mm. Abbreviations – prenas: prenasal component of the infra-orbital canal; suborb: suborbital component of the infra-orbital canal; nasal: nasal component of the infra-orbital canal; orbnas: orbito-nasal component of the infra-orbital canal; subros: subrostral component of the supra-orbital canal; angular: angular component of the hyomandibular canal; jugular: jugular component of the hyomandibular canal; subpleur: subpleural component of the hyomandibular canal; subtub: subpleural tubules; d-subtub: dichotomous branching of the subpleural tubules.

edge of the disc. Lateral line canal patterns for various stingray taxa are shown in Figures 1 to 4.

2. $^{(1.0)}$ *Anterior tubules of the subpleural loop* extend from the hyomandibular canal towards the anterior border of the disc. *Urobatis* and *Urotrygon* show conspicuous dichotomous branching at the tips of their subpleural tubules (Fig. 1C, D). In all other taxa examined, including the primary outgroup *Plesiobatis* (Fig. 1A), this condition was not observed. Distinct dichotomous branching is therefore coded as the derived state for character 2.

The presence of extended anterior subpleural tubules may be an apomorphy for stingrays. In all other batoids diagrammed in Garman (1888) and Chu & Wen (1979), subpleural tubules were not observed to extend towards the anterior edge of the disc (but see Garman's [1888] plate 24 of *Rhinobatos*). Chu & Wen's (1979) figure of *Urolophus aurantiacus* Müller & Henle shows no anterior or lateral subpleural tubules. However, specimens of *Urolophus aurantiacus* and *Urolophus cruciatus* (Lacépède) examined for this study, clearly possessed anterior pleural tubules.

3. $^{(1.0)}$ *The subpleural component of the hyomandibular canal* in *Dasyatis*, Indo-West Pacific *Himantura*, and *Gymnura*, closely follows the anterior edge of the disc, passing approximately halfway or further towards the lateral border of the pectoral fin, while giving off many subpleural tubules (usually 30 + )(Figs. 2B, 3). The subpleural in these taxa also shows a distinctive lateral 'hook' after moving away from the edge of the disc (Figs. 2B, 3) In the pelagic myliobatoid rays, the subpleural component of the hyomandibular extends over 3/4 the distance to the lateral edge of the disc, usually gives off many tubules (30 + ), and is highly indented at the posterolateral border (Fig. 2C). This indentation creates two distinct subpleural loops or spaces. In all other stingrays examined, including the outgroup *Plesiobatis*, the subpleural component of the hyomandibular does not closely follow the anterior edge of the disc, gives off few anterior tubules (less than 30), and traces a somewhat semicircular path along its lateral border (Figs 1, 2A, 4). The condition seen in *Dasyatis*, Indo-West Pacific *Himantura*, and *Gymnura* is coded as the derived character state (3[1]) and is considered intermediate to the derived condition in *Myliobatis*, *Aetomylaeus*, *Aetobatus*, *Rhinoptera*, *Mobula*, and *Manta* (3[2]) (see Fig. 2 for ordering hypothesis). The subpleural component in *Paratrygon* (Fig. 4C), while giving off a large number of

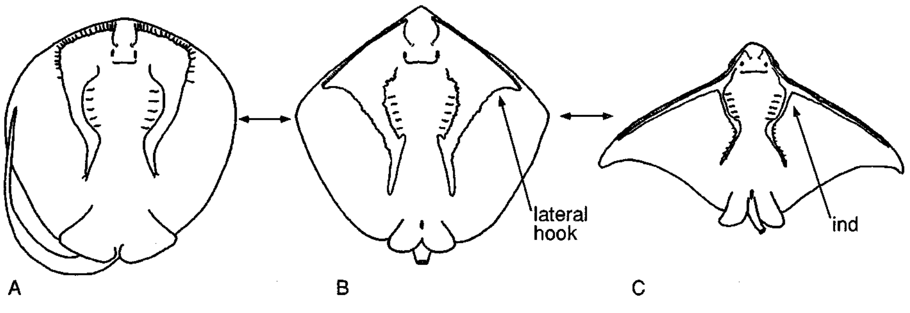

Figure 2. Ventral view of subpleural component of the hyomandibular canal (other canals not shown), and hypothesized transitions between character states for character 3. A, *Himantura pacifica* (ROM 66838); B, *Himantura uarnak* (after Chu and Wen, 1979, figure 54); C, *Aetomylaeus maculatus* (Gray) after Chu and Wen, 1979, figure 61). Abbreviations – lateral hook; lateral hook of the subpleural; ind: indentation of the subpleural.

subpleural tubules and travelling nearly to the lateral edge of the disc, does not show the lateral 'hook' of state 3[1] and is coded as the plesiomorphic condition, 3[0].

*4.* $^{(1.0)}$ *The suborbital component of the infra-orbital canal* passes to the ventral surface of the disc near the midline, halfway between the tip of the snout and the front of the neurocranium (in most benthic taxa), and thereafter shows a variety of modifications in different stingrays. In *Plesiobatis, Urolophus, Urobatis, Urotrygon* (Fig. 1), amphi-American *Himantura, Taeniura*, some *Dasyatis* species (Fig. 3A,B), *Gymnura*, and the pelagic myliobatoid rays, the suborbital component extends laterally and posteriorly, then loops to connect to one or more of the subrostral component of the supra-orbital, the nasal component of the infra-orbital, and the angular component of the hyomandibular. In *Potamotrygon* and *Plesiotrygon* (Fig. 4A,B), the suborbital has a distinct forward loop that is considered a derived state (4[1]). In *Paratrygon* (Fig. 4C), the suborbital takes part in extensive branching to cover the anterior aspect of the

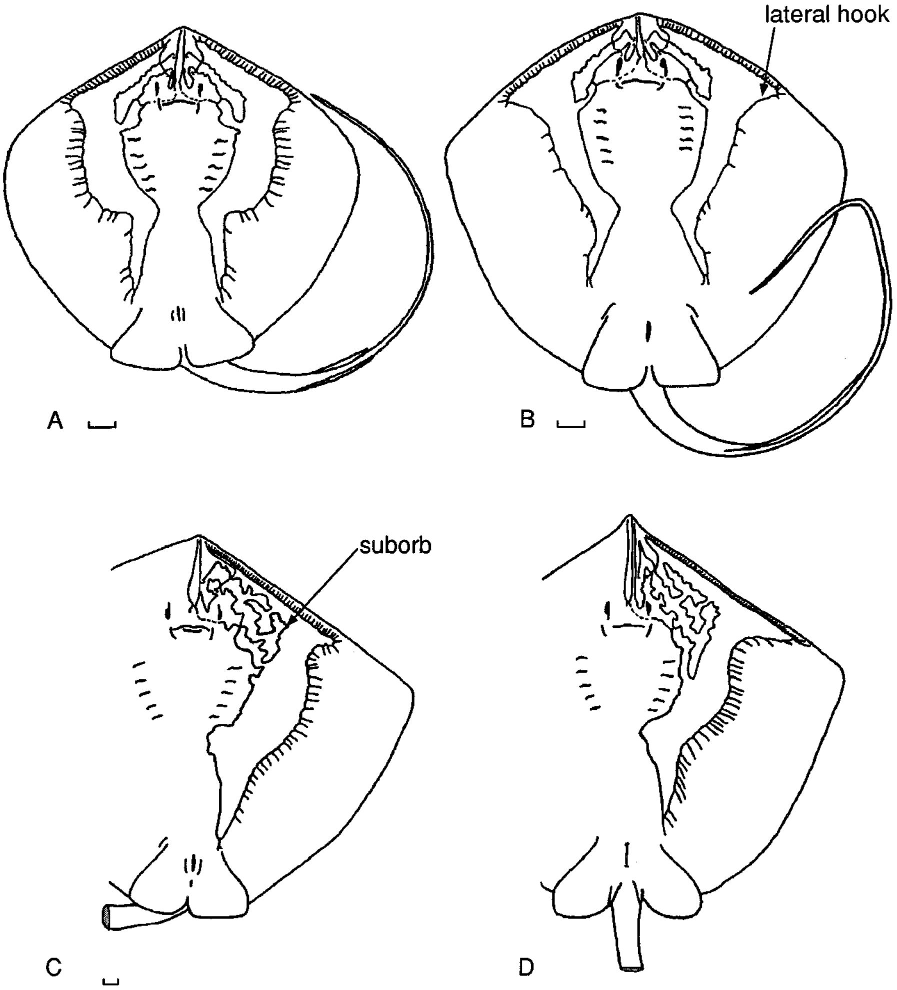

Figure 3. Ventral lateral line canals of *Dasyatis* species. A, *Dasyatis sabina* (Leseuer) (ROM 46549); B, *Dasyatis say* (Leseuer) (TCWC 5818.1); C, *Dasyatis longus* (Garman) (ROM 66840); D, *Dasyatis guttata* (Bloch & Schneider) (after Garman, 1888, plate 41). Scale bar = 10 mm. See Figs 1 and 2 for abbreviations.

disc in a ‘web’ or plexus of canals; this condition is considered another derived state (4[2]). *Himantura uarnak, H. gerrardi* (Gray) (Chu & Wen, 1979: figs 54, 55), *Dasyatis guttata* and *D. longus* (Fig. 3C,D) show extensive reticulation and looping of the suborbital to cover a considerable area. This reticulation is considered a third derived state — 4[3]. *Himantura imbricata* shows yet another pattern: the suborbital is arranged in multiple loops to create a number of distinct compartments (Garman, 1888: plate 39; Chandy, 1957: fig.58); this species is not included as a distinct OTU and was therefore not coded for this unique character. No obvious order of transformation between derived states was evident; thus this character was run unordered in all analyses.

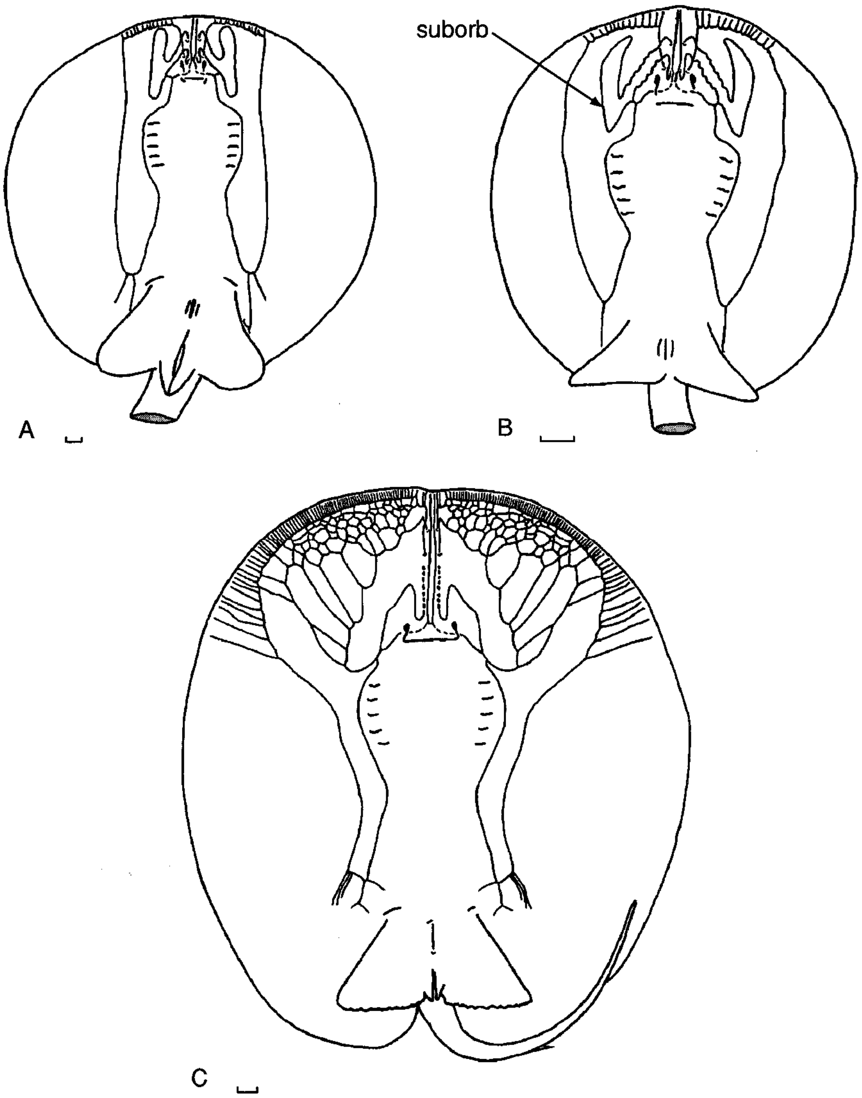

Figure 4. Ventral lateral line canals of Potamotrygonidae. A, *Potamotrygon motoro* (Natterer) (ROM 66844); B, *Plesiotrygon iwamae* (ROM 65366); C, *Paratrygon aireba* (Müller & Henle) (ROM 66843). Scale bar = 10 mm.

Note that a potential apomorphy for stingrays must be the penetration of the disc (dorso-ventrally) by the suborbital component of the infra-orbital canal near the midline. In outgroups to stingrays, the suborbital passes dorso-ventrally near the edge of the disc.

*The subrostral component of the supra-orbital canal* approaches the midline and runs alongside the prenasal component of the infra-orbital canal. Garman (1888) reported that in *Potamotrygon* and *Paratrygon*, the subrostral canal, as it approaches the anterior edge of the disc, changes from a tube-like structure to a row of closed rings connected by tissue. These appear similar to the vesicles of Savi found on the ventral surface of torpedoes, and may represent an obsolescent canal condition. Garman (1888) also reported their presence in *Urobatis halleri* and *Urobatis jamaicensis* and Chu & Wen (1979) indicated their appearance in *Himantura uarnak*, *H. gerrardi*, and *Dasyatis micropthalmus*. In the present study they were observed in a number of taxa, including species of *Urotrygon* and *Dasyatis*. The presence of these vesicles probably represents an apomorphic condition (they do not appear to be present in *Plesiobatis* or any of the secondary outgroups); however, their nearly universal occurrence in at least one species of most genera, coding difficulties, and possible intra-specific variation precluded their use in this analysis.

### *Neurocranium*

The neurocranium was not intensively investigated for this study; however, characters from the phylogenetic and anatomical literature were examined and used in the cladistic analysis. The neurocranium is somewhat box-like in shape and contains or supports the brain and the olfactory, visual, and auditory organs. The anterior ethmoid region contains two large nasal capsules; the middle, or orbital region includes large openings for the orbits; and the posterior otico-occipital region contains the auditory capsules.

*The nasal capsules* are paired spheres which enclose the olfactory organs. Nishida (1990) considers the ventrolateral expansion of the nasal capsules to be an

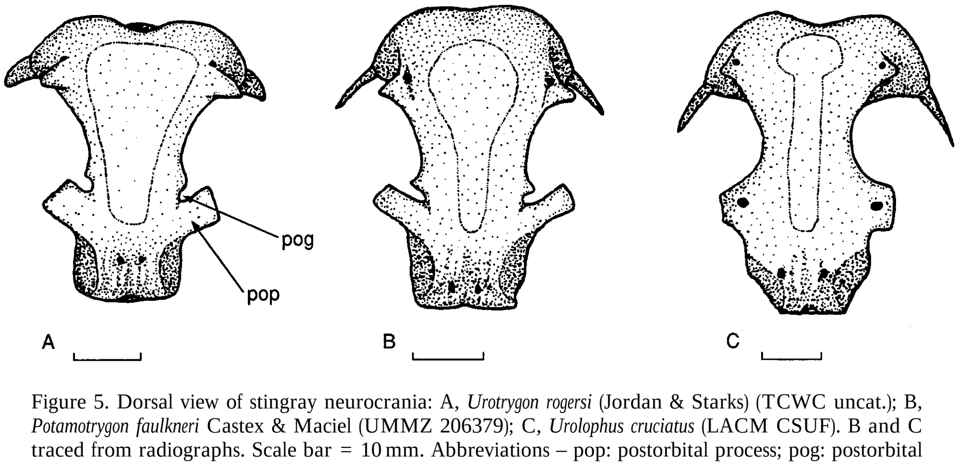

Figure 5. Dorsal view of stingray neurocrania: A, *Urotrygon rogersi* (Jordan & Starks) (TCWC uncat.); B, *Potamotrygon faulkneri* Castex & Maciel (UMMZ 206379); C, *Urolophus cruciatus* (LACM CSUF). B and C traced from radiographs. Scale bar = 10 mm. Abbreviations – pop: postorbital process; pog: postorbital groove.

apomorphy of stingrays to the exclusion of *Plesiobatis* and *Hexatrygon*. However, Miyake (1988) provides a figure that shows ventrolateral expansion in *Plesiobatis* (as *Urotrygon daviesi*). Examination of Miyake's specimen for this study appears to corroborate his view, and suggests that the variation in lateral to ventro-lateral expansion of the capsules is difficult to quantify. Accordingly, this character was not used in the analysis.

*5.* $^{(1.0)}$ *The nasal curtain*, an external morphological feature, is formed by the fusion of the anteromedial nasal flaps in stingrays (Bell, 1993). In *Plesiobatis* (Nishida, 1990: fig. 57a) and *Hexatrygon* (Heemstra & Smith, 1980: fig. 6), the nasal curtain fails to reach posteriorly to the anterior border of the mouth, however, it does so in all other stingrays. The extension of the nasal curtain to the anterior border of the mouth is considered the derived state (5[1]) (Nishida, 1990). The polarization of this character is based on Nishida's (1990) observations of non-myliobatoid outgroups.

*6.* $^{(1.0)}$ *The anterior process of the neurocranium* In *Rhinoptera, Mobula* and *Manta*, extends over the nasal capsules, while it does not in other stingrays and the outgroups. The presence of this extension is considered the derived character state (6[1]) (Nishida, 1990). Nishida (1990) also reports that the pre-orbital process is poorly developed in *Rhinoptera, Mobula*, and *Manta*. However, this character may be related to the anterolateral extension of the neurocranium and is not used separately here.

*7.* $^{(1.0)}$ *The optic nerve foramen* is located laterally on the neurocranium and is the largest foramen in the orbital region. In *Urolophus*, this foramen is considerably larger than in other stingray taxa and outgroups, occupying lengthwise about one third of the orbital region (Miyake, 1988); also, it is directly adjacent to the eye stalk. This condition is considered the derived state (7[1]).

*8.* $^{(1.0)}$ *The interorbital region* is relatively narrow in most stingrays and the outgroups. In *Mobula* and *Manta*, the interorbital region is wider than the length of the neurocranium, and this is considered the derived character state (8[1])(Nishida, 1990).

*9.* $^{(1.0)}$ *The lateral margin of the postorbital process* is elongate and forms a cylindrical protuberance in *Aetobatus, Rhinoptera, Mobula*, and *Manta*, but not in any other stingray taxa, including the outgroups. The presence of a cylindrical protuberance is considered the derived state (9[1])(Nishida, 1990).

*10.* $^{(1.33)}$ *A deep lateral groove in the postorbital process*, in most stingrays, allows the passage of the infra-orbital lateral line canal, separating the postorbital process into an anterior and a posterior component (Fig. 5A, B); this condition is assigned state 10(1)(Miyake, 1988; Nishida, 1990). In *Plesiobatis, Urolophus* (Fig. 5C), *Aetobatus, Aetomylaeus, Rhinoptera, Mobula*, and *Manta* the infra-orbital canal passes through a foramen in the process — this condition is designated state 10[0]. Both conditions appear in the outgroup (the groove in *Hexatrygon*, and the foramen in *Plesiobatis*), which is therefore coded as polymorphic. Skates (and other non-stingray batoids) have minimally developed postorbital processes, usually with slight indentations on the lateral aspect. The homology between this indentation and the deep postorbital groove of most stingrays is unclear.

### *Mandibular arch and hyomandibular*

The mandibular arch consists of the paired, dorsoventrally flattened palatoquadrate and mandibular (or Meckelian) cartilages, which support numerous rows of teeth or large pavement-like tooth plates. The jaw itself receives support from the hyomandibular, which descends anterolaterally from its articulation with the neurocranium.

*The connection between the hyomandibular and mandibular arch* is one of two main types in stingrays according to Nishida (1990). In the outgroups *Hexatrygon* and *Plesiobatis*, and in *Gymnura*, *Aetoplatea*, *Aetomylaeus*, *Mobula*, and *Manta*, the hyomandibular articulates directly with the mandibular cartilage (Nishida, 1990). In other taxa (Fig. 6A–I), the connection, although variable in length, is through a separate component. However, some OTU's appear to show multiple states; *Myliobatis* species show both direct and indirect articulations (Garman, 1913: plate 73; Nishida, 1990: fig. 20j). Also the condition in the outgroup *Plesiobatis* is in question, since Nishida's figure appears to show an indirect articulation through a ligament (Nishida, 1990: fig. 20a). Accordingly, this character was not used in the phylogenetic analysis.

*11.* $^{(0.5)}$ *Hyomandibular accessory cartilages* are small, usually rod-like elements associated with various aspects of the hyomandibular. In *Myliobatis* and *Aetomylaeus* (Nishida, 1990: fig. 22a) and *Rhinoptera* (Garman, 1913: plate 74), an anterior hyomandibular accessory cartilage extends from the anterior tip of the hyomandibular. This condition is not seen in other stingrays and is considered the derived state (11[1]) (Nishida, 1990).

A dorsolateral hyomandibular accessory cartilage is located, in some stingrays, approximately halfway along the hyomandibular's extension between jaw and neurocranium. This element was reported (as HAC-2) in *Dasyatis centroura* (Mitchill), *Urobatis halleri*, and *U. maculatus* Garman by Nishida (1990: fig. 22a). Garman (1913: plates 69–71) shows similar cartilages in *Urobatis jamaicensis* (labelled supraspiracular), *Potamotrygon constellata* (Vaillant) and *Paratrygon* (labelled postspiracular), and *Dasyatis guttata* (unlabelled); Holmgren (1940) noted the presence of a similarly positioned prochondrial rod in embryonic *Urobatis halleri*. In this study, such cartilages were observed in cleared and stained specimens of *Potamotrygon motoro* and *Urobatis halleri* (they are not evident in radiographs). The apparently scattered distribution of this feature throughout various stingray taxa devalues its possible usefulness as a phylogenetic character. However, the homology of the cartilage in different groups is yet to be established. In *Urobatis*, the tube-shaped dorsolateral HAC may be a derivative of the envelope for a sensory canal (Holmgren, 1940); in *Paratrygon* (and *Potamotrygon*) it may represent fused remnants of mandibular or hyoid gill rays, as suggested by its wider, flat shape. Until more developmental studies can be undertaken, the possible phylogenetic relevance of the dorsolateral HAC will, as Holmgren (1940: 192) puts it, remain 'quite obscure'.

*12.* $^{(1.0)}$ *The angular(s)* are small cartilages occurring in the ligament that connects the hyomandibular to the mandibular arch. In amphi-American *Himantura*, these elements consist of a collection of variously sized cartilages embedded in a matrix of connective tissue (Fig. 6G, H). This condition was not observed in any other stingray taxa, and is considered a derived state 12(1).

In all species of *Potamotrygon* examined, and as originally shown by Garman (1913)

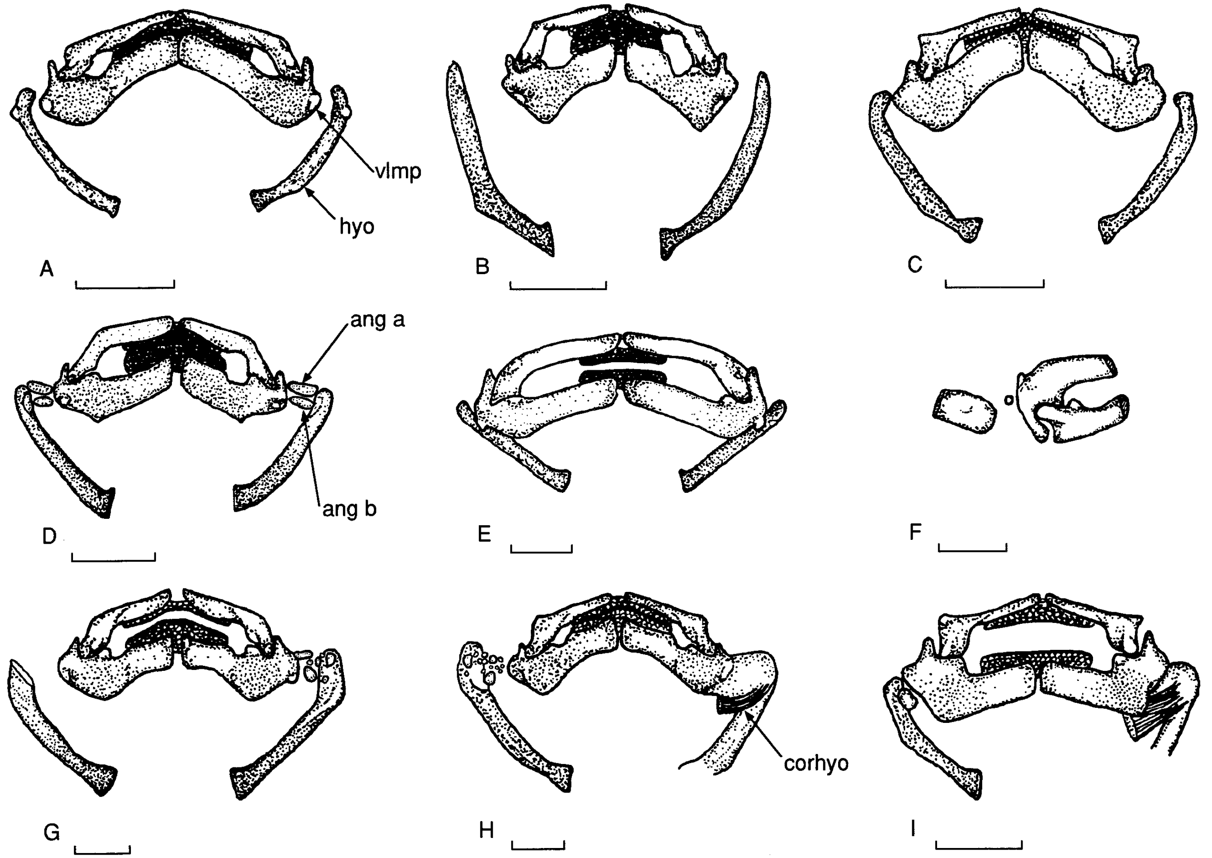

Figure 6. Ventral view of mandibular arch and hyomandibular cartilages of stingrays. A: *Himantura jenkinsii* (Annandale) (ROM 23011); B: *Taeniura lymma* (ROM 50295); C: *Urobatis maculatus* (USNM 11951); D: *Paratrygon aireba* (ROM 66843); E: *Paratrygon aiereba* (ROM 66843); F: *Paratrygon aireba* (ROM 66843)–lateral view of hyomandibular and jaw (pulled slightly apart); G: *Himantura schmardae* (Werner) (ROM 66845); H: *Himantura pacifica* (Beebe & Tee-Van) (ROM 66838); I: *Urolophus cruciatus* (LACM CSUF). Left side of G obscured. Right side of H and I shows attachment of coracohyoideus. Scale bar = 10 mm. Abbreviations – vlmp: ventrolateral madibular process; hyo: hyomandibular, ang a: angular a; ang b; angular b; corhyo: coracohyoideus.

and discussed by Holmgren (1943), two discrete angular cartilages were found to be present on each side of the jaw (Fig. 6D). The cartilage located anteriorly in the ligament (angular-a) is connected directly to both the hyomandibular and mandibular. The posterior cartilage (angular-b) is often smaller and, in radiographs of larger specimens, appears to 'float' in the connective ligament rather than articulate with other cartilages directly. *Plesiotrygon* has an extremely robust, spool-shaped, angular-a, but does not appear to have a second angular (Lovejoy, 1993: fig. 8c). In *Paratrygon* (Fig. 6E), the distance between the hyomandibular and mandibular is considerably decreased; however, a small angular cartilage was found in the connective ligament (Fig. 6F). The presence of a robust and elongate angular cartilage that appears to play a functional role in the articulation between hyomandibular and mandibular is considered a derived state (character 12[2]) for *Potamotrygon* and *Plesiotrygon. Paratrygon* is coded as missing data for this character because the homology of its 'angular' is unknown. The close relationship between the hyomandibular and mandibular in this species has altered the potential functional role of such a cartilage.

The angular elements in amphi-American *Himantura* appear to play a similar functional role to the angular(s) of *Potamotrygon* and *Plesiotrygon*. In all these taxa, the hyomandibular / mandibular link is more rigid than a simple ligamentous connection. Indeed, the strengthening of the hyomandibular / mandibular connection with multiple small cartilages is considered the intermediate state (12[1]) between the ligamentous condition (12[0]) and the presence of robust angular cartilages (12[2]). Such an ordering hypothesis is strengthened by the presence, in *Himantura schmardae*, of cartilages that are similar in shape, and may be homologous with the angular-a of *Potamotrygon* and *Plesiotrygon*.

McEachran (pers. comm.) and Miyake (1988: 443) suggested that a close phylogenetic relationship between potamotrygonids and *Taeniura lymma* is supported by "…the occurrence of the 'a' cartilage between the Meckelian and hyomandibular cartilages…". Carvalho (pers. comm.) also noticed an area of slight chondrification in the hyomandibular / mandibular connective ligament of a cleared and stained *T. lymma* specimen (AMNH 44079SW). However, for the present study, radiographs of two specimens, and dissection of a third, failed to reveal the presence of a discrete angular cartilage (Fig. 6B). Although chondrification within the ligament may sometimes occur in this species, this condition is not considered homologous to the discrete angular cartilage(s) of *Potamotrygon* and *Plesiotrygon*, which is visible in radiographed and cleared and stained specimens of all sizes. *Taeniura* is therefore coded as having the plesiomorphic condition of a ligament or direct articulation between the hyomandibular and mandibular.

*13.* $^{(0.5)}$ *A cartilaginous element near the anterior tip of the hyomandibular* was observed in several stingray taxa. Garman (1913: plates 73–75) figured such cartilages in *Myliobatis*, *Aetobatus*, *Rhinoptera*, and *Mobula*. In the present study, small square cartilages were evident in *Urolophus* (Fig. 6I). These elements are shaped differently and do not appear to share the functional role of the angular cartilages of *Potamotrygon*, *Plesiotrygon*, and amphi-American *Himantura*. In the latter taxa, the angulars exist as discrete entities interposed between the mandibular and hyomandibular, while in the former groups, the 'angular cartilages' appear to share a functional role with the anteriomedial aspect of the hyomandibular. This function is evidenced, in *Urolophus*, by the insertion of the coracohyomandibularis on both the hyomandibular and the separate cartilage (Fig. 6I). Also, the hyomandibular appears to maintain its direct or ligamentous connection to the mandibular. In *Potamotrygon, Plesiotrygon*, and amphi-American *Himantura*, the coracohyomandibularis inserts on the hyomandibular (Fig. 6H). *Myliobatis, Aetobatus, Rhinoptera, Mobula*, and *Urolophus* are tentatively coded as sharing a derived state of a separate character (13[1]), however, the pelagic stingrays were not closely examined in the present study and their condition may not truly be homologous with that seen in *Urolophus*.

*The ventrolateral process of the mandibular* projects from the mandibular cartilage near its attachment to the hyomandibular. Garman's (1913) figures of pelagic stingrays show an apparently well-developed ventrolateral process. Observation of radiographs, cleared and stained, and dissected specimens revealed the ventrolateral process to be well-developed in potamotrygonids, *Dasyatis, Taeniura*, and *Himantura*, (Fig. 6A,B,D,E,G,H) but poorly developed in *Urolophus, Urobatis*, and *Urotrygon* (Fig. 6C,I). Outgroup taxa (Heemstra & Smith, 1980; Nishida, 1990) also lack a well-developed process. Unfortunately, this character shows continuous variation and could not be objectively coded (as Nishida [1990] found). However, a trend towards increased development of the ventrolateral process appears to be shared by non-Urolophid taxa.

*14.* $^{(0.5)}$ *Symphysial fusion of the mandibular and palatoquadrate cartilages* occurs in *Aetomylaeus, Rhinoptera, Mobula, Manta* (Nishida, 1990), *Aetobatus*, and some species of *Myliobatis* (Garman, 1913: plate 73), but not in any other stingray or outgroup taxon. This fusion is considered the derived state (14[1])(Nishida, 1990).

*15.* $^{(0.5)}$ *Thickening of the mandibular cartilage* near the symphysis, occurs in *Myliobatis* (Fig. 8A), *Aetomylaeus, Aetobatus*, and *Rhinoptera*, but not in any other stingray taxon. This thickening is considered the derived state (15[1])(Nishida, 1990).

*16.* $^{(1.0)}$ *A wing-like process on the mandibular cartilage* is found in *Myliobatis, Aetomyleus, Aetobatus, Rhinoptera, Mobula*, and *Manta*, but not in any other stingray taxon. The presence of the wing-like process is considered derived (16[1])(Nishida, 1990).

*17.* $^{(0.5)}$ *Teeth* in Stingrays can be organized into two categories. In *Myliobatis, Aetomylaeus, Aetobatus*, and *Rhinoptera*, the teeth of the upper and lower jaws are arranged in flattened bands to create a broad, uniform surface. In all other stingrays, and the outgroups, teeth are small, usually cusped, and arranged in multiple strips along the jaws. The arrangement of teeth into flattened, pavement-like bands is considered the derived character state (17[1])(Nishida, 1990).

### *Branchial arches*

The branchial arches consist of a series of cartilages that provide support for the gills and pharynx. A central medial plate lies dorsal to the heart and afferent arteries, and connects to 5 (or 6 for *Hexatrygon*) pairs of ceratobranchials (Fig. 7). The medial plate in stingrays is probably an ontogenetic composite of the second to fifth hypobranchials and basibranchials (Miyake & McEachran, 1991). Anteriorly, in benthic stingrays, the pseudohyal is connected to the first ceratobranchial and projects laterally. The first hypobranchial articulates with the pseudohyal and extends anteriorly to articulate with the basihyal. Posteriorly, the first four ceratobranchials project laterally and dorsally to articulate with the epibranchials; the fifth ceratobranchial articulates firmly with the anteromedial aspect of the scapulocoracoid. The first three epibranchials articulate medially with pharyngobranchials, which in turn articulate with the occipital region of the neurocranium and the anterior end of the first synarcual. The fourth epibranchial articulates directly to the scapulocoracoid above the attachment of the fifth ceratobranchial (Miyake & McEachran, 1991; Nishida, 1990).

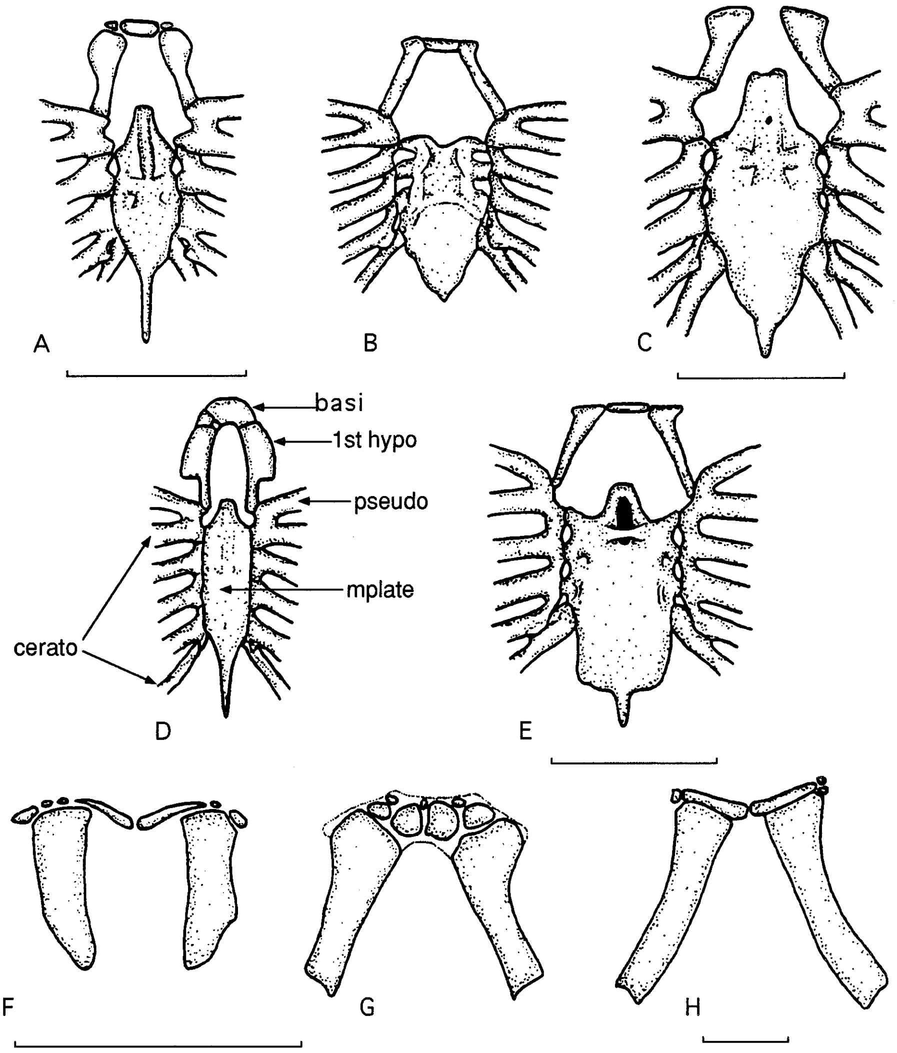

Figure 7. A–E. Ventral view of stingray ventral branchial arch cartilages. A, *Urobatis halleri* (FMNH 42601); B, *Plesiobatis daviesi* (after Miyake & McEachran, 1991: fig. 9a); C, *Urotrygon sp.* (FMNH 93737); D, *Taeniura lymma* (after Garman, 1913: plate 71); E, *Gymnura micrura* (FMNH 89990). F–H. Ventral view of stingray basihyal and hypobranchial cartilages. F, *Potamotrygon motoro* (FMNH 94503); G, *Plesiotrygon iwamae* (after Rosa *et al.*, 1987, fig. 8b); H, *Plesiotrygon iwamae* (ROM 65366). H traced from radiograph. Scale bar = 10 mm. Abbreviations – basi: basihyal; 1st hypo: 1st hypobranchial; pseudo: pseudohyal; mplate: medial plate; cerato: ceratobranchials.

*Bridges over the aorta and afferent branchial vessels* are formed by projections from the medial plate in a number of stingray taxa, including *Plesiobatis, Hexatrygon, Urobatis, Urotrygon, Urolophus, Gymnura*, and various potamotrygonids (Miyake & McEachran, 1991). In other stingrays (dasyatids and pelagic myliobatoids), these structures are absent. Presumably, the presence of bridges should be considered the plesiomorphic state, since they are found in the primary outgroups; the derived state would thus be shared by dasyatids and pelagic stingrays. However, the presence and extent of the medial plate bridges vary within species, thus this condition is not considered suitable for phylogenetic analysis.

*18.* $^{(0.05)}$ *The basihyal* is a thin cartilage that lies anterior and perpendicular to the long axis of the medial plate, and articulates laterally with the first hypobranchial. It is variably present (segmented or unsegmented) or absent in the taxa examined. In *Plesiobatis* (Nishida, 1990: fig. 26), *Hexatrygon* (Heemstra & Smith, 1980: fig. 10), *Urolophus* (Miyake & McEachran, 1991: fig. 9), *Gymnura* (Fig. 7E), and *Aetoplatea*, the basihyal is present and unsegmented. This is considered the plesiomorphic state for stingrays. In *Urotrygon* (Fig. 7C), *Aetobatus*, *Rhinoptera*, *Mobula* and *Manta* (Nishida, 1990: fig. 28), the basihyal is absent, and this is considered a derived state (18[2]) (Nishida, 1990). *Myliobatis* has a small cartilaginous element anterior to the medial plate that may represent either the reduced basihyal or first hypobranchial; *Myliobatis* is coded as having an unknown state for character 18. Finally, in all other taxa, including *Dasyatis, Himantura, Taeniura, Urobatis*, and the potamotrygonid genera, the basihyal is present and segmented into a variable number of distinct components (Fig. 7A, D and F–H). The degree of segmentation of the basihyal may vary dramatically within species (compare Fig. 7G,H) and is not incorporated into the coding scheme. The segmentation of the basihyal, however, is considered a derived state (18[1]), and is ordered as intermediate between the plesiomorphic, unsegmented basihyal presence, 18[0], and the more derived absence 18[2].

*Fusion of the ceratobranchials* to one another, and to the pseudohyal varies between different stingray taxa. Nishida (1990) suggested that the fusion of the pseudohyal to the first ceratobranchial was an apomorphy for stingrays. However, Miyake & McEachran (1991) pointed out that the lack of fusion between these two elements had been reported for *Hexatrygon bickelli* (Heemstra & Smith, 1980), *Dasyatis kuhlii* (Müller & Henle), and *Taeniura forksall*. The most common condition within stingrays appears to be the fusion of the pseudohyal to the first ceratobranchial, and the lack of fusion between the rest of the ceratobranchials (Fig. 7A–D). This is the state observed in most *Urolophus*, *Urobatis*, and *Urotrygon* species, some dasyatids (Miyake & McEachran, 1991: figs. 8,9), and *Plesiotrygon iwamae* (Rosa *et al*., 1987: fig. 8b). In *Paratrygon aireba* (Rosa, 1985), *Dasyatis americana* Hildebrand & Schroeder, and *Urotrygon micropthalmum* (Miyake & McEachran, 1991: figs. 8,9) the pseudohyal and the first two ceratobranchials are fused together. *Potamotrygon constellata, P. magdallena* (Valenciennes), *P. motoro*, and *P. yepezi* Castex & Castello (Rosa, 1985), *Dasyatis guttata*, and *D. zugei* (Müller & Henle) (Garman, 1913: plate 71) and *Gymnura, Myliobatis*, and *Mobula* (Nishida, 1990: fig. 26, 28) have the pseudohyal fused to the first four ceratobranchials. The plesiomorphic state for this character is probably the lack of fusion between all elements (ceratobranchials and the pseudohyal are unfused in outgroups to stingrays). However, the distribution of potential apomorphic states is complex, with fusion patterns apparently varying within genera and species;

difficulty in determining appropriate states for several taxa precluded the use of this potential character.

### *First synarcual cartilage*

The first synarcuum of stingrays consists of an elongate tube-like cartilage composed of several fused vertebrae (Fig. 8). Anteriorly, it articulates with the neurocranium, and posteriorly, it articulates with the intersynarcual vertebrae in the region of the pectoral girdle. Dorsally, a medial crest projects from the synarcuum and runs from its anterior end to the posterior region where the suprascapular has been fused. Lateral stays project dorsolaterally and extend posteriorly along the ventral aspect of the synarcuum. Posteriorly, the scapulocoracoid articulates via two large facets to the lateral aspect of the first synarcuum, and anteriorly, the pharyngobranchials articulate via small facets to the lateral base of the first synarcuum. Spinal nerve foramina penetrate the lateral aspect of the synarcuum along its length.

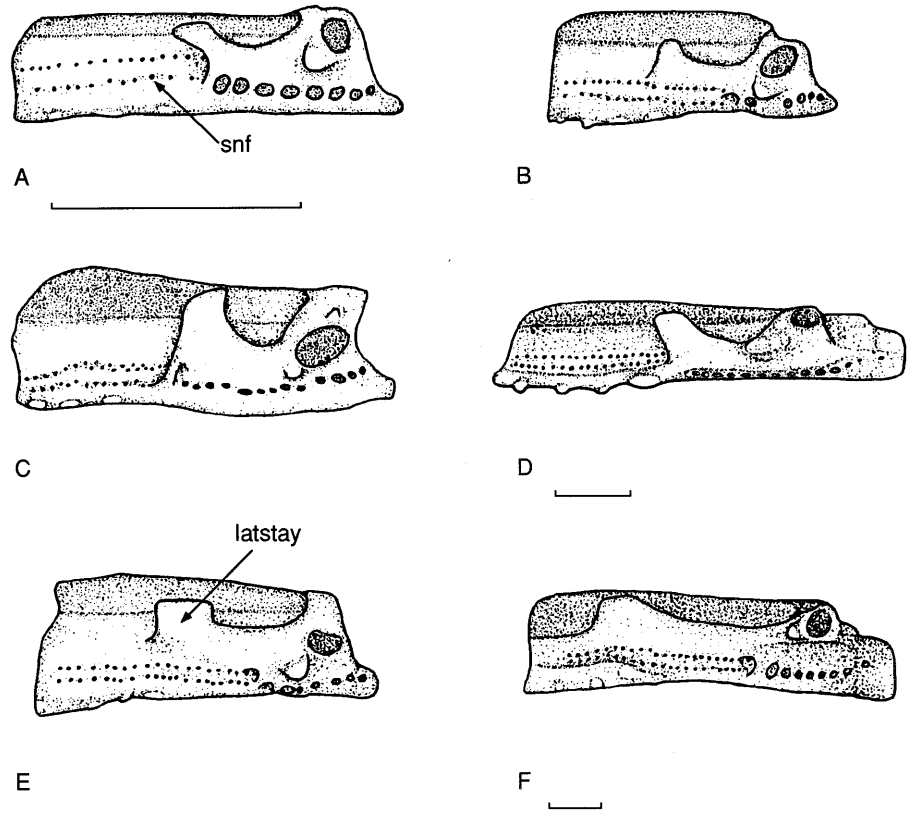

Figure 8. Lateral view of the first synarcual cartilage of stingrays. A, *Urobatis halleri* (FMNH 42601); B, *Urobatis jamaicensis* (AMNH 30385); C, *Dasyatis americana* (AMNH 30607); D, *Paratrygon aireba* (ROM 66843); E, *Potamotrygon sp.* (AMNH 38138 SW); F, *Plesiotrygon iwamae* (ROM 65366). Scale bar = 10 mm. Abbreviations – snf: spinal nerve foramina; latstay: lateral stay.

*19.* $^{(0.5)}$ *The base of the lateral stay* is pierced to a variable extent by foramina for the passage of spinal nerves. In some taxa, including *Urobatis jamaicensis* (Fig. 8B), *Potamotrygon* sp. (Fig. 8E), *Potamotrygon yepezi* (Nishida, 1990: fig. 38c), *P. motoro* and *P. constellata* (Garman, 1913: plate 55), and *Plesiotrygon iwamae* (Fig. 8F), the anterior base of the lateral stay joins the synarcuum dorsal to the spinal nerve foramina. It is not pierced by foramina except posteriorly. In all other taxa examined, including the outgroup *Plesiobatis* (Nishida, 1990: fig. 38a), the anterior base of the lateral stay joins the synarcuum ventral to the spinal nerve foramina, and thus the lateral stay is pierced along its entire length by larger foramina (Fig. 8A,C,D). The connection of the anterior base of the lateral stay above the spinal nerve foramina is considered a derived state (19[1]). In *Aetobatus*, *Rhinoptera*, and *Mobula*, the base of the lateral stay is considerably reduced, precluding assessment of the condition; these taxa were coded as having unknown states for character 19.

In all secondary outgroups diagrammed by Garman (1913) and Nishida (1990), the base of the lateral stay is not penetrated by foramina. The presence of foramina in the lateral stay may thus be an apomorphy for stingrays, but more outgroups need to be examined before it is accepted.

*20.* $^{(1.0)}$ *The lateral stay* is present in most stingrays, but appears to be absent or considerably reduced in *Gymnura* (Nishida, 1990: fig. 38). This loss or reduction is considered the derived state (20[1]). The lack of a lateral stay in *Mobula* may be related to the absence of the base for the lateral stay and, therefore, is not automatically considered homologous with the condition seen in *Gymnura*; the character is coded as inapplicable to *Mobula*. Note also that Nishida (1990: fig. 39) considers a lateral stay present in *Rhinoptera*; however, it projects from the suprascapular process and may not be homologous with the lateral stays in other stingrays (which project from the base of the synarcual cartilage). Further investigation of synarcual modifications in pelagic myliobatoid stingrays should provide additional characters (e.g. the reduction of the base of the lateral stay is probably apomorphic for *Aetobatus, Rhinoptera, Mobula*, and *Manta*).

*21.* $^{(1.0)}$ *The degree of lateral projection of the lateral stay* varies among stingrays. In *Urotrygon* species and Pacific coast *Urobatis* species, the lateral stay is thin and arcs laterally outwards. In all other stingray taxa examined, including *Urobatis jamaicensis*, the lateral stay projects anteriorly and only slightly laterally. The lateral arc of the lateral stay is considered the derived condition (21[1]); however, more taxa need to be examined (particularly *Urolophus* species) to confirm the validity of this character.

*22.* $^{(1.0)}$ *The width of the first synarcuum* is approximately equal to its height in most stingrays. In *Paratrygon*, however, the synarcuum is considerably wider than it is tall (Rosa, 1985), and this is considered the derived state (22[1]) (Rosa, 1985).

### *Scapulocoracoid and pectoral fin*

The scapulocoracoid consists of several fused anatomical elements. The coracoid is a strong bar that lies ventral and perpendicular to the long axis of the body. Laterally, it curves dorsally and supports the pectoral fin and a scapular process. The scapular process articulates dorsally with the suprascapular cartilage (which is fused to the first synarcuum). The lateral aspect of the scapulocoracoid has condyles for articulation with the propterygium, the mesopterygium, and the metapterygium, and has a number of foramina for the passage of nerves and attachment of muscles (Fig. 9).

*Scapulocoracoid foramina* vary in number in different stingray taxa. Most species, including the outgroup *Plesiobatis*, have four (anterodorsal, anteroventral, posterodorsal, posteroventral). However, according to Nishida (1990), in *Urolophus, Urobatis,* and *Urotrygon*, the posterodorsal foramen is absent. For this study, a minute posterodorsal foramen was observed in *Urotrygon rogersi* (Fig. 9A), and *Urobatis jamaicensis*. This character may therefore vary within OTU's, and was not used in this analysis.

*23.* $^{(0.5)}$ *The scapular process* contains laterally either a fossa or a foramen (Fig. 9A–C), which passes through to the medial side in most stingray taxa (Miyake, 1988; Nishida, 1990). In the outgroup *Plesiobatis*, and in *Urolophus* (Miyake, 1988), *Potamotrygon, Paratrygon,* and *Plesiotrygon* (Fig 9D–F), neither a fossa nor a foramen is present. The fossa/foramen is also missing in *Gymnura* and *Aetoplatea*, however in these genera, its absence may be correlated with the huge expansion of the anterodorsal foramen. The presence of fossa or foramina on the scapular process is considered the derived state (23[1]). The absence in *Gymnura* and *Aetoplatea* may be a consequence of other morphological changes, and is thus coded as inapplicable.

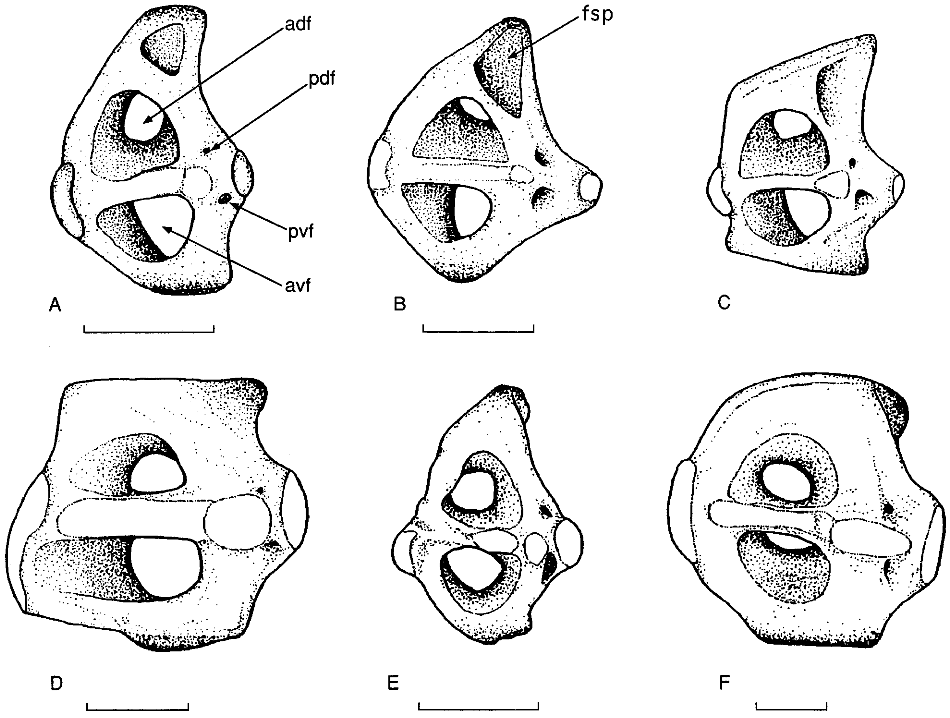

Figure 9. Lateral view of stingray scapulocoracoids. A, *Urotrygon rogersi* (TCWC uncat.); B, *Dasyatis sabina* (TCWC 2591.1); C, *Taeniura lymma* (after Miyake, 1988: Fig 91j); D, *Paratrygon aireba* (ROM 66843); E, *Potamotrygon yepezi* (after Nishida, 1990: fig. 30c); F, *Plesiotrygon iwamae* (ROM 65366). Scale bar = 10 mm. Abbreviations – adf: anterodorsal foramen; pdf: posterodorsal foramen; pvf: posteroventral foramen; avf: anteroventral foramen; fsp: foramen of the scapular process.

*24.* (1.0) *Epibranchial and ceratobranchial articulations* are located on the anteromedial aspect of the scapulocoracoid. Nishida (1990) reports that in *Myliobatis, Aetobatus, Rhinoptera, Mobula,* and *Manta,* these articulations are distinctly separated. In all other taxa, including outgroups, the condyles are adjacent. Separation of the articulations for the epibranchial and ceratobranchial cartilages is the derived state (24[1]) (Nishida, 1990).

*25.* (1.0) *The pectoral propterygium* arcs anteriorly to articulate with the antorbital cartilage from the nasal capsule. In some taxa, the first segmentation of the propterygium occurs before, or at, the middle of the nasal capsule. This condition was observed in *Urolophus, Urotrygon, Urobatis, Plesiotrygon, Paratrygon, Potamotrygon, Taeniura,* and amphi-American *Himantura* (Fig. 10A,C,D,E; Lovejoy, 1993: fig. 13a,c,d). In other taxa, such as *Dasyatis,* Indo-West Pacific *Himantura, Gymnura,* and *Myliobatis,* segmentation occurs at the anterior tip or beyond the nasal capsule (Fig. 10B). *Plesiobatis* appears to possess the latter condition, while the propterygium of *Hexatrygon bickelli* is first segmented approximately 3/4 of the way along the nasal capsule (Heemstra & Smith, 1980: fig.10). Secondary outgroups show propterygia segmented much earlier (at a level with the occipital region of the neurocranium). The outgroups are thus considered ambiguous with respect to polarization, and are coded as unknown. *Rhinoptera, Mobula,* and *Manta* could not be assessed for this character due to extensive modifications of the propterygia and were also coded as unknown.

*26.* (1.0) *Articulations between the propterygium and various pectoral cartilages* vary to some extent in stingray taxa. As the propterygium curves posteriorly, it articulates first with the procondyle and next with the lateral aspect of the scapulocoracoid anterior to the

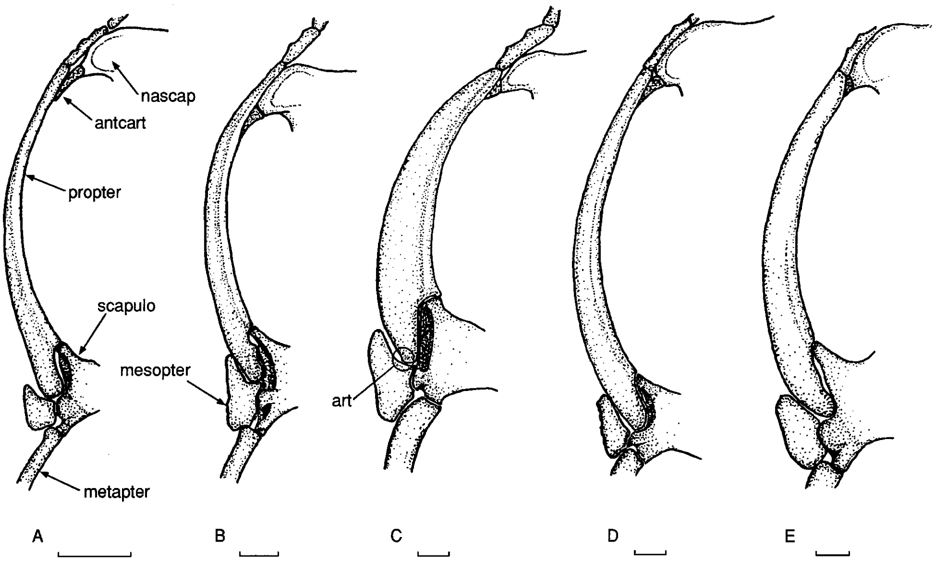

Figure 10. Ventral view of propterygium and its articulation with the neurocranium and scapulocoracoid. A, *Urobatis concentricus* (Osburn & Nichols) (ROM 66839); B, *Dasyatis guttata* (ROM 25096); C, *Paratrygon aireba* (UMMZ 211261); D, *Potamotrygon motoro* (UMMZ 207766); E, *Plesiotrygon iwamae* (ROM 65366). Scale bar = 10 mm. Abbreviations – nascap: nasal capsule; antcart: antorbital cartilage; propter: propterygium; scapulo: scapulocoracoid; metapter: metapterygium; mesopter: mesopterygium; art: articulation between propterygium and mesopterygium.

mesocondyle. In most stingrays, including the outgroups *Hexatrygon* (Heemstra & Smith, 1980: fig. 11) and *Plesiobatis* (Nishida, 1990: fig. 30a) the posterior tip of the propterygium also articulates with the anteromedial aspect of the mesopterygium (Fig. 10A–C). However, in all *Potamotrygon* species examined, and in *Plesiotrygon iwamae*, this articulation is absent, possibly because of lateral expansion of the meta- and mesocondylar region of the scapulocoracoid (Fig. 10D,E). This absence of an articulation between the anterior tip of the propterygium and the mesopterygium is considered the derived state (26[1]).

*27.* $^{(1.0)}$ *The mesopterygium,* in most stingrays, is a single element that articulates medially with the scapulocoracoid and laterally with pectoral-fin radials. In *Gymnura*, and *Myliobatis*, the mesopterygium consists of several distinct components, all of which articulate with the scapulocoracoid (Nishida, 1990: fig. 31 A,B,C). The latter condition is not exhibited by outgroup taxa, and is considered a derived state (27[1]). In *Aetobatus*, *Rhinoptera*, and *Mobula*, the mesopterygium appears to be absent or fused with the scapulocoracoid, allowing the fin radials to articulate more directly with the pectoral girdle (Garman, 1913: plate 73–75). This condition is considered another derived state (27[2]). An ordering hypothesis for this character is based on the progressive fragmentation and disappearance (or consolidation with the scapulocoracoid) of the mesopterygium, and considers state 27[1] the intermediate between 27[0] and 27[2].

*28.* $^{(1.0)}$ *The cephalic lobes*, composed of the rostral pectoral radials, and separated from the rest of the pectoral fin, are found only in *Aetobus, Aetomylaeus, Rhinoptera, Mobula,* and *Manta* (Nishida, 1990). The presence of cephalic lobes is considered derived (28[1]) (Nishida, 1990).

### *Pelvic girdle*

The pelvic girdle is relatively simple in stingrays, consisting of a puboischiadic bar with a number of processes.

*29.* $^{(0.5)}$ *The prepelvic process* extends anteriorly from the medial aspect of puboishiadic bar. In most stingrays, including the outgroup taxa *Hexatrygon* and *Plesiobatis*, the prepelvic process is moderately to minimally developed (see Fig. 11 A–E). However, in *Potamotrygon, Paratrygon, Plesiotrygon*, and the pelagic stingrays *Rhinoptera* and *Mobula*, the prepelvic process is greatly elongated (Fig. 11F; Garman, 1913: plate 54). This extension of the prepelvic process is considered the derived state (29[1]). Boeseman (1948: 32) reported, for *Himantura schmardae*, "…the occurrence of a well developed median triangular flat point or process, directed rostrad, on the pelvis… and seems somewhat too well developed for the genus *Dasyatis*". However, examination of specimens of amphi-American *Himantura* for this study show the prepelvic process to be within the range of other dasyatids (Fig. 11E), an opinion shared by Bigelow & Schroeder (1953).

*30.* $^{(1.0)}$ *The puboischiadic bar* is arched anteriorly in all stingrays examined. In *Aetobatus*, *Rhinoptera*, and *Mobula*, the puboishiadic bar is extremely arched, forming a distinct horseshoe shape (Garman, 1913: plate 54). In all other stingrays examined, including the outgroups, comparable arching is not observed. The extreme arching of the puboishchiadic bar is therefore considered the derived state (30[1]).

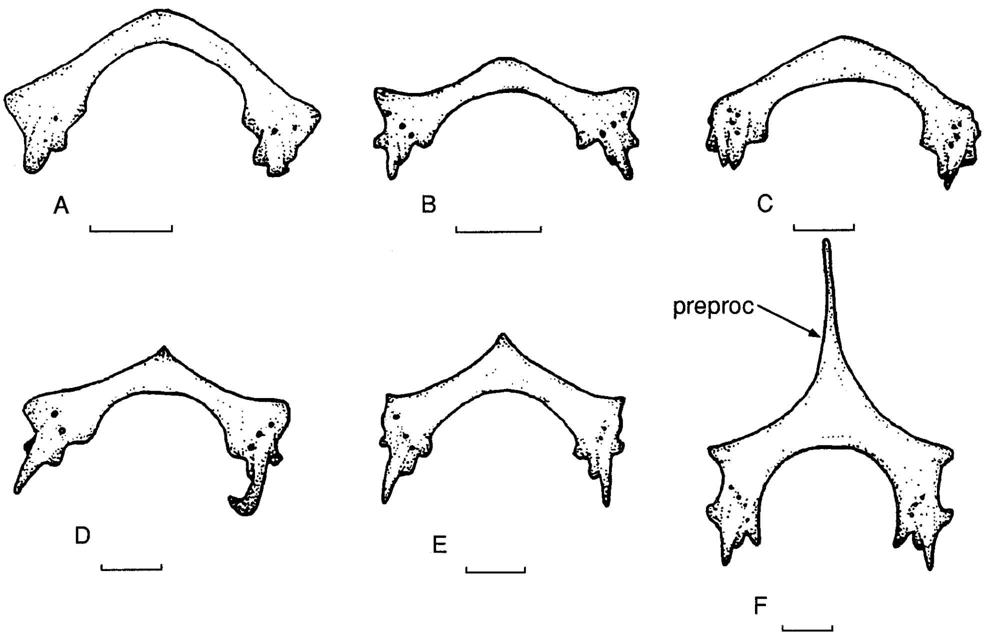

Figure 11. Ventral view of stingray pelvic girdles of. A, *Plesiobatis daviesi* (BPBM 30909); B, *Urobatis maculatus* (USNM 11951); C, *Dasyatis americana* (ANSP 103931); D, *Taeniura lymma* (ROM 39404); E, *Himantura schmardae* (ANSP 103478); F, *Paratrygon aireba* (UMMZ 211261). Scale bar = 10 mm. Abbreviation – preproc: prepelvic process.

### *Vertebrae*

An apomorphic character for stingrays is the presence of a second synarcuum of fused vertebrae, which lies posterior to the first synarcuum and several intersynarcual vertebrae. The second synarcuum is in turn followed by monospondylous vertebrae, and then several diplospondylous vertebrae.

*31.* $^{(1.0)}$ *Diplospondylous vertebrae* in most stingrays begin at the level of the pelvic girdle. In *Hexatryon*, *Plesiobatis*, and the more distant outgroups, and in *Urolophus*, *Urobatis*, and *Urotrygon*, these vertebrae continue to the tip of the tail (Fig. 12A,B). In other stingray taxa, diplospondylous vertebrae terminate in the region of the caudal spine; the rest of the tail is supported by an unsegmented, cartilaginous rod (Fig. 12C,D). The presence of this unsegmented rod is considered the derived state (31[1]).

Nishida (1990) initially proposed this character, however, he decided against using it in his analysis because he detected the plesiomorphic state (distinct vertebrae to the tip of the tail) in *Gymnura micrura* (Bloch & Schneider). A radiograph of *G. micrura* for this study appears to confirm Nishida's observation; however, rather than rejecting the entire character, *Gymnura* was coded as having an unknown state. This coding decision was based on Nishida's uncertainty of the appropriate state for *Gymnura*, despite his examination of several species.

*32.* $^{(0.5)}$ *The dorsal fin* is missing in most stingray taxa, but is present in several pelagic stingray genera including: *Aetoplatea*, *Myliobatis*, *Aetobatus*, *Aetomylaeus*, *Rhinoptera*, *Mobula*, and *Manta* (Nishida, 1990). A small dorsal fin is also present in some *Urolophus* species (McKay, 1966; Dixon, 1969; Last & Gomon, 1987). Although dorsal fins are

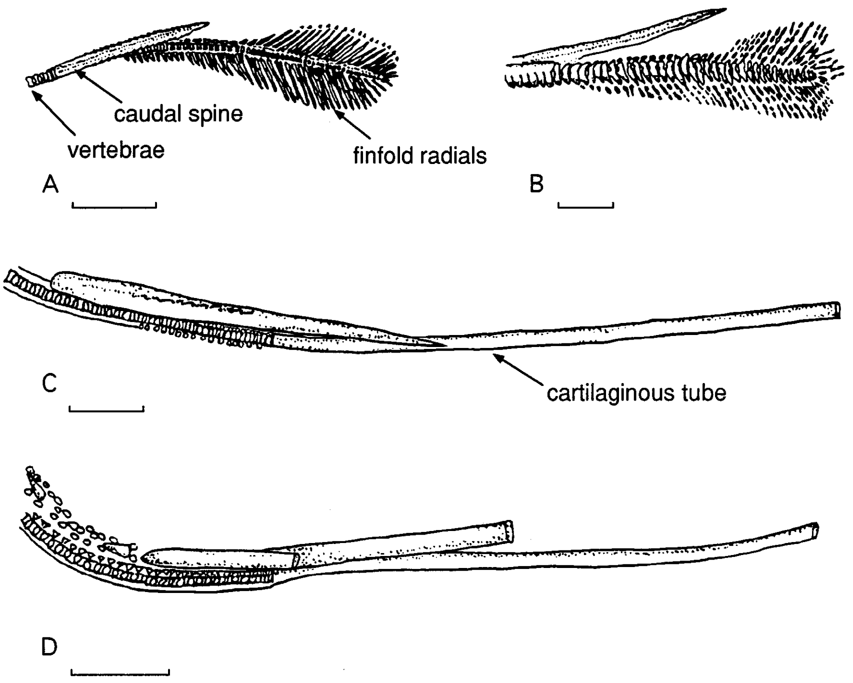

Figure 12. Posteriormost distinguishable vertebrae of stingrays. A, *Urobatis jamaicensis* (ROM 28276); B, *Urolophus testaceus* (Müller & Henle) (after Nishida, 1990: fig. 41); C, *Potamotrygon motoro* (ROM 26182); D, *Himantura jenkinsii* (ROM 23011). Scale bar = 10 mm.

present in most secondary outgroups, the presence of a dorsal fin is considered a derived character (32[1]) within stingrays, because of its absence in *Hexatrygon* and *Plesiobatis. Urolophus* is coded as polymorphic.

*33.* $^{(1.0)}$ *The caudal fin* is supported by distinct cartilaginous radials, and is present in *Urolophus, Urobatis,* and *Urotrygon* (Fig. 12A,B) Radials are also seen in *Hexatrygon* (Heemstra & Smith, 1980:1), but not in *Plesiobatis* (although "neural and haemal arch components are fringed with a few small radials" [Miyake, 1988: 239]). The absence of cartilaginous finfold radials in all other stingrays is considered the derived state (33[1]).

### *Ventral cranial musculature*

The ventral muscles of the cranial region are involved with movements of the mandibular and hyomandibular arches. Terminology here follows the most recent and complete study by Miyake *et al.* (1992a), and includes, in most benthic stingrays the following paired muscles: the depressor rostri, which originates as a flat sheet from the strong superficial fascia covering the gill area, extends anterolaterally to become aponeurotic before the adductor mandibulae, and inserts on the lateral border of the nasal capsule and the adjacent propterygium; the depressor mandibulae, which originates ventrally from the superficial fascia and dorsally from the depressor hyomandibulae, and inserts on Meckel's cartilage; the depressor hyomandibulae, which originates ventromedially from its antimere and/or the superficial fascia, and extends dorsolaterally to insert on the hyomandibular; the coracomandibularis, a long cylindrical muscle which originates near the coracoarcualis between the gill arches, extends anteriorly, and inserts on the posteroventral aspect of Meckel's cartilage; the coracohyoideus, a short cylindrical muscle which originates from the basihyal and hypobranchial cartilages, and extends posteriorly to insert onto the wall of the first gill arch; and the coracohyomandibularis, which consists of two major components: the anteriormost originates medially, dorsal to the hypobranchial cartilages, while the posteriormost originates from fascia near the heart, but both components join to extend laterally where they insert on the hyomandibular. More detailed descriptions of these and other cranial muscles, particularly the adductor mandibulae complex, are available in Miyake (1988) and Miyake *et al.* (1992a).

*34.* $^{(1.0)}$ *The depressor rostri* originates from variable positions in the superficial ventral fascia; however, these differences were impossible to quantify, and could not be considered in the analysis. Nishida (1990) noticed that the aponeurosis of the anterior aspect of the depressor rostri does not occur in *Gymnura, Aetomylaeus*, or *Manta*. In these taxa, the muscle inserts directly on the pectoral proterygium, rather than by a tendon. This direct insertion is considered the derived state (34[1]) (Nishida, 1990).

*The depressor mandibulae* is variously shaped in stingray taxa (and absent from *Hexatrygon* [Nishida, 1990]). In most species examined, it is somewhat triangular, with a broad origin, and a narrow insertion. In *Potamotrygon orbignyi*, it is thin and strap-like

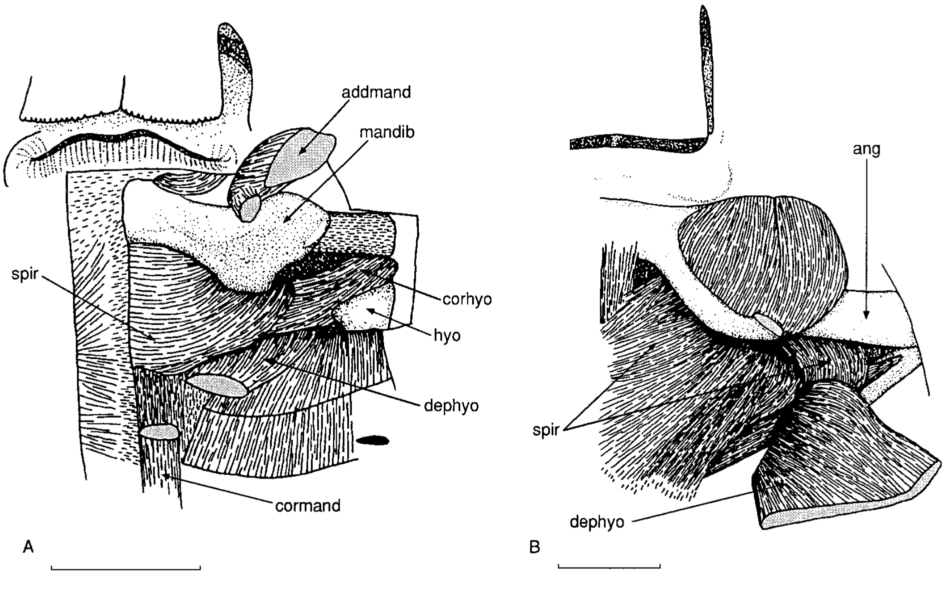

Figure 13. Ventral mandibular musculature of stingrays. A, *Taeniura lymma* (ROM 39404); B, *Potamotrygon orbignyi* (Castelnau) (ROM 26182) In A&B, the depressor rostri and depressor mandibulate have been removed, and the depressor hyomandibulae is folded back. Scale bar = 10 mm. Abbreviations – addmand: adductor mandibulae; mandib: mandibular cartilage; corhyo: coracohyomandibularis; hyo: hyomandibular; dephyo: depressor hyomandibulae; cormand: coracomandibularis; spir: spiracularis; ang: angular cartilage.

in shape, with a narrow insertion and origin. The latter shape, however, was not evident in *Potamotrygon magdalenae* (Miyake *et al*. 1992b: fig. 15). In *Dasyatis sabina*, some fibres from the depressor mandibulae were intermixed ventromedially with the depressor hyomandibular, and laterally with the constrictor hyoideus ventralis, however this condition was not observed in *Dasyatis longus*. Neither of these patterns were considered suitable for character coding.

*35.* $^{(1.0)}$. *The adductor mandibulae* consists of a complex of muscles closely associated with the palatoquadrate and Meckel's cartilage. In the pelagic stingrays *Myliobatis*, *Aetomylaeus, Aetobatus*, *Rhinoptera*, *Mobula*, and *Manta*, a component of the adductor mandibulae extends posteromedially, dorsal to the coracomandibularis, to join its antimere. Nishida (1990) considered this muscle to be the depressor mandibulae; however, the interpretation of Miyake (1988) and Miyake *et al.* (1992a) that it represents part of the adductor mandibulae is followed here. In either case, the distinctive extension of this muscle is not observed in any other stingray taxon, and is considered the derived state (35[1]) (Nishida, 1990).

*36.* $^{(0.5)}$ *The spiracularis* in most benthic stingrays originates dorsally from the otic region and extends ventrally along the prespiracular wall. It then divides into two components, one of which inserts on the hyomandibular, the other proceeds deeper to insert on the posterior surface of Meckel's cartilage. In *Taeniura lymma*, the deeper component of the spiracularis sends fibres beyond Meckel's cartilage to insert near the midline dorsal to the paired coracomandibularis (Fig. 13A). This extension of the spiracularis is considered a derived state (36[1]). In *Potamotrygon*, *Plesiotrygon iwamae*, and amphi-American *Himantura*, a component of the spiracularis also extends beyond Meckel's cartilage towards the midline (Fig. 13B). However, in these taxa the spiracularis inserts ventral to the coracomandibularis. The spiracularis thus lies directly dorsal to the depressor hyomandibulae. Additionally in these taxa, another component of the spiracularis extends ventral to the anterolateral edge of the depressor hyomandibulae to run posteromedially where it inserts near the midline. This component is fused to the posterolateral aspect of the depressor mandibulae (Fig. 14A,B). This complex condition of the spiracularis is considered another derived state (36[2]). An ordered transformation series can thus be constructed, based on the consecutive elaboration of the spiracularis: the condition seen in *Taeniura* is considered intermediate to the derived condition in *Potamotrygon, Plesiotrygon*, and amphi-American *Himantura*.

Miyake (1988) and Miyake *et al.* (1992a) observed the extension of the spiracularis in *Potamotrygon magdalena* and *Taeniura lymma*; however, they did not mention the differences between derived states discussed here. Kesteven (1942) observed an extension of the spiracularis similar to that in *Taeniura lymma* in *Dasyatis brevicaudatus* (Hutton), with fibres inserting into a median raphe (and onto the basihyal and first hypobranchial) presumably dorsal to the coracomandibularis. *Dasyatis brevicaudatus* could not be incorporated in the present study, however, Kesteven's finding suggests that examination of the spiracularis in other dasyatids could yield interesting results. Specimens of *Dasyatis longus, D. brevis* (Garman), *D. sabina*, and *D. say* observed for this study all showed the plesiomorphic condition of the spiracularis, thus *Dasyatis* was coded as having state 36[0].

*37.* $^{(1.0)}$ *The coracohyoideus* is a short cylindrical muscle in most benthic stingrays. Nishida (1990) described the coracohyoideus as connected at the midline in

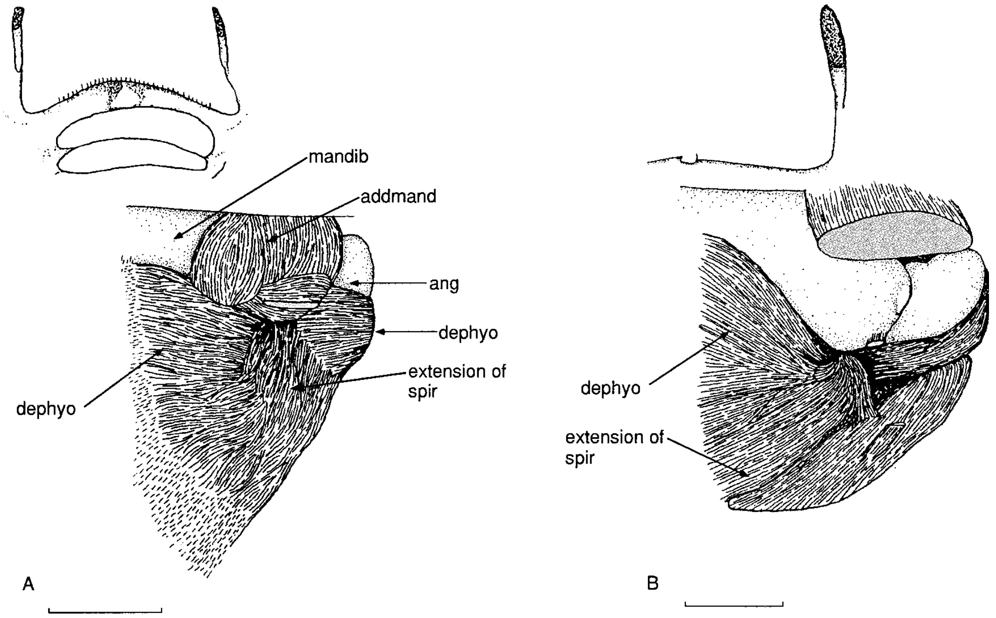

Figure 14. Ventral mandibular musculature of stingrays. A, *Plesiotrygon iwamae* (ROM 65366); B, *Himantura pacifica* (ROM 66838). In A&B the depressor rostri and depressor mandibulae have been removed. Scale bar = 10 mm. For abbreviations, see Fig.13.

*Myliobatis, Aetomylaeus, Aetobatus, Rhinoptera, Mobula,* and *Manta*. Miyake (1988) and Miyake *et al.* (1992) consider the coracohyoideus to be absent in these taxa (they describe a 'y' muscle in its place). Nishida's (1990) terminology is followed here, and the midline connection of the coracohyoideus, absent in all other stingrays, is considered the derived state (37[1]) (Nishida, 1990).

In the specimens of *Uroytrygon* and *Urobatis* examined, the coracohyoideus appears to consist of two distinct components, a longer and thicker, medial one which extends from the basihyal (or anterior edge of the hypobrancial in *Urotrygon*), and a shorter lateral segment which extends from the hypobranchial (to the more medial aspect in *Urotrygon*). This 'bilobed' aspect of the coracohyoideus may be a potential apomorphy for these taxa, however, partially subdivided coracohyoideii were seen in some other taxa, complicating coding attempts. Observations of more taxa are necessary before this character is considered.

## *Physiology*

Physiological characters are yet to be exploited as a source of phylogenetic information in stingrays (except by Rosa, 1985), although some authors have made preliminary forays in this direction.

*38.* $^{(1.0)}$ *Urea retention* is evident in most stingray taxa, including the freshwater species *Dasyatis garouaensis* (Stauch & Blanc) (Thorson & Watson, 1975) and the outgroups *Hexatrygon* and *Plesiobatis*. However, in *Paratrygon, Potamotrygon,* and *Plesiotrygon*, urea is not retained in the blood, and this condition is considered a derived state (38[1]) (Rosa, 1985).

### *Embryology*

Stingrays are considered aplacentally viviparous (Breder & Rosen, 1966; Wourms, 1977; Hamlett, 1989) with embryos initially receiving nutrients from a yolk (lecithotrophic), and subsequently from the uterine wall. Trophonemata (uterine villi) exude nutrient-rich 'uterine milk' (embryotrophic material) for the embryo. Some of these trophonemata enter the spiracles of the embryo.

*39.* $^{(1.0)}$ *A small spiracular lobe or tentacle.* is found on the inner margin of the spiracle in embryonic *Urobatis* and *Urotrygon* (Bigelow & Schroeder,1953; Miyake, 1988), but is resorbed before or shortly after birth. La-Marca (1963) investigated the structural significance of the lobe in *Urobatis jamaicensis*, concluding that it may serve to direct trophonemata into the spiracle. He also detected a muscular attachment of the lobe to the pharynx, which might serve to generate a pumping of 'uterine milk'. The embryonic spiracular lobe is missing in all other taxa, including primary and secondary outgroups, thus its presence in *Urobatis* and *Urotrygon* is considered derived (39[1]) (Miyake, 1988).

*The ovaries and uteri* in mature female stingrays are variably developed. In species of *Dasyatis* and *Himantura*, usually only the left ovary and uterus is functional (see Snelson *et al*. [1988] for *D. sabina*; Snelson, Williams-Hooper & Schmid [1989] for *D. say*; Thorson [1983] for *D. guttata*; and Wood-Mason & Alcock [1892] for *Himantura bleekeri* [Blyth]). A similar condition appears to be present in *Myliobatis* (Wood-Mason & Alcock, 1892); however, only a few specimens were examined. In *Urobatis, Urotrygon* and *Potamotrygon*, the right ovary is inoperative, but both oviducts and uteri are functional (though not equally so); see Babel (1967) for *Urobatis halleri*, and Thorson, Langhammer, & Oetinger (1983) for *Potamotrygon motoro* and *P. constellata*. A specimen of *Urotrygon nana* Miyake & McEachran (ROM 66837) collected by the author off the Pacific coast of Costa Rica had two embryos in the left, and one in the right uterus. *Gymnura* also appears to have two functional uteri (Wood-Mason & Alcock, 1891). Unfortunately, the reproductive systems of *Hexatrygon* and *Plesiobatis* are unavailable to polarize these potential characters. In skates, the right ovary and both oviducts are functional (Wourms, 1977) suggesting (1) that the functioning of the left ovary may be an apomorphy for stingrays and (2) that the non-functional right uterus may be a character of *Dasyatis* and Indo-West Pacific *Himantura*. Certainly, more data are necessary before such characters can be used confidently; however, the similarity discussed by Thorson (1983) between *Urobatis* and *Potamotrygon* in terms of uterine function is most probably the result of a shared plesiomorphic state (based on the analyses presented herein).

## PHYLOGENETIC ANALYSES

Analysis of the character matrix (Table 1) was performed using the branch and bound search algorithm of PAUP 3.1; the single cladogram resolved is shown in

TABLE 1. Character matrix for stingray genera. In PAUP analysis: '?' (unknown state) and 'i' (inapplicable character) coded as missing data (?); '&' coded as polymorphic (0,1) – see text.

| | | | | |
|---|---|---|---|---|
| Hexatrygon / Plesiobatis | 0 0 0 0 0 0 0 0 0 & | 0 0 0 0 0 0 0 0 0 0 | 0 0 0 0 ? 0 0 0 0 0 | 0 0 0 0 0 0 0 0 0 0 |
| Urolophus | 0 0 0 0 1 0 1 0 0 0 | 0 0 1 0 0 0 0 0 0 0 | 0 0 0 0 0 0 0 0 0 0 | 0 & 0 0 0 0 0 0 0 0 |
| Atlantic Urobatis | 0 1 0 0 1 0 0 0 0 1 | 0 0 0 0 0 0 0 1 1 0 | 0 0 1 0 0 0 0 0 0 0 | 0 0 0 0 0 0 0 0 0 1 |
| Pacific Urobatis | 0 1 0 0 1 0 0 0 0 1 | 0 0 0 0 0 0 0 1 0 0 | 1 0 1 0 0 0 0 0 0 0 | 0 0 0 0 0 0 0 0 0 1 |
| Urotrygon | 0 1 0 0 1 0 0 0 0 1 | 0 0 0 0 0 0 0 2 0 0 | 1 0 1 0 0 0 0 0 0 0 | 0 0 0 0 0 0 0 0 0 1 |
| Paratrygon | 0 0 0 2 1 0 0 0 0 1 | 0 ? 0 0 0 0 0 1 0 0 | 0 1 0 0 0 0 0 0 1 0 | 1 0 1 0 0 0 0 0 1 0 |
| Potamotrygon | 0 0 0 1 1 0 0 0 0 1 | 0 2 0 0 0 0 0 1 1 0 | 0 0 0 0 0 1 0 0 1 0 | 1 0 1 0 0 2 0 1 0 |
| Plesiotrygon | 0 0 0 1 1 0 0 0 0 1 | 0 2 0 0 0 0 0 1 1 0 | 0 0 0 0 0 1 0 0 1 0 | 1 0 1 0 0 2 0 1 0 |
| amphi-Am. Himantura | 0 0 0 0 1 0 0 0 0 1 | 0 1 0 0 0 0 0 1 0 0 | 0 0 1 0 0 0 0 0 0 0 | 1 0 1 0 0 2 0 0 0 |
| Taeniura | 0 0 0 0 1 0 0 0 0 1 | 0 0 0 0 0 0 0 1 0 0 | 0 0 1 0 0 0 0 0 0 0 | 1 0 1 0 0 1 0 0 0 |
| IWP Himantura | 0 0 1 3 1 0 0 0 0 1 | 0 0 0 0 0 0 0 1 0 0 | 0 0 1 0 1 0 0 0 0 0 | 1 0 1 0 0 0 0 0 0 0 |
| Dasyatis 1 | 0 0 1 3 1 0 0 0 0 1 | 0 0 0 0 0 0 0 1 0 0 | 0 0 1 0 1 0 0 0 0 0 | 1 0 1 0 0 0 0 0 0 0 |
| Dasyatis 2 | 0 0 1 0 1 0 0 0 0 1 | 0 0 0 0 0 0 0 1 0 0 | 0 0 1 0 1 0 0 0 0 0 | 1 0 1 0 0 0 0 0 0 0 |
| Gymnura | 1 0 1 0 1 0 0 0 0 1 | 0 0 0 0 0 0 0 0 0 1 | 0 0 i 0 1 0 1 0 0 0 | ? 0 1 1 0 0 0 0 0 |
| Myliobatis | 0 0 2 0 1 0 0 0 0 1 | 1 0 1 & 1 1 1 ? 0 0 | 0 0 1 1 1 0 1 0 0 0 | 1 1 1 0 1 0 1 0 0 |
| Aetobatus | 0 0 2 0 1 0 0 0 1 0 | 0 0 1 1 1 1 1 2 ? 0 | 0 0 1 1 1 0 2 1 0 1 | 1 1 1 0 1 0 1 0 0 |
| Rhinoptera | 0 0 2 0 1 1 0 0 1 0 | 1 0 1 1 1 1 1 2 ? 0 | 0 0 1 1 ? 0 2 1 1 1 | 1 1 1 0 1 0 1 0 0 |
| Mobula | 0 0 2 0 1 1 0 1 1 0 | 0 0 1 1 0 1 0 2 ? i | 0 0 1 1 ? 0 2 1 1 1 | 1 1 1 0 1 0 1 0 0 |

Figure 15. Apomorphies and homoplasies based on ACCTRAN optimization are marked at each node; ACCTRAN optimization, which 'accelerates' character transformation early in the tree, was chosen because it prefers single origins and losses over multiple origins. For some characters, other equally parsimonious optimizations may exist, although these do not affect the topology of the tree. In the following text, only characters that change unambiguously on branches are discussed (unambiguous changes = changes that in all possible optimizations occur on a particular branch); homoplastic characters exhibiting reversal are indicated by a '$^{-}$', and multiple independent acquisitions by a '$^{+}$'.

Stingrays, to the exclusion of *Hexatrygon* and *Plesiobatis*, are grouped by character 5(1), extension of the nasal curtain to the anterior border of the mouth. The family Urolophidae is evidently paraphyletic — *Urolophus* falls out as the basal clade, subsequent to the outgroup, to all other stingrays. Characters 10(1)$^{+}$, lateral groove in the postorbital process; 18(1)$^{+-}$, fragmentation of the basihyal; and 23(1)$^{-}$, presence of foramen or fossa in the scapular process, support a stingray clade to the exclusion of *Urolophus* and the outgroups. *Urolophus* itself is diagnosed by 7(1), increase in size of the optic nerve foramen, and 13(1)$^{+}$, presence of a cartilaginous element functionally related to the anterior tip of hyomandibular. The next separable group consists of the amphi-American taxa, *Urobatis* and *Urotrygon*. This clade is supported by 2(1), dichotomous branching of the anterior subpleural tubules, and 39(1), presence of a spiracular tentacle. Within this group, the Caribbean species *Urobatis jamaicensis* (diagnosed by 19[1]$^{+}$, connection of base of the lateral stay above spinal nerve foramina) is basal to *Urotrygon* and Pacific *Urobatis*, a clade supported by 21(1) lateral arching of the lateral stay. *Urotrygon* is further diagnosed by 18(2),$^{+}$ absence of the basihyal cartilage.

A clade consisting of potamotrygonids, dasyatids, gymnura, and pelagic myliobatoid stingrays is supported by characters 31(1), presence of an unsegmented cartilaginous rod in tail, and 33(1), absence of cartilaginous fin-fold radials. Within this large clade, a monophyletic group composed of potamotrygonids, amphi-American *Himantura*, and *Taeniura* is defined by 36(1)$^{-}$, ventral extension of the spiracularis muscle. Potamotrygonids and their sister group, amphi-American

*Himantura*, are further supported by character 12(1), presence of multiple angular elements between the hyomandibular and mandibular cartilages.

The family Potamotrygonidae is monophyletic based on a number of characters: $23(0)^{-}$, absence of fossa or foramen in the scapular process; $29(1)^{+}$, extension of the prepelvic process; and 38(1), suppression of urea retention. Within the potamotrygonids, the basal taxon *Paratrygon* is diagnosed by characters 22(1), increased width of the first synarcuum; $36(0)^{-}$, loss of spiracular muscle extension. A group composed of *Plesiotrygon* and *Potamotrygon* is supported by characters $19(1)^{+}$, connection of base of the lateral stay above spinal nerve foramina, and 26(1), absence of an articulation between the propterygium and mesopterygium.

Dasyatids (excluding amphi-American *Himantura* and *Taeniura*), *Gymnura*, and pelagic myliobatoid rays make up a monophyletic group defined by characters 3(1), subpleural component of the hyomandibular canal with a distinct lateral hook and closely following anterior border of disc, and 25(1) pectoral propterygium extended beyond the nasal capsule. Within this group, *Dasyatis* 1, *Dasyatis* 2 + Indo-West Pacific *Himantura*, and *Gymnura* + pelagic myliobatoid rays diverge as a trichotomy. *Dasyatis* 2 and Indo-West Pacific *Himantura* are characterized by 4(3), extensively reticulated suborbital component of the infra-orbital canal. *Gymnura* and pelagic myliobatoid rays are monophyletic based on 27(1), division of the mesopterygium into several discrete components. *Gymnura* is diagnosed by characters 1(1), extensively branched tubules of the subpleural loop; $18(0)^{-}$, unsegmented basihyal; 20(1), absence of a lateral stay; and 34(1) depressor rostri inserted directly on the propterygium.

Pelagic myliobatoid stingrays are decisively monophyletic based on nine character changes: 3(2), presence of two distinct subpleural loops; $13(1)^{+}$, presence of a cartilaginous element functionally related to the anterior tip of the hyomandibular; $15(1)^{-}$, thickening of the mandibular cartilage; 16(1), presence of a wing-like process on the mandibular cartilage; $17(1)^{-}$, teeth arranged in flattened pavement-like bands; 24(1), separation of the articulations for the epibranchial and ceratobranchial cartilages; 32(1), presence of a dorsal fin; 35(1), posteromedial extension of the adductor mandibulae; and 37(1), midline connection of the coracohyoideus. *Myliobatis*, the basal clade within pelagic myliobatoids, is diagnosed by character $11(1)^{+}$, presence of an anterior hyomandibular accessory cartilage.

A clade consisting of *Aetobatus*, *Rhinoptera*, and *Mobula* receives support from characters 9(1), lateral margin of postorbital process prolonged into cylindrical projection; $10(0)^{+}$, absence of lateral groove in postorbital process; 27(2), mesopterygium absent or fused with scapulocoracoid; 28(1), presence of cephalic lobes; and 30(1), extreme arching of ischiopubic bar. *Aetobatus* diverges next, and *Rhinoptera* and *Mobula* are defined as a clade by 6(1), presence of an anterior process on the neurocranium, and $29(1)^{+}$, presence of an elongate prepelvic process. *Rhinoptera* is defined by character $11(1)^{+}$, presence of an anterior hyomandibular accessory cartilage; *Mobula* is diagnosed by 8(1), expanded interorbital region; $15(0)^{-}$ narrowing of the mandibular cartilage; and $17(0)^{-}$, small teeth arranged in rows along the jaws.

Additional phylogenetic analyses were performed incorporating manipulations of the basic data set. Exclusion of the only unordered character provided a single tree with a slightly decreased CI (0.79). The topology remained largely unaltered; however, with character 4(3) no longer providing support for the clade composed of

*Dasyatis* 1 and Indo-West Pacific *Himantura*, these taxa were drawn into the polytomy of *Dasyatis* 2 and *Gymnura* + pelagic myliobatoid rays.

Running the analysis with unordered multistate characters resulted in 8 equally parsimonious trees. The basic tree topology was maintained in all these cladograms; however, in some cases, resolution of the sister group to potamotrygonids was lost. Amphi-American *Himantura* and *Taeniura* were sometimes positioned basal to, or in a clade with the remaining daysatids + *Gymnura* + pelagic myliobatoid rays. In all cases, potamotrygonids remained a monophyletic group.

## DISCUSSION

### *General systematics*

Phylogenetic hypotheses provide the requisite framework for examining the validity of previously suggested groups and their interrelationships. In this study, the family Urolophidae, accepted by Bigelow & Schroeder (1953) as consisting of the genera *Urolophus* (including *Urobatis*), *Trygonoptera* (for Indo-West Pacific species with a small dorsal fin — not used in this study) and *Urotrygon*, is evidently paraphyletic. The characters that group stingrays to the exclusion of *Urolophus* show reversals in some taxa, suggesting that caution may be required in interpreting this branch. However, the reversals occur in widely separated parts of the tree, and are at least three nodes away from the original changes.

Garman's (1913) decision, most recently supported by Miyake (1988) and McEachran (in press, pers. comm.), to separate *Urolophus* into two groups, an Indo-West Pacific genus *Urolophus*, and an amphi-American genus *Urobatis*, is supported by the present investigation. Furthermore, a group composed of the amphi-American genera, *Urobatis* and *Urotrygon*, is monophyletic based on two synapomorphies. Within this clade, the western Atlantic species *Urobatis jamaicensis* is positioned basal to *Urotrygon* and eastern Pacific *Urobatis* species (making *Urobatis* a paraphyletic genus). However, the apomorphy that groups *Urotrygon* and *Urobatis* to the exclusion of *U. jamaicensis* (laterally splayed lateral process of the synarcuum), needs to be more thoroughly investigated, particularly in *Urolophus* species (few specimens of which were available for the present study).

An additional character which may be of importance in resolving relationships within a *Urobatis/Urotrygon* clade is the condition of the basihyal cartilage. Miyake & McEachran (1991) reported an unsegmented basihyal in *Urobatis jamaicensis* (the plesiomorphic condition, also exhibited by *Urolophus*). The presence of this state in this species would allow two possible transformations of the basihyal character. In one, the basihyal is plesiomorphically unsegmented until the node supporting Pacific *Urobatis* and *Urotrygon*, at which point it becomes segmented, and is subsequently lost in *Urotrygon*. This transformation would provide additional evidence for the basal position of *U. jamaicensis*. The alternative transformation allows segmentation of the basihyal to occur at the node following the divergence of *Urolophus*, and a reversal to the plesiomorphic unsegmented condition in *U. jamaicensis*. These scenarios, of course, depend on the actual state exhibited by *U. jamaicensis*, and since Garman (1913, plate 69) shows *U. sloani* ( = *U. jamaicensis*) with a segmented basihyal, this derived condition was used for the analysis. Further examination of specimens should shed some light on the appropriate character state for this species, and further collection and analysis of appropriate characters for ‘urolophids’ will allow a more complete understanding of these basal stingrays.

Potamotrygonids were determined to be a monophyletic group, and are positioned within a larger clade with *Taeniura* and amphi-American *Himantura*. The relationships within this group are discussed in more detail in later sections.

Dasyatidae, a family previously thought to contain *Himantura*, *Taeniura*, *Dasyatis*, and a few other genera not used in this analysis, such as *Hypolophus* and *Urogymnus* (Bigelow & Schroeder, 1953; J.S. Nelson, 1984), were found to be polyphyletic. Some members are more closely related to potamotrygonids (*Taeniura* and amphi-American *Himantura*), while others may not be monophyletic without the inclusion of *Gymnura* and the pelagic myliobatoid rays. Genera within the dasyatids are also poly/paraphyletic. Amphi-American *Himantura* species are most closely related to potamotrygonids, whereas Indo-West Pacific *Himantura* species are grouped with a subset of *Dasyatis*. *Dasyatis* could potentially be a monophyletic group with Indo-West Pacific *Himantura*, but no character to support such a clade was found during this study. *Taeniura* may also be a polyphyletic genus. Preliminary observations of a specimen of *Taeniura meyeni* Müller & Henle, indicate that it may be more closely related to *Dasyatis* and Indo-West Pacific *Himantura* than to *Taeniura lymma*.

These findings echo the sentiments of many ray systematists who have cautioned that the family Dasyatidae and dasyatid genera, particularly *Dasyatis* and *Himantura* may not reflect natural groups (Compagno & Roberts, 1982, 1984; Miyake, 1988; Ishihara, 1990; Nishida & Nakaya, 1990). The problem appears to stem from taxonomic work which placed considerable emphasis solely on tail fin-fold characters to distinguish groups. This tradition was partially initiated by Garman (1913) (but see also Müller & Henle, 1841; Günther, 1870). Although Garman pointed out that “…*for convenience* the species of the genus (*Dasyatis*) may be arranged by means of the cutaneous folds on the tail…” (Garman, 1913: 375 emphasis and brackets mine), later workers used such criteria almost exclusively to define groups and elevate these convenient subgenera (Fowler, 1941) to generic status (Bigelow & Schroeder, 1953). A phylogenetic hypothesis reveals the effects of the taxonomic confusion surrounding ‘dasyatid’ stingrays, but also provides a strong framework for future efforts to disentangle the relationships of these fishes. Lateral line canal patterns should provide a valuable pool of phylogenetic characters for this endeavour.

A potential problem with the monophyly of *Dasyatis* 1, *Dasyatis* 2 and Indo-West Pacific *Himantura*, is the unresolved relationship of these taxa to *Gymnura* and pelagic myliobatoid rays. Some *Dasyatis* taxon(a) or Indo-West Pacific *Himantura* species may share a common ancestor with this group. This aspect of the phylogenetic hypothesis differs from that of Nishida (1990) and Rosa (1985), in which *Gymnura* and pelagic myliobatoid rays were considered the sister group, or basal to all other stingrays.

In this phylogeny, like that of Nishida (1990), *Gymnura* is considered the sister taxon to pelagic myliobatoid rays. However, Nishida’s hypothesis differs in that *Myliobatis* and *Aetobatus* are considered sister taxa, and are supported by 4 characters. Of these, however, the thickening of the mandibular cartilage (character 15 in this study; 92 in Nishida’s), teeth in flattened pavement-like bands (character 17; 93 in Nishida’s), are also shared by *Rhinoptera* (and must be independently evolved in this clade) but missing in *Mobula* and *Manta*. They can, therefore, be optimized as reversals (in *Mobula* and *Manta*) rather than parallelisms, and do not provide decisive support for an *Aetobatus* + *Myliobatis* clade. Of the other characters, presence of articulations between neighbouring propterygia (Nishida’s #82) could not be verified for the current analysis and was not used; however, the derived state for this character is shared by *Gymnura, Aetoplatea, Myliobatis, Aetobatus* and *Aetomylaeus* (Nishida, 1990) and, therefore, also does not provide definite support for *Aetobatus* + *Myliobatis*, depending on the optimization used. In the phylogeny presented here, 5 unambiguous changes (4 of which are non-homoplastic) define a clade composed of *Aetobatus, Rhinoptera*, and *Mobula*, and this is considered a more likely topology.

### *Monophyly of the Potamotrygonidae*

*Paratrygon aireba* was certainly the most enigmatic freshwater stingray examined. While sharing the elongate prepelvic process of potamotrygonids, *Paratrygon* lacks the distinct modifications of the spiracularis muscle, and has undergone such structural modifications in the lateral line canals and the jaw cartilages, that characters from these systems which might have defined potamotrygonids as a group are unrecognizable. Because of this, under certain conditions, the family Potamotrygonidae as traditionally defined, is not diagnosable as a monophyletic unit.

In the primary analysis, neotropical freshwater stingrays were supported as monophyletic by several characters. The integrity of this clade remained unaltered even when all multistate characters were considered unordered. However, character 38(1), suppression of urea retention, is almost certainly correlated with the freshwater environment of these rays. The use of a single, possibly ecologically correlated character was considered a relatively conservative approach; Rosa (1985) used four freshwater-related characters to help define the Potamotrygonidae. These included: (1) complete adaptation to freshwater, and reduced tolerance to salt water, (2) suppression of urea retention, (3) reduction of rectal gland, and (4) reduction of ampullae of Lorenzini and associated canals to a microampullary system. Nevertheless, in order to trace the evolution of freshwater adaptation in a completely non-circular manner, some authors (e.g. Coddington, 1988) have advocated the exclusion of such definite ecology-correlated characters (however, see Kluge & Wolf [1993] for an alternative view).

Character 38 was excluded from a branch and bound analysis using PAUP 3.1, and three equally parsimonious topologies were resolved (Fig. 16). The topologies differ only in the position of *Paratrygon* relative to other rays. In two trees, Potamotrygonidae is no longer a monophyletic group — with *Paratrygon* located in a polytomy with (Fig. 16A), or basal to stingray clades to the exclusion of *Urolophus, Urotrygon, Urobatis*, and the outgroup (Fig. 16B). In these cases, the optimization of freshwater adaptation suggests that multiple invasions of South America may have taken place with *Paratrygon* entering first, followed by the *Potamotrygon/Plesiotrygon* ancestor. The third tree produced (Fig. 16C) is identical to the original (Fig. 15), and suggests that the evolution of freshwater tolerance in neotropical stingrays has happened only once.

Is there a way of choosing from the alternative scenarios provided by the three different trees? Closer examination of the characters involved suggests that there may be. In the Figure 16C tree, two unambiguous character changes provide definite support for a potamotrygonid clade: 23(0), absence of a fossa or foramen in the scapular process (a reversal to the plesiomorphic condition), and 29(1), presence of an extended prepelvic process (independently evolved by *Rhinoptera* and *Mobula*). These characters are shared by all three potamotrygonid genera. *Paratrygon*, however, does

TABLE 2. Step matrix for alternate ordering of character 36. Loss of either derived state (36[1] or 36[2]) requires only a single step.

| | To state: | | |
|---|---|---|---|
| From state: | 0 | 1 | 2 |
| 0 | 0 | 1 | 2 |
| 1 | 1 | 0 | 1 |
| 2 | 1 | 1 | 0 |

not share either derived state of character 36: the extension of the spiracularis, which groups *Taeniura* (state 36[1]), and amphi-American *Himantura*, *Potamotrygon* and *Plesiotrygon* (state 36[2]) together. *Paratrygon*, therefore, must lose this character if grouped with the other freshwater stingray taxa, and the transformation of state 36(2) to 36(0) requires 2 steps (if DELTRAN optimized, the grouping of *Paratrygon* with *Potamotrygon* and *Plesiotrygon* requires two independent gains of state 36[2], however this is considered a less likely alternative). The reversal of the spiracularis character, in *Paratrygon*, to the plesiomorphic state requires an equivalent number of steps as the evolution of states 23(0) and 29(1), allowing the other topologies, which move *Paratrygon* away from the other freshwater stingrays, to be equally parsimonious alternatives. These trees necessitate the independent evolution of states 23(0) and 29(1) (Fig. 16A,B).

It seems reasonable that the evolution of two discrete states in unrelated characters 23 and 29 provides a more robust phylogenetic signal than the reversal to a plesiomorphic condition of character 36. The ordering of the latter character requires a transition through an intermediate state, requiring two steps. However, the reversal in *Paratrygon* may represent only a single evolutionary event. To test the effect of decreasing the influence of the spiracularis reversal, character 36 was ordered using a step matrix so that the change from state 36(2) to 36(0) required only a single step (see table 2). When the analysis was rerun using this modification, only a single solution, identical to the primary cladogram (Fig. 15), was resolved.

The data as originally coded, to the exclusion of ecologically correlated character 38, provide three equally parsimonious trees. However, in two of these, independent evolution of two separate states is required to explain the results. In the third, a single reversal from derived state 36(2) directly to plesiomorphic state 36(0) could explain the pattern. The latter hypothesis, which upholds the monophyly of potamotrygonids, is considered more parsimonious because it involves a single change rather than two. This exercise, however, is only required if ecological characters are excluded from the analysis; a considerable suite of freshwater-correlated features do support the monophyletic status of potamotrygonids (Rosa, 1985; Rosa *et al.*, 1987) and imply a unique freshwater adaptive event.

### *Relationships within Potamotrygonidae*

Castex (1967a, b) made preliminary attempts to assemble groups within *Potamotrygon*, based on tooth and denticle formations. Rosa (1985) and Rosa *et al.* (1987) provided the only phylogenetic hypotheses for intergeneric potamotrygonid

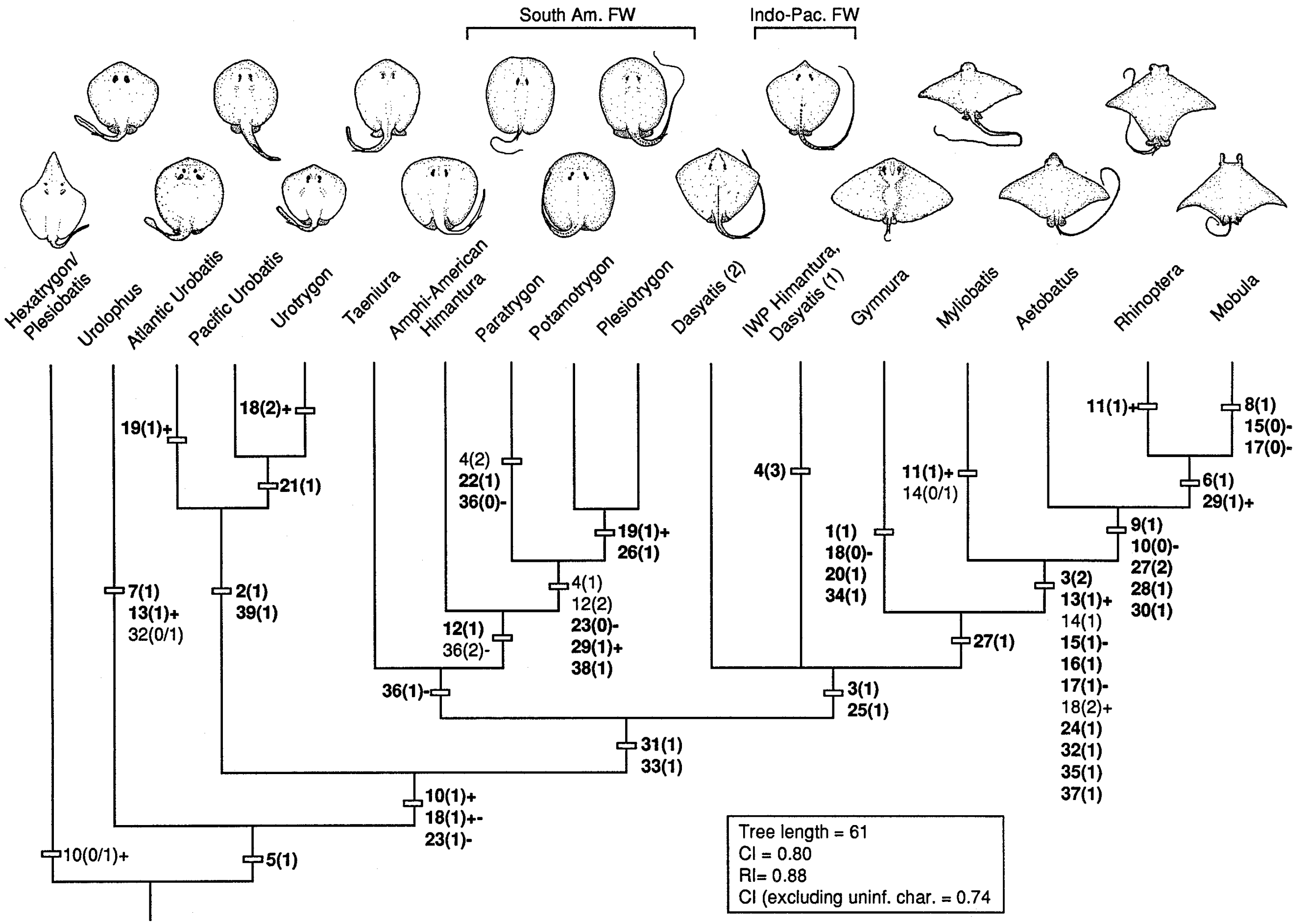

Figure 15. Single most parsimonious tree produced by phylogenetic analysis of stingray taxa. Homoplasy indicated by ‘ + ’ (independent gain) or ‘–’ (reversal). Some ray silhouettes after Castello & Yagolkowski (1969); Compagno (1977); Heemstra & Smith (1980); McEachran & Capapé (1984).

relationships. In their tree, they resolved *Potamotrygon* and *Paratrygon* as sister taxa, with *Plesiotrygon* as the basal member of the clade, hence its name. Rosa (1985) submits that *Paratrygon* exhibits the greatest amount of anagenetic change (witness the

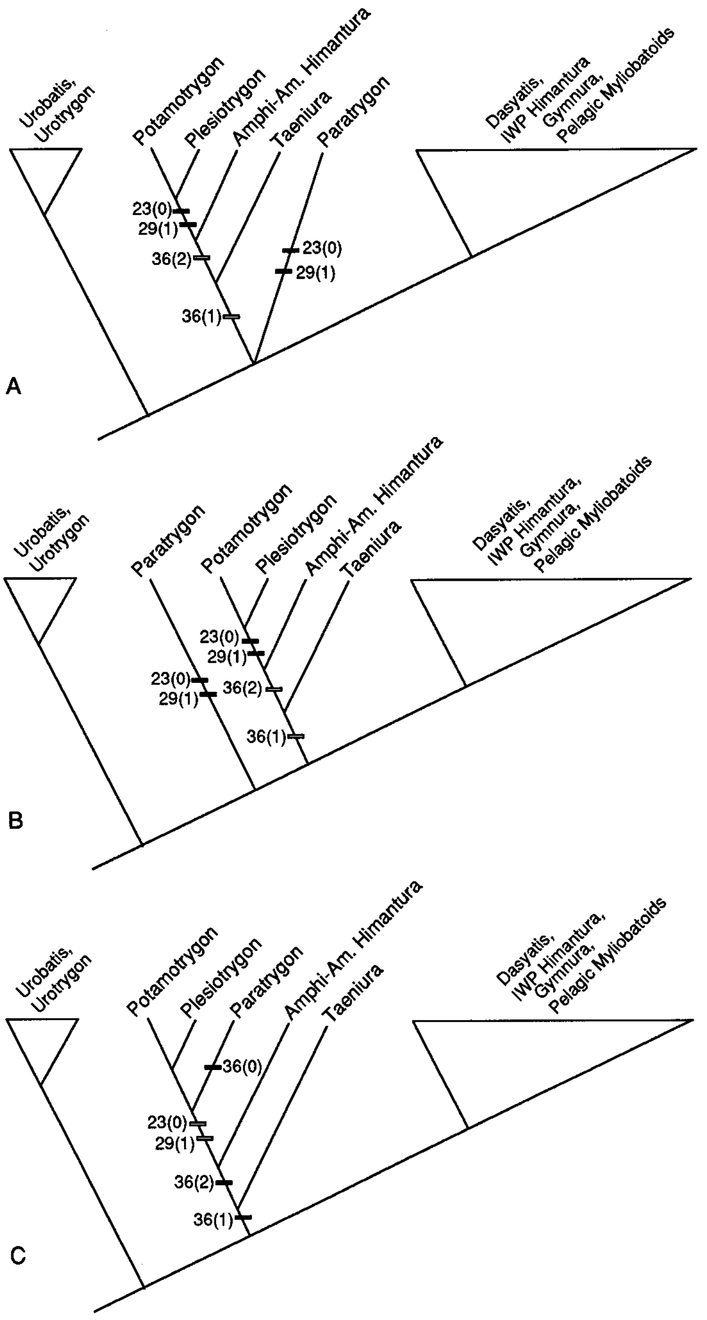

Figure 16. Three most parsimonious trees produced when character 38 (urea retention) is excluded from the analysis. The partial distributions of characters 23, 29 and 36 are mapped on the topologies. Closed bars = homoplastic state changes; open bars = non-homoplastic state changes for this part of the cladogram only (all three characters are homoplastic when the whole cladogram is considered).

unique subpleural lateral line canal plexus) and based on overall similarity, *Potamotrygon* and *Plesiotrygon* would probably be considered sister taxa. He quite rightly rejects this phenetic approach, and bases the sister-group relationship between *Potamotrygon* and *Paratrygon* on shared derived characters.

The hypothesis presented here, however, suggests that *Paratrygon* is basal to a clade composed of *Potamotrygon* and *Plesiotrygon* (Fig. 15). A close inspection of Rosa's (1985) and Rosa *et al*'s (1987) analyses reveal why this difference occurs. In Rosa *et al*. (1987: 456), the families Urolophidae and Hexatrygonidae form the sister group to potamotrygonids based on a single shared character: "the presence of cartilaginous supporting elements (radials) in the caudal finfold". However, while embryonic *Potamotrygon* specimens have this character (Rosa, 1985), adult *Plesiotrygon, Paratrygon* and *Potamotrygon* species lack caudal finfold radials. This problem was dealt with by hypothesizing another character for potamotrygonids: "ontogenetic loss of the caudal finfold radials". The sister group status of urolophids and hexatrygonids relative to potamotrygonids was thus based on the presence of a single character in embryonic members of one potamotrygonid genus. Radials are absent in embryonic *Paratrygon*, and embryonic *Plesiotrygon* species are yet to be examined, but probably also lack radials, as the tail of *Plesiotrygon* is very similar to that of most Dasyatids. Furthermore, outgroup analysis of caudal finfold radials reveal their presence to represent a plesiomorphic condition and thus be of little significance in defining groups.

The use of urolophids as the primary outgroup to potamotrygonids affected the polarizations of Rosa's characters. Rosa (1985) provided a list of seven apomorphies supporting a *Paratrygon* + *Potamotrygon* clade: (1) high modal number of pectoral-fin radials; (2) low modal number of pelvic-fin radials; (3) pelvic fins dorsally covered by disc; (4) reduction of tail length; (5) high number of branchial rays on ventral pseudohyoid; (6) fusion of inner margins of first two ceratobranchials; and (7) reduction of the postventral fenestra of scapulocoracoid (numbers do not correspond to Rosa's [1985]). Of these, characters 1 and 3 are completely reversed in polarity when amphi-American *Himantura* is considered the outgroup; characters 1, 2 and 5 have overlapping ranges which greatly limits their phylogenetic utility; character 6 varies extensively within operational taxonomic units, and possibly within species; and character 7 was found to inaccurately portray states possessed by ingroup taxa. These characters are discussed in more detail below.

The relatively high numbers of pectoral-fin radials (1) in *Paratrygon* and *Potamotrygon*, when repolarized, are found to be plesiomorphically similar to amphi-American *Himantura* and the more distant relative *Taeniura. Plesiotrygon*, using Rosa's state definitions, would be considered to have the derived condition of relatively low radial counts. This character however shows overlapping ranges in different taxa, and would require statistical procedures to validate states. Such procedures, and their coding methods (Archie, 1985; Goldman, 1988), and the use of quantitative data in general, have been critiqued (e.g. Pimentel & Riggins, 1987). Furthermore, numbers of pectoral-fin radials may be correlated with the absolute size of the disc. Owing to these difficulties, pectoral-fin radial counts were not used in the present analysis.

Pelvic-fin radials (2) are subject to the same difficulties as the previous character. Also, the ranges of this character are almost completely overlapping. The pelvic fins (3) are described by Rosa (1985: 417) "as more or less covered by the disc" in *Dasyatis, Himantura, Taeniura, Potamotrygon*, and *Paratrygon*, or exposed behind the disc in

*Gymnura*, *Hexatrygon*, *Urotrygon*, *Urolophus*, *Urobatis*, and *Plesiotrygon*. The use of amphi-American *Himantura* as an outgroup for this character suggests that *Potamotrygon* and *Paratrygon* share the plesiomorphic state and *Plesiotrygon* shows the derived reversal. Observation of stingray illustrations, however, suggests that differences in pelvic fin coverage are difficult to objectively quantify. In Bigelow & Schroeder (1953: figs 99, 90, 88), the pelvic fins of *Urobatis jamaicensis* appear to be more covered by the disc than those of *Dasyatis say* or *D. sabina*. Consequently, this character was not used in the present analysis.

Potamotrygonids exhibit a full range of stingrays tails (4): *Potamotrygon* has a short (less than 2 × disc width) tail with dorsal and ventral finfolds, *Paratrygon* has a short (1.6–1.9 × disc width) distally filiform tail with no finfolds and *Plesiotrygon* has a long (2.0–5.5 × disc width) distally filiform tail with a ventral membranous finfold (Rosa, 1985). Although using the decidedly short-tailed urolophids as the outgroup, Rosa considered the long tail of *Plesiotrygon* plesiomorphic, and the shorter tails of *Potamotrygon* and *Paratrygon* as synapomorphic. The tail length ranges of all potamotrygonids overlap with the amphi-American *Himantura* outgroup (approximately 2 × disc width [Meek & Hildebrand, 1923; Beebe & Tee Van, 1941; Bigelow & Schroeder, 1953;]) making a polarity assessment difficult; however, the long tail of *Plesiotrygon* might be regarded as autapomorphic. It should be noted that the absence of finfolds on the distally filiform tail of *Paratrygon* probably represents the plesiomorphic condition shown by amphi-American *Himantura*; the tail of *Plesiotrygon* (although with a finfold) is also distally filiform, as are the tails of *Potamotrygon* embryos (unlike the embryonic tails of urolophids).

The numbers of branchial rays on the ventral pseudohyoid (5) show overlapping ranges in potamotrygonids and are subject to the critiques of quantitative characters. The use of ceratobranchial fusion characters (6) has been previously discussed and is not considered a reliable source of phylogenetic information.

Rosa suggests that the reduction of the posteroventral foramen in the scapulocoracoid (7) represents a synapomorphy for *Potamotrygon* and *Paratrygon*. Dissection of specimens for this study revealed that small posteroventral scapulocoracoid foramina are widespread among stingray taxa, and that the foramen in *Plesiotrygon* is at least the same size as the foramen in *Paratrygon* and *Potamotrygon* (see Fig. 9).

The characters proposed by Rosa (1985) and Rosa *et al.* (1987) to support a sister group relationship between *Potamotrygon* and *Paratrygon* are problematic due to the difficulties outlined above. However, characters supporting a *Potamotrygon* + *Plesiotrygon* clade were found during the present study; these include: 19(1), the connection of the anterior base of the lateral stay above the spinal nerve foramina, and 26(1), the absence of an articulation between the propterygium and mesopterygium. The topology suggested by these characters is considered a more likely explanation of evolutionary relationships within potamotrygonids.

### *The sister group to Potamotrygonidae*

The classic apomorphy for Potamotrygonidae, the extended prepelvic process, was not discerned in any other benthic stingrays (although it was determined to have evolved convergently in some pelagic myliobatoids). The unique lateral line canal traits, the suborbital plexus of *Paratrygon* and the suborbital loop of *Potamotrygon* and

*Plesiotrygon*, also failed to betray the relationships of freshwater stingrays to their marine relatives. The peculiar extensions of the spiracularis muscle, however, and the development of specialized cartilages between the hyomandibular and mandibular arch, reveal genealogical ties between the amphi-American taxa, *Himantura schmardae* and *Himantura pacifica*, and the neotropical freshwater stingrays.

As mentioned above, however, the situation is complicated by *Paratrygon*. This freshwater ray has undergone considerable modification of the mandibular cartilages and musculature, and does not appear to share the characters that group amphi-American *Himantura*, *Potamotrygon*, and *Plesiotrygon* together. This creates instability in the node that groups the potamotrygonids and amphi-American *Himantura* as a clade; the branch has a decay index of only 1 (i.e. in a strict consensus tree of all the topologies one step longer than the most parsimonious solution, the node collapses [Bremer, 1988]). This occurs partly because *Paratrygon* can escape the potamotrygonid and *Himantura* clade with little increase in tree length, an effect contributed to by character 36. Due to the ordering of this character (extension of the spiracularis), *Paratrygon* must undergo a two step reversal in order to be grouped with the other potamotrygonids and amphi-American *Himantura* (see above: Monophyly of the Potamotrygonidae). If the ordering of the spiracularis character is modified, using a step matrix, to allow the change from state 36(2) to 36(0) to require only a single step (Table 2), support for amphi-American *Himantura* as the sister group to potamotrygonids increases. With this modification, the decay index for the potamotrygonid/amphi-American *Himantura* clade increases to two; furthermore, the clade is present in 92% of the trees that are two or less steps longer than the most parsimonious solution (Table 3).

For comparison, Brooks *et al.*'s (1981) hypothesis of *Urobatis* as the sister group to freshwater rays was tested by constraining monophyly between potamotrygonids and various *Urobatis* combinations (i.e. Potamotrygonidae and Pacific *Urobatis*, Potamotrygonidae and Atlantic *Urobatis*, and Potamotrygonidae and Atlantic and Pacific *Urobatis*) and searching for the shortest tree. In all cases, optimal solutions required at least five additional steps, or six if the step matrix ordering of the spircularis character is used (Table 3).

Thus, while the hypothesis of amphi-American *Himantura* as sister group to the

TABLE 3. Frequencies of selected taxa as the sister group to Potamotrygonidae in optimal and sub-optimal trees. Calculations were performed with character 36 ordered or defined by step matrix. Numbers represent percentage of the trees that contain the clade in question.

| | Amphi-American Himantura | | Urobatis* | |
|---|---|---|---|---|
| Extra steps | Ordered | Step matrix | Ordered | Step matrix |
| 0 (optimal) | 100 | 100 | 0 | 0 |
| ≤1 | 65 | 100 | 0 | 0 |
| ≤2 | 37 | 92 | 0 | 0 |
| ≤3 | 29 | 77 | 0 | 0 |
| ≤4 | 25 | 67 | 0 | 0 |
| ≤5 | 21 | 55 | ≤0.1 | 0 |
| ≤6** | <20 | <50 | <0.1 | <0.1 |

*Frequency of any of three possibilities; (Atlantic Urobatis, (Potamotrygonidae)), (Pacific Urobatis, (Potanotrygonidae)), or (Atlantic Urobatis, Pacific Urobatis, (Potamotrygonidae)).

**All trees could not be retained dur to computational limitations; frequencies were estimated from tree length frequency distributions and searches with monophyly constraints.

neotropical freshwater stingrays should be tested with additional evidence, it remains a significantly more parsimonious explanation of the morphological data than the *Urobatis* alternative. The ecological and biogeographic data relevant to this hypothesis are discussed below.

## *Ecology*

Since the only benthic stingrays known to enter freshwaters are members of the family Dasyatidae (now hypothesized to be paraphyletic), an initial hypothesis was that potamotrygonids would be most closely related to a freshwater-invading dasyatid taxon. The other possibility, that potamotrygonids were derived from Urolophidae (also hypothesized to be paraphyletic), was considered less parsimonious in an ecological sense. In a dispersal scenario, an obligate marine taxon would have to both develop *de novo* the capability of invading freshwater, and invade South America while leaving no trace of ancestral marine taxa with these capabilities. The vicariance version of the urolophid hypothesis (Brooks *et al.*, 1981) would require an explanation of why an obligate marine urolophid, rather than a freshwater-tolerant dasyatid species, survived when trapped in an incipient freshwater habitat. The analysis undertaken here supports the first alternative — that complete adaptation to freshwater in potamotrygonids proceeded through an intermediate condition of euryhalinity. This is evident from a consideration of the salinity tolerance of amphi-American *Himantura*, the sister group to neotropical freshwater rays.

*H. schmardae* has been reported from brackish and freshwater Venezuelan, Columbian, and Mexican river mouths and lagoons (Thorson, Brooks & Mayes, 1983; Castro-Aguirre, 1978), and Fernández-Yépez & Espinosa (1970a,b) reported overlapping ranges from *Himantura schmardae* and *Potamotrygon yepezi* in freshwaters of Lake Maracaibo. Thorson *et al* (1983: 678) go on to suggest that "…the range of salinity tolerance and the range of urea levels of *H. schmardae* clearly characterize it as a brackish water form, preferring intermediate to low salinities." (although the Caribbean distribution of the species suggests that it must occasionally penetrate fully marine waters). Thus, as the sister group to potamotrygonids, at least one of the two species of amphi-American *Himantura* demonstrates the initial stages of complete freshwater tolerance (unfortunately, little evidence is available concerning the salinity tolerance of *H. pacifica*). The parsimonious conclusion is that the ancestor of both amphi-American *Himantura* and Potamotrygonidae was freshwater tolerant. This ecological/physiological reconstruction provides a reasonable rationale for how a marine species was able to disperse into the rivers of South America, or to survive a vicariance event trapping it in incipient freshwaters.

A broader view of stingray phylogeny suggests that freshwater tolerance has evolved multiple times in independent lineages. *Taeniura*, the sister group to amphi-American *Himantura* and potamotrygonids, appears to be a strictly marine reef fish. Its basal position relative to euryhaline and freshwater taxa implies that freshwater tolerance evolved subsequent to its divergence. The Indo-West Pacific freshwater-invading or freshwater-resident taxa, *Hypolophus sephen*, *Dasyatis laosensis* Roberts & Karnasuta, *Himantura krempfi* (Chabanaud), *H. signifer*, *H. fluviatilis* (Hamilton-Buchanon), and *H. chaophraya* Monkolprasit & Roberts (Compagno & Roberts, 1982; Taniuchi, 1979; Roberts & Karnasuta, 1987; Monkolprasit & Roberts, 1990; Taniuchi *et al.*, 1991) appear to lie within the Indo-West Pacific *Himantura* + *Dasyatis*

1 clade (see Fig. 15). Independent freshwater tolerance probably evolved at least once within this group. The affinities of the West African freshwater stingrays, *Dasyatis garouaensis* and *D. ukpam* (Smith) (Compagno & Roberts, 1984) are largely unknown, but these taxa probably represent another independent freshwater invasion. Additionally, Feibel (1993) has outlined a freshwater colonization by stingrays of the Turkana Basin in East Africa, between 1.9 and 1.3 Myr ago. Better biogeographic understanding of these events awaits finer phylogenetic resolution within the *Dasyatis* and *Himantura* clades.

## *Biogeography*

The two species of amphi-American *Himantura, H. schmardae*, and *H. pacifica* have distributions centred, respectively, on the Atlantic and Pacific coasts of central America (Fig. 17A). *Himantura schmardae* is known from Surinam to Tabasco, Mexico, and Trinidad, Jamaica, and Cuba (Bigelow & Schroeder, 1953; Castro-Aguirre, 1978); *H. pacifica* has been recorded from Costa Rican waters (Beebe & Tee-Van, 1941; Lopez & Bussing, 1982) and Mar Muerto and Laguna Continental, Oaxaca, Mexico (Castro-Aguirre, 1978), but not from the more southern waters of Pacific South America. The distribution of amphi-American *Himantura* can be compared to expectations for various hypotheses about the origins of their hypothesized sister group, the Potamotrygonidae.

Brooks *et al*. (1981) proposed an Andean orogeny vicariance hypothesis for the origins of potamotrygonids, whereby an ancestral stingray population from the Pacific is trapped in a shallow inland sea, and survives progressive desalinization to become a component of the freshwater neotropical ichthyofauna. An expected correlate of this hypothesis is the distribution of the sister taxon of potamotrygonids along the Pacific coast of South America. However, this is a prediction not fulfilled by the range of amphi-american *Himantura. H. pacifica*, as mentioned above, has not been recorded further South than Central America. Thus, to explain the current distributions, Brooks *et al*.'s Pacific-origin scenario would have to assume the disappearance of the potamotrygonid ancestor along Pacific South America after it gave rise to its freshwater relatives.

Brooks (1992: 592) also submits that:

> The geography of South America prior to the mid-Miocene epoch differed in three significant ways from what we see today: Africa and South America were joined (i.e. there was no Atlantic Ocean at the mouth of the Amazon), the Andes began sweeping upward from the south beginning in the early Cretaceous and moving northward, and the Amazon river flowed into the Pacific Ocean until the mid-Miocene epoch, when it was blocked by Andean orogeny, becoming an inland sea and eventually opening to the Atlantic Ocean. Thus, if potamotrygonids are a relatively old component of neotropical freshwater diversity east of the Andes, they must have come from the Pacific Ocean, which is today west of the Andes.

This perspective considerably underestimates the age of the Atlantic Ocean, which was opening between Africa and North America during the middle to late Jurassic. The proto-Caribbean was also open by this stage (Pindell & Dewey, 1982; Pindell, 1985), and the rift between Africa and South America was well-developed by the middle to late Cretaceous (e.g. Howarth, 1981; Smith, Hurley & Briden, 1981; Maisey, 1991). A marine potamotrygonid ancestor could therefore have been distributed along northern South America by 145 Myr ago, and along the Eastern coast by 90 Myr. Clearly, possible freshwater stingray fossils from the Miocene of Peru (Frailey, 1986) do not limit the origin of potamotrygonids to the Pacific: potamotrygonid ancestors had a vast amount of time (50 Myr + ) to make their way from nearly any coast to the interior of the continent.

The orogenesis of the Andes, and a possible 'reversal of flow' of the Amazon, are alluring palaeogeological components of the Brooks *et al.* (1981) hypothesis, however, there are other possible vicariance scenarios for the origins of neotropical freshwater rays. Northern South America experienced frequent marine introgressions up until the late Miocene, evidenced by marine deposits in basins of northern Ecuador, Colombia, Venezuela, and the Guyanas (Harrington, 1962; Bellizzia, de Bellizzia & Munoz, 1981; Megard, 1989; Hoorn, 1994a, b). Such incursions may have reached as far south as Peru and Brazil, as evidenced by deposits with fossil marine molluscs (Nuttall, 1990), pollen (Hoorn, 1993), and ostracods (Sheppard & Bate, 1980). The gradual retreats of these systems could have isolated stingrays in remnant marine or brackish lakes. The subsequent desalinization of these habitats, and their integration with continental river systems, may have provided appropriate conditions for the evolution of freshwater species. Assuming the origin of the potamotrygonids took place along the northern edge of South America does not require additional hypotheses to explain the present distribution of the sister group. Additionally, the parasite data presented by Brooks *et al.* (1981), show a strong relationship between neotropical freshwater and Western Atlantic (Caribbean) areas (Lovejoy, 1993; Straney, 1982). However, as Rosa (1985) points out, the question of an Atlantic versus Pacific origin for the potamotrygonids is probably irrelevant, as the Panamanian isthmus was open from late Paleocene to early Pliocene (White, 1986) and continental seaways (Harrington, 1962) allowed free circulation of marine fauna between the Atlantic and Pacific.

Although a synapomorphy was not proposed for the two amphi-American *Himantura* species, they are morphologically quite similar, and probably represent sister taxa (presence of quadriradiate dermal tubercles is a potential character). If this is the case, area relationships constructed for the potamotrygonid + amphi-American *Himantura* clade (Fig. 17B) parallel those of *Cetengraulis* anchovies (and *Anchovia*) provided by G.J. Nelson (1984) (Fig. 17C). In both cases, a neotropical freshwater group is basal to an Eastern Pacific and Western Atlantic sister pair. The more recent Atlantic/Pacific split can be correlated with the formation of the isthmus of Panama, while the earlier divergence could be connected to a more ancient event, such as Andean orogeny and/or marine introgressions into Northern South America (Fig. 17B). The question of Atlantic versus Pacific potamotrygonid origins is unanswerable from such area cladograms, and as discussed above, is inappropriate since both oceans were connected before the Pliocene. The most probable reconstruction suggests that an ancestral taxon, distributed along northern South America, gave rise to potamotrygonids, and was subsequently divided by the isthmus of Panama.

The hypothesis that *Paratrygon* represents the basal taxon within Potamotrygonidae provides little resolution for ideas concerning the biogeographic origins of neotropical freshwater rays. *Paratrygon*'s broad distribution in Bolivia, eastern Peru, northern Brazil, and Venezuela (Rosa, 1985) is congruent with both an Andean orogeny or a more northern vicariance scenario, perhaps involving the Orinoco drainage. If potamotrygonids have originated in northern South America, an area cladogram from a more resolved potamotrygonid phylogeny might show basal taxa distributed in the northern South America (e.g. Orinoco, Magdalena) basins. This pattern would conflict with the South to North derivation proposed by Brooks *et al.* (1981) and critiqued by Rosa (1985), but might parallel the area relationships derived from phylogenies of the curimatid genus *Potamorhina* (Vari, 1984) and the subfamily Glandulocaudinae (Weitzman & Fink, 1985). In these groups, northern taxa (from the Maracaibo/Magdalena basins) are basal to more widespread groups in the Amazon river system (Vari, 1988; Vari & Weitzman, 1990).

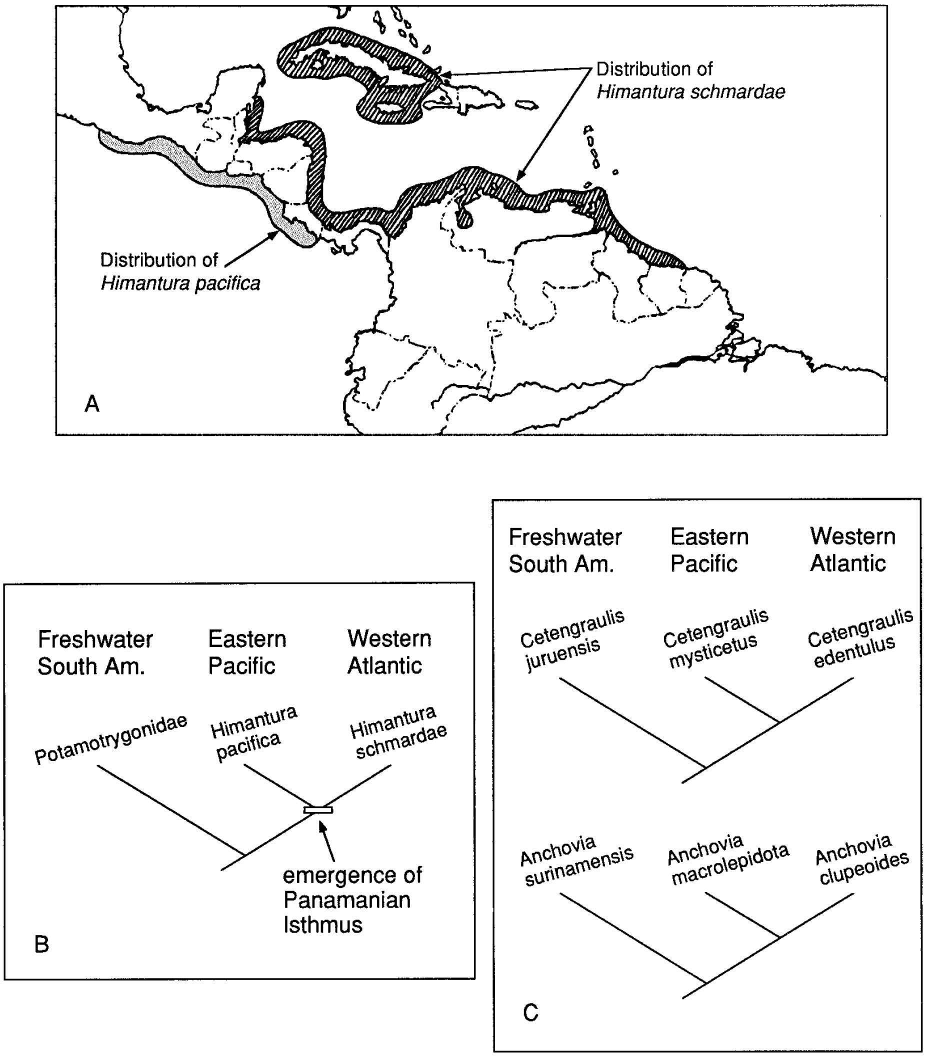

Figure 17. A, distribution of amphi-American *Himantura*, estimated from the literature (see text). Note that *H. pacifica* has not been reported farther South than Costa Rica. B, area cladogram for potamotrygonids and amphi-American *Himantura*, the position of the split caused by the Panamanian Isthmus suggests that entry to neotropical freshwaters took place prior to this event. C, area cladograms for two clades of anchovies (after G. Nelson [1984]).

Brooks *et al.* (1981) and Brooks & Deardorff (1988) supported their idea of a circum-Pacific connection for the potamotrygonids with distributional data for *Urolophus, Urobatis,* and *Urotrygon.* The biogeography of these basal clades is no longer of direct relevance to the origins of potamotrygonids. However, the positioning of Atlantic *Urobatis* as the sister taxon to Pacific *Urobatis* and *Urotrygon* may lend credence to Rosa's (1985) hypothesis of a Tethyan track for the group (based in part on urolophid fossils from Europe).

The position of *Taeniura* (distributed primarily in the Indo-West Pacific) as sister group to potamotrygonids + amphi-American *Himantura* initially seems to suggest a trans- or circum-Pacific area relationship. However, *Taeniura grabata* (Geoffroy St. Hilaire) (not available for this study) is also known from the west coast of Africa and the Mediterranean (Fowler, 1934; McEachran & Capapé, 1984), leaving open the possibility of an Atlantic or Tethyan connection. *T. grabata* is very poorly known, yet potentially a key species in the biogeographic puzzle. It requires a thorough assessment of its relationships to other rays.

## CONCLUSION

The construction of a robust phylogeny for stingrays has provided a reasonable framework for evaluating evolutionary scenarios concerning the group. Ideally, additional historical information could be retrieved from palaeontological data. However stingrays like most cartilaginous fishes, have a relatively depauperate fossil record (aside from dentition). Potamotrygonid fossils have been reported from Miocene deposits in Peru (Frailey, 1986), Miocene deposits in Colombia (Lundberg, pers. comm.), Tertiary deposits in Argentina (Larrazet, 1886), and Upper Tertiary deposits in Ethiopia (Arambourg, 1947). In all cases, assignment of such fossils (consisting of tail spines and dermal tubercles) to Potamotrygonidae has been based solely on zoogeographical and palaeoecological inference rather than morphological evidence (see Thorson & Watson [1975] for a critique of the latter two reports). Well-preserved fossil stingrays have been retrieved from the Eocene Green River Formation of Wyoming (see Grande [1980] and references therein). The phylogenetic affinities of these freshwater rays are unknown; if they are found to comprise a monophyletic group with potamotrygonids, a radical reevaluation of current biogeographical hypotheses will no doubt be required. Further resolution of biogeographic events surrounding the invasion of South American by marine fishes awaits detailed phylogenetic analyses of additional taxa; the nexus of these studies promises to enhance historical understanding of this component of the neotropical ichthyofauna.

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## APPENDIX

### *Material examined*

Acronyms of museums and institutions are as follows: AMNH: American Museum of Natural History, New York, New York; ANSP: Academy of Natural Sciences, Philadelphia, Pennsylvania; BPBM: Bernice P. Bishop Museum, Honolulu, Hawaii; CAS: California Academy of Sciences, San Francisco, California; FMNH: Field Museum of Natural History, Chicago, Illinois; LACM: Los Angeles County Museum of Natural History, Los Angeles, California; ROM: Royal Ontario Museum, Toronto, Ontario; TCWC: Texas Cooperative Wildlife Collection, Texas A&M University, College Station Texas; UCR: Museo de Zoología, Universidad de Costa Rica, Ciudad Universitaria, Costa Rica; UMMZ: Museum of Zoology, University of Michigan, Ann Arbor, Michigan; USNM: National Museum of Natural History, Smithsonian Institution, Washington, D.C. * = cleared and stained specimen.

*Aetobatus narinari*: FMNH 4830 (Bermuda), ROM 66835 (Costa Rica)
*Dasyatis americana*: ANSP 103931 (Colombia), ROM 23655 (Tobago)
*Dasyatis brevis*: LACM 31759-16 (Baja, Mexico)
*Dasyatis centroura*: ROM 23893, ROM 42578 (off South Carolina)
*Dasyatis guttata*: ANSP 153736 (Florida), ROM 25096 (Guyanas)
*Dasyatis longus*: ROM 66847, ROM 66840 (both from Costa Rica)
*Dasyatis sabina*: ANSP 49827 (Florida), ROM 46549 (Gulf of Mexico)
*Dasyatis say*: FMNH 40223* (Texas), TCWC 5818.1 (Gulf of Mexico), ROM 42595 (off North Carolina)
*Dasyatis violacea*: ROM 25636, ROM 43223 (both Atlantic)
*Dasyatis purpureus*: ROM 43958 (Chagos Archipelago)
*Gymnura micrura*: FMNH 89990* (Surinam), USNM 222598 (Guianas)
*Himantura pacifica*: ROM 66838 (Costa Rica)
*Himantura schmardae*: ANSP 103470 (Trinidad/Tobago), ROM 66845 (Guyana)
*Himantura jenkinisii*: ROM 23011 (Singapore)
*Hypolophus sephen*: LACM 38130–88
*Myliobatis californicus*: USNM 26781, USNM 27064 (California)
*Myliobatis longirostris*: USNM 222686 (Mexico)

*Paratrygon aireba*: ROM 66843 (Venezuela), UMMZ 204840 (Bolivia), UMMZ 211261 (Venezuela), USNM 264005 (Peru)

*Plesiobatis (Utrotrygon daviesi)*: BPBM 24578, BPBM 30909 (both from Hawaiian Islands)

*Plesiotrygon iwamae*: FMNH 94500 (Ecuador), ROM 65366 (Brazil)

*Potamotrygon castexi*: ANSP 142483 (Peru)

*Potamotrygon falkneri*: DBAV.UERJ 718.3 via AMNH, UMMZ 206379 (Paraguay)

*Potamotrygon histrix*: UMMZ 208058 (Paraguay)

*Potamotrygon humerosa*: ANSP 94842 (Brazil)

*Potamotrygon magdalenae*: UMMZ 211755

*Potamotrygon motoro*: FMNH 94503* (Ecuador), ROM 66844,m UMMZ 207766 (Paraguay), UMMZ 220137, USNM 167713 (Peru)

*Potamotrygon orbignyi*: ROM 26182 (Guyana), UMMZ 211262 (Venezuela), USNM 257090 (Venezuela)

*Potamotrygon schroederi*: UMMZ 220135

*Potamotrygon signata*: ANSP 69344 (Brazil)

*Potamotrygon yepezi*: USNM 205276 (Venezuela)

*Rhinoptera bonasus*: FMNH 565 (Florida)

*Rhinoptera quadriloba*: FMNH (Texas)

*Taeniura lymma*: ANSP 103814 (Australia), ANSP 128768 (New Guinea), ROM 39404 (Australia), ROM 50295

*Taeniura meyeni*: LACM 44314-1, LACM 44001-2, LACM 43679-1 (all from Galapagos Islands), USNM 222646 (Somalia)

*Urobatis jamaicensis*: ANSP 101660 (Bahamas), ROM 28276 (Nassau)

*Urobatis concentricus*: ROM 66839 (Costa Rica)

*Urobatis halleri*: USNM 181313 (Baja, Mexico)

*Urobatis maculatus*: LACM 42299-1 (San Filipe, Mexico), USNM 119751 (Concepcion Bay, Mexico)

*Urobatis tumbesensis*: AMNH 44021 (Ecuador)

*Urolophus aurantiacus*: USNM 26543 (Japan)

*Urolophus cruciatus*: LACM CSUF (Victoria, Australia)

*Urolophus fuscus*: USNM 151756 (Japan)

*Urolophus testaceus*: LACM W55-190-7 (western Australia)

*Urotrygon asterias*: FMNH 93737* (El Salvador)

*Urotrygon aspidura*: CAS 51835 (Panama)

*Urotrygon chilensis*: ROM 66832, ROM 66833, ROM 66836, ROM 66842 (all from Costa Rica)

*Urotrygon micropthalmum*: FMNH 90096 (Guianas-Brazil), USNM 222693 (Surinam)

*Urotrygon munda*: LACM 30745-11 (Golfo de Nicoya, Costa Rica), UCR 2216-14 (El-Rompio, Panana), UCR 463-5 (Golfo de Nicoya, Costa Rica), UCR 559-11 (Corinto, Costa Rica), USNM 220612 (El Salvador)

*Urotrygon nana*: ROM 66837 (Costa Rica)

*Urotrygon reticulata*: USNM 321478 (Panama)

*Urotrygon rogersi*: LACM W50-57 (Sonora [Gulf], Mexico), TCWC uncat (Ecuador), USNM 181322 (Baja, Mexico)