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Facial and opercular muscles in the Anguilliformes (Elopomorpha: Teleostei): Comparative anatomy and phylogenetic implications for the basal position of *Protanguilla*

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Abstract

The teleost order Anguilliformes, true eels, comprises more than 1000 described species in 20 families, commonly known as eels, congers, morays, and gulper eels. Comprehensive studies of Anguilliformes are limited, resulting in a lack of consensus for morphology-based phylogenetic hypotheses. A detailed morphological analysis of the cephalic and opercular myology offers a promising new source of characters to help elucidate the intrarelationships of Anguilliformes. Our study is the most extensive myological analysis for the group and includes 97 terminal taxa, with representatives from each of the 20 families of Anguilliformes plus outgroup clades. Results demonstrate that muscle characters inform phylogenetic relationships within Anguilliformes, and we propose two new synapomorphies for all extant members, including *Protanguilla palau*, the "living fossil"—*adductor mandibulae* originating on the parietal (vs. restricted to suspensorium) and *segmentum mandibularis* absent (vs. present). Exceptions for the first condition characterize highly modified saccopharyngoids, and for the second one, Notacanthidae. More importantly, we suggest three new synapomorphies for the remaining extant anguilliforms (except in highly modified saccopharyngoids)—*adductor mandibulae* originates on the frontals (vs. frontals naked), *adductor mandibulae stegalis* is separated from the *rictalis* (vs. *ricto-stegalis* fused into a single piece), and the *levator operculi* inserts on the lateral surface of the opercle (vs. medial surface of the opercle). Our phylogenetic optimization strongly corroborates the hypothesis that *Protanguilla* is the sister group of all other extant eels. A further goal of this paper is to clearly document the substantive conflicts between the available molecular data and the extensive and diverse morphological evidence.

KEYWORDS
cranial muscle, eels, myology

1 | INTRODUCTION

> At once he became an enigma. One side or the other of his nature was perfectly comprehensible, but both sides together were bewildering.
>
> Jack London, The Sea Wolf

The Elopomorpha include Elopiformes (tarpons), Albuliformes (bonefishes), Notacanthiformes (spiny eels), and Anguilliformes (eels).

Of those, Anguilliformes (sensu Johnson et al., 2012) are the richest and the most heterogeneous, with 1043 species in 20 families and 161 genera (Fricke et al., 2022) encompassing about 95% of elopomorph species. Anguilliformes occur in freshwater, brackish, and marine habitats, ranging from shallow shore waters (reef, rocky, and soft bottoms) to bathypelagic and abysopelagic (Hasting et al., 2015; Helfmann et al., 2009; J. S. Nelson et al., 2016).

With the discovery and description of Protanguillidae, Johnson et al. (2012) proposed 15 synapomorphies for the Anguilliformes (including Cretaceous and extant forms) based primarily on external morphology and osteology and placed *Protanguilla* as the sister group of all other extant anguilliforms. That hypothesis was corroborated in only one of two equally supported molecular analyses presented in that study. Additionally, Johnson et al. (2012) and Springer and Johnson (2015) provided a total of 34 synapomorphies for Anguilliformes; however, 19 of those are derived from soft tissue or unknown in fossil forms (Table 1). Results of subsequent molecular studies conflict with morphological data and do not support a basal position of *Protanguilla*. The molecular hypotheses of Tang and Fielitz (2012), Santini et al. (2013), Chen et al. (2013), and Poulsen et al. (2018) placed *Protanguilla palau* as the sister group of Synaphobranchidae, as did one of the two of Johnson et al. (2012). We represented Protanguillidae and Synaphobranchidae taxa by *P. palau* and *Synaphobranchus affinis* in Figure 1a,b. The molecular hypotheses imply that trenchant features of *Protanguilla*, such as the presence of premaxilla, symplectic, metapterygoid, separated upper hypurals, and gill rakers, are all homoplastic and have evolved independently in *Protanguilla* or have been lost twice in extant eels.

To date, only three studies have used muscle characters as the primary source of evidence in understanding anguilliform intrarelationships (Eagderi, 2010; G. J. Nelson, 1967; Springer & Johnson, 2004, 2015). G. J. Nelson (1967) explored muscles associated with gill arches in five eel families (Congridae, Muraenidae, Anguillidae, Chlopsidae, and Moringuidae), and Springer and Johnson (2004) gave detailed descriptions of the dorsal gill-arch muscles of Congridae, Anguillidae, and Synaphobranchidae. Eagderi (2010) compared the *segmentum facialis* of *Musculus adductor mandibulae* anatomy in eight families (Synaphobranchidae, Anguillidae, Moringuidae, Muraenidae, Heterenchelyidae, Congridae, Nettastomatidae, and Eurypharyngidae), focusing on ecological adaptations with no phylogenetic context. Most recently, Springer and Johnson (2015) compared the gill-arch muscles of *Protanguilla* to what they believed to be primitive extant eels (Congridae, Anguillidae, Serrivomeridae, and Synaphobranchidae) and other elopomorphs (Elopiformes, Albuliformes, and Notacanthiformes), suggesting seven new gill-arch muscle synapomorphies of extant Anguilliformes, and two characters for "advanced eels" (all eels except *Protanguilla*).

Despite substantial yet episodic progress represented by earlier contributions, facial, opercle, and gill muscles in anguilliforms remain incompletely explored. Previous studies have relied on a relatively limited comparative framework of three to eight families (Eagderi, 2010; G. J. Nelson, 1967; Springer & Johnson, 2004, 2015). Facial and opercular muscles have proved highly informative in anatomical descriptions of several subgroups of bony fish, such as Notacanthiformes (Greenwood, 1977), Teleostei (Datovo & Vari, 2014; Winterbottom, 1974a), Tetraodontiformes (Winterbottom, 1974b), Stromateiformes (Pastana et al., 2021), Gymnotiformes (Peixoto & de Pinna, 2022), and Actinopterygii (Datovo and Rizzato, 2018). There is every reason to believe that similarly, valuable information awaits discovery in these myological complexes of anguilliforms.

In this study, we investigated the facial and opercular muscle system of Anguilliformes on a comparative basis substantially broader than that of previous studies, considered all morphological evidence, and compared them to the placement of *Protanguilla* in molecular hypotheses. Our focus has been on exploring new morphological variations relevant to this placement, and we present several newly discovered facial and opercular muscle characters that bear on this and the diagnosis of extant Anguilliformes.

2 | MATERIALS AND METHODS

We dissected specimens of 83 species (102 specimens) in 20 families and 71 genera of anguilliforms, plus 11 genera of other Elopomorpha. Examined material is listed in Section 2.2.

We divided the nonopercular cephalic muscles into three main complexes: *adductor mandibulae* (*segmentum mandibularis* and *facialis*), *levator arcus palatini*, and *adductor hyomandibulae*. Opercular muscles are divided into *levator operculi*, *dilatator operculi*, and *adductor operculi*. Extrinsic and intrinsic eyes muscles, gill and trunk muscles, and *ramus mandibularis trigeminus* nerve were outside the scope of the present contribution.

For the myological comparative study, specimens were double-stained for cartilage and bone before dissection following the protocol of Datovo and Bockmann (2010). Dissections removed the epithelium, ligaments, blood vessels, and connective tissues from the pectoral girdle region toward the head using forceps. Subsequently, nasal organs and eyes were removed and preserved for future studies. One side of the lower jaw was removed by freeing it from the neurocranium, hyomandibular, and opercular ligament, preserving the *dilatator operculi*, *levator arcus palatini*, *adductor hyomandibulae*, and *levator operculi* fibers. A complete description of the facial segment of *M. adductor mandibulae* arrangement and a list of the characters is given in Section 3.

For osteological comparative studies, specimens were cleared and stained for bone and cartilage following the protocol of Taylor and Van Dyke (1985).

Osteological nomenclature follows Patterson (1975) and Johnson et al. (2012). Myological terminology follows Winterbottom (1974a), Datovo and Vari (2013), and Datovo and Rizzato (2018).

Photographs were taken with a Canon EOS Rebel T7i DSLR Camera or Zeiss Axiocam attached to a Zeiss SteREO Discovery V12, and focus stacking used the Combine ZP program.

TABLE 1 Proposed morphological synapomorphies of Anguilliformes

	Reference	Morphological character	Groups
1	Johnson et al. (2012)	Ethmoid fused with vomer	Ex, C
2	Johnson et al. (2012)	Pterotic extends anteriorly above prootic to contact pterosphenoid	Ex, C
3	Johnson et al. (2012)	Dermopalatine and autopalatine absent	Ex, C
4	Johnson et al. (2012)	Pectoral girdle displaced posteriorly	Ex, C
5	Johnson et al. (2012)	First pharyngobranchial absent and pharyngobranchials without uncinate processes	Ex, C
6	Johnson et al. (2012)	Gill arches free from braincase and displaced posteriorly	Ex, C
7	Johnson et al. (2012)	Opercular series characterized by a distinctive pattern	Ex, C
8	Johnson et al. (2012)	Uppermost branchiostegals curving dorsally behind and often slightly above the opercle	Ex, C
9	Johnson et al. (2012)	Posterior ceratohyal is almost equal to or longer than anterior ceratohyal	Ex, C
10	Johnson et al. (2012)	Branchiostegals are more numerous on the posterior than on the anterior ceratohyals	Ex, C
11	Johnson et al. (2012)	Posteriormost one to four branchiostegals with spatulate expansions distally	Ex, C
12	Johnson et al. (2012)	Dorsal part of the suture between anterior and posterior ceratohyals deflected posteriorly	Ex, C
13	Johnson et al. (2012)	Interhyal absent in adults	Ex, C
14	Johnson et al. (2012)	Angular, articular, and retroarticular fused into a single bone	Ex, C
15	Johnson et al. (2012)	Two pairs of upper pharyngeal tooth plates are present and autogenous	Ex, C
16	Johnson et al. (2012)	Endopterygoid absent	Ex
17	Johnson et al. (2012)	Scales on the body absent (or, when present, nonimbricate), embedded and arranged in “basket-weave fashion”	Ex
18	Johnson et al. (2012)	One or no hypohyals	Ex
19	Johnson et al. (2012)	Dorsal and anal fins confluent with caudal fin	Ex
20	Johnson et al. (2012)	Caudal fin rays of fewer than eight in each lobe	Ex
21	Johnson et al. (2012)	Posttemporal absent	Ex
22	Johnson et al. (2012)	Epurals absent	Ex
23	Johnson et al. (2012)	Pyloric caeca absent	Ex, U
24	Johnson et al. (2012)	Nostrils widely separated, the posterior one just anterior to the orbit	Ex, U
25	Johnson et al. (2012)	Gill membranes united across the isthmus, openings restricted	Ex, U
26	Springer and Johnson (2015)	*Levator interni* 3 absent	Ex
27	Springer and Johnson (2015)	*Musculus pharyngobranchialis* 2-*epibranchialis* 1 present	Ex
28	Springer and Johnson (2015)	One of the two *pharyngoclavicularis*	Ex
29	Springer and Johnson (2015)	*Rectus ventralis* 3 and 4 present	Ex
30	Springer and Johnson (2015)	*Rectus dorsalis* 2 present	Ex
31	Springer and Johnson (2015)	*Rectus communis* absent	Ex
32	Springer and Johnson (2015)	*Levator internus* 2 insertion includes UP4	Ex
33	Springer and Johnson (2015)	Hypobranchial 3 cartilaginous or absent	Ex
34	Springer and Johnson (2015)	Accessory element at distal end of ceratobranchial 4 absent	Ex
35	Current study	*Adductor mandibulae* originates on parietal	Ex
36	Current study	*Adductor mandibulae* lacks *segmentum mandibularis*	Ex

Note: C includes Cretaceous forms; Ex—extant forms only; U—state unknown for Cretaceous forms.

FIGURE 1 Lateral view of the left side of (a) *Protanguilla palau* (Anguilliformes: Protanguillidae) NSMT-P 98249 (176 mm standard length) and (b) *Synaphobranchus affinis* (Anguilliformes: Synaphobranchidae) USNM 198623 (325 mm total length).

TABLE 2 Proposed morphological synapomorphies of extant Anguilliformes, excluding *Protanguilla palau*

	Reference	Morphological character	Groups	
1	Johnson et al. (2012)	Premaxilla absent	Ex	J1
2	Johnson et al. (2012)	Symplectic fused to quadrate	Ex	J2
3	Johnson et al. (2012)	Metapterygoid absent	Ex	J3
4	Johnson et al. (2012)	Upper hypurals fused	Ex	J4
5	Johnson et al. (2012)	Gill rakers absent	Ex, C	J5
6	Johnson et al. (2012)	More than 90 vertebrae	Ex, C	J6
7	Johnson et al. (2012)	Pterotic approaches anterior margin of pterosphenoid	Ex, C	J7
8	Springer and Johnson (2015)	Basibranchial 3–4 and hypobranchial 3 complex	Ex, U	S1
9	Springer and Johnson (2015)	*Rectus ventralis 4 communis* present	Ex, U	S2
10	Current study	*Adductor mandibulae* originates on frontal	Ex, U	E1
11	Current study	*Adductor mandibulae* divided into *rictalis* and *stegalis*	Ex. U	E2
12	Current study	*Levator operculi* inserted on the lateral surface of the opercle	Ex, U	E3

Note: C includes Cretaceous forms; Ex—extant forms only; U—state unknown for Cretaceous forms.

2.1 | Phylogenetic inference

Table 1 lists 34 synapomorphies proposed by Johnson et al. (2012), Springer and Johnson (2015), and the current study for extant anguilliforms and clarifies which are also present, absent, or unknown for Cretaceous forms. Table 2 lists 12 of those characters which were mapped (optimized) on two recent and most significant phylogenetic topologies: Johnson et al. (2012) and Santini et al. (2013). The resulting cladograms summarize the hypotheses of relationships based on combined morphological and molecular data (Johnson et al., 2012; Figure 2a) and solely molecular (Santini et al., 2013; Figure 2b). We selected those two topologies due to conflicting placements of *P. palau*. Johnson et al. (2012) placed as the sister group of all other extant anguilliforms (Figure 2a), and Santini et al. (2013) placed Protanguillidae and Synaphobranchidae as sister groups, together representing the sister group of the remaining anguilliforms (Figure 2b).

We proposed solving the character reconstruction ambiguity in this study of maximum parsimony optimization. In the case of characters with ambiguous state distributions, we adopted ACCTRAN optimization (de Pinna, 1991; Swofford & Maddison, 1987), which favors state changes toward the root of the tree (Figure 2a,b).

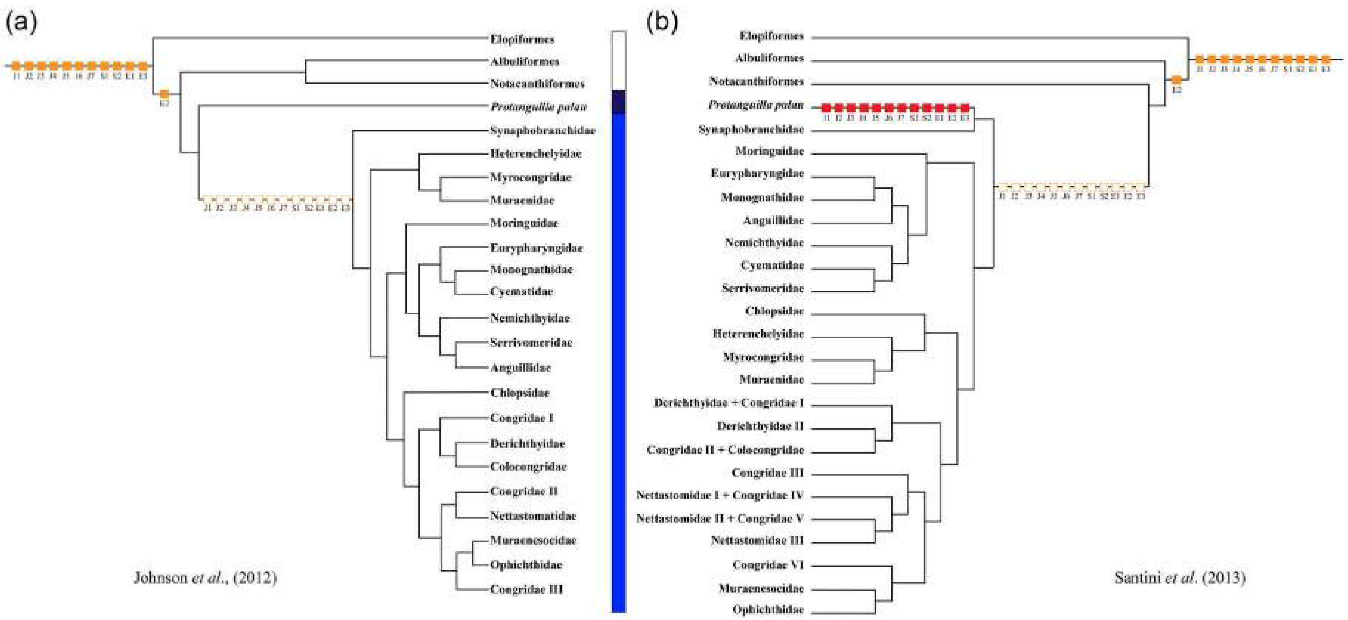

FIGURE 2 Main hypotheses of internal relationships based on morphological and molecular analysis: Johnson et al. (2012) on the left side (a), and molecular results of Santini et al. (2013) on right side (b). Facial muscle characters were optimized in maximum parsimony analysis. Numbers associated with nodes follow the order of the characters listed in Section 3. Presence and absence states are marked as solid and hollow orange blocks, respectively. Solid red squares represent the reversal states. The bar in the middle represents: dark blue, Protanguillidae; light blue, remaining eels, and white correspond to the outgroup.

2.2 | Materials examined

Institutional abbreviations follow Sabaj (2020, 2022) as described in the text. Specimen length is expressed as total length (TL).

Elopiformes: Elopidae: *Elops saurus*, Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ) 40531, 1 of 8, 273 mm TL, Rio de Janeiro, Brazil; *Elops affinis*, USNM 321767, 1 of 7, 130 mm TL, Pacific Panamá.

Albuliformes: Albulidae: *Pterothrissus gissu*, NSMT 74776, 2 of 5, 265 mm TL, Honshu, Japan; *P. gissu*, NSMT 91485, 1 of 5, 205 mm TL, Honshu, Japan; *Albula vulpes*, MNRJ 37303, 1 of 18, 126 mm TL, Rio de Janeiro, Brazil; *A. vulpes*, USNM 218871, 1 of 11, 87 mm TL, Coco Island, Mauritius.

Notacanthiformes: Halosauridae: *Halosaurus pectoralis*, USNM 317567, 1 of 7, 506 mm TL, Walters Shoal, Indian Ocean; *Halosauropsis macrochir*, VIMS 3110, 1 of 8, 552 mm TL, Northwest Atlantic Ocean; *Aldrovandia affinis*, USNM 319542, 1 of 5, 460 mm TL, Gulf of Mexico, United States; *Aldrovandia phalacra*, VIMS 8390, 1 of 4, 361 mm TL, *Halosaurus guentheri*, USNM 214336, 2 of 4, 220-430 mm TL, Gulf of Mexico, United States; *A. affinis*, USNM 455369, 1, C&S, 370 mm TL, Gulf of Mexico, United States.

Nothacanthidae: *Notacanthus sexspinis*, NSMT 66835, 1 of 3, 587 mm TL, New Zealand; *Polyacanthonotus rissoanus*, VIMS 4552, 1 of 5, 418 mm TL; *Notacanthus spinosus*, USNM 421321, 1, 241 mm TL, Pacific Panamá; *Lipogenys gillii*, MCZ 38072, 1, damaged, United States; *Lipogenys gillii*, MCZ 37613, 1, 350 mm TL, Canada.

Anguilliformes: Synaphobranchidae: *Synaphobranchus* sp., MNRJ 26716, 1 of 21, 430 mm TL, Espirito Santo, Brazil; *Synaphobranchus kaupii*, USNM 444949, 1, 195 mm TL, Virginia, United States; *Synaphobranchus* sp., USNM 316662, 1 of 10, damaged, South Carolina, United States; *Simenchelys parasitica*, USNM 31727, 1, 309 mm TL, Off Martha's Vineyard; *Dysomma anguillare*, ANSP 158368, 1, 365 mm TL, Philippine Islands; *Dysommina rugosa*, MZUSP 86463, 1 of 12, 288 mm TL, São Paulo, Brazil; *Meadia abyssalis*, MUSP 84460, 1, 467 mm TL, off Brazil, Western south Atlantic; *Diastobranchus capensis*, MNRJ 26783, 1 of 3, 780 mm TL, Bahia, Brazil; *S. parasitica*, SIO 05-11, 1 of 78, 123 mm TL, Darwin Seamount, Pacific Ocean; *Ilyophis nigeli*, NSMT 49061, 2 of 20, 280–314 mm TL, Honshu, Japan; *S. parasitica*, MNRJ 40772, 1 of 11, 144 mm TL, Rio de Janeiro, Brazil. Heterenchelyidae: *Pythonichthys asodes*, UF 228629, 1 of 3, 253 mm TL, Panama; Myrocongridae: *Myroconger nigrodentatus*, CAS 86426, 1, 240 mm TL, Galápagos Islands, Ecuador; Muraenidae: *Anarchias seychellensis*, MCZ 162323, 1, 65 mm TL, Kiribati; *Muraena lentiginosa*, CAS 214486, 1 of 9, 88 mm TL, Jalisco, Mexico; *Strophidon sathete*, USNM 217007, 1, 620 mm TL, Papua New Guinea; *Rhinomuraena quaesita*, USNM 338049, 1, 730 mm TL, Tonga Islands; *Uropterygius concolor*, CAS 218255, 1 of 11, 105 mm TL, Fiji; *Anarchias allardicei*, CAS 201807, 1 of 29, 98 mm TL, Micronesia; *Enchelycore nigricans*, ZUEC 10962, 1, 266 mm, Bahia, Brasil; *Monopenchelys acuta*, USNM 31284, 1 of 8, 150 mm TL, Dominica; *Echidna nebulosa*, USNM 312133, 1 of 6, 276 mm TL, Taiwan; *Gymnothorax vicinus*, MZUSP 11900, 1 of 15, 241 mm TL, Bahia, Brazil; *Echidna catenata*, MZUSP 46483, 1 of 2, 545 mm TL, Pernambuco, Brazil; *Gymnomuraena zebra*, SIO 62-8, 1 of 7, 149 mm TL, Mexico; Nemichthyidae: *Nemichthys scolopaceus*, ANSP 158461, 1 of 7, 513 mm TL, Ecuador; *Avocettina infans*, UF 215159, 1 of 2, 361 mm TL, Liberia; *N. scolopaceus*, USNM 358874, 1 of 5, 700 mm TL, Atlantic Ocean; *Labichthys carinatus*, ANSP 112054, 1, Damaged, Bahamas; *A. infans*, USNM 219558, 1 of 2, 719 mm TL, Pacific Hawaii; *N. scolopaceus*, USNM 383142, 1 of 3, 930 mm TL, off United States, Western North Atlantic; *N. scolopaceus*, USNM 447921, 1, 640 mm TL, off United States, Western North Atlantic; *N. scolopaceus*, USNM 454801, 1, 670 mm TL, C&S, off United States, Western North Atlantic; *A. infans*, USNM 454802, 1, C&S, 420 mm TL, Pacific Hawaii; *Labichthys yanoi*, ANSP 124929, 1, C&S, 390 mm, South Australia, Indian Ocean. Colocongridae: *Coloconger meadi*, USNM 443687, 1, 333 mm TL, Suriname; *C. meadi*, UF 211407, 1 of 2, 249 mm TL, Nicaragua; *C. meadi*, USNM 198761, 1, 380 mm TL, Venezuela; Derichthyidae: *Derichthys serpentinus*, USNM 444953, 1, 112 mm TL, Bermuda; *D. serpentinus*, SIO 73-132-26, 1, 104 mm TL, Oahu: off Waianae; *Nessorhamphus danae*, SIO 73-166, 1 of 3, 105 mm TL, Pacific Ocean; Nettastomatidae: *Nettastoma melanurum*, USNM 405025, 1, 505 mm TL, Cape Verde; *N. melanurum*, MNRJ 26821, 2 of 5, 505-512 mm TL, Bahia, Brazil; *Facciolella equatorialis*, SIO 91-72, 1 of 33, 296 mm TL, off Point Loma; *Venefica procera*, MNRJ 26806, 1 of 22, 1091 mm TL, Bahia, Brazil; *Hoplunnis tenuis*, USNM 27115, 1, 320 mm TL, Suriname; Moringuidae: *Neoconger vermiformis*, USNM 444957, 1 of 2, 95 mm TL, Canal Zone, Panama; *Moringua raitaborua*, USNM 406625, 1 of 9, 225 mm TL, Negros Occidental, Philippines; *Moringua edwardsi*, MZUSP 11810, 1 of 20, 263 mm TL, Bahia, Brazil; Muraenesocidae: *Oxyconger leptognathus*, NMMB P022865, 1 of 6, 270 mm TL, Kaohsiung, Taiwan; *Muraenesox cinereus*, CAS 51089, 1 of 10, 235 mm TL; *M. cinereus*, USNM 431418, 1, Damaged, Ba Ria Vung Tau, Vietnam; Serrivomeridae: *Serrivomer beanii*, USNM 444955, 1, 321 mm TL, Bermudas; *Serrivomer* sp., USNM 443689, 1, 610 mm TL, North Pacific Ocean; *Serrivomer* sp., USNM 447922, 1, 293 mm TL, North Pacific Ocean; *Serrivomer schmidti*, MNRJ 26850, 1 of 7, 576 mm TL, Bahia, Brazil; *Stemonidium hypomelas*, ANSP 152332, 1 of 2, 300 mm TL, Hawaii, United States; Anguillidae: *Anguilla rostrata*, MCP 28163, 1 of 7, 190 mm TL, Maryland, United States: *A. rostrata*, USNM 444959, 1, 133 mm TL, Roatan, Honduras; *A. rostrata*, USNM 190998, 1 of 5, Damaged, North Carolina, United States; *A. marmorata*, USNM 191271, 1 of 4, 178 mm TL, Hualien County, Taiwan; Chlopsidae: *Kaupichthys* sp., USNM 444952, 1 of 2, 97 mm TL, Rotuma, Fiji; *Chilorhinus suensoni*, MZUSP 18431, 1 of 18, 116 mm TL, Bahia, Brazil; *Kaupichthys japonicus*, USNM 364736, 1 of 11, 110 mm TL, Solomon Islands; *Kaupichthys hyoproroides*, ANSP 190028, 1 of 3, 176 mm TL, Great Bahama Bank, Bahamas; Cyematidae: *Cyema atrum*, SIO 66-546, 1 of 2, 96 mm TL, SW of Cortez Bank, United States; *Neocyema* sp., MCZ 165900, 1, 103 mm TL, United States; *C. atrum*, MCZ 47843, 1, 109 mm TL, South Pacific; *C. atrum*, USNM 208060, 1 of 3, 35 mm TL, South Pacific; Eurypharyngidae: *Eurypharynx pelecanoides*, SIO 73-43, 1 of 4, 350 mm TL, California; *E. pelecanoides*, USNM 348531, 1, 505 mm TL, off Western Sahara, Eastern North Atlantic; *E. pelecanoides*, USNM 215621, 1, C&S, 690 mm TL, Hawaiian islands, Eastern North Pacific; Congridae: *Macrocephenchelys brevirostris*, USNM 443688, 1, 370 mm TL, Taiwan; *Xenomystax congroides*, USNM 444950, 1 of 2, 325 mm TL, Lesser Antille, Virgin Islands; *Macrocephenchelys brachialis*, NMMB P025654, 1 of 5, 236 mm TL, Pingtung, Taiwan; *Rhynchoconger gracilior*, FSBC 31507, 1 of 5, 361 mm TL, Florida, United States; *Gavialiceps taeniola*, ANSP 113805, 1 of 4, 725 mm TL, Indian ocean; *Conger cinereus*, USNM 115969, 1 of 19, 345 mm TL, Tutuila, American Samoa; *Gorgasia punctata*, CAS 20752, 1 of 93, 289 mm TL, Nayarit, Mexico; *Heteroconger camelopardalis*, MZUSP 57611, 1 of 3, 304 mm TL, Pernambuco, Brazil; *Heteroconger klausewitzi*, USNM 316033, 1 of 6, 215 mm TL, Galapagos Islands, Ecuador; *Acromycter perturbator*, MNRJ 27115, 1 of 16, 260 mm TL, Rio de Janeiro, Brazil; *Conger orbignianus*, MZUSP 11950, 1 of 4, 510 mm TL, Rio Grande do Sul, Brazil; *Bathycongrus dubius*, MZUSP 12290, 1 of 15, 222 mm TL, Brazil; *Ariosoma* sp., MZUSP 12060, 1 of 14, 305 mm TL, São Paulo, Brazil; *Congriscus megastoma*, NSMT 57574, 3 of 14, 259 mm TL, Shikoku Tosa Bay, Japan; *Gnathophis ginanago*, NSMT 65815, 1 of 5, 330 mm TL, East China Sea; *Bathyuroconger vicinus*, MNRJ 26813, 1 of 4, 745 mm TL, Bahia, Brazil; *C. megastoma*, USNM 150199, 1 of 3, 330 mm TL, Japan; *Pseudophichthys splendens*, USNM 195904, 1 of 27, 240 mm TL, off United States, Western North America. Ophichthidae: *Ophichthus gomesii*, MNRJ 32607, 1 of 3, 368 mm TL, Rio de Janeiro, Brazil; *Myrichthys ocellatus*, MZUSP 12027, 1 of 10, 464 mm TL, Bahia, Brazil; *Stictorhinus potamius*, MNRJ 30419, 1 of 65, 275 mm TL, Rio Tocantis, Brazil; *Bascanichthys* sp., MZUSP 12008, 1 of 3, 365 mm TL, Bahia, Brazil; *Letharchus velifer*, MZUSP 12020, 1 of 40, 197 mm TL, Bahia, Brazil; *Pseudomyrophis frio*, MZUSP 12309, 1, 214 mm TL, Brazil; *Ahlia egmontis*, MZUSP 11997, 1 of 14, 142 mm TL, Bahia, Brazil; *Myrophis punctatus*, MZUSP 60779, 1 of 10, 204 mm TL, Bahia, Brazil; *Yirrkala misolensis*, NMMB P027671, 1 of 2, 361 mm TL, Taiwan; *Neenchelys mccoskeri*, NMMB P027671, 1 of 3, 310 mm TL, Donggang, Pingtung, Taiwan; *Echelus uropterus*, NSMT 63505, 1 of 3, 350 mm TL, East China Sea; *Schismorhynchus labialis*, USNM 444954, 1 of 2, 142 mm TL, Tonga Islands, Tonga; *Callechelys catostoma*, USNM 444951, 1, 540 mm TL, Eniwetok Atoll, Marshall Islands; Protanguillidae: *P. palau*, USNM 396051, 1, 141 mm TL, Palau; *P. palau*, USNM 443818, 1 of 2, 90 mm, Palau; Monognathidae: *Monognathus jesperseni*, MCZ 164702, 1, 124 mm, United States. *Monognathus rosenblatti*, SIO 86-42, 1 of 4, 48 mm TL, No Central Gyre; *M. rosenblatti*, SIO 86-43, 2 of 11, 50–51 mm TL, No Central Gyre; Saccopharyngidae: *Saccopharynx ampullaceus*, 1, 298 mm TL, 40°43′ N/66°36′ W, Atlantic Ocean: slope Water, United States.

3 | RESULTS

As described by Johnson et al. (2012), *Protanguilla* exhibits several remarkably unexpected plesiomorphic structures relative to extant anguilliforms (e.g., the presence of a premaxilla). The most parsimonious interpretation of these features is that they have been lost in all other extant anguilliforms. The relaxed molecular-clock analysis of anguilliforms estimated that *Protanguilla* diverged from the remainder of the order approximately 220 Myr, which, together, shows a

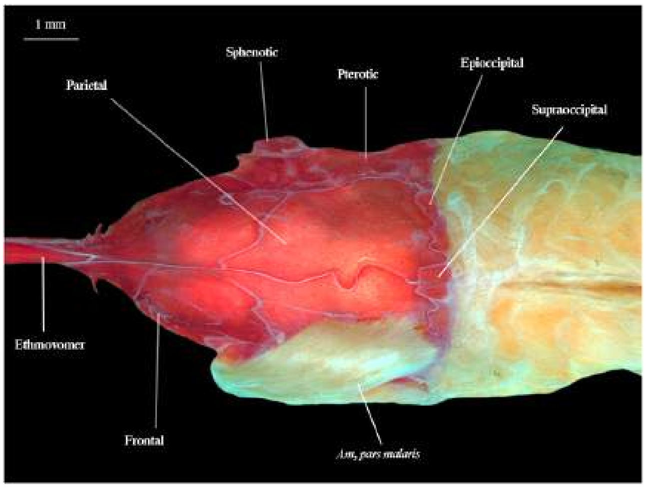

FIGURE 3 Dorsal view of the neurocranium of *Protanguilla palau* (Anguilliformes: Protanguillidae) USNM 443818 (90 mm total length). AM, *adductor mandibulae*.

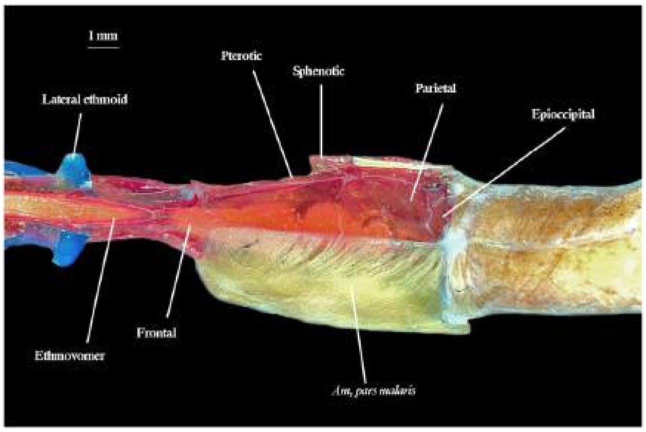

FIGURE 4 Dorsal view of the neurocranium of *Hoplunnis tenuis* (Anguilliformes: Nettastomatidae) USNM 444956 (320 mm total length). AM, *adductor mandibulae*.

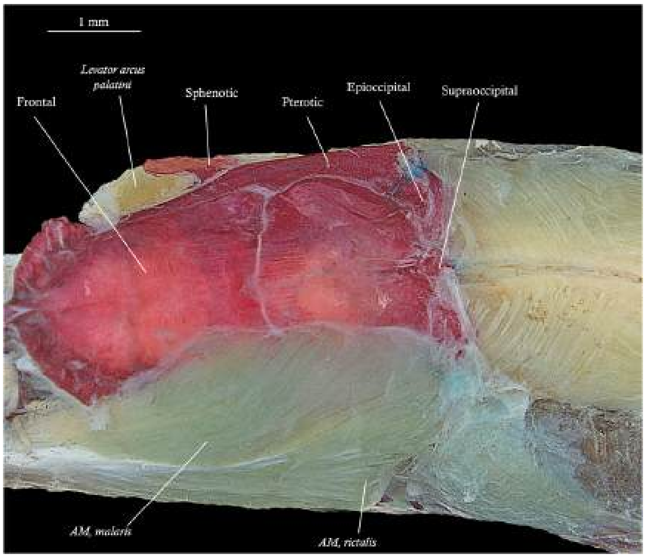

FIGURE 5 Dorsal view of the neurocranium of *Acromycter perturbator* (Anguilliformes: Congridae) MNRJ 27115 (260 mm total length). AM, *adductor mandibulae*.

multitude of plesiomorphic characters for the group, led the authors to suggest that it is a "living fossil."

In the cladogram (Figure 2), characters listed by Johnson et al. (2012) and Springer and Johnson (2015) are indicated by orange (plesiomorphic), white (synapomorphy), and red (reversals) squares. The characters are numbered as in Johnson et al. (2012, pp. 6–7) and Springer and Johnson (2015, pp. 618–619), and so designated by the first letter of the author's surname as in Table 2, where they are described as follows: premaxilla absent versus present (J1); symplectic fused versus free from quadrate (J2); metapterygoid absent versus present (J3); upper hypurals fused versus free (J4); gill rakers absent versus present (J5); vertebrae more versus fewer than 90 (J6); pterotic does not versus does approach anterior margin of pterosphenoid (J7); *rectus ventralis 4 communis* present versus absent (S1); and configuration of basibranchial 3, basibranchial 4, and hyobranchial 3 complex (S2; see Springer & Johnson, 2015, pp. 618–619). Newly discovered facial and opercular musculature synapomorphies of extant eels (unknown for Cretaceous forms), lacking in *Protanguilla*, are labeled 1–3 and described as follows:

- *E1—Adductor mandibulae* originates on the frontal (Figures 3–11, character E1—Table 2 and Figure 2). This most robust portion of the *segmentum facialis* of *M. adductor mandibulae* originates on the frontals in all eels, except *Protanguilla*. In most cases, it covers the top of the head entirely, the few exceptions being colocongrids, *A. perturbator* (Congridae), and *D. anguillare* (Synaphobranchidae). In those exceptions, the *adductor mandibulae* also originate on the frontal, but does not cover the top of the head. In most derived saccopharyngoid eels, the *adductor mandibulae* are restricted to the suspensorium.
- *E2—Adductor mandibulae* is divided into *pars epistegalis* and *substegalis* (Figure 12; character E2—Table 2 and Figure 2). Almost all anguilliforms bear two subsections of the "hidden" part of the *adductor mandibulae*. The *pars stegalis* is covered entirely by the *malaris* and *rictalis*, and it is separated into two sections, except in *Protanguilla*, where the *rictalis* and *stegalis* are undivided. The undivided *ricto-stegalis* of *Protanguilla* is similar to that of basal elopomorphs, except *Elops*, where the *segmentum mandibularis* is undivided. An undivided *adductor mandibulae* is otherwise seen only in the most phylogenetically distal saccopharyngoid eels, that is, eurypharyngids, monognathids, and saccopharyngids.
- *E3—Levator operculi* is inserted on the lateral surface of the opercle (Figures 6–10 and 13; character E3—Table 2 and Figure 2). In all elopomorphs, the *levator operculi* originates on the posterior portion of the pterotic. However, its insertion exhibits two different arrangements. In *Protanguilla* and basal elopomorphs, the *levator operculi* inserts on the medial surface of the opercle. By contrast, in the remaining eels, it inserts on the lateral face of the opercle.

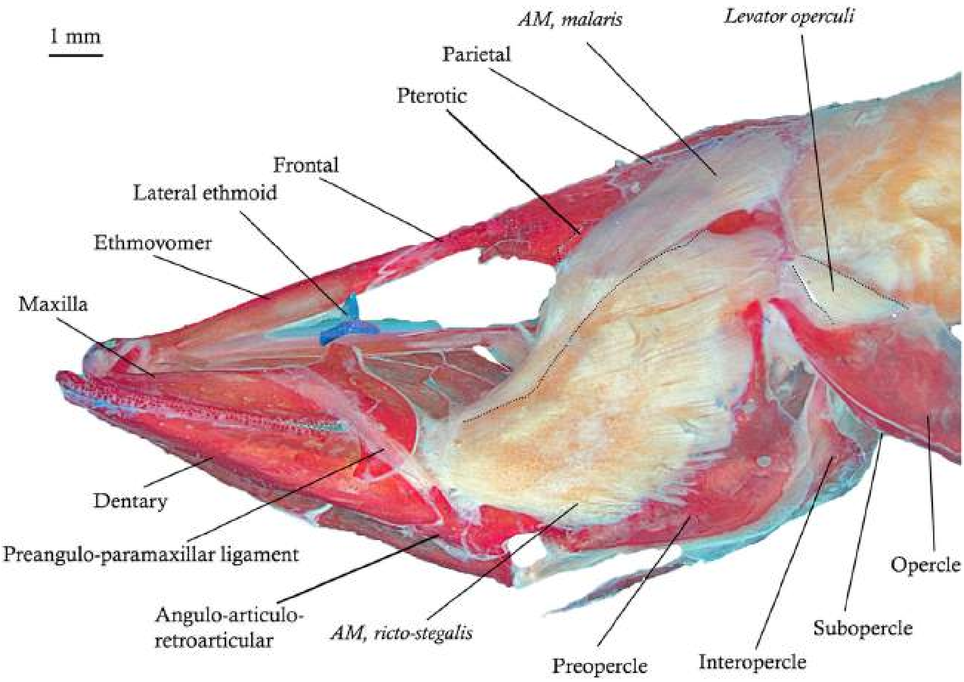

FIGURE 6 Lateral view of left side of the *segmentum facialis* of the *adductor mandibulae* of *Protanguilla palau* (Anguilliformes: Protanguillidae) USNM 443818 (90 mm total length). AM, *adductor mandibulae*.

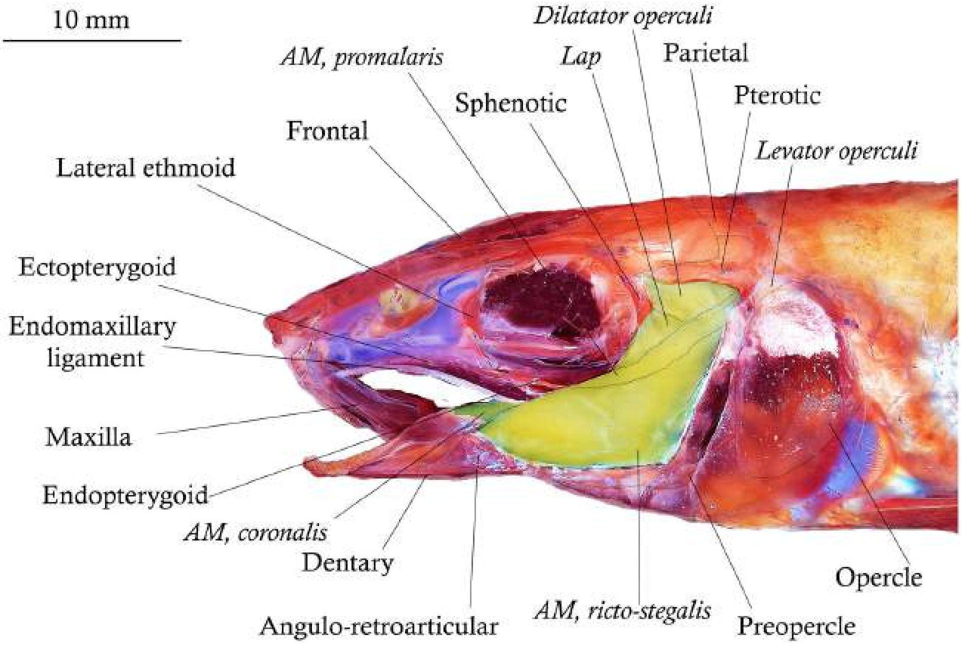

FIGURE 7 Lateral view of the left side of the *segmentum facialis* of the *adductor mandibulae* of *Albula vulpes* (Albuliformes: Albulidae) USNM 218871 (87 mm total length). AM, *adductor mandibulae*; LAP, *levator arcus palatini*.

Two additional new synapomorphies of Anguilliformes are not mapped because they are not relevant to the goal of this study (see more in Table 1), but they are mentioned because of their potential importance for future larger-scale studies. Both are exclusive to extant eels (unknown for Cretaceous forms), including *Protanguilla*, and absent in other elopomorphs:

- *Adductor mandibulae* originates on the parietal (Figures 3–11 and Table 1). In Anguilliformes, the dorsal portion of the *adductor*

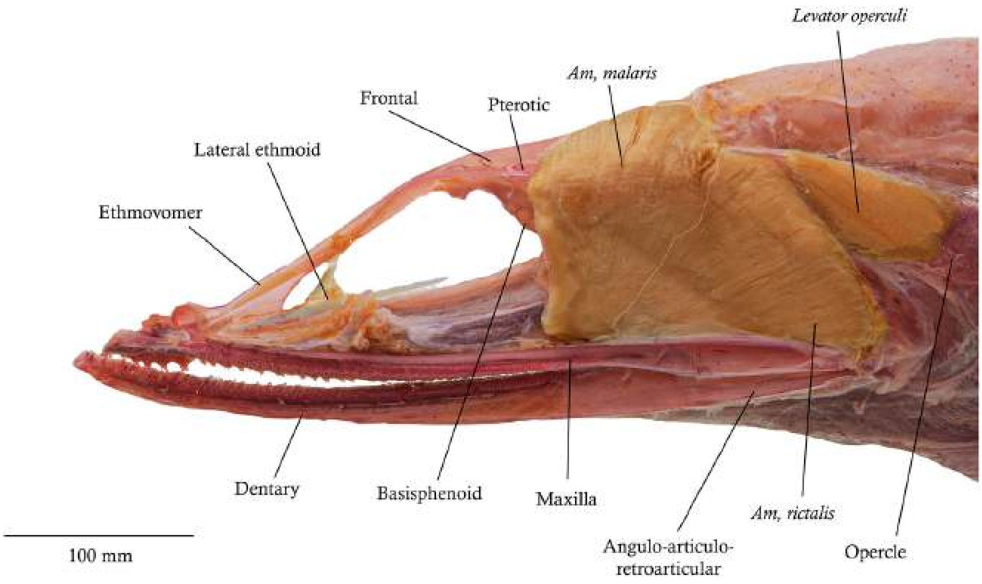

FIGURE 8 Lateral view of the left side of the *segmentum facialis* of the *adductor mandibulae* of *Synaphobranchus kaupii* (Anguilliformes: Synaphobranchidae) USNM 444949 (195 mm total length). AM, *adductor mandibulae*.

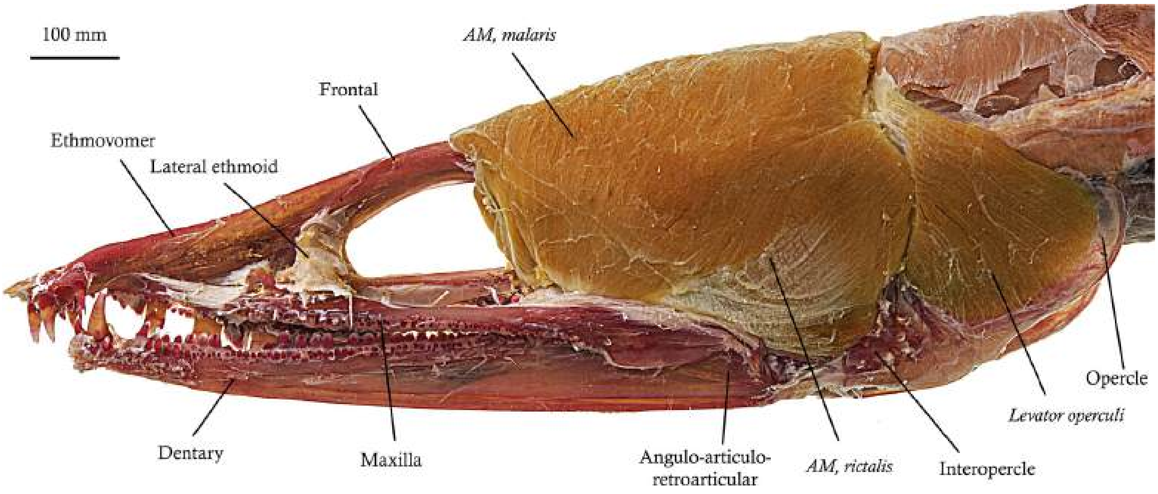

FIGURE 9 Lateral view of the left side of the *segmentum facialis* of the *adductor mandibulae* of *Muraenesox cinereus* (Anguilliformes: Muraenesocidae) USNM 431418 (specimen damaged). AM, *adductor mandibulae*.

mandibulae originates on the dorsal face of the parietal, in contrast to the condition in basal elopomorphs where the muscle originates on the hyomandibular. The exception in eels occurs in phylogenetically distal saccopharyngoids (eurypharyngids, monognathids, and saccopharyngids).

– *Adductor mandibulae* lacks the *segmentum mandibularis* (Figure 12 and Table 1). In all anguilliforms, the *adductor mandibulae segmentum facialis* inserts through a mandibular tendon directly onto the dentary. In the remaining elopomorphs, except notacanthids, the *adductor mandibulae facialis* inserts on the *segmentum mandibularis* through the intersegmental aponeurosis.

4 | DISCUSSION SUMMARY OF NEW CHARACTERS AND RESPECTIVE CLADES

Monophyly of the Anguilliformes has rarely been questioned (see Obermiller & Pfeiler, 2003; Robins, 1989) and is corroborated by numerous recent studies (Belouze, 2002; Chen et al., 2013; Forey et al., 1996; Johnson et al., 2012; Santini et al., 2013; Smith, 1984; Springer & Johnson, 2015). Johnson et al. (2012) and Springer and Johnson (2015) proposed a total of 34 morphological synapomorphies based on osteological, gill arch-muscle, and external morphology (Table 1), and we added two additional synapomorphies based on

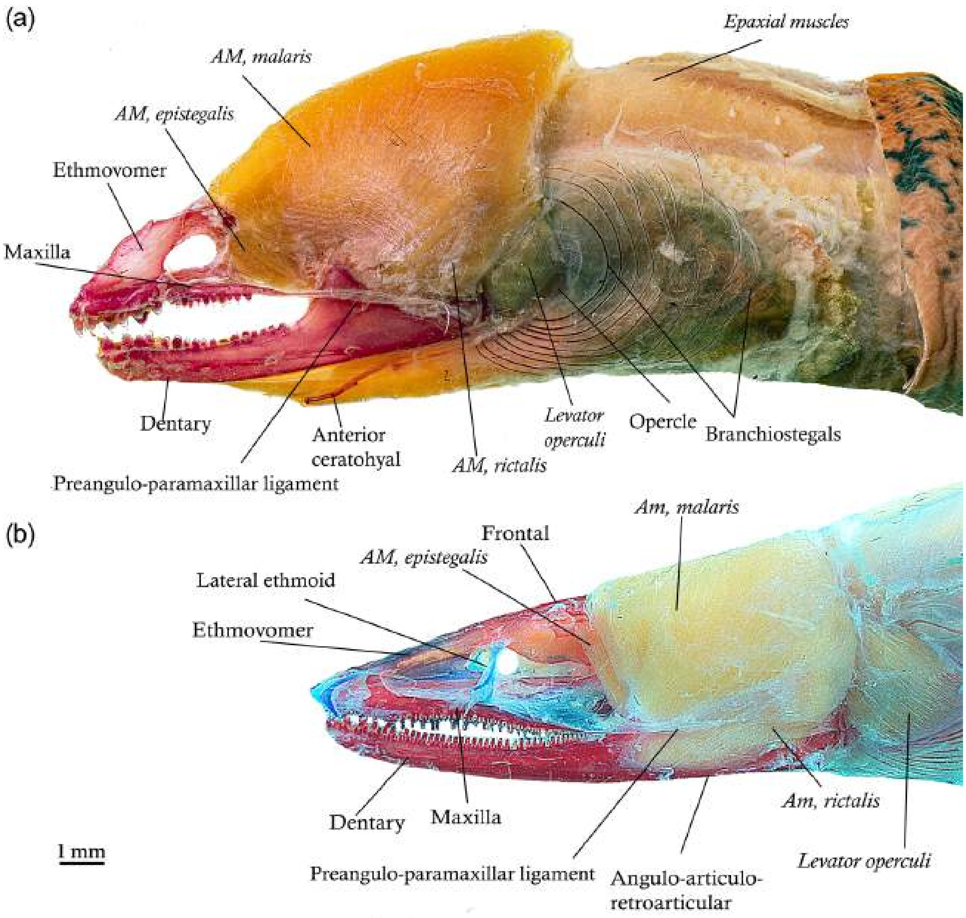

FIGURE 10 Lateral view of the left side of the *segmentum facialis* of the *adductor mandibulae* of (a) *Echidna nebulosa* (Anguilliformes: Muraenidae) USNM 312133 (276 mm total length) and (b) *Kaupichthys japonicus* (Elopomorpha: Anguilliformes: Chlopsidae) USNM 364736 (110 mm total length). AM, *adductor mandibulae*.

the facial and opercular muscles examined in this study. The latter two are as follows: absence of the *adductor mandibulae segmentum mandibularis* and *adductor mandibulae* originating on the parietal (Table 1, 35–36 numbers, respectively; neither character was mapped on the cladogram). Additionally, we propose three new synapomorphies for the "advanced eels" excluding *Protanguilla*, as follows: *Adductor mandibulae* originate on the frontal, *adductor mandibulae* divided into *pars epistegalis* and *substegalis*, and *levator operculi* inserts on the lateral surface of the opercle (Table 2).

A primary goal of this study was to investigate new morphological evidence from the facial and opercular musculature of Anguilliformes in a phylogenetic context and present it in two or more case studies. The first of these (herein) is focused on the placement of the putative "living fossil eel," *P. palau*. As noted above, we discovered three synapomorphies that corroborate the conclusion of Johnson et al. (2012, p. 9) that "…*Protanguilla* appears to represent an ancient independent evolutionary lineage within anguilliforms, whose placement within the basal anguilliforms is difficult in a molecular phylogenetic context." In light of that, we decided to reiterate the previously proposed relevant, morphologically diverse characters

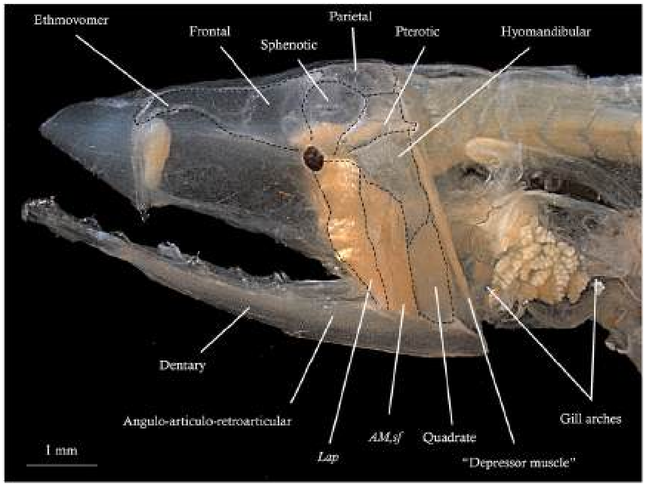

FIGURE 11 Lateral view of the left side of the *segmentum facialis* of the *adductor mandibulae* of *Monognathus rosenblatti* (Anguilliformes: Monognathidae) SIO 86-43 (50 mm total length). AM, sf, *adductor mandibulae*, *segmentum fascialis*; lap, *levator arcus palatini*.

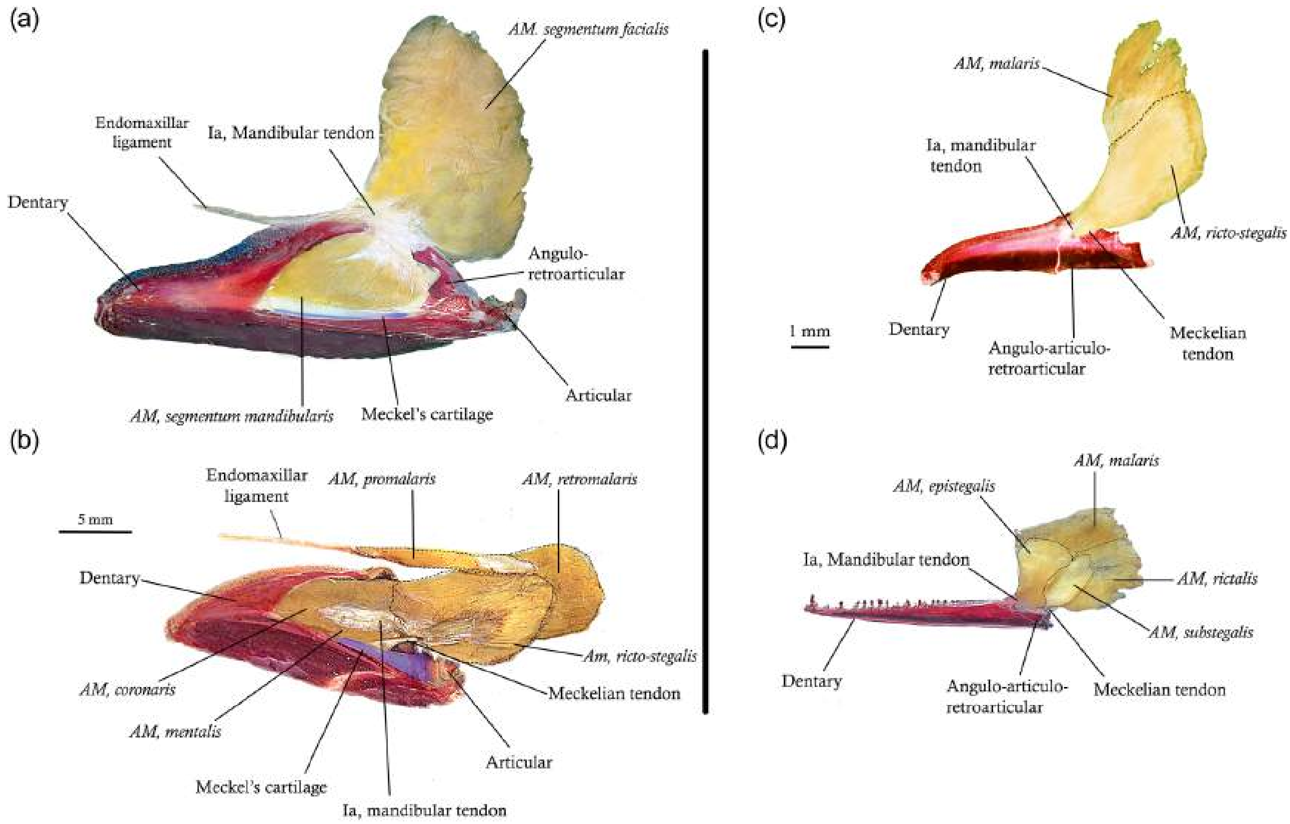

FIGURE 12 *Segmentum facialis* of the *adductor mandibulae* and lower jaw structures of (a) *Elops saurus* (Elopiformes: Elopidae) MNRJ 40531 (273 mm total length); (b) *Aldrovandia phalacra* (Notacanthiformes: Halosauridae) VIMS 8390 (361 mm total length); (c) *Protanguilla palau* (Anguilliformes Protanguillidae) USNM 443818 (90 mm total length); and (d) *Hoplunnis tenuis* (Elopomorpha: Anguilliformes: Nettastomatidae) USNM 444956 (320 mm total length). AM, *adductor mandibulae*; Ia, intersegmental aponeurosis.

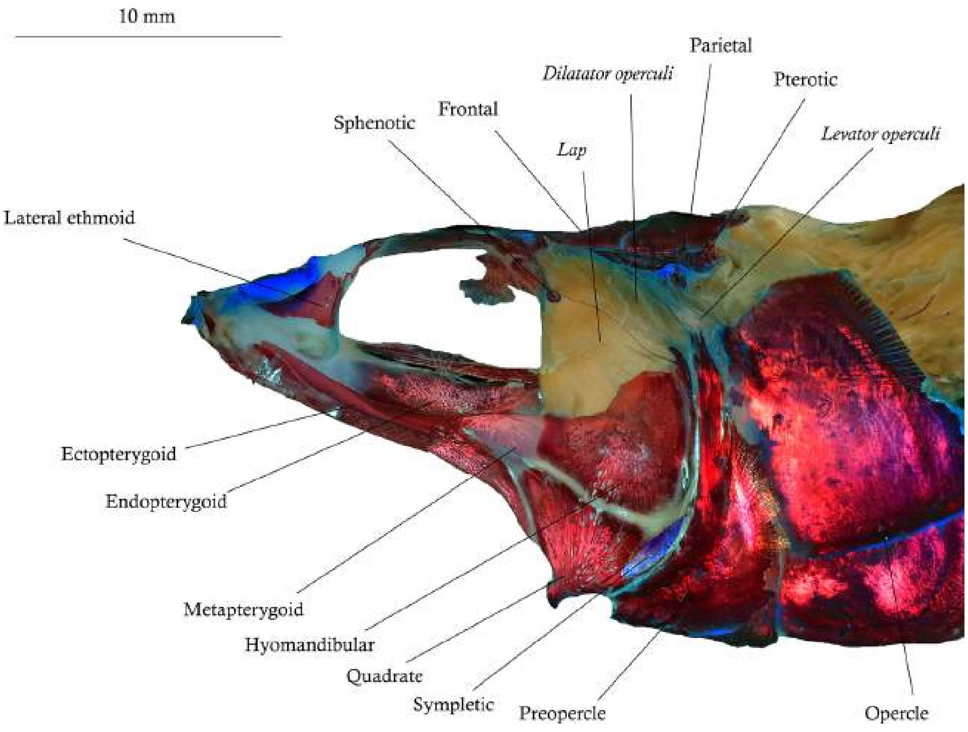

FIGURE 13 Lateral view of the left side of the *levator arcus palatini*, *dilatator operculi*, and *levator operculi* of *Elops saurus* (Elopiformes: Elopidae) USNM 321767 (130 mm standard length). The lower jaw and the *segmentum facialis* of the *adductor mandibulae* were removed. AM, *adductor mandibulae*; LAP, *levator arcus palatini*.

(Table 2), and explicitly test these by optimizing them on the two conflicting hypotheses (Figure 2). The 12 reversals required to accept the molecular hypothesis emanate from nine putatively independent osteological plesiomorphies of the jaws, suspensorium, gill arches, vertebral column, caudal skeleton, and braincase, and three of the gill-arch and facial and opercular muscles. The implausibility that all these morphological characteristics are plesiomorphically absent in eels and reappear only in *Protanguilla* is self-evident.

AUTHOR CONTRIBUTIONS

Vinicius C. Espíndola: Investigation; writing – original draft; writing – review and editing; supervision; methodology; conceptualization; project administration; software; data curation; resources. **G. David Johnson**: Methodology; investigation; conceptualization; funding acquisition; writing – original draft; writing – review and editing; software; data curation; validation; resources. **Mario C. C. De Pinna**: Visualization; writing – review and editing; writing – original draft; investigation; conceptualization; software; methodology; data curation; validation; resources.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no data sets were generated or analyzed during the current study.

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