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# A New Endogean, Dwarf, and Troglomorphic Species of Swamp Eel of the Genus *Ophisternon* (Synbranchiformes: Synbranchidae) from Costa Rica: Evidence from Comparative Mitogenomic and Anatomical Data

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and Melanie L. J. Stiassny[2]

**A new highly troglomorphic and diminutive swamp eel inhabiting muddy subsoil in remnants of a tropical rainforest in the Caribbean versant of Costa Rica is described. Comparative anatomical and mitogenomic data support the distinctiveness of the new species and its placement in the genus *Ophisternon*. The new species is unique among Neotropical congeners in having: 1) a greatly elevated number of precaudal vertebrae, 2) proportionally longer and larger premaxillary, dentary, palatine, and ectopterygoid teeth, 3) palatine and ectopterygoid teeth in a single row, and 4) a small, narrow, and crescent-shaped gill membrane opening. A novel phylogenetic hypothesis of synbranchid relationships proposed herein, derived from comparative mitogenomic data, adds to a body of evidence demonstrating that *Ophisternon* is not monophyletic (with respect to *Synbranchus*). This phylogeny, however, strongly supports the monophyly of Neotropical *Ophisternon*, with the new species resolved as sister to a clade consisting of *Ophisternon infernale* + *Ophisternon aenigmaticum*. A pattern of northwestern lineage dispersal and cladogenesis within the Neotropical clade of *Ophisternon* after its divergence from *Synbranchus* is inferred from our phylogenetic results and present-day species distributions. Our findings also reinforce the notion that the classification of synbranchid fishes is in dire need of a systematic and comprehensive revision, particularly with respect to the limits and composition of the genera with presence in the Neotropics.**

**Se describe una nueva anguila de pantano diminuta y altamente troglomorfa que habita el subsuelo fangoso en remanentes de una selva tropical en la vertiente Caribe de Costa Rica. Datos anatómicos y mitogenómicos comparados respaldan el carácter distintivo de la nueva especie y su ubicación en el género *Ophisternon*. La nueva especie es única entre sus congéneres neotropicales por presentar: 1) un número muy elevado de vértebras precaudales, 2) dientes premaxilares, dentarios, palatinos y ectopterigoideos proporcionalmente más largos y grandes, 3) dientes palatinos y ectopterigoideos en una sola fila, y 4) la abertura de la membrana branquial pequeña, estrecha, y en forma de media luna. Una nueva hipótesis de relaciones filogenéticas entre sinbránquidos aquí propuesta, derivada de datos mitogenómicos comparados, se suma a un conjunto de evidencia que demuestra que *Ophisternon* no es monofilético (con respecto a *Synbranchus*). Esta filogenia, sin embargo, apoya firmemente la monofilia de las especies neotropicales de *Ophisternon*, con la nueva especie resuelta como hermana de un clado que consiste en *Ophisternon infernale* + *Ophisternon aenigmaticum*. A partir de nuestros resultados filogenéticos y las distribuciones actuales de las especies, se infiere un patrón de dispersión y cladogénesis de linajes en dirección noroeste para el clado de las especies neotropicales de *Ophisternon* tras su divergencia de *Synbranchus*. Nuestros hallazgos además refuerzan la noción de que la clasificación de los peces sinbránquidos necesita urgentemente una revisión sistemática y exhaustiva, particularmente con respecto a los límites y la composición de los géneros con presencia en el Neotrópico.**

THE family Synbranchidae is a lineage of highly modified percomorph fishes colloquially known as swamp eels due to their morphological resemblance to true eels (Anguilliformes) and the burrowing habits—in swamps and marshes—of many included species (Rosen and Greenwood, 1976; Nelson et al., 2016). Swamp eels are found on all southern continents except Antarctica, in freshwater and sometimes estuarine habitats (Rosen and Greenwood, 1976). Because of their limited tolerance to high salinities (as secondary freshwater fishes)—although see Tyler and Feller (1996)—attempts to explain their near global distribution have historically relied on Gondwanan vicariance scenarios (Rosen, 1975, 1978; Mar-Silva et al., 2022). More recently, however, a time-scaled phylogeny of all major synbranchiform lineages has challenged the vicariance hypothesis in favor of pan-global rafting (Harrington et al., 2024). The taxonomic history of swamp eels has been no less convoluted, afflicted by numerous nomenclatural changes and limited resolution of phylogenetic relationships. The absence of significant meristic and morphometric variation, partly due to their highly modified morphology (lack of paired fins and scales, vestigial unpaired fins), has posed serious challenges to our understanding of synbranchid generic- and species-level taxonomy and consequently of phylogenetic relationships

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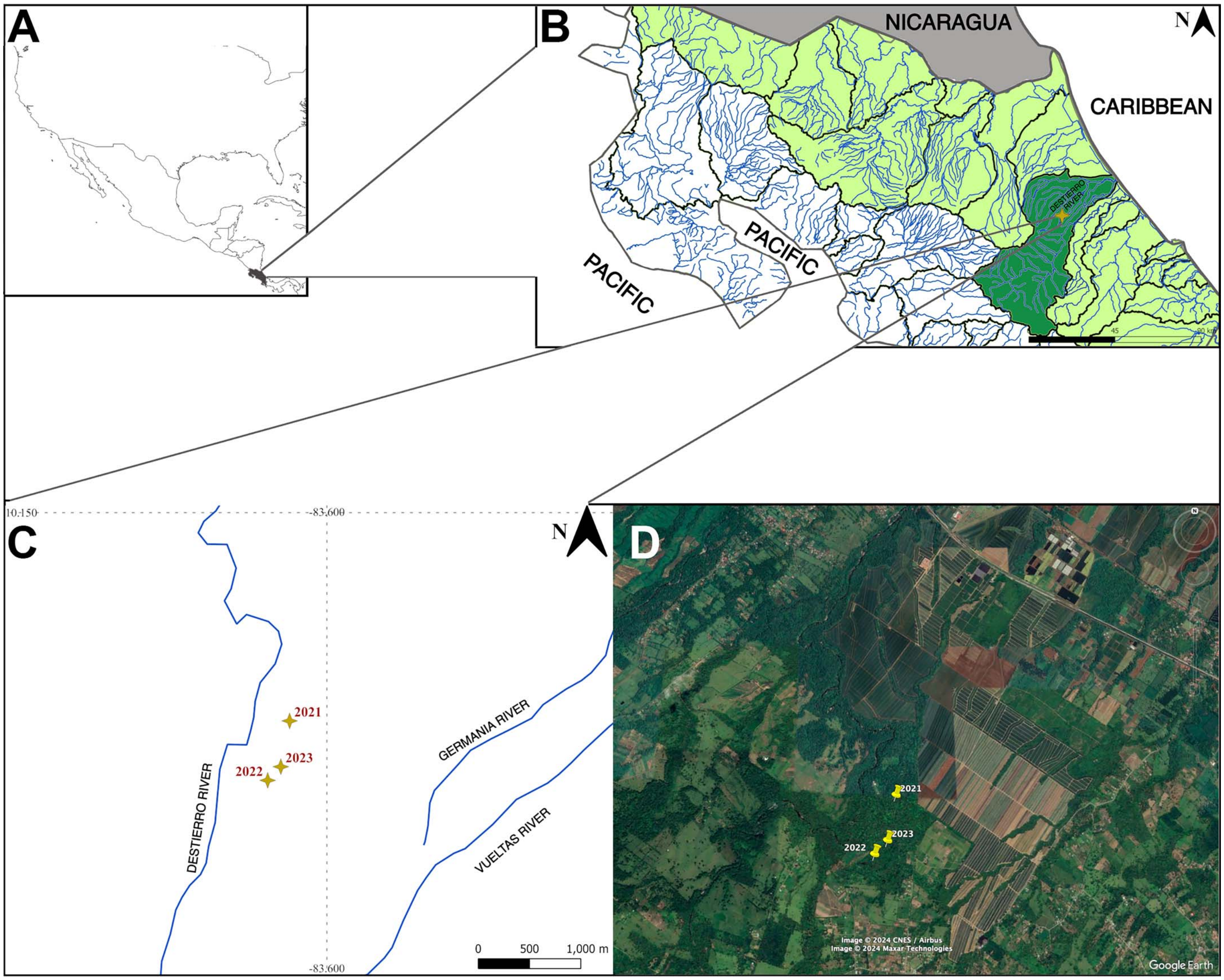

**Fig. 1.** Geographic distribution of *Ophisternon berlini*, new species. Location of the type locality at the (A) continental scale (highlighting Costa Rica), (B) national scale (northern Cost Rica, with Pacific versant basins in white, the Reventazón-Parismina drainage basin highlighted in dark green, and the remaining Atlantic versant basins highlighted in light green), and local scale via (C) map and (D) satellite image (from Google Earth; images copyright 2024 CNES/Airbus and 2024 Maxar Technologies). Point localities from the different collecting events in the premises of Las Brisas Nature Reserve (located on the east bank of the Destierro River) can be seen in C and D. The aerial satellite photograph (D) displays the striking differences in land use and land cover between the reserve (well-preserved secondary forest) and the surrounding areas (mainly agricultural). Basemap layers of major rivers and drainage basins of Costa Rica downloaded from the Ríos de Costa Rica ArcGIS Hub (https://hub.arcgis.com/datasets/geotec::ríos-de-costa-rica/about) and the Sistema Nacional de Información Territorial de Costa Rica (https://www.snitcr.go.cr), respectively.

(Rosen and Greenwood, 1976; Favorito-Amorim and Menezes, 1993, 1998; Favorito et al., 2005).

As currently recognized, Synbranchidae comprises 27 valid species classified in seven genera: *Macrotrema*, *Monopterus*, *Ophichthys*, *Ophisternon*, *Rakthamichthys*, *Synbranchus*, and *Typhlosynbranchus*. Neotropical swamp eels are restricted to the genera *Synbranchus* (4 spp.) and *Ophisternon* (2 spp.). While *Synbranchus* is endemic to Central and South America, *Ophisternon* also includes species in other continents: one in Africa, one in Asia, and two in Oceania (Fricke et al., 2024). The Neotropical species of *Ophisternon* are *O. infernale*, troglobitic and endemic to the cenotes and submerged caves of the Yucatán Peninsula karst aquifer (Hubbs, 1938; Arroyave et al., 2019), and *O. aenigmaticum*, distributed from southern Mexico to Honduras, including Belize and Guatemala (Rosen and Greenwood, 1976). Several records of synbranchids from the Antilles, Costa Rica, and northern South America have been tentatively regarded as *O. aenigmaticum*, effectively broadening the extent of occurrence of *Ophisternon* in the Neotropics (Rosen and Greenwood, 1976; Sáenz Sánchez et al., 2006; Fricke et al., 2024). However, it has also been argued that these geographic outliers could represent either undescribed species or misidentified specimens of the partially sympatric *Synbranchus marmoratus*, and that *O. aenigmaticum* is in fact restricted to the central portion of continental Middle America (Rosen and Greenwood, 1976; Bussing, 1998; Miller, 2005; Perdices et al., 2005).

Distinguishing between *Ophisternon* and *Synbranchus* based on external morphology is indeed not straightforward. Superficially, these genera look very much alike, and the main external character used to differentiate them is the shape of the gill opening. In both genera, the branchiostegal membrane is

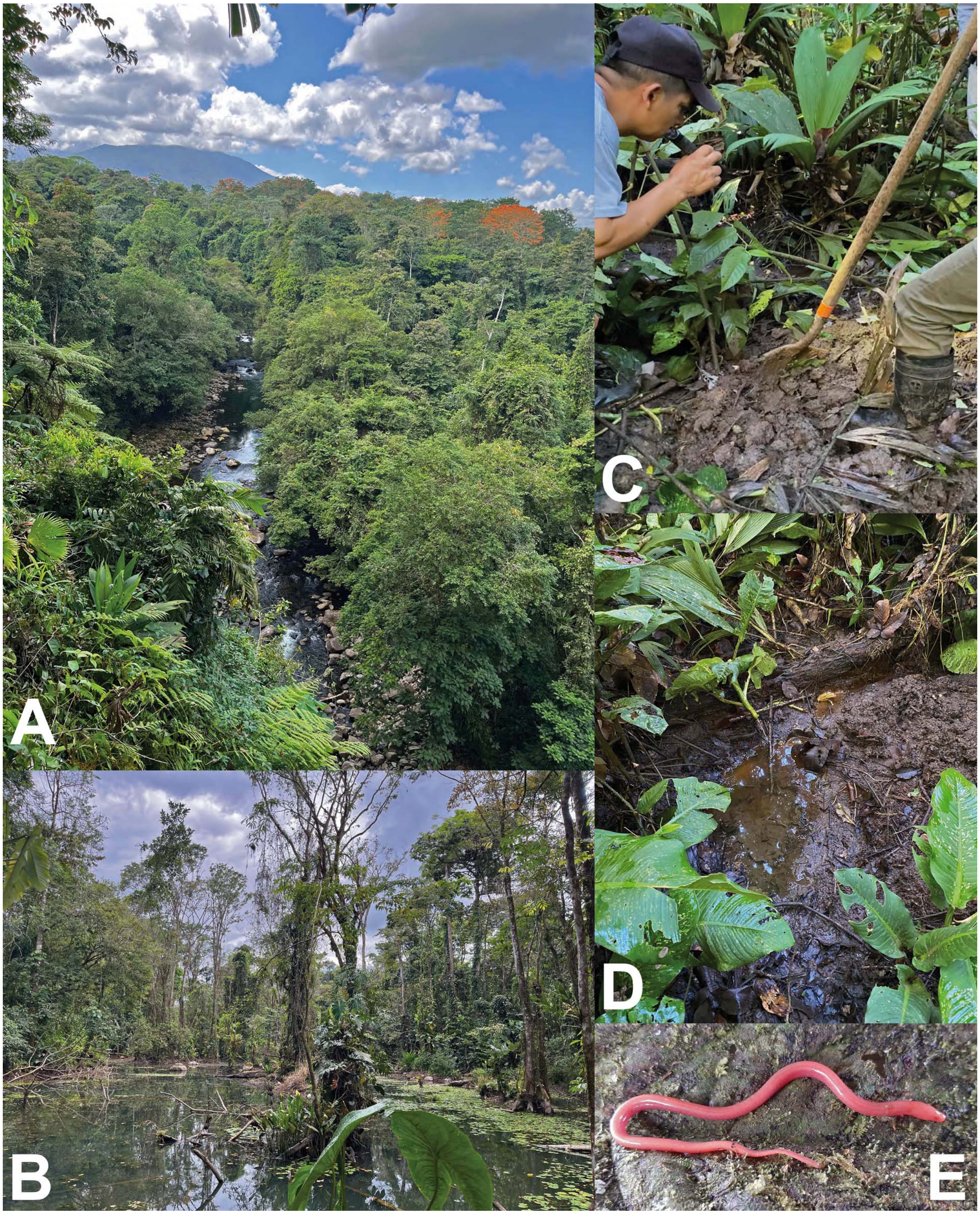

**Fig. 2.** Type locality of *Ophisternon berlini*, new species. (A) Vantage point view of the Destierro River within the premises of Las Brisas Nature Reserve near collecting sites. (B) One of the artificial lentic bodies of water inside the reserve near collecting sites. (C, D) Photographs of the 2021 collecting site (10°7′53.22″N, 83°36′11.95″W) displaying the muddy nature of the soil at the time of collection. (E) Live specimen of *Ophisternon berlini*, new species, freshly dug out from the abovementioned site.

united and free from the isthmus, with the gill opening said to be slit-like in *Ophisternon* versus pore-like in *Synbranchus* (Rosen and Greenwood, 1976). This distinction, nonetheless, is far from clear in many specimens. Osteologically, however, they are easily distinguished by the position of the pectoral girdle relative to the skull and postcranial vertebrae. In *Synbranchus*, the supracleithrum is posteriorly displaced to the level of the fifth vertebra and disconnected from a posttemporal bone that is reduced to a simple rod. Conversely, in *Ophisternon*, the shoulder girdle is connected to the skull by a forked posttemporal joining the supracleithrum at the level of the third vertebra (Rosen and Greenwood, 1976; Britz et al., 2022).

Both *Ophisternon* and *Synbranchus* have long and complex taxonomic histories, dating back to the late 18th and mid-19th centuries, respectively (Rosen and Greenwood, 1976). Two species of *Synbranchus* have been described in the 21st century (Favorito et al., 2005; Sabaj et al., 2022), but no new Neotropical species of *Ophisternon* have been described in almost 50 years, since the description of *O. aenigmaticum* by Rosen and Greenwood (1976). The fortuitous recent discovery of troglomorphic and diminutive swamp eels buried in muddy soil in a nature reserve in Costa Rica prompted us to pursue an investigation into the taxonomic identity of these specimens. The unusual morphology of this swamp eel, being externally very different from the only synbranchid eel known to occur in this region of the world, *S. marmoratus* (Bussing, 1998), suggested that it could correspond to an undescribed species. Based on comparative anatomical and mitogenomic evidence, we concluded that this population corresponds to an undescribed species of *Ophisternon*. Here we present a formal description of this new taxon.

## MATERIALS AND METHODS

***Specimen collection and preservation.***—Excavation work in June 2021 in a swampy area in the premises of Las Brisas, a private nature reserve located on the Central Volcanic Range of Costa Rica (Fig. 1), resulted in the discovery of two specimens of diminutive and troglomorphic swamp eels buried in the mud at approximately 50 cm depth (Fig. 2). Subsequent attempts at finding more of these unusual eels in the area were carried out in April 2022 and February 2023, resulting in the unearthing of five additional specimens, for an overall total of seven individuals from three closely adjacent collecting sites (Fig. 1). Blocks of mud containing live swamp eels were extracted on site and transported to a laboratory setting where specimens were removed from the mud and transferred to water (Fig. 3). Specimens were handled and euthanized with the anesthetic tricaine mesylate (MS-222) prior to preservation in accordance with recommended guidelines for the use of fishes in research (UFR Committee, 2014). Some specimens were tissued (tail clips) postmortem for genetic studies. Tissues were preserved in 96% ethanol and eventually cryopreserved at –20°C. After tissuing, voucher specimens were formalin fixed and a week later transferred gradually to 70% ethanol for long-term storage in the fish collections of the Universidad de Costa Rica (UCR) and the American Museum of Natural History (AMNH). Collection (R-SINAC-SE-DT-PI-003-2021; R-SINAC-SE-DT-PI-029-2023) and exportation (PE-CUSBSE-038-2023) permits were issued by the Sistema Nacional de Áreas de Conservación (SINAC). Access permit to genetic resources (CBio-54-2022-#359) was extended by the Comisión Institucional de Biodiversidad of the UCR.

***Comparative material.***—According to our current understanding of the diversity, systematics, and distribution of Neotropical synbranchids, the species most relevant for comparisons in the context of this species description are the Neotropical species of *Ophisternon* (*O. aenigmaticum* and *O. infernale*) and the species of *Synbranchus* present in Costa Rica (*Synbranchus marmoratus*). Additional comparative material examined includes the Australian Blind Cave Eel, *Ophisternon candidum*, and the Asian Swamp Eel, *Monopterus albus*. See Material Examined section for the complete list of specimens and the preparation/data type (in parentheses). Institutional abbreviations follow Sabaj (2020).


**Fig. 3.** Unearthing of specimens of *Ophisternon berlini*, new species. Photograph displaying the process of pulling out live specimens of the new species from a block of mud collected at the type locality.

***Morphometrics.***—Because of the highly specialized and modified morphology of swamp eels, they lack most external meristic and morphometric landmarks commonly used in taxonomic studies of teleostean fishes, particularly those involving median and paired fins. Consequently, the following measurements were recorded: 1) total length (from tip of snout to tail tip), 2) pre-anal length (from posterior border of anus to tip of snout), 3) post-anal length (from posterior border of anus to tail tip), 4) head length (from tip of the snout to the anterior rim of the gill opening in the ventral midline), 5) head width (distance between lateral borders of head, at posterior border of branchial opening), 6) body depth (at posterior border of branchial opening), 7) preorbital length (from anterior tip of snout to anterior border of the underlying orbit), 8) least interorbital width (shortest distance between dorsal borders of underlying eyes), 9) anterior to posterior narial distance (anterior to posterior nares), and 10) internarial distance between posterior nares. Due to tail damage to some specimens (precluding measurement of total length and post-anal length), our measurements follow a slight modification of the systematic procedures of recent synbranchid species descriptions (Favorito et al., 2005; Britz et al., 2016, 2018; Sabaj et al., 2022). All morphometric data were recorded with a digital caliper to the nearest 0.1 mm.

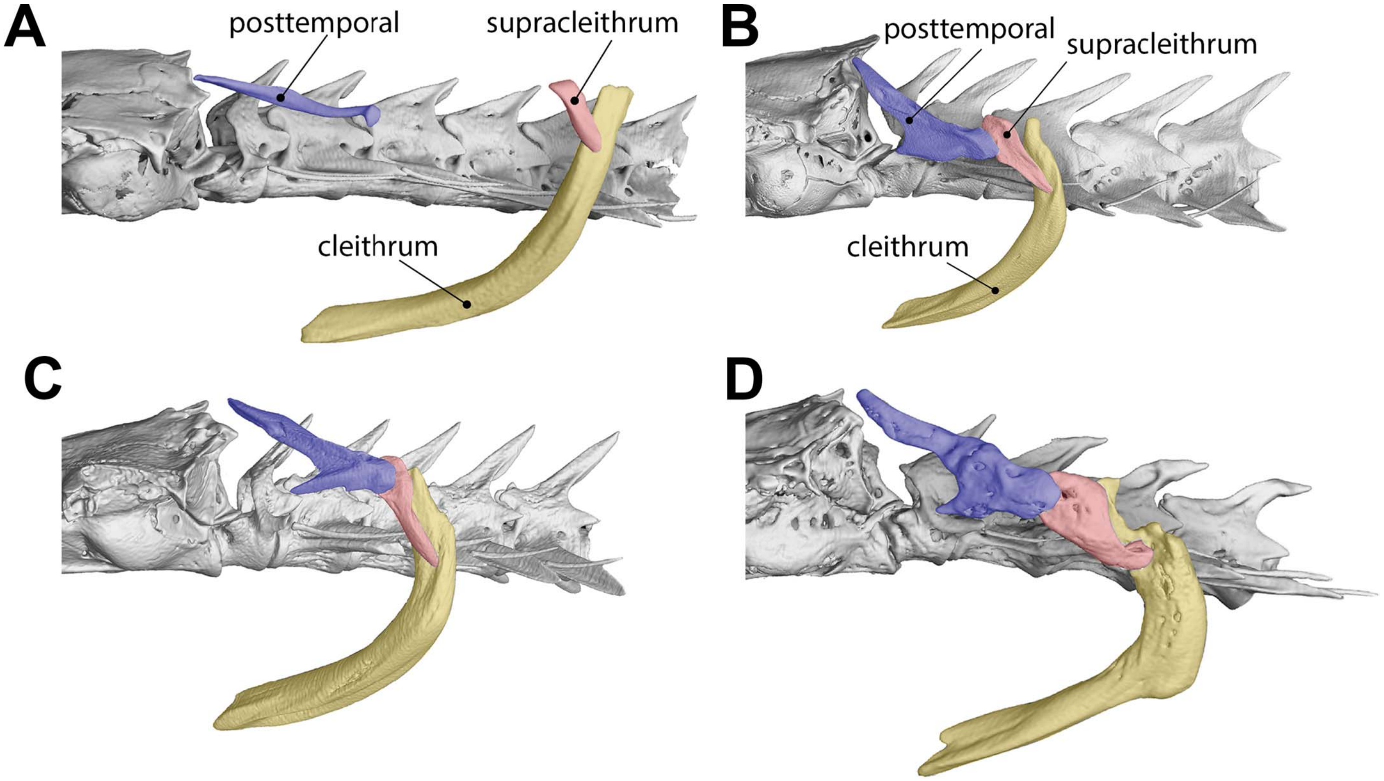

**Fig. 4.** Comparative osteology of the pectoral girdle. Posterior neurocrania and anterior vertebrae and pectoral girdles of: (A) *Synbranchus marmoratus* (AMNH 74542), (B) *Ophisternon berlini*, new species (holotype, AMNH 281169), (C) *Ophisternon aenigmaticum* (holotype, AMNH 32410), and (D) *Ophisternon infernale* (AMNH 277199). Not to scale.

***Skeletal anatomy.***—Osteological data were generated via micro-computed tomography (μCT) and from cleared and stained specimens (CS). Our osteological surveys focused on detecting taxonomically informative variation in elements of the skull (neurocranium, oral jaws, suspensoria, hyoid apparatus, and branchial basket), pectoral girdle, and vertebral column. Specimens were μCT scanned using a General Electric Phoenix v|tome|x with a 180kV Nano Tube at resolutions ranging from 5.7 to 28.5 μm, with beam energy set between 100–110 kV and 180–200 mA. Scanning was undertaken at the Microscopy and Imaging Facility at the AMNH, reconstructed using Phoenix datos|x (General Electric, Wunstorf, Germany), rendered using VGStudio Max version 2023.4 (Volume Graphics, Heidelberg, Germany), and edited using Adobe Photoshop 2024.

***Sequencing of new complete mitochondrial genomes.***—Voucher specimens CNPE-IBUNAM 24368 (tag JA1368) and AMNH 281171 (tag JA2005) were used to generate the first complete mitochondrial genomes of *O. aenigmaticum* and the new species described herein, respectively. DNA extraction and shearing, library preparation, Illumina sequencing, and mitogenome assembly and annotation procedures follow Mar-Silva et al. (2022), except for the use of the mitogenome of *O. infernale* (OM388306) as reference genome during annotation.

***Phylogenetic analysis.***—With the aim of assessing the phylogenetic placement and degree of genetic divergence of the new species with respect to the remaining Neotropical species of *Ophisternon* and other species of synbranchids with available complete mitochondrial genomes (Miya et al., 2001, 2003; White et al., 2020; Mar-Silva et al., 2022), comparative mitogenomic data were analyzed in a phylogenetic framework. The ingroup consisted of the new species (GenBank Accession PP975746), all other Neotropical species of *Ophisternon* (*O. infernale* [OM388306], *O. aenigmaticum* [PP975747]), the Australian *O. candidum* (MT436449), one of the four species of *Synbranchus*, *S. marmoratus* (AP004439), and the Asian *Ophichthys cuchia* (NC056403) and *Monopterus albus* (NC003192). The Marbled Spiny Eel, *Mastacembelus armatus* (NC023977), was used as outgroup and root. A phylogeny based on the concatenated alignment of all 13 mitochondrial protein-coding genes (PCGs) was inferred following the procedures of Mar-Silva et al. (2022).

## ***Ophisternon berlini*, new species**

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Berlin's Bloodworm Eel
Figures 2–11, Table 1

***Holotype.***—AMNH 281169, 163 mm TL, Costa Rica, Limón, Siquirres, Reventazón-Parismina drainage basin, Destierro River sub-basin, swampy area within the premises of Las Brisas Nature Reserve, semidry muddy soil near artificial permanent ponds, 10°7′34.16″N, 83°36′18.97″W, 247 masl, Freddy Perez, Juan Chávez, and Erick Berlin, 25 April 2022.

***Paratypes.***—AMNH 281170 (1 tissue voucher [UCR-CTP 5731], 1 alc, 167+ mm TL [terminal elements of tail missing]), female, Costa Rica, Limón, Siquirres, Reventazón-Parismina drainage basin, Destierro River sub-basin, swampy area within

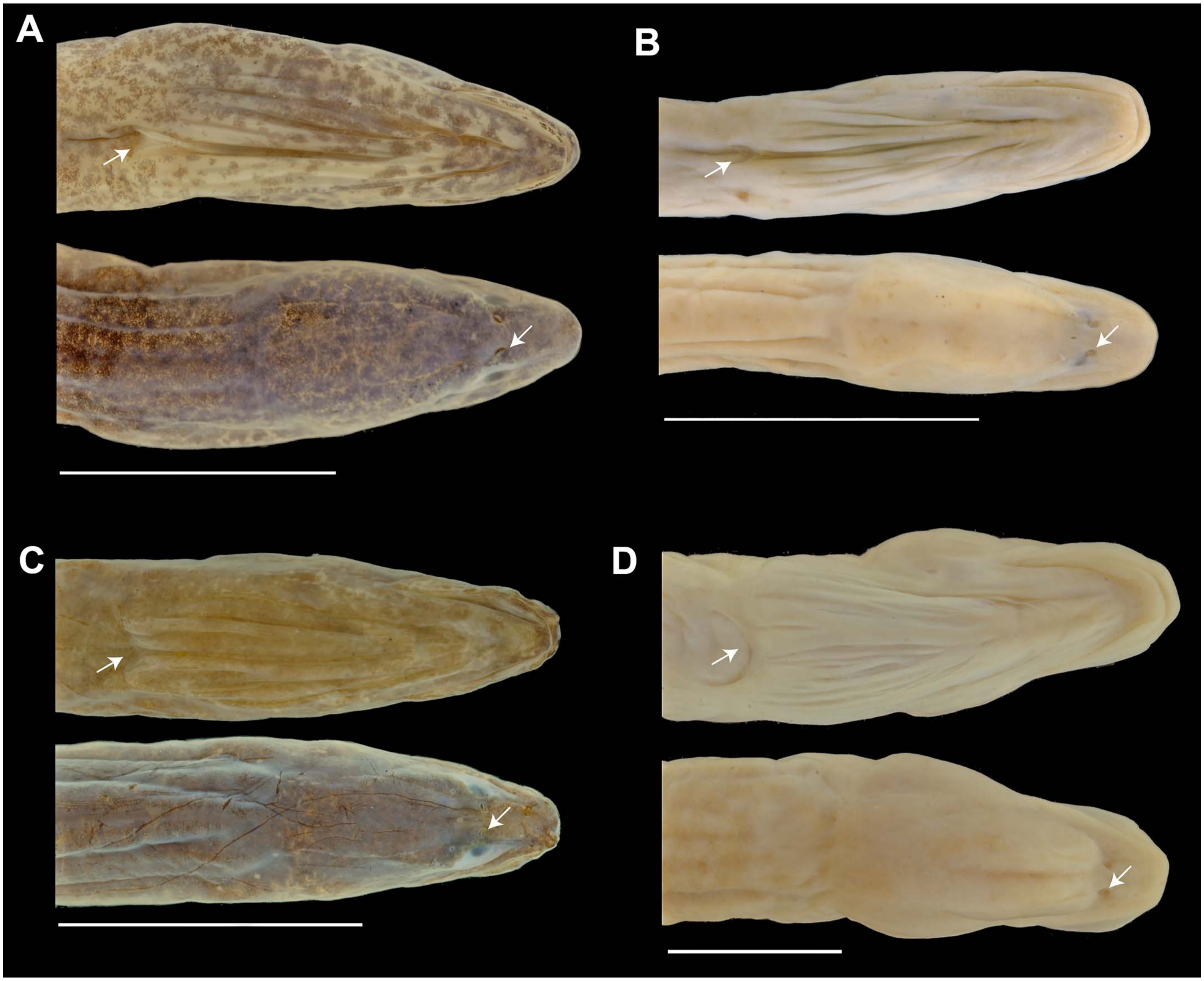

**Fig. 5.** Comparative (external) morphology of the head. Dorsal and ventral view of the head of: (A) *Synbranchus marmoratus* (AMNH 281172), (B) *Ophisternon berlini*, new species (paratype, AMNH 281170), (C) *Ophisternon aenigmaticum* (paratype, AMNH 32408), and (D) *Ophisternon infernale* (AMNH 32405). Scale bars = 1 cm, arrows indicate branchial openings and right posterior nares.

the premises of Las Brisas Nature Reserve, 10°7′53.22″N, 83°36′11.95″W, 235 masl, Freddy Perez, Juan Chávez, and Erick Berlin, 18 June 2021; AMNH 281171 (2 tissue vouchers [JA2005, JA2006], 2 alc, 144–162 mm TL), Costa Rica, Limón, Siquirres, Reventazón-Parismina drainage basin, Destierro River sub-basin, swampy area within the premises of Las Brisas Nature Reserve, 10°7′38.53″N, 83°36′14.76″W, Freddy Perez, Juan Chávez, and Erick Berlin, 6 February 2023; UCR 3321-001 (2 alc, 134–159 mm TL), Costa Rica, Limón, Siquirres, Reventazón-Parismina drainage basin, Destierro River sub-basin, swampy area within the premises of Las Brisas Nature Reserve, 10°7′53.22″N, 83°36′11.95″W, 235 masl, Freddy Perez, Juan Chávez, and Erick Berlin, 18 June 2021; UCR 3322-001 (1 alc, 142 mm TL), same data as holotype.

***Diagnosis.***—*Ophisternon berlini* is assigned to the genus *Ophisternon* by having a pectoral girdle connected to the skull by a forked posttemporal joining the supracleithrum at the level of the third vertebra vs. posteriorly displaced to the level of the fifth vertebra with posttemporal reduced to a simple rod and disconnected from the supracleithrum in *Synbranchus* (Fig. 4) and a branchiostegal membrane opening slit-like vs. small pore-like in *Synbranchus* (Fig. 5). *Ophisternon berlini* is unique among its Neotropical congeners in having: 1) an elevated number of precaudal vertebrae: ~100 vs. ~65 in *O. infernale* and ~75 in *O. aenigmaticum*; as the number of total vertebrae is similar among Neotropical species of *Ophisternon* (~140), the precaudal/caudal vertebrae ratio is significantly higher in the new species (Fig. 6); 2) a distinctive oral dentition characterized by longer and larger (and therefore less numerous), conical premaxillary (<50), dentary (<30), palatine (<10), and ectopterygoid (<20) teeth (Fig. 7); 3) palatine and ectopterygoid teeth in a single row vs. two or three irregular rows becoming a single row posteriorly on ectopterygoid (Fig. 7); and 4) a small and narrow (acutely angled) crescent-shaped gill membrane opening, occupying about 20% of the head's ventral surface vs. wider and larger (Fig. 5). Its troglobitic phenotype, characterized by depigmented skin (pink when alive and cream when preserved) and extremely reduced, subcutaneous eyes (Fig. 8), further distinguishes *O.*

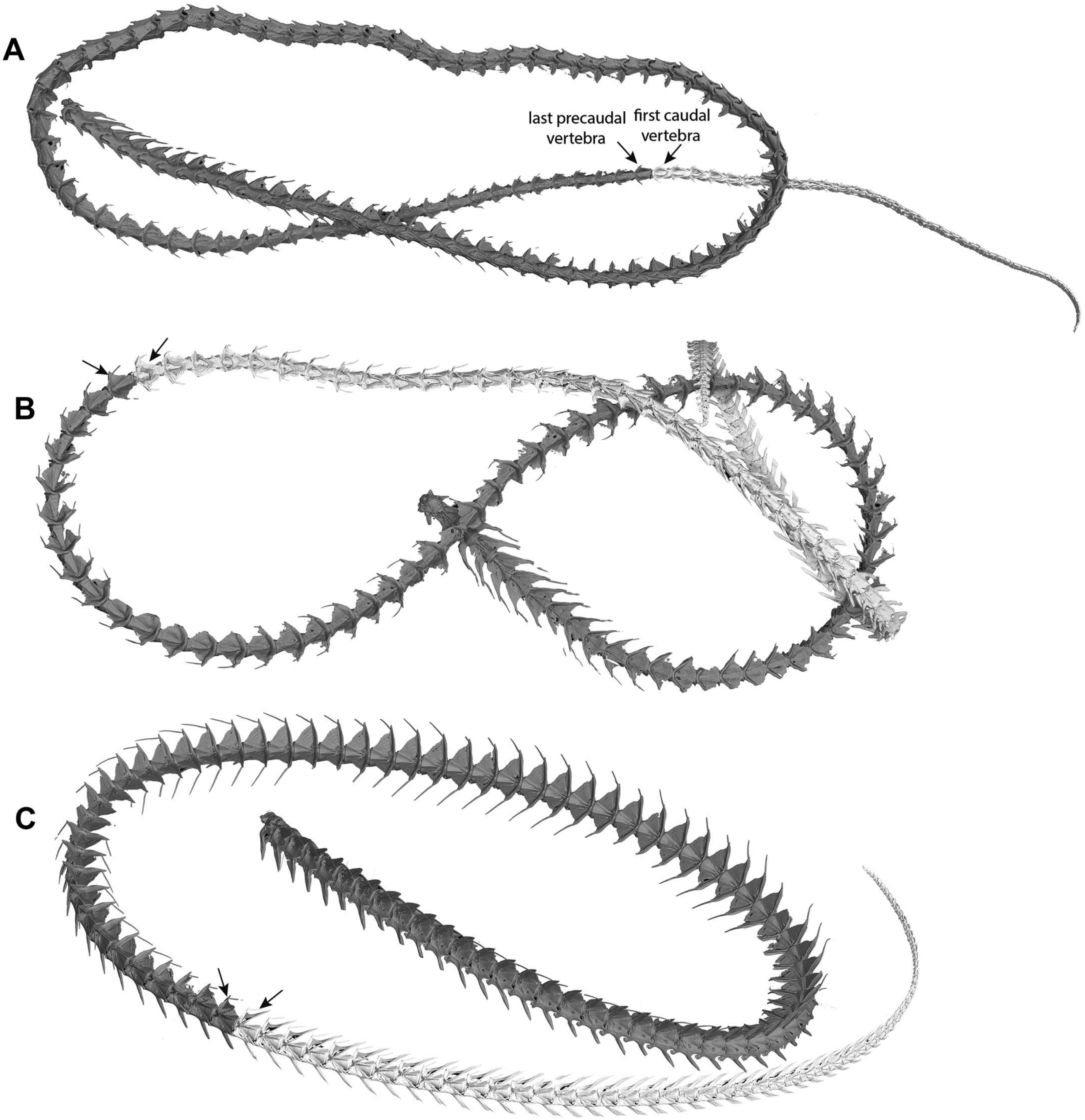

**Fig. 6.** Comparative osteology of the vertebral column. Vertebral column of: (A) *Ophisternon berlini*, new species (paratype, AMNH 281171), (B) *Ophisternon infernale* (AMNH 272952), and (C) *Ophisternon aenigmaticum* (holotype, AMNH 32410). Precaudal and caudal vertebrae dark- and light-colored, respectively. Not to scale.

*berlini* from other Neotropical synbranchids except for *O. infernale*, a species geographically restricted to the cenotes and submerged caves of the Yucatán Peninsula in Mexico, >1,200 km northwest in linear distance from the collection sites of *O. berlini*. *Ophisternon berlini* is further distinguished from *O. infernale* in lacking a neurocranial crest (Fig. 9) and in having a single tooth row on the palatine (vs. three rows; Fig. 7), elongate (vs. shorter) gill rakers, and stouter (vs. slenderer) fifth ceratobranchials and toothplates (Fig. 10).

***Description.***—For general appearance (including overall body shape and coloration) see Figure 8. Morphometric data derived from the type series are presented in Table 1. Body worm-like, small (maximum size c. 170 mm TL), and extremely slender (maximum body width 2.3% of TL). Head pointed but slightly rounded and blunt anteriorly, with small nuchal hump resulting from expansion of the adductor mandibulae musculature over the neurocranial roof, most developed in (presumed) males (e.g., Fig. 8A). Anterior nostril at

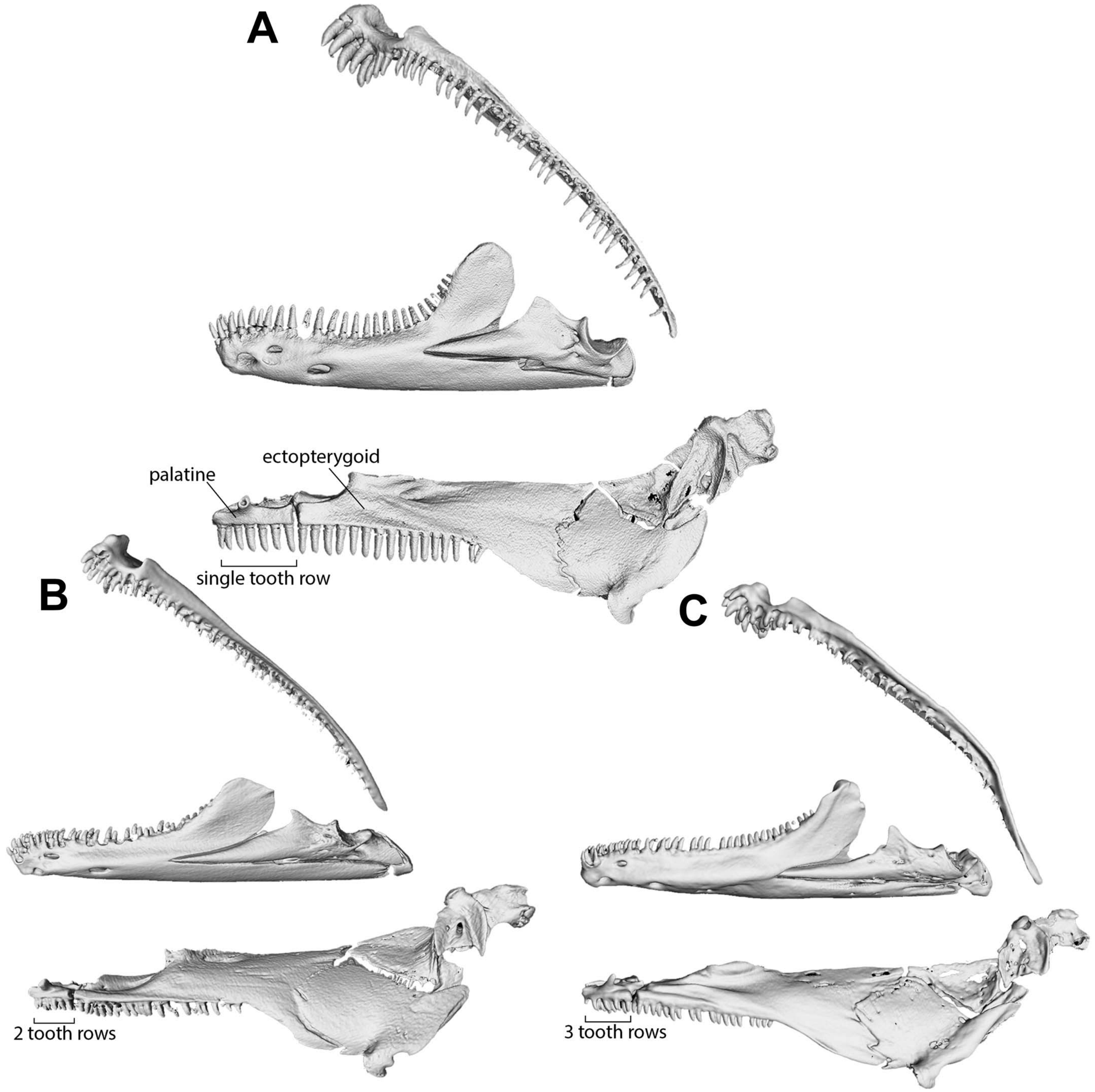

**Fig. 7.** Comparative osteology highlighting differences in dentition. Isolated premaxilla, lower jaw, and suspensorium (left side) of: (A) *Ophisternon berlini*, new species (holotype, AMNH 281169), (B) *Ophisternon aenigmaticum* (holotype, AMNH 32410), and (C) *Ophisternon infernale* (AMNH 272953). Not to scale.

snout tip; posterior nostril situated anterior to eye. Eyes very small and subcutaneous, covered with thick skin. Mouth subterminal. Premaxilla with several rows of large, conical, and slightly recurved teeth at expanded anterior tip, and a single row of teeth along most of ventral margin. Maxilla edentate. Dentary teeth large and arranged in a single row. Total number of vertebrae 142 (99–101 precaudal, 41–43 caudal). Six fully ossified branchiostegal rays extending posteriorly and reaching vertical through cleithrum. Branchiostegal membrane forming folds between branchiostegal rays. Gill opening ventral, small (occupying about 20% of head width), and lunate (acutely angled). Lateral line absent. Laterosensory system greatly reduced, externally visible as 2–3 tiny pores on the lower jaw and the preopercle.

***Coloration.***—In life, uniformly light/translucent pink, with epaxial and hypaxial musculature visible. Reduced and subcutaneous eyes visible as tiny dark spots. In alcohol, uniformly whiteish/pale cream (Fig. 8); body darker at branchial basket and around some sections of the digestive tract (possibly corresponding to clotted blood and undigested food items visible due to body translucency, respectively). Coloration like that of

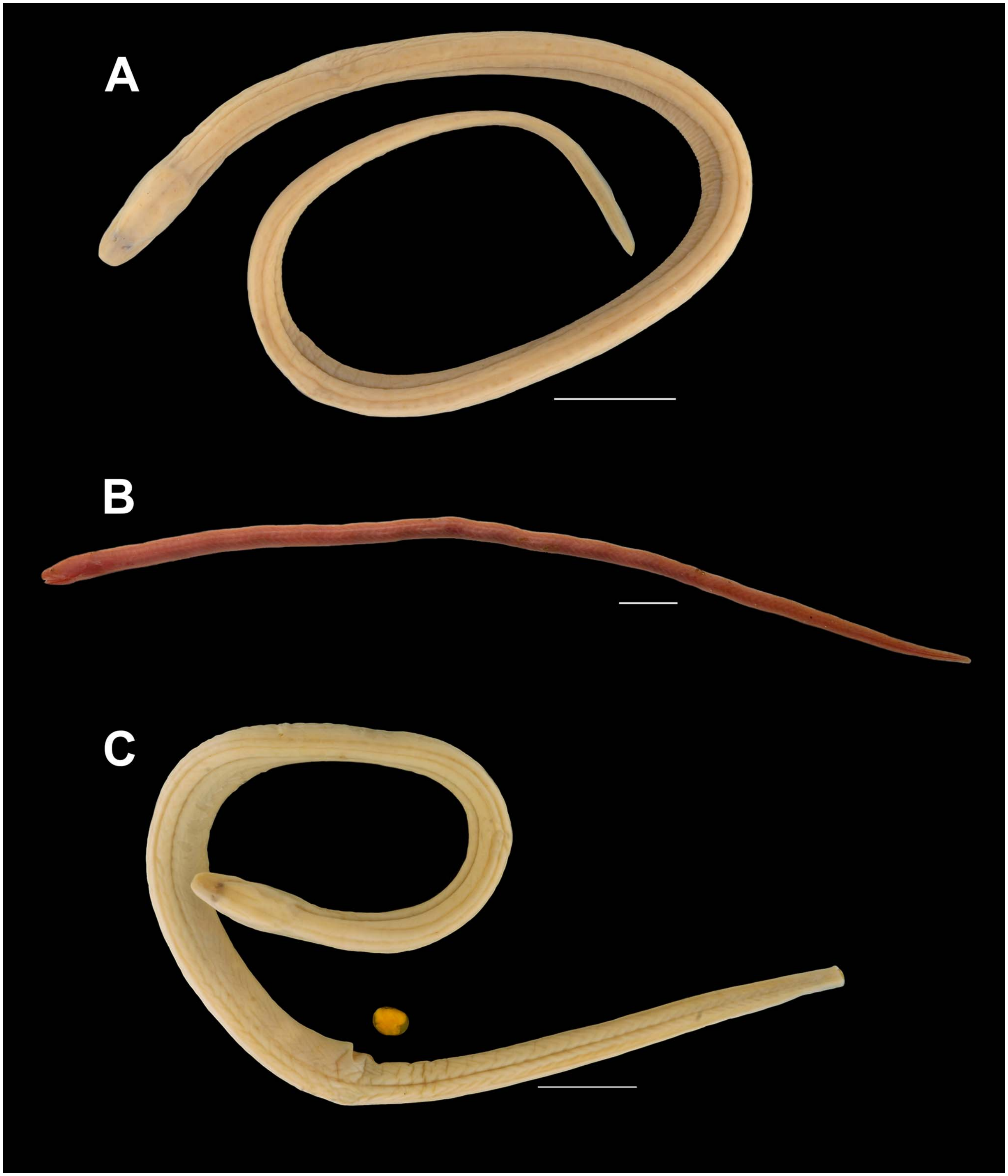

**Fig. 8.** External morphology of *Ophisternon berlini*, new species, in life and preservation. Holotype (AMNH 281169): (A) preserved and (B) immediately postmortem. (C) Paratype (AMNH 281170, female) preserved, including one of the eggs removed from ovary. Scale bars = 1 cm.

other troglomorphic synbranchids such as the congenerics *O. infernale* and *O. candidum*.

***Distribution and habitat.***—*Ophisternon berlini* is only known from specimens from three point localities in a swampy area within the premises of Las Brisas Nature Reserve, a tropical rainforest estate primarily devoted to biodiversity conservation and research, located near the town of La Alegría in Siquirres, Limón, Costa Rica, on the northeastern slopes of the Turrialba Volcano (Figs. 1, 2). These point localities are located within 200–600 m of each other, no further than 400 m from the Río Destierro, the main lotic system in the area (Reventazón-

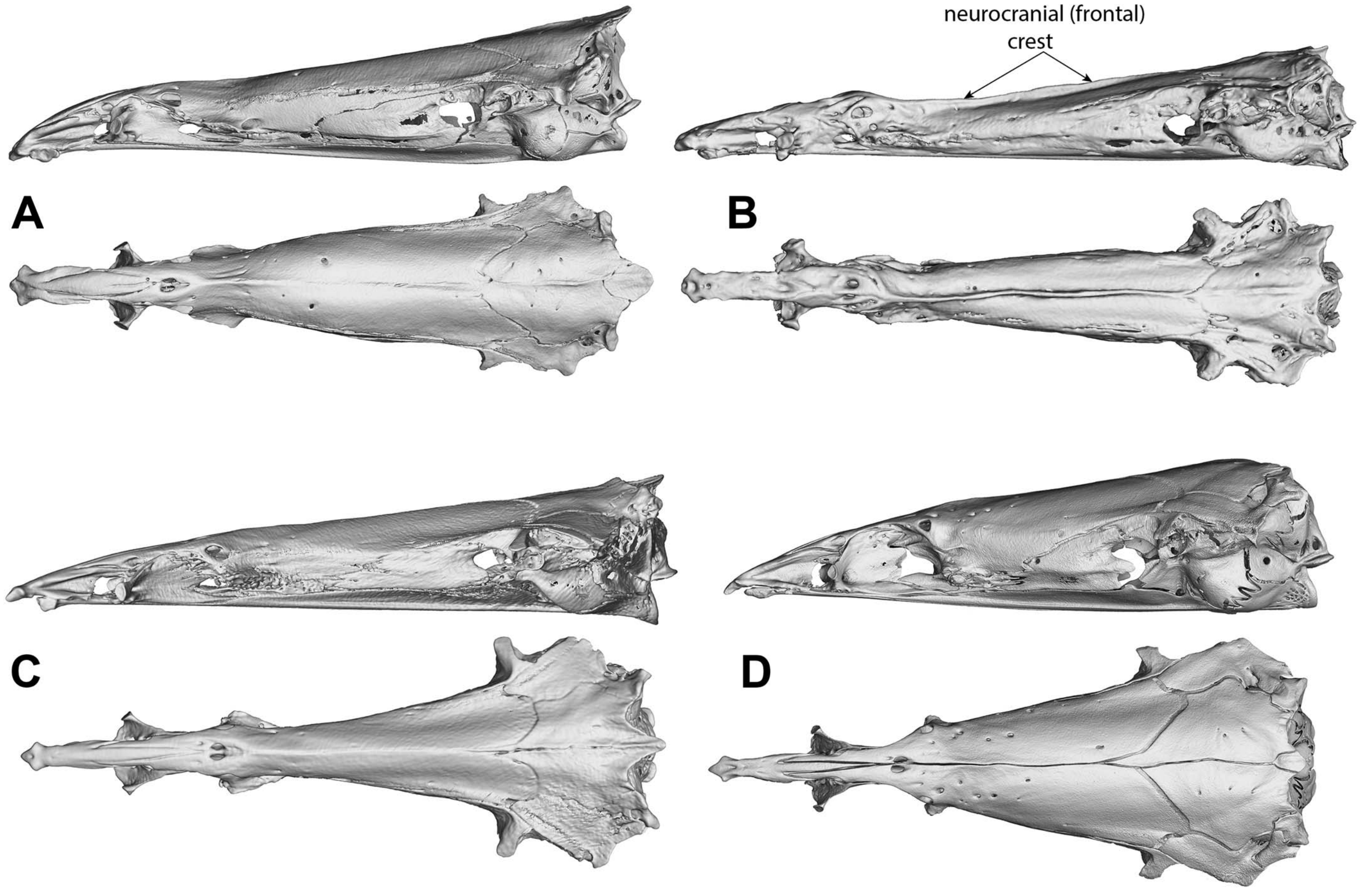

**Fig. 9.** Comparative osteology of the neurocranium. Neurocrania in lateral and dorsal views of: (A) *Ophisternon berlini*, new species (paratype, AMNH 281171, 162 mm TL), (B) *Ophisternon infernale* (AMNH 272953, 240 mm TL), (C) *Ophisternon aenigmaticum* (holotype, AMNH 32410, 358 mm TL), and (D) *Ophisternon aenigmaticum* (paratype, AMNH 32408, 154 mm TL). Not to scale.

Parismina drainage basin), and in proximity to a few artificial ponds. These ponds were created by the landowner to recreate lentic aquatic environments within his property to encourage their use by native fauna and flora, thus contributing to their preservation. The reserve offers a confined area of secondary but highly protected and conserved forest surrounded by a highly modified landscape dominated by crop fields (Fig. 1D). *Ophisternon berlini* appears to be an endogean species restricted to living in soil. All specimens from the type series were found buried in the mud at around 50–75 cm under the surface, in the subsoil layer. Some specimens were found together with *Caecilia volcani*, a relatively common caecilian in the reserve, and a species previously thought to be endemic to Panama but since 2017 confirmed to also occur in Costa Rica (Kubicki and Arias, 2017).

***Etymology.***—The specific epithet (a noun in the genitive case) is a patronym honoring Mr. Erick Berlin, a strong supporter of conservation and scientific research of Costa Rican biodiversity, discoverer of the population of swamp eel herein formally described, and owner of La Brisas, a private nature reserve that contains the type locality of the new species.

***Remarks.***—With a size range of just 114–c. 170 mm TL, specimens in the type series are considerably smaller than sexually mature individuals of both congeners (Rosen and Greenwood, 1976). However, dissection of one of the paratypes of *O. berlini* (AMNH 281170) revealed that a single ovary, containing about 15 large eggs, filled the visibly distended abdominal cavity (Fig. 8C), indicating that *O. berlini* is mature at a very small size. Completion of maturation at a small size (predisplacement) is evident also in a comparison of the neurocrania of *O. berlini* and *O. aenigmaticum* in individuals of similar total lengths. As indicated in Figure 9, the neurocranium of *O. berlini* (Fig. 9A) is fully ossified and "adult-like," while that of *O. aenigmaticum* at the same size (Fig. 9D) is clearly still developing with open sutures and a domed neurocranial roof, contrasting with the neurocranial morphology of mature specimens of that species (Fig. 9C). Taken together, these data indicate that *O. berlini* may correctly be a true dwarf species. Besides the abovementioned diagnosis, *O. berlini* could be easily recognized by the fact that, being sympatric only with *S. marmoratus*, it is the only swamp eel from the region that is endogean (i.e., soil-dwelling), dwarf, and troglomorphic.

***Newly generated synbranchid mitochondrial genomes.***—The complete mitochondrial genomes of *O. berlini* (GenBank Accession PP975746; AMNH 281171, paratype) and *O. aenigmaticum* (GenBank Accession PP975747; CNPE-IBUNAM 24368) are 16,997 and 16,834 bp long, respectively. Both mitogenomes display the typical length, composition, and arrangement of synbranchiform mitochondrial genomes, consisting of 37 genes, namely, 13 PCGs (12 on the H-strand and only ND6 on the L-strand), 2 rRNAs (both on the H-strand), 22

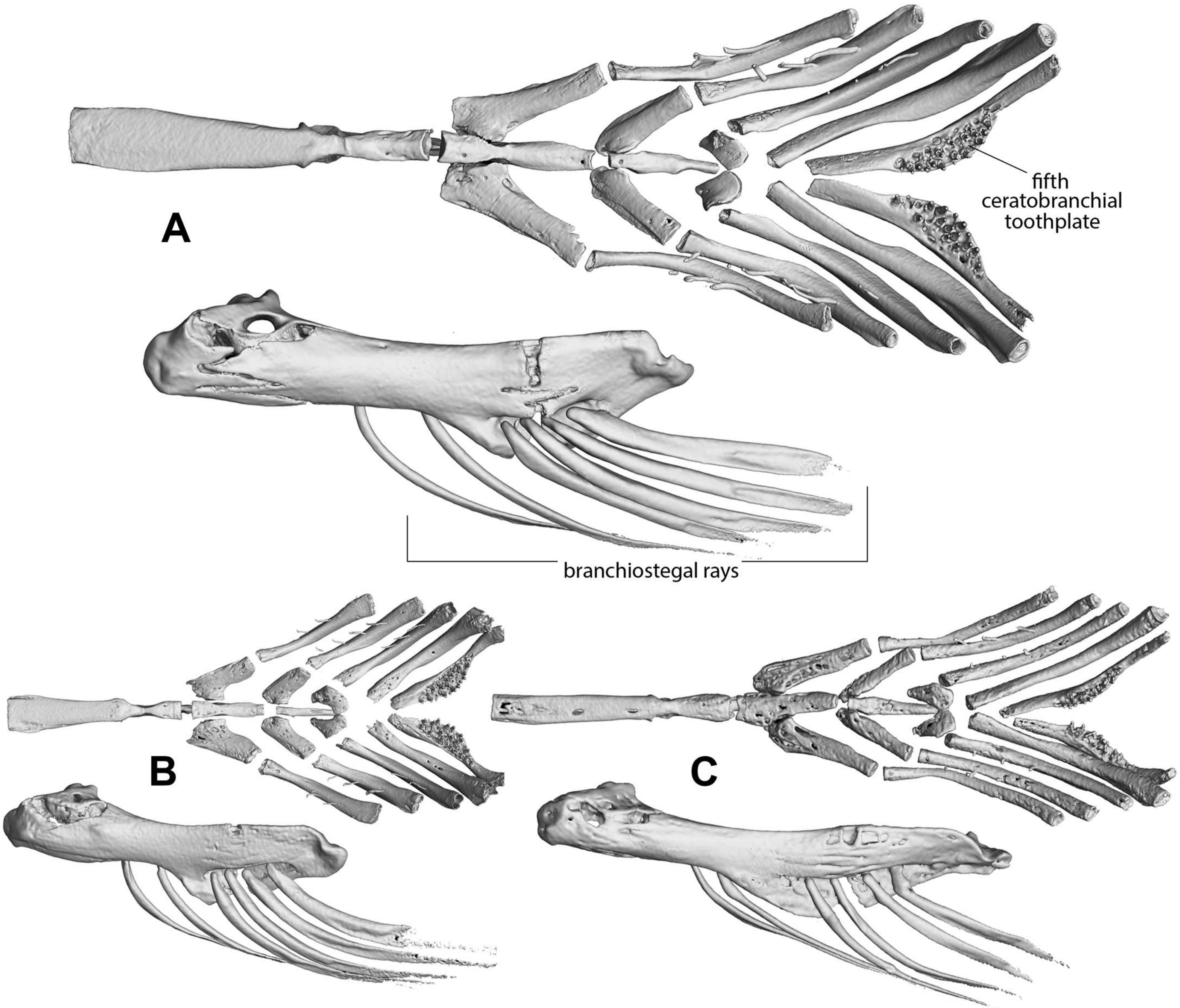

**Fig. 10.** Comparative osteology of the pharynx and hyoid apparatus. Gill arches (dorsal view) and left hyoid bar of: (A) *Ophisternon berlini*, new species (holotype, AMNH 281169), (B) *Ophisternon aenigmaticum* (holotype, AMNH 32410), and (C) *Ophisternon infernale* (AMNH 32405). Not to scale.

tRNAs (14 on the H-strand and eight on the L-strand), and one Control Region (D-loop). Notably, incomplete stop codons were detected in six and seven PCGs for *O. berlini* and *O. aenigmaticum*, respectively. The non-coding region D-loop is longest in *O. berlini* (1,270 bp) and flanked by tRNAPro and tRNAPhe in both species. Detailed information on the structure and organization of these newly generated mitogenomes is presented in Table 2.

***Genetic divergence and phylogenetic placement of* O. berlini.—** Concatenation of the 13 mitochondrial PCGs resulted in a DNA sequence data matrix totaling 11,424 aligned base pairs. A molecular phylogeny inferred from these comparative mitogenomic data (Fig. 11) resolved *O. berlini* as sister to a clade consisting of *O. infernale* + *O. aenigmaticum*. The genus *Ophisternon*, however, is rendered paraphyletic by the inferred position of *S. marmoratus*, nested between the Australian *O. candidum* and a clade consisting of all Neotropical species of *Ophisternon*, to which it is sister. Notably, all nodes in the phylogeny are strongly supported (bootstrap values = 100). Comparison of DNA sequence data from legacy markers commonly used in molecular systematic studies of fishes, such as COI, Cyt *b*, and ND2, revealed high levels of genetic divergence between *O. berlini* and its congeners, certainly much higher than expected for conspecifics, being typically <5–10% (Table 3).

## DISCUSSION

Swamp eels are notable for a recurrent (convergent) evolution of troglomorphism, the condition wherein an organism exhibits a peculiar phenotype characterized most notably by the reduction or complete loss of integumentary pigmentation and eyes (regressive traits), presumed to be an adaptation to life in lightless environments such as caves—hence the name; *troglo* = cave-dwelling (Wilkens, 1982; Romero

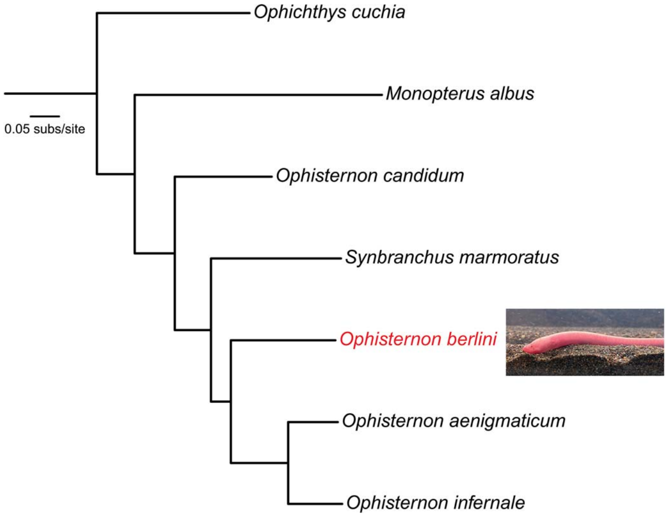

**Fig. 11.** Phylogenetic relationships among synbranchid species with available complete mitochondrial genomes (including all Neotropical species of *Ophisternon*), inferred from a concatenated alignment of all 13 mitochondrial PCGs. Outgroup (*Mastacembelus armatus*) not shown. Bootstrap support = 100 for all nodes. See Data Accessibility for tree file.

**Table 1.** Morphometric data for *Ophisternon berlini*, new species. *Maximum total and post-anal length do not consider data from the largest paratype (AMNH 281170), as its tail tip was cut during tissuing.

| | Holotype | *n* | Range | Mean |
|---|---|---|---|---|
| Total length (mm) | 163.0 | 5 | 114.0–167.0* | 145.6 |
| % of Total length | | | | |
| Pre-anal length | 66.2 | 6 | 64.8–74.5 | 71.3 |
| Post-anal length | 33.7 | 5 | 25.4–35.2* | 30.1 |
| Head length | 7.1 | 5 | 7.0–7.3 | 7.2 |
| Head width | 1.7 | 6 | 1.8–2.3 | 2.0 |
| Body depth | 2.1 | 5 | 2.2–2.4 | 2.2 |
| % of Head length | | | | |
| Preorbital length | 18.1 | 6 | 15.1–20.1 | 18.1 |
| Interorbital width | 10.3 | 6 | 7.0–11.1 | 9.2 |
| Ant-post narial distance | 18.1 | 6 | 14.2–19.2 | 17.2 |
| Internarial distance | 6.0 | 6 | 4.7–6.2 | 5.6 |

and Paulson, 2001; Giachino and Vailati, 2017). In fact, more than a third of global synbranchid species diversity is highly troglomorphic, including half of the species of *Ophisternon* (the Neotropical *O. berlini* and *O. infernale*, and the Australian *O. candidum*), the Malayan *Macrotrema caligans*, all the species of the Indian endemic *Rakthamichthys* (*R. digressus*, *R. indicus*, *R. mumba*, *R. rongsaw*, and *R. roseni*), and of the African genus *Typhlosynbranchus* (*T. boueti* and *T. luticolus*).

Complete darkness, however, is not unique to hypogean habitats, but also can occur in endogean (soil) environments. As a result, troglomorphism has also evolved in some members of the endogean fauna (Culver and Pipan, 2014; Giachino and Vailati, 2017). Most troglomorphic synbranchid eels (and other fishes for that matter) are nonetheless hypogean, restricted to groundwaters such as those from aquifers and submerged caves. Remarkably, *O. berlini*, which currently appears to be restricted to a subsoil habitat in a rather small swampy terrain in a remnant tropical rainforest in Costa Rica, is the latest addition to the very exclusive list of soil-dwelling fishes; the other only known species of endogean fishes—also synbranchid eels—are the Indian *R. rongsaw* and the Cameroonian *T. luticolus* (Britz et al., 2016, 2018; Proudlove and Elliott, 2018). Despite their geographically discontinuous multicontinental distribution, the external morphological resemblance among endogean synbranchid eels is impressive. This phenotype, characterized by reduced and subcutaneous eyes and a remarkable pink-red coloration in life (resulting from lack of pigmentation and highly vascularized skin), coupled with a small body size, are presumed adaptations to the endogean environment. Although troglomorphic animals tend to be rather small, endogean species—such as *O. berlini*—have much smaller and slenderer bodies than hypogean ones, presumably to better address the physiological and functional challenges of their almost semi-terrestrial and fossorial lifestyles (Giachino and Vailati, 2017). Dwarfism in *O. berlini* is therefore believed to be an adaptation to its edaphic habitat and fossorial lifestyle.

Synbranchidae is arguably a taxon suffering from both Linnean and Wallacean shortfalls (i.e., considerable gaps in our knowledge of the species-level diversity and their geographic distributions, respectively, *sensu* Lomolino and Heaney [2004]), a situation that has been exposed for the Neotropical component of the family in the context of the most recent (*Synbranchus*) species descriptions (Favorito et al., 2005; Sabaj et al., 2022) and a phylogeographic/phylogenetic study focused on Middle American populations of *S. marmoratus* and *O. aenigmaticum* (Perdices et al., 2005). Synbranchid systematics is challenging not only for the conservative external morphology of these fishes but also for their highly cryptic habits, rendering them difficult to find and collect. This is especially true for soil-dwelling populations, considering that these habitats are not particularly easy to efficiently sample and that, understandably, are almost never surveyed by ichthyologists. The discovery of a new species of endogean swamp eel from the Neotropics represents some progress, although modest, toward overcoming the abovementioned taxonomic and distributional shortfalls in the group. Perhaps more importantly, this discovery opens the exciting possibility that future targeted and systematic surveys in similar habitats in the region (or elsewhere) will result in range extensions and/or the detection of undescribed synbranchid diversity.

Comparative analysis of mtDNA demonstrates that the distinctiveness of *O. berlini* transcends an exclusively morphological diagnosis, extending to a very strong genetic differentiation from its congeners (Table 3; Fig. 11). For the case of the quintessential DNA barcode marker for animals, the mitochondrial gene COI, uncorrected pairwise genetic distances between the *O. berlini* and its congeners vastly exceed the traditionally employed ~3% sequence divergence heuristic threshold for conspecifics (Hebert et al., 2003; Pereira et al., 2011), further supporting its recognition as a distinct species (Table 3). The resultant mtDNA phylogeny (Fig. 11), although based on a partial sampling of total synbranchid diversity, is arguably the most taxonomically comprehensive phylogeny for the Neotropical component and adds to a body of evidence from recent molecular phylogenetic studies (Mar-Silva et al., 2022; Harrington et al., 2024) highlighting the non-monophyly of *Ophisternon*. These studies, regardless of degree of taxonomic coverage, agree that *Ophisternon* is rendered paraphyletic by the phylogenetic position of *S. marmoratus*. Our results, nonetheless, strongly support the monophyly of Neotropical

**Table 2.** General arrangement and associated features of the complete mitochondrial genomes of (A) *Ophisternon berlini*, new species (GenBank Accession PP975746) and (B) *Ophisternon aenigmaticum* (GenBank Accession PP975747). Intergenic space (IGS) is coded with (+) and overlapping with (−). AA = Amino acid, H = Heavy strand, L = Light strand.

| Locus | Type | One-letter code | Start | End | Length (bp) | Strand | # of AA | Anticodon | Start codon | Stop codon | IGS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (A) *Ophisternon berlini* | | | | | | | | | | | |
| tRNA$^{\mathrm{Phe}}$ | tRNA | F | 1 | 69 | 69 | H | | GAA | | | 0 |
| 12s rRNA | rRNA | | 70 | 1024 | 955 | H | | | | | 0 |
| tRNA$^{\mathrm{Val}}$ | tRNA | V | 1025 | 1096 | 72 | H | | TAC | | | 0 |
| 16s rRNA | rRNA | | 1097 | 2763 | 1667 | H | | | | | 0 |
| tRNA$^{\mathrm{Leu}}$ | tRNA | L | 2764 | 2836 | 73 | H | | TAA | | | 0 |
| ND1 | Protein-coding | | 2896 | 3861 | 966 | H | 321 | | ATG | TAG | 59 |
| tRNA$^{\mathrm{Ile}}$ | tRNA | I | 3876 | 3945 | 70 | H | | GAT | | | 15 |
| tRNA$^{\mathrm{Gln}}$ | tRNA | Q | 3957 | 4027 | 71 | L | | TTG | | | 12 |
| tRNA$^{\mathrm{Met}}$ | tRNA | M | 4027 | 4096 | 70 | H | | CAT | | | −1 |
| ND2 | Protein-coding | | 4097 | 5140 | 1044 | H | 348 | | ATG | TA- | 0 |
| tRNA$^{\mathrm{Trp}}$ | tRNA | W | 5141 | 5212 | 72 | H | | TCA | | | 0 |
| tRNA$^{\mathrm{Ala}}$ | tRNA | A | 5214 | 5282 | 69 | L | | TGC | | | 1 |
| tRNA$^{\mathrm{Asn}}$ | tRNA | N | 5284 | 5356 | 73 | L | | GTT | | | 1 |
| tRNA$^{\mathrm{Cys}}$ | tRNA | C | 5396 | 5461 | 66 | L | | GCA | | | 40 |
| tRNA$^{\mathrm{Tyr}}$ | tRNA | Y | 5462 | 5528 | 67 | L | | GTA | | | 0 |
| COXI | Protein-coding | | 5530 | 7116 | 1587 | H | 528 | | GTG | AGA | 1 |
| tRNA$^{\mathrm{Ser}}$ | tRNA | S | 7112 | 7182 | 71 | L | | TGA | | | −4 |
| tRNA$^{\mathrm{Asp}}$ | tRNA | D | 7188 | 7260 | 73 | H | | GTC | | | 6 |
| COXII | Protein-coding | | 7265 | 7955 | 691 | H | 230 | | ATG | T– | 5 |
| tRNA$^{\mathrm{Lys}}$ | tRNA | K | 7956 | 8027 | 72 | H | | TTT | | | 0 |
| ATPase8 | Protein-coding | | 8029 | 8196 | 168 | H | 55 | | ATG | TAA | 1 |
| ATPase6 | Protein-coding | | 8187 | 8869 | 683 | H | 227 | | ATG | TA- | −9 |
| COXIII | Protein-coding | | 8870 | 9653 | 784 | H | 261 | | ATG | T– | 0 |
| tRNA$^{\mathrm{Gly}}$ | tRNA | G | 9654 | 9722 | 69 | H | | TCC | | | 0 |
| ND3 | Protein-coding | | 9723 | 10070 | 348 | H | 116 | | ATG | T– | 0 |
| tRNA$^{\mathrm{Arg}}$ | tRNA | R | 10071 | 10139 | 69 | H | | TCG | | | 0 |
| ND4L | Protein-coding | | 10140 | 10436 | 297 | H | 98 | | ATG | TAA | 0 |
| ND4 | Protein-coding | | 10430 | 11810 | 1381 | H | 460 | | ATG | T– | −6 |
| tRNA$^{\mathrm{His}}$ | tRNA | H | 11811 | 11879 | 69 | H | | GTG | | | 0 |
| tRNA$^{\mathrm{Ser}}$ | tRNA | S | 11880 | 11945 | 66 | H | | GCT | | | 0 |
| tRNA$^{\mathrm{Leu}}$ | tRNA | L | 11945 | 12016 | 72 | H | | TAG | | | −1 |
| ND5 | Protein-coding | | 12018 | 13853 | 1836 | H | 611 | | ATG | TAA | 1 |
| ND6 | Protein-coding | | 13850 | 14371 | 522 | L | 173 | | ATG | TAG | −3 |
| tRNA$^{\mathrm{Glu}}$ | tRNA | E | 14373 | 14441 | 69 | L | | TTC | | | 1 |
| Cyt b | Protein-coding | | 14444 | 15583 | 1140 | H | 379 | | ATG | TAA | 2 |
| tRNA$^{\mathrm{Thr}}$ | tRNA | T | 15586 | 15659 | 74 | H | | TGT | | | 2 |
| tRNA$^{\mathrm{Pro}}$ | tRNA | P | 15659 | 15727 | 69 | L | | TGG | | | −1 |
| D-loop | Non-coding | | 15728 | 16997 | 1270 | H | | | | | 0 |
| (B) *Ophisternon aenigmaticum* | | | | | | | | | | | |
| tRNA$^{\mathrm{Phe}}$ | tRNA | F | 1 | 68 | 68 | H | | GAA | | | 0 |
| 12s rRNA | rRNA | | 69 | 1013 | 945 | H | | | | | 0 |
| tRNA$^{\mathrm{Val}}$ | tRNA | V | 1014 | 1087 | 74 | H | | TAC | | | 0 |
| 16s rRNA | rRNA | | 1088 | 2754 | 1667 | H | | | | | 0 |
| tRNA$^{\mathrm{Leu}}$ | tRNA | L | 2755 | 2828 | 74 | H | | TAA | | | 64 |
| ND1 | Protein-coding | | 2892 | 3878 | 987 | H | 321 | | ATG | TAG | 33 |
| tRNA$^{\mathrm{Ile}}$ | tRNA | I | 3911 | 3980 | 70 | H | | GAT | | | 8 |
| tRNA$^{\mathrm{Gln}}$ | tRNA | Q | 3989 | 4059 | 71 | L | | TTG | | | −1 |
| tRNA$^{\mathrm{Met}}$ | tRNA | M | 4059 | 4128 | 70 | H | | CAT | | | 0 |
| ND2 | Protein-coding | | 4129 | 5172 | 1044 | H | 348 | | ATG | TA- | 0 |
| tRNA$^{\mathrm{Trp}}$ | tRNA | W | 5173 | 5242 | 70 | H | | TCA | | | 1 |
| tRNA$^{\mathrm{Ala}}$ | tRNA | A | 5244 | 5312 | 69 | L | | TGC | | | 1 |
| tRNA$^{\mathrm{Asn}}$ | tRNA | N | 5314 | 5386 | 73 | L | | GTT | | | 35 |
| tRNA$^{\mathrm{Cys}}$ | tRNA | C | 5421 | 5486 | 66 | L | | GCA | | | 0 |
| tRNA$^{\mathrm{Tyr}}$ | tRNA | Y | 5487 | 5554 | 68 | L | | GTA | | | 1 |

**Table 2.** Continued.

| Locus | Type | One-letter code | Start | End | Length (bp) | Strand | # of AA | Anticodon | Start codon | Stop codon | IGS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| COXI | Protein-coding | | 5556 | 7142 | 1587 | H | 528 | | GTG | AGA | −4 |
| tRNA$^{Ser}$ | tRNA | S | 7138 | 7208 | 71 | L | | TGA | | | 2 |
| tRNA$^{Asp}$ | tRNA | D | 7211 | 7281 | 71 | H | | GTC | | | 2 |
| COXII | Protein-coding | | 7284 | 7974 | 691 | H | 230 | | ATG | T– | 0 |
| tRNA$^{Lys}$ | tRNA | K | 7975 | 8047 | 73 | H | | TTT | | | 1 |
| ATPase8 | Protein-coding | | 8049 | 8216 | 168 | H | 55 | | ATG | TAA | −9 |
| ATPase6 | Protein-coding | | 8207 | 8889 | 683 | H | 227 | | ATG | TA- | 0 |
| COXIII | Protein-coding | | 8890 | 9673 | 784 | H | 261 | | ATG | T– | 0 |
| tRNA$^{Gly}$ | tRNA | G | 9674 | 9742 | 69 | H | | TCC | | | 0 |
| ND3 | Protein-coding | | 9743 | 10090 | 348 | H | 116 | | ATG | — | 0 |
| tRNA$^{Arg}$ | tRNA | R | 10091 | 10159 | 69 | H | | TCG | | | 0 |
| ND4L | Protein-coding | | 10160 | 10456 | 297 | H | 98 | | ATG | TAA | −6 |
| ND4 | Protein-coding | | 10450 | 11830 | 1381 | H | 460 | | ATG | T– | 1 |
| tRNA$^{His}$ | tRNA | H | 11831 | 11899 | 69 | H | | GTG | | | 1 |
| tRNA$^{Ser}$ | tRNA | S | 11900 | 11966 | 67 | H | | GCT | | | −1 |
| tRNA$^{Leu}$ | tRNA | L | 11966 | 12038 | 73 | H | | TAG | | | 1 |
| ND5 | Protein-coding | | 12040 | 13875 | 1836 | H | 611 | | ATG | TAA | −3 |
| ND6 | Protein-coding | | 13872 | 14393 | 522 | L | 173 | | ATG | TAA | 1 |
| tRNA$^{Glu}$ | tRNA | E | 14395 | 14463 | 69 | L | | TTC | | | 2 |
| Cyt b | Protein-coding | | 14466 | 15606 | 1141 | H | 379 | | ATG | T– | 0 |
| tRNA$^{Thr}$ | tRNA | T | 15607 | 15681 | 75 | H | | TGT | | | −1 |
| tRNA$^{Pro}$ | tRNA | P | 15681 | 15749 | 69 | L | | TGG | | | 0 |
| D-loop | Non-coding | | 15750 | 16834 | 1085 | H | | | | | 0 |

*Ophisternon* and its sister-group relationship with *S. marmoratus*. Likewise, our results imply that a sister-group relationship between *O. infernale* and *S. marmoratus* inferred from mitogenomic data by Mar-Silva et al. (2022) was simply an artifact of incomplete taxon sampling. Although some authors have cast doubt on the monophyly of *Synbranchus* (Favorito-Amorim and Menezes, 1993, 1998; Favorito et al., 2005; Perdices et al., 2005), this particular hypothesis has yet to be tested in the context of a comprehensive sampling of Neotropical synbranchid species. All in all, it is undeniable that the classification of synbranchid fishes is in dire need of a systematic revision, particularly with respect to the limits and composition of the genera with presence in the Neotropics (i.e., *Ophisternon* and *Synbranchus*). The systematics of Old World synbranchids, conversely, has been the subject of recent studies that resulted in an improved taxonomy and the corresponding nomenclatural rearrangements (Britz et al., 2020, 2021).

A biogeographic consequence of the discovery of *O. berlini* is that, by pushing south the range of *Ophisternon* in the Neotropics (from Guatemala to Costa Rica), it significantly widens its area of sympatry with *Synbranchus*, previously thought to be limited to a narrow stretch in the vicinity of the Motagua fault in Guatemala (Rosen and Greenwood, 1976). It should be noted that specimens of *S. marmoratus* were collected in ponds adjacent to the type locality of *O. berlini*, which confirms the sympatry of these species. From a historical biogeographic perspective, our phylogenetic findings make sense considering present-day species distributions and imply a pattern of northwestern lineage dispersal and cladogenesis in the Neotropical clade of *Ophisternon* after its divergence from *Synbranchus*. Extinction assumptions aside, our mtDNA topology, coupled with current knowledge of present-day distributions, imply that the most recent common ancestor (MRCA) of Neotropical species of *Ophisternon* was probably distributed in Central America—after diverging from the lineage leading to *Synbranchus*—and that the MRCA of *O. aenigmaticum* and *O. infernale* diverged from the lineage leading to *O. berlini* also in Central America but possibly further north. Our phylogeny is also consistent with the hypothesis of a rather recent origin of the Yucatán-endemic and cave-dwelling *O. infernale* after diverging from *O. aenigmaticum* around the northernmost latitude of the distribution of the genus in the Neotropics (Mar-Silva et al., 2022).

**Table 3.** Matrix of percentage uncorrected *p*-distances showing levels of genetic divergence among the Neotropical synbranchids sampled in this study for three of the most commonly used mitochondrial legacy markers in molecular systematics studies of fishes (COI/Cyt *b*/ND2).

| | *O. berlini* | *O. aenigmaticum* | *O. infernale* | *O. candidum* | *S. marmoratus* |
|---|---|---|---|---|---|
| *O. berlini* | — | | | | |
| *O. aenigmaticum* | 15.0/21.2/22.7 | — | | | |
| *O. infernale* | 14.2/20.9/23.0 | 9.1/14.5/16.1 | — | | |
| *O. candidum* | 17.1/25.2/25.2 | 17.7/22.6/22.9 | 16.6/22.3/22.9 | — | |
| *S. marmoratus* | 17.9/31.4/26.4 | 17.9/22.1/25.7 | 17.5/21.2/24.9 | 18.2/23.2/24.4 | — |

## MATERIAL EXAMINED

*Monopterus albus*: AMNH 41579, 1 fluid, 166 mm TL, Indonesia, Borneo, Sungai tekam, small tributary kapuas river ca. 10 km upstream of sanggau (CS).

*Ophisternon aenigmaticum*: AMNH 32410, holotype, 1 alc, 349 mm TL, Guatemala, Alta Verapaz, Woodland pool ca. 13 km southwest Sebol (µCT); AMNH 32408, paratypes, 3 alc, 84–158 mm TL, Guatemala, Izabal, Río Dulce, south shore just east of Río Ciénaga (µCT); CNPE-IBUNAM 24368, 1 alc, Mexico, Chiapas, Río La Venta at Cascada El Aguacero (mtDNA).

*Ophisternon candidum*: AMNH 32404, 1 fluid, 256 mm TL, Australia, Western Australia, east side of northwest cape, from neds well to mowbowra creek (CS); WAM P.34817-001, Australia, Western Australia, Bungaroo Study Area (mtDNA from White et al. [2020]).

*Ophisternon infernale*: UMMZ 116093, holotype, 1 alc, 325 mm TL, Mexico, Yucatán, Hoctún, between Mérida and Chichén Itza (µCT osteological data from Britz et al. [2022]); AMNH 32405, 1 fluid, 183 mm TL, Mexico, Yucatán, Grutas de Tzab-Nah, 2 km south of Tecoh (CS); AMNH 272952, 1 alc, 277 mm TL, Mexico, Yucatán, Huhí, Cenote Kan-Chin (µCT); AMNH 272953, 1 alc, 240 mm TL, Mexico, Yucatán, Cenote Pet Hu (µCT); AMNH 277199, 1 alc, 273 mm TL, Mexico, Yucatán, Cenote del Pochote, 10 km NW Muna (µCT); CNPE-IBUNAM 23285, 1 alc, Mexico, Yucatán, Huhí, Cenote Kan-Chin (mtDNA).

*Synbranchus marmoratus*: AMNH 74542, 1 alc, 205 mm TL, Venezuela, Río Negro, Mawarinuma tributary, 5 km east of Cerro de Neblina base camp (µCT); AMNH 281172, 1 alc, 168 mm TL, Costa Rica, Limón, Siquirres, Reventazón-Parismina drainage basin, Destierro River sub-basin, artificial ponds in the premises of Las Brisas Nature Reserve (µCT).

## DATA ACCESSIBILITY

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