

A New Record and Species of Pygmy Pipehorse of the Genus *Cylix* (Teleostei, Syngnathidae) from South Africa and the African Continent

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Cylix nkosi, new species, of the formerly monotypic pygmy pipehorse genus *Cylix*, is described on the basis of the female holotype and the male paratype collected between 14–50 m over inshore warm-tropical coral reefs from KwaZulu-Natal, South Africa. The new taxon possesses derived characters consistent with the diagnosis of the type species *Cylix tupareomanaia* from Aotearoa New Zealand, including a prominent supraoccipital bone bearing a highly derived bony protuberance and the presence of distinct midventral head spines. The new species is distinguished from its congener primarily by the distinct shapes of the supraoccipital protuberance, midventral head spines, and the dorsomedial crest-like ridge on the first trunk ridge. *Cylix nkosi*, new species, can be further differentiated by genetic divergence in the mitochondrial COI gene from *C. tupareomanaia* and the morphologically similar members of the Indo-Pacific pygmy pipehorse genera *Acentronura* and *Idiotropiscis* (estimated uncorrected *p*-distances of 10.0% *C. tupareomanaia*, 16.6% *A. breviperula*, 20.6% *A. tentaculata*, 18.1% *I. australe*, and 18.8% *I. lumnitzeri*, respectively). *Cylix nkosi*, new species, is the first confirmed record of the genus in South Africa and the African continent. In addition, the occurrence of *C. nkosi*, new species, in the western Indian Ocean represents a substantial expansion of the geographical distribution of the genus beyond its original type locality in New Zealand.

THE family Syngnathidae is a large and morphologically distinctive group of predominantly small-bodied and cryptic marine fishes represented by more than 300 species in 57 genera (Dawson, 1985; Hamilton et al., 2017; Fricke et al., 2023). Members are widely distributed in temperate and tropical habitats among mostly shallow coastal areas of the Atlantic and Indo-Pacific Oceans, including soft sediment habitats, coral and rocky reefs, lagoons, mangroves, estuaries, seagrass beds, and algal flats (Dawson, 1985). Members of this family, comprising the seahorses, pipefishes, pipehorses, pygmy pipehorses, and seadragons, are distinguished from other marine fish groups by a suite of unique morphological characteristics, including elongated snouts, fused toothless jaws, thick plates of bony armor, male brooding, and behavioral and morphological adaptations for crypsis (Dawson, 1985).

The Indo-Pacific pygmy pipehorses, a group comprising three closely related and morphologically conserved genera, *Acentronura*, *Cylix*, and *Idiotropiscis* collectively form a low component of the marine ichthyofauna of shallow vegetated coastal habitats and coral and rocky reefs. These diminutive syngnathids have been observed in mixed habitats consisting of encrusting coralline algae, bryozoans, sponges, solitary corals, fucoid and turf algae, and seagrasses (Fraser-Brunner and Whitley, 1949; Dawson, 1984, 1985; Kuitert, 2004; Gomon, 2007; Browne et al., 2008; Short and Trnski, 2021). *Acentronura*, *Cylix*, and *Idiotropiscis* are united by synapomorphic characters, including a ventrally angled head at approximately 25° from the principal body axis, an enclosed brood pouch, brood pouch plates, a prehensile

tail, and the absence of a caudal fin (Dawson, 1985; Short and Trnski, 2021). Dawson (1985) provided data for differentiating the species of the genera *Acentronura* and *Idiotropiscis* based on meristic and morphometric characters, which Short and Trnski (2021) expanded to include novel morphological and neurocranial diagnostic characters for the differentiation at the generic and species level of *Acentronura*, *Cylix*, and *Idiotropiscis*.

The inshore marine ichthyofauna of South Africa is characterized by a morphologically diverse and species-rich group of warm-temperate southeast Atlantic and subtropical-tropical Indo-Pacific syngnathids represented by 27 species in 12 genera (Claassens et al., 2022). Among this group, *Acentronura tentaculata* is the only recorded species of pygmy pipehorse occurring in South Africa and the western Indian Ocean. Additionally, the South African Indian Ocean coast exhibits a high degree of endemism with three syngnathid species of conservation concern. These species include the endangered temperate estuarine species *Hippocampus capensis* (Bell et al., 2003; Lockyear et al., 2006; Claassens and Hodgson, 2018; Claassens and Harasti, 2020; Claassens et al., 2022), critically endangered *Syngnathus watermeyer* (Dawson, 1985; Cowley and Whitfield, 2001; Mwale, 2006; Vorwerk et al., 2007; Mwale et al., 2014; Claassens et al., 2022), and the pygmy seahorse *H. nalu* (Short et al., 2020; Claassens et al., 2022).

In 2009, a putative new member of *Cylix* was photographically recorded at the type locality as *H. nalu*, specifically at 2 Mile Reef in Sodwana Bay, KwaZulu-Natal, South Africa (Short et al., 2020). It was provisionally identified as a

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member of the seahorse genus *Hippocampus* by a local scuba diver on iNaturalist.org (<https://www.inaturalist.org/observations/11120680>, 11120683). Subsequently, in 2017, individuals corresponding to the new member and exhibiting distinct variations in body coloration were observed at a depth of 18–20 m at 2 Mile Reef by Christo van Jaarsveld of SeaXplore. A female and a male specimen were acquired for diagnosis to conduct a comprehensive study of the new species. The second and third authors collected the female specimen at 2 Mile Reef, Sodwana Bay, in 2018. The male specimen was acquired through an assessment of sub-Saharan syngnathid biodiversity (Claassens et al., 2022) that included the examination of vouchered specimens of pygmy pipehorses collected from various coastal regions in East Africa, at the South African Institute for Aquatic Biodiversity (SAIAB). Among the specimens in the SAIAB collection, a single male specimen exhibiting a fully developed enclosed brood pouch, obtained from the Kosi River Mouth, Kosi Bay, KwaZulu-Natal, South Africa at a depth of 50 m in 1987, was identified as the new representative of *Cylix* previously discovered in Sodwana Bay.

Morphological examination of the female (Sodwana Bay) and male (Kosi Bay) specimens revealed that these diminutive pygmy pipehorses, although highly similar to *C. tupareomanaia* in morphological appearance, can be distinguished by the distinct shapes of the highly derived supraoccipital bony protuberances and midventral head and first trunk ring spines, as revealed by micro-computed tomography and molecular sequence data. The taxonomic study presented herein provides a formal description of the species, designated as *C. nkosi*, new species. Additionally, it establishes the first confirmed record of *Cylix* in South Africa and the African continent. As a result, this finding contributes significantly to our knowledge of the distribution and diversity of the genus.

MATERIALS AND METHODS

The female specimen of *C. nkosi* (SAMC F041935), new species, was collected in 2018 by hand via scuba diving at 22 m depth in Sodwana Bay, KwaZulu-Natal, South Africa (Figs. 1–3). The specimen was subsequently fixed and preserved in 90% ethanol and deposited in the fish collection of the Iziko South African Museum (SAM), Cape Town, South Africa. The male specimen (SAIAB 39737, <https://www.gbif.org/occurrence/1230372893>), originally identified as *Acentronura mossambica* and reidentified by the author as *Cylix nkosi*, new species, was collected in 1987 by trawl in 50 m depth at the mouth of the Kosi River, Kosi Bay, KwaZulu-Natal, South Africa (Fig. 1). It is housed in the fish collection of SAIAB, Makhanda, South Africa.

Morphological analyses.—Head and body measurements follow Short et al. (2018) and are expressed as a percent of standard length (SL) or head length (HL). Osteocranial and postcranial skeletal terminology follow Short and Trnski (2021). External morphological characters were documented using a dissecting microscope and high-resolution digital images, which were taken using a Canon E05 5D with a 50 mm lens. Computed tomography (CT) scans of select specimens were obtained to examine osteological characters of the axial skeleton of type specimens at the University of New England, NSW, Australia CT Scanner

Facility using a GE v|tome|x S industrial micro-CT scanner with a voxel resolution (slice thickness) of 8 µm. The resulting CT scan data were visualized and rendered in Horos (<https://www.horosproject.org>).

Molecular analyses.—Genomic DNA was extracted from the right eye of the 95% ethanol-fixed holotype of *C. nkosi* (SAMC F041935), new species, using a DNeasy Blood and Tissue Extraction Kit (Qiagen, Inc.) in accordance with the manufacturer's protocols and initial tissue digestion overnight at 56°C. A shotgun library was prepared from the extracted DNA using an Illumina Nextera DNA Flex library prep kit and sequenced using a MiSeq and 2×150 v3 reagent kit at the Ramaciotti Centre for Genomics (UNSW Sydney, Australia).

The mitochondrial genome was assembled by initially mapping reads to a reference 655 bp COI fragment from *Hippocampus kuda* (GenBank: EU930325) in Geneious v 10.05 (<https://www.geneious.com>). When no reads could be further extracted from the shotgun dataset, sequences with a minimum average phred quality score of 30 were filtered out using BBDuk and used to assemble the complete mitochondrial genome with the De Novo Assemble function in Geneious. The mitogenome was annotated by comparison to the *Hippocampus kuda* mitochondrial genome (AP005985) and the Find ORFs function in Geneious using the Vertebrate Mitochondrial translation table. The complete mitochondrial genome of *C. nkosi*, new species, is available from GenBank under accession number OM022101.

Genetic distances (uncorrected *p*-distances) were calculated based on COI sequences using MEGA v. 7.0.26 (Kumar et al., 2008).

Cylix nkosi, new species

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Common Name: Sodwana Pygmy Pipehorse

Figures 1–9; Tables 1–3

Holotype.—SAMC F041935, 45.9 mm SL, female, 2 Mile Reef, Sodwana Bay, KwaZulu-Natal, South Africa, 27°30'46.6"S, 32°41'10.4"E, 22 m depth, 18 October 2018, L. Claassens, R. Smith, scuba diving.

Paratype.—SAIAB 39737, 46.6 mm SL, male, the mouth of the Kosi River, Kosi Bay, KwaZulu-Natal, South Africa, 26°53'54.0"S, 32°55'30.0"E, field station ZA 10, R.V. Meiring Nande, trawl, 50 m depth, 7 June 1987.

Diagnosis.—*Cylix nkosi* differs from *C. tupareomanaia* in possessing a supraoccipital bone bearing a highly derived and distinct diamond-shaped bony protuberance (SP; vs. cup-like bony protuberance; Table 3) oriented anteriorly, rhombus-shaped in anterodorsal view, divided transversally into two sections by a ridge; knob-shaped midventral spine (CSS; vs. udder-shaped bony protuberance; Table 3) on the cleithral symphysis; blunt midventral spine (MVFTRS; vs. conical midventral spine; Table 3) on the first trunk ring between the pectoral-fin bases; thick dorsomedial crest-like ridge (FTRC; vs. thin and rugose crest-like ridge; Table 3) extending over the length of the first trunk ridge; median frontal spine (FS) at convergence of anterior edges of the



Fig. 1. *Cylix nkosi*, types, preserved. (A) SAMC F041935, female, holotype, 45.9 mm SL, 2 Mile Reef, Sodwana Bay, KwaZulu-Natal, South Africa. (B) SAIAB 39737, male, paratype, 46.6 mm SL, Kosi River, Kosi Bay, KwaZulu-Natal, South Africa. Photographs by Graham Short. Scale bars = 5 mm.

supraoccipital bone, anterior margin straight-edged; rugose double lateral head spine (DHS) present below supraoccipital v; three supraorbital extensions or spines (ORE), anterior two a double spine and equal in size, the posteriormost spine large and oblong in shape. The following characters are also diagnostic, although not unique to the species: cleithrum continuous middorsally; supracleithrum present; anterior and posterior nuchal plates absent, large gap present between the supraoccipital and cleithrum; small blunt midlateral spines proximally on operculum; two oval-shaped spines on cleithral ring, one spine (CS1) present on anterior to ventral third of pectoral-fin base, the second spine (CS2) present at the ventral extent of the head; small

spine (PLS) present posterolaterally on the pelvic-fin base; four subdorsal spines, forming a square, the dorsal two enlarged, the ventral two small in size.

Description.—Meristic and morphometric characters for the type specimens are listed in Table 1. Superior trunk and tail ridges discontinuous below dorsal-fin base, lateral tail ridge present, inferior trunk ridge ends at the anal ring, lateral trunk ridge confluent with the inferior tail ridge. Body slender; head large relative to body, angled ventrally approximately 25° from the principal body axis, the dorsal profile pyramidal in lateral aspect, rising steeply from snout to elevated and prominent supraoccipital bone; supraoccipital

Table 1. Counts and proportional measurements for type specimens of *Cylix nkosi* and *C. tupareomanaia*. Ratios are separated by a colon and are listed as percent of SL, HL, or SnL. Abbreviations: CH (coronet height), DL (dorsal-fin length), HD (head depth), HL (head length), OD (orbital diameter), PL (pectoral-fin length), PO (post-orbital length), SL (standard length), SnD (snout depth), SnL (snout length), TaL (tail length), TD4 (trunk depth between the 4th and 5th trunk rings), TD9 (between the 9th and 10th trunk rings), TrL (trunk length).

	<i>Cylix nkosi</i>	<i>Cylix nkosi</i>	<i>Cylix tupareomanaia</i>
Voucher number	SAMC F04193	SAIAB 39737	Short and Trnski, 2021
Type	Holotype	Paratype	Holotype
Sex	Female	Male	Male
SL (mm)	45.9	46.6	31.4
Trunk rings	14	14	13
Tail rings	35	35	36
Subdorsal trunk rings	3	3	3
Dorsal-fin rays	15	15	14
Anal-fin rays	4	4	4
Pectoral-fin rays	14	14	14
In % of SL			
HL:SL	16.1	16.9	19.6
TrL:SL	34.3	28.9	28.4
DL:SL	5.6	6.1	5.3
PL:SL	2.9	2.8	3.1
TaL:SL	49.6	52.2	52.2
TD4:SL	7.2	7.3	7.7
TD9:SL	6.8	7.6	6.8
SnD:SnL	36.9	43.0	52.5
In % of HL			
HD:HL	68.3	62.5	59.3
SnL:HL	36.6	32.4	32.6
OD:HL	21.9	25.2	16.3
PO:HL	42.8	42.1	42.7
CH:HL	38.2	50.0	48.2
HL:TrL	47.1	58.5	69.2

bone bearing a highly derived and distinct diamond-shaped bony protuberance (SP) oriented anteriorly well behind the eye, rhombus-shaped in anterodorsal view, divided transversally into two sections by a ridge (Figs. 6–9; referred to as the supraoccipital crest in Short and Trnski, 2021); cleithrum continuous middorsally; supracleithrum present; anterior and posterior nuchal plates absent (though overlooked by Short and Trnski, 2021 in their description of *C. tupareomanaia* due to the highly derived morphology of the neurocranial bones, we now note the absence of the posterior nuchal plate represents a synapomorphy of *Cylix*); diamond-shaped bony protuberance on supraoccipital equal in height to cleithrum, forming a large gap between the two bones; thick dorsomedial crest-like ridge present on the first trunk ridge (FTRC) directly behind the cleithrum; gill opening anterior to and supported by the cleithrum; rim of orbit elevated dorsolaterally and strongly ventrally, fluted with rugose sculpturing; opercular ridge low, angled dorsally toward gill opening; swelling of gular region posteroventrally of eye, forming a transverse pair of blunt protuberances; pectoral-fin base without distinct ridges on a strongly elevated ventrolateral bulge; dorsal-fin origin on 12th trunk ring, fin base elevated; trunk in lateral view narrowest at 1st and 2nd trunk rings where angle of head forms from body axis, broadest at 5th trunk ring; principal body ridges distinct and moderately elevated; tail rings of uniform depth over most of length, becoming progressively shorter and smaller near posterior tip; tail prehensile; scutella not evident.

Enlarged spine (SnS1) on dorsal midline of snout on the ethmoid area, at confluence with the anterior ends of supra-orbital ridges, its height extended well above level of nares; two small middorsal spines (SnS2, SnS3) on the mesethmoid bone anterior to the enlarged spine on the snout; three supraorbital extensions or spines (ORE), the anterior two spines a merged double spine and equal in size, the posteriormost spine large and oblong in shape; distinct median frontal spine (FS) at convergence of anterior edges of the supraoccipital bone, protruding forward, anterior margin of spine straight-edged; lateral head spines present, rugose double lateral head spine (DHS) directly below the supraoccipital protuberance, small blunt midlateral spines proximally on operculum; knob-shaped midventral spine (CSS) on the cleithral symphysis; blunt midventral spine (MVFTRS) on the first trunk ring between the pectoral-fin bases (Figs. 2, 7, 9); two oval-shaped spines on cleithral ring, moderate-sized spine (CS1) anterior to ventral third of pectoral-fin base, large lobe-shaped spine (CS2) at ventral extent of head; small spine present (PLS) posterolaterally on the pectoral-fin base (Figs. 2, 7); superior trunk ridge spines absent; lateral trunk ridges with small blunt spines on each trunk ridge starting at the 2nd trunk ridge; inferior trunk ridges with small blunt spines starting on the 2nd trunk ridge; subdorsal spines four, superior trunk ridge ending with two prominent subdorsal spines, superior tail ridge commencing with two small reduced subdorsal spines, in alignment with larger subdorsal spines above; superior tail ridge spines absent.

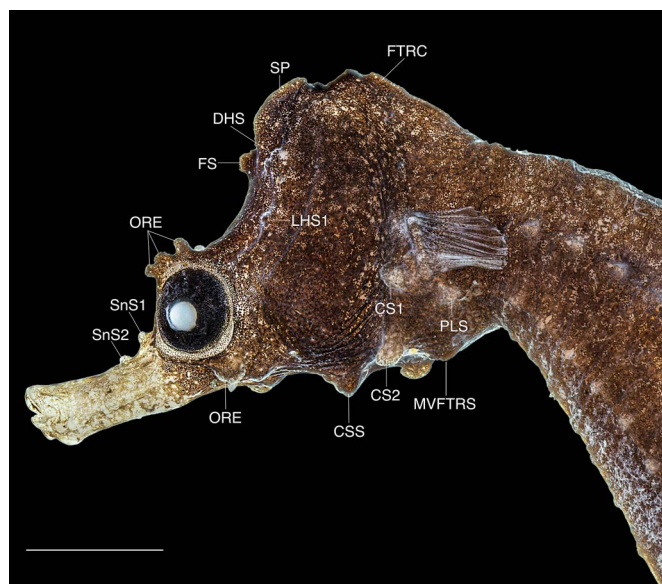


Fig. 2. Lateral view of the head of *Cylix nkosi*, SAMC F041935, female, holotype, highlighting positions of diagnostic characters, including the supraoccipital protuberance and medioventral conical spines on the cleithral symphysis and the first trunk ring between the pectoral-fin bases. Abbreviations: CS, cleithral spines; CSS, medioventral spine on the cleithral symphysis; DHS, double head spine; FS, frontal spine; FTRC, first trunk ring crest; LHS, lateral head spine; MVFTRS, medioventral spine on first trunk ring between the pectoral-fin bases; ORE, orbital rim extension; PLS, posterolateral spine on pectoral-fin base; SnS, snout spines; SP, supraoccipital protuberance. Scale bar = 5 mm.

Male paratype SAIAB 39737 exhibits brood pouch dimorphism (Figs. 1, 4, 6). Brood pouch positioned along the ventral midline of the tail below the anteriormost six tail rings. It is enclosed by six arcuate bony pouch plates (Fig. 6), which extend ventrolaterally from the anterior ventral plate ridges of the tail, progressively reducing in size posteriorly. Second to fifth brood pouch plates consist of double arcuate bony extensions merged into one bony extension. Anterior- and posteriormost pouch plates consist

of single bony extensions. The posteriormost pouch plate diminutive in size.

Coloration.—Holotype in life (Fig. 3), head, trunk, and tail reddish brown; ventrolateral margin of trunk brown; dorsum of head speckled with translucent red dots; snout white. Thin red filaments are present on the dorsum of the trunk. Other individuals of *C. nkosi* observed at the type location exhibit background coloration uniformly solid red, mustard yellow, or light brown, respectively (Fig. 4A–D), which apparently relates to the coloration of local sessile invertebrate growth. In alcohol, the holotype is dark brown in coloration (Fig. 1). Fins hyaline. Coloration of preserved paratype in Figure 1. Specimen lacks pigment in head, trunk, and tail, light tan in coloration.

Distribution and habitat.—*Cylix nkosi* is currently recorded from two locations in KwaZulu-Natal, South Africa: Sodwana Bay and Kosi Bay (Fig. 5). These two sites are situated within the boundaries of the iSimangaliso Wetland Park, a UNESCO World Heritage Site that extends from Lake St Lucia in the south to the Mozambique border in the north, encompassing the north coast of the KwaZulu-Natal province. The new species likely has a wider distribution along the tropical Indian Ocean coast of East Africa and remains undetected, perhaps attributable to its cryptic behavior and diminutive size, low abundance throughout its range, a rarity in the regions where surveys have been conducted, or misidentification as members of *Acentronura* or *Hippocampus*. Future ichthyofaunal surveys, as well as observations by recreational scuba divers, may confirm its occurrence in northern regions such as Mozambique, Tanzania, Kenya, and offshore areas towards Madagascar.

Africa's southernmost coral reefs are situated in northern KwaZulu-Natal Province, South Africa, which are dominated chiefly by soft coral and branching stony corals of the genus *Acropora* and *Pocillopora* (Riegl et al., 1996; Schleyer and Celliers, 2002, 2003; Schleyer et al., 2005,



Fig. 3. *Cylix nkosi* in situ, SAMC F041935, female, holotype, 45.9 mm SL, 2 Mile Reef, Sodwana Bay, KwaZulu-Natal, South Africa, 22 m depth. (A) Lateral view of the body. (B) Anterolateral view of the head highlighting the diamond-shaped supraoccipital protuberance pair on the head. Abbreviation: SP, supraoccipital protuberance. Photographs by Richard Smith.

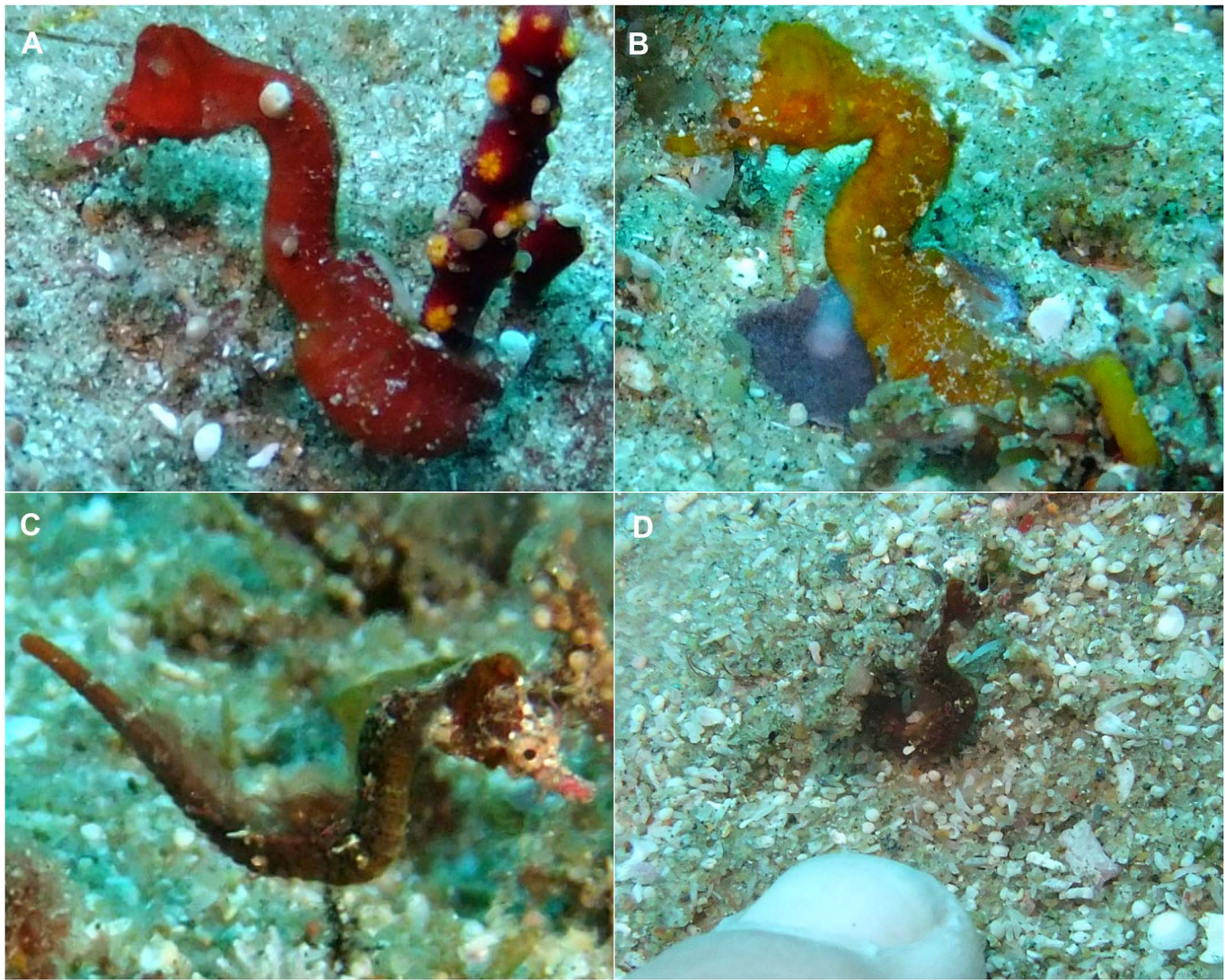


Fig. 4. *Cylix nkosi* in situ, 2 Mile Reef, Sodwana Bay, KwaZulu-Natal, South Africa, 22 m depth: (A) male, pregnant, red coloration; (B) male, pregnant, yellow coloration; (C) female, brown coloration; (D) female, brown coloration. Photographs © Christo van Jaarsveld (SeaXplore), used with permission.

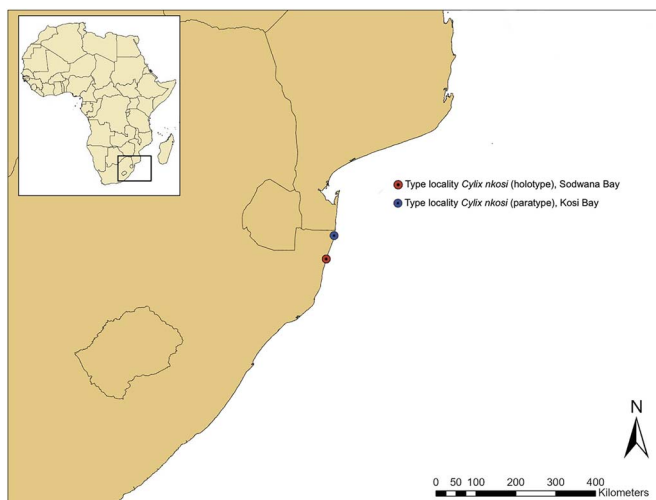


Fig. 5. Distribution of *Cylix nkosi* in South Africa. Red dot = type locality of holotype SAMC F041935 and location of photographed (non-collected) individuals of *C. nkosi*, located at 2 Mile Reef, Sodwana Bay, KwaZulu-Natal, South Africa; blue dot = type locality of paratype SAIAB 39737, located at Kosi Bay, KwaZulu-Natal, South Africa.

2008; Porter and Schleyer, 2017, 2019; Schleyer and Porter, 2018). The coast of KwaZulu-Natal is regularly subjected to open ocean and episodic storm swells that cause the breakage and dislodgement of these stony corals (Riegl et al., 1996; Schleyer and Porter, 2018; Porter and Schleyer, 2019). At the type locality in Sodwana Bay, the marine ecosystem consists of flat sandstone-based coral reefs in addition to unique topographic features comprising low pinnacles, shallow drop-offs, and sandy gullies (Ramsey, 1994). The holotype and other observed individuals of *C. nkosi* in situ (Fig. 4A–D) were observed in protected sandy basins in otherwise exposed areas with accumulated debris near flat rocky reefs and observed loosely associating with sandy worm casts, sponges, and small octocorals (Fig. 4), using the sessile invertebrates as a holdfast for their prehensile tails. The holotype was not observed in close proximity to other individuals of *C. nkosi* at the time of collection, although their cryptic nature may have prevented other individuals from being observed.

Etymology.—The species epithet is derived from the Nguni or Zulu term for chief due to the crown-like nature of the

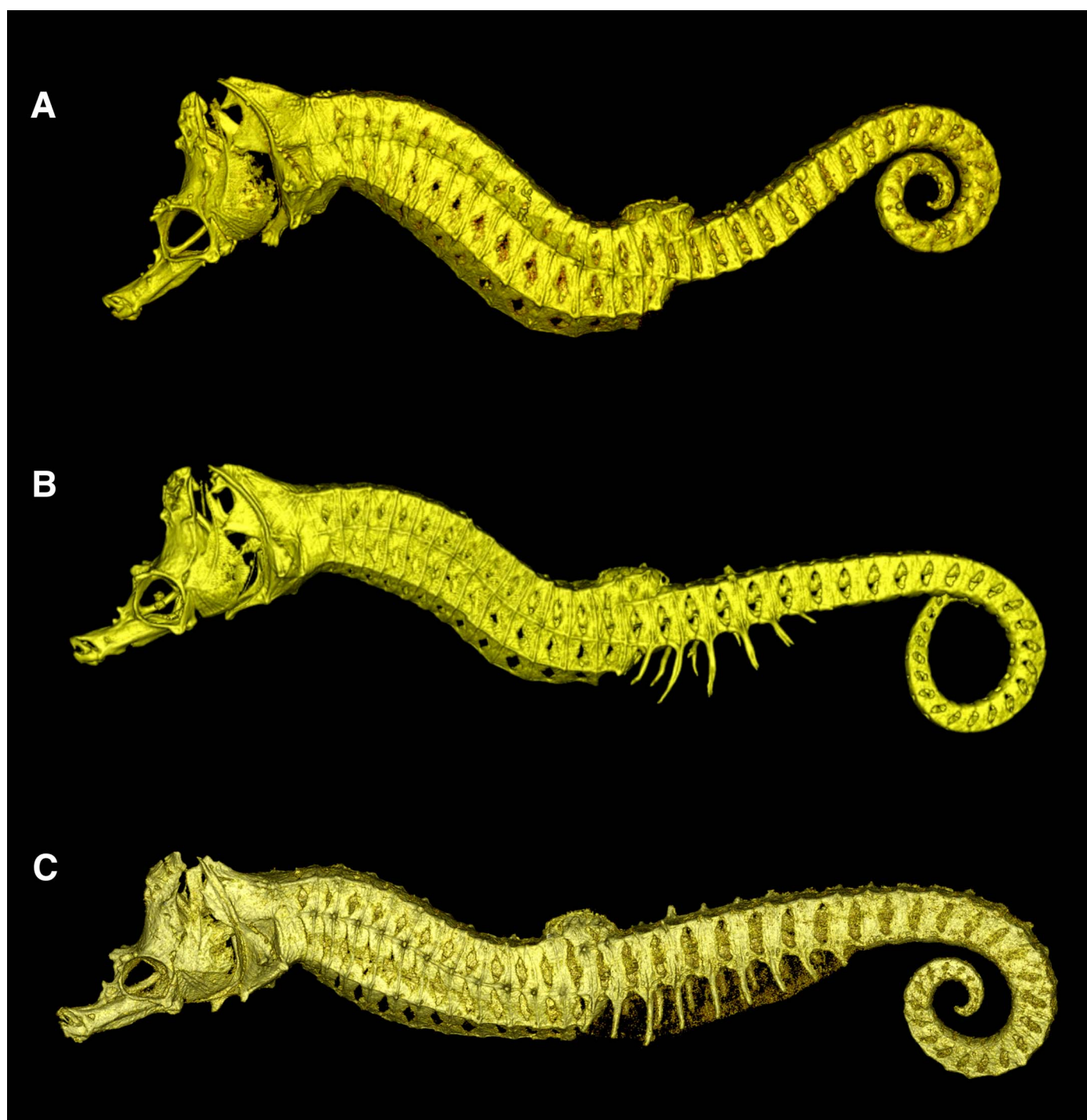


Fig. 6. Lateral view of CT scanned skeletons of preserved specimens of (A) *Cylix nkosi*, SAMC F041935, female, holotype, 45.9 mm SL, (B) *C. nkosi*, SAIAB 39737, male, paratype, 46.6 mm SL, and (C) *Cylix tupareomanaia*, NMNZ P.046322, male, paratype, 55.5 mm SL.

highly derived bony protuberance on the supraoccipital bone. A noun in the genitive. New English Names: Sodwana Pygmy Pipehorse is proposed here for *Cylix nkosi*.

DISCUSSION

Morphological comparisons.—*Cylix nkosi* and *C. tupareomanaia* share numerous derived characters, including angled head, rim of orbit elevated dorsad and ventrad, slender

body, body ridge configurations, prominent supraoccipital, continuous cleithrum, midventral spines on the cleithral symphysis and on the first trunk ring between the pectoral-fin bases, dorsomedial crest-like ridge on the first trunk ridge, two spines on the cleithral ring, spine present posterolaterally on the pelvic-fin base, four subdorsal spines, presence of dimorphic brood pouch plates in males that consist of arcuate bony extensions located under the anteriormost tail rings, prehensile tail, and absence of a caudal fin (Figs. 6–9; Table 2). In addition, the two congeners share certain morphological

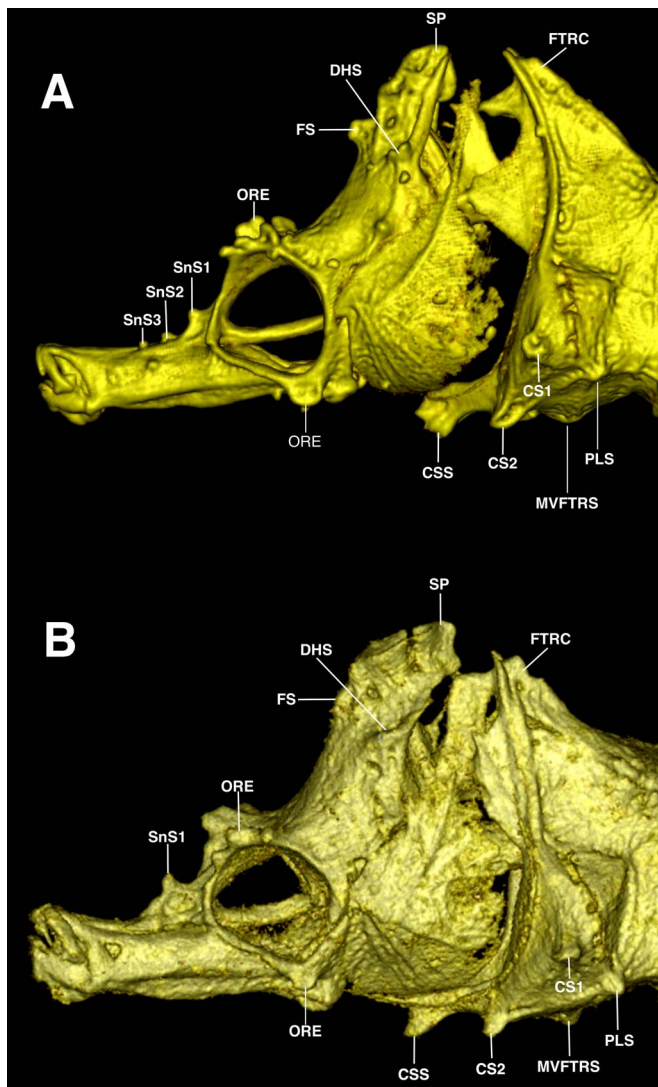


Fig. 7. Lateral view of CT scanned neurocranium of (A) *Cylix nkosi*, SAMC F041935, female, holotype and (B) *C. tupareomanaia*, NMNZ P.046322. Positions of diagnostic characters are highlighted, including the supraoccipital protuberance and medioventral spines on the cleithral symphysis and the first trunk ring between the pectoral-fin bases. Abbreviations: CS, cleithral spines; CSS, medioventral conical spine on the cleithral symphysis; DHS, double head spine; FS, frontal spine; FTRC, first trunk ring crest; LHS, lateral head spine; MVFTRS, medioventral spine on first trunk ring between the pectoral-fin bases; ORE, orbital rim extension; PLS, posterolateral spine on pectoral-fin base; SnS, snout spines; SP, supraoccipital protuberance.

features of the neurocranium, including a prominent supraoccipital bone bearing a highly derived bony protuberance and absence of anterior and posterior nuchal plates as revealed by micro-computed tomography (Figs. 6–8; Table 2).

Although exhibiting a high degree of morphological similarity in external appearance, a suite of anatomical features of the head and first trunk ring readily distinguish the two members of *Cylix* from each other (Figs. 6–9; Table 3), including the shape of the supraoccipital bony protuberance, which differs markedly in morphology in the form of complex anatomical shapes (diamond-like in *C. nkosi* vs. cup-like in *C. tupareomanaia*); the shape of the midventral spines on the cleithral symphysis (knob-like in *C. nkosi* vs. udder-like in

C. tupareomanaia); the shape of the midventral spines on the first trunk ring between the pectoral-fin bases (blunt in *C. nkosi* vs. conical in *C. tupareomanaia*); the shape of the dorsomedial crest-like ridge on the first trunk ring (thick in *C. nkosi* vs. short and rugose in *C. tupareomanaia*). The members can be further distinguished by the number and shape of the male brood pouch plates. *Cylix nkosi* possesses six plates in the form of double arcuate extensions, whereas *C. tupareomanaia* possesses eight plates in the form of single arcuate extensions (Fig. 6).

Mitochondrial genome and genetic distances.—A total of 7,259,439 forward and reverse reads were recovered from the shotgun library of which 15,273 were used to assemble the mitogenome of *C. nkosi* (coverage: mean = 117.1, SD = 20.3). The mitogenome of *C. nkosi* is 16,534 bp, consisting of 13 protein-coding genes, two rRNA genes, 22 tRNA genes, and a control region (D-loop). Table 4 summarizes uncorrected *p*-distances at the mitochondrial CO1 gene between *C. nkosi* and the previously sequenced pygmy pipehorses *C. tupareomanaia*, *A. breviperula*, *A. tentaculata*, *I. australe*, and *I. lumnitzeri* (Hamilton et al., 2017; Short and Trnski, 2021). *Cylix nkosi* differs from *C. tupareomanaia* by 10.0%, *A. breviperula* by 16.6%, *A. tentaculata* by 20.6%, *I. australe* by 18.1%, and *I. lumnitzeri* by 18.8%. According to the Reece et al. (2010) study, the reported mitochondrial DNA (mtDNA) clock rates in marine teleosts suggest a divergence rate of approximately 1.2% per million years. Applying this rate indicates that the divergence between *C. nkosi* and *C. tupareomanaia* may have occurred approximately 8.3 million years ago.

Biogeography of *Cylix*, *Acentronura*, and *Idiotropiscis*.—*Cylix* comprises two recognized species that occur in the Southern Hemisphere. *Cylix nkosi* occurs along the northeast coast of South Africa in the Western Indian Ocean, whereas *C. tupareomanaia* occurs off the North Island of Aotearoa New Zealand in the South Pacific (Short and Trnski, 2021). A representative of *Cylix* was recorded in Fiji, located in the Western Pacific, by the liveboard scuba diving company Nai'a in 2010. These records can be found at the following online sources: <https://inaturalist.ala.org.au/observations/34691744> and https://www.youtube.com/watch?v=RowELvDg7jY&ab_channel=Saminfiji. Furthermore, a recent observation of *Cylix* was recorded at Mafia Island, Tanzania in 2022, expanding the known distribution of the genus in eastern Africa (<https://inaturalist.ala.org.au/observations/143646333>). Similarly, a representative of *Cylix* was recently recorded in the Maldives in March 2023, marking the first confirmed observation of the genus in the Northern Hemisphere. (<https://www.inaturalist.org/observations/150749541>).

Although *Cylix* exhibits a wide distribution spanning the Indo-Pacific region, from the Western Indian Ocean to the Central and South Pacific, its members are at present recorded as short-range endemics restricted to their respective type localities. This restricted range is likely a result of morphological factors such as their diminutive size and adaptations for crypsis. Additional contributing factors may include geographical isolation, ocean currents, and specific habitat preferences (Smith, 1963; Briggs, 1999; Randall, 2001; Letourneur et al., 2004; Gordon et al., 2010). The distribution of *Cylix* encompasses

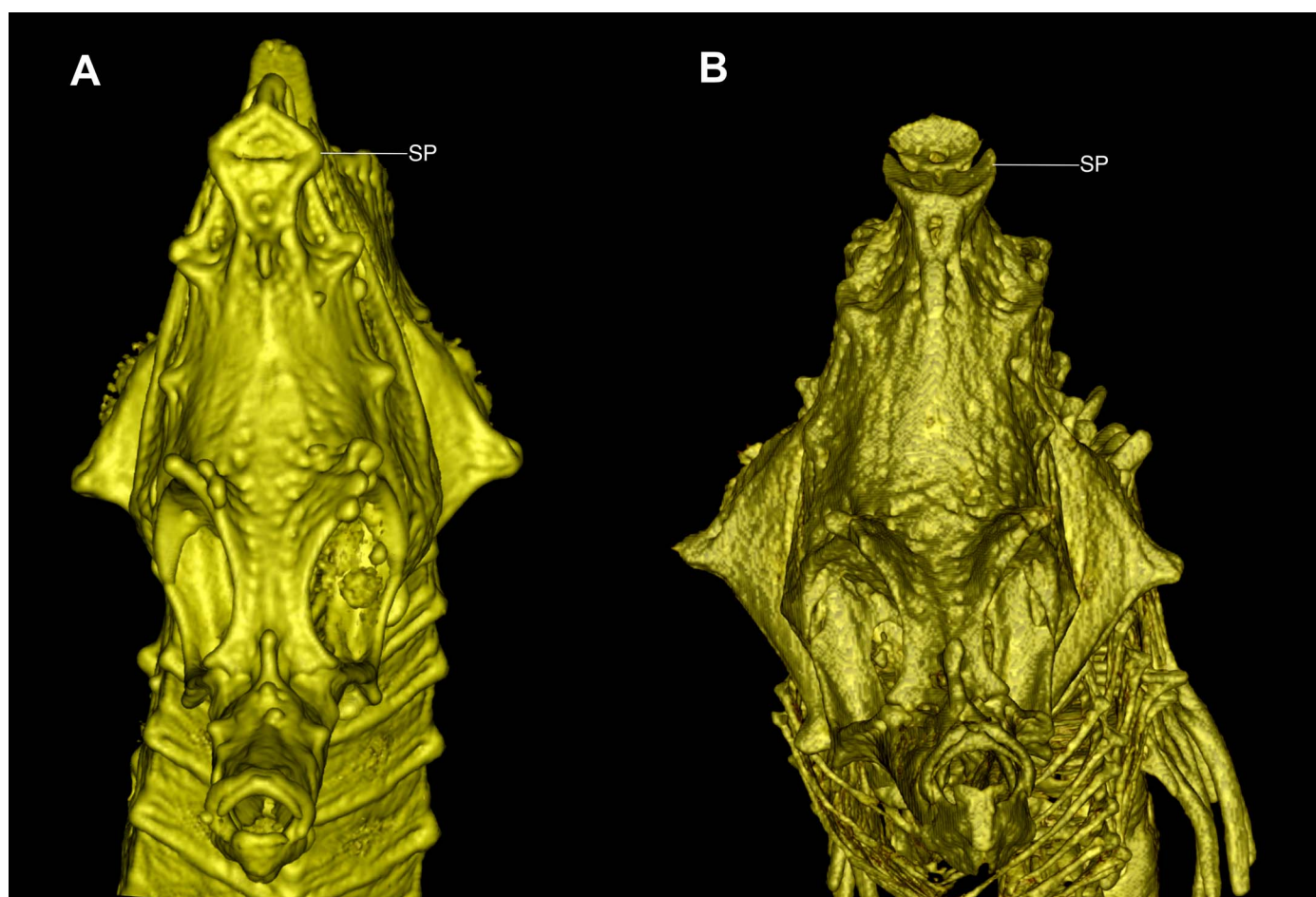


Fig. 8. Anterior view of CT scanned neurocranium highlighting supraoccipital protuberances: (A) *Cylix nkosi*, SAMC F041935, female, holotype; (B) *C. tupareomanaia*, NMNZ P.046322. In *C. nkosi*, the supraoccipital protuberance is diamond-shaped, while in *C. tupareomanaia*, it is bifurcated and cup-like. Abbreviation: SP, supraoccipital protuberance.

three well-established marine biogeographic provinces, all recognized as biodiversity hotspots for nearshore marine ichthyofauna: the tropical western Indian Ocean (based on specimen records), Indo-Polynesian Provinces (based on photographic observations), and the temperate Auckland Province (based on specimen records; Smith, 1963; Briggs, 1974, 1999; Gordon et al., 2010; Briggs and Bowen, 2012; Kulbicki et al., 2013; Toonen et al., 2016). Marine biogeographic provinces of the Indo-Pacific, based on distinct marine floras and faunas, are defined by >10% endemism and recognized habitat discontinuities in coastal and shallow marine habitats (Briggs, 1974; Brown and Lomolino, 1998; Bellwood and Wainwright, 2002; Robertson et al., 2004; Floeter et al., 2008; Briggs and Bowen, 2012; Kulbicki et al., 2013; Bowen et al., 2016; Samoilys et al., 2022). In addition, *Cylix* spans both the warm-temperate and tropical regions of the Indo-Pacific, which distinguishes it from most of the 57 currently described syngnathid genera (Dawson, 1985; Fricke et al., 2023). Seven other genera, including *Campichthys*, *Festucalex*, *Hippocampus*, *Maroubra*, *Nannocampus*, *Syngnathus*, and *Urocampus* (Dawson, 1985; Kuitert, 2004; Short and Trnski, 2021; Araki and Motomura, 2022), share this distribution across both regions of the Indo-Pacific. It is important to note that *Cylix* was only identified within the past decade,

so our knowledge of its distribution and behaviors remains limited.

The three members of *Idiotropiscis* are restricted to the Southern Hemisphere and recorded exclusively in Australia within the temperate Southeastern and tropical Southwestern Australian Provinces (Dawson, 1985; Kuitert, 2004; Briggs and Bowen, 2012; Toonen et al., 2016; Short and Trnski, 2021). *Idiotropiscis lumnitzeri* is a short-range endemic restricted to New South Wales (NSW) where it occurs from Bawley Point in the south to Cabbage Tree Bay, Manly. *Idiotropiscis australe* occurs over a broader range extending from the Fleurieu Peninsula in South Australia (<https://www.inaturalist.org/observations/17644477>, <https://www.inaturalist.org/observations/87369954>) to southern Western Australia (https://www.inaturalist.org/observations?place_id=6827&subview=map&taxon_id=89079; Dawson, 1985). Types specimens of *I. laronae* are recorded in northern Western Australia (Dawson, 1984, 1985), within the southern extent of the Indo-Polynesian Province.

In contrast, members of *Acentronura* exhibit a wide distribution that extends across both the Northern and Southern Hemispheres. This genus occupies a significant range within the Indo-Pacific region, encompassing four distinct marine biogeographic provinces (Dawson, 1985;

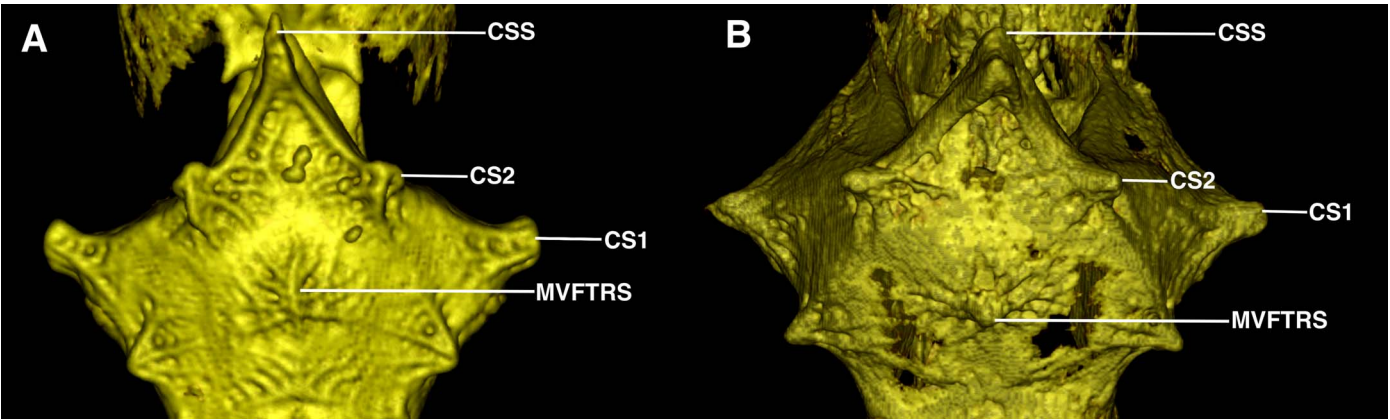


Fig. 9. Ventral view of CT scanned first trunk ring highlighting the medioventral spines on the cleithral symphysis and the first trunk ring between the pectoral-fin bases: (A) *Cylix nkosi*, SAMC F041935, female, holotype; (B) *C. tupareomanaia*, NMNZ P.046322. Abbreviation: CS, cleithral spines; CSS, medioventral spine on the cleithral symphysis; MVFTRS, medioventral spine on first trunk ring between the pectoral-fin bases.

Table 2. Shared morphological derived characters among the type specimens of *C. nkosi* and *C. tupareomanaia*.

	<i>Cylix nkosi</i>	<i>Cylix nkosi</i>	<i>Cylix tupareomanaia</i>
Voucher number	SAMC F04193	SAIAB 39737	Short and Trnski, 2021
Type	Holotype	Paratype	Holotype
Head angled ventrad 25°		present	
Superior and inferior trunk and tail ridges		discontinuous	
Supraoccipital bony protuberance		present	
Anterior nuchal plate		absent	
Posterior nuchal plate		absent	
Dorsomedial crest-like ridge on first trunk ring		present	
Large rugose lateral head spine		present	
Small lateral head spines		present	
Supraoccipital spine		present	
Snout spines		present	
Midventral spine on cleithral symphysis		present	
Midventral spine on first trunk ring		present	
Cleithrum confluent		present	
Cleithral spine on the first trunk ring between the pectoral-fin bases		present	
Small spine ventroposterior to pectoral-fin base		present	
Subdorsal spines		present	
Orbital rim extensions		present	
Swelling of gular region ventroposterior to eye		present	
Superior trunk ridge spines		present	
Superior tail ridge spines		present	

Table 3. Comparison of morphological features that distinguish the type specimens of *C. nkosi* from *C. tupareomanaia*.

	<i>Cylix nkosi</i>	<i>Cylix nkosi</i>	<i>Cylix tupareomanaia</i>
Voucher number	SAMC F04193	SAIAB 39737	Short and Trnski, 2021
Supraoccipital bony protuberance	Diamond-shaped	Diamond-shaped	Cup-shaped
Midventral spine on the cleithral symphysis	Knob-shaped	Knob-shaped	Udder-shaped
Midventral spine on the first trunk ring between the pectoral-fin bases	Blunt	Blunt	Conical
Crest-like ridge on first trunk ring	Thick	Thick	Thin and rugose
Number of male brood pouch plates	6	6	8

Short and Trnski, 2021). *Acentronura breviperula* is distributed throughout the Indo-Polynesian Province, extending into the northern boundaries of the Southeastern Australian Province (specifically, Stradbroke Island, QLD;

Richard Smith, pers. obs., 2020). *Acentronura tentaculata* occurs within the Indo-Polynesian and Western Indian Ocean Provinces (Dawson, 1985), while *A. gracilissima* is restricted to the Sino-Japanese Province in central and southern Japan

Table 4. Uncorrected genetic distances (*p*-distances) summary between *C. nkosi*, *C. tupareomanaia*, and members of *Acentronura* and *Idiotropiscis* based on cytochrome c oxidase I (COI) sequences analyzed in this study.

	GenBank	Species	1	2	3	4	5	6
1	OM022101	<i>C. nkosi</i>						
2	MH30620	<i>C. tupareomanaia</i>	0.100					
3	KY066067	<i>A. breviperula</i>	0.166	0.195				
4	KY066068	<i>A. tentaculata</i>	0.206	0.204	0.144			
5	MH30620	<i>I. australe</i>	0.181	0.179	0.097	0.131		
6	KY066120	<i>I. lumnitzeri</i>	0.188	0.184	0.146	0.146	0.141	

(Dawson, 1985). Given these findings, it is highly probable that targeted surveys within the aforementioned marine biogeographic provinces across the Indo-Pacific would uncover numerous additional species records for *Cylix*.

DATA ACCESSIBILITY

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