A New Cryptic Species of Polymixia (Teleostei, Acanthomorpha, Polymixiiformes, Polymixiidae) Revealed by Molecules and Morphology

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A New Cryptic Species of *Polymixia* (Teleostei, Acanthomorpha, Polymixiiformes, Polymixiidae) Revealed by Molecules and Morphology

Terry C. Grande[1] and Mark V. H. Wilson[1,2]

A new and previously cryptic fish species, *Polymixia hollisterae*, new species, proposed common name ''Bermuda Beardfish,'' is described from two voucher specimens collected in 1997 at a depth of about 280 fm or 512 m on the NW slope of the Bermuda Platform, and a third voucher collected by a midwater trawl in the north-central Gulf of Mexico. The species belongs to the paracanthopterygian acanthomorph genus *Polymixia*, family Polymixiidae, order Polymixiiformes, and is named after pioneering ichthyologist, ocean explorer, and conservationist Gloria E. Hollister (Anable). It is distinguished from all other species in the genus by its greater dorsal and anal fin heights (19% and 14% of SL, respectively), its extremely long pectoral and pelvic fins (22% and 14% of SL, respectively), its relatively large eye diameter (34% of total head length), and its morphologically distinctive first anal-fin radial. It has a unique pigmentation pattern on the dorsal, anal, and caudal fins consisting of narrow, dark black patches near the tips of the longest few soft dorsal- and anal-fin rays, and a caudal-fin margin with a dark black fringe covering the distal fourth of the fin. Based on an earlier molecular study that revealed it to be a cryptic species, *P. hollisterae*, new species, is closely related to *P. japonica*, but it differs by 20–33 nucleotides in a total alignment of 4,983 nuclear and mitochondrial sites. The new species shares a distinctive scale morphology with *P. japonica* and *P. nobilis* (the type species); an intermediate number of dorsal-fin rays (V,31–32), like *P. japonica* (V,31–34); a distinctive preopercle shape with *P. japonica*, *P. lowei*, and *P. berndti*; and a low number of pyloric caeca with *P. lowei* and *P. berndti*. Pored lateral-line scales number 35, an intermediate number compared to reported counts of other species (31–39). The three supraneurals are sigmoid-shaped like those of *P. nobilis*, while dorsal-fin proximal radials interfinger with neural spines in a unique pattern. Body shape, as studied with measurement proportions and multivariate morphometrics, is distinguished by a relatively large head, large eyes, long jaws, and a more streamlined pre-dorsal body profile than its congeners. Further research and additional collecting will be needed to ascertain the geographic distribution and conservation status of the new species.

*P*OLYMIXIA (order Polymixiiformes) is an enigmatic fish genus, phylogenetically positioned at or near the base of the Paracanthopterygii, a group that also includes the orders Percopsiformes, Zeiformes, Stylephoriformes, and Gadiformes (Betancur-R et al., 2013; Borden et al., 2013, 2019; Grande et al., 2013, 2018; Hughes et al., 2018). Known as the beardfishes for their distinguishing pair of long hyoid barbels, species of *Polymixia* have been collected from mesophotic (40–129 m), rariphotic (130–309 m; Baldwin et al., 2018), and aphotic depths (>309 m), mostly over the outer margins of continental shelves and over continental slopes and flanks of seamounts (submarine and present-day islands) in the Atlantic, Pacific, and Indian Oceans (Borden et al., 2019).

Species of this genus are generally demersal fishes as adults, inhabiting depths of about 100–700 m; the type species, *P. nobilis*, is thought to prefer areas with hard bottoms (García-Mederos et al., 2010) but some others appear to prefer soft substrates (e.g., *P. lowei*; Baumberger et al., 2010). Some species have been observed to trail their barbels close to the seafloor (e.g., *P. lowei*: Baumberger et al., 2010; *P. japonica* observed in aquarium: Tsujita, 2020) or actively touch and/or probe the sediment surface with their barbels (*P. japonica*: JAMSTEC, 2014). Ono (1982) described the unique intrinsic muscular system and innervation of the barbels in *P. lowei* and *P. japonica*, while Kim et al. (2001) described the muscles controlling the base of the barbel in three Pacific species of *Polymixia*. Smaller adult specimens (<36 cm fork length) of *P. nobilis*, studied in waters off the Canary Islands, were found

to have stomach contents that included mainly crustaceans such as pandalid shrimps, whereas larger individuals had ingested multiple species of small fishes, crustaceans, and occasionally squids and octopuses, in decreasing order of frequency (García-Mederos et al., 2010). A spawning aggregation containing thousands of *P. lowei* was recorded in a 413 m depth sinkhole near the Florida Keys using a crewed submersible (Baumberger et al., 2010).

Although 14 nominal species of *Polymixia* have been described, ten have traditionally been considered valid in recent decades: *Polymixia nobilis*—the type species (Atlantic Ocean), *P. berndti* (Pacific and Indian Oceans), *P. busakhini* (Indian and southwestern South Pacific Oceans), *P. fusca* (Arabian Gulf region), *P. japonica* (Western North Pacific), *P. longispina* (Western North Pacific and Eastern Indian Oceans), *P. lowei* (Western Atlantic), *P. salagomeziensis* (Eastern South Pacific), *P. sazonovi* (Western North Pacific), and *P. yuri* (Eastern South Pacific; Kotylar, 1982, 1984, 1991, 1993). Taxonomic authorities for all nominal species are given in Borden et al. (2019). All species of *Polymixia* are phenotypically broadly similar in color patterns and general body shape, with most meristics overlapping, making species identifications difficult. All species share 29 total vertebrae, five dorsal-fin spines, four anal-fin spines, and seven pelvic-fin rays. In addition, all species possess antorbital and orbitosphenoid bones in the skull, a large supraoccipital crest, a forward extension of the supratemporal fossa, subocular shelves on all infraorbitals, two hyomandibular condyles, two supramaxillae, three sets of intermuscular

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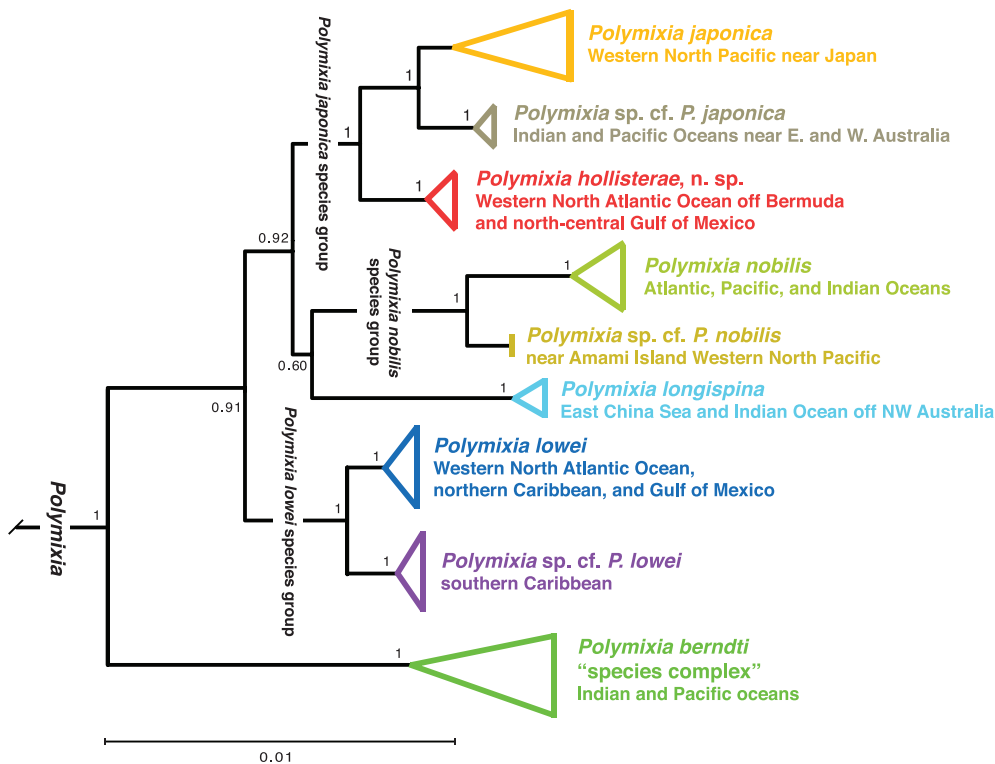
Fig. 1. Phylogeny of species and species groups in the genus *Polymixia* based on Bayesian results using nuclear and mitochondrial loci from Borden et al. (2019). The new Bermuda species is a member of the *P. japonica* species group, sister to *P. japonica* + *Polymixia* cf. *P. japonica*. The triangle representing each species has its vertical edge proportional to the number of genetic samples studied and its left apex located at the earliest split among all of its samples (compare with Borden et al., 2019: fig. 4).

bones or ligaments, and a full spine on preural centrum 2 (Grande et al., 2013). The morphological similarity among species in many instances resulted in incorrect identifications or identifications based on locality, with researchers not realizing that some species might have broader geographical distributions.

Borden et al. (2019) recently examined the taxonomic composition, phylogenetics, and geographic distributions of species of *Polymixia* based on five nuclear and two mitochondrial loci (results summarized here in Fig. 1). Six nominal species for which DNA samples were available were included in their study, but nine species-level clades were recovered. Five of these represent previously recognized species (*P. nobilis*, *P. berndti*, *P. japonica*, *P. longispina*, and *P. lowei*), although several were found to have revised geographic distributions. Evidence also suggested that *P. busakhini* is a junior synonym of *P. nobilis*, leaving five valid species of the six that were included by Borden et al. (2019). In addition, four of the nine species-level clades recognized by them appeared to be unnamed. One of the unnamed species-level clades (the subject of this study) was based on samples from Bermuda, originally identified as *P. lowei* by Smith-Vaniz et al. (1999) and catalogued as such in the Bermuda Aquarium Museum and Zoo (BAMZ) collection. In the phylogeny of Borden et al. (2019), this clade was recovered in the *P. japonica* species group, which also included *P. japonica* from Japanese waters and two specimens of another unnamed species from waters off eastern and Western Australia. The "Bermuda clade" is genetically distinct from *P. japonica* by 20–33 nucleotides and from the unnamed Australian species by 26–29 nucleotides in the total alignment of 4,983 sites (Borden et al., 2019).

In addition, Borden et al. (2019) examined morphological characters previously suggested by Kotlyar (1982, 1993). They concluded that the "Bermuda clade" is similar to *P.*

japonica and *P. nobilis* in having a unique scale morphology where the ctenus-like spines of its spinoid scales (Roberts, 1993) are arranged in a wedge configuration (Kotlyar, 1982, 1993). This clade is also similar to *P. japonica* in the number of dorsal-fin rays and the rounded posterior margin of the preopercle. *Polymixia nobilis,* on the other hand, has a much higher dorsal-fin ray number than both the new species and *P. japonica*, and exhibits a uniquely pointed preopercular margin.

Here we formally describe this "Bermuda clade" as a new species of *Polymixia*, based on three voucher specimens: adult specimens from Bermuda for two of the three genetic samples analyzed by Borden et al. (2019), and a third voucher identified by its barcode sequence—a small juvenile from the Gulf of Mexico that is missing the posterior third of its body as a result of tissue sampling for DNA. The two specimens from Bermuda were located and borrowed by the authors from BAMZ. The new species is compared with other species in the genus, including *P. japonica*, its presumed closest relative among named species, as well as with specimens of *P. lowei* and *P. nobilis* that have also been collected both in Bermuda waters and the Gulf of Mexico. The new species is distinct not only on the basis of its molecular sequence, but also by previously unrecognized anatomical, meristic, and morphometric characters unique among its congeners, confirming it as new.

MATERIALS AND METHODS

Measurements specific to this study.—BD, maximum body depth measured vertically from the origin of the dorsal fin; HDH, head height measured vertically from ventral body margin at the anteroventral tip of the preopercle to the dorsal body margin at the tip of the supraoccipital; POHL, preopercular head length measured from the tip of the snout horizontally to the posterior margin of the preopercle; SL,

standard length measured from the tip of the snout to the end of the hypural plate; THL, total head length measured from the tip of the snout to the posterior margin of the opercle.

Meristics, measurements, and specimen deposition.—Length data (e.g., eye diameter, pre-dorsal length) were measured on alcohol specimens where possible or from radiographs. Ratios or proportions are expressed as percentages of the SL, BD, POHL, or THL. Fin-ray counts were made from both alcohol specimens and radiographs. Scale counts were obtained directly from alcohol specimens. Standard measurements and counts were made from the left side of the specimen following Hubbs and Lagler (2004). Additional scale counts and measurements specific for *Polymixia* followed Kotlyar (1982, 1993).

Osteological features of the type material were observed by means of radiographs made in the Division of Fishes, FMNH or μCT scans made at Loyola Chicago. The paratype from Bermuda was dissected to count the number of pyloric caeca. The skeletal and internal anatomy of comparative material was examined by means of alcohol specimens, cleared and double-stained specimens (Dingerkus and Uhler, 1977), and radiographs. One important dried skeleton of *P. nobilis* from the Canary Islands deposited in the collections of the Academy of Natural Sciences of Philadelphia (ANSP) was examined.

The holotype of the new species is deposited in the fish collections of the BAMZ. The paratype from Bermuda is deposited in the collections of FMNH. The second paratype from the Gulf of Mexico, which is just the third known specimen of the species, is deposited in the collections of the MCZ. Both Bermuda specimens of the new species, as well as multiple Bermuda specimens of *P. lowei* (BAMZ collections), together with the single Bermuda specimen of *P. nobilis* from Challenger Bank (ANSP 124292), were originally caught by sport and/or commercial fishermen using vertical long lines and subsequently donated to the museums for scientific study.

Morphometric analysis.—Multivariate morphometric analyses were conducted to assess the body-shape variation within and among species of *Polymixia*, and to compare the body form of the new species with those of its closest relatives. Fin shapes could not be assessed morphometrically (except for lengths of the longest rays) because fins in the alcohol-preserved specimens were at different degrees of compression or spread when imaged. The majority of 2D landmarks for multivariate morphometrics were taken from high-resolution digital images of alcohol-preserved specimens, most of them newly imaged for this study. A few images of rare species were obtained from museum and biodiversity web sites (see comparative material). Altogether, 34 landmarks for each of 27 specimens were digitized by means of the PointPicker plugin (Thévenaz, 2016) using ImageJ v. 2.2.0 (Rasband, 2010–2020). Morphometric analyses used MorphoJ version 1.06d (Klingenberg, 2011). Landmarks were subjected to Procrustes fit aligned by principal axes. A covariance matrix was generated from the Procrustes coordinates and subjected to Principal Component Analysis (PCA). Shape changes corresponding to each of the first four principal components of the PCA were examined. Shape differences along the principal axes were visualized using wireframe diagrams. The

landmarks for most specimens were also used to calculate linear measurements and measurement ratios for comparisons among species. Landmarks specified in pixel coordinates were used to calculate linear measurements in pixel units and subsequently converted to millimeters based on ruler scales included within the images.

Polymixia hollisterae, new species

urn:lsid:zoobank.org:act:475AE39F-7A68-4519-870E-3228F9893AD8
Order Polymixiiformes Rosen and Patterson, 1969
Family Polymixiidae Bleeker, 1859
Genus *Polymixia* Lowe, 1836
Proposed common name: Bermuda Beardfish
Figures 2–6, Table 1

Polymixia lowei (in part), Smith-Vaniz et al., 1999: BAMZ 1997-159-006 (2, 170–180).

Holotype.—(Figs. 2A, 4A, 5A, B, 6A) BAMZ 1997-159-006, 173.0 mm SL, 224.0 mm TL, Bermuda, "outside Eastern Blue Cut" near the NW edge of the Bermuda Platform, 32°25′01″N, 64°56′43″W, 280 fathoms (512 m), hook and line (vertical long line), collected and donated by Craig Soares and Richard Allen, 1 August 1997.

Paratypes.—(Figs. 2B, 4B, 5C, 6B) FMNH 145004, 185.0 mm SL, 240.0 mm TL, collecting locality, date, depth, method, and collectors same as holotype. (Fig. 3) MCZ 174218, small juvenile, 20 mm SL, original catalog no. DeepEnd Consortium DPND 1320, original identification *P. lowei*, sample i.d. DP02-11Aug15-MOC10-SW-3D-017-N0, midwater trawl 10 m² MOCNESS, 3 mm mesh, depth range 0–1502.5 m, in water of maximum depth 2500 m, towed between 27°0′13″N, 88°29′39″W and 26°52′15″N, 88°31′22″W, 11 August 2015.

Diagnosis.—A species of *Polymixia* distinguished from all other species in the genus based upon the following unique characters. Measurements are based on adult specimens: eye diameter (EYD) larger than in other species of the genus, averaging 46.4% of POHL vs. 36%–44% in congeners; head longer relative to SL than in congeners; POHL averaging 25.4% of SL vs. 22%–24% in others; THL averaging 34.4% of SL; dorsal and anal fin heights greater than in other species of *Polymixia*, longest dorsal and anal rays averaging 20.6% and 15.2% of SL, respectively, vs. 14–17% and 11–13% in congeners; pectoral (PFL) and pelvic fins (VFL) relatively longer than in other species, averaging 22.1% and 15% of SL, respectively, vs. 18–20% and 12–13% in congeners. The first anal radial is distinctive: the main shaft of the radial is noticeably straighter as opposed to significantly curved in other species; the anterior process is relatively narrow and nearly horizontal with respect to the axis of the body (5° from axis vs. 10–25° in congeners) and makes an angle of about 38° with the main shaft, greater than the angles of approximately 25–32° seen in other species of *Polymixia*. In addition, the new species exhibits a unique pigmentation pattern on the dorsal, anal, and caudal fins. The pattern consists of narrow, dark black patches near the tips of the longest soft dorsal- and anal-fin rays (vs. much broader, dark gray patches in congeners), and a caudal-fin margin with a dark black, continuous fringe covering the distal fourth of almost all caudal-fin rays (vs. more diffuse patches only near

Fig. 2. The type specimens of *Polymixia hollisterae*, new species, from Bermuda. (A) Holotype, BAMZ 1997-159-006, 173 mm SL. (B) Paratype, FMNH 145004, 185 mm SL. Scale bars = 1 cm.

the tips of the dorsal and sometimes the ventral caudal lobe in congeners). Like *P. japonica* and *P. nobilis*, the new species exhibits wedge-shaped rows of ctenus-like spines on its spinoid scales. This is contrary to the vertical rows of spines near the posterior edge of each scale in *P. fusca, P. lowei, P. berndti,* and *P. longispina*.

Etymology.—The new species is named in honor of Gloria E. Hollister (Anable), B.S., M.S. (1900–1988), pioneering ichthyologist, key member of the William Beebe bathysphere expeditions in Bermuda, world record holder for deep-sea descent by a woman, leader of tropical zoological expedition, Red Cross Blood Bank pioneer, and ground-breaking conservationist. Further details of her contributions are given in Appendix 1.

Molecular evidence.—*Polymixia hollisterae* was, until now, a true cryptic species. Its existence was not suspected when experts initially identified the holotype and Bermuda paratype as *P. lowei* (Smith-Vaniz et al., 1999), whereas the Gulf of Mexico paratype was initially identified as *P. nobilis* and later reidentified as *P. lowei* (T. T. Sutton, DeepEnd Consortium, pers. comm., 2020). Muscle tissues were removed by earlier investigators for DNA extraction from the right side of the holotype and the Bermuda paratype. The posterior third of the body and tail had been removed from

the much smaller Gulf of Mexico paratype as a barcoding tissue sample (Fig. 3B).

The phylogeny of species clades in the genus *Polymixia* (Fig. 1) adopted here is the Bayesian analysis of mitochondrial and nuclear sequences by Borden et al. (2019: fig. 4). GenBank numbers for the sequences analyzed are given in that paper. The analysis by Borden et al. (2019) resolved a unique clade with high support values composed of three samples from Bermuda representing the new species, with relationships to other species clades as shown in Figure 1.

Two of the Bermuda samples from Borden et al. (2019) are represented in a distinct cluster in the neighbor-joining (NJ) identification 'trees' for *Polymixia* from the Barcode of Life Database (BOLD Systems; Ratnasingham and Hebert, 2007), based on the mitochondrial COI locus (Borden et al., 2019: fig. S1). It is not possible based on present information to determine which of the two Bermuda samples in the BOLD cluster belongs to the holotype and which to the Bermuda paratype. A third sample in the barcode cluster is the Gulf of Mexico paratype of *P. hollisterae* (Fig. 3), with locality listed in BOLD as "United States." The available barcode sequence of the Gulf of Mexico paratype is identical to that of one of the two Bermuda type specimens.

Description.—See Table 1 for measurements and meristics of both the holotype and the very similar Bermuda paratype. Damage to the body and fins of the Gulf of Mexico paratype
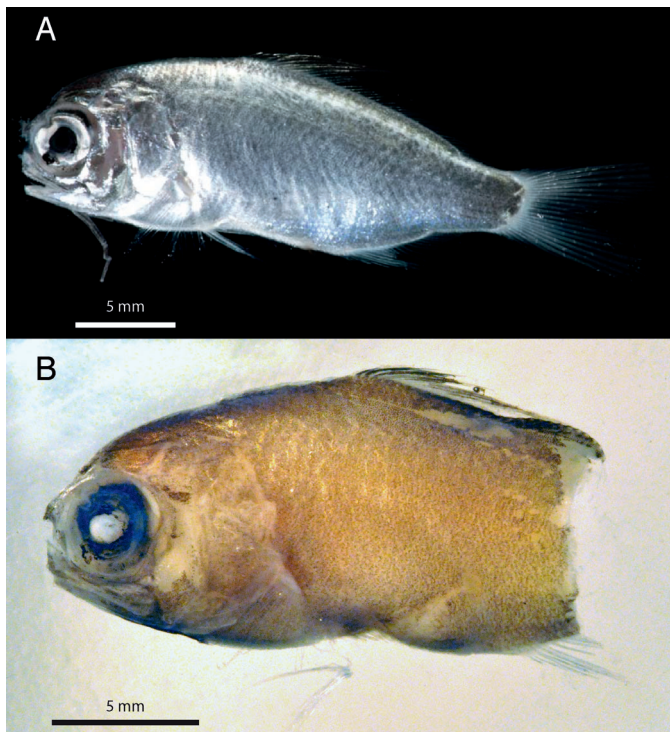
Fig. 3. The second paratype of *Polymixia hollisterae*, new species, from the north-central Gulf of Mexico, MCZ 174218, 20 mm SL (formerly DeepEnd Consortium specimen DPND 1320). (A) The image taken aboard ship shortly after collection in 2015, photo by Danté Fenolio, © DeepEnd, used with permission. (B) The existing specimen, lacking the posterior one-third of the body and tail owing to tissue sampling for DNA. Scale bars = 5 mm (estimated from the specimen's original standard length).

precluded obtaining many of the meristic or morphometric data from the specimen.

Detailed measurements and meristics of the adult type specimens are given in Table 1. The holotype (SL 173 mm) and Bermuda paratype (SL 185 mm) have elongated and laterally compressed bodies (Fig. 2) with body depths of 37% and 36.4% of SL. The predorsal lengths are 46% and 45% of SL. The caudal peduncle lengths are 15% and 14.5% of SL. The caudal peduncle depths are 10.1% and 10.2% of SL. The THL are 34% and 35% of SL. The POHL are 25.1% and 25.7% of SL. The HDH are 32.3% and 34.9% of SL. Snout lengths are 23.3% and 21.6% of POHL or 17% and 16% of THL. This species has an extremely large eye compared to its congeners (Figs. 2, 4, 5B), 45% and 47% of POHL or 34% and 35% of THL. There are five large circumorbital bones, each with a laterally projecting flange bordering the orbit. Pores of the circumorbital sensory canal are seen immediately behind or beneath the flange (Figs. 5B, 6).

The Gulf of Mexico paratype is a small juvenile that is missing about one-third of its body and tail. Measurements estimated in ImageJ from the shipboard image (Fig. 3A), using the original record of its standard length (20 mm) for scale, are as follows: caudal peduncle length 16% of SL; caudal peduncle depth 2.5 mm (12.5% of SL); length of dorsal-fin base 46.5% of SL; length of anal-fin base 18% of SL. Measurements obtained directly from the existing specimen are as follows: body depth at dorsal origin 34% of SL; predorsal length 39% of SL; total head length 30% of SL;

preopercular head length 19% of SL); head height 30% of SL; snout length 10.5% of POHL or 7% of THL; eye diameter 63% of POHL or 40% of THL.

Scales in *P. hollisterae* are of spinoid type, as in other species of *Polymixia* (Roberts, 1993: fig. 11). Flank scales anterior to the dorsal fin have their ctenus-like spines arranged in a wedge pattern, like those of *P. japonica* and *P. nobilis* (Kotlyar, 1993). In the juvenile paratype, the spines are few but in a triangular patch with anterior apex, interpreted as an incipient wedge. The snout is blunt and devoid of scales, while large scales are positioned on the opercle and preopercle. Scales are smaller on the ventral side of the fish, increasing in size dorsally. The largest scales are positioned on the trunk between the lateral line and the dorsal fin. Small scales are arranged along the base of the dorsal and anal fins. The lateral line is continuous, with 35 pored scales in both Bermuda type specimens. The last pored lateral-line scale is at the posterior margin of the hypural plate, just anterior to the caudal-fin rays. Scales do not extend onto the caudal-fin rays. Following Kotlyar (1982, 1993), the number of scales in a vertical row from the origin of the dorsal fin to the lateral line (his S1; S1S herein) is 5, the number of scales in an oblique row from the origin of the dorsal fin to the lateral line (his S2; S2S herein) is 10, and the number of scales in an oblique row from the lateral line to the origin of the anal fin (his S3; S3S herein) is 14. The measured distances (Table 1) corresponding to S1 (S1D herein) represent 31% and 30% of BD for the Bermuda types and 40% for the juvenile paratype, and for S2 (S2D herein) they represent 52% and 51% of BD for the Bermuda types and 44% for the juvenile paratype. The vertical distances from the origin of the anal fin to the lateral line (AO–LL) represent 59% and 57% of BD, respectively and 48.5% for the juvenile paratype.

This species, like other species of *Polymixia* (e.g., Kotlyar, 1993; Borden et al., 2019), has 29 total vertebrae, 10 of which are abdominal (defined as lacking a complete haemal arch) and 19 of which are caudal vertebrae (Fig. 4). Kotlyar (1982, 1984, 1991, 1993) counted abdominal vs. caudal vertebrae differently, apparently using the first haemal spine to mark the first caudal vertebra. Using Kotlyar's criterion, there would be 12 abdominals and 17 caudals in both Bermuda types. Ossified epipleural intermusculars (see Patterson and Johnson, 1995 for details) are borne on the ribs of vertebrae 9–12, and on the haemal spines of vertebrae 13 through about 19 (Fig. 4). Ossified epineural intermusculars are borne on the neural arches or spines of vertebrae 2 through about 19. Ossified epicentrals, the third set of intermusculars, first recognized in *Polymixia* by Patterson and Johnson (1995) and further described by Gemballa and Britz (1998), can be seen in the radiographs on several of the abdominal vertebrae, whereas on other vertebrae they most likely are ligamentous; a precise count even of the ossified ones is not possible in the radiographs. The homology of the single pair of large intermuscular bones borne on the first vertebra has been controversial. Patterson and Johnson (1995) argued that it was an epineural displaced ventrally, but Gemballa and Britz (1998) made a convincing case that it is an enlarged and heavily ossified epicentral. Its function remains unknown.

The first two caudal vertebrae in *P. hollisterae* (three in *P. nobilis* and at least one specimen of *P. yuri*: see details below in Comparisons) bear ribs in addition to having a closed haemal canal. The third caudal vertebra (vertebra 13) in *P.*

Fig. 4. X-radiographs of the type specimens of *Polymixia hollisterae*, new species, from Bermuda. (A) Holotype, BAMZ 1997-159-006, 173 mm SL. (B) Paratype, FMNH 145004, 185 mm SL. Scale bars = 1 cm.

hollisterae is the first one with a haemal spine, which is distinctively shaped in being expanded in the midline and prolonged into a needle-like posteroventral process (Fig. 4). According to some conventions (Kotlyar, 1993; Patterson and Johnson, 1995), this would be the first caudal vertebra. This enlarged haemal spine is associated with and lies immediately posterior to the enlarged first proximal anal radial, but they do not appear to be in contact with each other.

As in all species of *Polymixia*, neural spines are present on all vertebrae anterior to compound centrum PU1+U1 (Fig. 4). Four neural spines are anterior to the first proximal dorsal radial (Figs. 4, 7). Neural and haemal spines that interdigitate with dorsal and anal proximal radials have their distal third expanded antero-posteriorly (i.e., they are paddle- or oar-shaped; Figs. 4, 7). A trait that differs among individuals and among species is that six neural spines are counted in radiographs between the last dorsal-fin radial and the epurals in the holotype of *P. hollisterae*, but seven in the Bermuda paratype, while seven haemal spines occur between the last anal radial and the parhypural in both specimens (Fig. 4).

The mouth is large and terminal (Figs. 2, 5B, 6). The maxilla reaches to beneath the posterior margin of the orbit. The upper jaw lengths on the adult types are 73% and 71% of POHL, while the lower jaw lengths are 77% and 75% of POHL, respectively. On the juvenile paratype they are 71% of POHL for the upper and 84% for the lower jaw. On each

maxilla there are two flat supramaxillae that abut but do not overlap each other and do not appear to be moveably articulated (Figs. 5B, 6), as is typical in *Polymixia*; the anterior one is about half the size of the posterior one. The dentition consists of tiny sandpaper-like teeth on the upper and lower jaws, vomer, and palatines. Teeth on the dentary of the adult types (Fig. 6) form a narrow, sinuous band with soft tissue on either side, different from those of the premaxilla, which form a broad band that extends to the outer margin of that bone. We were able to count about 30 pyloric caeca in the Bermuda paratype of *P. hollisterae*.

A pair of long hyoid barbels is present (Figs. 5B, C, 6), their posterior tips not quite reaching the pelvic-fin base. The barbels of the holotype as preserved are coiled (Figs. 5B, 6A) and cannot be measured accurately, but those of the Bermuda paratype are nearly straight, extend posteriorly (Figs. 5C, 6B), and are 55.6 mm in length (30% of SL). The small juvenile paratype's barbels are damaged but what remains of one is 2.9 mm long (14.5% of SL). Seven branchiostegal rays are present on each side in all species of *Polymixia*, although they are not easily counted in the specimens of *P. hollisterae*. In other species, the first three are very small and modified to support the hyoid barbel of the same side (Starks, 1904; Zehren, 1979; Ono, 1982; Grande et al., 2013; Borden et al., 2019). There are three large, spathiform branchiostegal rays per side arising from
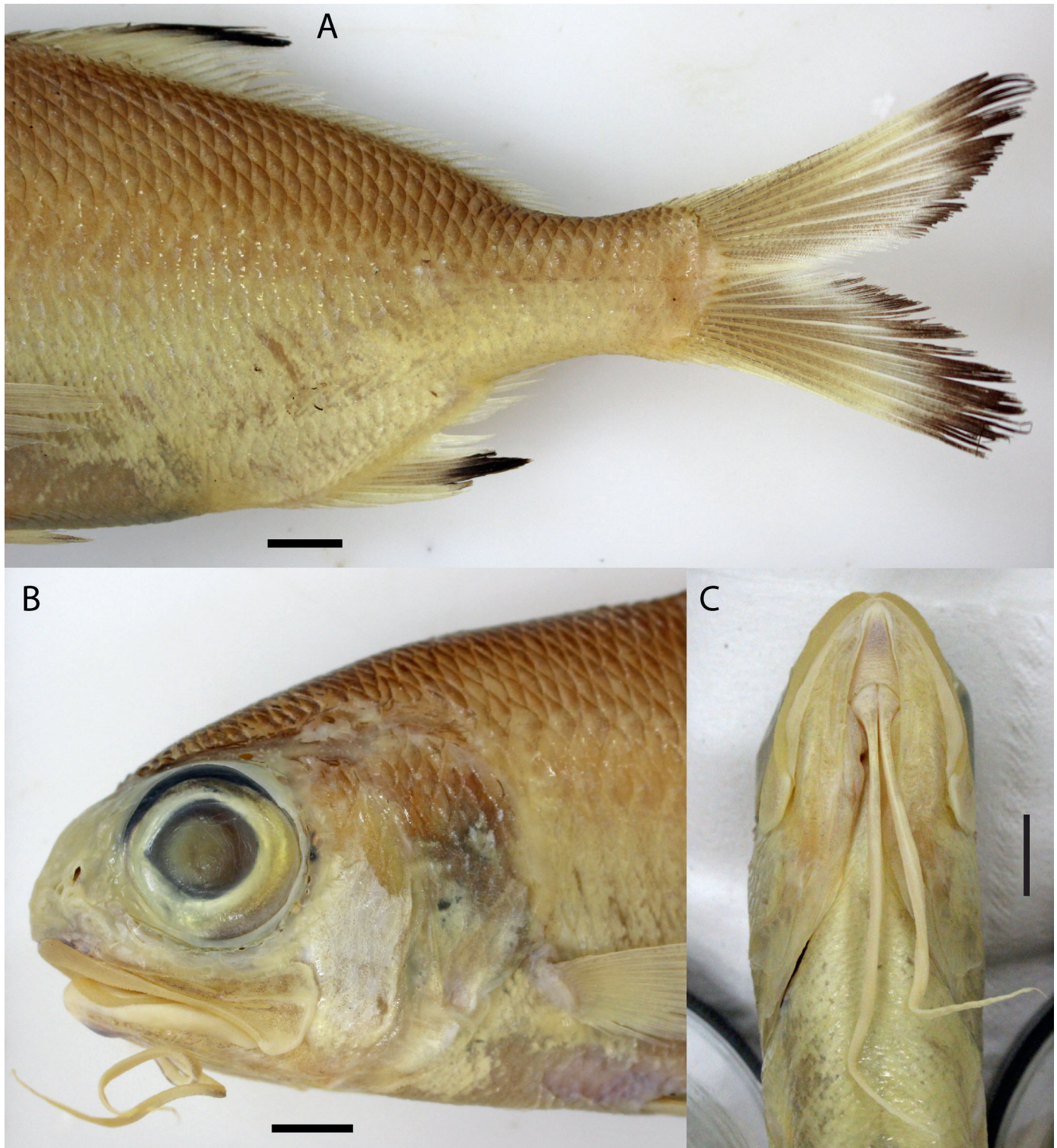
Fig. 5. Closeup views of *Polymixia hollisterae*, new species. (A) Caudal region and median fins of the holotype, BAMZ 1997-159-006, 173 mm SL. (B) Lateral view of the head of the holotype. (C) Ventral view of the head showing the hyoid barbels of the Bermuda paratype, FMNH 145004, 185 mm SL. The base of each barbel contains three small, modified branchiostegals (Ono, 1982). Scale bars = 1 cm.

the anterior ceratohyal and one from the posterior ceratohyal in all species of *Polymixia*. The posterior margin of the preopercle is rounded, with no indentation along its lower posterior margin and no pointed posteroventral projection (Fig. 2), a condition similar to that in *P. japonica* (Borden et al., 2019).

The lengths of the dorsal-fin base (DFB) in the holotype and Bermuda paratype represent 42.7% and 40.9% of SL, respectively, and 46.5% of SL in the juvenile paratype. The origin of the dorsal fin, denoted by the base of the small first fin spine, is vertically above vertebra 8 (Figs. 4, 7D, E) in both adult specimens. The dorsal fin in the holotype has five
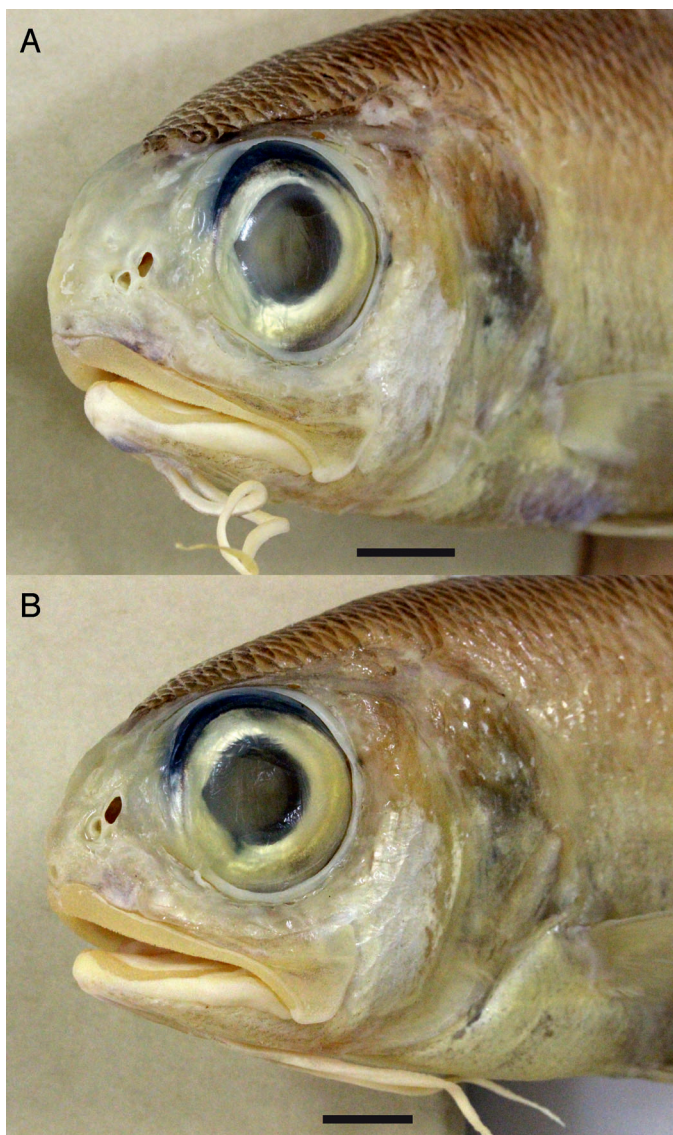
Fig. 6. Oblique views of the head of the type specimens of *Polymixia hollisterae*, new species. (A) Holotype, BAMZ 1997-159-006, 173 mm SL. (B) Bermuda paratype, FMNH 145004, 185 mm SL. Scale bars = 1 cm. Note the sinuous band of teeth on the dentary in both specimens, and the broad band of teeth on the premaxilla.

spines and 32 soft fin rays (V, 32), all soft rays being branched and the last one doubled but counted as one (Figs. 2A, 4A). The Bermuda paratype has one fewer soft ray (Figs. 2B, 4B). Both it and the juvenile paratype also have five spines. The first dorsal-fin radial supports one small spine. Each subsequent fin spine is progressively longer than the one before. The longest soft ray is number two, measuring 19.5% of SL in the holotype, 21.5% of SL in the Bermuda paratype (Fig. 4), and 27.5% of SL in the juvenile paratype. The next five rays in both Bermuda types are also noticeably longer than more posterior rays, after which the remaining rays become just slightly shorter in length in a regular progression (Figs. 2, 4).

There are three supraneurals (Figs. 4, 7D, E), the first inserting anterior to the first neural spine, the second between the first and second neural spines, and the third between the third and fourth neural spines. Thus, there is no supraneural between neural spines three and four in either

Bermuda type specimen. Dorsal-fin proximal radials are long, nearly straight, and anterior radials overlap significantly with the distal portions of neural spines (Figs. 4, 7D, E). Each of the first 15–17 proximal radials has an expanded lateral flange as well as a prominent anterior and posterior midline flange, whereas the more posterior radials are more rod-like. The interdigitation patterns in *P. hollisterae* and other examined species are summarized in Figure 8. In both Bermuda specimens, the first dorsal radial inserts between neural spines 4 and 5 and the second between neural spines 5 and 6. Beginning with the third and fourth radials, two radials insert between adjacent neural spines. Only one radial, the ninth, inserts between neural spines 9 and 10, followed by two each of the next ten radials inserting between adjacent neural spines. In both specimens, three radials insert between neural spines 15 and 16. The more posterior patterns of interdigitation differ slightly between the two specimens.

The lengths of the anal-fin bases (AFB) represent 16.9% and 16.3% of SL in the Bermuda types and 18% of SL in the juvenile type. The origin of the anal fin, denoted by the base of the first anal-fin spine, is directly below vertebra 18 (Fig. 4). The anal fin has four spines and 15 soft rays (IV,15) in both specimens. The juvenile paratype's anal-fin spines and rays are damaged. Each anal spine is progressively longer than the preceding one. All soft rays are branched and the last one is doubled and counted as one. The longest soft rays (the first and second) are each 13.8% of SL in the holotype, but subsequent rays are progressively shorter. The longest ray in the Bermuda paratype is 16.5% of SL. The distance between the vent and the anal-fin origin (AN–AF) is 8% of SL in the holotype and 6% of SL in the Bermuda paratype.

Anal-fin proximal radials slightly interdigitate with haemal spines. The proximal radials are less expanded than dorsal-fin proximal radials, except for the greatly enlarged first anal radial (Figs. 4, 9C, D, 10C, D), which has a general shape that is unique for the genus but also has a specific morphology that is diagnostic for the new species. This specific morphology was confirmed by CT scan in the Gulf of Mexico juvenile paratype (Fig. 11). In the new species, the main shaft of the first radial is nearly straight and inclined at an angle of about 43° to the vertebral column. The anterodorsal tip of the first radial ends just anterior to but does not contact the expanded first haemal spine of vertebra 13. The anterior process of the radial is more nearly horizontal than in other species, making an angle of about 38° with the shaft and about 5° with the vertebral column. In the angle between the two processes is a thin-walled, conical cavity that has a relatively greater volume than that of other species in the genus.

The caudal fin is forked and its fin-ray formula is vi 9,9 v, with two upper and two lower procurrent rays closest to the principal rays being segmented; principal rays include one unbranched ray in each lobe, the intervening principal rays being branched. This pattern is consistent with those of all species of *Polymixia* as far as known. The inner fin rays of the dorsal and ventral lobes frequently overlap distally (Figs. 2, 4, 5A). The caudal-fin skeleton is similar to that in other species of the genus (Grande et al., 2013), consisting of three epurals, six autogenous hypurals, and two uroneurals (Fig. 4). A full spine on preural centrum 2 is present as in other paracanthopterygians (Borden et al., 2013, 2019; Grande et al., 2013, 2018).

Table 1. Measurements, counts, and selected morphological characters for the type specimens of *Polymixia hollisterae*, new species. Lengths are given in millimeters; —, data unavailable; §, length estimated from the ship-board image using original SL of 20 mm for scale.

Character	Holotype BAMZ 1997-159-006	Paratype FMNH 145004	Adult types average	Small paratype MCZ 174218
Total length (TL)	224	240	232	§ 25.7
Standard length (SL)	173	185	179	20
Total vertebral centra	29	29	29	—
Abdominal centra based on lack of haemal arch	10	10	10	—
Caudal centra based on presence of haemal arch	19	19	19	—
Abdominal centra based on lack of haemal spine	12	12	12	—
Caudal centra based on presence of haemal spine	17	17	17	—
Neural spines after last dorsal-fin radial	6	7	6.5	—
Haemal spines (including parhypural) after last anal-fin radial	8	8	8	—
Caudal-fin rays	vi 9,9 v	vi 9,9 v	vi 9,9 v	—
Dorsal-fin spines, soft rays	V,32	V,31	V,31.5	V,—
Anal-fin spines, soft rays	IV,15	IV,15	IV,15	—
Pored lateral line scales	35	35	35	—
Pelvic-fin rays	7	7	7	—
Pectoral-fin rays	17	17	17	17
Scales from dorsal origin vertically to lateral line (S1S)	5	5	5	—
Scales diagonally from dorsal origin to lateral line (S2S)	10	10	10	—
Scales from anal origin vertically to lateral line (S3S)	14	12	13	—
Pyloric caeca	—	~30	—	—
Distance from dorsal origin vertically to lateral line (S1D)	19.5	20.0	19.8	2.7
Distance from dorsal origin diagonally to lateral line (S2D)	33.3	34.5	33.9	3.0
Distance from anal origin vertically to lateral line (AO–LL)	37.3	38.3	37.8	3.3
Greatest body depth (BD; at dorsal-fin origin)	63.5	67.5	65.5	6.8
Predorsal length (PDL)	79.6	83.6	83.1	7.8
Length of dorsal-fin base (DFB)	73.8	75.6	74.7	§ 9.3
Height of dorsal fin (LDR)	33.7	39.9	36.8	5.5
Length of anal-fin base (AFB)	29.3	30.1	29.7	§ 3.6
Height of anal fin (LAR)	23.8	30.5	27.2	—
Length of pelvic fin (VFL)	25.0	28.7	26.9	§ 3.0
Length of pectoral fin (PFL)	35.8	43.2	39.5	3.1
Pectoral fin to caudal base (PF–CB)	109	118.2	113.6	§ 13.7
Pectoral fin to pelvic-fin origin (PF–VF; pectoral–ventral)	10.5	11.4	11	§ 10.7
Pelvic fin to caudal base (VF–CB)	101.5	116.9	109.2	§ 12.5
Pelvic fin to anal-fin origin (VF–AF; ventral–anal)	50	55.6	52.8	§ 5.6
Depth of caudal peduncle (CPD)	17.5	18.9	18.2	2.5
Length of caudal peduncle (CPL; anal insertion to caudal base)	25.7	26.8	26.3	§ 3.2
Vent to anal-fin origin (AN–AF)	14.4	15	14.7	§ 1.2
Eye (orbit) diameter (EYD)	19.9	22.3	21.1	2.4
Length of snout (SNL)	10.1	10.3	10.2	0.4
Preopercular head length (POHL)	43.4	47.5	45.5	3.8
Total head length (THL; snout to posterior edge of opercle)	58.8	64.5	61.7	6.0
Height/depth of head (HDH; at tip of supraoccipital)	55.8	56.3	56.1	6.0
Length of upper jaw (UJL)	31.8	33.8	32.8	2.7
Length of lower jaw (LJL)	33.6	35.5	34.6	3.2
Length of barbels (LB)	—	55.6	—	2.9+
Barbels reaching pelvic-fin base?	No	No	No	No
Scale spinoid morphology	Wedge	Wedge	Wedge	Wedge
Shape of posteroventral edge of preopercle	Rounded	Rounded	Rounded	Rounded

The pectoral fin has a splint at the base of its leading (anterodorsal) edge and 17 principal soft rays in all three types. The lengths of the soft rays increase from ventral to dorsal. The longest (dorsalmost) ray and the shortest (ventralmost) ray are segmented but not branched, while all other rays are branched. The pectoral fin is relatively long in the adult specimens, with PFL 21% of SL in the holotype, 24% of SL in the Bermuda paratype, and 15.5% in the Gulf of Mexico juvenile paratype. The pectoral fin is supported by

five narrow radials contained within a narrow fleshy base (Figs. 2, 4, 5B) that is located directly ventral to vertebra 5 (Fig. 4). The distance between the pectoral-fin origin and the end of the hypurals (PF–CB) is 63% of SL in the holotype and 64% of SL in the Bermuda paratype.

The anterior tip of the pelvic girdle is directly ventral to vertebra 4, and the origin of the pelvic fin is ventral to vertebra 7 (Fig. 4). The distance between the pelvic-fin origin and the posterior end of the hypural plate (VF–CB) is 59% of
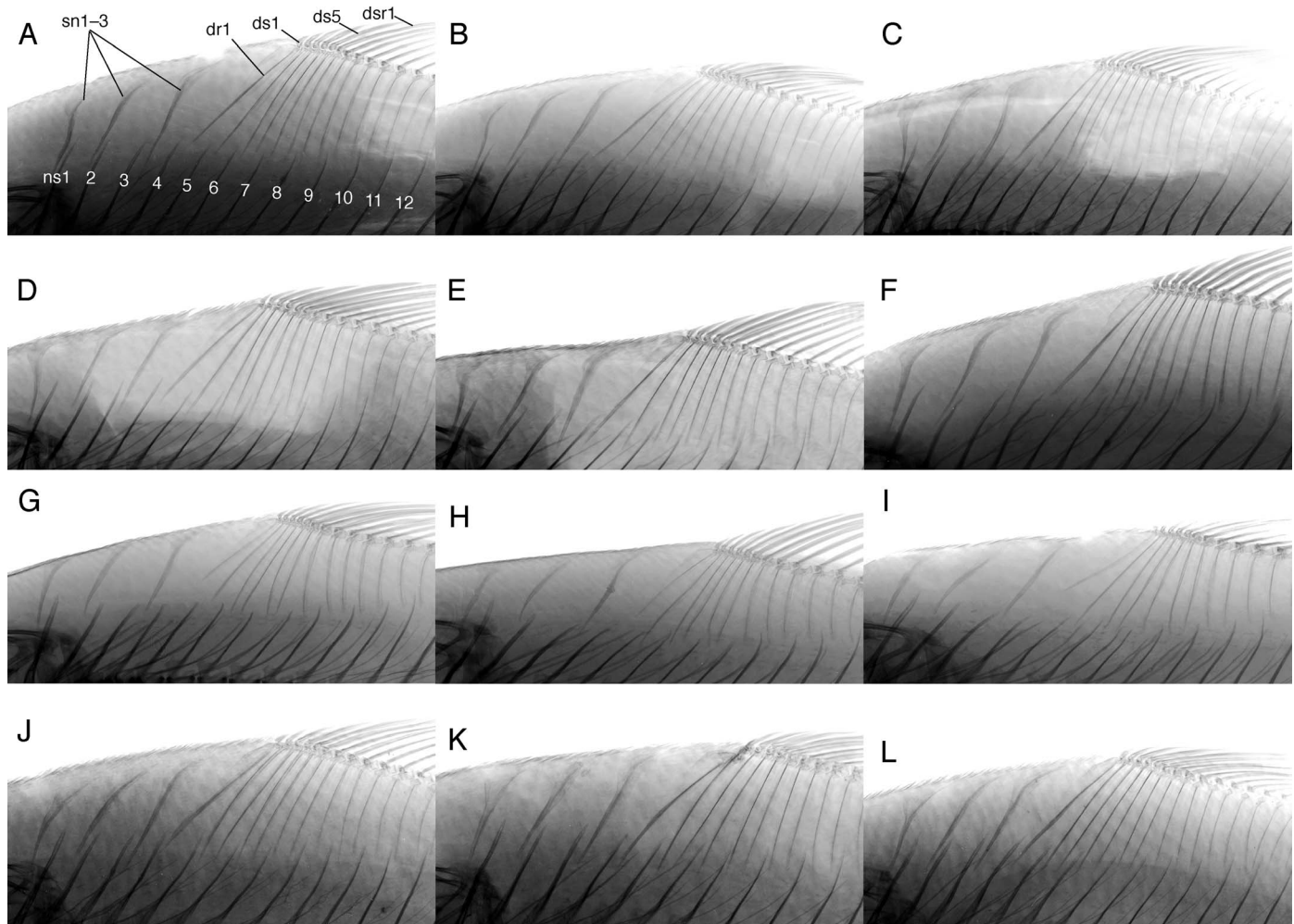
Fig. 7. X-radiographs of supraneurals and proximal dorsal radials in several species of *Polymixia*. (A–C, F) *Polymixia nobilis*. (A) FMNH 142335, 243 mm SL, Madeira. (B) FMNH 142336, 266 mm SL, Madeira. (C) FMNH 142337, 175 mm SL, Madeira. (F) ANSP 124292, 290 mm SL, Bermuda. (D) *Polymixia hollisterae*, new species, holotype, BAMZ 1997-159-006, 173 mm SL, Bermuda. (E) *Polymixia hollisterae*, new species, paratype, FMNH 145004, 185 mm SL, Bermuda. (G–I) *Polymixia lowei*. (G) BAMZ 1989-047-003, 210 mm SL, Bermuda. (H) BAMZ 1989-047-006, 226 mm SL, Bermuda. (I) USNM RAD 2126927. (J–L) *Polymixia japonica*, Sea of Japan. (J) FMNH 63860-1, 153 mm SL. (K) FMNH 63860-2, 144 mm SL. (L) FMNH 63860-3, 146 mm SL. Abbreviations: dr, dorsal-fin proximal radials (numbered); ds, dorsal-fin spines (numbered); dsr, dorsal-fin soft rays (numbered); ns, neural spines (numbered); sn, supraneurals (numbered).

SL in the holotype and 63% of SL in the Bermuda paratype. The number of pelvic-fin rays is 7, a number that is consistent among all species of *Polymixia*. The VFL is 14.5% of SL in the holotype and 15.5% of SL in the Bermuda paratype. The leading pelvic ray is segmented but not branched. The second pelvic-fin ray is the longest, and the remaining rays are progressively shorter. The pectoral fin extends as far posteriorly as does the pelvic fin. A similar condition is seen in *P. japonica*.

Coloration in alcohol.—Body coloration in alcohol in the Bermuda type specimens is not uniform, being light tan on the belly, progressively darker toward the lateral line, and further darkened to a medium brown mid-dorsally. The darkest body pigmentation (a dark brown) is on the upper scale-covered part of the head, beside the base of the dorsal fin, and on the upper part of the caudal peduncle (Figs. 2, 5A). Little or no pigmentation is present on the snout or paired fins, but the dorsal rim of the eye and orbit is pigmented black (Figs. 5, 6). No other external part of the eye or orbit is noticeably pigmented in alcohol. The most conspicuous pigmentation is present on the dorsal, anal, and caudal fins (Figs. 2, 5A) and sets this species of *Polymixia* apart from all others. Deep black pigmentation is present on the distal parts of the first five soft rays of the dorsal fin, forming a narrow dark patch at the anterodorsal tip of the fin. The first soft ray is the most heavily pigmented, with a slight reduction of pigmentation on rays 2–5. The pigmentation of rays 1–3 begins at the midpoint of each ray, while dark pigment is present on the distal third of rays 4–5. Pigmentation of the anal fin is very similar to that on the dorsal fin but more restricted in area, occurring on the first 4 soft rays, again forming a dark patch at the tip of the fin. Although these fin rays are slightly pigmented proximally, the pigment darkens to virtually black on the distal one-half or one-third of each ray. The proximal parts of the caudal rays appear brown to gray, but the distal ¼ of the caudal-fin rays is pigmented with a black margin that occurs on all the principal rays of the fin, except for a few of the shortest rays

Number of dorsal-fin radials inserting between adjacent neural spines		4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	13-14	14-15	15-16	16-17	17-18	18-19	19-20	20-21	21-22	22-23	Total
Polymixia japonica	FMNH 63858 small 2 Japan	1	2	2	2	1	2	2	2	2	2	2	3	2	2	2	2	2	0	0	33
	FMNH 63858 small 1 Japan	1	2	2	2	1	2	1	2	3	2	2	3	2	2	2	3	3	1	0	36
	FMNH 63858 large 1 Japan	1	2	2	2	1	2	2	2	2	2	2	3	2	3	2	2	2	2	0	36
	FMNH 120897 1 of 3 Taiwan	1	2	2	2	1	2	2	2	2	3	2	2	2	3	2	2	4	1	0	37
	FMNH 55422 1 Japan	1	2	2	2	1	2	2	2	2	3	2	2	2	2	2	2	3	3	0	37
	FMNH 55422 2 Japan	1	2	2	2	1	2	2	2	2	2	3	2	2	2	2	3	3	2	0	37
	FMNH 63860-1 Japan	1	2	2	2	1	2	1	2	3	2	2	3	2	2	2	2	2	4	0	37
	FMNH 63860-3 Japan	1	2	2	2	1	2	2	2	2	2	2	2	3	2	3	2	4	1	0	37
	FMNH 63860-2 Japan	1	2	1	2	2	2	1	2	3	2	2	3	2	2	2	3	2	3	0	37
Polymixia hollisterae, n. sp.	FMNH 145004, paratype, Bermuda	1	1	2	2	2	1	2	2	2	2	2	3	2	2	2	3	3	1	0	35
	BAMZ 1997-159-006, holotype, Bermuda	1	1	2	2	2	1	2	2	2	2	2	3	2	3	2	2	3	2	0	36
Polymixia nobilis	FMNH 142235 Madeira	1	1	2	2	2	2	2	2	3	2	3	2	2	3	2	3	2	3	1	40
	FMNH 142236 Madeira	1	1	2	2	2	2	2	2	2	3	2	3	2	2	3	2	3	3	0	39
	FMNH 142237 Madeira	1	1	2	2	2	2	2	2	3	2	3	2	2	3	2	2	3	3	1	40
	ANSP 124292 Bermuda	1	1	2	2	2	2	2	2	2	3	2	3	2	3	2	2	3	3	2	41
	ANSP 90603 (formerly *P. japonica*) Hawaii	1	1	2	2	2	2	2	2	2	3	2	2	3	2	2	3	3	4	0	40
	FMNH 64695 (C&S) Caribbean	1	2	2	2	1	2	2	2	2	2	3	2	3	2	2	3	3	4	0	40
Polymixia yuri	FMNH 96566 Nazka Ridge, SE Pacific	1	1	2	2	2	2	2	2	3	2	2	2	3	2	3	3	3	3	0	40
Polymixia lowei	BAMZ 1989-047-006 Bermuda	1	1	2	2	1	2	1	2	2	1	3	2	2	2	2	2	3	2	0	33
	BAMZ 1989-047-003 Bermuda	1	1	2	2	1	2	2	1	2	2	3	2	2	2	2	2	4	1	0	34
Polymixia berndti	ANSP 88844 (formerly *P. japonica*) Hawaii	1	1	2	2	2	1	2	2	2	2	2	2	2	2	2	2	3	2	0	34
	FMNH 120894 (voucher) Taiwan	1	1	2	2	2	1	2	2	2	2	2	2	1	3	2	2	3	0	0	32
	FMNH 120894 1 of 4 Taiwan	1	1	2	2	2	1	2	2	2	2	2	2	3	1	2	2	3	1	0	33
	FMNH 120894 (C&S) 1 Taiwan	1	1	2	2	2	1	2	2	2	2	2	2	2	2	2	2	4	2	0	35
	FMNH 120894 4 of 4 Taiwan	1	1	2	2	2	2	1	2	2	2	2	2	2	2	2	3	3	1	0	34
	FMNH 120894 3 of 4 Taiwan	1	1	2	2	2	1	2	2	3	2	2	2	2	2	2	3	3	1	0	35
	FMNH 120894 (C&S) 2 Taiwan	1	1	1	2	2	2	2	2	2	2	2	3	2	2	2	2	4	0	0	34
	FMNH 120895 (voucher) Taiwan	1	2	1	2	2	2	1	2	2	2	2	2	2	2	2	2	4	0	0	33

Fig. 8. Summary of the patterns of interdigitation of dorsal proximal radials between neural spines in selected species and specimens of *Polymixia*. The number of radials between adjacent neural spines is shown beginning with neural spines 4 and 5 and ending at or before neural spines 22 and 23. Data are taken from whole-fish radiographs except for one cleared and stained specimen of *P. nobilis*.

in the middle of the fin. This black fringe is not seen in congeners.

The Gulf of Mexico paratype is a small juvenile (Fig. 3) that lacks most of the distinctive pigmentation seen in the adult type specimens from Bermuda. The body and fins are mostly silvery in the shipboard image (Fig. 3A). Pigmentation of the anal fin appears to be mostly absent. The dorsal-fin spines and a few anterior dorsal-fin soft rays have a darker but not continuous pigmentation. The caudal fin is missing in the existing voucher (Fig. 3B) and difficult to see, but not obviously pigmented, in the ship-board image (Fig. 3A).

Habitat and distribution.—*Polymixia hollisterae* is known so far from only two localities. The adult specimens are from the NNW flank of the Bermuda Platform, which is an eroded remnant of an ancient oceanic volcano complex (Fig. 12). Both specimens were collected near or on the seafloor at the same place, on the same date, and at the same depth of approximately 280 fm or 512 m (Smith-Vaniz et al., 1999). This locality is different from the known distributions of the two other species of *Polymixia* that occur in Bermuda waters (Fig. 12). Specimens of *P. lowei* from Bermuda are so far only known (Fig. 12) from the southeastern flank of the platform (off the southeastern shore of Bermuda near Devonshire Bay and off the former Sonesta Beach) at depths of 150–250 fm (274–457 m). The single known specimen of *P. nobilis* from the Bermuda region was taken on the flank of Challenger Bank (Fig. 12), the closest submarine rise 24 km or 14 nautical miles southwest of the Bermuda islands, at a depth of 200 fm (366 m).

The small, juvenile Gulf of Mexico paratype was taken in a midwater trawl at an unknown depth between 0 and 1,502.5 m, in water with a maximum depth of 2,500 m. The middle of the trawl's track was about 232 km (130 nautical miles) SSE (bearing 157.5°) from the mouth of the main channel of the Mississippi River. The track was 16 km in length (8.7 nautical miles) and had a bearing of 191°.

Comparisons

Molecular and morphological characters.—The molecular results of Borden et al. (2019) demonstrate that *Polymixia hollisterae* is distinct from all other species of *Polymixia*. Although phylogenetically closest to *P. japonica* and an unnamed species from Australia (Fig. 1; Borden et al., 2019: fig. 4), *P. hollisterae* differs genetically from *P. japonica* by 20–33 nucleotides in the total alignment of 4,983 sites (Borden et al., 2019). *Polymixia hollisterae* is also genetically distinct from the type species *P. nobilis* and from *P. lowei* by 57–63 and 42–48 nucleotides, respectively (Borden et al., 2019: fig. 7). The latter two species also occur in Bermuda waters. The new species differs the most genetically from the *P. berndti* species complex (phylogenetically the earliest branching clade in the genus, known from the Pacific and Indian Oceans) by 80–93 nucleotides out of 4,983 (Borden et al., 2019).

Borden et al. (2019) discussed four morphological characters, originally proposed by Kotylar (1993), for which Borden et al. (2019) examined possible phylogenetic signal among species of *Polymixia*. First, *Polymixia hollisterae* shares with *P. nobilis* and *P. japonica* a distinctive and presumably derived scale morphology in that the ctenus-like spines are arranged in a wedge pattern along the posterior margin of the scale. The cteni of all other species of *Polymixia* are arranged in vertical rows along the posterior margin.

Second, the new species differs from *P. nobilis* in the number of dorsal-fin rays, and is more similar to *P. japonica* in this respect. *Polymixia nobilis* exhibits 33–38 dorsal-fin rays, while *P. japonica* has 30–35 and *P. hollisterae* has 31–32 rays in the dorsal fin. Kotylar (1993) considered the condition found in *P. japonica* to be an intermediate number of rays, whereas *P. lowei* (26–32) and *P. berndti* (26–31) have the lowest counts.
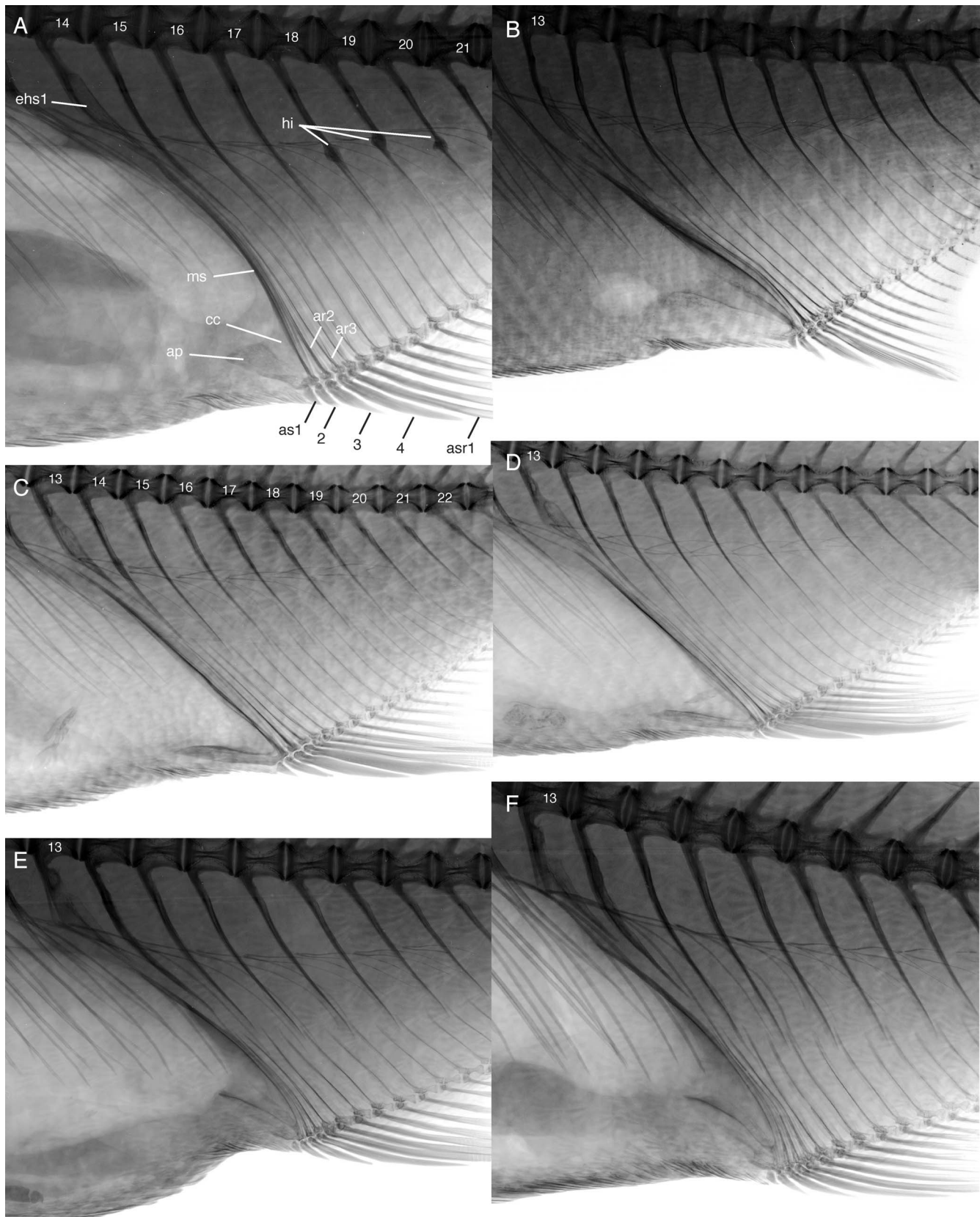
Fig. 9. X-radiographs of anal-fin radials and associated vertebrae in selected species of *Polymixia*, all from Bermuda except for (B) which is from the Sea of Japan. (A) *P. nobilis*, ANSP 124292, 290 mm SL. (B) *P. japonica*, FMNH 63860-1, 153 mm SL. (C) *Polymixia hollisterae*, new species, paratype, FMNH 145004, 185 mm SL. (D) *Polymixia hollisterae*, new species, holotype, BAMZ 1997-159-006, 173 mm SL. (E) *Polymixia lowei*, BAMZ 1989-047-003, 210 mm SL. (F) *Polymixia lowei*, BAMZ 1989-047-006, 226 mm SL. Abbreviations: ap, anterior process of first anal-fin proximal radial; ar, anal-fin proximal radial (numbered); as, anal-fin spines (numbered); asr, anal-fin soft rays (numbered); cc, conical cavity of first anal radial; ehs1, expanded first haemal spine; hi, probable healed injury of haemal spines; ms, main shaft of first anal-fin radial.
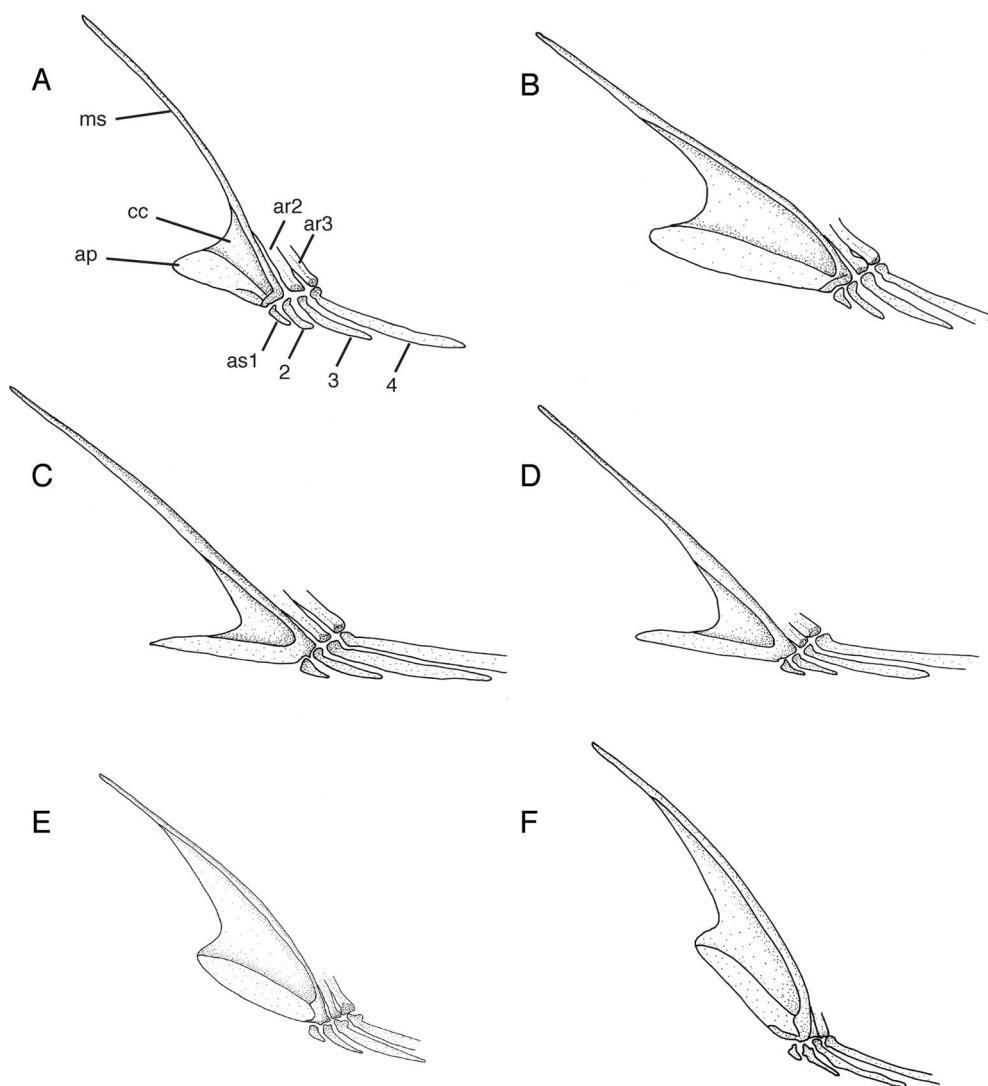
Fig. 10. Drawings of first anal-fin proximal radials in selected species of *Polymixia* (compare with the radiographs in Fig. 9). (A) *P. nobilis*, ANSP 124292, 290 mm SL, Bermuda. (B) *P. japonica*, FMNH 63860-1, 153 mm SL, Sea of Japan. (C) *P. hollisterae*, new species, paratype, FMNH 145004, 185 mm SL, Bermuda. (D) *P. hollisterae*, new species, holotype, BAMZ 1997-159-006, 173 mm SL, Bermuda. (E) *P. lowei*, BAMZ 1989-047-003, 210 mm SL. (F) *P. lowei*, BAMZ 1989-047-006, 226 mm SL. Compare with the radiographs in Figure 9. Abbreviations: see caption for Figure 9.

The counts in *P. hollisterae* overlap both with the intermediate range of *P. japonica* and the lower range of *P. lowei* and *P. berndti*.

Third, similar to *P. japonica*, *P. lowei*, and *P. berndti*, but different from the presumably more derived condition in *P. nobilis*, the new species shares a similar preopercle shape (Kotlyar, 1993; Borden et al., 2019). In these species, the posteroventral margin of the preopercle is rounded, never extending to a point as it is in *P. nobilis*.

Fourth, according to Kotlyar (1993), the species *Polymixia nobilis*, *P. yuri,* and *P. sazonovi* are distinct from all other species in having a very high number of pyloric caeca (more than 100). Using counts reported by Kotlyar (1993), the number of pyloric caeca in the other known species of *Polymixia* is 65 or fewer. Our examination of the paratype of *P. hollisterae* (FMNH 145004) revealed a pyloric caeca count of about 30, somewhat fewer than that of *P. japonica* (48–65) and within the range of both *P. lowei* (27–30) and *P. berndti* (28–48).

In addition to the four characters discussed above, the coloration in alcohol distinguishes *P. hollisterae* from all other known congeners. The closest similarity is probably to *P. japonica*, in which there is a broad, dark, pigmented area on the distal half of the anterior dorsal-fin soft rays, broader and not as dark (in alcohol) as that in *P. hollisterae*. *Polymixia japonica* has a much fainter and more diffuse pigmentation on the anal fin and a more diffuse and much paler pigmentation usually restricted to the tip of the dorsal lobe and sometimes the tip of the ventral lobe of the caudal fin.

Supraneurals.—The supraneurals in *Polymixia* (Figs. 4, 7) are shaped like mountaineering ice picks with the pick's point facing posteriorly within the dorsal body musculature and the shaft corresponding to the pick's handle. The shaft of the first supraneural inserts anterior to the first neural spine, the second between the first and second neural spines, and the third supraneural between neural spines three and four. There is no supraneural between the second and third neural spines in any specimen of any of the species examined radiographically. The supraneurals differ somewhat among examined species (Fig. 7). For example, the shaft of the first supraneural has a sigmoid curvature in *P. nobilis* (Fig. 7A–C, F) and *P. hollisterae* (Fig. 7D, E) but has a posteriorly concave curvature in *P. lowei* (Fig. 7G–I) and *P. japonica* (Fig. 7J–L). Supraneurals in *P. lowei* do not extend as far ventrally between neural spines as they do in the other species examined (Fig. 7).
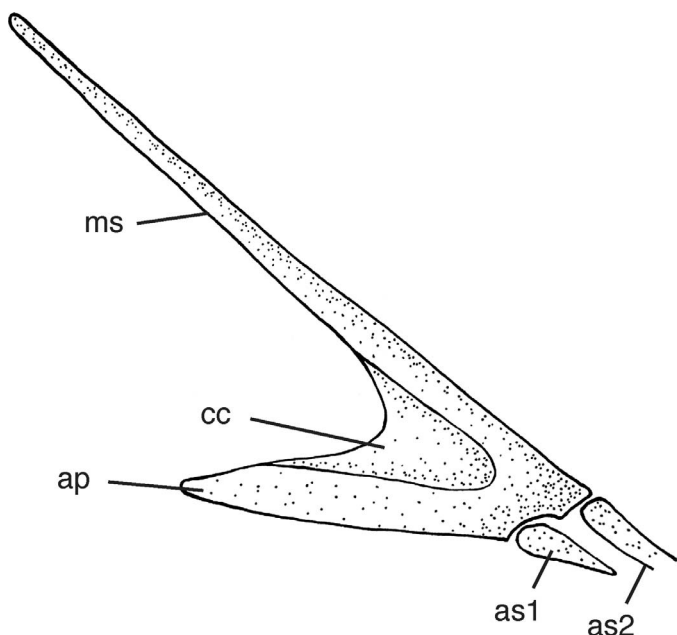
Fig. 11. Drawing of the first anal-fin radial of the second paratype of *Polymixia hollisterae*, new species, MCZ 174218, north-central Gulf of Mexico, from μCT scan data. Note the nearly straight main shaft and the nearly horizontal anterior process, like those of the Bermuda holotype and paratype (Fig. 10C, D). These diagnostic traits confirm morphologically its correct identification to species. Abbreviations: see caption for Figure 9.

Dorsal radial interdigitation with neural spines.—Dorsal-fin proximal radials interdigitate with neural spines, the overlap between spines and radials being greater in *P. hollisterae* and slightly less so in *P. japonica*, and lesser in the other species examined (Fig. 7). There are also differences among the species in the number of radials between adjacent neural spines, with distinctive patterns moving along the vertebral column. All species examined have one radial between neural spines 4 and 5 (Figs. 7, 8). However, in almost all specimens examined of *P. japonica*, there are two radials between spines 5 and 6, 6 and 7, and 7 and 8, then one between spines 8 and 9 and one between spines 9 and 10. In the other examined species, there is a single radial between spines 5 and 6. In both specimens of *P. hollisterae,* there is one radial between each of spines 4 and 5 and 5 and 6, followed by two each between spines 6 and 7, 7 and 8, and 8 and 9, and one between spines 9 and 10 (Figs. 4, 7). These sequences (Fig. 8) can be summarized as *P. japonica* 1-2-2-2-1-2-, *P. hollisterae* 1-1-2-2-2-1-, *P. nobilis* 1-1-2-2-2-2- (in most specimens), and *P. lowei* 1-1-2-2-1-2- (in the two specimens from Bermuda that we examined radiographically). The pattern is more variable in *P. berndti* (Fig. 8), with four of the specimens examined showing 1-1-2-2-2-1-, but with variant patterns of 1-1-2-2-2-2- (in two), 1-0-2-2-2-1-, 1-1-1-2-2-2-, and 1-2-1-2-2-2- (in one each). Thus *P. hollisterae* differs in the pattern of interdigitation of the anterior dorsal-fin proximal radials from *P. japonica*, *P. nobilis*, and *P. lowei*, and from about half of examined specimens of *P. berndti*. In all examined species, the
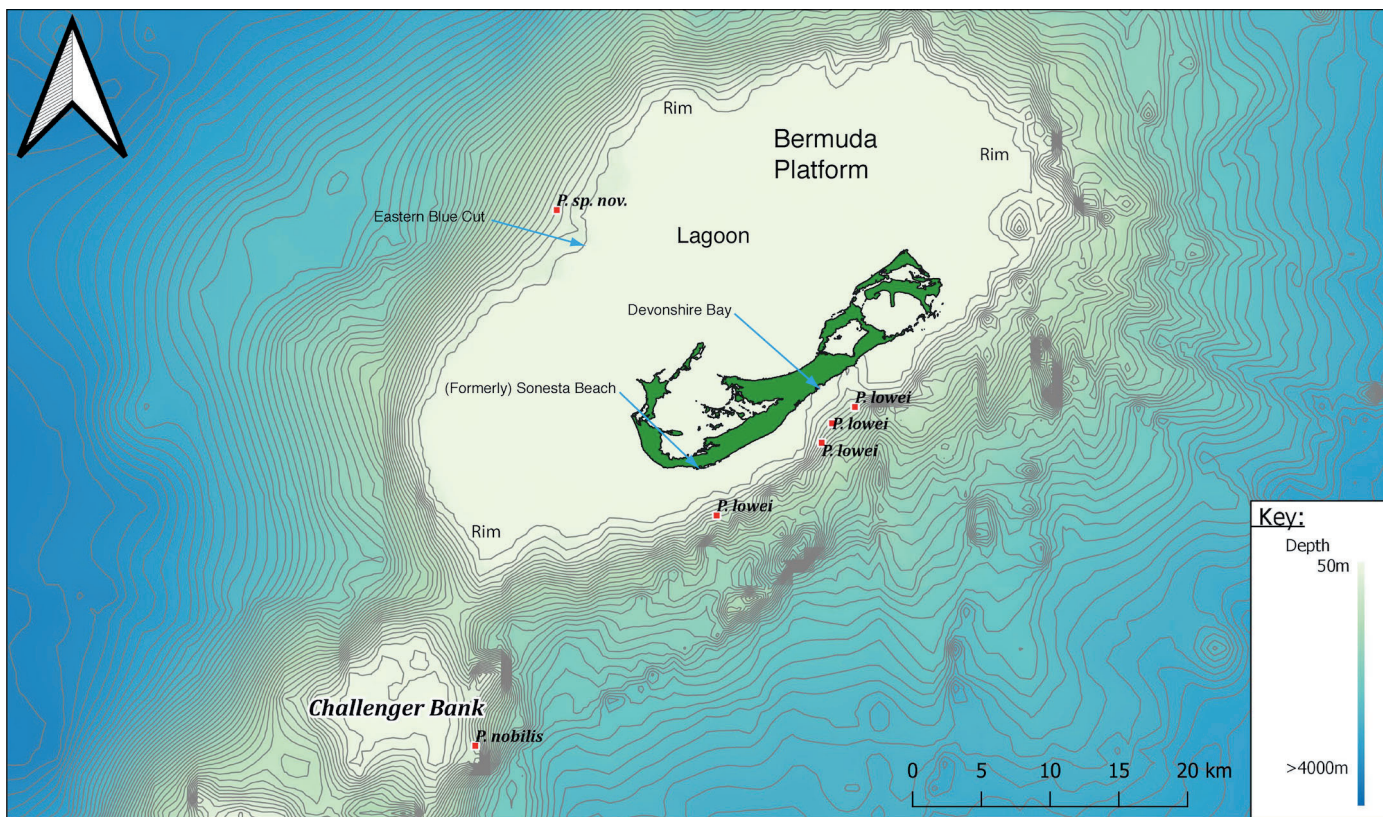
Fig. 12. Bathymetric map of the Bermuda Platform, Challenger Bank, and adjacent submarine slopes, with collecting localities for three species of *Polymixia*. "*P.* sp. nov." denotes *Polymixia hollisterae*, new species, caught "outside Eastern Blue Cut" off the NW edge of the Bermuda Platform. The more frequently caught *Polymixia lowei* has only been taken in two areas off the SE shore of Bermuda, and the single known specimen of *Polymixia nobilis* was taken on the slope of Challenger Bank, 24 km (14 nautical miles) southwest of the Bermuda Islands. Base map by L. Doughty (BAMZ).

interdigitation pattern of more posterior dorsal radials shows more individual variation, but there is no specimen of any species with an identical pattern overall to that of either specimen of *P. hollisterae* (Fig. 8).

First haemal arch.—The first caudal vertebra is often defined as the anteriormost one with a closed haemal arch; this is vertebra 11 in all specimens of the genus *Polymixia* for which we could verify the presence or absence of a closed arch. Defined this way, there are ten precaudal centra and 19 caudal centra in all specimens where the character could be assessed. However, in most species, the anterior two caudal vertebrae (vertebrae 11–12), thus defined, support well-developed ribs and lack a haemal spine. In *P. nobilis* (five specimens; e.g., Fig. 9A) and *P. yuri* (one specimen), the anterior three caudal vertebrae (vertebrae 11–13) have a complete haemal arch but no spine.

First haemal spine.—In all species, the first haemal spine is shorter than subsequent spines and expanded in the midline (Fig. 9). It lies just posterior to the dorsal tip of the first anal-fin proximal radial and differs slightly in shape among examined species. In *P. hollisterae*, *P. japonica*, *P. lowei*, and *P. berndti*, the first vertebra with a well-developed haemal spine is vertebra number 13. Only in *P. nobilis* and the single specimen of *P. yuri* available to us is the first haemal spine found on vertebra 14. This is the case for the three examined specimens of *P. nobilis* from the type locality of Madeira, as well as the single dried skeleton from the Canary Islands and the single known specimen of *P. nobilis* from Bermuda (Fig. 9A).

First proximal anal-fin radial.—In all species of *Polymixia*, the first proximal anal-fin radial as seen in radiographs, cleared-and-stained specimens, and a single dried skeleton inserts just anterior to the first haemal spine. The radial and spine approach each other closely but do not appear to be in contact (Fig. 9). The first anal proximal radial has a characteristic shape in *Polymixia* and it also has distinctive features in each of the examined species (Figs. 4, 9, 10).

The main shaft of the radial varies in curvature; it is nearly straight in *P. hollisterae*, moderately curved in *P. japonica* and *P. nobilis*, and strongly curved in *P. lowei* (Figs. 9, 10). The shaft of the radial is even more strongly curved in *P. berndti* (not shown here). The angle that the main shaft makes with the vertebral column varies (Fig. 9) from a high value of about 50° in *P. nobilis*, through intermediate values of about 42° in *P. lowei*, 40° in *P. hollisterae*, and 35° in *P. japonica*, to a low of about 25° in *P. berndti*. In addition, the anterior process of the radial differs in the angle it makes with the main shaft. The largest angle among examined species (about 38°) occurs in *P. hollisterae*, in which the anterior process lies close to the ventral body wall and is only 5° from being parallel to the body axis (Fig. 4), whereas in other species the anterior process is directed anterodorsally to varying degrees (Figs. 9, 10).

Morphometrics.—Altogether, 34 landmarks for each of 27 specimens representing five species of *Polymixia* were digitized and used for morphometric analysis. The principal components analysis based on Procrustes coordinates yielded PC1 through PC4 explaining 28.3%, 19.2%, 11.7%, and 9.7% of the variance in the data. Only the first two principal components were needed to separate the two adult speci-

mens of *P. hollisterae* from the other four species (Fig. 13), with PC 2 giving the greatest separation and specimens of the other four species occupying mostly non-overlapping regions of the morphospace. The most extreme scores on PC 2 are for the holotype of *P. hollisterae* and one of our specimens of *P. lowei* from Bermuda, although *P. hollisterae* scores higher and *P. lowei* lower on PC 1.

Morphometric differences among the species are illustrated by the wireframe drawings (Fig. 13). These results illustrate that there are important differences among most of the examined species in the relative size of the head, eyes, and jaws, and also in the dorsal outline of the body. *Polymixia hollisterae* has a relatively large head, large eyes, and long jaws, and in addition its pre-dorsal body outline is more streamlined than in most other species (Fig. 13).

Body proportions.—Similar differences are seen in body proportions (measurement ratios), which are shown in Figure 14 and were calculated from the landmark pixel locations (point to point). Compared to the other four species analyzed morphometrically, *P. hollisterae* has the greatest average eye diameter and snout length as a ratio of preopercular head length, as well as the greatest ratio of preopercular head length to standard length, and conversely it has the smallest ratio of pectoral fin to caudal base distance, pelvic fin to caudal base distance, and pelvic fin to anal-fin origin distance, all as ratios of standard length (Fig. 14). Among proportions not analyzed morphometrically, *P. hollisterae* also has the greatest average ratio in the lengths of the longest dorsal ray and longest anal ray, as well as in the lengths of the pectoral and pelvic fins to standard length. Overall, *P. hollisterae* appears to be the most streamlined of all species of *Polymixia*, suggesting that it is among the fastest-swimming species of the genus.

DISCUSSION

The species of the genus *Polymixia* are difficult to identify without detailed study. A significant number of museum specimens and DNA barcode samples were shown by Borden et al. (2019) to be misidentifications, many of them possibly influenced by faulty assumptions about geographic distributions. In addition, cases of cryptic species, sibling species, and probable taxonomic synonymies were discovered by Borden et al. (2019). The clearest case of a cryptic species uncovered in their study is the subject of the present paper. As demonstrated here, the close inspection of actual specimens, examination of radiographs, and analysis of morphometric landmarks reveal diagnostic external and internal characters, including color pattern, body proportions, and unique osteological features, all of which establish *P. hollisterae* as a new species that is recognizable beyond its molecular signature.

Little is known about the biology of *P. hollisterae* and its congeners, but museum collection data provide some clues to the life histories of these elusive fishes. Individual specimens have sometimes been caught at mesophotic depths (40–129 m), but most records (e.g., *P. nobilis* off Cape Verde Archipelago in Menezes et al., 2004) of the genus are from rariphotic (130–500 m) and aphotic (>500 m) depths (to about 800 m).

Preflexion larvae of species of *Polymixia* are unknown (Lyczkowski-Schultz, 2005); however, records of early flexion
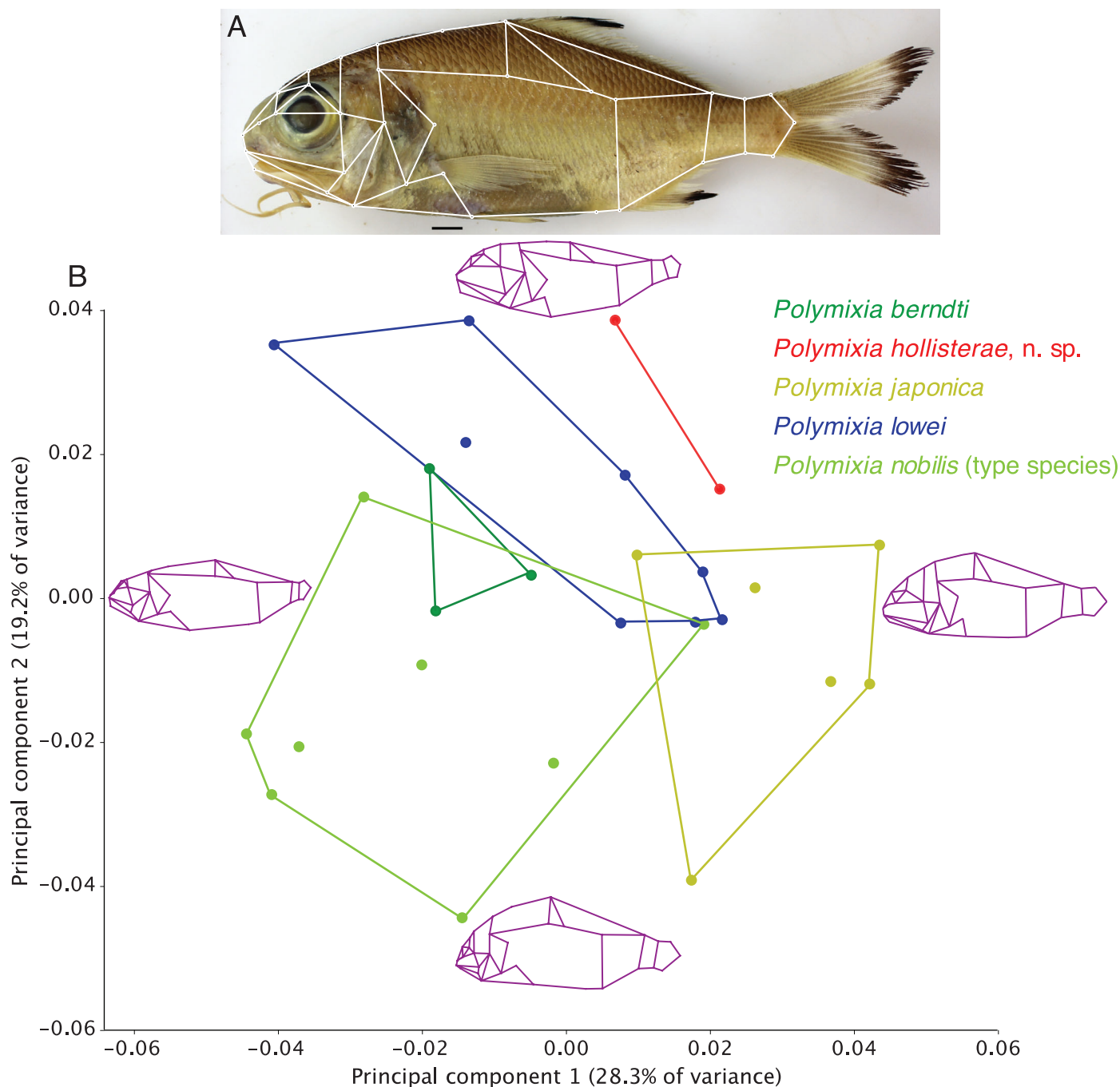
Fig. 13. Results of the Principal Components Analysis of Procrustes coordinates for 34 landmarks from 27 adult specimens belonging to five species of *Polymixia*. (A) Wireframe representation of the 34 landmarks digitized for the analysis superimposed on an image of the holotype of *Polymixia hollisterae*, new species. (B) Graph of component scores for PC 1 and PC 2, showing evidence of the distinct body form of *Polymixia hollisterae*, new species. The four wireframe cartoons illustrate the variation along both principal axes in the analyzed species.

and post-flexion larval stages as small as 4.1 mm in length are reported (e.g., Baldwin and Johnson, 1995; Lyczkowski-Schultz, 2005; Fahay, 2007). Early juvenile stages include the 20 mm SL Gulf of Mexico paratype of *P. hollisterae* described in the present paper.

Larger specimens of various species are usually caught individually, suggesting that adult *Polymixia* have a solitary life history stage, while smaller specimens can more often be caught in a single net haul, indicating that schooling might be common for smaller individuals. The large spawning aggregation of *P. lowei* reported by Baumberger et al. (2010)

demonstrates that there is at least one life history event where adults gather in large numbers.

The discovery of *Polymixia hollisterae* in Bermuda waters makes Bermuda a center of species diversity for the genus, remarkably so considering its small geographic area. A few areas have been reported to have three or more species, such as the Japanese Archipelago plus Taiwan and vicinity with *P. japonica*, *P. berndti*, *P. longispina*, and *Polymixia* cf. *P. nobilis* (Borden et al., 2019), but that area is far larger. Up to three species have also been identified in collections from the Hawaiian Islands (*P. berndti*, *P. japonica*, and *P. nobilis*).

Species	*P. japonica*	*P. hollisterae*, n. sp.	*P. nobilis*	*P. lowei*	*P. berndti*
n	7–10	2	8	5–6	3–10
Statistic	AVG	AVG	AVG	AVG	AVG
Standard Length (SL) (mm)	134	179	230	163	113
S1 Distance (S1D) / Body Depth (BD)	0.298	0.285	0.292	0.242	0.259
S2 Distance (S2D) / Body Depth (BD)	0.393	0.431	0.403	0.375	0.376
Anal Origin to Lateral Line (AO–LL) / Body Depth (BD)	0.582	0.539	0.573	0.601	0.576
Length of Dorsal Fin Base (DFB) / Standard Length (SL)	0.422	0.404	0.410	0.399	0.417
Length of Longest Dorsal Ray (LLDR) / Standard Length (SL)	0.159	0.193	0.137	0.165	0.169
Length of Anal Fin Base (AFB) / Standard Length (SL)	0.174	0.175	0.177	0.186	0.175
Length of Longest Anal Ray (LLAR) / Standard Length (SL)	0.126	0.143	0.114	0.108	0.120
Pelvic Fin Length (VFL) / Standard Length (SL)	0.118	0.143	0.130	0.120	0.133
Pectoral Fin Length (PFL) / Standard Length (SL)	0.186	0.223	0.184	0.193	0.195
Body Depth (BD) / Standard Length (SL)	0.363	0.358	0.370	0.354	0.337
Pectoral Fin to Caudal Base (PF-CB) / Standard Length (SL)	0.665	0.660	0.695	0.665	0.665
Pelvic Fin to Caudal Base (VF-CB) / Standard Length (SL)	0.643	0.630	0.649	0.633	0.639
Pelvic Fin to Anal Fin Origin (VF-AF) / Standard Length (SL)	0.298	0.293	0.314	0.300	0.342
Depth of Caudal Peduncle / Standard Length (SL)	0.109	0.098	0.102	0.094	0.098
Length of Caudal Peduncle / Standard Length (SL)	0.191	0.178	0.177	0.160	0.160
Diameter of Orbit (EYD) / Preopercular Head Length (POHL)	0.438	0.445	0.365	0.411	0.417
Snout Length (SNL) / Preopercular Head Length (POHL)	0.290	0.304	0.296	0.288	0.286
Preopercular Head Length (POHL) / Standard Length (SL)	0.232	0.255	0.216	0.244	0.233
Head Depth (HDH) / Standard Length (SL)	0.266	0.272	0.239	0.264	0.281
Length of Upper Jaw (UJL) / Preopercular Head Length (POHL)	0.747	0.691	0.666	0.698	0.758
Length of Lower Jaw (LJL) / Preopercular Head Length (POHL)	0.746	0.749	0.736	0.733	0.762

Fig. 14. Comparison of average proportions among adult specimens of five species of *Polymixia*, calculated using lengths derived from the morphometric landmark coordinates. Averages for each proportion and species are colored orange if they are the highest ratio among the five species and blue if they are the lowest in the comparison. *Polymixia hollisterae*, new species, has the greatest number of extreme values (12: 8 highest, 4 lowest) of the five species.

Although the holotype of *P. berndti* was collected off Honolulu, Hawaii, a different specimen from Hawaii that was originally identified as *P. berndti* is more likely *P. nobilis* (USNM FIN 31943), and specimens from Hawaii identified as *P. japonica* are more likely to represent *P. berndti* based on our examination (e.g., ANSP 88844). Thus, confirmed species in the much larger area of the Hawaiian Islands are two (*P. berndti* and *P. nobilis*), not three. Therefore, the diversity of *Polymixia* in the small area around Bermuda appears to be virtually unique.

The identification of only one juvenile of *Polymixia hollisterae* in the Gulf of Mexico and two adults from Bermuda waters might be the result of disparate collecting techniques, thus underestimating the population numbers in these locations. For example, in Bermuda, conservation regulations restrict fishing only to hook and line, and vertical long-line fishing for deeper fishes is not common in recent years (S. R. Smith, pers. comm.). It is also possible that suitable habitat for adults of *P. hollisterae* is limited to only one flank of the Bermuda Platform. In the case of the small juvenile paratype from the Gulf of Mexico (MCZ 174218), perhaps it was carried by ocean currents into the northern Gulf. Regardless, that single record in the northern Gulf of Mexico means that *P. hollisterae* is not restricted to Bermuda. It remains unknown if in the Gulf of Mexico there are corresponding adults and reproducing populations.

Now that the existence of *P. hollisterae* is known and can be recognized by both molecular and morphological traits, re-identification of museum specimens will provide insight into this species' range and distribution patterns. This once cryptic species is also clearly a prime candidate for recognition in environmental DNA samples, since it can be reliably identified by its barcode sequence (Borden et al., 2019). The realization that what was once recognized as *Polymixia lowei* is actually something different will aid in conservation efforts. The first step in any conservation effort is an understanding of the biodiversity of the region. Species studies such as the one presented here are paramount in that effort. In addition, future focused studies dealing with the species composition of *Polymixia,* such as the study of *P. hollisterae* presented here, will add more pieces to the grand puzzle of understanding the diversity, unique morphology, unusual behaviors, and ultimately the evolutionary history of the genus *Polymixia*, all of which in turn are arguably keys to a better understanding of the evolution of acanthomorph fishes.

MATERIAL EXAMINED

Institutional abbreviations follow Sabaj (2020). Key: alcohol, stored in ethanol; CS, cleared and stained for bone and cartilage; rad, radiographs; image, photos.

Polymixia berndti: 22 spec., 73–340 mm SL: FMNH 95583 (alcohol), 120894 (voucher, alcohol), 120895 (voucher, alcohol), 120896 (alcohol); USNM 389346 (alcohol, CS).

Polymixia japonica: 23 spec., 109–238 mm SL: ANSP 88844 (alcohol; this specimen has been identified as *P. berndti*), 90603 (alcohol); FMNH 55422 (alcohol), 63858 (alcohol), 63859 (alcohol), 63860 (3, alcohol), 63861 (alcohol), 120897 (voucher, alcohol); USNM 398535 (voucher, alcohol; this specimen was identified as *P. berndti* by Borden et al., 2019).

Polymixia lowei: 67 spec., 65–175 mm SL: ANSP 105710 (alcohol), 144889 (alcohol); BAMZ 1984-047-006, 1989-047-003 (alcohol); KU 30367 (CS skull only); MCZ 39186 (CS), 39415 (alcohol), 39770 (alcohol), 45907 (CS); UF 36330 (alcohol), 40083 (alcohol), 40263 (alcohol), 44346 (alcohol, CS), 127145 (alcohol), 127151 (CS), 184751 (CS); USNM 185284 (alcohol, CS), 185401 (rad), 323212 (photo), 398653 (3 of 4, alcohol).

Polymixia nobilis: 12 spec., 104–300 mm SL: ANSP 78251 (dry skeleton), 124292 (alcohol, rad); FMNH 64695, 142235–37 (alcohol, CS, rad); UF 231494 (alcohol); USNM 398653 (1 of 4, CS), USNM RAD118739–001 (rad).

Polymixia sazonovi: 1 spec. (photo).

Polymixia yuri: 1 spec., 175 mm SL: FMNH 96566 (alcohol).

DATA ACCESSIBILITY

ACKNOWLEDGMENTS

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APPENDIX 1

Gloria E. Hollister Anable

Polymixia hollisterae is named in honor of Gloria Elaine Hollister (1900–1988) for her landmark contributions to deep-sea research, ichthyology, tropical exploration, conservation (Berra, 1988; Moore, 2014; Nature Conservancy, 2020), and pioneering work for the Red Cross Blood Bank during WWII.

Hollister graduated with a degree in zoology from Connecticut College (B.S. 1924) and a master's from Columbia University (M.S. 1925), working with W. K. Gregory among others. During this time, she participated in an expedition to British Guiana, and records suggest that she met William Beebe for the first time. After graduation from Columbia, she worked in cancer biology at the Rockefeller Institute, and then in 1928 she was recruited to Beebe's new Department of Tropical Research (DTR) at the New York Zoological Society (NYZS, now the Wildlife Conservation Society).

As a key member of William Beebe's deep-sea bathysphere explorations in the waters off Bermuda from 1928 to 1940, Hollister's main responsibilities were surface-to-bathysphere communication and the recording of the conversations to and from the bathysphere, as well as ichthyological identifications and research (Strochlic, 2020a, 2020b). Some of her dive transcripts are reproduced in Beebe's (1934) book *Half Mile Down*, and all formed the basis for Else Bostelmann's artistic renderings of the deep-sea fishes, thus inspiring a generation of young naturalists (Krause, 2018).

Hollister's scientific expertise was most important in the field of ichthyology. She co-authored descriptions of new fish species from the West Indies (Beebe and Hollister, 1933, 1935), including the Shortstripe Goby, *Elacatinus chancei*

(Beebe and Hollister, 1933), and her ichthyological knowledge allowed her to understand and accurately report what Beebe saw in the deep sea. She also descended in the bathysphere herself, setting records for depth of descent by a woman—410 feet (125 m) in 1930 and 1,208 feet (368 m) in 1934—the latter a record that stood for decades.

Working in the team's laboratory on Nonsuch Island (now a nature preserve) in Bermuda (https://www.nationalgeographic.com/history/2020/03/these-women-unlocked-the-mysteries-of-the-deep-sea/), and between field seasons at the DTR in New York, Hollister perfected and published the first reliable method of clearing and staining fishes for study of their osteology. She called the results "Fish Magic" (Hollister, 1930). The fishes' flesh was cleared with potassium hydroxide and glycerine, and bone was stained with Alizarin Red S (Hollister, 1934). She then applied the technique to study and publish descriptions of the caudal skeletons of many Bermuda fishes (Hollister, 1936, 1937a, 1937b, 1940, 1941).

Hollister frequently gave public lectures on natural history, exploration, and the bathysphere, with titles such as "With Beebe in Bermuda" and "My Jungle Days" (Anonymous, 1932: https://digital.lib.uiowa.edu/islandora/object/ui%3Atc_44379_44375). Funds from her public lectures were used for the bathysphere expeditions and for a planned expedition into the jungles of (what was then) British Guiana; Hollister led the expedition in 1936, using a small airplane and reaching as far as Kaieteur Falls, along the way studying the native fauna such as the Golden Tree Frog and the Rainbow Tanager.

In 1941, she left DTR and joined the American Red Cross to help organize its first blood donor project; the blood was badly needed for shipment to Britain, and after Pearl Harbor also for injured U.S. soldiers, airmen, and sailors. She became the public face of the Red Cross and blood donation through the Red Cross Speaker's Bureau in Washington D.C.

Hollister married Anthony Anable in 1941. After the end of the war, she led a citizens' effort to save from imminent destruction a natural area near the New York–Connecticut border called the Mianus River Gorge. In collaboration with the Nature Conservancy, Hollister and like-minded conservationists purchased the Gorge, which became the first ever land purchase project of The Nature Conservancy, and later became the first U.S. National Natural Landmark to be officially registered. Among her later honors were the Gold Medal of Connecticut College (1970) and the Outstanding Achievement Award (1981) of the Society of Women Geographers.