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Review of the hagfishes (Myxinidae) from the Galapagos Islands, with descriptions of four new species and their phylogenetic relationships

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Hagfishes are an ancient group of benthic marine craniates that are found in deep or cold waters around the world. Among the 83 valid species, four are described from the Galapagos Islands: *Eptatretus bobwisneri*, *E. grouseri*, *E. mccoskeri* and *Rubicundus lakeside.* During a recent expedition to the archipelago, six species of hagfishes were collected, including four undescribed species of the genera *Eptatretus* (***Eptatretus goslinei* sp. nov.**) and *Myxine* (***Myxine greggi* sp. nov.**, ***M. martinii* sp. nov.** and ***M. phantasma* sp. nov.**). In this paper, we provide a review of the eight species of hagfishes from the Galapagos Islands, including new diagnoses and an identification key for all species. *Myxine phantasma* is remarkable in that it is the only species of *Myxine* known to completely lack melanin-based pigments. Our species delineations were based on both morphological and molecular analyses. A phylogenetic hypothesis based on molecular data suggests that Galapagos hagfishes arose from multiple independent colonisations of the islands from as many as five different ancestral lineages. The large number of endemic hagfishes in the geologically young Galapagos Islands suggests that there is much global hagfish diversity yet to be discovered.

ADDITIONAL KEYWORDS: Ecuador – *Eptatretus* – *Myxine* – Myxiniformes – systematics.

INTRODUCTION

Hagfishes (Myxinidae) comprise a monophyletic group of jawless, cartilaginous, eel-like, basal craniates with an entirely marine distribution, inhabiting the cool or deep parts of the oceans of both hemispheres. They usually occupy burrows located in soft, mud-bottom habitats, with some species occurring in shallow waters (Mincarone & Soto, 2001; Fernholm & Vinding, 2012) and others down to at least 2743 m (Wisner & McMillan, 1990). Recent studies have indicated that some species are also closely associated with deep-sea coral reefs (Mincarone & McCosker, 2004; Fernholm & Quattrini, 2008, Kuo *et al.*, 2010), hydrothermal vents (Møller & Jones, 2007) and cold seeps (Polanco-Fernandez & Fernholm, 2014). The latitudinal range of hagfishes extends from the Arctic Circle (Wisner & McMillan, 1995) to the South Shetland Islands, near Antarctica (Norman, 1937), but most species are found in temperate and tropical waters.

Our current understanding of the systematics of hagfishes recognizes the order Myxiniformes, which contains the monophyletic family Myxinidae. The family is subdivided into the subfamilies Eptatretinae, Myxininae and Rubicundinae (Fernholm *et al.*, 2013). The number of recognized genera in Eptatretinae

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has been historically controversial. Recent molecular phylogenetic studies found that both *Paramyxine* Berg, 1940 and *Quadratus* Wisner, 1999 must be considered junior synonyms of *Eptatretus* Cloquet, 1819 (e.g. Kuo *et al.*, 2003; Chen *et al.*, 2005; Fernholm *et al.*, 2013; Zintzen *et al.*, 2015) and that at least four species previously described in *Eptatretus* must be placed in the monophyletic genus *Rubicundus* Fernholm *et al.*, 2013.

In spite of being a well-known area in terms of biodiversity, geology and evolutionary history, the Galapagos Islands have only recently been explored for hagfishes. All four species known so far, *Eptatretus bobwisneri* Fernholm *et al.*, 2013, *E. grouseri* McMillan, 1999, *E. mccoskeri* McMillan, 1999 and *Rubicundus lakeside* (Mincarone & McCosker, 2004), were collected by the submersible *Johnson Sea Link* during two expeditions conducted in 1995 and 1998 by the California Academy of Sciences (CAS), Harbor Branch Oceanographic Institute (HBOI) and the National Museum of Natural History (USNM) (McMillan, 1999; Mincarone & McCosker, 2004).

During a recent deep-sea expedition conducted around the Galapagos Islands, six species of hagfishes were collected, four of them undescribed. Here we describe a new species of *Eptatretus* and three new species of *Myxine* from that expedition, and we provide more detailed descriptions for *E. bobwisneri* and *E. mccoskeri*. New diagnoses for all hagfishes from the Galapagos are also provided. In addition, a phylogenetic hypothesis for Galapagos hagfishes based on molecular data is proposed and discussed.

MATERIAL AND METHODS

Specimen collection

All specimens were collected between 26 May and 11 June 2019 on vessels chartered from Puerto Ayora, Santa Cruz Island. Three one-day trips near Santa Cruz Island were undertaken on the vessel *Valeska Yamile*, and a seven-day expedition to the islands of Isabela and Fernandina was mounted on the vessel *Queen Mabel*. Hagfishes were collected using traps constructed from 5-gal plastic buckets, each fitted with two plastic conical eel traps and baited with yellowfin tuna (*Thunnus albacares* Bonnaterre, 1788). A total of 25 trap deployments were made, with seven of them successful at catching hagfish. Typical times on the bottom ranged from 1.5 to 3 h. Depth of each station was indirectly estimated based on a bathymetric digital elevation model of the Galapagos Islands (NOAA, 2017). Institutional abbreviations, including those of the comparative materials (Supporting Information, Appendix S1), follow Sabaj (2020), except MCCDRS – Marine Collection of the Charles Darwin Research Station (Puerto Ayora, Galapagos, Ecuador).

Video collection

In situ video of hagfishes was collected using three different baited remote underwater video (BRUV) rigs. The first was a tripod-style rig based on a design provided by Sam Owen at MarAlliance (www.maralliance.org). This rig, which was originally designed for shallow deployments where the final position could be adjusted by divers, was problematic in our deep deployments, especially in areas of high current and jagged rock bottoms, both of which are common in the Galapagos. A second rig was constructed during the expedition by modifying a 5-gal plastic bucket trap. A light and camera mount was added via a ½-in diameter threaded aluminium rod, which passed through the centre of the entire trap. The rod was secured at the base of the trap with washers and nuts and protruded 40 cm above the trap lid. A GoPro camera and an LED light source, each enclosed in a high-pressure housing, were secured to the rod using stainless steel hose clamps. During a subsequent trip in January 2020, a third aluminium BRUV design was used, which had a lower vertical profile and improved stability on the bottom, as well as two light mounts and an improved field of view unobstructed by a trap.

Hagfish morphology

Methods for measurements and counts follow those of Fernholm & Hubbs (1981) and McMillan & Wisner (1984, 2004), except the preventral length, which is measured from the front of the rostrum to the origin of ventral finfold. Terminology of nasal sinus papillae follows Mok (2001). Length of specimens (in mm) is given as total length (TL), defined as the distance from the front of the rostrum to the posterior margin of the caudal finfold. All other measurements are given as percentage of TL. Counts of gill pouches (GP), gill apertures (GA) and cusps were taken from both sides of the specimens, while the slime pore counts were taken only from the left side. Photos of the teeth were obtained with a Leica M205 FA stereo microscope.

Polymerase Chain Reaction (PCR) and sequencing

Following field collection and photographic vouchering, fresh hagfish specimens were euthanized with an overdose of MS-222 anaesthetic (powder; tricaine methanesulfonate, Catalogue No. NC0135573; Western Chemical Inc., Ferndale, WA, USA). Immediately post-mortem, ~ 0.5 cm^3 of muscle was dissected and stored in 95% EtOH for 22 voucher specimens.

Our molecular analyses are based on two mitochondrial markers (16S and *COI*), because

sequence data for these loci are publicly available for many hagfish species. DNA was extracted from muscle tissue for each voucher using the DNeasy Blood and Tissue kit (Qiagen). We obtained 16S amplicons for each voucher using universal primers (Palumbi *et al.*, 1991) and One*Taq* (NEB) (PCR cycle parameters: 94 °C for 2 min; 30 cycles of 94 °C for 30 s, 50 °C for 1 min, 68 °C for 1 min; 68 °C for 10 min; 4 °C hold). We obtained *COI* amplicons for each voucher sample using universal primers (Ward *et al.*, 2005) also using One*Taq* (NEB) (PCR cycle parameters: 94 °C for 2 min; 30 cycles of 94 °C for 30 s, 52 °C for 1 min, 68 °C for 1 min; 68 °C for 10 min; 4 °C hold). Polymerase chain reaction (PCR) results were assayed using gel electrophoresis on a 1% agarose gel. Sanger sequencing was conducted following standard protocols (Eurofins; Lancaster, PA).

In addition to Sanger sequencing data, we also used an Illumina DNA barcode sequencing approach, where amplicons derived from the above were ligated with sequencing adapters (Illumina) and voucher-specific barcodes (Illumina) and sequenced on a HighSeq 2500 (Illumina). In doing so, we were able to confirm all 16S sequences and most *COI* sequences derived from Sanger sequencing. We were also able to obtain additional *COI* sequence data for three voucher DNA samples that proved difficult using Sanger sequencing. In total we obtained 16S Sanger sequences from all voucher DNA samples, *COI* Sanger sequences for 17 voucher DNA samples and three additional *COI* sequences from Illumina sequencing. All sequences have been deposited on Genbank (Supporting Information, Appendix S2). A superscript note along the text (material examined) indicates the specimen sequenced for *COI* and/or 16S. Species names from GenBank were updated following Fernholm *et al.* (2013).

Phylogenetic analyses

Sequence data for the individual loci were aligned separately using the *linsi* option in MAFFT (Katoh & Standley, 2013). Multiple sequence alignments for each locus were then concatenated using custom computational scripts yielding a concatenated alignment of 1204 nucleotides (16S positions 1–442; *COI* positions 443–1084). We analysed our concatenated dataset using the MFP+MERGE option in IQTREE (Minh *et al.*, 2020). This option first finds best-fit models for each sequence partition and then estimates the maximum-likelihood tree under that modelling scheme, which was TIM2+F+I+G4 for 16S and TN+F+I+G4 for *COI*. We assessed nodal support by generating 1000 approximate likelihood ratio test and ultrafast bootstrap replicates (Minh *et al.*, 2020). Phylogenetic results were visualized using GGTREE (Yu, 2020).

RESULTS

Taxonomy

In seven successful deployments in the vicinity of the islands Santa Cruz, Fernandina, and Isabela (Fig. 1), we collected a total of 112 hagfish specimens representing six species, two of which were previously described (*Eptatretus bobwisneri* and *E. mccoskeri*). Redescriptions or original descriptions for all Galapagos hagfish species are provided below.

Rubicundus lakeside (Mincarone & McCosker, 2004)

Lakeside hagfish

(Figs 1, 2E; Tables 1–3)

Eptatretus lakeside Mincarone & McCosker, 2004: 163, figs 1, 3a (original description; type locality: Galapagos Islands, NW Fernandina Island, off Cabo Douglas, 00°17'30"S, 91°39'36"W, 762 m; holotype: CAS 201880).

Eptatretus Eptatretus lakeside. – Møller & Jones, 2007: 63.

Eptatretus lakeside. – Fernholm & Quattrini, 2008: 126. – McCosker & Rosenblatt, 2010: 172. – Knapp *et al.*, 2011: 404. – Ruiz *et al.*, 2011: 30.

Rubicundus lakeside. – Fernholm *et al.*, 2013: 302. – Cruz-Mena & Angulo, 2015: 326. Zintzen *et al.*, 2015: 377. – Angulo & Del Moral-Flores, 2016: 115.

Material examined: CAS 201880, holotype (275 mm), Galapagos, NW Fernandina Island, off Cabo Douglas, 00°17'30"S, 91°39'36"W, 762 m depth, submersible *Johnson Sea Link*, baited minnow trap, David Pawson, Godfrey Merlen, 17 July 1998.

Diagnosis: *Rubicundus lakeside* differs from all congeners [*R. eos* (Fernholm, 1991) from Challenger Plateau, west of New Zealand, *R. lopheliae* (Fernholm & Quattrini, 2008) from off North Carolina, USA and *R. rubicundus* (Kuo, Lee & Mok, 2010) from Taiwan] by having a 3/3 multicusp pattern of teeth (vs. 3/3-4 in *R. lopheliae* and 3/2 in *R. rubicundus*), 15 prebranchial pores (vs. 26 in *R. eos* and 19–21 in *R. lopheliae*), 50 trunk pores (vs. 75–77 in *R. eos* and 62 in *R. rubicundus*), 19 tail pores (vs. 26 in *R. eos*), 88 total pores (vs. 128–130 in *R. eos* and 100–102 in *R. rubicundus*) and by having two bilaterally symmetrical nasal-sinus papillae (vs. no nasal-sinus papillae in *R. eos* and *R. lophelia*) (Fernholm, 1991; Mincarone & McCosker, 2004; Fernholm & Quattrini, 2008; Kuo *et al.*, 2010).

Description: Body morphology, measurements and counts provided by Mincarone & McCosker (2004).

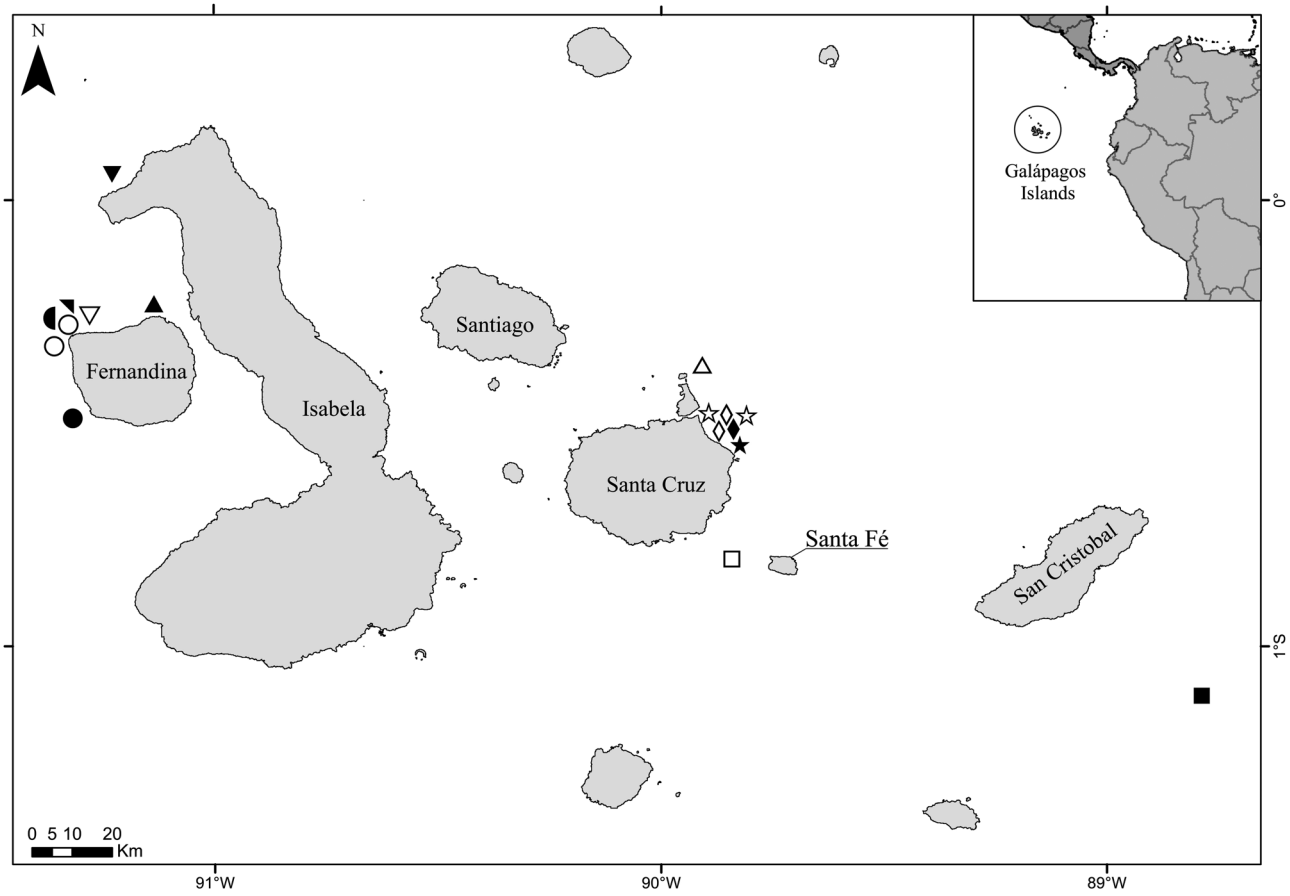

Figure 1. Distribution of hagfishes from the Galapagos Islands: (◖) *Rubicundus lakeside*, (●) *Eptatretus bobwisneri*, (▼) *Eptatretus goslinei*, (▲) *Eptatretus grouseri*, (■) *Eptatretus mccoskeri*, (★) *Myxine greggi*, (◥) *Myxine martinii*, (♦) *Myxine phantasma*. Black symbols indicate type localities. Some overlapping symbols were moved slightly for clarity.

Distribution and habitat: Galapagos Islands: known only from the holotype, collected off Cabo Douglas, NW Fernandina Island, 762 m depth (Fig. 1).

EPTATRETUS BOBWISNERI FERNHOLM *ET AL.*, 2013

BOB WISNER'S HAGFISH

(FIGS 1, 2A, 3A, 5, 6A; TABLES 1–3)

Eptatretus bobwisneri Fernholm *et al.*, 2013: 302. Replacement for *Eptatretus wisneri* McMillan, 1999, preoccupied by *Paramyxine wisneri* Kuo *et al.*, 1994. – Cruz-Mena & Angulo, 2015: 325. – Angulo & Del Moral-Flores, 2016: 100.

Eptatretus wisneri McMillan, 1999: 116 [original description; type locality: Galapagos Islands, 00°28.0'S, 91°37.2'W, depth 1848 ft (563 m); type-series: holotype, CAS 86429; paratype, SIO 97-76 (1)]. – Mok *et al.* 2001: 1026. – McMillan & Wisner, 2004: 55. – Mincarone & McCosker, 2004: 166. – McCosker & Rosenblatt, 2010: 172. – Mincarone & Fernholm, 2010: 795. – Knapp *et al.*, 2011: 404. – Ruiz *et al.*, 2011: 30. – Mincarone & McCosker, 2014: 347.

Eptatretus Eptatretus wisneri. – Møller & Jones, 2007: 64.

Material examined: CAS 86429, holotype (355 mm), SW Fernandina Island, Cabo Hammond, 00°27'56"S, 91°37'33"W, 563 m depth, submersible *Johnson Sea Link*, baited minnow trap, John E. McCosker, 14 November 1995. SIO 97-76, paratype, 1 (316 mm), NW Fernandina Island, Cabo Douglas, 00°17.5'S, 91°38.9'W, 512 m depth, submersible *Johnson Sea Link*, baited minnow trap, John E. McCosker, 16 November 1995. SIO 19-80$^{COI,\ 16S}$, 2 (164–190 mm), NW Fernandina Island, Cabo Douglas, 00°17'37.94"S, 91°39'18.10"W, 557 m depth, *Queen Mabel*, sta. G20, baited trap, Douglas Fudge *et al.*, 6 June 2019, 9:51–12:40 h.

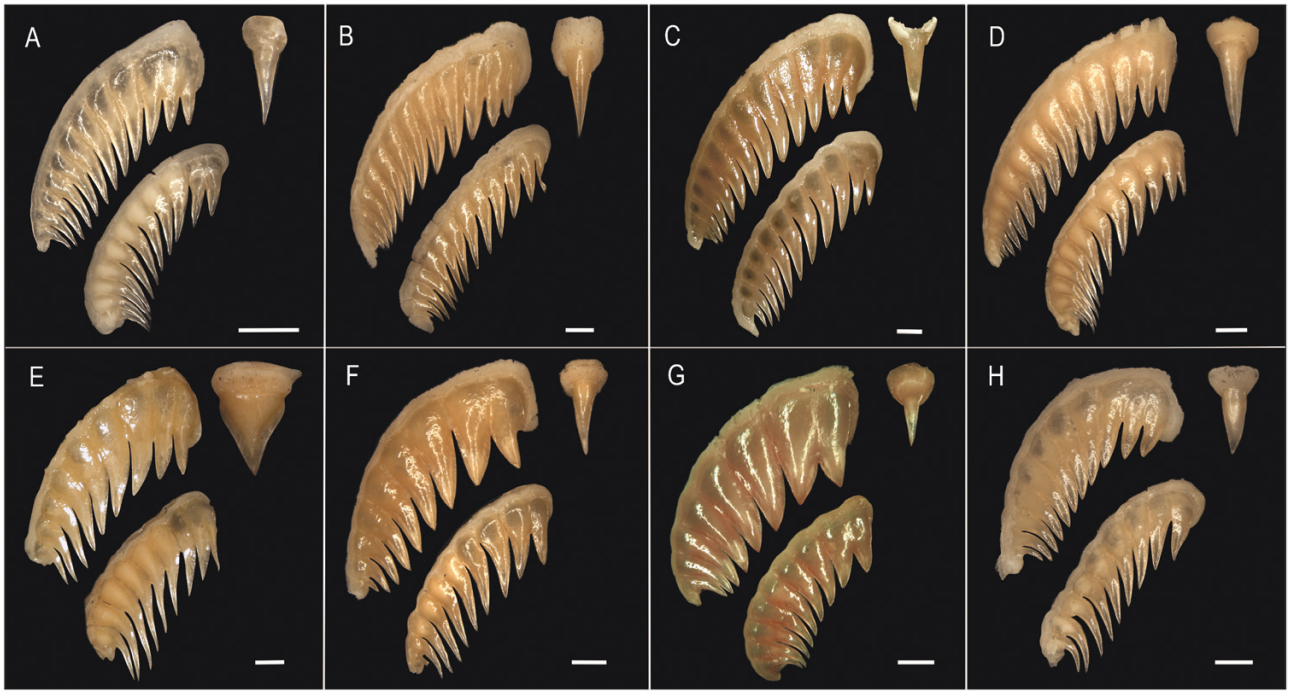

Figure 2. Anterior and posterior row of teeth (left series) and palatine tooth of the hagfishes from the Galapagos Islands: A, *Eptatretus bobwisneri* (SIO 19-80, 164 mm TL); B, *Eptatretus goslinei* (MCCDRS 9402, 305 mm TL); C, *Eptatretus grouseri* (CAS 201882, 420 mm TL); D, *Eptatretus mccoskeri* (SIO 19-81, 310 mm TL); E, *Rubicundus lakeside* (CAS 201880, 275 mm TL); F, *Myxine greggi* (MCCDRS 9401, 468 mm TL); G, *Myxine martinii* (SIO 19-80, 361 mm TL); H, *Myxine phantasma* (MCCDRS 9400, 365 mm TL). Scale bars: 1 mm.

Diagnosis: *Eptatretus bobwisneri* differs from all congeners, except *E. gomoni* Mincarone & Fernholm, 2010 from Western Australia, *E. indrambaryai* Wongratana, 1983 from Thailand, *E. luzonicus* Fernholm *et al.*, 2013 from the Philippines, *E. octatrema* (Barnard, 1923) from South Africa and *E. okinoseanus* (Dean, 1904) from Japan and Taiwan, by having eight pairs of gill apertures well-spaced and arranged in a near straight line, and a 3/2 multicusp pattern of teeth. *Eptatretus bobwisneri* differs from these congeners by having: 44–47 total cusps (vs. 50 in *E. gomoni* and 38–40 in *E. octatrema*); 9–11 prebranchial pores (vs. 12–13 in *E. gomoni*, 22–26 in *E. octatrema* and 13–17 in *E. okinoseanus*); 43–47 trunk pores (vs. 57–58 in *E. gomoni*, 63–68 in *E. octatrema* and 54–61 in *E. okinoseanus*); 73–76 total pores (vs. 91–93 in *E. gomoni*, 77–82 in *E. indrambaryai*, 84–88 in *E. luzonicus*, 104–117 in *E. octatrema* and 87–97 in *E. okinoseanus*); no nasal-sinus papillae (vs. one single nasal-sinus papilla in *E. luzonicus*, two bilaterally symmetrical nasal-sinus papillae in *E. octatrema*) (Wongratana, 1983; Mok, 2001; McMillan & Wisner, 2004; Mincarone & Fernholm, 2010; Mincarone & McCosker, 2014; Mincarone, 2017).

Description: Body elongated, subcylindrical at prebranchial and branchial regions, laterally compressed at trunk and strongly compressed at tail. Rostrum bluntly rounded. Nasal-sinus papillae absent. Eyespots conspicuous. Three pairs of barbels on head, first two pairs subequal in size (1.6–2.0% TL) and adjacent to opening of nasopharyngeal duct; third pair longer (2.4–2.7% TL) and immediately adjacent to mouth. Ventral finfold absent or low (1 mm high), beginning within anterior third of trunk, extending backwards to cloaca. Caudal finfold well developed, thin, extending around tail to dorsal surface, ending about over cloaca.

Body proportions (in percentage of TL; description of the holotype followed by the range for paratype and non-type specimens in brackets): preocular length 6.2 (6.3–6.7); prebranchial length 18.9 (19.5–22.8); branchial length 12.1 (8.9–10.4); preventral length 46.5 (45.8–52.2); trunk length 53.5 (48.4–55.3); tail length 17.7 (14.7–16.5); body width at PCD 6.5 (5.3–7.9); body depth at PCD (6.3–8.2); body depth including VFF 8.0 (5.4–8.7); body depth excluding VFF 7.7 (4.9–8.7); body depth at cloaca 6.5 (5.8–7.9); tail depth 8.3 (7.8–8.5).

Table 1. Meristic and morphometric characters for species of *Eptatretus* and *Rubicundus* from the Galapagos Islands

Species	*E. bobwisneri*		***E. goslinei***		*E. grouseri*		*E. mccoskeri*		*R. lakeside*
N	holotype	3	holotype	17 paratypes	holotype	3	holotype	6	holotype
	CAS 86429	range	SIO 19-78	range	CAS 86428	range	CAS 86431	range	CAS 201880
Total length TL (mm)	355	164–316	300	234–360	370	138–420	310	284–310	275
Gill apertures [a]	8 + 8	8–8	7 + 7	7–7	5 + 5	5–5 [b]	8 + 8	8–8 [c]	5 + 5
Gill pouches [a]	8 + 8	8–8	7 + 7	7–7	5 + 5	5–5 [b]	8 + 8	8–8 [c]	5 + 5
Nasal-sinus papillae	0	0	2	2	0	0	2	2	2
Cusps									
Multicusps (ant./post.)	3/2	3/2	3/3	3/3	3/2	3/2	3/3	3/3	3/3
Anterior unicusps [a]	9 + 9	9–10	11 + 9	9–12	9 + 9	9–10	10 + 10	8–10	6 + 6
Posterior unicusps [a]	8 + 9	8–9	10 + 10	8–11	8 + 8	8–9	9 + 10	8–10	6 + 6
Total cusps	45	44–47	52	47–58	44	46–48	51	44–51	36
Slime pores, left side									
Prebranchial	9	9–11	11	10–12	12	11–12	13	13–16	15
Branchial	7	7–7	6	6–6	4	4–5	7	6–7	4
Trunk	43	45–47	42	37–43	46	42–48	43	38–43	50
Tail	14	12–13	13	10–13	15	13–14	10	10–12	19
Total pores	73	75–76	72	65–72	77	71–79	73	69–75	88
Measurements as % of TL									
Preocular length	6.2	6.3–6.7	6.3	5.3–7.7	5.1	5.1–6.7	7.1	5.6–6.3	5.6
Prebranchial length	18.9	19.5–22.8	23.0	20.0–24.8	20.3	21.0–22.2	25.8	22.6–26.1	24.7
Branchial length	12.1	8.9–10.4	10.0	9.2–12.5	8.1	6.3–6.5	10.0	8.8–13.5	6.2
Preventral length	46.5	45.8–52.2	50.0	38.5–55.7	–	49.3–52.4	51.6	38.7–53.4	33.8
Trunk length	53.5	48.4–55.3	52.3	49.0–55.0	57.0	54.0–55.7	49.7	49.3–56.1	50.9
Tail length	17.7	14.7–16.5	15.7	14.2–17.9	14.6	16.9–17.5	15.8	10.6–16.9	18.2
Body width at PCD	6.5	5.3–7.9	7.0	5.6–8.1	5.9	5.4–6.3	7.3	6.9–8.6	5.0
Body depth at PCD	–	6.3–8.2	8.3	6.6–9.4	–	–	–	6.6–8.4	–
Body depth incl. VFF	8.0	5.4–8.7	9.3	7.7–11.7	6.8	7.9–9.0	8.2	7.7–9.3	7.2
Body depth excl. VFF	7.7	4.9–8.7	8.7	7.6–10.7	6.8	7.9–8.8	8.2	7.4–9.1	6.4
Body depth at cloaca	6.5	5.8–7.9	8.3	6.7–9.2	5.7	6.4–6.5	7.3	6.8–14.5	5.4
Tail depth	8.3	7.8–8.5	10.0	7.7–11.0	6.8	7.6–7.9	8.9	7.1–9.8	6.0

[a] Left + right side for single specimen.
[b] One specimen (SIO 97-77, 138 mm TL) with six gill pouches and apertures.
[c] One specimen (MCCDRS 9397, 304 mm TL) with seven gill pouches and apertures.

Table 2. Frequency of cusps for species of *Eptatretus* and *Rubicundus* from the Galapagos Islands

	Anterior unicusps									Posterior unicusps						
	6	7	8	9	10	11	12	*n*		6	7	8	9	10	11	*n*
Eptatretus bobwisneri				6	2			8				3	5			8
Eptatretus goslinei				6	18	9	3	36				3	18	13	2	36
Eptatretus grouseri				6	2			8				4	4			8
Eptatretus mccoskeri			4	4	6			14				4	8	2		14
Rubicundus lakeside	2							2		2						2
	Total cusps															
	36	~	44	45	46	47	48	49	50	51	52	53	54	~	58	*n*
Eptatretus bobwisneri			1	1		2										4
Eptatretus goslinei						1	2	1	3	2	5	1	2		1	18
Eptatretus grouseri			2		1		1									4
Eptatretus mccoskeri			1		2		1		1	2						7
Rubicundus lakeside	1															1

Counts (description of the holotype followed by the range for paratype and non-type specimens in brackets): multicusp pattern 3/2; anterior unicusps 9 (9–10); posterior unicusps 8 (8–9); total cusps 45 (44–47). Prebranchial pores 9 (9–11); branchial pores 7 (7); trunk pores 43 (45–47); tail pores 14 (12–13); total pores 73 (75–76).

Eight pairs of gill pouches corresponding to eight pairs of gill apertures. Gill apertures well-spaced and linearly arranged. Last branchial duct confluent with pharyngocutaneous duct on left side, forming a larger aperture. Posterior tip of dental muscle reaches gill pouches 4–5. Ventral aorta branches at gill pouch 6.

Colour (in life): body medium to dark brown, head slightly lighter; mouth and surrounding area white; barbels with white tip; very distinct eyespots; gill apertures with white margins; ventral finfold and caudal finfold with white margins (Fig. 3). Colour in alcohol similar to that described for live specimens.

Distribution and habitat: Galapagos Islands: known only from four specimens collected in two localities off Fernandina Island: Cabo Hammond and Cabo Douglas, at depths from 512 to 577 m (Fig. 1).

EPTATRETUS GOSLINEI SP. NOV.

GOSLINE'S HAGFISH

(FIGS 1, 2B, 3B, 5; TABLES 1–3)

Zoobank registration. urn:lsid:zoobank.org:act:A0F33266-3F54-4B7E-936B-0766CA231683

Holotype: SIO 19-78, 300 mm, NW Isabela Island, between Cabo Berkeley and Punta Flores, 00°01'54.47"N, 91°33'02.12"W, 467 m depth, *Queen Mabel*, sta. G23, baited trap, Douglas Fudge *et al.*, 7 June 2019, 12:20–15:00 h.

Paratypes: MCCDRS 9403$^{COI,\ 16S}$, 14(234–347 mm) and SIO 19–79$^{COI,\ 16S}$, 2(305–360 mm), taken with the holotype. MCCDRS 9402$^{COI,\ 16S}$, 1(305 mm), NW Fernandina Island, Cabo Douglas, 00°17'43.73"S, 91°39'19.61" W, 478 m depth, Queen Mabel, sta. G21, baited trap, Douglas Fudge *et al.*, 6 June 2019, 15:06–16:52 h.

Diagnosis: Eptatretus goslinei differs from all congeners, except *E. astrolabium* Fernholm & Mincarone, 2010 from Papua New Guinea, *E. caribbaeus* Fernholm, 1982 from the Caribbean Sea, *E. carlhubbsi* McMillan & Wisner, 1984 from Hawaii, Wake, and Tinian islands, *E. cirrhatus* (Forster in Bloch & Schneider, 1801) from south-eastern Australia and New Zealand, *E. cryptus* Roberts & Stewart, 2015 from New Zealand, *E. goliath* Mincarone & Stewart, 2006 from New Zealand, *E. laurahubbsae* McMillan & Wisner, 1984 from Juan Fernández Island, *E. menezesi* Mincarone, 2000 from southern Brazil and *E. strahani* McMillan & Wisner, 1984 from the Philippines and western Australia, by having seven pairs of gill apertures well spaced and arranged in a near straight line. *Eptatretus goslinei* differs from these seven-gilled congeners by having: 9–12 anterior unicusps (vs. 13–17 in *E. carlhubbsi* and *E. laurahubbsae*); 47–58 total cusps (vs. 61–71 in *E. carlhubbsi*, 61–68 in *E. laurahubbsae*); 65–72 total pores (vs. 83–84 in *E. astrolabium*, 79–85 in *E. caribbeaus*, 93–110 in *E. carlhubbsi*, 82–91 in *E. cirrhatus*, 78–86 in *E. cryptus*, 89–95 in *E. goliath*, 97–105 in *E. laurahubbsae*, 86–94 in *E. menezesi*, 76–86 in *E. strahani*); and by having two bilaterally

Table 3. Frequency of slime pores (left side) for species of *Eptatretus* and *Rubicundus* from the Galapagos Islands

	Prebranchial pores																	
	9	10	11	12	13	14	15	16										*n*
Eptatretus bobwisneri	1	2	1															4
Eptatretus goslinei		4	10	4														18
Eptatretus grouseri			1	3														4
Eptatretus mccoskeri					2	1	2	2										7
Rubicundus lakeside							1											1
	Branchial pores																	
	4	5	6	7														*n*
Eptatretus bobwisneri				4														4
Eptatretus goslinei			18															18
Eptatretus grouseri	1	3																4
Eptatretus mccoskeri			1	6														7
Rubicundus lakeside	1																	1
	Trunk pores																	
	37	38	39	40	41	42	43	44	45	46	47	48	49	50				*n*
Eptatretus bobwisneri							1		2		1							4
Eptatretus goslinei	1	1	3	5	4	3	1											18
Eptatretus grouseri						2				1		1						4
Eptatretus mccoskeri		2		1	1	1	2											7
Rubicundus lakeside														1				1
	Tail pores																	
	10	11	12	13	14	15	16	17	18	19								*n*
Eptatretus bobwisneri			2	1	1													4
Eptatretus goslinei	4	10	2	2														18
Eptatretus grouseri				1	2	1												4
Eptatretus mccoskeri	2	4	1															7
Rubicundus lakeside										1								1
	Total pores																	
	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	~	88	*n*
Eptatretus bobwisneri									1		2	1						4
Eptatretus goslinei	2		3	4	6	1		2										18
Eptatretus grouseri							1		1				1		1			4
Eptatretus mccoskeri					1			2	1		3							7
Rubicundus lakeside																	1	1

symmetrical nasal-sinus papillae (vs. no nasal-sinus papillae in *E. astrolabium* and *E. caribbeaus*) (Fernholm, 1982; McMillan & Wisner, 1984; Mincarone, 2000; Mok, 2001; Mincarone & Stewart, 2006; Fernholm & Mincarone, 2010; Mincarone & Fernholm, 2010; Zintzen *et al.*, 2015).

Description: Body elongated, subcylindrical at prebranchial and branchial regions, laterally compressed at trunk and strongly compressed at tail. Rostrum bluntly rounded. Eyespots conspicuous. Two small, bilaterally symmetrical nasal-sinus papillae in the dorsal surface of the nasal sinus (difficult to observe in some specimens). Three pairs of barbels on head, first two pairs subequal in size (1.3–2.4% TL) and adjacent to opening of nasopharyngeal duct; third pair longer (2.0–3.2% TL) and immediately adjacent to mouth. Ventral finfold absent (occasionally represented by a white line) or low (1–2 mm high), beginning within anterior third of trunk, extending

Figure 3. Fresh specimens of *Eptatretus* from Galapagos: A, *Eptatretus bobwisneri* (SIO 19-80, 190 mm TL); B, *Eptatretus goslinei* (holotype, SIO 19-78, 300 mm TL); C, *Eptatretus mccoskeri* (SIO 19-81, 310 mm TL). Scale bars: 5 cm.

backwards to cloaca. Caudal finfold well developed, thin, extending around tail to dorsal surface, ending about over cloaca.

Body proportions (in percentage of TL; description of the holotype followed by range of paratypes in brackets): preocular length 6.3 (5.3–7.7); prebranchial length 23.0 (20.0–24.8); branchial length 10.0 (9.2–12.5); preventral length 50.0 (38.5–55.7); trunk length 52.3 (49.0–55.0); tail length 15.7 (14.2–17.9); body width at PCD 7.0 (5.6–8.1); body depth at PCD 8.3 (6.6–9.4); body depth including VFF 9.3 (7.7–11.7); body depth excluding VFF 8.7 (7.6–10.7); body depth at cloaca 8.3 (6.7–9.2); tail depth 10.0 (7.7–11.0).

Counts (description of the holotype followed by range of paratypes in brackets): multicusp pattern 3/3; anterior unicusps 11 (9–12); posterior unicusps 10 (8–11); total cusps 52 (47–58). Prebranchial pores 11 (10–12); branchial pores 6 (6); trunk pores 42 (37–43); tail pores 13 (10–13); total pores 72 (65–72).

Seven pairs of gill pouches corresponding to seven pairs of gill apertures. Gill apertures well-spaced and linearly arranged. Last branchial duct confluent with pharyngocutaneous duct on left side, forming a larger aperture. Posterior tip of dental muscle reaches gill pouches 4–6. Ventral aorta branches at gill pouch 5–6.

Colour (in life): body dark brown (almost black in some specimens); mouth with white margin; barbels with white tip; very distinct eyespots; gill apertures with white margins; ventral finfold appears only as a pale line or, when present, with white margin; caudal finfold occasionally with white margin (Fig. 3). Colour in alcohol similar to that of live specimens.

Distribution and habitat: Galapagos Islands: known from 18 specimens collected in two localities off north-western Isabela (between Cabo Berkeley and Punta Flores), and north-western Fernandina (Cabo Douglas), at depths from 467 to 478 m (Fig. 1).

Etymology: This species is named for Dr John M. Gosline (1944–2016), Professor in the Department of Zoology at the University of British Columbia (UBC), Vancouver, Canada, who pioneered work on the biomechanics of hagfish slime.

Remarks: Images of an unidentified species of *Eptatretus* were obtained off the north-eastern coast of Santa Cruz Island during the second expedition to the Galapagos in January 2020 (Fig. 6). Although no specimens were collected, the colour pattern and the number of gill apertures observed in video are similar to those of *E. goslinei*.

EPTATRETUS GROUSERI MCMILLAN, 1999

GROUSER'S HAGFISH

(FIGS 1, 2C; TABLES 1–3)

Eptatretus grouseri McMillan, 1999: 114, fig. 2a [original description; type locality: Galapagos Islands, 00°14.6'S, 91°26.6'W, 2370 ft (722 m); type series: holotype, CAS 86428; paratype, SIO 97-77 (1)].

Eptatretus grouseri. – Mok *et al.*, 2001: 1026. – Mincarone & McCosker, 2004: 164, figs 2, 3b. – Fernholm & Quattrini, 2008: 126. – McCosker & Rosenblatt, 2010: 172. – Knapp *et al.*, 2011: 404. – Ruiz *et al.*, 2011: 30. – Cruz-Mena & Angulo, 2015: 325. – Angulo & Del Moral-Flores, 2016: 103. – Mincarone, 2017: 802.

Eptatretus Eptatretus grouseri. – Møller & Jones, 2007: 63.

Material examined: CAS 86428, holotype (370 mm), NE Fernandina Island, Punta Espinosa, 00°14'36"S, 91°26'36"W, 722 m depth, submersible *Johnson Sea Link*, baited minnow trap, J. E. McCosker, R. G. Gilmore, 17 November 1995. SIO 97-77 (ex CAS 86428), paratype, 1(138 mm), taken with holotype. CAS 201882, 2(315–420 mm), Seymour Island, 00°21'42"S, 90°15'00"W, 648 m depth, submersible *Johnson Sea Link*, baited minnow trap, C. Baldwin, J. E. McCosker, 25 July 1998.

Diagnosis: Eptatretus grouseri differs from all congeners, except *E. aceroi* Polanco-Fernandez & Fernholm, 2014 from Colombia, *E. profundus* (Barnard, 1923) from South Africa, *E. wandoensis* Song & Kim, 2020 from South Korea and *E. wayuu* Mok *et al.*, 2001 from Colombia, by having five pairs (six pairs in one specimen of *E. grouseri*) of gill apertures arranged in a straight line and a 3/2 multicusp pattern of teeth. *Eptatretus grouseri* differs from these five-gilled congeners by having: 44–48 total cusps (vs. 58 in *E. aceroi*, 40–43 in *E. wandoensis* and 41–43 in *E. wayuu*); 11–12 prebranchial pores (vs. 44 in *E. aceroi*, 14–18 in *E. wandoensis* and 24 in *E. wayuu*); 42–48 trunk pores (vs. 107 in *E. aceroi*, 48–51 in *E. profundus* and 38–40 in *E. wayuu*); 13–15 tail pores (9–11 in *E. wandoensis*); 71–79 total pores (vs. 174 in *E. aceroi* and 81–86 in *E. profundus*) (Mok *et al.*, 2001; Polanco-Fernandez & Fernholm, 2014; Mincarone, 2017; Song & Kim, 2020).

Description: Body morphology, measurements and counts provided by McMillan (1999) and Mincarone & McCosker (2004).

Distribution and habitat: Galapagos Islands: known from four specimens collected in two localities: off Fernandina Island (Punta Espinosa), at 722 m depth, and off north-eastern Seymour Island, at 648 m depth (Fig. 1).

EPTATRETUS MCCOSKERI MCMILLAN, 1999

MCCOSKER'S HAGFISH

(FIGS 1, 2D, 3C, 5, 6C; TABLES 1–3)

Eptatretus mccoskeri McMillan, 1999: 115, fig. 2d–g [type locality: Galapagos Islands, 01°06.3'S, 89°06.9'W, depth 704 ft (correct depth 660 ft/201 m); type series: holotype, CAS 86431; paratypes, SIO 97-75 (2), USNM 344905 (1)].

Eptatretus mccoskeri. – Mok, 2001: 356. – Mok *et al.*, 2001: 1026. – McMillan & Wisner, 2004: 55. – Mincarone & McCosker, 2004: 166. – Møller & Jones, 2007: 64. – McCosker & Rosenblatt, 2010: 172. – Mincarone & Fernholm, 2010: 797. – Knapp *et al.*, 2011: 404. – Ruiz *et al.*, 2011: 30. – Mincarone & McCosker, 2014: 347. – Cruz-Mena & Angulo, 2015: 325. – Angulo & Del Moral-Flores, 2016: 105.

Eptatretus Eptatretus mccoskeri. – Møller & Jones, 2007: 64.

Material examined: CAS 86431, holotype (310 mm), off SE San Cristobal Island, 01°06'19"S, 89°06'56"W, 201 m depth, submersible *Johnson Sea Link*, baited minnow trap, John E. McCosker, 16 November 1995. SIO 97–75, paratype (290 mm), and USNM 344905, paratype (284 mm), taken with the holotype. MCCDRS 9397$^{COI,\ 16S}$, 2(303–304 mm) and SIO 19-81$^{COI,\ 16S}$, 2 (310–310 mm), between Santa Cruz and Santa Fé islands, 00°48'00"S, 90°10'12"W, 155 m depth, *Valeska Yamile*, sta. G1, baited trap, Douglas Fudge *et al.*, 26 May 2019, 08:01–10:06 h.

Diagnosis: Eptatretus mccoskeri differs from all congeners, except *Eptatretus poicilus* Zintzen & Roberts, 2015 from New Zealand, by having eight pairs (seven pairs in one specimen of *E. mccoskeri*) of gill apertures well-spaced and arranged in a near straight line, and a 3/3 multicusp pattern of teeth. *Eptatretus mccoskeri* differs from *E. poicilus* by its number of trunk pores (38–43 vs. 45–50), number of total pores (69–75 vs. 78–86) and by its colour pattern (body purple to purplish brown vs. body strongly mottled with pale brown, dark brown and white-beige) (McMillan, 1999; Zintzen *et al.*, 2015).

Description: Body elongated, subcylindrical at prebranchial and branchial regions, laterally compressed at trunk and strongly compressed at tail. Rostrum bluntly rounded. Two bilaterally symmetrical nasal-sinus papillae in the dorsal surface of the nasal sinus. Eyespots conspicuous. Three pairs of barbels on head: first two about equal in size (1.5–2.2% TL) and adjacent to opening of nasopharyngeal duct; third pair longer (1.9–2.9% TL) and immediately adjacent to mouth. Ventral finfold absent (occasionally represented by a white line) or low (1–2 mm high), beginning within anterior 1/3 of trunk, extending backwards to cloaca. Caudal finfold well developed, thin, extending around tail to dorsal surface, ending about over cloaca.

Body proportions (in percentage of TL; description of the holotype followed by range of paratypes in brackets): preocular length 7.1 (5.6–6.3); prebranchial length 25.8 (22.6–26.1); branchial length 10.0 (8.8–13.5); preventral length 51.6 (38.7–53.4); trunk length 49.7 (49.3–56.1); tail length 15.8 (10.6–16.9); body width at PCD 7.3 (6.9–8.6); body depth at PCD (6.6–8.4); body depth including VFF 8.2 (7.7–9.3); body depth excluding VFF 8.2 (7.4–9.1); body depth at cloaca 7.3 (6.8–14.5); tail depth 8.9 (7.1–9.8).

Counts (description of the holotype followed by range of paratypes in brackets): multicusp pattern 3/3; anterior unicusps 10 (8–10); posterior unicusps 9 (8–10); total cusps 51 (44–51). Prebranchial pores 13 (13–16); branchial pores 7 (6–7); trunk pores 43 (38–43); tail pores 10 (10–12); total pores 73 (69–75).

Eight pairs of gill pouches corresponding to eight pairs of gill apertures (one specimen with seven pairs of gill pouches and apertures). Gill apertures

well-spaced and linearly arranged. Last branchial duct confluent with pharyngocutaneous duct on left side, forming a larger aperture. Posterior tip of dental muscle reaches gill pouches 5–6. Ventral aorta branches at gill pouches 6–7.

Colour (in life): body purple to purplish brown; mouth with white margin; barbels white; eyespots visible, but not prominent; gill apertures with white margins; ventral finfold occasionally lighter than body; caudal finfold with white margin (Fig. 3). Colour in alcohol darker than that described for live specimens.

Distribution and habitat: Galapagos Islands: known from seven specimens collected in two localities: off south-eastern San Cristobal Island, on the top of a seamount at about 201 m depth; and between Santa Cruz and Santa Fé islands, at 155 m depth (Fig. 1).

MYXINE GREGGI SP. NOV.

GREGG'S HAGFISH

(FIGS 1, 2F, 4A, 5, 6D; TABLES 4–6)

Zoobank registration. urn:lsid:zoobank.org:act:309DFFDD-9C79-42F6-AB51-4B43E1BAFB6C

Holotype: SIO 19-82, 408 mm, off NE Santa Cruz Island, 00°30'57.68"S, 90°10'17.90"W, 688 m depth, *Valeska Yamile*, sta. G24, baited trap, Douglas Fudge *et al*., 11 June 2019, 07:59–11:10 h.

Paratypes: MCCDRS 9404, 11 (293-445 mm) and SIO 19–83, 1 (515 mm), taken with the holotype. MCCDRS 9399$^{COI,\ 16S}$, 1 (457 mm), off NE Santa Cruz Island, 00°29'21.27"S, 90°10'22.48"W, 789 m depth, *Valeska Yamile*, baited trap, Douglas Fudge *et al*., 29 May 2019, 10:16–11:37h. MCCDRS 9401$^{COI,\ 16S}$, 10 (181–485 mm) and SIO 19-84$^{COI,\ 16S}$, 1 (437 mm), off north-eastern Santa Cruz Island, 00°29'46.47"S, 90°12'00.73"W, 815 m depth, *Valeska Yamile*, sta. G5, baited trap, Douglas Fudge *et al*., 29 May 2019, 09:21–12:39h.

Diagnosis: *Myxine greggi* differs from all congeners, except *M. affinis* Günther, 1870 and *M. australis* Jenyns, 1842 from southern South America, *M. glutinosa* Linnaeus, 1758 from the eastern North Atlantic and Mediterranean, *M. limosa* Girard, 1859 from the western North Atlantic, *M. hubbsi* Wisner & McMillan, 1995 from the eastern Pacific, *M. hubbsoides* Wisner & McMillan, 1995 from Chile, *M. jespersenae* Møller *et al.*, 2005 from Greenland and Iceland, *M. knappi* Wisner & McMillan, 1995 from southern Argentina, *M. kuoi* Mok, 2002 from Taiwan, *M. mcmillanae* Hensley, 1991 from the Gulf of Mexico and Caribbean Sea, *M. paucidens* Regan, 1913 from Japan, *M. sotoi* Mincarone, 2001 from southern Brazil, and *M. martinii* from the Galapagos, by having six pairs of gill pouches and a 2/2 multicusp pattern of teeth. *Myxine greggi* differs from these congeners by having: 34–40 total cusps (vs. 30–32 in *M. kuoi*, 42–88 in *M. mcmillanae* and 26 in *M. paucidens*); 22–26 prebranchial pores (vs. 30–31 *M. hubbsoides*, 28–37 in *M. jespersenae*, 30–38 in *M. knappi* and 28–38 in *M. sotoi*); 58–66 trunk pores (vs. 68–71 in *M. hubbsoides* and 52–54 in *M. martinii*); 91–102 total pores (vs. 111–116 in *M. hubbsoides* and 107–121 in *M. jespersenae*); and by having one single conspicuous nasal-sinus papilla in the mid-dorsal surface of the nasal sinus (vs. two bilaterally symmetrical nasal-sinus papillae in *M. jespersenae*). *Myxine greggi* can be also distinguished from the congeners with six-gill pouches by its colour pattern (body dark brown with white head vs. body entirely pigmented, without white head, in *M. affinis*, *M. australis*, *M. hubbsoides*, *M. knappi*, *M. kuoi* and *M. paucidens*). *Myxine greggi* can be further distinguished from *M. hubbsi* by having a well-developed ventral finfold [3–7 mm high vs. vestigial to low (1–2 mm high)] (Hensley, 1991; Wisner & McMillan, 1995; Mincarone, 2001; Mok, 2001, 2002; McMillan & Wisner, 2004; Møller *et al.*, 2005).

Figure 4. Fresh specimens of *Myxine* from Galapagos: A, *Myxine greggi* (holotype, SIO 19-82, 408 mm TL); B, *Myxine martinii* (holotype, SIO 19-85, 381 mm TL); C, *Myxine phantasma* (holotype, SIO 19-86, 510 mm TL). Scale bars: 5 cm.

Description: Body elongated, subcylindrical at prebranchial and branchial regions, laterally compressed at trunk and strongly compressed at tail. Rostrum triangular with rounded tip. One single conspicuous nasal-sinus papilla in the mid-dorsal surface of the nasal sinus. Eyespots absent. Three pairs of barbels on head: first two about equal in size (0.6–1.4% TL) and adjacent to opening of nasopharyngeal duct; third pair longer (1.0–1.9% TL) and immediately adjacent to mouth. Ventral finfold well developed (3–7 mm high), beginning within anterior 10% of trunk, extending backward to the cloaca. Caudal finfold thin, rounded, beginning immediately posterior to edge of cloaca, extending around tail to dorsal surface, ending about over cloaca.

Body proportions (in percentage of TL; description of the holotype followed by range of paratypes in brackets): prebranchial length 25.7 (23.2–28.3); preventral length 27.5 (24.9–31.1); trunk length 62.3 (56.9–64.8); tail length 12.0 (8.7–13.9); body width at PCD 2.5 (2.6–3.6); body depth at PCD 3.6 (3.3–4.4); body depth including VFF 5.4 (3.3–5.5); body depth excluding VFF 3.7 (2.9–5.0); body depth at cloaca 3.4 (2.6–4.3); tail depth 4.2 (2.9–4.8).

Counts (description of the holotype followed by range of paratypes in brackets): multicusp pattern 2/2; anterior unicusps 6 (6–8); posterior unicusps 7 (7–8); total cusps 34 (34–40). Prebranchial pores 24 (22–26); trunk pores 66 (58–66); tail pores 10 (9–12); total pores 100 (91–102).

Six pairs of gill pouches, with efferent branchial ducts on either side combined into a single external gill aperture posterior to the gill pouches. Gill aperture on the left side confluent with the pharyngocutaneous duct aperture. Dental muscle overlies the first pair of gill pouches. Ventral aorta not branched.

Colour (in life): body dark brown, its dorsal region lighter than ventral (more evident in juveniles); head white, becoming gradually darker backwards; mouth and barbels white; gill apertures with white margin; ventral finfold the same colour as or even darker than body; caudal finfold the same colour as body (Fig. 4). Colour in alcohol similar to that described for live specimens.

Distribution and habitat: Galapagos Islands: known from 25 specimens collected in three stations off north-eastern Santa Cruz Island, between 688 and 815 m depth (Fig. 1).

Etymology: This species is named for John Gregg, founder and president of the Western Flyer Foundation. John is an ardent supporter of marine biology research and a hagfish enthusiast. He joined the team during part of the Galapagos expedition and was on the boat when the specimens were collected.

MYXINE MARTINII SP. NOV.

MARTINI'S HAGFISH

(FIGS 1, 2G, 4B, 5; TABLES 4–6)

Zoobank registration. urn:lsid:zoobank.org:act:5765A69C-0676-4067-B433-ED64FBDBD684

Holotype: SIO 19-85$^{COI,\ 16S}$, 381 mm, NW Fernandina Island, Cabo Douglas, 00°17'37.94" S, 91°39'18.10"W, 557 m depth, *Queen Mabel*, sta. G20, baited trap, Douglas Fudge *et al.*, 6 June 2019, 09:51–12:40 h.

Paratypes: SIO 19-80$^{COI,\ 16S}$, 1 (361 mm), taken with the holotype.

Diagnosis: Myxine martinii differs from all congeners, except *M. affinis* Günther, 1870 and *M. australis* Jenyns, 1842 from southern South America, *M. glutinosa* Linnaeus, 1758 from the eastern North Atlantic and Mediterranean, *M. limosa* Girard, 1859 from the western North Atlantic, *M. hubbsi* Wisner & McMillan, 1995 from the eastern Pacific, *M. hubbsoides* Wisner & McMillan, 1995 from Chile, *M. jespersenae* Møller *et al.*, 2005 from Greenland and Iceland, *M. knappi* Wisner & McMillan, 1995 from southern Argentina, *M. kuoi* Mok, 2002 from Taiwan, *M. mcmillanae* Hensley, 1991 from the Gulf of Mexico and Caribbean Sea, *M. paucidens* Regan, 1913 from Japan, *M. sotoi* Mincarone, 2001 from southern Brazil, and *M. greggi* from the Galapagos, by having six pairs of gill pouches and a 2/2 multicusp pattern of teeth. *Myxine martinii* differs from these congeners by having: 34 total cusps (vs. 38–46 in *M. affinis*, 38–44 in *M. jespersenae*, 30–32 in *M. kuoi*, 42–48 in *M. mcmillanae*, 26 in *M. paucidens* and 34–44 in *M. sotoi*); 26–27 prebranchial pores (vs. 30–31 *M. hubbsoides*, 28–37 in *M. jespersenae*, 30–38 in *M. knappi* and 28–38 in *M. sotoi*); 52–54 trunk pores (vs. 57–79 in *M. affinis*, 57–73 in *M. hubbsi*, 68–71 in *M. hubbsoides*, 57–58 in *M. kuoi*, 60–76 in *M. mcmillanae*, 61–73 in *M. sotoi* and 58–66 in *M. greggi*); 91 total pores (vs. 94–124 in *M. affinis*, 111–116 in *M. hubbsoides*, 107–121 in *M. jespersenae*, 98–126 in *M. knappi*, 95–100 in *M. kuoi*, 101–113 in *M. mcmillanae* and 101–119 in *M. sotoi*); and by having one single conspicuous nasal-sinus papilla in the mid-dorsal surface of the nasal sinus (vs. two bilaterally symmetrical nasal-sinus papillae in *M. jespersenae*). *Myxine martinii* can be also distinguished from congeners with six-gill pouches by its colour pattern (body dark brown with white head vs. body entirely pigmented, without white head, in *M. affinis*, *M. australis*, *M. hubbsoides*, *M. knappi*, *M. kuoi* and *M. paucidens*). *Myxine martinii* can be further distinguished from *M. greggi* and *M. hubbsi* by having a white ventral finfold (vs. same colour or darker than body). In *Myxine martinii*, the gill aperture on the

Table 4. Meristic and morphometric characters for species of *Myxine* from the Galapagos Islands

Species	***M. greggi***		***M. martinii***		***M. phantasma***	
N	holotype	24 paratypes	holotype	1 paratype	holotype	61 paratypes
	SIO 19-82	range	SIO 19-85	SIO 19-80	SIO 19-86	range
Total length TL (mm)	408	181–515	381	361	510	178–480
Gill apertures [a]	1 + 1	1–1	1 + 1	1 + 1	1 + 1	1–1
Gill pouches [a]	6 + 6	6–6	6 + 6	6 + 6	6 + 6	6–6
Nasal-sinus papillae	1	1	1	1	2	2
Cusps						
Multicusps (ant./post.)	2/2	2/2	2/2	2/2	3/2	3/2
Anterior unicusps [a]	6 + 6	6–8	6 + 6	6 + 6	8 + 8	7–10
Posterior unicusps [a]	7 + 7	7–8	7 + 7	7 + 7	7 + 7	7–9
Total cusps	34	34–40	34	34	40	38–46
Slime pores, left side						
Prebranchial	24	22–26	26	27	19	18–24
Trunk	66	58–66	54	52	65	64–75
Tail	10	9–12	11	12	8	6–10
Total pores	100	91–102	91	91	92	90–106
Measurements as % of TL						
Prebranchial length	25.7	23.2–28.3	31.0	30.2	23.5	23.0–26.4
Preventral length	27.5	24.9–31.1	36.2	33.2	24.1	24.1–29.2
Trunk length	62.3	56.9–64.8	56.0	57.6	65.7	59.3–68.2
Tail length	12.0	8.7–13.9	13.0	12.2	11.2	9.0–12.4
Body width at PCD	2.5	2.6–3.6	4.2	3.9	3.7	2.1–3.8
Body depth at PCD	3.6	3.3–4.4	5.0	5.5	4.7	2.8–4.7
Body depth including VFF	5.4	3.3–5.5	4.5	5.0	5.0	4.2–6.4
Body depth excluding VFF	3.7	2.9–5.0	3.9	4.4	4.2	3.2–5.6
Body depth at cloaca	3.4	2.6–4.3	4.2	3.9	4.3	2.9–4.5
Tail depth	4.2	2.9–4.8	5.0	4.7	4.3	4.1–5.6

[a]Left + right side for single specimen.

left side is not confluent with the pharyngocutaneous duct aperture, but separated by a very short distance (*c*. 1 mm). This character is only observed in *Myxine paucidens* and some specimens of *Myxine capensis* Regan, 1913 (a species with seven pairs of pouches from southern Africa) (Hensley, 1991; Wisner & McMillan, 1995; Mincarone, 2001; Mok, 2001, 2002; McMillan & Wisner, 2004; Møller *et al.*, 2005; Mincarone *et al.*, 2011).

Description: Body elongated, subcylindrical at prebranchial and branchial regions, laterally compressed at trunk and strongly compressed at tail. Rostrum triangular with rounded tip. One single conspicuous nasal-sinus papilla in the mid-dorsal surface of the nasal sinus. Eyespots absent. Three pairs of barbels on head: first two about equal in size (1.0–1.2% TL) and adjacent to opening of nasopharyngeal duct; third pair longer (1.6–1.8% TL) and immediately adjacent to mouth. Ventral finfold low (< 1 mm high), beginning within anterior 10% of trunk, extending backward to the cloaca. Caudal finfold thin, rounded, beginning immediately posterior to edge of cloaca, extending around tail to dorsal surface, ending about over cloaca.

Body proportions (in percentage of TL; description of the holotype followed by paratype in brackets): prebranchial length 31.0 (30.2); preventral length 36.2 (33.2); trunk length 56.0 (57.6); tail length 13.0 (12.2); body width at PCD 4.2 (3.9); body depth at PCD 5.0 (5.5); body depth including VFF 4.5 (5.0); body depth excluding VFF 3.9 (4.4); body depth at cloaca 4.2 (3.9); tail depth 5.0 (4.7).

Counts (description of the holotype followed by paratype in brackets): multicusp pattern 2/2; anterior unicusps 6 (6); posterior unicusps 7 (7); total cusps 34 (34). Prebranchial pores 26 (27); trunk pores 54 (52); tail pores 11 (12); total pores 91 (91).

Table 5. Frequency of cusps for species of *Myxine* from the Galapagos Islands

	Anterior unicusps						Posterior unicusps				
	6	7	8	9	10	*n*	7	8	9		*n*
Myxine greggi	8	33	3			44	30	14			44
Myxine martinii	4					4	4				4
Myxine phantasma		9	76	37	2	124	58	61	5		124
	Total cusps										
	34	35	36	37	38	39	40	41	42	43	44	45	46	*n*
Myxine greggi	2	1	11	4	3		1							22
Myxine martinii	2													2
Myxine phantasma					2	3	15	8	10	18	3	1	2	62

Table 6. Frequency of pores (left side) for species of *Myxine* from the Galapagos Islands

	Prebranchial pores																							
	18	19	20	21	22	23	24	25	26	27														*n*
Myxine greggi					5	7	10	2	1															25
Myxine martinii									1	1														2
Myxine phantasma	1	2	6	12	18	13	10																	62
	Trunk pores																							
	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	*n*
Myxine greggi							1	1		3	2	2	3	5	6										23
Myxine martinii	1		1																						2
Myxine phantasma													2	7	12	21	7	6	3	2	1			1	62
	Tail pores																							
	6	7	8	9	10	11	12																	*n*
Myxine greggi				2	14	8	1																	25
Myxine martinii						1	1																	2
Myxine phantasma	5	14	24	18	1																			62
	Total pores																							
	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106							*n*
Myxine greggi		1		1		1	4	2	5	3	4	1	1											23
Myxine martinii		2																						2
Myxine phantasma	1		1	1	5	8	12	7	11	5	5	1	2	2			1							62

Six pairs of gill pouches, with efferent branchial ducts on either side combined into a single external gill aperture posterior to the gill pouches. Gill aperture on the left side not confluent with the pharyngocutaneous duct aperture, but separated by a very short distance (< 1 mm). Dental muscle overlies the first pair of gill pouches. Ventral aorta not branched.

Colour (in life): body dark brown; ventral region between rows of slime pores lighter than body; tip of head, mouth and barbels white; gill apertures with white margin; ventral finfold white; caudal finfold the same colour as body (Fig. 4). Colour in alcohol similar to that described for live specimens.

Distribution and habitat: Galapagos Islands: known only from two specimens collected off Cabo Douglas, north-western Fernandina, at 557 m depth (Fig. 1).

Etymology: This species is named for Dr Frederic (Ric) Martini, who for many years taught at the Shoals Marine Laboratory (University of New Hampshire, Cornell University) and introduced many students to

the wonders of hagfish through his lectures and his research publications.

MYXINE PHANTASMA SP. NOV.

GHOST HAGFISH

(FIGS 1, 2H, 4C, 5, 6E; TABLES 4–6)

Zoobank registration: urn:lsid:zoobank.org:act:3A877CFC-03B5-40F8-8190-E90EEE79025C

Holotype: SIO 19-86, 510 mm, off NE Santa Cruz Island, 00°30’57.68”S, 90°10’17.90”W, 688 m depth, *Valeska Yamile*, sta. G24, baited trap, Douglas Fudge *et al.*, 11 June 2019, 07:59–11:10 h.

Paratypes: MCCDRS 9405$^{COI,\ 16S}$, 54 (178–426 mm) and SIO 19-83$^{COI,\ 16S}$, 2 (442–480 mm), taken with the holotype. MCCDRS 9398$^{COI,\ 16S}$, 1 (206 mm), off NE Santa Cruz Island, 00°29’21.27”S, 90°10’22.48”W, 789 m depth, *Valeska Yamile*, baited trap, Douglas Fudge *et al.*, 29 May 2019, 10:16–11:37 h. MCCDRS 9400$^{COI,\ 16S}$, 3 (187–365 mm), off NE Santa Cruz Island, 00°29’46.47”S, 90°12’00.73”W, 815 m depth, *Valeska Yamile*, baited trap, Douglas Fudge *et al.*, 29 May 2019, 09:21–12:39 h.

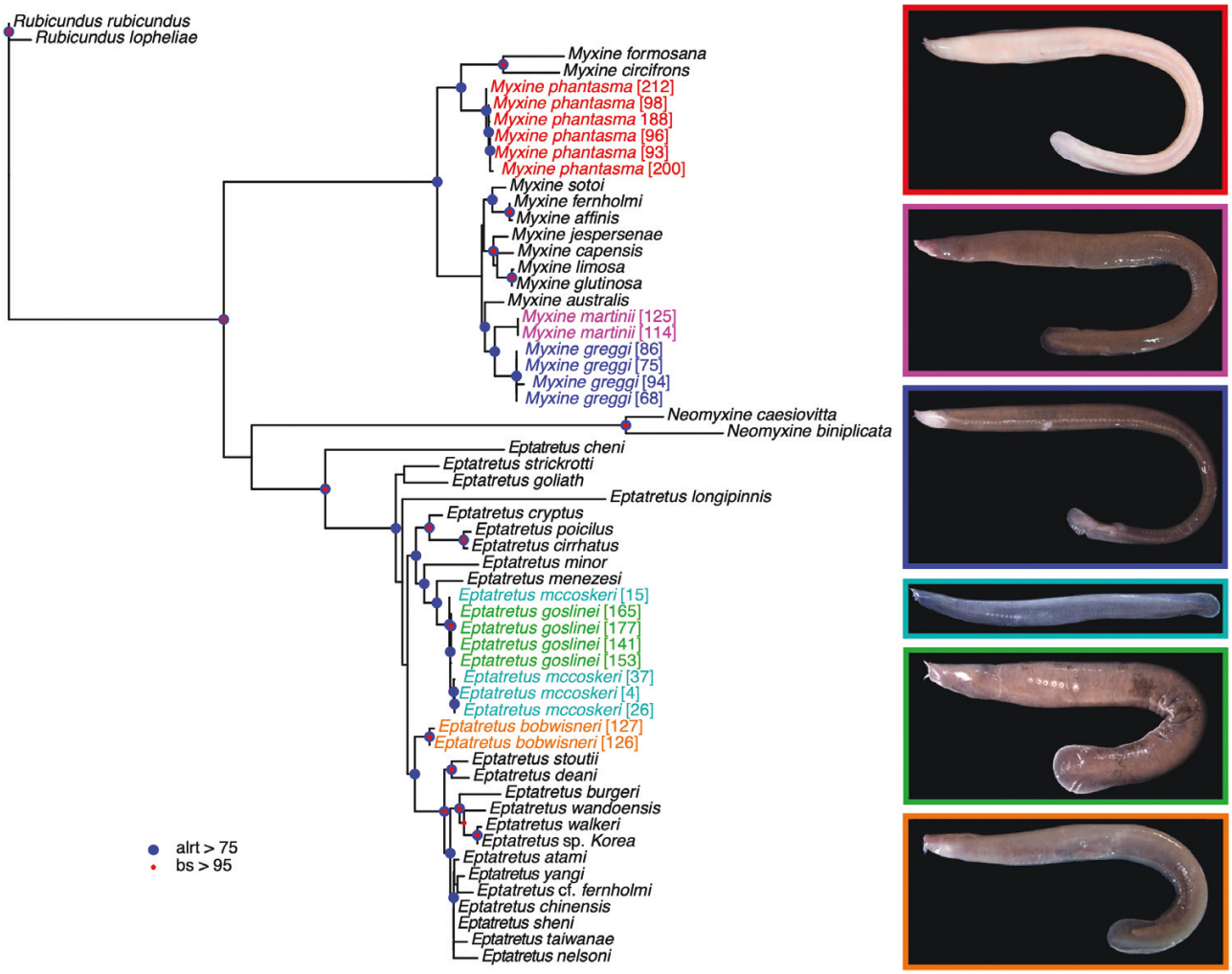

Figure 5. Phylogeny of hagfishes, including new data for six species from Galapagos. Publicly available and new 16S and *COI* data (Supporting Information, Appendix S2) were used to estimate phylogeny under the MFP-merge option in iqtree (Minh *et al.*, 2020). Galapagos hagfishes fall into five distinct clades of *Myxine* and *Eptatretus* species suggesting multiple, independent colonization events. Individual isolates are indicated in brackets. Nodes that receive greater than 0.75 alrt score are indicated with a blue circle. Nodes that receive greater than 95% ultrafast bootstrap support are indicated with a red circle.

Diagnosis: *Myxine phantasma* differs from all congeners, except *M. debueni* Wisner & McMillan, 1995 from the Strait of Magellan, Chile, *M. fernholmi* Wisner & McMillan, 1995 from the Falkland Islands and *M. garmani* Jordan & Snyder, 1901 from Japan, by having six pairs of gill pouches and a 3/2 multicusp pattern of teeth. *Myxine phantasma* differs from these congeners by having: 18–24 prebranchial pores (vs. 27–29 in *M. garmani*), 64–75 trunk pores (vs. 76 in *M. debueni*, 80–83 in *M. fernholmi* and 52–61 in *M. garmani*), 6–10 tail pores (vs. 12–13 in *M. garmani*), 90–106 total pores (vs. 113–121 in *M. fernholmi*); and by having two bilaterally symmetrical nasal-sinus papillae in the dorsal surface of the nasal sinus (vs. one single nasal-sinus papilla in *M. debueni*). In addition, *M. phantasma* has a totally unpigmented body, which makes its skin transparent in live specimens. This unique character has been not described for any other species of *Myxine* (Wisner & McMillan, 1995; Mok, 2001; McMillan & Wisner, 2004).

Description: Body elongated, subcylindrical at prebranchial and branchial regions, laterally compressed at trunk and strongly compressed at tail. Rostrum triangular with rounded tip. Two conspicuous, bilaterally symmetrical nasal-sinus papillae in the dorsal surface of the nasal sinus. Eyespots absent. Three pairs of barbels on head: first two about equal in size (0.7–1.6% TL) and adjacent to opening of nasopharyngeal duct; third pair longer (1.3–2.2% TL) and immediately adjacent to mouth. Ventral finfold well developed (2–6 mm high), beginning within anterior 10% of trunk, extending backward to the cloaca. Caudal finfold thin, rounded, beginning immediately posterior to edge of cloaca, extending around tail to dorsal surface, ending about over cloaca.

Body proportions (in percentage of TL; description of the holotype followed by paratype in brackets): prebranchial length 23.5 (23.0–26.4); preventral length 24.1 (24.1–29.2); trunk length 65.7 (59.3–68.2); tail length 11.2 (9.0–12.4); body width at PCD 3.7 (2.1–3.8); body depth at PCD 4.7 (2.8–4.7); body depth including VFF 5.0 (4.2–6.4); body depth excluding VFF 4.2 (3.2–5.6); body depth at cloaca 4.3 (2.9–4.5); tail depth 4.3 (4.1–5.6).

Counts (description of the holotype followed by paratype in brackets): multicusp pattern 3/2; anterior unicusps 8 (7–10); posterior unicusps 7 (7–9); total cusps 40 (38–46). Prebranchial pores 19 (18–24); trunk pores 65 (64–75); tail pores 8 (6–10); total pores 92 (90–106).

Six pairs of gill pouches, with efferent branchial ducts on either side combined into a single external gill aperture posterior to the gill pouches. Gill aperture on the left side confluent with the pharyngocutaneous duct aperture. Dental muscle overlies the first pair of gill pouches. Ventral aorta not branched.

Colour (in life): body overall pinkish due mostly to the underlying muscle and blood vessels coloration; skin transparent, without pigmentation; muscles, gill pouches, liver and slime glands visible through the skin (Fig. 4). Colour (in alcohol): body overall whitish to light beige. Specimens filmed *in situ* reveals a subtle blue tinge on the tail when illuminated with white light (Fig. 6).

Distribution and habitat: Galapagos Islands: known from 62 specimens collected in three stations off north-eastern Santa Cruz Island, between 688 and 815 m depth (Fig. 1).

Etymology: From the Greek φάντασμα, ghost, which refers to the transparent skin and lack of melanin-based pigmentation in this species. It is a noun in apposition.

PHYLOGENY

Our phylogenetic results are consistent with previous analyses of similar data from hagfishes (Kuo *et al.*, 2003; Kuo *et al.*, 2010; Fernholm *et al.*, 2013; Zintzen *et al.*, 2015; Song & Kim, 2020). With just two loci and ~1 kb of sequence alignment data, we expected limited support for some nodes. For instance, our phylogenetic analyses do not resolve the position of the *Neomyxine* species, nor do they support several more recent bifurcations among several eptatretid clades. However, by focusing our attention on only those nodes that receive greater than 0.75 alrt and/or 95% ultrafast bootstrap support (http://www.iqtree.org/doc), our results are sufficient to conclude the following regarding the evolutionary history of Galapagos hagfishes.

The eight species described thus far from the Galapagos (*Rubicundus lakeside*, *Eptatretus mccoskeri*, *E. bobwisneri*, *E. grouseri*, *E. goslinei*, *Myxine greggi*, *M. martinii* and *M. phantasma*) represent five distinct clades (four described here and *Rubicundus* previously described morphologically), which most likely suggests multiple, relatively recent colonization events of hagfishes to the Galapagos. Our data also suggest that species diversification may have occurred in the Galapagos following the introduction of the ancestor of *M. martinii* and *M. greggi*, as well as after the arrival of the ancestor of *E. mccoskeri* and *E. goslinei*. While the Galapagos endemics *E. mccoskeri* and *E. goslinei* are clearly differentiated morphologically, the molecular data do not have the power to resolve these two species, indicating an early stage of speciation.

Observations from baited remote underwater video (BRUV)

Information provided by BRUV revealed a rich and apparently healthy benthic environment. We obtained video at depth of five Galapagos hagfish species (Fig. 6). The only species that we caught but failed to film live was *M. martinii*. The video data provide us with information about the colour and behaviour at depth of hagfishes, as well as the nature of the habitat at each collection station, particularly those off north-eastern Santa Cruz (near Gordon Rocks) and off north-western Fernandina (Cabo Douglas). Bottom type near Gordon Rocks ranged from flat, sandy, naked bottom, covered by only thin sediments to a structured, irregular, rocky bottom, inhabited by a great variety of corals, shrimps, isopods, crabs, sea urchins, brittle stars and fishes. Among the fishes reliably identified from the video are: the broadnose sevengill shark *Notorynchus cepedianus* (Péron, 1807) (Hexanchidae), recently reported for the first time in the Galapagos by Buglass *et al.* (2020), the mottled scorpionfish *Pontinus clemensi* Fitch, 1955 (Scorpaenidae) and the snailfish *Paraliparis* sp. (Liparidae). Unidentified fishes of the families Ophichthidae, Macrouridae and Moridae were also observed. At Cabo Douglas, the bottom was a mix of rocks of different sizes and shapes, resulting in an irregular and structured habitat. Due to the BRUV design used near Cabo Douglas, most of the field of view was obstructed by the trap and the images of the fauna were limited. Nevertheless, it was still possible to observe some unidentified species of crabs (Majidae and Xanthidae) and fishes (Moridae and Macrouridae). The BRUV used near Gordon Rocks gave a less obstructed view of the bottom and fauna (Fig. 6B–E).

DISCUSSION

Over the course of ten days at sea, six species of hagfishes were collected, which included two previously described species from the genus *Eptatretus* (*E. mccoskeri* and *E. bobwisneri*) and four new species. Three of these were species from the genus *Myxine* (*M. greggi*, *M. martinii* and *M. phantasma*), which are the first reports of any *Myxine* in the Galapagos, as well as a new member of the genus *Eptatretus* (*E. goslinei*). Despite several fishing attempts along the northern coast of Fernandina Island, we were not able to collect additional specimens of *R. lakeside* (known only from the holotype) and *E. grouseri* (known from four specimens), originally described from Cabo Douglas and Punta Espinosa, respectively. The four new species of hagfish described here plus the four previously described by McMillan (1999) and Mincarone & McCosker (2004), brings the total to eight species known from the Galapagos Islands, with all of these species appearing to be endemic.

Our data add new information about the distribution of hagfishes in the Galapagos to the discoveries from the 1995 and 1998 expeditions (McMillan, 1999; Mincarone & McCosker, 2004). Due to the limited amount of

Key to the hagfish species from the Galapagos Islands

1a. Multiple (five to eight) pairs of gill apertures; caudal fin rays bifurcated at tip 2
1b. One pair of gill apertures; caudal fin rays not bifurcated 6
2a. Body pinkish; nasopharyngeal duct cylindrical, tube-like, slightly projecting; 36 total cusps; 88 total pores ..... *Rubicundus lakeside*
2b. Body dark, nasopharyngeal duct not elongated; 44–58 total cusps; 65–79 total pores 3
3a. Five (one specimen with six) pairs of gill apertures ..... *Eptatretus grouseri*
3b. Seven or eight pairs of gill apertures 4
4a. Seven pairs of gill apertures ..... *Eptatretus goslinei*
4b. Eight (one specimen of *E. mccoskeri* with seven) pairs of gill apertures 5
5a. 3/2 multicusp pattern; 9–11 prebranchial pores; no papillae in the dorsal surface of the nasal sinus ..... *Eptatretus bobwisneri*
5b. 3/3 multicusp pattern; 13–16 prebranchial pores; two bilaterally symmetrical nasal-sinus papillae in the dorsal surface of the nasal sinus ..... *Eptatretus mccoskeri*
6a. Body whitish (skin transparent in live specimens); 3/2 multicusp pattern; two bilaterally symmetrical nasal-sinus papillae in the dorsal surface of the nasal sinus ..... *Myxine phantasma*
6b. Body brown; 2/2 multicusp pattern; one single mid-dorsal nasal-sinus papilla in the dorsal surface of the nasal sinus 7
7a. 58–66 trunk pores; prebranchial length 23–28 % TL; ventral finfold brown to black ..... *Myxine greggi*
7b. 52–54 trunk pores; prebranchial length 30–31 % TL; ventral finfold white ..... *Myxine martinii*

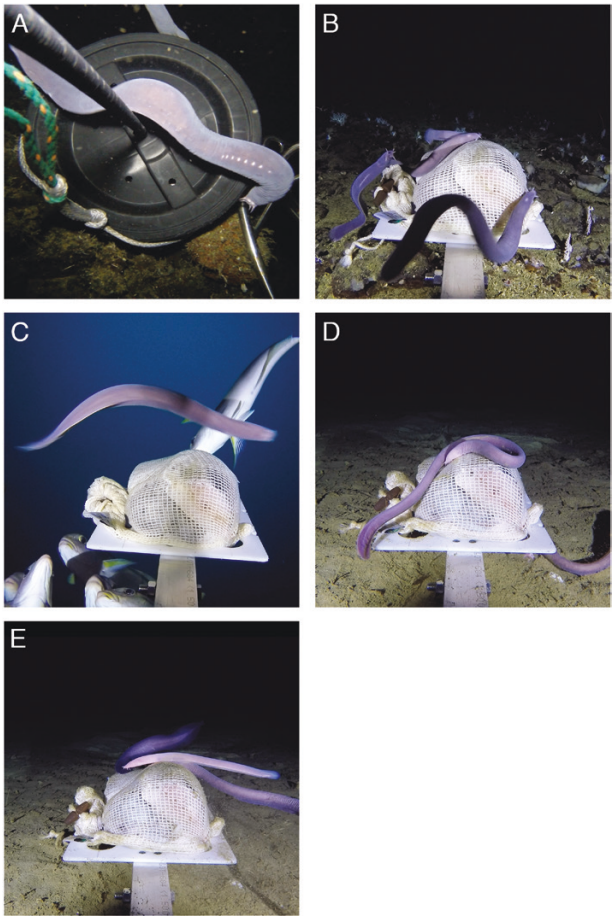

Figure 6. Still images of five species of Galapagos hagfishes from baited remote underwater video (BRUV): A, *Eptatretus bobwisneri* near a 5-gal bucket trap off north-western Fernandina Island (00°17'37.6"S, 91°39'02.8"W, 502 m depth), showing its characteristic eight gill apertures; B, four individuals of *Eptatretus* sp., filmed off north-eastern Santa Cruz Island (00°31'37.6"S, 90°11'14.7"W, 370 m depth); C, the only individual of *Eptatretus mccoskeri* caught on video, between Santa Cruz and Santa Fé islands (00°47'43.0"S, 90°09'29.9"W, 150 m depth); D, *Myxine greggi*, off north-eastern Santa Cruz Island (00°30'17.1"S, 90°10'02.3"W, 803 m depth); E, a small individual of *Myxine phantasma*, appearing in front of a larger *Myxine greggi*, and the snailfish *Paraliparis* sp., off north-eastern Santa Cruz Island (00°30'17.1"S, 90°10'02.3"W, 803 m depth).

sampling from only a small number of islands, it is difficult at this point to make generalizations about how the eight species are geographically and vertically distributed. Four hagfish species occur at Cabo Douglas (*R. lakeside*, *E. bobwisneri*, *E. goslinei* and *M. martinii*), but it is not clear if the hagfish diversity at this site is exceptional in the Galapagos, or the result of increased fishing effort there. *Eptatretus grouseri* currently has the widest range, with individuals collected 133 km apart at two sites: Punta Espinosa, at the north-east corner of Fernandina Island, and north of Santa Cruz Island. Specimens of *E. mccoskeri* were caught 122 km apart at two sites: halfway between Santa Cruz and Santa Fé islands, and south-east of San Cristobal Island. *Eptatretus goslinei* was caught in two locations separated by 38 km on the north-western coasts of Fernandina and Isabela islands. *Eptatretus goslinei* is the only species caught close to Isabela, although it is likely that the three other species found near Fernandina also occur along the Isabela coast given the close proximity of these islands. Specimens of *E. bobwisneri* were collected at three sites on the western coast of Fernandina, all within a range of 20 km. All other species are known from only one location: *R. lakeside* and *M. martinii* from Cabo Douglas, north-western Fernandina; and *M. greggi* and *M. phantasma* from off north-eastern Santa Cruz Island.

Our data for *M. greggi* and *M. phantasma* extend the maximum depth for collected Galapagos hagfish specimens to 859 m, from a previous maximum of 762 m for *R. lakeside* (Mincarone & McCosker, 2004). *Myxine greggi* and *M. phantasma* seem to be ecologically associated because they were always trapped and filmed together near Gordon Rocks (off north-eastern Santa Cruz) during both expeditions (May–June 2019 and February 2020). McMillan (1999) reports an observation from a submersible of a hagfish at a depth of 884 m near San Salvador (Santiago) Island during the expedition in 1995, although this specimen was not captured. In the same expedition, *E. mccoskeri* was captured at 201 m, and our collections extend the range for this species up to 155 m, making it by far the shallowest hagfish known in the Galapagos. While it is tempting to speculate about whether depth is an important component of the niches, collection efforts so far have not been standardized to make any solid conclusions on this front. In the eastern North Pacific, *Eptatretus stoutii* and its closest living relative, *E. deani*, occur over similar geographical ranges, but at different depths, with *E. deani* found at greater depths (107–2743 m) than *E. stoutii* (16–966 m) (Wisner & McMillan, 1990). A similar situation has been observed for two sympatric hagfishes from South Africa, where *E. hexatrema* is reported from the surface to at least 400 m, while *E. profundus* occurs from 490 to 1150 m depth (Fernholm & Vinding, 2012; Mincarone, 2017). Of the four species collected near Cabo Douglas along the north-west coast of Fernandina, the species at the two depth extremes, *E. goslinei* (467–478 m) and *R. lakeside* (762 m), are unlikely to overlap in their depth ranges. In contrast, the two species in the middle of the depth range, *M. martinii* and *E. bobwisneri*, clearly overlap in their depth ranges, because some of them were captured together in the same trap. Similarly, the two species collected near Gordon Rocks, off north-western

Santa Cruz, *M. greggi* and *M. phantasma*, were always captured together, both on video (Fig. 6E) and in the traps. In our expedition, the deepest traps we set were at ~1000 m, and we were unable to sample deeper than this due to equipment limitations. Given that depths of ~4000 m occur close to several islands of the Galapagos, and that hagfishes have been collected at depths as great as 2743 m (Wisner & McMillan, 1990) and observed at even greater depths (Martini, 1998), it is likely that more species of deep-water hagfishes remain to be discovered there.

The large number of Galapagos hagfish species (about 9% of described world diversity) raises the question of how and why this area is a diversity hotspot for this group. One possibility is that hagfish diversity correlates with a diversity of suitable benthic habitats in the Galapagos, which include rocky volcanic bottoms, deep coral reefs, scattered areas with soft sediment, and hydrothermal vents (Møller & Jones, 2007; Fernholm & Quattrini, 2008). With hagfishes adapted mainly to a scavenging lifestyle, perhaps the remarkably productive Galapagos Islands provide abundant and diverse resources that helped drive hagfish diversification there. Analysis of stomach content data will be required to test this hypothesis.

The high diversity of hagfishes of the Galapagos also raises the intriguing possibility that other chains of islands and/or seamounts might harbour similar amounts of diversity in this clade. If this is the case, then worldwide hagfish diversity may be far greater than current estimates. Currently, 87 valid species of hagfishes, including those described herein, are known, but undoubtedly a great number of undescribed species remain to be discovered. In addition, several species are known only from the holotype or a few specimens deposited in fish collections and basic aspects of their biology and ecology remain largely unknown. In this context, local deep-sea surveys have contributed to the understanding of the diversity and distribution of rare or poorly documented species.

The phylogenetic analysis (Fig. 5) suggests that hagfishes colonized the Galapagos from at least five different sources: four colonizations indicated by the current data and one colonization represented by *Rubicundus lakeside*. Although we do not have molecular data for *R. lakeside*, it is likely that this species arose from a species that shares a recent common ancestor with one of the three other *Rubicundus* species (*R. eos*, *R. lopheliae* and *R. rubicundus*). Although *M. greggi* and *M. phantasma* appeared together in traps and on video, molecular data suggest that they are not sister-taxa. Based on its position in the phylogeny, *M. phantasma* likely arose from a Pan-Pacific ancestor that also gave rise to *M. circifrons* and *M. formosana*. The closest relative of *Myxine greggi* is *M. martinii*, which suggests that these two species arose from a single colonizing species of *Myxine*, and judging from their position in the tree, that ancestor likely originated in the South Atlantic. This sister-group relationship of *M. greggi* and *M. martinii* is also supported by morphological evidence; both species are very similar in their measurements, counts and pigmentation pattern. Similarly, given the close similarity of their 16S and *COI* genes, *E. mccoskeri* and *E. goslinei* are likely still diverging in the Galapagos. The position of these two species in the tree suggests that they may be descended from a common ancestor that originated in the Caribbean and moved into the Pacific, possibly before the formation of the Panama land-bridge. While the land bridge is believed to have formed well before extant Galapagos Islands were formed (Hoorn & Flantua, 2015), it was almost certainly not there when the hotspot that formed the Galapagos Islands appeared, about 20 Mya. Although the molecular markers 16S and *COI* are unable to distinguish *E. mccoskeri* and *E. goslinei* as separate species, the morphology supports this distinction, with the most significant difference being the number of gill apertures and gill pouches (seven in *E. goslinei*, eight in *E. mccoskeri*). Based on its position in the tree, *E. bobwisneri* likely arose from an eptatretid ancestor of Pacific origin.

While our phylogeny (Fig. 5) represents the most current estimation of hagfish phylogeny, the present data, based on only two markers, are inadequate to resolve the deeper nodes in the hagfish tree. Phylogenomic analyses of *Eptatretus* and *Myxine* are forthcoming, but a critical need still exists for additional sampling of *Rubicudus* and *Neomyxine* Richardson & Jowett, 1951. Further, most hagfish collection efforts have focused on coastal or near coastal waters. Mid-basin sampling has been only rarely conducted (Møller & Jones, 2007), but is required if we are to understand the true dimensions of hagfish biodiversity.

The high rate of endemism among Galapagos hagfishes is remarkable when compared to the rate for all Galapagos fishes, which is only about 13% (79 endemics out of a total of 592 species) (Tirado-Sanchez *et al.*, 2016). One possible explanation might be related to the fact that most marine fishes have a planktonic larval stage and hagfishes do not. Small, planktonic larvae allow many marine species to disperse numerous offspring over vast distances, whereas the hagfishes, with their large, yolky eggs and direct-developing embryos, are far more limited when it comes to dispersal. Because hagfishes typically remain closely associated with the bottom, deep water between islands could pose barriers to gene flow that do not exist for species that produce planktonic larvae that can easily drift from one island to the next. Our results and analysis suggest that future expeditions to the Galapagos, especially near islands that have

yet to be explored and in waters deeper than 1000 m, are likely to reveal even more new species and provide further insights into the ecology and evolution of the hagfishes.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Appendix S1. List of comparative materials.
Appendix S2. Molecular data and GenBank accessions used in phylogenetic analyses.