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A new species of *Ptychochromis* from southeastern Madagascar (Teleostei: Cichlidae)

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Abstract

We describe a new species in the endemic Malagasy cichlid genus *Ptychochromis*. *Ptychochromis mainty*, new species, is known from four individuals, all collected in the Fort Dauphin region of southeastern Madagascar, and shares a palatine morphology (eastern-type palatine) with other eastern congeners. *Ptychochromis mainty* is distinguished from all congeners by a nearly uniform dark brown to black pigmentation pattern in preservation and by the presence of a relatively continuous and expansive black longitudinal midlateral blotch in life, extending from the posterior margin of the opercle to the caudal peduncle. The new species is further distinguished from other eastern *Ptychochromis* species by having minimal or no overlap of the first supraneural with the dorsoposterior region of the supraoccipital crest (vs. marked overlap). We present a molecular-based phylogeny for all available *Ptychochromis* species, which supports the hypothesis that *P. mainty* is a distinct taxon. The new species is recovered as the sister taxon to *P. grandidieri* within a clade comprising species with an eastern-type palatine morphology. We present a geometric morphometric analysis that provides additional evidence to distinguish *P. mainty* from congeners.

Key words: Cichlidae, Madagascar, *mainty*, new species, phylogeny, Ptychochrominae, *Ptychochromis*

Introduction

Ptychochromis is endemic to Madagascar and members of the genus are distributed throughout eastern, far northern and northwestern Madagascar. Species of *Ptychochromis* occur in both fresh and brackish-water habitats, and are generally restricted to lowland rivers, streams, and shallow floodplain lakes. *Ptychochromis* currently comprises nine valid species (Stiassny & Sparks, 2006; Sparks & Stiassny, 2010), and all but one species (*P. grandidieri*) exhibit quite restricted geographic ranges (Fig. 1). Members of the genus historically occurred in southwestern Madagascar (viz., *P. onilahy* Stiassny & Sparks, 2006), however, based on a number of unsuccessful recent surveys to collect *P. onilahy* in the region, those populations are now considered likely to be extinct. Herein we describe a new species of *Ptychochromis* from extreme southeastern Madagascar, use geometric morphometrics to compare variation in overall body shape among *Ptychochromis* species with eastern-type palatines [the "eastern group" of Stiassny and Sparks (2006)], and report on the phylogenetic placement of the new species within the genus.

Material and methods

A morphological assessment of *P. mainty* included meristic counts and linear body measurements, following Sparks (2002, 2003) and Stiassny and Sparks (2006), and these data are presented in Table 1. Further observations were made from a cleared and stained (C&S) suspensorium, suborbital series and branchial basket isolated from a *P. mainty* specimen (AMNH 241979, paratype), following a Ridewood dissection (Bemis *et al*., 2004). Specimens used for comparative analyses were cleared and stained using a modified protocol based on Taylor and Van Dyke

(1985). Additionally, both digital and standard radiographs were used for osteological comparisons. Institutional abbreviations are as listed in Leviton *et al.* (1985) and Sabaj Pérez (2014).

We used geometric morphometrics to compare variation in overall body shape to reveal potential differences between *Ptychochromis mainty* and other *Ptychochromis* species with eastern-type palatines. Geometric morphometrics is a landmark-based method for comparing shapes among specimens (Zelditch *et al*. 2004). We assessed body shape with a set of 15 fixed landmarks and 43 sliding semilandmarks, which define points along an outline (see Gunz & Mitteroecker, 2013). Landmarks were digitized using version 2.17 of the program *tpsDig2* (Rohlf, 2013). Shapes were aligned and visualized as deformation grids, computed with thin-plate spline transformations in the *geomorph* package (Adams & Otarola-Castillo, 2013) in R (R Core Team, 2013). To provide an appropriate comparison, reducing impacts of allometry, we included only large juveniles and adult individuals, which generally matched the size range available for the new species. The shapes of *P. mainty* specimens were statistically compared to that of *P. ernestmagnusi* (the most superficially similar species in overall body shape) and also to *P. grandidieri* (the closest relative). Shape comparisons were made with a Procrustes ANOVA, implemented in *geomorph* with significance based on a 10,000-replicate permutation test.

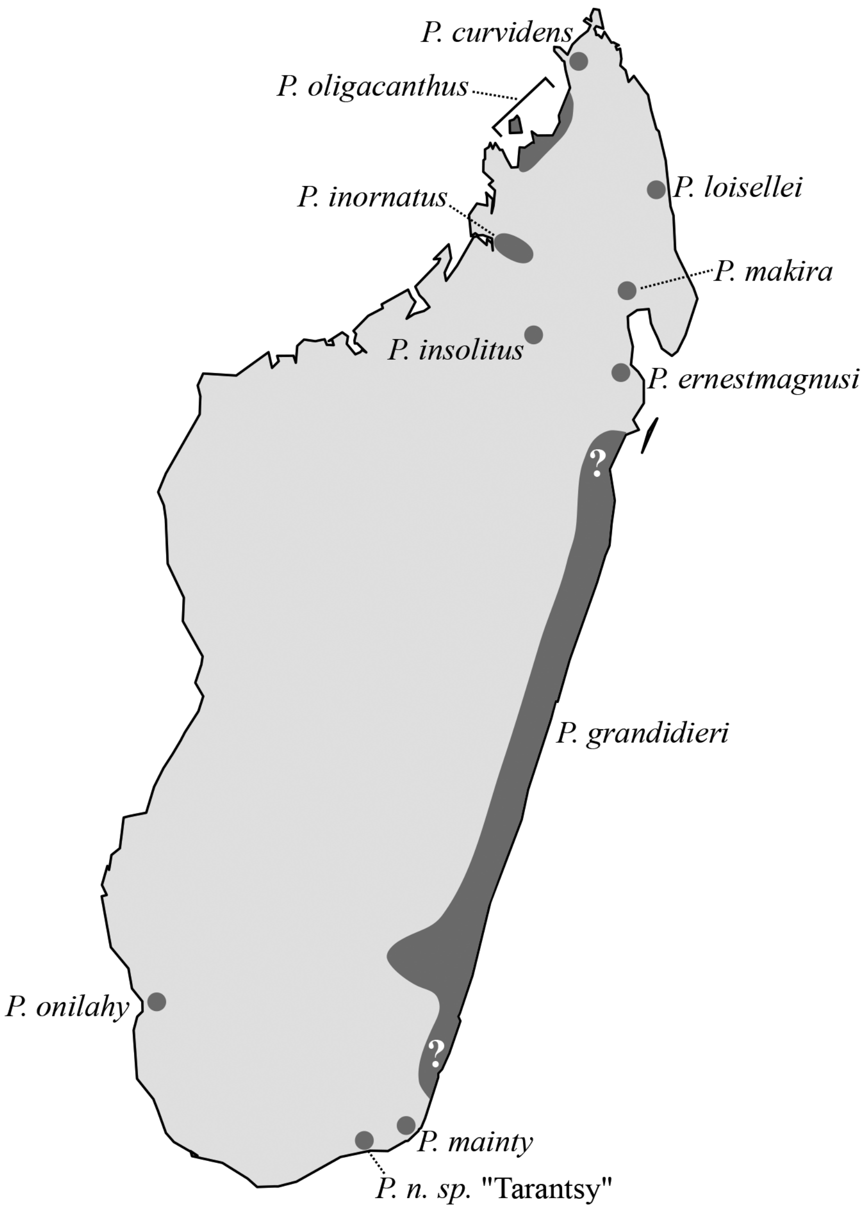

FIGURE 1. Map of Madagascar, showing known geographic ranges of *Ptychochromis* species.

Representatives from seven of 10 currently recognized species of *Ptychochromis*, including the new species described here, were sampled for phylogenetic analyses. *Katria katria* was included as the outgroup based on the findings of Sparks (2004) and Sparks and Smith (2004a), which resolved this species as the sister taxon to a monophyletic *Ptychochromis*, a conclusion further supported by Stiassny and Sparks (2006) and McMahan *et al.* (2014). Four genes/gene fragments, including nuclear and mitochondrial DNA (16S, COI, H3, Tmo-4C4) previously used by Sparks and Smith (2004a) to investigate the phylogeny and biogeography of cichlid fishes, comprise the molecular markers used in this study to infer the phylogenetic placement of the newly described species within *Ptychochromis*. Sequencing of the new species follows the general procedures for DNA extraction, amplification, and purification, along with primers and thermal profiles detailed in Sparks and Smith (2004a). The remaining comparative DNA sequence data were generated by previous authors (Sparks, 2004; Sparks & Smith, 2004a; Stiassny & Sparks, 2006) and were readily available from Genbank. Contig assembly and sequence editing of newly generated sequences was performed using Geneious Pro version 7.1.5 (Biomatters, available from http://www.geneious.com/). IUPAC nucleotide ambiguity codes were used to represent heterozygous sites. The resulting sequences were trimmed to exclude primer regions and examined for appropriateness using BLASTx (Altschul *et al.*, 1990). Each gene was aligned using MUSCLE (Edgar, 2004) under default parameters as implemented in Geneious, followed by concatenation of individual alignments. All sequences were checked for stop codons and for miscalled amino acids by examining translation alignments. The concatenated alignment was analyzed using RAxML v.8 (Stamatakis, 2014) through the CIPRES Science Gateway v.3.3 (Miller *et al.*, 2010) under the GTRGAMMA model using full ML optimization for the tree search and 1000 rapid bootstrap searches to assess nodal support (Stamatakis *et al.*, 2008). Voucher catalog/tissue numbers and GenBank accession numbers for gene sequences generated and included in this study are listed in Table 2.

Systematic account

Ptychochromis mainty, new species

Figures 1–10; Tables 1–2

Holotype. AMNH 238825, 140.4 mm SL, Fort Dauphin region, Taolagnaro, Madagascar, coll. P.V. Loiselle, 13 September 2006.

Paratypes. AMNH 265944, 1 ex., 148.8 mm SL, data as for holotype. AMNH 241978, 1 ex., 101.3 mm SL, Fort Dauphin region, Taolagnaro, Madagascar, coll. A. Saunders *et al*., 2007. AMNH 241979, 1 ex., partial C&S, 100.5 mm SL, Fort Dauphin region, Taolagnaro, Madagascar, coll. A. Saunders *et al*., 2007.

Diagnosis. A *Ptychochromis* with palatine morphology typical of species on Madagascar's east coast (i.e., "eastern-type palatine" of Stiassny & Sparks, 2006). Distinguished from all congeners by uniform dark brownish to black coloration in preservation and in life by expansive, continuous (or nearly so), longitudinal black blotch on midlateral flank extending from posterior margin of opercle to caudal peduncle (versus absent in *P. inornatus*, *P. onilahy*, *P. curvidens*, *P. loisellei*, *P. insolitus*, or comprising multiple distinct midlateral blotches or bars in *P. ernestmagnusi*, *P. makira*, *P. grandidieri*, and *P. oligacanthus*). *Ptychochromis mainty* displays little or no overlap of anterior supraneural relative to supraoccipital crest, versus marked overlap in some eastern congeners (*P. makira* and *P. ernestmagnusi*). In comparison to *P. grandidieri*, body depth as a percentage of SL in *P. mainty* considerably less for similarly sized individuals and slope of head in profile less steep. New species further distinguished from *P. grandidieri* by fewer and more sparsely arranged teeth on second pharyngobranchial toothplate (Pb2) of upper pharyngeal jaw (vs. numerous, densely arranged teeth).

Description. Morphometric and meristic data presented in Table 1. Shallow bodied, with elongated caudal peduncle (Fig. 2). Body overall convex in shape, most notably in dorsal profile. Ventral margin between pelvic fin and vent nearly flat to slightly convex. Prominent point along ventral margin at first anal spine due to moderate ventral sloping between vent and first anal spine and strong dorsal sloping along anal fin toward the caudal peduncle. Profile of head flat and rising at roughly 40 degrees from midline. Slight hump visible from supraoccipital crest in larger specimens.

Vertebral count 27 in all examined specimens, with 14 pre-caudal and 13 caudal vertebrae.

Oral jaws isognathous with dentition present across most of premaxillary arcade and covering about 2/3 of dentary. Dentition distally expanded and bicuspid anteriorly. Posterior teeth weakly bicuspid or flat, and rarely unicusped with pointed distal end. Outer row of teeth larger than inner rows. Three inner rows of teeth on anteriormost portion of dentary, reducing to single row of outer teeth posteriorly. Up to four inner rows of teeth on anterior portion of premaxilla, reducing to single row of outer teeth posteriorly.

TABLE 1. Morphological and meristic data for *Ptychochromis mainty*, new species. Linear measurements (mm) are provided as percent of standard length (SL) or percent of head length (HL), unless noted differently. Values in parentheses indicate number of specimens examined. Holotype counts denoted by the letter H.

		P. mainty			
Character	N	Holotype	Range	Mean	SD
Standard length (mm)	4	140.4	100.5−148.8	122.7	
Percentage of SL					
Head length	4	33.7	33.7−38.0	35.7	1.84
Body depth	4	41.4	41.4−45.5	43.3	1.78
Predorsal length	4	39.7	39.7−44.6	42.3	2.02
Preanal length	4	73.4	73.4−75.8	74.5	0.98
Prepelvic length	4	41.9	41.9−44.6	43.4	1.22
Head width (max.)	4	18.0	17.0−18.4	17.7	0.64
Caudal peduncle length	4	16.6	14.9−16.6	15.7	0.69
Caudal peduncle width	4	3.9	3.8−6.4	4.8	1.23
Caudal peduncle depth	4	16.1	15.8−16.5	16.0	0.35
Pectoral-fin length	4	28.8	28.8−32.0	30.2	1.68
Pelvic-fin length	4	31.0	28.5−40.1	34.3	5.45
Percentage of HL					
Snout length	4	44.8	39.8−44.8	42.9	2.24
Orbit diameter	4	33.4	31.1−34.8	33.0	1.54
Upper-jaw length	4	37.1	34.7−38.3	36.4	1.62
Lower-jaw length	4	42.0	38.5−42.0	40.8	1.58
Interorbital width	4	29.5	27.7−31.9	29.5	1.74
Preorbital depth	4	27.8	24.1−27.8	26.5	1.70
Caudal peduncle length/depth	4	1.0	0.9−1.0	1.0	0.04
Caudal peduncle length/width	4	4.2	2.5−4.2	3.4	0.80
Scales in lateral line	4	28 (1), 30 (2,H), 31 (1)			
Scales: lateral line to dorsal fin	4	5 (3,H), 6 (1)			
Scales: pectoral to pelvic bases	4	4			
Gill rakers (lower limb 1st arch)	4	10			
Vertebrae (pre-caudal + caudal)	4	14+13 = 27			
Dorsal fin	4	XIII 11 (1), XIII 12 (2,H), XIII 13 (1)			
Anal fin	4	III 8 (3,H), III 9 (1)			

FIGURE 2. *Ptychochromis mainty*, AMNH 238825, holotype, 140.4 mm SL (scale bar = 1cm). Photo taken on right side due to specimen bending.

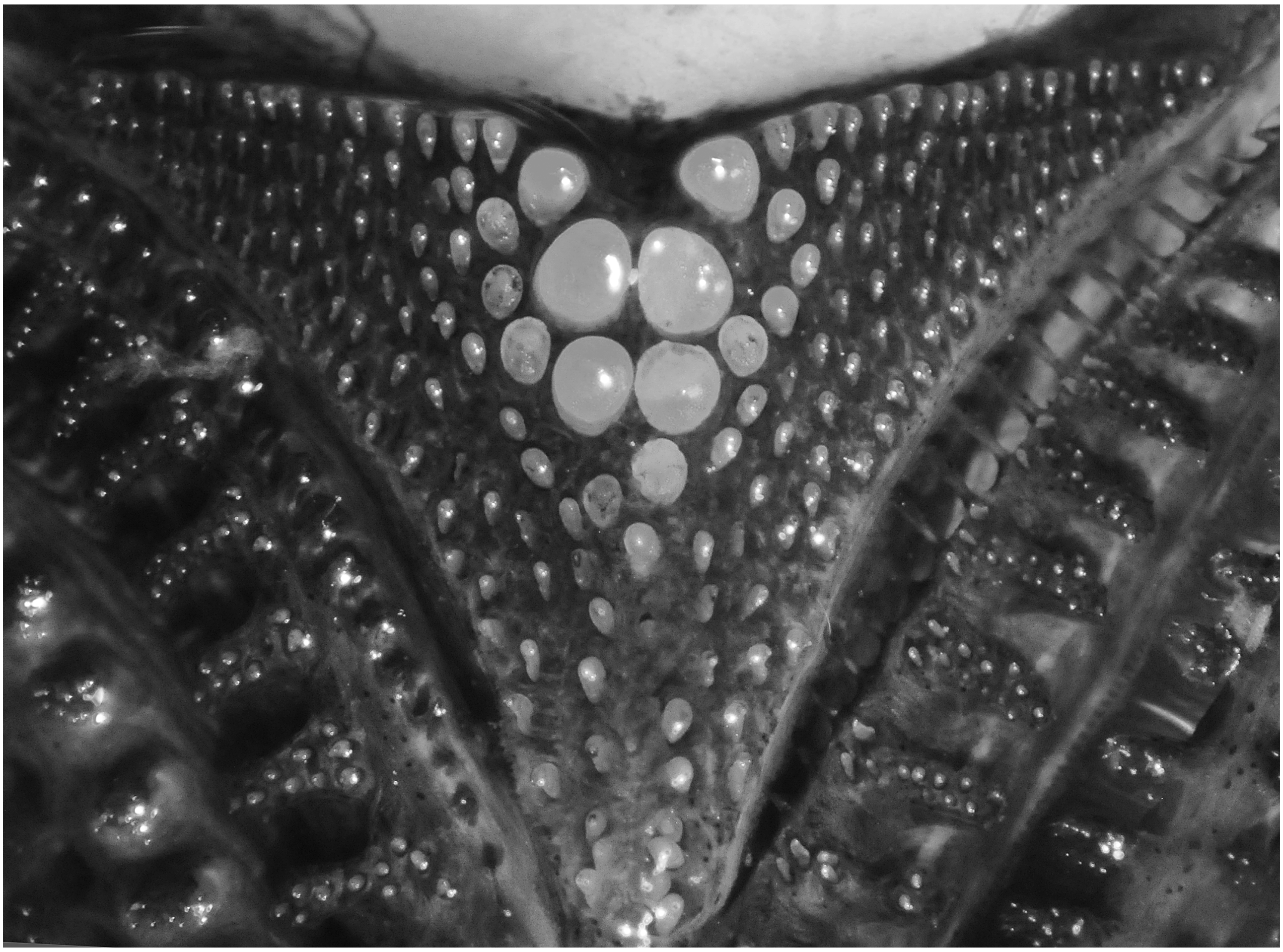

FIGURE 3. Lower pharyngeal jaw (LPJ) in dorsal view, representing fused 5th ceratobranchials in *Ptychochromis mainty*. Tooth-bearing rakers on 4th gill arch are also visible (lateral to LPJ). AMNH 241979, paratype, 100.5 mm SL.

Robust lower pharyngeal jaw (LPJ), consisting of medially fused 5th ceratobranchials, with interdigitating suture uniting both halves. Posteromedially, both LPJ and upper pharyngeal jaw (UPJ) with enlarged molariform dentition (Fig. 3). Remaining dentition on LPJ and UPJ comparatively small and biscuspid, with well-developed posterior cusp and poorly developed anterior cusp. Second pharyngobranchial toothplate (Pb2) with three to four rows of elongate, sparsely arranged hooked and biscupid teeth (Fig. 4). Presence of free second epibranchial toothplate. Teeth on second epibranchial toothplate bicuspid, with one cusp strongly hooked and other cusp very poorly developed. Non-molariform teeth on third pharyngobranchial toothplate, decreasing in size laterally, but increasing in intensity of hooked appearance. Fourth upper toothplate with sparsely arranged hooked and bicuspid teeth that decrease in size and development toward plate's outer margin.

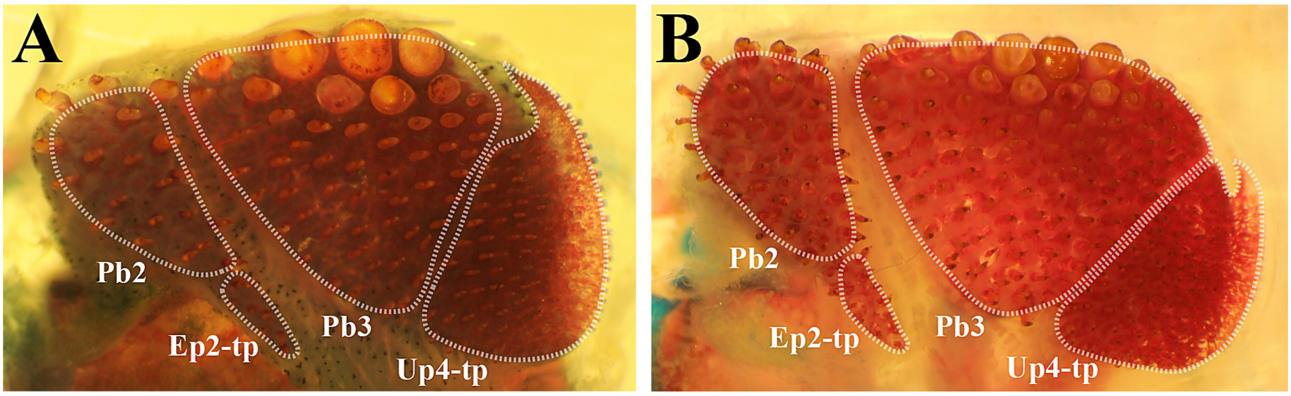

FIGURE 4. Ventral view of upper pharyngeal jaw (UPJ) in (**A**) *Ptychochromis mainty* (AMNH 241979, paratype) and (**B**) *Ptychochromis grandidieri* (AMNH 88018). Jaw elements pictured are the pharyngobranchial toothplates (Pb2 & Pb3), the second epibranchial toothplate (Ep2-tp) and the fourth upper toothplate (Up4-tp).

Gill rakers number 10 on lower limb of first gill arch, not including raker in angle of arch. Tooth-bearing rakers present on lower limb of first gill arch. Raker dentition conical with occasional weak curvature. Seven to eight elongate epibranchial gill rakers. Rakers increasingly robust and with more numerous teeth from second gill arch to fourth.

Flank squamation comprised of large, regularly imbricate, weakly ctenoid scales. Anteriormost occurrence of ctenii near first dorsal spine and just posterior to pectoral fin base, and continuing posteriorly to origin of caudal fin. Chest scales in larger specimens reduced in size and weakly embedded. Embedded cycloid scales present on nape and head. Cycloid scales present on opercle. Preopercle asquamate. Cheek scales cycloid, comprising up to four rows. Anterior half of interorbital area, snout, anterior cheek and lachrymal asquamate. Scales on caudal fin ctenoid at base, and become increasingly smaller, cycloid and embedded posteriorly. Lateral line scales with well-developed pores and numbering 28, 30 (mode) and 31. Four scales between pectoral and pelvic fin bases. Five (mode) or six scales diagonally between dorsal-fin origin and upper lateral line. No scales extending onto dorsal- or anal-fin membranes.

Dorsal fin with XIII spines and 11 to 13 soft rays. Anal fin with III spines and eight or nine soft rays. First anal spine much shorter in length than the second and third. Soft rays of dorsal and anal fins elongate, producing tapered appearance in both. Anal-fin rays extend approximately to origin of caudal fin, although in one smaller specimen (AMNH 241978) first three soft rays extend well posterior of caudal base. Pectoral fin elongate, with rays increasing in length dorsally and extending posteriorly to anterior origin of lower lateral line. Pelvic fin long and extending approximately to first anal-fin spine when adpressed, but more elongate in AMNH 242978, extending to middle of anal fin. Caudal fin emarginate, with somewhat rounded and equally sized upper and lower lobes.

Miscellaneous osteology and anatomy. Infraorbital series comprising seven elements. Lachrymal with four pores; fourth pore directly adjacent to anterior pore of second infraorbital. Second infraorbital expanded. Second infraorbital excluded from margin of orbit by third infraorbital. First supraneural flattened dorsally and with little or no overlap with posterior portion of supraoccipital crest. Second supraneural smaller than first or absent (Fig. 5C), and more varied in shape, with forms either having anterior extension with flattened dorsal surface (Fig 5D) or no anterior projection. Less developed or absent second supraneurals present in larger specimens (AMNH 238825, 140.4 mm SL and AMNH 265944, 148.8 mm SL). Laterosenory canals on dentary and preopercle well developed with large pores.

TABLE 2. GenBank accession numbers and voucher numbers for specimens used in molecular phylogenetic analysis.

Taxon	Voucher Specimen/Tissue #	GenBank Accession #			
		16S	COI	H3	Tmo-4C4
Katria katria	UMMZ PNR MLJS 1991	AY263814	AY263880	AY662915	AY662840
Ptychochromis grandidieri	UMMZ JSS 94-26	AY263811	AY263878	AY662916	AY662841
Ptychochromis inornatus	UMMZ JSS 94-19	AY263812	AY263875	AY662917	AY662842
Ptychochromis insolitus	UMMZ 237066	AY662725	AY662777	AY662922	AY662847
Ptychochromis loisellei	AMNH uncat.	AY662723	AY662776	AY662920	AY662845
Ptychochromis makira	AMNH 237132	AY662724	AY662775	AY662921	AY662846
Ptychochromis oligacanthus "Nosy Be"	UMMZ JSS 96-20	AY263813	AY263873	AY662918	AY662843
Ptychochromis oligacanthus "northwest"	UMMZ uncat.	AY662722	AY662774	AY662919	AY662844
Ptychochromis mainty	AMNH 241978	KT981945	KT981941	KT981943	KT981947
Ptychochromis mainty	AMNH 241979	KT981946	KT981942	KT981944	KT981948

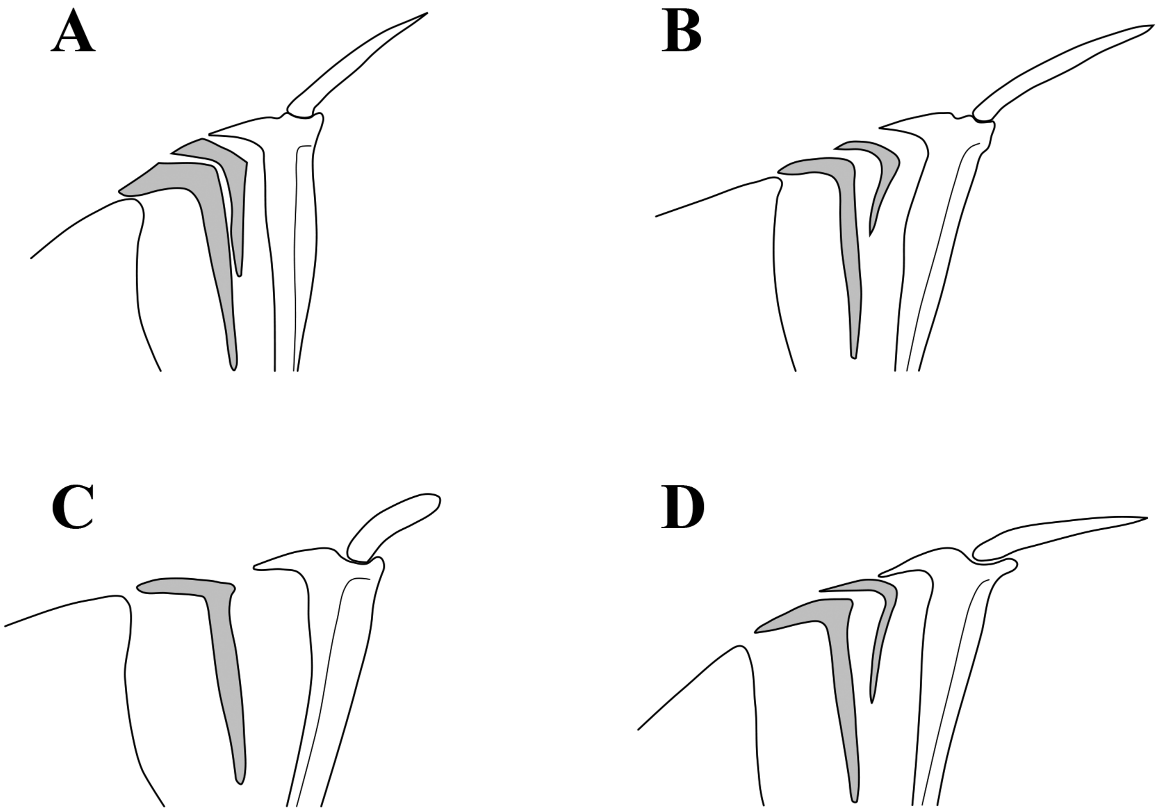

FIGURE 5. Variation in supraneural morphology for (**A**) *Ptychochromis ernestmagnusi* (AMNH 249489, paratype), (**B**) *Ptychochromis grandidieri* (UMMZ 237312), (**C**) *Ptychochromis mainty* (AMNH 238825, holotype) and (**D**) *Ptychochromis mainty* (AMNH 241979, paratype).

Coloration in life (Fig. 6). No images available from type series illustrating live coloration. Photos of live *P. mainty*, collected from Fort Dauphin region of southeastern Madagascar, provided by A. Saunders. Ground coloration light to dark olive green. One expansive longitudinal black blotch present on midlateral flank, and sometimes one or two smaller blotches also on caudal peduncle. Primary blotch starting just posterior to opercle and continuous to caudal peduncle, but sometimes with break approximately at a vertical through anterior insertion of anal fin. This pattern contrasts with that of conspecifics, including *P. ernestmagnusi*, *P. makira*, *P. grandidieri* and *P. oligacanthus*, where several distinct midlateral blotches or bars are present. In individuals with darker ground coloration, ventral half of body has non-uniform yellow to greenish-yellow coloration. Base color of fins same as body. Yellow coloration present on margin of dorsal fin, associated with distal portion of spines and sometimes also present on tips of soft rays. Small patches of yellow coloration also present near distal ends of dorsal and ventral caudal fin rays. Head color uniform and same as base body coloration. Additional images of specimens from Lake Taransty (*Ptychochromis* n. sp. "Tarantsy") and of an unidentified *Ptychochromis* from west of Fort Dauphin region show fishes with expansive, but less continuous midlateral blotches (de Rham and Nourissat 2004, pp. 47–49), which could potentially represent color variants of *P. mainty*.

FIGURE 6. *Ptychochromis mainty*. Live specimen collected from the Fort Dauphin region of Madagascar and photographed by Alex Saunders.

Coloration in preservation (Fig. 2). Base color of body dark brown, with ventral half just slightly lighter than dorsal. In smaller individuals (AMNH 241978, 101.3 mm SL and AMNH 241979, 100.5 mm SL), ventral half of body pale brown and markedly lighter than dorsal half. Darkest pigment (i.e., black) occurs midlaterally and is most prominent directly posterior of opercle, although it does not contrast greatly with ground coloration of body. This darkened area corresponds to large, black midlateral blotch present in life. Posterior half of lower jaw cream to light brown in color. Pectoral fin radials dark, but membrane cream colored, giving fin a light overall appearance. Some fading of pelvic fin at medial base, becoming light brown to nearly white in color. Anal fin slightly (holotype) to mostly cream colored near base posteriorly. Vent clearly stands out from ventral margin of body and is white to cream in color.

Distribution and habitat (Fig. 1). Known only from Fort Dauphin region near Taolagnaro in extreme southeastern Madagascar. Another, presumably distinct, population of *Ptychochromis*, referred to as *P.* sp. "Tarantsy", is known from Lake Tarantsy, roughly 45 km southwest of Fort Dauphin, but its relationship to *P. mainty* and other members of the genus is currently unknown (de Rham & Nourissat, 2004).

Diet. Diet is largely unknown, although a radiograph of a *P. mainty* paratype (AMNH 241978) reveals that the stomach contains gastropod shells (Fig. 7).

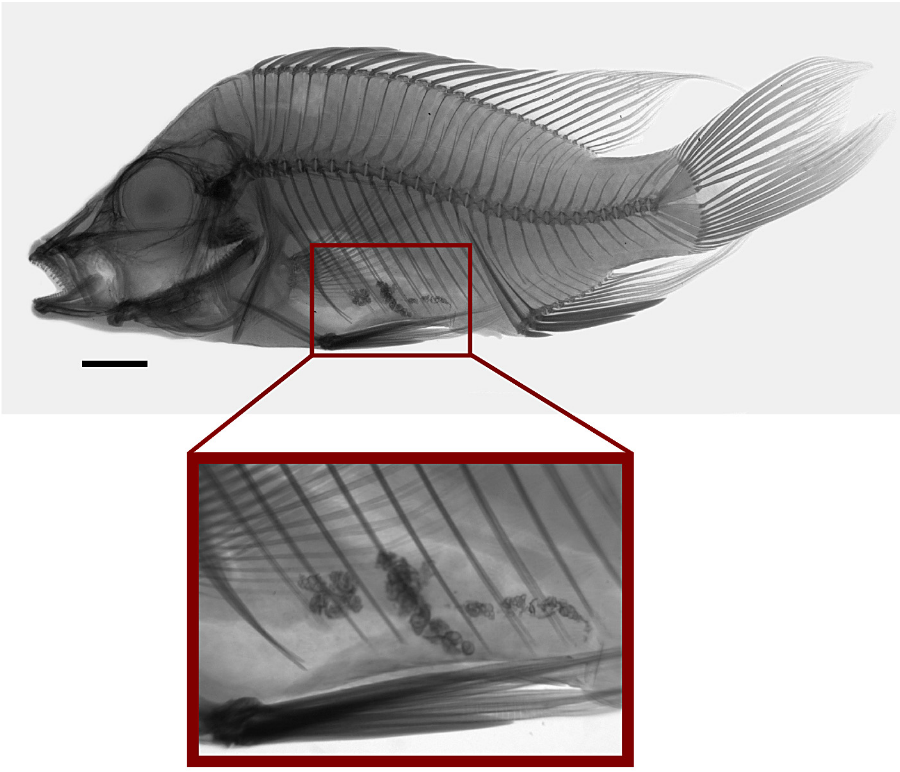

FIGURE 7. Radiograph of *Ptychochromis mainty*, AMNH 241978, paratype, showing gastropod shells in the gut. Scale bar = 1cm.

Conservation Status. Madagascar's freshwater biotas are severely threatened due to widespread habitat destruction, overfishing, and the introduction of numerous exotic species. This situation is exacerbated by high levels of endemicity and greatly restricted ranges (often comprising a single river system or lake) for most of the island's freshwater fishes (Sparks & Stiassny, 2003; Sparks & Smith, 2004b; Sparks, 2008). In fact, over half of Madagascar's native freshwater fish species are categorized as endangered, critically endangered or extinct (Benstead *et al*., 2003; Sparks & Stiassny, 2008; Irwin *et al.*, 2010), and the situation has become far more dire in recent years (JSS, pers. obs.). The region from which the type series of *P. mainty* was collected has been poorly surveyed for freshwater fishes, and the conservation status of the new species remains unknown. We attempted to survey the region from which the type series was collected during an expedition to Madagascar in April of 2014, but were unable to gain access to forested regions in the area with suitable habitat due to permitting issues.

Etymology. Named after the Malagasy word for black, *mainty*, referring to the species' uniform dark pigmentation pattern in preservation and large black midlateral blotch in life.

Discussion and comparisons

The phylogeny presented in Figure 8 is resolved into strongly supported clades, except for the node supporting placement of *Ptychochromis makira* as the sister taxon to the remaining east coast species (viz., *P. grandidieri*, *P. loisellei*, and *P. mainty*, new species). The new species is recovered as a distinct taxon and sister to *P. grandidieri*, a relationship further corroborated by a high bootstrap value (B=100). Biogeographically this makes sense, as the southern limit of the range of *P. grandidieri*, which is widely distributed along the east coast of Madagascar, terminates just north of the range of *P. mainty* (Fig. 1).

Linear measurements of *P. mainty* revealed morphological differences with congeners based on morphometric data reported in Stiassny and Sparks (2006) and Sparks and Stiassny (2010). Head length in *P. mainty* (35.7 ± 1.84 SD) was longer than west coast *Ptychochromis* species *P. curvidens* (31.2 ± 0.62 SD) and *P. insolitus* (33.4, holotype), as well as the eastern species *P. makira* (32.1 ± 0.12 SD). In addition, *P. mainty* (43.3 ± 1.78 SD) was more shallow bodied than *P. curvidens* (49.7 ± 3.07 SD) and *P. makira* (48.5 ± 0.58 SD), but was deeper bodied than *P. insolitus* (48.5, holotype). Among east coast congeners, the upper jaw length in the new species (36.4 ± 1.62 SD) was longer than that of *P. loisellei* (27.6 ± 3.34 SD) and *P. grandidieri* (29.3 ± 3.22 SD). Orbit diameter in *P. mainty* (33.0 ± 1.54 SD) was larger than that of *P. makira* (26.6 ± 1.40 SD) and *P. loisellei* (27.8 ± 1.73 SD). Lateral line scales in *P. mainty* numbered 28–31, versus 34–37 in *P. ernestmagnusi*, 33 in *P. makira* and 33–34 in *P. onilahy*.

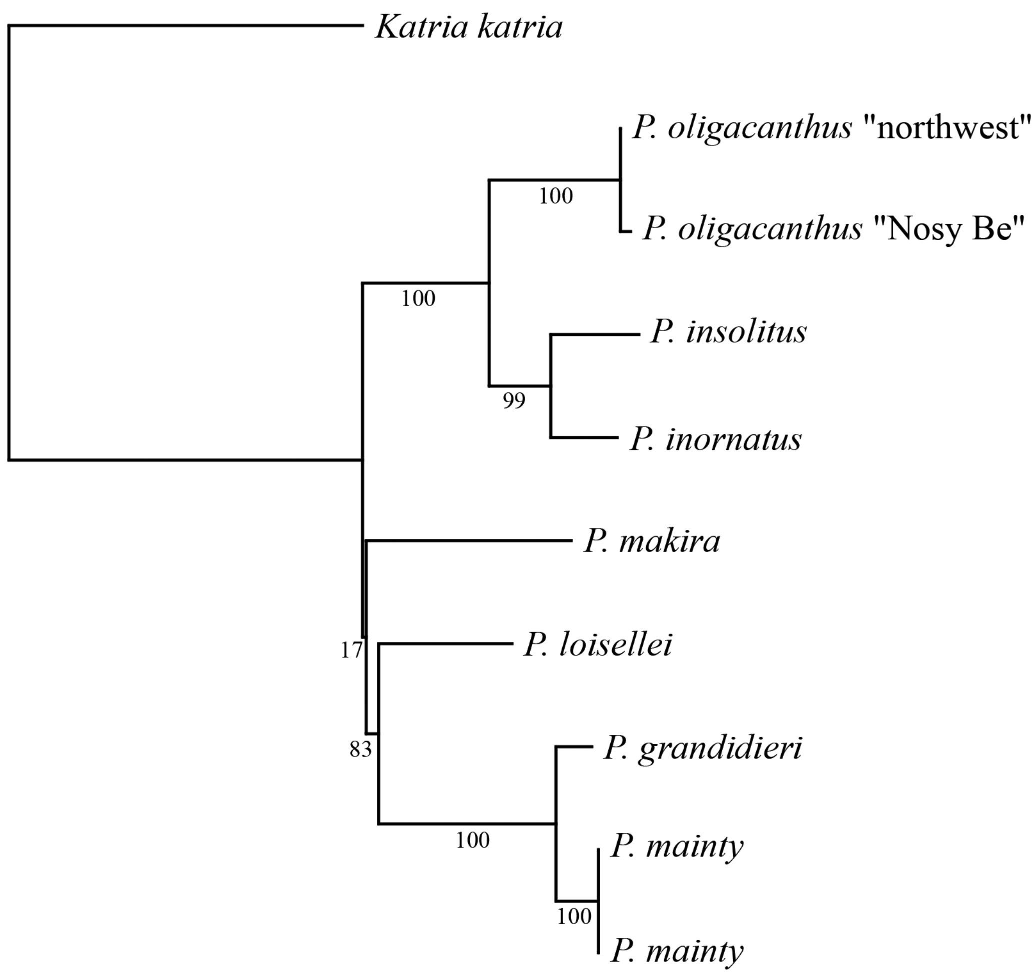

FIGURE 8. Maximum likelihood phylogeny for all available species of *Ptychochromis*, including *P. mainty*, new species. Bootstrap support values are shown at the nodes.

Ranges of linear measurements in the new species often overlapped with those of *P. gradidieri*. This could reflect similar overall morphologies in closely related species, but is also likely due to large variance in *P. grandidieri* measurements arising from potential allometric influences (i.e. no small juveniles of the new species were available for inclusion). A comparison of more similarly sized individuals (i.e., those specimens used for geometric morphometric analyses) revealed that *P. mainty* was among the most shallow bodied species of *Ptychochromis*, and had a body depth/SL that was well below that of *P. grandidieri* (Fig. 9).

Geometric morphometrics of overall body shape revealed the relative position of the new species within the morphospace of eastern Madagascar congeners (Fig. 10). Whereas additional materials may allow for more robust comparisons, *P. mainty* did appear to represent a distinctive morphological entity and it also defined one extreme of the primary axis of shape variation (PC1). Overall, *P. mainty* was more shallow bodied than other *Ptychochromis* species and the lateral flank was anteroposteriorly compressed, compared to increasingly expanded and deep-bodied species like *P. curvidens* (see shapes of warped grids in Fig. 10). In addition, the caudal peduncle in *P. mainty* was longer and the head also displayed a relative expansion that resulted in an elongated and gently sloping head profile. Of the eastern *Ptychochromis* species, *P. ernestmagnusi* was most close in shape to *P. mainty* (Fig.

10), but there were significant shape differences between the two species (n=10, df=1, R^2=0.34, p=0.009). In addition, the shape of *P. mainty* was different from the closely related species *P. grandidieri* (n=16, df=1, R^2=0.40, p<0.0001).

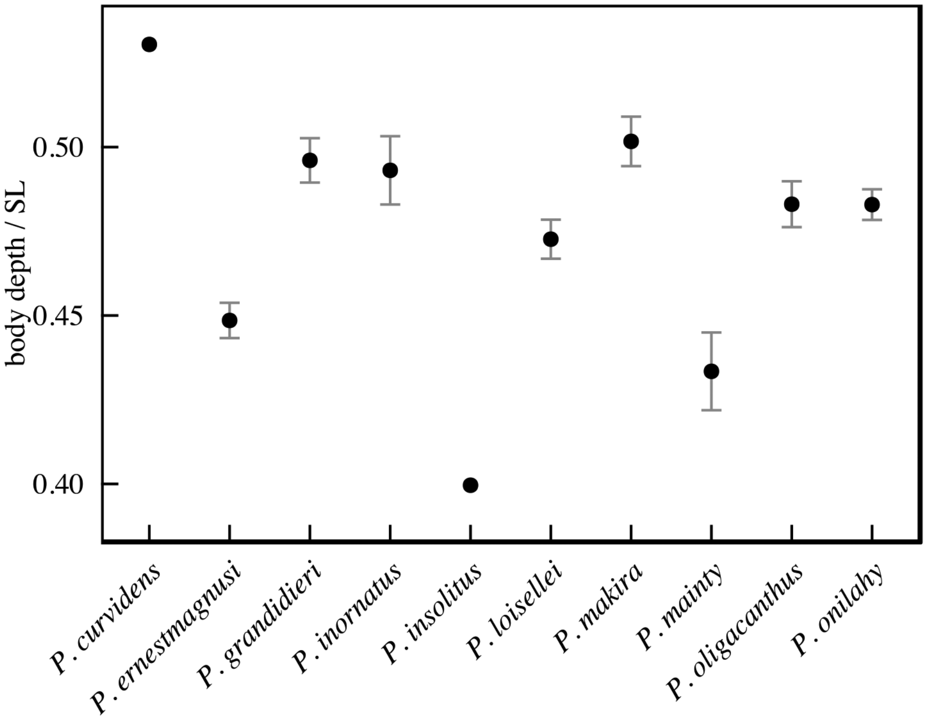

FIGURE 9. Body depth/SL for *Ptychochromis* species, with error bars showing standard error. Individuals included in analysis were of a similar size range to the new species, with no small juveniles.

Comparative materials: Values after catalog numbers represent specimens examined and not necessarily the total number of specimens in each lot.

Ptychochromis curvidens: AMNH 237133, paratype, 1 ex., northern Madagascar, Antsiranana (Diego Suarez) Province, Andranofanjava, Andranofanjava-Sandriapiana River system, PDR-JCN-99-X-9, P. de Rham and J.-C. Nourissat.

Ptychochromis ernestmagnusi: AMNH 249490, holotype, Mananara (du nord) River at Antanambaobe Village (16° 16′01″S, 49° 40′ 01″E), Madagascar, 120 m a.s.l., MG 10-06, coll. P.V. Loiselle. AMNH 249488, paratypes, 5 ex., Mananara (du nord) River at Antanibaolina Village (16° 15′ 01″S, 49 ° 40′ 41″E), Madagascar, 108 m a.s.l., MG 09-06, coll. P.V. Loiselle. AMNH 249489, paratype, 1 ex., data as for holotype.

Ptychochromis grandidieri: MNHN A.4147, holotype, Madagascar, region of high forests, Humblot and Grandidier. See discussion in Sparks (2003) regarding the locality of the holotype. AMNH 228067, 3 ex., southeastern Madagascar, Lopary, Mananizo River, PVL-98-06, P.V. Loiselle and local fishermen. AMNH 228072, 3 ex., southeastern Madagascar, Ampataka village, Sahambavy River, PVL-98-07, P.V. Loiselle. AMNH 235518, 2 ex., eastern Madagascar, Mananjary, estuary of river Mananjary behind marketplace, ca. 1 kilometer from sea, MJS-PNR-88-22 MAD, M.J. Stiassny, P.N. Reinthal, and local fishermen. AMNH 248791, 1 ex., eastern Madagascar, Sakonila River at Tsorsambo village, PVL-MG-06-18, P.V. Loiselle. AMNH 88018, 4 ex., incl. 1 ex. C&S, southeastern Madagascar, Mananjary, estuary of Mananjary River, (21º05′S 48º27′E), MJS-PNR-88-22 MAD, M.J. Stiassny, P.N. Reinthal, and local fishermen. AMNH 88076, 1 ex., eastern Madagascar, Vatomandry, (19° 20′S, 49° 00′E), MJS-PNR-88-41 MAD, M.J. Stiassny and P.N. Reinthal. AMNH 88092, 2 ex., eastern Madagascar, Mahanoro, Pangalanes canal north of Mangoro River, MJS-PNR-88-46 MAD, M.J. Stiassny and P.N. Reinthal. UMMZ 237312, 1 ex. C&S, southeastern Madagascar, Manombo Special Reserve. PNR96-3, P.N. Reinthal

Ptychochromis inornatus: UMMZ 237063, paratypes, 4 ex., northwestern Madagascar, Antalaha Province, northeast of Antsohihy, Ankofia drainage, Anjingo River (14° 50′ 41.0″S, 48° 14′ 38.3E), JSS 94-19, J. S. Sparks, K. J. Riseng, and local Malagasy guides. UMMZ 237065, paratype, 1 ex., northwestern Madagascar, Antalaha Province, northeast of Antsohihy, Ankofia drainage, Lake Andrapongy (14° 41′ 49.3″S, 48° 07′ 54.3″E), JSS 94-21, J. S. Sparks and K. J. Riseng.

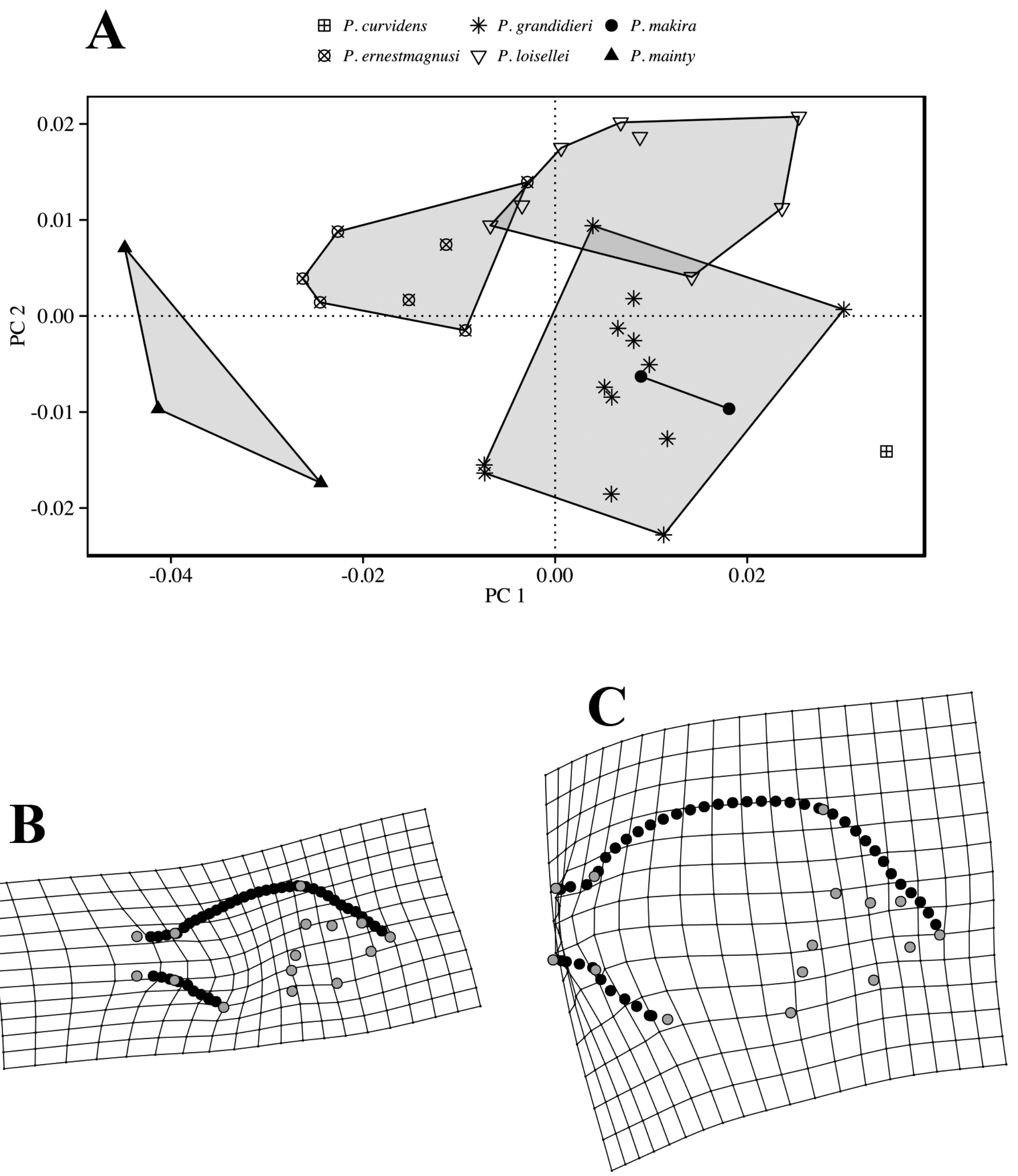

FIGURE 10. (**A**) Principle components (PC) 1 and 2, displaying variation in body shape for east coast *Ptychochromis* species. Visualization of shape variation is provided as warped grids of (**B**) the largest and (**C**) the smallest PC 1 values relative to mean shape. The largest PC 1 values are set by the shallow-bodied *P. mainty* and the smallest by the deep-bodied *P. curvidens*. Fixed landmarks are shown in gray and sliding semilandmarks are in black.

Ptychochromis insolitus: UMMZ 237066, holotype, juvenile; northeastern Madagascar, Antalaha Province, near town of Mandritsara, Sofia drainage basin, Amboaboa (= Ambomboa) River (15° 50′ 1″S; 48° 42′ 51″E), JSS96-25, J. S. Sparks and K. J. Riseng.

Ptychochromis loisellei: AMNH 232462, holotype, male, northeastern Madagascar, Antalaha Province, north of Sambava, Mahanara River at Antsirabe-Nord, just upstream of bridge over route N-5 (13° 58.49′S; 49° 57.81′E), PVL-01-29, P.V. Loiselle and local fishermen. AMNH 231249, paratype, 1 ex., northeastern Madagascar, Antalaha Province, main channel of the Mahanara River at Antsirabe-Nord, at bridge on Route N-5 (13° 38.49′S 49° 57.81′E), PVL-00-07, P.V. Loiselle. AMNH 231258, paratypes, 2 ex., northeastern Madagascar, Antalaha Province, main channel of the Mahanara River at Antsirsabe-Nord, at bridge on Route N-5 (13° 58.49′S 49°

57.81′E), PVL-00-12, P.V. Loiselle. AMNH 232458, paratype, 1 ex., northeastern Madagascar, Antalaha Province, Mahanara River, ca. 4 km northwest of Antsirabe-Nord (13° 57.30′S 49° 56.20′E), PVL-01-27, P.V. Loiselle and local fishermen. AMNH 251904, 3 ex., 251904 Mahanara du Nord River at Antsirabe-Nord, northeastern Madagascar, Antalaha Province, main channel of the Mahanara River at Antsirsabe-Nord, at bridge on Route N-5 (13° 58.51′S 49° 57.18′E), PVL-MG-07-13, P.V. Loiselle.

Ptychochromis makira: AMNH 237131, holotype, northeastern Madagascar, Antalaha Province, north of Maroansetra, near town of Marovonona, Antainambalana River, JSS-WLS-KLT-03-XI-1, purchased from local fishermen by Augustin Sarovy, J. S. Sparks, W. L. Smith, and K. L. Tang. AMNH 237132, paratype, 1 ex. (C&S in part), data as for holotype.

Ptychochromis oligacanthus: AMNH 215522, 4 ex., northwestern Madagascar, Lake Bemapaza, Nosy Be, PVL-94-XI, P.V. Loiselle. AMNH 215523, 1 ex., northwestern Madagascar, Lakes Djabala and Ampombilava, Nosy Be. PVL-94-XI A, P.V. Loiselle. AMNH 230699, 3 ex., northwestern Madagascar, Lake Andjavibe, Nosy Be, PVL-95-X-12, P.V. Loiselle and O. Lucanus. AMNH 232399, 1 ex., northwestern Madagascar, Lake ampombilava, PVL-01-05, P.V. Loiselle from local woman.

Ptychochromis onilahy: MNHN 1962-0201, holotype, southwestern Madagascar, Province of Tulear, Onilahy River, A. Kiener. AMNH 237130, paratype, 1 ex. (C&S in part), data as for holotype. MNHN 2006-0780, paratypes, 3 ex., data as for holotype.

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References

Adams, D.C. & Otarola-Castillo, E. (2013) Geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution*, 4, 393–399.
http://dx.doi.org/10.1111/2041-210X.12035

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) Basic local alignment search tool. *Journal of Molecular Biology*, 215, 403–410.
http://dx.doi.org/10.1016/S0022-2836(05)80360-2

Bemis, W.E., Hilton, E.J., Brown, B., Arrindell, R., Richmond, A.M., Little, C.D., Grande, L., Forey, P.L. & Nelson, G.J. (2004) Methods for preparing dry, partially articulated skeletons of Osteichthyans, with notes on making Ridewood dissections of the cranial skeleton. *Copeia*, 2004, 603–609.
http://dx.doi.org/10.1643/CI-03-054R1

Benstead, J.P., de Rahm, P.H., Gattolliat, J., Gibson, F., Loiselle, P.V., Sartori, M., Sparks, J.S. & Stiassny, M.L.J. (2003) Conserving Madagascar's Freshwater Diversity. *Bioscience*, 53, 1101–1111.
http://dx.doi.org/10.1641/0006-3568(2003)053[1101:CMFB]2.0.CO;2

de Rham, P. & Nourissat, J. (2004) *The endemic cichlids of Madagascar*. French Cichlid Association, St. Julia, 191 pp.

Edgar, R.C. (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics*, 5, 113.
http://dx.doi.org/10.1186/1471-2105-5-113

Gunz, P. & Mitteroecker, P. (2013) Semilandmarks: a method for quantifying curves and surfaces. *Histrix*, 24, 103–109.

Irwin, M.T, Wright, P.C., Birkinshaw C., Fisher, B.L., Gardner, C.J., Glos, J., Goodman, S.M., Loiselle, P., Rabeson, P.,

Raharison, J.-L., Raherilalao M.J., Rakotondravony, D., Raselimanana, A., Ratsimbazafy, J., Sparks, J.S., Wilmé, L., Jörg, U. & Ganzhorn, J.U. (2010) Patterns of species change in anthropogenically disturbed forests of Madagascar. *Biological Conservation*, 143, 2351–2362.
http://dx.doi.org/10.1016/j.biocon.2010.01.023

Leviton, A.E., Gibbs, R.H., Heal, E. & Dawson, C.E. (1985) Standards in herpetology and ichthyology: Part I. Standard symbolic codes for institutional resource collections in herpetology and ichthyology. *Copeia*, 1985, 802–832.

McMahan, C.D., Chakrabarty, P., Sparks, J.S., Smith, W.L. & Davis, M.P. (2013) Temporal patterns of diversification across global cichlid biodiversity (Acanthomorpha: Cichlidae). *PLoS ONE*, 8 (8), e71162.
http://dx.doi.org/10.1371/journal.pone.0071162

Miller, M., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *In*: *Gateway Computing Environments Workshop (GCE) IEEE*, 1–8, 80.
http://dx.doi.org/10.1109/gce.2010.5676129

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from: http://www.R-project.org/ (accessed 3 November 2015)

Rohlf, F.J. (2013) tpsDIG2, v2.17. Freeware. Available from: http://life.bio.sunysb.edu/morph (accessed 3 November 2015)

Sabaj Pérez, M.H. (Ed.) (2014) Standard symbolic codes for institutional resource collections in herpetology and ichthyology: an Online Reference, Version 5.0 (22 September 2014) American Society of Ichthyologists and Herpetologists, Washington, D.C. Electronically accessible. Available from: http://www.asih.org/resources (accessed 3 November 2015)

Sparks, J.S. (2002) *Ptychochromis inornatus*, a new cichlid (Teleostei: Cichlidae) from northwestern Madagascar, with a discussion of intrageneric variation in Ptychochromis. *Copeia*, 2002, 120–130.
http://dx.doi.org/10.1643/0045-8511(2002)002[0120:PIANCT]2.0.CO;2

Sparks, J.S. (2003) Taxonomic status of the Malagasy cichlid *Ptychochromis grandidieri* Sauvage 1882 (Teleostei: Cichlidae), including a molecular phylogenetic analysis of the ptychochromine cichlids. *Ichthyological Exploration of Freshwaters*, 14, 317–328.

Sparks, J.S. (2004) Molecular phylogeny and biogeography of the Malagasy and South Asian cichlids (Teleostei: Perciformes: Cichlidae). *Molecular Phylogenetics and Evolution*, 30, 599–614.
http://dx.doi.org/10.1016/S1055-7903(03)00225-2

Sparks, J.S. (2008) Phylogeny of the cichlid subfamily Etroplinae and taxonomic revision of the Malagasy cichlid genus *Paretroplus* (Teleostei: Cichlidae). *Bulletin of the American Museum of Natural History*, 314, 1–151.
http://dx.doi.org/10.1206/314.1

Sparks, J.S. & Smith, W.L. (2004a) Phylogeny and Biogeography of cichlid fishes (Teleostei: Perciformes: Cichlidae). *Cladistics*, 20, 501–507.
http://dx.doi.org/10.1111/j.1096-0031.2004.00038.x

Sparks, J.S. & Smith, W.L. (2004b) Phylogeny and biogeography of the Malagasy and Australasian rainbowfishes (Teleostei: Melanotaenioidei): Gondwanan vicariance and evolution in freshwater. *Molecular Phylogenetics and Evolution*, 33, 719–734.
http://dx.doi.org/10.1016/j.ympev.2004.07.002

Sparks, J.S. & Stiassny, M.L.J. (2003) Introduction to the freshwater fishes. *In*: Goodman, S.M. & Benstead, J.P. (Eds.), *The natural history of Madagascar*. University of Chicago Press, Chicago, pp. 849–863.

Sparks, J.S. & Stiassny, M.L.J. (2008) Les poissons d'eaux douce. *In*: Goodman, S.M. (Ed.), *Paysages Naturels et Biodiversité de Madagascar*. Publications Scientifiques Muséum national d'Histoire naturelle (MNHN), Paris, pp. 283–309.

Sparks, J.S. & Stiassny, M.L.J. (2010) A new species of Ptychochromis from northeastern Madagascar (Teleostei: Cichlidae), with an updated phylogeny and revised diagnosis for the genus. *Zootaxa*, 2341, 33–51.

Stamatakis, A. (2014) RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. *Bioinformatics*, 30 (9), 1312–1313.
http://dx.doi.org/10.1093/bioinformatics/btu033

Stamatakis, A., Hoover, P., Rougemont, J. (2008) A rapid bootstrap algorithm for the RAxML Web servers. *Systematic Biology*, 57, 758–771.
http://dx.doi.org/10.1080/10635150802429642

Stiassny, M.L.J. & Sparks, J.S. (2006) Phylogeny and taxonomic revision of the endemic Malagasy genus *Ptychochromis* (Teleostei: Cichlidae), with the description of five new species and a diagnosis for *Katria*, new genus. *American Museum Novitates*, 3535, 1–55.
http://dx.doi.org/10.1206/0003-0082(2006)3535[1:PATROT]2.0.CO;2

Taylor, W.R. & Van Dyke, G.C. (1985) Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybium*, 9, 107–119.

Zelditch, M.L., Swiderski, D.L., Sheets, H.D. & Fink, W.L. (2004) *Geometric Morphometrics for Biologists: A Primer*. Elsevier Academic Press, San Diego, CA, 443 pp.