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A giant anguilliform leptocephalus *Thalassenchelys foliaceus* Castle & Raju 1975 is a junior synonym of *Congriscus maldivensis* (Norman 1939)

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A single specimen of giant leptocephalus *Thalassenchelys foliaceus* Castle & Raju 1975 was caught in subtropical waters of the western North Pacific Ocean. Mitochondrial *coI* gene sequence divergence between *T. foliaceus* and *Congriscus maldivensis* (Norman 1939) was 0·64 ± 0·27% (mean ± S.E.), and the myomere and vertebral counts of these species were similar, indicating *T. foliaceus* is a junior synonym of *C. maldivensis*.

Key words: Anguilliformes; *coI* gene; DNA; myomere; synonym; vertebral counts.

Anguilliform leptocephali of the genus *Thalassenchelys* Castle & Raju 1975 are remarkably large and peculiarly shaped eel larvae. Two species were described by Castle and Raju (1975), of which *Thalassenchelys coheni* Castle & Raju 1975 reaches a larger size and has more myomeres than *Thalassenchelys foliaceus* Castle & Raju 1975. The former species is reported to occur in the temperate area of the North Pacific Ocean (Castle & Raju, 1975; Shimokawa *et al.*, 1995; Takahashi *et al.*, 2008; Hanke *et al.*, 2014; Shubin & Koinov, 2014; Kurogi *et al.*, 2016), and the latter is found in tropical to subtropical areas of Indo-west Pacific (Castle & Raju, 1975). Adult forms of these leptocephali were unknown, and the family placement was not clarified for decades. Based on the relatively short intestine and extremely deep body, Castle & Raju (1975) proposed the Chlopsidae as a family candidate for *Thalassenchelys*. Based on phylogenetic analysis using partial mitochondrial DNA sequences, Obermiller & Pfeiler (2003) suggested the leptocephali belong to the family Serrivomeridae, while López *et al.* (2007) have placed the genus in the family Colocongridae. Phylogenetic

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analysis using whole mtDNA sequences (Inoue *et al.*, 2010), however, did not support any of the abovementioned hypotheses. Recently, Kurogi *et al.* (2016) revealed that *T. coheni* is a junior synonym of the bigmouth conger *Congriscus megastomus* (Günther 1877) thereby belonging to the family Congridae, because these two species share nearly identical mitochondrial DNA sequences, and vertebral and myomere counts. Kurogi *et al.* (2016) also suggested that the adult form of *T. foliaceus* could be conspecific with (and a junior synonym of) *Congriscus maldivensis* (Norman, 1939), based on the meristic characteristics and distribution records of the larval and adult forms, but no specimen of *T. foliaceus* was available for genetic analysis. In 2015, during a research cruise by R.V. *Kaiyo-Maru* (Fisheries Agency of Japan) in a subtropical area of the western North Pacific Ocean, a single leptocephalus was caught, with meristic characteristics and body shape similar to *T. foliaceus*. This paper reports the first mtDNA sequence data for *T. foliaceus* and provides molecular and morphological evidence that *T. foliaceus* is a junior synonym of *C. maldivensis*.

Catch information of a leptocephalus and Congridae is shown in Table I. A large leptocephalus (Fig. 1) was caught in the Philippine Sea by Isaacs-Kidd mid-water trawl (IKMT) net of the R.V. *Kaiyo-Maru*, with step-wise towing at 180, 120 and 30 m for 20 min each, during 0100 to 0220 hours on 10 October 2015. This individual was frozen on board and transferred to the laboratory. After collecting a small piece of muscle tissue for DNA analysis, this leptocephalus (SNFR2081) was fixed in 8% neutralized formalin in sea water and deposited in the Seikai National Research Institute, Fish Specimens Collection, National Fisheries Research Agency, Nagasaki, Japan, along with the ethanol preserved tissue sample. Specimens of *C. maldivensis* (Fig. 2) were caught off northern Western Australia using a demersal beam trawl net of the R.V. *Southern Surveyor*, CSIRO, Hobart, Australia. These specimens were frozen and transferred to the CSIRO and Museum Victoria, Melbourne, Australia, fish collections. After photographing and collecting a small piece of muscle tissue from selected specimens for DNA analysis, these eels were fixed in 10% formalin and deposited in the Australian National Fish Collection, CSIRO (CSIRO H 6584-05, H 6592-09, H 6595-01, H 6595-02), and the Museum Victoria fish collection (NMV A 29709-007, A 29730-026, A 29713-018) (Table I).

Amplification of a portion of the *coI* mitochondrial gene of the leptocephalus was undertaken using the PCR conditions as described previously (Kurogi *et al.*, 2016). The *coI* region of nine specimens of *C. maldivensis* was amplified using the Fish-BCL (5′-TCAACYAATCAYAAAGATATYGGCAC-3′) and Fish-BCH (5′-ACTTCYGGGTGRCCRAARAATCA-3′) of Baldwin *et al.* (2009).

Direct nucleotide sequencing was performed using the PCR primers above; nucleotide sequence data (625–664 bp) were deposited in DNA database of Japan (DDBL), European Molecular Biology Laboratory (EMBL) and GenBank under accession numbers of LC102204, GU673079–GU673084, KU674793–KU674795 (Table I). These sequences were compared with previously reported *coI* sequences of 39 *C. megastomus* including 32 leptocephali (formerly *T. coheni*) (Inoue *et al.*, 2010; Chen *et al.*, 2014; Kurogi *et al.*, 2016). Using MEGA 6 (Tamura *et al.*, 2013), the gamma-corrected Kimura's two parameter (K2P) distance was selected as the best-fit model for nucleotide substitution.

The leptocephalus (Fig. 1) has a large and deep body with rounded tail, somewhat cylindrical head, and no pigmentation: total length (L_T) 192·2 mm, head length

Table I. Catch and GenBank accession information of a leptocephalus of *Thalassenchelys foliaceus* caught by R.V. *Kaiyo-Maru* and adult eels of *Congriscus maldivensis* caught by R.V. *Southern Surveyor*

Species	Specimen registration details*	Accession number	L_T (mm)	Latitude	Longitude	Depth (m)	date
T. foliaceus	SNFR2081	LC102204	192·2	16·11′–16·07′ N	132° 00′–131° 59′ E	180–0	10 October 2015
C. maldivensis	CSIRO H 6584-05 (BW-A4610)	GU673079	190	13° 16′ 21″ S	123° 21′ 24″ E	390–394	6 July 2007
C. maldivensis	CSIRO H 6595-01 (BW-A4611)	GU673080	314	12° 29′ 57″ S	123° 25′ 00″ E	397–405	6 July 2007
C. maldivensis¶	CSIRO H 6595-02 (BW-A4612)	GU673081	262	12° 29′ 57″ S	123° 25′ 00″ E	397–405	6 July 2007
C. maldivensis§	CSIRO H 6595-02 (BW-A4613)	GU673082	185	12° 29′ 57″ S	123° 25′ 00″ E	397–405	6 July 2007
C. maldivensis¶	CSIRO H 6592-09 (BW-A4614)	GU673083	233	12° 36′ 57″ S	123° 26′ 12″ E	403–419	7 July 2007
C. maldivensis§	CSIRO H 6592-09 (BW-A4615)	GU673084	227	12° 36′ 57″ S	123° 26′ 12″ E	403–419	7 July 2007
C. maldivensis	NMV A 29709-007 (BW-A12979)	KU674793	165	16° 36′ 19″ S	120° 34′ 30″ E	407–414	30 June 2007
C. maldivensis	NMV A 29730-026 (BW-A12980)	KU674794	330	12° 29′ 57″ S	123° 25′ 00″ E	397–405	6 July 2007
C. maldivensis	NMV A 29713-018 (BW-A13007)	KU674795	247	15° 35′ 27″ S	120° 49′ 07″ E	399–399	1 July 2007

L_T, total length.

*Corresponding Barcode of Life (BOLD; http://www.barcodinglife.com/) numbers are given in parenthesis.

¶ largest and § the smallest individuals within a registered specimen lot.

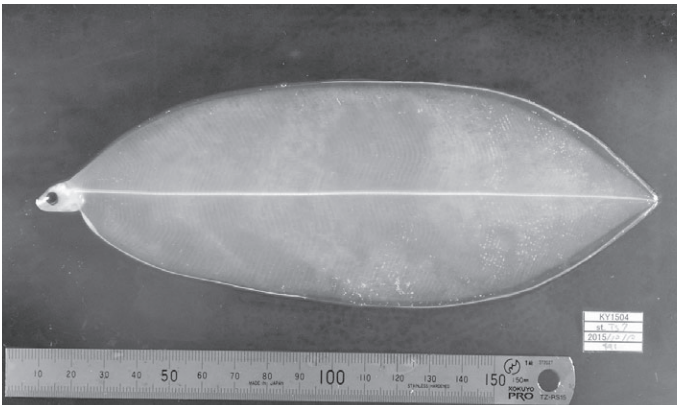

FIG. 1. Lateral view of *Thalassenchelys foliaceus*: SNFR2081, 192·2 mm total length.

13·4 mm (6·9% of L_T), predorsal length 85·6 mm (44·5%), pre-anal length 80·6 mm (41·9%), and greatest body depth 70·6 mm (36·7%). The meristic characters are given in Table II. Among these characteristics, the total number of myomeres, pre-anal myomeres, and the number of myomeres anterior to the position of the last vertical blood vessel (LVBV) are consistent with *T. foliaceus* as described by Castle & Raju (1975). In contrast, the total, pre-anal and LVBV myomere counts are different from *T. coheni* described previously (Castle & Raju, 1975; Shimokawa *et al.*, 1995; Shubin & Koinov, 2014; Kurogi *et al.*, 2016).

A lateral view of *C. maldivensis* (CSIRO H 6595–01) (Fig. 2) shows no dark-coloured area or spot on the body, in contrast with the other congeneric species (*C. megastomus* and *Congriscus marquesaensis* Karmovskaya 2004) (Asano, 1962; Karmovskaya, 2004; Kurogi *et al.*, 2016). The L_T of the nine individuals of *C. maldivensis* studied here ranged from 165 to 330 mm. Total and trunk vertebral counts were 138–141 and 48–49, respectively, corresponding to those of

FIG. 2. Lateral view of *Congriscus maldivensis*: CSIRO H 6595-01 (BW-A4611), 314·0 mm total length. Scale bar = 50 mm.

TABLE II. Meristic comparison between leptocephali of *Thalassenchelys* and adult eels of *Congriscus*

					Number of myomeres or vertebrae					
Species	Reference	*n*	L_T (mm)	Pectoral fin rays	Total	Trunk	Pre-anal	Postanal	At FVBV	At LVBV
Thalassenchelys										
T. foliaceus	Present study	1	192·2	17	143		55	88	10	51
T. foliaceus	Castle & Raju (1975)	25	34·5–228·0	15, 18[a]	142–153		55–62	84–92	–	50–58
T. coheni	Castle & Raju (1975)	18	147·0–304·0	–	152–163		67–74	83–92	–	55–67
T. coheni	Shimokawa *et al.* (1995)	4	121·5–250·0	–	153–157		69–71	83–87	10–12	61–64
T. coheni	Shubin & Koinov (2014)	4	190·0–270·0	–	152–160		66–72	85–89	10–12	61–64
T. coheni	Kurogi *et al.* (2016)	20	106·0–273·0	–	152–158		64–69[e]	–	–	59–63[f]
Congriscus										
C. maldivensis	Present study	9	165–330	18[b]	138–141[c]	48–49[c]				
C. maldivensis	Castle (1968)	1	340	19	148	47				
C. maldivensis	Karmovskaya (2004)	41	175·0–370·0	15–20	137–152	47–52				
C. megastomus	Asano (1962)	71	221·0–345·5	16–20	150–159	54–59				
C. megastomus	Castle & Raju (1975)	?	?	–	150–159	54–59				
C. megastomus	Karmovskaya (2004)	10	208·0–485·0	19–20	153–157	58–60				
C. megastomus	Kurogi *et al.* (2016)	13	196·0–348·0	17–19[d]	153–162	55–60				
C. marquesaensis	Karmovskaya (2004)	4	222·0–273·0	22	158–164	55–57				

FVBF, first vertical blood vessel; LVBV, last vertical blood vessel; L_T, total length; *n*, number of individuals examined.

[a] Fifteen indistinct rays from a leptocephalus and 18 rays from a metamorphic individual; [b] based on four individuals; [c] based on six individuals; [d] based on nine individuals; [e] based on 19 leptocephali; [f] based on 16 leptocephali.

TABLE III. Per cent K2P distances (mean ± S.E.) within (on the diagonal) and between (below the diagonal) species of *Thalassenchelys foliaceus*, *Congriscus maldivensis* and *C. megastomus*. Values in parentheses is the number of fishes examined

	T. foliaceus	*C. maldivensis*	*C. megastomus*
T. foliaceus (1)	–		3·07 ± 0·67*
C. maldivensis (9)	0·64 ± 0·27	0·28 ± 0·12	
C. megastomus (39)	3·11 ± 0·71	3·07 ± 0·68	0·35 ± 0·10

*Distance between *T. foliaceus* + *C. maldivensis* and *C. megastomus*.

C. maldivensis reported elsewhere and differing from *C. megastomus* and *C. marquesaensis* described in previous studies (Asano, 1962; Castle & Raju, 1975; Karmovskaya, 2004; Kurogi *et al.*, 2016).

No insertions, deletions or stop codons were observed in the 10 nucleotide sequences determined in the present study. Mean ± S.E. K2P distance within species was low (0·28 ± 0·12% in *C. maldivensis*, 0·35 ± 0·1% in *C. megastomus*) (Table III). Mean ± S.E. K2P distance between *T. foliaceus* and *C. maldivensis* is 0·64 ± 0·27%, well within the range of intra-specific divergence in the fish *coI* gene (Ward *et al.*, 2005; Kochzius *et al.*, 2010). Mean ± S.E. K2P distance between *T. foliaceus* and *C. megastomus* is 3·11 ± 0·71%, that between *C. maldivensis* and *C. megastomus* is 3·07 ± 0·68%, and that between *T. foliaceus* plus *C. maldivensis* and *C. megastomus* is 3·07 ± 0·67%, well within the range of interspecific divergence in the fish *coI* gene (Ward *et al.*, 2005; Kochzius *et al.*, 2010). *Thalassenchelys foliaceus* and *C. maldivensis* form a cohesive cluster, substantially diverging from that of *C. megastomus* (Fig. 3).

Based on the variation in morphometric and meristic data noted by Karmovskaya (2004), *C. maldivensis* may be a species complex but, as genetic data are lacking from the type locality and other areas, the leptocephalus was compared here with specimens from the nearest populations with available genetic data. Results obtained by morphological and genetic analyses performed in the present study indicate that *T. foliaceus* is a larval form and a junior synonym of *C. maldivensis*. The results of this study reject the previous hypotheses that leptocephali of *Thalassenchelys* may be neotenic species or the adults are short-lived (Mecklenburg *et al.*, 2002). Although Castle & Raju (1975) noted that the myomere counts in two species of *Thalassenchelys* and vertebral counts in two species of *Congriscus* Jordan & Hubbs 1925 are concordant with one another, they dismissed *Congriscus* as a candidate for the adult form of *Thalassenchelys*. This was due to Asano's (1962) report that leptocephali of *C. megastomus* have much lower body height (as a proportion of L_T) than *Thalassenchelys*. To date, almost all leptocephali of *Thalassenchelys* have been caught in the mid to near-surface layer (Castle & Raju, 1975; Shimokawa *et al.*, 1995; Shubin & Koinov, 2014; Kurogi *et al.*, 2016; present study), while leptocephali of *C. megastomus* described by Asano (1962) were caught by deep-sea trawl together with the adult form. This indicates that leptocephali described by Asano (1962) may be the final stage of metamorphosis having considerably reduced body height.

Catch locations of adult forms of *C. maldivensis* are concordant with those of the leptocephali (Castle & Raju, 1975; Karmovskaya, 2004; Kurogi *et al.*, 2016; present study). A North Pacific wide distribution of leptocephali of *C. megastomus* (formerly

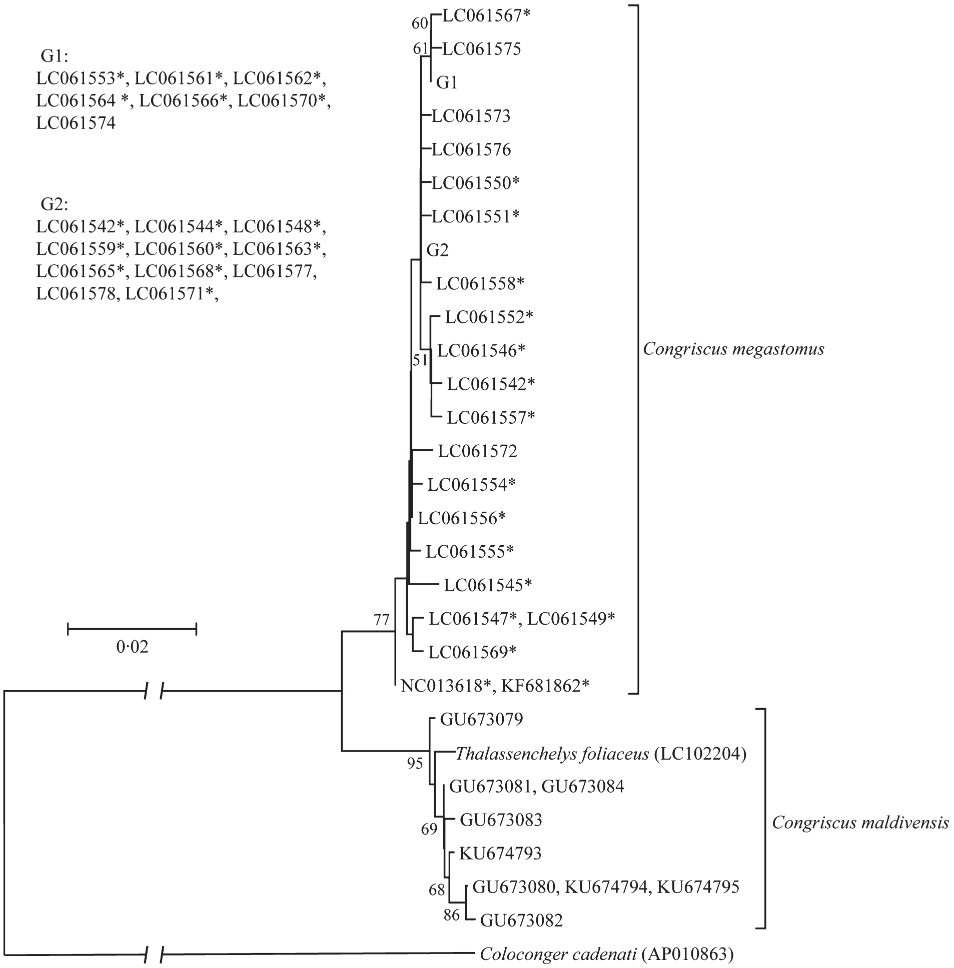

FIG. 3. Neighbour-joining tree of Kimura-2-parameter distances of cytochrome oxidase subunit I (*coI*) sequences of *Congriscus megastomus*, *Congriscus maldivensis* and *Thalassenchelys foliaceus*. *, larval forms of *C. megastomus* (*i.e. Thalassenchelysi coheni*). *Coloconger cadenati* (AP010863) is used as an out group. Bootstrap values of >50% (out of 1000 replicates) are shown at the nodes.

T. coheni) has been confirmed previously (Castle & Raju, 1975; Shimokawa *et al.*, 1995; Shubin & Koinov, 2014; Kurogi *et al.*, 2016), while adult forms of this species are not reported from central-to-eastern North Pacific (Kurogi *et al.*, 2016). The present study notes that some locality data published by Karmovskaya (2004) for *C. megastomus* is incorrect. The latitudes for the R.V. *Vityaz* specimens should have been north of the equator rather than south (E. Karmovskaya pers. comm.). This error was perpetuated in the map presented by Kurogi *et al.* (2016) representing these localities as southern Australia, rather than the North Pacific. No specimens of *C. megastomus* have been found in Australian waters.

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References

Asano, H. (1962). Studies on the congrid eels of Japan. *Bulletin of Misaki Marine Biological Institute of Kyoto University* **1,** 1–143.

Baldwin, C. C., Mounts, J. H., Smith, D. G. & Weigt, L. A. (2009). Genetic identification and color descriptions of early life-history stages of Belizean *Phaeoptyx* and *Astrapogon* (Teleostei: Apogonidae) with comments on identification of adult *Phaeoptyx*. *Zootaxa* **2008,** 1–22.

Castle, P. H. J. (1968). The congrid eels of the western Indian Ocean and the Red Sea. *Ichthyological Bulletin, Rhodes University* **33,** 685–726.

Castle, P. H. J. & Raju, N. S. (1975). Some rare leptocephali from the Atlantic and Indo-Pacific Oceans. *Dana Report* **85,** 1–25.

Chen, J.-N., López, J. A., Lavoué, S., Miya, M. & Chen, W.-J. (2014). Phylogeny of the Elopomorpha (Teleostei): evidence from six nuclear and mitochondrial markers. *Molecular Phylogenetics and Evolution* **70,** 152–161.

Hanke, G. F., Peden, A. E. & Bedard, J. M. (2014). New records of spiny eels (Albuliformes), true eels (Anguilliformes), and bobtail eels (Saccopharyngiformes) in British Columbia, Canada. *Northwestern Naturalist* **95,** 63–76.

Inoue, J. G., Miya, M., Miller, M. J., Sado, T., Hanel, R., Hatooka, K., Aoyama, J., Minegishi, Y., Nishida, M. & Tsukamoto, K. (2010). Deep-ocean origin of the freshwater eels. *Biology Letters* **6,** 363–366.

Karmovskaya, E. S. (2004). Benthopelagic bathyal conger eels of families Congridae and Nettastomatidae from the Western Tropical Pacific, with descriptions of ten new species. *Journal of Ichthyology* **44,** S1–S32.

Kochzius, M., Seidel, C., Antoniou, A., Botla, S. K., Campo, D., Cariani, A., Vazquez, E. G., Hauschild, J., Hervet, C., Hjörleifsdottír, S., Hreggvidsson, G., Kappel, K., Landi, M., Magoulas, A., Marteinsson, V., Nölte, M., Planes, S., Tinti, F., Turan, C., Venugopal, M. N., Weber, H. & Blohm, D. (2010). Identifying fishes through DNA barcodes and microarrays. *PLoS ONE* **5,** e12620.

Kurogi, H., Chow, S., Yanagimoto, T., Konishi, K., Nakamichi, R., Sakai, K., Ohkawa, T., Saruwatari, T., Takahashi, M., Ueno, Y. & Mochioka, N. (2016). Adult form of a giant anguilliform leptocephalus *Thalassenchelys coheni* Castle and Raju 1975 is *Congriscus megastomus* (Günther 1877). *Ichthyological Research* **63,** 239–246.

López, J. A., Westneat, M. W. & Hanel, R. (2007). The phylogenetic affinities of the mysterious anguilliform genera *Coloconger* and *Thalassenchelys* as supported by mtDNA sequences. *Copeia* **2007,** 959–966.

Mecklenburg, C. W., Mecklenburg, T. A. & Thorsteinson, L. K. (2002). *Fishes of Alaska*. Bethesda, MD: American Fisheries Society.

Obermiller, L. E. & Pfeiler, E. (2003). Phylogenetic relationships of elopomorph fishes inferred from mitochondrial ribosomal DNA sequences. *Molecular Phylogenetics and Evolution* **26,** 202–214.

Shimokawa, T., Amaoka, K., Kajiwara, Y. & Suyama, S. (1995). Occurrence of *Thalassenchelys coheni* (Anguilliformes; Chlopsidae) in the West Pacific Ocean. *Japanese Journal of Ichthyology* **42,** 89–92.

Shubin, A. O. & Koinov, A. A. (2014). New findings of leptocephali *Thalassenchelys coheni* (Chlopsidae/Colocongridae) in the Northwestern Part of the Pacific Ocean. *Journal of Ichthyology* **54,** 214–215.

Takahashi, M., Mochioka, N., Shinagawa, S., Yatsu, A. & Nakazono, A. (2008). Distribution patterns of leptocephali in the Kuroshio-Oyashio transitional region of the western North Pacific. *Fisheries Oceanography* **17,** 165–177.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30,** 2725–2729.

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R. & Hebert, P. D. N. (2005). Barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B* **360,** 1847–1857.