

Supplementary file

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Evolution of bidirectional sex change and gonochorism in fishes of the gobiid genera *Trimma*, *Priolepis*, and *Trimmatom*

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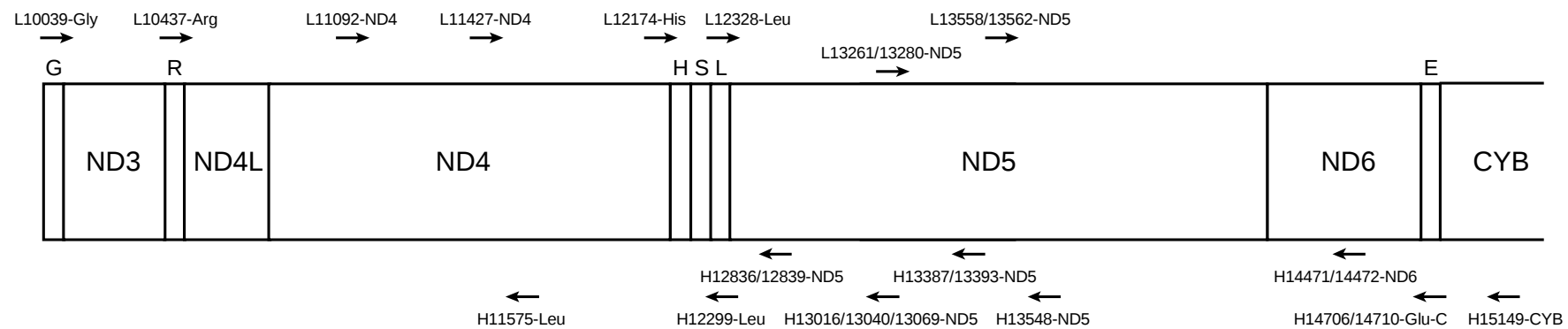
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Supplementary Table 1 A list of the species used in phylogenetic analysis and determination of sexuality. For non-catalogued specimens, locality or source is shown. CBM, Natural History Museum and Institute, Chiba; CMNH, Coastal Branch of Natural History Museum and Institute, Chiba; NMST, National Science Museum; ROM, Royal Ontario Museum; YCM, Yokosuka City Museum; KPM, Kanagawa Prefectural Museum of Natural History. *conspecific with *Trimmatom* sp. by Akihito et al. (2013)

Species	Accession number (Registration number)	Specimens for determination of sexuality (mm in TL : male; female)
Ingroup		
<i>Trimma annosum</i>	AB854445(CBM-ZF11489)	ROM45977 (23.0; 21.0)
<i>T. benjamini</i>	AB854447(ROM64354)	ROM4612 (25.0; 24.0)
<i>T. caudomaculatum</i>	AB854465	Amami Isl., Japan (32.0; 29.0)
<i>T. caesiura</i>	AB854419(CBM-ZF11460)	YCM-P34185 (36.0; 30.5)
<i>T. cana</i>	AB854449(ROM74764)	ROM74894 (24.0; 23.5)
<i>T. emeryi</i>	AB854421(CBM-ZF11462)	YCM-P36315 (22.0,22.5; 19.5, 20.5)
<i>T. fangi</i>	AB854453	ROM85152 (26.5; 26.0)
<i>T. flammeum</i>	AB854454	ROM59781 (26.0; 25.0)
<i>T. flavatram</i>	AB854432(CBM-ZF11475)	Amami Isl., Japan (female 25.0, 26.5)
<i>T. fucatum</i>	AB854455(ROM68769)	ROM58769 (25.5; 25.0)
<i>T. gigantum</i>	AB854456(ROM80658)	ROM88104 (33.0; 39.0)
<i>T. grammistes</i>	AB854422(CBM-ZF11463)	
<i>T. hayashii</i>	AB854431(CBM-ZF11474)	Amami Isl., Japan (24.0; 20.0)
<i>T. kudoii</i>	AB854434(CBM-ZF11477)	
<i>T. lantana</i>	AB854457	ROM83136 (22.0; 24.0)
<i>T. macrophthalmia</i>	AB854424(CBM-ZF11465)	YCM-P37070 (20.0, 21.0; 19.0, 19.0)
<i>T. maiandros</i>	AB854430(CBM-ZF11473)	Amami Isl., Japan (22.0, 21.5)
<i>T. marinae</i>	AB854423(CBM-ZF11464)	Amami Isl., Japan (22.0; 25.0)
<i>T. milta</i>	AB854474(ROM80399)	Amami Isl., Japan (26.0; 23.0, 23.5)
<i>T. nasa</i>	AB854458(ROM80347)	ROM74780 (18.5; 21.5)
<i>T. naudei</i>	AB854425(CBM-ZF11466)	YCM-P41276 (33.5; 32.0, 35.0)
<i>T. necopinum</i>	AB854459	ROM52450 (26.5; 24.0)
<i>T. okinawae</i>	AB854426(CBM-ZF11467)	
<i>T. preclarum</i>	AB854460(ROM80021)	ROM 83062 (24.5; 24.0)
<i>T. rubromaculatum</i>	AB854436(CBM-ZF11479)	Ornament fish shop (22.0, 26.0; 19.0)
<i>T. sheppardi</i>	AB854427(CBM-ZF11468)	YCM-P28333 (24.0; 23.0)
<i>T. stobbsi</i>	AB854461(ROM63937)	ROM46052 (18.5; 20.0)
<i>T. striatum</i>	AB854462(ROM74959)	YCM-P37166 (26.0, 28.0; 24.5, 27.0)
<i>T. tauroculum</i>	AB854463(ROM80354)	ROM80027 (21.0; 21.5)
<i>T. taylori</i>	AB854428(CBM-ZF11469)	Amami Isl., Japan (27.0; 30.0)
<i>T. yanagitai</i>	AB854433(CBM-ZF11476)	
<i>Priolepis akihitoi</i>	AB854442(CBM-ZF11484)	
<i>P. borea</i>	LC021306/7 (CMNH-ZF016029)	
<i>P. cincta</i>	AB854437(CBM-ZF11480)	
<i>P. fallacincta</i>	AB854438(CBM-ZF11481)	
<i>P. hipoliti</i>	LC021302	
<i>P. inhaca</i>	AB854439(CBM-ZF11482)	
<i>P. latifascima</i>	AB854470(CBM-ZF12223)	
<i>P. semidoliata</i>	AB854440/1(CBM-ZF-11483)	
<i>Trimmatom</i> sp.*	AB854443(CBM-ZF11485)	Amami Isl., Japan (25.0); Kuchierabu Isl., Japan (23.0)
<i>Trimmatom pharus</i>	AB854471(CBM-ZF12297)	Iriomote Isl., Japan (17.0), YCM-P 44761 (17.0, 18.5), KPM-NI 5522 (15.5) ; Sesoko Isl., Japan (21.0), YCM-P 44761 (17.5, 19.5), KPM-NI 5071(16.0), 5076(17.0)
Gobiid outgroup		
<i>Bathygobius fuscus</i>	AB854444(CBM-ZF-11488)	Sakurajima, Kagoshima, Japan (female 78.0, 97.3)
<i>Bollmannia boqueronsis</i>	LC021303	
<i>Cabillus tongarevae</i>	AB854467 (NMST-P 75536)	
<i>Cabillus</i> sp.	AB854468 (CBM-ZF-12221)	Iriomote Isl., Japan (44.8; 44.0)
<i>Gollogobius olivaceus</i>	LC021304 (CBM-ZF 12215)	
Non-gobiid outgroup		
<i>Eleotris acanthopoma</i>	AP004455	
<i>Rhyacichthys aspro</i>	AP004454	



Supplementary Fig. 1 Schematic representation of the ND4/ND5 gene region and relative positions of the newly designed and previously published polymerase chain reaction primers (see Supplementary Table 2). Transfer RNA genes are represented by a single-letter code

Supplementary Table 2 Primers are designated by their 3' ends, which correspond to the position on the human mitochondrial genome (Anderson et al. 1981) by convention. L and H denote heavy and light strands, respectively. Degenerate positions of sequences are denoted using IUPAC codes

Primer	Sequence (5'–3')	Reference
Long PCR primers		
L10039-Gly-G	TTCCAATYWCM TGGTYTTGG	This study
L10437-Arg-G	TATTTTAATTAAAATAKT TGR TTTTCG	This study
H14706-Glu-G	ACAACGRTGG TTTTTC AAG	This study
H14710-Glu-C	CTTGTAGTTGAATWACAACGGTGGT TYTTC	Miya et al. (2006)
H15149-CYB	GGTGGCKCCTCAGAAGGACATTTGKCCTCA	Miya and Nishida (2000)
Short PCR primers		
L10437-Arg-G	TATTTTAATTAAAATAKT TGR TTTTCG	This study
L11092-ND4-G	TKTTCTATG TKATGTTTGARGC	This study
L11427-ND4-G	CCMAARGCSCATGTW GARGC	This study
L12174-His-G	TTTAAMKAAAACATTAGATTGTGATTC	This study
L12328-Leu-C	AACTCTTGGTGCAAMTCCAAG	Miya et al. (2006)
L12936-ND5	AACTCMTGGGAGATTCAACAA	Miya and Nishida (2000)
L13261-ND5-G	GCKTTTTC AACWTCAAGCCA	This study
L13280-ND5-M	CARCTWGGCCTAATRATRG T	This study
L13558-ND5-G	TCMTACCTMAACGCCTGAGC	This study
L13562-ND5	TCTTACCTAAACGCCTGAGCCCT	Miya and Nishida (1999)
H11575-ND4-G	ATKAGKGAYTTKAGGTCTGTTTG	This study
H12299-Leu-G	GCACCAAGAGTTTTTGTTCC	This study
H12836-ND5-G	GCKGCKGTGTTKGCGTCKGC	This study
H12839-ND5-G	AGKGCTGCWGTGTTKGCR TC	This study
H13016-ND5	AGTCAKGGGTGGAGKCCRAATTG	Miya and Nishida (1999)
H13040-ND5-G	GGYGTWGGRCCCTCCATKGC	This study
H13069-ND5	GTGCTGGAGTGKAGTAGGGC	Miya and Nishida (1999)
H13387-ND5-G	TTKCGGATGTCYTGYTCRTC	This study
H13393-ND5-C	CCTATTTTKCGGATGTCTTG YTC	Miya et al. (2006)
H13548-ND5-G	GTKAGGGA KAGGGCTCAGGCGTT	This study
H14471-ND6	GCTTTGGCKGCKGAGCCTT	Minegishi et al. (2005)
H14472-ND6-G	GKTTTGTCTTATTCKKCRGC	This study
H14706-Glu-G	ACAACGRTGG TTTTTC AAG	This study
H14710-Glu-C	CTTGTAGTTGAATWACAACGGTGGT TYTTC	Miya et al. (2006)

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