

Ecography

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Supplementary material

1 **Legends for supplementary material**

2 Table A1. List of examined material of putative species of subgenus *Sardinella* used
3 for morphological comparisons.

4 Table A2. Information and references for the primers used through this study.

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6 *Sardinella*, used in this study for phylogenetic and species delimitation analyses.

7 Table A4. Additional *COI* sequences of *Sardinella* mined from BOLD
8 (BIN:AAB7268) and used in this study for the population structure and haplotype
9 distribution analyses.

10 Table A5. Average genetic distances matrices (in %) for each gene partition.

11 Figure A1. Bayesian Inference (BI) phylogenetic tree for the geographical samples of
12 subgenus *Sardinella*, employed from nuclear intron *S7* gene. BI significant values
13 (>0.9) are displayed above nodes; Maximum Likelihood (ML) bootstrap support
14 values below nodes (>80). Accession vouchers are summarized in table A3, except
15 for *Sardinella hualiensis* which is indicated within the figure.

Table A1. List of examined material of putative species of subgenus *Sardinella* used in this study for morphological comparisons.

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MED->POW	0.7
POW ->WIO	53.3
POW ->MED	8.6
POW -> AOW	24.1

AOW, Atlantic Ocean – West; IOW, Indian Ocean –
West; MED – Mediterranean Sea; POW, Pacific
Ocean - West

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Table A2. Information for the primers used for PCR and sequencing through this study

Morphospecies	Region	Country	Locality	No. of specimens	Museum Catalog	25
Short head	Atlantic Ocean – Central west	USA	Florida	1	HUJ 14313	
		USA	North Carolina	5	USNM391423	26
	Atlantic Ocean - Southwest	Argentina	Mar del Plata	2	UNMDP 1310,1311	
		Brazil	Rio de Janeiro	12	-	27
		Brazil	Rio de Janeiro	3	MNHN 3380, 3746, 5484	
	Atlantic Ocean - East	Angola	-	2	SAIAB 65899, 66093	28
		Benin	-	1	HUJ 14334	
		Senegal	Dakar	1	HUJ 2036	29
		Spain	Tenerife	1	HUJ 19225	
	Mediterranean Sea	France	Toulon	1	MNHN 3745	30
		Greece	Rhodes Is.	10	SMNHNTAU P.15067	
		Israel	Ashdod	20	SMNHNTAU P.14293, 14325	31
		Italy	Messina	1	MNHN A9824	
		Spain	Tarragona	10	SMNHNTAU P.15035	32
		Turkey	Mersin	12	SMNHNTAU P.15039	
Pacific Ocean - Northwest	Japan	Kagoshima	6	HUJ 20297	33	
	Philippines	Batangas	1	USNM 403459		
	Philippines	Manila	9	SMNHNTAU P.15498	34	
	Philippines	Manila	3	HUJ 2126		
	Philippines	Sorsogon	1	USNM 408914	35	
	Taiwan	Taipei	5	SMNHNTAU P.15394		
Long head	Indian Ocean - Central	India	Malabar	1	MNHN 3744	36
		India	Puducherry	1	MNHN 3743	
		Sri Lanka	Negombo	3	SAIAB 187360	37
Indian Ocean - West	Kenya	Mombasa	20	SMNHNTAU P. 15054, 15085	38	
Pacific Ocean - Southwest	Indonesia	West Papua	2	MAGNT S. 15252-001		

Acronyms –USNM, Smithsonian National Museum of Natural History, Washington; HUJ, Hebrew University of Jerusalem, Jerusalem; MNHN, Muséum National d'Histoire Naturelle, Paris; UNMDP, Universidad Nacional de Mar del Plata, Mar del Plata; SAIAB, South African Institute for Aquatic Biodiversity, Grahamstown; SMNHNTAU, the Steinhardt Museum of Natural History, Tel Aviv University; MAGNT, Museum and Art Gallery of the Northern Territories, Darwin

Gene	Primers sequences	Annealing temp (°C)	References
COI	Fish F2 Forward – 5'-TCGACTAATCATAAAGATATCGGCAC-3'	54	Ward et al. (2005)
	Fish F2 Reverse – 5'-ACTTCAGGGTGACCGAAGAATCAGAA-3'	54	Ward et al. (2005)
Cytb	CytbH Forward – 5'-GTGACTTGAAAAACCACCGTTG-3'	50	Song et al. (1998)
	CytbL Reverse – 5'-AATAGGAAGTATCATTCGGGTTTGATG-3'	50	Taberlet et al. (1992)
16S	16Sar Forward – 5'-CGCCTGTTTATCAAAAACAT-3'	45	Palumbi (1996)
	16Sbr Reverse – 5'-CCGGTCTGAACTCAGATCACGT-3'	45	Palumbi (1996)
S7	S7RPEX1F Forward - 5' - TGGCCTCTTCCTTGGCCGTC - 3'	51	Chow and Hazama (1998)
	S7RPEX2R Reverse - 5' - AACTCGTCTGGCTTTTCGCC - 3'	51	Chow and Hazama (1998)

References

- Chow, S. and Hazama, K. 1998. Universal PCR primers for S7 ribosomal protein gene introns in fish. *Mol. Ecol.* 7: 1255-1256.
- Palumbi, S. 1996. Nucleic acids II: the polymerase chain reaction. *Mol. Syst.* 2: 205-247.
- Song, C. B., Near, T. J. and Page, L. M. 1998. Phylogenetic relations among Percid fishes as inferred from mitochondrial Cytochrome b DNA sequence data. *Mol. Phylogenet. Evol.* 10: 343-353.
- Taberlet, P., Meyer, A. and Bouvet, J. 1992. Unusually large mitochondrial variation in populations of the blue tit *Parus caeruleus*. *Mol. Ecol.* 1: 27-36.
- Ward, R. D. et al. 2005. DNA barcoding Australia's fish species. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 360: 1847-1857.

Table A3. Taxon sampling and accession vouchers for putative species of subgenus *Sardinella*, used in this study for phylogenetic and species-delimitation analyses.

Morphospecies	Region	Locality	Accession vouchers			
			<i>COI</i> (BOLD)	<i>Cytb</i> (GenBank)	<i>I6S</i> (GenBank)	<i>S7</i> (GenBank)
Short-head	Atlantic Ocean	Rio de Janeiro, Brazil	NPRS(021-030)-15	KR020874-83	KR056095-104	KR020932, KR020933
		Mar del Plata, Argentina	NPRS(089-090)-15	KR020899-900	KR056105-6	-
	Mediterranean Sea	Cádiz ¹ , Spain	NPRS(063-064)-15	KR020872-3	KR056107-8	KR020936
		Tarragona, Spain	NPRS(036-043)-15	KR020863-71	KR056107-16	KR020939
		Rhodes, Greece	NPRS(053-062)-15	KR020835-44	KR056117-26	-
		Mersin, Turkey	NPRS(044-052)-15	KR020854-62	KR056127-32	KR020938
		Ashdod, Israel	NPRS(068-079,091)-15	KR020884-98	KR056133-38	-
	Pacific Ocean	Manila, Philippines	NPRS(080-088)-15	KR020921-29	KR056139-47	KR020940, KR020941
		Taipei, Taiwan	NPRS(031-035)-15	KR020849-53	KR056148-52	KR020930
Kagoshima, Japan		NPRS(065-067)-15	KR020845-48	KR056153-56	-	
Long-head	Indian Ocean	Mombasa, Kenya	NPRS(001-009)-15	KR020908-15	KR056157-69	KR020931, KR020934
		Kerala, India	NPRS(010-020)-15	KR020901-07, KR020916-20	KR056169-75	KR020935, KR020937
<u>Outgroup representatives</u>						
<i>Sardinella albella</i>	Indian Ocean	Mombasa, Kenya	IPSAR040-14	KJ566786	KP307727	-
<i>Sardinella fimbriata</i>	West Pacific	Manila, Philippines	IPSAR085-14	KP307685	KP307746	KM518737
<i>Sardinella gibbosa</i>	Indian Ocean	Ashdod, Israel	IPSAR004-14	KJ566800	KP307736	KM518734, KM518734
<i>Sardinella maderensis</i>	Mediterranean	Ashdod, Israel	IPSAR100-14	KP307699	KP307745	-
<i>Sardina pilchardus</i>	Mediterranean	Ashdod, Israel	NPRS093-16	FR851437*	KX371633	AY650226
<i>Sardinops sagax</i>	Indian Ocean	Port Elizabeth, South Africa	NPRS092-16	EU552565*	KX371632	-
<i>Herklotsichthys quadrimaculatus</i>	Indian Ocean	Mombasa, Kenya	NPRS094-16	-	KX371634	KM518736

¹ Considered in this study as Mediterranean region due to its close proximity to the Gibraltar Strait

* Mined sequences from GenBank

Table A4. Additional *COI* sequences of *Sardinella* mined from BOLD (BIN:AAB7268) and used in this study for the population structure and haplotype distribution analyses.

Region	Locality	<i>n</i>	Accession numbers
Atlantic Ocean - Northwest	North Carolina, USA	5	BCOLL413-08 - 417-08
	Florida, USA	1	SMSA188-09
	Tobago, Trinidad and Tobago	1	TOBA248-09
Atlantic Ocean - Southwest	Sao Paulo, Brazil	5	MFSP98-09 - 101-09, 1999-11
Atlantic Ocean - East	Angola	5	HVDBF476-12 – 480-12
Mediterranean Sea	Jaffa, Israel	6	JFS200-14 – 205-14
Indian Ocean	Mumbai, India	5	WLIND739-07 – 743-07
Pacific Ocean - Northwest	Penghu county, Taiwan	3	FTWS277-09, 773-09, 923-09
	Guangdong, China	4	FSCS327-06, 329-06, 413-07, 417-07
	Metro Manila, Philippines	4	BPS011-10, 012-10, 014-10, 015-10
Pacific Ocean - Southwest	Bali, Indonesia	7	FOAJ673-09 – 674-09, FOAJ723-09 – 727-09
	Perth, Australia	1	FOAF167-07

Table A5. Average genetic distances matrices (in %) for each gene* partition of *Sardinella aurita*. Standard deviations are presented in upper diagonal

<i>16S</i>	Rio de Janeiro	Mar del Plata	Cádiz	Tarragona	Rhodes	Mersin	Ashdod	Kagoshima	Taipei	Manila	Kerala	Mombasa
Brazil, Rio de Janeiro ¹		0.07	0.20	0.20	0.20	0.20	0.18	0.20	0.22	0.20	0.21	0.20
Argentina, Mar del Plata ¹	0.12		0.24	0.25	0.24	0.24	0.22	0.24	0.26	0.24	0.26	0.25
Spain, Cádiz ¹	0.33	0.34		0.05	0.08	0.03	0.05	0.23	0.24	0.23	0.24	0.23
Spain, Tarragona ¹	0.41	0.43	0.09		0.10	0.06	0.07	0.23	0.25	0.23	0.25	0.24
Greece, Rhodes ¹	0.40	0.41	0.10	0.19		0.08	0.10	0.23	0.25	0.23	0.25	0.24
Turkey, Mersin ¹	0.36	0.37	0.03	0.11	0.13		0.05	0.23	0.25	0.23	0.24	0.23
Israel, Ashdod ¹	0.36	0.37	0.09	0.17	0.18	0.11		0.22	0.23	0.22	0.23	0.22
Japan, Kagoshima ¹	0.36	0.39	0.39	0.47	0.46	0.42	0.42		0.09	0.05	0.26	0.25
Taiwan, Taipei ¹	0.50	0.52	0.52	0.60	0.59	0.55	0.55	0.22		0.09	0.25	0.24
Philippines, Manila ¹	0.35	0.36	0.36	0.45	0.43	0.39	0.39	0.06	0.19		0.26	0.25
India, Kerala ²	0.43	0.44	0.44	0.53	0.51	0.47	0.47	0.49	0.53	0.46		0.09
Kenya, Mombasa ²	0.38	0.40	0.40	0.49	0.46	0.43	0.43	0.45	0.51	0.42	0.12	
<i>COI</i>	Rio de Janeiro	Mar del Plata	Cádiz	Tarragona	Rhodes	Mersin	Ashdod	Kagoshima	Taipei	Manila	Kerala	Mombasa
Brazil, Rio de Janeiro ¹		0.14	0.30	0.29	0.27	0.30	0.28	0.37	0.35	0.38	0.37	0.37
Argentina, Mar del Plata ¹	0.37		0.30	0.29	0.28	0.30	0.28	0.36	0.34	0.37	0.37	0.37
Spain, Cádiz ¹	0.83	0.75		0.04	0.04	0.02	0.04	0.47	0.44	0.47	0.45	0.45
Spain, Tarragona ¹	0.88	0.81	0.09		0.06	0.04	0.06	0.47	0.44	0.47	0.45	0.44
Greece, Rhodes ¹	0.83	0.75	0.12	0.21		0.05	0.07	0.44	0.41	0.44	0.42	0.42
Turkey, Mersin ¹	0.84	0.77	0.02	0.11	0.14		0.04	0.47	0.44	0.47	0.45	0.45
Israel, Ashdod ¹	0.86	0.79	0.13	0.21	0.23	0.14		0.45	0.42	0.45	0.43	0.43
Japan, Kagoshima ¹	1.38	1.31	1.77	1.83	1.69	1.79	1.74		0.17	0.15	0.39	0.39
Taiwan, Taipei ¹	1.22	1.15	1.61	1.66	1.51	1.62	1.57	0.74		0.12	0.30	0.30
Philippines, Manila ¹	1.26	1.19	1.65	1.71	1.56	1.67	1.62	0.58	0.51		0.38	0.38
India, Kerala ²	1.20	1.14	1.60	1.65	1.51	1.61	1.56	1.34	0.90	1.15		0.06
Kenya, Mombasa ²	1.26	1.19	1.65	1.71	1.56	1.67	1.62	1.38	0.95	1.20	0.21	
<i>Cytb</i>	Rio de Janeiro	Mar del Plata	Cádiz	Tarragona	Rhodes	Mersin	Ashdod	Kagoshima	Taipei	Manila	Kerala	Mombasa
Brazil, Rio de Janeiro ¹		0.15	0.42	0.43	0.42	0.43	0.42	0.51	0.47	0.49	0.43	0.44
Argentina, Mar del Plata ¹	0.31		0.45	0.45	0.45	0.46	0.45	0.54	0.50	0.51	0.45	0.46
Spain, Cádiz ¹	0.97	1.10		0.05	0.07	0.11	0.03	0.41	0.39	0.37	0.40	0.40
Spain, Tarragona ¹	1.04	1.13	0.07		0.09	0.12	0.06	0.41	0.40	0.37	0.41	0.41
Greece, Rhodes ¹	1.13	1.26	0.20	0.27		0.13	0.09	0.36	0.34	0.34	0.36	0.36

Turkey, Mersin ¹	1.14	1.28	0.17	0.24	0.36		0.11	0.42	0.40	0.38	0.42	0.42
Israel, Ashdod ¹	1.03	1.16	0.09	0.16	0.27	0.26		0.39	0.37	0.35	0.39	0.39
Japan, Kagoshima ¹	1.59	1.72	1.05	1.12	1.03	1.22	1.07		0.16	0.21	0.24	0.22
Taiwan, Taipei ¹	1.28	1.42	0.84	0.91	0.84	1.01	0.87	0.43		0.19	0.17	0.16
Philippines, Manila ¹	1.64	1.78	1.10	1.18	1.13	1.28	1.13	0.78	0.68		0.24	0.22
India, Kerala ²	1.08	1.22	0.90	0.97	0.93	1.07	0.93	0.66	0.38	0.85		0.12
Kenya, Mombasa ²	1.14	1.27	0.91	0.98	0.93	1.08	0.94	0.59	0.35	0.80	0.31	

<i>S7</i>	South-west Atlantic	Mediterranean	Western Pacific	Indian Ocean
South-west Atlantic ¹ (<i>n</i> =2)		0.69	0.62	0.39
Mediterranean ¹ (<i>n</i> =3)	2.18		0.16	0.32
Western Pacific ¹ (<i>n</i> =3)	1.96	0.36		0.33
Indian Ocean ² (<i>n</i> =4)	1.20	0.98	1.05	

* *I6S*, *COI*, and *S7* matrices were computed using Tamura-Nei model; *cytb* matrix using Tamura-3-Parameters model;

1– Short-head morphospecies; 2 – Long-head morphospecies

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Nuclear S7 gene tree

