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Inter- specific and subspecific genetic divergences of freshwater eels, genus *Anguilla* including a recently described species, *A. luzonensis*, based on whole mitochondrial genome sequences

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Abstract—We examined general characteristics and genetic divergences of whole mitochondrial genome sequences for all 19 species/subspecies of the genus *Anguilla* including a recently described new species, *A. luzonensis*. The entire genome ranged from 16,550 (*A. borneensis*) to 16,833 base pairs (*A. nebulosa labiata*), and general characteristics such as length of each gene and gene order were very conservative across the genus and also similar with other anguilliform and bony fishes. Pairwise comparisons of sequence differences showed that interspecific divergences did not overlap with intersubspecific variations and found a clear hierarchical structure in the genus *Anguilla*, in which the two subspecies of *A. australis* and *A. nebulosa* had slight and the smallest differences among all species/subspecies pairs, followed by the two subspecies of *A. bicolor*, the *A. anguilla* and *A. rostrata* pair, and the other species pairs in order. *A. luzonensis* showed considerable sequence differences from all other species in all genes and was appeared to be genetically close to *A. interioris*. The present study analyzed the whole mitochondrial genome of *A. luzonensis* for the first time and genetic divergences for all anguillid species and subspecies, and provided basic information for revision and reaching agreement about the taxonomy of the genus *Anguilla* using molecular characteristics, which have been discussed recently.

Key words: *Anguilla*, *Anguilla luzonensis*, taxonomy, mitochondrial DNA, interspecies and inter-subspecies variations

Introduction

Recent remarkable progress in molecular genetics has given rise to various questions in traditional taxonomy of many organisms based on morphological characters. The inclusive taxonomy of freshwater eels of the genus *Anguilla* by Ege (1939) has long been widely accepted. However, the use of molecular genetic information as a taxonomic character has found several possible flaws in Ege (1939)'s taxonomy. Dijkstra and Jellyman (1999) demonstrated no significant genetic divergence between two subspecies of *A. australis* using partial nucleotide sequences of control region of mitochondrial DNA (mtDNA), and thus suggested that these subspecies should be considered as a single species, although a microsatellite study lately suggested that this subspecies designation is likely valid (Shen and Tzeng 2007). Aoyama et al. (1999a) used 12S and 16S ribosomal RNA (rRNA) and cytochrome *b* (cyt *b*) gene sequences and found some *A. interioris* among individuals that were collected from New Guinea and were identified as *A. celebesensis* according to Ege (1939), showing that morphological characters were not sufficient to distinguish different species that are genetically recognized.

Watanabe et al. (in press) recently described a new species of the genus *Anguilla*, *A. luzonensis*, for the first time in seventy years after Ege (1939)'s revision. However, they examined the specimens only morphologically, and taxonomic status of this new species in genetic aspects has not yet been evaluated. MtDNA sequences have been frequently used for phylogenetic analyses and population genetic studies, which has consequently contributed to evaluate traditional taxonomy of animals and to establish many molecular species identification methods. The aim of the present study was to evaluate interspecific and intersubspecific genetic divergences of all species/subspecies of the genus *Anguilla*, including *A. luzonensis*, by determining whole mitochondrial genome sequence of the new species for the first time and analyzing it along with sequences of all anguillid species, in order to provide basic information about their taxonomy and species identification.

Materials and Methods

Mitochondrial DNA sequences

The present study followed the taxonomy and all species names of the genus *Anguilla* by Ege (1939), which has been the basis of studies of freshwater eels, in order to avoid confusion in comparisons with other literatures.

An adult eel of *A. luzonensis* was collected at a tributary of the Cagayan River in northern Luzon in Philippines on January 29, 2008, which was deposited in the Fish Collection of National Science Museum, Tokyo (registered number NSMT-P 90001, Watanabe et al. in press). Species identification based on morphological observation in detail for this specimen was reported elsewhere (Watanabe et al. in press). Small tissue was stored in 99% ethanol until DNA extraction by a classical phenol/chloroform/isoamyl alcohol method after digestion by proteinase *K*. Entire mtDNA was first purified by a long PCR technique (Cheng et al. 1997) using a total of five fish-versatile primers (L12321-Leu, S-LA-16S-H, L8343-Lys, H12293-Leu, AnUn-CYB-H; Miya and Nishida 2000, Inoue et al. 2001a, Minegishi et al. 2005). PCR conditions were as same as Minegishi et al. (2005). Long PCR products were then diluted 10 times with sterilized TE buffer for subsequent PCRs. Using the diluted long PCR products as template DNA, nested PCRs were conducted with a total of 75 fish-versatile primers under reaction conditions as described in Minegishi et al. (2005). For detailed locations and sequences of all primers used, see Miya and Nishida (2000), Ishiguro et al. (2001), Inoue et al. (2001a) and Minegishi et al. (2005).

For comparisons of genetic diversity of mtDNA among anguillid species, we used whole mtDNA nucleotide sequences for other 18 species and subspecies of the genus *Anguilla*, which were determined by Inoue et al. (2001a) and Minegishi et al. (2005) (DDBJ/EMBL/GenBank Accession nos. AB038556, AP007233 - AP007249).

Genetic variation investigations

Each gene was identified by referring to the mtDNA sequence of *A. japonica* (Inoue et al. 2001a). Individual gene sequences for all 19 species and subspecies were manually aligned using DNASIS version 3.7 (Hitachi Software Engineering) and MacClade (Maddison and Maddison 2000) after automatic alignment with Clustal X (Thompson et al. 1997) as needed. Number of substitutions and genetic distances were calculated for all possible species/subspecies pairs at each gene with PAUP* 4.0b10 (Swofford 1998).

Results

General characteristics of mitochondrial genome of the genus *Anguilla*

The complete mtDNA sequence of *A. luzonensis* was deposited in DDBJ/EMBL/GenBank (Accession No. AB469437). The entire mtDNA genome of 19 *Anguilla* species and subspecies ranged from 16,550 (*A. borneensis*, a synonym of *A. malgumora*) to 16,833 (*A. nebulosa labiata*, a synonym of *A. bengalensis labiata*) base pairs (bp) and *A. luzonensis* showed a total of 16,635 bp (Table 1). Whole mtDNA genomes of all anguillid species contained 13 protein-coding genes, two rRNA genes, 22 transfer RNA (tRNA) genes and a non-coding region general to vertebrates (Boore 1999). All genes were encoded by the H strand, except NADH dehydrogenase subunits (ND) 6 gene and eight tRNA genes. Their gene order was identical to those of other typical bony fishes (e.g., Johansen and Bakke 1996, Hurst et al. 1999, Inoue et al. 2000, Broughton et al. 2001).

Length of each gene, base composition, codon usage in protein-coding genes, estimated secondary structures of two rRNA and 22 tRNA genes were highly conserved across the genus, and also very similar to those of other fishes and typical vertebrates as reported in *A. japonica* (Inoue et al. 2001a). In contrast, a huge variation in lengths of control region was observed, which ranged from 828 (*A. borneensis*) to 1107 bp (*A. nebulosa labiata*), with a maximum difference of 279 bp. Apart from termination-associated sequence (TAS, Doda et al. 1981) and conservative sequence block (CSB-S, -II, -III; Walberg and Clayton 1981), control region was extremely variable among species so that a reliable alignment could not be obtained.

Genetic divergences of anguillid species/subspecies

Pairwise sequence differences among all 19 anguillid species and subspecies including *A. luzonensis* ranged as follows: 0–5.71% for 12S rRNA, 0.18–4.82% for 16S rRNA, 0.15–11.42% for ATPase subunits (ATP) 6, 0–11.91% for ATPase8, 0–8.48% for cytochrome c oxidase subunit (CO) I, 0–6.22% for COII, 0.26–8.28% for COIII, 0–10.35% for cyt *b*, 0.51–9.26% for ND1, 0.29–13.11% for ND2, 0–12.31% for ND3, 0.36–10.57% for ND4, 0–12.12% for ND4L, 0.49–10.10% for ND5, 0.19–9.39% for ND6, 0–3.71% for combined tRNAs (Table 2, 3, Fig. 1). Nucleotide divergences of two rRNA and tRNA genes in genus *Anguilla* were relatively smaller than those of 13 protein-coding genes. The minimum sequence differences between species ranged from 0.26% (tRNA) to 4.21% (cyt *b*) and the maximum was from 3.64% (16S rRNA) to 13.11% (ND2) (Table 2, 3). The *A. anguilla* and *A. rostrata* pair constantly presented the minimum interspecific sequence differences among all possible previously recognized species pairs except for 12S rRNA, ATP6,

Table 1. Length of each mitochondrial gene sequences of all species and subspecies of the genus *Anguilla*.

Species/subspecies	mtDNA gene (base pairs)																
	12S	16S	ATP6	ATP8	COI	COII	COIII	cyt *b*	ND1	ND2	ND3	ND4	ND4L	ND5	ND6	tRNAs	CR	total
A. anguilla	949	1708	683	168	1593	691	785	1140	972	1045	349	1381	297	1842	522	1565	964	16 683
A. australis australis	947	1709	.	.	.	.	.	.	.	.	.	.	.	.	.	.	966	16 686
A. australis schmidti	.	1709	.	.	.	.	.	.	.	.	.	.	.	.	.	.	958	16 682
A. bicolor bicolor	948	1704	.	.	.	.	.	.	.	.	.	.	.	.	.	1564	978	16 700
A. bicolor pacifica	948	1704	.	.	.	.	.	.	.	.	.	.	.	.	.	1564	976	16 693
A. borneensis	948	1707	.	.	.	.	.	.	.	.	.	.	.	.	.	1566	828	16 550
A. malgumora	948	1710	.	.	.	.	.	.	.	.	.	.	.	.	.	.	978	16 700
A. dieffenbachi	947	1711	.	.	.	.	.	.	.	.	.	.	.	.	.	1564	961	16 687
A. interioris	948	1712	.	.	.	.	.	.	.	.	.	.	.	.	.	.	982	16 713
A. japonica	946	1704	.	.	.	.	.	.	.	.	.	.	.	.	.	.	967	16 685
A. marmorata	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1564	1020	16 745
A. megastoma	947	1709	.	.	.	.	.	.	.	.	.	.	.	.	.	.	987	16 714
A. mossambica	950	1710	.	.	.	.	.	.	.	.	.	.	.	.	.	1566	971	16 693
A. nebulosa labiata	948	1710	.	.	.	.	.	.	.	.	.	.	.	.	.	1564	1107	16 833
A. nebulosa nebulosa	948	1710	.	.	.	.	.	.	.	.	.	.	.	.	.	1564	982	16 707
A. obscura	948	1709	.	.	.	.	.	.	.	.	.	.	.	.	.	1564	981	16 704
A. reinhardti	.	1711	.	.	.	.	.	.	.	.	.	.	.	.	.	.	967	16 690
A. rostrata	947	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	962	16 679
A. luzonensis	949	1711	.	.	.	.	.	.	.	.	.	.	.	.	.		908	16 635

The same lengths as *A. anguilla* were shown as dot. Underlines show maximum and minimum lengths.

Table 2. Sequence divergences (%) of each mitochondrial gene between previously described species, between previously described subspecies, and between *A. luzonensis* and other anguillid 18 species/subspecies

Gene	Between previously described species	Between previously described subspecies	Between *A. luzonensis* and other 18 species/subspecies
12S rRNA	0.95–5.71	0–0.53	0.63–5.70
16S rRNA	1.11–4.82	0.18–0.59	1.70–4.71
ATP6	3.66–11.42	0.15–1.32	3.37–10.4
ATP8	1.79–11.91	0–1.19	2.38–8.93
COI	2.95–8.48	0–1.13	3.64–7.78
COII	1.74–6.22	0–0.87	2.46–5.21
COIII	2.04–8.28	0.26–1.91	3.06–7.64
cyt *b*	4.21–10.35	0–2.11	3.60–9.04
ND1	3.09–9.26	0.51–1.75	3.81–8.64
ND2	3.64–13.11	0.29–3.64	3.73–9.67
ND3	3.15–12.03	0–2.01	2.87–12.32
ND4	3.84–10.57	0.36–1.88	4.27–10.14
ND4L	2.69–12.12	0–1.01	3.37–10.10
ND5	3.26–10.10	0.49–1.95	3.96–9.28
ND6	2.68–9.39	0.19–2.11	2.30–7.85
tRNAs	0.26–3.64	0–0.51	0.77–3.71

cyt *b*, ND3 and ND6, whereas maximum differences were found in various species pairs, for example between *A. australis* schmidtii and *A. borneensis* in 16S rRNA, *A. anguilla* and *A. neblosa labiata* in COI, *A. megastoma* and *A. rostrata* in cyt *b* (Table 2, 3).

Genetic variations between subspecies were generally smaller than those between species. The maximum sequence differences between subspecies ranged from 0.51% (tRNA) to 3.64% (ND2), and the minimum was from 0 (12S rRNA, ATP8, COI, COII, cyt *b*, ND3, ND4L, tRNA) to 0.51% (ND1) (Table 2, 3). The subspecies pairs having the smallest differences were the two subspecies of *A. australis* and *A. nebulosa*, and either of these two subspecies pair had identical nucleotide sequences in eight of these 15 genes. The two subspecies of *A. bicolor* were the most diverged among three pairs of subspecies, and demonstrated similar sequence differences with *A. anguilla* and *A. rostrata* pair in some cases such as the ND2 and COIII gene (Table 2, 3, Fig. 1).

The new species, *A. luzonensis*, showed considerable sequence differences from all other species in all genes. The smallest differences were found with *A. interioris* among all species pairs, ranging from 0.63% (12S rRNA) to 4.27% (ND4), except 16S (with *A. nebulosa nebulosa*), ATP8 (with *A. nebulosa nebulosa*), COIII (with two subspecies of *A. nebulosa*), ND3 (with *A. obscura*) and ND6 (with *A. marmorata*). Genetic divergence between *A. luzonensis* and *A. interioris* was similar with that between *A. anguilla* and *A. rostrata*, except 12S rRNA, ATP6, cyt *b*, ND4 and ND6 that were slightly smaller than those between *A. anguilla* and *A. rostrata* (Table 2, 3, Fig. 1).

Table 3. Pairwise sequence differences of each mitochondrial gene of all anguilld species and subspecies including *A. luzonensis* (above, %; absolute number, below)

(A) 12S rRNA

	Angu	Auau	Ausc	Bibi	Bipa	Born	Cele	Dief	Ine	Japo	Marm	Mega	Moss	Nela	Nene	Obsc	Rein	Rost	Luzo
Angu		2.86	2.85	2.75	2.54	2.54	2.85	2.43	2.43	2.54	2.85	2.64	4.55	3.38	3.38	2.96	2.86	1.06	2.53
Auau	27		0.21	3.38	3.28	3.60	3.70	2.33	3.49	4.02	3.38	3.49	5.29	4.02	4.02	3.59	3.60	2.75	3.28
Ausc	27	2		3.38	3.27	3.60	3.70	2.33	3.49	4.02	3.38	3.49	5.07	3.80	3.80	3.59	3.60	2.75	3.27
Bibi	26	32	32		0.53	3.07	2.11	3.49	1.80	2.54	2.01	2.64	5.39	2.11	2.11	1.48	2.22	2.85	1.80
Bipa	24	31	31	5		3.17	2.01	2.96	1.48	2.44	1.69	2.11	5.07	2.01	2.01	1.37	2.12	2.54	1.48
Born	24	34	34	29	30		3.38	3.39	3.49	3.49	4.12	3.70	4.44	3.91	3.91	3.38	3.71	2.64	4.02
Cele	27	35	35	20	19	32		3.28	1.90	2.54	2.32	2.64	5.71	2.22	2.22	1.80	2.01	3.17	2.32
Dief	23	22	22	33	28	32	31		2.54	3.49	2.86	2.96	4.44	3.49	3.49	3.07	2.96	2.11	2.96
Inte	23	33	33	17	14	33	18	24		2.12	0.95	1.80	5.39	1.37	1.37	0.95	1.80	2.64	0.63
Japo	24	38	38	24	23	33	24	33	20		2.22	2.65	5.29	2.22	2.22	2.22	2.01	2.75	2.01
Marm	27	32	32	19	16	39	22	27	9	21		2.11	5.49	1.69	1.69	1.48	2.01	3.17	0.95
Mega	25	33	33	25	20	35	25	28	17	25	20		5.18	2.54	2.54	2.11	2.54	2.64	1.80
Moss	43	50	48	51	48	42	54	42	51	50	52	49		5.71	5.71	5.71	5.28	4.12	5.70
Nela	32	38	36	20	19	37	21	33	13	21	16	24	54		0	1.27	1.90	3.38	1.58
Nene	32	38	36	20	19	37	21	33	13	21	16	24	54	0		1.27	1.90	3.38	1.58
Obsc	28	34	34	14	13	32	17	29	9	21	14	20	54	12	12		1.91	2.96	1.16
Rein	27	34	34	21	20	35	19	28	17	19	19	24	50	18	18	18		2.86	2.01
Rost	10	26	26	27	24	25	30	20	25	26	30	25	39	32	32	28	27		2.75
Luzo	24	31	31	17	14	38	22	28	6	19	9	17	54	15	15	11	19	26	

Abbrebiations are as follows; Angu, *A. anguilla*; Auau, *A. australis australis*; Ausc, *A. australis schmidtii*; Bibi, *A. bicolor bicolor*; Bipa, *A. bicolor pacifica*; Born, *A. borneensis*; Cele, *A. malgumora*; Dief, A. dieffenbachii; Inte, *A. interioris*; Japo, *A. japonica*; Marm, *A. marmorata*; Mega, *A. megastoma*; Nela, *A. nebulosa labiata*; Nene, *A. nebulosa nebulosa*; Obsc, *A. obscura*; Rein, *A. reinhardtii*; Rost, *A. rostrata*; Luzo, *A. luzonensis*.

(B) 16S rRNA

	Angu	Auau	Ausc	Bibi	Bipa	Born	Cele	Dief	Ine	Japo	Marm	Mega	Moss	Nela	Nene	Obsc	Rein	Rost	Luzo
Angu		3.29	3.52	3.72	3.66	3.66	3.06	3.05	3.64	3.36	3.36	3.35	2.88	3.65	3.59	3.41	3.23	1.11	3.94
Auau	56		0.23	4.48	4.36	4.59	4.06	3.17	4.40	4.24	4.23	4.40	3.46	4.29	4.23	4.29	4.28	3.58	4.63
Ausc	60	4		4.53	4.42	4.82	4.11	3.34	4.45	4.47	4.35	4.40	3.64	4.29	4.23	4.34	4.34	3.82	4.69
Bibi	63	76	77		0.59	4.61	3.54	4.30	2.17	3.72	2.59	3.95	4.19	2.47	2.41	2.17	3.95	3.78	2.52
Bipa	62	74	75	10		4.79	3.48	4.24	2.23	3.60	2.65	4.01	4.19	2.59	2.41	2.23	4.01	3.60	2.58
Born	62	78	82	78	81		4.36	4.59	4.71	4.32	4.66	4.59	3.71	4.60	4.66	4.36	4.77	4.06	4.71
Cele	52	69	70	60	59	74		3.99	3.29	3.72	3.54	2.64	3.59	3.59	3.41	3.12	3.47	3.30	3.59
Dief	52	54	57	73	72	78	68		4.16	4.24	4.00	4.17	3.28	4.17	4.11	3.93	3.93	3.28	4.40
Inte	62	75	76	37	38	80	56	71		3.65	1.99	3.70	3.70	1.70	1.64	2.05	3.93	3.94	1.70
Japo	57	72	76	63	61	73	63	72	62		3.71	3.60	3.71	3.60	3.54	3.65	3.83	3.42	3.88
Marm	57	72	74	44	45	79	60	68	34	63		3.89	4.12	2.59	2.41	2.40	4.12	3.54	2.64
Mega	57	75	75	67	68	78	45	71	63	61	66		3.70	3.89	3.83	3.88	3.93	3.47	3.64
Moss	49	59	62	71	71	63	61	56	63	63	70	63		3.83	3.77	3.94	3.58	3.35	4.11
Nela	62	73	73	42	44	78	61	71	29	61	44	66	65		0.18	2.23	3.71	3.95	2.05
Nene	61	72	72	41	41	79	58	70	28	60	41	65	64	3		2.05	3.65	3.89	1.99
Obsc	58	73	74	37	38	74	53	67	35	62	41	66	67	38	35		3.41	3.65	2.40
Rein	55	73	74	67	68	81	59	67	67	65	70	67	61	63	62	58		3.58	3.99
Rost	19	61	65	64	61	69	56	56	67	58	60	59	57	67	66	62	61		4.12
Luzo	67	79	80	43	44	80	61	75	29	66	45	62	70	35	34	41	68	70	

(C) ATP6

	Angu	Auau	Ausc	Bibi	Bipa	Born	Cele	Dief	Ine	Japo	Marm	Mega	Moss	Nela	Nene	Obsc	Rein	Rost	Luzo
Angu		6.88	6.74	9.81	9.66	8.49	8.93	7.17	9.22	8.49	9.96	9.52	9.66	10.40	10.25	10.10	8.93	4.39	9.37
Auau	47		0.73	9.66	8.93	8.20	9.22	6.88	9.66	8.79	9.96	9.52	8.79	11.42	11.42	10.25	9.96	7.32	9.52
Ausc	46	5		9.81	9.37	8.49	9.37	7.03	9.66	8.79	10.10	9.66	8.79	11.42	11.42	10.25	10.10	7.47	9.52
Bibi	67	66	67		1.32	10.10	7.17	8.64	4.10	7.32	6.30	8.93	10.10	6.74	6.88	4.83	7.47	9.81	5.27
Bipa	66	61	64	9		9.81	7.61	8.49	4.39	7.47	6.74	9.37	9.96	6.88	7.03	5.56	7.91	9.37	5.27
Born	58	56	58	69	67		8.49	7.91	9.52	9.08	10.10	8.79	8.93	10.10	9.96	9.66	8.49	8.35	9.96
Cele	61	63	64	49	52	58		8.20	6.44	7.47	7.17	5.56	8.64	8.64	8.49	7.17	7.17	9.08	7.17
Dief	49	47	48	59	58	54	56		8.20	9.52	9.37	9.52	6.74	10.54	10.40	9.08	7.76	6.88	8.79
Inte	63	66	66	28	30	65	44	56		7.76	5.56	7.47	9.08	4.83	4.69	3.66	6.88	9.66	3.37
Japo	58	60	60	50	51	62	51	65	53		9.81	9.22	9.96	9.81	9.66	9.22	8.05	8.64	7.76
Marm	68	68	69	43	46	69	49	64	38	67		8.49	10.10	6.88	6.74	6.15	8.20	10.98	6.15
Mega	65	65	66	61	64	60	38	65	51	63	58		9.22	9.08	8.93	8.35	8.05	9.81	7.91
Moss	66	60	60	69	68	61	59	46	62	68	69	63		10.84	10.69	10.25	8.64	9.08	10.25
Nela	71	78	78	46	47	69	59	72	33	67	47	62	74		0.15	6.30	9.08	11.13	6.59
Nene	70	78	78	47	48	68	58	71	32	66	46	61	73	1		6.15	8.93	10.98	6.44
Obsc	69	70	70	33	38	66	49	62	25	63	42	57	70	43	42		8.49	10.69	5.12
Rein	61	68	69	51	54	58	49	53	47	55	56	55	59	62	61	58		9.08	7.76
Rost	30	50	51	67	64	57	62	47	66	59	75	67	62	76	75	73	62		10.40
Luzo	64	65	65	36	36	68	49	60	23	53	42	54	70	45	44	35	53	71	

(D) ATP8

	Angu	Auau	Ausc	Bibi	Bipa	Born	Cele	Dief	Ine	Japo	Marm	Mega	Moss	Nela	Nene	Obsc	Rein	Rost	Luzo
Angu		6.55	6.55	8.93	8.93	5.95	8.93	4.76	7.74	11.91	8.33	7.74	5.36	7.14	6.55	5.95	6.55	2.38	6.55
Auau	11		0	10.12	9.52	8.93	6.55	5.95	8.33	11.31	8.33	7.74	7.74	8.33	7.74	7.14	6.55	6.55	7.74
Ausc	11	0		10.12	9.52	8.93	6.55	5.95	8.33	11.31	8.33	7.74	7.74	8.33	7.74	7.14	6.55	6.55	7.74
Bibi	15	17	17		1.19	6.55	7.14	8.33	7.14	7.74	5.36	7.14	9.52	5.36	4.76	4.17	5.95	9.52	5.95
Bipa	15	16	16	2		5.36	6.55	7.14	5.95	7.74	5.36	6.55	8.93	5.36	4.76	4.17	5.36	10.12	5.95
Born	10	15	15	11	9		7.74	4.76	5.95	9.52	6.55	7.14	5.95	6.55	5.95	5.36	6.55	8.33	7.14
Cele	15	11	11	12	11	13		6.55	6.55	7.14	5.36	3.57	7.14	5.36	4.76	2.98	3.57	7.74	4.76
Dief	8	10	10	14	12	8	11		4.76	9.52	6.55	6.55	5.36	5.36	4.76	4.17	4.17	7.14	4.76
Inte	13	14	14	12	10	10	11	8		9.52	5.36	5.95	7.74	4.17	3.57	4.17	5.36	10.12	3.57
Japo	20	19	19	13	13	16	12	16	16		8.33	9.52	11.91	7.14	6.55	5.95	7.14	10.71	8.93
Marm	14	14	14	9	9	11	9	11	9	14		6.55	8.93	3.57	2.98	3.57	5.36	10.71	2.98
Mega	13	13	13	12	11	12	6	11	10	16	11		4.76	6.55	5.95	4.17	4.76	8.93	5.95
Moss	9	13	13	16	15	10	12	9	13	20	15	8		7.74	7.14	6.55	5.95	7.74	7.14
Nela	12	14	14	9	9	11	9	9	7	12	6	11	13		0.60	2.38	4.17	9.52	2.98
Nene	11	13	13	8	8	10	8	8	6	11	5	10	12	1		1.79	3.57	8.93	2.38
Obsc	10	12	12	7	7	9	5	7	7	10	6	7	11	4	3		1.79	7.14	2.98
Rein	11	11	11	10	9	11	6	7	9	12	9	8	10	7	6	3		7.79	3.57
Rost	4	11	11	16	17	14	13	12	17	18	18	15	13	16	15	12	13		8.93
Luzo	11	13	13	10	10	12	8	8	6	15	5	10	12	5	4	5	6	15	

(E) COI

	Angu	Auau	Ausc	Bibi	Bipa	Born	Cele	Dief	Ine	Japo	Marm	Mega	Moss	Nela	Nene	Obsc	Rein	Rost	Luzo
Angu		6.97	6.97	7.78	7.66	8.29	8.22	6.34	7.72	8.16	7.91	7.60	7.41	8.48	8.35	7.85	8.10	2.95	7.72
Auau	111		0	7.72	8.04	7.28	7.53	5.21	7.85	7.72	7.03	7.03	6.72	7.72	7.35	7.22	7.22	6.53	7.60
Ausc	111	0		7.72	8.04	7.28	7.53	5.21	7.85	7.72	7.03	7.03	6.72	7.72	7.35	7.22	7.22	6.53	7.60
Bibi	124	123	123		1.13	7.78	6.53	7.16	4.83	6.72	5.02	6.03	7.35	4.96	4.71	3.58	6.72	7.85	5.09
Bipa	122	128	128	18		7.91	6.53	6.97	4.71	6.72	4.96	5.96	7.60	4.96	4.83	3.33	6.91	7.60	4.96
Born	132	116	116	124	126		7.78	7.22	7.78	7.66	7.22	7.97	7.66	7.78	7.41	7.97	7.97	7.91	7.41
Cele	131	120	120	104	104	124		6.65	5.90	6.03	5.52	4.77	7.78	6.22	5.96	5.59	5.96	8.10	6.22
Dief	101	83	83	114	111	115	106		6.53	6.72	6.22	6.15	6.22	6.84	6.59	6.40	7.16	5.78	6.72
Inte	123	125	125	77	75	124	94	104		5.34	4.14	5.65	7.60	4.52	4.27	4.08	6.15	7.66	3.64
Japo	130	123	123	107	107	122	96	107	85		5.27	5.52	8.16	5.96	5.71	5.84	6.22	8.04	5.65
Marm	126	112	112	80	79	115	88	99	66	84		5.46	7.28	4.39	4.14	3.89	5.59	7.53	4.27
Mega	121	112	112	96	95	127	76	98	90	88	87		6.91	6.03	5.90	5.71	5.71	7.60	5.78
Moss	118	107	107	117	121	122	124	99	121	130	116	110		8.04	7.66	7.60	7.60	7.03	7.47
Nela	135	123	123	79	79	124	99	109	72	95	70	96	128		0.38	4.58	6.40	8.10	4.21
Nene	133	117	117	75	77	118	95	105	68	91	66	94	122	6		4.33	6.03	7.85	3.83
Obsc	125	115	115	57	53	127	89	102	65	93	62	91	121	73	69		6.59	7.47	4.65
Rein	129	115	115	107	110	127	95	114	98	99	89	91	121	102	96	105		7.97	6.34
Rost	47	104	104	125	121	126	129	92	122	128	120	121	112	129	125	119	127		7.78
Luzo	123	121	121	81	79	118	99	107	58	90	68	92	119	67	61	74	101	124	

(F) COII

	Angu	Auau	Ausc	Bibi	Bipa	Born	Cele	Dief	Ine	Japo	Marm	Mega	Moss	Nela	Nene	Obsc	Rein	Rost	Luzo
Angu		3.33	3.47	4.20	4.49	5.21	4.05	4.34	5.21	3.62	4.34	4.92	5.21	4.63	4.63	5.07	5.07	1.74	4.34
Auau	23		0.15	4.92	5.21	4.49	4.63	3.62	5.64	4.63	4.63	4.92	5.64	4.92	4.92	5.79	5.36	3.62	4.49
Ausc	24	1		5.07	5.36	4.63	4.49	3.76	5.79	4.78	4.78	5.07	5.79	5.07	5.07	5.93	5.50	3.76	4.63
Bibi	29	34	35		0.87	5.64	3.91	5.36	3.76	3.76	3.76	5.36	5.64	2.89	2.89	3.76	5.07	4.34	2.89
Bipa	31	36	37	6		6.22	4.05	5.36	4.05	4.20	3.91	5.21	5.93	3.18	3.18	3.76	4.92	4.63	3.47
Born	36	31	32	39	43		5.07	5.36	5.50	3.62	4.92	5.36	5.93	5.21	5.21	5.79	4.92	4.78	4.92
Cele	28	32	31	27	28	35		4.63	4.05	3.91	3.18	2.46	4.92	4.49	4.49	4.20	3.47	4.05	3.18
Dief	30	25	26	37	37	37	32		5.64	4.78	4.78	4.78	4.92	5.07	5.07	5.64	5.21	4.34	4.78
Inte	36	39	40	26	28	38	28	39		3.91	4.20	5.50	5.93	3.04	3.04	4.49	5.79	5.36	2.46
Japo	25	32	33	26	29	25	27	33	27		2.89	5.21	5.21	3.62	3.62	4.92	4.78	3.91	3.91
Marm	30	32	33	26	27	34	22	33	29	20		4.49	4.78	3.76	3.76	4.05	4.49	4.63	3.33
Mega	34	34	35	37	36	37	17	33	38	36	31		5.79	5.93	5.93	5.64	3.62	4.63	4.92
Moss	36	39	40	39	41	41	34	34	41	36	33	40		5.64	5.64	5.50	5.07	5.36	5.21
Nela	32	34	35	20	22	36	31	35	21	25	26	41	39		0	4.34	5.36	4.78	2.61
Nene	32	34	35	20	22	36	31	35	21	25	26	41	39	0		4.34	5.36	4.78	2.61
Obsc	35	40	41	26	26	40	29	39	31	34	28	39	38	30	30		5.21	5.07	4.05
Rein	35	37	38	35	34	34	24	36	40	33	31	25	35	37	37	36		4.49	5.21
Rost	12	25	26	30	32	33	28	30	37	27	32	32	37	33	33	35	31		4.63
Luzo	30	31	32	20	24	34	22	33	17	27	23	34	36	18	18	28	36	32	

(G) COIII

	Angu	Auau	Ausc	Bibi	Bipa	Born	Cele	Dief	Ine	Japo	Marm	Mega	Moss	Nela	Nene	Obsc	Rein	Rost	Luzo
Angu		7.39	7.52	7.39	6.75	7.77	7.77	7.01	7.01	6.62	6.50	7.39	7.77	6.62	6.88	7.26	7.64	2.04	7.13
Auau	58		0.38	6.24	6.24	7.13	6.24	5.73	6.12	6.75	5.61	6.75	7.01	6.37	6.12	5.86	5.99	6.37	6.12
Ausc	59	3		6.37	6.37	7.26	6.37	5.86	6.24	6.88	5.73	6.88	7.13	6.50	6.24	5.99	6.12	6.50	5.99
Bibi	58	49	50		1.91	6.12	6.37	6.24	2.68	5.10	3.82	5.73	7.01	3.95	3.95	3.82	5.61	6.88	3.95
Bipa	53	49	50	15		5.99	5.86	5.99	2.68	5.22	3.31	5.99	6.75	3.69	3.69	3.57	5.61	6.24	3.95
Born	61	56	57	48	47		7.26	6.62	6.12	7.39	6.37	7.01	8.28	6.24	6.50	6.12	6.75	6.88	6.37
Cele	61	49	50	50	46	57		6.88	4.84	5.73	4.71	3.69	7.90	4.97	4.71	5.35	5.99	7.52	5.22
Dief	55	45	46	49	47	52	54		5.73	6.50	6.24	7.13	6.62	5.86	5.86	5.99	6.50	5.99	5.86
Inte	55	48	49	21	21	48	38	45		5.10	2.55	4.33	6.50	3.19	3.19	2.93	5.35	6.50	3.44
Japo	52	53	54	40	41	58	45	51	40		5.10	5.73	6.88	4.33	4.33	5.10	4.59	6.88	5.48
Marm	51	44	45	30	26	50	37	49	20	40		4.71	7.01	3.19	3.19	2.42	4.97	6.50	3.19
Mega	58	53	54	45	47	55	29	56	34	45	37		6.88	4.97	5.22	4.84	5.10	7.13	5.48
Moss	61	55	56	55	53	65	62	52	51	54	55	54		7.01	7.01	7.39	6.37	7.39	7.64
Nela	52	50	51	31	29	49	39	46	25	34	25	39	55		0.26	3.82	5.10	6.37	3.06
Nene	54	48	49	31	29	51	37	46	25	34	25	41	55	2		3.82	5.10	6.62	3.06
Obsc	57	46	47	30	28	48	42	47	23	40	19	38	58	30	30		5.22	7.01	3.31
Rein	60	47	48	44	44	53	47	51	42	36	39	40	50	40	40	41		6.88	5.22
Rost	16	50	51	54	49	54	59	47	51	54	51	56	58	50	52	55	54		6.62
Luzo	56	48	47	31	31	50	41	46	27	43	25	43	60	24	24	26	41	52	

(H) cyt *b*

	Angu	Auau	Ausc	Bibi	Bipa	Born	Cele	Dief	Ine	Japo	Marm	Mega	Moss	Nela	Nene	Obsc	Rein	Rost	Luzo
Angu		7.98	7.90	9.39	9.12	8.33	9.91	6.93	8.86	9.21	8.68	10.26	8.86	9.21	9.21	8.86	8.86	4.21	8.51
Auau	91		0.18	8.95	8.60	7.72	8.33	5.70	8.16	8.60	7.98	8.51	7.46	8.60	8.60	9.39	7.54	7.98	8.86
Ausc	90	2		8.86	8.51	7.81	8.25	5.61	8.07	8.68	7.90	8.42	7.46	8.51	8.51	9.30	7.46	7.90	8.77
Bibi	107	102	101		2.11	9.04	7.11	7.46	5.97	8.60	6.40	8.33	8.77	6.67	6.67	6.32	7.19	8.77	6.84
Bipa	104	98	97	24		8.86	7.19	7.37	5.79	8.25	6.58	8.68	9.04	6.67	6.67	6.14	7.37	8.86	6.75
Born	95	88	89	103	101		8.60	6.93	7.63	8.42	7.90	9.56	8.25	8.60	8.60	8.25	7.98	8.68	8.16
Cele	113	95	94	81	82	98		6.14	6.58	6.58	6.49	6.14	9.47	6.58	6.58	7.54	6.49	9.12	7.37
Dief	79	65	64	85	84	79	70		6.93	7.11	7.37	7.81	7.11	6.93	6.93	7.63	6.67	6.84	7.11
Inte	101	93	92	68	66	87	75	79		7.37	4.30	7.46	8.68	5.00	5.00	5.61	6.23	8.95	3.60
Japo	105	98	99	98	94	96	75	81	84		6.67	7.37	8.77	6.93	6.93	7.98	5.79	8.86	7.54
Marm	99	91	90	73	75	90	74	84	49	76		7.19	8.42	5.35	5.35	5.88	5.09	8.42	5.09
Mega	117	97	96	95	99	109	70	89	85	84	82		9.12	7.63	7.63	8.51	7.54	10.35	8.60
Moss	101	85	85	100	103	94	108	81	99	100	96	104		9.12	9.12	9.30	7.98	8.51	9.04
Nela	105	98	97	76	76	98	75	79	57	79	61	87	104		0	5.97	6.23	8.77	5.18
Nene	105	98	97	76	76	98	75	79	57	79	61	87	104	0		5.97	6.23	8.77	5.18
Obsc	101	107	106	72	70	94	86	87	64	91	67	97	106	68	68		6.93	8.60	5.53
Rein	101	86	85	82	84	91	74	76	71	66	58	86	91	71	71	79		8.68	6.67
Rost	48	91	90	100	101	99	104	78	102	101	96	118	97	100	100	98	99		8.77
Luzo	97	101	100	78	77	93	84	81	41	86	58	98	103	59	59	63	76	100	

(I) ND1

	Angu	Auau	Ausc	Bibi	Bipa	Born	Cele	Dief	Ine	Japo	Marm	Mega	Moss	Nela	Nene	Obsc	Rein	Rost	Luzo
Angu		5.86	5.97	8.54	8.13	8.33	8.44	6.79	8.23	9.26	8.85	8.44	7.72	8.23	8.64	8.54	7.51	3.09	8.64
Auau	57		0.51	7.61	7.92	7.00	7.41	6.17	7.51	8.33	8.23	7.31	7.72	7.20	7.41	7.82	7.10	6.48	7.61
Ausc	58	5		7.72	8.13	7.10	7.82	6.28	7.61	8.13	8.33	7.10	7.82	7.41	7.61	8.03	7.31	6.79	8.03
Bibi	83	74	75		1.75	8.95	7.41	7.61	6.17	7.41	6.38	7.72	8.33	5.56	5.76	4.94	6.28	8.23	6.28
Bipa	79	77	79	17		8.85	7.10	7.92	5.86	7.00	6.38	7.72	8.33	5.45	5.66	4.22	5.97	7.82	5.86
Born	81	68	69	87	86		6.89	6.89	7.72	9.26	8.44	8.03	9.05	7.92	8.33	8.44	8.75	7.82	8.33
Cele	82	72	76	72	69	67		7.10	6.38	7.20	7.20	5.45	9.16	7.10	7.31	6.58	6.69	7.41	6.69
Dief	66	60	61	74	77	67	69		6.48	7.72	7.31	7.82	6.48	6.69	7.10	6.89	7.20	5.76	7.00
Inte	80	73	74	60	57	75	62	63		7.20	5.66	5.97	8.13	5.25	5.45	5.14	7.10	7.72	3.81
Japo	90	81	79	72	68	90	70	75	70		7.92	7.31	8.44	7.31	7.31	6.28	7.31	7.82	7.92
Marm	86	80	81	62	62	82	70	71	55	77		7.51	8.23	4.73	5.25	5.86	6.58	8.23	5.45
Mega	82	71	69	75	75	78	53	76	58	71	73		8.95	6.48	6.69	7.20	7.41	7.61	6.58
Moss	75	75	76	81	81	88	89	63	79	82	80	87		8.03	8.54	7.72	7.92	7.51	7.92
Nela	80	70	72	54	53	77	69	65	51	71	46	63	78		1.03	5.04	6.17	7.10	5.14
Nene	84	72	74	56	55	81	71	69	53	71	51	65	83	10		5.25	6.17	7.51	5.35
Obsc	83	76	78	48	41	82	64	67	50	61	57	70	75	49	51		6.48	7.41	5.35
Rein	73	69	71	61	58	85	65	70	69	71	64	72	77	60	60	63		7.31	6.58
Rost	30	63	66	80	76	76	72	56	75	76	80	74	73	69	73	72	71		8.13
Luzo	84	74	78	61	57	81	65	68	37	77	53	64	77	50	52	52	64	79	

(J) ND2

	Angu	Auau	Ausc	Bibi	Bipa	Born	Cele	Dief	Ine	Japo	Marm	Mega	Moss	Nela	Nene	Obsc	Rein	Rost	Luzo
Angu		7.18	7.08	10.14	11.10	8.71	8.04	8.80	8.61	7.94	9.47	7.37	10.81	7.94	7.85	8.80	8.61	3.64	8.80
Auau	75		0.29	8.71	10.05	7.46	6.41	6.60	6.89	6.79	7.94	6.89	8.52	6.70	6.60	6.99	7.56	7.08	7.37
Ausc	74	3		8.71	10.05	7.56	6.51	6.51	6.99	6.70	8.04	6.99	8.42	6.79	6.70	7.08	7.46	6.99	7.46
Bibi	106	91	91		3.64	10.05	8.52	10.62	7.18	8.52	7.94	7.27	11.87	7.18	7.27	6.41	8.80	10.81	6.99
Bipa	116	105	105	38		10.62	9.57	11.58	8.04	9.38	9.19	8.52	13.11	8.23	8.33	7.66	10.24	11.48	8.04
Born	91	78	79	105	111		8.04	9.00	6.99	8.04	7.94	7.27	9.28	6.89	6.99	8.04	8.42	8.61	7.85
Cele	84	67	68	89	100	84		8.33	6.03	5.65	6.41	5.07	9.95	5.84	5.74	5.84	6.89	8.04	5.74
Dief	92	69	68	111	121	94	87		8.61	8.33	9.09	9.00	9.86	8.52	8.61	9.00	9.28	8.42	8.90
Inte	90	72	73	75	84	73	63	90		6.70	4.79	5.55	9.67	4.40	4.31	4.69	6.89	8.80	3.73
Japo	83	71	70	89	98	84	59	87	70		7.08	5.84	8.42	6.51	6.41	6.79	7.08	7.66	6.12
Marm	99	83	84	83	96	83	67	95	50	74		6.60	10.43	4.69	4.59	5.07	7.85	9.38	5.07
Mega	77	72	73	76	89	76	53	94	58	61	69		9.09	4.98	4.88	5.93	6.12	8.13	5.36
Moss	113	89	88	124	137	97	104	103	101	88	109	95		9.67	9.38	9.86	9.95	10.81	9.67
Nela	83	70	71	75	86	72	61	89	46	68	49	52	101		0.29	5.36	6.60	8.33	3.92
Nene	82	69	70	76	87	73	60	90	45	67	48	51	98	3		5.26	6.70	8.23	3.83
Obsc	92	73	74	67	80	84	61	94	49	71	53	62	103	56	55		7.37	8.52	4.98
Rein	90	79	78	92	107	88	72	97	72	74	82	64	104	69	70	77		8.90	7.18
Rost	38	74	73	113	120	90	84	88	92	80	98	85	113	87	86	89	93		9.09
Luzo	92	77	78	73	84	82	60	93	39	64	53	56	101	41	40	52	75	95	

(K) ND3

	Angu	Auau	Ausc	Bibi	Bipa	Born	Cele	Dief	Ine	Japo	Marm	Mega	Moss	Nela	Nene	Obsc	Rein	Rost	Luzo
Angu		10.32	10.03	10.32	10.60	9.46	10.32	8.02	8.88	8.88	10.32	9.46	11.46	10.32	10.32	9.46	8.60	3.15	9.74
Auau	36		0.29	11.46	10.89	10.89	11.18	6.59	9.74	10.89	8.88	10.03	11.75	10.03	10.03	9.74	8.88	9.46	9.46
Ausc	35	1		11.18	10.60	10.60	10.89	6.88	9.46	10.60	8.60	9.74	11.46	9.74	9.74	9.46	8.60	9.17	9.17
Bibi	36	40	39		2.01	12.03	9.74	9.74	4.01	8.88	4.87	8.60	10.32	5.16	5.16	4.87	7.45	8.88	4.87
Bipa	37	38	37	7		10.60	8.88	9.17	4.59	8.88	4.59	8.31	9.46	5.44	5.44	4.01	7.16	9.74	5.44
Born	33	38	37	42	37		10.89	8.88	12.03	10.32	11.75	11.18	9.74	11.75	11.75	10.60	10.32	10.32	12.32
Cele	36	39	38	34	31	38		8.88	7.74	8.02	8.31	6.59	10.60	7.45	7.45	8.02	5.73	9.46	9.17
Dief	28	23	24	34	32	31	31		8.60	7.16	8.31	8.02	9.46	8.31	8.31	7.74	5.16	7.74	8.31
Inte	31	34	33	14	16	42	27	30		7.74	4.01	8.31	9.74	3.73	3.73	3.44	6.59	8.02	3.44
Japo	31	38	37	31	31	36	28	25	27		8.88	6.88	10.03	7.16	7.16	7.74	6.30	8.60	7.45
Marm	36	31	30	17	16	41	29	29	14	31		8.31	9.46	4.30	4.30	4.01	6.59	8.88	3.73
Mega	33	35	34	30	29	39	23	28	29	24	29		10.32	7.45	7.45	8.02	6.02	9.17	8.02
Moss	40	41	40	36	33	34	37	33	34	35	33	36		8.88	8.88	8.88	8.88	10.32	9.17
Nela	36	35	34	18	19	41	26	29	13	25	15	26	31		0	3.73	6.02	9.46	3.44
Nene	36	35	34	18	19	41	26	29	13	25	15	26	31	0		3.73	6.02	9.46	3.44
Obsc	33	34	33	17	14	37	28	27	12	27	14	28	31	13	13		6.59	8.60	2.87
Rein	30	31	30	26	25	36	20	18	23	22	23	21	31	21	21	23		8.31	6.59
Rost	11	33	32	31	34	36	33	27	28	30	31	32	36	33	33	30	29		8.31
Luzo	34	33	32	17	19	43	32	29	12	26	13	28	32	12	12	10	23	29	

(L) ND4

	Angu	Auau	Ausc	Bibi	Bipa	Born	Cele	Dief	Ine	Japo	Marm	Mega	Moss	Nela	Nene	Obsc	Rein	Rost	Luzo
Angu		10.28	10.21	9.49	9.27	10.57	9.63	8.40	8.98	8.69	8.98	9.34	9.41	10.36	10.36	8.98	8.76	4.49	9.63
Auau	142		0.36	9.20	9.20	9.34	8.76	7.02	8.83	8.62	8.98	8.55	9.92	10.50	10.28	8.55	8.91	8.98	9.70
Ausc	141	5		9.20	9.20	9.56	8.76	7.02	8.69	8.69	8.83	8.40	9.99	10.50	10.28	8.55	8.98	8.91	9.70
Bibi	131	127	127		1.88	8.91	7.24	8.18	5.43	7.68	6.23	7.82	9.12	6.23	6.08	4.92	7.10	8.69	5.65
Bipa	128	127	127	26		8.98	6.88	7.82	5.00	7.75	5.50	7.17	9.34	5.65	5.50	4.06	7.02	8.18	5.00
Born	146	129	132	123	124		9.20	9.12	8.47	9.56	9.20	9.78	9.99	9.63	9.34	9.34	9.27	10.21	10.14
Cele	133	121	121	100	95	127		8.04	7.24	7.17	7.17	6.08	8.76	8.47	8.04	7.31	6.95	8.40	7.53
Dief	116	97	97	113	108	126	111		8.18	8.04	8.18	8.18	8.62	9.20	9.27	7.68	7.53	7.68	8.18
Inte	124	122	120	75	69	117	100	113		6.66	3.84	6.66	8.91	5.58	5.43	4.06	6.37	8.40	4.27
Japo	120	119	120	106	107	132	99	111	92		6.81	6.73	9.12	8.69	8.76	6.81	6.52	7.24	7.31
Marm	124	124	122	86	76	127	99	113	53	94		7.17	8.91	5.29	5.21	4.13	7.60	8.26	5.21
Mega	129	118	116	108	99	135	84	113	92	93	99		9.27	8.47	8.11	6.81	6.37	8.76	7.60
Moss	130	137	138	126	129	138	121	119	123	126	123	128		9.56	9.70	9.12	8.91	9.34	9.34
Nela	143	145	145	86	78	133	117	127	77	120	73	117	132		0.72	5.29	8.33	9.70	6.37
Nene	143	142	142	84	76	129	111	128	75	121	72	112	134	10		5.00	8.18	9.56	6.37
Obsc	124	118	118	68	56	129	101	106	56	94	57	94	126	73	69		7.39	8.55	4.71
Rein	121	123	124	98	97	128	96	104	88	90	105	88	123	115	113	102		8.40	7.60
Rost	62	124	123	120	113	141	116	106	116	100	114	121	129	134	132	118	116		9.20
Luzo	133	134	134	78	69	140	104	113	59	101	72	105	129	88	88	65	105	127	

M) ND4L

	Angu	Auau	Ausc	Bibi	Bipa	Born	Cele	Dief	Ine	Japo	Marm	Mega	Moss	Nela	Nene	Obsc	Rein	Rost	Luzo
Angu		7.07	7.07	8.42	8.75	8.42	7.07	5.39	8.75	8.42	8.08	6.06	10.44	8.08	8.08	9.43	8.42	2.69	7.41
Auau	21		0	8.08	8.42	10.10	5.72	7.41	8.08	8.42	7.41	5.39	8.75	7.41	7.41	8.08	5.72	7.41	8.08
Ausc	21	0		8.08	8.42	10.10	5.72	7.41	8.08	8.42	7.41	5.39	8.75	7.41	7.41	8.08	5.72	7.41	8.08
Bibi	25	24	24		1.01	8.08	7.41	8.08	4.38	7.41	4.04	5.39	9.76	3.37	3.37	2.69	7.07	9.43	4.04
Bipa	26	25	25	3		8.75	8.08	8.42	5.05	7.41	4.38	5.72	10.10	3.70	3.70	3.37	7.41	9.09	4.38
Born	25	30	30	24	26		8.75	8.08	8.75	9.09	8.75	7.41	12.12	9.09	9.09	9.43	8.42	9.43	9.09
Cele	21	17	17	22	24	26		6.40	6.40	5.72	6.40	4.38	7.74	6.40	6.40	6.40	5.39	7.74	7.07
Dief	16	22	22	24	25	24	19		8.08	6.40	6.40	4.04	8.08	7.41	7.41	8.42	7.74	6.73	7.41
Inte	26	24	24	13	15	26	19	24		8.08	3.37	5.39	10.10	3.37	3.37	5.05	7.07	8.75	3.37
Japo	25	25	25	22	22	27	17	19	24		6.73	6.40	8.42	6.40	6.40	7.07	7.74	7.74	7.74
Marm	24	22	22	12	13	26	19	19	10	20		4.71	8.42	3.37	3.37	4.38	5.72	8.08	3.37
Mega	18	16	16	16	17	22	13	12	16	19	14		8.08	4.71	4.71	5.72	5.72	6.73	5.39
Moss	31	26	26	29	30	36	23	24	30	25	25	24		8.75	8.75	9.43	9.76	10.77	10.10
Nela	24	22	22	10	11	27	19	22	10	19	10	14	26		0	4.38	7.07	8.75	3.37
Nene	24	22	22	10	11	27	19	22	10	19	10	14	26	0		4.38	7.07	8.75	3.37
Obsc	28	24	24	8	10	28	19	25	15	21	13	17	28	13	13		7.07	10.44	5.05
Rein	25	17	17	21	22	25	16	23	21	23	17	17	29	21	21	21		8.08	7.07
Rost	8	22	22	28	27	28	23	20	26	23	24	20	32	26	26	31	24		7.41
Luzo	22	24	24	12	13	27	21	22	10	23	10	16	30	10	10	15	21	22	

(N) ND5

	Angu	Auau	Ausc	Bibi	Bipa	Born	Cele	Dief	Ine	Japo	Marm	Mega	Moss	Nela	Nene	Obsc	Rein	Rost	Luzo
Angu		8.42	8.58	10.10	9.72	9.72	9.66	7.17	9.50	8.47	9.66	9.07	8.96	9.56	9.50	9.12	8.69	3.26	9.28
Auau	155		0.49	9.07	9.28	8.31	8.25	6.24	8.74	7.76	8.69	8.69	8.47	9.18	9.39	8.74	8.36	8.58	8.58
Ausc	158	9		9.12	9.45	8.74	8.58	6.35	8.80	8.09	8.90	8.90	8.63	9.28	9.50	8.74	8.58	8.74	8.80
Bibi	186	167	168		1.95	9.45	7.44	8.63	5.21	7.66	5.92	7.38	8.90	6.08	6.24	5.32	7.06	10.10	5.54
Bipa	179	171	174	36		9.61	7.66	8.63	4.78	7.82	5.81	7.55	8.58	5.97	6.14	5.00	6.79	9.83	5.38
Born	179	153	161	174	177		8.80	8.14	9.12	8.52	8.69	9.66	9.45	9.39	9.61	9.50	8.52	9.88	9.18
Cele	178	152	158	137	141	162		7.87	7.17	6.84	7.22	5.97	8.96	7.44	7.55	7.71	6.90	9.72	6.95
Dief	132	115	117	159	159	150	145		8.25	7.06	7.60	7.55	7.22	8.31	8.52	8.09	7.76	7.76	7.60
Inte	175	161	162	96	88	168	132	152		7.66	4.72	6.73	8.74	4.67	4.72	5.00	7.06	9.61	3.96
Japo	156	143	149	141	144	157	126	130	141		6.84	7.17	8.58	7.87	8.09	7.76	6.90	8.80	7.06
Marm	178	160	164	109	107	160	133	140	87	126		6.68	8.20	5.05	5.21	5.97	6.84	9.61	4.78
Mega	167	160	164	136	139	178	110	139	124	132	123		8.25	7.00	7.22	7.55	7.06	9.34	7.00
Moss	165	156	159	164	158	174	165	133	161	158	151	152		8.96	9.18	8.36	7.93	9.01	8.42
Nela	176	169	171	112	110	173	137	153	86	145	93	129	165		0.49	5.86	7.28	9.61	4.18
Nene	175	173	175	115	113	177	139	157	87	149	96	133	169	9		6.03	7.49	9.66	4.34
Obsc	168	161	161	98	92	175	142	149	92	143	110	139	154	108	111		7.55	9.56	5.86
Rein	160	154	158	130	125	157	127	143	130	127	126	130	146	134	138	139		8.47	6.90
Rost	60	158	161	186	181	182	179	143	177	162	177	172	166	177	178	176	156		9.28
Luzo	171	158	162	102	99	169	128	140	73	130	88	129	155	77	80	108	127	171	

(O) ND6

	Angu	Auau	Ausc	Bibi	Bipa	Born	Cele	Dief	Ine	Japo	Marm	Mega	Moss	Nela	Nene	Obsc	Rein	Rost	Luzo
Angu		6.13	5.94	6.90	6.51	7.47	5.94	5.36	6.90	8.43	7.28	6.32	8.05	6.32	5.94	6.90	7.09	2.87	6.90
Auau	32		0.19	7.47	7.28	7.47	7.28	7.28	7.28	9.20	8.43	7.66	6.71	7.09	6.71	7.28	7.66	6.71	7.28
Ausc	31	1		7.28	7.09	7.28	7.09	7.09	7.09	9.00	8.24	7.47	6.51	6.90	6.51	7.09	7.47	6.51	7.09
Bibi	36	39	38		2.11	8.24	6.51	7.47	2.87	8.43	4.60	6.13	6.51	5.36	4.98	3.64	6.32	7.47	4.02
Bipa	34	38	37	11		7.28	6.32	7.28	2.68	7.85	4.22	5.94	5.36	4.60	4.22	2.68	5.75	7.47	3.45
Born	39	39	38	43	38		8.24	6.51	6.90	8.24	7.09	7.85	7.47	7.85	7.47	7.47	8.24	8.81	6.32
Cele	31	38	37	34	33	43		6.90	5.94	7.85	6.32	4.79	7.09	5.94	5.56	6.32	6.13	7.47	5.56
Dief	28	38	37	39	38	34	36		6.71	8.24	8.05	7.28	7.85	8.05	7.66	6.90	6.90	6.51	6.90
Inte	36	38	37	15	14	36	31	35		7.47	3.83	5.94	6.51	4.41	4.02	2.68	5.36	7.85	2.68
Japo	44	48	47	44	41	43	41	43	39		7.85	7.09	9.39	8.05	7.66	7.47	7.66	9.39	7.09
Marm	38	44	43	24	22	37	33	42	20	41		6.90	7.47	4.60	4.22	3.83	7.47	8.24	2.30
Mega	33	40	39	32	31	41	25	38	31	37	36		7.28	6.51	6.13	6.32	6.13	7.66	5.56
Moss	42	35	34	34	28	39	37	41	34	49	39	38		7.47	7.09	6.71	8.05	8.62	6.71
Nela	33	37	36	28	24	41	31	42	23	42	24	34	39		0.58	4.22	6.13	8.05	4.22
Nene	31	35	34	26	22	39	29	40	21	40	22	32	37	3		3.83	5.75	7.66	3.83
Obsc	36	38	37	19	14	39	33	36	14	39	20	33	35	22	20		5.94	7.66	2.68
Rein	37	40	39	33	30	43	32	36	28	40	39	32	42	32	30	31		8.43	5.75
Rost	15	35	34	39	39	46	39	34	41	49	43	40	45	42	40	40	44		7.85
Luzo	36	38	37	21	18	33	29	36	14	37	12	29	35	22	20	14	30	41	

(P) tRNAs

	Angu	Auau	Ausc	Bibi	Bipa	Born	Cele	Dief	Ine	Japo	Marm	Mega	Moss	Nela	Nene	Obsc	Rein	Rost	Luzo
Angu		2.05	2.05	2.62	2.49	2.11	2.62	1.79	2.94	2.94	2.56	2.56	2.94	3.07	3.00	2.75	2.75	0.26	3.01
Auau	32		0	3.13	3.00	2.49	2.68	2.05	2.81	3.20	2.69	2.49	3.26	3.20	3.13	2.81	3.07	1.92	3.13
Ausc	32	0		3.13	3.00	2.49	2.68	2.05	2.81	3.20	2.69	2.49	3.26	3.20	3.13	2.81	3.07	1.92	3.13
Bibi	41	49	49		0.51	3.07	2.05	3.20	1.34	2.43	1.22	1.73	3.32	1.47	1.41	1.22	2.56	2.49	1.54
Bipa	39	47	47	8		3.20	2.17	3.14	1.34	2.56	1.09	1.98	3.07	1.34	1.28	1.22	2.56	2.37	1.54
Born	33	39	39	48	50		3.07	2.69	3.39	3.39	3.13	3.00	3.26	3.64	3.58	3.39	3.32	1.98	3.58
Cele	41	42	42	32	34	48		2.75	1.92	2.11	1.47	0.96	2.81	2.17	2.11	2.05	2.05	2.49	1.86
Dief	28	32	32	50	49	42	43		3.13	3.01	2.88	2.69	2.82	3.26	3.20	3.14	2.88	1.66	3.27
Inte	46	44	44	21	21	53	30	49		2.62	0.90	1.73	3.45	1.15	1.09	0.90	2.30	2.81	0.77
Japo	46	50	50	38	40	53	33	47	41		2.24	1.92	3.58	2.62	2.56	2.49	2.75	2.81	2.50
Marm	40	42	42	19	17	49	23	45	14	35		1.41	3.13	0.90	0.83	0.90	2.37	2.43	0.96
Mega	40	39	39	27	31	47	15	42	27	30	22		3.13	1.98	1.92	1.85	2.11	2.43	1.79
Moss	46	51	51	52	48	51	44	44	54	56	49	49		3.58	3.52	3.58	3.32	2.81	3.71
Nela	48	50	50	23	21	57	34	51	18	41	14	31	56		0.32	0.90	2.81	2.94	1.28
Nene	47	49	49	22	20	56	33	50	17	40	13	30	55	5		0.83	2.75	2.88	1.22
Obsc	43	44	44	19	19	53	32	49	14	39	14	29	56	14	13		2.69	2.62	1.02
Rein	43	48	48	40	40	52	32	45	36	43	37	33	52	44	43	42		2.62	2.56
Rost	4	30	30	39	37	31	39	26	44	44	38	38	44	46	45	41	41		3.01
Luzo	47	49	49	24	24	56	29	51	12	39	15	28	58	20	19	16	40	47	

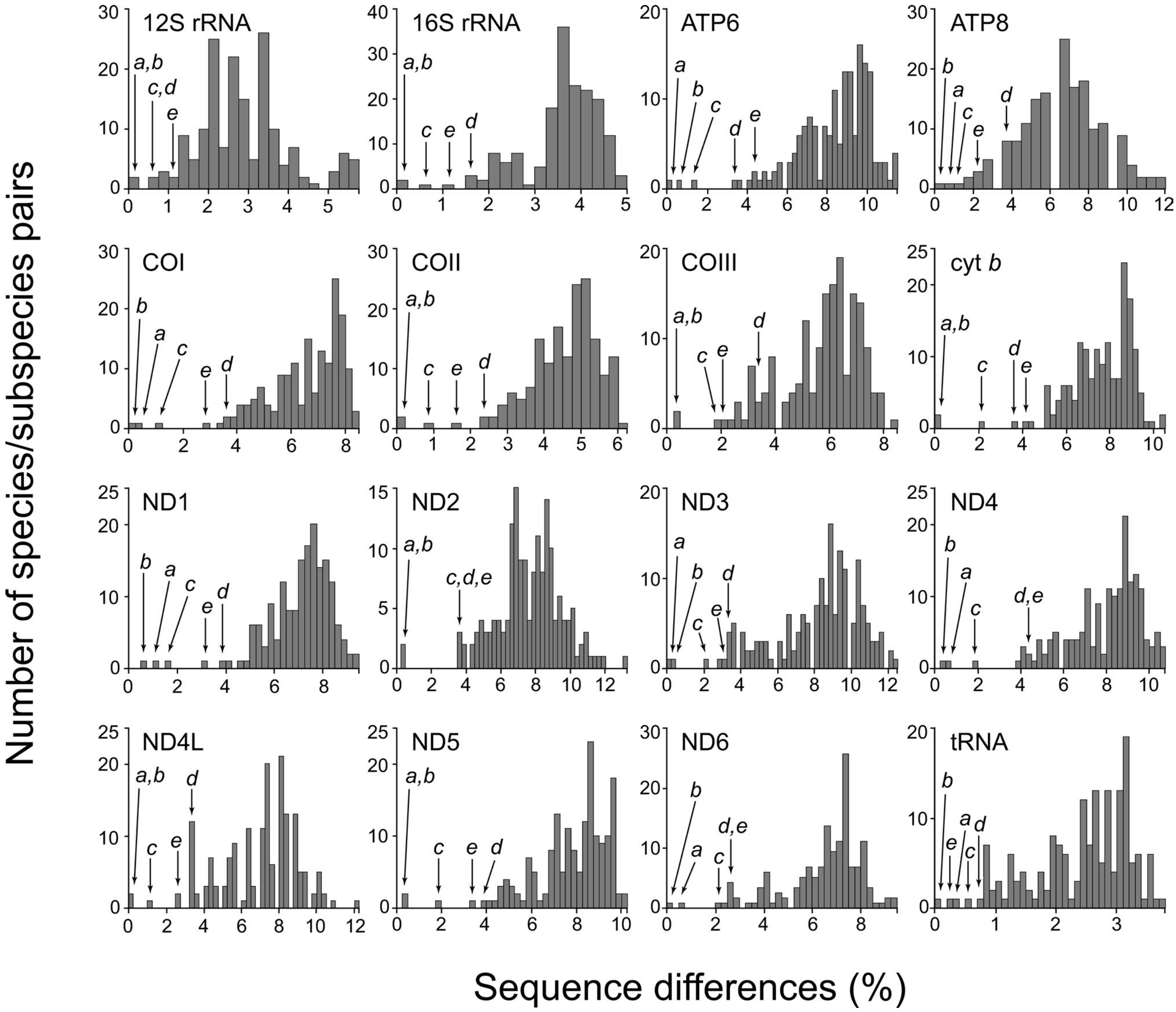

Fig. 1. Distributions of sequence differences for all 19 species and subspecies of the genus *Anguilla*, including *A. luzonensis*, for 13 protein-coding, two rRNA genes and concatenated tRNA genes of mitochondrial genome. In each panel, a shows comparisons between the two subspecies of *A. nebulosa*, *b* for the two subspecies of *A. australis*, *c* for the two subspecies of *A. bicolor*, *d* for the *A. luzonensis* and *A. interioris* pair, and *e* includes the *A. anguilla* and *A. rostrata* pair.

Discussion

The present study examined whole mtDNA sequences of all species and subspecies in the genus *Anguilla* along with a newly described species for the first time, and found anguillid species to have typical mtDNA characteristics comparing to other vertebrates and bony fishes as reported in *A. japonica* (Inoue et al. 2001a). It was also revealed that basic characteristics of mtDNA such as length of each gene and gene order are common among all anguillid eels. This suggests that general mtDNA characteristics are likely very conservative at a genus level. In contrast, gene order rearrangement in mitochondrial genomes has been reported in various higher leveled taxa like families, orders, and classes (e.g., Macey et al. 1997, Boore 1999, Inoue et al. 2001b, Peng et al. 2005, Perseke et al. 2008). In this case, such particularity may be also shared among closely related species, for example among their congeners.

The mitochondrial genomes of the genus *Anguilla* demonstrated clear divergences between species and subspecies. This indicated that the all previously recognized 18 species/subspecies of the genus and recently described *A. luzonensis* diverged enough to be considered as different species as they stand so far based on their morphological characters. In 16S rRNA and cyt *b* genes, which are most frequently used as taxonomic characters and for genetic species identification in this genus, sequence differences found in this study were almost same as reported in Aoyama et al. (2001), except cyt *b* between the two subspecies of *A. bicolor* (4.6%). Since this difference between the two studies was unlikely caused by misidentification of species, a possible explanation is that the two subspecies of *A. bicolor* have already diverged at species level. Indeed, sequence differences of ND2 and tRNA between two subspecies of *A. bicolor* reached interspecific divergence level, and COIII also showed divergences that were close to interspecific variations (Table 2). Genetic divergence status of the *A. bicolor* subspecies will be determined by examining their sequence differences carefully with more specimens.

Among all possible species pairs, *A. anguilla* and *A. rostrata* were found to have the smallest divergence as has been well known. In the present study, the mtDNA divergence between *A. luzonensis* and *A. interioris* was appeared to be roughly same as between *A. anguilla* and *A. rostrata*. On the other hand, genetic differences between the two subspecies of *A. australis* were very slight as previously suggested (Aoyama et al. 1999b, 2000, 2001, Watanabe et al. 2004, 2005). Moreover, it was revealed that the genetic variation between two subspecies of *A. nebulosa* is also as small as that of *A. australis*. Therefore, according to mtDNA divergences, there is likely a clear hierarchical structure in the genus *Anguilla* that the *A. australis* and *A. nebulosa* subspecies had the smallest differences in the genus, then the *A. bicolor* subspecies, *A. anguilla* and *A. rostrata* pair, *A. luzonensis* and *A. interioris* pair, and the other species pairs in order.

The present study showed the genetic variations of whole mitochondrial genome sequences for all 19 species and subspecies of the genus *Anguilla* and suggested that *A. luzonensis* is likely genetically valid as a new anguillid species. The inter-species and subspecies genetic divergence of the whole mtDNA sequences revealed in this study could provide the fundamental information for the future revision and agreement of the taxonomy of the genus *Anguilla* based on the molecular characteristics of each species or subspecies.

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