

Supplementary materials

Table S1. Primers used for the amplification in the mitochondrial genomes of *Saurogobio dabryi* and *S. punctatus* in this study.

Primer	Size (bp)	Annealing temperature (°C)	Primer sequence (5'-3')
<i>S. dabryi</i>			
Sd01	1977	58	F: TCGTGGCAGACATACTGATCC R: TGGGTTGTGCCCATTGTC
Sd02	434	57	F: CGCCAGGGTACTACGAGCAT R: TTACGACTTGCCTCCCCTTG
Sd03	1527	58	F: CAGCTTACCCTGTGAAGGCA R: CCGCTTCTGCACGGATAGAT
Sd04	1702	58	F: GAAAGGGAAGGAAGCTCGGCA R: CAGGAGGGCAGCTTTGGTTA
Sd05	1875	58	F: GGCCCTATCAAGCCTAGCAG R: GGTACGGGTAAAAGGCCCA
Sd06	1473	55	F: CCCTTGACCCAACCCTCTTA R: AGCGCCTAAAATCGATGAAACA
Sd07	1861	57	F: ACTAATGATCGGAGCGCCTG R: TCGAAATTGGCCCCGGTGTA
Sd08	1714	56	F: CCGTTCTGCCAGCTGTAATC R: AGGCTCAGAAAAACCCGAGG
Sd09	919	57	F: CCAAGCACACGCATTTCACA R: TCAAAATGAGACGACGGCCA
Sd10	411	58	F: AGCTGCTGCCTGATATTGACAC R: GTTCCTGCTGGGTTGTGGAG
Sd11	1825	57	F: TGGCCGTCGTCTCATTTTGA R: ATCAGTTGTATAGGGCGGCG
Sd12	1683	58	F: GGCTCCCTGCCACTTTTAGT R: GACCAATATCCCCTGCTCGG
Sd13	1418	57	F: GGGCGTCGGTATTATGTCCTT R: GGGGGTTAAGTCGTGGTACTC
Sd14	449	57	F: GTTGCCACCAAAGTCGACC R: TGGGGAAGTCGTTCTGTTGC
Sd15	1679	58	F: ACCCTGACCTTAGCCATCCT R: CCGATGATGACGTAGGGGTG
Sd16	421	58	F: TTTGCCTACGCCATCCTACG R: GCCAGGGGTGGGAGTTAAAA
<i>S. punctatus</i>			
Sp01	1130	57	F: TCCTGGCATATGGTTACTGGTG R: GGTGAGGTTTAAACGGGGGTT
Sp02	1261	55	F: ACCCTAGCTGAAACAAACCG R: GTGAGAAATGCTGCTGAGGTC
Sp03	1449	59	F: CCCTCATCATCCAAACAGCCC R: GTCTACGGATGCTCCGGCA
Sp04	929	55	F: TCATTCTCATCCCCCTAGCAG R: GACAAGGATAACAGGATTCGGTG

Table S2. Characteristics of the mitochondrial genomes of two gudgeon species *S. dabryi*1-4 and *S. punctatus*.

Gene /Element	Position		Size (bp)	Start codon	Termination codon	Amino acid	Anti-codon	Intergenic region ^a	Strand
	Start	End							
<i>tRNA-Phe</i>	1	69	69				GAA	0	H
<i>12S rRNA</i>	70/70/69	1029/1028/1028	960/959/960					2/2/1	H
<i>tRNA-Val</i>	1032/1031/1030	1103/1102/1102	72/72/73				TAC	0	H
<i>16S rRNA</i>	1104/1103/1103	2794/2790/2790	1691/1688/1688					0	H
<i>tRNA-Leu</i>	2795/2791/2791	2870/2866/2866	76				TAA	0/1/1	H
<i>ND1</i>	2871/2868/2868	3845/3842/3842	975	ATG	TAG/TAA/TAA	324		4	H
<i>tRNA-Ile</i>	3850/3847/3847	3921/3918/3918	72				GAT	-2	H
<i>tRNA-Gln</i>	3920/3917/3917	3990/3987/3987	71				TTG	1	L
<i>tRNA-Met</i>	3992/3989/3989	4060/4057/4057	69				CAT	0	H
<i>ND2</i>	4061/4058/4058	5107/5104/5104	1047	ATG	TAA	348		-1	H
<i>tRNA-Trp</i>	5107/5104/5104	5177/5174/5174	71				TCA	2	H
<i>tRNA-Ala</i>	5180/5177/5177	5248/5245/5245	69				TGC	1	L
<i>tRNA-Asn</i>	5250/5247/5247	5322/5319/5319	73				GTT	1	L
<i>OL</i>	5324/5321/5321	5354/5351/5351	31					-1	
<i>tRNA-Cys</i>	5354/5351/5351	5421/5418/5418	68				GCA	2	L
<i>tRNA-Tyr</i>	5424/5421/5421	5491/5488/5488	68				GTA	1	L
<i>COXI</i>	5493/5490/5490	7043/7040/7040	1551	GTG	TAA	516		0	H
<i>tRNA-Ser</i>	7044/7041/7041	7114/7111/7111	71				TGA	3	L
<i>tRNA-Asp</i>	7118/7115/7115	7189/7186/7186	72				GTC	13	H
<i>COXII</i>	7203/7200/7200	7893/7890/7890	691	ATG	T	230		0	H
<i>tRNA-Lys</i>	7894/7891/7891	7969/7966/7966	76				TTT	1	H
<i>ATP8</i>	7971/7968/7968	8135/8132/8132	165	ATG	TAA	54		-7	H
<i>ATP6</i>	8129/8126/8126	8812/8809/8809	684	ATG	TAA	227		-1	H
<i>COXIII</i>	8812/8809/8809	9596/9593/9591	785/785/783	ATG	TA	261		-1	H
<i>tRNA-Gly</i>	9596/9593/9593	9666/9663/9663	71				TCC	0	H
<i>ND3</i>	9667/9664/9664	10017/10014/10014	351	ATG	TAG	116		-2	H
<i>tRNA-Arg</i>	10016/10013/10013	10085/10082/10082	70				TCG	0	H
<i>ND4L</i>	10086/10083/10083	10382/10379/10379	297	ATG	TAA	98		-7	H
<i>ND4</i>	10376/10373/10373	11757/11754/11754	1382/1382/1383	ATG	TA/TA/TAG	460		0/0/-1	H
<i>tRNA-His</i>	11758/11755/11755	11827/11823/11823	70/69/69				GTG	0	H
<i>tRNA-Ser</i>	11828/11824/11824	11896/11892/11892	69				GCT	-1	H
<i>tRNA-Leu</i>	11896/11892/11892	11968/11964/11964	73				TAG	0	H
<i>ND5</i>	11969/11965/11965	13804/13800/13800	1836	ATG	TAA	611		-4	H
<i>ND6</i>	13801/13797/13797	14322/14318/14318	522	ATG	TAG	173		0	L
<i>tRNA-Glu</i>	14323/14319/14319	14391/14387/14387	69				TTC	4	L
<i>Cyt b</i>	14396/14392/14392	15536/15532/15532	1141	ATG	T	380		0	H
<i>tRNA-Thr</i>	15537/15533/15533	15608/15604/15604	72				TGT	-1	H
<i>tRNA-Pro</i>	15608/15604/15604	15677/15673/15673	70				TGG	0	L
<i>D-loop</i>	15678/15674/15674	16604/16601/16601	927/928/927					0	H

^a: Numbers correspond to the nucleotides separating adjacent genes, with a negative number indicating an overlap. Characteristics of the mitochondrial genomes of *S. dabryi* 1 and 2 are the same, while *S. dabryi* 3 and 4 are the same. The same information of mitogenomes of *S. punctatus*, *S. dabryi* 1 and 2, and *S. dabryi* 3 and 4 was displayed only once (e.g., position, size, etc.), and their different information was spaced by '/'. H represents the heavy strand; L represents the light strand.

Table S3. Likelihood ratio tests and parameter estimates under branch model for genes between *Gobiobotia* subgroup and *Pseudogobio* subgroup.

Branch model	Ln L	Parameter estimates	df	2Δln L	LRT P-value
<i>ND1</i>					
M0 (one ratio)	-13070.404278	$\omega = 0.02117$	95		
two-ratio vs. one-ratio	-13070.287762	$\omega_0 = 0.02123, \omega_1 = 0.01298$	96	0.23303	0.62928
<i>ND2</i>					
M0 (one ratio)	-14976.612797	$\omega = 0.05512$	95		
two-ratio vs. one-ratio	-14973.495841	$\omega_0 = 0.05554, \omega_1 = 0.00010$	96	6.23391	0.01253*
<i>COXI</i>					
M0 (one ratio)	-15518.739888	$\omega = 0.01328$	95		
two-ratio vs. one-ratio	-15517.368435	$\omega_0 = 0.01342, \omega_1 = 0.00010$	96	2.74291	0.09769
<i>COXII</i>					
M0 (one ratio)	-6930.292859	$\omega = 0.02918$	95		
two-ratio vs. one-ratio	-6929.858710	$\omega_0 = 0.02929, \omega_1 = 0.00010$	96	0.86830	0.35143
<i>ATP8</i>					
M0 (one ratio)	-1675.341011	$\omega = 0.08210$	95		
two-ratio vs. one-ratio	-1675.340997	$\omega_0 = 0.08210, \omega_1 = 0.00010$	96	0.00008	0.99578
<i>ATP6</i>					
M0 (one ratio)	-8503.534227	$\omega = 0.03789$	95		
two-ratio vs. one-ratio	-8502.641000	$\omega_0 = 0.03832, \omega_1 = 0.01142$	96	1.78645	0.18136
<i>COXIII</i>					
M0 (one ratio)	-7836.226237	$\omega = 0.01622$	95		
two-ratio vs. one-ratio	-7836.187618	$\omega_0 = 0.01627, \omega_1 = 0.01225$	96	0.07724	0.78108
<i>ND3</i>					
M0 (one ratio)	-4374.089883	$\omega = 0.04266$	95		
two-ratio vs. one-ratio	-4372.192776	$\omega_0 = 0.04350, \omega_1 = 0.00010$	96	3.79421	0.05143
<i>ND4L</i>					
M0 (one ratio)	-3148.370909	$\omega = 0.01633$	95		
two-ratio vs. one-ratio	-3147.816331	$\omega_0 = 0.01658, \omega_1 = 0.00010$	96	1.10916	0.29227
<i>ND4</i>					
M0 (one ratio)	-18170.870087	$\omega = 0.03608$	95		
two-ratio vs. one-ratio	-18162.398440	$\omega_0 = 0.03690, \omega_1 = 0.00010$	96	16.94329	0.00004**
<i>ND5</i>					
M0 (one ratio)	-24859.463921	$\omega = 0.05941$	95		
two-ratio vs. one-ratio	-24858.850342	$\omega_0 = 0.05965, \omega_1 = 0.03116$	96	1.22716	0.26796
<i>ND6</i>					
M0 (one ratio)	-7192.734989	$\omega = 0.04308$	95		
two-ratio vs. one-ratio	-7190.171432	$\omega_0 = 0.04366, \omega_1 = 0.00010$	96	5.12711	0.02355*
<i>Cyt b</i>					
M0 (one ratio)	-13607.932219	$\omega = 0.01588$	95		
two-ratio vs. one-ratio	-13606.929245	$\omega_0 = 0.01601, \omega_1 = 0.00010$	96	2.00595	0.15668

ω_1 dN/dS value of foreground branch, ω_0 dN/dS value of background branch, significant difference LRT values displayed as bold.

Table S4. Likelihood ratio tests and parameter estimates under branch model for genes between *Saurogobio* tribe and *Pseudogobio* tribe.

Branch model	Ln L	Parameter estimates	df	2Δln L	LRT P-value
<i>ND1</i>					
M0 (one ratio)	-10226.236203	$\omega = 0.02322$	77		
two-ratio vs. one-ratio	-10226.086036	$\omega_0 = 0.02336, \omega_1 = 0.01652$	78	0.30033	0.58367
<i>ND2</i>					
M0 (one ratio)	-11816.720079	$\omega = 0.05169$	77		
two-ratio vs. one-ratio	-11816.485173	$\omega_0 = 0.05200, \omega_1 = 0.03921$	78	0.46981	0.49307
<i>COXI</i>					
M0 (one ratio)	-12439.962281	$\omega = 0.01678$	77		
two-ratio vs. one-ratio	-12437.675806	$\omega_0 = 0.01709, \omega_1 = 0.00010$	78	4.57295	0.03248*
<i>COXII</i>					
M0 (one ratio)	-5393.742869	$\omega = 0.03296$	77		
two-ratio vs. one-ratio	-5391.320766	$\omega_0 = 0.03422, \omega_1 = 0.00010$	78	4.84421	0.02774*
<i>ATP8</i>					
M0 (one ratio)	-1339.215153	$\omega = 0.09622$	77		
two-ratio vs. one-ratio	-1335.289131	$\omega_0 = 0.10294, \omega_1 = 0.00010$	78	7.85204	0.00508**
<i>ATP6</i>					
M0 (one ratio)	-6575.914508	$\omega = 0.04144$	77		
two-ratio vs. one-ratio	-6574.649547	$\omega_0 = 0.04043, \omega_1 = 0.10905$	78	2.52992	0.11171
<i>COXIII</i>					
M0 (one ratio)	-6142.780360	$\omega = 0.01602$	77		
two-ratio vs. one-ratio	-6142.760513	$\omega_0 = 0.01609, \omega_1 = 0.01346$	78	0.03969	0.84208
<i>ND3</i>					
M0 (one ratio)	-3316.874050	$\omega = 0.04635$	77		
two-ratio vs. one-ratio	-3316.825163	$\omega_0 = 0.04664, \omega_1 = 0.03730$	78	0.09777	0.75452
<i>ND4L</i>					
M0 (one ratio)	-2452.044738	$\omega = 0.01830$	77		
two-ratio vs. one-ratio	-2451.398316	$\omega_0 = 0.01718, \omega_1 = 0.03719$	78	1.29284	0.25552
<i>ND4</i>					
M0 (one ratio)	-14262.679574	$\omega = 0.03659$	77		
two-ratio vs. one-ratio	-14250.507866	$\omega_0 = 0.03438, \omega_1 = 0.13475$	78	24.34342	0.00000**
<i>ND5</i>					
M0 (one ratio)	-19625.971940	$\omega = 0.05962$	77		
two-ratio vs. one-ratio	-19625.970971	$\omega_0 = 0.05960, \omega_1 = 0.06051$	78	0.00194	0.96489
<i>ND6</i>					
M0 (one ratio)	-5707.620951	$\omega = 0.04103$	77		
two-ratio vs. one-ratio	-5707.320732	$\omega_0 = 0.04066, \omega_1 = 0.07768$	78	0.60044	0.43841
<i>Cyt b</i>					
M0 (one ratio)	-10671.529345	$\omega = 0.01562$	77		
two-ratio vs. one-ratio	-10671.528422	$\omega_0 = 0.01561, \omega_1 = 0.01602$	78	0.00185	0.96573

ω_1 dN/dS value of foreground branch, ω_0 dN/dS value of background branch, significant difference LRT values displayed as bold.

Table S5. Likelihood ratio tests and parameter estimates under branch model for genes between *Saurogobio* branch A and *Saurogobio* branch B.

Branch model	Ln L	Parameter estimates	df	2Δln L	LRT P-value
<i>ND1</i>					
M0 (one ratio)	-3641.316842	$\omega = 0.02468$	23		
two-ratio vs. one-ratio	-3638.250992	$\omega_0 = 0.02787, \omega_1 = 0.00566$	24	6.13170	0.01328*
<i>ND2</i>					
M0 (one ratio)	-3806.798366	$\omega = 0.04086$	23		
two-ratio vs. one-ratio	-3805.125359	$\omega_0 = 0.04424, \omega_1 = 0.01697$	24	3.34602	0.06737
<i>COXI</i>					
M0 (one ratio)	-4793.481054	$\omega = 0.00614$	23		
two-ratio vs. one-ratio	-4793.047187	$\omega_0 = 0.00675, \omega_1 = 0.00155$	24	0.86772	0.35159
<i>COXII</i>					
M0 (one ratio)	-1993.069087	$\omega = 0.02593$	23		
two-ratio vs. one-ratio	-1989.617875	$\omega_0 = 0.03049, \omega_1 = 0.00010$	24	6.90242	0.00861**
<i>ATP8</i>					
M0 (one ratio)	-487.999297	$\omega = 0.01040$	23		
two-ratio vs. one-ratio	-487.993329	$\omega_0 = 0.10497, \omega_1 = 0.08977$	24	0.01194	0.91299
<i>ATP6</i>					
M0 (one ratio)	-2306.423393	$\omega = 0.03557$	23		
two-ratio vs. one-ratio	-2301.548351	$\omega_0 = 0.04238, \omega_1 = 0.00010$	24	9.75008	0.00179**
<i>COXIII</i>					
M0 (one ratio)	-2498.814955	$\omega = 0.01911$	23		
two-ratio vs. one-ratio	-2498.575447	$\omega_0 = 0.02026, \omega_1 = 0.01195$	24	0.47902	0.48887
<i>ND3</i>					
M0 (one ratio)	-1225.251294	$\omega = 0.03817$	23		
two-ratio vs. one-ratio	-1225.142061	$\omega_0 = 0.03925, \omega_1 = 0.02344$	24	0.21846	0.64022
<i>ND4L</i>					
M0 (one ratio)	-921.854944	$\omega = 0.01055$	23		
two-ratio vs. one-ratio	-921.047231	$\omega_0 = 0.01247, \omega_1 = 0.00010$	24	1.61542	0.20373
<i>ND4</i>					
M0 (one ratio)	-5130.798044	$\omega = 0.03624$	23		
two-ratio vs. one-ratio	-5130.538317	$\omega_0 = 0.03516, \omega_1 = 0.04494$	24	0.51944	0.47108
<i>ND5</i>					
M0 (one ratio)	-6627.971808	$\omega = 0.04967$	23		
two-ratio vs. one-ratio	-6625.941905	$\omega_0 = 0.05270, \omega_1 = 0.02498$	24	4.05980	0.04392*
<i>ND6</i>					
M0 (one ratio)	-1963.919329	$\omega = 0.04847$	23		
two-ratio vs. one-ratio	-1963.619015	$\omega_0 = 0.05022, \omega_1 = 0.02959$	24	0.60062	0.43834
<i>Cyt b</i>					
M0 (one ratio)	-3818.85911	$\omega = 0.01710$	23		
two-ratio vs. one-ratio	-3816.21860	$\omega_0 = 0.00463, \omega_1 = 0.01974$	24	5.28102	0.02156**

ω_1 dN/dS value of foreground branch, ω_0 dN/dS value of background branch, significant difference LRT values displayed as bold.

Table S6. Likelihood ratio tests and parameter estimates under branch model for genes between *Abbottina* branch and *Pseudogobio* branch.

Branch model	Ln L	Parameter estimates	df	2Δln L	LRT P-value
<i>ND1</i>					
M0 (one ratio)	-7518.696299	$\omega = 0.02193$	53		
two-ratio vs. one-ratio	-7517.808878	$\omega_0 = 0.02127, \omega_1 = 0.03932$	54	1.77484	0.18278
<i>ND2</i>					
M0 (one ratio)	-8944.606264	$\omega = 0.05518$	53		
two-ratio vs. one-ratio	-8944.233752	$\omega_0 = 0.05464, \omega_1 = 0.08174$	54	0.74502	0.38806
<i>COXI</i>					
M0 (one ratio)	-9314.778132	$\omega = 0.02153$	53		
two-ratio vs. one-ratio	-9294.747944	$\omega_0 = 0.01682, \omega_1 = 0.10972$	54	40.06038	0.00000**
<i>COXII</i>					
M0 (one ratio)	-4125.069803	$\omega = 0.03616$	53		
two-ratio vs. one-ratio	-4111.124550	$\omega_0 = 0.02904, \omega_1 = 0.29584$	54	27.89051	0.00000**
<i>ATP8</i>					
M0 (one ratio)	-1010.913454	$\omega = 0.10370$	53		
two-ratio vs. one-ratio	-1009.862737	$\omega_0 = 0.09772, \omega_1 = 0.00010$	54	2.10143	0.14716
<i>ATP6</i>					
M0 (one ratio)	-4894.718069	$\omega = 0.04032$	53		
two-ratio vs. one-ratio	-4892.834453	$\omega_0 = 0.03859, \omega_1 = 0.10655$	54	3.76723	0.05227
<i>COXIII</i>					
M0 (one ratio)	-4471.979860	$\omega = 0.01446$	53		
two-ratio vs. one-ratio	-4469.624864	$\omega_0 = 0.01305, \omega_1 = 0.04798$	54	4.70999	0.02999*
<i>ND3</i>					
M0 (one ratio)	-2430.146856	$\omega = 0.04978$	53		
two-ratio vs. one-ratio	-2430.140460	$\omega_0 = 0.04959, \omega_1 = 0.05341$	54	0.01279	0.90995
<i>ND4L</i>					
M0 (one ratio)	-1800.628248	$\omega = 0.02048$	53		
two-ratio vs. one-ratio	-1800.627156	$\omega_0 = 0.02054, \omega_1 = 0.01970$	54	0.00218	0.96273
<i>ND4</i>					
M0 (one ratio)	-10309.086615	$\omega = 0.03304$	53		
two-ratio vs. one-ratio	-10309.078653	$\omega_0 = 0.03297, \omega_1 = 0.03457$	54	0.01592	0.89958
<i>ND5</i>					
M0 (one ratio)	-14771.747150	$\omega = 0.06229$	53		
two-ratio vs. one-ratio	-14771.214791	$\omega_0 = 0.06292, \omega_1 = 0.04479$	54	1.06472	0.30214
<i>ND6</i>					
M0 (one ratio)	-4212.686902	$\omega = 0.03586$	53		
two-ratio vs. one-ratio	-4212.587061	$\omega_0 = 0.03548, \omega_1 = 0.04541$	54	0.19968	0.65498
<i>Cyt b</i>					
M0 (one ratio)	-7985.636450	$\omega = 0.011473$	53		
two-ratio vs. one-ratio	-7978.701576	$\omega_0 = 0.01304, \omega_1 = 0.06821$	54	13.86975	0.00020**

ω_1 dN/dS value of foreground branch, ω_0 dN/dS value of background branch, significant difference LRT values displayed as bold.

Table S7. Likelihood ratio tests and parameter estimates under branch model for genes between *Pseudogobio* subbranch and *Biwia* complex subbranch.

Branch model	Ln L	Parameter estimates	df	2Δln L	LRT P-value
<i>ND1</i>					
M0 (one ratio)	-6335.605590	$\omega = 0.01705$	45		
two-ratio vs. one-ratio	-6331.666605	$\omega_0 = 0.01812, \omega_1 = 0.00010$	46	7.87797	0.00500**
<i>ND2</i>					
M0 (one ratio)	-7451.713537	$\omega = 0.04238$	45		
two-ratio vs. one-ratio	-7448.487509	$\omega_0 = 0.04437, \omega_1 = 0.01449$	46	6.45206	0.01108*
<i>COXI</i>					
M0 (one ratio)	-7414.361712	$\omega = 0.00324$	45		
two-ratio vs. one-ratio	-7413.365700	$\omega_0 = 0.00349, \omega_1 = 0.00010$	46	1.99202	0.15813
<i>COXII</i>					
M0 (one ratio)	-3159.454633	$\omega = 0.00637$	45		
two-ratio vs. one-ratio	-3158.854110	$\omega_0 = 0.00571, \omega_1 = 0.01530$	46	1.20105	0.27311
<i>ATP8</i>					
M0 (one ratio)	-753.278370	$\omega = 0.07171$	45		
two-ratio vs. one-ratio	-751.210852	$\omega_0 = 0.07901, \omega_1 = 0.00010$	46	4.13504	0.04200*
<i>ATP6</i>					
M0 (one ratio)	-3942.486680	$\omega = 0.01993$	45		
two-ratio vs. one-ratio	-3937.148100	$\omega_0 = 0.02206, \omega_1 = 0.00010$	46	10.67716	0.00108**
<i>COXIII</i>					
M0 (one ratio)	-3796.674702	$\omega = 0.01084$	45		
two-ratio vs. one-ratio	-3794.849736	$\omega_0 = 0.01169, \omega_1 = 0.00010$	46	3.64993	0.05607
<i>ND3</i>					
M0 (one ratio)	-2015.242887	$\omega = 0.03711$	45		
two-ratio vs. one-ratio	-2013.471095	$\omega_0 = 0.04009, \omega_1 = 0.00752$	46	3.54358	0.05978
<i>ND4L</i>					
M0 (one ratio)	-1484.739787	$\omega = 0.01017$	45		
two-ratio vs. one-ratio	-1484.626002	$\omega_0 = 0.00968, \omega_1 = 0.01714$	46	0.22757	0.63333
<i>ND4</i>					
M0 (one ratio)	-8693.683271	$\omega = 0.02845$	45		
two-ratio vs. one-ratio	-8693.542602	$\omega_0 = 0.02878, \omega_1 = 0.02343$	46	0.28134	0.59583
<i>ND5</i>					
M0 (one ratio)	-12520.054865	$\omega = 0.05645$	45		
two-ratio vs. one-ratio	-12519.576542	$\omega_0 = 0.05729, \omega_1 = 0.04483$	46	0.95665	0.32803
<i>ND6</i>					
M0 (one ratio)	-3509.282412	$\omega = 0.02552$	45		
two-ratio vs. one-ratio	-3509.273967	$\omega_0 = 0.02565, \omega_1 = 0.02392$	46	0.01689	0.89660
<i>Cyt b</i>					
M0 (one ratio)	-6758.273817	$\omega = 0.00889$	45		
two-ratio vs. one-ratio	-6757.963837	$\omega_0 = 0.00858, \omega_1 = 0.01307$	46	0.61996	0.43106

ω_1 dN/dS value of foreground branch, ω_0 dN/dS value of background branch, significant difference LRT values displayed as bold.

Table S8. Parameter estimates and log-likelihood values under models among sites for *ND5* gene between *Abbottina* branch and *Pseudogobio* branch.

Model	Ln L	Estimates of parameters			Model compared	LRT <i>P</i> -value	Positively selected sites
M3	-14458.922389	p ₀ =0.72050	p ₁ =0.23722	p ₂ =0.04228	M0 vs. M3	0.00000	[] Not Allowed
M0	-14785.592850	ω ₀ =0.00685	ω ₁ =0.15515	ω ₂ =0.61383			
M2a	-14592.358120	p ₀ =0.93540	p ₁ =0.06460	p ₂ =0.00000	M1a vs. M2a	0.99999	[] Not Allowed 30 D 0.896, 34 Q 0.972 *, 525 H 0.997 **
M1a	-14592.358111	ω ₀ =0.03877	ω ₁ =1.00000	ω ₂ =3.24359			
		p ₀ =0.93540	p ₁ =0.06460				
		ω ₀ =0.03877	ω ₁ =1.00000				
M8	-14467.224202	p ₀ =0.98091	p ₁ =0.19951	q=2.23544	M7 vs. M8	0.00000	Not Allowed
M7	-14499.292618	(p ₁ = 0.01909)	ω= 1.00000				
M8a	-14467.224202	p=0.20943	q=1.75932		M8a vs. M8	1.00000	Not Allowed
		p ₀ =0.98091	p=0.19951	q=2.23541			
		(p ₁ = 0.01909)	ω= 1.00000				

Table S9. Parameter estimates and log-likelihood values under models among sites for *ND2*, *ND4*, and *ND5* genes between *Pseudogobio* subbranch and *Biwia* complex subbranch.

Genes	Model	Ln L	Estimates of parameters			Model compared	LRT P-value	Positively selected sites
<i>ND2</i>			$p_0=0.86577$	$p_1=0.13423$	$p_2=0.00000$			
	M3	-7308.238087	$\omega_0=0.01348$	$\omega_1=0.25621$	$\omega_2=64.42707$			[]
	M0	-7414.093565		$\omega_0=0.04379$		M0 vs. M3	0.00000	Not Allowed
			$p_0=0.97213$	$p_1=0.02787$	$p_2=0.00000$			
	M2a	-7333.751619	$\omega_0=0.03069$	$\omega_1=1.00000$	$\omega_2=31.00040$			[]
			$p_0=0.97213$	$p_1=0.02787$		M1a vs. M2a	1.00000	Not Allowed
	M1a	-7333.751619	$\omega_0=0.03069$	$\omega_1=1.00000$				
			$p_0=0.98539$	$p_1=0.16621$	$q=2.74693$			221 T 0.543, 237 S 0.623, 274 D 0.960 *, 277 A 0.631
<i>ND4</i>	M8	-7290.676459	($p_1=0.01461$)	$\omega=1.00000$				
	M7	-7318.842293	$p=0.18332$	$q=2.19708$		M7 vs. M8	0.00000	Not Allowed
			$p_0=0.98539$	$p=0.16621$	$q=2.74693$			
	M8a	-7290.676459	($p_1=0.01461$)	$\omega=1.00000$		M8a vs. M8	0.99804	Not Allowed
			$p_0=0.91824$	$p_1=0.07521$	$p_2=0.00655$			
	M3	-8468.764243	$\omega_0=0.00606$	$\omega_1=0.26197$	$\omega_2=1.16976$			[]
	M0	-8633.391256		$\omega_0=0.02861$		M0 vs. M3	0.00000	Not Allowed
			$p_0=0.95833$	$p_1=0.04167$	$p_2=0.00000$			
<i>ND5</i>	M2a	-8518.424204	$\omega_0=0.01256$	$\omega_1=1.00000$	$\omega_2=34.58410$			[]
			$p_0=0.95833$	$p_1=0.04167$		M1a vs. M2a	0.99999	Not Allowed
	M1a	-8518.424203	$\omega_0=0.01256$	$\omega_1=1.00000$				
			$p_0=0.97983$	$p_1=0.03903$	$q=0.37962$			
	M8	-8493.093615	($p_1=0.02017$)	$\omega=1.00000$				26 A 0.975 *, 189 D 0.602
	M7	-8521.337900	$p=0.04116$	$q=0.32453$		M7 vs. M8	0.00000	Not Allowed
			$p_0=0.97983$	$p=0.03849$	$q=0.37107$			
	M8a	-8493.093599	($p_1=0.02017$)	$\omega=1.00000$		M8a vs. M8	0.99549	Not Allowed
<i>ND5</i>			$p_0=0.85138$	$p_1=0.14862$	$p_2=0.00000$			
	M3	-12304.348203	$\omega_0=0.01534$	$\omega_1=0.32130$	$\omega_2=57.22185$			[]
	M0	-12534.632508		$\omega_0=0.05744$		M0 vs. M3	0.00000	Not Allowed
			$p_0=0.94188$	$p_1=0.04465$	$p_2=0.01347$			
	M2a	-12366.032902	$\omega_0=0.03359$	$\omega_1=1.00000$	$\omega_2=1.00000$			[]
			$p_0=0.94188$	$p_1=0.05812$		M1a vs. M2a	1.00000	Not Allowed
	M1a	-12366.032902	$\omega_0=0.03359$	$\omega_1=1.00000$				30 K 0.819, 34 P 0.952 *, 277 E 0.553, 525 S 0.988 *, 526 L 0.667
			$p_0=0.97416$	$p_1=0.19584$	$q=2.69821$			
<i>ND5</i>	M8	-12299.346989	($p_1=0.02584$)	$\omega=1.00000$				
	M7	-12332.341160	$p=0.20253$	$q=1.79323$		M7 vs. M8	0.00000	Not Allowed
			$p_0=0.97416$	$p=0.19584$	$q=2.69826$			
	M8a	-12299.346988	($p_1=0.02584$)	$\omega=1.00000$		M8a vs. M8	0.99887	Not Allowed