Relaxation of Selective Constraint on the Ultra-Large Mitochondrial Genomes of Arcidae (Mollusca: Bivalvia)

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Abstract The mitochondrial genomes (mitogenomes) are purportedly under selection for smaller size to improve their replication and translation efficiency. However, the mitogenomes of Arcidae species are larger than those of other bivalves, and are among the largest metazoan mitogenomes reported to date. In order to explore the differences of base composition and selective constraints between the large and small mitogenomes, we compared the mitogenomes of 9 large arcid mitogenomes and 77 small bivalves mitogenomes. Base composition analyses indicated that Arcidae mitogenomes have significantly greater GC skews in both their whole genomes and coding sequences. This result suggests that the replication of the large mitogenomes in Arcidae may be slower than those in other bivalves, exposing the parental strand to deamination for a longer time. Selection pressure analyses showed that the mitochondrial protein-coding genes of Arcidae species have significantly higher Ka/Ks ratios than other bivalves, suggesting that they have accumulated more nonsynonymous nucleotide substitutions. Seven protein-coding genes (*atp6, cox1-3, nad1, nad4* and *nad5*) show significant difference for Ka/Ks ratios between the Arcidae and non-Arcidae groups. However, these divergences are not observed in the nuclear gene within histone H3. From these observations, we concluded that the large mitogenomes of Arcidae species experienced more relaxed selective constraints. As some Arcidae species are more tolerant to hypoxia that can lead to low metabolic rate, the relaxed selective constraints of mitogenomes may be energy-related. This study provides new insights into the evolution of Arcidae mitogenomes.

Key words Arcidae; mitochondrial genome; genome size; relaxed selective constraint

1 Introduction

The mitochondrial genomes (mitogenomes) of most bilaterian animals include a standard set of 13 protein-coding genes (PCGs), 2 ribosomal RNA (rRNA) genes, 22 transfer RNA (tRNA) genes, and an A+T-rich region (Boore, 1999). Although there are exceptions, most mitogenomes size are from 14 to 17 kb. Typically, few intergenic nucleotides exist except for a single large non-coding region, which were thought to contain elements that control the initiation of replication and transcription of the mitogenome (Boore, 1999; Lavrov, 2007). This consistency in gene content across distantly related lineages, as well as the lack of intergenic spacers, suggests that the mitogenome is under selection for compact size (Rand and Harrison, 1986). Compared with nuclear genome, the mitogenome has several advantages including conserved gene content, maternal inheritance, lack of extensive recombination, and relatively high nucleotide substitution rates (Boore, 1999; Curole and

Kocher, 1999; Gissi *et al*., 2008). These advantages make it a good model for the studies of evolutionary genomics (Saccone *et al*., 1999; Gissi *et al*., 2008; Cameron, 2014).

Molluscs, especially bivalves, usually display an extraordinary amount of variation in mitogenome structure and size, even with differences in the closely related species (Gissi *et al*., 2008; Simison and Boore, 2008). The size of the bivalve mitogenomes are highly variable, ranging from 14622 in *Lanternula elliptica* (Park and Ahn, 2015) to 46985 bp in *Scapharca broughtonii* (Liu *et al*., 2013) in length. Several bivalve species have showed large sizes of mitogenomes (greater than 20 kb). The mitogenome size of the deep sea scallop *Placopecten magellanicus* is up to 40725 bp (Smith and Snyder, 2007) and the mitogenome of Zhikong scallop *Chlamys farreri* is 21695 bp (Xu *et al*., 2011). The mitogenome size of Manila clam *Venerupis philippinarum* is 22676 bp in female type and 21441 bp in male type (Passamonti and Scali, 2001). The mitogenome of *Bryopa lata*, Clavagellidae, is at least 31969 bp long (Williams *et al*., 2017). The size of Arcidae mitogenomes are more unusual, of which the largest mitogenome size is 46985 bp (*Scapharca broughtonii*) (Liu *et al*., 2013). The

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bivalve species have a sedentary lifestyle, with low metabolic rate (Sun *et al.*, 2017). The previous researches proposed that the large mitogenome of bivalves perhaps experience weak purifying selection, which may be correlated with their low metabolic rates (Strotz *et al.*, 2018; Kong *et al.*, 2020).

Ark shells are among the oldest bivalve lineages, reaching back to the lower Ordovician which is about 450 Myr (Morton *et al.*, 1998). The species of Arcidae are globally distributed, predominantly in the tropical shallow waters and warm temperate seas, containing approximately 260 species and 31 genera (Oliver and Holmes, 2006). Mitogenomes of ark shell species are among the largest metazoan mitogenomes reported to date, ranging from 18 to 56 kb in length (Kong *et al.*, 2020). It has been argued that animal mitogenomes are characterized by a tightly packed collection of conserved genes and other functional elements, accompanying with drastic mitogenome size reduction in evolutionary history of animal (Burger *et al.*, 2003; Schneider and Ebert, 2004; Signorovitch *et al.*, 2007). Arcidae presented a challenge to the point of selection favoring compact genomes by virtue of large mitogenome size. In our previous studies, we found the mitogenome size is positively correlated with the combined length of *cox1*, *cox2* and *cox3*, the length of *Cytb*, and the combined length of *rrnS* and *rrnL* (Sun *et al.*, 2016). Researchers have found the inverted repeat sequences might facilitate the mitogenome expansions (Kong *et al.*, 2020). It has been believed that metabolic rates influence the selective constraints acting on the mitogenome, and purify selection act on small genome size (Rand, 1993). However, the natural selection act on the large mitogenome of Arcidae remains unexplored.

The large size of Arcidae mitogenomes lead to several questions. Firstly, whether the nucleotide compositions of large Arcidae mitochondrial DNA (mtDNA) are different from those of other bivalve species with small ones? Secondly, what's the difference in substitution rate of mtDNA between Arcidae and other bivalve species? Thirdly, do all the mitochondrial genes of Arcidae experience the same selection pressure? In order to address these questions, we conducted a comparative genomic analysis and test the roles of the evolutionary constraints on the mtDNA of Arcidae to provide a complete view of molecular evolution in the mtDNA.

2 Materials and Methods

2.1 Source of Data

The mtDNA sequences (Table 1) and nuclear gene (histone H3) (Table 2) of bivalves were downloaded from GenBank. All the mitochondrial protein-coding genes were extracted from each mitogenome.

2.2 Base Composition

AT and GC skew were calculated according to the formula defined by Perna and Kocher (1995), AT skew$=(A-T)/(A+T)$ and GC skew$=(G-C)/(G+C)$, which provides an index of compositional asymmetry between strands. Skews were calculated for all sites, and also for fourfold degenerate sites, which are expected to be less constrained (Reyes *et al.*, 1998). We then compared these measures of nucleotide skew between Arcoidae and non-Arcoidae taxa. All statistical analyses were performed with IBM SPSS Statistics, release 19.0.0.1.

Table 1 The mtDNA sequences of bivalves downloaded from GenBank

Species	Accession no.	Ka/Ks	Ka	Ks	atp6	cox-1	cox-2	cox-3	cytb	nd1	nd2	nd3	nd4	nd4l	nd5	nd6
Tegillarca granosa	KJ607173	0.0552	0.0492	0.8918	0.0284	0.0447	0.1107	0.0247	0.0255	0.0517	0.0967	0.0196	0.0526	0.0418	0.0895	0.0641
Scapharca kagoshimensis	KF750628	0.1188	0.0741	0.6240	0.0203	0.0478	–		0.1438	0.2349	0.0874	0.9466	0.2834	0.0357	0.0414	0.2071	0.1105
Scapharca broughtonii	AB729113	0.0901	0.0147	0.1635	0.6263	0.0883	0.0418	–	–	0.0303	0.0745	–	–	0.0360	0.0918	–
Anadara vellicata	KP954700	0.0558	0.0654	1.1734	0.0909	0.0125	0.0738	0.0351	0.0362	0.1339	0.9985	0.0479	0.0710	0.0372	0.0714	0.1068
Trisidos kiyoni	KU975161	0.0434	0.2155	4.9697	0.1043	0.0265	0.0330	0.1235	0.0262	0.0664	0.1905	0.1165	0.0882	0.0341	0.0353	0.0794
Potiarca pilula	KU975162	0.0681	0.1364	2.0028	0.0466	0.0352	0.1266	0.0497	0.0507	0.0445	0.0048	0.0448	0.0862	0.3316	0.0824	0.0692
Arca navicularis	MG641752	0.0531	0.2711	5.1060	0.0828	0.0320	0.0642	0.1479	0.1805	0.0839	0.0456	0.0690	0.0838	0.1316	0.0613	0.1800
Barbatia virescens	MF374794	0.0845	0.0865	1.0236	0.0745	0.0325	0.1254	0.0754	0.0512	0.0521	0.0853	–	0.0853	0.2313	0.0863	0.0741
Anadara consociata	MG641753	0.0654	0.0717	1.0961	0.0887	0.0230	0.1783	0.0506	0.0200	0.0353	0.1255	0.0528	0.0381	0.0191	0.0735	0.0870
Crassostrea hongkongensis	FJ841963	0.0433	0.0156	0.3608	0.0190	0.0067	0.0001	0.0236	0.0468	0.0453	0.0890	0.4552	0.0638	0.0187	0.0846	0.0561
Crassostrea iredalei	FJ841967	0.0571	0.0510	0.8927	0.0338	0.0091	0.0121	0.0872	0.0868	0.0743	0.1286	0.0664	0.0835	0.0140	0.0656	0.0651
Crassostrea sikamea	FJ841966	0.0513	0.0158	0.3073	0.0394	0.0001	0.0001	0.0194	0.0537	0.0266	0.0546	0.1069	0.0661	0.0001	0.1571	0.0583
Crassostrea angulata	FJ841965	0.0481	0.0027	0.0569	0.0001	0.0001	0.0001	0.1289	0.0317	0.0598	0.2178	0.3846	0.0432	0.2252	0.0913	0.0677
Crassostrea ariakensis	FJ841964	0.0851	0.0439	0.5154	0.0036	0.0122	0.0038	0.0722	0.1402	0.0550	0.2060	0.1997	0.0935	0.1377	0.1070	0.3430
Crassostrea virginica	AY905542	0.0161	0.0669	4.1576	0.0209	0.0116	0.0155	0.0465	0.0271	0.0201	0.0322	0.0334	0.0130	0.0937	0.0438	0.0128
Crassostrea gigas	KJ855241	0.1020	0.0063	0.0615	0.5212	0.0125	0.0001	0.0215	0.0401	0.2728	0.1779	0.0503	0.1310	0.0001	0.2280	0.3055
Crassostrea gasar	KR856227	0.0124	0.0578	4.6675	0.0208	0.0099	0.0026	0.0077	0.0173	0.0145	0.0283	0.0783	0.0144	0.0285	0.0528	0.0503
Crassostrea nippona	HM015198	0.0621	0.0383	0.6162	0.0289	0.0185	0.0427	0.1081	0.0546	0.0350	0.1143	0.0565	0.0826	0.0508	0.1148	0.0694
Ostrea lurida	KC768038	0.0506	0.0301	0.5939	0.0053	0.0049	0.0103	0.0864	0.0530	0.0556	0.0414	0.0883	–	–	0.0586	0.1849
Ostrea edulis	JF274008	0.0362	0.0429	1.1847	0.0222	0.0468	0.0260	0.0512	0.0793	0.0138	–	0.0215	0.0303	0.0271	0.0263	0.0665
Saccostrea mordax	FJ841968	0.0230	0.0324	1.4093	0.0293	0.0132	0.0056	0.0374	0.0287	0.0179	0.0659	0.0247	0.0263	–	0.0229	0.0399
Saccostrea cucullata	KP967577	0.0245	0.0263	1.0749	0.0158	0.0008	0.0191	0.0191	0.0234	0.0181	0.0515	0.0013	0.0212	0.0071	0.0562	0.1323
Mimachlamys nobilis	FJ415225	0.0400	0.0378	0.9460	0.0708	0.0134	0.0648	0.0378	0.0397	0.0167	0.0419	0.1007	0.0272	0.0065	0.0644	0.0567
Mimachlamys senatoria	KF214684	0.0261	0.0227	0.8678	0.0273	0.0129	0.0280	0.0242	0.0192	0.0126	0.0511	0.7871	0.0197	0.0203	0.0589	0.0226
Placopecten magellanicus	DQ088274	0.0545	0.1395	2.5600	0.1003	0.0310	0.0557	0.0453	0.0539	0.0315	0.0820	0.0780	0.0654	0.1249	0.1613	0.1204

(*continued*)

Species	Accession no.	Ka/Ks	Ka	Ks	atp6	cox-1	cox-2	cox-3	cytb	nd1	nd2	nd3	nd4	nd4l	nd5	nd6
Chlamys farreri	EU715252	0.0447	0.0635	1.4216	0.0919	0.0160	0.0688	0.0228	0.0640	0.0291	0.1366	0.0387	0.0374	0.0428	0.0729	0.0382
Mizuhopecten yessoensis	AB271769	0.0453	0.0571	1.2599	0.0529	0.0189	0.0532	0.0673	0.0564	0.0271	0.0809	0.0303	0.0326	0.0473	0.0454	0.0835
Argopecten irradians	EU023915	0.0443	0.0340	0.7672	0.0065	0.0006	0.0887	0.0001	0.0087	–	0.0278	0.6156	0.0135	0.0099	0.0439	–
Argopecten purpuratus	KF601246	0.0240	0.0171	0.7128	0.0511	0.0031	0.0157	0.0001	0.0205	0.0372	–	–	0.0581	–	0.0142	0.0405
Argopecten ventricosus	KT161261	0.0238	0.0061	0.2578	0.0094	0.0083	0.0146	0.0136	0.0408	0.0108	0.1215	0.0262	0.0087	0.0234	0.0373	0.3601
Pinctada margaritifera	HM467838	0.0512	0.0648	1.2652	0.2361	0.0034	0.0963	0.0416	0.0429	0.0374	0.0354	0.0053	0.0518	0.0232	0.0419	0.0952
Pinctada maxima	GQ452847	0.0420	0.0596	1.4187	0.0689	0.0137	0.0321	0.0107	0.0168	0.0361	–	0.0493	0.0155	0.0456	0.0071	0.0460
Mytilus trossulus	GU936625	0.0217	0.0119	0.5481	0.0152	0.0035	0.0026	0.0001	0.0113	0.0145	0.0683	–	0.0494	0.0170	0.0424	0.0682
Mytilus californianus	GQ527172	0.0209	0.0100	0.4802	0.0340	0.0063	0.0078	0.0020	0.0128	0.0323	0.0367	0.0027	0.0186	0.0202	0.0390	0.0266
Mytilus edulis	AY484747	0.0042	0.0173	4.1351	0.0001	0.0167	0.0001	–	0.7360	0.0001	0.4733	0.0001	0.2050	0.0001	0.0530	–
Mytilus galloprovincialis	AY497292	0.0589	0.0006	0.0105	0.0001	0.0001	0.0537	0.0001	0.0001	0.0001	0.2241	0.0001	0.0476	0.0001	0.4803	0.0001
Mytilus coruscus	KJ577549	0.0406	0.0274	0.6756	0.0060	0.0023	0.0033	0.0076	0.0086	0.0204	0.0453	0.2561	0.1965	0.0226	0.0556	0.0245
Brachidontes exustus	KM233636	0.0187	0.0803	4.3003	0.0709	0.0101	0.0659	0.0300	0.0057	0.0208	0.0388	0.0121	0.0633	0.0275	0.0362	0.0509
Perna viridis	JQ970425	0.0328	0.1890	5.7562	0.0037	0.0154	0.0436	0.0477	0.0470	0.0233	0.0176	0.0200	0.0377	0.0017	0.0442	0.0547
Perna perna	KM655841	0.0151	0.0801	5.3151	0.0402	0.0106	0.1130	0.0475	0.0013	0.0246	0.0164	0.0257	0.0366	0.0657	0.0140	0.0247
Musculista senhousia	GU001954	0.0512	0.2326	4.5476	0.0687	0.0203	0.0706	0.0709	0.0287	0.0702	0.0792	0.0052	0.0777	0.0105	0.0540	0.0585
Meretrix petechialis	EU145977	0.0803	0.0001	0.0009	0.0000	0.0001	0.0001	0.0000	0.5377	–	0.1904	0.0002	0.0002	0.0000	0.0000	0.0001
Meretrix lamarckii	GU071281	0.0920	0.0059	0.0638	0.0780	0.0457	0.2167	0.1385	0.0828	0.0066	0.1492	0.0612	0.0442	0.1590	0.0790	0.0142
Meretrix meretrix	GQ463598	0.0332	0.0000	0.0005	0.2694	0.0000	0.0000	0.0001	0.0001	0.0000	0.0940	0.1328	0.0001	0.0001	0.1120	0.0588
Meretrix lusoria	GQ903339	0.0602	0.0008	0.0129	0.0370	0.0251	0.0961	0.0195	0.0499	0.0222	0.0985	0.0001	0.2169	0.1643	0.0792	0.2989
Meretrix lyrata	KC832317	0.0573	0.0031	0.0541	–	0.0473	0.0760	0.1010	0.0476	0.0259	0.1848	0.0084	0.0438	0.0793	0.0350	0.0995
Paphia euglypta	GU269271	0.0274	0.0010	0.0375	0.0138	0.0085	0.0448	0.0227	0.0176	0.0327	0.0880	0.0178	0.0762	0.0001	0.0277	0.0646
Paphia undulata	JF969278	0.0276	0.0010	0.0345	0.0197	0.0088	0.0325	–	0.0113	0.0316	0.0492	0.0106	0.0141	–	0.0361	0.0311
Paphia textile	JF969277	0.0282	0.0008	0.0298	0.0208	0.0062	0.0703	0.0048	0.0213	0.0273	0.0315	0.0066	0.0395	0.0146	0.0479	0.0987
Paphia amabilis	JF969276	0.0600	0.0025	0.0411	0.0222	0.0598	0.0411	0.0369	0.0672	0.0266	0.0408	0.0270	0.0224	–	0.0693	0.1035
Venerupis philippinarum	AB065375	0.0357	0.0078	0.2178	0.0598	0.0073	0.0228	0.0332	0.0277	0.0503	0.0608	0.0861	0.0227	1.1707	0.0070	0.3303
Saxidomus purpuratus	KP419933	0.0239	0.0031	0.1295	0.0216	0.0169	0.0567	0.0204	0.0452	0.0318	0.1331	0.0966	0.0174	0.0028	0.0184	0.1631
Solenaia oleivora	KF296320	0.0556	0.0016	0.0286	0.0426	0.0017	0.0373	0.0269	0.0368	0.0359	0.1227	0.0841	0.0649	0.0559	0.0574	0.0617
Solen strictus	JN786377	0.0331	0.0011	0.0326	0.0938	0.0042	0.0044	–	0.0334	0.0279	0.1669	0.0571	0.0048	0.0135	0.0138	0.0348
Solen grandis	HQ703012	0.0183	0.0005	0.0263	0.0160	0.0010	–	0.0055	0.0123	0.0188	0.0505	0.0109	0.0078	0.0057	0.0072	0.0179
Solenaia carinatus	KC848654	0.0542	0.0014	0.0251	0.0621	0.0055	0.0319	0.0445	0.0706	0.0395	0.1073	0.1692	0.0374	0.0356	0.0602	0.0910
Lucinella divaricata	EF043342	0.0094	0.0004	0.0424	0.1626	0.0053	0.0046	0.0027	0.0159	0.0209	0.0647	0.0040	0.0091	0.0233	0.0041	0.2772
Loripes lacteus	EF043341	0.0105	0.0006	0.0553	0.0124	0.0001	0.0085	0.0017	0.0102	0.0817	0.0926	0.0099	0.0096	0.0178	0.0009	0.0389
Acanthocardia tuberculata	DQ632743	0.0481	0.0087	0.1819	0.0097	0.0208	0.0074	0.0157	0.0211	0.0478	0.1627	0.0234	0.0751	0.4939	0.1067	0.2459
Fulvia mutica	AB809077	0.0382	0.0065	0.1699	0.0364	0.0192	0.0072	0.0339	0.0213	0.0555	0.0041	0.0026	0.0377	0.1013	0.0500	0.3063
Tridacna squamosa	KP205428	0.0624	0.0568	0.9115	0.0054	0.0058	0.0142	0.0487	0.0218	0.3568	0.3273	0.5336	0.1362	0.7820	0.0161	–
Semele scabra	JN398365	0.0185	0.0022	0.1184	0.0081	0.0075	0.0157	0.0167	0.0342	0.0598	0.0711	0.0082	0.0214	0.0453	0.0069	0.0745
Nuttallia olivacea	JN398364	0.0176	0.0024	0.1339	0.0559	0.0121	0.0101	0.0112	0.0253	0.0400	0.0916	0.0129	0.0201	0.0359	0.0128	0.0258
Soletellina diphos	JN398363	0.0134	0.0010	0.0723	0.0137	0.0087	0.0359	0.0905	0.0197	0.0293	0.0881	0.1082	0.0122	0.0265	0.0167	0.0341
Moerella iridescens	JN398362	0.0151	0.0013	0.0839	0.0241	0.0061	0.0096	0.0031	0.0070	0.0229	0.0085	0.0346	0.0259	0.0015	0.0233	0.0728
Solecurtus divaricatus	JN398367	0.0169	0.0012	0.0705	0.0202	0.0128	0.0206	0.0990	0.0422	0.0579	0.0764	0.0308	0.0198	0.0445	0.0022	0.0400
Sinonovacula constricta	EU880278	0.0385	0.0071	0.1852	0.0165	0.0170	0.0056	0.0139	0.0433	0.0856	0.1151	0.1165	0.0141	0.0168	0.0556	0.1046
Coelomactra antiquata	JQ423460	0.0122	0.0007	0.0609	0.0006	0.0030	0.0389	0.0067	0.0180	0.0178	0.0509	0.1096	0.0104	0.0583	0.0185	0.0123
Mactra chinensis	KJ754823	0.0128	0.0007	0.0565	0.0025	0.0010	0.0316	0.0029	0.0167	0.0313	0.0582	0.0054	0.0038	0.0112	0.0269	0.0523
Lutraria rhynchaena	HG799089	0.0278	0.0039	0.1406	0.1280	0.0081	0.0222	0.0312	0.0306	0.0820	0.0507	0.0316	0.0172	0.1027	0.0447	0.1188
Arctica islandica	KF363951	0.0248	0.0034	0.1386	0.0310	0.0173	0.0093	0.0075	0.0232	0.0169	0.0535	0.0245	0.0270	0.0165	0.0367	0.1801
Calyptogena magnifica	KR862368	0.0650	0.0181	0.2790	0.1005	0.0383	0.0891	0.0394	0.0681	0.0818	0.1309	0.0365	0.0207	0.0513	0.1166	0.1257
Mya arenaria	KJ755996	0.0905	0.0363	0.4006	0.1179	0.0732	0.0445	0.1039	0.1126	0.0038	0.2250	0.1928	0.0663	0.0367	0.0564	0.4209
Hiatella arctica	DQ632742	0.0990	0.0441	0.4451	0.2868	0.0464	0.1977	0.0127	0.1309	0.0811	0.1201	0.5193	0.1167	–	0.0894	0.3495
Panopea generosa	KM580067	0.0095	0.0005	0.0503	0.0232	0.0030	0.1959	0.0005	0.0399	0.0289	0.0491	0.0068	0.0294	0.0013	0.0071	0.0371
Panopea globosa	KM580068	0.0080	0.0004	0.0457	0.0039	0.0011	0.0160	0.0024	0.0319	0.0295	0.0961	0.0087	0.0348	0.1062	0.0148	0.0591
Anodonta anatina	KF030964	0.0543	0.0015	0.0279	0.0496	0.0090	0.0212	0.0185	0.0405	0.0548	0.0510	0.0301	0.0877	0.1430	0.0665	0.0957
Anodonta arcaeformis	KF667530	0.0766	0.0003	0.0039	0.1123	0.0145	0.0001	0.0001	0.0442	0.1493	0.1044	0.1981	0.1005	0.3934	0.0886	0.0330
Anodonta lucida	KF667529	0.0819	0.0039	0.0473	0.1482	0.0110	0.0603	0.0380	0.0580	0.0938	0.1221	0.1266	0.0470	0.0760	0.1020	0.1669
Anodonta euscaphys	KP187851	0.0923	0.0007	0.0079	0.1726	0.0891	–	0.1114	0.1095	0.0805	0.3326	0.0001	0.4194	–	0.1384	0.2573
Hyriopsis cumingii	HM347668	0.0596	0.0008	0.0126	0.1685	0.0001	0.0234	0.0119	0.0430	0.1631	0.1192	0.0211	0.0873	–	0.0698	0.0650
Hyriopsis schlegelii	HQ641406	0.0459	0.0005	0.0115	0.0198	0.0035	0.0531	0.0105	0.0319	0.0351	0.0596	–	0.0475	0.0001	0.0514	0.0674
Utterbackia imbecillis	HM856637	0.0754	0.0029	0.0380	0.1575	0.0081	0.0267	0.0421	0.1127	0.0677	0.0885	0.0812	0.0905	0.1471	0.1252	0.1197
Utterbackia peninsularis	HM856635	0.0496	0.0102	0.2065	0.0177	0.0191	0.0416	0.0020	0.1196	0.0822	0.1027	0.1512	0.0209	0.3393	0.0371	0.0389
Unio pictorum	HM014130	0.0561	0.0018	0.0313	0.0910	0.0092	0.0333	0.0171	0.0524	0.0699	0.0522	0.0714	0.0500	0.1272	0.0630	0.1158
Unio douglasiae	KM657954	0.0519	0.0015	0.0295	0.0713	0.0085	0.0027	0.0087	0.0712	0.0250	0.0597	0.0245	0.0608	0.0354	0.0560	0.0873

Note: –, not available.

Table 2 The nuclear gene (histone H3) of bivalves downloaded from GenBank

Species	Accession no.	Ka/Ks	Species	Accession no.	Ka/Ks
Crassostrea ariakensis	KM460854	0.0001	*Scapharca subcrenata*	JN974603	0.0001
Crassostrea gigas	KM460855	0.0001	*Scapharca broughtonii*	JN974601	0.0001
Crassostrea virginica	KM460872	0.0001	*Scapharca inaequivalvis*	JN974599	0.0001
Crassostrea nippona	KM460859	0.0001	*Scapharca cornea*	JN974597	0.0001
Ostrea circumpicta	KM460864	0.0298	*Scapharca gubernaculum*	JN974594	0.0001
Ostrea denselamellosa	KM460862	0.0771	*Anadara antiquata*	JN974592	0.0001
Saccostrea kegaki	KM460867	0.0001	*Anadara crebricostata*	JN974591	0.0001
Pinctada martensi	JN974634	0.0001	*Anadara vellicata*	JN974589	0.0001
Pinctada margaritifera	KU763362	0.0001	*Mytilisepta virgata*	MK642879	0.0001
Argopecten purpuratus	EU379525	0.0001	*Modiolus modiolus*	HF545159	0.0001
Pseudamussium peslutrae	EU379529	0.0001	*Bathymodiolus manusensis*	HF545158	0.0001
Argopecten gibbus	EU379497	0.0001	*Bathymodiolus boomerang*	HF545135	0.0001
Mimachlamys asperrima	HM540086	0.0001	*Bathymodiolus brevior*	HF545132	0.0001
Arca sp.	JN974630	0.0001	*Mytilus edulis*	AY377769	0.0001
Barbatia virescens	KT757878	0.0001	*Trachycardium egmontianum*	KR422792	0.0001
Barbatia fusca	JN974626	0.0001	*Vasticardium angulatum*	KR422797	0.0001
Trisidos kiyonoi	JN974623	0.0001	*Vasticardium assimile*	KR422799	0.0001
Arca navicularis	JN974618	0.0001	*Lyrocardium lyratum*	KR422775	0.0001
Barbatia lima	JN974613	0.0001	*Laevicardium serratum*	KR422774	0.0001
Scapharca globosa	JN974584	0.0001	*Laevicardium pictum*	KR422772	0.1361
Arca ventricosa	AF416858	0.0001	*Keenocardium blandum*	KR422767	0.0001
Barbatia lacerata	JN974611	0.0001	*Fulvia undatopicta*	KR422766	0.0001
Anadara pilula	JN974608	0.0001	*Fulvia nienkeae*	KR422763	0.0001
Tegillarca granosa	JN974607	0.0001	*Fulvia mutica*	KR422761	0.0001
Tegillarca nodifera	JN974605	0.0001			

2.3 Estimation of Nonsynonymous/Synonymous Substitutions Ratios (Ka/Ks)

The maximum-likelihood phylogenetic relationships were reconstructed based on nucleotide sequences of twelve protein-coding genes using RAxML v.7.0.4 (Stamatakis, 2006). The twelve-partitioned nucleotide sequences were aligned with ClustalX (Thompson *et al.*, 1997). The ratios of nonsynonymous to synonymous substitutions (Ka/Ks) were estimated for each branch using CodeML implemented in the PAML package (Yang, 2007). Model 1 was used, which allows a free Ka/Ks ratio. Only Ka/Ks and Ks values of the external branches were selected in the following analyses, *i.e.*, deleterious mutations (Ka/Ks) between modern species and their most recent ancestors. The statistical analyses were performed with IBM SPSS Statistics, release 19.0.0.1.

3 Results and Discussion

3.1 Relationship Between Genome Size and Nucleotide Composition in Arcidae

Arcidae species poss larger mitogenomes than that found in typical animals, challenging the conventional hypothesis that a compact mitogenome is a common feature among all animals. The increased size of Arcidae mitogenome is due to the presence of long noncoding regions. Genomic coverage by mitochondrial noncoding regions can reach up to 71% (33046 bp) for *S. kagoshimensis* (Sun *et al.*, 2014). Larger size molecules are usually considered to be at a selective disadvantage simply because they take

longer time to replicate, leaving fewer copies to be transmitted (Boyce *et al.*, 1989). On the contrary, smaller sized mtDNA molecules are with replicative or selective advantage (Boyce *et al.*, 1989).

In order to explore if the large Arcidae mtDNA can affect replication mechanics, we compared the nucleotide skew of whole genome sequences (P_{mtDNA}), the protein-coding genes at all codon positions (P_{123}), and the fourfold codon positions (P_{4FD}) between Arcidae species and the other bivalve species (Table 3). AT skews for the P_{mtDNA} are the same between Arcidae and non-Arcidae groups (Mann-Whitney U-test, $P = 0.904$, Fig.1A); however, the GC skews of Arcidae species are significantly greater than that of the non-Arcidae group ($P = 0.003$, Fig.1B). The GC skews of P_{123} in Arcidae species are also significantly greater than those of non-Arcidae group ($P = 0.001$, Fig.1C), while AT skews do not differ ($P = 0.076$, Fig.1D). This pattern is similar when analysis is restricted to the P_{4FD}, which are presumed to be under weaker selection, with the trend toward greater GC skew in Arcidae species ($P = 0.001$, Fig.1E) and little difference in AT skew ($P = 0.048$, Fig.1F).

The asymmetric mechanism of mtDNA replication, in which the parental strand is exposed to mutation while it is in a single-stranded state, can account for the strong compositional asymmetry observed in mitogenomes (Reyes *et al.*, 1998). According to this hypothesis, a possible explanation for the marked nucleotide skew in Arcidae species migth be that the mitogenome replication in Arcidae is slower than those in other bivalves, exposing the parental strand to deamination for a longer time. Thus, the data of the compositional asymmetry of Arcidae and non-Ar-

Table 3 The bivalves mitochondrial genomes included in the analysis of strand asymmetry in nucleotide composition

Species	Accession no.	mtDNA		P$_{123}$		P$_{4FD}$	
		AT skew	GC skew	AT skew	GC skew	AT skew	GC skew
Tegillarca granosa	KJ607173	−0.14	0.41	−0.29	0.43	−0.35	0.72
Anadara vellicata	KP954700	−0.12	0.42	−0.28	0.40	−0.24	0.60
Scapharca kagoshimensis	KF750628	−0.10	0.11	−0.28	0.31	−0.33	0.38
Scapharca broughtonii	AB729113	−0.17	0.36	−0.26	0.33	−0.26	0.43
Trisidos kiyonoi	KU975161	−0.30	0.45	−0.39	0.43	−0.56	0.65
Potiarca pilula	KU975162	−0.15	0.42	−0.27	0.40	−0.25	0.66
Arca navicularis	MG641752	−0.23	0.37	−0.32	0.36	−0.33	0.56
Anadara consociata	MH535977	−0.15	0.46	−0.31	0.47	−0.39	0.73
Barbatia virescens	MF374794	−0.31	0.43	−0.44	0.38	−0.65	0.60
Crassostrea hongkongensis	EU266073	−0.13	0.21	−0.20	0.19	−0.11	0.37
Crassostrea iredalei	FJ841967	−0.10	0.20	−0.20	0.18	−0.10	0.35
Crassostrea sikamea	FJ841966	−0.13	0.21	−0.22	0.19	−0.16	0.33
Crassostrea angulata	FJ841965	−0.13	0.20	−0.22	0.18	−0.16	0.32
Crassostrea ariakensis	FJ841964	−0.13	0.21	−0.22	0.19	−0.15	0.34
Crassostrea virginica	AY905542	−0.13	0.16	−0.22	0.14	−0.14	0.23
Crassostrea nippona	HM015198	−0.10	0.20	−0.20	0.18	−0.08	0.32
Crassostrea gigas	AF177226	−0.13	0.20	−0.22	0.19	−0.15	0.25
Crassostrea gasar	KR856227	−0.13	0.17	−0.22	0.15	−0.13	0.21
Ostrea lurida	KC768038	−0.13	0.18	−0.21	0.18	−0.17	0.28
Ostrea edulis	JF274008	−0.14	0.20	−0.20	0.18	−0.12	0.31
Saccostrea mordax	FJ841968	−0.15	0.21	−0.22	0.19	−0.14	0.40
Saccostrea cucullata	KP967577	−0.15	0.19	−0.22	0.18	−0.15	0.25
Mimachlamys nobilis	FJ415225	−0.25	0.31	−0.34	0.33	−0.39	0.41
Mimachlamys senatoria	KF214684	−0.28	0.32	−0.34	0.33	−0.44	0.40
Placopecten magellanicus	DQ088274	−0.27	0.40	−0.43	0.41	−0.61	0.62
Chlamys farreri	EU715252	−0.18	0.34	−0.31	0.32	−0.31	0.51
Mizuhopecten yessoensis	AB271769	−0.20	0.25	−0.30	0.27	−0.25	0.33
Argopecten irradians	EU023915	−0.26	0.24	−0.35	0.26	−0.38	0.28
Argopecten purpuratus	KF601246	−0.28	0.30	−0.38	0.32	−0.49	0.45
Argopecten ventricosus	KT161261	−0.27	0.28	−0.37	0.30	−0.45	0.38
Pinctada margaritifera	HM467838	−0.23	0.36	−0.32	0.36	−0.37	0.40
Pinctada maxima	GQ452847	−0.31	0.45	−0.39	0.48	−0.55	0.64
Mytilus trossulus	GU936625	−0.09	0.23	−0.16	0.24	0.01	0.45
Mytilus californianus	GQ527172	−0.11	0.26	−0.17	0.27	−0.02	0.50
Mytilus edulis	AY484747	−0.11	0.25	−0.17	0.25	−0.03	0.46
Mytilus galloprovincialis	AY497292	−0.11	0.24	−0.17	0.24	−0.01	0.46
Mytilus coruscus	KJ577549	−0.11	0.26	−0.17	0.26	−0.06	0.49
Brachidontes exustus	KM233636	−0.17	0.20	−0.24	0.19	−0.24	0.18
Perna viridis	JQ970425	−0.19	0.39	−0.26	0.38	−0.24	0.78
Perna perna	KM655841	−0.19	0.20	−0.27	0.22	−0.38	0.25
Musculista senhousia	GU001954	−0.17	0.23	−0.23	0.23	−0.24	0.21
Meretrix petechialis	EU145977	−0.26	0.39	−0.34	0.38	−0.55	0.58
Meretrix lamarckii	GU071281	−0.28	0.43	−0.37	0.39	−0.63	0.65
Meretrix lyrata	KC832317	−0.27	0.45	−0.37	0.40	−0.59	0.60
Meretrix lusoria	GQ903339	−0.26	0.42	−0.35	0.39	−0.57	0.57
Meretrix meretrix	GQ463598	−0.25	0.39	−0.34	0.37	−0.55	0.51
Paphia euglypta	GU269271	−0.15	0.35	−0.22	0.36	−0.16	0.56
Paphia textile	JF969277	−0.14	0.37	−0.25	0.38	−0.21	0.60
Paphia amabilis	JF969276	−0.11	0.29	−0.20	0.30	−0.15	0.42
Paphia undulata	JF969278	−0.15	0.36	−0.25	0.37	−0.16	0.48
Venerupis philippinarum	AB065375	−0.13	0.35	−0.21	0.36	−0.21	0.43
Saxidomus purpuratus	KP419933	−0.22	0.35	−0.31	0.33	−0.35	0.57
Solenaia oleivora	KF296320	−0.22	0.37	−0.28	0.16	−0.25	0.21
Solen grandis	HQ703012	−0.30	0.39	−0.40	0.41	−0.50	0.59
Solen strictus	JN786377	−0.31	0.37	−0.41	0.39	−0.52	0.51
Solenaia carinatus	KC848654	−0.22	0.39	−0.27	0.17	−0.20	0.22

(*to be continued*)

(*continued*)

Species	Accession no.	mtDNA		P$_{123}$		P$_{4FD}$	
		AT skew	GC skew	AT skew	GC skew	AT skew	GC skew
Lucinella divaricata	EF043342	−0.24	0.33	−0.31	0.32	−0.31	0.31
Loripes lacteus	EF043341	−0.23	0.32	−0.31	0.33	−0.31	0.46
Acanthocardia tuberculata	DQ632743	−0.18	0.17	−0.25	0.18	−0.11	0.25
Fulvia mutica	AB809077	−0.13	0.28	−0.24	0.29	−0.16	0.38
Tridacna squamosa	KP205428	−0.12	0.19	−0.19	0.26	−0.05	0.34
Semele scabra	JN398365	−0.23	0.43	−0.32	0.43	−0.36	0.62
Nuttallia olivacea	JN398364	−0.15	0.32	−0.21	0.33	−0.10	0.54
Soletellina diphos	JN398363	−0.26	0.37	−0.33	0.38	−0.45	0.60
Moerella iridescens	JN398362	−0.22	0.35	−0.30	0.32	−0.36	0.34
Solecurtus divaricatus	JN398367	−0.29	0.38	−0.37	0.39	−0.55	0.50
Sinonovacula constricta	EU880278	−0.23	0.36	−0.32	0.37	−0.36	0.63
Coelomactra antiquata	JQ423460	−0.20	0.30	−0.29	0.29	−0.27	0.50
Mactra chinensis	KJ754823	−0.22	0.26	−0.32	0.25	−0.36	0.40
Lutraria rhynchaena	HG799089	−0.28	0.40	−0.37	0.40	−0.47	0.60
Arctica islandica	KF363951	−0.16	0.30	−0.24	0.31	−0.16	0.43
Calyptogena magnifica	KR862368	−0.20	0.39	−0.29	0.39	−0.35	0.64
Mya arenaria	KJ755996	−0.13	0.32	−0.20	0.33	−0.25	0.28
Hiatella arctica	DQ632742	−0.15	0.29	−0.22	0.31	−0.28	0.29
Panopea generosa	KM580067	−0.21	0.38	−0.29	0.39	−0.31	0.33
Panopea globosa	KM580068	−0.27	0.44	−0.35	0.45	−0.38	0.40
Anodonta anatina	KF030964	−0.15	0.30	−0.24	0.18	−0.21	0.20
Anodonta arcaeformis	KF667530	−0.12	0.27	−0.24	0.18	−0.18	0.26
Anodonta lucida	KF667529	−0.13	0.28	−0.23	0.16	−0.19	0.16
Anodonta euscaphys	KP187851	−0.12	0.26	−0.28	0.18	−0.16	0.19
Hyriopsis cumingii	HM347668	−0.23	0.36	−0.30	0.21	−0.27	0.30
Hyriopsis schlegelii	HQ641406	−0.23	0.35	−0.31	0.22	−0.29	0.35
Utterbackia imbecillis	HM856637	−0.15	0.28	−0.23	0.18	−0.20	0.21
Utterbackia peninsularis	HM856635	−0.13	0.26	−0.19	0.13	−0.15	0.18
Unio pictorum	HM014130	−0.19	0.32	−0.25	0.19	−0.25	0.22
Unio douglasiae	KM657954	−0.18	0.32	−0.25	0.19	−0.24	0.21
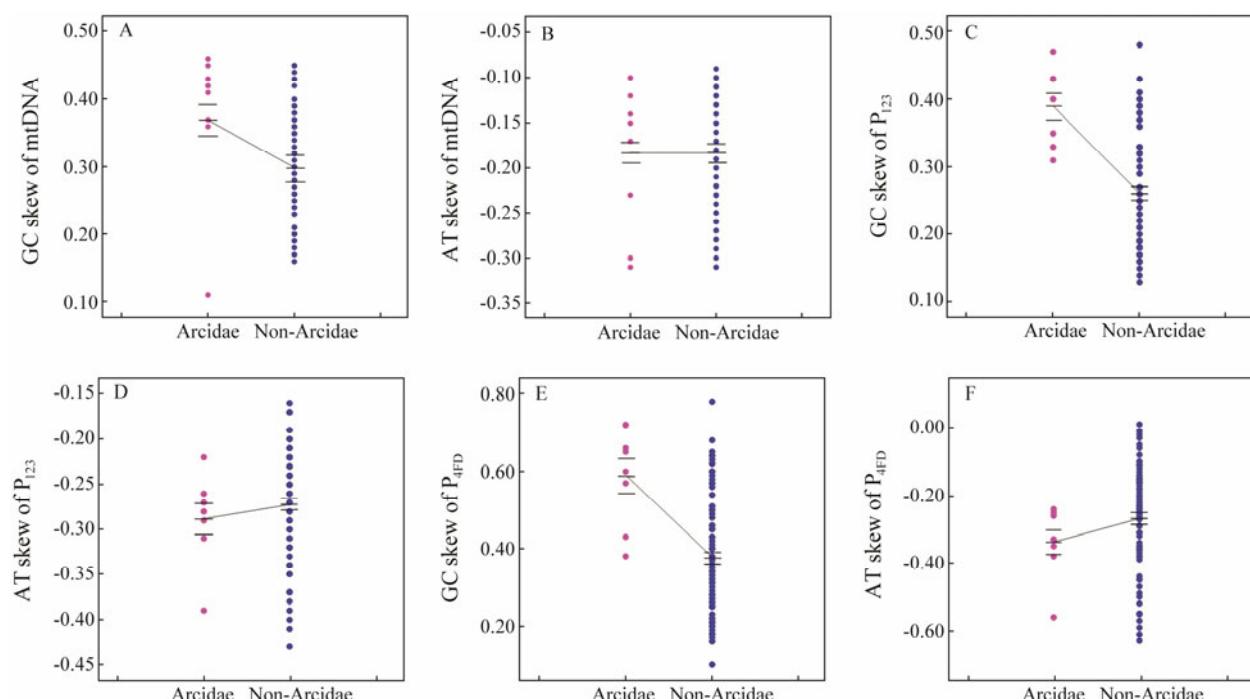
Fig.1 Nucleotide skew values of the whole mtDNA and protein-coding genes of Arcidae and non-Arcidae bivalve mitochondrial genomes. A, GC skews for mtDNA; B, AT skews for mtDNA; C, GC skews for all sites (P$_{123}$); D, AT skews for all sites (P$_{123}$); E, GC skews for the fourfold degenerate sites (P$_{4FD}$); F, AT skews for the fourfold degenerate sites (P$_{4FD}$). The average values of each group are indicated along with standard error bars.

cidae group indicated that the presence of the exceptional long no-coding regions may affect replication mechanics. However, the large size of mitogenomes in the Arcidae species does not mean a significant replicative disadvantage. The exceptional long no-coding regions may provide additional replication initiation signals, which can increase the number of genome replicates per template genome (Jiang *et al.*, 2007; Eberhard and Wright, 2016). This is really advantageous if the replication of Arcidae mtDNA is particularly slow, as reflected by the marked nucleotide skew that were found in Arcidae mitogenomes. One way to test this idea is to map replication initiation sites to see whether Arcidae mitogenomes have more replication initiation zones in the non-coding regions.

3.2 Relaxed Selective Constraint on Large Mitogenomes of Arcidae

Previous studies have shown that the mitogenomes are under selection for smaller size, which can cause higher replication and translation efficiency (Rand, 1993). According to this hypothesis, the large mitogenomes of Arcidae species may under different selective constraint compared with small mitogenomes in other bivalves. In order to explore this difference, we assembled a data set of 86 mitogenomes of bivalves and constructed the Maximum Likelihood (ML) phylogenetic tree (Fig.2).

The ratio of nonsynonymous (change in amino acid) and synonymous (silent) substitutions (Ka/Ks) is generally used to measure the selective constraints acting on the protein-coding sequences. The mitochondrial data set of bivalves (listed in Supplementary Table 1) were first divided into 'Arcidae (large mitogenome)' and 'non-Arcidae (small mitogenome)' groups to represent groups with different mitogenome sizes (Fig.2). The Arcidae group has a significant higher mean value of Ka/Ks (0.0705) than the non-Arcidae group (0.0421) ($P=0.002$, Mann-Whitney U-test, Fig.3A). The mean value for Ka is also significantly different between Arcidae and non-Arcidae groups (0.1094 *vs.* 0.0238, $P<0.001$, Fig.3B), suggesting that the mitochondrial protein-coding genes of Arcidae accumulate more nonsynonymous mutations compared with other bivalves. Considering that the divergences of synonymous mutation rate may bias the results, we compared the average Ks between Arcidae and non-Arcidae groups. The mean value of Ks in Arcidae group (1.8945) is significantly higher than that of the non-Arcidae group (0.7524; $P=0.003$). The greater Ks may result in a smaller Ka/Ks ratio in Arcidae group, making the results more conservative. Therefore, our analyses suggest that the higher Ka/Ks values in Arcidae group are not simply originated from the divergences in synonymous mutation rates.

To identify which mitochondrial protein-coding genes are most affected by the selective constraints, we tested the Ka/Ks ratio for each of the 12 mitochondrial genes (Table 1). Seven protein-coding genes (*atp6, cox1–3, nad1, nad4* and *nad5*) show significantly higher Ka/Ks ratios in Arcidae species (Fig.4). This result suggests that these genes may have experienced more relaxed functional con-

straints.

In order to determine whether the Ka/Ks variations depend upon the mitogenome size, or they just reflect a general pattern of molecular evolution for bivalves, we repeated the above analysis for histone H3, a nuclear gene from 49 bivalves, which is independent of mitogenome size (listed in Table 2). However, the Ka/Ks ratio of histone H3 gene is not significantly different between the two groups (0.0001 *vs.* 0.0085, $P=0.142$).

An alternative hypothesis to explain this finding is that the selective constraints are relaxed on the large mitogenomes of Arcidae species. Because mitochondria play a crucial role in energy generation, mitochondrial genes are more sensitive to the energy-related selective pressures. Higher rates of nonsynonymous substitutions in mtDNA genes may lead to more radical amino acid substitutions (Hanada *et al.*, 2007), resulting a reduction in electron-transferring respiratory chain activity (Weber *et al.*, 1997; Brown *et al.*, 2000). Previous study has showed that low metabolic rates is correlated with relaxed selective constraints on mitochondrial genes (Chong and Mueller, 2012). Based on this hypothesis, Arcidae species may be more likely to survive and reproduce with lower metabolic requirements than other bivalves under similar environment. This coincides with the biological characteristics of Arcidae species. Some Arcidae species are more tolerant to asphyxiation as they can more economically consume oxygen, such as the arcid clam *Scapharca kagoshimensis*, which can adapt well to oxygen content change in the water (Anistratenko and Khaliman, 2006; Soldatov *et al.*, 2009). When the organisms expose to environmental hypoxia, the energy production in mitochondria is slowed, and metabolic rate will be suppressed (Richards, 2011). We thus deduced that the relaxation of selective constraint on large mitogenomes of arcid species is related to their low metabolic rates. The relaxation of selective constraints contributes to generate 'new' (adapted) mitochondrial genes, and positive selection is the basis of adaptive evolution (Shen *et al.*, 2010). Thus the positive selection may have occurred on some mitochondrial genes in Arcidae species to generate the adapted genes.

4 Conclusions

In the present study, we conducted a comparative analysis of 86 bivalve mitogenomes (including 9 arcid mitogenomes) to explore the differences of base composition and selective constraints between the large mitogenomes in Arcidae and small ones in other bivalves. Arcidae mitogenomes have significantly greater GC skews in their coding sequences. The mitochondrial protein-coding genes of Arcidae species had significant higher Ka/Ks than other bivalves. Seven protein-coding genes (*atp6, cox1-3, nad1, nad4* and *nad5*) are most affected by the selective constraints. These divergences are not observed in the nuclear gene (histone H3). The replication of the large mitogenomes in Arcidae may be slower than those in other bivalves. The large mitogenomes of Arcidae experienced more relaxed selective constraints, which is supposed to
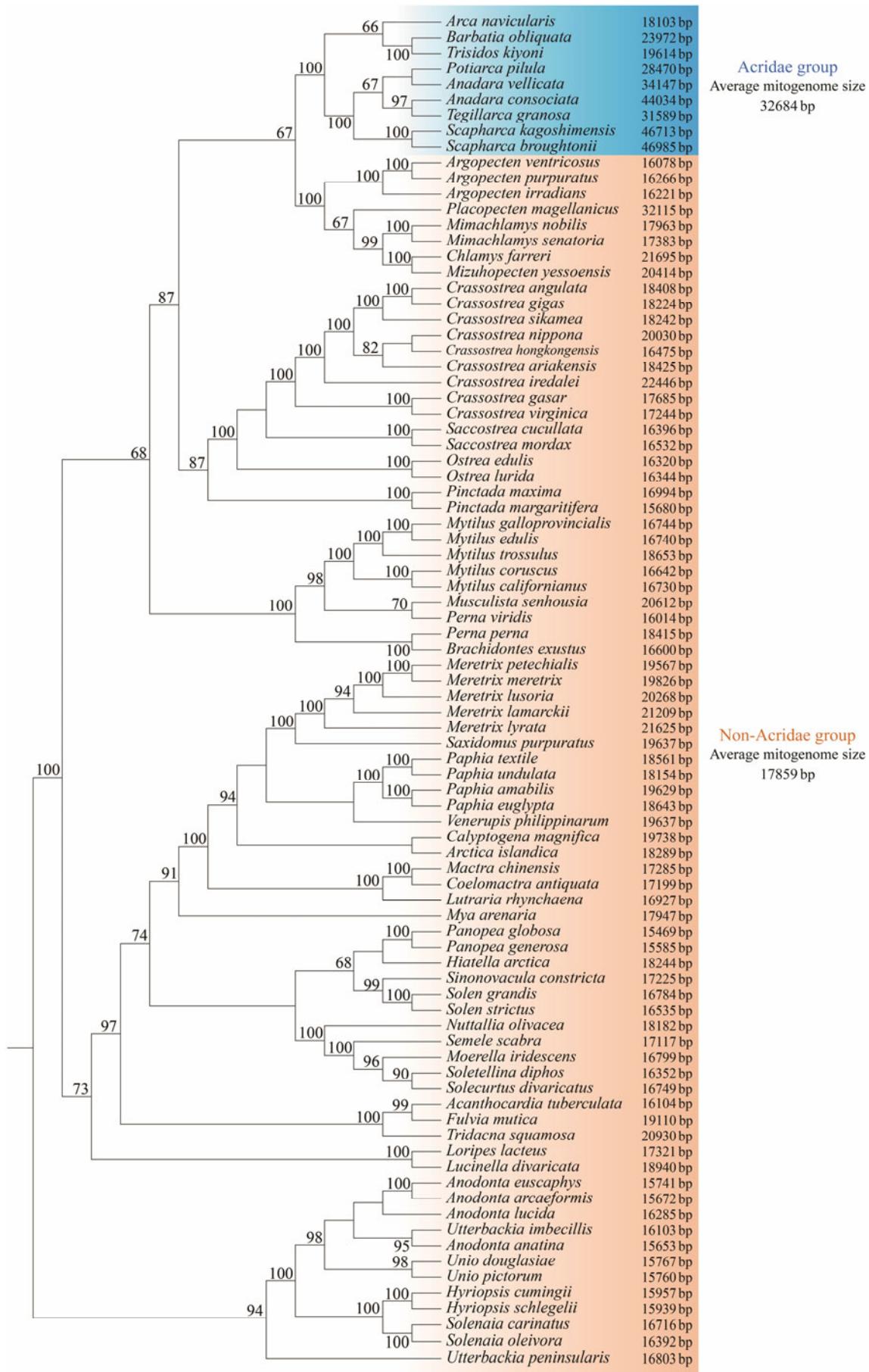
Fig.2 Bivalve phylogenetic tree constructed from 12 mitochondrial protein-coding genes with the Maximum Likelihood method. Arcidae species are marked in blue. The mitogenome size of each bivalve species are indicated. The average mitogenome size of Arcidae and non-Arcidae groups are presented.
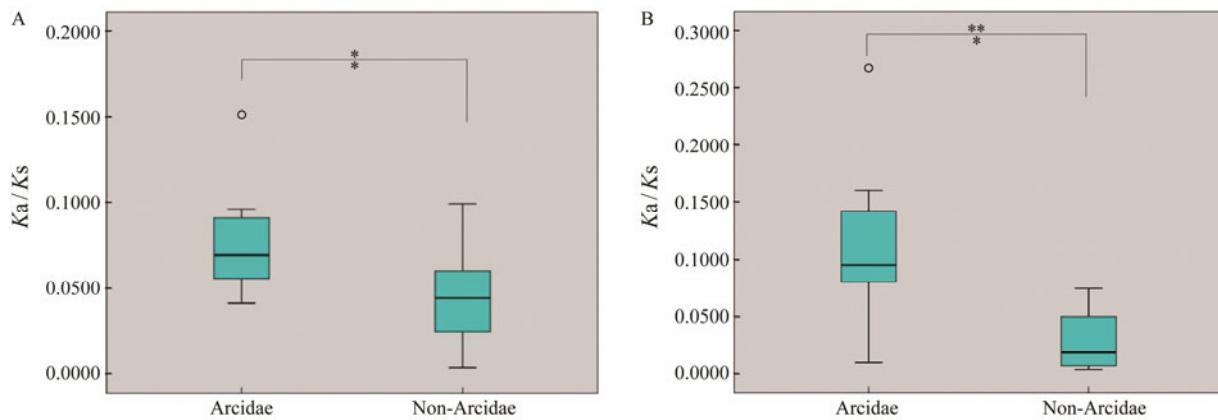
Fig.3 Comparisons of *K*a/*K*s ratios (A) and *K*a (B) between Arcidae and non-Arcidae groups. *$0.01<P<0.05$, **$0.001<P<0.01$, ***$P<0.001$.
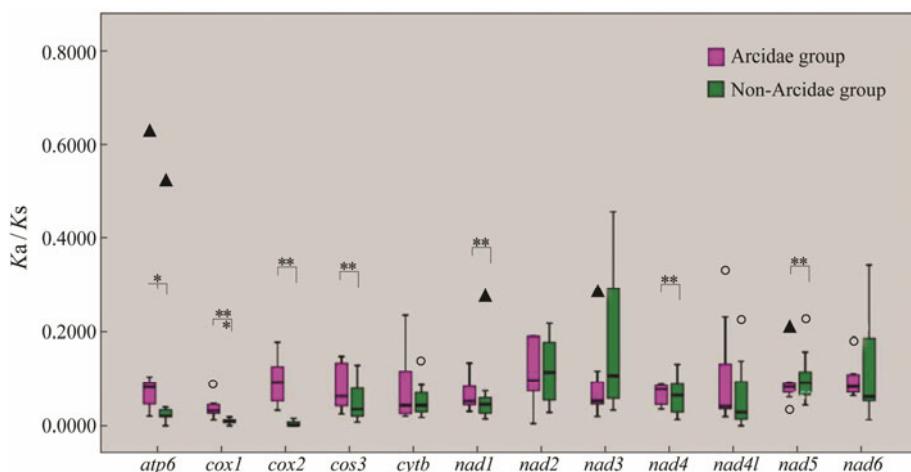

Fig.4 Comparisons of *K*a/*K*s ratios of the 12 mitochondrial protein-coding genes between Arcidae and non-Arcidae groups. *$0.01<P<0.05$, **$0.001<P<0.01$, ***$P<0.001$.

be related to their low metabolic rates, a response to hypoxia exposure.

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