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, 266003

: (*Sanguinolaria*) , , , (COI 16S rRNA), (H3 28S rRNA) , 3 9 61 4 T12(COI) , GC AT , AT , GC 4 (COI 16S rRNA H3 28S rRNA) 5.073 3.042 1.564 1.480, 0.4, 4 , 9 (Pi<0.05) (Hd<0.5) COI 0~0.016, 0.087~0.331, (*Sanguinolaria ovalis*) (*Sanguinolaria chinensis*) , 0.087 (maximum likelihood, ML) (Bayesian inference, BI) , 3 , , ,

: ; COI; 16S rRNA; H3; 28S rRNA;
: S917 **:** A **:** 1005 8737 (2018)05 0936 13

(*Sanguinolaria*) , (Mollusca) (Bivalvia) (*Nutallia*) (Venerioda) (Tellinacea) , (Psammobiidae) 15 [1-2], ( ), [8-9], , , [3-4] , [5-7] , , , [1] 3 14 , , (*Psammotaea*)12 , (*Nutallia*) 1 (*Sanguinolaria olivacea*), [10], COI 16S rRNA (*Hainania*)1 [11], H3 (*Sanguinolaria tchangsii*) [2] 10 28S rRNA

: 2017-11-07; **:** 2017-12-28.
: (2016ZDJS06A06); (201762014).
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[12-14] , , 9 ,
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, [15] ,
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[3,11], ,
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1.1

COI 16S rRNA, 2 28S rRNA
H3 , 1 , 2003—2017 ,

1

Tab. 1 List of *Sanguinolaria* samples used in this study with sampling locality and numbers

sampling locality	species								
	S. acuta	*S. chinensis*	*S. diphos*	*S. elongata*	*S. minor*	*S. ovalis*	*S.tchangsii*	*S. virescens*	*S. olivacea*
Putian, Fujian Province, China			3						
Changle, Fujian Province, China		1				5			
Zhanjiang, Guangdong Province, China	3								
Beihai, Guangxi Province, China	7			4	1		5	3	
Haikou, Hainan Province, China				4	6				
Lingshui, Hainan Province, China				2				3	
Sanya, Hainan Province, China			1	3					
Wenchang, Hainan Province, China								2	
Lianyungang, Jiangsu Province, China									5
Qingdao, Shandong Province, China									1
Rizhao, Shandong Province, China									2

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, 95 %

1.2 DNA

[16], 10 mg , DNA DNA , 1×TE 50 μL 1% DNA ; nanodrop DNA , 100 ng/μL

1.3 PCR DNA

DNA , 1031F/ 1031R COI , O-F/O-R D-F/D-R COI , PEF/PER 16S rRNA (2) PCR 50 μL : 5 μL 10×PCR buffer, 8 μL dNTP(10mmol/L), 1 μL (10 μmol/L), 1 μL (10 μmol/L), 1 μL DNA(100 ng/μL), 0.5 μL *Taq* (5 U/μL, TaKaRa), PCR 50 μL PCR : 94℃ 3 min, 32 : 94 ℃ 30 s, 50 ℃ (LCO1490/HCO2198 48℃) 45 s, 72℃ 45 s, 72℃ 7 min PCR 2 μL 1.5% ,

1.4

DNAstar 5.01 SeqMan (http://www.DNASTAR.com) , , BLAST(https://blast.ncbi.nlm.nih.gov/Blast.cgi) , BioEdit v7.0 [21] CLUSTAL W[22] MEGA 5.0 [23] 4 kimura2-paramter 9 DnaSp 5.0 [24] DAMBE [25] COI 16S rRNA H3 28S rRNA SequenceMatrix1.7.8 [26] PAUP 4.0b10 [27] 4 jMODELTEST[28]

2 PCR

Tab. 2 The sequences of the PCR primers used in this study

locus	primer	(5′–3′) sequence(5′–3′)	/℃ annealing temperature	reference
COI	1031F	AGGATGATAATGCGAACTGA	50	this study
	1031R	GCGTGCGAAATAATACCAA	50	this study
	O-F	GATTATTACCTCCTGCTATTGC	50	this study
	O-R	CCTCTTACGAACTACACCTC	50	this study
	LCO1490	GGTCAACAAATCATAAAGATATTGG	48	Folmer [17]
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	48	Folmer [17]
	D-F	TCACAAGGATATTGGTAGGT	50	this study
	D-R	ATGTAGTTCGTTGGGGAG	50	this study
16S rRNA	PEF	CTAAGGTAGCATAATAGTTCGTCCC	50	this study
	PER	GCAAACCCTAGCCTCAAATAAG	50	this study
	ar	CGCCTGTTTATCAAAAACAT	50	Palumbi[18]
	br	CCGGTCTGAACTCAGATCACGT	50	Palumbi[18]
28S rRNA	28sC1	ACCCGCTGAATTTAAGCAT	50	Hassouna [19]
	28sD2	TCCGTGTTTCAAGACGG	50	Hassouna [19]
H3	H3F	ATGGCTCGTACCAAGCAGACVGC	50	Colgan [20]
	H3R	ATATCCTTRGGCATRATRGRGAC	50	Colgan [20]

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(*Ruditapes philippinarum*) (Bayesian inference, BI) (maximum likelihood, ML) , BI MrBayes 3.1.2[29] , ML PHYML version 2.4.3[30]

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2.1 4

PCR , COI 16S rRNA H3 28S rRNA 61 244 , COI 16S rRNA H3 28S rRNA 557~1482 bp 323~521 bp 272 bp 666 bp(3) CLUSTAL W , 387 bp COI, 272 bp H3 , (gap) 16S rRNA 336 bp 28S rRNA 690 bp

9 COI 16S rRNA H3 28S rRNA , 9

3

Tab. 3 List of sequences obtained for this study with GenBank accession numbers

genus	species	ID ID of specimen	GenBank GenBank accession no.			
			COI	16S rRNA	H3	28S rRNA
Sanguinolaria	*acuta*	acuta.bh1	MG517136	MG517014	MG517197	MG517075
		acuta.bh2	MG517137	MG517015	MG517198	MG517076
		acuta.bh3	MG517138	MG517016	MG517199	MG517077
		acuta.bh4	MG517139	MG517017	MG517200	MG517078
		acuta.bhbt1	MG517130	MG517008	MG517191	MG517069
		acuta.bhbt2	MG517131	MG517009	MG517192	MG517070
		acuta.bhbt3	MG517132	MG517010	MG517193	MG517071
		acuta.zj1	MG517133	MG517011	MG517194	MG517072
		acuta.zj2	MG517134	MG517012	MG517195	MG517073
		acuta.zj3	MG517135	MG517013	MG517196	MG517074
Sanguinolaria	*chinensis*	chinensis.cl1	MG517140	MG517018	MG517201	MG517079
Sanguinolaria	*diphos*	diphos.pt1	MG517141	MG517019	MG517202	MG517080
		diphos.pt2	MG517143	MG517021	MG517204	MG517082
		diphos.pt3	MG517144	MG517022	MG517205	MG517083
		diphos.sy1	MG517142	MG517020	MG517203	MG517081
Sanguinolaria	*elongata*	elongata.bh1	MG517154	MG517032	MG517215	MG517093
		elongata.bh2	MG517155	MG517033	MG517216	MG517094
		elongata.bh3	MG517156	MG517034	MG517217	MG517095
		elongata.bh4	MG517157	MG517035	MG517218	MG517096
		elongata.hkdm1	MG517145	MG517023	MG517206	MG517084
		elongata.hkdm2	MG517146	MG517024	MG517207	MG517085
		elongata.hkdm3	MG517147	MG517025	MG517208	MG517086
		elongata.hkdm4	MG517148	MG517026	MG517209	MG517087
		elongata.hn1	MG517149	MG517027	MG517210	MG517088
		elongata.hn2	MG517150	MG517028	MG517211	MG517089
		elongata.hnsy1	MG517151	MG517029	MG517212	MG517090
		elongata.hnsy2	MG517152	MG517030	MG517213	MG517091
		elongata.hnsy3	MG517153	MG517031	MG517214	MG517092

(to be continued)

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(3 Tab. 3 continued)

genus	species	ID ID of specimen	GenBank GenBank accession no.			
			COI	16S rRNA	H3	28S rRNA
Sanguinolaria	*minor*	minor.bh1	MG517159	MG517037	MG517220	MG517098
		minor.ls1	MG517158	MG517036	MG517219	MG517097
		minor.ls2	MG517160	MG517038	MG517221	MG517099
		minor.ls3	MG517161	MG517039	MG517222	MG517100
		minor.ls4	MG517162	MG517040	MG517223	MG517101
		minor.ls5	MG517163	MG517041	MG517224	MG517102
		minor.ls6	MG517164	MG517042	MG517225	MG517103
Sanguinolaria	*olivacea*	olivacea.jm1	MG517165	MG517043	MG517226	MG517104
		olivacea.lyg1	MG517168	MG517046	MG517229	MG517107
		olivacea.lyg2	MG517169	MG517047	MG517230	MG517108
		olivacea.lyg3	MG517170	MG517048	MG517231	MG517109
		olivacea.lyg4	MG517171	MG517049	MG517232	MG517110
		olivacea.lyg5	MG517172	MG517050	MG517233	MG517111
		olivacea.rz1	MG517166	MG517044	MG517227	MG517105
		olivacea.rz2	MG517167	MG517045	MG517228	MG517106
Sanguinolaria	*ovalis*	ovalis.cl1	MG517173	MG517051	MG517234	MG517112
		ovalis.cl2	MG517174	MG517052	MG517235	MG517113
		ovalis.cl3	MG517175	MG517053	MG517236	MG517114
		ovalis.cl4	MG517176	MG517054	MG517237	MG517115
		ovalis.cl5	MG517177	MG517055	MG517238	MG517116
Sanguinolaria	*tchangsii*	tchangsii.bhbt1	MG517178	MG517056	MG517239	MG517117
		tchangsii.bhbt2	MG517179	MG517057	MG517240	MG517118
		tchangsii.bhbt3	MG517180	MG517058	MG517241	MG517119
		tchangsii.bhbt4	MG517181	MG517059	MG517242	MG517120
		tchangsii.bhbt5	MG517182	MG517060	MG517243	MG517121
Sanguinolaria	*virescens*	virescens.bh1	MG517183	MG517061	MG517244	MG517122
		virescens.bh2	MG517184	MG517062	MG517245	MG517123
		virescens.bh3	MG517185	MG517063	MG517246	MG517124
		virescens.ls1	MG517188	MG517066	MG517249	MG517127
		virescens.ls2	MG517189	MG517067	MG517250	MG517128
		virescens.ls3	MG517190	MG517068	MG517251	MG517129
		virescens.wc1	MG517186	MG517064	MG517247	MG517125
		virescens.wc2	MG517187	MG517065	MG517248	MG517126

2 COI 16S rRNA 4 , 2
GC 41.17% 41.01%, AT (T) 2 ,
, GC , (C) , 28S rRNA
38.95% 38.64% 2 H3 28S rRNA GC , (A)
GC 59.37% 63.96%, 3 , (G)
AT , H3 GC , 3
62.92%, 28S rRNA GC **2.2**
, 65.47%, 64.98% 4 4 5

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4 COI 16 S rRNA H3 28S rRNA

Tab. 4 Nucleotide frequencies of COI 16S rRNA H3 and 28S rRNA gene fragments

gene	/% base composition				GC /% GC content
	(T)	(C)	(A)	(G)	
COI	38.82	17.98	20.01	23.19	41.17
16S rRNA	30.02	13.94	28.98	27.07	41.01
H3	17.21	31.20	23.41	28.17	59.37
28S rRNA	16.95	28.24		35.72	63.96

5 COI 16S rRNA H3 28S rRNA

Tab. 5 Genetic diversity parameters of COI, 16S rRNA, H3 and 28S rRNA gene fragments

gene	number of haplotypes	haplotype diversity	average nucleotide difference	nucleotide diversity
16S rRNA	20	1.0000	52.5842	0.4045
28S rRNA	14	0.9130	37.7546	0.0569
COI	23	0.9399	63.7721	0.4141
H3	22	0.9388	18.3620	0.0673

16S rRNA 130 ,
129 1 ,
/ 26 ,
R=3.042 28S rRNA 154
, 128 26
, / 21 ,
R=1.480
, COI 154
, 153 1
, / ,
R=5.073; H3 56
, 52 4
, / ,
R=1.564 COI H3
, ,
3
COI 9
,
0~0.016, 0.087~0.331,
, 0.087;
8 ,
, 0.258; 8
, , 0.219(
6) 16S rRNA
0.001~0.011, 0.008~
0.393, , 0.008;
8 ,
, 0.318; 8
, , 0.245 (
7) H3
0~0.009, 0.004~0.140,
, 0.004;
8 ,
, 0.129; 8
, , 0.091 (8)
28S rRNA
0~0.019, 0.006~
0.191, 8 ,
0.136;
8 ,
, 0.064 (9)

4

1 , COI
90.70%, GTR
[21], COI
14.06%, H3
53.85%,
3.89%, GTR
, 16S rRNA
28S rRNA
,
COI [22]
SequenceMatrix COI
16S rRNA H3 28S
rRNA , gap 1555 bp
, T12(data T12),
PAUP 4.0b10 T12 4
, P=0.096, T12
[23]

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6 COI 9

Tab. 6 Average genetic distances based on COI gene fragments of nine *Sanguinolaria* species

	species	1	2	3	4	5	6	7	8	intraspecies genetic distance
1	*S. acuta*									0.000
2	*S. chinensis*	0.193								n/c
3	*S. diphos*	0.226	0.176							0.001
4	*S. elongata*	0.192	0.175	0.224						0.004
5	*S. minor*	0.170	0.191	0.225	0.169					0.004
6	*S. olivacea*	0.258	0.269	0.331	0.278	0.284				0.001
7	*S. ovalis*	0.216	0.087	0.164	0.196	0.216	0.314			0.005
8	*S. tchangsii*	0.245	0.236	0.244	0.219	0.249	0.274	0.261		0.000
9	*S. virescens*	0.206	0.209	0.235	0.178	0.189	0.265	0.196	0.275	0.016

7 16S rRNA 9

Tab. 7 Average genetic distances based on 16S rRNA gene fragments of nine *Sanguinolaria* species

	species	1	2	3	4	5	6	7	8	intraspecies genetic distance
1	*S. acuta*									0.004
2	*S. chinensis*	0.119								n/c
3	*S. diphos*	0.127	0.101							0.003
4	*S. elongata*	0.218	0.199	0.188						0.000
5	*S. minor*	0.186	0.208	0.190	0.248					0.011
6	*S. olivacea*	0.318	0.383	0.377	0.393	0.363				0.002
7	*S. ovalis*	0.138	0.088	0.090	0.181	0.201	0.387			0.001
8	*S. tchangsii*	0.256	0.245	0.257	0.290	0.294	0.325	0.252		0.007
9	*S. virescens*	0.169	0.192	0.186	0.157	0.208	0.368	0.165	0.251	0.005

8 H3 9

Tab. 8 Genetic average distances based on H3 gene fragments of nine *Sanguinolaria* species

	species	1	2	3	4	5	6	7	8	intraspecies genetic distance
1	*S. acuta*									0.002
2	*S. chinensis*	0.020								n/c
3	*S. diphos*	0.016	0.011							0.000
4	*S. elongata*	0.069	0.082	0.069						0.013
5	*S. minor*	0.051	0.062	0.050	0.062					0.009
6	*S. olivacea*	0.137	0.146	0.137	0.129	0.138				0.002
7	*S. ovalis*	0.024	0.004	0.015	0.077	0.058	0.141			0.000
8	*S. tchangsii*	0.100	0.104	0.096	0.109	0.102	0.135	0.100		0.000
9	*S. virescens*	0.031	0.034	0.030	0.082	0.049	0.141	0.038	0.091	0.000

2.3

ML BI T12 2 , 9 , , , GTR+I+G ML BI 7 ,

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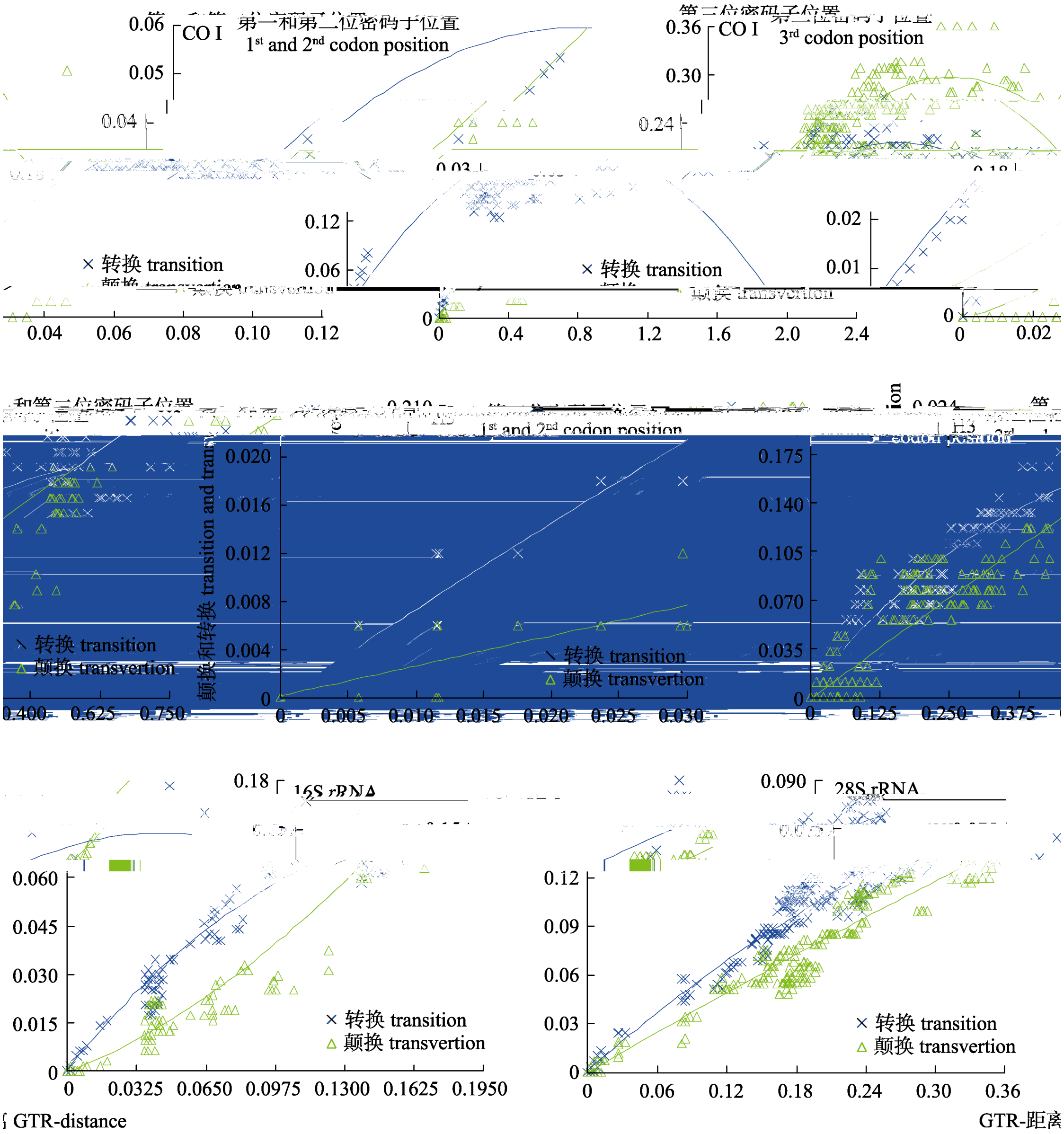
9 28S rRNA 9

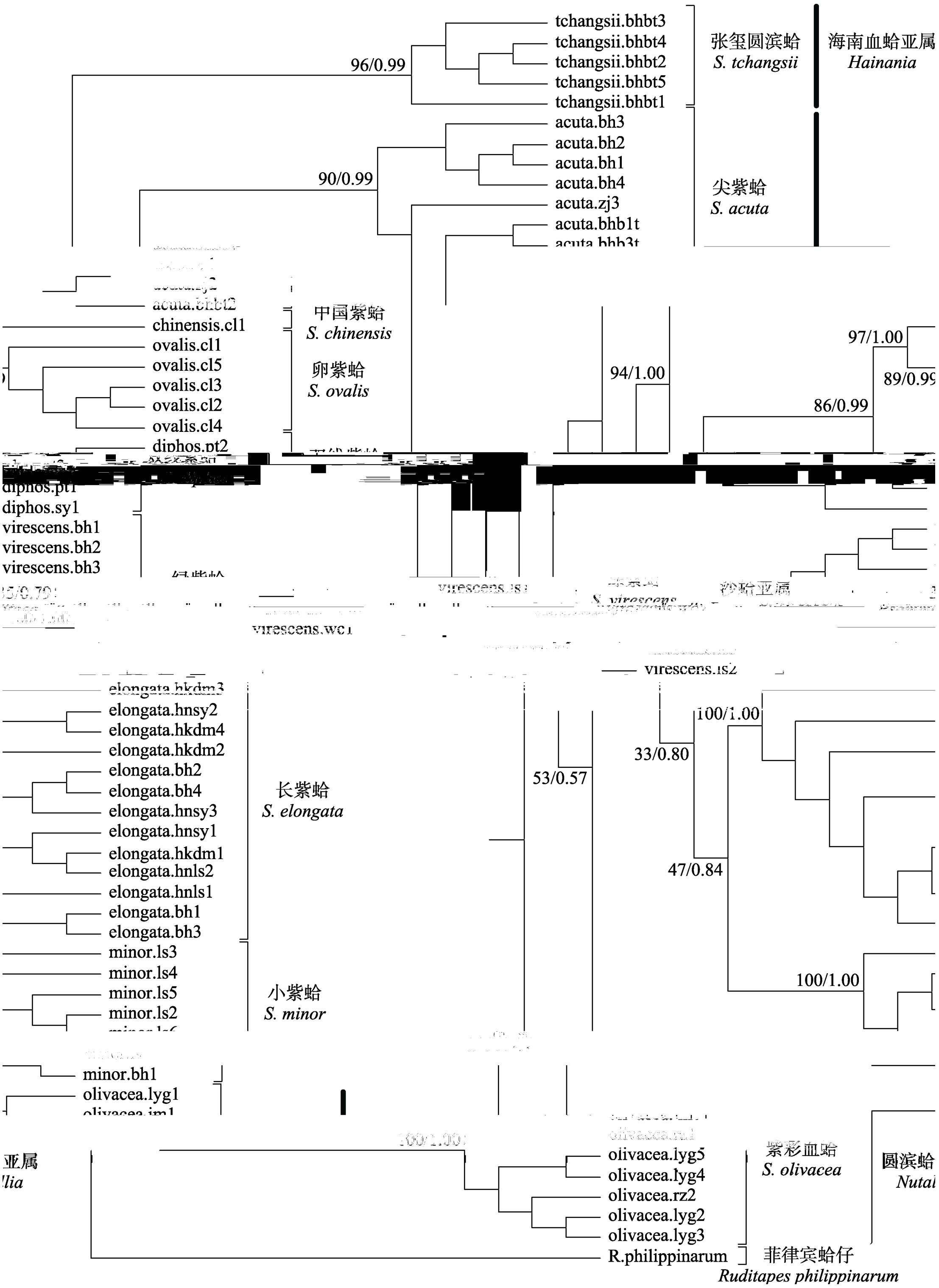
Tab. 9 Genetic average distances based on 28S rRNA gene fragments of nine *Sanguinolaria* species

	species	1	2	3	4	5	6	7	8	intraspecies genetic distance
1	*S. acuta*									0.000
2	*S. chinensis*	0.067								n/c
3	*S. diphos*	0.015	0.064							0.000
4	*S. elongata*	0.048	0.095	0.043						0.002
5	*S. minor*	0.053	0.102	0.048	0.048					0.019
6	*S. olivacea*	0.144	0.191	0.140	0.146	0.144				0.000
7	*S. ovalis*	0.009	0.057	0.006	0.039	0.046	0.136			0.000
8	*S. tchangsii*	0.065	0.120	0.067	0.080	0.082	0.144	0.067		0.001
9	*S. virescens*	0.018	0.067	0.015	0.037	0.046	0.136	0.009	0.064	0.000



1 COI 16S rRNA H3 28S rRNA

Fig. 1 Saturation analysis plot for the COI, 16S rRNA, H3 and 28S rRNA transition and transversion substitutions of genus *Sanguinolaria*


tchangsii.bhbt3
tchangsii.bhbt4
tchangsii.bhbt2
tchangsii.bhbt5
tchangsii.bhbt1
96/0.99
张玺圆滨蛤
S. tchangsii
海南血蛤亚属
Hainania
acuta.bh3
acuta.bh2
acuta.bh1
acuta.bh4
acuta.zj3
acuta.bhb1t
90/0.99
尖紫蛤
S. acuta
chinensis.cl1
中国紫蛤
S. chinensis
ovalis.cl1
ovalis.cl5
ovalis.cl3
ovalis.cl2
ovalis.cl4
卵紫蛤
S. ovalis
diphos.pt2
diphos.pt1
diphos.sy1
virescens.bh1
virescens.bh2
virescens.bh3
97/1.00
89/0.99
94/1.00
86/0.99
virescens.ls1
virescens.wc1
virescens.ls2
elongata.hkdm3
elongata.hnsy2
elongata.hkdm4
elongata.hkdm2
elongata.bh2
elongata.bh4
elongata.hnsy3
elongata.hnsy1
elongata.hkdm1
elongata.hnls2
elongata.hnls1
elongata.bh1
elongata.bh3
长紫蛤
S. elongata
53/0.57
100/1.00
33/0.80
47/0.84
minor.ls3
minor.ls4
minor.ls5
minor.ls2
小紫蛤
S. minor
100/1.00
minor.bh1
olivacea.lyg1
olivacea.lyg5
olivacea.lyg4
olivacea.rz2
olivacea.lyg2
olivacea.lyg3
紫彩血蛤
S. olivacea
圆滨蛤
R.philippinarum
菲律宾蛤仔
Ruditapes philippinarum

2 T12 9

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Fig. 2 Phylogenetic trees of nine *Sanguinolaria* species based on data T12
Numbers on the trees represent the values of supporting values and posterior probability values.

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{[+(+)]+[+(
+)]+ }

3

9 COI
16S rRNA GC AT ,
AT ,
, DNA
[24] DNA
, R (TS/TV)
[25] Desalle [26] , ,
,
, , *R*
; , ,
, *R* Holmquist[27]
0.4, 4
R ,

, ,

[28]

DNA
, 16S rRNA
COI ,
8 COI
16S rRNA ;

7 ,
COI 16S
rRNA COI

, 16S rRNA

[29]

, 16S rRNA
, COI
,

4 , E 6* OH¡t8=3GDô &2ÝçN± ½A/iR» IàŸ¡Ã` l x0ÙP0

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[1, 8],

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COI

(0.087),

10 ,

[36]

(COI 16S rRNA) (H3 28S rRNA)

9 61

, 9

(Pi<0.05) (Hd<0.5); COI

0~0.016

, 0.087~0.331;

3 ,

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Phylogenetic relationships among *Sanguinolaria* species in the coastal waters of China

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Abstract: Fragments of the mitochondrial gene (COI and 16S rRNA) and the nuclear marker (H3 and 28S rRNA) regions are suitable for phylogenetic relationship analysis. The phylogenetic relationships among nine *Sanguinolaria* species (*Sanguinolaria acuta*, *S. chinensis*, *S. diphos*, *S. ovalis*, *S. virescens*, *S. elongata*, *S. minor*, *S. olivacea,* and *S. tchangsii*) collected from the coastal areas of China was analyzed through the sequencing of COI, H3, 16S rRNA, and 28S rRNA genes. The two mitochondrial genes, COI *and* 16S rRNA, had an obvious AT-skewness, and their GC content were 41.17% and 41.01%, respectively, showing a certain degree of base preference. The overall transition/transversion bias of the four fragments (COI 5.073, 16S rRNA 3.042, H3 1.564, and 28S rRNA 1.480) were much higher than the critical value (0.4), which could offer effective phylogenetic information. As the genetic diversity parameters of all four markers in nine *Sanguinolaria* species had higher values ($Pi<0.05$, $Hd<0.5$) than most bivalve species, it is suggested that the *Sanguinolaria* species may have long history in the localities, and sufficient phylogenetic information. Among the nine *Sanguinolaria* species, the genetic average distances based on the COI gene were 0.087–0.331, and the genetic distances within species were 0–0.016. The minimum genetic distance was between *S. ovalis* and *S. chinensis*, which was much higher than the average value (0.005) between most species of bivalves. The saturation of the nucleotide substitution process in the four markers was calculated as well. The third codon positions of the COI gene's transition and transversion substitutions reached the point of saturation, which means that the third codon positions of the COI gene could not provide effective information. The combined data set T12, including the four molecular markers without the third codon positions of COI, totaled 1555 bp in length. Phylogenetic trees constructed by the Maximum Likelihood (ML) and Bayesian Inference (BI) methods were consistent, and cluster analysis based on the data set T12 showed three separate lineages, corresponding to three morphologically defined subgenera: *Hainania*, *Nutallia*, and *Psammotaea*. Combining morphological and molecular evidence, the results support that *S. ovalis* and *S. chinensis* has the closest phylogenetic relationship. The study sheds light on the genetic diversity and phylogenetic relationship of *Sanguinolaria* species occurring along the coast of China, which may be useful in genetic resource protection and sustainable use.

Key words: *Sanguinolaria*; COI; 16S rRNA; H3; 28S rRNA; phylogenetic relationship

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