



Molecular Phylogenetic of *Oithona* based on mitochondrial COI and 18S gene

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Study purposes

The genus *Oithona* is one of small size cyclopoid copepods which widely distributed and occurs in all kinds of marine environments (estuarine, coastal and oceanic waters). However, identification of *Oithona* based on micro-morphological characters is extremely difficult and time-consuming. Previous fragmentary taxonomic studies of *Oithona* in the South China Sea, were based solely on morphology. Here, we used DNA barcoding and DNA taxonomy to investigate the species diversity of the genus *Oithona* in the South China Sea. We also performed tests on DNA taxonomy (ABGD and GMYC) and built phylogenetic tree base on 18S and COI sequence with the same gene sequence data deposited in GenBank.

Table 1 List of species in *Oithona* examined, with location coordinates, Genbank scession

species	Location & ID	Longitude	Latitude	Sequence ID	Accession number	Sequence source
<i>O. dissimilis</i>	Japan	127.42° E	26.22° N	OD62	AB604162.1	GenBank
				OD63	AB604163.1	GenBank
				OD64	AB604164.1	GenBank
<i>O. oculata</i>	Hawaii	157.51° W	21.29° N	OO42	KC594142.1	GenBank
				OO43	KC594143.1	GenBank
				OO44	KC594144.1	GenBank
<i>O. simplex</i>	Hawaii	157.42° W	21.25° N	OX50	KC594150.1	GenBank
<i>O. frigida</i>	Southern Ocean	0.07° W	62.02° S	OF38	KU982938.1	GenBank
	South China Sea NS1	116.06° E	12.51° N	SP61	-	This study
<i>O. tenuis</i>	South China Sea NS2	110.09° E	10.54° N	SP62	-	This study
	South China Sea NS3	111.01° E	12.55° N	SP63	-	This study
	South China Sea NS4	115.11° E	9.59° N	SP64	-	This study
	South China Sea NS5	113.23° E	21.01° N	SP65	-	This study
	Mediterranean Sea	7.32° E	43.69° N	OP59	KU982959.1	GenBank
<i>O. plumifera</i>	South China Sea NS6	111.56° E	17.25° N	OP1	-	This study
	South China Sea NS7	113.19° E	14.52° N	OP2	-	This study
	South China Sea NS8	117.07° E	14.56° N	OP3	-	This study
	South China Sea NS9	108.56° E	18.14° N	OP4	-	This study
	South China Sea NS9	108.56° E	18.14° N	OP5	-	This study
<i>O. similis</i>	Korea Strait	127.59° E	34.05° N	OS70	JN230870.1	GenBank
	North Sea	3° E	53° N	OS71	JN230871.1	GenBank
				OS45	KT208745.1	GenBank
<i>O. setigera</i>	South China Sea NS10	109.42° E	17.20° N	OG1	-	This study
	South China Sea NS11	111.08° E	10.55° N	OG2	-	This study
<i>O. fallax</i>	South China Sea NS11	111.16° E	10.53° N	SP71	-	This study
	South China Sea NS12	115° E	11.61° N	SP72	-	This study
<i>O. longispina</i>	South China Sea NS13	111.09° E	14.53° N	SP41	-	This study
	South China Sea NS11	111.03° E	10.62° N	SP42	-	This study
	South China Sea NS8	117.12° E	14.52° N	SP43	-	This study
	South China Sea NS5	113.21° E	21.41° N	SP44	-	This study
	South China Sea NS11	111.11° E	10.52° N	SP45	-	This study
<i>O. attenuata</i>	Kaneohe Bay	157.47° W	21.25° N	OA39	KC594139.1	GenBank
				OA40	KC594140.1	GenBank
<i>O. nana</i>	Helgoland	8.45° E	55.03° N	ON56	KU982956.1	GenBank
<i>Oithona</i> sp.	California	117.89° W	33.60° N	OSP13	ZPC08013	Bold Systems
<i>O. davisae</i>	Korea Strait	Unpublished		OD88	KR048988.1	GenBank

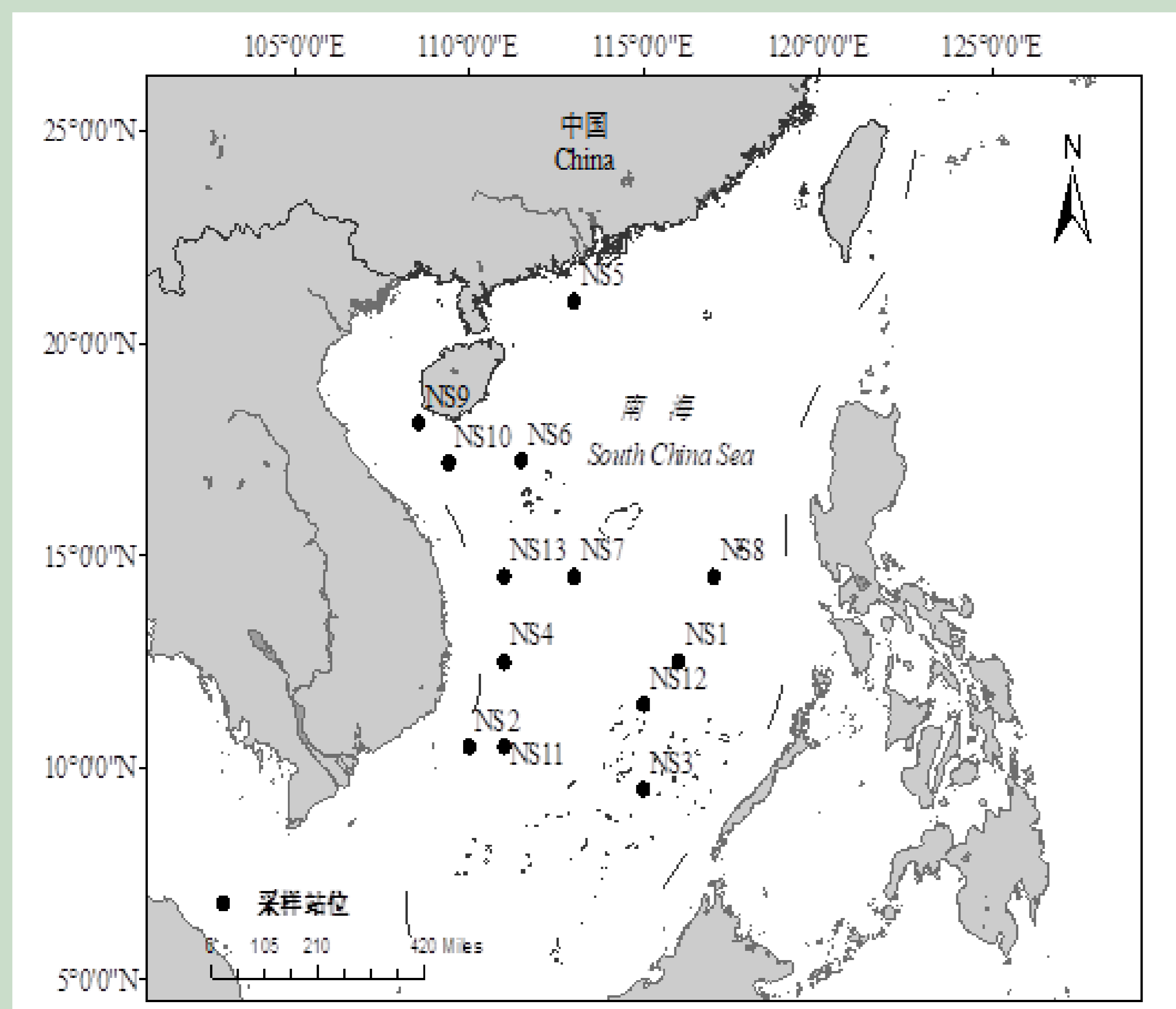


Fig.1 Location of sampling sites in South China Sea

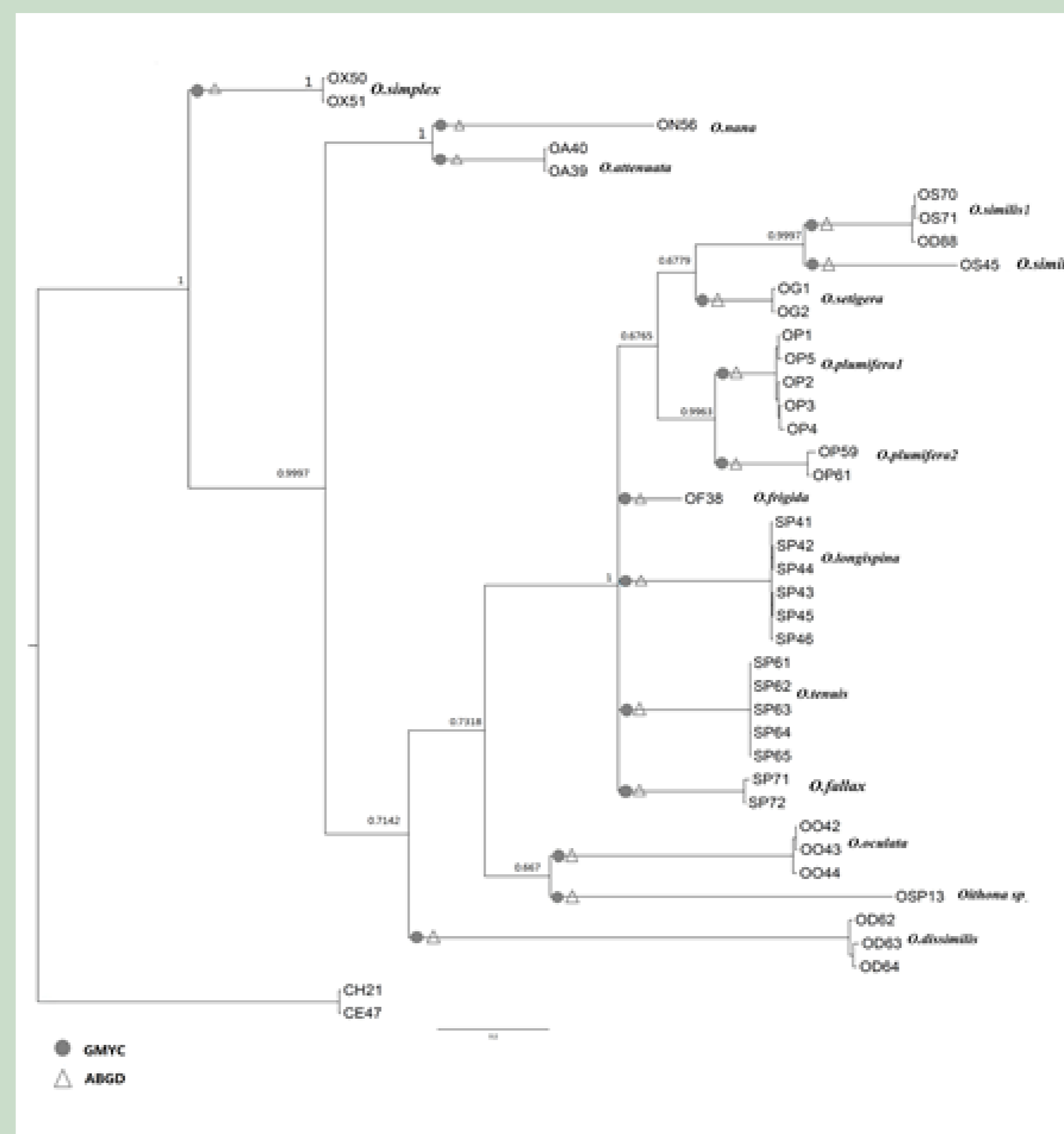


Fig. 2 Bayes phylogenetic tree of *Oithona* base on mitochondria COI gene sequences. Numbers on the nodes are Bayesian posterior probabilities, results of DNA taxonomy from ABGD and GMYC are shown on the node.

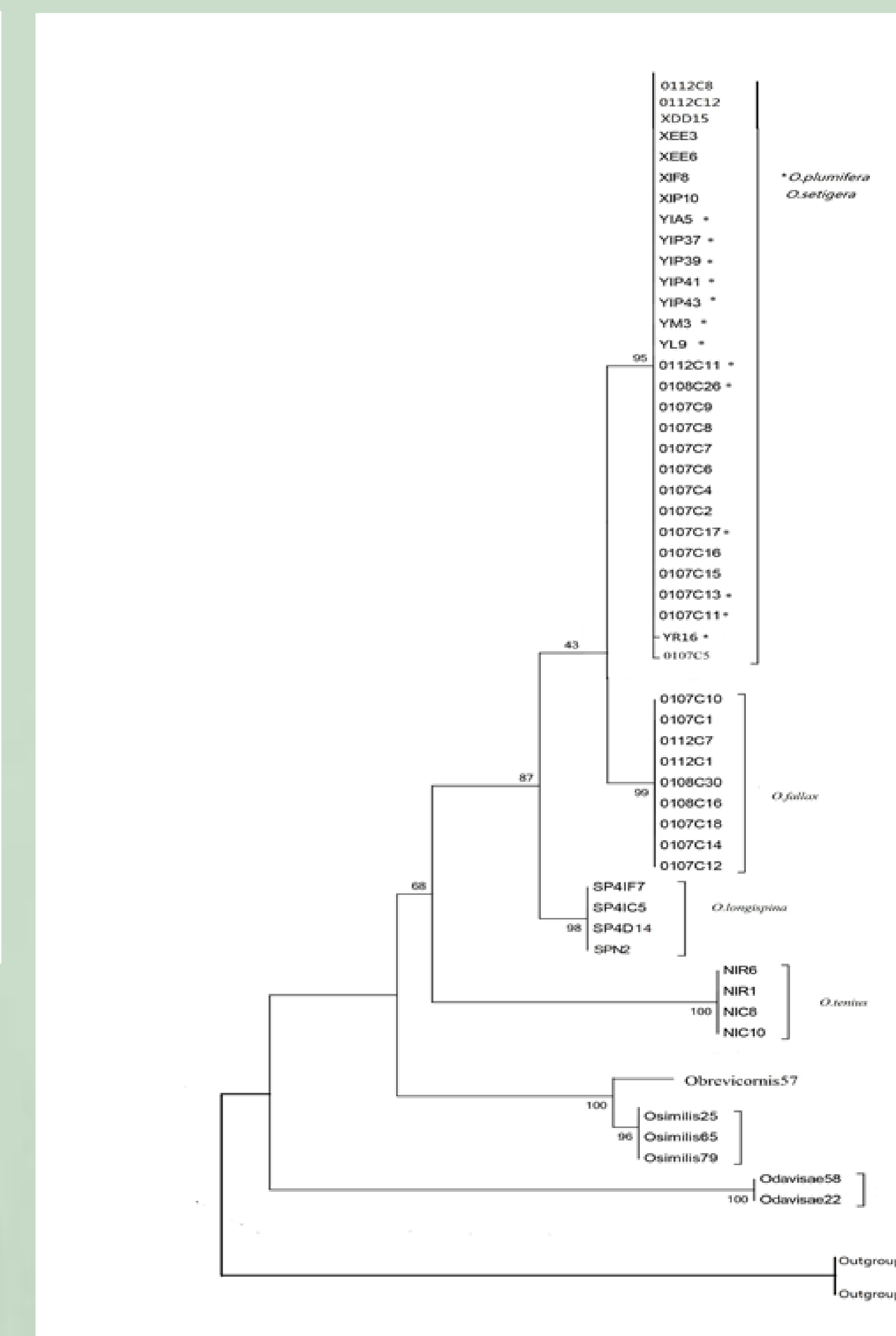


Fig. 3 Maximum Likelihood phylogenetic tree of *Oithona* base on mitochondria 18S rDNA gene sequences. Bootstrap values are shown on the node.

Table 2 The genetic distance within (on the diagonal) and between (below the diagonal) 15 *Oithona* in Kimura 2-parameter model

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	0.009±0.003														
2	0.387±0.036	0.004±0.002													
3	0.413±0.038	0.341±0.035	0.007±0.003												
4	0.324±0.031	0.332±0.032	0.381±0.035												
5	0.395±0.037	0.229±0.026	0.379±0.037	0.381±0.036											
6	0.362±0.034	0.277±0.029	0.305±0.031	0.336±0.033	0.296±0.029										
7	0.383±0.037	0.369±0.036	0.380±0.036	0.374±0.036	0.382±0.036	0.356±0.035									
8	0.345±0.034	0.395±0.037	0.338±0.033	0.335±0.034	0.398±0.036	0.334±0.033	0.294±0.030	0.002±0.002							
9	0.445±0.042	0.391±0.039	0.397±0.039	0.435±0.041	0.393±0.037	0.365±0.036	0.435±0.042	0.363±0.034							
10	0.374±0.035	0.283±0.029	0.363±0.034	0.341±0.034	0.271±0.029	0.238±0.026	0.349±0.033	0.330±0.031	0.322±0.031	0.003±0.001					
11	0.373±0.036	0.267±0.028	0.361±0.035	0.377±0.035	0.299±0.030	0.211±0.024	0.380±0.037	0.323±0.032	0.346±0.035	0.233±0.026	0.004±0.003				
12	0.367±0.034	0.244±0.027	0.365±0.035	0.328±0.032	0.278±0.030	0.254±0.027	0.407±0.036	0.377±0.037	0.369±0.036	0.245±0.027	0.269±0.029	0.004±0.003			
13	0.375±0.035	0.272±0.028	0.352±0.035	0.368±0.035	0.283±0.028	0.183±0.023	0.366±0.034	0.367±0.035	0.392±0.038	0.244±0.027	0.248±0.028	0.252±0.027	0.009±0.003		
14	0.325±0.032	0.254±0.027	0.335±0.033	0.286±0.029	0.221±0.025	0.177±0.022	0.345±0.035	0.356±0.035	0.425±0.042	0.244±0.027	0.244±0.028	0.225±0.026	0.226±0.025	0.002±0.002	
15	0.368±0.036	0.265±0.028	0.349±0.033	0.378±0.037	0.271±0.028	0.248±0.028	0.403±0.038	0.353±0.036	0.369±0.038	0.290±0.030	0.266±0.029	0.221±0.025	0.186±0.023	0.248±0.026	0.016±0.006

1 *O. dissimilis*, 2 *O. similis*, 3 *O. oculata*, 4 *O. simplex*, 5 *O. similis*, 6 *O. feigida*, 7 *O. nana*, 8 *O. attenuata*, 9 *Oithona* sp., 10 *O. longispina*, 11 *O. tenuis*, 12 *O. setigera*, 13 *O. plumifera*, 14 *O. fallax*, 15 *O. plumifera*

Results

The results of delimitation using ABGD and GMYC model were consistent with morphological approach. The average transition/transversion ratio (si/sv) of COI gene was 1.44. Transition and transversion mainly occur at third site. The average transition/transversion ratio of 18S rDNA gene sequence is 2.96, which is higher than COI gene sequence. 18S rDNA gene was found to be highly conserved samples. There are differences in base composition between 18S rDNA gene and COI gene. COI gene show strong (A+T) bias with the average content of A, T, C, G is 24.5%, 37.6%, 19.4%, 18.4%. The content of A+T (62.1%) is higher than content of G+C (37.8%). However, 18S rDNA gene sequences were diffidence from mitochondria COI gene. 18S rDNA gene sequences were conservative with low frequency of variable site and high frequency invariable site. The content of base show nearly equal with the average content of A, T, C, G is 25.2%, 24.3%, 23.2%, 27.2%.

Conclusions

Our study, the first of its kind in the South China Sea, revealed nine species of *Oithona*, using DNA barcoding as a tool to help delineate species and their distribution pattern. The genetic divergence based on COI data sets within species was 0-1.6% while among species varies within a large range, from 17.7% to 44.5%. However, the genetic divergence based on 18S gene is 0.1% -5%. Phylogenetic trees showed that each species was clustered together as a monophyletic group. *O. simplex* first separated from others species indicated pioneer speciation in *Oithona*. Two cryptic species were found in *O. similis* and *O. plumifera*, which were from South China Sea and Mediterranean, Korea Strait and North Sea, the K2P genetic divergence were 18.6% and 22.9%, respectively.