Mitochondrial DNA

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SHORT COMMUNICATION

New insight into the phylogeny of *Sinonovacula* (Bivalvia: Solecurtidae) revealed by comprehensive DNA barcoding analyses of two mitochondrial genes

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Abstract

The present study was undertaken to clarify the genetic relationships of *Sinonovacula* through comprehensive DNA barcoding analyses of *COI* and *16S rRNA* genes. For both genes, the K2P distances between individuals of *Sinonovacula* and individuals of other genera belonging to Tellinoidea were much bigger than those between *Sinonovacula* and genera of Solenoidea. On the Bayesian tree of combined data, *Sinonovacula* and *Cultellus* formed a well supports monophylic clade. An extremely high matching rate of CAs between *Sinonovacula* and the reference family Cultellidae was found. Thus, we suggest transferring *Sinonovacula* from Solecurtidae to Cultellidae, as a sister group of *Cultellus*.

Keywords

16S rRNA, *COI*, DNA barcoding, phylogeny, *Sinonovacula*

History

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Introduction

DNA barcoding proposed by Hebert et al. (2003) is using a short stretch of the mitochondrial gene to identify species and analyze phylogenetic relationships (Frezal & Leblois, 2008). Most methods of DNA barcoding can be broadly divided into two main categories: tree-based and character-based. Tree-based methods can be further divided into two classes. One is the distance-based method, which converts DNA sequences into pairwise genetic distances and then classifies taxa based on the degree of divergences (Hebert et al., 2003). The other is monophyly-based method, which constructs a gene tree and identifies independently evolving clade as taxon (Zou et al., 2011). The character-based algorithm delimits taxa by identifying a unique combination of diagnostic nucleotides (at least 3 states) (DaSalle et al., 2005).

Sinonovacula, an extant genus of Venerida, Heterodonta, Bivalvia, contains two known species: *Sinonovacula constricta* (Lamarck, 1818) and *Sinonovacula rivularis* (Huang & Zhang, 2007). Both of them are economically and ecologically important clams (Feng et al., 2010; Huang & Zhang, 2007). However, the taxonomy and phylogeny of *Sinonovacula* has been debated for decades but has not reached a unanimous view by far. Herein we analyzed the mitochondrial DNA cytochromec oxidase subunit I gene (*COI*) and the 16 small-subunit ribosomal RNA (*16S rRNA*) genes of 20 tellinoidean and solenoidean species to recover the phylogeny of *Sinonovacula*.

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Materials and methods

Ethics statement

All the studied species were not endangered or protected. The locations are not privately-owned or protected in any way, thus no specific permissions were required for them.

Specimen sampling

A total of 102 individuals consisting of 68 from tellinoidean, 19 from solenoidean, and 15 from cardioideans (as outgroups) were sequenced. All samples were collected from 26 geographically wide localities along the coast of China from 2002 to 2011.

DNA extraction, amplification and sequencing

DNA was extracted from small pieces of adductor muscle tissues following a phenol-chloroform procedure modified by Li et al. (2002). Isolated DNA was resuspended in 1% TE buffer and stored at −20 °C until used. *COI* and *16S* genes were amplified by polymerase chain reaction (PCR) using three pairs of primers: LCO1490/HCO2198 (Folmer et al., 1994), COXF-ALT/COXR-ALT (Mikkelsen et al., 2006) and 16Sar/16Sbr (Palumbi, 1996). PCR was implemented in a 50 μL mix containing 2 U Taq DNA polymerase (Takara), about 100 ng template DNA, 1 μM forward and reverse primers, 200 μM of each dNTP, 1× PCR buffer, and 2 mM $MgCl_2$. All PCRs were carried out by the following thermocycler programme: 94 °C for 3 min, 35 cycles of 94 °C for 45 s, 44–56 °C for 1 min and 72 °C for 1 min, then 72 °C for 10 min for extension.

PCR products were first visualized on 1.5% agarose gels with ethiam bromide and then purified by EZ Spin Column DNA Gel Extraction kit (Sangon Biotech, Chedun, Shanghai, China). The purified products were sequenced using ABI PRISM 3730 (Applied Biosystems, Foster, CA). The sequences were edited manually using SEQMAN software (DNAstar, Madison, WI)

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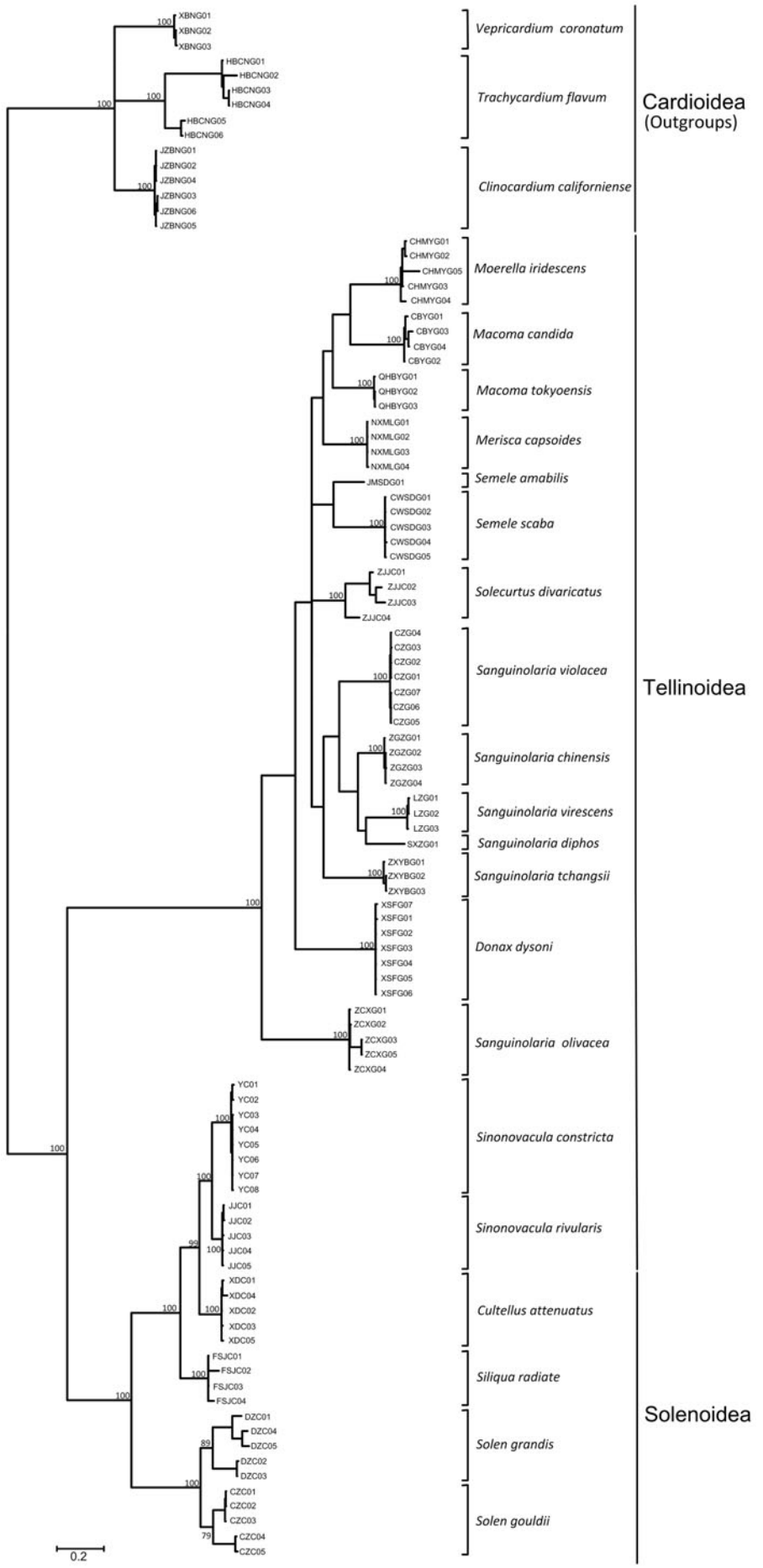

Figure 1. Bayesian tree of COI + 16S data of tellinoideans and solenoideans with Cardioidea as outgroups. The posterior probabilities were shown when $\geq$0.7.

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(DNASTAR 7.2.1) and aligned by Clustal W (Thompson et al., 1994) in BioEdit 7.0.9 (Hall, 1999).

Tree-based barcoding analysis

Pairwise genetic distances were calculated using Kimura's 2-parameter (K2P) model in MEGA 4.0 (Tamura et al., 2007) for both genes. The Bayesian tree of the combined data (*COI + 16S* genes) was performed in MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). The optimal evolution model was HKY + I + G determined by jModelTest 0.11 (Posada & Buckley, 2004). The parameters set for the Bayesian tree were: random trees = 2; mcmc ngen = 10000000; samplefreq = 1000; sump burnin = 2500.

Character-based barcoding analysis

For the character-based analysis, the characteristic attribute organization system (CAOS) (Sarkar et al., 2008) was applied. CAOS identified pure unique diagnostics, termed as ''characteristic attributes'' (CAs), at the target-branching nodes. Herein, sequences of 14 tellinoideans and 4 solenoideans were selected to constitute a reference dataset. Phylogenic trees of the reference sequences were produced in PAUP v4.0b10 (Swofford, 2002) using the K2P model and incorporated into NEXUS files with the DNA data matrix in MacClade v4.06 (Maddison & Maddison, 2009), respectively. Then the datasets were executed in P-Gnome to identify CAs on the family level. After identifying CAs of the reference families, we calculated the matching rate of CA sites between *Sinonovacula* and each family.

Results

Genetic divergences between *Sinonovacula* and other genera belonging to Tellinoidea ranged from 45.2% to 58.8% (mean distance of 50.1%) for *COI* gene, and from 39.3% to 49.3% (mean distance of 44.1%) for *16S rRNA* gene. The highest genetic distances happened between *Sinonovacula* and *Solecurtus*. Divergences between *Sinonovacula* and solenoidean genera ranged from15.0% to 31.4% (average distance of 23.4%) for *COI* gene and from 5.0% to 29.5% (average distance of 18.7%) for *16S rRNA* gene. The lowest genetic distances were found between *Sinonovacula* and *Cultellus*.

In Bayesian tree with Cardioidea as outgroups, all individuals of *Sinonovacula* firstly clustered together on the species-level separately with 100% supports, then grouped with *Cultellus attenuatus* as a sister group nesting within *Siliqua radiate* on the family level with well supports, and finally formed a highly supported clade with Solenidae, distant from Solecurtus of Tellinoidea (Figure 1).

A sufficient number of diagnostic positions were identified for each reference family, 10–46 for *COI* gene and 15–66 for *16S* gene (Table 1). Matching rate between *Sinonovacula* and Cultellidae at the CA sites was 90.9% for *COI* gene and 97.0% for *16S*. While *Sinonovacula* shares few characters (<3 CAs) with other families, the matching rate is less than 20.0% for both genes.

Table 1. Matching rate of CA sites between *Sinonovacula* and each family.

	Number of CAs		Matching rate	
Family	*COI*	*16S*	*COI*	*16S*
Psammobiidae	14	15	0.071	0.133
Tellinidae	11	23	0.182	0.087
Semelidae	14	37	0.143	0.054
Donacidae	10	28	0.200	0.036
Solecurtidae	33	15	0.000	0.067
Cultellidae	44	66	0.909	0.970
Solenidae	46	63	0.022	0.000

Discussion

Sinonovacula was originally classified in *Solen* by Linné. Then it was transferred to Tellinoidea by Graham (1934) on the basis of anatomic characteristics, which was fully supported by Yonge (1959). This decision was also accepted by Vokes (1980) who identified *Sinonovacula* as a genus of Solecurtidae. Taylor et al. (2007) reconstructed the phylogeny of Heterodonta using sequences of 18S and 28S rRNA genes, and found that *Sinonovacula* clustered into Solenoidea. The results of Yuan et al. (2012) based on gene arrangements of the mitochondrial genomes of six heterodont bivalves indicated that *S. constrictais* had a closer relationship with *Solen grandis*.

Our results on various DNA barcoding analyses of multiple mitochondrial genes revealed new insight into the phylogeny of *Sinonovacula*. Genetic distances between individuals of *Sinonovacula* and individuals of other genera belonging to Tellinoidea were much bigger than those between *Sinonovacula* and genera of Solenoidea. Thus, the degree of genetic similarity between *Sinonovacula* and genera of Solenoidea was significant, especially with *Cultellus*. In the Bayesian tree, *Sinonovacula* formed a monophyletic clade with *Cultellus* and *Siliqua* (Figure 1), which implicated that they had a common ancestor. CAOS demonstrated that the matching rate between *Sinonovacula* and Cultellidae was extremely high (Table 1), i.e. they shared more than 3 CAs. We concluded that *Sinonovacula* had a closer phylogenetic relationship with *Cultellus* rather than *Solecurtus* on the molecular data. Thus, we suggest transferring *Sinonovacula* from Solecurtidae to Cultellidae, as a sister group of *Cultellus*.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper. The study was supported by research grants from Fundamental Research Funds for the Central Universities, and National Natural Science Foundation of China (41276138, 31372524).

Data Accessibility: Detail information of samples would be given by the corresponding author. GenBank numbers of the sequences: JN859860-JN860027.

References

DeSalle R, Egan MG, Siddall M. (2005). The unholy trinity: Taxonomy, species delimitation and DNA barcoding. Philos T Roy Soc B 360: 1905–16.

Feng B, Dong L, Niu D, Meng S, Zhang B, Liu D, Hu S, Li J. (2010). Identification of immune genes of the Agamaki Clam (Sinonovacula constricta) by sequencing and bioinformatic analysis of ESTs. Mar Biotechnol 12:282–91.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotech 3:294–9.

Frezal L, Leblois R. (2008). Four years of DNA barcoding: Current advantages and prospects. Infect Genet Evol 8:727–36.

Graham A. (1934b). The structure and relationship of lamellibranchs possessing a cruciform muscle. Proc Roy Soc Edinb 54:158–87.

Hall TA. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–8.

Hebert PDN, Cywinska A, Ball SL, deWaard JR. (2003). Biological identifications through DNA barcodes. Pro R Soc Lond B 270:313–21.

Huang R, Zhang YF. (2007). A new species of the genus *Sinonovacula*. Taiwan Strait 26:115–20.

Li Q, Park C, Kijima A. (2002). Isolation and characterization of microsatellite loci in the Pacific abalone, Haliotis discus hannai. J Shellfish Res 21:811–15.

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Maddison WP, Maddison DR. (2009). Mesquite: A modular system for evolutionary analysis. Version 2.71. Available at: http://mesquiteproject.org (Accessed 7 September 2009).

Mikkelsen PM, Bieler R, Kappner I, Rawlings T. (2006). Phylogeny of *Veneroidea* (Mollusca: Bivalvia) based on morphology and molecules. Zool J Linn Soc (Lond) 148:439–521.

Palumbi SR. (1996) Nucleic acids II: The polymerase chain reaction. Massachusetts: Sinauer and Associates Inc.

Posada D, Buckley TR. (2004). Model selection and model averaging in phylogenetics: Advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Syst Biol 53:793–808.

Ronquist F, Huelsenbeck JP. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–4.

Sarkar IN, Planet PJ, Desalle R. (2008). CAOS software for use in character-based DNA barcoding. Mol Ecol Res 8:1256–9.

Swofford DL. (2002). PAUP: Phylogenetic analysis using parsimony (and other methods). Version 4.0. Massachusetts: Sinauer Associates.

Tamura K, Dudley J, Nei M, Kumar S. (2007). Mega 4: Molecular evolutionary genetics analyses (mega) software version 4.0. Mol Biol Evol 24:1596–9.

Taylor JD, Williams ST, Glover EA, Dyal P. (2007). A molecular phylogeny of heterodont bivalves (Mollusca: Bivalvia: Heterodonta): New analyses of *18S* and *28S* rRna genes. Zool Scr 36:587–606.

Thompson JD, Higgins DG, Gibson TJ. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–80.

Vokes HE. (1980). Genera of the Bivalvia: A systematic and bibliographic catalogue. New York: Paleontological Research Institution Press.

Yonge CM. (1959). On the structure, biology and systematic position of *Pharus Legumen* (L.). J Mar Biol Assoc UK 38:277–90.

Yuan Y, Li Q, Yu H, Kong L. (2012). The complete mitochondrial genomes of six Heterodont bivalves (Tellinoidea and Solenoidea): Variable gene arrangements and phylogenetic implications. PLoS One 7:e32353.

Zou S, Li Q, Kong L, Yu H, Zheng X. (2011). Comparing the usefulness of distance, monophyly and character-based DNA barcoding methods in species identification: A case study of Neogastropoda. PLoS One 6: e26619.

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