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※ 應用粒線體 DNA 探討雙殼綱動物的演化和分類 ※
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摘要

依據粒線體之 16S RNA 基因和 CO I 基因部分序列的系統發生關係重建雙殼綱簾蛤科（Veneridae）分類系統。共採用 21 個簾蛤科動物進行分析，其中 11 種和兩種外群動物（*Corbucula* sp.和 *Nucula* sp）採自台灣，其餘 8 種的基因序列來自 GCG 的基因庫。實驗得到約 550bp 之 16S RNA 基因和約 650bp 之 CO I 基因部分序列，二者均有 33 個基因型。系統發生樹以 neighbor-joining (NJ), maximum parsimony (MP) methods 和 bootstrap test 檢測可信度，結果發現簾蛤科動物並非單係群，因為蜆科動物在系統樹中無法與其分離。*Tapes literatus*, *Tapes philippinarum*, *Rudittapes variegatus*, and *Rudittapes philippinarum* 四種外型相近的種，從遺傳距離、基因形式和系統樹結果發現應為同一個種。大型文蛤和小型文蛤雖然外型大小有明顯區別，但在遺傳距離、基因形式和系統樹結果中顯示，其仍為同一種。而大型環文蛤和小型環文蛤不僅外型大小有明顯區別，其遺傳距離、基因形式和系統樹分析結果，均顯示其為兩個不同的種，且親緣關係非常的遠。

關鍵字：雙殼綱、簾蛤科、系統發育、分類。.

Abstract

The phylogenetic relationship based on partial sequences of the cytochrome c oxidase subunit I gene and the 16S RNA gene in mtDNA to reconstuct the systematics of the Veneridae Family in Bivalvia. Nucleotide sequences of 21 species of venerid clams were used in this study, including 11 examined species and 2 outgroup species colleted from Taiwan, 8 species from Genebank. About 550 bp of 16S RNA gene and 650 bases of *COI* gene were sequenced to obtain 33 haplotypes in each gene. Phylogenetic trees were established by neighbor-joining (NJ), maximum parsimony (MP) methods and their bootstrap test using *Corbucula* sp., *Nucula* sp. and *Mytilus* sp. as the outgroup. From genetic distances and all phylogenetic trees pointed that venerid clams perhaps not be the monophyletic groups for *Corbucula* sp. and *Nucula* sp. not separating from other taxa of Veneridae. A surprised results of *Tapes literatus*, *Tapes philippinarum*, *Rudittapes variegatus*, and *Rudittapes philippinarum,* these 4 species with similar valve morphs, were suggested to be only one species. The specimens of different sizes in *Meretrix lusoria* species supposed to be the same species. The specimens of

different sizes in *Cyclina sinensis* species supposed to belong the two different species.

Key words: Bivalvia, Veneridae, phylogeny, classification.

Introduction

The Veneridae, including more than 500 living species, is the largest and diverse family in Bivalvia. Usually thick-valved, equivalved, and isomyarian. There are three cardinal teeth in each hinge teeth, but anterior teeth sometimes weak or absent and posterior teeth sometimes absent too. Valves show concentric or sometimes radial sculptures. The systematics of venerid clams is very controversial and discrepancy in different taxonomic characters which were selected by different taxonomists (Pelseneer, 1911; Purchon, 1959; Newell, 1965; Morton, 1979). Classification must base on many characters to provide the evidence from which relationship between organisms is inferred (Mayr and Ashlock, 1991). Taxonomic work should rely on abundant experiences to determine the suitable and homologous morphological characters from lots of attributes in organism. Venerid clams adapt to diverse ecological environments which may have strongly influenced in the valve morph, therefore many characters are not useful in showing really infinities (Morton, 1979).

Molecular phylogeny are much more suitable for evolutionary studies than morphological and physiological data for many reasons, one of them is homology assessment is easier with molecular data than with morphological traits (Graur and Li, 1999). Four main factors, i.e. small genomic size, present in all eukaryotic cells, maternal inheritance, and rapid variation (Barton and Jones, 1983; Bermingham *et al.*, 1986; Brown *et al.*, 1979; Dawid and Blackler, 1972), allow the mtDNA be a new instrument for solving problems of evolutionary and systemic relationships (Avise, 1989). Especially, different regions of genes in mitochondrial genome present different variation and qualities. Thus, mtDNA may be sufficiently variable for detecting taxa at different taxonomic categories (Aquadro *et al.*, 1984; Barton and Jones, 1983; Brown *et al.*, 1979; Brown and Simpson, 1981; Moritz *et al.*, 1987).

For example, A-T rich region usually was used to analyze relationships between or among populations or species (Mack *et al.*, 1986; Mortiz *et al.*, 1987);

cytochrome b for species or genus (Bowen *et al.*, 1993), and the sequence coding for the small subunit rRNA (s-rRNA) or for the large subunit rRNA (l-rRNA) genes for families or the other higher taxonomic levels, such as for Bovidae systematics and evolution (Allard *et al.*, 1992), for the phylogeny of tick taxa (Black and Piesman, 1994), for the phylogenetic study of *Mytilus* (Hoeh *et al.*, 1997), for the phylogeny of the family Cichlidae (Farias *et al.*, 1999; 2000). The large subunit of rRNA genes of mitochondrial genome in animal have a high degree of homology and structural conservation relative to most protein-coding RNA. The mitochondrial genomes of all animal cells appear to be the same basic pattern (Attrardi, 1985). Therefore, the l-rRNA gene of the mtDNA becomes an effective and common tool for studies of genetics or evolution for systematics. In this study, we derived the new systematics in Veneridae via the partial sequences of 16S rRNA gene and *CO* I gene in mtDNA to reconstruct the phylogenetic relationships.

MATERIALS AND METHODS

Samples including the small specimens of *Cyclina sinensis* (Cys B), the large specimens of *Cyclina sinensis* (Cys), the small specimens of *Meretrix lusoria* (Mel), the large specimens *Meretrix lusoria* (Melt), of *Tapes literatus* (Tal), *Gomphina veneriformis* (Gov), *Paphia undulata* (Pau), *Rudittapes variegatus* (Ruv), *Rudittapes philippinarum* (Rup), *Corbicula fluminea* (Cof and corbicula) ,one unknown species (unknown), were collected from the West and North coasts of Taiwan. Collection was made between March and October in 2000. The samples were stored at -20 °C or in 70% ethanol until used. Each species was selected 2 to 3 samples, and their muscles were gathered for isolating mtDNA.

MtDNA was extracted following basically the protocol of Jiang et al., (1997) with only minor modifications. Polymerase chain reaction and PCR directed sequencing To design these primers, we compared published sequences for invertebrates (Clary and Wolstenholme 1985; Jacobs et al.,1988), and vertebrates (Bibb, 1981) mtDNAs and searched for highly conserved regions in l-rRNA genes. 16S L1 (5'-CCAGTCAGTCCACCAAATTAA-3') and 16S R1 (5'-GGCTTGATTTATGATTTTTGCC-3'). DNA extraction, amplification, and sequencing. The reaction mixture (50 μl in total volume) contained 6 μl (100~500ng) of genomic DNA, 1 μl of each primer (10 pm/μl), 0.6 μl dNTPs

(25mM), 0.4 μl Taq DNA polymerase (5units, Super Taq polymerase, HT Biotechnology Cambridge, UK), 5 μl reaction buffer (10mM pH8.3 Tris-HCl, 50 mM KCl, and 1.5 mM MgCl2) and added 37 μl of distilled H2O. Amplification was carried out for 32 cycles as follows: 1 min 10 sec denaturation at 95℃, 1 min 30 sec annealing at 50℃, and 2 min extension at 72℃, followed by 10min extension at 72℃. PCR products were purified with the Qia-quick PCR purification kit (QIAGEN, Promega) and directly sequenced using an automated DNA sequencer (ABI PRISM 377 DNA Sequencer, PE Applied Biosystems). All sequences were aligned via Pileup program in GCG package and Clustal W program (Thompson et al. 1994) in BioEdit (version 4.7.8, Hall 1999) computer software and corrected by eye in GeneDoc (Nicholas *et al.*, 1997) computer software. The phylogenetic trees were constructed by neighbor-joining (NJ) method (Saitou and Nei, 1987) in Clustal W (Thompson *et al.*, 1994) and by maximum parsimony (MP) method using a heuristic search algorithm with the 50 % majority-rule consensus in PAUP (Swofford 1993). In the NJ method, the numbers of nucleotide substitution per site were estimated for multiple substitutions by Kimura's (1980) two-parameter method.

Results and Discussion

About 550 bp of 16S RNA gene and 650 bases of *CO*I gene were sequenced to obtain 33 haplotypes in each gene. Divergence of the partial sequence in 16S rRNA (among Genus from 0.20 to 1.20; within Genus from 0.20 to 1.15; among species from 0.00 to 0.02) and *CO* I (among species from 0.33 to 1.10; within Genus from 0.120 to 0.89; among species from 0.00 to 0.01) were higher than in the other invertebrates. The evolution rate in 16S rRNA gene is faster than *CO* I gene in this study.

A+T composition of the partial sequence in 16S rRNA (from 0.61 to0.75)and *CO* I (from 0.64-0.68) present the high A+T rich condition and 16S rRNA gene seems more diverse than *CO* I gene. The percentage of A+T base composition in venerid clams is the similar phenomenon as in the other mollusk (from 0.61-0.74) (Jiang *et al.*, 1997).

Phylogenetic trees were established by neighbor-joining (NJ), maximum parsimony (MP) methods and their bootstrap test using *Corbucula* sp., *Nucula* sp. and *Mytilus* sp. as the outgroups to reconstruct the systematics of the venerid

clams. The patterns and the distributions of different taxa in phylogenetic trees which constructed by partial sequence in 16S rRNA or *CO* I are very similar. Both of them present that Veneridae Family in Bivalvia perhaps not be a monophyletic group for the outgroup of *Corbucula* sp. and *Nucula* sp. not separating from other taxa of Veneridae, but mixed up with them. According to the classification of Purchon (1959) and Newell (1965), Corbuculidae. is the sister group of Veneridae, and the Nuculanidae is the ancient group compared with Veneridae. The site of Nuculanidae taxon is at the side of tree, so we can accept the possible of relationships with venerid clams. But the Corbicualidae taxon from the valve morph to lifestyle is very similar to venerid clams, maybe they exist more closely relationships than we think.

Tapes literatus, *Tapes philippinarum*, *Rudittapes variegatus*, and *Rudittapes philippinarum* are very similar in valve morph and live mix up with each other in the field. The characters of classification among them base on color patterns, valve shapes, and sculptures, but usually cannot easily distinguish them when you collected them at the same time and locality. From the nucleotide sequences, genetic distance (from 0.00 to 0.01) and the phylogenetic tree, we suggested *Tapes literatus*, *Tapes philippinarum*, *Rudittapes variegatus*, and *Rudittapes philippinarum* should be only one species.

Specimens of *Meretrix lusoria* existed two kinds of sizes and seems very different. The large size specimens are bigger than small size about two times but with almost the same appearances. After the sequence analysis (genetic distance: 0.000-0.002) and phylogenetic tree, we suggested that the specimens of different sizes in *Meretrix lusoria* supposed to be the same species. But we still could find that their mtDNA still has differentiation phenomenon.

The specimens of different sizes in *Cyclina sinensis* usually collect together, but they have very significant various appearance. The shell of the small size specimens is yellow color with light purple color at outer circle. The shell of the large size specimens is light yellow color with outer coat existing short hair. The small size specimens is the young stage and large size specimens is the mature stage. But from our study via sequence analysis (genetic distance: 0.88-1.11) and phylogenetic tree (they are separated into different lineages), we supposed them to belong the two different species.

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