

# The solitary ascidians *Polycarpa* spp. in Taiwan

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## Abstract

The species of *Polycarpa* (Styelidae: Pleurogona: Ascidiacea) are solitary ascidians that generally occur on the surface of coral reefs or rocks in shallow water and have affinities with the tropical fauna (Kott, 1985). The adult body is entirely embedded within a leathery tunic which is usually covered with sand, algae, sponges and smaller colonial ascidians, and the obvious features for discovering them in the field are the two siphons. They have unbranched tentacles around the inlet of the branchial sac, and four folds on each side of the branchial sac. More than one unbranched gonads are located on both sides of body, either upright/prostrate on the body wall or embedded into the body wall. The sessile *Polycarpa* ascidians are important members in coral reef communities since they provide habitats for other organisms, such as algae, bivalves, polychaetes and echinoderms. There are about 40 species of *Polycarpa* recorded in the world (Kott, 1985 and Monniot, *et al*, 1991), eight of them are discovered in Japan, but only one species is previously recorded in Taiwan (Chen and Dai, 1998). The molecular phylogeny of the Tunicata (Stach and Turbeville, 2002) and suborder Aplousobranchiata (Turon and Lopez-Legentil, 2004) showed that the phylogeny of some genera, such as *Molgula*, *Botryllus*, are clear, but only one species representing *Polycarpa* (*P. pomaria*) was included. The objectives of this study are to discover how many species of *Polycarpa* in Taiwan, and to study their molecular phylogeny.

A total of 92 individuals of *Polycarpa* were collected by scuba diving at depths between 2 and 20 m from northern and southern Taiwan (northern: Yeliu, Shenao, Longdong, and Yanliao; southern: Wanlitong, NPP III Outlet, Houbihu, and Tiaoshi) in 2005 and 2006. Samples were identified according to Kott and Monniot's descriptions based on the gonad positions, dorsal tubercle slits, stomach length, endocarps, and spherical vesicles observed under a dissecting microscope. DNA was extracted, and a portion of the cytochrome C oxidase subunit I (COI) of mitochondrial gene and the nuclear ribosomal internal transcribed spacer (ITS) were amplified by polymerase chain reactions and sequenced. These sequences were applied to phylogenetic reconstruction using Neighbor joining (NJ), Maximum parsimony (MP), Maximum likelihood (ML), and Bayesian methods. The haplotype network analysis

was conducted using the program TCS, which implements the statistical parsimony algorithm to provide a 95 % confidence set for the relationship of haplotypes.

In the present study, six *Polycarpa* species (one from Okinawa) were discovered (Fig. 1). The most common species in northern and southern Taiwan were *P. rima* and *P. argentata*, respectively. *P. argentata*, *P. sp.1*, and *P. sp.2* are only found in southern Taiwan. The proportions of *P. rima* were 93% and 23% in our samples collected from northern and southern Taiwan respectively. Furthermore, the population density of *P. rima* in northern Taiwan was higher than that in southern Taiwan. The phylogenetic relationship of five *Polycarpa* species were reconstructed with the COI gene (606 bp) and the combination of COI and ITS sequences (1200 bp) by NJ, MP, ML, Bayesian criteria. These species were separated obviously with high bootstrap values and were consistent with the morphological distinctions. The phylogenetic trees show that there are three clades in *P. rima* with high bootstrap confidence (Fig. 2). The number of nucleotide differences among the three clades are more than 5%, equals to the differences between two species. This pattern is similar to that showed in the TCS network analysis. These results indicate that there may be cryptic species within *Polycarpa rima*.

## References

- Chen YT, Dai CF (1998) Sexual reproduction of the ascidian *Polycarpa cryptocarpa kroboja* from the northern coast of Taiwan. *Acta Oceanographica Taiwanica* 37: 201-210.
- Kott P (1985) The Australian ascidiacea part 1, phlebobranchia and stolidobranchia. *Mem. Qd Mus.* 23: 1-440.
- Monniot C, Monniot F, Laboute P (1991) Coral reef ascidians of New Caledonia. Orstom, Paris.
- Stach T, Turbeville JM (2002) Phylogeny of Tunicata inferred from molecular and morphological characters. *Molecular Phylogenetic and Evolution* 25: 408-428.
- Xavier T, Lopez-Legentil S (2004) Ascidian molecular phylogeny inferred from mtDNA data with emphasis on the Aplousobranchiata. *Molecular Phylogenetic and Evolution* 33: 309-320.

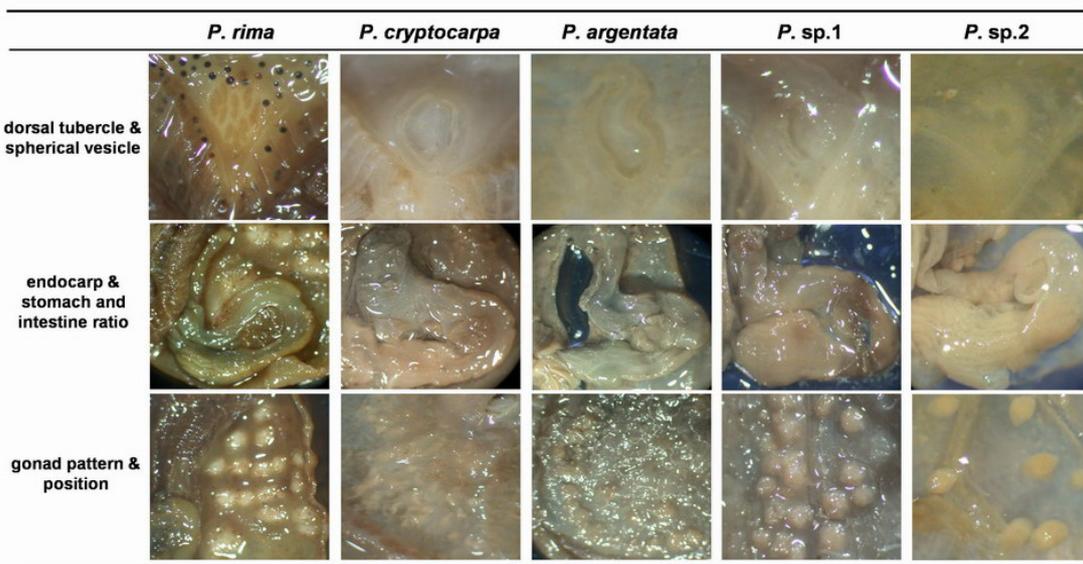


Figure 1. The morphological characters of the Polycarpa species.

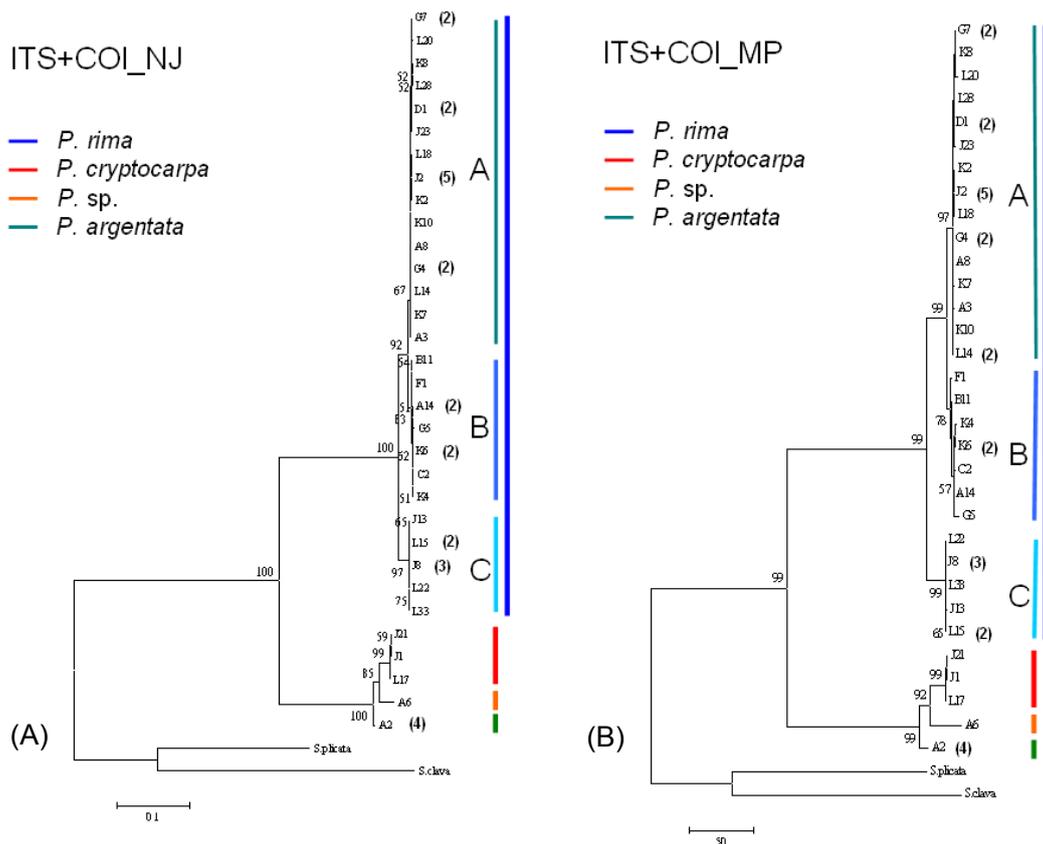


Figure 2. The Neighbor-Joining tree (A) and maximum parsimony tree (B) derived from the combination sequence of ITS sequences and partial COI mtDNA (total 1200bp). Numbers above the branches indicate the bootstrap support percentages, and numbers in the parentheses represent the number of samples that have identical sequences.