

## Molecular phylogenetic evidence for host switching in chemoautotrophic symbionts of deep-sea *Calyptogena* clams

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*Calyptogena* clams are living in deep-sea chemosynthetic habitats and globally distributed in seeps and hydrothermal vents. They are nutritionally dependent on chemoautotrophic sulfur oxidizing bacteria, which are harbored within their gill epithelial cells. The *Calyptogena* symbionts are thought to be vertically transmitted via clam's egg to the next generation. Both host and symbiont are thought to coevolve, because topologies of the phylogenetic trees of them form a mirror image. However, their phylogenetic trees have not been robust enough for analyzing their coevolutional relationship, because of using partial gene sequences of host (mitochondrial *cox1* and *rrnL* genes) and symbiont (16S rRNA gene). The possibility of lateral acquisition of the symbiont has been reported in some *Calyptogena* lineages. To improve the phylogenetic trees of *Calyptogena* clams and of symbiont, we sequenced the mitochondrial genomes of *Calyptogena* clams, and several their symbiont genes, and analyzed the phylogenetic trees by using the concatenated sequences.

Mitochondrial genomes of *C. phaseoliformis*, *C. okutanii* and *C. fossajaponica* were sequenced. Based on these mitochondrial genome sequences, primer sets for PCR of mitochondrial genes of other *Calyptogena* clams were designed. Using them, 11 mitochondrial genes (*cox1*, *cox2*, *cox3*, *nad1*, *nad3*, *nad4*, *nad5*, *cytb*, *atp6*, *atp8* and *rrnL*) of other 8 *Calyptogena* species (*C. fausta*, *C. kawamurai*, *C. kilmeri*, *C. laubieri*, *C. nautilei*, *C. pacifica*, *C. soyoae*, *C. stearnsii*) were amplified by PCR and sequenced. Eight genes (16S rRNA, 23S rRNA, *uvrA*, *uvrD*, *mfd*, *groEL*, *groES* and *gyrB*) of symbionts of these *Calyptogena* clams were also sequenced. Phylogenetic trees of clams and symbionts were constructed by maximum likelihood and Bayesian analysis based on concatenated 11 mitochondrial and 8 symbiont genes, respectively.

The reliabilities of phylogenetic trees of the hosts and their symbionts were significantly improved by using the concatenated genes sequences (Fig.1). Bootstrap values and posterior probabilities of internal nodes were better supported than those of the previous phylogenetic trees using partial gene sequences. Topological congruence of host and symbiont that was supported by bootstrap value (100%) and posterior probabilities (1.0), was shown in *C. okutanii*, *C. soyoae*, *C. kilmeri*, *C. pacifica* and *C. fausta*. These results suggested that these symbionts were cospeciated with their host clams (green boxes in Fig.1). Although the topologies of host and symbiont were congruent with *C. fossajaponica* and *C. phaseoliformis*, there were the low bootstrap values and low posterior probabilities in the host clade.

Topological incongruence between host and symbiont trees was shown in *C. kawamurai* - *C. laubieri* clade and *C. nautilei* - *C. stearnsii* clades (Fig.1). Congruence of topologies was rejected by approximately unbiased test using sitewise log-likelihoods (red branches in Fig.1). This result suggested that these symbionts have not cospeciated with their host clams. Host switching of the symbionts in the clades of *C. kawamurai* - *C. laubieri* and *C. nautilei* - *C. stearnsii* were examined by coevolution software, which compared the topologies of host and symbiont. Host switching is the event that symbiont is transferred from a host to a new host in a different lineage during speciation. The host switching of symbiont between *C. kawamurai* and *C. laubieri* was suggested by this software. Moreover, both clams are living in different depths of the same area (blue box on Fig.1). However, this software did not suggest the host switching of symbionts between *C. nautilei* and *C. stearnsii*. They are living in different areas. In this study, we show the phylogenetic relationships of cospeciation and non-cospeciation species with the symbionts among examined 11 *Calyptogena* species. It was suggested that topological incongruence of host and symbiont trees in clade of *C. kawamurai* - *C. laubieri* may be due to the host switching

Keywords: symbiosis, deep-sea *Calyptogena* clams, coevolution, host switching

BPT24-07

Room:213

Time:April 29 11:00-11:15

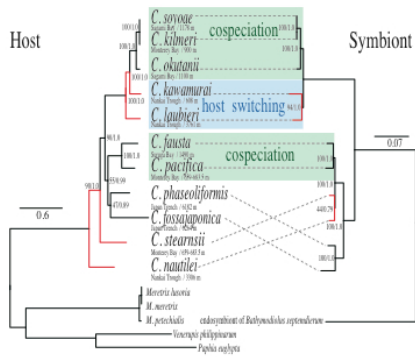


Fig1. cospeciation and host swithing on *Calyptogenia* clam and symbiont trees.  
 Numbers in the nodes correspond to maximum likelihood bootstrap values and posterior probabilities.  
 Reg branches correspond to topological congruence rejected by approximately unbiased test using  
 sitewise log-likelihoods.