More than one species? Exploring the possibility of cryptic species in the giant clam Tridacna maxima using mitochondrial COI sequences

Tamayo Joseph Ricky, Kochzius Marc, Huyghe Filip, Dissanayake Kushlani, Akbal Sezgi

Marine Biology, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussel, Belgium
E-mail: joseph.ricky.tamayo@vub.be

Giant clams from the subfamily *Tridacninae* are ecologically significant species currently threatened both by global change and other anthropogenic activities. Currently, there are 12 accepted extant species of giant clams distributed in the Indo-Pacific, all listed under Annex II of the Convention on International Trade in Endangered Species and Flora (CITES). To effectively conserve this group, a consistent and clear species description and delimitation is needed. Several phylogeographic studies using COI sequences and nuclear markers have shown deep divergent lineages in the small giant clam *Tridacna maxima*. In this study, we performed species delimitation using COI sequence of 200 *T. maxima* samples from the Red Sea, Madagascar, East Africa, Maldives, Eastern Indian Ocean, Indo-Malay Archipelago, South China Sea, Western Pacific, Central Pacific, and South Pacific. Within *T. maxima,* a mean Kimura 2-Parameter (K2P) genetic distance of 6 % was observed. We employed four different species delimitation methods: Automatic Barcode Gap Discovery (ABGD), Assemble Species by Automatic Partitioning (ASAP), Bayesian Poisson Tree Process (bPTP), and Generalized Yule Mixed Coalescent methods (GMYC). ABGD yielded 15 operational taxonomic units (OTUs) when prior maximal distance was between 0.28 % to 0.46 %, and eight species when it was 0.77 % to 2.15 %. Similar to the latter, ASAP identified eight OTUs as the best partition. GMYC and bPTP (using default parameters) obtained 18 and 29 species, respectively. To determine the validity of an OTU, three criteria were applied: (i) the OTU should consist of more than one sample, (ii) the OTU should be monophyletic, and (iii) all the delimitation methods identified them as putative species. In consensus, the delimitation methods showed four deeply divergent OTUs within *T. maxima*. We detected three OTUs in the Pacific: OTU1 is composed of samples from the Indo-Malay Archipelago and South China Sea; OTU2 comprised of samples from the Central Pacific and South Pacific; and OTU3 is found in the Western Pacific. The fourth OTU (OTU4) is composed of samples from the Indian Ocean and the Red Sea. K2P distance values ranged from the smallest value of 3.38 % between OTU1 and OTU4 to 10.16 % between OTU2 and OTU3. These results align with earlier findings of a morphologically undistinguishable cryptic species within the *T. maxima* complex, resurrected as *T. noae*, in the Pacific Ocean. They indicate that more cryptic speciation might be present within *T. maxima,* more specifically in the WIO, the Red Sea and the Central Indian Ocean.Further investigation of possible additional OTUs is recommended to provide a more comprehensive description of genetic diversity within *T. maxima*.

Keywords
Phylogenetic Analysis; Species Boundaries; Phylogeny