Captive Hybridization of the Giant Clams *Tridacna maxima* (Röding, 1798) and *Tridacna noae* (Röding, 1798)

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CAPTIVE HYBRIDIZATION OF THE GIANT CLAMS TRIDACNA MAXIMA (RÖDING, 1798) AND TRIDACNA NOAE (RÖDING, 1798)

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ABSTRACT The giant clam Tridacna noae is a recently resurrected taxon distinguished from Tridacna maxima on the basis of reproductive isolation, mitochondrial DNA differences, and mantle morphology and ornamentation; however, the morphological characteristics used to distinguish the two species are not consistent with genetic assignment throughout their zone of overlap. This has led to the suggestion that hybridization occurs and constituted a need to reexamine the reproductive isolation of T. noae and T. maxima. This study provides evidence that sympatric populations of T. maxima and T. noae in the center of their range overlap and can hybridize and produce juveniles. Breeding trials using T. maxima and T. noae for both maternal and paternal hybrid crosses were conducted to compare larval and early juvenile development. The fertilization success and survival of both hybrid crosses suggest potential for T. maxima and T. noae hybrids to exist in nature. On this basis, the previously reported reproductive isolation between T. maxima and T. noae may not be apposite across their zone of overlap. Future genetic, population demographic, and ecological studies should consider the possibility of hybridization between T. maxima and T. noae.

KEY WORDS: Papua New Guinea, mariculture, invertebrate, tridacnidae, Tridacna maxima, Tridacna noae

INTRODUCTION

Giant clams (Cardiidae: Tridacnidae) are highly emblematic, heavily targeted coral reef species throughout their Indo-Pacific distribution, from East Africa and the Red Sea to the central Pacific Ocean (bin Othman et al. 2010). Generalized depletion of natural populations throughout their range due to exploitation for meat (Lucas 1994, Kinch 2002), curios (Usher 1984, Heslinga 1996), and the marine aquarium trade (Kinch & Teitelbaum 2010, Mies et al. 2017) has prompted the listing of all Tridacnidae in Appendix II of the Convention on International Trade in Endangered Species since 1985 (UNEP-WCMC 2016a, 2016b) and on the International Union for Conservation of Nature Redlist since 1986 (IUCN 2016). Given their multiple anthropogenic uses and ecological significance, there is substantial merit in conserving giant clams (Neo et al. 2015a). Contemporary taxonomic knowledge, indicative of reproductive isolation, remains fundamental for achieving giant clam conservation and for facilitating captive production to reduce pressures on wild populations (bin Othman et al. 2010, Borsa et al. 2015, Johnson et al. 2016).

Su et al. (2014) recently resurrected the teardrop giant clam Tridacna noae (Röding 1798) from synonymy with Tridacna maxima (Röding 1798) on the basis of reproductive isolation in culture, genetic distance, and mantle morphology and ornamentation. Recent studies of giant clam populations in Ningaloo, Western Australia, however, indicate that mantle morphology and ornamentation conflicted with genetic assignment for populations of these two species (Johnson et al. 2016). This has brought controversy to the taxonomic definition of T. noae (Johnson et al. 2016). The present taxonomic definition of T. noae (Su et al. 2014) relies on mitochondrial sequences to allow diagnosis of this taxon relative to all other extant Tridacna species (Borsa et al. 2015); however, effective differentiation on the basis of mitochondrial sequences relies on the assumption of reproductive isolation. The maternal inheritance of mitochondrial DNA (Sato & Sato 2013) could mask very recent or current genetic exchange between two species through hybridization and fail to detect the presence of nonfertile hybrid populations. This has been proposed as a possible explanation for the observed variation in mantle morphology and ornamentation within T. noae, but was subsequently discounted given the absence of evidence for hybridization (Borsa et al. 2015).

The geographic range of Tridacna noae extends from Kiritimati in the Central Pacific to Christmas Island in the Indian Ocean to Taiwan (Huelsken et al. 2013, Borsa et al. 2014, Militz et al. 2015, Neo & Low 2017), broadly overlapping with the morphologically similar Tridacna maxima (Fig. 1; bin Othman et al. 2010). Sympatry of the two species has been confirmed in several locations in the Pacific (Huelsken et al. 2013, Borsa et al. 2014, Militz et al. 2015) with local populations showing similar distributions in response to environmental gradients (Militz et al. 2015). The overlap in range combined with the overlap of localized distributions of T. noae and T. maxima provides potential for gametes from either species to encounter one another (Neo et al. 2015b). While Su et al. (2014) reported reproductive isolation between T. maxima and T. noae in culture, their observations were restricted to giant clams sourced near Taiwan. This does not preclude the possibility that these species can hybridize elsewhere within their range, especially given that multiple, distinct phylogenetic lineages of both T. maxima and T. noae persist across their ranges (Huelsken et al. 2013, Hui et al. 2016).

This study assessed whether morphologically distinct Tridacna maxima and Tridacna noae from New Ireland Province, Papua New Guinea (PNG), can successfully cross-fertilize to produce hybrid juveniles. Specifically, the larval development of both maternal and paternal hybrid crosses was examined and compared with pure-strain T. maxima and T. noae. Comparisons of shell morphometrics and shape are made between strains for the first month of development.

MATERIALS AND METHODS

A series of four breeding trials were conducted at the National Fisheries Authority Nago Island Mariculture and
Research Facility at Kavieng to compare larval and early juvenile development of *Tridacna maxima*, *Tridacna noae*, and both maternal and paternal hybrids of the two species. Adult clams used as brood stock were sourced from fringing reefs around the outer barrier islands of the Kavieng lagoonal system (2°36′ S, 150°46′ E) of New Ireland Province in PNG. The outer barrier islands are where densities of both *T. maxima* and *T. noae* are highest within the lagoonal system (Militz et al. 2015). This location is at the center of the range overlap for *T. maxima* and *T. noae* (Fig. 1). Brood stock were identified by mantle morphology and coloration (Fig. 2), which are still considered to be useful means of distinguishing between *T. noae* and *T. maxima* in the Western-Pacific region (Su et al. 2014, Borsa et al. 2014, 2015).

Collection of adult clams involved removing an individual clam and a portion of substratum to which its byssal threads were anchored. Clams were held in insulated containers filled with seawater and transported (<10 km) by boat to the Nago Island Mariculture and Research Facility. Adult *Tridacna maxima* and *Tridacna noae* were held separately in 2,000-L raceways and provided with a continual flow of unfiltered seawater sourced from the fringing reef surrounding the island research facility. Spawning was initiated within 5 days of collection. Individual clams were isolated in 50-L holding vessels filled with 1-μm filtered seawater (FSW). Spawning was induced by injecting 0.7–1.0 mL of a 2-mM serotonin solution (5-hydroxytryptamine creatinine sulfate complex) into the gonad by hypodermic needle insertion through the mantle (Braley 1985, Crawford et al. 1986). This method of spawning induction is superior to alternative methods (e.g., heat stress) trialed previously with *T. noae* (Southgate et al. 2016, 2017).

Two sets of *Tridacna noae* and *Tridacna maxima* clams were used in this study (Fig. 2). For all trials, both eggs and sperm were collected from *T. maxima* and *T. noae* within 30 min of serotonin injection. Given that tridacnines are hermaphroditic (Braley 1992, Lucas 1994), care was taken to collect sperm and eggs separately from individual clams. As all clams released sperm and eggs in sequence after serotonin injection, spermatozoa were collected first before the clams were cleaned in FSW and transferred to a new holding vessel containing FSW before spawning eggs. A sample of water containing spawned eggs (>1,000 eggs) was removed from each holding vessel and lightly aerated to monitor for any self-fertilization at 4 h postfertilization. Remaining eggs were fertilized with spermatozoa within 10 min of egg release.

![Figure 1](image1.png)  
**Figure 1.** Geographic range of *Tridacna maxima* (light shading) and *Tridacna noae* (dark shading). The black triangle identifies where this research was conducted. Distribution of *T. noae* adapted from Borsa et al. (2014) with additional range extension information from Neo and Low (2017) and the authors’ unpublished data (2017) for Hainan Island, China. Distribution of *T. maxima* adapted from bin Othman et al. (2010).

![Figure 2](image2.png)  
**Figure 2.** Adult breeding clams used in the (A) *Tridacna noae*/*Tridacna maxima* and (B) *T. maxima*/*T. noae* hybrid crosses. In both photos, *Tridacna noae* is on the left. From left to right antero-posterior measurements of adult clams were as follows: (A) 20 and 28 cm, (B) 18.5 and 23 cm.
Fertilized eggs from all trials were stocked into 2,000-L cylindrical larval rearing tanks filled with FSW at a density of 0.64 eggs mL$^{-1}$. Fertilization success was determined at 4 h postfertilization from three replicate counts of 100 eggs for each trial. Fertilized eggs were identified as those having passed first cleavage (Southgate et al. 2016). The rearing of embryos and larvae closely followed the “extensive” methods of Braley (1992). In short, no food was provided to larvae, and no water exchange occurred until 15 days postfertilization, when a 100% daily water exchange using 10-μm filtered sea water was begun. Giant clam larvae can be successfully cultured without provision of microalgae (Lucas 1994) and earlier research in this laboratory has confirmed this for Tridacna noae (authors’ unpublished data). For the first month of development, water temperatures, measured hourly by Hobo temperature loggers, were maintained between 28.56 and 32.60°C (7 ± SE, 30.72 ± 0.01), with salinity, measured daily using a refractometer, maintained between 35 and 36 for all trials. Larval rearing tanks were provided with gentle aeration and exposed to natural sunlight reduced through transparent polycarbonate roof sheets. Zooxanthellae, involved in 50% light transmittance shade-cloth. Zooxanthellae, sunlight reduced through transparent polycarbonate roof sheets.

0.01), with salinity, measured daily using a refractometer, among the different trials using a permutational-analysis of variance based on Euclidean distances with the statistical pseudo statistics. Two-sample \( t \)-tests, where equal variances were not assumed, were used to evaluate survival at 30 days postfertilization for both hybrid strains in comparison with pure-strain \( T. \) noae.

RESULTS

Examination of 1,000 unfertilized eggs from each trial confirmed that the spawning procedures were effective in preventing self-fertilization. No self-fertilized eggs were observed. To estimate the upper limit of the frequency of self-fertilization, the 95% binomial confidence interval was calculated based on the sample size of 1,000 eggs. The upper confidence limit gives 0.37% as the maximum frequency of self-fertilization for all trials.

The diameter of unfertilized eggs spawned by the Tridacna maxima broodstock showed similarities to unfertilized eggs of Tridacna noae among trials (Table 1). By contrast, \( T. \) maxima spermatozoa were significantly larger (including both the head and flagellum) than \( T. \) noae spermatozoa in all trials (Table 1). Mean (±SE) fertilization success of \( T. \) maxima (88.0% ± 3.2%), \( T. \) noae (94.7% ± 1.2%), and the \( T. \) noae/\( T. \) maxima\textsuperscript{2} cross (89.3% ± 1.8%) were statistically similar (Fig. 3). The \( T. \) maxima/\( T. \) noae\textsuperscript{2} cross had a much lower fertilization success of 40.7% ± 3.2% (Fig. 3; \( F_{\text{pseudo}} = 101.3, P = 0.003 \)).

Larval development of both hybrid strains progressed in a manner typical of tridacines, with larvae progressing through trochophore, veliger, and pediveliger stages before metamorphosing into juvenile clams (Fig. 4). Differentiation of the developing larval strains was not possible on the basis of shell size (i.e., APM or DVM) or shape (i.e., APM/DVM ratio). Where differences between strains did occur, they were not consistent and could not reliably distinguish a particular strain (Table 1). Only after metamorphosis, at 15 days postfertilization, could all juvenile clam strains be distinguished by differences in shell size (Table 1). Despite differences in shell size, all strains had similar shell shape (\( F_{\text{pseudo}} = 2.20, P = 0.07; \) Table 1). Even at 30 days postfertilization similarities remained apparent, with Tridacna maxima and Tridacna noae juveniles having shell valves of similar shape, although more elongated than both hybrid strains (Table 1). Size differences were apparent at 30 days postfertilization with strains arising from \( T. \) maxima eggs being significantly larger than the two strains arising from \( T. \) noae eggs (Table 1).

Survival (±SE) of Tridacna maxima/\( T. \) noae\textsuperscript{2} hybrids at 30 days postfertilization was the highest (0.50% ± 0.14% of stocked eggs) among all trials, despite this cross having the lowest fertilization success. This hybrid had much higher survival than the \( T. \) noae/\( T. \) maxima\textsuperscript{2} hybrids (0.08% ± 0.02% of stocked eggs) at 30 days postfertilization (\( t_{(2, 9.5)} = 2.87, P = 0.02 \)). In comparison with the hybrids, survival of \( T. \) noae juveniles at 30 days postfertilization was 0.07% ± 0.05% of stocked eggs, which was statistically similar to the \( T. \) noae/\( T. \) maxima\textsuperscript{2} hybrid (\( t_{(2, 5.8)} = 0.16, P = 0.88 \)) and substantially lower than the \( T. \) maxima/\( T. \) noae\textsuperscript{2} hybrid (\( t_{(2, 11.2)} = 2.80, P = 0.02 \)).

DISCUSSION

The taxonomic status of Tridacna noae has been the subject of much debate because of its resurrection to species level in 2014 (Penny & Willan 2014, Su et al. 2014, Borsa et al. 2015, Johnson et al. 2016). Reproductive isolation, consistent genetic differences, or substantial morphological differences between taxa are expected when defining species as separately evolving metapopulation lineages, but the extent to which these factors occur depend upon whether speciation is complete or only
**TABLE 1.**
Morphological measurements (mean ± SE in μm) of larval and early juveniles for *Tridacna maxima*, *Tridacna noae*, and both maternal and paternal hybrids.

<table>
<thead>
<tr>
<th>Stage (time)</th>
<th>Measurement</th>
<th><em>T. maxima</em></th>
<th><em>T. noae</em></th>
<th><em>T. maxima</em></th>
<th><em>T. noae</em></th>
<th><em>T. noae</em></th>
</tr>
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<tbody>
<tr>
<td>Sperm</td>
<td>Length</td>
<td>11.77 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.09 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.50 ± 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.15 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.92 ± 0.09&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Flagellum</td>
<td>Length</td>
<td>40.25 ± 0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.99 ± 0.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.94 ± 0.41&lt;sup&gt;c&lt;/sup&gt;</td>
<td>36.14 ± 0.95&lt;sup&gt;d&lt;/sup&gt;</td>
<td>13.71 ± 0.89&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Unfertilized egg</td>
<td>Diameter</td>
<td>92.35 ± 0.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94.33 ± 0.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90.79 ± 0.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>97.44 ± 1.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>101.14 ± 0.47&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>D-veliger (24 h)</td>
<td>APM</td>
<td>147.60 ± 0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>150.27 ± 0.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>143.07 ± 0.42&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>148.40 ± 0.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>146.32 ± 0.58&lt;sup&gt;d&lt;/sup&gt;</td>
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<td></td>
<td>DVM</td>
<td>121.67 ± 0.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>126.46 ± 0.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>117.62 ± 0.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>122.75 ± 0.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>118.12 ± 0.74&lt;sup&gt;c&lt;/sup&gt;</td>
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<td></td>
<td>Ratio</td>
<td>1.21 ± 0.005&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.19 ± 0.008&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.22 ± 0.004&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.21 ± 0.004&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.24 ± 0.006&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Midstage veliger (72 h)</td>
<td>APM</td>
<td>168.29 ± 1.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>177.03 ± 0.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td>172.51 ± 1.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>174.65 ± 0.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>165.02 ± 2.54&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
<td>DVM</td>
<td>143.26 ± 1.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>148.25 ± 0.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>145.66 ± 0.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>144.91 ± 0.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>140.48 ± 1.30&lt;sup&gt;c&lt;/sup&gt;</td>
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<td></td>
<td>Ratio</td>
<td>1.18 ± 0.008&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.19 ± 0.004&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.18 ± 0.004&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.21 ± 0.004&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.19 ± 0.009&lt;sup&gt;abc&lt;/sup&gt;</td>
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<tr>
<td>Pediveliger (144 h)</td>
<td>APM</td>
<td>186.44 ± 1.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>183.81 ± 1.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>187.31 ± 0.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td>186.10 ± 0.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>176.50 ± 0.97&lt;sup&gt;c&lt;/sup&gt;</td>
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<td></td>
<td>DVM</td>
<td>161.33 ± 1.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>154.33 ± 1.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>162.09 ± 0.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>159.97 ± 0.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>151.86 ± 1.01&lt;sup&gt;c&lt;/sup&gt;</td>
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<td></td>
<td>Ratio</td>
<td>1.16 ± 0.005&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.19 ± 0.005&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.16 ± 0.004&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.16 ± 0.003&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.16 ± 0.005&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Juveniles (15 days)</td>
<td>APM</td>
<td>306.95 ± 6.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>258.73 ± 6.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>269.73 ± 5.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>194.03 ± 3.14&lt;sup&gt;d&lt;/sup&gt;</td>
<td>211.26 ± 4.73&lt;sup&gt;e&lt;/sup&gt;</td>
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<td></td>
<td>DVM</td>
<td>270.71 ± 6.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>222.80 ± 5.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>235.70 ± 4.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>167.95 ± 2.81&lt;sup&gt;d&lt;/sup&gt;</td>
<td>185.06 ± 3.76&lt;sup&gt;e&lt;/sup&gt;</td>
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<td></td>
<td>Ratio</td>
<td>1.14 ± 0.008&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.16 ± 0.009&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.15 ± 0.009&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.16 ± 0.005&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.14 ± 0.006&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Juveniles (30 days)</td>
<td>APM</td>
<td>607.53 ± 19.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>588.08 ± 19.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>431.61 ± 9.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>523.48 ± 19.18&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
<td>DVM</td>
<td>502.45 ± 16.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>510.01 ± 17.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>383.42 ± 8.90&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>1.16 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.13 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.20 ± 0.009&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NA</td>
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</table>

Different superscript letters denote statistical significance (*P* < 0.05) between trials for a given measurement.

* Data obtained from Southgate et al. (2016).

incipient (de Queiroz 2007). In the context of tridacine populations in the Taiwanese archipelago, *T. noae* was found to be reproductively isolated in culture, genetically different, and morphologically distinct from *Tridacna maxima* (Su et al. 2014, Borsa et al. 2015).

This study provides conflicting evidence, indicating a lack of reproductive isolation between *Tridacna noae* and *Tridacna maxima* in culture. Hybrid larvae from both maternal and paternal crosses metamorphosed into juvenile clams with survivorship at 30 days postfertilization comparable with pure-strain tridacine culture (this study, Braley et al. 1988, Waters et al. 2016). Despite *T. noae* being cited as reproductively isolated from *T. maxima* (Su et al. 2014, Borsa et al. 2015), such claims are not supported by a published study with detailed culture methodology (see Su et al. 2013, Su et al. 2014). This greatly limits the opportunity to examine the extent to which abiotic factors (i.e., broodstock conditioning, spawning induction, fertilization, and larval husbandry) of the used culture method may have influenced the disparate survival reported here and by Su et al. (2014). It is well established that the abiotic factors listed previously influence early survival of tridacines in culture (Braley 1992, Waters et al. 2016, Southgate et al. 2017).

The geographical source location of brood stock clams may also have influenced the disparate survivorship. Clams spawned by Su et al. (2014) were sourced from Dongsha in the South China Sea and transported to Penghu, Taiwan for experimental use (Su et al. 2013). This locale is part of a distinct *Tridacna maxima* monophyletic group encompassing the Indonesian

![Figure 3. Mean (±SE) fertilization success of *Tridacna maxima*, *Tridacna noae*, and both maternal and paternal hybrid crosses. Different superscript letters denote statistical significance (*P* < 0.05).](image-url)
throughflow, Singapore, and Taiwan that differs from a second monophyletic group encompassing the Solomon Islands, the Great Barrier Reef, the Torres Strait, and Western New Guinea (Nuryanto & Kochzius, 2009, Huelsken et al. 2013, Hui et al. 2016). Given that this study sourced brood stock close to the Solomon Islands (New Ireland Province, PNG; Fig. 1), the adult *T. maxima* clams used here likely came from this second group. Huelsken et al. (2013) also found a similar divergence for

**Figure 4.** Larval developmental stages of the *Tridacna maxima*/*Tridacna noae* (A, C, E, G) and *T. noae*/*T. maxima* (B, D, F, H) hybrids: (A, B) D-stage veligers, (C, D) midstage veligers, (E, F) pediveligers, and (G, H) metamorphosed juveniles 15 days postfertilisation. Developmental stages were photographed when collecting data collated in Table 1. Scale bars to 50 μm.
their *Tridacna* sp., later identified as *Tridacna noae* (Borsa et al. 2014), indicating different evolutionary lineages between Taiwanese and the Solomon Islands’ populations. Taking the results of Su et al. (2014) at face value it would appear that the Taiwanese phylogenies of *T. maxima* and *T. noae* are reproductively isolated, whereas the Western Pacific phylogenies of *T. maxima* and *T. noae* are capable of hybridizing, as demonstrated in this study. The extent to which these phylogenies differ physiologically, particularly in relation to gamete ultrastructure (Keys & Healy 1999, 2000), has yet to be investigated and limits explanatory discussion.

The genetic implications of successful hybridization between *Tridacna maxima* and *Tridacna noae* are dependent upon two factors: (1) whether hybridization of *T. maxima* and *T. noae* occurs in nature and (2) whether juvenile hybrid clams can successful develop into reproductively viable adults. Similar distributions of *T. maxima* and *T. noae* over environmental gradients and coexistence of local populations (Borsa et al. 2014, Militz et al. 2015) would suggest a possibility for their gametes to encounter one another in the environment (Neo et al. 2015b). The successful cross-fertilization and survival of hybrid clams further suggests that *T. maxima* and *T. noae* hybrids could persist in nature. The potential for this merits investigation after genetic/phenotypic evaluation of these hybrid strains that would enable their detection in the field. Prior field studies conducted in this region of study have failed to detect phenotypic intermediates of *T. maxima* and *T. noae* (Borsa et al. 2014, Militz et al. 2015), suggesting that if hybrids do exist in the natural population they phenotypically resemble one of the parental species, or exist at low abundance, precluding prior detection.

It is also presently unclear if *Tridacna maxima* and *Tridacna noae* hybrids can successfully develop into fertile adults. Unfortunately, because of the slow growth rates of tridacnines, it will not be possible to assess the fertility of the hybrid strains produced in this study for several years (Nash et al. 1988). The genetic distinctness of *T. maxima* from *T. noae*, where studied (Huelsken et al. 2013, Grulois et al. 2015, Johnson et al. 2016), would suggest against recent or continuous genetic exchange between the two species. This indicates that if hybrids do persist in nature they are unlikely to be fertile. A genetic study of these hybrid strains will be required to establish the extent to which hybridization may account for the intraspecific genetic differentiation within *T. maxima* and *T. noae* (Huelsken et al. 2013, Hui et al. 2016) and is presently being undertaken by the authors.

Published studies documenting the capacity for tridacnines to hybridize are limited (Alcazar 1988) and this is the first study to demonstrate hybridization among the *Tridacna*. Recent molecular phylogenies showed that *Tridacna noae* has more genetic similarity to *Tridacna squamosa* (Lamarec, 1819) and *Tridacna crocea* (Lamarec, 1819) than *Tridacna maxima* (Huelsken et al. 2013, Grulois et al. 2015, Johnson et al. 2016). Given the successful hybridization between *T. maxima* and *T. noae*, it reasons that there should also be scope for the more closely related *T. squamosa* and *T. crocea* to hybridize with *T. noae*. Yet, preliminary trials in this laboratory to assess the feasibility of maternal and paternal hybrid crosses of *T. noae* and *T. squamosa*, failed to yield competent larvae capable of surviving beyond 72 h postfertilization (authors’ unpublished data). Thus, genetic relatedness alone appears unable to predict the capacity for hybridization among tridacnines.

Previously, hybridization among the tridacnines has only been reported for *Hippopus*. Alcazar (1988) reported that *Hippopus porcellanus* eggs were successfully fertilized by *Hippopus hippopus* sperm. The embryogenesis of hybrids was similar to pure-strain larvae except in the progression of development. Hybrids initially developed faster than pure-strain larvae during the trochophore and veliger stages, but developmental differences evened out with the onset of metamorphosis. Similar variation was observed in this study with the shell size of hybrid larvae differing from those of pure-strain larvae during the velger stages before evening out during the pediveliger stage, immediately before metamorphosis. Postmetamorphosis, the early juvenile growth of the *Tridacna maxima*/*Tridacna noae* hybrid exceeded that of *T. noae* juveniles but was comparable with that of *T. maxima* juveniles.

In summary, this study has demonstrated that interspecific hybridization can occur between morphologically identified *Tridacna maxima* and *Tridacna noae*, in culture. Both maternal and paternal crosses resulted in larvae that successfully metamorphosed into juvenile clams. The comparable survival of hybrid clams with pure strain tridacnines in culture (this study, Braley et al. 1988, Waters et al. 2016) suggests that there is potential for these hybrids to exist in nature. On this basis, future genetic, population demographic, and ecological studies should consider the possibility of hybridization among tridacnines. As the current cohorts of hybrid clams develop, research in this laboratory will extend to an examination of phenotypic expression combined with genetic evaluation for both crosses to enable their detection in the field.

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