

Article

Comparative Analysis of Eye Traits and Visual Resolution Among Three Hatchery-Bred Giant Clams (*Tridacna crocea*, *T. squamosa*, *T. maxima*)

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Simple Summary

Giant clams are marine mollusks renowned for their immense size and colorful mantles. However, scientists have limited knowledge about how these eyes differ between species or how clearly they can see. This study investigated three common species: *Tridacna crocea*, *Tridacna squamosa*, and *Tridacna maxima*. We measured fundamental traits such as eye count and diameter, and determined how clearly they can see by observing their reactions to striped grating patterns. We found significant differences: *Tridacna maxima* possessed the highest number of eyes, whereas *Tridacna squamosa* had the largest eyes. *Tridacna crocea* exhibited the highest visual resolution. All three species possess simple “pinhole” eyes that lack lenses. We suggest that these specific ocular variations evolved to help each species survive in its particular habitat, influenced by environmental factors like water depth. These findings are valuable as they provide new insights into how simple visual systems adapt to different ecological niches.

Abstract

Bivalves possess a diverse array of photoreceptive organs that are significant for their evolutionary success and systematic classification. Giant clams are the largest bivalve mollusks, with mantle tissue permanently extended in nature to maintain symbiosis with zooxanthellae and perceive environmental cues. Eyes serve as critical sensory organs for these organisms, yet the structural and functional characteristics of tridacnine eyes remain inadequately understood. This study systematically investigated the ocular traits and visual resolution of three ecologically distinct giant clam species (*Tridacna crocea*, *T. squamosa*, *T. maxima*) using morphometric analysis, hematoxylin-eosin (HE) staining, transmission electron microscopy (TEM), and grating stimulation assays. Significant interspecific differences were observed in eye count, diameter, and pupil-to-eye ratio (PER): *T. maxima* exhibited the highest mean eye count (221 ± 8), *T. squamosa* the largest mean eye diameter

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(0.490 ± 0.082 mm), and *T. crocea* the highest mean PER (0.363 ± 0.041). Eyes were numerically symmetric on the left and right mantles but positionally asymmetric, showing random distribution patterns along the mantle margin without fixed corresponding locations across species. All three species possessed typical pinhole eyes lacking lenses and retinas, primarily composed of filler cells, receptor cells, and sparse neurons, with symbiotic zooxanthellae distributed in the surrounding mantle tissue. Grating stimulation assays revealed resolvable stripe periods of $5.82\text{--}11.64^\circ$ (*T. crocea*), $8.62\text{--}13.16^\circ$ (*T. squamosa*), and $10.15\text{--}12.26^\circ$ (*T. maxima*), confirming *T. crocea* as the species with the highest visual resolution. These ocular variations are inferred to reflect adaptive evolution driven by ecological niches and habitat-specific factors (water depth or light intensity), while the simplified pinhole morphology is consistent with their sedentary lifestyle and metabolic dependence on symbiotic zooxanthellae. These ocular variations provide potential morphological markers for the systematic classification of Tridacninae and offer valuable insights for researchers studying the evolutionary plasticity of bivalve visual systems.

Keywords: giant clam eyes; pinhole eyes; evolution; vision; structure; visual acuity

1. Introduction

Giant clams (Tridacninae) comprise the largest bivalve mollusks globally, with shell lengths ranging from approximately 15 cm to over 1 m [1]. Giant clams harbor symbiotic zooxanthellae within their unique tubular system, which provides a stable microenvironment for the algae. This symbiotic relationship necessitates prolonged exposure to light, requiring the shells to remain open for extended periods. Consequently, giant clams face heightened environmental challenges, including increased risk of predation, physical damage, and exposure to fluctuating environmental conditions [2,3]. To mitigate these risks, giant clams have evolved numerous pallial eyes that perceive environmental changes and detect potential threats, triggering rapid mantle retraction and shell closure.

The eye serves as the primary sensory organ for vision, and evolutionary processes have generated a diverse array of ocular morphologies in nature to adapt to different ecological demands. Representative structural categories include primitive eyes, cup eyes, ocelli, multiple ocelli, compound eyes, pinhole eyes, camera-type eyes, and reflective eyes [4]. Simulations of aquatic animal eye evolution conducted by Nilsson and Pelger [5] reveal that the primitive eye consists of a simple cup-shaped structure containing a single photoreceptor cell and one pigment cell. To achieve higher spatial resolution, evolutionary trajectories initially involve the longitudinal deepening of the ocular structure to form cup eyes, which can better distinguish light direction. Subsequently, pigment cells narrow the aperture to produce pinhole eyes, which improve image sharpness by reducing light scattering. Finally, the emergence of a lens facilitates the development of camera-type eyes, enabling precise light focusing and high-resolution imaging.

Bivalves have evolved a diverse array of photoreceptive organs essential for habitat selection, predator avoidance, and the synchronization of biological rhythms [6]. While larvae often utilize transient ocelli for settlement [7–10], adults primarily possess cephalic or pallial eyes. Cephalic eyes are evolutionarily conserved yet rare [11,12]. In contrast, pallial eyes—characteristic of giant clams—evolved independently across multiple lineages, including the Pteriida, Pectinidae, and Cardioidea. These organs demonstrate significant morphological plasticity, appearing on the outer, middle, or inner mantle folds depending on the taxon [13–16]. Consequently, ocular traits such as eye count, position, and structure are increasingly valued as diagnostic taxonomic characters, providing critical insights into bivalve phylogeny.

Despite the diversity of bivalve visual systems, research on the eyes of giant clams remains relatively limited. The eyes of Tridacninae are classified as pallial eyes, which are numerous and distributed along the outer mantle folds, representing a unique adaptation for their photosymbiotic lifestyle. Upon detecting moving objects or sudden changes in light intensity, these organisms rapidly retract their mantles and close their shells tightly to avoid potential harm. Specifically, Land [17] pioneered the functional analysis of *Tridacna maxima* eyes, identifying them as pinhole-type structures capable of basic spatial resolution. However, comparative studies on the ocular traits and visual capabilities of different giant clam species are still lacking. Besides, theoretical models of pinhole eyes suggest that image quality depends on the geometry of the pupil and eye cup, where a higher Pupil-to-Eye Ratio (PER) is predicted to correlate with superior optical performance and visual resolution. Yet, empirical evidence systematically comparing these traits across *Tridacna* species is absent, hindering our comprehensive understanding of their evolutionary adaptation strategies. In the present study, we systematically characterized the ocular structures, basic eye parameters, and visual angles of three ecologically important giant clam species (*T. crocea*, *T. squamosa*, and *T. maxima*). They are taxonomically classified under the Phylum Mollusca, Class Bivalvia, Order Venerida, Family Tridacnidae, and genus *Tridacna* [18]. These species exhibit distinct vertical zonation and life history strategies, serving as an ideal model for comparative sensory ecology. Confined to shallow reef flats at depths of 0 to 5 m, *T. crocea* adopts an endolithic lifestyle by fully embedding itself within calcareous substrates to withstand high wave energy [1]. *T. maxima* predominantly colonizes high-energy reef crests down to 10 m, relying on robust byssal attachment and partial boring for stability. Research indicates its light compensation depth of approximately 16 m aligns closely with its maximum depth limit, characterizing it as a strict photoautotroph restricted by light availability [19]. In contrast, *T. squamosa* demonstrates the broadest depth range, extending to 42 m and settling on sandy or rubble substrates along reef slopes. Despite this deep distribution, its light compensation depth remains surprisingly shallow at roughly 9 m. This discrepancy implies that individuals in deeper waters must supplement their energy budget through heterotrophic filter-feeding to survive in low-light regimes [1,19]. We hypothesize that such distinct variations in ambient light intensity, substrate type, and predator guilds have driven divergent adaptations in their visual systems. These findings are expected to provide foundational data for understanding the evolution and ecological adaptation of the bivalve visual system, as well as insights into the interaction between giant clams and their habitats.

2. Materials and Methods

2.1. Specimen Selection and Basic Parameter Measurement

All specimens were obtained from hatchery-reared populations in Sanya City of Hainan Province in China. During the experiment, water quality parameters were maintained at stable levels: water temperature 26–28 °C, salinity 33–35 ppt, and pH 8.0–8.2. The one-year-old individuals of *T. crocea*, *T. squamosa*, and *T. maxima* utilized in this study exhibit significant external morphological differences as manifested in the mantle coloration, pattern, and shell structures (Figure 1). One-year-old specimens of *T. crocea*, *T. squamosa*, and *T. maxima* were randomly selected for this study. To determine eye diameter and pupil size, we examined 5 randomly chosen individuals per species using a stereomicroscope (S8APO/MC170HD, Leica, Wetzlar, Germany). Subsequently, 6 eyes were randomly sampled from each individual. Microscopic images were acquired and processed using Leica Application Suite V4.6 software to conduct the necessary measurements. For counting the number of eyes, 30 individuals were randomly selected from each species, and the number of eyes present on the left and right mantles was tallied independently.

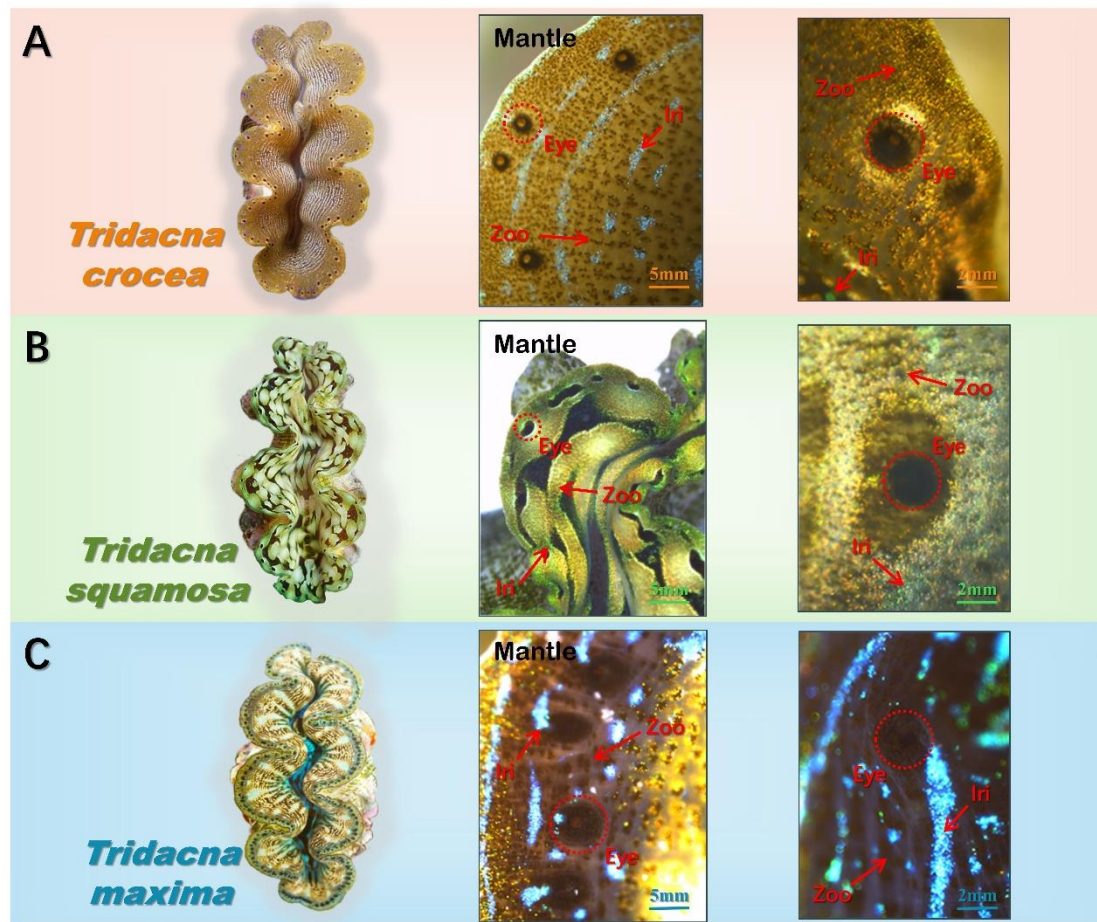


Figure 1. Comparative external morphology and ocular distribution of three giant clam species. The panels illustrate the morphological characteristics of one-year-old (A) *T. crocea*, (B) *T. squamosa*, and (C) *T. maxima*. Left column: View of the whole organism. Middle column: Enlarged view of the mantle tissue showing the distribution of eyes (Eye) and iridophores (Iri). Right column: High-magnification close-up of a single eye and surrounding pigmented structures.

2.2. Collection and Preparation of HE Staining Samples

All experiments were conducted in accordance with local animal care and use guidelines. Experimental specimens were anesthetized in a 4% magnesium chloride-seawater solution for 2 h to minimize stress and ensure complete extension of the mantle tissue [20]. Once the mantles were fully extended, the eyes and adjacent mantle tissue (approximately 5 mm × 5 mm) were carefully excised using sterile surgical scissors and immediately placed in Bouin's solution for 24 h preservation at room temperature. This was followed by sequential processes of dehydration through a graded ethanol series (70%, 80%, 90%, 95%, and 100% ethanol, 30 min per gradient), clearing in xylene (twice, 15 min each), embedding in paraffin wax at 60 °C, sectioning into 5 µm thick slices using a microtome (RM2235, Leica, **Wetzlar**, Germany), and mounting on glass slides. Finally, the sections were stained with hematoxylin-eosin (HE) staining solution following standard protocols: hematoxylin staining for 5 min, differentiation with 1% hydrochloric acid-ethanol for 30 s, eosin staining for 3 min, dehydration, clearing, and mounting with neutral balsam. Stained sections were observed and imaged using a light microscope (DM4000B, Leica, **Wetzlar**, Germany).

2.3. Collection and Preparation of TEM Samples

After anesthesia as described above, the eyes and adjacent mantle tissue of giant clam specimens were rapidly excised and immediately placed in a 2.5% glutaraldehyde fixative solution (pH 7.2–7.4) for storage at 4 °C. Subsequently, samples were postfixed in 1% phosphate-buffered osmium tetroxide (OsO₄, Servicebio, Wuhan, China) (0.1 mol/L) for 2 h at room temperature. Tissues were dehydrated through a graded ethanol series and embedded in EMBed-812 resin. Ultrathin sections ranging from 60 nm to 80 nm were then stained with uranyl acetate (Servicebio, Wuhan, China) and lead citrate (Servicebio, Wuhan, China), and observed on a Hitachi HT7700 TEM (Hitachi, Tokyo, Japan).

2.4. Experimental Setup for Grating Stimulation Assays

The experimental methodology followed the protocol established by Land[17]. The experiments were conducted indoors under subdued natural daylight to simulate the ambient light environment of the clams' natural habitat and ensure the eyes were light-adapted. Care was taken to avoid direct sunlight and reflections on the screen surface. The schematic diagram of the experimental setup is presented in Figure 2. Stimuli employed 80% high-contrast square-wave gratings with variable periods. These gratings face downward directly above the aquarium housing the clams. The minimum distance between the clam mantles and the screen was measured accounting for the position of the air–water interface. This distance was utilized to calculate the angle subtended by the grating width at the clam eyes. These dimensions ensured that the screen fully covered the entire mantle of each clam. Additionally, a full-spectrum LED light source (wavelength 400–700 nm) was used to provide consistent illumination, with the luminance of the white regions on the screen maintained at 400 nits.

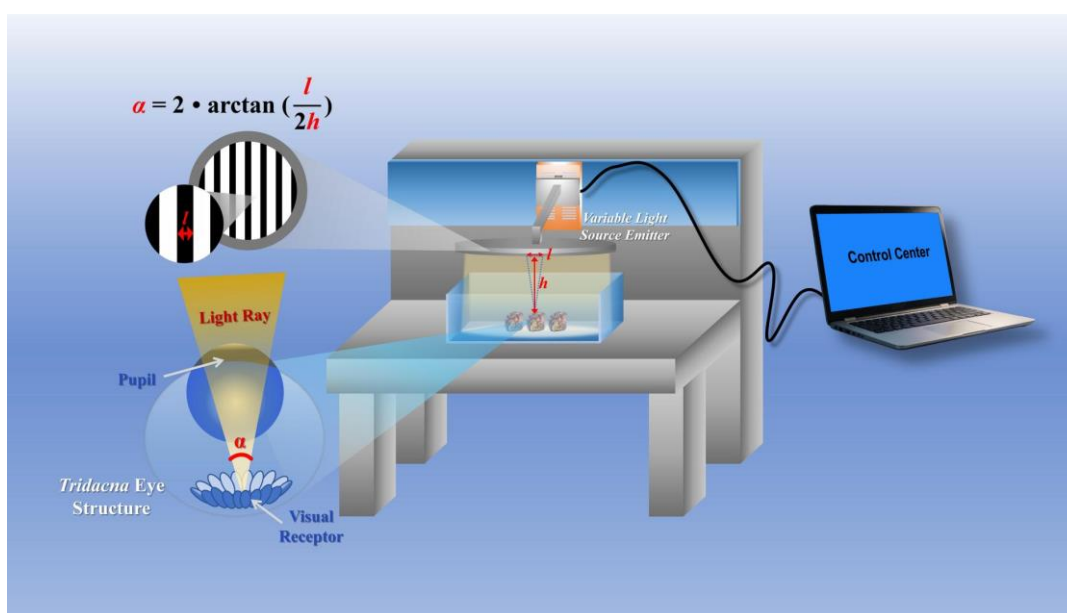


Figure 2. Schematic representation of the behavioral assay setup used to determine visual resolution. The system consists of a computer-controlled variable light source and a screen displaying high-contrast (80%) square-wave gratings positioned directly above the aquarium. The visual angle (α) subtended by the grating stripe at the clam's eye was calculated using the formula $\alpha = 2 \times \arctan(l/2h)$, where l represents the width of a single stripe (one black or one white bar) and h denotes the distance from the screen to the mantle surface (corrected for the air–water interface). Clam responses (siphon retraction or shell closure) were monitored to determine visual thresholds.

2.5. Statistics Analysis

Data processing and statistical analyses were performed using Microsoft Excel 2024 and SPSS version 27.0. Homogeneity of variance was assessed using Levene's test. For data adhering to normal distribution and homogeneity of variance, one-way ANOVA followed by Duncan's multiple range test was used. For data with heterogeneous variance, Welch's ANOVA followed by Games–Howell post hoc test was employed. Results were considered statistically significant when probability (p) values were less than 0.05. Figures were generated using Origin 2024.

3. Result

3.1. Basic Eye Parameters of 3 Giant Clams

Biometric measurements yielded mean shell lengths of 27.69 ± 3.16 mm for *T. crocea*, 39.54 ± 3.62 mm for *T. squamosa*, and 34.61 ± 4.48 mm for *T. maxima*. Regarding ocular parameters (Figure 3), significant interspecific variations were observed in the total number of eyes per individual. Specifically, *T. maxima* exhibited the highest eye count (221 ± 8), which was significantly higher than *T. crocea* (75 ± 11) and *T. squamosa* (60 ± 8) ($p < 0.001$). We also analyzed the distribution of eyes on the left and right mantles to assess symmetry. Paired t -tests revealed no significant differences in eye count between the two sides for *T. crocea* ($t(29) = 1.181$, $p = 0.247$), *T. squamosa* ($t(29) = 0.967$, $p = 0.341$), and *T. maxima* ($t(29) = -0.175$, $p = 0.862$).

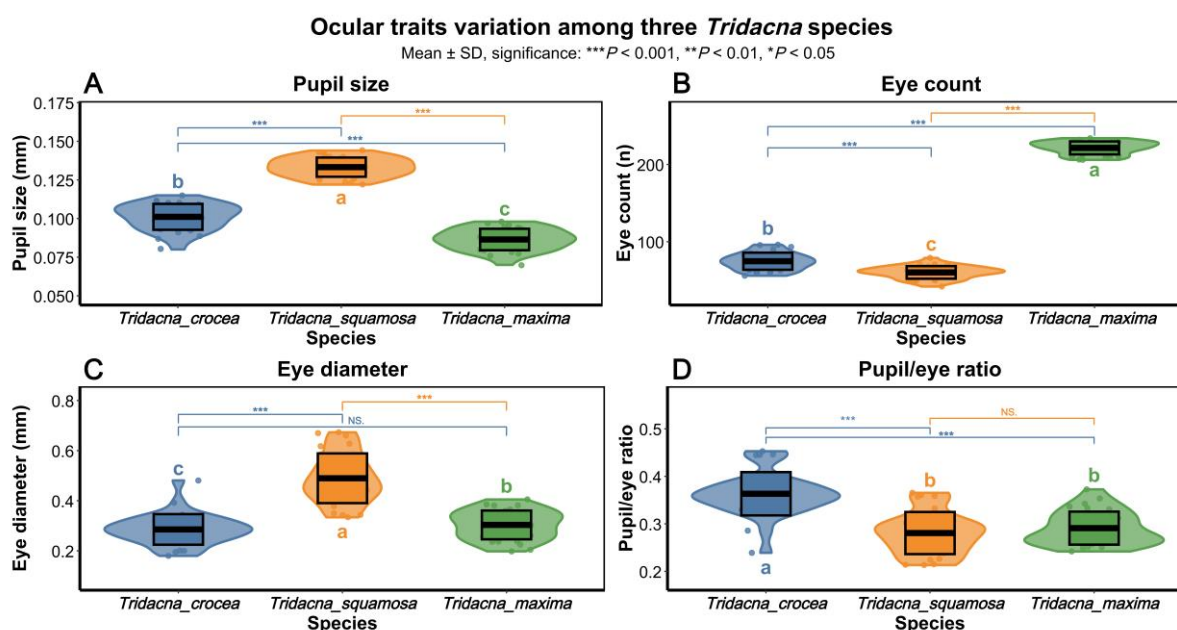


Figure 3. Quantitative analysis of ocular traits among *T. crocea*, *T. squamosa*, and *T. maxima*. Violin plots display the distribution of (A) pupil size, (B) total eye count per individual, (C) eye diameter, and (D) pupil-to-eye ratio (PER) ($n = 30$ individuals for eye count; $n = 5$ individuals, 30 eyes total for eye diameter and pupil size). The black box within each violin plot represents the interquartile range, and the horizontal line indicates the median. Data are presented as mean $\pm$ standard deviation (SD). Different lowercase letters (a, b, c) indicate statistically significant differences between species (where $a > b > c$; Duncan's multiple range test, $p < 0.05$). Asterisks denote significance levels: *** $p < 0.001$; NS indicates no significant difference.

Distinct variation in eye diameter per individual was observed among the 3 giant clams. *T. squamosa* exhibited significantly larger eye diameters compared to the other

species. In contrast, *T. crocea* and *T. maxima* showed no significant difference in this trait. Specifically, *T. squamosa* (0.490 ± 0.099 mm) was significantly larger than both *T. crocea* (0.285 ± 0.061 mm) and *T. maxima* (0.303 ± 0.057 mm) ($p < 0.001$).

Regarding pupil size, *T. squamosa* also displayed the largest value (0.133 ± 0.006 mm), significantly exceeding *T. crocea* (0.101 ± 0.008 mm) and *T. maxima* (0.086 ± 0.007 mm) ($p < 0.001$).

Additionally, this study calculated the PER for each clam individual. *T. crocea* showed the highest PER (0.368 ± 0.077), differing significantly from the other two species ($p < 0.001$), while no significant difference in PER was found between *T. squamosa* (0.284 ± 0.061) and *T. maxima* (0.296 ± 0.067) ($p > 0.05$).

3.2. HE-Stained Sections of Eyes from the 3 Giant Clams

Standardized sectioning methods were applied to the eyes of all 3 species. Cross-sections were prepared along the maximum transverse plane of the eyes and adjacent mantle tissue. The morphological appearance of the histological sections is illustrated in Figure 4. All eye cavities were oval-shaped with specific diameters measuring approximately 145.13 μ m for *T. crocea*, 139.82 μ m for *T. squamosa*, and 130.53 μ m for *T. maxima*. From the histological sections, filler cells, receptor cells, and a small number of neuronal cells were observed. Specifically, filler cells were concentrated in the central ocular region while receptor cells surrounded them in a ring-like pattern. Additionally, neuronal cells were distributed near the eye cavities. There were differences in the proportional ratio of filler cells to receptor cells among the 3 species, which may be attributed to variations in the relative depth during sectioning.

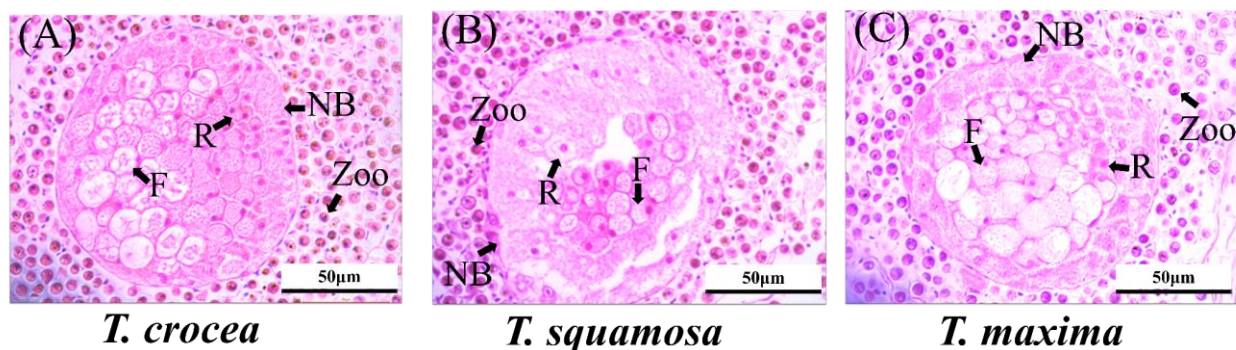


Figure 4. Histological organization of giant clam eyes examined via Hematoxylin-Eosin (HE) staining. Representative cross-sections of eyes from (A) *T. crocea*, (B) *T. squamosa*, and (C) *T. maxima*. The eyes are encapsulated, distinct structures lacking a lens. Arrows indicate key anatomical components: F, filler cells occupying the central region; R, receptor cells arranged peripherally around the filler cells; NB, nerve bundles exiting the eye cup; and Zoo, symbiotic zooxanthellae distributed in the adjacent mantle tissue.

3.3. Basic Structure of Eyes from the 3 Giant Clams Under TEM

TEM revealed the detailed ultrastructure of the eyes in *T. crocea*, *T. squamosa*, and *T. maxima* (Figure 5). At low magnification, the eyes of all three species presented a conserved oval morphology embedded within the mantle tissue. The ocular interior was predominantly occupied by filler cells, with receptor cells located peripherally and symbiotic zooxanthellae distributed in the adjacent tissue, highlighting the close spatial relationship between the visual and symbiotic systems.

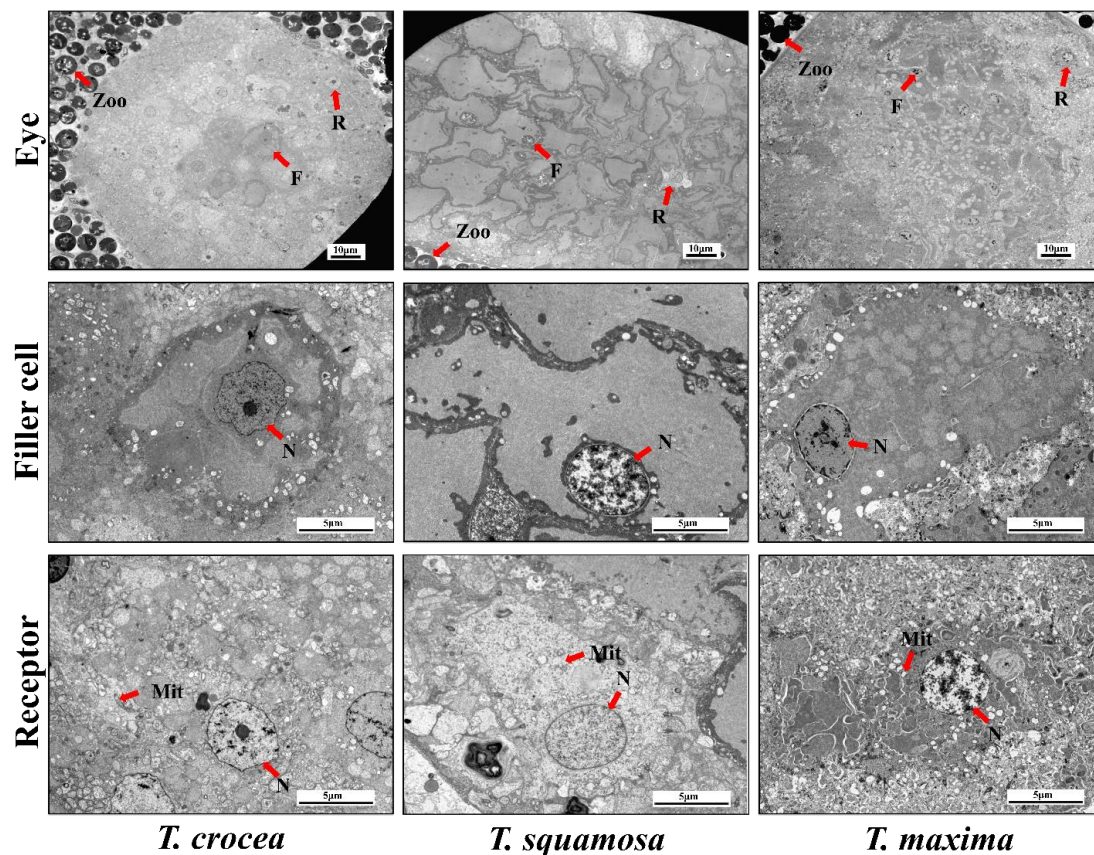


Figure 5. Ultrastructural organization of the eyes in three giant clam species visualized by Transmission Electron Microscopy (TEM). Columns correspond to *T. crocea*, *T. squamosa*, and *T. maxima*, respectively. Top row (Eye): Low-magnification overview showing the general arrangement of filler cells (F) and receptor cells (R) within the eye, with symbiotic zooxanthellae (Zoo) visible in the adjacent mantle tissue. Middle row (Filler cell): High-magnification views of filler cells, each containing a prominent nucleus (N). Note the distinct cell membranes in *T. squamosa* compared to the vacuolated cytoplasm in the other two species. Bottom row (Receptor): High-magnification views of receptor cells, characterized by the presence of a nucleus (N) and a high density of mitochondria (Mit) in the cytoplasm, indicating high metabolic activity associated with light perception.

High-magnification analysis revealed distinct interspecific variations in filler cells. In *T. squamosa*, filler cells exhibited compact organization with well-defined plasma membranes. In contrast, the filler cells of *T. crocea* and *T. maxima* displayed less distinct boundaries and contained numerous cytoplasmic vacuoles of varying sizes, potentially indicating differences in intracellular fluid regulation or light-scattering properties. Despite these structural variances, the nuclei of filler cells in all species were consistently spherical and centrally located, featuring prominent, electron-dense nucleoli.

Crucially, ultrastructural examination of the receptor cells identified a high density of mitochondria within the cytoplasm across all three species. These organelles were abundant and closely associated with the nucleus. The enrichment of mitochondria suggests that the photoreceptor cells in giant clams are metabolically active. This high energy production is likely required to drive the ion pumps and signal transduction pathways necessary for maintaining membrane potential and generating rapid neural responses to shadow stimuli.

3.4. Investigation of Spatial Resolution via Grating Stimulation Assays

Responses to grating stimuli were observed by adjusting the grating width. We established a response intensity scoring standard shown in Figure 6, where a score of 0 indicated no response. A score of 0.5 denoted the initiation of siphon and mantle contraction, while a score of 1 represented complete mantle contraction and shell closure. Additionally, to minimize observer bias, behavioral responses were recorded independently by two observers, and the final response score was averaged.

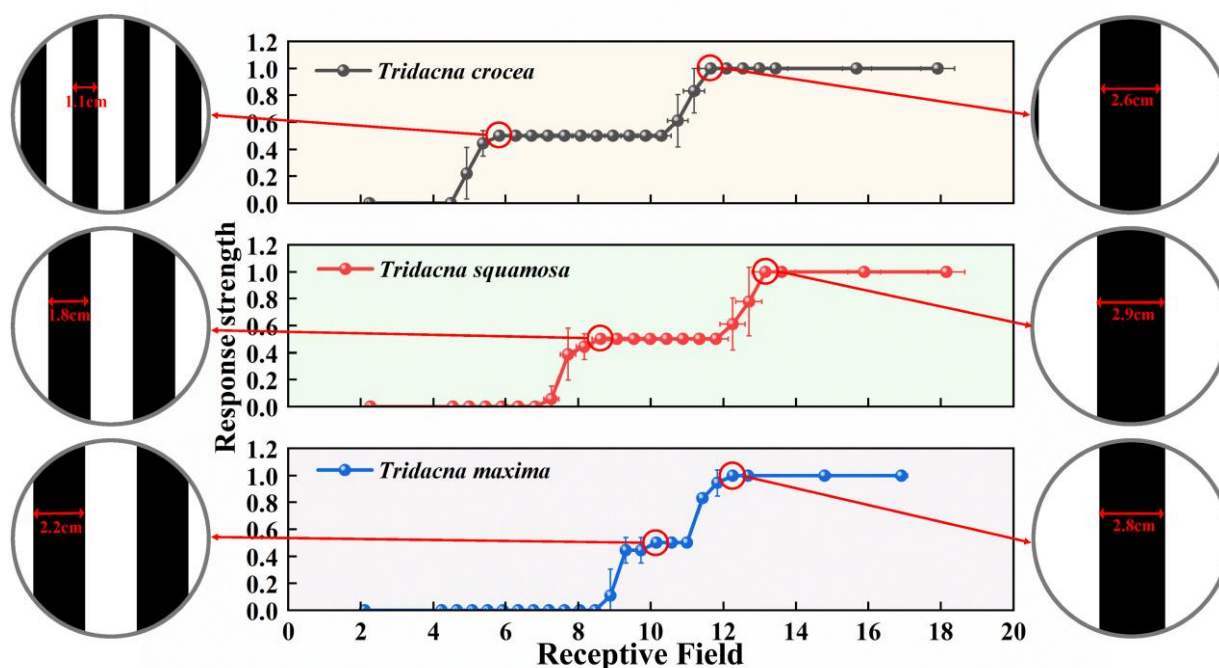


Figure 6. Determination of spatial resolution thresholds using grating stimulation assays. Response intensity curves for (Top) *T. crocea*, (Middle) *T. squamosa*, and (Bottom) *T. maxima* plotted against the receptive field angle. Response strength ranges from 0 (no response) to 1 (full shell closure). The left red circles indicate the threshold visual angle at which a response was first initiated, while the right red circles mark the angle at which the maximum response was achieved. Circular insets illustrate the specific grating widths corresponding to these critical threshold points for each species.

Experimental results indicated distinct response thresholds for each species. *T. crocea* initiated a response when the receptive field angle projected onto the receptors was approximately 5.82°. It achieved a full response at approximately 11.64°. Similarly, *T. squamosa* initiated a response at approximately 8.62° and achieved a full response at 13.16°. Finally, *T. maxima* initiated a response at approximately 10.15° and achieved a full response at 12.26° (pairwise comparison $p < 0.05$ against both *T. squamosa* and *T. maxima*).

4. Discussion

The three giant clam species (*T. crocea*, *T. squamosa*, and *T. maxima*) in this study were classified as possessing pinhole eyes based on structural characteristics. A defining feature of pinhole eyes is the absence of a lens, which precludes active light focusing. Additionally, individual photoreceptor cells receive photons randomly from various directions, as there is no lens to direct light to specific photoreceptors. This phenomenon reduces image quality and introduces noise, causing significant blurring of details in low-contrast images [21]. However, pinhole eyes have the advantage of a large depth of field, allowing objects at different distances to be imaged simultaneously, which is beneficial for sedentary organisms like giant clams that need to detect threats from various distances.

In the present study, the significant interspecific differences in eye count observed in these 1-year-old juveniles, reared under identical hatchery conditions, suggest that eye number is a genetically fixed trait specific to each species, rather than being driven by differences in shell length. Increasing sampling points in the visual environment via a greater number of eyes may represent an oversampling strategy employed by these animals, although specific benefits remain to be fully elucidated. In distributed visual systems, a larger number of overlapping optical units theoretically provides enhanced sensitivity and improves the signal-to-noise ratio of visual signals by integrating information from multiple eyes. Furthermore, it may ensure complete visual coverage of the environment, eliminating blind spots [5]. Eye count likely affects functions such as visual field range and neural processing latency: increased eye count enables animals to obtain more precise stereoscopic vision by comparing signals from adjacent eyes, which aids in distance perception [22]. Additionally, some organisms have evolved two distinct visual system strategies: one involves developing high-sensitivity systems by reducing eye number and increasing photoreceptor volume to capture more photons in low-light environments; the alternative strategy achieves high-resolution systems by increasing eye number and decreasing photoreceptor volume to improve spatial sampling [23]. Audino demonstrated that mobile scallop species possess significantly more eyes than sedentary ones, suggesting that increased visual sampling is essential for detecting predators in dynamic habitats [24]. *T. maxima*, which has a larger adult body size and may face more complex environmental threats, exhibited the highest eye count, which aligns with this strategy.

Eye size directly determines the capacity for receptor and nerve cells, thereby influencing image quality. Collectively, eye and pupil size reflect evolutionary trajectories adapted to specific habitat conditions. Invertebrate eye evolution typically follows a progressive sequence from cup eyes to pinhole eyes and finally to camera-type eyes [4]. Specifically, with the formation of pinhole eyes, the amount of incident light entering the eye through the aperture decreases compared to cup eyes. Individual photoreceptor cells then randomly receive photons from different directions, which in turn leads to reduced image quality and the occurrence of noise. Furthermore, insufficient incident light causes significant blurring of details in low-contrast images. When optical blurring fails to eliminate noise, organisms can only further improve imaging resolution through the evolution of a lens [21]. However, giant clams have retained the pinhole eye structure, which may be due to their sedentary lifestyle and metabolic reliance on symbiotic zooxanthellae, reducing the selective pressure for high-resolution vision.

Previous research indicates that the actual performance of pinhole eyes in *Nautilus* and *Tridacna* aligns closely with the theoretical optical optimum [17]. Theoretical models of pinhole eyes suggest that image quality depends on the geometry of the pupil and eye cup, where a higher PER is predicted to correlate with superior optical performance and visual resolution. This theoretical optimum is intrinsically linked to the dimensions of the pupil and eye: a larger PER allows more light to enter the eye, improving light-gathering capacity, while a smaller PER reduces light scattering, improving image sharpness. Therefore, the PER serves as a reliable proxy for evaluating the functional performance of pinhole eyes. In the present study, *T. crocea* exhibited the largest PER, which was significantly higher than that of *T. squamosa* and *T. maxima*. This morphological advantage is consistent with results from grating stimulation assays, where *T. crocea* demonstrated the highest visual resolution, indicating that the PER is indeed closely related to visual function in giant clams.

Variations in eye count, eye diameter, pupil size, and PER were observed among *T. crocea*, *T. squamosa*, and *T. maxima*, and these variations are speculated to be primarily driven by differences in their habitats. Specifically, the three species exhibit distinct habitat preferences: *T. crocea* inhabits tropical shallow-sea coral reef environments at water

depths of 0.5 m to 3.5 m, where light intensity is high and environmental conditions are relatively stable [25]. *T. squamosa* resides in subtidal coral reefs or rocky seabeds at water depths greater than 20 m, where light intensity is low and the environment is darker [26]. *T. maxima* is distributed in coral reefs and lagoon shoals at water depths less than 10 m, with moderate light intensity [27]. Among them, *T. squamosa* occupies the deepest habitat, and its low-light environment favors a reduction in eye count and an increase in photoreceptor volume to enhance light sensitivity, which is consistent with the high-sensitivity strategy proposed by Blest [23]. In contrast, *T. crocea* and *T. maxima* inhabit shallower waters with sufficient light, and consequently, they tend to increase eye count and decrease photoreceptor volume to develop high-resolution visual systems, enabling them to detect small changes in the environment. Multiple eyes allow the three giant clams to detect objects from various directions, and upon detecting an approaching object, the clams rapidly contract their mantles and close their shells tightly to avoid predation.

Furthermore, differences in eye characteristics between *T. crocea* and *T. maxima* may relate to adult body size. The adult body size of *T. maxima* is approximately twice that of *T. crocea*, and a larger body size may expose the clams to more complex environmental challenges and greater environmental pressure (e.g., more predators, larger home ranges). This drives the evolution of more eyes to achieve higher-resolution visual function, ensuring comprehensive environmental monitoring.

Statistical analysis involved counting eyes on the left and right mantles of each individual separately, and the results showed no significant difference in the number of eyes between the left and right mantles of the three species. This indicates that the eyes of giant clams exhibit numerically symmetric distribution patterns despite their incomplete positional symmetry. Numerical symmetry ensures balanced visual perception on both sides of the body, which is important for accurate direction detection, while positional asymmetry may be a result of random distribution during development, with no significant impact on visual function.

These interspecific variations in ocular morphology strongly support the hypothesis of adaptive plasticity driven by environmental constraints. *T. squamosa*, which typically inhabits deeper waters with attenuated light intensity, exhibited the largest absolute pupil size. This enlargement likely serves to maximize photon capture to function effectively in dim environments, inevitably sacrificing some degree of image sharpness due to increased blur circles. Conversely, *T. crocea* resides in shallow, high-light intertidal zones where photon abundance is not a limiting factor. Consequently, it has evolved the highest Pupil-to-Eye Ratio (PER) and the finest visual resolution. This adaptation allows *T. crocea* to prioritize spatial acuity over sensitivity, enhancing its ability to detect potential predators against the complex, bright background of the reef flat. Furthermore, *T. maxima*, often found in intermediate depths with a sessile, exposed lifestyle, possessed significantly more eyes than the other species. Given the restricted acceptance angle of individual pin-hole eyes, this proliferation of ocular organs likely functions to maximize total visual field coverage. By increasing the density of visual sampling points along the mantle, *T. maxima* achieves omnidirectional environmental monitoring, ensuring robust motion detection from all directions to compensate for its vulnerability to predation.

Histological and ultrastructural analysis revealed numerous zooxanthellae in the mantle tissue surrounding the eyes, confirming the stable symbiotic relationship between giant clams and zooxanthellae. The host (giant clam) provides a safe habitat along with inorganic salts and carbon dioxide required for photosynthesis, while zooxanthellae synthesize organic matter through photosynthesis, supplying up to 90% of the energy demand for the host [28,29]. This symbiotic relationship is crucial for the survival and growth of giant clams, and the proximity of zooxanthellae to the eyes may also play a role in light perception, as the algae can absorb specific wavelengths of light, potentially

modifying the light environment detected by the clam's eyes. Further observations revealed no significant differences in core ocular structure among the three species: each eye is mainly composed of filler cells in the anterior-middle region and receptor cells at the base, with sparse neuronal cells for signal transmission. The filler cells occupying the eye cavity likely serve a dual function: providing structural support to maintain the optimal pinhole geometry and acting analogously to a vitreous body. Their clear cytoplasm may facilitate light transmission and reduce internal scattering, thereby enhancing the image quality on the primitive retina. Neuron cells were clearly observable in histological sections of *T. crocea*, which may be related to its higher visual resolution. None of the three species possesses differentiated lenses, which prevents light refraction and classifies them as typical pinhole eyes. High magnification revealed the absence of a retinal structure; instead, a simple photosensitive system composed of photoreceptor cells and neurons enables blurred visual imaging. This eye structure is relatively primitive among mollusks. For example, cephalopod eyes, such as those of squids, are camera-type eyes exhibiting convergent evolution with vertebrate eyes [30]. However, comparative studies have shown important anatomical and histological differences: the vertebrate retina is inverted (photoreceptors located at the back of the retina), while the cephalopod retina is everted (photoreceptors located at the front); furthermore, the vertebrate retina possesses a multilayered structure containing several types of photoreceptors, neurons, and glial cells [31], while the cephalopod retina consists of two nuclear layers separated by a basal membrane containing photoreceptors and support cells [32]. Scallops possess a unique visual system comprising up to 200 reflecting eyes, each containing a concave mirror rather than a lens to focus light, and the hierarchical organization of the multilayered mirror regulates image formation from component guanine crystals at the nanoscale to complex three-dimensional morphology at the millimeter level [33]. Compared with squids and scallops, giant clams have not evolved such complex eye structures, presumably because giant clams are sedentary organisms relying mainly on zooxanthellae for energy, which eliminates the need for active predation or rapid predator avoidance, thereby reducing the requirement for a complex visual system.

Our results are consistent with research by Land [17] on *T. maxima*: specifically, clams showed no response to static stimuli but exhibited a strong response to gratings with sudden phase changes (moving stimuli). Land [17] also revealed that the receptor receptive field angle of *T. maxima* was approximately consistent with the visual angle estimated via anatomical methods. Using the grating threshold established in that experiment, we derived receptive field angles for the three species: approximately 10.94° for *T. crocea*, 13.61° for *T. squamosa*, and 14.01° for *T. maxima*. Precise determination of receptor receptive field angles for these three species requires combined analysis with anatomical estimates, as the grating stimulation assay reflects the overall visual response of the organism, while anatomical methods can directly measure the receptive field of individual photoreceptor cells. Results indicate that *T. crocea* possesses higher visual resolution compared to *T. maxima* and *T. squamosa*, which is consistent with its higher PER and more receptor cells. Conversely, no significant difference was observed between *T. squamosa* and *T. maxima* in visual resolution, which may be due to their similar PER values. Notably, all three species in this study exhibited higher visual resolution compared to the *T. maxima* specimens described by Land [17]. A discrepancy exists regarding the visual resolution of *T. maxima* between the two studies, and the higher resolution observed in the present study may be attributed to differences in the geographical origin of the sampled populations (Land's study used specimens from the Great Barrier Reef, while the present study used specimens from Hainan, China) or differences in environmental conditions during rearing (hatchery-reared vs. wild-caught).

However, the visual resolution of these three giant clams is still far lower than that of scallops in the Family Pectinidae. Scallops have a unique eye morphology featuring a double-layered retina, a reflector, and a differentiated lens structure, which enables them to resolve objects 2° across, providing a level of protection unavailable to animals relying solely on a shadow response [34]. Nevertheless, if giant clams responded to all stimuli in their visual environment, they would spend most of their life with their shells closed, which would hinder photosynthesis by symbiotic zooxanthellae and reduce energy intake. It is therefore plausible that their relatively simple optical system acts as a filter to prevent such excessive responses, only triggering defensive behaviors when stimuli exceed a certain threshold (large moving objects or sudden light changes), which balances the need for protection and energy acquisition.

5. Conclusions

Data from this study revealed significant differences in key eye parameters among one-year-old *T. crocea*, *T. squamosa*, and *T. maxima*, including eye count, diameter, pupil size, and PER. *T. maxima* had the highest eye count, *T. squamosa* had the largest eye diameter and pupil size, and *T. crocea* had the highest PER and visual resolution. However, all three species shared a common characteristic: the number of eyes on the left and right mantles was symmetric, while their positions were not fully symmetric. These parameter differences are hypothesized to represent evolutionary adaptations to respective habitats and environmental conditions, such as variations in water depth and light intensity. Furthermore, anatomical observation combined with histological and ultrastructural analyses confirmed that the eyes of all three species are of the pinhole type, lacking a lens and retina structure, and primarily composed of filler cells, receptor cells, and sparse neuronal cells. Symbiotic zooxanthellae were detected in the surrounding mantle tissue, highlighting the close relationship between the visual system and symbiosis. Additionally, grating stimulation assays quantified the receptive field angles of eye receptors to characterize spatial resolution, with specific receptive field angles of approximately 10.94° (*T. crocea*), 13.61° (*T. squamosa*), and 14.01° (*T. maxima*). These findings provide comprehensive insights into the structure and function of the visual system in giant clams, contributing to a better understanding of the evolutionary and ecological adaptation mechanisms of bivalves. Future research could focus on the molecular mechanisms underlying photoreception in giant clams, as well as the impact of environmental factors (light intensity, temperature) on the development and function of their eyes. However, this study has certain limitations. The use of hatchery-reared individuals may not fully reflect the phenotypic plasticity seen in wild populations subject to different environmental pressures. Future research should aim to compare these findings with wild specimens and integrate ocular morphology with molecular phylogenetics to further refine the systematic classification of the Tridacninae subfamily.

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References

1. Neo, M.L.; Wabnitz, C.C.C.; Braley, R.D.; Heslinga, G.A.; Fauvelot, C.; Wynsberge, S.V.; Andréfouët, S.G.E.; Waters, C.; Tan, A.S.-H.; Gomez, E.D.; et al. Giant Clams (Bivalvia: Cardiidae: Tridacninae): A Comprehensive Update of Species and Their Distribution, Current Threats and Conservation Status. In *Oceanography and Marine Biology*; Hawkins, S.J., Evans, A.J., Dale, A.C., Firth, L.B., Hughes, D.J., Smith, I.P., Eds.; CRC Press: Boca Raton, FL, USA, 2017; pp. 87–387, ISBN 978-1-315-27227-6.
2. Yonge, C.M. Mode of life, feeding, digestion and symbiosis with zooxanthellae in the tridacnidae. *Sci. Rep./Great Barrier Reef Exped.* **1928–29** **1936**, *1*, 283–321.
3. Yonge, C.M. Functional morphology and evolution in the Tridacnidae (Mollusca: Bivalvia: Cardiacea). *Rec. Aust. Mus.* **1981**, *33*, 735–777. <https://doi.org/10.3853/j.0067-1975.33.1981.196>.
4. Chen, C.F.; Yao, H.Y. The diversity and consistency of the evolution of the eyes. *J. Biology* **2023**, *40*, 104. <https://doi.org/10.3969/j.issn.2095-1736.2023.01.104>.
5. Nilsson, D.E.; Pelger, S. A Pessimistic Estimate of the Time Required for an Eye to Evolve. *Proc. Biol. Sci.* **1994**, *256*, 53–58. <https://doi.org/10.1098/rspb.1994.0048>.
6. Land, M.F.; Nilsson, D.E. *Animal Eyes*; Oxford University Press: New York, NY, USA, 2012.
7. Baker, S.M.; Mann, R. Description of Metamorphic Phases in the Oyster *Crassostrea Virginica* and Effects of Hypoxia on Metamorphosis. *Mar. Ecol. Prog. Ser.* **1994**, *104*, 91–99.
8. Hodgson, C.A.; Burke, R.D. Development and Larval Morphology of the Spiny Scallop, *Chlamys Hastata*. *Biol. Bull.* **1988**, *174*, 303–318. <https://doi.org/10.2307/1541956>.
9. Cragg, S.M. *Biology and Ecology of Scallop Larvae*; Developments in Aquaculture and Fisheries Science Elsevier: Amsterdam, The Netherlands, 2016.
10. Cole, H.A. The Fate of the Larval Organs in the Metamorphosis of *Ostrea Edulis*. *J. Mar. Biol. Assoc. UK* **1938**, *22*, 469–484. <https://doi.org/10.1017/S0025315400012376>.
11. Morton, B. The Evolution of Eyes in the Bivalvia: New Insights. *Am. Malacol. Bull.* **2008**, *26*, 35–45. <https://doi.org/10.4003/006.026.0205>.
12. Zaixso, H. Nervous System and Receptors in the Ribbed Mussel, *Aulacomya Atrata* (Bivalvia: Mytilidae). *Rev. biol. Mar oceanogr.* **2003**, *38*, 43–56.
13. Audino, J.A.; Serb, J.M.; Marian, J.E.A.R. Left–Right Asymmetry of the Visual System in the Scallop *Nodipecten Nodosus* (Bivalvia: Pectinidae). *J. Molluscan Stud.* **2023**, *89*, eyad007. <https://doi.org/10.1093/mollus/eyad007>.
14. Sumner-Rooney, L. ‘Distributed’ Vision and the Architecture of Animal Visual Systems. *J. Exp. Biol.* **2023**, *226*, jeb245392. <https://doi.org/10.1242/jeb.245392>.
15. Malkowsky, Y.; Götz, M.-C. Impact of Habitat and Life Trait on Character Evolution of Pallial Eyes in Pectinidae (Mollusca: Bivalvia). *Org Divers Evol.* **2014**, *14*, 173–185. <https://doi.org/10.1007/s13127-013-0165-z>.
16. Audino, J.A.; Serb, J.M.; Marian, J.E.A.R. Hard to Get, Easy to Lose: Evolution of Mantle Photoreceptor Organs in Bivalves (Bivalvia, Pteriomorpha). *Evolution* **2020**, *74*, 2105–2120.
17. Land, M.F. The Spatial Resolution of the Pinhole Eyes of Giant Clams (*Tridacna Maxima*). *Proc. R. Soc. Lond. B* **2002**, *270*, 185–188. <https://doi.org/10.1098/rspb.2002.2222>.

18. Harzhauser, M.; Mandic, O.; Piller, W.E.; Reuter, M.; Kroh, A. Tracing Back the Origin of the Indo-Pacific Mollusc Fauna: Basal Tridacninae from the Oligocene and Miocene of the Sultanate of Oman. *Palaeontology* **2008**, *51*, 199–213. <https://doi.org/10.1111/j.1475-4983.2007.00742.x>.
19. Jantzen, C.; Wild, C.; El-Zibdah, M.; Roa-Quiaoit, H.A.; Haacke, C.; Richter, C. Photosynthetic Performance of Giant Clams, *Tridacna Maxima* and *T. Squamosa*, Red Sea. *Mar. Biol.* **2008**, *155*, 211–221. <https://doi.org/10.1007/s00227-008-1019-7>.
20. Ninwichian, P.; Khumrak, T.; Chaikereee, P.; Phuwan, N. Effects of Agricultural-Grade Magnesium Chloride as an Anesthetic Agent and a Gonad Sampling Technique on White Scar Oysters (*Crassostrea Belcheri*). *An. Acad. Bras. Ciênc.* **2025**, *97*, e20241089. <https://doi.org/10.1590/0001-3765202520241089>.
21. Dawkins, R. *The Blind Watchmaker*; Reprint; Longman Scientific & Techn: Essex, UK, 1987.
22. Opell, B.D.; Ware, A.D. Changes in Visual Fields Associated with Web Reduction in the Spider Family Uloboridae. *J. Morphol.* **1987**, *192*, 87–100. <https://doi.org/10.1002/jmor.1051920202>.
23. Blest, A.D. Retinal Mosaics of the Principal Eyes of Jumping Spiders (Salticidae) in Some Neotropical Habitats: Optical Trade-Offs between Sizes and Habitat Illuminances. *J. Comp. Physiol.* **1985**, *157*, 391–404. <https://doi.org/10.1007/BF00618129>.
24. Audino, J.A.; Adams, D.C.; Serb, J.M. Variation in Eye Abundance among Scallops Reveals Ontogenetic and Evolutionary Convergence Associated with Life Habits. *Evolution* **2022**, *76*, 1607–1618. <https://doi.org/10.1111/evo.14541>.
25. Goh, G.; Todd, P. The Distribution and Status of Giant Clams (Family Tridacnidae)—A Short Review. *Raffles B Zool.* **2010**, *58*, 103–111.
26. Richter, C.; Roa-Quiaoit, H.; Jantzen, C.; Al-Zibdah, M.; Kochzius, M. Collapse of a New Living Species of Giant Clam in the Red Sea. *Curr. Biol.* **2008**, *18*, 1349–1354. <https://doi.org/10.1016/j.cub.2008.07.060>.
27. Huang, K. *Illustrated Handbook of Marine Mollusks in China*; China Ocean Press: Beijing, China, 2008.
28. Hirose, E.; Iwai, K.; Maruyama, T. Establishment of the Photosymbiosis in the Early Ontogeny of Three Giant Clams. *Mar. Biol.* **2006**, *148*, 551–558. <https://doi.org/10.1007/s00227-005-0119-x>.
29. Lucas, J.S. Giant Clams. *Curr. Biol.* **2014**, *24*, R183–R184. <https://doi.org/10.1016/j.cub.2013.11.062>.
30. Koenig, K.M.; Gross, J.M. Evolution and Development of Complex Eyes: A Celebration of Diversity. *Development* **2020**, *147*, dev182923. <https://doi.org/10.1242/dev.182923>.
31. Bejarano-Escobar, R.; Blasco, M.; Martín-Partido, G.; Francisco-Morcillo, J. Molecular Characterization of Cell Types in the Developing, Mature, and Regenerating Fish Retina. *Rev. Fish. Biol. Fisheries* **2014**, *24*, 127–158. <https://doi.org/10.1007/s11160-013-9320-z>.
32. Imarazene, B.; Andouche, A.; Bassaglia, Y.; Lopez, P.-J.; Bonnaud-Ponticelli, L. Eye Development in *Sepia Officinalis* Embryo: What the Uncommon Gene Expression Profiles Tell Us about Eye Evolution. *Front. Physiol.* **2017**, *8*, 613. <https://doi.org/10.3389/fphys.2017.00613>.
33. Palmer, B.A.; Taylor, G.J.; Brumfeld, V.; Gur, D.; Shemesh, M.; Elad, N.; Oshero, A.; Oron, D.; Weiner, S.; Addadi, L. The Image-Forming Mirror in the Eye of the Scallop. *Science* **2017**, *358*, 1172–1175. <https://doi.org/10.1126/science.aam9506>.
34. Land, M.F. Activity in the Optic Nerve of *Pecten Maximus* in Response to Changes in Light Intensity, and To Pattern and Movement in the Optical Environment. *J. Exp. Biol.* **1966**, *45*, 83–99. <https://doi.org/10.1242/jeb.45.1.83>.

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