Press Release

Keio University

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Keio University

Utilizing the "Stealth" of the Invasive Ascidian
- Towards establishing a new model organism for bioimaging -

A research team including students and faculty at the Keio University has taken the first step toward making *Ascidiella aspersa* (also known as the European sea squirt) a new model organism for bioimaging research by defining their developmental stages and constructing a three-dimensional embryological image resource. The team included Haruka M. Funakoshi and Takumi T. Shito, second-year master's students at Keio's Graduate School of Science and Technology along with Professor Kotaro Oka and Associate Professor Kohji Hotta of the Faculty of Science and Technology.

A. aspersa is an ascidian belonging to the class of chordates—the closest relatives of vertebrates. *A. aspersa* is a well-known invasive species that reproduces invasively not only in Japan but around the world, inflicting damage to shellfish aquaculture operations by attaching themselves to scallops. Despite this, embryos of this species are so transparent that they can transmit 90% of visible light, making scientists investigate their potential as a possible model organism for bioimaging research. However, until now there has been no standard developmental table for this species, an essential element in furthering such research.

As a first step to establishing *A. aspersa* as a model organism, researchers referenced the world standard developmental table of *Ciona intestinalis*,[1] defining 28 different developmental stages for *A. aspersa* that span from fertilized egg to hatched larva. Additionally, they developed a web-based three-dimensional embryological image of the developmental table viewable by anyone in the world. This resource contains an astounding collection of over 3,000 cross-sectional and 3D images of the organism taken via confocal laser scanning microscopy.

The *A. aspersa* 3D image resource constructed in this study is indispensable for connecting different omics data to each developmental stage's spatiotemporal hierarchy, and is expected to aid in forming a system-level understanding of the embryonic development and phylogenic evolution of chordates—a classification that includes humans. If research on this species continues to expand the research foundation for *A. aspersa*, scientists believe that it could connect to potential measures to prevent these ascidians from attaching to and harming scallops.

The results of this study were published in the online edition of the Swiss scientific journal *Frontiers in Cell and Developmental Biology* on December 17, 2021.

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1. Research Background

Bioimaging—a technique that visualizes the dynamics of various molecules in living organisms and is facilitated by the remarkable development of science and technologies such as microscopes and various fluorescent probes—has contributed greatly to life science research. The embryonic transparency of laboratory organisms is incredibly important, especially when conducting image studies using living organisms. This is because if an embryo is opaque, it is difficult to capture the depth of the embryo, leading to an underutilization of imaging technology.

Ascidians of the genus *Phallusia* have transparent embryos and are already being used as laboratory organisms in Europe. They are not, however, found in other regions such as Japan and the United States. For this study, researchers searched the Japanese coast comprehensively for species with transparent embryos suitable for imaging. As a result, they found that the ascidian with the clearest embryo in Japan was the non-native *A. aspersa*, reported to be attached in large numbers to farmed scallops, seriously damaging the scallop aquaculture industry (Kanamori, 2016; Shito *et al*., 2020).

Several factors attest to the viability of *A. aspersa* as a world-class model organism. One is that, in addition to their high embryological transparency in the visible light range (light transmittance of about 90% or equivalent to that of a glass window), this species can translate mRNA prior to egg fertilization, allowing researchers to use florescent protein probes to monitor early stages of development. Another factor is that, as it is an invasive species, it can be found living in areas uninhabited by ascidians of the genus *Phallusia*, making it readily available worldwide.

For these reasons, this research group aims to establish this species as a new model organism suitable for imaging by taking advantage of the embryonic transparency, or "stealth," of *A. aspersa*. The study began by first defining what would be established as the world standard developmental table for *A. aspersa*.

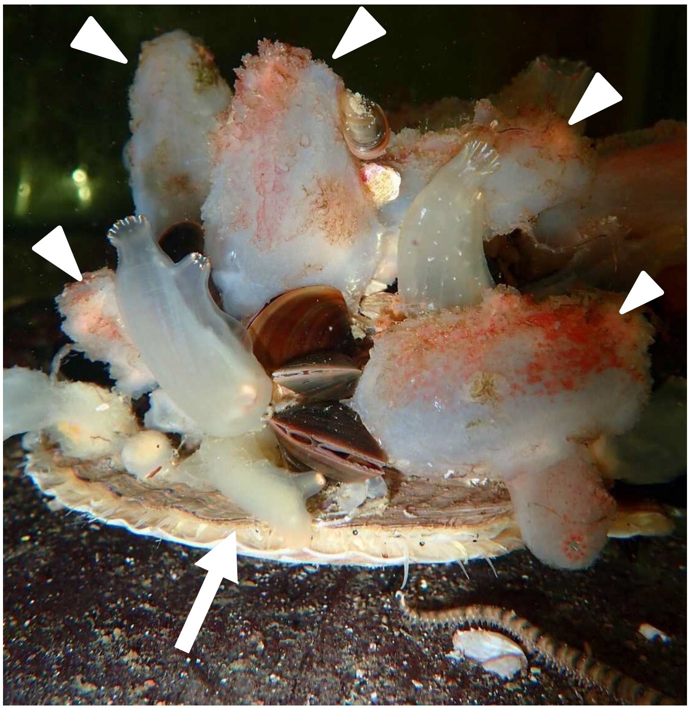

Figure 1: ***Ascidiella aspersa*** **(triangles) on a scallop (arrow)**
As pictured, various organisms, including ascidians and mussels, attach to farmed scallops, inhibiting their growth. *A. aspersa* can be identified by their distinctive reddish coat.

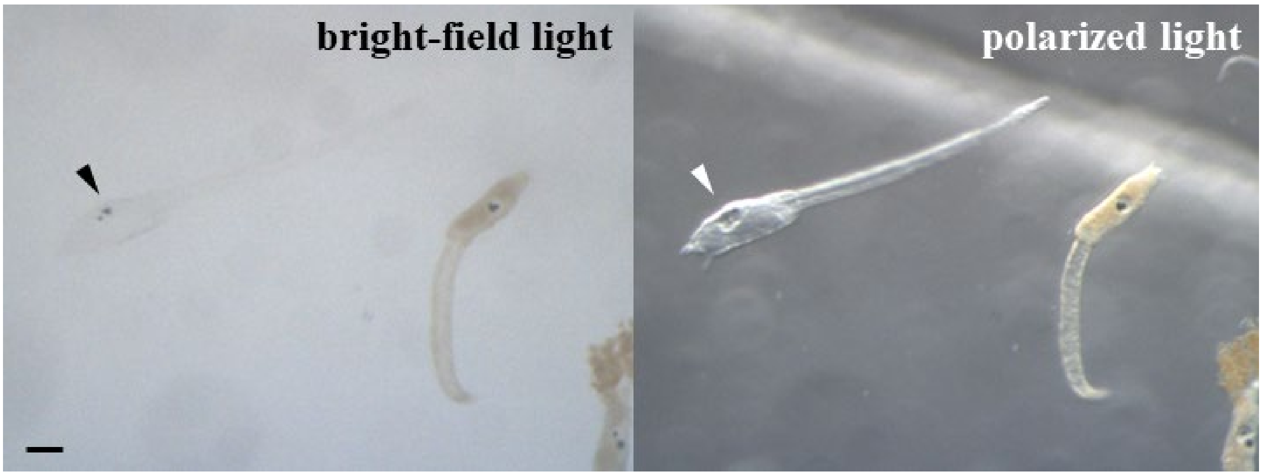

Figure 2: ***Ascidiella aspersa*** **and** ***Ciona intestinalis*** **larvae photographed under bright-field and polarized light** (from Shito *et al.*, 2020).
Ciona are visible in all lighting conditions, whereas, in bright-field lighting, *A. aspersa* larvae (left) are "stealthy" in that only the eye (triangle) is visible.

2. Research details

Ascidians are the closest relatives to vertebrates and are a valuable model organism for understanding vertebrate development and evolution due to their small number of constituent cells and well-explored cell lineage. Among them, the species primarily used in research are *Ciona intestinalis* and *Halocynthia roretzi*. Comparatively, invasive *Ascidiella aspersa* have nearly transparent embryos (Shito *et al.*, 2020), pointing to their promise as a model organism for bioimaging research. However, scientists had not completed a standardized developmental table had yet to be defined and lacked in anatomical knowledge. Thus, as the first step to establishing *A. aspersa* as a model organism, researchers referenced the standardized developmental table of *Ciona intestinalis* type A (Hotta *et al.*, 2007) to define its standard developmental stages (Fig. 3). The developmental process from fertilized egg to hatched larva was classified into 28 developmental stages (stages 1-26).[2]

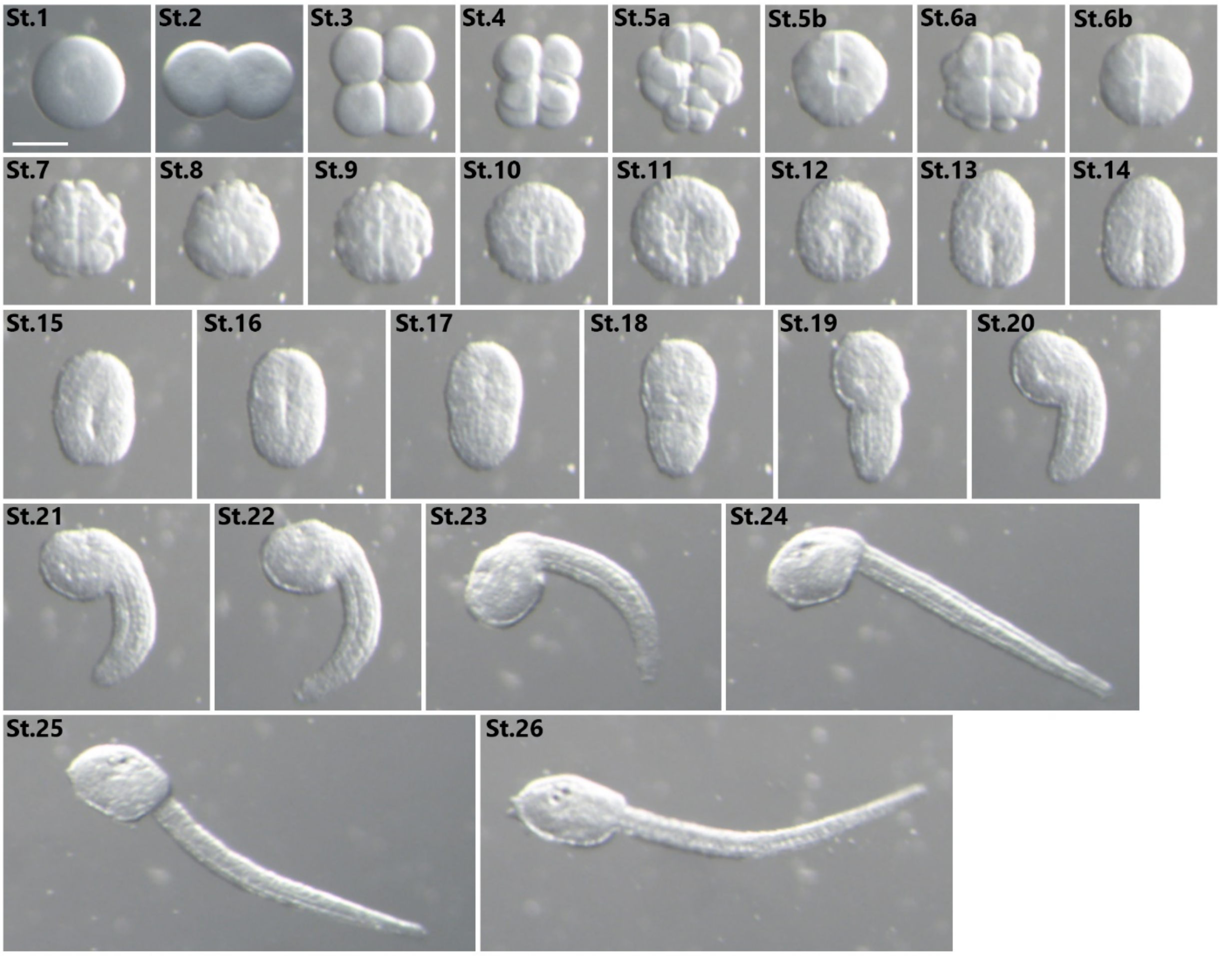

Figure 3: Defining the developmental stages of the *Ascidiella aspersa* embryo, which transmits 90% of visible light.

Researchers then photographed *A. aspersa* embryos at each of the 28 defined developmental stages using confocal laser scanning microscopy. Based on more than 3,000 cross-sectional images, they created a 3D image that enables observation of the detailed structure of the embryo from any angle (Fig. 4). These cross-sectional and 3D images also contain additional information such as cell lineage, tail length, trunk length, and other criteria for defining developmental stages.

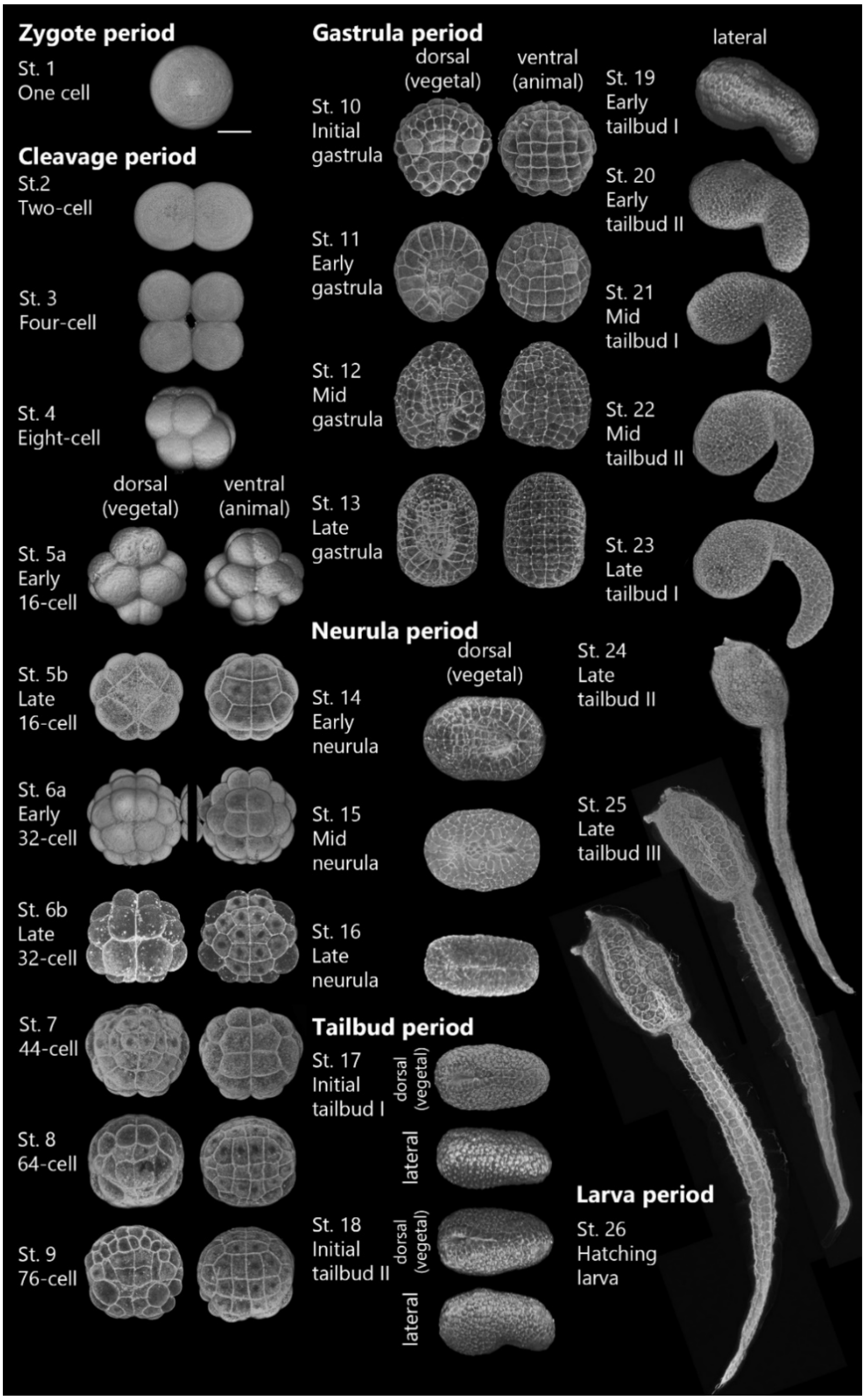

Figure 4: Embryological images of each developmental stage constructed in 3D from more than 3,000 confocal microscopy laser scanning images

All images, definitions of developmental stages, and annotation information obtained in this study have been compiled into an online digital image resource RAMNe (Resources of *Ascidiella aspersa* Morphology Network-based), which can be viewed by anyone from anywhere in the world (Fig. 5).

Figure 5: Top page of the *Ascidiella aspersa* 3D database "RAMNe" developed in this study
URL: https://www.bpni.bio.keio.ac.jp/RAMNe/latest/index.html

Researchers also compared cleavage patterns, embryonic morphology, and developmental rates between *Ascidiella aspersa* and *Ciona intestinalis* in a cross-species analysis. The results showed that cleavage patterns and the developmental time up to the neurula period in *A. aspersa* were remarkably conserved in comparison to *Ciona*. On the other hand, the ratio of the trunk and tail length in the tailbud period were smaller than *Ciona*, indicating a relatively short tail. This may be related to the fact that the number of cells that make up the caudal epidermis of *A. aspersa* is lower than that of *Ciona*. Researchers also found that the timing of the tail bend in *A. aspersa* is earlier than in *Ciona*. These differences may form the basis for *A. aspera's* invasive nature.

Finally, researchers performed two imaging experiments taking advantage of the areas in which *A. aspera* excels: its embryological transparency and early translation of mRNA. In the first experiment, researchers stained the cell membranes of *A. aspera*, and demonstrated that it is possible to capture 3D images of the entire embryo while it is still alive.[3] The second experiment demonstrated the noteworthy ability of *A. aspera* to translate foreign mRNA in early stages of development. (Fig. 6). The experiment also showed that with *A. aspera*, fluorescent protein sensors can be used to observe the wave-like propagation of the increase in Ca^{2+} concentration at the moment of fertilization.[4]

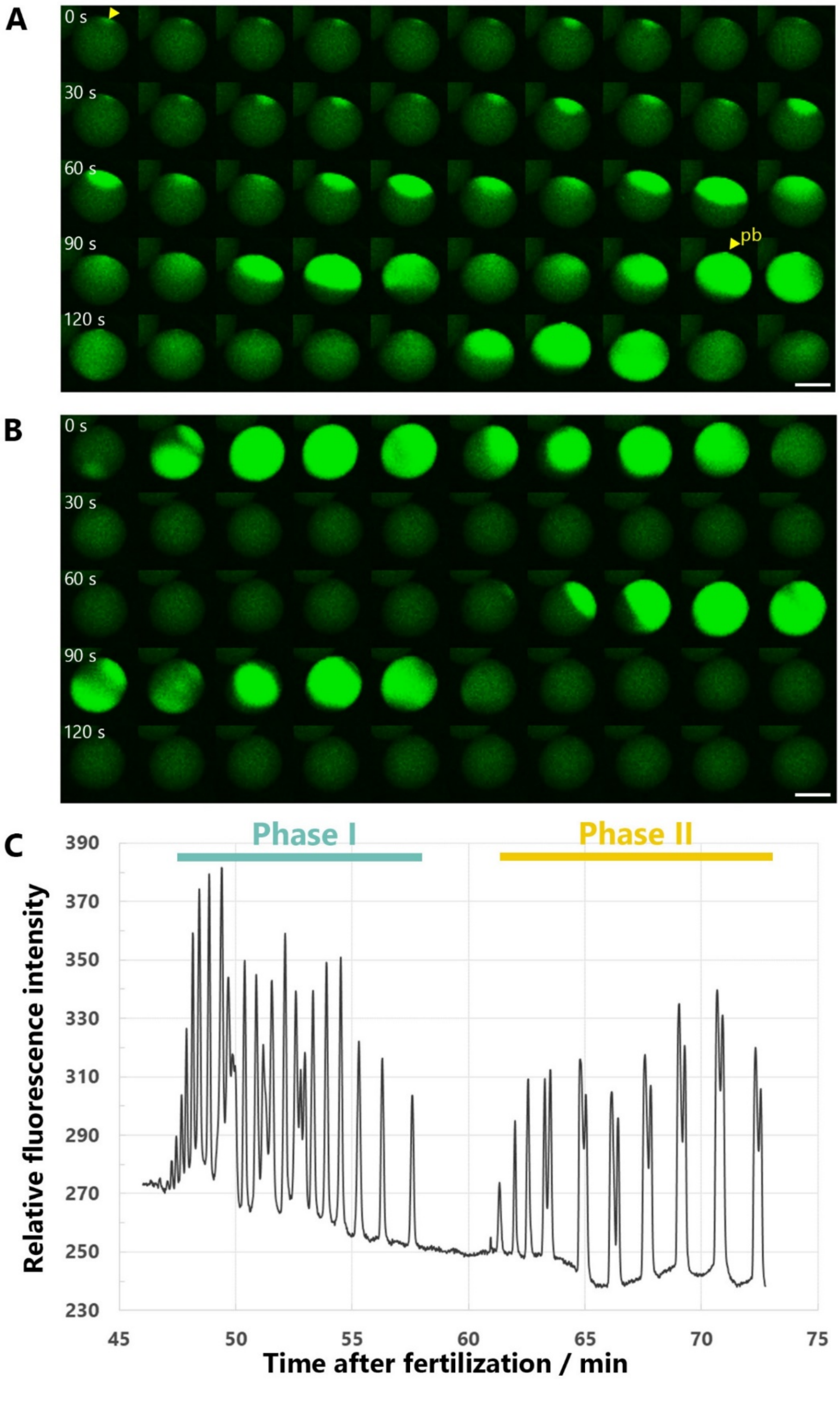

Figure 6: Injection of GCaMP6s mRNA results in two Ca^{2+} oscillations at fertilization
A: First Ca^{2+} oscillation directly following fertilization, B: Second Ca^{2+} oscillation, C: Graph showing change in brightness during oscillations

3. Future Developments

In the future, in order to make *Ascidiella aspersa* easier to utilize in experiments, the researchers from this project hope to consolidate the information required for life science research such as its genome and transcriptome data. In addition, interspecific comparisons with *Ciona intestinalis* revealed that *A. aspersa* has a relatively short tail and a different arrangement of adhesive organs.[5] These characteristics may be connected to the invasive nature of this species against scallops and other marine life handled in aquaculture operations. While this study deals with the lifecycle up to hatched larvae, scientists are also considering defining further developmental stages for swimming larvae, metamorphosis, and even juveniles in the hopes that further research into such stages will help mitigate damage to aquaculture operations.

In addition to these potential avenues, researchers will continue to provide embryological and anatomical information on *A. aspersa* to other researchers around the world through the 3D embryological image resource RAMNe. The team also hopes that this fascinating organism will become the new model organism of choice for understanding development and evolution at cellular and systematic levels.

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Details of Journal Article

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Database Information

Resources of *Ascidiella aspersa* Morphology Network-based; R*A*MNe

https://www.bpni.bio.keio.ac.jp/RAMNe/latest/index.html

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Makoto Kanamori, Katsuhisa Baba, Yasuko Konda, Seiji Goshima (2014). Distribution of the invasive ascidian Ascidiella aspersa (Müller, 1776) (Urochordata, Ascidiacea) in Hokkaido. *Japanese Journal of Benthology*, p. 23-31, issue I, volume 69)
https://doi.org/10.5179/benthos.69.23

Shito, T. T., Hasegawa, N., Oka, K., and Hotta, K. (2020). Phylogenetic Comparison of Egg Transparency in Ascidians by Hyperspectral Imaging. *Sci. Rep.* 10, 1–12.
https://doi.org/10.1038/s41598-020-77585-y

Hotta, K., Mitsuhara, K., Takahashi, H., Inaba, K., Oka, K., Gojobori, T. and Ikeo, K. (2007), A web-based interactive developmental table for the ascidian *Ciona intestinalis*, including 3D real-image embryo reconstructions: I. From fertilized egg to hatching larva. Dev. Dyn., 236: 1790-1805.
https://doi.org/10.1002/dvdy.21188

Explanation of Terms and Reference Materials

[1] *Ciona intestinalis*
One of the ascidian model organisms used in research worldwide
Scientific name: *Ciona intestinalis* type A *(Ciona robusta*)

[2] Developmental process from fertilized egg to hatched larva (video)
https://figshare.com/articles/media/Video1_Developmental_Table_and_Three-Dimensional_Embryological_Image_Resource_of_the_Ascidian_Ascidiella_aspersa_MP4/17255246

[3] 3D shot of an *Ascidiella aspersa* embryo (video)
https://figshare.com/articles/media/Video2_Developmental_Table_and_Three-Dimensional_Embryological_Image_Resource_of_the_Ascidian_Ascidiella_aspersa_MP4/17255249

[4] Ca^{2+} oscillation during fertilization (video)
https://figshare.com/articles/media/Video3_Developmental_Table_and_Three-Dimensional_Embryological_Image_Resource_of_the_Ascidian_Ascidiella_aspersa_MP4/17255252

[5] Adhesive organs
An organ used by ascidian larvae to attach to rocks and other substrates and metamorphose

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