

## Dataset Paper

# Longitudinal Observation of Japanese Lancelet, *Branchiostoma japonicum*, Metamorphosis

Takao Kaji,<sup>1</sup> Yoichi Hoshino,<sup>2</sup> Yasuhisa Henmi,<sup>3</sup> and Kinya Yasui<sup>1</sup>

<sup>1</sup> Department of Biological Sciences, Graduate School of Science, Hiroshima University, 1-3-1 Kagamiyama, Higashihiroshima, Hiroshima 739-8526, Japan

<sup>2</sup> Department of Virology, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan

<sup>3</sup> Center for Marine Environment Studies, Kumamoto University, 39-1 Kurokami 2-chome, Kumamoto, Kumamoto 860-8555, Japan

Correspondence should be addressed to Kinya Yasui; [furaha@sci.hiroshima-u.ac.jp](mailto:furaha@sci.hiroshima-u.ac.jp)

Received 4 April 2012; Accepted 8 May 2012

Academic Editors: M. Kurokawa, K. Terakado, and S. Tochinai

Copyright © 2013 Takao Kaji et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The lancelet (amphioxus) performs metamorphosis and produces minute and ciliate pelagic larvae commonly found in other metamorphic marine invertebrates. During larval life and metamorphosis, however, the animal displays interesting combination of features not found in other animals such as long coexistence of ciliate and muscular locomotion and no change in feeding behavior. The uniqueness of lancelet metamorphosis can provide important data to understand the evolutionary history of this animal as well as the metamorphosis broadly appeared in metazoans. Although lancelet metamorphosis has been studied, all previous studies depended on cross-sectional observations. To get serial data on metamorphic events, we performed longitudinal observations on the Japanese lancelet under the culture condition and confirmed the following: (1) there were individual variations of the duration of metamorphosis from 15 to 27 days; (2) growth was arrested for a month and the maximum reduction of the body length (2.2%–3.2%) occurred when gill slits became paired; (3) during rather long duration of metamorphosis, the oral transformation and the division of the gill pores by tongue bar were completed within two to four days. Our observations suggest that the duration and mode of lancelet metamorphosis depend mainly on intrinsic requirements rather than on extrinsic selective pressures.

## 1. Introduction

Profound change of body plan under the same genomic background during life cycle is common in metazoans. It is called metamorphosis and found in at least some species of most extant phyla [1]. Metamorphosis of lancelets (amphioxus) has been noticed from the first embryological study of Kowalevsky [2] and interested the following scientists such as Lankester and Willey [3], Willey [4], and van Wijhe [5]. Like other marine invertebrates, lancelets change their life mode from pelagic to benthic passing through metamorphosis. However, their diet and feeding method are not changed principally. Lancelet ontogeny uniquely displays a long-term coexistence of cilia- and muscle-driving locomotion before metamorphosis [6, 7]. The metamorphosis takes place after

pelagic larval life, the duration of which depends on the environmental condition and species [8–10], and animals after metamorphosis settle into sand substratum.

Metamorphic changes mainly occur in the pharyngeal region in lancelets. Metapleural folds appear first on the ventral trunk on both sides and fused ventrally covering the original body surface in the pharyngeal region and abdominal region anterior to the iliocolonic ring [3]. Larval mouth that occupies from the second to the ninth myomere wide on the left body surface just before metamorphosis is shrunken forward during metamorphosis and finally transformed into the velum as a median organ [4, 11]. Not only the mouth but also the digestive system as a whole undergoes remarkable modification. A single row of gill pores, which expand from the midventral to the right side, get their counterparts above

them on the right side, and both rows of gill pores are destined to the left-right pairwise positioning. During the pairing, each gill pore is divided into two by a tongue bar. Endostyle originally developed on the right side of the pharynx also moves to the ventral floor and expands longitudinally. At the junction between the esophagus and stomach, the budding of hepatic diverticulum appears ventrally.

Although the aforementioned morphological changes are comprehensive and occur during relatively short period, lancelet metamorphosis is distinct from that of animals producing ciliate larvae such as lophotrochozoans. The latter animals completely change body plan, locomotion mode, and feeding behavior with metamorphosis as a trigger. In marine environment, as intermediate state during the transition of life mode causes higher risk to predation, the duration of metamorphosis is presumed to be optimally shortened [12]. In chordates, all three members, cephalochordates, urochordates, and vertebrates, especially anuran amphibians, display metamorphosis, but each metamorphosis is distinct from others among chordate taxa and, except urochordates, also from that of other invertebrates. As a gene coding thyroid hormone receptor has been found in lancelets [13], a mechanism similar to that of anurans that takes weeks to months to complete metamorphosis [14] was expected, and it has been revealed that a thyroid hormone derivative (triiodothyroacetic acid) can induce lancelet metamorphosis [15]. To advance studies on the lancelet metamorphosis, we have to understand basic time course of the metamorphosis based on longitudinal observations. We report here individual metamorphosis observed longitudinally under the culture condition.

## 2. Methodology

First generation of laboratory progeny of the Japanese lancelet (now assigned to *Branchiostoma japonicum* [16]) fertilized on 23 and 24 July 2011 at the Marine Station of Kumamoto University, Japan, was maintained as described previously [17]. When metamorphic signs were observed in some precocious individuals at the end of August, about 200 animals were maintained in a small plastic container with 500 mL filtered seawater. From these, five individuals that were developing metapleural folds were reared one by one in 92 mm Petri dish with filtered seawater. The animals were fed daily with 20 mL and a few milliliters of well-grown culture medium of a diatome *Chaetoceros gracilis* for the plastic container and Petri dish, respectively. Seawater and plastic wares were changed every three days. The atmospheric temperature close to the culture dishes during the observation is shown in Figure 2, which can be regarded as being equivalent to water temperature because of small volume of culture dishes. Light-dark cycle was under the natural regime. Separately maintained five animals were photographed under the microscope daily from 17 September to 17 October 2011, and then body length measurement of the living animals with objective micrometer was started from 22 September and continued until 12 November 2011. The digital images were processed to produce montage for each animal with Adobe Photoshop CS4. Totally 31 images for each were used for

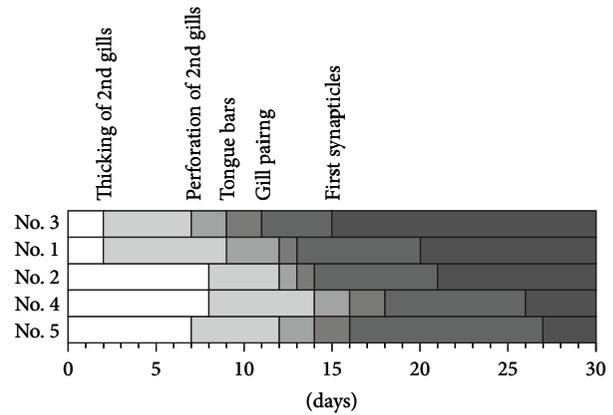


FIGURE 1: Time course of individual metamorphosis in *Branchiostoma japonicum* at temperature fluctuated between 21.5 and 24.0°C. Note the individual variation of the duration and rapid expansion of ciliate surface by both opening of secondary gill series and division of each gill pore with tongue bar. The number of the animals corresponds to that in Figure 2.

making QuickTime movies by using Apple iMovie (Dataset Item 3 (Video)). The measurement of body length was also performed on the montage images.

Timing of metamorphosis was as follows. The observations were carried out directly on the living animals under the microscope and on the montage images. On 17 September, all individuals subjected to longitudinal observation were developing atrial chamber that had covered the posterior nine to ten primary gill pores (future left-side gill pores). The duration of metamorphosis showed individual variations under the culture condition. From the first observation of the thickened epithelium of the secondary gills (right-side gills), which is the most easily identifiable landmark in the early phase of metamorphosis, to that of elliptical window-like perforation of the secondary gills, it took four to seven days, and from the budding of tongue bar in the secondary gill pore to the pairing of left and right gill slits, it took one to two days. Then it took further four to eleven days to the formation of primary synapticles between branchial and tongue bars, at which metamorphosis is completed and thereafter animals are called juveniles [11]. In total, the fastest individual took 13 days and the slowest 20 days from the thickening of the secondary gills to the primary synapticle formation between gill and tongue bars (Figure 1).

The metamorphosis of lancelets is comparable only in habitat change from plankton to benthos with other metamorphic marine invertebrates, although there is no consistent account on the larval behavior. The larval behavior has been variously described as entirely planktonic [4], predominately planktonic under culture condition [18], vertical migrant between the sea bottom and surface with a day cycle [19, 20], and dweller on the sea bottom until the 6-gill stage and then becomes planktonic [8]. Under the culture condition, however, we observed that larvae stayed on the bottom in the glass tank frequently throughout their life. This suggests that the Japanese lancelet in larval life inhabits in the water

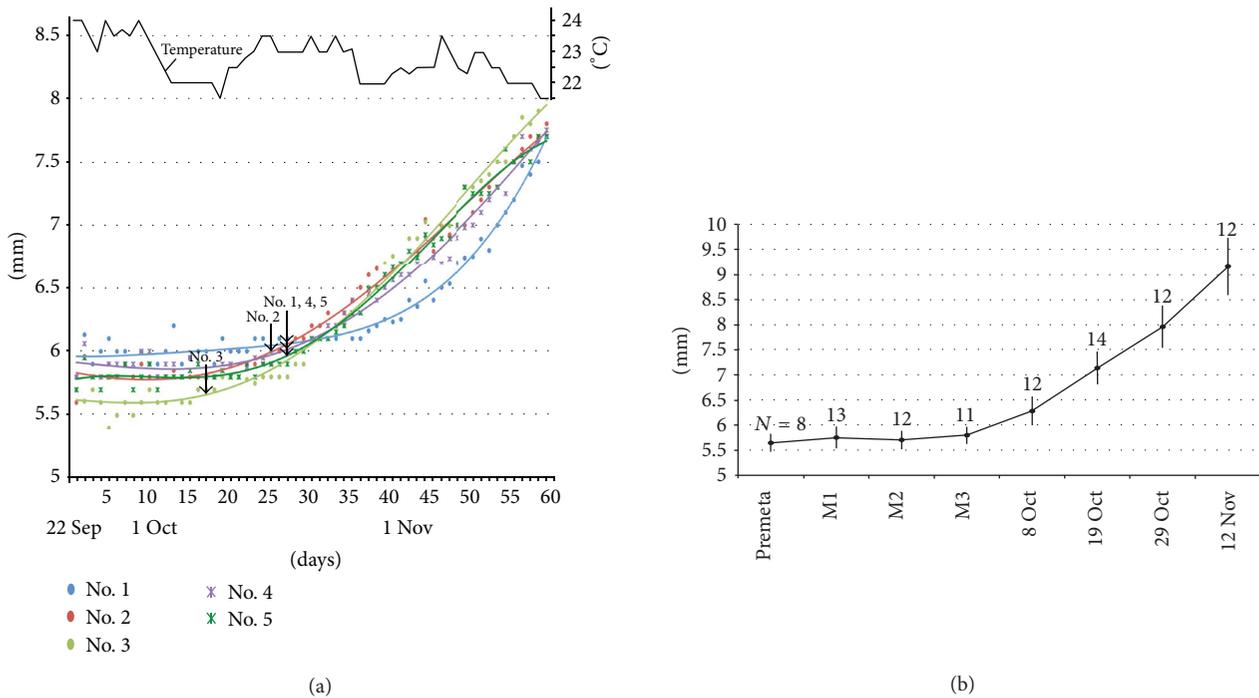


FIGURE 2: Growth curves and culture temperature. (a) Growth curves of five individuals and culture temperature drawn from Dataset Item 1 (Table). Arrows with specimen numbers denote when the specimen grows longer than the size at the beginning of observation. Growth lines are approximated by fourth-order regression curves. All specimens show growth arrest during metamorphosis. (b) Growth curve obtained by a cross-sectional sampling grouped according to developmental stages drawn from Dataset Item 2 (Table). All specimens again display growth arrest during metamorphosis. In the sampling at the postmetamorphic stage, well-grown animals were selected to avoid mixture with younger stages, which causes size difference at the postmetamorphic stages between the isolated culture (a) and pooled culture (b). Each measurement shows the mean  $\pm$  SD. Premeta: premetamorphic stage; M1: thickening of secondary gill series; M2: bisection of secondary gill pores; M3: pairing of gill slits.

column and on the bottom surface for a long period before metamorphosis, and it is interesting to know how long lancelets need for metamorphosis. Since all previous studies on lancelet metamorphosis are based on cross-sectional observations, there is little reliable data on this issue, in which the Florida lancelet took eight days at 22.5°C and about five days at 30°C under a culture condition [10]. In the present study, as we started the observation when the animals developed atrium partially, it is not known how many days the studied animals took for metamorphosis exactly. However, animals with slow metamorphosis took 27 days and the fastest animal 15 days from the start of the observation to the formation of the primary synapticles under the conditioned temperature between 21.5 and 24.0°C.

Lancelet larvae develop a large mouth occupying about a fifth of the body length and large gill openings during pelagic life. To change from pelagic to benthic, lancelet larvae required the strengthening and protection of the oral and pharyngeal regions because they settle into sand substratum when they are about 5 mm long. To meet this requirement, the mouth and gill pores are covered with preoral hoods and atrium during metamorphosis, respectively. At that time, however, the mouth changes to a very small opening and the number of gill pores reduces to about half [4, 11]. These changes seem to hurt effective intake of seawater. To

minimize this transitional discordance, lancelets develop the secondary gill series rapidly and divide all gill pores by tongue bar producing about twice large area for branchial cilia that create water flow. During a long duration of metamorphosis compared with that of most marine invertebrates, which is usually a rapid process as hours or a few days [14], the modification of the mouth and gill system took only two to four days.

Lancelet larvae are small and ciliate like the pelagic larva of most marine invertebrates. A difference from the others is found in that they develop functional muscles at the early larval stage [7, 11]. Against inherent danger for lancelet larvae as pointed out for other small pelagic larvae, there seems to be nothing to protect them in considering the anatomy and behavior. Lancelet metamorphosis thus seems to follow the *need for speed* hypothesis to avoid risk [14]. However, the duration of the metamorphosis is not so short unlike other marine invertebrates. Rapid changes found in the mouth and branchial system seem to correspond with intrinsic requirements. Locomotion and feeding behavior that are subjected to external selection pressures do not change substantially between pre- and postmetamorphosis in lancelets. The uniqueness of lancelet metamorphosis will provide a research model to test what factors can determine the duration of metamorphosis [21].

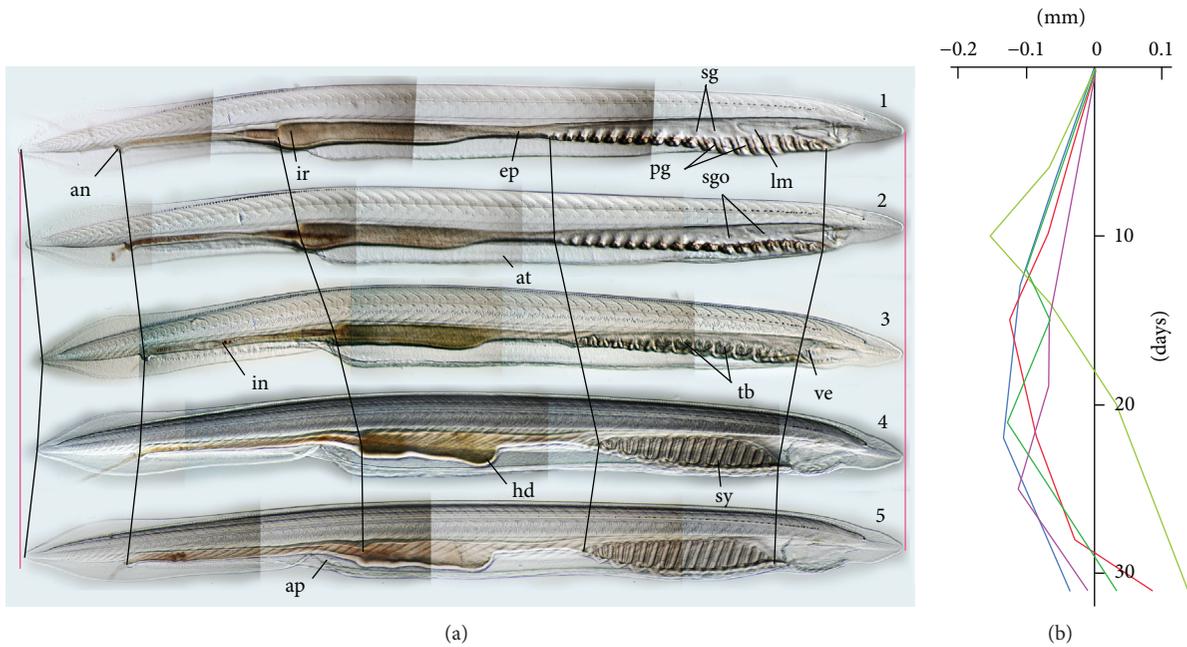


FIGURE 3: Size reduction and change of digestive system. (a) Chronological change of the specimen number 1 in Figure 2(a). Note the remarkable reduction of the pharyngeal length and moderate reduction of the esophagus and midgut region as well as remarkable elongation of the intestine. (b) Reduction of body length in five individuals compared with the length at the beginning of the observation. Line colors are the same as those in Figure 2(a). Reduction of the body length is maximized when left and right gill rows become paired. Animal images numbered 1–5 are as follows: 1, on 17 September; 2, on 25 September; 3, on 30 September; 4, on 9 October; and 5, on 17 October. an: anus; ap: atriopore; at: atrium; ep: esophagus; hd: hepatic diverticulum; in: intestine; ir: iliocolonic ring; lm: larval mouth; pg: primary gills; sg: secondary gills; sgo: secondary gill openings; sy: synapticle; tb: tongue bars; ve: velum.

Growth during metamorphosis was as follows. It has been pointed out that the European lancelet *Branchiostoma lanceolatum* stopped growing or reduced the body length during metamorphosis [5, 22, 23]. In contrast, the Florida lancelet *B. floridae* was reported to continue growing [10]. Although some of these studies were based on animals under the laboratory condition, none performed longitudinal observation. To confirm whether or not the Japanese lancelet reduces the body length during metamorphosis, for five individuals we have traced longitudinally the change of the body length in living state under the dissection microscope. The result was that the Japanese lancelet did not grow during metamorphosis and reduced the size slightly (Dataset Items 1 and 2 (Table); Figures 2 and 3). In the measurement on the individual images, compared with the size at the beginning of the observation (forming atrium), the size reduction was from 2.2% to 3.2% of the body length being observed when gill slits became paired (Figure 3(b)). As the body length of the premetamorphic stage is less than 6.0 mm, it seems to be rather difficult to depict exact size reduction by cross-sectional observation (Table 1). An interesting observation is that regardless of the fluctuated culture temperature, animals started metamorphosis when their body size was smaller than others tended to perform metamorphosis quickly and grew faster after metamorphosis (Figure 2), which was also recognized in animals maintained in the 500 mL container, though not quantified.

As gill pores were disappearing, the length of the pharynx was shortened and the esophagus was slightly elongated. This compensation was not balanced with the actual reduction of the pharynx, and thus the gut was pulled anteriorly (Figure 3). In this modification of the alimentary canal, the iliocolonic ring was moved anteriorly, which was located posterior to the atriopore at the onset of metamorphosis and anterior to it by the final stage of metamorphosis. The reduction of the pharyngeal length and the anterior shift of the anterior gut thus were compensated by the elongation of the intestine (Figure 3). When the budding of the hepatic diverticulum occurred, the anterior gut region was reduced further even when the body length started growing again. The elongation of the intestine alone compensated this gut modification. A chronological change during metamorphosis of a single animal for a month is shown in Dataset Item 3 (Video) that has been produced from the montage images taken daily.

Contrary to the European lancelet, the Florida lancelet did not show a stop of growth or shrinkage of the body size during metamorphosis [10]. It is not known whether growth arrest found in the present study and pointed out in the European species depends on environmental factors such as temperature and food availability or growth arrest is a fixed character in some lancelet species. In the present culture method, the growth arrest continued for a month. Individual variations in the onset of metamorphosis exceed

TABLE 1: Number, Mean, and SD of body lengths (mm) of staged living individuals measured with objective micrometer on the date indicated, which were selected from pooled culture in a container.

Stages	Premetamorphosis 27/09/2011	M1 24/09/2011	M2 24/09/2011	M3 23/09/2011	Juvenile 8/10/2011	Juvenile 19/10/2011	Juvenile 29/10/2011	Juvenile 12/11/2011
Number	8	13	12	11	12	14	12	12
Mean	5.65	5.75	5.71	5.8	6.28	7.14	7.96	9.17
SD	0.18	0.22	0.19	0.18	0.29	0.33	0.42	0.56

M1: thickening of secondary gill series; M2: bisection of secondary gill pores; M3: pairing of gill slits.

this period [17], and cross-sectional random sampling may hide the growth arrest.

### 3. Dataset Description

The dataset associated with this Dataset Paper consists of 3 items which are described as follows.

*Dataset Item 1 (Table).* Body lengths (mm) of studied living individuals measured with objective micrometer and culture temperature ( $^{\circ}\text{C}$ ).

*Column 1:* Days of Culture

*Column 2:* Date

*Column 3:* Temperature ( $^{\circ}\text{C}$ )

*Column 4:* Individual Number 1 (mm)

*Column 5:* Individual Number 2 (mm)

*Column 6:* Individual Number 3 (mm)

*Column 7:* Individual Number 4 (mm)

*Column 8:* Individual Number 5 (mm)

*Dataset Item 2 (Table).* Body lengths (mm) of staged living individuals measured with objective micrometer on the date indicated which were selected from pooled culture in a container. In the table, M1 indicates thickening of secondary gill series; M2, bisection of secondary gill pores; and M3, pairing of gill slits.

*Column 1:* Premetamorphosis Stage on 27/09/2011 (mm)

*Column 2:* M1 Stage on 24/09/2011 (mm)

*Column 3:* M2 Stage on 24/09/2011 (mm)

*Column 4:* M3 Stage on 23/09/2011 (mm)

*Column 5:* Juvenile Stage on 8/10/2011 (mm)

*Column 6:* Juvenile Stage on 19/10/2011 (mm)

*Column 7:* Juvenile Stage on 29/10/2011 (mm)

*Column 8:* Juvenile Stage on 12/11/2011 (mm)

*Dataset Item 3 (Video).* Morphological change of Individual number 3 during metamorphosis from 17 September to 17 October. This video has been produced from 31 montage images taken one image every day by using Apple iMovie.

### 4. Concluding Remarks

Although the growth pattern during larval and postlarval periods in lancelets is heavily affected with environmental conditions [10, 17], our first longitudinal observation of lancelet metamorphosis has found features that seem likely to be relatively irrelevant to external condition. The duration of metamorphosis varies among individuals even under a constant culture condition. Growth was arrested until when paired gill slits started to increase in number (called the third gill formation), and the maximum reduction of the body length was observed when gill slits became paired. It is an open question that whether the growth arrest can depend on external factors or not. Lancelet metamorphosis is a long process compared with that of other marine invertebrates [14]. During this period, however, the oral transformation and the bisection of each gill pore took place within a few days. The reduction of pharyngeal and midgut length was solely compensated by elongation of the intestine, which may be related to digestive efficiency [24]. These characters suggest that internal requirements rather than predation pressures are important for the duration and mode of lancelet metamorphosis.

The lancelet body plan displays some contradicting features. Its streamlined shape with massive myomeres makes us imagine a very active life like that of predatory vertebrates. However, lancelets depend completely on filter feeding like sessile animals with some exceptional cases in larval life [20] and settled lancelets stay in sand substratum inactively throughout their life ([25] and our observations in the culture system). Although we do not know how their body form and life mode have been acquired historically, the lancelet lineage seems to have diverged from the common stock with vertebrates by the Early Cambrian [26]. The genomic study on the Florida lancelet gives a possibility that the lancelet body form has rather been stable since its divergence [27]. The most remarkable feature of the lancelet metamorphosis is the transformation from a left-right asymmetry to a symmetric appearance. As mitochondrial DNA sequence comparison suggests that the asymmetric feature is a primitive character in lancelets [28], it is interesting to know how and, if possible, when the lancelet ancestor acquired metamorphosis. Despite the fact that lancelets seem to have no effective strategies, predation pressures did not affect strongly the feature of the lancelet metamorphosis, which is an interesting contrast with other marine invertebrates. Lancelets thus may retain the original metamorphic processes and provide unique clues to study the evolution of metamorphosis.

## Dataset Availability

The dataset associated with this Dataset Paper is dedicated to the public domain using the CC0 waiver and is available at <http://dx.doi.org/10.7167/2013/839671/dataset>.

## Conflict of Interests

There is no conflict of interests in financial gain.

## Acknowledgments

The authors thank A. Maenaka of Hiroshima University and Y. Hayashida of Kumamoto University for maintaining the lancelet colony and also thank H. Shimasaki of Kumamoto University for boating to collect wild lancelets in the Amakusa Sea. Alga for feeding is being cultured at the Gene Science Division of the Natural Science Center for Basic Research and Development, Hiroshima University, with helps of I. Yamashita and C. Hikosaka.

## References

- [1] S. M. Degnan and B. M. Degnan, "The initiation of metamorphosis as an ancient polyphenic trait and its role in metazoan life-cycle evolution," *Philosophical Transactions of the Royal Society B*, vol. 365, no. 1540, pp. 641–651, 2010.
- [2] A. Kowalevsky, "Entwickelingsgeschichte des *Amphioxus lanceolatus*," *L'Académie Impériale des Sciences de St. Pétersburg*, vol. 11, no. 4, pp. 1–17, 1867.
- [3] E. R. Lankester and A. Willey, "The development of the atrial chamber of amphioxus," *Quarterly Journal of Microscopic Science*, vol. 31, no. 123, pp. 445–466, 1890.
- [4] A. Willey, "The later larval development of amphioxus," *Quarterly Journal of Microscopic Science*, vol. 32, no. 126, pp. 183–234, 1891.
- [5] J. W. van Wijhe, "On the metamorphosis of *Amphioxus lanceolatus*," *Koninkijke Akademie van Wetenschappen te Amsterdam*, vol. 16, pp. 574–583, 1914.
- [6] F. S. Chia, J. Buckland-Nicks, and C. M. Young, "Locomotion of marine invertebrate larvae: a review," *Canadian Journal of Zoology*, vol. 62, no. 7, pp. 1205–1222, 1984.
- [7] M. D. Stokes, "Larval locomotion of the lancelet *Branchiostoma floridae*," *Journal of Experimental Biology*, vol. 200, no. 11, pp. 1661–1680, 1997.
- [8] J. E. Webb, "The ecology of Lagos Lagoon. III. The life history of *Branchiostoma nigeriense* Webb," *Transactions of the Royal Society B*, vol. 241, no. 683, pp. 335–353, 1958.
- [9] J. H. Wickstead, "Chordata: acrania (Cephalochordata)," in *Reproduction of Marine Invertebrates*, A. C. Giese and J. S. Pearse, Eds., vol. 2 of *Entoprocts and Lesser Coelomates*, pp. 282–319, Academic Press, New York, NY, USA, 1975.
- [10] M. D. Stokes and N. D. Holland, "Embryos and larvae of a lancelet, *Branchiostoma floridae*, from hatching through metamorphosis: growth in the laboratory and external morphology," *Acta Zoologica*, vol. 76, no. 2, pp. 105–120, 1995.
- [11] M. Urata, N. Yamaguchi, Y. Henmi, and K. Yasui, "Larval development of the oriental lancelet, *Branchiostoma belcheri*, in laboratory mass culture," *Zoological Science*, vol. 24, no. 8, pp. 787–797, 2007.
- [12] G. Williams, *Adaptation and Natural Selection*, Princeton University Press, Princeton, NJ, USA, 1966.
- [13] L. Z. Holland et al., "The amphioxus genome illuminates vertebrate origins and cephalochordate biology," *Genome Research*, vol. 18, no. 7, pp. 1100–1111, 2008.
- [14] M. G. Hadfield, "Why and how marine-invertebrate larvae metamorphose so fast," *Seminars in Cell and Developmental Biology*, vol. 11, no. 6, pp. 437–443, 2000.
- [15] M. Paris, H. Escriva, M. Schubert et al., "Amphioxus postembryonic development reveals the homology of chordate metamorphosis," *Current Biology*, vol. 18, no. 11, pp. 825–830, 2008.
- [16] Q. J. Zhang, J. Zhong, S. H. Fang, and Y. Q. Wang, "*Branchiostoma japonicum* and *B. belcheri* are distinct lancelets (Cephalochordata) in Xiamen waters in China," *Zoological Science*, vol. 23, no. 6, pp. 573–579, 2006.
- [17] K. Yasui, M. Urata, N. Yamaguchi, H. Ueda, and Y. Henmi, "Laboratory culture of the oriental lancelet *Branchiostoma belcheri*," *Zoological Science*, vol. 24, no. 5, pp. 514–520, 2007.
- [18] M. D. Stokes and N. D. Holland, "Ciliary hovering in larval lancelets (= amphioxus)," *Biological Bulletin*, vol. 188, no. 3, pp. 231–233, 1995.
- [19] J. H. Wickstead and Q. Bone, "Ecology of acraniate larvae," *Nature*, vol. 184, no. 4702, pp. 1849–1851, 1959.
- [20] J. E. Webb, "On the feeding and behaviour of the larva of *Branchiostoma lanceolatum*," *Marine Biology*, vol. 3, no. 1, pp. 58–72, 1969.
- [21] P. T. Walsh, J. R. Downie, and P. Monaghan, "Plasticity of the duration of metamorphosis in the African clawed toad," *Journal of Zoology*, vol. 274, no. 2, pp. 143–149, 2008.
- [22] A. Willey, *Amphioxus and the Ancestor of the Vertebrates*, Macmillan, London, UK, 1894.
- [23] J. H. Wickstead, "*Branchiostoma lanceolatum* larvae: some experiments on the effect of thiouracil on metamorphosis," *Journal of the Marine Biological Association*, vol. 47, no. 1, pp. 49–59, 1967.
- [24] E. J. W. Barrington, "The digestive system of *Amphioxus (Branchiostoma) lanceolatum*," *Philosophical Transactions of the Royal Society B*, vol. 228, pp. 269–312, 1937.
- [25] H. Ueda and K. Sakaki, "Effects of turbation of the Japanese common lancelet *Branchiostoma japonicum* (Cephalochordata) on sediment condition: laboratory observation," *Plankton and Benthos Research*, vol. 2, no. 3, pp. 155–160, 2007.
- [26] D. G. Shu, H. L. Luo, S. Conway Morris et al., "Lower Cambrian vertebrates from south China," *Nature*, vol. 402, no. 6757, pp. 42–46, 1999.
- [27] N. H. Putnam, T. Butts, D. E. K. Ferrier et al., "The amphioxus genome and the evolution of the chordate karyotype," *Nature*, vol. 453, no. 7198, pp. 1064–1071, 2008.
- [28] T. Kon, M. Nohara, Y. Yamanoue, Y. Fujiwara, M. Nishida, and T. Nishikawa, "Phylogenetic position of a whale-fall lancelet (Cephalochordata) inferred from whole mitochondrial genome sequences," *BMC Evolutionary Biology*, vol. 7, article 127, 2007.



**Hindawi**

Submit your manuscripts at  
<http://www.hindawi.com>

