First description of developmental processes in *Sclerodactyla multipes* (Echinodermata: Holothuroidea: Dendrochirotida) from Misaki, Sagami Bay, Japan

**HISANORI KOHTSUKA**¹, KOHEI OGUCHI², YUSUKE YAMANA³ & MASANORI OKANISHI¹,*

¹Misaki Marine Biological Station, The University of Tokyo, 1024 Koajiro, Misaki, Miura, Kanagawa 238-0225, Japan
²National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan
³Wakayama Prefectural Museum of Natural History, 370-1 Funo, Kainan, Wakayama 642-0001, Japan

Received 15 September 2020; Accepted 30 April 2021 Responsible Editor: Shinji Shimode
doi: 10.3800/pbr.16.228

**Abstract:** More than 100 individuals of sea cucumber larvae were collected in the Japanese coastal sea of Moroiso, Sagami Bay, Kanagawa Prefecture, central-eastern Japan, in January 2018. Based on an obtained sequence of mitochondrial 16S rRNA gene region of one juvenile, it was identified as *Sclerodactyla multipes* by BLAST search with 0.3% genetic distance. The developmental process of the *S. multipes* was observed for three months, in which time, they grew from 250 µm to about 4 mm in length; here they showed distinct tentacles and dermal ossicles. Detailed morphological features of this species were described based on stereomicroscopic, fluorescence and SEM observations for the first time. This is the first description of life history through planktonic larva to juveniles in the family Sclerodactylidae.

**Key words:** Sea cucumber, 16S rRNA, SEM, fluorescence microscope, larvae

**Introduction**

Although embryological studies on sea cucumbers (Echinodermata: Holothuroidea) have been provided for several commercially useful species, such as *Apostichopus armata* (Selenka, 1867), *Athyonidium chilensis* (Semper, 1868), and *Isostichopus fuscus* (Ludwig, 1875) (Inaba & Maruyama 1988, Hamel et al. 2003, Guisado et al. 2012, Eguchi 2015, Huang et al. 2018), data for non-commercial species are inadequate (Inaba & Maruyama 1988). The order Dendrochirotida is a suspension-feeding sea cucumber world-wide distributed on the sea floor. The accumulated biological knowledge of this order is important for fisheries management and aquaculture of sea cucumbers, but its earliest life stages are scarcely studied (Gianasi et al. 2018). Recently, studies on the earliest life stages of 61 species in 32 genera of 5 families have been conducted, but only 15 species have been studied following the process of growth from larvae to juvenile (Table 1). The genus *Sclerodactyla* (Sclerodactylidae: Dendrochirotoda: Actinopoda) includes *Sclerodactyla briareus* (Lesueur, 1824) from the western Atlantic and *S. multipes* (Théel, 1886) from the western Pacific (Théel 1886, Hendler et al. 1995, Imaoka 1995, Kuramochi 2012). In these species, although a brief text-based description of development in *S. briareus* has been published (Oshihama 1925), no embryological study accompanied by photographs has been reported in this genus.

In this study, doliolaria and pentactula forms of the larvae of sea cucumbers were collected by towing plankton nets at the sea surface in Moroiso Bay, Miura Peninsula, Kanagawa Prefecture, Japan. We identified this species by DNA sequences based on 16S rRNA gene region, and documented the growth of its larvae using multiple observation methods.

**Methods**

One hundred and ten individuals were collected on 5 January 2018, using a plankton net (diameter 60 cm, mesh size 0.33 mm, net length 150 cm) towed at the sea surface for 5 min at Moroiso Inlet (35°09.405′N,139°36.345′E), Miura Peninsula, Kanagawa Prefecture (Fig. 1). Larvae of sea cucumbers were housed in a cylindrical...
Table 1. Summary of developmental studies of dendrochirotid sea cucumbers. “—” means no data.

| Order            | Family        | Species                        | Type of reproduction | Spawning | Unfertilized egg | Fertilized egg | Time and maximum size | Remarks                     | Reference                      |
|------------------|---------------|--------------------------------|----------------------|----------|------------------|-----------------|-----------------------|-----------------------------|--------------------------------|
| Dendrochirotida  | Asla lefevrei | (Barrois, 1882) | Lecithotrophic larva | Jan–May  | o                | O               | 400–650 µm           | 6 days, 700 µm              | Costelloe 1985, 1988, Thorsen 1946 |
| Cucumaria       |                |                                |                      |          |                  |                 |                       | Siluria                    |                                |
|                  |                |                                |                      |          |                  |                 |                       | 7 days, 689 µm              | Grisado et al. 2012           |
|                  | Athyonidium    | chilensis (Semper, 1868)       | Lecithotrophic larva | —        | O                | O               | 360 µm               | 5 days, 433 µm             | Deichmann 1922                |
|                  | Benthiophylophorus    | conchil-eugan (Pouartals, 1868) | Lecithotrophic larva | May      | —                | —               | —                     | —                           | Thomson 1878                  |
|                  | Cladodactyla    | crocea crocea (Lesson, 1830)  | Brooding             | Jan–Apr  | O                | 700 µm          | 9.5–10.75 days, 725 µm, Doliolaria | 18–21 days, 700–1,125 µm | McEuen 1987, 1988             |
|                  | Cucumaria      | falkis Ludwig, 1875           | Lecithotrophic larva | O        | O                | O               | 8 days, 1,500 µm, Vitellaria | 21 days, 689 µm             |                                |
|                  | falkis         | frontosa (Gunnerus, 1767)     | Lecithotrophic larva | Mar, June| O                | 900 µm          | 9–11 days, 1,300 µm | 46 days, 1,400 µm         | —                            | Gianasi et al. 2018, Hamel & Mercier 1996, So et al. 2011 |
|                  |               | georgiana (Lampert, 1886)     | Brooding             | —        | —                | 1,000 µm        |                       | —                           | Vaney 1925                     |
|                  |               | ijimai Ohshima, 1915          | Brooding             | ——       | 550 µm           | —               |                       | —                           | Hyman 1955, Ohshima 1915      |
|                  |               | japonica Semper, 1868         | Brooding             | Apr–Jun, Sep–Oct | O               | 500 µm          | —                     | —                           | Levin 1995                     |
|                  |               | johini Vaney, 1914           | Brooding             | —        | O                | 1,200 µm        | —                     | —                           | Hyman 1955, Vaney 1925        |
|                  |               | minia (Brandt, 1835)         | Lecithotrophic larva | O        | O                | 400–500 µm      | 3.5 days, 675–765 µm, Doliolaria | 29–30 days, 875–1,230 µm | —                            | McEuen 1987                   |
|                  |               | piperata (Stimpson, 1864)    | Lecithotrophic larva | O        | O                | 480–601 µm      | 3.5 days, 675–765 µm, Doliolaria | 22–25 days, 925–1,325 µm | —                            | McEuen 1987                   |
|                  |               | pseudosquarata Deichmann, 1938 | Brooding             | mid Dec–early Jan | O               | 916–1,237 µm      | 400–601 µm, Doliolaria | Just observed | Gonochoric. McEuen 1987, Rutherford 1977 |
|                  |               | vaneyi Cherbonnier, 1949      | Brooding             | —        | —                | —               | —                     | —                           | Hyman 1955                    |
|                  |               | pipet (Vaney, 1914)          | Brooding             | —        | —                | —               | —                     | —                           | Hyman 1955                    |
|                  |               | syracusanus (Grube, 1840)    | —                    | Nov–Dec  | —                | —               | —                     | —                           | Lo Bianco 1899               |
|                  |               | inbonecus incubans (Cherbonnier, 1972) | Brooding             | Apr      | 500 µm           | —               | —                     | —                           | Cherbonnier 1972              |
|                  |               | Leptopentacta panamica Deichmann, 1941 | Brooding             | —        | Just?            | —               | —                     | —                           | Deichmann 1941                |
|                  |               | Lissothuria antillensis Paxson, 1967 | Brooding             | all      | 400 µm           | —               | —                     | —                           | Miller 1985                   |
|                  |               | Neoamphicyclus luidus Hickman, 1962 | Brooding             | Dec      | 370 µm           | —               | —                     | —                           | Hickman 1978                  |
Table 1. Continued.

| Order          | Family                | Species                                             | Type of reproduction | Spawning Period | Unfertilized egg | Fertilized egg | Time and maximum size | Remarks                                      | Reference                      |
|----------------|-----------------------|-----------------------------------------------------|----------------------|-----------------|------------------|---------------------|--------------------------------------------|---------------------------------|
|                |                       |                                                     |                      |                 | Observation     | Observation Size   | Floating larva | Pentactula | Juvenile |                                      |                                |
| Ocnus glacialis (Ljungman, 1879) | Brooding            | — —                                              | — —                  | — —             | 700 µm           | —                   | —              | —          | —          | Mortensen 1894, Ludwig 1900, Hyman 1955, Vanney 1925 |                                |
| Ocnus plancti (Brandt, 1835) | Lecithotrophic larva | Mar–Apr                                           | — —                  | 500 µm          | —                | —                   | —              | —          | —          |                                      |                                |
| Paralepoptactula elongata (Düben & Koren, 1846) | Lecithotrophic larva | Jan                                               | —                   | —               | 250–350 µm       | Doliolaria         | Just observed 22–25 days, 925–1,325 µm | In vitro observation. | Chia & Buchanan 1969 |
| Pawsonia saxicola (Brady & Robertson, 1871) | Lecithotrophic larva | Mar–Apr                                           | — 500 µm           | —               | —                | —                   | —              | —          | —          | Orton 1914, Thorson 1946             |                                |
| Paralepoptactula elongata (Düben & Koren, 1846) | Brooding            | Nov–Dec                                           | —                   | 1,500 µm        | —                | —                   | —              | —          | —          | Hyman 1955, Simpson 1982           |                                |
| Pseudocnus grubii (von Marenzeller, 1874) | Brooding            | Nov–Dec                                           | —                   | —               | 300–400 µm       | —                   | —              | —          | —          | Lo Bianco 1899                      |                                |
| Pseudocnus lamperti (Ohshima, 1915) | Brooding            | Nov–Dec                                           | —                   | 877–1,087 µm    | —                | —                   | —              | —          | —          | Hermaphroditic. Hyman 1955, Ohshima 1915 |                                |
| Pseudocnus lubricus (Clark, 1882) | Lecithotrophic larva | June–Aug                                          | —                   | —               | —                | —                   | —              | —          | —          | The pentactula has 5 tentacles. McEuen 1987, Engstrom 1982 |                                |
| Pseudocnus macquariensis (Dendy, 1897) | Brooding            | May–June                                          | —                   | 1,800 µm        | —                | —                   | —              | —          | —          | Hermaphroditic. Simpson 1982        |                                |
| Pseudocnus costi (Vaney, 1908) | Brooding            | May–June                                          | —                   | —               | —                | —                   | —              | —          | —          | Hyman 1955                           |                                |
| Stereodermum imbricata (Ohshima, 1915) | Brooding            | May–June                                          | —                   | 1,200 µm        | —                | —                   | —              | —          | —          | Hyman 1955, Ohshima 1915            |                                |
| Trachythyone parva (Ludwig, 1875) | Brooding            | Nov–Dec                                           | —                   | 877–1,078 µm    | —                | —                   | —              | —          | —          | Hermaphroditic. Hyman 1955, Ohshima 1915 |                                |
| Phyllophoridae |                       |                                                     |                      |                 | —                | —                   | —              | —          | —          | McEuen 1987                          |                                |
| Pentamima chelis (Ludwig, 1886) | Brooding            | May–June                                          | —                   | 339–439 µm      | 2.25 days, 550 µm, Doliolaria | 6–7.5 days, 490–600 µm | 17 days? | —          | —          |                                |                                |
| Pentamima pulcherima (Ayers, 1852) | Lecithotrophic larva | May–June                                          | —                   | —                | —                | —                   | —              | —          | —          | McEuen 1988                          |                                |
| Phyllophorus (Phyllophorus) bernardianus (Grube, 1840) | Lecithotrophic larva | Winter                                            | —                   | 512–560 µm      | —                | —                   | —              | —          | —          | Hyman 1955, Ludwig 1898             |                                |
| Thyone benti (Deichmann, 1937) | Lecithotrophic larva | Winter                                            | —                   | —                | —                | —                   | —              | —          | —          | McEuen 1987                          |                                |
| Thyone inermis (Heller, 1868) | Brooding            | Oct–Nov                                           | —                   | —                | —                | —                   | —              | —          | —          | Lo Bianco 1899                      |                                |
| Psolidae |                       |                                                     |                      |                 | —                | —                   | —              | —          | —          | Wootton 1949                        |                                |
| Lissothira nutiens (Clark, 1901) | Brooding            | all                                               | —                   | 600 µm          | —                | —                   | —              | —          | —          | Hyman 1955                          |                                |
| Lissothira ornata Verrill, 1867 | Brooding            | —                                                | —                   | —                | —                | —                   | —              | —          | —          | Hyman 1955                          |                                |
| Psolidium incanum Ekman, 1925 | Brooding            | May–June                                          | —                   | 640 µm          | —                | —                   | —              | —          | —          | Hyman 1955, Ekman 1925              |                                |
Table 1. Continued.

| Order                  | Family            | Species                        | Type of reproduction | Spawning     | Unfertilized egg | Fertilized egg | Time and maximum size | Remarks                          | Reference                              |
|------------------------|-------------------|--------------------------------|----------------------|--------------|------------------|------------------|------------------------|------------------------------------|----------------------------------------|
|                        |                   |                                |                      |              |                  |                  |                        |                                    |                                        |
|                        |                   |                                |                      |              |                  |                  |                        |                                    |                                        |
|                        |                   |                                |                      |              |                  |                  |                        |                                    |                                        |
|                        |                   |                                |                      |              |                  |                  |                        |                                    |                                        |
| Psolium bullatum       | Ohshima, 1915     | Lecithotrophic larva           |                      |              | ○                | ○                | 293–354 μm             | 22 days, 670 μm                    | Mean Euen 1987, McEuen & Chia 1991     |
|                        |                   |                                |                      |              |                  |                  |                        |                                    |                                        |
| Polus antarcticus      | (Philippi, 1857)  | Brooding                       |                      |              | ○                | 700 μm           | 4 days, 600 μm, Doliolaria | 11–12 days, 730–805 μm             | Mean Euen 1987, Johnson & Johnstone 1950, Young & Chia 1982, McEuen & Chia 1991 |
|                        |                   |                                |                      |              |                  |                  |                        |                                    |                                        |
| Polus chitonoides      | Clark, 1901       | Lecithotrophic larva           | Mar–May              | ○            | ○                | 570–708 μm        | 5 days, 445 μm, Doliolaria | 26–32 days, 730–805 μm             | In vitro observation. Hyman 1955, Théel 1886 |
|                        |                   |                                |                      |              |                  |                  |                        |                                    |                                        |
| Polus ephippifer       | Thomson, 1876     | Brooding                       | Jan–Feb              | ○            | ○                |                  | 293–354 μm             | 4 days, 600 μm, Doliolaria | The settled juveniles survived for 50 weeks. Hyman 1955, Théel 1886 |
|                        |                   |                                |                      |              |                  |                  |                        |                                    |                                        |
| Polus figulus          | Ekman, 1925       | Brooding                       |                      |              | ○                | 640 μm           | 10–11 days, 445 μm Doliolaria | 7.75–8.5 days, 445 μm              | In vitro observation. Hyman 1955, Ekman 1925 |
|                        |                   |                                |                      |              |                  |                  |                        |                                    |                                        |
| Polus granulosus       | Vanay, 1906       | Brooding                       |                      |              | ○                | 500 μm           | 3 days, 445 μm Doliolaria | 2–15 days, 700 μm, 4,000 μm         | In vitro observation. Hyman 1955, Vaney 1925 |
|                        |                   |                                |                      |              |                  |                  |                        |                                    |                                        |
| Polus patagonicus      | Ekman, 1925       | Brooding                       | Feb–Mar              | ○            | ○                | 887 μm           | Just observed         | Just observed 7 months, 1,941 μm    | Brooding under the sole. Gimenez & Penchazadeh 2010 |
|                        |                   |                                |                      |              |                  |                  |                        |                                    |                                        |
| Polus lawrencei        | Martinez & Penchazadeh, 2017 | Brooding                       |                      |              | ○                |                  | 350 μm                | 10–11 days, 350 μm Vitrallaria | Hermaphroditic. Runnström & Runnström 1919, Thorson 1946, Pearse 1909 |
|                        |                   |                                |                      |              |                  |                  |                        |                                    |                                        |
| Polus punctatus        | Ekman, 1925       | Brooding                       |                      |              | ○                | 640 μm           | 2 days, Vitrallaria   | 10–11 days, 300 μm Vitrallaria    | Hermaphroditic. Runnström & Runnström 1919, Thorson 1946, Pearse 1909 |
|                        |                   |                                |                      |              |                  |                  |                        |                                    |                                        |
| Sclerodactyla briareus | Lesueur, 1824     | Lecithotrophic larva           |                      |              | ○                |                  | 2–15 days, 700 μm    | 15–194 days, 4,000 μm             | Hermaphroditic. Oshima 1925           |
|                        |                   |                                |                      |              |                  |                  |                        |                                    |                                        |
| Sclerodactyla multipes | (Théel, 1886)     | Lecithotrophic larva           | Jan?                 | ○            | ○                | 350 μm           | At least 1 day, 250 μm Doliolaria | 15–194 days, 4,000 μm             | Hermaphroditic. Oshima 1925           |
|                        |                   |                                |                      |              |                  |                  |                        |                                    |                                        |
| Ypsilothuriidae        |                   |                                |                      |              | ○                |                  | 2–15 days, 700 μm, 4,000 μm | 15–194 days, 4,000 μm             | This study                            |
|                        |                   |                                |                      |              |                  |                  |                        |                                    |                                        |

231

First description of developmental processes in Sclerodactyla multipes (Echinodermata: Holothuroidea:Dendrochirotida) from Misaki, Sagami Bay, Japan.
polystyrene water tank with a diameter of 300 mm and a height of 150 mm, and with a water volume of 10 L. The larvae were kept in a water bath with natural seawater. The water was changed every other day. Water temperatures were adjusted to that of the natural seawater, ranging from 11.8 °C to 24.0 °C, during 5 January 2018 to 17 July 2018 (194 days). The temperature of the surface seawater where the larvae were collected was 12 °C. Since this was the same as the water temperature in the laboratory, the larvae were reared in natural seawater. A small synchronous motor for agitating the water was attached to the tank (Fig. 2). The food provided for planktonic larvae of the sea cucumber was mainly the planktonic diatom Chaetoceros calcitrans (Paulsen) Takano, 1968, and after the larvae settled on the bottom, biological films collected in the intertidal zone of Moroiso Inlet at the laboratory were also provided as food.

For molecular analysis, DNA was extracted from the whole body of one individual (fixed in 99% ethanol) using the DNeasy Blood and Tissue extraction kit (Qiagen) according to manufacturer’s protocol. A partial sequence of the mitochondrial 16S rRNA gene region was amplified by PCR using primers employed by Palumbi (1996). PCR conditions followed Okanishi & Fujita (2013). The PCR products were separated from excess primers and oligonucleotides using Exo-SAP-IT (GE Healthcare), following the manufacturer’s protocol. All samples were sequenced bidirectionally and sequence products were run on a 3730xI DNA Analyzer (Thermo Fisher Scientific). The accession number of the sequence deposited in the DNA Data Bank of Japan (DDBJ) is LC577887.

Living larvae were photographed with a digital camera (OLYMPUS XZ7) mounted on a dissecting microscope. For fluorescence observations, samples were fixed with 4% Paraformaldehyde (PEA) for 30 minutes and washed with phosphate-buffered saline for 15 minutes × 3 times. The nuclei (DNA) and cytoskeletons (F-actin) were stained with 4′,6-diamidino-2-phenylindole (DAPI) (Sigma, St. Louis, MO, USA) and rhodamine-phalloidin (Invitrogen, Paisley, UK), respectively, for 1 h at room temperature, and then washed with Phosphate-buffered Saline + Tween 20 (PBT). After staining, samples were washed three times with PBT. The samples were observed with a fluorescence microscope BZ-9000E, KEYENCE, Japan. Image processing was done using the image analysis software BZ-II Analyzer, KEYENCE, Japan. For SEM observations, minute dermal ossicles were extracted by immersing the tissue in domestic liquid bleach (approximately 5% sodium hypochlorite solution) and dissolving the soft parts. After air-drying, the ossicles were fixed to SEM stubs with double-sided tape, their surfaces sputter-coated with platinum-palladium, and observed with a JSM 5200LV SEM.

Results

For molecular identification, we obtained 528 bp of a mitochondrial 16S rRNA gene region for the examined larva. A BLAST search of the sequence on the DDBJ website (http://blast.ddbj.nig.ac.jp/blastn?lang=ja) yielded Sclerodactyla multipes (accession no. MG586798) as the closest
First description of developmental processes in *Sclerodactyla multipes* (Echinodermata: Holothuroidea:Dendrochirotida) from Misaki, Sagami Bay, Japan.

Sequence with 0.3% K2P genetic distance.

The larvae of *S. multipes* examined in this study were about 250 $\mu$m in body length when collected (Fig. 3A–C). SEM observations showed that the cilia had already disappeared, and a pair of early primary podia (Fig. 3B) and five primary tentacles (Fig. 3C) had developed. Therefore, the larvae were identified as doliolaria larvae (late stage) because they were actively swimming, and had not yet

---

**Fig. 3.** Larval and juvenile development of *Sclerodactyla multipes*. A: doliolaria larva (dissecting microscope). B: doliolaria larva (SEM). The 5 primary tentacles and primary podia are emerging. (pt) primary tentacles. (ptf) primary tube feet. C: tentacles. * 5 tentacles. D: settled pentactula. (ten) tentacles. (tf) tube feet. E–H: pentactula. same individual. E: stereo microscope image. F: SEM. G: fluorescence microscope image (blue: DAPI, red: phalloidin). H: light microscope image. (oss) ossicle. I: 15-day pentactula. J: ossicle of 15-day pentactula. K: 30-day juvenile. L: 60-day juvenile. M: ossicles of 60-day juvenile. overlap in a tiled manner. N: 90-day juvenile. O: ossicles of 90-day juvenile also appears inside podia. P: 150-day juvenile. Scale bars: A–B, D, 50 $\mu$m; E–I, 100 $\mu$m; K, L, N, P, 1 mm.
settled to the seafloor. On the second day after collection (d.a.c.), the larvae became pentaculæ (Fig. 3D–H). The length of the body was 300 to 350 µm, and the body was equipped with one layer of reticulated micro-ossicles. One primary podia and five primary tentacles were extended and the larvae began an active benthic life (Fig. 3D, E, G). At this stage, phalloidin signals (i.e., well-developed actin filaments) were observed in the podia and primary tentacles (Fig. 3G). On the 15th d.a.c., the body length was over 700 µm and some branching was observed on the five primary tentacles (Fig. 3I). The ossicles in the body were flatter and more prominent (Fig. 3J). By the 30th d.a.c., the body length reached a body length of 3.5 mm (Fig. 3N). The dorsal ossicles were single-layered and were scattered. The tentacles began to have distinct ossicles (Fig. 3O). Despite continuous observation, no significant growth of the ossicles was observed. At the 150th d.a.c., the body length exceeded 4 mm (Fig. 3P). The podia became longer and increased in number (16 on ventral and 18 on dorsal side). Although these individuals were kept alive for 194 days, we could not identify them to species using morphological criteria.

**Discussion**

Although the larvae could not be reared to the adult stage in this study, molecular analysis showed that the genetic distance between larvae and *Sclerodactyla multipes* (MG586798) was 0.3%. This falls within the range of intraspecific differences (less than 0.5%) for 16S in holothurians (e.g., Kim et al. 2013, Vergara et al. 2018). Because the morphology of the *S. multipes* recorded in Genbank has not been reported, the possibility of misidentification cannot be ruled out. However, it is reasonable to assume that our larvae were indeed *S. multipes* as registered in Genbank (MG586798, sampling locality unidentified).

Currently, *Sclerodactyla briareus* and *S. multipes*, from the western Atlantic and western Pacific respectively (Théel 1886, Hendler et al. 1995, Imaoka 1995, Kuramochi 2012) are known in this genus. *S. multipes* has been recorded from Yokohama in Tokyo Bay and Monbetsu in Hokkaido (Imaoka 1995), and this study is the first to report this species from the vicinity of Misaki in Sagami Bay, assuming that the identification according to Genbank is correct.

The only known embryological record of this genus is a brief text-based study for *S. briareus* (Lesueur 1824) which documented that this species is lecithotrophic without a planktonic larval stage (Oshima 1925). Referring to that study, McEdward & Miner (2001) speculate that this species has pelagic, lecithotrophic and direct development. This study is the first documentation of the growth process of larvae of *S. multipes*.

In this study, larvae of *S. multipes* were successfully reared for approximately 200 days and grown to a maximum size of 4 mm in length. Regarding their growth rates, the body lengths reach about 700 µm in 15 days, 1 mm in 30 days, 2 mm in 60 days, and 3.5 mm in 90 days. When compared to other species within the order Dendrochirotida, these values are similar to other species with lecithotrophic larvae, which reached about 700 µm to 1 mm in 22–45 days, i.e., *Athyoniidium chilensis* (Semper, 1868), *Cucumaria falax* Ludwig, 1875, *Cucumaria frondosa* (Gunnerus, 1767), *Cucumaria miniata* (Brandt, 1835), *Cucumaria piperata* (Stimpson, 1864), *Paraleptopentacta elongata* (Düben & Koren, 1846), *Psolidium bullatum* Oshima, 1915, and *Psolus chitonoides* Clark, 1901 (Table 1).

In *Apostichopus japonicus* (Selenka, 1867) of the order Aspidochirotirotida, the larvae grow to 4 mm in length after 60 days of rearing (Inaba & Maruyama 1988), suggesting that the growth of the order Dendrochirotirotida is relatively slow. This might correlate to the ecological difference between Aspidochirotirotida (deposit-feeding) and Dendrochirotirotida (suspension-feeding) (Massin 1982).

*Sclerodactyla briareus* has a larval stage that is an egg yolk-nutrient lecithotrophic form (Oshima 1925), while the current species *S. multipes*, has a different developmental pattern with respect to its planktonic larva stage. However, with regard to the reproduction types of the 61 dendrochirotid species, including *S. multipes*, lecithotrophic larvae (17 species) and brooding (33 species) predominate, and these types occur in a polyphyletic manner within in each lineage (Table 1). Thus, the reproduction types are highly plastic within this order, and it is not surprising that the types were different even within the genus *Sclerodactyla*. In this study, we were unable to directly observe the exact spawning date of *S. multipes*, but we assume that the spawning occurred probably in early January, which was near the collection date of the floating larvae. When compared with the 30 dendrochirotid species for which the spawning periods are known, there is no similarity between the spawning periods and lineages. Assuming spawning of *S. multipes* occurs in January, this differs from other species of the family Sclerodactylidae (Table 1).

The lack of knowledge on the development of sea cucumbers, especially in the order Dendrochirotida, is probably due to difficulty in encouraging spawning, and to the difficulty in rearing larvae. Additionally, the lack of readily visible taxonomic characters in larvae makes it difficult to identify the species. In the order Dendrochirotida, only 13 species have been described through the planktonic larval stage to juveniles, and in the family Sclerodactylidae, planktonic larva to doliolaria of *Eupentacta fraudatrix* (Dyakonov & Baranova, 1958) has been observed, but the juveniles of sclerodactylids have never been observed (Ta-
ble 1). In this study, we reared large numbers of the larvae of \textit{S. briaereus}, obtained from the plankton and we identified the species based on sequences of the 16S mitochondrial DNA region, rearing them for a long period of time in a waterfont laboratory to understand their developmental process. This is the first description of life history through planktonic larva to juveniles in the family Sclerodactylidae.

Acknowledgements

We thank Dr. David L. Pawson of the National Museum of Natural History, Smithsonian Institution, and Mr. James Scott Walker for their careful and critical reading of the manuscript. We take this opportunity to express our gratitude to Michiyoshi Kawabata (MMBS), for her cooperation in sampling and larval management, Dr. Toru Miura for his assistance in using the SEM and fluorescence microscopes, and Dr. So Ishida of the Osaka City Museum of Natural History, Osaka, Japan, for registration of the examined specimens.

References

Chaffee C (1982) Birth by the viviparous holothroid \textit{Pachychone rubra} (Clark, 1901). In: \textit{International Echinoderm Conference}, Tampa Bay (ed Lawrence JM). A. A. Balkema, Rotterdam, pp. 465–466. 

Cerbonnier G (1972) \textit{Neocnus incubans}, nouveau genre et nouvelle espèce d’holothurie dendrochirote incubatrice de Méditerranée. C R Acad Scie Série D 275: 225–227.

Chia FS, Buchanan JB (1969) Larval development of \textit{Cucumaria}. J Mar Biol Assoc UK 49: 151–159.

Clark HL (1946) The echinoderm fauna of Australia. Its composition and its origin. Publ Carnegie Inst 566: 1–567.

Colwin LH, Colwin AL (1956) The acrosome filament and sperm entry in \textit{Thyone briaereus} (Holothuria) and \textit{Asterias}. Biol Bull 110: 243–257.

Costelloe J (1985) The annual reproductive cycle of the holothurian \textit{Aslia lefevrei} (Dendrochirotida: Echinodermata). Mar Biol 88: 155–165.

Costelloe J (1988) Reproductive cycle, development and recruitment of two geographically separated populations of the dendrochirote holothurian \textit{Aslia lefevrei}. Mar Biol 99: 535–545.

Deichmann E (1941) The Holothuroidea collected by the Velero

Deichmann E (1922) On some cases of multiplication by fission in holothurians. Vid Medd Dansk Naturh Inst Oceanogr 439: 73–84.

Engstrom NA (1982) Brooding behavior and reproductive biology of a subtidal Puget Sound sea cucumber, \textit{Cucumaria lubrica} (Clark, 1901) (Echinodermata: Holothuroidea) In: \textit{International Echinoderms Conference}, Tampa Bay (ed Lawrence JM). A.A. Balkema, Rotterdam, pp. 447–450.

Gianasi BL, Hamel J-F, Mercier A (2018) Morphometric and behavioural changes in the early life stages of the sea cucumber \textit{Cucumaria frondosa}. Aquaculture 490: 5–18.

Hamel JF, Mercier A (1996) Early development, settlement, growth and spatial distribution of the sea cucumber \textit{Cucumaria frondosa} (Echinodermata: Holothuroidea). Can J Fish Aquat Sci 53: 253–271.

Hamel JF, Ycaza-Hidalgo R, Mercier A (2003) Larval development and juvenile growth of the Galápagos sea cucumber \textit{Isostichopus fuscus}. SPC Bec Inf Bull 18: 3–8.

Hendler G, Miller JE, Pawson DL, Kier PM (1995) Sea stars, sea urchins and allies: Echinoderms of Florida and the Caribbean. Smithsonian Press, Washington, 390 pp.

Hickman VV (1978) Notes on three species of Tasmanian sea cucumbers including one species that broods its young in the coelome (Holothuroidea: Phyllophoridae, Caudinidae). Pap Proc R Soc Tasmania 112: 29–37.

Huang W, Luo D, Yu ZH, Ren CH, Jiang X, Luo P, Chen T, Hu CQ (2018) Spawning, larval development and juvenile growth of the tropical sea cucumber \textit{Holothuria leucospilota}. Aquaculture 488: 22–29.

Hyman LH (1955) The Invertebrates, vol. 4. Echinodermata. McGraw-Hill, New York, 763 pp.

Iwaoka T (1995) Holothuroidea. In: \textit{Guide to Seashore Animals of Japan with Color Pictures and Keys}, vol. II (ed Nishimura S). Hoikusha, Osaka, pp. 553–572. (In Japanese)

Inaba D, Maruyama Y (1988) Holothuroidea. In: \textit{Invertebrate Embryology (last volume)} (eds Dan K, Sekiguchi K, Ando I, S). Hoikusha, Osaka, pp. 553–572. (In Japanese)

Johnson MW, Johnson LT (1950) Early life history and larval development of some Puget Sound echinoderms. Contr Scripps Inst Oceanogr 498: 73–84.

Kim SW, Kerr AM, Pauley G (2013) Colour, confusion, and crossing: resolution of species problems in \textit{Bohadschia} (Echinodermata: Holothuroidea). Zool J Linn Soc 168: 81–97.

Kuramochi T (2012) Taxonomy and Morphology. In: \textit{Holothurian-Biology, Aquaculture and Culture} (eds Takahashi A, Okumura S). Seizando, Tokyo, pp. 1–17. (In Japanese)

Lesueur CA (1824) Description of several new Species of \textit{Holothuria}. J Acad Nat Sci Phila 4: 155–163.

Levin V (1995) Japanese sea cucumber \textit{Cucumaria japonica} in the far eastern seas of Russia. SPC Bec Inf Bull 7: 18–19.

Lo Bianco S (1899) Notizie biologiche riguardanti specialmente il periodo di maturità sessuale degli animali del golfo di Na-
