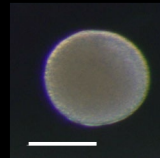

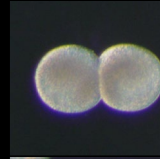


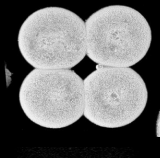
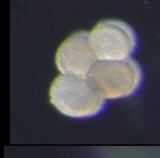



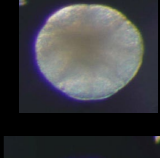
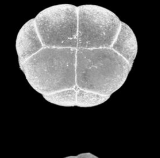
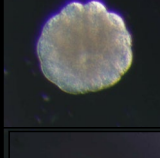
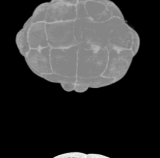
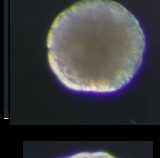
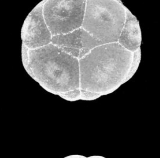
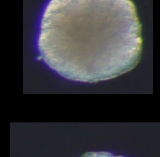

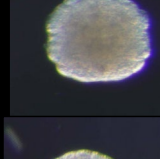
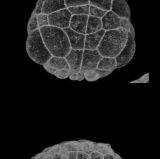
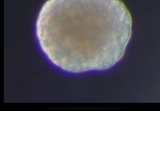

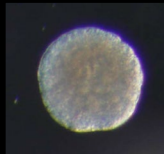
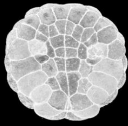
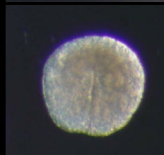
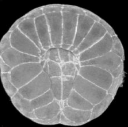
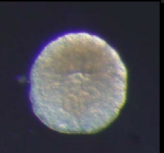
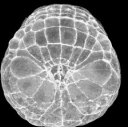
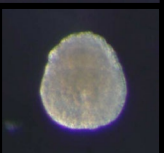


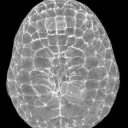
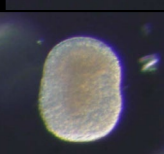

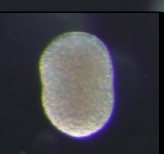



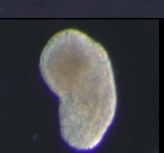







Developmental Table of *Ciona* (Hotta et al., 2017)




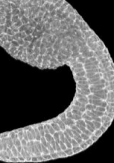

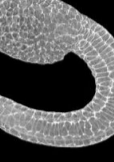

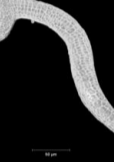
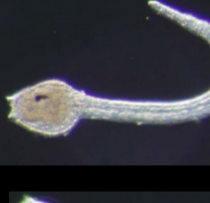

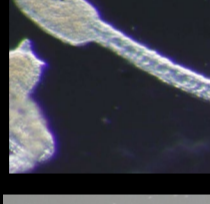
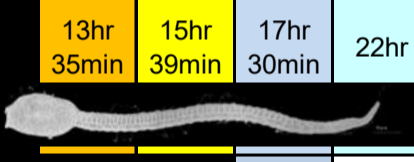


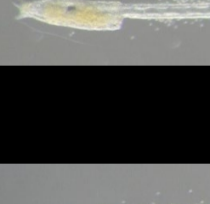

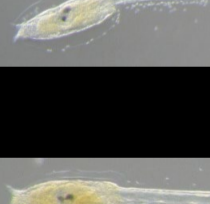
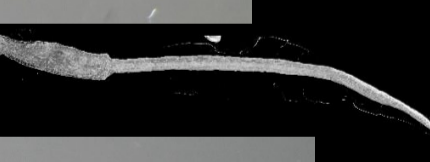



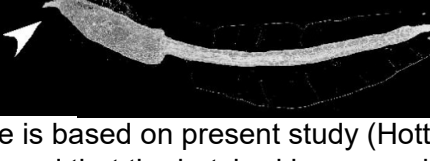
Periods of embryogenesis	Stage Name		Normal Image	Confocal 3D image	22°C	20°C	18°C	16°C	Description
	Stage	Cell Count							
I. Zygote period	St. 1	1 cell			0min	0min	0min	0min	Zygote, fertilized egg
II. Cleavage period	St. 2	2 cell			1hr	1hr	1hr	75min	Two cell-stage embryo
	St. 3	4 cell			84min	87min	87min	1hr 40min	Four cell-stage embryo
	St. 4	8 cell			1hr 39min	1hr 51min	1hr 54min	2hr 15min	Eight cell-stage embryo
	St. 5a	early 16 cell			2hr 3min	2hr 15min	2hr 21min	2hr 39min	Early sixteen-cell stage embryo
	St. 5b	late 16 cell			2hr 16min	2hr 33min	2hr 39min	3hr	Late sixteen-cell stage embryo
	St. 6-a	early 32 cell			2hr 36min	2hr 47min	3hr 6min	3hr 27min	Early thirty two-cell stage embryo
	St. 6-b	late 32 cell			2hr 45min	2hr 57min	3hr 12min	3hr 39min	Late thirty two-cell stage embryo
	St. 7	44cell				3hr 9min	3hr 21min		Fourty four-cell stage embryo. The vegetal side of the embryo is very round.
	St. 8	64 cell			3hr 27min		4hr	4hr 27min	Sixty four-cell stage embryo. Embryo has a square shape seen form the top, with bulging B7.4 cells.
	St. 9	76 cell			3hr 51min		4hr 12min	4hr 39min	Seventy six cell stage embryo. The vegetal side of the embryo is very flat

Developmental Table of *Ciona* (Hotta et al., 2017)

Periods of embryogenesis	Stage Name		Normal Image	Confocal 3D image	22°C	20°C	18°C	16°C	Description
III. Gastrula Period	St. 10	110 cell, initial gastrula			3hr 57min	4hr 9min	4hr 33min	5hr 11min	Gastrulation starts with the apical constriction of A7.1 blastomeres.
	St. 11	early gastrula			4hr 9min	4hr 21min	4hr 54min	5hr 27min	The notochord has invaginated. The vegetal side of the embryo has a horseshoe shape.
	St. 12	mid gastrula			4hr 33min	5hr 9min	5hr 39min	6hr 28min	Six-row neural plate stage. The blastopore is still central and open.
	St. 13	late gastrula			4hr 57min		5hr 55min	7hr 3min	The blastopore is in posterior position and nearly closed. The embryo elongates anteriorly. The neural plate has more than 6 rows and the A-line neural rows (I and II) start to curve (neurulation begin). The large b6.5 progeny are coming together at the midline.
IV. Neurula Period	St. 14	early neurula			5hr 31min	6hr 3min	6hr 21min	7hr 39min	A-line neural plate forms a gutter lined by b6.5 descendants. The embryo has a diamond shape. The gutter is not closed.
	St. 15	mid neurula			6hr 4min	6hr 27min	6hr 48min	8hr 35min	The neural tube has formed on most of its length. The embryo has an oval shape. The a-line neural plate also forms a gutter.
	St. 16	late neurula			6hr 25min	7hr 3min	7hr 24min	9hr 51min	The neural tube starts to form in the posterior territories. The embryo elongates.
V. Tailbud Period	St. 17	initial tailbud I			6hr 52min	7hr 55min	8hr 27min	10hr 27min	First indication of a separation between tail and trunk territories. The tail is not bent and has the same length as the trunk. Any notochord cells not finished intercalation. Tail/Trunk=1.0
	St. 18	initial tailbud II			7hr 31min	8hr 27min	8hr 50min	10hr 51min	The tail is clearly separated from the trunk. Tail and trunk have same length. Neuropore still open, a-line neurulation. Tail/Trunk=1.1
	St. 19	early tailbud I			7hr 40min	8hr 45min	9hr 19min	11hr 15min	The tail bends about 40° and is slightly longer than the trunk. A few anterior most notochord cells begin to intercalate and linear. Tail/Trunk=1.2
	St. 20	early tailbud II			8hr 6min	9hr 3min	9hr 30min	11hr 39min	Neuropore closed, tail bent by 60°, neurulation complete. Tail/Trunk=1.3

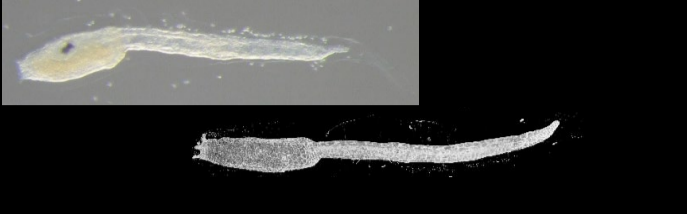
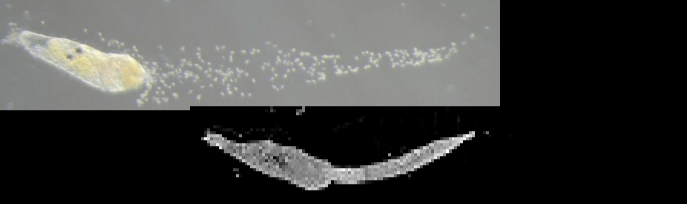
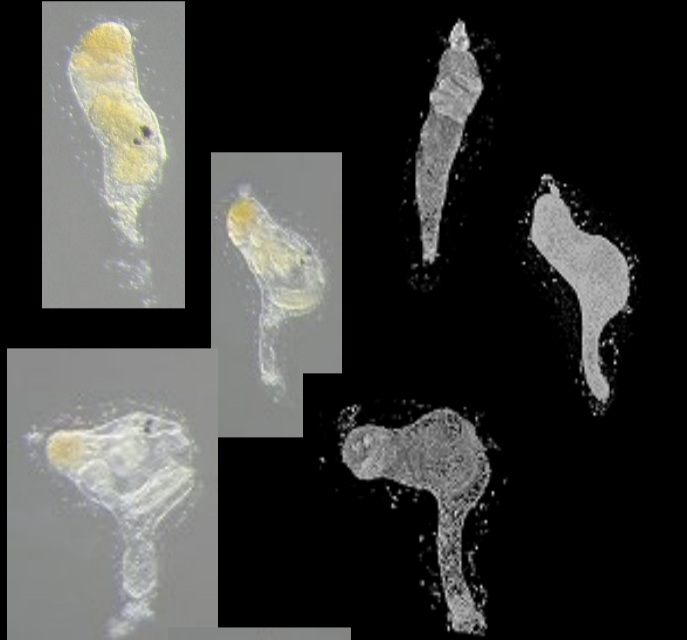
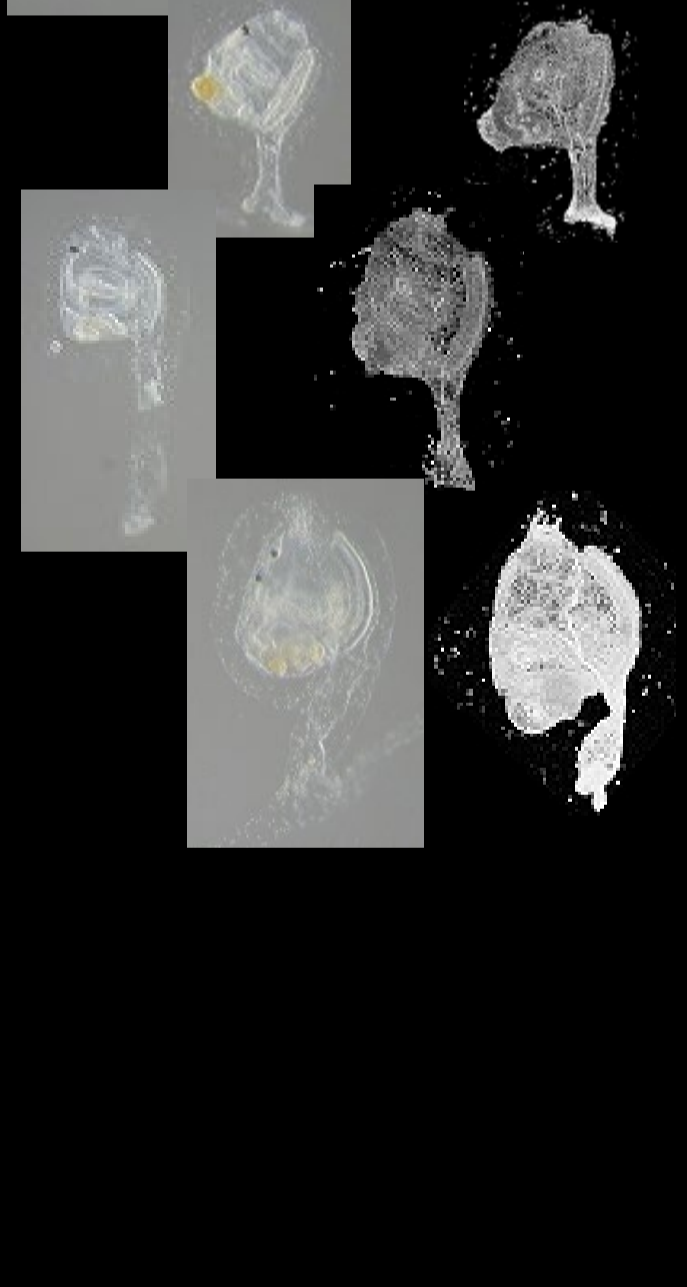
The developmental table from stage 1 to stage 26 is based on Hotta et al., Dev Dyn. 2007.

Developmental Table of *Ciona* (Hotta et al., 2007; Hotta et al., 2020)

Periods of embryogenesis	Stage Name		Normal Image	Confocal 3D image	22°C	20°C	18°C	16°C	Description
continued V. Tailbud Period	St. 21	mid tailbud I			8hr 46min	9hr 33min	10hr 2min	12hr 15min	Tail 1 1/2 times longer than trunk and curve ventrally (90°). Intercalation of notochord cells just finished. Tail/Trunk=1.6
	St. 22	mid tailbud II			9hr 29min	9hr 57min	10hr 54min	13hr 3min	The body adopts a half circle shape. Tail twice as long as trunk. Tail/Trunk=1.9
	St. 23	late tailbud I			10hr 11min	10hr 45min	11hr 54min	14hr	Initiation of the pigmentation of the otolith. Tail strongly curved with tip close to the anterior end of the trunk. Tail/Trunk=2.1
	St. 24	late tailbud II			11hr 23min	12hr 4min	13hr 27min	16hr 27min	Notochord vacuolation begins, palps start to be visible at the front end of the embryo. Tail straightens. Tail/Trunk=3.4
	St. 25	late tailbud III			12hr 23min	14hr 5min	15hr 54min	19hr 3min	Ocellus melanization. All notochord cells have vacuoles. Tail bent dorsally. Tail/Trunk=3.9
VI. Larva Period	St. 26	hatching larva			13hr 35min	15hr 39min	17hr 30min	22hr	Hatching, spherical trunk shape, immature papillae with pyramidal shape, irregular tail movements
	St. 27	early swimming larva					17.5- 20 hr		Spindle-like trunk shape, regular tail movements and swimming behaviour
	St. 28	mid swimming larva					20-22 hr		Elongated papillae and expansion of their basal part, squared trunk, spherical test cells, cilia in epidermal sensory neurons recognizable, preoral lobe recognizable
	St. 29	late swimming larva					22-24 hr		Longer and narrower head with respect to St. 28, trunk profile squared at transition between trunk and tail
VII. Adhesion Period	St. 30	adhesion					24-27 hr		Curved papillae, otolith and ocellus remnants recognizable
VIII. Tail absorption Period	St. 31	early tail absorption					27 hr		Beginning of tail absorption, tail bending at the transition between trunk and tail, otolith and ocellus remnants recognizable.

From stage 27 to stage 37, developmental table is based on present study (Hotta et al., 2020). The duration of larval swimming differs among individuals. Matsunobu et al. (2015) showed that the hatched larva requires at least three or four hours to get competence to commence metamorphosis. So the time after fertilization after Larva Period was broad.

Developmental Table of *Ciona* (Hotta et al., 2020)

Periods of embryogenesis	Stage Name		Normal Image / Confocal 3D image	18°C	Description
(continued) VIII. Tail absorption Period	St. 32	mid tail absorption		28 hr	50% of tail absorbed into trunk; tail shrunk and thickened, otolith and ocellus remnants recognizable
	St. 33	late tail absorption		29 hr	Tail completely resorbed, papillae no more recognizable, otolith and ocellus remnants recognizable
IX. Body axis rotation Period	St. 34	early body axis rotation		30-36 hr	Beginning of body axis rotation (angle between the stalk and the endostyle more than 0°), outer tunic compartment and outer cuticle layer no more present, tunic cells recognizable in definitive tunic, otolith and ocellus remnants recognizable.
	St. 35	mid body axis rotation		36-45 hr	Body axis rotation of about 30°-60°, one pair of gill-slit recognizable, otolith and ocellus remnants recognizable
	St. 36	late body axis rotation		45-60 hr	Two pairs of gill-slit open, body axis rotation of about 80°-90°, filtering and feeding activity present, otolith and ocellus remnants recognizable, heart beating
X. Juvenile Period	St. 37	early juvenile I		60-72 hr	Body axis rotation completed, stomach swollen, otolith and ocellus remnants recognizable
	St. 38	mid juvenile I		72-96 hr (3-4 days)	Larval tail remnants totally adsorbed
	St. 39	late juvenile I		96-144 hr (4-6 days)	Additional gill slit begin to open, appearance of stomach, gut and neural gland
	St. 40	early juvenile II		7dpf	Gonad in form of oval vesicle (corresponding to Stage 6 in Chiba et. al., 2004)
	St. 41	mid juvenile II		after 7dpf	Atrial siphon begins to fuse (corresponding to Stage 7 in Chiba et. al., 2004)

Please notify Kohji Hotta (khotta@bio.keio.ac.jp), if you find any incorrect information. From stage 27 to stage 37, developmental table is based on present study (Hotta et al., 2020).