Title

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Distinction of Two Morphotypes of *Turritopsis nutricula* Medusae (Cnidaria, Hydrozoa, Anthomedusae) in Japan, with Reference to their Different Abilities to Revert to the Hydroid Stage and their Distinct Geographical Distributions

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Abstract. By field sampling and laboratory rearing during 1976–2003, *Turritopsis nutricula* has been collected from almost the entire coast of Japan, from Okinawa Prefecture to Hokkaido, including offshore islands such as the Nansei Is., Tsushima I., and the Ogasawara Is. Combining all previous records of this species in Japanese waters since 1909 with recent records and observations, two morphotypes of medusae can be distinguished by umbrellar size and the number and arrangement of tentacles. The previously known type, here termed the “large type”, has many tentacles, up to 196, in two or more rows. It is distributed in northern Japan (Hokkaido and N Honshu) and in the Seto Inland Sea. The “small type”, a newly recognized morphotype in the present paper, has fewer than 82 tentacles in only one row and occurs in southern Japan (S Honshu, Kyushu, and the Nansei Islands). Life history studies have demonstrated rejuvenation in both morphotypes, although not as complete as the process in Mediterranean specimens, which are morphologically similar to the small type. In Japanese waters, the small type can revert to the hydroid more frequently than the large type. Rejuvenation of the small type over two generations was observed for the first time, and indefinite rejuvenation may be possible under optimal culture conditions.

Key words: *Turritopsis nutricula*, medusa, hydroid, life cycle, morphotypes, geographical distribution, repeated rejuvenation, Japan.

Introduction

Medusae of the genus *Turritopsis* have been known from Japan since Maas (1909) described them for the first time. They are principally known from northern and central Japan, and Uchida (1927b) referred them to *Turritopsis nutricula* McCrady, 1857. The hydroid of this species has also been reported a few times in Japanese waters, ranging from Kyushu to Hokkaido (Hirohito, 1969, 1988; Yamada and Nagao, 1971).

Recent systematic studies on this species conducted by me, predominantly in southern Japan, have revealed that mature or subadult medusae belong to two morphotypes. In the present report, the differences in the morphology and geographical distribution (Kubota, 2005a, b) of these two forms are documented, including new observations and a review of all previous reports on this genus in Japan. In addition, the ability of each morphotype to revert to the young hydroid stage was assessed. Life cycle reversion, first reported by Bavestrello et al. (1992) based on the Italian population, is a unique feature of at least some *Turritopsis* populations, and the animal is therefore potentially immortal together with another medusa, *Laodicea undulata* (Forbes and Goodsir, 1851) which has recently noted as the second species of immortal animal (Piraino et al., 2004;
Shin Kubota

De Vito et al., in preparation; Kubota, unpublished data).

Materials and Methods

From 1976 to 2003 many specimens of Turritopsis nutricula were examined in the living state soon after collection. Many medusae were collected by towing a plankton net (30 cm in diameter, 55 cm long, 334 μm in mesh size) in harbors, ports, and bays from Hokkaido in the north to various islands of Okinawa Prefecture in the south (Fig. 1; Appendix).

Medusae from Ago Bay, Mie Prefecture, were cultured in a 60 cc polystyrene container in the laboratory in Sapporo, Hokkaido, and all other cultured specimens, including cultured hydroids, were kept at Shirahama, Wakayama Prefecture. Cultures were kept in daily changes of filtered natural seawater at about 22°C and fed with newly hatched Artemia nauplii. Mature medusae were sometimes cultured until they degenerated, and morphological changes were observed under a binocular microscope. Rejuvenated hydroids reverted from medusae were also cultured similarly at Shirahama, but some were kept in running seawater in the laboratory and fed with Artemia nauplii and/or natural food arriving with the seawater. Observations on rejuvenation were repeated as many times as possible.

Results and Discussion

(1) Collection records of medusae and hydroids in Japan

For medusae of Turritopsis nutricula, new locality records and additional collection records from previously known localities are mapped in Fig. 1 and listed in the Appendix along with all relevant

Table 1. Records of mature or subadult medusae, as well as youngest-stage medusae, of Turritopsis nutricula in Japan, obtained in the sea or in the laboratory by culture.

<table>
<thead>
<tr>
<th>Locality (Site no. in Fig. 1)</th>
<th>Collected month and year</th>
<th>Developmental stage</th>
<th>Umbrellar diameter/height (mm)</th>
<th>No. of tentacles and rows</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akkeshi (1)</td>
<td>IX-1962</td>
<td>mature*</td>
<td>6.5-7.5/6.5-7.5*</td>
<td>125-187 (-)*</td>
<td>Yamada &amp; Nagao, 1971</td>
</tr>
<tr>
<td>Akkeshi (1)</td>
<td>VII-1970</td>
<td>youngest**</td>
<td>1.1-1.2/1.2-1.3**</td>
<td>12 (-)*</td>
<td>Yamada &amp; Nagao, 1971</td>
</tr>
<tr>
<td>Akkeshi (1)</td>
<td>VII-1976</td>
<td>youngest**</td>
<td>-</td>
<td>11-12 (1)</td>
<td>Present study</td>
</tr>
<tr>
<td>Oshoro (2)</td>
<td>V1</td>
<td>adult*</td>
<td>3.5/4*</td>
<td>80&lt; (2)*</td>
<td>Uchida, 1925</td>
</tr>
<tr>
<td>Oshoro (2)</td>
<td>1923-1927</td>
<td>adult*</td>
<td>-8/-9*</td>
<td>120 (2)*</td>
<td>Uchida, 1927b</td>
</tr>
<tr>
<td>Iwaki (6)</td>
<td>VII-2002</td>
<td>subadult*</td>
<td>7.0/6.8***</td>
<td>196 (2)**</td>
<td>Kubota &amp; Mizutani, 2003</td>
</tr>
<tr>
<td>Misaki (9)</td>
<td>X-1904</td>
<td>mature*</td>
<td>-5/-9*</td>
<td>120-150 (several)*</td>
<td>Maas, 1909</td>
</tr>
<tr>
<td>Hayama (near 8/9)</td>
<td>-</td>
<td>youngest**</td>
<td>0.6/0.7**</td>
<td>10-12 (1)**</td>
<td>Hirohito, 1988</td>
</tr>
<tr>
<td>Ago Bay (13)</td>
<td>IX-1984</td>
<td>female**17</td>
<td>2.9/-</td>
<td>41 (1)*</td>
<td>Present study</td>
</tr>
<tr>
<td>Tanabe Bay (14)</td>
<td>IX-2001, VII-2003</td>
<td>female*</td>
<td>2.5-2.9/-</td>
<td>28-37 (1)*</td>
<td>Present study</td>
</tr>
<tr>
<td>Kaga, Oshima I. (16)</td>
<td>VII-2002</td>
<td>female*</td>
<td>-4/-</td>
<td>99 (2)*</td>
<td>Kubota et al., 2003</td>
</tr>
<tr>
<td>Kagoshima (23)</td>
<td>1906</td>
<td>female*</td>
<td>-4/-</td>
<td>89-91 (2)*</td>
<td>Bigelow, 1913</td>
</tr>
<tr>
<td>Kagoshima (22)</td>
<td>XI-2002</td>
<td>subadult*</td>
<td>4.4/4.1***</td>
<td>82 (1)**</td>
<td>Chikuchi-shin &amp; Kubota, 2003b</td>
</tr>
<tr>
<td>Nishinoomote, Tanegashima I. (25)</td>
<td>VIII-2002</td>
<td>youngest**</td>
<td>0.50-0.68/-</td>
<td>8-12 (1)**</td>
<td>Present study</td>
</tr>
<tr>
<td>Tomari Port, Okinawa I. (30)</td>
<td>III-2003</td>
<td>male*20</td>
<td>2.0/-</td>
<td>23 (1)*</td>
<td>Present study</td>
</tr>
</tbody>
</table>

*: not described/no data.
*: specimens collected in the sea.
#: specimens matured in culture, and their ages in days after collection.
*: specimens released in the laboratory from hydroids collected in the sea.
**: specimens released in the laboratory from the rejuvenated hydroids derived from medusae collected in the sea.
***: maximum values.
references. For details concerning the specimens from each site, see Tables 1-3. For hydroids, new collection records, previous records, and records of rejuvenated hydroids [in brackets] are mapped in Fig. 4 and listed in the Appendix together with all relevant references.

Medusae of this species have been collected from waters almost all around Japan, but very rarely in offshore waters of the Nansei Islands despite frequently surveys there during research cruises of the “Toyoshio-maru” from Hiroshima University (Kubota, 2005b, unpublished data). Most of the specimens collected by me during in the entire study were immature small medusae (Fig. 1, Table 1, Appendix).

In 2002 well-grown (=subadult) or spent medusae
Fig. 2. Examples of morphological constancy in laboratory-reared medusae of the small morphotype of *Turritopsis nutricula* after maturation (F/M: female/male; for localities 13 and 30, see Fig. 1).

were collected from the coast of Fukushima Pref., northern Japan (Kubota and Mizutani, 2003) and from some stations in the Seto Inland Sea (Kubota et al., 2003). These finds all correspond to the previous known "large morphotype" (Uchida, 1925; Yamada and Nagao, 1971).

About 200 large, subadult individuals collected in one sample (a kind of a swarm) in Kagoshima Bay in 2002 (Chikuchishin and Kubota, 2003b) belonged to a different morphotype as described below. They included the largest specimens of this "small morphotype". Besides recent collections of mature medusae from the sea in southern Japan, cultivation of newly released medusae originating from various localities has provided other adult medusae that can be assigned to the new morphotype (Table 1, Figs 1–3). The morphology of this type remains constant after gonad maturation, as has been observed in specimens of both sexes from Ago Bay in Mie Prefecture and Tomari Port in Okinawa Island, Okinawa Prefecture. Their life span is maximally 2.5 months (Fig. 2).

In both morphotypes, tentacle number is correlated with umbrella size, but the morphological distinction between the two types is only clear in mature or subadult medusae (Figs 1–3, Tables 1–2). The large morphotype has the tentacles in two rows (or more), while the small morphotype has one row only. Local variation was noticed in the small morphotype. The two forms are well separated geographically: the large morphotype occurs in cooler, northern waters and the Seto Inland Sea, whereas the small morphotype is found in warmer, southern waters (Fig. 1).
Table 2. Records of hydroids of *Turritopsis nutricula* in Japan. Hydroids collected in the sea are listed in the upper part, and rejuvenated ones derived from medusae are listed in the lower part.

<table>
<thead>
<tr>
<th>Locality (Site no in Fig. 4)</th>
<th>Collection month and year</th>
<th>Depth (m)</th>
<th>Colony height (max. mm)</th>
<th>Condition of hydrocaulus</th>
<th>No. of tentacles (max.)</th>
<th>Medusa buds</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ushibuka (6)</td>
<td>VII-1966</td>
<td>no data</td>
<td>20</td>
<td>polysiphonic</td>
<td>no data</td>
<td>present</td>
<td>Hirohito, 1969</td>
</tr>
<tr>
<td>Izu Oshima I. (4)</td>
<td>VII-1977</td>
<td>30-75</td>
<td>no data</td>
<td>no data</td>
<td>no data</td>
<td>absent</td>
<td>Hirohito, 1983</td>
</tr>
<tr>
<td>Akkeshi (1)</td>
<td>VII-1970</td>
<td>3</td>
<td>22</td>
<td>monosiphonic</td>
<td>19</td>
<td>present</td>
<td>Yamada &amp; Nagao, 1971</td>
</tr>
<tr>
<td>off Hayama (3)</td>
<td>V-1930-IX-1936</td>
<td>45</td>
<td>20</td>
<td>polysiphonic</td>
<td>20</td>
<td>present</td>
<td>Hirohito, 1988</td>
</tr>
<tr>
<td>Nishinoomote, Tanegashima I. (8)</td>
<td>2002</td>
<td>Lab. 2.1</td>
<td>monosiphonic</td>
<td>15</td>
<td>present</td>
<td>Present study</td>
<td></td>
</tr>
<tr>
<td>Tomari Port, Okinawa I. (9)</td>
<td>2003</td>
<td>no data</td>
<td>monosiphonic</td>
<td>no data</td>
<td>absent</td>
<td>Present study</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Reversability to the hydroid stage from the medusae in the two morphotypes of *Turritopsis nutricula* in Japan.

<table>
<thead>
<tr>
<th>Locality (Site no in Fig. 4)</th>
<th>Type of medusae</th>
<th>No. and state of maturity of medusae examined</th>
<th>No. of medusae rejuvenated</th>
<th>Reversibility (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iwaki (2)</td>
<td>Large</td>
<td>19 subadult</td>
<td>2</td>
<td>10.5</td>
<td>Kubota &amp; Mizutani, 2003</td>
</tr>
<tr>
<td>Tanabe Bay (5)</td>
<td>Small</td>
<td>8 young</td>
<td>5</td>
<td>62.5</td>
<td>Present study</td>
</tr>
<tr>
<td>Tanabe Bay (5)</td>
<td>Small</td>
<td>1 female</td>
<td>0</td>
<td>0.0</td>
<td>Present study</td>
</tr>
<tr>
<td>Kagoshima (7)</td>
<td>?Small</td>
<td>4 young</td>
<td>1</td>
<td>25.0</td>
<td>Chikuchishin &amp; Kubota, 2003a</td>
</tr>
<tr>
<td>Kagoshima (7)</td>
<td>Small</td>
<td>11 subadult</td>
<td>1</td>
<td>9.1</td>
<td>Chikuchishin &amp; Kubota, 2003b</td>
</tr>
<tr>
<td>Nishinoomote, Tanegashima I. (8)</td>
<td>Small</td>
<td>1 young</td>
<td>1</td>
<td>100.0</td>
<td>Present study</td>
</tr>
<tr>
<td>Tomari Port, Okinawa I. (9)</td>
<td>Small</td>
<td>9 young</td>
<td>8</td>
<td>88.9</td>
<td>Present study</td>
</tr>
</tbody>
</table>

All data combined: Large morphotype (2) | 19 subadult | 2 | 10.5 | Kubota & Mizutani, 2003 |
Small morphotype (5, 7-9) | 22 young | + 11 subadult | + 1 female | 16 | 47.1 | Chikuchishin & Kubota, 2003a, b; Present study |

No distinct difference could be found between the youngest (1-day-old) medusae released in the laboratory from hydroids collected from any locality except for medusae from Akkeshi, Hokkaido, which are sometimes slightly larger than the small morphotype in southern Japan (Table 1: ##; Kubota, 2005a). Knowledge of the growth process in both morphotypes should be clarified in the future in order to be able to distinguish them earlier.

Although records of the hydroid from nature are scarce, the known finds cover most of the Japanese Islands (Fig. 4, Table 2), which indicates a wide geographical distribution of this developmental stage, comparable to that of the medusa stage. Hirohito (1969) recorded colonies with polysiphonic stems, but all other material from both the field and the laboratory had only monosiphonic stems. The taxonomic importance of this character needs to be evaluated in the future.

(2) Reversability of the medusae to the hydroid

As Table 3 and Fig. 4 show, medusae of the two morphotypes can revert to the hydroid after degeneration of the medusan body. Usually one medusa transforms into one hydroid colony. The small form, which closely resembles the medusa from the Mediterranean Sea (Piraino et al., 1996), does not rejuvenate in all cases, unlike the Italian population. The small Japanese morphotype can revert more easily back to the polyp stage than the large type,
but several pieces of one large-form medusa can revert to the hydroid stage, thus generating colonies of clonal origin (Kubota and Mizutani, 2003).

The first observation of a repeated rejuvenation was made on a small, immature medusa collected from southern Japan (Tanegashima I., Kagoshima Pref.) at the end of May, 2002 (Fig. 4: site 8). It is conceivable that this rejuvenation could continue indefinitely, leading to the immortal life of that animal. That young medusa first reverted to the hydroid stage two days after collection, aboard the research ship (“Toyoshio-maru”, Hiroshima Univ.). Then, within two months, the hydroid grew up into a well-developed colony consisting of several tens of zooids and began to release many young medusae. These young medusae could again revert to polyps.
All the secondarily rejuvenated colonies, 10 in number, were accidentally lost at the end of June, 2003.

In both morphotypes of the medusae, the manubrium sometimes remained after the disintegration of the rest of the medusa, but this part never reverted to the hydroid, as has been observed by me in a leptomedusa, *Eutima japonica* Uchida, 1925 (Kubota, 1983). Nonetheless, the manubria were able to feed on *Artemia* nauplii for several months (Kubota and Mizutani, 2003; Kubota, present study on a male medusa from Okinawa I.), and the lifespan of the medusa was thus prolonged to twice the usual duration. I have observed such partial survival in other hydromedusae (Kubota, unpublished data).

Piraino *et al.* (1996) demonstrated that a manubrium isolated from an intact medusa of *T. nutricula* did not rejuvenate, which suggests that a combination of several kinds of tissues of the medusa is required for rejuvenation. A manubrium alone of any hydromedusae evidently cannot transform into the young hydroid stage, even if kept under optimal conditions.

(3) Taxonomic remarks

According to Yamada and Nagao (1971), who observed the life cycle of the large form of Japanese *Turritopsis*, and Hirohito (1988), who adopted the opinion of Uchida (1927a, b, 1930, 1938a, b, 1940), there is only one species *Turritopsis*, namely *Turritopsis nutricula* Macrady, 1857. Kramp (1961) and Bouillon and Boero (2000) also adopted this view. Until recently, only the large form of *Turritopsis* was known from Japan; juvenile medusae from southern Japan (e. g. Fig. 1: sites 14, 19) were also attributed to it.

Of all the earlier records, only the medusae from Misaki described by Maas (1909) as *Turritopsis nutricula* var. *pacific* had several rows of tentacles, whereas all others had the tentacles in two rows. This difference can be explained by intraspecific variation.

The small morphotype also shows some variations as the present study demonstrates. Through my recent field collections and laboratory rearing of many specimens from various localities, particularly from southern Japan, the small form can be distinguished clearly from the large form found in northern Japan. The small form was first found in 1984, a laboratory-reared individual collected from Ago Bay, Mie Pref. (Fig. 2), paralleling with the intermediate type of bivalve-inhabiting hydrozoan, a so-called a missing-link in evolution (Kubota, 1984). Later similar finds of the small form of *Turritopsis* were made at other localities in southern Japan, e. g. Okinawa Island in Okinawa Prefecture and Tanabe Bay in Wakayama Prefecture (Table 1; Figs 2, 3; Kubota, unpublished data).

The characteristics of this small form agree well with those of laboratory-reared *Turritopsis* medusae found in Italy. Some recently collected individuals from Kagoshima Bay, Kyushu (Chikuchishin and Kubota, 2003b), were exceptionally large for this morphotype, however, thus being somewhat intermediate with respect to the large morphotype. The causes of this cline-like variation in the small form should be clarified in the future by examining more populations from intervening regions.

Whether these two or more morphotypes reflect mere intraspecific variation or the presence of two or more biological species must be confirmed by experimental methods, such as DNA sequence comparison. Also, confirmation of the occurrence of the large morphotype in Kagoshima Bay is required, there being no record since the first report by Bigelow (1913).

Laboratory-reared Italian medusae can revert completely to the hydroid; all 4001 individuals examined rejuvenated regardless of developmental stage (Piraino *et al.*, 1996, 2004; Kubota, 2000). In contrast to this complete reversion observed in Mediterranean animals, reversion is not perfect or occurs at low frequency in Japanese populations, although both morphotypes are able to rejuvenate (Table 3). This reduced ability is possibly due to Japanese populations being genetically more heterogeneous.

In Japanese waters, the hydroids and the medusae of *Turritopsis* are widely distributed. Two forms of medusae can be distinguished, and they have a dis-
junct distribution. Both the medusa forms can rejuvenate to the hydroid and two rounds of rejuvenation have been observed in the laboratory for the first time. However, rejuvenation success was not 100% as in the Mediterranean medusae, which are morphologically similar to the small morphotype of Japan.

Revision of the taxonomic status of these two (or more?) morphotypes is postponed until more biological studies have been carried out. Schuchert (2004) and Bouillon et al. (2004) recognize two distinct Turritopsis species in the Mediterranean Sea, T. nutricula McCrady, 1857 and T. dohrii (Weismann, 1883), and it cannot be excluded that the Japanese form is similarly polytypic.

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Appendix. List of collection sites of medusae and hydroids of Turritopsis nutricula in Japan, with site numbers corresponding to Figs 1 and 4, respectively (see Table 1).

Medusae (Fig. 1): new records and new collections from previously known localities. Abbreviations: F/M, mature female/male medusae; m, immature medusae.

Hydroids (Fig. 4): new and previous records, and records of rejuvenated hydroids [in brackets].
References


Piraino, S., Boero, F., Aeschbach, F. and Schmid, V., 1996. Reversing the life cycle: medusae transforming into polyps and cell transdifferenti-


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