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Abstract

In spite of considerable research effort on the "immortal jellyfish" *Turritopsis dohrnii* (Weismann, 1883), a comprehensive account including the ontogenetic stages throughout its normal life cycle is still missing. Here, we report the development of the medusa morph, with description of four consecutive stages distinguished by the morphology of the umbrellar apex and the number of marginal tentacles. Medusae reared at two different temperatures (14 and 25°C) showed identical morphological features, but with shorter developmental time at higher temperature. Additional information on the morphology of the polyp stage is also provided. The implications for an easy morphological identification of *T. dohrnii* medusae from other congeneric species are discussed.

Keywords: *Hydromedusae, plankton, life cycle, morphological characterization, Oceaniidae*

Introduction

The genus *Turritopsis* McCrady, 1857 includes species of the family Oceaniidae (Hydrozoa: Cnidaria) that have hydroid colonies with adnate side-branches and which produce medusae with a peduncle-like mass formed of vacuolated gastrodermal cells (Schuchert 2004; Bouillon et al. 2006). In its current usage, *Turritopsis* shares morphological similarities at the medusa, polyp, or both stages with three other oceaniid taxa: *Oceania* Kölliker, 1853 auct., *Corydendrium* van Beneden, 1844, and *Turritopsoides* Calder, 1988. *Turritopsis*, however, is easily separated from the latter two genera on the basis of the production of medusae, lacking in *Corydendrium* and *Turritopsoides* (Calder 1988a,b; Bouillon et al. 2006). The distinction from *Oceania* is less clear, and since the polyp stage of this genus is too poorly known to allow any useful comparison with *Turritopsis*, the two genera have been separated exclusively on the basis of morphological characters of the medusa, such as the nematocyst clusters along the mouth (sessile in *Turritopsis*, stalked in *Oceania*) and the morphology of the pseudopeduncle (gelatinous in *Oceania*, formed by vacuolated cells in *Turritopsis*) (Schuchert 2004; Bouillon et al. 2006). The medusae of these genera, however, resemble each other quite closely, and it may be the case that more information on morphological and molecular variation will lead to merging *Turritopsis* and *Oceania* into one single taxon (Schuchert 2004).

The taxonomic relationships inside of the genus *Turritopsis* are by no means settled and the species boundaries need to be clarified by further research, especially on life cycles. In addition, many records of several nominal species of *Turritopsis* exist under the name *Turritopsis nutricula* McCrady, 1857, the type species of the genus. Confusion arose in the literature mainly because *T. nutricula* had long been regarded as a circumglobal species with which most of the other nominal species of *Turritopsis* were synonymous. Morphological and life-history studies (Schuchert 2004) as well as molecular investigations (Miglietta et al. 2007) provided complementary evidence that populations from the Northeastern Atlantic, Mediterranean, New Zealand, and Japan do not belong to *T. nutricula*, thus increasing the potentially valid *Turritopsis* species to 10. These species are widely

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distributed in temperate and tropical waters, sometimes being quite conspicuous in the medusa stage due to the color of the manubrium, as is the case for *T. rubra* (Farquhar, 1895) from New Zealand and *T. polycirrha* (Keferstein, 1862) from the English Channel (both as *T. nutricula* in Schuchert 1996; Russell 1953, respectively).

The Mediterranean representative of the genus, *Turritopsis dohrnii* (Weismann, 1883), has been receiving much public attention because its medusa is able to metamorphose back into the polyp stage, which is why this species, initially referred to as *T. nutricula*, has been nicknamed "the immortal jelly-fish" (Bavestrello et al. 1992; Piraino et al. 1996, 2004; Carlà et al. 2003). This unusual life-history feature would allow *T. dohrnii* to easily survive through long journeys in cargo ships and ballast waters, making it a potential invader outside of its native distributional range (Miglietta & Lessios 2009). The discovery of reverse development in *T. dohrnii* was the starting point for a considerable research effort on the cellular mechanisms underlying medusozoan reverse development (Piraino et al. 2004; De Vito et al. 2006; Schmich et al. 2007), and it attracted attention from a large scientific community working on aging, regenerative and cell differentiation biology (Quiquand et al. 2009; Haines et al. 2013; Sánchez-Alvarado & Yamanaka 2014; Sinkovics 2015). Despite this, knowledge on the morphological changes during the life cycle of this species is still scant and the adult mature medusa of this species has been observed only twice under laboratory conditions (Piraino et al. 1996; Carlà et al. 2003), and another two times in nature (Neppi & Stiasny 1913 as *T. nutricula*; Miglietta & Lessios 2009). The original description of *Turritopsis dohrnii* (as *Dendroclava dohrnii*) by Weismann (1883) was based on the hydroid stage and the medusa buds only. Schuchert (2004) considered the Mediterranean species of *Turritopsisis* as distinct from *T. nutricula*, and referred it to the taxon described by Weismann as *Dendroclava dohrnii*, hence *Turritopsis dohrnii* (Weismann, 1883). Later accounts of this species focused more on ontogeny reversal than on the description of the whole life cycle. The present study provides the first description of the complete development of *T. dohrnii* medusae, from liberation to maturity, and aims to test the effects of temperature changes on growth and gonad differentiation. Additional information on the morphology of the polyp stage is also provided.

Material and methods

Hydroid colonies of *Turritopsis dohrnii* were collected on 2 September 2013 by SCUBA diving in Santa Caterina, Lecce (40°8′30.01″N, 17°58′43.98″E), off the Italian coasts of the Ionian Sea (approximately 2 m depth). In the laboratory, colonies including polyps with medusa buds were separated, transferred to glass bowls, and reared in 0.45-μm-filtered seawater (FSW) at constant temperature (25°C). Bowls were checked several times throughout the following 24 h to detect the liberation of medusae. In the meantime, the morphological characteristics of the colonies were recorded. Newly released medusae were immediately transferred to glass beakers (1000 mL) with FSW (salinity 37 psu) and kept in constant temperature chambers at either 14°C (n = 100) or 25°C (n = 100) with a 12 h light/12 h dark photoperiod. Water circulation and uniform aeration were provided by means of a plastic plate connected to a slow-speed motor (5–6 rpm) through a silicon tub, in order to avoid the production of air bubbles that could damage the medusae. A second cohort of newly liberated medusae was obtained on 23 September 2013 and reared at 14°C (n = 30) and 25°C (n = 30).

Medusae of both cohorts were measured, photographed, and fed with 2-day-old *Artemia salina* nauplii every 2 days, adding excess numbers of nauplii to the glass bowls containing the medusae. Seawater was replaced 2 h after each feeding session. Variations in umbrella, manubrium, and peduncle shape and size, and other anatomical features such as occurrence of manubrial pads, shape and color of gonads, number and shape of tentacles, and position and color of ocelli were recorded daily. In addition, every 2 days, three medusae from each temperature treatment were anaesthetized in a 1:1 solution of 7% magnesium chloride ($MgCl_2$) solution and seawater, and preserved in a 4% formaldehyde solution in seawater. Samples of fertile and infertile colonies were preserved in the same way. Nematocyst types and their distribution were determined by light microscopy on squash preparations (Östman et al. 1991). Only capsules of undischarged nematocysts were measured, and differences between their length and width related to medusa ontogeny were investigated through one-way analyses of variance (ANOVA). In addition, two-way ANOVA comparisons were used to test the effect of temperature on the umbrella morphology and developmental time of *Turritopsis dohrnii* medusae.

Results

Taxonomic accounts

Turritopsis dohrnii (Weismann, 1883)
(Tables I, II; Figures 1–3)

Table I. Measurements of the polyp stage of *Turritopsis dohrnii* (Weismann, 1883). n = 9 for characters a–d, n = 27 for characters e–m. SD = standard deviation.

	Mean ± SD (range)
a. Colony height (mm)	8.56 ± 5.36 (3.00–18.00)
b. Hydrocaulus length (mm)	7.44 ± 5.55 (3.00–17.00)
c. Diameter at the base of hydrocaulus (mm)	0.73 ± 0.21 (0.50–1.00)
d. Diameter at the distal end of hydrocaulus (mm)	0.46 ± 0.07 (0.30–0.50)
e. Hydranth length (mm)	0.88 ± 0.09 (0.68–1.20)
f. Hydranth width (mm)	0.28 ± 0.07 (0.11–0.40)
g. Number of tentacles per polyp	18 ± 5 (11–23)
h. Length of fully extended tentacles (mm)	0.61 ± 0.06 (0.50–0.70)
i. Diameter of medusa buds (mm)	0.43 ± 0.06 (0.35–0.52)
j. Desmonemes: length of capsule (μm)	5.57 ± 0.09 (5.0–6.0)
k. Desmonemes: width of capsule (μm)	3.48 ± 0.65 (2.5–4.0)
l. Microbasic eurytele: length of capsule (μm)	8.38 ± 0.65 (8.0–9.0)
m. Microbasic eurytele: width of capsule (μm)	3.02 ± 0.84 (2.7–3.7)

Table II. Measurements of the medusa stage of *Turritopsis dohrnii* (Weismann, 1883). Measurements expressed as mean ± standard deviation (SD; range), in mm.

|	Newly liberated medusa	Eight-tentacle stage	Twelve-tentacle stage	Sixteen-tentacle mature stage
a. Umbrella height	0.93 ± 0.12 (0.70–1.10)	1.35 ± 0.26 (0.90–1.60)	1.55 ± 0.16 (1.22–1.88)	2.43 ± 0.33 (1.78–2.99)
b. Umbrella width	0.78 ± 0.07 (0.71–0.94)	0.89 ± 0.17 (0.50–1.30)	1.51 ± 0.21 (1.10–1.88)	2.06 ± 0.29 (1.48–2.63)
c. Manubrium length	0.40 ± 0.09 (0.28–0.58)	0.50 ± 0.04 (0.28–0.58)	0.69 ± 0.11 (0.48–0.91)	0.93 ± 0.25 (0.43–1.43)
d. Number of tentacles	8	8	12	16
e. Length of fully extended tentacles	2.19 ± 0.20 (1.90–2.60)	3.04 ± 0.20 (1.90–3.40)	3.80 ± 0.09 (3.62–3.98)	5.03 ± 0.52 (3.99–6.06)
Measured medusae (n)	80	72	48	12
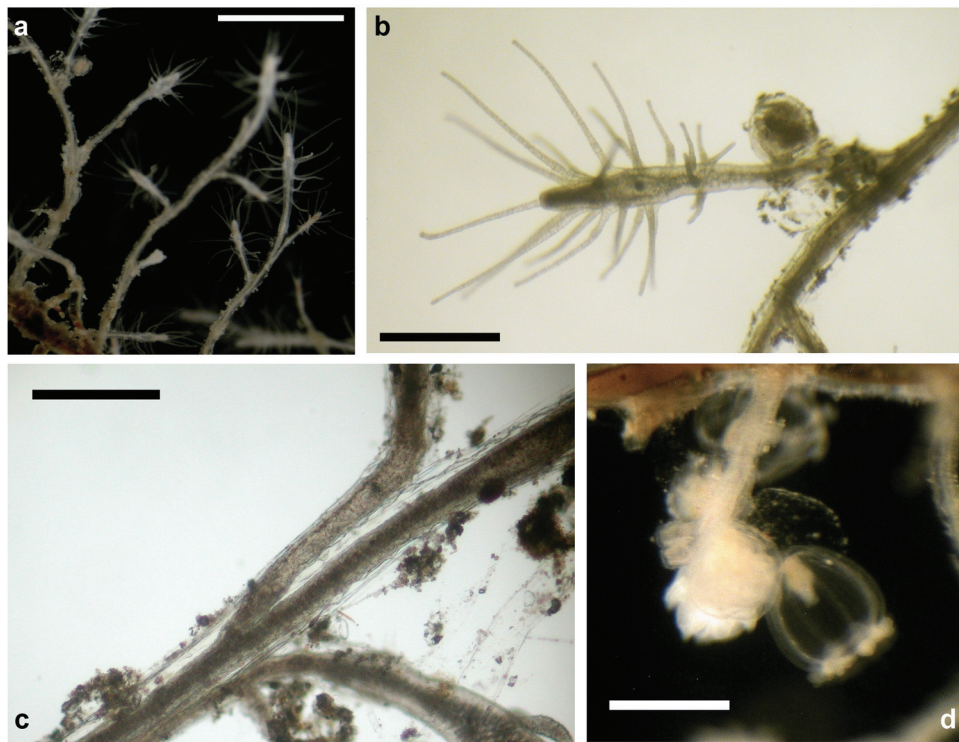
Figure 1. Polyp stage of *Turritopsis dohrnii* (Weismann, 1883). (a) Portion of a colony. (b) Polyp with young medusa bud. (c) Basal adnate part of hydrocladia. (d) Almost completely developed medusa bud. Scale bars: A = 1 mm; b–d = 0.4 mm.

Figure 2. Morphological details of the medusae of *Turritopsis dohrnii* (Weismann, 1883). (a) Masses of vacuolated cells forming the pseudopeduncle (aboral view) in an eight-tentacled medusa. (b) Lateral view of the pseudopeduncle and manubrium of a 12-tentacled medusa. (c) Marginal bulbs with adaxial ocelli of a mature medusa. Scale bars: A = 0.1 mm; b = 0.2 mm; c = 0.05 mm.

Hydroid stage. Colonies of *T. dohrnii* were growing on rocky overhangs, both directly on rock and on a variety of biogenic substrates including tubes of serpulid polychaetes, encrusting bryozoans, barnacles, coralline red algae, and the hydrocaulus of a *Eudendrium racemosum* colony. Most colonies of *T. dohrnii* were fertile at the time of sampling. See Gravili et al. (2015) for a description of the hydroid stage. The cnidome of the polyp includes desmonemes (5.0–6.0 µm long, 2.5–4.0 µm wide) and microbasic euryteles (8.0–9.0 µm long, 2.7–3.7 µm wide), present in both the tentacles and the hydranth body. Additional details on the measurements and morphological variation of the examined colonies are given in Table I.

Newly liberated medusa. Umbrella almost spherical, transparent, slightly higher than wide, with irregularly scattered cnidocysts over most of the exumbrella; mesoglea of similar thickness apically and laterally; manubrium cruciform in transverse section, brownish, extending to 1/3–1/2 of the subumbrellar cavity, with four interradial yellow pads occupying almost half of its length; mouth with four inconspicuous lips, each with a continuous row of sessile cnidocyst (microbasic euryteles) clusters; pseudopeduncle (Bouillon et al. 2006) inconspicuous but present, transparent, with four compact masses of vacuolated cells situated above digestive part of manubrium and overtopping the proximal end of the four radial canals; eight tentacles, with somewhat swollen tips; marginal bulbs each with one adaxial ocellus, red to rust colored, laterally seen as if mounted on a small protuberance; no trace of gonads. Besides the oral microbasic euryteles (6.7–9.2 µm long, 4.0–4.4 µm wide), the cnidome of the newly liberated medusae includes desmonemes (4.5–5.0 µm long, 2.7–4.3 µm wide).

Remarks. Small *T. dohrnii* medusae began pulsating before being released and finally swam away from the parent colony thanks to frequent contractions of the umbrella. Liberated medusae displayed a strong positive phototropism at this stage, but they would not feed even if presented with small pieces of dissected *Artemia* nauplii. Furthermore, the presence of living prey or nauplii homogenates in the water did not elicit any feeding response in these animals.

Eight-tentacled medusa. Umbrella slightly bell shaped, transparent, higher than wide; mesoglea thicker at the apex than laterally; manubrium cruciform in transverse section, brownish, extending to 1/3–1/2 of the subumbrellar cavity, without interradial pads; mouth with four inconspicuous lips, each with a continuous row of sessile cnidocyst clusters; pseudo-peduncle small but conspicuous, transparent, with four compact masses of vacuolated cells situated above digestive part of manubrium and overtopping the proximal end of the four radial canals; eight tentacles, with somewhat swollen tips; marginal bulbs each with one adaxial ocellus, similar to the one in newly released medusae; no trace of the gonads was observed. Cnidome: desmonemes (4.3–5.3 µm long, 3.6–4.1 µm wide) and microbasic euryteles (7.2–9.7 µm long, 3.9–4.0 µm wide).

Remarks. After liberation, the medusae increased in size; the top of the umbrella modified from rounded to slightly conical, developing a pseudo-peduncle and losing the manubrial pads. During these first days of life, the number of tentacles remains unchanged. Two-day-old medusae started to feed on live nauplii: only a single, small-sized nauplius per medusa, but killing many more with the tentacles. No significant morphological differences were detected between the 14 and 25°C medusae at this and the following stages or between individuals from different cohorts (see below). Thus, the descriptions and measures provided herein include combined information from the two batches of reared medusae. Moreover,
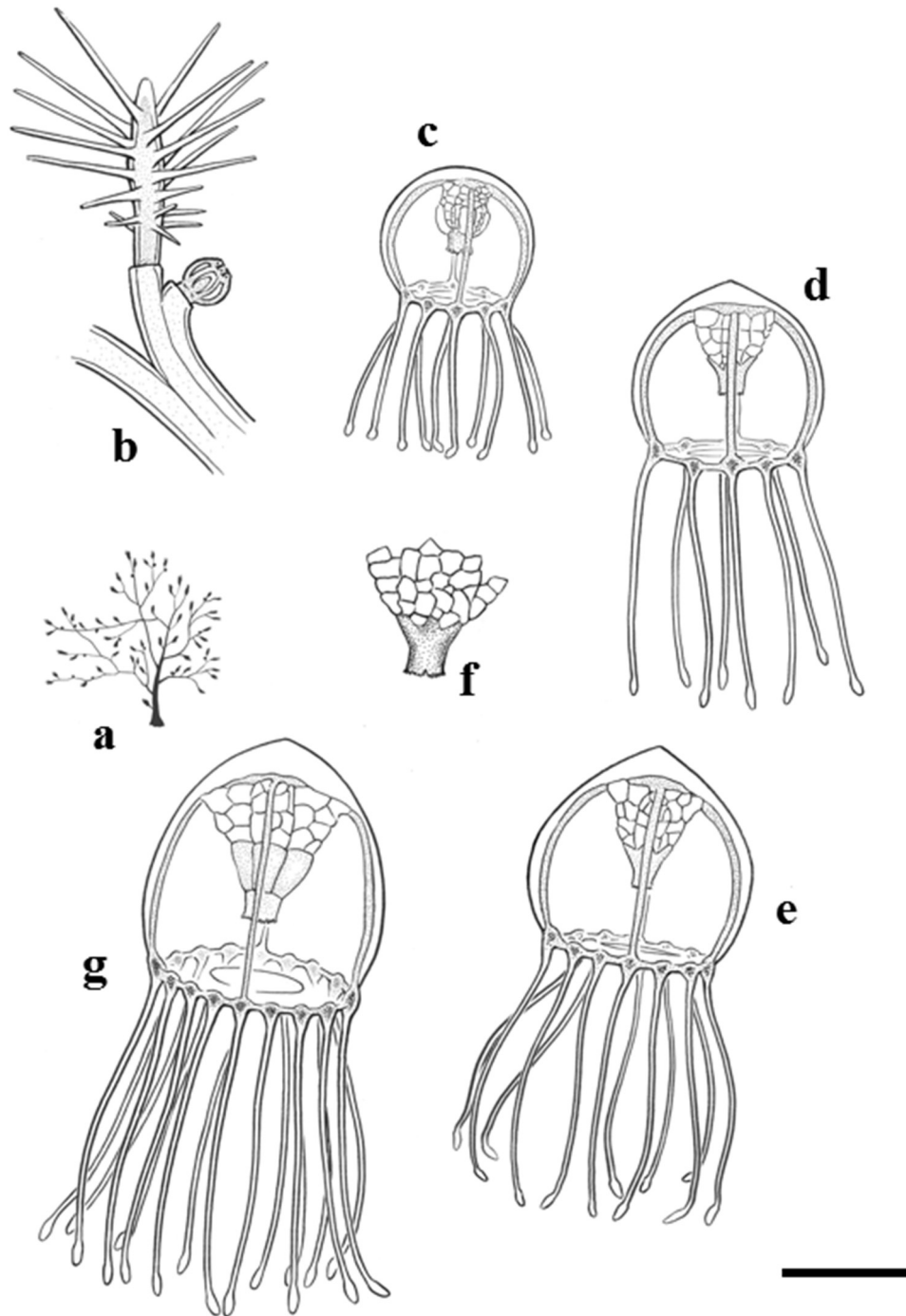
Figure 3. Life cycle of *Turritopsis dohrnii* (Weismann, 1883); all stages drawn from living animals. (a) Colony outline. (b) Polyp with medusa bud. (c) Newly liberated medusa. (d) Eight-tentacled medusa. (e) Twelve-tentacled medusa. (f) Manubrium of a 16-tentacled medusa. (g) Sixteen-tentacled mature adult medusa. Scale bar: a = 6.0 mm; b = 0.5 mm; c–g = 1.0 mm.

nematocyst length remains constant through the development of the medusae of *Turritopsis dohrnii*, as no significant differences were observed in the length of desmonemes [$F_{(3, 20)}$ = 0.49, p = 0.693] or microbasic euryteles [$F_{(3,}$ 20) = 1.12, p = 0.366] among distinct developmental stages.

Twelve-tentacled medusa. Umbrella bell shaped, transparent, almost as high as wide; mesoglea thicker at

the apex than laterally; manubrium cruciform in transverse section, brownish, extending to 1/2 of the subumbrellar cavity, without manubrial pads; mouth with four inconspicuous lips, each with a continuous row of sessile cnidocyst (microbasic euryteles) clusters; pseudopeduncle well-developed, transparent, with four masses of vacuolated cells of different sizes; four radial canals and one circular canal; 12 tentacles, four perradial and eight adradial, each with a swollen tip; marginal bulbs each with one adaxial ocellus, similar to those in previous stages; no gonads. Cnidome: desmonemes (4.4–5.3 μm long, 3.0–3.9 μm wide) and microbasic euryteles (6.5–9.1 μm long, 3.9–4.5 μm wide).

Remarks. Reaching the 12-tentacle developmental stage, *T. dohrnii* medusae further developed the pseudopeduncle, and the general size of the body increased. Twelve-tentacled medusae were able to feed on even the largest nauplii, and often they would try to ingest more than one prey at a time.

Sixteen-tentacled mature medusa. Umbrella bell shaped, transparent, higher than wide; mesoglea thicker apically than laterally; manubrium cylindrical, with cruciform base in transverse section, brownish but acquiring an orange tonality when digesting *Artemia* nauplii, extending to 1/2–1/3 of the subumbrellar cavity; mouth with four inconspicuous lips, each with a continuous row of evident, sessile cnidocyst (microbasic euryteles) clusters; pseudopeduncle well developed, transparent, pyramidal, with four perradial masses of vacuolated cells of different sizes, larger ones at the base of the peduncle; four radial canals and a circular canal; 16 tentacles, each with a swollen tip; marginal bulbs each with one adaxial ocellus, rust colored, on a small protuberance, some small, developing marginal bulbs may also be present (up to two of them were observed in the examined medusae); all specimens were male, with the gonads developing as four interradial masses, dull orange to brownish, located on the proximal end of the manubrium at the junction between this and the pseudopeduncle. Cnidome: desmonemes (4.5–4.9 μm long, 2.5–3.9 μm wide) and microbasic euryteles (7.3–9.3 μm long, 3.8–4.5 μm wide).

Remarks

At the final stage of growth, the medusae developed an extra set of four tentacles (bringing the total up to 16) and reached maturity. In addition to the appearance of the gonads (male in all the examined specimens), in this stage the pseudo-peduncle and umbrella shape reached their final morphological conformation. All the medusae began to show signs of exhaustion and stopped feeding at days 35–37. For medusae reared at 25°C, this happened after what seemed to be an event of gamete liberation (the appearance of the gonads changed from opaque to translucent and they shrank), although no traces of spermatozoa were found in the examined water.

Effects of temperature on medusa ontogeny

The incubation of different medusa cohorts at different experimental temperatures produced no

Table III. Summary of the statistical testing [two-way analysis of variance (ANOVA) comparisons] of the effect of cohort and temperature on the umbrella morphology and developmental time of *Turritopsis dohrnii* medusae. Statistically significant results (P < 0.01) are highlighted in grey. SS = sum of squares; DF = degrees of freedom; MS = mean squares; F = F statistic; P = probability value.

Umbrella diameter	Eight-tentacled stage					Twelve-tentacled stage					Sixteen-tentacled stage				
Source	SS	DF	MS	F	P	SS	DF	MS	F	P	SS	DF	MS	F	P
Cohort (Co)	0.003	1	0.003	0.04	0.849	0.021	1	0.021	0.20	0.655	0.113	1	0.113	1.78	0.193
Temperature (Te)	0.018	1	0.018	0.22	0.637	0.053	1	0.053	0.52	0.475	0.008	1	0.008	0.12	0.728
Co × Te	0.005	1	0.005	0.07	0.799	0.403	1	0.403	3.92	0.054	0.340	1	0.340	5.37	0.028
Residual	44902	56	0.080			45217	44	0.103			17,738	28	0.063		
Total	45164	59				49992	47				22,347	31			

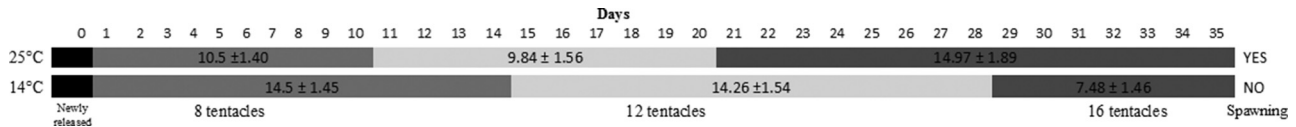| Developmental time	Eight-tentacled stage					Twelve-tentacled stage				
Source	SS	DF	MS	F	P	SS	DF	MS	F	P
Cohort	0.150	1	0.150	0.31	0.579	0.021	1	0.021	0.03	0.856
Temperature	580167	1	580167	120.63	**0.001**	585208	1	585208	93.92	**0.001**
Co × Te	0.150	1	0.150	0.31	0.579	0.021	1	0.021	0.03	0.856
Residual	269333	56	0.481			274167	44	0.623		
Total	852500	59				859792	47			

Figure 4. Differences in time spent by *Turritopsis dohrnii* medusae in each morphological stage from liberation to death according to temperature. Numbers inside the bars are mean number of days ± standard deviation (SD).

detectable differences between individuals at the same developmental stage reared at different temperatures or from different cohorts, in spite of a reduced developmental rate in medusae reared at 14°C (Table III; Figure 4). The survival of medusae was not evidently affected by temperature, with 15% of the individuals completing the life cycle at both 14 and 25°C. Further observations on the effect of temperature on the development of the medusae include the detection of gonadic primordia already at the 12-tentacled stage reared at 25°C, while four 16-tentacled medusae reared at 14°C failed to completely develop the gonadic tissue by the end of the experiment.

Discussion

This study provides a complete description of the morphological changes undergone by the medusae of *Turritopsis dohrnii* throughout development from liberation to sexual maturation and it aims to support future identification of *T. dohrnii* medusae based on morphologically consistent characteristic features.

The low number of tentacles in adult *T. dohrnii* medusae represents one of the least variable characters useful for the morphological diagnosis of this species in Mediterranean waters. As pointed out by Schuchert (2004) and Miglietta et al. (2007), the number of tentacles recorded in the adult medusae of the other species of *Turritopsis* is consistently higher than the highest number of tentacles ever observed in a *T. dohrnii* medusa (32, according to Neppi & Stiasny 1913), except for *T. fascicularis* Fraser, 1943, *T. minor* (Nutting, 1905) and *T. chevalense* (Thornely, 1904), whose medusa stage has never been observed. Adult *T. pleurostoma* (Péron and Lesueur, 1810) and *T. lata* von Lendenfeld, 1885 medusae have been reported to have 32 and more than 20 tentacles, respectively, but the validity of the former species is somewhat dubious and there are other morphological characteristics that would allow for the distinction of *T. dohrnii* from both species; namely the size (medusae larger than 30 mm are characteristic of *T. pleurostoma*) and the structure of the peduncle (vacuolated cells confined

to proximal part of radial canals and along peduncle are diagnostic of *T. lata*). Furthermore, the one-time finding of 32-tentacled adult *T. dohrnii* medusae by Neppi and Stiasny (1913) has never been repeated, all subsequent observations of mature medusae corresponding to specimens with less than 24 tentacles, and even this number may prove to be too high to be considered common in this species: after examining about 4000 medusae, Piraino et al. (1996) stated that medusae with more than 16 tentacles were never observed in their laboratory rearings, as also corroborated by further investigations (Carlà et al. 2003).

While adult *T. dohrnii* medusae consistently develop fewer tentacles than all other known *Turritopsis* medusae, some variation exists in this character between populations of this species inside and outside of its native range. Miglietta and Lessios (2009) suggest that this variation (they found only eight-tentacled adult medusae in their survey of Panamanian waters, while Mediterranean mature medusae usually have 16 tentacles) may be a response to different climatic conditions experienced by temperate and tropical populations. The latter authors go on to say that this phenomenon could be attributable to a process of heterochrony in the introduced tropical populations of the species, a scenario that is in agreement with our observations on the modifications of the life cycle of *T. dohrnii* linked to temperature variations.

The number of tentacles in newly released medusae of *T. dohrnii* shows even less variation. Both in this study and in that of Piraino et al. (1996) and Carlà et al. (2003), only eight-tentacled newly released medusae were observed; although some records of newly released medusae with 12 tentacles exist elsewhere (Schuchert 2004). Together with the low number of tentacles, the morphology of the manubrium and mouth in the mature medusae of *T. dohrnii* may be diagnostic of the species. The lips of mature *T. dohrnii* medusae are small, inconspicuous, and hard to see even in actively feeding animals, in contrast with what is found in the adult of other species of *Turritopsis* with known medusa stage, where the lips have been described and illustrated: they are recurved or fringed and rather well

developed (Russell 1953; Kramp 1961; Schuchert 1996, 2004).

Our observations allowed us to note some important differences with the descriptions given so far of the medusae of *T. dohrnii*. For instance, the presence of dark spots in the middle of the gonads turned out to be a variable and not well-defined character. In the specimens analyzed in this studio, the presence of dark spots in the middle of the gonads was not constant. The presence of such spots has been scarcely documented in the scientific literature, mentioned only by Neppi and Stiasny (1913), and restated later in the descriptions of Schuchert (2004) and Miglietta et al. (2007). Schuchert (2004) gives this character a potential for differentiation from other species of the genus (where the presence of such points has never been registered), although he states that it needs to be re-evaluated. Here, we argue that this character has no robust diagnostic value, since brown spots were observed only in one individual and seemed to vanish after gametes were released. On the other hand, it is confirmed that the tips of the tentacles are always swollen in the specimens of this species, even when they are completely relaxed, and that the masses of vacuolated cells of the pseudopeduncle form four distinct groups. Similarly, our observations provide further evidence that medusa size never exceeds 3 mm, and that the top of the umbrella changes from rounded to slightly conical when the medusae still are in the eight-tentacled stage. The color of the manubrium is confirmed to be brownish, as suggested by Schuchert (2004), but our observations revealed that this character is variable depending on food items and digestion stage (in this case, the manubrium appeared slightly brown when it was empty, and acquired an orange color after ingesting *Artemia* nauplii).

Only male specimens were observed in the present study, but the mechanism underlying this phenomenon could not be clarified from our experiments. Most likely, the presence of an all-male cohort may be the result of environmentally dependent sex determination, at either the polyp or the medusa stage. Temperature-dependent sex determination in Hydrozoa has been shown to occur in several species of *Hydra* (both gonochoristic and hermaphroditic) (Littlefield 1994; Nishimiya-Fujisawa & Kobayashi 2012) and *Clytia* (Carré & Carré 2000). Alternatively, food availability together with temperature affects the sex ratio in species of *Hydra*, leading to a dominance of males or females in laboratory populations according to an interaction of the two factors (Kaliszewicz & Lipińska 2013). Furthermore, field observations in populations of

Eudendrium racemosum and *Eudendrium glomeratum* agree with a model of sex-determination dependent on environmental conditions (most likely temperature) for benthic hydrozoans in Mediterranean temperate waters, as they show the dominance of males or females in the sea is related to the sea water conditions (Arillo et al. 1989; Di Camillo et al. 2012). All of our colonies were growing at the same temperature conditions *in situ*, and they were all placed at the same temperature until medusa liberation in the laboratory; thus, it is possible that the determination of sex towards male specimens occurred already at the moment of release and regardless of culturing conditions of the medusae. A parallel situation has been observed in colonies of *Clytia hemisphaerica*, where medusae liberated at 15°C developed into males regardless of the temperature at which they were raised (either 15 or 24°C; Carré & Carré 2000).

Morphological differences observed between medusae reared at 14 and 25°C are attributable to the fact that at higher temperature the individuals have higher metabolic rates and therefore medusae grown in cold temperatures took longer to develop gonads and, on average, reached maturity with a larger body size. The presence of gonads was observed in some medusae reared at 24°C even in a 12-tentacled stage, while medusae reared at 14°C only showed the presence of gonadic primordia. Previous studies showed that *T. dohrnii* medusae reach sexual maturity after 25–30 days at 20°C, and after 18–22 days at 22°C, always at a 16-tentacle stage with a bell height of about 1.8–2 mm (Piraino et al. 1996; Carlà et al. 2003). In the present study, the tendency of reaching sexual maturity at early stages with increasing temperatures is confirmed.

Unlike medusae, the polyp colonies of *T. dohrnii* are indistinguishable from those belonging to the other species of the genus based only on morphological evidence. Furthermore, the polyp stage of the other medusa-producing genus of the Oceaniidae, *Oceania*, is not known from the field. Our current knowledge on the morphology of the polyps of several species of *Turritopsis* (such as *T. pleurostoma, T. lata, T. pacifica* and *T. polycirrha*) is scarce or lacking, and no set of characters has been identified as diagnostic of any of the other, better known species. Colony structure (erect vs. stolonal), degree of ramification of the colony (profusely branched vs. sparingly branched) and number of cenosarc tubes inside the hydrocaulus (monosiphonic vs. polysiphonic) have all been used as somewhat useful characters to discriminate the polyp stage of the species of *Turritopsis*, but a great deal of variation occurs in these characters even between colonies of the same

population, as observed here for *T. dohrnii*. Variation of colony structure and degree of ramification are likely to depend also on development and environmental factors, making it unreliable to use these characters as diagnostic for the identification of polyp colonies in *Turritopsis*. Schuchert (2004) noticed that *T. dohrnii* colonies tend to be larger than those of *T. nutricula*, but further information is needed to evaluate whether size may provide a clue for the differentiation of both species. According to Calder (1988a), the colonies of *T. dohrnii* are polysiphonic while those of *T. nutricula* are monosiphonic, but our observations and those of other authors (Migotto 1996; Schuchert 2004) show that both monosiphonic and polysiphonic colonies occur in the former species.

Understanding the life cycles of the remaining species of *Turritopsis* will help solve the problem of delimitation of species within the genus and will allow for a better comparison with the results of this study. We hope that further studies in this field will help clarify the limits of the species in *Turritopsis*, because we believe historical inconsistencies in the genus classification could be resolved once the life cycles of all species are better known. Particular attention should be paid to species whose polyp or medusa stage is unknown.

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References

Arillo A, Bavestrello G, Boero F. 1989. Circannual cycle and oxygen consumption in *Eudendrium glomeratum* (Cnidaria, Anthomedusae): Studies on a shallow water population. Marine Ecology 10:289–301. DOI:10.1111/j.1439-0485.1989.tb00074.x.

Bavestrello G, Sommer C, Sara M. 1992. Bi-directional conversion in *Turritopsis nutricula* (Hydrozoa). Scientia Marina 56:137–140.

Bouillon J, Gravili C, Pagès F, Gili J-M, Boero F. 2006. An introduction to Hydrozoa. Mémoires Du Muséum National d'Histoire Naturelle 194:1–591.

Calder DR. 1988a. *Turritopsoides brehmeri*, a new genus and species of athecate hydroid from Belize (Hydrozoa: Clavidae). Proceedings of the Biological Society of Washington 101:229–233.

Calder DR. 1988b. Shallow-water hydroids of Bermuda. The Athecatae. Life Sciences Contributions of the Royal Ontario Museum, Canada 148:1–107.

Carlà EC, Pagliara P, Piraino S, Boero F, Dini L. 2003. Morphological and ultrastructural analysis of *Turritopsis nutricula* during life cycle reversal. Tissue Cell 35:213–222. DOI:10.1016/S0040-8166(03)00028-4.

Carré D, Carré C. 2000. Origin of germ cells, sex determination, and sex inversion in medusae of the genus *Clytia* (Hydrozoa, Leptomedusae): The influence of temperature. Journal of Experimental Zoology 287:233–242. DOI:10.1002/1097-010X(20000801)287:3<233::AID-JEZ5>3.0.CO;2-F.

De Vito D, Piraino S, Schmich J, Bouillon J, Boero F. 2006. Evidence of reverse development in Leptomedusae (Cnidaria, Hydrozoa): the case of *Laodicea undulata* (Forbes and Goodsir 1851). Marine Biology 149:339–346.

Di Camillo CG, Betti F, Bo M, Martinelli M, Puce S, Vasapollo C, Bavestrello G. 2012. Population dynamics of *Eudendrium racemosum* (Cnidaria, Hydrozoa) from the North Adriatic Sea. Marine Biology 159:1593–1609. DOI:10.1007/s00227-012-1948-z.

Gravili C, De Vito D, Di Camillo CG, Martell L, Piraino S, Boero F. 2015. The non-Siphonophoran Hydrozoa (Cnidaria) of Salento, Italy with notes on their life-cycles: An illustrated guide. Zootaxa 3908:1–187. DOI:10.11646/zootaxa.3908.1.1.

Haines DD, Juhasz B, Tosaki A. 2013. Management of multicellular senescence and oxidative stress. Journal of Cellular and Molecular Medicine 17:936–957. DOI:10.1111/jcmm.2013.17.issue-8.

Kaliszewicz A, Lipińska A. 2013. Environmental condition related reproductive strategies and sex ratio in hydras. Acta Zoologica 94:177–183. DOI:10.1111/azo.2013.94.issue-2.

Kramp PL. 1961. Synopsis of the medusae of the world. Journal of the Marine Biological Association of the United Kingdom 40:1–469.

Littlefield CL. 1994. Cell-cell interactions and the control of sex determination in *Hydra*. Seminars in Developmental Biology 5:13–20. DOI:10.1006/sedb.1994.1003.

Miglietta MP, Lessios HA. 2009. A silent invasion. Biological Invasions 11:825–834. DOI:10.1007/s10530-008-9296-0.

Miglietta MP, Piraino S, Kubota S, Schuchert P. 2007. Species in the genus *Turritopsis* (Cnidaria, Hydrozoa): A molecular evaluation. Journal of Zoological Systematics and Evolutionary Research 45:11–19. DOI:10.1111/jzs.2007.45.issue-1.

Migotto AE. 1996. Benthic shallow-water hydroids (Cnidaria, Hydrozoa) of the coast of São Sebastião, Brazil, including a checklist of Brazilian hydroids. Zoologische Verhandelingen 306:1–125.

Neppi V, Stiasny G. 1913. Die hydromedusan des Golfes von Triest. Arbeiten Des Zoologischen Institutes Der Universität Wien 20:23–92.

Nishimiya-Fujisawa C, Kobayashi S. 2012. Germline stem cells and sex determination in *Hydra*. The International Journal of Developmental Biology 56:499–508. DOI:10.1387/ijdb.123509cf.

Östman C, Kem WR, Piraino S. 1991. Nematocysts of the Mediterranean hydroid *Halocordyle disticha*. Hydrobiologia 216/217:607–613. DOI:10.1007/BF00026520.

Piraino S, Boero F, Aeschbach B, Schmid V. 1996. Reversing the life cycle: Medusae transforming into polyps and cell transdifferentiation in *Turritopsis nutricula* (Cnidaria, Hydrozoa). Biological Bulletin 190:302–312. DOI:10.2307/1543022.

Piraino S, De Vito D, Schmich J, Bouillon J, Boero F. 2004. Reverse development in Cnidaria. Canadian Journal of Zoology 82:1748–1754. DOI:10.1139/z04-174.

Quiquand M, Yanze N, Schmich J, Schmid V, Galliot B, Piraino S. 2009. More constraint on ParaHox than Hox gene families in early metazoan evolution. Developmental Biology 328:173–187. DOI:10.1016/j.ydbio.2009.01.022.

Russell FS. 1953. The medusae of the British Isles: Anthomedusae, Leptomedusae, Limnomedusae, Trachymedusae and Narcomedusae. London: Cambridge University Press. Vol. 530. pp. 35 plates.

Sánchez-Alvarado A, Yamanaka S. 2014. Rethinking differentiation: Stem cells, regeneration, and plasticity. Cell 157:110–119. DOI:10.1016/j.cell.2014.02.041.

Schmich J, Kraus Y, De Vito D, Graziussi D, Boero F, Piraino S. 2007. Induction of reverse development in two marine Hydrozoans. The International Journal of Developmental Biology 51:45–56.

Schuchert P. 1996. The marine fauna of New Zealand: Athecate hydroids and their medusae (Cnidaria: Hydrozoa). New Zealand Oceanographic Institute Memoir 106:1–159.

Schuchert P. 2004. Revision of the European athecate hydroids and their medusae (Hydrozoa, Cnidaria): Families Oceanidae and Pachycordylidae. Revue Suisse De Zoologie 111:315–369. DOI:10.5962/bhl.part.80242.

Sinkovics JG. 2015. The cell survival pathways of the primordial RNA–DNA complex remain conserved in the extant genomes and may function as proto-oncogenes. European Journal of Microbiology and Immunology 5:25–43. DOI:10.1556/EuJMI-D-14-00034.

Weismann A. 1883. Die Entstehung der Sexualzellen bei den Hydromedusen. Zugleich ein Beitrag zur Kenntnis des Baues und der Lebenserscheinungen dieser Gruppe. Jena: Fischer. pp. i–xiii, 1–295.