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# Sexual reproduction of the soft coral *Dendronephthya castanea* (Alcyonacea: Nephtheidae)

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*Dendronephthya castanea* Utinomi, 1952 is a member of the family Nephtheidae, and dominates shallow waters adjacent to the southern part of Jejudo Island, Korea. This species is a gonochoric internal brooder with a sex ratio of 1:1, and releases planulae around the time of the full and new moon from July to September, when the seawater temperature peaks. The gametogenic cycle is annual, and oogenesis (12 months) is longer than spermatogenesis (4–5 months). No difference in reproductive features including sexuality, sex ratio, gametogenesis and gametogenic cycles was found between the sympatric species *D. castanea* and *D. gigantea*, and there was no temporal reproductive isolation. Investigation of the morphological taxonomy and molecular biology of these species indicates that they have very similar or identical traits, suggesting an absence of speciation and a need for taxonomic reclassification.

Keywords: sexual reproduction; gametogenesis; *Dendronephthya castanea*; soft coral; Anthozoa

## Introduction

Soft corals of the order Alcyonacea comprising five families, 79 genera and approximately 1145 species are fleshy mass octocorals with no skeletal axis (Daly et al. 2007), and are distributed from tropical to temperate regions. They are ecologically important and abundant members of the biodiversity in coral reef and other communities. These soft corals have three possible modes of sexual reproduction: broadcasting of gametes, internal brooding, and external surface brooding (Benayahu and Loya 1983; Farrant 1986; Benayahu et al. 1990; Benayahu 1991). The reproductive mode differs among the various families; most species of the family Alcyoniidae use the broadcast mode of reproduction, although the family Xeniidae shows only brooding behavior. In terms of sexuality, gonochorism predominates among soft coral species, although a few species show hermaphroditism (Benayahu et al. 1990; Benayahu 1991; Achituv et al. 1992; McFadden and Hochberg 2003; Hwang and Song 2007). The patterns of reproduction among alcyonaceans are summarized in Table 1.

Relative to the families Alcyoniidae and Xeniidae there is limited information available regarding sexual reproduction in the family Nephtheidae, even though this family contains almost half of the known soft coral species (approximately 500). In particular, sexual reproductions has been described for only three species of the genus *Dendronephthya* (Dahan and Benayahu 1997; Choi and Song 2007; Hwang and Song 2007), despite this genus having the majority of species (approximately 250) in the family Nephtheidae (Daly et al. 2007) and inhabiting tropical to temperate regions (Fabricius and Alderslade 2001).

Among the 250 species of the genus *Dendronephthya*, nine have been reported to occur in Korean waters (Song 1976; Rho and Song 1977). These are particularly abundant at 5–40 m depth adjacent to the southern coast of Jejudo Island, where the sympatric species *D. castanea* and *D. gigantea* dominate at 10–25 m depth; however, it is difficult to distinguish them because of their similar morphology (Figure 1). In this study we investigated the taxonomy of these two species by examining the reproductive features of *D. castanea*, including sexuality, reproductive mode, gametogenesis and the reproductive cycle, and compared these features with those previously documented for *D. gigantea*.

## Materials and methods

### Collection of specimens

Jejudo Island (Figure 1) is located in the temperate zone but has a somewhat subtropical climate because its coast is affected by the Tsushima Warm Current, which branches from the Kuroshio Current. Seawater temperature in this area ranges from 13 to 28°C (Korea Hydrographic and Oceanographic Administration; KHOA) depending on the season. *D. castanea* mainly inhabits rock surfaces at 5–30 m depth. Small samples of *D. castanea* (3–5 cm long) were cut from randomly selected colonies (height > 30 cm) at depths of 10–30 m (accessed using SCUBA) in Munseom Island between July 2003 and August 2007. Following collection the samples were anesthetized with menthol, fixed in 4–5% (v/v) neutral formalin diluted with seawater, and preserved in 70% (v/v) ethanol.

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Table 1. Reproductive pattern documented in the literature on Alcyonacea.

| Taxa | Number of species | Sexuality | | | | Mode of reproduction | | | | References |
|---|---|---|---|---|---|---|---|---|---|---|
| | | G | H | G/H | P | BS | EB | IB | U | |
| Alcyoniidae | 39 | 37 | 1 | – | 1 | 27 | 2 | 8 | 2 | 1,3,4,7,8,9,10,11, 15, 16 |
| *Alcyonium* | 11 | 9 | 1 | – | 1 | 5 | 1 | 4 | 1 | 1,3,8,9 |
| *Anthomastus* | 2 | 2 | – | – | – | – | – | 2 | – | 7, 16 |
| *Cladiella* | 1 | 1 | – | – | – | 1 | – | – | – | 4 |
| *Discophyton* | 1 | 1 | – | – | – | – | – | 1 | – | 9 |
| *Lobophytum* | 7 | 7 | – | – | – | 7 | – | – | – | 3, 11 |
| *Parerythropodium* | 1 | 1 | – | – | – | – | 1 | – | – | 3 |
| *Sarcophyton* | 5 | 5 | – | – | – | 5 | – | – | – | 3,10, 15 |
| *Sinularia* | 9 | 9 | – | – | – | 9 | – | – | – | 3 |
| *Thrombophyton* | 2 | 2 | – | – | – | – | – | 1 | 1 | 9 |
| Nephtheidae | 6 | 6 | – | – | – | 3 | 1 | 2 | – | 2,3,6,12,13,14 |
| *Litophyton* | 1 | 1 | – | – | – | – | – | 1 | – | 3 |
| *Scleronephthya* | 1 | 1 | | | | 1 | | | | 14 |
| *Dendronephthya* | 3 | 3 | – | – | – | 2 | – | 1 | – | 6,12,13 |
| *Capnella* | 1 | 1 | – | – | – | – | 1 | – | – | 2 |
| Xeniidae | 24 | 18 | 5 | 1 | – | – | 1 | 16 | 7 | 3,4,5 |
| *Xenia* | 15 | 12 | 3 | – | – | – | – | 10 | 5 | 3,4,5 |
| *Heteroxenia* | 4 | 1 | 2 | 1 | – | – | – | 4 | – | 3 |
| *Anthelia* | 2 | 2 | – | – | – | – | – | 1 | 1 | 3,4 |
| *Sympodium* | 1 | 1 | – | – | – | – | – | 1 | – | 3,4 |
| *Efflatounaria* | 1 | 1 | – | – | – | – | 1 | – | – | 3 |
| *Cespitularia* | 1 | 1 | – | – | – | – | – | – | 1 | 4 |

References: (1) Sebens 1983, (2) Farrant 1986, (3) Benayahu et al. 1990, (4) Benayahu 1991, (5) Achituv et al. 1992, (6) Dahan and Benayahu 1997, (7) Cordes et al. 2001, (8) McFadden et al. 2001, (9) McFadden and Hochberg 2003, (10) Schleyer et al. 2004, (11) Fan et al. 2005, (12) Choi and Song 2007, (13) Hwang and Song 2007, (14) Hwang and Song 2009, (15) Hellström et al. 2010, (16) Mercier and Hamel 2011.
BS, broadcast spawner; EB, external brooder; G, gonochoric; H, hermaphroditic; G/H, gonochoric or hermaphroditic; IB, internal brooder; P, parthenogenetic; U, unknown.

### Dissection

The preserved specimens were dissected using a stereo-microscope (Semi SV-6, Carl Zeiss) to investigate the sexuality of each colony and the external features of gametes. The gametogenic cycle was also investigated on a monthly basis by measuring the average length of the longest and shortest axes of > 30 gametes from each colony using an image analyzer (Motic Image Plus 2.0 instrument, Motic China Group Co.); the average axis length was calculated to enable comparisons among months. The seawater temperature near the collection site was recorded almost daily by the Korea Hydrographic and Oceanographic Administration (KHOA) throughout the study period.

### Images and data analysis

Images of living colonies in situ were taken using a digital camera (5060-WZ, Olympus) with an under-water housing (Patima-7070, Patima Uw_Eng Co. Ltd.), and images of the collected specimens were obtained using a digital camera (G-7, Canon) prior to fixation. All images of gametes were obtained using the Olympus camera attached to a stereomicroscope (Semi SV-6 or SV-11, Carl Zeiss) or a light microscope (BH-2, Olympus).

The overall sex ratio was determined by counting the number of female and male colonies during the study period; more accurate ratios were calculated using the colonies from June to August throughout the study period, when oocytes and spermaries were observed together. Deviation from a 1:1 sex ratio was tested using the $\chi^2$ goodness-of-fit test with the significance level set at $P < 0.05$. Statistical analysis was carried out using SPSS version 17.

### Results

### Sexuality and sex ratio

All the *D. castanea* colonies examined microscopically were gonochoric. Among the total of 157 colonies examined during the study period 87 were female, 44 were male, and 26 contained small or no gametes (Figure 2). Female colonies were significantly more abundant than male colonies (sex ratio of 2:1), in contrast to the expected 1:1 ratio (including inactive colonies: $\chi^2 = 31.132$, df = 2,
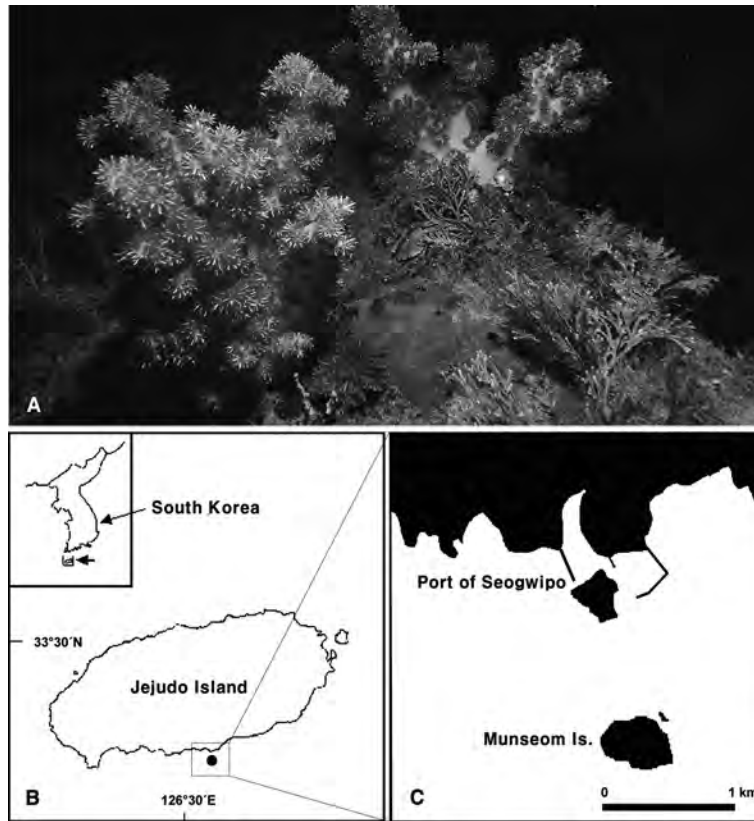
Figure 1. Colonies of sympatric species of *Dendronephthya* and map of study site. A. Colonies of *D. castanea* (left) and *D. gigantea* (right). B. Jejudo Island located in the southern part of Korea. C. Munseom Island located off the port of Seogwipo.

$P \leq 0.001$; excluding inactive colonies: $\chi^2 = 14.115$, df $= 1$, $P \leq 0.001$). However, male colonies were observed mainly from late spring or early summer to autumn, while female colonies were present throughout the year. Thus, only those colonies collected between June and August were used to calculate the sex ratio, excluding possible male colonies with small or no gametes. Among the 76 colonies of *D. castanea* collected between June and August in 2003–2007, 41 were female, 32 were male, and three were sexually inactive. These data indicated a female to male sex ratio of 1.3:1, which was not significantly different from the expected 1:1 ratio ($\chi^2 = 1.110$, df $= 1$, $P = 0.292$).

### Gametogenesis (gonad development)

Gametogenic development in *D. castanea* was similar to that of other alcyonaceans; the gametes originated in mesenterial filaments within the polyp cavity. Immature gametes initially connected by pedicles to the mesenteries were gradually transferred to the gastrovascular cavity, where they subsequently detached from the mesenteries as they matured. The spherical oocytes were evident by the presence of a well-developed follicular layer and a prominent nucleus with a single nucleolus. The oocytes tended to change color as they matured, from transparent to beige to ivory, and finally to vivid orange. The spermaries were initially transparent but became beige in color with maturity.

The gametes of *D. castanea* were classified into a number of oogenic (five) and spermatogenic (four) stages, depending on maturity (Table 2) and the processes involved (Figure 3). In the oogenic stage, the primary oocytes (stage I) were $\leq 45$ μm in diameter (mean size $35.3 \pm 6.9$ μm; mean $\pm$ SD; $n = 54$), were clustered within the mesenterial filaments, had large distinct nuclei, and were transparent as a consequence of the absence of cytoplasm. In stage II the nuclei of the oocytes were located on one side, and the color changed to beige as a result of the accumulation of cytoplasm. At this stage the oocytes became individually connected by pedicels to the mesenteries in the gastrovascular cavity, and their diameter ranged from 46 to 100 μm (mean $79.9 \pm 14.1$ μm; $n = 925$). In stage III yolk synthesis (vitellogenesis) began. Yolk bodies accumulated around the nuclei in the oocytes, resulting in a color change from beige to ivory. The maturing oocytes (diameter range 101–180 μm; mean $130.5 \pm 20.4$ μm; $n = 1947$) became detached from the
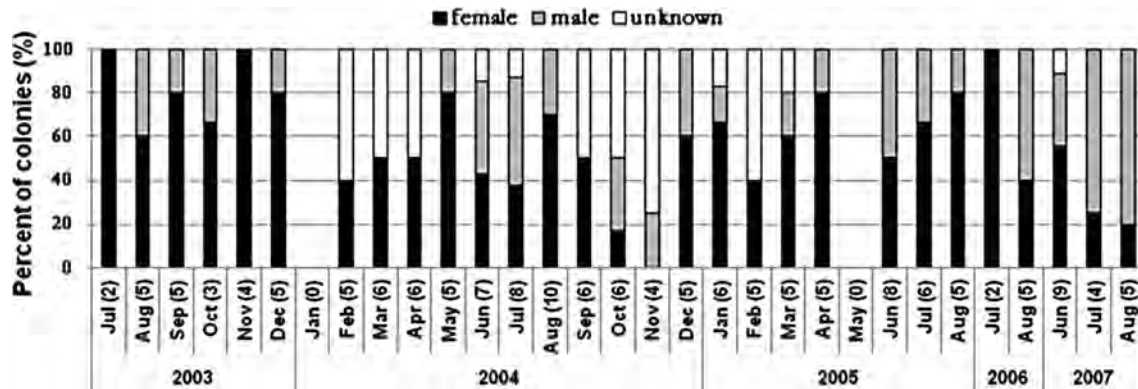
Figure 2. *Dendronephthya castanea*. Percentage of colonies containing ooctyes, spermaries, and no gametes from 2003 to 2007. Number of colonies used in each month are indicated in parentheses. Colonies with no gamete are mentioned as unknown.

mesenteries and entered a cavity covered by well developed follicular layers. In stage IV the oocyte size rapidly increased because of ongoing yolk synthesis, and the follicular layers became thicker. Synthesized yolk bodies were apparent throughout the oocytes, resulting in a color change to orange. Late vitellogenic oocytes ranged from 181 to 299 μm in diameter (mean $235.3 \pm 34.8$ μm; $n = 724$). In stage V the mature oocytes were filled with yolk, resulting in a color change to vivid orange. These oocytes were $\geq 300$ μm to 490 μm in diameter (mean $355.8 \pm 37.6$ μm; $n = 601$).

In stage I of spermatogenesis, the immature spherical spermaries were embedded in mesenteries containing clusters of spermatogonia, and their boundaries were indistinct. The spermaries were transparent in color and $\leq 45$ μm in diameter (mean $34.1 \pm 7.1$ μm; $n = 67$). In stage II the boundaries of the spermaries were distinct and they moved into the cavity, where they remain attached to the mesenteries by pedicles. The spermaries were opaque and filled with developing spermatocytes, and ranged in diameter from 46 to 160 μm (mean $110.6 \pm 30.6$ μm; $n = 497$). In stage III the spermatocytes were arranged peripherally, resulting in hollow centers, and began to develop into spermatids. The spermaries changed from opaque to beige in color as the spermatids accumulated, and late in this stage the spermaries and mesenteries disconnected. The diameter of the sperm-

aries in this stage ranged from 161 to 269 μm (mean $211.9 \pm 29.4$ μm; $n = 746$). In stage IV the spermaries were mature, with diameters $\geq 270$ to 431 μm (mean $308.2 \pm 32.9$ μm; n = 226). Late in this stage, the spermaries were deep beige colored and not spherical, and the spermatids metamorphosed into spermatozoa.

### Annual reproductive cycle

*D. castanea* had a single annual reproductive cycle with a clear difference between females and males. While spermaries were only detected from late spring to early autumn (typically from May to September), oocytes were observed throughout most of the year during the study period; the exception was November 2004, when no female colonies were collected.

In females stage I and II oocytes were found throughout most of the study period, although their relative frequency changed seasonally (Figure 4). In 2003 the sum of the relative frequencies of these two stages was comparatively low ($0.1 \pm 0.04$; mean $\pm$ SD; n = 5) between July and November, but peaked in December and remained high until the following March (mean $0.4 \pm 0.03$; $n = 3$). A similar pattern was evident in the successive years to August 2005. The frequency of these two stages declined sharply to 0.07 in April 2004, and remained low until late August (mean $0.1 \pm 0.08$;

Table 2. *Dendronephthya castanea*. Range and mean diameter of gametes, which depends on stages ($n =$ number of gametes at each stage).

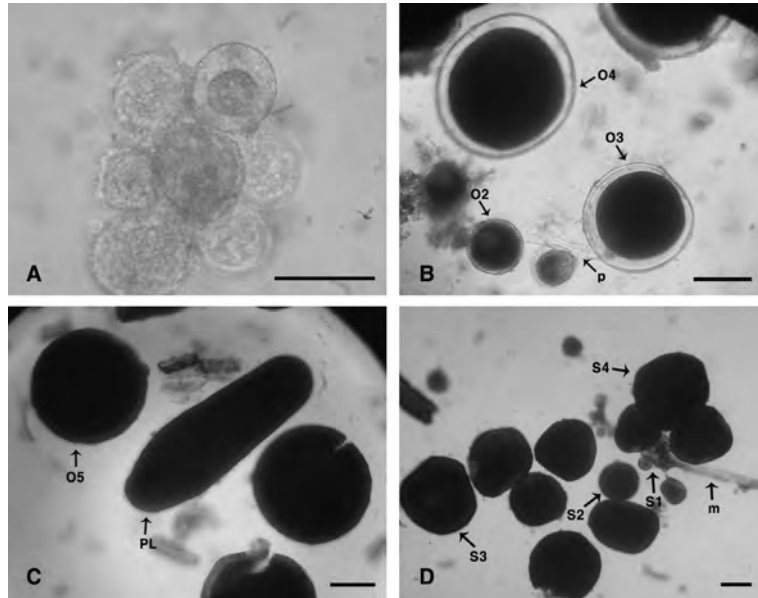| Stage | Oocytes | | | Spermaries | | |
|---|---|---|---|---|---|---|
| | Range (μm) | Mean $\pm$ SD (μm) | $n$ | Range (μm) | Mean $\pm$ SD (μm) | $n$ |
| I | $\leq 45$ | $35.3 \pm 6.9$ | 54 | $\leq 45$ | $34.1 \pm 7.1$ | 67 |
| II | 46–100 | $79.9 \pm 14.1$ | 925 | 46–160 | $110.6 \pm 30.6$ | 497 |
| III | 101–180 | $130.5 \pm 20.4$ | 1947 | 161–269 | $211.9 \pm 29.4$ | 746 |
| IV | 181–299 | $235.3 \pm 34.8$ | 724 | $\geq 270$ | $308.2 \pm 32.9$ | 226 |
| V | $\geq 300$ | $355.8 \pm 37.6$ | 601 | | | |

Figure 3. *Dendronephthya castanea.*. Development of gametes. A. Cluster of stage I oocytes. B. Stage II oocyte connected to mesentery by pedicle and stage III and stage IV enveloped with follicular layer. C. Fully matured stage V oocyte and planula. D. Cluster of stage I spermaries connected to mesentery and stage II to stage IV spermaries. Scale bar = 50 μm (A) and 100 μm (B–D). m, mesentery; O1, stage I oocyte; O2, stage II oocyte, O3, stage III oocyte, O4, stage IV oocyte, O5, stage V oocyte, p, pedicle, PL, planula, S1, stage I spermary, S2, stage II spermary, S3, stage III spermary; S4, stage IV spermary.

$n = 6$). In September the frequency increased (peaking at 0.8 during October) and remained high until the following March (mean $0.5 \pm 0.18$; $n = 7$), but between April and August 2005 the frequency of these two oocyte stages was low ($0.1 \pm 0.04$; $n = 4$). Throughout every year in the study stage I and II oocytes increased in frequency and then declined. In particular, the frequency of stages I and II was sharply increased in December 2003 and September 2004, when stage IV and V oocytes were no longer observed. The frequency of stage III oocytes also fluctuated throughout the year. From 2003 to 2005 their frequency was high prior to and following summer (mean $0.6 \pm 0.18$; $n = 17$), and low during summer (July and August; mean $0.1 \pm 0.03$; $n = 7$). The frequency of this stage peaked in April 2004 and 2005 (0.9 and 0.7, respectively), and then decreased steadily to August ($<$ 0.1). In stage IV late vitellogenic oocytes were mainly observed between April and September during the study period (mean frequency $0.3 \pm 0.15$, $n = 14$), and tend to be present at high frequencies ($0.4 \pm 0.08$; $n = 9$) during summer, with a peak in June. Fully mature stage V oocytes were found from June to November, were abundant in July and August (mean frequency $0.5 \pm 0.04$; $n = 7$), and peaked in frequency in August from 2003 to 2005; after this the stage IV oocytes peaked in frequency. Planulae and mature oocytes were observed within the cavity in August 2004, suggesting the reproductive mode was internal brooding.

In males, all the four spermary stages were observed together during brief periods that fluctuated somewhat among the study years from 2003 to 2005 (Figure 5). The sum of the relative frequencies of stages I and II peaked in May 2004 (1.0) and June 2005 (0.7), but in 2003 the frequency peak observed in December was an outlier caused by observation of only two immature spermaries. Maturing (stage III) and mature (stage IV) spermaries were mainly observed from June to August during the study period, with mean frequencies of $0.5 \pm 0.18$ ($n = 7$) and $0.2 \pm 0.16$ ($n = 7$), respectively.

***Planulation***

The release of internally brooded planulae from the mother colonies of *D. castanea* was observed in both field and aquarium specimens from July to August 2003, in August 2004, and from August to September in 2006, when mature oocytes (stage V) were largely found (Figure 3C). In the field, release of ciliated orange planulae occurred during the full and new moon periods in July and August 2003, during the last quarter of the moon in August 2004, and during the new and full moon in August and September 2006, respectively. Planulation of *D. castanea* was observed to co-occur with planulation of *D. gigantea*, and the planulae were not morphologically distinguishable. In aquaria the planulae were released continuously for
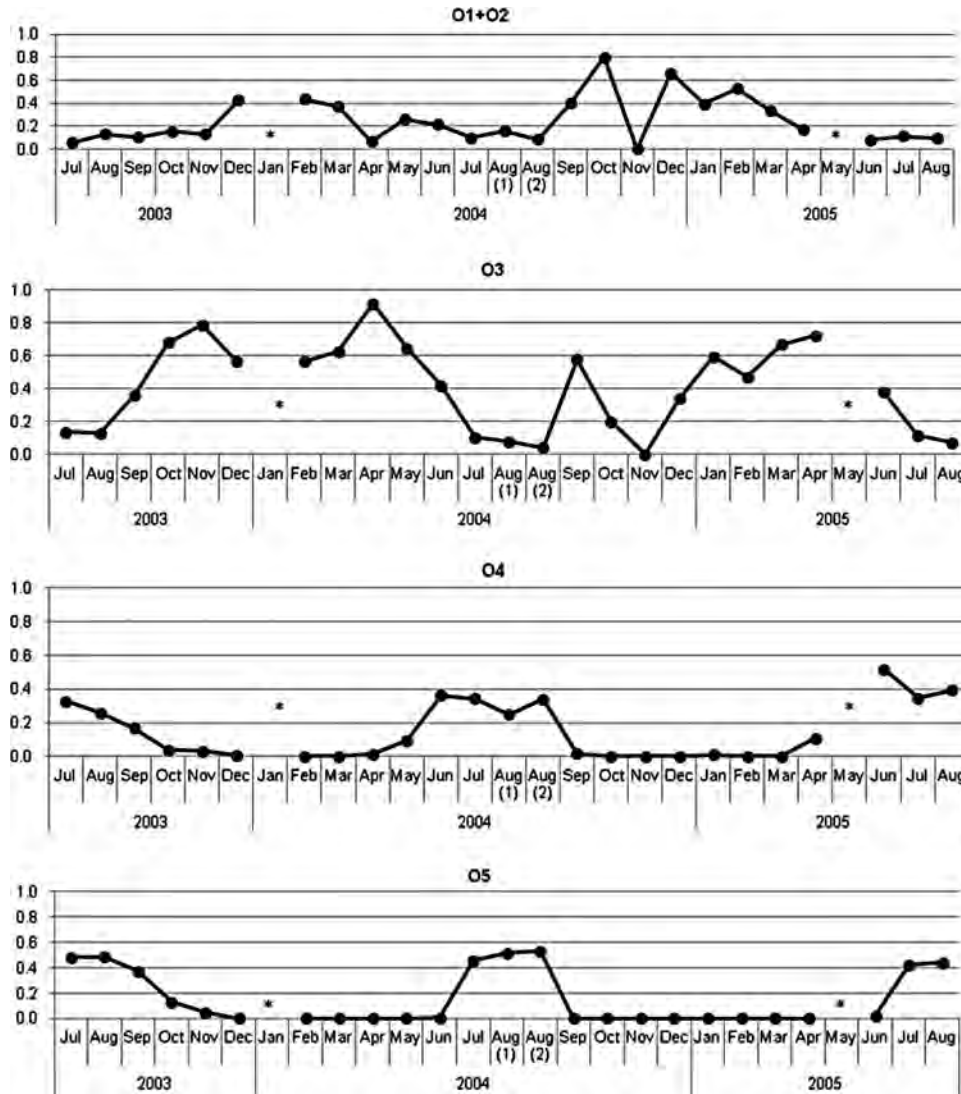
Figure 4. *Dendronephthya castanea*. Monthly frequency of each oogenic stage from July 2003 to August 2005 ($n = 4251$ oocytes). O1, stage I oocytes; O2, stage II oocytes, O3, stage III oocytes, O4, stage IV oocytes, O5, stage V oocytes, *, month with no collection.

## Discussion

Including the present study, reproduction of four species of the genus *Dendronephthya* has been reported, and three of them have been described from Jejudo Island (North Pacific Ocean) and one has been described from Eilat, in the Red Sea (Dahan and Benayahu 1997; Choi and Song 2007; Hwang and Song 2007). The reproductive features of these four species are summarized in Table 3.

All colonies of *D. castanea* examined in this study were gonochoric, as previously reported for most octocorals including soft corals and gorgonians (Kinzie

1970; Grigg 1977; Benayahu et al. 1990; Brazaeu and Lasker 1990; Achituv et al. 1992; Lasker et al. 1996; Zeevi and Benayahu 1999; Orejas et al. 2007; Seo et al. 2008). Several members of the Alcyoniidae and Xeniidae are hermaphroditic, and a low level of hermaphroditism has been reported in the gonochoric species *Sarcophyton glaucum* (Sebens 1983; Benayahu et al. 1990; Benayahu 1991; Schleyer et al. 2004), but all the species belonging to the family Nephtheidae are gonochoric (Farrant 1986; Benayahu et al. 1990; Dahan and Benayahu 1997; Choi and Song 2007; Hwang and Song 2007, 2009).

With respect to sex ratio, female colonies of *D. castanea* were more abundant than male colonies during the entire study period (ratio 2:1), but during the breeding period (June to August) the sex ratio was
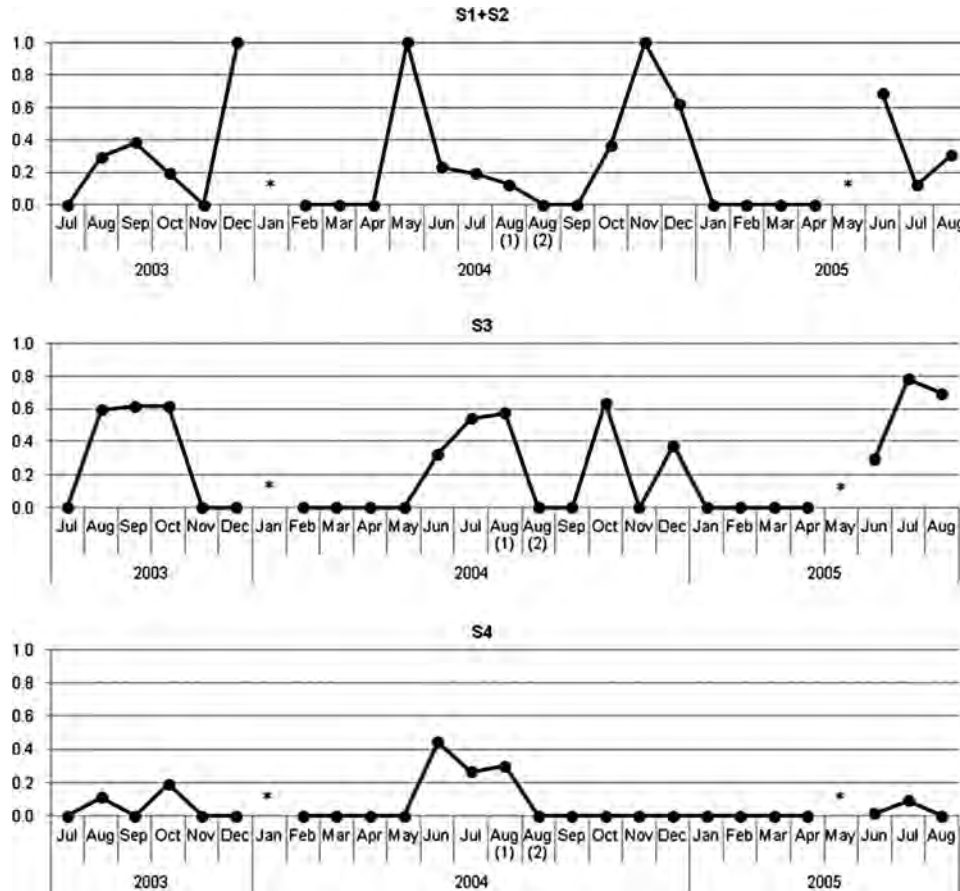
Figure 5. *Dendronephthya castanea*. Monthly frequency of each spermatogenic stage from July 2003 to August 2005 (n = 1,536 speramries). S1, stage I spermaries, S2, stage II spermaries; S3, stage III spermaries; S4, stage IV spermaries;*, month with no collection.

1:1. This pattern was also observed in the sympatric species *D. gigantea*. However, in spawners the sex ratio is biased towards females in *D. hemprichi*, but is almost equal in *D. spinulosa* (unpublished data). Choi and Song (2007) reported that in *D. suensoni* the sex ratio is biased towards females, but reproductively inactive colonies were more abundant than the male colonies during their study, suggesting the possibility of an equal ratio. Other octocoral spawners that occur in Jejudo Island, including *Anthoplexaura dimorpha* and *Calicogorgia granulosa*, showed a sex ratio biased towards females, but *Scleronephthya gracillimum* showed an almost equal ratio (Cho 2008; Seo et al. 2008; Hwang and Song 2009). These studies strongly suggest no correlation between sex ratio and mode of reproduction in the soft corals. The sex ratios in some gorgonian species differ depending on the geographical region. For example, the sex ratio of *Corallium rubrum* is 1:1 in Costa Brava (Spain), but is biased towards females in Italy, and for both *Paramuricea clavata* and *Eunicella singularis* the ratio is 1:1 at the Medes Island, while at Cape of Palos it is biased towards males for *P. clavata*

and females for *E. singularis* (Santangelo et al. 2003; Tsounis et al. 2006; Gori et al. 2007). The sex ratio within a population may be related to population density rather than the reproductive mode for achieving optimal fertilization (Brazeau and Lasker 1990; Santangelo et al. 1993; Tsounis 2005). *D. castanea* is extremely dominant in the study area together with *D. gigantea*, and the sympatric broadcasting species *S. gracillimum* is also very abundant (Hwang and Song 2009).

The reproductive mode of both *D. castanea* and *D. gigantea* is internal brooding. The patterns of gamete formation and arrangement in *D. castanea* are similar to those of other soft corals (Benayahu 1991; Cordes et al. 2001; McFadden and Hochberg 2003; Hwang and Song 2007, 2009). The size of gametes in the oogenic stages was almost the same for *D. castanea* and *D. gigantea*, while the gametes in each of the spermatogenic stages were somewhat larger in *D. castanea* (Table 3; Hwang and Song 2007). The size of dendronephthyan gametes does not show a strong correlation with the mode of reproduction. The mature oocytes of the brooders *D. castanea* and *D. gigantea* ($356 \pm 38$ μm and $346 \pm 30$ μm,

Table 3. Comparison of reproductive features among four dendronephthyans

| Reproductive features | D. castanea | D. gigantea | D. hemprichi | D. suensoni |
|---|---|---|---|---|
| Sexuality | G | G | G | G |
| Sex ratio of female to male (whole/specific period) | 2:1/1.3:1 (Jun to Aug) | 1.5:1/0.9:1 (Jun to Aug) | 3:2 (year-round) | 2:1 |
| Mode of reproduction | IB | IB | BS | BS |
| Oogenic/spermatogenic period (month) | 12/4–5 | 12/3–5 | Spermatogenesis rather rapid | 12/6 |
| Size of matured oocyte/spermary (mean) (μm) | 300–490(356) /270–431(308) | 300–480(346) / < 220(256) | 260–500/–400 | > 190 (249)/ > 160 (226) |
| Gametogenic cycles/year | 1 | 1 | Year-round | 1 |
| Timing of planulation or spawning | Jul–Sep | Jul–Sep | Year-round | Sep–Oct |
| References | Present work | Hwang and Song 2007, present work | Dahan and Benayahu 1997 | Choi and Song 2007 |

BS, broadcast spawner; G, gonochoric; IB, internal brooder; U, unknown.

respectively) are larger than in the spawner *D. suensoni* (249 ± 36 μm), but the mature oocytes of *D. hemprichi* are > 260 μm (maximum 500 μm), and other tropical and subtropical corals including *Anthelia glauca*, *Sarcophyton glaucum*, *S. elegans* and *Lobophytum pauciflorum* have mature oocytes > 500 μm in size, suggesting a latitudinal gradient effect on the size of gametes (Benayahu and Loya 1986; Dahan and Benayahu 1997; Kruger et al. 1998; Fan et al. 2005; Hellström et al., 2010). In the case of *C. rubrum* the gamete size varies depending on the depth and region, with gamete diameters being greater in shallow water and at low latitudes, suggesting the influence of a temperature gradient (Tsounis 2006).

The gametogenic cycle was seasonal, and a difference between female and male colonies was apparent, with longer oogenesis (biorhythms of 12 months) and shorter spermatogenesis (4–5 months). This is generally consistent with gametogenesis observed in octocorals (Benayahu and Loya 1986; Brazaeu and Lasker 1990; Hwang and Song 2007, 2009; Seo et al. 2008; Hellström et al., 2010), although spermaries and oocytes are present together throughout the year in some soft corals (Kruger et al. 1998; Fan et al. 2005). The gametogenic cycle of *D. castanea* was directly correlated with seasonal factors including seawater temperature, with marked fluctuations in the monthly mean diameter of gametes associated with changing seawater temperature throughout the study period (Figure 6). Planulation events were also observed and others may have taken place when the seawater temperature reached the annual peak between August and September. The regulation of sexual reproduction by this environmental factor is known in sympatric soft corals and the corals of temperate regions (Harii et al. 2001; Neves and Pires 2002; Vermeij et al. 2004; Choi and Song 2007; Hwang and Song 2007, 2009). The survival

and settlement of planulae is also enhanced in warmer water, resulting in increased reproductive success (Ben-David-Zaslow and Benayahu 1996; Nozawa and Harrison 2000; Fan et al. 2005). Planulation in *D. castanea* was also correlated with full and new moon phases of the lunar cycle, as has been reported for *D. gigantea* and many other soft corals (Kruger et al. 1998; Schleyer et al. 2004; Hwang and Song 2007).

*D. castanea* differs morphologically from *D. gigantea* in colony growth form and anthocodial grade, which are major classification characters for the genus *Dendronephthya*. All specimens in this study had the same anthocodial grade, coinciding with the original description of *D. castanea* from Korean waters, but the specimens had a glomerate and not an umbellate form, which differs from previous records (Utinomi 1952; Rho and Song 1977). The holotype of *D. castanea* by Utinomi (1952) and specimens described by Rho and Song (1977) were very small ( < 5 cm in height) relative to our specimens ( > 30 cm), and Utinomi (1952) described different branching of the glomerate form in a large colony (paratype). In addition, the growth form may be misleading as it can vary depending on the size and condition (expanded or contracted) of the colony. Therefore, it was not surprising that our specimens were morphologically identified not as a variant of *D. gigantea* but as *D. castanea*, although they were glomerate. However, we did not find differences between *D. castanea* and *D. gigantea* in terms of reproductive features including sexuality, reproductive mode, and the timing of reproduction (suggesting reproductive isolation) throughout the study. In addition, although the evolution of mitochondrial DNA sequences is slow in the Anthozoa by the mismatch repair gene (msh1) and the relatively recent divergence times among octocorals (Pont-Kingdon et al. 1995; Shearer et al. 2002; Kim et al. 2008), the mitochondrial DNA sequences (18,730 bp) of
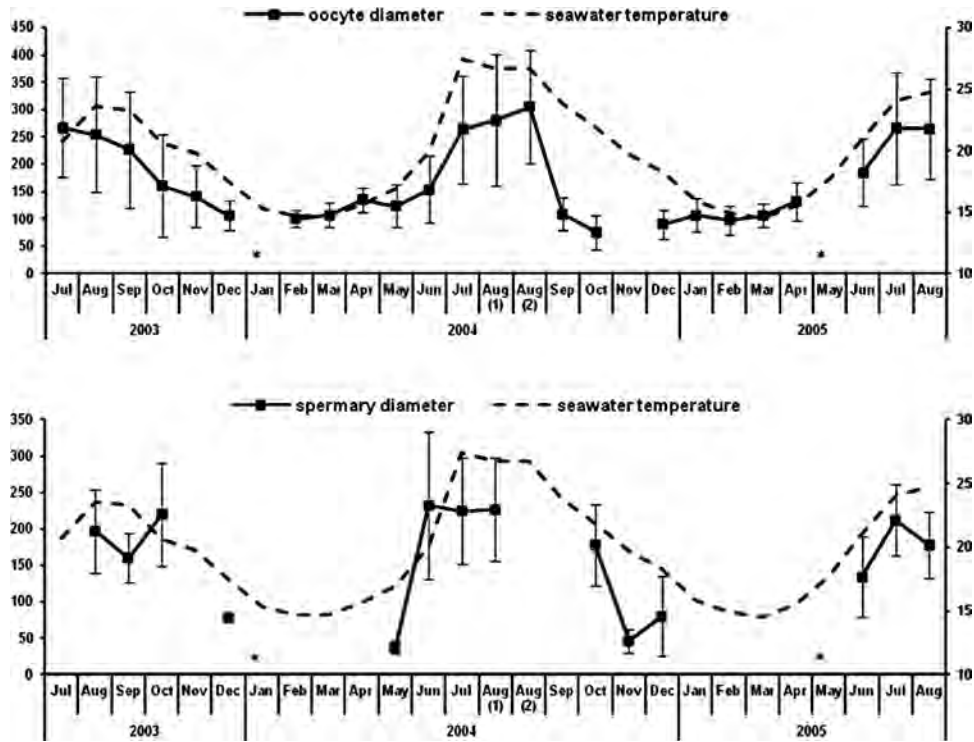
Figure 6. *Dendronephthya castanea*. Monthly mean diameter of oocytes and spermaries according to the seawater temperature (error bar = SD). Left and right axes indicate mean diameter and seawater temperature, respectively. *, months with no collection.

these two species are identical (Kim 2008). Thus, these several studies strongly indicate the absence of speciation between *D. castanea* and *D. gigantea*, and consequently suggest that *D. castanea* be incorporated into the species *D. gigantea*.

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