**ORIGINAL PAPER**

# A silent invasion

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**Abstract** Invasions mediated by humans have been reported from around the world, and ships' ballast water has been recognized as the main source of marine invaders worldwide. Some invasions have dramatic economic and ecological consequences. On the other hand, many invasions especially in the marine realm, can go unnoticed. Here we identify a human mediated, worldwide introduction of the hydrozoan species *Turritopsis dohrnii*. The normal life cycle of hydrozoans involves the asexual budding of medusae from colonial polyps. Medusae of *Turritopsis*, however, when starved or damaged, are able to revert their life cycle, going back to the polyp stage through a process called transdifferentiation. They can thus easily survive through long journeys in cargo ships and ballast waters. We have identified a clade of the mitochondrial 16S gene in *Turritopsis* which contains individuals collected from Japan, the Pacific and Atlantic coasts of Panama, Florida, Spain, and Italy differing from each other in only an average of 0.31% of their base-pairs. Fifteen individuals from Japan, Atlantic Panama, Spain, and Italy shared the same haplotype. *Turritopsis dohrnii* medusae, despite the lack of genetic differences, are morphologically different between the tropical and temperate locations we sampled, attesting to a process of phenotypic response to local conditions that contributes to making this grand scale invasion a silent one.

**Keywords** Invasive species · Morphological response · Hydrozoa · *Turritopsis* · Medusa

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## Introduction

Invasions mediated by humans have been reported from around the world, and ships' ballast water has been recognized as the main source of marine invaders worldwide (Carlton 1989; Carlton and Geller 1993, Cohen and Carlton 1998). They are often recognized for their ecological impact on native species and represent an opportunity to gain crucial insight into ecological and evolutionary processes (Sax et al. 2007). Some invasions have dramatic economic and ecological consequences. Examples include the invasion of the American ctenophore *Mnemiopsis* into the Black Sea, which caused the collapse of anchovy fishery in 1990 (Brodeur et al. 2002), of the brown tree snake (*Boiga irregularis*) in Guam, which caused the extinction of many bird

species (Savidge 1987), and of the zebra mussel (*Dreissena polymorpha)* in the North American Great Lakes, whose fouling activity on underwater machinery causes millions of dollar of damage every year (Pimentel et al. 2005). Despite the attention that invasions have received, exotic species, especially in the marine realm, can go unnoticed. This is due to several reasons: inconspicuousness of the invasive organism, unrecognized impact on the native species assemblages, or morphological differentiation of the invasive populations from the source population. Instances of morphological modifications of a species in the invaded area can be the outcome of phenotypic plasticity or rapid adaptive evolutionary divergence (see Huey et al. 2000).

Molecular data have been a useful tool for recognizing exotic species (Holland et al. 2004; Holland 2000), but the degree to which invasive species may go unnoticed is still a mostly unaddressed issue. Moreover, documented cases of invaders showing new morphological features in the invaded range are rare and mostly restricted to terrestrial species. Adaptive differentiation in color and size was demonstrated in the house sparrow in North America and the Hawaiian Islands after its introduction from Europe (Johnston and Selander 1964). Evolutionary diversification was documented in *Drosophila suboscura* after its introduction to the New World (Huey et al. 2000). Size increases in invading populations have been documented in marine organisms (Grosholz and Ruiz 2003). However, a phenotypic response of the basic morphology of an invasive species to the exotic environment has not, to our knowledge, yet been reported in the marine realm.

Hydromedusae are inconspicuous members of the phylum Cnidaria, yet they represent one of the most widespread and diverse components of gelatinous plankton. They are produced from benthic colonies of polyps by asexual budding (See Fig. 1). Starved or damaged medusae of most hydrozoan species perish, but those of the genus *Turritopsis* can undifferentiate into a benthic cyst that subsequently reverts into a new polyp, capable of asexually releasing new medusae (Bavestrello et al. 1992, Piraino et al. 1996, 2004) (Fig. 1). The capability of reversing the life cycle, also known as transdifferentiation, grants *Turritopsis* potential immortality (Piraino et al.

1996). Thus, for species of *Turritopsis*, the medusae of which can survive extreme environments and lack of food by reverting into an undifferentiated cluster of cells, the probability of being transported through ballast water outside their native range is potentially very high.

Here we compare mitochondrial haplotypes of *Turritopsis* collected around the world to assess the degree to which genetic exchange occurs between distant oceans and seas. Morphological data of the medusa stage are also used to determine local response of the medusae to different environmental conditions in which they may find themselves.

## Material and methods

### Field collection and morphological analyses

To assess the invasiveness of *Turritopsis* in oceans around the world we collected medusae and polyps from the Atlantic and Pacific coasts of Panama, and from Florida during 2006–7. We sequenced a 600 bp segment of the mitochondrial 16S gene in these samples and analyzed them along with previously published ones from Southern Japan (Okinawa), Northern Japan (Kagoshima and Fukushima Prefectures), Italy (Apulia), New Zealand, Tasmania, Spain (Mallorca and Andalucia) and the Eastern United States (Massachusetts) (Miglietta et al. 2007) (Appendix 1).

Medusae and polyps of the genus *Turritopsis* were collected from Panama Bay (eastern Pacific) (weekly form February 2006 to April 2007), Bocas del Toro, off the Atlantic coast of Panama (monthly form February 2006 to April 2007), Galeta, on the Atlantic coast of Panama (June 2006) and Ft. Pierce, Florida (May and October 2006). Polyps were collected while SCUBA diving or snorkeling; medusae by towing a 0.85 µm mesh plankton net (Aquaticeco, model number: DNP8). Samples and localities are listed in appendix 1. Morphology of the medusae was examined in the laboratory using a stereo microscope. Number of tentacles of the adult and juvenile medusae was recorded for each individual. When fertile colonies were collected by SCUBA diving, newly released medusae were analyzed as they detached from the colonies in the laboratory. The
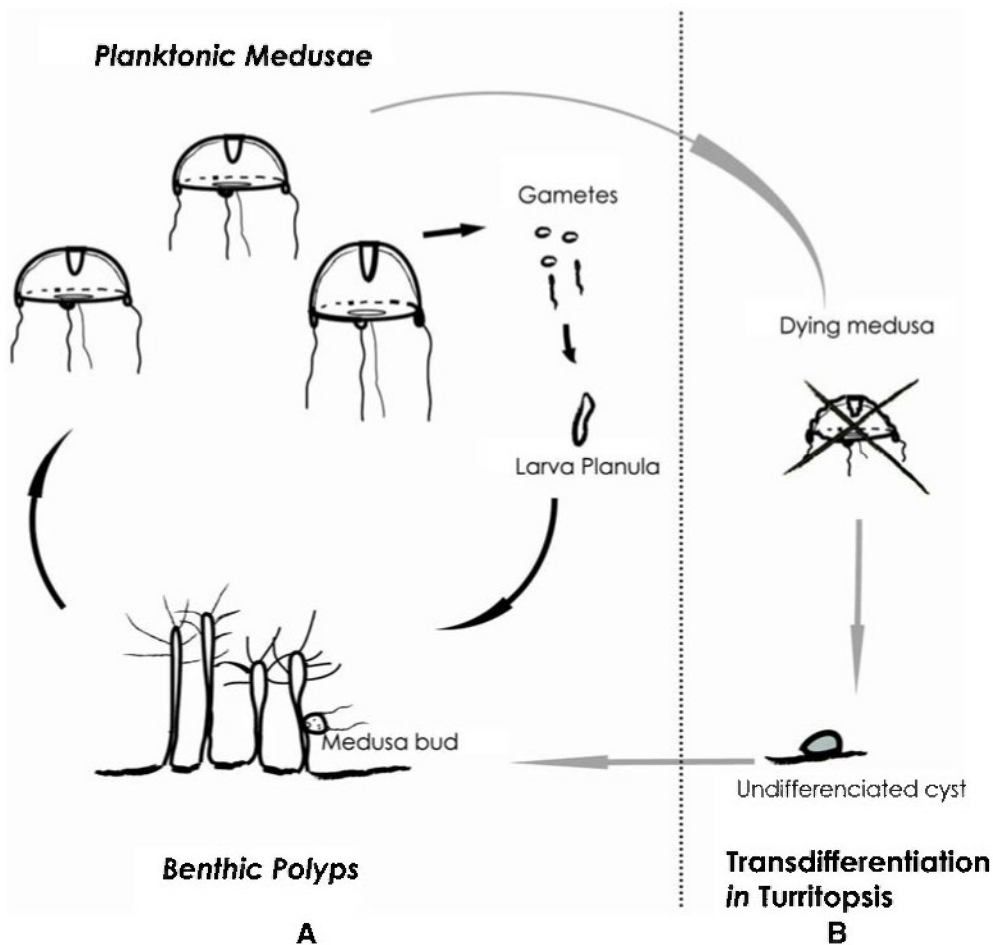
**Planktonic Medusae**

Gametes

Dying medusa

Larva Planula

Medusa bud

Undifferenciated cyst

**Benthic Polyps**

**Transdifferentiation in Turritopsis**

**A**

**B**

**Fig. 1** **a** Basic hydrozoan life cycle. Benthic, colonial polyps asexually produce medusa buds that will develop into fully formed planktonic medusae. The adult medusae release eggs and sperm in the water column, then die. The resulting larva will settle on the appropriate substrate to metamorphose into a new polyp. **b** Transdifferentiation in *Turritopsis*. Starved or damaged medusae of *Turritopsis* can de-differentiate into a benthic cyst that subsequently reverts into a new polyp colony, capable of asexually releasing new medusae

number of tentacles was also recorded in these individuals.

PCR, sequencing and sequence alignment

Total genomic DNA was extracted from ethanol-preserved specimens (polyps or medusae) following an adapted version of the protocol described by Oakley and Cunningham (2000) or by using the QIAamp 96 DNA Blood Kit. A 600 bp fragment of the mitochondrial 16S gene was amplified using the Polymerase Chain Reaction (PCR). Primers used were SHA 5′-ACGGAATGAACTCAAATCATG T-3′ and SHB 5′-TCGACTGTTTACCAAAAACA TA-3′ (Cunningham and Buss 1993). The mitochondrial 16S gene is a useful genetic marker, routinely used for species level identification in the Hydrozoa (Miglietta et al. 2007, Schuchert 2005, Cunningham and Buss 1993). PCR reactions were set as following: 1.5 μl of each 10 μM primers, 2.5 μl of 10x buffer, 2.5 μl of 25 mM MgCl, 2.5 μl of 10 mM dNTP, 0.3 μl of Taq in a total volume of 25 μl. Amplification took place under the following PCR conditions: 1 min at 94°C, then 35 cycles of 94°C for 15 s, 50°C for 1:30 min and 72°C for 2:30 min, with a final extension at 72°C for 5 min. PCR products were

purified using exoSAP-it digestion carried out using 0.8 μl of 10 μg/μl Exo and 1.5 μl of 1 μg/μl SAP in 20 μl of the PCR reaction. Samples were incubated at 37°C for 30 min. and, then at 80°C for 15 min. The purified PCR product was run on a 2% agarose gel stained with ethidium bromide to assay its quantity and quality (i.e. accessory bands). The purified PCR product was used as a template for double stranded sequencing using the amplification primers. DNA sequencing was performed using an ABI 3130 XL automated DNA sequencer.

The sequences were edited using SEQUENCHER v. 2.4 (Gene Codes) and aligned using ClustalX (Thompson et al. 1997). Alignments were confirmed and edited by eye in MACCLADE v. 4.05 (Maddison and Maddison 2000). Sequences were deposited in GenBank under accession numbers EU624348–EU624393.

Phylogenetic analysis

A total of 23 new sequences from Pacific and Atlantic Panama and Florida were analyzed along with a total of 23 published sequences of *Turritopsis* from Southern and Northern Japan, Italy, New Zealand, Spain, Tanzania, and the Eastern United States (Miglietta et al. 2007). Phylogenetic analyses were conducted with Maximum Parsimony (MP), Maximum Likelihood (ML) and Neighbor Joining (NJ) methods. MP heuristic searches and NJ analyses were performed with PAUP* version 4.0b10 for Macintosh (Swofford 2002) and ML heuristic searches both with PAUP* v. 4.0b10 and GARLI 0.951 (Zwickl 2006). Support for individual nodes was assessed using 100 (ML) or 1000 (MP and NJ) bootstrap replicates. For ML and NJ analyses, the model for best nucleotide substitution was selected using the hierarchical criterion as implemented in Modeltest 3.7 (Posada and Crandall 1998). The best-fit model was HKY (Hasegawa et al. 1985) + G + I with gamma correction (alpha = 0.4542).

Within and between group Kimura 2-parameter average distances were calculated in MEGA 3.1 (Kumar et al. 2004). Groups were defined as the seven clades of mtDNA resulting from the phylogenetic analyses, each of which is assumed to represent a separate species of *Turritopsis* (Fig. 2).

Results

The tree topology from the ML analysis in GARLI (Fig. 2) was identical to the ML tree, the NJ and the MP trees in PAUP*. The NJ, MP and ML bootstrap analyses recovered similar bootstrap supports for all the nodes (Fig. 2). The genus comprises 7 distinct clades (Fig. 2), three of which have been identified, respectively, as *Turritopsis dohrnii* (initially described from Italy), *T. rubra* (New Zealand and Tasmania and Northern Japan), *T. nutricula* from the NE coast of the United States. Four clades are still undescribed: *T.* sp.1 was collected in Bocas del Toro (Atlantic Panama); *T.* sp.2 has been reported from Japan and is morphologically identical to *T. dohrnii* from the Mediterranean Sea (Miglietta et al. 2007), *T.* sp.3 was found in Andalucia, Spain (Miglietta et al. 2007) and *T.* sp.4 in Bocas del Toro, Atlantic Panama.

The molecular analysis of *Turritopsis* shows a compact clade (0.31% within species diversity by Kimura 2- parameter distance) containing individuals from Apulia, Italy, from Mallorca, Spain (Mediterranean Sea), from Okinawa (Japan, Pacific Ocean), from Bocas del Toro and Galeta (Atlantic coast of Panama,), from Panama Bay (Pacific coast of Panama) and from Fort Pierce (Atlantic coast of Florida) (Fig. 2). This mitochondrial DNA clade belongs to *Turritopsis dohrnii* (see Schuchert 2006). Individuals possessing indistinguishable haplotypes were found at Bocas del Toro, Panama (10 individuals), Japan (3 individuals), and the Mediterranean Sea (2 individuals). When placed into a worldwide phylogeny of *Turritopsis*, this geographically heterogeneous clade formed a well-supported monophyletic unit (Neighbor Joining, Maximum Parsimony and Maximum Likelihood Bootstrap support of 100%) distinct from the other presumed species of this genus. By way of contrast, between-species divergence in the genus, ranging from 1.39 to 10.13% (Kimura 2-parameter distance), is at least one order of magnitude larger than within-clade divergence in *T. dohrnii* (Table 1).

A total of 259 adult medusae (see Table 2) from Bocas del Toro and Galeta (Atlantic Panama), and the Bay of Panama (Pacific Panama) were examined, and all of them showed 8 tentacles. Three mature
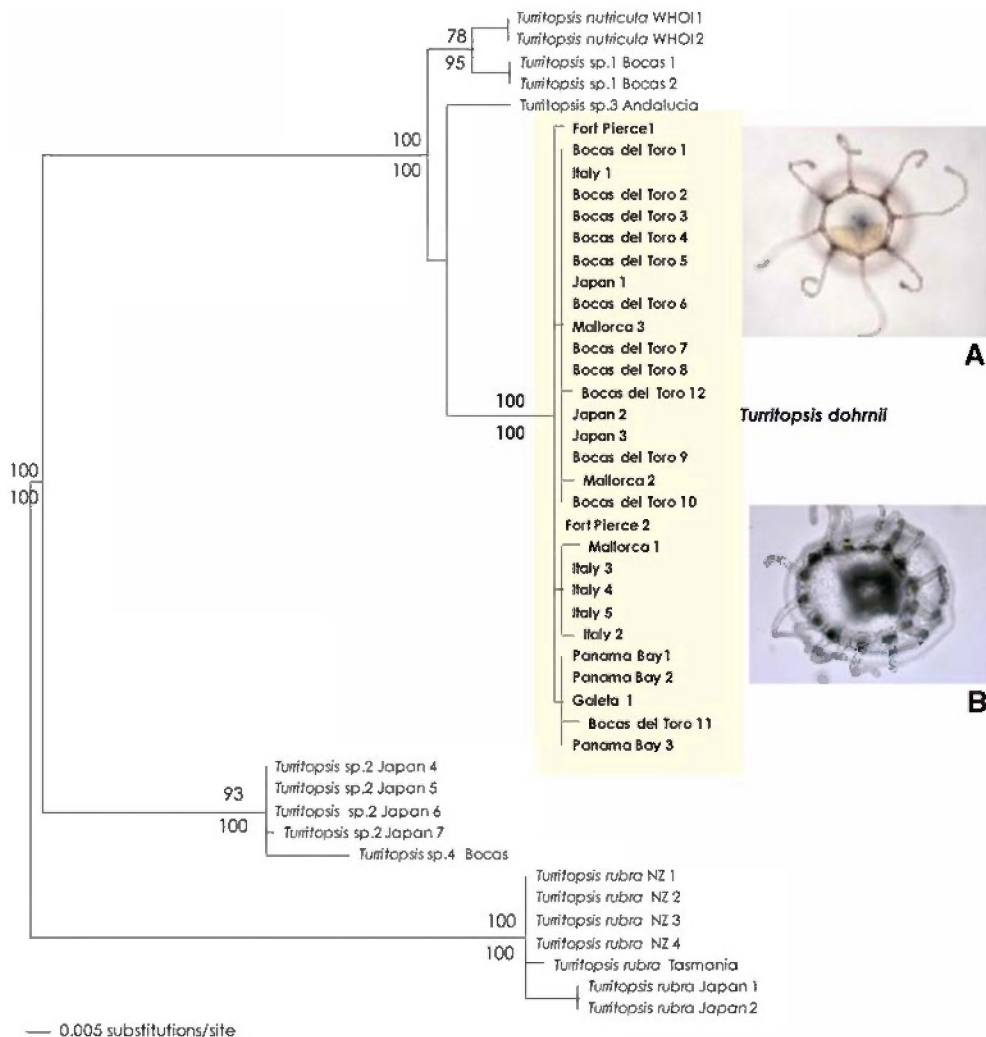
**Fig. 2** Maximum Likelihood tree of the genus *Turritopsis* based on 600 bp of the 16S mitochondrial RNA gene. For each node the Maximum Parsimony (below the node) and Maximum Likelihood (above the node) bootstrap supports are reported (100 replicates in ML, 1000 in MP). The clade corresponding to *Turritopsis dohrnii* is shaded. It is composed of individuals from Bocas del Toro and Galeta (Atlantic Panama), Bay of Panama (Pacific Panama), Mallorca (Mediterranean Sea—Spain), Apulia (Mediterranean sea—Italy), Fort Pierce (Atlantic—Florida) and Okinawa (Japan). On the right side the two medusa morphs: **a** adult medusa with 8 tentacles from Panama Bay; **b** adult medusa with 19 tentacles from Florida

*T. dohrnii* medusae collected in Florida had 12, 15 and 19 tentacles.

## Discussion

With the use of molecular tools we recognize a clade of *Turritopsis* that comprises, within 0.31% genetic diversity (Kimura 2-parameter), individuals from Apulia, Italy and Mallorca, Spain (Mediterranean Sea), from Okinawa (Japan, Pacific Ocean), from Bocas del Toro and Galeta (Atlantic coast of Panama), from Panama Bay (Pacific coast of Panama) and from Fort Pierce (Atlantic coast of Florida). The close genetic similarity between individuals of *Turritopsis dohrnii* from distant localities is consistent with its being an invasive species that has spread in the world's oceans. Although the life cycle of this species comprises a medusa that is planktonic and could potentially be broadly dispersed, its life cycle cannot account for such

**Table 1** Within and between group (species) Kimura 2-parameter distances in *Turritopsis*.

|  | [1] (%) | [2] (%) | [3] (%) | [4] (%) | [5] (%) | [6] (%) | [7] (%) |
|---|---|---|---|---|---|---|---|
| [1] *T. nutricula* | 0 | – | – | – | – | – | – |
| [2] *T.* sp.3 Spain | 3.13 (17) | n/c | – | – | – | – | – |
| [3] *T.* sp.1—Atlantic | 1.39 (8) | 2.57 (14) | 0 | – | – | – | – |
| [4] *T. dohorni*/invasive | 3.65 (20) | 3.13 (16.4) | 4.04 (21.4) | 0.31 (1.6) | – | – | – |
| [5] *T.* sp.2 Japan | 7.92 (43.5) | 8.1 (42) | 8.11 (44.5) | 8.28 (42.9) | 0.09 (0.5) | – | – |
| [6] *T.* sp.4—Atlantic | 8.26 (45) | 8.78 (46) | 8.26 (45) | 8.95 (46.1) | 1.62 (9.25) | n/c | – |
| [7] *T. rubra* | 10.06 (54) | 9.74 (50.8) | 10.06 (54.9) | 10.13 (52.8) | 8.85 (48.4) | 9.66 (52.1) | 0.59 (3.4) |

Between group average distance values are below the diagonal; within group diversity values along the diagonal (both in %). In parentheses: Average differences expressed in number of variable nucleotides

**Table 2** Medusae of *Turritopsis dohrnii* collected in Panama during 2006 and 2007.

| Locality | N. of Medusae | Average n. of Tentacles | Standard Deviation |
|---|---|---|---|
| *Panama Bay* | 225 | 8 | 0 |
| *Bocas del Toro, Colon* | 27 | 8 | 0 |
| *Galeta* | 7 | 8 | 0 |
| *Tot.* | 259 | 8 | 0 |

Number of medusae observed in each locality, average number of tentacles, and standard deviation

low within-clade genetic diversity spread over such a wide geographic range. Studies on other Cnidaria suggest that the presence of the planktonic medusae does not translate into genetic homogeneity (See Dawson and Jacobs 2001, Govindarajan et al. 2005, Boero and Bouillon 1993). More specifically, it has been shown that the non-invasive, cosmopolitan hydrozoan species *Obelia geniculata* (despite the dispersal potential of a fully functional and long-lived planktonic medusa) displays significant genetic structure in the same fragment of the 16S mitochondrial RNA gene used here (Govindarajan et al. 2005). In the world-wide phylogeny of *O. geniculata* that comprises samples from various localities in the Pacific and Atlantic ocean, three distinct clades could be identified (Govindarajan et al. 2005). Each of these clade contained haplotypes unique to each location. Divergence between clades of *Obelia* ranged from 2.3 to 2.7% (Kimura 2-parameter distance). The potential immortality of *Turritopsis dorhnii* medusae is not likely to confer additional dispersal potential to the species by currents, because the cysts into which the medusae metamorphose are benthic. Moreover, the capability of transdifferentiation has also been recorded in *Turritopsis* sp. 2 from Japan (Fig. 2), yet this species has remained confined to a single locality. Thus, the observed geographic spread of low within-clade genetic diversity found in *T. dohrnii*, can only be explained as the outcome of recent gene flow around the globe, and the most likely method of such conveyance is travel of individuals between the hemispheres in ballast waters of cargo ships (as medusae) or on ships hulls (as polyps).

As the volume of global trade increases, the rate of establishment of exotic species is also expected to become more frequent (Cohen and Carlton 1998; Mooney and Cleland 2001). Despite this increasingly strong trend, a limited number of world-wide marine invaders have been recognized thus far. *Turritopsis dohrnii* thus represents one of very few reported cases of an invertebrate as a global invader. The pattern of its spreading is similar to that observed in the invasive bryozoan *Bugula neritina* (Mackie et al. 2006), a single haplotype of which was found in various localities in the Pacific (Australia, Hong Kong, Hawaii, California) and Atlantic (Curacao, England, and the Atlantic coast of the United States).

The capability of *T. dohrnii* medusae to reverse their life cycle makes this species an excellent hitchhiker in ballast waters. However, at least one of the sampling locations (Bocas del Toro) is located 300 km from the Atlantic entrance of the Panama Canal, far away from any major harbors or shipping lanes. The invading trajectory of *T. dohrnii* is thus expanding beyond the main ship traffic routes.

Despite the lack of variation in 16S, the mature medusae of this species show local morphological

differences. *Turritopsis dohrnii* medusae from tropical waters of Bocas del Toro, Galeta and Panama Bay always have eight tentacles (total of 259 medusae examined, see Table 2). Italian and Japanese *Turritopsis* are two of the best-studied hydrozoan systems in the world, and thus plenty of reports on their morphology are available. Mature medusae from the Mediterranean Sea have an average of 16 tentacles, with a minimum of 12 and a maximum of 24 (4000 medusae observed by Piraino et al. 1996, Piraino pers. comm.). Medusae in Japan have 14–24 tentacles (hundreds of medusae observed in Japan over a century, see Kubota 2005 for a review) and are thus very similar to the Mediterranean ones (Miglietta et al. 2007). Three mature *T. dohrnii* medusae we collected in Florida had 12, 15 and 19 tentacles and thus were similar to the forms from Italy and Japan. The rest of our genetic sample from Florida consists of polyps.

Our data demonstrate that medusae of *Turritopsis dohrnii* have spread across the world's oceans. Its medusae show no morphological variation within the tropical local populations but exhibit well-defined differences between the temperate and tropical localities herein studied, thus showing a process of local phenotypic response. That the two tropical populations (Atlantic and Pacific Panama) do not show morphological differences suggests the possibility that tentacle number is a response to similar climatic conditions. Consistent with this conclusion is the observation that the Italian population is morphologically similar to the Japanese population. However, in Japan native *Turritopsis* sp. 2 and invading *T. dohrnii* are found in sympatry and look very similar (Miglietta et al. 2007), so the identification of the exotic species is possible only by molecular means.

Despite the difference in tentacle numbers, no other morphological differences were noted between the populations. The number of tentacles in Hydroidomedusae is known to be a plastic character and to increase with age (or growth) (Bouillon et al. 2006). Newly released *Turritopsis* medusae recorded from Panama and Florida had 8 tentacles, the same number as all newly born medusae of *Turritopsis* from elsewhere (see in particular Piraino et al. 1996,

Schuchert 2006, Kubota 2005 for Mediterranean and Japanese medusae). Whereas medusae from Italy, Japan and Florida grow into adults with more tentacles, mature medusae from Bocas del Toro, Galeta and the Bay of Panama are retaining their juvenile features, possibly through a process of heterochrony (i.e. change in the timing of gonad development versus somatic development, as defined by Gould (1977)).

Changes in size have been recorded in the European green crab *Carcinus maenas* after its introduction in the West Coast of the United States (Grosholz and Ruiz, 1996) and in 11 additional species of marine invertebrates out of 19 investigated by Grosholz and Ruiz (2003). A change in reproductive mode was recorded in the sea anemone *Diadumene lineata,* which outside its native range reproduces only asexually (Fukui 1995). However, a case of basic morphological change of a marine invasive species in the introduced environment, like the one observed for the *Turritopsis* medusae, has never been previously reported.

The implication of our results is twofold: they identify a worldwide marine invader, and document a rapid process of local morphological response. They also provide insight in the presence of invasive species that can go unnoticed due to their rapid morphological change in a new geographic area. Although studies of global scope are expensive and logistically difficult, case studies that result from collaborations between scientists in different locations around the world are needed in order to draw general conclusions on the frequency of large-scale invasions (Zabin et al. 2007). Wordwide "silent invasions" like the one observed in *Turritopsis* may be more common than previously thought.

## Appendix

Examined material: sequence name as it appears on the tree (Fig. 1), localities, collection dates, type of material (polyp or medusa) and GenBank accession numbers. Sequences from Miglietta et al. (2007) are shaded

| Sequence Name | Species Identification | Locality | Date | Material | GenBank Accession Number |
|---|---|---|---|---|---|
| *Turritopsis* sp.1 Bocas1 | *Turritopsis* sp.1 | Atlantic, Panama, Bocas del Toro | 9-Apr-06 | Medusa | EU624351 |
| *Turritopsis* sp.1 Bocas2 | *Turritopsis* sp.1 | Atlantic, Panama, Bocas del Toro | 9-Apr-06 | Medusa | EU624352 |
| *Turritopsis* sp.4 Bocas | *Turritopsis* sp.4 | Atlantic, Panama, Bocas del Toro | 1-Oct-07 | Polyps | EU624379 |
| Fort Pierce 1 | *Turritopsis dohrnii* | Atlantic, Florida, Fort Pierce | 27-Apr-06 | Polyps | EU624353 |
| Bocas del Toro 1 | *Turritopsis dohrnii* | Atlantic, Panama, Bocas del Toro | 6-Jan-06 | Medusa | EU624354 |
| Bocas del Toro 2 | *Turritopsis dohrnii* | Atlantic, Panama, Bocas del Toro | 24-Jul-2006 | Polyps | EU624356 |
| Bocas del Toro 3 | *Turritopsis dohrnii* | Atlantic, Panama, Bocas del Toro | 21-Apr-06 | Polyps | EU624357 |
| Bocas del Toro 4 | *Turritopsis dohrnii* | Atlantic, Panama, Bocas del Toro | 20-May-06 | Polyps | EU624358 |
| Bocas del Toro 5 | *Turritopsis dohrnii* | Atlantic, Panama, Bocas del Toro | 18-Nov-05 | Polyps | EU624359 |
| Bocas del Toro 6 | *Turritopsis dohrnii* | Atlantic, Panama, Bocas del Toro | 4-Feb-06 | Polyps | EU624369 |
| Bocas del Toro 7 | *Turritopsis dohrnii* | Atlantic, Panama, Bocas del Toro | 20-May-06 | Polyps | EU624371 |
| Bocas del Toro 8 | *Turritopsis dohrnii* | Atlantic, Panama, Bocas del Toro | 21-May-06 | Medusa | EU624373 |
| Bocas del Toro 9 | *Turritopsis dohrnii* | Atlantic, Panama, Bocas del Toro | 18-Nov-05 | Polyps | EU624391 |
| Bocas del Toro 10 | *Turritopsis dohrnii* | Atlantic, Panama, Bocas del Toro | 1-Dec-07 | Polyps | EU624393 |
| Fort Pierce 2 | *Turritopsis dohrnii* | Atlantic, Florida, Fort Pierce | 12-Oct-06 | Polyps | EU624361 |
| Panama Bay 1 | *Turritopsis dohrnii* | Pacific, Panama, Panama Bay | 20-Apr-07 | Medusa | EU624366 |
| Panama Bay 2 | *Turritopsis dohrnii* | Pacific, Panama, Panama Bay | 20-Apr-07 | Medusa | EU624367 |
| Galeta 1 | *Turritopsis dohrnii* | Atlantic, Galeta, Panama | 5-Jun-06 | Medusa | EU624368 |
| Bocas del Toro 11 | *Turritopsis dohrnii* | Atlantic, Panama, Bocas del Toro | 1-Oct-07 | Polyps | EU624372 |
| Bocas del Toro 12 | *Turritopsis dohrnii* | Atlantic, Panama, Bocas del Toro | 27-Jul-06 | Polyps | EU624374 |
| Panama Bay 3 | *Turritopsis dohrnii* | Pacific, Panama, Panama Bay | 7-Jul-06 | Medusa | EU624390 |
| Mallorca 1 | *Turritopsis dohrnii* | Mediterranean, Mallorca, Cala Murada | 15-Jul-97 | Polyps | EU624362 |
| Mallorca 2 | *Turritopsis dohrnii* | Mediterranean, Mallorca, Cala Murada | 16-Aug-00 | Polyps | EU624392 |
| Mallorca 3 | *Turritopsis dohrnii* | Mediterranean, Mallorca, Cala Murada | 22-Aug-99 | Polyps | EU624370 |

**Appendix** continued

| Japan 1 | *Turritopsis dohrnii* | Japan Okinawa Island | Early March 2003 | Polyps | EU624360 |
|---|---|---|---|---|---|
| Japan 2 | *Turritopsis dohrnii* | Japan | 7-Nov-02 | Polyps | EU624387 |
| Italy 1 | *Turritopsis dohrnii* | Mediterranean, Italy, Apulia | Nov-02 | Polyps | EU624355 |
| Japan 3 | *Turritopsis dohrnii* | Japan, Okinawa Island | Early March 2003 | Polyps | EU624388 |
| Italy 2 | *Turritopsis dohrnii* | Mediterranean, Italy, Apulia | 7-Nov-02 | Polyps | EU624389 |
| Italy 3 | *Turritopsis dohrnii* | Mediterranean, Italy, Apulia | 7-Nov-02 | Polyps | EU624363 |
| Italy 4 | *Turritopsis dohrnii* | Mediterranean, Italy, Apulia | 7-Nov-02 | Polyps | EU624364 |
| Italy 5 | *Turritopsis dohrnii* | Mediterranean, Italy, Apulia | 7-Nov-02 | Polyps | EU624365 |
| *Turritopsis nutricula* WHOI 1 | *Turritopsis nutricula* | USA, MA, Woods Hole | 1-Oct-01 | Polyps | EU624348 |
| *Turritopsis nutricula* WHOI 2 | *Turritopsis nutricula* | USA, MA, Woods Hole | 1-Oct-01 | Polyps | EU624349 |
| *Turritopsis* sp.2 Japan 4 | *Turritopsis* sp.2 | Japan - Kagoshima , Kyushu | 6-Nov-02 | Medusa | EU624375 |
| *Turritopsis* sp.2 Japan 5 | *Turritopsis* sp.2 | Japan - Kagoshima , Kyushu | 6-Nov-02 | Medusa | EU624376 |
| *Turritopsis* sp.2 Japan 6 | *Turritopsis* sp.2 | Japan - Kagoshima, Kyushu | 6-Nov-02 | Medusa | EU624377 |
| *Turritopsis* sp.2 Japan 7 | *Turritopsis* sp.2 | Japan - Tanabe Bay | 18-Jul-03 | Medusa | EU624378 |
| *Turritopsis* sp3. Andalucia | *Turritopsis* sp.3 | Mediterranean, Spain, Andalucia, Las Negras | 28-Jul-03 | Polyps | EU624350 |
| *Turritopsis rubra* NZ1 | *Turritopsis rubra* | New Zealand, Wellington Harbour | 12-Jul-02 | Polyps | EU624380 |
| *Turritopsis rubra* NZ2 | *Turritopsis rubra* | New Zealand, Hauraki Gulf | 29-Jul-02 | Medusa | EU624381 |
| *Turritopsis rubra* NZ3 | *Turritopsis rubra* | New Zealand, Hauraki Gulf | 29-Jul-02 | Medusa | EU624382 |
| *Turritopsis rubra* NZ4 | *Turritopsis rubra* | New Zealand, Hauraki Gulf | 29-Jul-02 | Medusa | EU624383 |
| *Turritopsis rubra* Tasmania | *Turritopsis rubra* | Australia, Tasmania, Hobart | 8-Jun-04 | Medusa | EU624385 |
| *Turritopsis rubra* Japan 1 | *Turritopsis rubra* | Japan –Fukushima Prefecture | 25-Jun-09 | Medusa | EU624384 |
| *Turritopsis rubra* Japan 2 | *Turritopsis rubra* | Japan–Fukushima Prefecture | 25-Jun-09 | Medusa | EU624386 |

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