

# Phenotypic diversity of *Thuridilla hopei* (Vérany, 1853) (Gastropoda Heterobranchia Sacoglossa). A DNA-barcoding approach

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## ABSTRACT

The sacoglossan *Thuridilla hopei* (Vérany, 1853) shows highly diverse chromatic patterns. Based on the morphological examination of specimens from different Mediterranean localities, we have observed that in spite of this great variability in colours of *T. hopei*, two major chromatic morphotypes are related to bathymetry. Specimens from deeper water exhibit blue darker and more uniform patterns than individuals from shallower water, which show a more variable, dashed and spotted arrangement of light blue, yellow, orange, white and black pigmentation. A molecular genetic analysis using the mitochondrial COI and 16S rDNA markers has confirmed that all these extremely different chromatic morphotypes belong to a single specific entity, i.e. *T. hopei*, a sacoglossan with a wide distribution, from Macaronesia in the Atlantic, to the easternmost Mediterranean Sea.

## KEY WORDS

Sacoglossa; *Thuridilla hopei*; colour morphotypes; Mediterranean Sea; Atlantic Ocean.

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## INTRODUCTION

The plakobranchid sacoglossan genus *Thuridilla* Bergh, 1872 is represented in the northeastern Atlantic and in the Mediterranean Sea by two species, *T. hopei* (Vérany, 1853) (Carmona et al., 2011; Malaquias et al., 2012) and the amphiatlantic *T. mazda* Ortea et Spinosa, 2000, recently recorded from the Azores (Malaquias et al., 2012). The brightly coloured *T. hopei* lives from the lower intertidal down to about 35 m depth, and generally is found crawling on hard substrate. It feeds suctorially on photophilous algae, in particular on *Derbesia tenuissima* (de Notaris) Crouan et Crouanand

*Cladophora vagabunda* (Linnaeus) van den Hoek (Marín & Ros, 2004; Händeler & Wägele, 2007; Händeler, 2011) and it retains functional algal chloroplasts from its food for few days (Marín & Ros, 1989, 2004). The aposematic chromatic pattern of this slug is related to the presence in its tissues of toxic compounds like the diterpenoids thuridillin A, B and C and nor-thuridillin (Gavagnin et al., 1993; De Rinaldis, 2012).

During the last decade, the high levels of intraspecific chromatic variation of *T. hopei* has been well documented in literature (Trainito, 2005; Händeler, 2011; Carmona et al., 2011 and references therein), as well as depicted in the Sea Slug

Forum (Australian Museum, Sydney, Available from <http://www.seaslugforum.net/>). It is worth of mentioning that the Macaronesian specimens of *T. hopei*, have long been ascribed to the Caribbean *T. picta* (Verrill, 1901), due to a similar colour pattern (see Cervera et al., 2004; Malaquias et al., 2009), until Carmona et al. (2011) demonstrated by genetic data that they belong to *T. hopei*.

We have observed and documented the chromatic variability of *T. hopei* at different Mediterranean localities, particularly along the Italian coast. Interestingly, we observed that the distribution of different chromatic phenotypes is related to bathymetry, as previously reported by Cattaneo-Vietti (1990) and Händeler (2011). Specimens from deeper water exhibit dark blue and more uniform patterns (“bluish” form according to Händeler, 2011: 37) than individuals from shallower water, which show a more dashed and spotted arrangement of light blue, yellow, orange, white and black pigmentation (“rosy” form according to Händeler, 2011: 37). Different colour types have been usually considered as chromatic morphotypes of *T. hopei*, despite the consistent chromatic differences linked to their bathymetry (Carmona et al., 2011).

Therefore, we wanted to either confirm or deny the conspecificity of “bluish” and “rosy” forms by genetic data, being the significance of chromatic differences difficult to evaluate based on morphological data alone. In this study we applied a molecular analysis, using two mitochondrial markers, the barcode COI and the 16S rDNA, to the different colour morphotypes of *T. hopei*, to test the hypothesis that “bluish” and “rosy” specimens of these snails belong to the same species and confirm the lusitanic distribution of this sacoglossan sea slug.

## MATERIAL AND METHODS

Collection data, vouchers and accession numbers are listed in Table 1. Italian specimens from several localities were collected by hand during SCUBA diving at different depth. Each specimen was photographed in situ and/or in aquarium, then fixed in ethanol.

A piece of tissue was dissected from the foot for DNA extraction, and the remaining animal was deposited at the Department of Biology and Biotechnologies “Charles Darwin” (La Sapienza Rome

University). DNA was extracted using a standard proteinase K phenol/chloroform method with ethanol precipitation, as reported in Oliverio & Mariottini (2001). A fragment of the mitochondrial 16S rDNA was amplified by PCR using the universal primers 16Sar-L and 16Sbr-H (Palumbi et al., 2001), while a fragment of the mitochondrial cytochrome oxidase I (COI) was amplified using the universal primers LCO1490 and HCO2198 (Folmer et al., 1994); for PCR conditions see Prkić et al. (2014). All amplicons were sequenced by Genechron Centre of Sequencing, ENEA (La Casaccia, Rome, Italy) or by European Division of Macrogen Inc. (Amsterdam, The Netherlands), using the same PCR primers.

Forward and reverse sequences were assembled and edited, and the resulting consensus sequences of each specimen were readily aligned by hand. BLAST search was always conducted for each sequence. Published sequences (COI and 16S) of *Thuridilla* were downloaded from the GenBank. Although the definition of a phylogenetic hypothesis for the genus *Thuridilla* was not within the aims of this paper, nevertheless, phylogenetic relationships among the *Thuridilla* sequences were inferred to have a phylogenetic framework for the estimation of the genetic distances: we used neighbour-joining (NJ) and maximum likelihood (ML) (both bootstrapped over 1000 replicates), by the software MEGA 5.0 (Tamura et al., 2011) and Bayesian Inference (BI) by the software MrBayes (with  $5 \times 10^6$  generations, and 25% burnin) by MrBayes 3.2.2 (Ronquist et al., 2012). Sequences of the Atlantic *Elysia timida* Risso, 1818, retrieved from the GenBank, were used as outgroup. Nodes in the phylogenetic trees were considered ‘highly’ supported with Bayesian posterior values  $\geq 96\%$  and bootstrap values  $\geq 80\%$ ; nodes with Bayesian posterior values of 90–95% and bootstrap values of 70–79% were considered ‘moderately’ supported (lower support values were considered not significant).

Genetic divergence among the barcode COI sequences was observed (p distance) and estimated using the Kimura-2-parameters nucleotide substitution model (K2p distance).

## RESULTS AND DISCUSSION

Animals were observed and sampled at seven localities of the Tyrrhenian Sea (Table 1).

Species	Voucher ID	Locality	COI	16S	References
<i>Elysia timida</i>	MNCN 15.05/53680	Menorca, Spain (MED)	HQ616847	-----	Carmona et al., 2011
<i>Elysia timida</i>	MNCN 15.05/53680	Menorca, Spain (MED)	-----	HQ616818	Carmona et al., 2011
<i>Thuridilla albopustulosa</i>	ZSM:20033615	NW-Sulawesi, Indonesia	-----	EU140889	Händeler & Wägele, 2007
<i>Thuridilla bayeri</i>	-----	-----	DQ471279	DQ480208	Bass, 2006
<i>Thuridilla bayeri</i>	-----	-----	-----	DQ480206	Bass, 2006
<i>Thuridilla bayeri</i>	-----	-----	-----	DQ480207	Bass, 2006
<i>Thuridilla bayeri</i>	-----	-----	-----	DQ480207	Bass, 2006
<i>Thuridilla bayeri</i>	ZSM:20033612	NW-Sulawesi, Indonesia	-----	EU140886	Händeler & Wägele, 2007
<i>Thuridilla carlsoni</i>	-----	Lizard Island, Australia	HM187640	-----	Wägele et al., 2010
<i>Thuridilla carlsoni</i>	-----	Lizard Island, Australia	-----	EU140877	Händeler & Wägele, 2007
<i>Thuridilla carlsoni</i>	-----	Lizard Island, Australia	-----	EU140878	Händeler & Wägele, 2007
<i>Thuridilla carlsoni</i>	-----	-----	-----	DQ480214	Bass, 2006
<i>Thuridilla gracilis</i>	-----	Lizard Island, Australia	HM187641	-----	Wägele et al., 2010
<i>Thuridilla gracilis</i>	-----	Hamahiga, Okinawa, Japan	AB758972	-----	Takano et al., 2013
<i>Thuridilla gracilis</i>	-----	Lizard Island, Australia	-----	EU140884	Händeler & Wägele, 2007
<i>Thuridilla gracilis</i>	-----	Lizard Island, Australia	-----	EU140885	Händeler & Wägele, 2007
<i>Thuridilla gracilis</i>	-----	Lizard Island, Australia	-----	EU140883	Händeler & Wägele, 2007
<i>Thuridilla gracilis</i>	-----	Hamahiga, Okinawa, Japan	-----	AB759041	Takano et al., 2013
<i>Thuridilla hoffae</i>	ZSM:20060224	Samoa	-----	EU140880	Händeler & Wägele, 2007
<i>Thuridilla hoffae</i>	-----	-----	-----	DQ480213	Bass, 2006
<i>Thuridilla hopei</i>	-----	Elba Is., Italy (MED)	-----	EU140881	Händeler & Wägele, 2007
<i>Thuridilla hopei</i>	CASIZ 184307	France (MED)	HQ616854	HQ616825	Carmona et al., 2011

Table 1. Voucher ID, collection localities and sequence accession numbers of *Thuridilla* and *Elysia* specimens. MED: Mediterranean; EA: Eastern Atlantic; WA: Western Atlantic (continued).

Species	Voucher ID	Locality	COI	16S	References
<i>Thuridilla hopei</i>	MNCN 15.05/53682	Menorca, Spain (MED)	HQ616849	HQ616820	Carmona et al., 2011
<i>Thuridilla hopei</i>	-----	Mataro, Baretta del Abre, Barcelona, Spain (MED)	-----	EU140882	Händeler & Wägele, 2007
<i>Thuridilla hopei</i>	MNCN/ADN 17015	Western Andalusia, Spain (EA)	HQ616855	HQ616826	Carmona et al., 2011
<i>Thuridilla hopei</i>	ZMBN 81680	Azores, Portugal (EA)	HQ616850	HQ616821	Carmona et al., 2011
<i>Thuridilla hopei</i>	ZMBN 81680	Azores, Portugal (EA)	HQ658123	-----	Carmona et al., 2011
<i>Thuridilla hopei</i>	MNCN 15.05/53685	Madeira, Portugal (EA)	HQ616853	HQ616824	Carmona et al., 2011
<i>Thuridilla hopei</i>	-----	Blanes, Cala St. Francesc, Spain (MED)	GQ996678	-----	Händeler et al., 2009
<i>Thuridilla hopei</i>	-----	Giglio Is., Italy (MED)	KC573743	-----	Krug et al., 2013
<i>Thuridilla hopei</i>	-----	Mataro, Baretta del Abre, Barcelona, Spain (MED)	GQ996677	-----	Händeler et al., 2009
<i>Thuridilla hopei</i>	-----	-----	AF249810	-----	Wollscheid-Len- geling et al., 2001
<i>Thuridilla hopei</i>	BAU1651.1	Giglio Is., Italy, 42°22'27"N 10°52'47"E, 30 m depth (MED)	KJ397547	KJ363910	Present study
<i>Thuridilla hopei</i>	BAU1651.2	Giglio Is., Italy, 42°22'27"N 10°52'47"E, 30 m depth (MED)	KJ397548	KJ363911	Present study
<i>Thuridilla hopei</i>	BAU1652	Le Formiche rocks, Italy, 42°34'28"N 10°52'58"E, 30 m depth (MED)	KJ397549	KJ363912	Present study
<i>Thuridilla hopei</i>	BAU1653.1	Sant'Agostino, Italy, 42°08'45"N 11°43'48"E, 25 m depth (MED)	KJ397550	KJ363913	Present study
<i>Thuridilla hopei</i>	BAU1653.2	Sant'Agostino, Italy, 42°08'45"N 11°43'48"E, 25 m depth (MED)	KJ397551	KJ363914	Present study
<i>Thuridilla hopei</i>	BAU1654	Giannutri Is., Italy, 42°15'07"N 11°07'04"E, 20 m depth (MED)	-----	KJ363915	Present study
<i>Thuridilla hopei</i>	BAU1655.1	Ponza Is., Italy, 40°52'52"N 12°58'02"E, 25 m depth (MED)	-----	KJ363916	Present study
<i>Thuridilla hopei</i>	BAU1655.2	Ponza Is., Italy, 40°52'52"N 12°58'02"E, 25 m depth (MED)	KJ397552	-----	Present study
<i>Thuridilla hopei</i>	BAU1656	Cape Circeo, Italy, 41°13'31"N 13°03'02"E, 14 m depth (MED)	-----	KJ363917	Present study
<i>Thuridilla hopei</i>	BAU1657.1	San Vito Lo Capo, Sicily, 38°10'02"N 12°46'11"E, 10 m depth (MED)	KJ397553	KJ363918	Present study

Table 1. Voucher ID, collection localities and sequence accession numbers of *Thuridilla* and *Elysia* specimens. MED: Mediterranean; EA: Eastern Atlantic; WA: Western Atlantic (continued).

Species	Voucher ID	Locality	COI	16S	References
<i>Thuridilla hopei</i>	BAU1657.1	San Vito Lo Capo, Sicily, 38°10'02"N 12°46'11"E, 10 m depth (MED)	KJ397553	KJ363918	Present study
<i>Thuridilla hopei</i>	BAU1657.2	San Vito Lo Capo, Sicily, 38°10'02"N 12°46'11"E, 10 m depth (MED)	KJ397554	KJ363919	Present study
<i>Thuridilla kathae</i>	-----	Lizard Island, Australia	-----	EU140879	Händeler & Wägele, 2007
<i>Thuridilla lineolata</i>	-----	Sulawesi, Indonesia	-----	EU140887	Händeler & Wägele, 2007
<i>Thuridilla livida</i>	-----	Merizo Clay's backyard, Guam	HM187636	-----	Wägele et al., 2010
<i>Thuridilla livida</i>	-----	Bile Bay, Guam	-----	HM187607	Wägele et al., 2010
<i>Thuridilla livida</i>	-----	-----	-----	DQ480211	Bass, 2006
<i>Thuridilla mazda</i>	UNAM 3027	Mexico	-----	HQ616836	Carmona et al., 2011
<i>Thuridilla neona</i>	-----	Lord Howe Is., Australia	KC573747		Krug et al., 2013
<i>Thuridilla neona</i>	-----	-----	-----	DQ480209	Bass, 2006
<i>Thuridilla picta</i>	ZMBN 83023	Bermuda (WA)	HQ616851	HQ616822	Carmona et al., 2011
<i>Thuridilla picta</i>	ZMBN 83023	Bermuda (WA)	HQ658125	-----	Carmona et al., 2011
<i>Thuridilla picta</i>	MNCN 15.05/53683	Colombia (WA)	HQ616861	HQ616832	Carmona et al., 2011
<i>Thuridilla picta</i>	MNCN 15.05/54991	Colombia (WA)	HQ616862	HQ616833	Carmona et al., 2011
<i>Thuridilla picta</i>	MNCN/ADN 17016	Cuba (WA)	HQ616852	HQ616823	Carmona et al., 2011
<i>Thuridilla ratna</i>	-----	-----	-----	AF249256	Wollscheid-Lengeling et al., 2001
<i>Thuridilla splendens</i>	-----	Kouri, Okinawa, Japan	AB758973	-----	Takano et al., 2013
<i>Thuridilla splendens</i>	-----	Kouri, Okinawa, Japan	-----	AB759042	Takano et al., 2013
<i>Thuridilla undula</i>	-----	-----	-----	DQ480210	Bass, 2006
<i>Thuridilla vatae</i>	-----	Vaisala lagoon, Savaii Island, Samoa	HM187637	-----	Wägele et al., 2010
<i>Thuridilla vatae</i>	ZSM:20060088	Samoa	-----	EU140888	Händeler & Wägele, 2007
<i>Thuridilla vatae</i>	-----	-----	-----	DQ480212	Bass, 2006

Table 1. Voucher ID, collection localities and sequence accession numbers of *Thuridilla* and *Elysia* specimens. MED: Mediterranean; EA: Eastern Atlantic; WA: Western Atlantic.

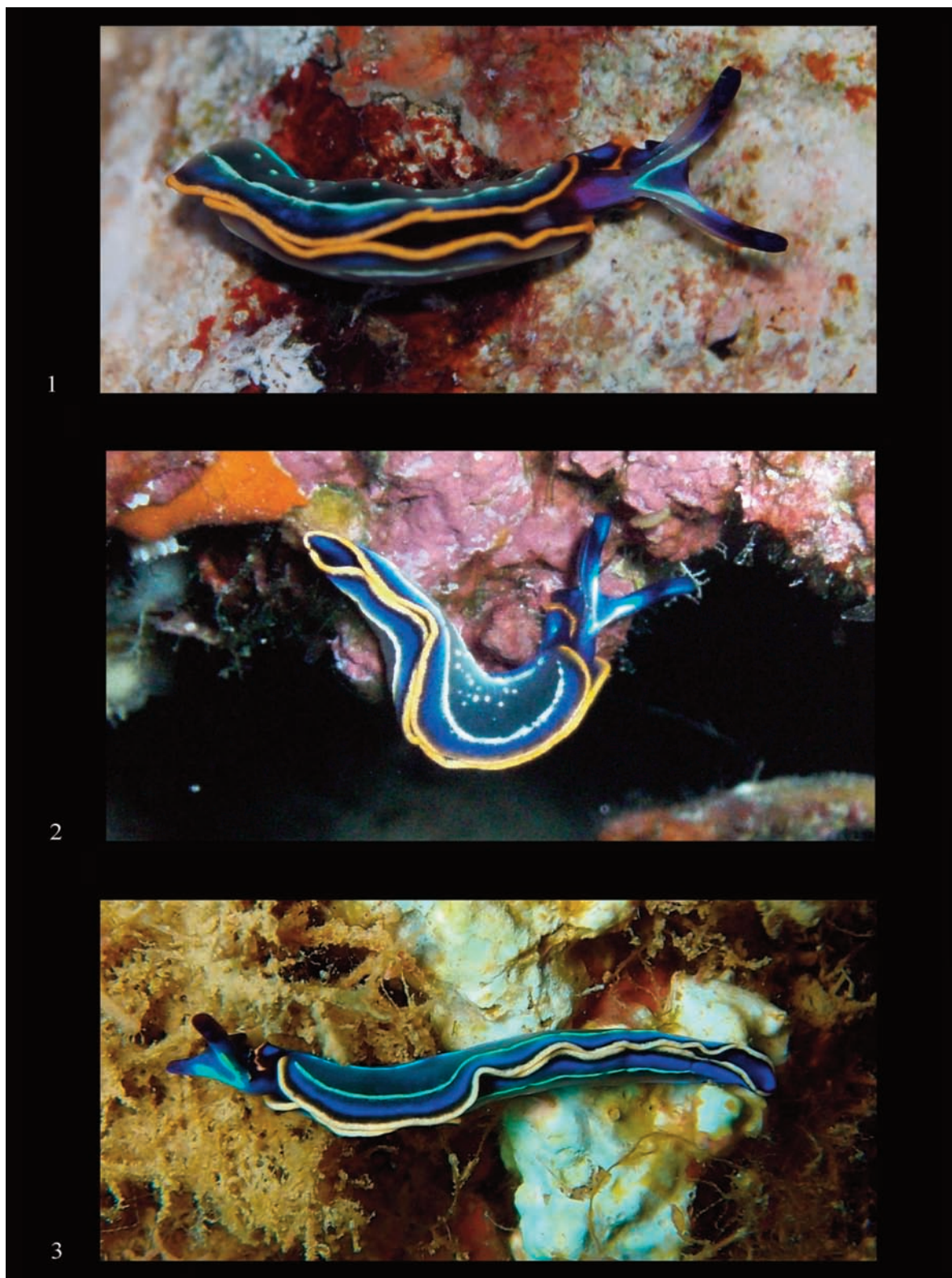


Figure 1. *Thuridilla hopei* “bluish” morphotype. Giannutri Is., Tuscany Archipelago, 42°15'07”N 11°07'04”E, Italy, 20 m depth. Figure 2. *T. hopei* “bluish” morphotype. St. Agostino, Latium coast, 42°08'45”N 11°43'48”E, Italy, 25 m depth. Figure 3. *T. hopei* “bluish” morphotype. Ponza is., Latium coast, 40°52'52”N 12°58'02”E, Italy, 25 m depth.



Figure 4. *Thuridilla hopei* "rosy" morphotype. Zannone Is., Latium coast, 40°57'19"N 13°03'19"E, Italy, 2 m depth. Figure 5. *T. hopei* "rosy" morphotype. San Vito Lo Capo, Sicily coast, 38°10'02"N 12°46'11"E, Italy, 10 m depth. Figure 6. *T. hopei* intermediate morphotype. St. Agostino, Latium coast, 42°08'45"N 11°43'48"E, Italy, 25 m depth.

According to the body colour, almost all specimens could be split into two main colour forms, the “bluish” (Figs. 1–3) and “rosy” (Figs. 4, 5) morphotypes. According to our data and other sources (see Table 2), slugs could be referred to: 1) the “bluish” form, showing a dark blue colour on the upper part of the rhinophores and living in deeper water (usually deeper than -25 m); 2) the “rosy” form, showing a wider white coloration of the rhinophores, including the upper portion, is a shallower water inhabitant (max depth recorded 15 m). Interestingly, the few specimens showing a colour pattern that was not easy to assign unambiguously (an example is depicted in Fig. 6), were collected at intermediate depths, from -20 to -25 m (Table 2; Fig. 7).

The phylogenetic analysis based on the partial sequences of COI and 16S mitochondrial genes, yielded similar trees (Figs. 8, 9). Since this work was not aimed to the definition of a molecular phylogenetic hypothesis for the entire genus *Thuridilla*, we will not discuss the phylogenetic details of the

trees. We just notice that according to the relationships among the sequences of specimens of the complex of *T. bayeri* (Marcus, 1965), including also the nominal taxa *T. gracilis* (Risbec, 1928), *T. ratna* (Marcus, 1965), *T. splendens* (Baba, 1949), this group is worthy of a genetic approach to clarify the status of the various taxa. The sequences ascribed to *T. hopei* and *T. picta* (Verrill, 1901) were more closely related to *T. neona* Gosliner, 1995 than to any other species of the genus (support range: 95–98%), similarly to what found by Gosliner et al. (1995) based on an anatomical dataset. The difference in our trees was that *T. hopei* and *T. picta* formed two reciprocally monophyletic clades more closely related each other (98–99%) than to *T. neona*. In all phylogenetic analyses (Figs. 8, 9) the specimens of *T. picta* from the Caribbean formed a highly supported clade (98–99%). All specimens from the eastern Atlantic also formed a highly supported clade (98–100%), corresponding to *T. hopei* as conceived in Carmona et al. (2011).

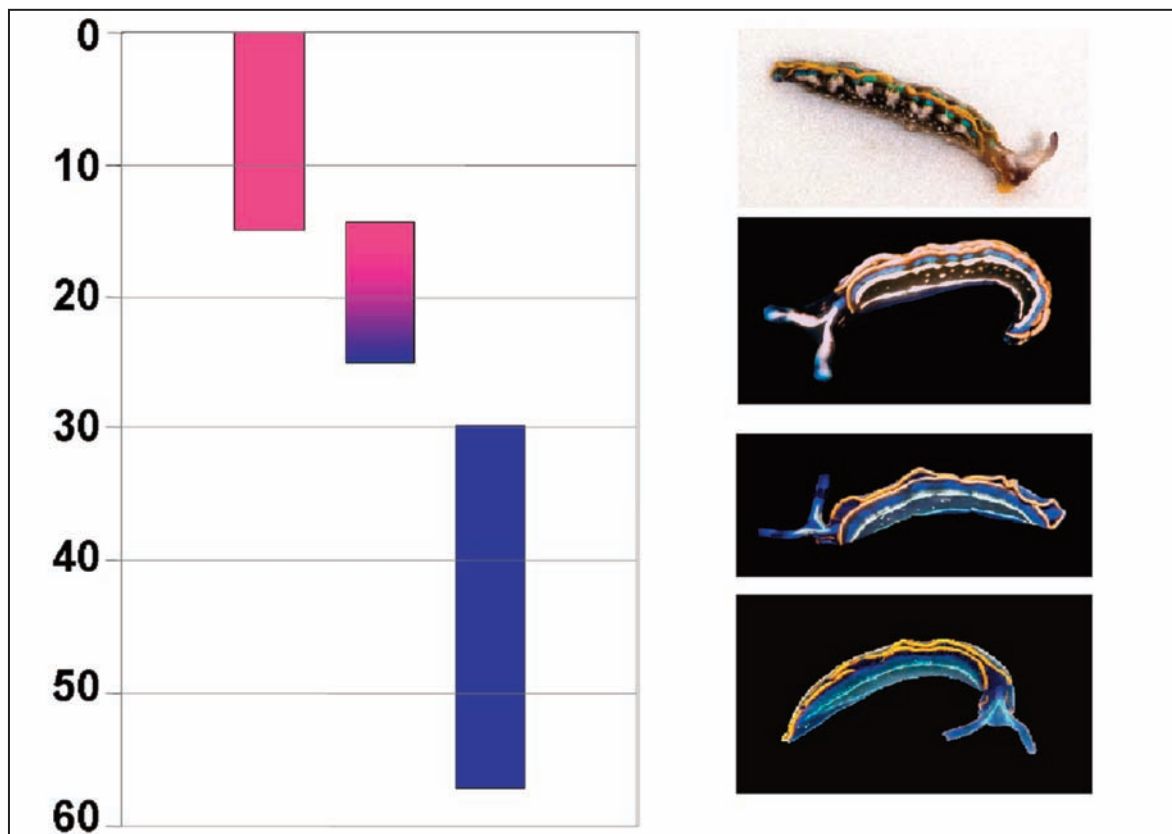


Figure 7. Depth ranges in meters of the ‘rosy’, ‘bluish’ and intermediate morphotypes, summarized from the data of Table 2.



<i>T. hopei</i> morphotypes	N° Specimens	Mediterranean records	Depth (meter)
Bluish	4	Le Formiche rocks, Italy, 42°34'28''N 10°52'58''E	30
	20	Giglio Is., Italy, 42°22'27''N 10°52'47''E	35
	10	Giannutri Is., Italy, 42°15'07''N 11°07'04''E	30
	15	St. Agostino, Italy, 42°08'45''N 11°43'48''E	25-30
	35	MPA "Secche di Tor Paterno", Italy, 41°36'00''N 12°19'00''E	25-35
	7	Ponza Is., Italy, 40°52'52''N 12°58'02''E	25-30
	1	Balun Is., Croatia ( <a href="http://www.seaslugforum.net/message/1934">http://www.seaslugforum.net/message/1934</a> )	57
	1	Area Marina Protetta "Secche di Tor Paterno", Italy, 41°36'00''N 12°19'00''E (Alberto Altomare, personal communication)	20-25
Intermediate	1	Giannutri Is., Italy, 42°15'07''N 11°07'04''E	20
	2	St. Agostino, Italy, 42°08'45''N 11°43'48''E	25
	1	Cap d'Antibes, France ( <a href="http://www.seaslugforum.net/message/21023">http://www.seaslugforum.net/message/21023</a> )	20
	1	Dive site "l'Ocell", Cerbere, France ( <a href="http://www.seaslugforum.net/message/7551">http://www.seaslugforum.net/message/7551</a> )	14
Rosy	13	"Secchitelle" Torvajonica, Italy, 41°34'15''N 12°19'09''E	10-14
	2	Torre Astura, Italy, 41°24'31''N 12°45'54''E	0.5
	1	off Cape Circeo, Italy, 41°13'31'' N 13°03'02''E	15
	1	Zannone Is., Italy, 40°57'19''N 13°03'19''E	2
	2	Santa Caterina, Italy, 40°08'23''N 17°59'12''E	2
	2	San Vito Lo Capo, Sicily, 38°10'02''N 11°46'11''E	10
	2	"La digue", Port-Leucate, France ( <a href="http://www.seaslugforum.net/message/14769">http://www.seaslugforum.net/message/14769</a> )	2
	1	Villafranche-sur-mer, France, ( <a href="http://www.seaslugforum.net/message/22520">http://www.seaslugforum.net/message/22520</a> )	5
	1	"Pierre qui tramole", Cap Croisette, France, ( <a href="http://www.seaslugforum.net/message/7304">http://www.seaslugforum.net/message/7304</a> )	15
	1	Kounoupele beach, Ileia, Greece ( <a href="http://www.seaslugforum.net/message/15307">http://www.seaslugforum.net/message/15307</a> )	0.3
	1	Bodrum, Turkey ( <a href="http://www.seaslugforum.net/message/17707">http://www.seaslugforum.net/message/17707</a> )	15
	1	Michmort beach, Israel ( <a href="http://www.seaslugforum.net/message/20048">http://www.seaslugforum.net/message/20048</a> )	2
	24	Split, Croatia (Jakov Prkić, personal communication)	0-1
	20	Murter Island - loc. Kosirina, Croatia (Alen Petani, personal communication)	0-1
	7	Turanj, Croatia (Alen Petani, personal communication)	0-1
	> 300	Biograd - loc. Bošana, Croatia (Alen Petani, personal communication)	0-2
	5	Zadar - loc. Karma, Croatia (Alen Petani, personal communication)	0.5-2
	> 100	Zaton - loc. Bilotinjak, Croatia (Alen Petani, personal communication)	0-2
	5	Vrsi, Croatia (Alen Petani, personal communication)	0-1
	10	Nin - loc. Sabunike, Croatia (Alen Petani, personal communication)	0.5-1
	5	Vir Island - loc. Pedinka, Croatia (Alen Petani, personal communication)	1-2
	1	Dive site "Yellow Wall", Capoliveri, Elba Is., Italy ( <a href="http://www.seaslugforum.net/message/1934">http://www.seaslugforum.net/message/1934</a> )	11
	1	Capo Figari, Sardinia, (Egidio Trainito, personal communication)	15
1	Divesite "Blue Grotto", Malta ( <a href="http://www.seaslugforum.net/message/1934">http://www.seaslugforum.net/message/1934</a> )	6	
1	"Secchitelle", Torvajonica, Italy (Alberto Altomare, personal communication)	10-15	
1	Brindisi, Italy (Vincenzo Marra, personal communication)	5-15	

Table 2. Mediterranean Records of *Thuridilla hopei* assigned to bluish-rosy or intermediate morphotypes, with their locality and bathymetry. Original records by the authors, personal communications or retrieved from the Internet.

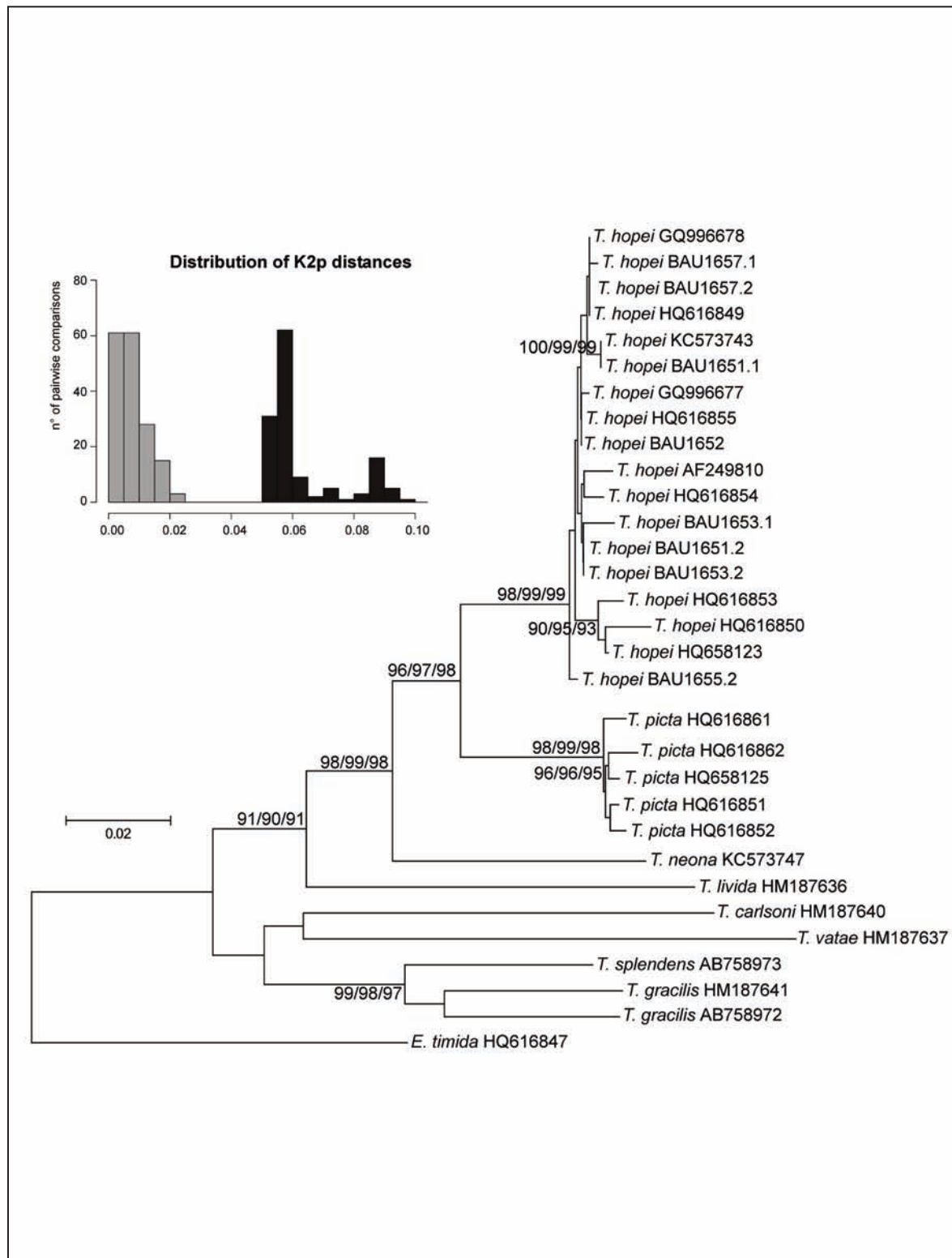


Figure 8. Maximum Likelihood tree based on the COI dataset (K2p model of evolution). Numbers at nodes are bootstrap on NJ and ML analyses (1000 replicates) and Bayesian posterior support ( $5 \times 10^6$  generations and 25% burnin). Only moderately or highly supported nodes are annotated. Scale is calibrated ML distance. The histogram shows the distribution of the pairwise estimated genetic distances (K2p) in intraspecific (left, light grey) and interspecific (right, dark grey) comparisons.

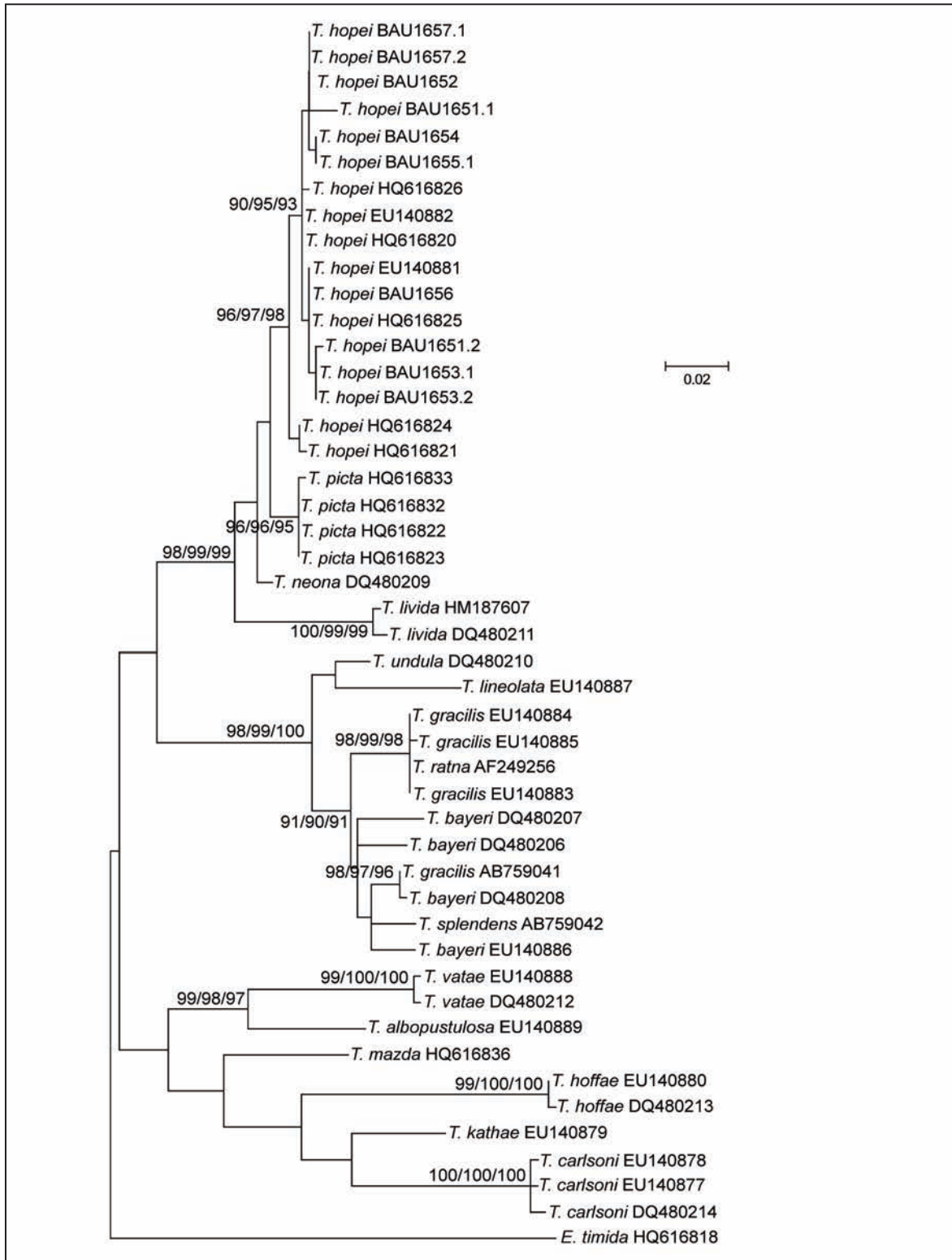


Figure 9. Maximum Likelihood tree based on the 16S dataset (K2p model of evolution). Numbers at nodes are bootstrap on NJ and ML analyses (1000 replicates) and Bayesian posterior support ( $5 \times 10^6$  generations and 25% burnin). Only moderately or highly supported nodes are annotated. Scale is calibrated ML distance.



Figure 10. *Thuridilla hopei* “bluish” morphotype and *Felimare tricolor*. St. Agostino, Latium coast, 42°08’45”N 11°43’48”E, Italy, 35 m depth. Figure 11. *T. hopei* “bluish” morphotype and *Felimare fontandraui*. Giglio Is., Tuscany Archipelago, 42°22’27”N 10°52’47”E, Italy, 30 m depth.

The interspecific K2p genetic distances estimated on the COI sequence alignment (Fig. 8; distance matrices available on request from the authors), excluding the comparisons with the outgroup, ranged from 5.1% to 20.8% (mean 11.6%). With the exception of the two specimens ascribed to *T. gracilis* (from a complex in need of revision, and with a distance of 6.8%), the K2p distances (COI) ranged from 0.5% to 0.9% (mean 0.7%) in the *T. picta* clade, and from 0% to 2.2% (mean 0.8%) in the *T. hopei* clade.

The largest distance values in the *T. hopei* clade were observed between the Macaronesian and the other eastern Atlantic and Mediterranean specimens (0.8%–2.2%). This is perfectly fitting a pattern with only two species involved in this clade, *T. picta* in the western and *T. hopei* in the eastern Atlantic, respectively, as previously proposed by Carmona et al. (2011).

Furthermore, our results do not support any taxonomic split of the “bluish” and “rosy” chromatic forms, which fall within the genetic variation of *T. hopei*. Interestingly, the “bluish” morphotype was often recorded at several Tyrrhenian localities (Figs. 10,11), in the same spots where also species of the family Chromodorididae of the blue chromatic group, such as *Felimare tricolor* (Cantraine, 1835) and *F. fontandraui* (Pruvot-Fol, 1951) were observed. Müllerian mimicry complexes have been described, involving similarly coloured toxic sea slugs, but sometimes including also polyclads (Gosliner, 2001: 166, fig. 1). In *T. hopei* the intraspecific variability of colour patterns, with somehow discrete morphotypes living at different depths, may be partly driven by mimicry coevolution.

The deeper water “bluish” morphotype observed in strict syntopy with the “blue” *Felimare* spp., might be a new Müllerian mimicry complex for the Mediterranean, where the sympatric species may have evolved the shared bright body colours and patterns convergently in the deeper waters (Gosliner, 2001). Conversely, no evidence has been so far collected for a similar mimicry complex involving the “rosy” morphotype in shallow waters. It is worth mentioning that the shallower specimens display a higher variability.

The Macaronesian specimens of *T. hopei*, could represent a third colour morphotype, since their colour pattern albeit similar to the “rosy” morphotype, yet shows a reduction of the dashed light-blue

spots at the edge of the parapodia, a larger black portion without the basal white line, the terminal portion of rhinophores with a coloured band consisting of light blue, black and orange rings. This “ringed-rhinophore” chromatism, which has been so far documented in a single Mediterranean case, from Israel (Table 2, <http://www.seaslugforum.net/message/20048>), may either represent a third discrete colour morphotype or fall within the high variability of the shallow water *T. hopei*.

Future studies will focus on the selective factors acting at different depths, to maintain these two colour forms in the Mediterranean Sea.

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