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RESEARCH NOTE

Tergipes tergipes (Förskal, 1775) (Gastropoda: Nudibranchia) is an amphiatlantic species

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Nearly 13% of ‘opisthobranch’ gastropods in the Atlantic Ocean are believed to show amphiatlantic distributions (García & Bertsch, 2009). This assumption, which has been based mainly on morpho-anatomical similarities, has rarely been tested within a molecular phylogenetic framework. During the past decade, molecular methods have frequently shown that cosmopolitan or widely distributed heterobranch ‘species’ consist of taxonomic complexes of multiple linages (Malaquias & Reid, 2008, 2009; Carmona *et al*., 2011, 2014a, b; Jörger *et al*., 2012; Ornelas-Gatdula *et al*., 2012; Pola *et al*., 2012; Krug *et al*., 2013).

At present, the aeolid genus *Tergipes* Cuvier, 1805 contains six species (Bouchet & Gofas, 2013): *T. antarcticus* Pelseneer, 1903; *T. brochi* Risso, 1818; *T. dicquemari* Risso, 1818; *T. edwardsii* Nordmann, 1844; *T. tergipes* (Förskal, 1775) and *T. valentini* (Elliot, 1907). However, the two species described by Risso (1818) have not been encountered in the past century. Regarding *T. valentini,* this species was transferred to *Cuthona* by Schrödl (2003) because it has more than a single ceras per row. However, this reassignment has been overlooked by later researchers (e.g. Valdés *et al*., 2012).

Tergipes tergipes is regarded as an amphiatlantic boreal species (Franz, 1970; Thompson & Brown, 1984; Templado *et al*., 1987; Bleakney, 1996). It has been reported from Iceland (Lemche, 1938; Platts, 1985) to the southern Iberian Peninsula, including Sweden, Denmark, The Netherlands, the British Isles and Atlantic coast of France (Schmekel & Portmann, 1982; Thompson & Brown, 1984; Cervera *et al*., 2004). Schmekel & Portman (1982), Betti (2011) and Martynov & Korshunova (2011) reported specimens of *T. tergipes* from the Mediterranean, Adriatic, and the Barents and the Black Seas respectively. The western Atlantic distribution of this species ranges from Newfoundland (Canada) to New Jersey (USA) (Bleakney, 1996). In addition, a single specimen of *T. tergipes* has been reported from Brazil (Marcus, 1957). Although the amphiatlantic distribution of this species has been generally accepted, Bleakney (1996) expressed doubt about the conspecificity of populations on either side of the Atlantic.

In this study we use molecular phylogenetic methods to test the hypothesis that the populations of *T. tergipes* on the eastern and western coastlines of the Atlantic Ocean are conspecific.

Samples were collected by SCUBA diving with standard sampling techniques for heterobranchs and obtained from wet collections housed at several museums, namely California Academy of Sciences, San Francisco, USA (CASIZ), Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN) and Zoological Museum of Moscow State University (ZMMU). Nineteen specimens of *T. tergipes* were studied. Thirty-four additional sequences were obtained from GenBank (see Table 1 for full list of samples, localities and vouchers), including one sequence of *T. antarcticus. Tritonia challengeriana* Bergh, 1884 was chosen as a distant outgroup because of its basal phylogenetic position within Cladobranchia (Pola & Gosliner, 2010). Tissue samples were taken from the foot. Extraction, amplification, purification and sequencing of portions of the COI, 16S rRNA and H3 genes followed the methods of Carmona *et al*. (2013, 2014b). Sequence reactions were run on a 3730XL DNA sequencer (Applied Biosystems). Sequences were verified by forward and reverse comparisons and have been deposited in GenBank (Table 1).

Sequences were assembled and edited with Geneious Pro v. 4.7.6 (Drummond *et al*., 2009), aligned in MAFFT (Katoh *et al*., 2009) and further checked using MacClade v. 4.06 (Maddison & Maddison, 2005). Uncorrected pairwise p-distance values between each taxon were calculated for the COI gene. The best-fit evolutionary model (GTR+I+G for COI and 16S and GTR+G for H3) was determined in MrModeltest v. 2.3 (Nylander, 2004), using the Akaike information criterion

Table 1. List of specimens used for phylogenetic analyses.

Family	Species	Locality	Voucher	Genbank accession numbers		
				COI	16S	H3
Tritoniidae	*Tritonia antarctica* Pfeffer, 1886	Bouvetoya, S Atlantic		HM162718	HM162643	HM162550
Dendronotidae	*Dendronotus venustus* MacFarland, 1966	Santa Monica, California		HM162709	HM162630	HM162536
Proctonotidae	*Janolus mirabilis* Baba & Abe, 1970	Philippines		HM162750	HM162674	HM162583
Facelinidae	*Favorinus elenalexiarum* García & Troncoso, 2001	Costa Rica, E Atlantic		HM162755	HM162679	HM162588
	Godiva quadricolor (Barnard, 1927)	South Africa		HM162692	HM162602	HM162508
	Phyllodesmium horridum (Macnae, 1954)	South Africa		HM162757	HM162681	HM162590
	Sakuraeoli senosimensis (Baba, 1930)	California		HM162758	HM162682	HM162591
Tergipedidae	*Catriona* sp. A	Hawaii		JQ997021	JQ996816	JQ996915
	Catriona sp. B	Peru		JQ997024	JQ996819	JQ996918
	Cuthona sp. 35	Philippines		JQ997026	JQ996820	JQ996921
	Cuthona sp. A	Philippines		JQ997019	JQ996814	JQ996913
	Tergipes antarcticus Pelseneer, 1903			GU227106		
	Tergipes tergipes (Förskal, 1775)	Barents Sea, Russia	ZMMU Op-378	KJ434080	–	KJ434098
		Barents Sea, Russia	ZMMU Op-379	KJ434081	–	KJ434099
		E Scheldt, Netherlands	MNCN 15.05/67226	KJ434070	KJ434055	–
		E Scheldt, Netherlands	MNCN 15.05/67227	KJ434071	KJ434056	KJ434087
		E Scheldt, Netherlands	MNCN 15.05/67228	KJ434072	KJ434057	KJ434088
		E Scheldt, Netherlands	MNCN 15.05/67229	KJ434073	KJ434058	KJ434089
		E Scheldt, Netherlands	MNCN 15.05/67230	–	KJ434059	KJ434090
		E Scheldt, Netherlands	MNCN 15.05/67231	KJ434074	KJ434060	KJ434091
		E Scheldt, Netherlands	MNCN 15.05/67232	–	KJ434061	KJ434092
		E Scheldt, Netherlands	MNCN 15.05/67233	KJ434069	KJ434054	KJ434085
		E Scheldt, Netherlands	MNCN 15.05/67234	–	–	KJ434086
		Maine, USA	CASIZ 182699	KJ434077	KJ434064	KJ434095
		Maine, USA	CASIZ 183940	KJ434078	KJ434065	KJ434096
		New Hampshire, USA	CASIZ 184192	KJ434079	KJ434066	KJ434097
		Swansea, Wales, UK	MNCN 15.05/67235	KJ434067	KJ434052	KJ434082
		Swansea, Wales, UK	MNCN 15.05/67236	KJ434068	KJ434053	KJ434083
		Swansea, Wales, UK	MNCN 15.05/67238	–	–	KJ434084
		Trieste, Italy	MNCN 15.05/67224	KJ434075	KJ434062	KJ434093
		Trieste, Italy	MNCN 15.05/67225	KJ434076	KJ4340623	KJ434094

EA, Eastern Atlantic; EP, Eastern Pacific; GB, GenBank.

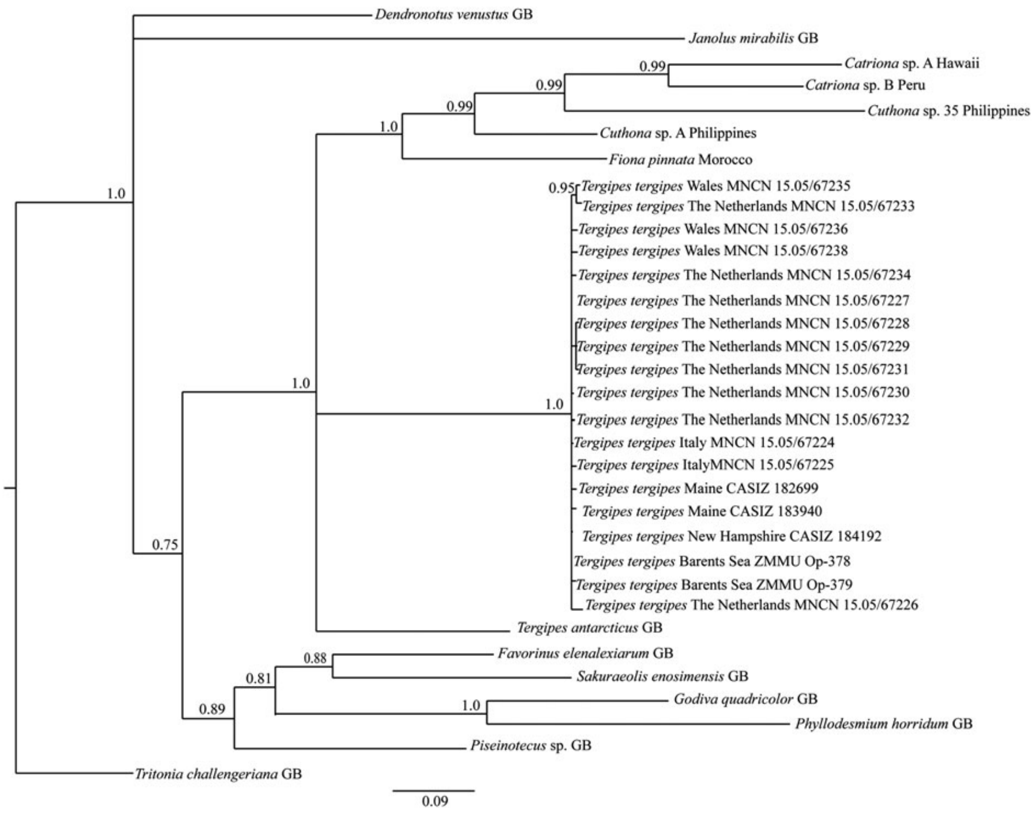

Figure 1. Phylogenetic hypothesis based on the combined dataset (H3+COI+16S) inferred by Bayesian analysis. Numbers above branches are posterior probabilities; values >0.95 indicate strong support. Specimens are listed in Table 1.

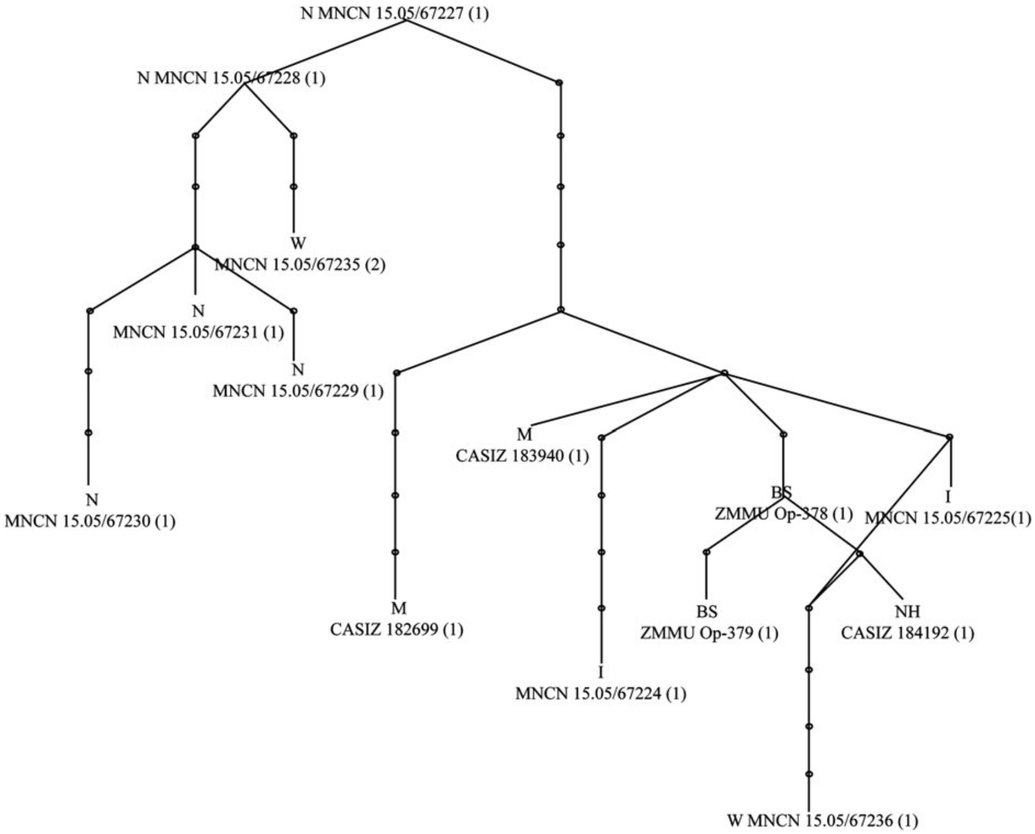

Figure 2. COI haplotype network for *Tergipes tergipes* in The Netherlands (N), Wales (W), Italy (I), the Barents Sea (BS), Maine (M) and New Hampshire (NH). Numbers in brackets indicate the haplotype frequency.

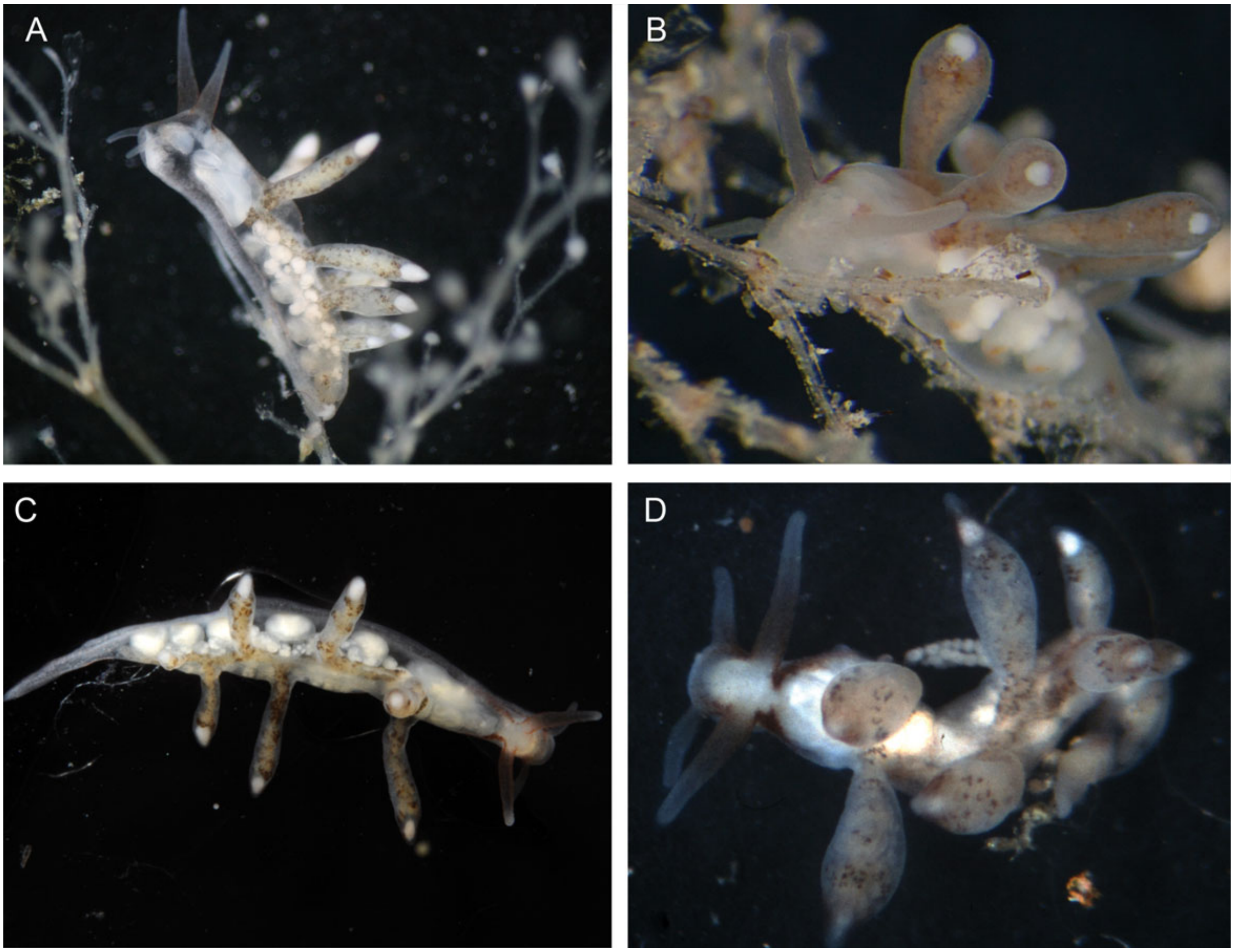

Figure 3. *Tergipes tergipes*. **A.** Wales (photo by David Kipling), 7 mm length. **B.** The Netherlands (photo by Peter H. van Bragt), 5 mm length. **C.** Barents Sea, Russia (photo by Tatiana Korshunova), 5 mm length. **D.** Maine (photo by Terrence M. Gosliner), 4 mm length.

(Akaike, 1974). MrBayes v. 3.1.2 (Ronquist & Huelsenbeck, 2003) was used for Bayesian inference analysis and to estimate posterior probabilities (PP) for node support with two runs of 5,000,000 generations each. Convergence was checked in TRACER v. 1.5 (Drummond & Rambaut, 2007) with a burn-in of 25%.

We also applied the Automatic Barcode Gap Discovery (ABGD) method to detect species-level clusters (Puillandre *et al.*, 2012a). ABGD is a distance-based method designed to detect the so-called 'barcode gap' in the distribution of pairwise distances calculated in a COI alignment (Puillandre *et al.*, 2012a, b). The web-based ABGD program (available at http://wwwabi.snv.jussieu.fr/public/abgd/) was employed with the default settings to generate a preliminary partition of sequences, using the COI alignment and excluding the outgroup. In addition an unrooted statistical parsimony network was generated for COI using TCS v. 1.21 (Clement *et al.*, 2000) with a 95% connection limit.

The combined dataset of three genes yielded a sequence alignment of 1,427 positions. Trees produced by separate analyses of H3, 16S and COI genes (not shown) showed the same topology and similar resolution as the three-gene tree (Fig. 1). All the specimens of *T. tergipes* clustered together in a single clade (PP = 1.0). The uncorrected p-distance for COI among the specimens ranged from 0.0% to 2.7% (between one specimen from The Netherlands and one from Italy). Applying the ABGD method resulted in a single partition with one group containing all the specimens of *T. tergipes* included in this study. The prior maximal distance was 0.001. Fourteen haplotypes were identified in the fifteen specimens sequenced for COI (Fig. 2). The haplotype network did not suggest any structure correlated with geographical origin. In addition, we did not find external morphological differences among the specimens studied (Fig. 3).

These results support the amphiatlantic status of *T. tergipes*, because the methods used here have not detected any population differentiation or structure. Todd (1981) highlighted the opportunist behaviour of this small aeolid, which preys on a wide range of hydroids. In addition, this species has been reported in estuarine areas of the North Sea (Swennen, 1961), indicating resistance to low salinities. In the Barents Sea this species has been found in fouling communities and on the drifting objects off the coast (Martynov *et al.*, 2006). These characteristics may explain the wide distribution of this species and highlight its ability to survive in a range of habitats.

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