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Molecular phylogeography of Mediterranean and Eastern Atlantic sponges of the genus *Aplysina*

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The relationships between these taxa were sorted out with the help of several molecular markers. No single marker proved to be efficient and informative enough, but the combination of information obtained from the mitochondrial gene of the **Cytochrome Oxidase I (COI)**, the **Internal Transcribed Spacer 2 (ITS2)** of ribosomal DNA, **18S rRNA** gene (18S), and a **minisatellite (Miac1)**, could provide significant insights on these relationships.

Aplysina spp. (Verongida: Aplysinidae) are common Demospongiae represented by 2 described species in the Mediterranean and nearby Atlantic. *A. aerophoba*, shallow and photophilic, is known from the entire Mediterranean and in the Macaronesian archipelagos, down to Senegal on the West African coast. *A. cavernicola*, almost identical except for a slightly different color and shape, usually lacks symbiotic Cyanobacteria, and has a very different ecology. *A. cavernicola* is sciaphilic, being often found at cave entrance or under shaded overhangs. It has been reported from the Mediterranean in the western basin to the Gibraltar Straits, in the Adriatic and the Aegean Seas. In some areas such as the NW basin, the two species have very contrasting ecologies which makes them easy to identify. However in areas such as the eastern basin or the Adriatic, the ecological transition is not so sharp and identification is sometimes difficult. Because the main difference lies in their ecology, it has been proposed by some authors to synonymize the 2 species, but the matter has remained unsettled. A third form of *Aplysina* (here mentioned as "n. sp.") has been found in the Atlantic from the Basque Country to the Algarve coast of Portugal and Madeira. It has the ecology of *A. cavernicola* but it is much smaller, encrusting, with long budding filaments. Very similar species of *Aplysina* occur in the Caribbean, across the Atlantic, where the diversity of Verongida is high. For comparative purposes, we could study Verongida from the Caribbean, Western Australia, and a putative *Aplysina aerophoba* from Senegal.

Sampling area	Taxon	COI	ITS2	18S	Miac1
Lebanon (Batroun)	<i>A. aerophoba</i>				X
Adriatic (Pula)	<i>A. aerophoba</i>	X			X
Adriatic (Dugi Otok)	<i>A. cavernicola</i>	X			X
Adriatic (Vis)	<i>A. cavernicola</i>	X	X		X
Coisica (Scandola)	<i>A. cavernicola</i>	X			X
Italy (Portovenere)	<i>A. cavernicola</i>				X
France (Villefranche)	<i>A. cavernicola</i>		X		X
France (Port-Cros)	<i>A. cavernicola</i>	X			X
France (Marseille)	<i>A. cavernicola</i>	X	X	X	X
France (Banyuls)	<i>A. cavernicola</i>	X			X
Catalogna (Medes Is.)	<i>A. cavernicola</i>	X	X	X	X
Basque Country (Fr.)	<i>A. cavernicola</i>	X	X	X	X
Portugal (Berlenga)	<i>Aplysina n. sp.</i>	X	X	X	X
Portugal (Algarve)	<i>Aplysina n. sp.</i>	X	X	X	X
Madeira	<i>Aplysina n. sp.</i>	X			X
Senegal	<i>A. aerophoba?</i>	X			X
Caribbean (Martinique)	<i>A. fistularis</i>	X	X		X
	<i>Aplysina sp. CAR</i>	X	X		X
	<i>Verongula rigida</i>	X	X		X
Western Australia	<i>Aplysina sp. WAM1</i>	X			X
	<i>Aplysina sp. WAM2</i>	X			X
	<i>Suberea laboutei</i>		X		X

The success of each marker was highly irregular. Some samples worked well for one and not for another. Cloning PCR products was necessary for COI, ITS2 and 18S. Several other samples (not listed here) did not amplify. Phylogenetic trees were constructed using different methods (only Neighbour Joining shown here) all producing congruent results.

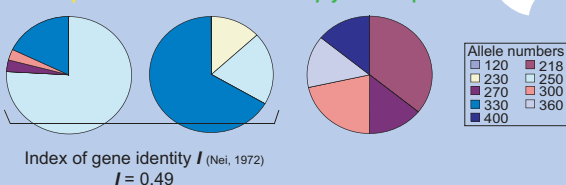


Mitochondrial COI proved to be rather poorly informative at the peri-specific level: it failed to discriminate the small *Aplysina n. sp.* from *A. aerophoba*, and the differentiation between *A. aerophoba* and *A. cavernicola* is very weak. The sample from Senegal appears not to be *A. aerophoba*, according to COI. It is placed close to the *A. fistularis* from the Caribbean. This will require further studies. COI consistently discriminates *A. cavernicola* from the group *A. aerophoba* / *Aplysina n. sp.* even when samples came from the same location. There are however two exceptions (black arrows) where Croatian, positively identified, *A. aerophoba* grouped with the *A. cavernicola* sequences.

ITS-2 results were difficult to analyse due to the presence of multiple copies in the genome. It also failed to adequately resolve the relationships between our 3 target taxa. However, our 3 taxa are well separated from the series of *Aplysina* species found in the Caribbean and California. Also, ITS2 brings *Suberea* (family Aplysinellidae) closer to *Aplysina* than to *Verongula* (both in Aplysinidae). Finally the GenBank *A. lacunosa* "soft" appears to be a *Verongula*.

18S results (not shown) were not clear cut and require further work. They suggest *Aplysina n. sp.* is indeed well separated from *A. aerophoba* and *A. cavernicola* but they failed to unambiguously discriminate between the latter two species.

Allele frequencies of minisatellite Miac 1



The Miac 1 minisatellite finally allowed us to get an answer concerning the three taxa of *Aplysina* present in the Mediterranean and nearby Atlantic: the three taxa must be 3 different valid species of *Aplysina*. Allele frequencies are indeed very different even when the sponges are found in sympatry. Although such results are preliminary (more is to be learned from microsatellites currently being obtained), the value of *I* calculated between *A. aerophoba* and *A. cavernicola* is more in the range known for interspecific identities rather than for intraspecific ones. Also, COI was interesting in providing a possible case of hybrids between the 2 species in the Adriatic, although this remains to be further confirmed. COI and ITS2 seem to be useful markers at the inter-specific or sometimes intra-specific levels for some groups of sponges. They are however not variable enough for some species groups of *Aplysina* spp.

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