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Congruence of three molecular DNA markers assays used to identify species within the blue mussel species - complex

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Abstract

Introduction:

The blue mussel species-complex is composed of several closely related species: *Mytilus edulis* (Linnaeus, 1758), *Mytilus galloprovincialis* (Lamarck, 1819) and *Mytilus trossulus* (Gould, 1850); and more recently *Mytilus chilensis* (Hupé, 1854). These mussel species are morphologically similar, and they hybridize wherever their geographical distributions overlap. The species identity and the composition of *Mytilus* spp. in any northern or southern hemisphere coastal zone became important because of their ecological and economical importance. Therefore, here we examine the congruence of three molecular markers to identify these *Mytilus* species.

Methods:

Mussels samples consistent of 30 individuals from four locations were collected in: Ría de Vigo, Spain; Bellevue, Canadá; Long Island, USA and 18 individuals from Port Elizabeth, South Africa.

Table 1

Location	Specie described in the scientific literature for that location	Samplłng date	Mussels sampled
Ría de Vigo (Spain)	*M. galloprovincialis*	2018	30
Bellevue (Canada)	*M. trossulus*	2017	30
Long Island (U.S.A.)	*M. edulis*	2017	30
Port Elizabeth (South Africa)	*M. galloprovincialis*	2017	18

The three molecular markers used to test congruence were: 1) Me15/16: these primers were developed by Inoue et al. (1995) and amplifies species-specific size fragments of the adhesive protein-coding gene in *Mytilus*. 2) 16s rRNA developed by Hilbish et al. 2000 and 3) COIxba developed by Fernadez-Tajes et al. (2011)

Figure 1

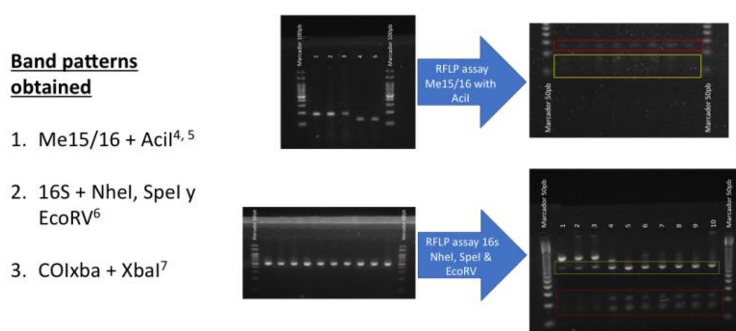

Figure1: Three molecular markers assays used to test the congruency among them to identify blue mussel species

Results:

The species identification for each molecular marker was tabulated for each species and location in Table 2. The nuclear marker Me 15/16 and the mitochondrial marker COIxba showed similar results in species identification among the locations sampled. No mussel hybrids were detected with the Me 15/16 nuclear marker, which corroborate the distribution pattern of the scientific literature composed of mainly pure species among the locations sampled. The same nuclear marker detected *Mytilus trossulus* only in Bellevue, Canadá, with some *Mytilus edulis* (Comesaña

et al, 1999), while only *Mytilus edulis* in Long Island.

Categoría taxonómica	Bellevue		Long Island		Ría de Vigo			Sudáfrica			Total		
	Me15/16	16s	Me15/16	16s	Me15/16	16s	COlxba	Me15/16	16s	COlxba	Me15/16	16s	COlxba
M. edulis	25	18	30	20	-	28	-	-	16	-	**55**	**82**	-
M. trossulus	5	12	-	8	-	-	-	-	-	-	**5**	**20**	-
M. galloprovincialis	-	-	-	-	30	2	30	18	2	18	**48**	**4**	**48**

Table 2: Frequency of species identified by each molecular marker used at the four locations

The mitocondrial marker 16S overestimated the number of *Mytilus trossulus* individuals mussels in Bellevue and Long Island, according to Zbawicka et al (2010), there is introgression of *Mytilus edulis* genes into *Mytilus trossulus*, which can explain the incongruencias found in these two localities sampled and analysed by the nuclear Me 15/16 and mitochondrial 16S markers. This phenomenon is also detected where according to Me 15/16 most of the mussels are *Mytilus galloprovincialis* such in Port Elizabeth and Rio de Vigo, which indicate a high level of introgression of *Mytilus edulis* into *Mytilus galloprovincialis* populations. In general we found low congruente among these molecular markers, maybe due to its different segregation patterns because the doubly uniparental inheritance of mitochondria on these mussels species, and the introgression because hybridization (Westfall, et al. 2010).

Low values of verisimilitude were observed, the 16s rRNA marker in comparison to the Me15/16, showed values of 0.789 for *M. edulis*, 1.529 in M. trossulus and 0.262 for *M. galloprovincialis*.