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Research Article

Differences in sperm ultrastructure between *Mytilus chilensis* and *Mytilus galloprovincialis* (Bivalvia, Mytilidae): could be used as a taxonomic trait?

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ABSTRACT. The sperm ultrastructure has been used to solve several systematic and phylogenetic problems in marine invertebrates. The sperm ultrastructure of the Chilean mussel *Mytilus chilensis* and *Mytilus galloprovincialis* corresponds to the ect-aquasperm type. Sperm from both taxa measured 55-60 µm between head (acrosome + nucleus), midpiece (only 5 mitochondria) and the flagellum which in its end piece has a smaller diameter tail. The differences between both taxa are clearly shown, in the structure of the acrosome and nucleus. Therefore, according to our results and those reported in the literature, we indicate that Chilean native mussel sperm is different from other species of the *Mytilus* complex (*M. trossulus*, *M. galloprovincialis* and *M. edulis*). These differences in sperm ultrastructure found in *M. chilensis*, are another trait that can be used to validate the taxonomic status of the species. Differences in sperm morphology are related with reproductive isolation, and probably will be useful to understand future data on speciation. Finally, we discussed the finding that *Mytilus galloprovincialis* sperm from Chile have an acrosome notoriously smaller than those reported for specimens from Europe and Africa, though they have a great similarity with specimens from Japan, as reported in the literature.

Keywords: mussel, *Mytilus chilensis*, *Mytilus galloprovincialis*, sperm, ect-aquasperm, taxonomy.

Diferencias en la ultraestructura espermática entre *Mytilus chilensis* y *Mytilus galloprovincialis* (Bivalvia, Mytilidae): ¿Se puede utilizar como un carácter taxonómico?

RESUMEN. La ultraestructura de los espermatozoides se ha utilizado para resolver varios problemas sistemáticos y filogenéticos en invertebrados marinos. La ultraestructura de los espermatozoides del mejillón chileno *Mytilus chilensis* y *Mytilus galloprovincialis* corresponde al tipo acuesperma. La medida de los espermatozoides de ambos taxa fue de 55-60 µm entre la cabeza (acrosoma + núcleo), sector medio (solo 5 mitocondrias) y el flagelo, que en su pieza final tiene una cola de menor diámetro. La diferencia entre ambos taxa se mostró claramente en la estructura del acrosoma y el núcleo. Por lo tanto, de acuerdo a nuestros resultados y a los de la literatura, se indica que el mejillón chileno es diferente a los del complejo *Mytilus* (*M. trossulus*, *M. galloprovincialis* y *M. edulis*). Estas diferencias en la ultraestructura de los espermatozoides que se encontraron en *M. chilensis*, son otro rasgo que se puede utilizar para validar el estatus taxonómico de las especies. Las diferencias en la morfología de los espermatozoides están relacionadas con el aislamiento reproductivo y probablemente serán de ayuda para entender futuros datos de especiación. Finalmente, se discute que los espermatozoides de *Mytilus galloprovincialis* que se encuentran en Chile tienen un acrosoma notablemente menor a los reportados en especímenes de Europa y África, aunque tienen una gran similitud con los especímenes de Japón, como se reporta en la literatura.

Palabras clave: mejillones, *Mytilus chilensis*, *Mytilus galloprovincialis,* espermatozoides, acuesperma, taxonomía.

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INTRODUCTION

Among the mollusks, bivalves of the Mytilidae family (Rafinesque, 1815) have external fertilization, therefore, the male gametes have a primitive morphological pattern type I or ect-aquasperm (Franzén, 1955; Jamieson & Rouse, 1989) with a conical head, underdeveloped midpiece and only one mitochondrial ring at the nuclear base, which has a single flagellum (Garrido & Gallardo, 1996; Kafanov & Drozdov, 1998).

Sperm morphology has been used to solve several systematic and phylogenetic problems in marine invertebrates (Franzén, 1955; Healy, 1988; Ferraguti & Gelder, 1991; Justine, 1991; Kafanov & Drozdov, 1998; Costa *et al.,* 2004; Tyurin & Drozdov, 2005), moreover, the ultrastructure of sperm has been used with taxonomic purposes in several species (Popham, 1979; Franzén, 1983; Howard *et al.,* 2009; Introíni *et al.,* 2010). Initially it was used in mammals and then it was confirmed as valid for other animals including bivalve mollusks (Drozdov & Reunov, 1986). For example, it has been used to identify and classify species of the genus *Mytella* (Bivalvia) (Soot-Ryen, 1955), considering the presence of the axial rod (Introini *et al.,* 2010), or to taxonomically differentiate species of the genus *Bathypolipus* (Cephalopoda) (Grimpe, 1921), using and comparing features of the acrosome (Roura *et al.,* 2010). Also, Yurchenko (2012) indicated the species-specific differences in the sperm ultrastructure within the Ostreidae (Rafinesque, 1815), which could be identified both ultrastructurally and morphometrically. Therefore, this type of study is an accurate tool for the identification of marine species, especially if there are classification problems. Studies have demonstrated, particularly in mytilids, that the ultrastructure of the spermatozoa has a high taxonomic value, considering that the sperm characteristics of a species do not differ among different populations, because it may limit pre-zygotic reproductive isolation (Drozdov & Reunov, 1986; Hodgson & Bernard, 1986a, 1986b, Garrido & Gallardo, 1996; Kafanov & Drozdov, 1998; Introini *et al.,* 2010).

Since 1976 there has been a significant increase in the taxonomic understanding of the *Mytilus* status, however, currently there is still confusion mainly regarding the species that live in the southern hemisphere of America. *Mytilus chilensis* (Hupé, 1854), a species endemic to Chile, found from Tirúa River (38°S) to Magellan Strait (53°S) (Hernández & González, 1976).

Although the taxonomic status of *Mytilus chilensis* has been recently reported (Ouagajjou *et al.,* 2011) and genetic identification protocols for the *Mytilus* complex (*M. edulis, M. trossulus, M. galloprovincialis* and *M. chilensis*) have been published (Santaclara *et al.,* 2006; Fernández-Tajes *et al.,* 2011; Ouagajjou *et al.,* 2011), there are still some studies indicating the opposite, classifying it as *Mytilus edulis chilensis* (McDonald *et al.,* 1991; Toro, 1998b), *Mytilus edulis platensis* (Borsa *et al.,* 2012) or *Mytilus galloprovincialis chilensis* (Cárcamo *et al.,* 2005). Moreover, recent studies indicated that the Chilean mussel could correspond to a southern hemisphere lineage of *M. galloprovincialis* (Westfall & Gardner, 2010). This southern hemisphere lineage of the blue mussel *Mytilus galloprovincialis* has been diverging in allopatry from northern hemisphere conspecifics for about 0.84-1.2 million years (Westfall & Gardner, 2013). Notably, to date there is no consensus on the taxonomic validity of the Chilean mussels; however, studies of the spermatozoa ultrastructure in the *Mytilus* complex, including the Chilean mussel that can be useful as a taxonomic trait, have not been performed so far. On the other hand, the exotic species *Mytilus galloprovincialis* (Lamarck, 1819) which was originated in the Mediterranean Sea has recently been reported in Chile, Biobío Region (Daguin & Borsa, 2000; Toro *et al.,* 2005) where it probably lives sympatrically with the Chilean native mussel. Moreover, it has been also recently demonstrated that laboratory crosses between these species generate viable hybrids, although larval survival differed with those from pure species (Toro *et al.,* 2012).

The aim of this study was to compare the ultrastructure of the spermatozoa of the endemic Chilean mussel with the exotic *Mytilus galloprovincialis* from Caleta Tumbes, to provide information on both, its biology and the controversial understanding of the so called *Mytilus* complex.

MATERIALS AND METHODS

Mature males individuals of the *Mytilus chilensis* and *Mytilus galloprovincialis* were collected in Calbuco (41°49'S, 73°06'W) and Caleta Tumbes (36°43'S, 73°08'W), respectively, during spring time when there was a higher frequency of mature indivi-duals (Oyarzún *et al.,* 2011). Both mussel populations were subjected to genetic analysis to establish by molecular markers the differentiation of both taxa (Santaclara *et al.,* 2006).

Samples for electron microscopy were obtained from freshly opened mussels. For transmission electron microscopy (TEM), small pieces of the testes were fixed in 2.5% glutaraldehyde in a 0.2-M phosphate buffer (pH 7.4) for 2 h at 4°C and post-

fixed in 1% osmium tetroxide in a phosphate buffer for 2 h at 4°C. The pieces were then dehydrated in an ethanol series and embedded in araldite resin. Ultrathin sections were obtained on a Sorvall MT-1Ultra-microtome, stained with uranyl acetate and lead citrate (Glauert, 1965) and examined in an Hitachi H-700 transmission electron microscope. For the scanning electron microscope (SEM) analyses, 100 µL of sperm suspension from each of the five selected individuals were mixed (following Garrido & Gallardo, 1996), and a drop of sperm suspension was placed on a cover glass, prefixed with 2.5% glutaraldehyde in a 0.2-M phosphate buffer (pH 7.4) for 2 h at 4°C, and then post-fixed with 1% osmium tetroxide in a phosphate buffer for 2 h at 4°C. The material was dehydrated in a graded ethanol series, critical point dried, coated with gold, and then observed and recorded with a Leo-420 scanning electron microscope.

Size (Table 1) was measured in 50 spermatozoa obtained from a mixed sample of 12 individuals per species on the images obtained in the SEM, using the program © Image-Pro Plus v.6.0 for Windows (image analysis software).

For statistical differences between the two taxa in total length, the acrosome and nucleus diameter, was performed Student *t*-test for independent samples (Sokal & Rohlf, 1995). The presumptions of homoscedasticity and normality of variables and residuals of the model were tested by a Levene test and a Shapiro-Wilk test, respectively. The analyses were run using the statistical program SPSS v.15.0.1 (SPSS ibérica, IBM Company, Chicago, IL, USA).

RESULTS

The length of Chilean mussel's spermatozoa was 60.1 ± 0.07 µm and for *Mytilus galloprovincialis* it was 55.3 ± 0.08 µm, these measures comprised the three sperm regions: head, midpiece and the flagellum which in its end piece has a smaller diameter tail. In addition, there were no differences in structures between the two species (Fig. 2). Statistical analysis showed significant differences in the total length ($t_{(98)}$ = 335.69, $P < 0.05$), length of the acrosome ($t_{(98)}$ = -29.83, $P < 0.05$) and the nucleus diameter ($t_{(98)}$ = 9.68, $P < 0.05$), between the two taxa analyzed.

The male gamete showed, in both taxa, a conical head with an acrosome of elongated shape, with four areas of different density (Fig. 2). Chilean mussels presented a well developed acrosome (2.3 µm length) but it was smaller than the one of *Mytilus galloprovincialis* (3.1 µm). It is possible to observe in both mytilids the presence of an axial rod, which is a subacrosomal filament located in the endonuclear channel that extends from the subacrosomal material to the rear end of the midpiece and had 70 µm diameter (Fig. 2). Also, *M. galloprovincialis* shows a round nucleus of 1.70 µm diameter which is different to the oval nucleus of Chilean mussels of 1.88 µm diameter (Table 1, Fig. 2). Both species presented an underdeveloped midpiece with a single mitochondrial ring (both taxa 100% = 5 mitochondria) in the nuclear base. Inside are located a pair of centrioles, one proximal (the one nearer the nucleus) and one distal (Fig. 2). The presence of a single flagellum (9+2 microtubules, surrounded by a plasma membrane) stands out, which has a midpiece and an end piece or tail (Fig. 1).

DISCUSSION

In this study, it was observed that the spermatozoa of both species had the same structures (Fig. 2) adjusting to the ect-aquasperm proposed by Jamieson & Rouse (1989). However, there were differences in the size and shape of some structures, mainly the acrosomal length and shape of the nucleus (Figs. 1, 2). These are considered species-specific taxonomic traits in the genus *Mytilus* (Popham, 1979; Crespo *et al.*, 1990; Garrido & Gallardo, 1996; Kafanov & Drozdov, 1998). Therefore, Chilean mussels (*M. chilensis*) showed an acrosome of 2.3 µm length with a greater basal widening than *M. galloprovincialis* (Figs. 1, 2). It also showed a higher content of subacrosomal material and flattened basal rings that overlap (hood) the upper area of the nucleus (Table 1; Figs. 2, 3). Similar results were found for this species in the Corral Bay (Garrido & Gallardo, 1996). Therefore, according to our results and those reported in the literature, there is evidence that Chilean mussel sperm morphology is different from those species from the *Mytilus* complex (*M. trossulus, M. galloprovincialis* and *M. edulis*) (Table 1, Fig. 3). Hodgson & Bernard (1986a, 1986b) indicated that the use of the sperm ultrastructure of *Mytilus edulis* and *M. galloprovincialis* can be trusted for taxonomic purposes, because comparing gametes of these species with those from England, Japan (Niijima & Dan, 1965), North America (Longo & Dornfeld, 1967), South Africa and Spain (Crespo *et al.*, 1990), they concluded that spermatozoa from mussels of all locations were species-specific (*i.e.*, in structure and size), giving fidelity to the published scheme (Fig. 3). Later, Kafanov & Drozdov (1998) in a comprehensive review, used these evidence for a phylogenetic classification of the Mytiloida order. On the other hand, Westfall & Gardner (2010) reported genetic

Table 1. Acrosome length and nuclear diameter (µm) of the spermatozoon of Chilean mussels (*Mytilus chilensis*), *M. edulis*, *M. galloprovincialis* and *M. trossulus* ($\overline{X}$ ± DS) (n = 50).

Species	Acrosome length	Nuclear diameter	Locality	References
Mytilus edulis	2.03 ± 0.30	1.61 ± 0.14	Plymouth, United Kingdoom	Hudgson & Bernard (1986a)
M. galloprovincialis	5.08 ± 0.13	1.76 ± 0.13	Plymouth, United Kingdoom	Hudgson & Bernard (1986a)
M. galloprovincialis	5.10 ± 0.17	1.57 ± 0.08	Bloubergstrand, South Africa	Hudgson & Bernard (1986b)
M. galloprovincialis	2.98 ± 0.08	1.78 ± 0.07	Gokasho Bay, Japan	Komaru *et al.* (1995)
M. galloprovincialis	5	1.5	Galicia, Spain	Crespo *et al.* (1990)
M. galloprovincialis	3.16 + 0.18	1.70 + 0.07	Caleta Tumbes, Chile	Present study
Mytilus chilensis	3.10 + 0.70	1.90 + 0.14	Corral Bay, Chile	Garrido & Gallardo (1996)
Mytilus chilensis	2.33 ± 0.11	1.88 ± 0.12	Calbuco, Chile	Present study
Mytilus trossulus	3.3	1.9	Not determined	Kafanov & Drozdov (1998)

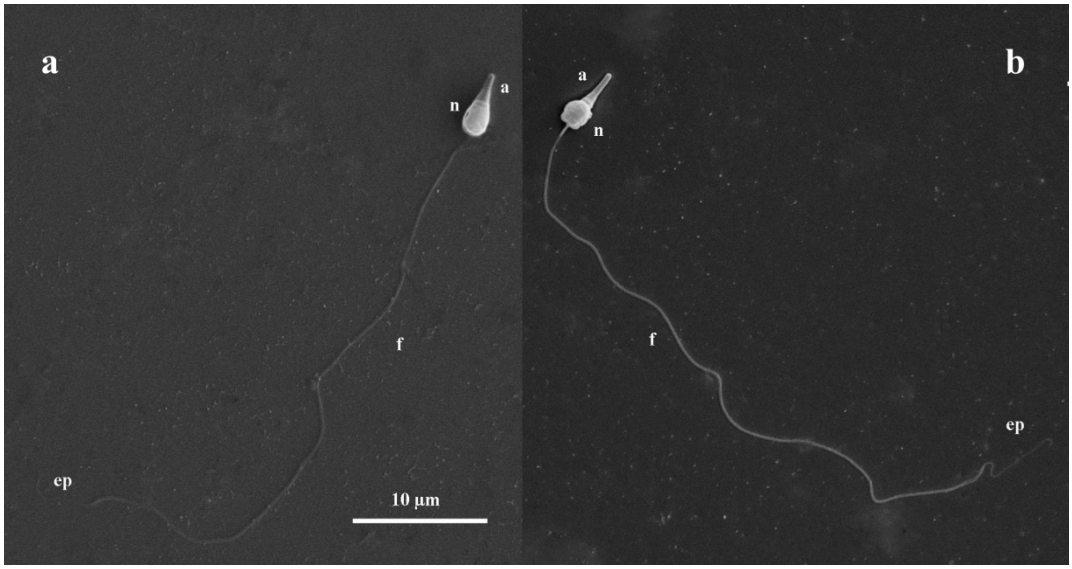

Figure 1. Scanning electron micrographs of the mature sperm of a) *Mytilus chilensis*, and b) *Mytilus galloprovincialis*. a: acrosome, n: nucleus, f: flagellum, ep: end piece or tail.

differences between species of *Mytilus* complex and Chilean mussel with 16S rRNA RFLP assay; similarly Ouagajjou *et al.* (2011) found similar results by using nine microsatellite. Thus, although between these four species, differences at the spermatic and genetic level exist, it is still necessary to understand the evolutionary history of Chilean mussel. It would be interesting to compare the morphology of spermatozoa with phylogenetic data to observe species-specific changes in the germ cells.

It is very important to elucidate the taxonomic status of the Chilean mussel, mainly because of its high importance as economic resource in the south of Chile (Oyarzún *et al.*, 2011; SERNAPESCA, 2012). In addition, the designation of origin, that is a geographical indication applied to food products (*e.g.*, mussels), have as starting point, the taxonomic status, an issue that Chile is approaching in some endemic species that will strengthening the product, considering in special the Chilean mussel which is actually in the third place among global mussel production (Freire *et al.*, 2007; FAO, 2012).

Previous studies have determined that spermatozoa of *Mytilus galloprovincialis* in populations of England, Spain and South Africa (Hodgson & Bernard, 1986a,1986b; Crespo *et al.*, 1990) are similar in structure, form and size regarding both the total and acrosomal lenght (50 µm and 5.1 µm, respectively) and the number of mitochondria (between 5 and 6). However, our results showed a significant difference in the acrosome length (3.1 µm) and in the number of mitochondria (100% = five) (Table 1). Komura *et al.*

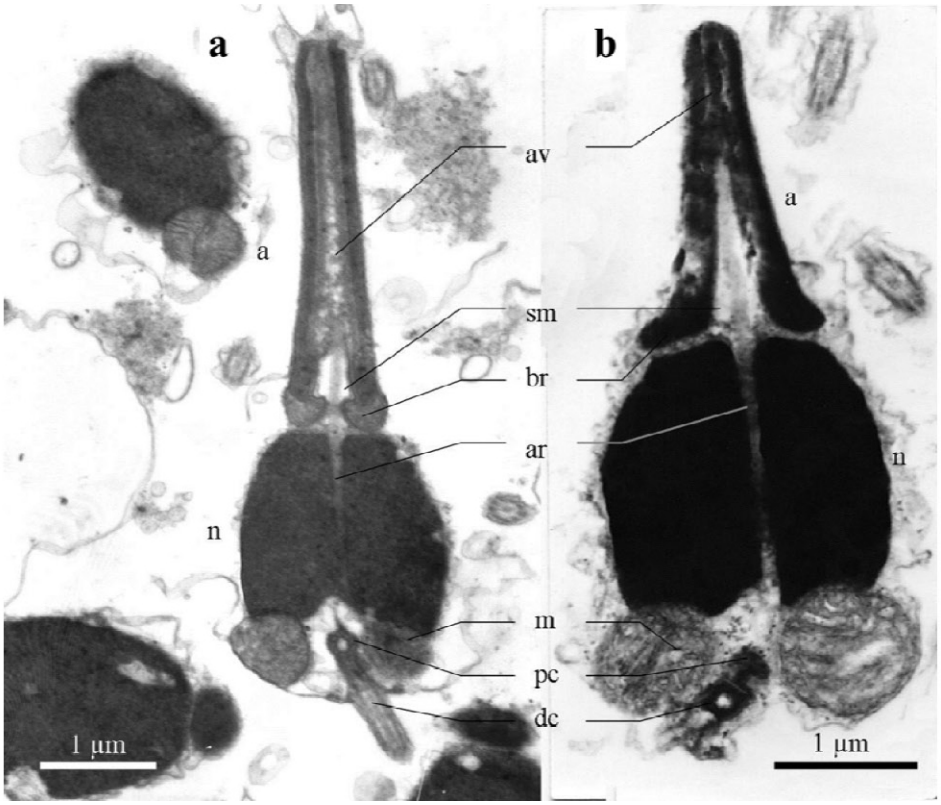

Figure 2. Mature sperm TEM micrographs in longitudinal section of a) *Mytilus galloprovincialis*, and b) *Mytilus chilensis*. a: acrosome, ar: axial rod, av: acrosomal vesicle, br: basal ring, dc: distal centriole, m: mitochondria, n: nucleus, pc: proximal centriole, sm: subacrosomal material.

(1995) reported in Japan, features and size in spermatozoa of this species, equal to those found in the present study (Table 1, Fig. 3). Accordingly, it is possible to infer that both populations (Japan and Chile) share sperm features that are different from those reported in Europe and South Africa. Unfortunately, the studies conducted in Europe and South Africa did not used molecular markers to identify taxa, and probably some mussels analyzed were hybrids; especially taking into account the existence of the hybrid zone in England (Wilhelm & Hilbish, 1998). Moreover, Briones *et al.* (2012) found differences in the acrosome length (between 1.01 µm and 2.52 µm) among populations of the intertidal mussel *Perumytilus purpuratus*, along a latitudinal gradient of ~2200 km, and indicated that is probably due to a speciation process. This process occurs due to differences in the acrosome and/or flagellum which produce prezygotic incompatibility (between sperms and eggs), because there are receptors located in these structures that are used for fertilization in some marine invertebrates, especially those with external fertilization (Pitnick *et al.,* 2009). In this context, although there have been published studies indicating gamete incompatibilities among species of the *Mytilus* complex (Bierne *et al.,* 2002; Rawson *et al.,* 2003), this is the first study that finds evidence which indicates quantitative differences between Chilean mussel sperm with other species of the *Mytilus* complex. However, the role that sperm morphology plays in fertilization is surprisingly poorly known (Howard *et al.,* 2009). While the role of the acrosomal protein M7 lysin on reproductive isolation in *Mytilus* is well understood, the mechanisms driving the evolution of this protein are not jet fully elucidated (Springer & Crespi, 2007; Hess *et al.,* 2012).

The difference in sperm ultrastructure found in *M. chilensis* with regard to *M. edulis* and *M. galloprovincialis* is another trait, which can be used to validate the taxonomic status of the former species. Clearly our results provide an interesting line of research to understand speciation and reproductive isolation, as a fundamental process in evolution (Mayr, 1969; Wiley, 1977). Considering the recent divergence among *Mytilus* species complex (in the Pleistocene between 0.84 to 1.2 mya (Gérard *et al.,* 2008)), this evidence

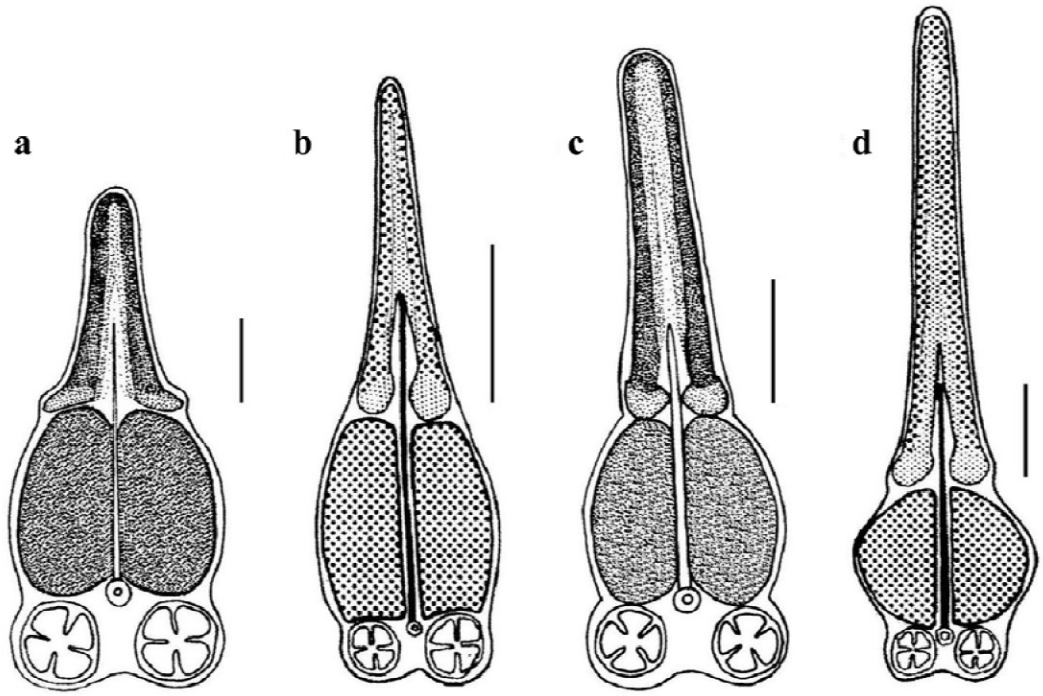

Figure 3. Structural pattern of spermatozoa of the a) Chilean mussels (present study), b) *M. edulis* (extracted image Hodgson & Bernard, 1986a, 1986b), c) *Mytilus galloprovincialis* (present study), and d) *Mytilus galloprovincialis* (extracted image Hodgson & Bernard 1986a, 1986b). We use the same format of the original schematics for better comparison.

could of help to understand the early morphological changes in the germ cells and could answer: (1) What are the morphological changes in sperm that produce a reproductive barrier? or, (2) What are the genetic and molecular underpinnings of prezygotic reproductive isolation?

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REFERENCES

Bierne, N., P. David, P. Boudry & F. Bonhomme. 2002. Assortative fertilization and selection at larval stage in the mussels *Mytilus edulis* and *M. galloprovincialis*. Evolution, 56: 292-298.

Borsa, P., V. Rolland & C. Daguin-Thiébaut. 2012. Genetics and taxonomy of Chilean smooth-shelled mussels, *Mytilus* spp. (Bivalvia: Mytilidae). C.R. Biol., 335: 51-61.

Briones, C., R. Guiñez, O. Garrido, P.A. Oyarzún, J.E. Toro & M. Pérez. 2012. Sperm polymorphism and genetic divergence in the mussel *Perumytilus purpuratus*. Mar. Biol., 159: 1865-1870.

Cárcamo, C., A.S. Comesaña, F.M. Winkler & A. Sanjuan. 2005. Allozyme identification of mussels (Bivalvia: *Mytilus*) on the Pacific coast of South America. J. Shellfish Res., 24: 1101-1115.

Costa, G.C., A.A. Garda, R.D. Teixeira, G.R. Colli & S.N. Báo. 2004. Comparative analysis of the sperm ultrastructure of three species of *Phyllomedusa* (Anura, Hylidae). Acta Zool., 85: 257-262.

Crespo, C.A., T. Garcia-Caballero, A. Beiras & J. Espinosa. 1990. Evidence from sperm ultrastructure that the mussel of Galician estuaries is *Mytilus galloprovincialis* Lamarck. J. Mollus. Stud., 56: 127-128.

Daguin, C. & P. Borsa. 2000. Genetic relationships of *Mytilus galloprovincialis* Lmk. Populations worldwide: evidence from nuclear-DNA markers. In: A. Crame, E. Harper & J. Taylor (eds.). Bivalve systematics and evolution. Geological Society of London, Special Publications, London, pp. 389-397.

Drozdov, A.L. & A.A. Reunov. 1986. The morphology of gametes of the blue mussel *Mytilus edulis* from the white and Japan seas and avancha inlet. Biol. Morya., 4: 52-55.

Fernández-Tajes, J., A. Longa, J. García-Gil, Y.W. Chiu, Y.S. Huang, J. Méndez & R.S. Lee. 2011. Alternative PCR-RFLP methods for mussel *Mytilus* species identification. Eur. Food Res. Technol., 233: 791-796.

Ferraguti, M. & S.R. Gelder. 1991. The comparative ultrastructure of spermatozoa from five Branchiobdellidans (Annelida: Clitellata). Can. J. Zool., 69: 1945-1956.

Food and Agriculture Organization (FAO). 2012. Cwp handbook of fishery statistical standards. Section J: Aquaculture http://www.fao.org/ fishery/cwp/handbook/J/en. Reviewed: 17 December 2013.

Franzén, A. 1955. Comparative morphological investigations into spermiogenesis among Mollusca. Zool. Bldr. Upps., 30: 399-456.

Franzén, A. 1983. Ultrastructural studies of spermatozoa in three bivalve species with notes on evolution of elongated sperm nucleus in primitive spermatozoa. Gamete Res., 7: 199-214.

Freire, R., J. Fernández-Tajes, M.J. López-Piñon & J. Méndez. 2007. Estrategias en el diagnóstico molecular para la identificación de especies comerciales de moluscos bivalvos. In: P. Martínez & A. Figueras (eds.). Genética y genómica en acuicultura. Publicaciones Científicas y Tecnológicas del Observatorio Español de Acuicultura, Madrid, pp. 113-154.

Garrido, O. & C.S. Gallardo. 1996. Ultrastructure of sperms in bivalve molluscs of the Mytilidae family. Invertebr. Reprod. Dev., 29: 95-102.

Gérard, K., N. Bierne, P. Borsa, A. Chenuil & J.-P. Feral. 2008. Pleistocene separation of mitochondrial lineages of *Mytilus* spp. mussels from northern and southern hemispheres and strong genetic differentiation among southern populations. Mol. Phylogenet. Evol., 49: 84-91.

Glauert, A.M. 1965. Section staining, cytology, autora diography and immunochemistry for biology specimens. In: D.H. Kay (ed.). Techniques for electron microscopy. Blackwell Scientific, Oxford, pp. 254-310.

Healy, J.M. 1988. Sperm morphology and its systematic importance in the Gastropoda. In: W.F. Ponder (ed.). Prosobranch phylogeny. Proceedings of a Symposium held at the 9th International Malacological Congress. Edinburgh, pp. 251-266.

Hernández, J.M. & L.E. González. 1976. Observaciones sobre el comportamiento de mitílidos chilenos en cultivo suspendido. I chorito (*Mytilus chilensis*), Hupe, 1854. Invest. Pesq., Chile, 22: 50.

Hess, A.K., M. Bartel, K. Roth, K. Messerschmidt, K. Heilmann, E. Kenchington, B. Michael & H. Stuckas. 2012. Expression of M6 and M7 lysin in *Mytilus edulis* is not restricted to sperm, but occurs also in oocytes and somatic tissue of males and females. Mol. Reprod. Dev., 79: 517-524.

Hodgson, A.N. & R.T.F. Bernard. 1986a. Observations on the ultrastructure of the spermatozoon of two mytilids from the south-west coast of England. J. Mar. Biol. Assoc. UK., 66: 385-390.

Hodgson, A.N. & R.T.F. Bernard. 1986b. Ultrastructure of the sperm and spermatogenesis of three species of Mytilidae (Mollusca, Bivalvia). Gamete Res., 15: 123-135.

Howard, D.J., S.R. Palumbi, L.M. Birge & M.K. Manier. 2009. Sperm and speciation. In: T.R. Birkhead, D.J. Hosken & S. Pitnick (eds.). Sperm biology: an evolutionary perspective. Elsevier, New York, pp. 367-403.

Introini, G.O., F.M. Maesteri, F.P.P. Leite & S.M. Recco-Pimentel. 2010. Sperm ultrastructure of *Mytella* (Bivalvia) populations from distinct habitats along the northern coast of São Paulo state, Brazil. Biocell., 34: 103-111.

Jamieson, B.G. & G.W. Rouse. 1989. The spermatozoa of the Polychaeta (Annelida): an ultrastructural review. Biol. Rev. Cambridge Philos. Soc., 64: 93-157.

Justine, J.L. 1991. Phylogeny of parasitic platyhelminthes: a critical study of synapomorphies proposed on the basis of the ultrastructure of spermiogenesis and spermatozoa. Can. J. Zool., 69: 1421-1440.

Kafanov, A.I. & A.L. Drozdov. 1998. Comparative sperm morphology and phylogenetic classification of recent mytiloidea (Bivalvia). Malacologia, 39: 129-139.

Komura, A., J. Scarpa & K. Wada. 1995. Ultrastructure of spermatozoa in induced tetraploid mussel *Mytilus galloprovincialis* (Lmk.). J. Shellfish Res., 14: 405-410.

Longo, F.G. & E.J. Dornfeld. 1967. The fine structure of spermatid differentiation in the mussel *Mytilus edulis*. J. Ultrastruct. Res., 20: 462-480.

Mayr, E. 1969. Principles of systematic zoology. McGraw-Hill, New York, 434 pp.

McDonald, J.H., R. Seed & R.K. Koehn. 1991. Allozymes and morphometric characters of 3 species of *Mytilus* in the northern and southern hemispheres. Mar. Biol., 111: 323-333.

Niijima, L. & J. Dan. 1965. The acrosome reaction in *Mytilus edulis*. I. Fine structure of the intact acrosome. J. Cell Biol., 25: 243-248.

Ouagajjou, Y., P. Presa, M. Astorga & M. Perez. 2011. Microsatellites of *Mytilus chilensis*: a genomic print of its taxonomic status within *Mytilus* sp. J. Shellfish Res., 30: 325-330.

Oyarzún, P.A., J.E. Toro, R. Jaramillo, R. Guiñez, C. Briones & M. Astorga. 2011. Ciclo gonadal del chorito *Mytilus chilensis* (Bivalvia: Mytilidae) en dos localidades del sur de chile. Lat. Am. J. Aquat. Res., 39: 512-525.

Pitnick, S., M.F. Wolfner & S.S. Suarez. 2009. Ejaculate-female and sperm-female interactions. In: T.R. Birkhead, D.J. Hosken & S. Pitnick (eds.). Sperm biology: an evolutionary perspective. Elsevier, New York, pp. 247-304.

Popham, J.D. 1979. Comparative spermatozoan morphology and bivalve phylogeny. Malacol. Rev., 12: 1-20.

Rawson, P.D., C. Slaughter & P.O. Yund. 2003. Patterns of gamete incompatibility between the blue mussels *Mytilus edulis* and *M. trossulus*. Mar. Biol., 143: 317-325.

Roura, A., A. Guerra, A.F. Gonzalez & S. Pascual. 2010. Sperm ultrastructure in *Bathypolypus bairdii* and *B. sponsalis* (Cephalopoda: Octopoda). J. Morphol., 271: 143-151.

Santaclara, F.J., M. Espiñeira, A.G. Cabado, A. Aldasoro, N. Gonzalez-Lavín & J.M. Vieites. 2006. Development of a method for the genetic identification of mussel species belonging to *Mytilus*, *Perna*, *Aulacomya*, and other genera. J. Agric. Food Chem., 54: 8461-8470.

Servicio Nacional de Pesca (SERNAPESCA). 2012. Anuario estadístico de pesca. Valparaiso. http://www.sernapesca.cl. Reviewed: 18 December 2012.

Sokal, R.R. & F.J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research. W.H. Freeman, New York, 937 pp.

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Springer, S.A. & B.J. Crespi. 2007. Adaptive gamete-recognition divergence in a hybridizing *Mytilus* population. Evolution, 61: 772-783.

Toro, J.E. 1998b. PCR-based nuclear and mtdna markers and shell morphology as an approach to study the taxonomic status of the chilean blue mussel, *Mytilus chilensis* (Bivalvia). Aquat. Living Resour., 11: 347-353.

Toro, J.E., J.A. Ojeda, A.M. Vergara, G.C. Castro & A.C. Alcapán. 2005. Molecular characterization of the Chilean blue mussel (*Mytilus chilensis* Hupé, 1854) demonstrates evidence for the occurrence of *Mytilus galloprovincialis* in southern Chile. J. Shellfish Res., 24: 1117-1124.

Toro, J.E., P.A. Oyarzún, C.S. Peñaloza, A. Alcapán, V. Videla, J. Tilleria, M. Astorga & V. Martínez. 2012. Production and performance of larvae and spat of pure and hybrid species of *Mytilus chilensis* and *M. galloprovincialis* from laboratory crosses. Lat. Am. J. Aquat. Res., 40: 243-247.

Tyurin, S.A. & A.L. Drozdov. 2005. Ultrastructure of sperm in *Mercenaria stimpsoni* and *Mactra chinensis* (Mollusca; Bivalvia) from de sea of Japan. Russ. J. Mar. Biol., 31: 391-395.

Westfall, K.M. & J.P.A. Gardner. 2010. Genetic diversity of southern hemisphere blue mussels (Bivalvia: Mytilidae) and the identification of non-indigenous taxa. Biol. J. Linn. Soc., 101: 898-909.

Wiley, E.O. 1977. The evolutionary species concept reconsidered. Syst. Biol., 27: 17-26.

Wilhelm, R. & T.J. Hilbish. 1998. Assessment of natural selection in a hybrid population of mussels: evaluation of exogenous *vs* endogenous selection models. Mar. Biol., 131: 505-514.

Yurchenko, O.V. 2012. Comparative ultrastructural study of spermatozoa in some oyster species from the Asian-Pacific coast. Micron, 43: 365-373.