Erroneous identification of the mussel, *Mytilus galloprovincialis* (Lamarck 1819) as the specie, *Mytilus chilensis* (Hupe 1854) in the Bay of Concepcion, Chile

Identificación errónea del mejillón, *Mytilus galloprovincialis* (Lamarck 1819) como la especie, *Mytilus chilensis* (Hupe 1854) en la Bahía de Concepción, Chile

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ABSTRACT

This communication informs that presence of the Chilean mussel, *Mytilus chilensis* (Hupe 1854) in the coast of the Bay of Concepcion (Chile) reported in the scientific literature is an erroneous identification, being *Mytilus galloprovincialis* (Lamarck 1819) the right species.

RESUMEN

Esta comunicación informa que la presencia de *Mytilus chilensis* (Hupe 1854) citada en la literatura científica para la costa de la Bahía de Concepción (Chile) no es correcta, siendo *Mytilus galloprovincialis* (Lamarck 1819) la identificación taxonómica válida.

The increasing interest to carry out mussel farming in the coasts of the Gulf of Arauco and Bay of Concepcion (Central Chile) requires knowing exactly what species of mussels are present in this coastal zone because aquaculture activity in this coastal region can be done only with organisms living in the zone. In the literature on the bivalves inhabiting the intertidal zone of this region, Aldea & Valdovinos (2005) mention to the Chilean mussel, *Mytilus chilensis* (Hupe 1854) in sampling carried out in 2001, as naturally present in the rocky shore of: i) Punta Parra Cove, Cocholgüe Cove and Port of Talcahuano within the Bay of Concepcion, ii) mouth of the Biobío river, iii) Tubul Cove and Punta Lavapié Cove inside the Gulf of Arauco. These localities represent a geographic range from S 36° 38' 43" to S 37° 09' 01".

However, the taxonomic identification made by these authors, is erroneous. The correct species corresponds

to the mussel, *Mytilus galloprovincialis* (Lamarck 1819) with local name of "choro araucano" in Chile. This mussel species shows a world-wide distribution (Westfall & Gardner 2010) and Castilla *et al.* (2005) indicated its presence at the Chiloe Island at Southern Chile and at Bay of Coliumo (Central Chile). The identification mistake was verified by examining the same mussel valves deposited by Aldea & Valdovinos (2005) in the Museum of Zoology of the Universidad de Concepción with the accessing code UCCC 29016 (Figure 1, Left). The morphologic comparison of the mussel shells studied by Aldea & Valdovinos (2005) with shells of *M. chilensis* and *M. galloprovincialis* validated genetically following the protocol of Santaclara *et al.* (2006) and deposited with accessing codes UCCC 1208 (Figure 1, Center) and UCCC 35932 (Figure 1, Right), respectively, shows the identification mistake done by these authors.

Morphological differences among shells of *Mytilus edulis*, *Mytilus galloprovincialis* and *Mytilus trossulus* deposited at the British Museum of Natural History of London by McDonald *et al.* (1991) with accession number 1299, 2309 and 2244, respectively, are showed in Figure 2 for comparison purposes. On base upon these materials, collected at Magdalen Island (Quebec, Canada), Posjet Bay (U.S.S.R) and Magadan (U.S.S.R), respectively, McDonald *et al.* (1991) named the “*Mytilus* Complex” considered as a standard on the *Mytilus* taxonomy.

To accurately verify the presence of *M. chilensis* in the same intertidal points worked in 2001 by Aldea & Valdovinos (2005), new mussel samplings were carried out by us on October of 2011 in the rocky shore intertidal zone within the Bay of Concepcion, at the localities of Parra Point Cove, Bellavista Beach and Cocholgüe Cove. The careful search for *Mytilus* spp. naturally settled on these intertidal points revealed only the presence of *M. galloprovincialis* forming

either isolated patches or single specimens coexisting with the other common intertidal mussel, *Perumytilus purpuratus* (Lamarck 1819). The *in situ* identification of *M. galloprovincialis* was endorsed later on in the Laboratory Genetic and Aquaculture of the Universidad of Concepción, applying the protocol of PCR-RFLP developed by Santaclara *et al.* (2006). This clear-cut assay genetic method is based on the amplification of the genes of the polyphenolic proteins that made the byssus of these bivalves, using the genetic markers Me15 and Me16 described earlier by Inoue *et al.* (1995). These genetic markers generate a fragment of 126 pairs of bases in *M. chilensis* and *M. galloprovincialis*, which are digested with endonuclease Aci I, according to Santaclara *et al.* (2006). The restriction site of the enzyme is only present in the DNA of *M. galloprovincialis* generating then two new fragments of 57 and to 69 pairs of bases but this result is not exhibited by *M. chilensis*, species that conserves the band of 126 pb once digested with Aci I. (Figure 3).

FIGURE 1. Left: *Mytilus chilensis*, identified by Aldea & Valdovinos (2205) (UCCC 29061); Center: *Mytilus galloprovincialis* (UCCC 35932); Right: *Mytilus chilensis* (UCCC1208).

FIGURA 1. Izquierda: *Mytilus chilensis*, identificado por Aldea & Valdovinos(2205)(UCCC29061);Centro:*Mytilus galloprovincialis* (UCCC 35932); Derecha: *Mytilus chilensis* (UCCC1208).

FIGURE 2. Mussel valves deposited at the British Museum of Natural History (London) by MacDonald *et al.* (1991).Keys: 2244, *Mytilus trossulus*; 2309, *Mytilus galloprovincialis*; 1299 , *Mytilus edulis*. Upper row, outside view of right valve; bottom row, inside view of the right valve.

FIGURA 2. Valvas de mitilidos depositados por MacDonald *et al.* (1991) en el Museo de Historia Natural de Londres. Claves: 2244, *Mytilus trossulus*; 2309, *Mytilus galloprovincialis*; 1299, *Mytilus edulis*. Fila superior, vista externa de valva derecha; fila inferior, vista interna de valva derecha.

FIGURE 3. PCR-RFLP of the polyphenolic protein gen with Aci I enzyme, obtained from *Mytilus* spp. samples. Line 50 bp: DNA ladder, Invitrogen; 1, PCR from *M. edulis*; 2, PCR from *M. chilensis*; 3, PCR from *M. galloprovincialis*; 4, RFLP from *M. edulis*; 5, RFLP from *M. chilensis*; 6, RFLP from *M. galloprovincialis*; 7-9 RFLP from *M. galloprovincialis* collected at Punta de Parra Cove; 10-12, RFLP from *M. galloprovincialis* collected at Bellavista Cove; 13-15 RFLP from *M. galloprovincialis* collected at Cocholgue Cove.

FIGURA 3. Perfil de Restricción (PCR-RFLP) del gen de la proteína polifenólica con la enzima Aci I, obtenidos con ejemplares de *Mytilus* spp. Línea 50 pb: Marcador de Peso Molecular (50 bp DNA ladder, Invitrogen); 1, producto de PCR de *M. edulis*; 2, producto de PCR de *M. chilensis*; 3, producto de PCR de *M. galloprovincialis*; 4, RFLP de *M. edulis* con la enzima Aci I; 5, RFLP de *M. chilensis* con la enzima Aci I; 6, RFLP de *M. galloprovincialis* con la enzima Aci I; 7-9 RFLP de ejemplares de *M. galloprovincialis* de Punta de Parra; 10-12, RFLP de ejemplares de *M. galloprovincialis* de Bellavista, Tomé; 13-15 RFLP de ejemplares de *M. galloprovincialis* de Cocholgue.

The first mention of the presence of *M. galloprovincialis* in Chilean coasts, specifically Dichato (Bay of Coliumo, Chile) was made by Daguin & Borsa (2000) applying molecular markers. Later on, Ruiz *et al.* (2005) and Toro *et al.* (2005) ratified this reference. Castilla *et al.* (2005) cited *M. galloprovincialis* as an exotic invasive species to Chile with presence at Castro and Yaldad (Chiloe Island; Southern Chile) and Dichato (Bay of Coliumo, Central Chile) following Toro *et al.* (1998), Daguin & Borsa (2000) and Hilbish *et al.* (2000). The manuscript of Castilla *et al.* (2005) was sent for publication on 2001 indicating that the main focus of distribution of *M. galloprovincialis* was the mussel farms at Chiloe Island but without evidence of local natural beds of this mussel species. Daguin (2000) and Daguin & Borsa (2000) based on nuclear DNA markers, pointed out that *M. galloprovincialis* could be introduced to Chile from the Mediterranean. However, it has to be noted that until Santaclara *et al.* (2006), adequate genetic molecular markers were not standardized to accurately separate *M. chilensis* from *M. galloprovincialis*. By this reason, samples of *M. chilensis* could be recognized as *M. galloprovincialis* in the studies carried out by Toro *et al.* (1998), Hilbish *et al.* (2000) and Daguin & Borsa (2000). Recently, Borsa *et al.* (2012) discuss the taxonomic status of *M. chilensis* pointing out that different *Mytilus* species might have been included under “*M. chilensis*” proposing that the native Chilean smooth-shelled *Mytilus*, should be assigned subspecific rank and named *M. edulis platensis* d’Orbigny

1846 (Borsa *et al.* 2012). The point that whether *M. chilensis* is an accepted species among the *Mytilus* complex, together with its geographical distribution along the Chilean coasts, needs to be clarified as soon as possible, since the massive aquaculture activities on this marine resource at southern Chilean coasts.

The presence of the mussel *M. galloprovincialis* at the coasts of the Gulf of Arauco, Bay of Concepcion and Bay of Coliumo was long time missed by artisanal fishermen and ecologists because its similar shell shape, size and shell color with juveniles of the mussel *Choromytilus chorus* (Molina 1782), a very abundant subtidal mussel in these coastal areas. The most notorious differences between both species, are the number and size of teeth located on the hinge line of the shell beneath the ligament, being only 1-2 and large on *Ch. Chorus* and more than 5 and small in *M. galloprovincialis*; and the color of the female gonad that it is dark-brown in *Ch. Chorus* and yellow-orange in *M. galloprovincialis*. In addition, samples of mussels collected from a long-line system for culturing the northern scallop, *Chlamys purpuratus* at Tongoy Bay (30°15’S) and from a *M. chilensis* farm at Chiloé Island (41°S) send to us for taxonomic identification, were genetically identified as *M. galloprovincialis* (Figure 4).

On the other hand, Hupé (1854) mentions and describes the mussel species, *Mytilus chorus*, *Mytilus chilensis*, *Mytilus*

dactyliformis, *Mytilus magellanicus*, *Mytilus orbignyana*, *Mytilus granulatus*, *Mytilus ovalis* and *Mytilus scalprum* at the Chilean coast. From the description given by Hupé (1854) and the details in the scientific drawings in the Plate 5 (Figure 5) it could be concluded that Hupé (1854) citation of *M. chorus* corresponds to *Choromytilus chorus* (named by Hupé “as a typical mussel from Concepcion Bay”); meanwhile, *M. chilensis* (Plate 5, fig. 4) is the actual species *M. chilensis* applying Santaclara *et al.* (2006) protocol; and *M. dactyliformis* (Plate 5, figs. 6, 6A) corresponds to *Semimytilus algosus* (Gould 1859). However, it is remarkable that *M. orbignyana*, cited by Hupé (1854) as living at Coquimbo coasts (30° S) shows quite similar shell-shape to the actual *M. galloprovincialis*. Furthermore, the figures 4, 5 and 5a in the Plate 5 of Hupé work (1854) evidence the same morphological differences that can be observed today between *M. chilensis* and *M. galloprovincialis*.

Since Aldea & Valdovinos (2005) are frequently cited as a confident source that *M. chilensis* is living at the Bay of Concepcion, it was necessary to clarify the erroneous identification of *M. chilensis* made by these authors.

However, it could be possible that in the near future, natural beds of *M. chilensis* could be found in the coastal zone of Trana and Llico Coves (inside Gulf of Arauco), Coliumo, Dichato and Burca Coves (inside Bay of Coliumo) established from commercial aquaculture activities started with *M. chilensis* seeds massively brought from Chiloé Island (Southern Chile) since 2009. All these commercial mussel farms were strongly affected by the 27/February tsunami, with the growing systems entangled on the beaches, and the mussels fully dispersed on the sandy and rocky bottoms. Actually, there are high concerns that *M. chilensis* seeds transported from the Chiloé Island to support mussel farming at Gulf of Arauco, Bay of Concepcion and Bay of Coliumo can be a biological hazard to this marine environment since transported collectors with mussel seeds could be contaminated with dinoflagellates and diatoms that produce toxic red tides that commonly occurs at the Chiloé Island, but so far have been not reported to the coastal waters of Gulf of Arauco, Bay of Concepcion and Bay of Coliumo.

Figure 4. Right valves of *M. galloprovincialis* collected at Bay of Tongoy (left) and Chiloé Island (right). Bar = 5 cm.

Figura 4. Valvas derechas de *M. galloprovincialis* colectadas en Bahía de Tongoy y en Isla de Chiloé. Barra = 5 cm.

Figure 5. Plate 5 in Gay (1854) showing the different species of bivalves described by Hupe (1854). 4: *Mytilus chilensis*; 5: *Mytilus orbignyanus*; 6: *Mytilus dactyliformis*; 7: *Mytilus granulatus*.

Figura 5. Lámina 5 en Gay (1854) mostrando las diferentes especies de bivalvos descritos por Hupe (1854). 4: *Mytilus chilensis*; 5: *Mytilus orbignyanus*; 6: *Mytilus dactyliformis*; 7: *Mytilus granulatus*.

ACKNOWLEDGMENTS

S. Ferrada and C. B. Canales-Aguirre are students in the Doctoral Program in Systematics and Biodiversity and were supported by UdeC Doctoral Fellowship. This work was funded by projects FONDEF D03I-1095 and INNOVA-CORFO 10CREC8535.

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Recibido: 21.06.12
Aceptado: 03.10.12