Phylogenetic and phylogeographic patterns of *Didymosphenia geminata* on invaded sites in Chile

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Understanding the population dynamics of invasive processes has become a pressing concern in a highly connected world faced with ongoing climate change and increased exotic species introduction. In Chile, the invasive freshwater benthic diatom *Didymosphenia geminata* (Lyngbye) Schmidt has become widespread, expanding across multiple river basins spanning over 3000 km in three years. Here we evaluate the phylogenetic and phylogeographic relationships of *D. geminata* samples collected throughout the invaded range in Chile, using specific rbcL gene sequence previously published. Genetic sequences for this marker were generated for 19 sample sites, and were compared with available freshwater diatom sequences, as well as with previously published rbcL gene sequences for *D. geminata*. We find that all genetic sequences collected within Chile present phylogenetic divergences from *D. geminata* samples collected in Siberia, as well as from samples of the genera Gomphonema, Cymbella and Encyonema. Thus, we validate the invasion by *D. geminata*, in agreement with existing morphological taxonomic criteria. In addition, a haplotype analysis showed a total of 13 haplotypes, two of which (haplotypes I and IX) found in 12 and 3 populations respectively, while each of the remaining haplotypes presents a single population. Thus, these results are consistent either with the introduction of multiple lineages, or with a rapid genetic differentiation in this invading freshwater diatom. Further genetic sampling, both within Chile and in countries that may have been potential sources of the invasion are needed to test these alternative hypotheses.
Phylogenetic and phylogeographic patterns of Didymosphenia geminata on invaded sites in Chile

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Abstract

Understanding the population dynamics of invasive processes has become a pressing concern in a highly connected world faced with ongoing climate change and increased exotic species introduction. In Chile, the invasive freshwater benthic diatom *Didymosphenia geminata* (Lyngbye) Schmidt has become widespread, expanding across multiple river basins spanning over 3000 km in three years. Here we evaluate the phylogenetic and phylogeographic relationships of *D. geminata* samples collected throughout the invaded range in Chile, using specific *rbcL* gene sequence previously published. Genetic sequences for this marker were generated for 19 sample sites, and were compared with available freshwater diatom sequences, as well as with previously published *rbcL* gene sequences for *D. geminata*. We find that all genetic sequences collected within Chile present phylogenetic divergences from *D. geminata* samples collected in Siberia, as well as from samples of the genera *Gomphonema*, *Cymbella* and *Encyonema*. Thus, we validate the invasion by *D. geminata*, in agreement with existing morphological taxonomic criteria. In addition, a haplotype analysis showed a total of 13 haplotypes, two of which (haplotypes I and IX) found in 12 and 3 populations respectively, while each of the remaining haplotypes presents a single population. Thus, these results are consistent either with the introduction of multiple lineages, or with a rapid genetic differentiation in this invading freshwater diatom. Further genetic sampling, both within Chile and in countries that may have been potential sources of the invasion are needed to test these alternative hypotheses.
Introduction

*Didymosphenia geminata* (Lyngbye) Schmidt is a freshwater benthic diatom, also known as “Rock snot” because it may attach to solid surfaces with polysaccharide stalks extruded from individual cells, forming a dense mucilaginous mat which may extend to form nuisance blooms that dominate riverine environments (Bothwell et al., 2009). *D. geminata* is considered native to the northern hemisphere (Kilroy, 2004), with the first nuisance bloom documented on Vancouver Island, British Columbia in the early 1990s (Bothwell et al., 2009). In Chile, *D. geminata* was first reported in 1962 (Díaz et al., 2012; AMAKAIK, 2016). However, no further records were described in the literature until the summer of 2010 in the Futaleufú basin, were the first nuisance bloom was reported in Río Espolón (approximately 43° S – 71°O), Región de Los Lagos (Díaz et al., 2012; AMAKAIK, 2016; Segura 2011). Within three years, *D. geminata* expanded its range to 3000 km, ranging from 38° S to 53° S and it is now considered a ‘plague’, as declared by the Chilean Subsecretaria de Pesca, Resolución Ex. 2064/2010 (Leone et al., 2014).

The most common way to identify *D. geminata* in Chile has been the taxonomic evaluation under microscope (Díaz et al., 2012; AMAKAIK, 2016) However, recent work by Jaramillo et al. (2015) applied DNA-barcoding based on *rbcL* and 18s rRNA genes to identify current genetic lineages of *D. geminata* found in 4 Chilean rivers, thereby providing evidence that genetic identification of this invasive diatom is feasible. Nevertheless, available genetic sequences for species of the *Didymosphenia* genus still remain scarce in reference databases such as GenBank (see Nakov et al. 2014, Jaramillo et al. 2015). Hence, complementing available
genetic information could improve the information available to allow the early
detection and monitoring of this invasive species particularly in uncontaminated
rivers, helping managers to make conservation decisions about places invaded by
*D. geminata* (Bothwell et al., 2009; Darling and Blum, 2007). In order to provide
additional genetic evidence that allows us to examine the pattern of genetic
diversity in this invasive species, we reconstructed intraspecific phylogeographic
relationships and analyzed the patterns of haplotype frequencies in this species. To
this end, we used specific ribulose-1,5-bisphosphate carboxylase/oxygenase large
subunit (*rbcL*) gene primers designed by Jaramillo et al., (2015) and examined the
genetic sequences of *D. geminata* samples from 19 infected sites in Chile.

Considering that molecular markers can provide valuable information about
biodiversity, relationships between environmental and may also allow the
reconstruction of changes in biodiversity through time, the goal of this paper was to
evaluate phylogenetic and phylogeographic relationships of *D. geminata* samples
collected throughout the invaded range in Chile

**Materials and methods**

To evaluate phylogenetic relationships between sites infected with *D. geminata*,
samples were collected between December of 2015 and January of 2016 from 19
sampling sites located from 38°S to 47°S in Central-Southern Chile (Table 1). At
each sampling site, at least 20 mL of biomass were taken from mats produced by
D. geminata. Genomic samples were conserved with ethanol 70% in 50mL conical plastic tubes and were cold stored until they were moved to Austral Biotech Investigation Center, usually between 2 days. Once arrived to the laboratory dependences, were stored at -80°C until processing. Also, at each sampling site a 1 cm³ volume of D. geminata mat was collected and fixed using Lugol as described in Díaz et al. (2012) and AMAKAIK (2016), and then transported to laboratory to carry out taxonomic confirmation under light microscope. Biosecurity procedures were conducted at each sampling site to prevent potential D. geminata contamination among rivers, following the procedures described by Diaz et al. (2011).

To extract total genomic DNA (gDNA) from mat samples, Powersoil® kit from MoBio Laboratories were used. The quality and quantity of genomic DNA was evaluated using agarose gel electrophoresis and Optizen POP Spectrophotometer (Mecasys). To amplify the specific sequence of rbcL gene from D. geminata, we used the primers designed by Jaramillo et al. (2015), with the following sequences: rbcl-F: 5’-ACC AAC AAC TGT ACC AGC GT-3’ and rbcl-R: 5’-TGG GAT GCT TCA TAC GCA GT-3’.

All PCRs were performed in a final volume of 20 µL, containing 1X PCR buffer, 4mM MgCl₂, 10mM of each dNTP, 0.2 U GoTaq® G2 Flexi DNA polymerase, primers 10 µM and 1 µL of gDNA. The amplification of rbcL gene was performed under the following conditions: denaturation initial 94°C x 2 min, 35 cycles, including an initial denaturation of 94°C x 30 seconds, alignment at 57°C x 30 seconds, extension at 72°C x 90 seconds, final extension at 72°C x 5 minutes. 5µL of each
PCR product was evaluated at agarose gel 2% with RedGel™ nucleotide staining, then, 15µL of PCR product was purified with E.Z.N.A.® Cycle Pure Kit D6493-01 from OMEGA bio-tek.

Purified PCR products were sequenced by Macrogen (http://dna.macrogen.com/eng/). All sequences obtained were evaluated at Chromas (http://technelysium.com.au/wp/chromas) to clean chromatograms. Preliminary identification of the obtained sequences was done by performing a nucleotide BLAST search (blastn) (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

To examine genetic diversity and establish phylogenetic relation between rbcL gene sequences of *D. geminata*, a phylogenetic analysis was carried out including previously described diatom sequences for this gene in *D. geminata*. Thus, we examined all the 19 sequences described in this work, together with the seven sequences reported in Chile from Jaramillo et al. (2015) and 2 sequences available for rbcL gene in GenBank, reported by Nakov et al. (2014). Also, we examined sequences available at GenBank/l for three additional genera of diatoms: *Gomphonema*, *Cymbella* and *Enchyonema*. As a result, we examined a total of 48 sequences corresponding to 20 species of diatoms. These sequences were used to construct phylogenetic relationships trees using three approaches. The first one corresponded to a phenetic neighbour joining tree (NJ), which grouped sequences according to their degree of similarity or genetic distance (Saitou and Nei, 1987). The following two trees were built by using cladistics approximations, where group sequences were formed based on the degree of similarity to the previously identified groups. The cladistics trees were built using maximum parsimony (MP)
and maximum likelihood (ML) (REFERENCE). It should be noted that the latter is a probabilistic method, which is why it was selected for the final interpretation of phylogenetic relationships. To perform diversity analysis or genetic variability at the population level, the sequences were aligned using the ClustalW routine in the software package Molecular Evolutionary Genetic Analysis version 7.0 for bigger datasets (MEGA7) (Kumar, Stetcher and Kimura 2016). Once aligned, the sequences were collapsed into haplotypes using the FaBox package (Villesen, 2007) (http://users-bir-c.au.dk/biopv/php/fabox/).

After the phylogenetic relationships identification of these species of Diatoms, sequences corresponding to samples obtained in the fluvial systems in Chile (7 reported in Jaramillo et al (2015) and 19 reported in this work) were analyzed to examine the population variability of *D. geminata* that cover the territory invasion of this specie in our country. To perform diversity analysis or genetic variability at the population level, the sequences were aligned using the ClustalW routine in the software package Molecular Evolutionary Genetic Analysis version 7.0 for bigger datasets (MEGA7) (Kumar, Stetcher and Kimura 2016). Once aligned, the sequences were collapsed into haplotypes using the FaBox package (Villesen, 2007) (http://users-bir-c.au.dk/biopv/php/fabox/). This allowed us to enumerate the amount of haplotypes presents a level of the basins sampled, and also to determine the population genetic diversity, through the calculation of the index of diversity of Shannon-Wiener: \[ H = - \sum_{i=1}^{S} p_i \ln(p_i) \] considering the index i along the haplotypes identified in the previous section.

To infer the historical processes that gave rise to sample populations, the
relationships between haplotypes and geography were examined by a network of haplotypes.

This network was built using the R pegas 0.9 module (Paradis 2010), in R software (R Development Core Team, 2014). The haplotype network is a bi-directional graph that connects the different haplotypes identified, using a model of infinite DNA sequence sites (that is, uncorrected distance or Hamming), taking into account the deletion or omission of missing data pairs (Templeton et al., 1992). The size of the nodes in the resulting network is proportional to the number of sequences for each given haplotype, and the identity of the sequences present in each haplotype is represented by colors.

In addition, the number of estimated mutations between linked haplotypes is represented by small points. Finally, to exam the geographic variation in the identity and frequency haplotype, the geographical location of all sequences was represent by color coding the membership of the sequences in different groups of haplotypes.

Results

Phylogenetic relationships

Figures 1 to 3 illustrate the results obtained by performing a phylogenetic analysis of the 48 diatom sequences using different algorithms to construct the tree of phylogenetic relationships. In Figure 1 we report the results obtained by using a
phenetic algorithm, specifically, nearest neighbor linkage. In this result it can be seen that the genera *Gomphonema*, *Cymbella* and *Encyonema* are clearly separated from most of the sequences of the genus *Didymoshpenia*. Exceptions to this separation are *Didymosphenia* sequences corresponding to *Didymosphenia geminata* with Genebank accession code KJ011820, and KJ011818, reported in GenBank ([https://www.ncbi.nlm.nih.gov/genbank/](https://www.ncbi.nlm.nih.gov/genbank/)) by Nakov et al. (2014), which both correspond to Lake Baikal, Siberia. Thus, the invasive *D. geminata* lineages observed in Chile do not correspond to the Siberian lineages described by Nakov et al. (2014).

This separation of the samples obtained in Chile with respect to the other sequences is also observed when examining the topologies obtained by the algorithms of maximum parsimony (cladistic analysis) and maximum likelihood (cladistic analysis) (Figures 2 and 3). Again, we also observed divergences between the siberian *D. geminata* sequences KJ011820 and KJ011818 with respect to those obtained in the present project and in the work of Jaramillo *et al.* (2015). Furthermore, despite the differences in phylogeny reported in our analysis, a consistent result is the observed separation of the samples collected in Chile with respect to the samples of *Gomphonema*, *Cymbella* and *Encyonema*.

**Diversity and Population Structure**

Regarding the population structure and its genetic variability, the results indicate that, independently of the method of analysis, a group of 15 to 18 sequences of
Didymosphenia geminata are grouped with a very low genetic distance between them. This suggests that there are a large number of the 26 Chilean samples that share very similar sequences. Regardless of the algorithm selected, separations can be seen in the remaining set of samples. These results indicate that there is an important group of populations that could belong to a single lineage. In turn, the remaining sequences could correspond to lineages that originated by mutation from a first introduction or may correspond to new lineages that have been introduced independently.

The analysis of haplotypes allowed to solve the high degree of similarity among the 26 sequences collected in Chile, particularly for those that do not appear as differentiated in the phylogenetic trees (Figures 1 to 3). Table 1 details information obtained for each sequence after using the FaBox software, including the result for the identification of haplotypes based on rbcL gene from Didymosphenia geminata in Chile. The analysis of haplotypes showed that there are two frequent haplotypes corresponding to haplotype I and IX, with 12 and 3 populations respectively, while each of the remaining haplotypes presents a single population (Table 2). When examining the genetic diversity for the different basins, there is a decrease along the latitudinal gradient, with the Bío Bío basin being the most diverse, with six haplotypes present, while the basins of the Puelo and Baker rivers being the least diverse, presenting only a single haplotype (Figure 4). This could indicate a source of invasion from the north to the south. In this sense, and considering only the number of observed haplotypes, a first hypothesis formulated would be that the observed genetic diversity could correspond to a single introduction of a diverse
population, with a point of origin in Bío Bío and later transfer or propagation towards the South. However, there are alternative interpretations or hypotheses. The first is that the high number of haplotypes could correspond to multiple introductions, subsequent to the original introduction. Alternatively, a number of these introductions could have given rise to new haplotypes, through mutations, resulting in greater diversity, as observed in the Bío Bío, Valdivia, Bueno, Palena and Aysén basins.

To examine to what degree the evidence obtained suggests one or another hypothesis, the degree of similarity between haplotypes was assessed by a network or graph analysis. Figure 5 depicts the haplotype network obtained from the sequences (Paradis 2010). This network corresponds to a bi-directional graph that connects the different haplotypes identified from the analyzed sequences. In the network, each node corresponds to a population or sequence, and is connected by a link to that (or those) haplotypes of greater similarity as to its genetic sequence. The graph uses an uncorrected or Hamming distance, corresponding to a model that assumes the existence of infinite DNA sequence sites (Templeton et al., 1992). This network represents the number of sequences for each haplotype given by the size of the node, also detecting by color the identity of the sequences present in each haplotype. The points located along each link represent the number of mutations between the haplotypes connected by said link.

When examining the haplotype network, it can be seen that haplotype I, which is
the most frequent, is separated from haplotypes III, XI and XII by a single mutation. Of these three, only haplotype III has very similar sequences, highlighting haplotype VIII (one difference mutation with III), haplotype VII (two difference mutations with III) and the IX population (two difference mutations with III). Therefore, it could be expected that haplotype I corresponds to an introduction and possibly IX also corresponds to an introduction. In order to identify these possibilities more clearly, the pattern of geographic distribution of these haplotypes was examined, given that space can significantly complement relationships. When examining the phylogeographic variation of the genetic diversity of the rbcL gene of *D. geminata* in Chile, we could see that the sequence I is widely distributed, while the others have a bounded distribution, with the exception of the sequence IX (Figure 6). However, the pattern of distance in number of mutations indicates that at least haplotypes XI and XII correspond to mutations of haplotype I. On the other hand, haplotypes II, IV, V, VI and XIII present great distances or accumulations of mutations with respect to their haplotypes closer and as well with to haplotype I, at least 4 of which are sequences of relatively isolated and little diverse populations.

All these antecedents suggest a potential for at least two events of invasion of the plague *D. geminata* in Chile, being able to reach up to ten events of introduction from different lineages.

**Discussion and Conclusion**

Regarding the genetic variability of *Didymosphenia geminata* samples reported in...
this work, we highlight three points that merit discussion. The first corresponds to
the quality and quantity of the results obtained, and their relevance in terms of
population screening as well as phylogenetic or estimation of relations between
diatom lineages. The second point corresponds to the genetic variability observed,
and the degree of resolution at the population and phylogenetic level, evidenced
mainly in the phylogenetic trees generated. Finally, the third point relates to the
implications of inferred haplotypes from the sequences studied, particularly for the
invasion and potential sources of this species in Chile.

With regard to the first point, it should be noted that the process of extraction and
purification of DNA for this group of organisms is a complex process and is
currently being actively researched worldwide. In this regard, our results
corroborate the validity and reliability of the \textit{rbcL} gene as a suitable molecular
marker to extract useful genetic information for the analysis of this specie.
Similarly, it should be noted that the results obtained contribute increasing the
amount of evidence available for this gene in the genus \textit{Didymosphenia} worldwide,
doubling the number of sequences reported of this gene. Both elements provide an
important degree of validity and reliability in the molecular protocol performed. In
this context, the variation introduced by our results on the estimation of the total
diatoms phylogeny, is a point of particular interest in the study of this group in
general. The great extent of the specie \textit{D. geminata} and its high invasive potential
require a comprehensive approach that considers in particular molecular markers
to elucidate kinship relationships, especially if it is desired to identify potential
sources of the different invading lineages, both in Chile and in other countries. On
the other hand, the validity and reliability of molecular markers such as *rbcL* will allow broaden the range of tools available for the taxonomy of this pest, helping to support existing identifications based on the taxonomy from morphological features.

A second important result is the clear difference between the samples collected in Chile, with respect to the sequences available from other species. In this sense, the results strongly suggest that the samples of *D. geminata* reported by Nakov et al. (2014) would not correspond to the population source of the current invasion of this specie in Chile. This makes relevant and necessary the development of additional sampling and sequencing efforts, both in our country and in potential sources, like in South America and in other continents. In addition, to successfully solving the genetic differences of *D. geminata* strains present in Chile, the three phylogenetic analysis methods used in this work coincide in indicating that a large number of the populations sequenced in our country correspond to sequences that are not very variable. Less than half of the remaining samples can be solved, presenting differences according to the agglomeration methods used. This indicates that there is an important degree of population diversification in this diatom specie.

Finally, we discuss the implications of this genetic variability for the interpretation and management of the invasion process of this pest. The presence of a high number of haplotypes, many of them very different, suggests that a number greater than 1 or 2 invasions could be feasible. However, studies with more molecular evidence are required, expanding the spectrum of genetic markers and the
populations to include, hopefully encompassing potentially population sources. In any case, the results indicate that the genetic structure of this specie is dominated by a dominant haplotype, defined as I, found in most of the basins studied. In turn, the large number of existing base substitutions for most of the remaining haplotypes suggests that a high number of lineages have invaded Chilean basins. In particular, at least 4 or 5 of these lineages are found in basins without other haplotypes. This evidence may be consistent several alternative hypotheses. First, the ongoing invasion could be explained by multiple invasion events, which would require long-distance dispersal events from diverse sources. Likely scenarios could include either transport from existing invaded sites in South America, or transport from other sites in North America. Alternatively, initial invasions could have contained a mixed set of genetic lineages, followed by long-distance dispersal events. In this regard, the spatial location of the different haplotypes strongly supports the existence of short- and large-scale transport mechanisms, given the geographical extension of the two most frequent haplotypes, and the large geographic dispersion of the rarer haplotypes.

On the other hand, an alternative mechanism that could account for the observed pattern of haplotype diversification could be the mutation of initial invasive lineages after they were introduced to Chilean river basins. This would require a large number of generations and a short generation time. A comprehensive review of available literature indicated that information on the generation times of freshwater diatoms is sparse (Kilroy 2004). Growth rates measured under laboratory culture
conditions have been found to vary considerably among species, as well as with temperature and light intensity, with generation times being quite rapid (< 30 h at temperatures between 12 and 20°C) in many cases (Baars, 1983). The large freshwater species *Pinnularia gibba* presented the shortest generation time recorded, with cells dividing every 13 h under the highest light – temperature (20°C) combination (Baars 1983). While there’s no specific information on the generation times of *Didymosphenia*, cell division every 30 h or less could lead to rapid accumulation of biomass, as well as eventually allowing rapid genetic change to accumulate. Again, elucidating these alternative hypotheses would require the analysis of a larger number of samples in the national territory, together with samples from other countries that may be potential sources for the Chilean invasion of *D. geminata*. 
References


Figure 1

Phylogenetic relationship of *Dydimosphenia geminata* and other related diatom species based on the *rbcL* gene.
Figure 2

Phylogenetic relationship of *Dydimosphenia geminata* and other related species of diatoms based on the *rbcL* gene.

The results obtained on the basis of cladistics algorithms of maximum parsimony are illustrated. For each branch, the percentages of bootstrap obtained are indicated. The value to the right of each branch indicates the later probabilities. The represented diatom genera are identified by the colors of the corresponding branch. In orange trees the species of the genus *Encyonema* are highlighted, whereas the species of *Gomphonema* are shown in green. Blue shows those sequences that correspond to species of *Cymbella*, and in red those sequences that correspond to species of the genus *Didymosphenia*. 
Figure 3

Phylogenetic relationship of *Dydimosphenia geminata* and other related species of diatoms based on the *rbcL* gene.

The results obtained are based on the probabilistic cladistics algorithm of maximum likelihood. The percentages of bootstrap obtained are indicated for each branch. The value to the right of each branch indicates the later probabilities. The represented diatom genera are identified by the colors of the corresponding branch. In orange trees the species of the genus *Encyonema* are highlighted, whereas the species of *Gomphonema* are shown in green. Blue shows those sequences that correspond to species of *Cymbella*, and in red those sequences that correspond to species of the genus *Didymosphenia*. 
Figure 4

Population genetic diversity of Dydimosphenia geminata in Chile.

Variation at the basin level for the diversity of haplotypes of the rbcL gene of Dydimosphenia geminata in Chile is illustrated. The number of haplotypes (A) and Shannon diversity (B) are illustrated for the 8 basins studied.
(A) Número de haplotipos

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<th>Biobío</th>
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<th>Puelo</th>
<th>Yelcho</th>
<th>Palena y Costeras, Limite X</th>
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(B) $H^*$

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Figure 5

Median binding network of the haplotypes of the *rbcL* gene of *Dydimosphenia geminata* in Chile.
Figure 6

Phylogeographic variation of the genetic diversity of the rbcL gene of Dydimosphenia geminata in Chile.
Table 1 (on next page)

Sampling sites, where samples were taken, including region, basin, river and geographic coordinates.

Each sample was designated with a Final Name for identification.
<table>
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<th>Region</th>
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<th>River</th>
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<td>Palena_050</td>
<td>43° 38' 57,546&quot; S</td>
<td>72° 1' 17,528&quot; W</td>
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<tr>
<td>Aysén_del_Gral._Carlos_Ibanez_del_Campo</td>
<td>R.Aysén</td>
<td>R.Norte</td>
<td>Norte_010</td>
<td>45° 14' 2,294&quot; S</td>
<td>71° 43' 19,44&quot; W</td>
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<tr>
<td>Aysén_del_Gral._Carlos_Ibanez_del_Campo</td>
<td>R.Aysén</td>
<td>R.Coyhaique</td>
<td>Aysén_050</td>
<td>45° 20' 41,333&quot; S</td>
<td>72° 27' 13,656&quot; W</td>
</tr>
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<td>Aysén_del_Gral._Carlos_Ibanez_del_Campo</td>
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<td>R.Coyhaique</td>
<td>Coyhaique_010</td>
<td>45° 32' 24,866&quot; S</td>
<td>71° 55' 3,102&quot; W</td>
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<tr>
<td>Aysén_del_Gral._Carlos_Ibanez_del_Campo</td>
<td>R.Aysén</td>
<td>R.Simpson</td>
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<td>45° 40' 14,788&quot; S</td>
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<td>Aysén_del_Gral._Carlos_Ibanez_del_Campo</td>
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<td>R.Pollux</td>
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<td>72° 3' 25,297&quot; W</td>
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<td>R.Aysén</td>
<td>R.Baker</td>
<td>Cochrane_010</td>
<td>47° 15' 19,08&quot; S</td>
<td>72° 32' 53,49&quot; W</td>
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PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27330v1 | CC BY 4.0 Open Access | rec: 8 Nov 2018, publ: 8 Nov 2018
Table 2 (on next page)

Location of the genetic sequence samples used to characterize the genetic diversity of *Didymosphenia geminata* in Chile.

The names of the basins and rivers and the geographical location of each sampled population are detailed. In the corresponding case, access masters are indicated for the Genbank database (*rbcL* gene) for each sample. The code of each population identifies the samples represented in Figures 1 to 4. Samples are sorted according to the results of haplotype analysis performed using the Pegas module (Paradis, 2010), indicating the haplotype corresponding to each one of them.
<table>
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<tr>
<th>Basin</th>
<th>River</th>
<th>Final Name</th>
<th>Lat (°S)</th>
<th>Long (°O)</th>
<th>Haplotype</th>
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<td>Llanquihue</td>
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Palena y C. Costeras, Límite X

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Palena y C. Costeras, Límite X

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1 Sequences reported by Jaramillo et al. (2015)