1 **Changes in sub-fossil chironomid assemblages in two Northern Patagonian lake systems associated with**

2 **the occurrence of historical fires**

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1   **Abstract**

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3   Patagonia is commonly seen as an exceptionally pristine area because of its wildlife and practically unpolluted

4   waters. However, during the twentieth century the burning of natural forests was one of the most important

5   human activities in Northern Chilean Patagonia. Some estimations indicate that three million hectares were

6   burned during the first three decades of the century being its impacts rarely assessed. Hence the objective of this

7   study was to evaluate the impacts of the historical fires in Lake Burgos (45º42'S) and Lake Thompson (45º38'S)

8   in Chilean Patagonia. The impact was measured by evaluating chironomid assemblage since they are sensitive

9   enough to be used as an indicator of aquatic ecosystem health. Fires have a direct and drastic effect on a lake

10   watershed but also indirectly affect a lake ecosystem, changing sedimentation patterns or increasing nutrient

11   inputs. In the studied lakes the periods with higher prevalence of fires were identified by charcoal analysis,

12   while organic matter and magnetic susceptibility allowed to confirm the pre-fire and post-fire periods. The

13   chironomid composition was evaluated through a PCA and an analysis of similarity (ANOSIM) to test the

14   significance among periods while a DCA was applied to the chironomid assemblage downcore to assess

15   compositional structure and taxa turnover. In Lake Burgos the ANOSIM test indicated significant differences

16   between the pre-fire and fire periods ($p<0.05$), while in Lake Thompson differences were not significant.

17   However, in Lake Thompson the PCA clearly separated the pre-fire from the fire period but not the fire from the

18   post-fire periods. In both lakes chironomid composition changed in relation to the period of higher prevalence of

19   fires, which in turn implies catchment changes, pollution, and other anthropogenic impacts. Particularly a

20   marked change in mesotrophic/eutrophic taxa was detected, reflecting an increase in nutrient input due to

21   deforestation. Our findings point out that the lacustrine ecosystems are still affected by the impact of fires and

22   the subsequent increase in nutrient supply that occurred almost fifty years ago. No sign of reverting to pre-

23   disturbance conditions was observed, which makes these lakes highly sensitive to current human-induced

24   impacts.

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Introduction

Patagonia has traditionally been described as a very pristine area with important reserves of wildlife (Brooks et al. 2006) and a large reservoir of freshwater resources (Rignot et al. 2003). Nevertheless, northern Patagonia is currently threatened by increasing human activities related to the development of the region (Vince 2010). However human activities in Patagonia are not new, being its ecosystems have been impacted by human activities over the past eighty years (Martinic 2005). Settlers started to colonize the Aysén region at the beginning of the twentieth century causing large fires that destroyed three million hectares between 1930 and 1950 (Martinic 2005). Hence the forest was progressively replaced by artificial prairies and after 1975 by some exotic plantations (Quintanilla 2005).

Fires are probably one of the most important and abrupt disturbances that can occur in a watershed. The direct effect of fire is obviously the loss of native vegetation, which increases runoff. Runoff enhances soil erosion, which promotes the input of sediment to aquatic ecosystems (Markgraf et al. 2007; Whitlock 2001). Among the indirect effects are the changes in water quality, mainly associated with a higher input of nutrients, which can lead to lake eutrophication. Eutrophication can rapidly affect the aquatic biota and the functioning of the aquatic system itself (McWethy et al. 2010; Philibert et al. 2003). Some studies have reported changes in diatom assemblages after the occurrence of fires, due to changes in pH and nutrients (Philibert et al. 2003). Besides, changes induced by fires on other aquatic organisms, like chironomids, whose response have rarely been investigated (Francis 2000; Tremblay et al. 2010).

One of the most frequently used approaches to track the fire history of a watershed is the analysis of charcoal stored in lake sediments (Long et al. 1998; Markgraft et al. 2007; McWethy et al. 2010; Whitlock 2001). During a forest fire the ignition of woody biomass produces charcoal particles that are transported by wind to lacustrine ecosystems, being preserved in the bottom sediments (Whitlock et al. 2008). It is possible to distinguish two kinds of charcoal: microcharcoal (<125-μm) and macrocharcoal (>125-μm; Markgraf et al. 2007; Whitlock and Larsen 2001). Unlike microcharcoal, macrocharcoal represents fires that occurred near the lake or in the basin because they cannot be dispersed more than 30 km (Clark 1988; Thevenon et al. 2010; Whitlock and Larsen 2001). It is therefore possible to identify past local fire events by counting macrocharcoal and looking for peaks of its concentration in lacustrine sediment sequences (Long et al. 1998; Higuera et al. 2010; Philibert et al. 2003).

Chironomidae (Insecta: Diptera) comprises one of the most abundant, diverse and representative aquatic insects being their larval stages an important link in the ecology of aquatic environments (Paggi 2001). In all the larval stages the chironomid develop a chitinous head capsule that is generally very well preserved in lake

sediments being very good indicators of past environmental changes (Walker et al. 1997). In southern South America chironomids have been successfully used as paleoclimate indicators, especially for detecting cold events in the Taitao peninsula (NW Patagonia; Massaferro et al. 2002) and also in recognizing the Huelmo Mascardi Cold Reversal (HMCR; Massaferro et al. 2009). In addition, they have been used to track the impact of tephra deposition on lacustrine ecosystems (Araneda et al. 2007).

In regards to anthropogenic impacts, chironomids have been used to infer trophic evolution of lakes (Brooks et al. 2001; Heiri and Lotter 2003), past levels of oxygen in lake waters (Quinlan and Smol 2001) and as quantitative indicators of salinity (Eggermont et al. 2006). In New Zealand McWethy et al. (2010) detected a noticeable change in chironomids associated with fires caused by the first European settlers. In Argentinean Patagonia Massaferro et al. (2005) found that the main fluctuations in chironomid assemblages in Lake Morenito were in response to human-related environmental disturbances of the first settlers established in the area, due to induced changes in the trophic state of the lake.

But what is the rationale of studying the response of biological proxies to environmental changes in Patagonia? As mentioned above, Patagonian ecosystems are undergoing increased pressure for development purposes. Estimating the response of these ecosystems to current environmental changes requires a good knowledge of the impact of historical fires. In addition, investigating the way in which fires can affect the chemistry of aquatic ecosystems and the global carbon dynamics is important since the frequency and intensity of fires is expected to increase as a consequence of global warming (Philibert et al. 2003).

Therefore, the goals of this study are (1) to identify the pre-disturbance periods composition of the chironomid assemblages in the sedimentary records of two Patagonian lakes; (2) to compare these results with the post-disturbance period to determine if there is an effective response of chironomid assemblages to forces such as fire, and 3) if possible, to assess the resilience capacity of lake ecosystems to such impacts.

Site description

The study area is located in Northern Patagonia in the Aysén region of Chile. Climate in the area corresponds to the supratemperature belt (Amigo and Ramírez 1998), characterized by humid conditions that present a steep precipitation gradient from the wet western to the dry eastern side of the Andes. Annual precipitation ranges from 3000 mm in the coastal zone (Puerto Aysén) to 611 mm in the eastern side of the Andes (Balmaceda airport; Romero 1985). The annual average precipitation of the study area is approximately to 1350 mm (Coyhaique city), which mainly falls as snow.

1   The first study site, Lake Burgos (45°42'35''S; 72°12'53''W), is located 25 km to the southwest of

2   Coyhaique (sector "Seis Lagunas") at an elevation of 379 m a.s.l. It has a surface area of 0.23 km^2 and a

3   maximum depth of 34 m (Fig. 1). The vegetation around the lake is composed of patches of *Nothofagus pumilio*

4   (Poepp. & Endl.) Krasser (Gajardo 1994). Most of the watershed is currently used for agriculture, supporting

5   farms and cattle activities. The second lake, Lake Thompson (45°38'26''S; 71°47'07''W), is located 20 km to the

6   southeast of Coyhaique, at an elevation of 750 m a.s.l. (Fig. 1) and is right on the Chile-Argentina border. It has

7   a surface area of 1.18 km^2 and a maximum depth of 15 m (Fig. 1). The vegetation in this area is mainly

8   composed of *Nothofagus antartica* (G. Forst.) Oerst. and *Berberis buxifolia* Lam., which are characteristic of the

9   forest-steppe ecotone (Gajardo 1994). Dead trees originating from the historical fires of the early 1900s are still

10   visible today in the Lake Thompson watershed. The watershed is currently used for minor cattle activity and

11   important reforestation activity using exotic species (*Pinus ponderosa*, P. Lawson & C. Lawson). Both lakes

12   catchments are mainly composed of basaltic and andesitic lavas and breccias although andesitic and rhyolitic

13   pyroclastic rocks can also occur (Sernageomin 2003).

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15   **Settlement and fire history**

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17   Settlement of Northern Chilean Patagonia in the Aysén river basin started at the beginning of the twentieth

18   century with the arrival of livestock companies (Martinic 2005). Pomar (1923) published a map of the

19   colonization in Northern Patagonia, which shows that the main settlements occurred in areas near Puerto Aysén,

20   Coyhaique and Balmaceda. In the 1930s the settlement process regained vigor following government pressure in

21   the law "Ley de Colonización de Aysén, Nº 4855", dictated in 1930 (Bizama et al. 2011).

22   Since the territory was mainly covered by forest, the settlers had to deforest it in order to transform it

23   into prairies for cattle grazing (Quintanilla 2005). The easiest and cheapest way to deforest was by burning. The

24   method used consisted of starting fires during the spring-summer season, after which the settlers accumulated

25   and dried biomass like branches and trees to initiate the fires in the next season. Settlers controlled the frequency

26   of fires, if the previous fire had not provided the desired results, the settlers would light a new fire in the same

27   area the following year. With the increase in the number of settlers, fires became widespread and continued until

28   the 1950s (Martinic 2005). Bizama et al. (2011) estimated that these fires provoked a loss of 23% of the original

29   vegetation.

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Methods

Sampling and sedimentological analysis

Sediment cores were retrieved from the deepest part of the two selected lakes using an Uwitec gravity corer. The core length reached 77 cm in Lake Burgos (core Burgos 06) and 172 cm in Lake Thompson (core Thompson 10). Once in the laboratory sediment cores were split lengthwise for non-invasive analysis, including X-ray radiography, magnetic susceptibility and visual description. Later the cores were sub-sampled at 1 cm intervals for analysis of organic content, chironomids and charcoal counting. The organic content of the sediment was estimated by loss on ignition (LOI) following the method described by Boyle (2002). Magnetic susceptibility was measured at 1-cm intervals using a Bartington MS2E sensor.

Chronology

The chronology of cores Thompson 10 and Burgos 06 were based on correlations to previously published age models obtained on parallel cores Thompson 08E and Burgos 07 (Bertrand et al. 2012). Correlations between the cores were based on magnetic susceptibility and organic content (LOI) profiles. The age models of sediment cores Thompson 08E and Burgos 07 were based on radiocarbon results only due to low activity of other radionuclides (^{210}Pb and ^{137}Cs). A detailed description of the age models is in Bertrand et al. (2012).

Charcoal

In Lake Burgos charcoal was analyzed from samples from core Burgos 06, which was later correlated with the dated core Burgos 07 (Fig. 2A). In Lake Thompson, charcoal data was obtained from core Thompson-09, which was correlated to the dated core Thompson-08 (Fig. 2B). In both cases magnetic susceptibility and LOI were used for cores correlation.

Sediment for charcoal analysis were sub-sampled at 1 cm intervals taking two or three ml of wet sediment which were disaggregated in a hot solution of KOH (10%) for 20 minutes at 70ºC (Lynch et al. 2003) and then sieved through a 125-µm mesh (Long et al. 1998; Whitlock 2001). Whitlock et al. (2008), indicated that the most representative interval of charcoal size is between 0.125-0.250 mm, therefore, we decided to work with > 0.125-mm fraction and to do not measure the size of the particles individually. This fraction was deposited in a Bogorov counting tray and particles were identified under a stereomicroscope at 32x magnification. Macrocharcoal concentration was estimated by dividing the number of particles by the volume of the sediment sample. In order to determine the charcoal accumulation rates (CHAR, in particles cm^{-2} yr^{-1}), the concentration

1   (particles^{-1} cm^{-3}) was divided by the deposition rate (yr cm^{-1}; Long et al. 1998).

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4   Chironomids

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6   Chironomids were analysed in cores Burgos 06 and Thompson-10, which were correlated with the dated cores

7   Burgos 07 and Thompson-10, respectively, using magnetic susceptibility and LOI (Fig. 2). Then four ml of wet

8   sediment were deflocculated in 10 % KOH for 15 minutes at 70º C and passed through a 90-μm sieve. The > 90

9   um fraction was later sorted in a Bogorov counting tray. The head capsules were picked out with entomological

10   tweezers, mounted in Hydromatrix, and identified using a Zeiss microscope (25, 40, or 100 x), following to

11   Wiederholm (1983), Paggi (2001) and Brooks et al. (2007). The relative abundance of each taxon was presented

12   as a percentage of the total abundance in each centimeter using TILIA and TILIA GRAPH and C2 v1.5 (Grimm

13   1987; Juggins 2007).

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15   Statistical analysis

16   To distinguish different associations along the profile, a Stratigraphically Constrained Sum-of-Squares cluster

17   analysis (CONISS) was applied to the percentage values of the chironomid assemblages using TILIA and TILIA

18   GRAPH (Grimm 1987) and C2 v1.5 (Juggins 2007) programs. The significance of changes among the periods

19   was done with an analysis of similarity (ANOSIM, Clarke and Warwick 2001) implemented in the package

20   Vegan in R (Oksanen et al. 2005). To distinguish possible groupings among the samples corresponding to the

21   pre-fires, fires and post-fires period, a Principal Components Analysis (PCA), was performed using CANOCO v.

22   4.5 (ter Braak and Smilauer 2002). A Detrended Correspondence Analysis (DCA) was also applied to the

23   chironomid assemblages in order to assess the compositional structure and taxa turnover throughout the profile

24   (Birks 1998; Langdon et al. 2004), using the package Vegan which was also used to estimate the Shannon

25   diversity index of the assemblage (Oksanen et al. 2005). The Shannon index was selected because it is a very

26   widely used index for comparing diversity between various habitats (Clarke and Warwick 2001).

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2 **Results**

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4 Sediment properties and dates

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6 The sediment record of Lake Burgos spans the last 157 years in the first 77 cm, giving a temporal resolution of

7 2.0 years cm^{-1}. In Lake Thompson the sediment record of the 172 cm extends back to the last 1570 cal. years.

8 The first 51 cm span the last 177 years, giving a temporal resolution of 3.5 years cm^{-1} for our period of interest

9 (Fig. 2). In Lake Burgos magnetic susceptibility shows important fluctuations from 77 to 25 cm, ranging

10 between 70 and 150 SI units (Fig. 2). A peak reaching 5000 SI units at 43 cm most likely represents a tephra

11 layer. From 25 cm to the surface, magnetic susceptibility is more stable than downcore, although an increase is

12 observed above 8 cm. Organic matter shows a similar pattern but it is reversed (Fig. 3A); it reaches a maximum

13 of 46% between 27 and 12 cm. A similar trend was observed in Lake Thompson (Fig. 3B). From 160 to 51 cm

14 magnetic susceptibility values are high (max 4500 SI units) and organic matter content is low (~10 %), while

15 above 51 cm magnetic susceptibility decreases and organic matter increases.

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18 Charcoal

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20 Figure 5 represent the Charcoal Accumulation Rate (CHAR) in Lake Burgos. Along the sediment record, the

21 charcoal particles occur only in the zone B-III (1980-2007). Charcoal particles in Lake Thompson occur in zones

22 T-II (1880-1990) and T-III (1990-2006). The zone T-II has the highest abundance of CHAR in the part of the

23 sediment record that corresponds to the fire period, peaking approximately between 1940 and 1960; in

24 agreement with the fires provoked by settlers. CHAR in zone T-III presents homogenous values of charcoal

25 particles that would represent the post-fire period in the most recent part of the sediment record.

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28 Chironomid assemblages

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30 *Lake Burgos*

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32 In order to stabilize the variance and reduce the "noise" of data, further analyses were based on square-root

33 transformed percentages. To avoid the influence of rare taxa, only those taxa with abundances higher than 2%

34 and present in at least two samples were considered (Brooks and Birks 2000; Millet et al. 2007). A total of 2,096

1 head capsules were found along the 70 cm sediment profile of Lake Burgos, corresponding to 31 taxa. The

2 Orthocladiinae subfamily represented 41.9% of the total abundance, followed by Chironominae with 40.1%

3 (25.4% Tribe Chironomini, 14.7% Tribe Tanytarsini), Tanypodinae with 14.4% and Podonominae with 3.6%. At

4 the taxa level, the most abundant were *Parakiefferiella* (18%), *Phaenopsectra* (12.8%), *Ablabesmyia* (7.7 %) and

5 *Limnophyes* (6.6%). Head capsule concentration was always above 5 per ml^{-1} in the entire profile, though the

6 concentration showed a noticeable increase from 28 cm to the upper part of the core, averaging 28 per ml^{-1} (Fig.

7 3A).

8   Two different groups of Tanytarsini (types A and B) were distinguished in the chironomid assemblages,

9 mainly based on differences in the shape of antennal pedestal (Fig. 4). An antennal pedestal with a low and

10 rounded spur characterizes Type A, whereas Type B has an antennal pedestal with a longer rounded spur. As this

11 very general classification of Tanytarsini most likely comprises more than two types, a research effort is ongoing

12 to obtain a more clear identification of such morphotypes (Massaferro; pers. commun.). CONISS of chironomid

13 assemblages show three different zones spanning the last 157 years (Fig. 5).

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15 Zone B-I (70-50 cm; 1870-1910 AD)

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17 Head capsule concentrations in this zone were always above 14 capsules per ml^{-1}; however, in some samples of

18 just 5 ml were more than 80 heads. The most abundant taxon is *Parakiefferiella*, which presents a maximum of

19 25% and is fairly constant along the entire zone. *Phaenopsectra* is the second most abundant taxon. It presents a

20 peak in abundance (20.8%) in the lower part of the zone, and it shows a slight decline towards the upper part of

21 the zone. Other important taxa in this zone are Tanytarsina B and *Ablabesmyia*, which are omnipresent but never

22 exceed 16% of the total abundance. *Apsectrotanypus* and *Riethia* are always below 14% of the abundance.

23 Shannon diversity is fairly constant, averaging a value of 2.4; however, there are some fluctuations at the

24 beginning of the zone (Fig. 5).

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26 Zone B-II (50-14 cm; 1910-1980 AD)

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28 This zone represents the maximum abundance of head capsules in the entire sedimentary profile, reaching a peak

29 of 38 heads per ml^{-1} at 28 cm. In this zone *Parakiefferiella* is the most abundant taxon, peaking (36%) at 24 cm

30 and decreasing rapidly towards the top of the zone. *Phaenopsectra* shows its maximum amount at 50 cm

31 (28.8%), after which it decreases until the end of the zone (12.1%). *Limnophyes* shows a fluctuating but globally

32 increasing trend, with levels close to 4% in the lower part of the zone and reaching 17.8% at the end of the zone.

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Other taxa like *Riethia* and *Ablabesmyia* show some peaks at 46 and 10 cm, with 14.9% and 17.7 %, respectively. Tanytarsina B decreases and in the end of the zone its abundance reaches 0%. Diversity fluctuates noticeably in comparison with the previous zone, reaching a minimum of 2.04 at 24 cm.

Zone B-III (14-0 cm; 1980-2005 AD)

This section represents the most recent part of the record, which also contains the maximum amount of charcoal particles (Fig. 5). In the lower part of this zone the number of head capsules is high and decreases toward the upper part of the zone. The lowest quantities were recorded at 12 cm (7 heads ml^{-1}). There is also a noticeable decrease in taxa that were abundant in the previous zones, such as *Parakiefferiella,* which shows low but stable levels. *Limnophyes* also present low numbers, decreasing from 12.8% in the lower part of the zone to 2.3% in its upper part. *Phaenopsectra* and *Eukiefferiella* are practically absent in this zone except for the sample at 6 cm, where it presents 1.6% of the total fossil abundance. In the case of *Stictochironomus,* it is absolutely absent in this zone.

Some taxa that were relatively rare or even absent in zones B-I and B-II show a noteworthy increase in B-III. Tanytarsina A reaches 30.6% in zone B-III, while *Ablabesmyia* increases from 4.2% to 17% (Fig. 5). *Apsectrotanypus* shows a similar trend, increasing from 1.6% to 12.8%. Simultaneously, *Cricotopus* increases from 0% to 8.3% and *Polypedilum* increases from 2.1% to 6.4%. In zone B-III, *Parapsectrocladius* shows a relatively stable presence, averaging 7%, which is very similar to the values obtained for zone B-I. Despite the changes described in each zone, diversity does not show drastic changes throughout the profile.

Lake Thompson

A total of 1885 head capsules from nineteen distinct taxa were retrieved from the 158 cm long sediment sequence (between 159-172 cm only a few head capsules were found). The Chironominae subfamily represents 88.7% (77% Tribe Chironomini, 11.7% Tribe Tanytarsini) of the total quantity, followed by Tanypodinae (7.2%), Orthocladiinae (4.0%) and Podonominae (0.1%). At the taxa level, the most abundant were *Chironomus plumosus* (48%), *Riethia* (14.2%), and Tanytarsina B (9.2 %).

The concentration of head capsules shows noticeable changes along the sequence. In the deeper part of the core, the concentration is very low. At times it is below 5 per ml^{-1} (Fig. 6), and there are less than 20 heads

total per sample. The concentrations above 53 cm show a marked increase, reaching a maximum of 20 heads per ml^{-1} at 8 cm. Checked with species accumulation curves the amount of 20 head capsules just catches a small proportion of the diversity, complicating inferences about climate or earlier human intervention. For this reason, and because the main objective of the study was to assess recent environmental changes, only the sequence from 53-0 cm (with a higher amount of heads) was considered for further analysis. In this segment the CONISS analysis distinguished three different zones, which represent approximately the last 178 years. The zones can be described as follows (Fig. 6).

Zone T-I (51-40 cm; 1830-1880 AD)

Head capsule concentrations present low values in the lower part of this zone, reaching a maximum at 46-48 cm. In this zone, the most abundant taxon is Tanytarsina B, with a level of 41% at 49 cm. *Polypedilum*, *Apedilum*, *Parakiefferiella*, and Tanytarsina A all show percentages lower than Tanytarsina B, even though in this zone they show their overall maximum quantity. Diversity in zone T-I is the highest of the entire profile, reaching a value of 2.21 at 43 cm.

Zone T-II (40-10 cm, 1880-1980 AD)

Head capsule amounts remain very stable in this zone and are always above 50 per sample, averaging 8 heads per ml^{-1}. This zone also marks an increase in taxa that had low levels in the previous zones, such as *Chironomus plumosus* and *Riethia,* which reach a level of 87.3% at 30 cm. Taxa like *Polypedilum*, Tanytarsina A and B and *Parakiefferiella* show an opposite trend in this zone, decreasing. Other taxa that are present in this zone, although low in concentration, are *Ablabesmyia*, *Limnophyes* and *Cricotopus*. Chironomid diversity is highly fluctuating in this zone, alternating between low (0.99) and high values (1.96). In contrast to the previous zone, zone T-II is characterized by a decrease in diversity (Fig. 6).

Zone T-III (10-0 cm, 1980-2007 AD)

This zone represents the most recent part of the sediment record and according to the amount of charcoal and the core chronology; it would correspond to the "post-fire" period. Head capsule concentration is relatively stable, though it decreases slightly towards the end of the zone. There is a noticeable change in this zone, which is

1   marked by the appearance and increase of taxa that were previously absent, such as *Chironomus anthracinus*

2   (maximum of 32.8% at 1 cm). *Parachironomus* and *Alotanypus* also exhibit small increases in comparison to the

3   previous zone. Nonetheless, levels of *C. plumosus* remain high, although they show a decreasing trend towards

4   the upper part of the zone, where *C. anthracinus* also becomes important. The diversity index shows a general

5   increasing trend toward the upper part of the zone.

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8   Response of chironomids to fire periods

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10   According to the charcoal profile, the fires period in Lake Burgos is found between 14-0 cm (1980-2005 AD;

11   Fig. 5). Only the pre-fire and fire periods are recorded in Lake Burgos, while the post-fires period is absent. It is

12   possible to detect a noticeable change in the assemblage composition between the pre-fire and the fire periods

13   best represented by the DCA scores (Fig. 5) and by the PCA biplot of Lake Burgos (Fig. 7). The ANOSIM test

14   delivered a R value of 0.88 and a p-value of 0.001 at 95% confidence intervals ($\alpha = 0.05$), indicating that there is

15   a statistically significant difference in the chironomid assemblages between both periods.

16   In Lake Thompson charcoal data indicates that the fire period is represented at 30-15 cm, corresponding

17   to the years 1930 to 1980 AD, being clearly distinguishable the pre-fire, fire and post-fire periods. Chironomid

18   assemblages also show important changes along the sediment sequence (Fig. 6), but unlike Lake Burgos, the

19   DCA scores show the most important change in the lower part of the core and not in the period corresponding to

20   the fire period.   The PCA biplot on chironomids and samples (Fig. 7) clearly separates the fauna of the pre-fire

21   period from those representing the fire period. However, it fails in distinguishing the post-fire period from the

22   fire period. The ANOSIM test gave a R value of 0.1, which is not significant at 95% confidence intervals (p-

23   value=0.07).

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Discussion

Chironomid composition

According to Brooks (2000), Massaferro et al. (2005) and others therein, there have been very few studies of Patagonian chironomids. This complicates any ecological interpretation due to the scarce knowledge of the ecology of chironomid taxa of southern South America and the lack of modern calibration sets (Verschuren and Eggermont 2006). However, as Massaferro et al. (2002) indicates, previous researchers (Brundin 1956; Saether 1975) showed that the ecological typology of chironomid genera has worldwide validity. This makes it possible to construct tentative inferences of southern South America, based on the ecology of Holartic fauna, which is well known.

Chironomid species composition and the diversity index of Lake Burgos and Lake Thompson show some differences. Lake Burgos recorded a higher number of taxa (25) than Lake Thompson (16) implying a higher value of the Shannon diversity index (2.44 versus 1.59). However, one of the most noticeable differences in the chironomid fauna between the two lakes is the high proportions of *C. plumosus* and *C. anthracinus* in Lake Thompson above 40 cm and the complete absence of both taxa in Lake Burgos. Ecological data from the Northern Hemisphere indicates a clear preference of these taxa for mesotrophic/eutrophic conditions (Brooks et al. 2007; Millet et al. 2007; Tremblay et al. 2010). This suggests that the eutrophication of Lake Thompson started at the beginning of the twentieth century and is still ongoing. Current water quality data of this lake reinforce such a statement because the amount of total nitrogen (0.49 mg l^{-1}) and total phosphorous (0.04 mg l^{-1}, Araneda unpublished data) are indicative of eutrophic conditions.

Despite changes in trophic-related taxa, it is also possible to distinguish a different proportion of littoral/semi-terrestrial taxa between the two lakes. Lake Burgos shows a relatively stable abundance of *Limnophyes* and *Parakiefferiella,* both are typical for littoral zones, and the latter is common in cold waters (Walker et al. 1992, Brooks et al. 2007). However, in Lake Thompson both taxa have very low abundances, occurring mainly in the lower part of the core and decreasing toward the recent past. Such differences could be explained by two factors that can act in parallel: lake morphometry and inter-specific competition. Lake Burgos has a proportionally higher amount of littoral zones than Lake Thompson, which could lead to a higher abundance of the association *Limnophyes/Parakiefferiella* and also semiterrestrial taxa (Fig. 5). With respect to competition, if the historical fires were able to trigger a rapid eutrophication process in Lake Thompson, a rapid ecological succession probably took place as well, represented by the dominance of the association *C. plumosus*/*C. anthracinus*.

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2   Response of chironomids to fire impacts

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4   There are few studies that evaluate the response of chironomids to fires that occurred in lacustrine watersheds.
5   To our knowledge, Francis (2001), McWethy et al. (2009), and Tremblay et al. (2010) are the only studies that
6   have addressed this issue until now.

7   At our study sites the forest fires that occurred in the lake watersheds are clearly represented by the
8   charcoal profiles. Lake Burgos only recorded the pre-fire and fire periods, while in Lake Thompson the pre-fire,
9   fire and post-fire periods are clearly represented (Figs. 6, 7). Lake Burgos has a proportionally bigger watershed
10   than Lake Thompson (Ad/Ao; ratio of the surface area of the drainage basin versus surface area of the lake; 21
11   for Lake Burgos versus 13 for Lake Thompson), but after the fires its watershed was rapidly converted to
12   pastures and used for livestock. This would explain why the sediment record does not give evidence of the post-
13   fire period. But despite some differences in morphometric parameters, the climatic settings in both lakes are
14   rather similar (rainfall, winds). Therefore the main factor explaining the difference in charcoal profile between
15   the lakes is the timing of the settlement process. Our results suggest that large forest fires started approximately
16   in 1920 AD in the region of Lake Thompson, while they appear to have started much later in the region of Lake
17   Burgos (Bizama et al. 2011; Figs. 5, 6). It is generally believed that the first settlers started to colonize from the
18   sea (Martinic 2005). Some evidence, however, suggests that a parallel "informal colonization" started from the
19   Argentinean side (Martinic 2005). Due to the easier environmental conditions of the eastern side of Northern
20   Chilean Patagonia —the open and windy pampa— the colonization from Argentina could have been faster and
21   more efficient. Since Lake Thompson is located close to the border of Argentina, it is expected that the settlers
22   impacted its watershed earlier and more intensively than the watershed of Lake Burgos.

23

24   Another factor that could have be responsible for the high prevalence of forest fires caused by the
25   settlers were the dry periods that characterized the beginning of the twentieth century in Patagonia (Holz and
26   Veblen 2011). However, the chironomid assemblage in both lakes does not seem to reflect a possible increase in
27   temperature. In Lake Thompson *Ablabesmyia*, *Apsectrotanypus*, *Polypedilum* and *Parachironomus* can be
28   interpreted as warm adapted taxa (Massaferro et al. 2009), but only *Parachironomus* increase slightly in the
29   upper part of the core and is also defined as an eutrophic taxon, then is not possible to clearly determine if its
30   changes are due to an increase in nutrients or an increase in temperature. In Lake Burgos *Polypedilum*,
31   *Apsectrotanypus*, *Ablabesmyia*, *Labrundinia*, *Parachironomus*, and *Cricotopus* can be indicative of warm
32   conditions (Massaferro et al. 2009). Nonetheless, only *Cricotopus* shows a slight increase in the upper part of

the core, but Williams et al. (2012) suggested that some genus of *Cricotopus* do not respond to temperature. Then it cannot be inferred an influence of current global warming on the chironomid assemblage of both lakes.

Specific changes in chironomid assemblages are clearly observed in the DCA1 scores (Figs. 5, 6). In Lake Burgos the largest change in the DCA1 score occurs in the phase with the highest concentrations of charcoal, during the main fire period (Fig. 5). In contrast, the most important change in the DCA1 score of Lake Thompson occurs in the deepest part of the core and not in the period with higher fires occurrences. This means that another factor was able to generate a more sensitive response of the chironomid assemblages of Lake Thompson. Below 51 cm the number of chironomids decreases rapidly and also the sediment properties change drastically (Fig. 3), decreasing organic content and increasing magnetic susceptibility. This suggests that this sediment could be composed of glacial clays (Fig. 3), probably associated with the manifestation of a Little Ice Age Type Event, which generated a noticeable impact on chironomid composition. Nevertheless, chironomids do not seem to reflect colder temperatures below 51 cm (absence of taxa adapted to low temperature). This issue needs further investigation, possibly by increasing the counting of head capsules for that period using larger samples. Another explanation of why chironomids are not reflecting a cold period could be due to the low taxonomic resolution of this study, where some taxa are grouped under the same type but probably some genera could have different temperature optima. Such a problem hopefully will be solved as the Southern South America taxonomy improves –in fact some effort is doing (Massaferro unpublished data), and by developing specific or local training set for temperature. However, increasing taxonomic resolution also implies some risks. Walker (2001) and Brooks et al. (2007) argue that there is an optimum with respect to taxonomic resolution, if this is too low it can cause a loss of information, but an increase in the apparent taxonomic resolution can also produce misidentification.

Regarding differences among periods, the PCA ordination for Lake Thompson clearly shows a difference between the fauna associated to the pre-fire period and the fauna associated to the fire period; however, no difference is observed between the fire and post-fires period (Fig. 7). This suggests that despite charcoal profile indicates that the fire period has ended; the lake and its biota are still experiencing the repercussions of the provoked changes. The ANOSIM test indicated that changes in chironomids of Lake Thompson seem to be less well expressed than those of Lake Burgos. There is however, a clear change towards mesotrophic/eutrophic taxa (*C. plumosus, C. anthracinus*) in the post-fire period, reinforcing the idea that there was a higher nutrient input in this lake after the fires.

The fact that chironomid assemblage is not returning to its pre-fires state in Lake Thompson could also be due to the nutrient enrichment after the fires, because probably was of such magnitude that it generates a total

dominance of eutrophic taxa and thus prevented any change in the assemblage composition. Nevertheless, Brooks et al. (2007) indicate that *Chironomus plumosus*-type is characteristic of strongly eutrophic lakes, while *Chironomus anthracinus*-type is more common in moderately eutrophic lakes. Therefore, the behavior of these two taxa in the more recent part of the core could be indicative of an incipient nutrient reduction in the lake.

Our results of Lake Burgos show a difference in chironomid assemblages associated to the pre-fire and fire periods, differing from the results of Tremblay et al. (2010), who suggest that fires do not have any effects on chironomid assemblages. We assume that this difference is due to the magnitude and extension of the anthropogenic fires. In the study area of Tremblay et al. (2010) the fires had an extension of 950 to 2000 ha. By contrast, three million ha of North Patagonian forest were burned during the settlement, with some watersheds being entirely burned using repeated forest fire ignition techniques (settler descendants, pers. commun.; Quintanilla 2005). Technical reports (IREN, 1979) of the Aysén area indicate that the watershed of Lake Burgos was completely burned after the big fires but was rapidly covered by pasture for livestock. In contrast, one third of the Lake Thompson watershed was burned, and even today is possible to observe dead trees, suggesting that the latter lake underwent a continuous nutrient input that leads to a noticeable current eutrophication process.

Francis (2001) also found important changes in the chironomid assemblages of Douglas Lake, Michigan, that coincides with the arrival of European settlers. The most distinctive change was an increase of eutrophic taxa like *Chironomus, Dicrotendipes, Glyptotendipes*, and *Polypedilum*, with a concomitant decrease in the abundance of *Sergentia*, which is described as a mesotrophic taxon that tolerates moderate oxygen deficiencies. These authors attribute such changes to heavy logging and fires that impacted littoral communities along with siltation and nutrient enrichment.

With respect to the resilience of lakes, and especially of chironomids, Heiri and Lotter (2003) indicate that chironomid assemblages display a considerable resilience to human-induced changes such as cattle activity, quickly returning to the pre-impact state. Scrimgeour et al. (2001), also indicate that benthic communities impacted by fires returns to pre-disturbance levels within 15–20 years.

McWethy et al. (2010), who relates chironomid responses and forest dynamics indicate that the native forests of New Zealand had little or no history of fire when European settlers arrived. They observed that after the most severe period of forest fires the recovery of the native forest was slow (centuries). This suggests the high vulnerability of this forest (formed also by *Nothofagus* spp.) to repeated fire. These observations are similar to our results from Patagonia, where forests formed by *Nothofagus pumilio* and *N. betuloides* [Mirbel] Oersted, K., present low natural restoration from the fires caused almost fifty years ago (Quintanilla 2005). Although some studies show that there was a natural background of fires before the arrival of the twentieth century settlers

1 in Patagonia, we hypothesize that the intensity of these early fires, which were also related to human occupation

2 (Tehuelches Indians), was of much lower magnitude and frequency than those of the twentieth century (Huber

3 and Markgraf 2003; Markgraf et al. 2007; Veblen et al. 1999), whose impacts surpassed the resilience capacity of

4 the affected lakes.

5 Our results of Lake Burgos, therefore, indicate that chironomids respond sensitively to fire impacts.

6 However, it is not possible to determine the resilience of the lake system to such changes since the charcoal

7 profile shows that fires were halted only recently, and chironomid communities do not reflect a change

8 associated to this period. The situation in Lake Thompson is a little different: anthropogenic fires were heavily

9 reduced during the last 20-30 years, but the chironomid fauna has not yet returned to pre-fire conditions. In fact,

10 it continues to show the eutrophication process without any sign of decline. Thus, it is possible to state that fire

11 impact considerably surpassed the resilience capacity of Lake Thompson since approximately 50 years after the

12 impact it still undergoing impact inertia. This means that these Patagonian lakes are highly sensitive to

13 anthropogenic activities of the last decades, and that even small changes −compared to the historical impacts−

14 are limiting the return to pre-disturbances conditions.

15

16

Conclusions

Subfossil chironomid records of two lakes in Northwestern Patagonia allow an evaluation of the impacts of the anthropogenic forest fires that occurred at beginning the twentieth century. In both sites chironomids seem to respond sensitively to the impacts generated by the forest fires, although significant differences between pre-fire and fire periods are only recorded in Lake Burgos. As indicated by other studies, the main effect of fires on lacustrine ecosystems is to increase the nutrient input. This change is clearly registered in the chironomid assemblage of Lake Thompson, which is dominated in its most recent part by *C. plumosus/C. anthracinus*, characteristic of mesotrophic/eutrophic conditions. Present-day levels of nutrients in the lake water confirmed such eutrophication state. In Lake Thompson, the DCA scores indicate that non fire-related factors affected the chironomid assemblages at the beginning of the record. According to our preliminary data, this could be due to the input of glacial sediment to the lake, probably during the Little Ice Age chronozone or another Neoglacial period, but to recognize clearly the impact of such event on chironomids assemblages requires to develop local transfer function for temperature. On the other hand, chironomids also suggest that the impact of fires in the recent part of the core was of such magnitude that the resilience capacity of the lake ecosystems was surpassed. Our data suggests that Patagonian watershed have not yet recovered from the historical fires, indicating that those ecosystems had little resilience to the severe burns occurred at beginning of the twentieth century. Finally, it is possible to conclude that these lakes and their watersheds are still under the effect of the fire impact, which makes them highly sensitive to the present-day human occupation; and that this continued anthropization precludes a return to pre-fire conditions.

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Figure captions:

Figure 1: (A) Map of the study area showing the location of lakes Burgos and Thompson. (B) Lake Thompson bathymetry. (C) Lake Burgos bathymetry.

Figure 2: Correlation between cores dated and cores analyzed through magnetic susceptibility and LOI. (A) Lake Burgos, (B) Lake Thompson.

Figure 3: Sedimentological properties of sediment cores lakes Burgos 06 (A) and Thompson 10 (B), including magnetic susceptibility, organic matter content (%LOI) chironomids head concentrations (head ml^{-1}) and total chironomid head capsules.

Figure 4: Ventral view of some chironomid larvae. (A) Tanytarsina type A, showing an antennal pedestal with no spur. (B) Tanytarsina type B with a prominent spur. (C) *Chironomus plumosus*-type and (D) *Chironomus anthracinus*-type.

Figure 5: Relative frequencies of chironomid assemblages of Lake Burgos. Charcoal profile, diversity index H', Semiterrestrial/Littoral chironomids, DCA scores and CONISS are also shown.

Figure 6: Relative frequencies of chironomid assemblages of Lake Thompson. Charcoal profile, diversity index H', Mesotrophic/Eutrophic chironomids, DCA scores and CONISS are also shown.

Figure 7: Biplot of the PCA analysis of chironomids records from lakes Burgos and Thompson. The empty squares represent the pre-fire periods, the black dots represent the fire periods and the empty dots represent the post-fire periods.
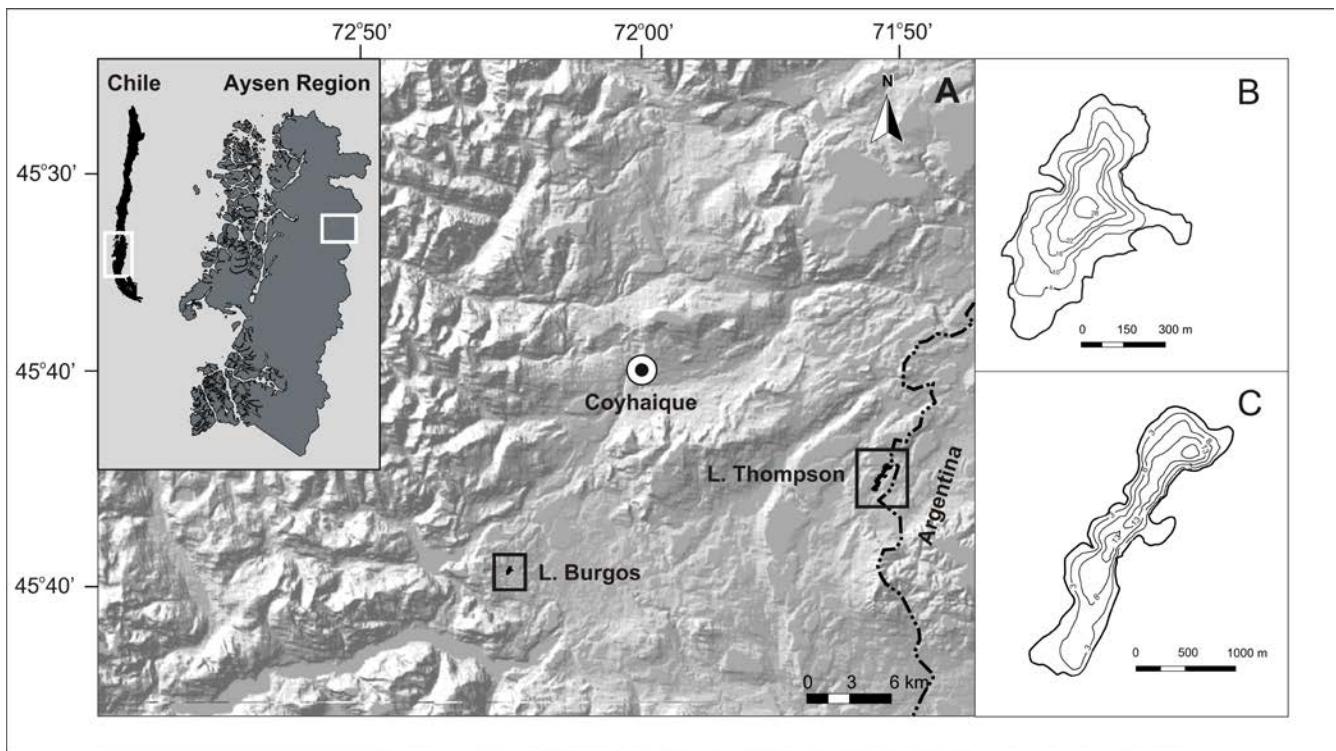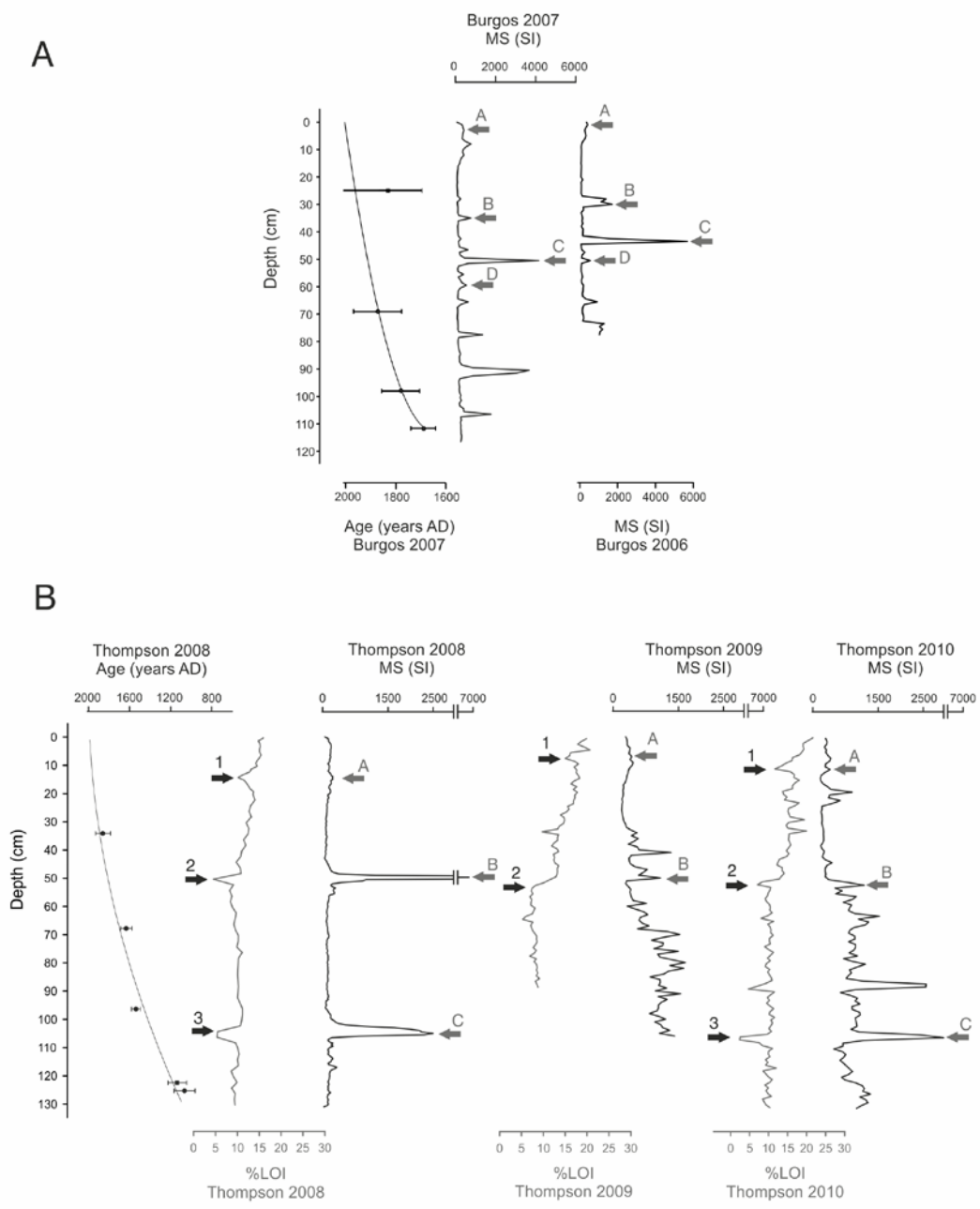
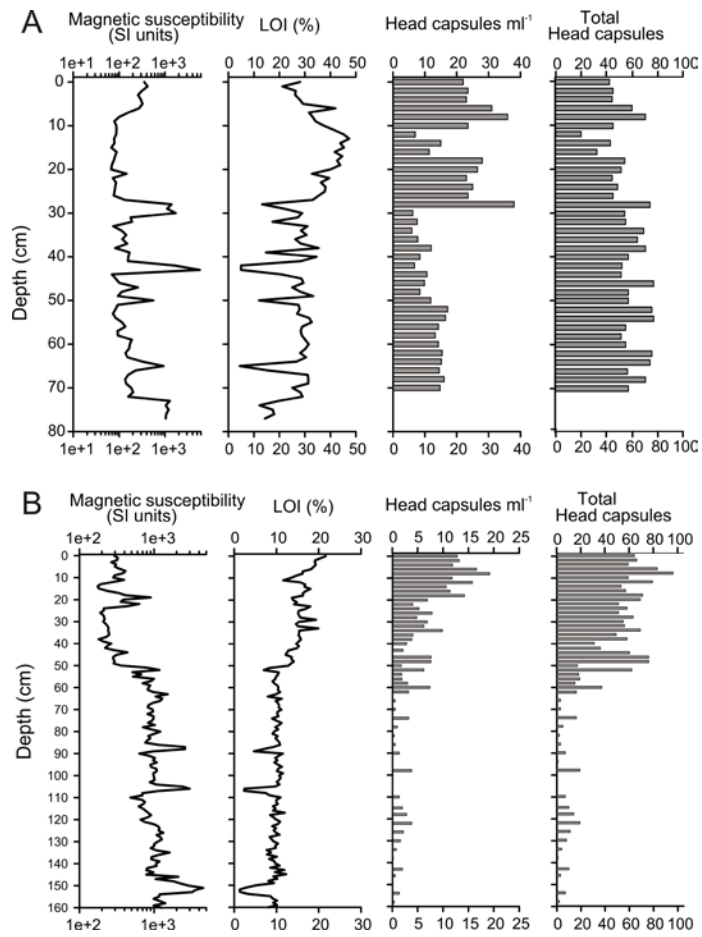Figure 1 *Araneda et al.*
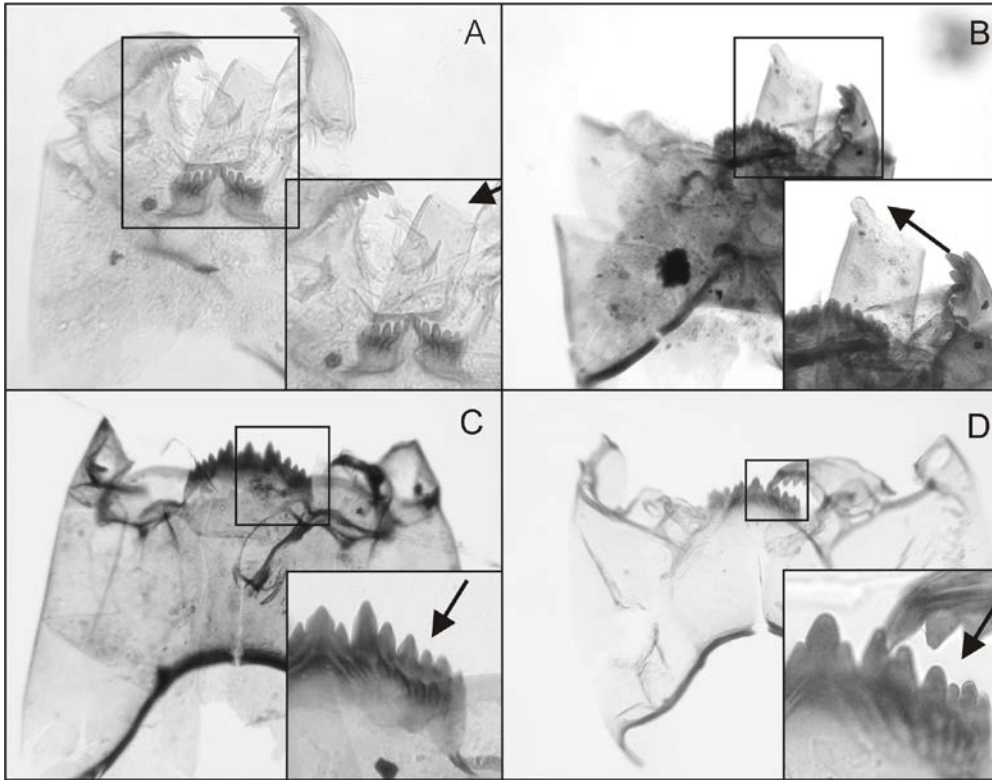
Figure 2 *Araneda et al*
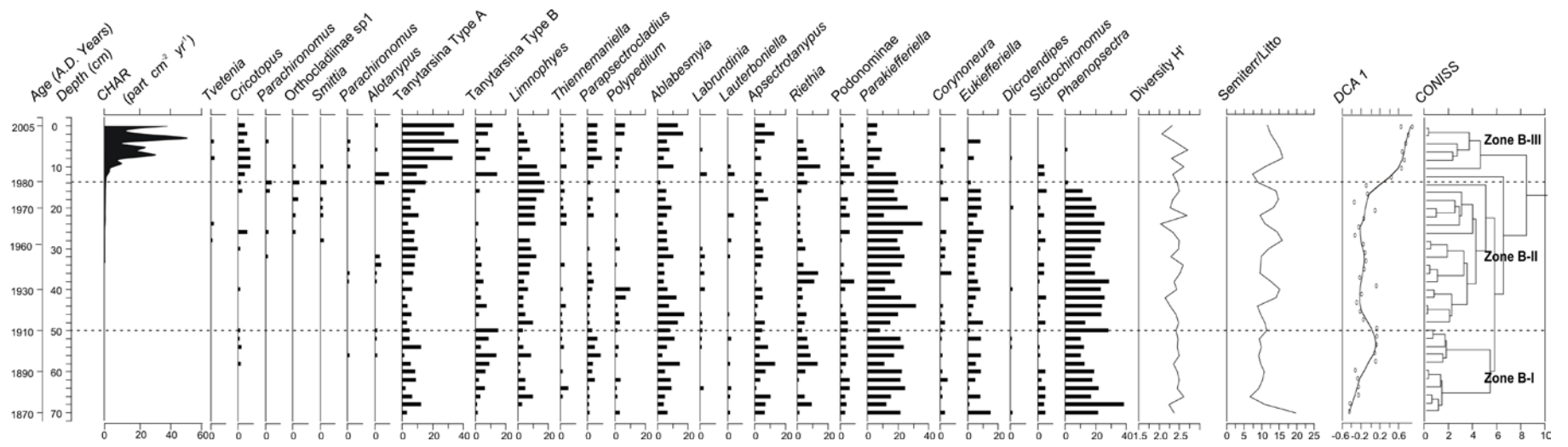
Figure 3 *Araneda et al.*
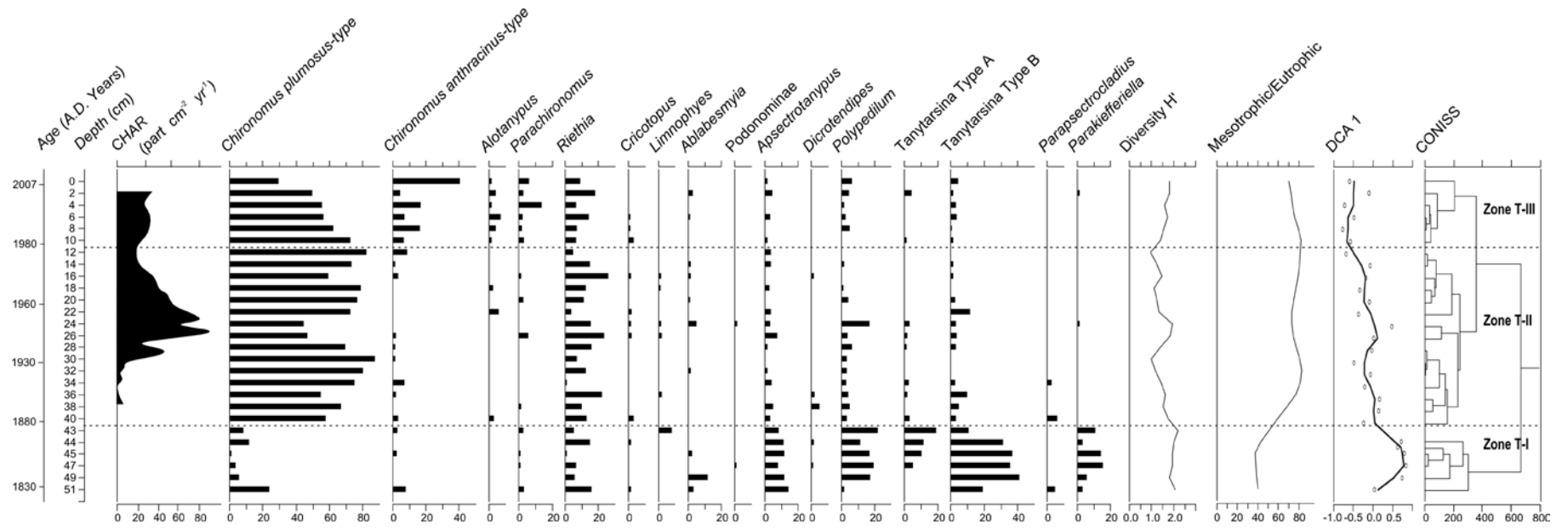
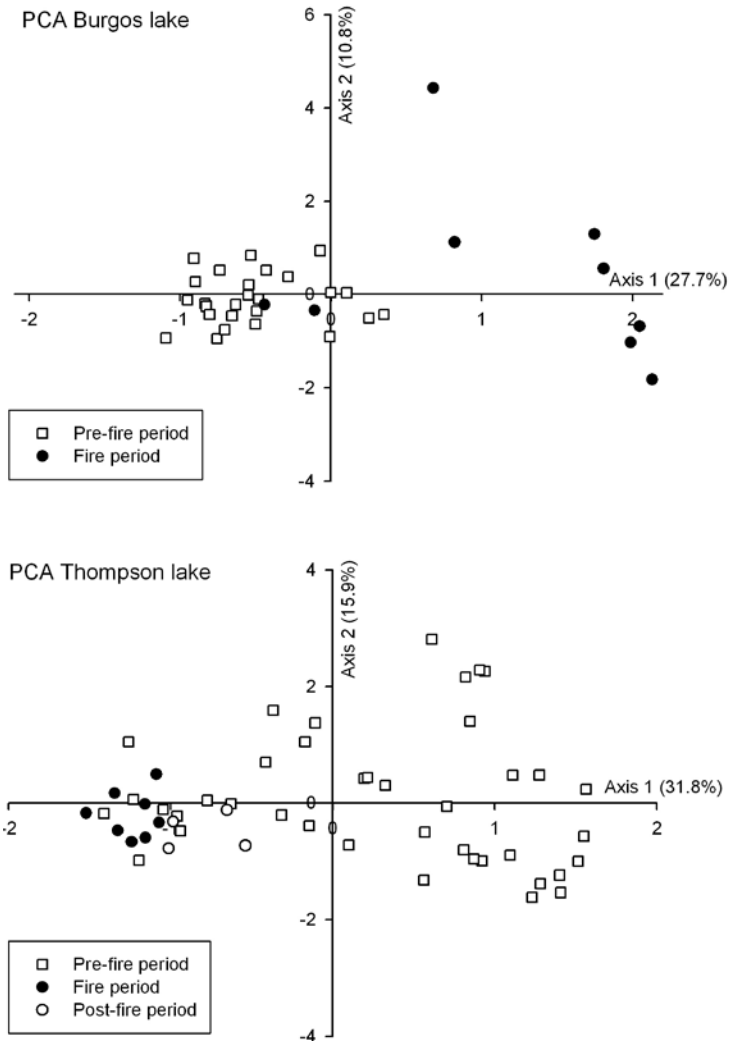Figure 4 *Araneda et al..*

Figure 5 *Araneda et al.*

Figure 6 *Araneda et al.*

PCA Burgos lake

PCA Thompson lake

Figure 7 *Araneda et al.*