

# Phylogenetic relationships and time-calibration of the South American fossil and extant species of southern beeches (*Nothofagus*)

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The genus *Nothofagus* is considered as one of the most interesting plant genera, not only for the living species but also due to the fossil evidence distributed throughout the Southern Hemisphere. Early publications postulated a close relationship between fossil and living species of *Nothofagus*. However, the intrageneric phylogenetic relationships are not yet fully explored. This work assesses the placement of fossil representatives of genus *Nothofagus*, using different search strategies (Equal Weight and Implied Weight), and it analyses relationships with the extant species from South America (Argentina and Chile). The relationships of fossil taxa with the monophyletic subgenera *Brassospora*, *Fuscospora*, *Lophozonia*, and *Nothofagus* and the monophyly of the clades corresponding to the four subgenera are tested. A time-calibrated tree is generated in an approach aiming at estimating the divergence times of all the major lineages. The results support the inclusion of most fossil taxa from South America into the subgenera of *Nothofagus*. The strict consensus tree shows the following species as closely related: *Nothofagus elongata* + *N. alpina*; *N. variabilis* + *N. pumilio*; *N. suberruginea* + *N. alessandri*; *N. serrulata* + *N. dombeyi*, and *N. crenulata* + *N. betuloides*. The species *N. simplicidens* shares a common ancestor with *N. pumilio*, *N. crenulata*, and *N. betuloides*. This contribution is one of the first attempts to integrate fossil and extant *Nothofagus* species from South America into a phylogenetic analysis and an approach for a time-calibrated tree.

**Key words:** Fagales, *Nothofagus*, fossil, extant, phylogeny, time-scaling, Cenozoic, South America.

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## Introduction

The Southern Hemisphere genus *Nothofagus* Blume, 1851 (southern beech) is one of the most interesting plant genera within the order Fagales due to the austral distribution of living species and the extensive fossil record of leaves, pollen grains, and wood (Ancibor 1991; Romero 1986; Hill 1992; Hill and Jordan 1993; Dutra and Batten 2000; Paull and Hill 2003; Poole 2002; Cantrill and Poole 2005; Poole and Cantrill 2006; Torres et al. 2009). Living and fossil members of this genus are distributed in the southern hemisphere of Antarctica (Dutra and Batten 2000; Cantrill and Poole 2012; Reguero et al. 2013), Australia, New Caledonia, New Guinea, New Zealand, Tasmania (Dettmann et al. 1990; Swenson et al. 2000), and South America (Dusén 1899; Romero 1986; Romero and Dibbern 1985). Vegetation

plant reconstructions using fossils provided evidence of past paleoenvironment and paleoclimate as the species of *Nothofagus* are good indicators of cool-temperate conditions (Cantrill and Poole 2012) during the Cretaceous and Paleogene (Romero 1986; Jordan and Hill 1994; Poole et al. 2001; Hinojosa et al. 2016).

Despite the growing amount of available morphological data in fossils, relationships among extinct species of *Nothofagus* are not yet fully explored (Hill 1992). Furthermore, there has been little general agreement about their evolutionary history and the classification scheme (Hill and Jordan 1993; Jordan and Hill 1999). Fossil leaves of Nothofagaceae are found in the most southern part of South America from the Late Cretaceous and throughout Paleogene, similar to the present day distribution of extant species (Dusén 1899; Berry 1938; Hünicken 1967; Torres

et al. 2009; Acosta and Premoli 2010; Premoli et al. 2011; Torres et al. 2013; Leppe et al. 2016a). The morphological study of the fossil leaves (Romero and Dibbern 1985; Vento et al. 2017) revealed unique features that are also currently present in the extant species of *Nothofagus* (Jones 1986; Tanai 1986; Premoli 1996; Heenan and Smiseen 2013). The previous comparisons of leaf imprints of fossil representatives of *Nothofagus* to their living counterparts are based on the description of morphological features and not backed by any phylogenetic analyses (Berry 1937b; Hünicken 1967; Romero and Dibbern 1985; Tanai 1986; Torres et al. 2009).

A systematics of extant species of *Nothofagus* was established by Steenis (1953) on the basis of the presence of the deciduous or evergreen habit, the vernation and cupular structure with two main divisions recognized: leaves deciduous, with plicate vernation and leaves persistent with no plicate vernation. An evolutionary scheme of leaf margin characters in the genera *Nothofagus* and *Fagus* was proposed by Melville (1982) though it was later criticized by Hill and Read (1991), who found some errors in the leaf character coding, probably because the illustrations were not based on cleared leaves methodology for specific details. An exhaustive revision of living and fossil distribution of genus *Nothofagus* with a detailed explanation about venation patterns and margin type have been published by Tanai (1986). Philipson and Philipson (1988) proposed a taxonomic classification considering its four different types of leaf vernation. Subsequently, Hill and Read (1991) proposed a new and more natural classification incorporating into a phylogenetic analysis characters from the cupule, leaf and cuticle morphology from living *Nothofagus* species, which resulted in the recognition of four subgenera: *Lophozonia*, *Fuscospora*, *Brassospora*, and *Nothofagus*. Phylogenetic analyses of extant species of genus *Nothofagus* (Cracraft 1975; Hill and Jordan 1993; Jordan and Hill 1999; Manos 1997; Premoli et al. 2011; Heenan and Smissen 2013) have shed some light on their evolutionary history. The use of fossil plants to establish better phylogenetic hypotheses has often been promoted with a trend toward using combined analysis of fossil and living taxa (Crane 1985; Nixon and Carpenter 1996; Doyle 2012; Hill 1991; Jordan and Hill 1999; Hill et al. 2015). Nevertheless, relationships between fossil and extant taxa are still poorly understood and only a few paleobotanical records from southern latitudes were incorporated into phylogenetic analyses for a better understanding of the evolutionary history of genus *Nothofagus* (Hill 1983). Early phylogenetic analyses, based on morphological characters, of this genus have provided a working classification (Hill and Read 1991) and interesting hypotheses about the relationships among species of *Nothofagus* (Hill and Jordan 1993) for both living and fossil taxa (Jordan and Hill 1999).

The aim of the present contribution is to investigate the relationships of South American (Argentina and Chile) fossil taxa of the genus *Nothofagus* (Nothofagaceae) with their subgenera: *Brassospora*, *Fuscospora*, *Lophozonia*, and *Nothofagus*. The monophyly of these four subgenera is also

tested, placing *Nothofagus* fossils in a phylogenetic tree. A time-scaling of the phylogenetic tree is estimated and discussed.

*Institutional abbreviations.*—BH, L.H. Bailey Hortorium, Ithaca, New York, USA; CPB, Museo de Paleontología, Córdoba, Argentina; IADIZA HRL, Ruíz Leal Herbarium, Mendoza, Argentina; and MACN, Museo Argentino de Ciencias Naturales, Buenos Aires, Argentina.

*Other abbreviations.*—BC, backbone constraint analysis; EW, equal weight; IW, implied weight; MPTs, Most Parsimonious Trees; FAD, First Appearance Data; LAD, Last Appearance Data.

## Material and methods

*Fossil and living material.*—The fossil material examined in this contribution was mainly collected by Hünicken (1967) and recently re-studied by Vento et al. (2017) and Vento and Prámparo (2018). All specimens are preserved as leaf impressions and housed at CPB. Additional fossil material of *Nothofagus* from Patagonia was revised at MACN and from the following literature sources: Dusén (1899, 1908); Berry (1937b); Fiori (1939); Romero and Dibbern (1985). Fossil specimens of *Nothofagus* (*Fagus*) *dicksonii* (Dusén, 1899) Tanai, 1986 described from South America (Dusén 1908; Berry 1937b; Fiori 1939) are fragmentary and consequently the descriptions and illustrations are not clear enough to observe diagnostic characters. For all these reasons *N. dicksonii* is not incorporated into our phylogenetic analyses.

Living species were examined from herbarium sheets held at BH, HRL, and MACN. In order to improve and better understand the phylogenetic relationships among the genera and species of Nothofagaceae, Australasian species were included, based on literature, living material at MACN, and the herbarium catalogue of the Royal Botanical Gardens available online at [www.kew.org](http://www.kew.org) (accessed on February 2017). Extant hybrids of *Nothofagus* were not considered in this contribution.

*Morphological evidence.*—Morphological characters 0–35, including fruit, flower and pollen features of the extant species were coded directly from Heenan and Smissen (2013). Morphological characters based on leaf morphology (36–45) (SOM 1, Supplementary Online Material available at [http://app.pan.pl/SOM/app63-Vento\\_Agrain\\_SOM.pdf](http://app.pan.pl/SOM/app63-Vento_Agrain_SOM.pdf)) were coded from South American fossil material and extant species. The fossil characters scored in this contribution were mainly based on the leaf margin and the venation pattern observations utilizing the following characters: type of leaf margin, teeth simple or composite, tooth spacing, teeth size, primary vein, number of secondary veins, secondary vein ending, apex morphology, base morphology and secondary veins, based mainly on the descriptions made by Tanai (1986) and Gandolfo and Romero (1992).

*Phylogenetic analysis.*—The phylogenetic analysis included both fossil and living taxa of the genus *Nothofagus* from Southern South America (Argentina and Chile). Representative living species from Southern Australia, New Zealand, and Tasmania were also included. Two outgroups were chosen for the phylogenetic analyses: *Fagus* Linnaeus, 1753 (Fagaceae) and *Betula pendula* Roth, 1788 (Betulaceae). *Nothofagus* species were previously included as members of the Fagaceae (Hill and Jordan 1993; Manos 1997; Jordan and Hill 1999) but were considered later to be more closely related to Betulaceae (Nixon 1982, 1989; Jones 1986; Dutra 1997; Premoli et al. 2011). The unique taxon for which the specimen approach was not applied is *Fagus*, which was coded as representing the genus as a whole with its polymorphic nature. The data matrix comprises a total of 46 morphological characters. All characters were informative and treated as non-additive. The phylogenetic analysis was performed using TNT v.1.5 parsimony software (Goloboff et al. 2008). The phylogenetic placement of leaf fossils of *Nothofagus* was tested using three different analytical approaches. We evaluated the results of these approaches and selected our evolutionary hypothesis based on current limitations of each method and considering the congruence of our results with the morphological and molecular available evidence. Firstly, we performed a traditional parsimony analysis under equal weights (EW). As a second alternative, we used a backbone constraint analysis (BC) using a molecular scaffold tree (see Springer et al. 2001 for details). This analysis was performed in order to minimize the negative effects of having too many missing data in parsimony analyses (Manos et al. 2007). The backbone constraint was made taking into account the topology of the consensus trees from Premoli et al. (2011), and Sauquet et al. (2012); the latter to incorporate the subgenus *Brassospora*. Finally, we used an implied weighting analysis (IW), exploring the tree topologies obtained with different concavity constants ( $k$ ) (Goloboff 1993). We used the command “xpiwe (\*)” to avoid that the missing entries generate too much homoplasy of the observed characters during the optimization of the MPTs (i.e., to receive a high fit). This command is comparable to use different values of  $k$  for each character according to its percentage of missing entries. Missing entries are assumed to have 50% of the homoplasy of observed entries as part of the extended implied weighting functions of TNT, that allow to assign lower values of  $k$  to those characters with more missing data, (i.e., down-weighting homoplasy more strongly), see Goloboff (2014) for details. For the IW analysis we explored the topologies of the strict consensus trees resulting from  $k = 1$  to  $k = 30$ . We used IterPCR (Pol and Escapa 2009) in order to identify unstable taxa. This methodology was implemented in TNT under the “pcrprune” command (Goloboff and Szumik 2015). We calculated Bremer support (Bremer 1994; Goloboff and Farris 2001) using up to 10 suboptimal trees, and Jackknife (Lanyon 1985) with removal probability at 0.36 and 500 replicates to measure branch support for

the EW and BC analyses. Such measures are indicated as frequency difference. Evaluation of branch support for the IW analysis was performed using Symmetric resampling (Goloboff et al. 2003) with change probability at 0.33 and 500 replicates (values are indicated as a frequency difference). Both, the EW and BC analyses were conducted using implicit enumeration option that finds the MPTs by means of an exact solution. Since implicit enumeration cannot be performed under extended implied weighting, the analysis under IW was performed using a traditional heuristic search. The search was on the base of Wagner trees with 500 random addition sequences, followed by the tree bisection reconnection (TBR) swapping algorithm, saving ten trees per replicate, and collapsing trees after the search. This was followed by a branch and bound search based on these trees from RAM.

*Time-scaling estimation.*—Divergence times using both fossil and extant taxa were estimated with R (R Core Team 2017) package Strap (Stratigraphic Tree Analysis for Paleontology) designed by Bell and Lloyd (2015). The calibration of the tree uses the first (FAD) and the last appearance datum (LAD) trusted record. The formatting matches that used in the R package paleotree (Bapst 2012) and allows for easy swapping of time-scaled trees between packages (Bell and Lloyd 2015). The minimum and maximum possible age should be considered in a calibrated tree using geochronological information of fossil taxa (Pol and Norell 2006). Therefore, to extract the temporal information from the extinct taxa, the age of each fossil was entered as non-contemporaneous date representing millions of years (Myr) before the present. This age is determined by biostratigraphic or by radiometric isotopic methods in the sequence in which the fossils were derived. The geological age of each fossil (FAD and LAS) was obtained directly from the literature (SOM 2). In order to plot the time-scaling tree against geologic time the function “geoscalePhylo” was used. The geologic time follows Gradstein et al. (2004) or the published time scales by the International Commission on Stratigraphy (Bell and Lloyd 2015). Command “StraPhyloCongruence” was used to assess a congruence of the tree and to calculate the supported measurements (SOM 3).

## Results

The EW analysis resulted in six MPT's of 138 steps. The strict consensus tree presents some polytomies, and the support values for most of the clades are low (SOM 4). The topology of this tree is not congruent with the knowledge of *Nothofagus* phylogeny, especially when considering the position of the species of subgenus *Fuscospora* which are nested within *Brassospora*. IterPCR improved the consensus by pruning the two taxa *Nothofagus alpina* (Poeppig and Endlicher, 1838) Oersted, 1871 and *Nothofagus elongata* (Dusén, 1899) Romero and Dibern, 1985. Conversely, the

BC analysis resulted in two MPTs of 506 steps, the strict consensus and branch support are showed in SOM 4. In this case, IterPCR did not find any improvement by pruning taxa from consensus. The topology of the strict consensus tree is in agreement with the current knowledge but the support values are still low. Finally, the analyses using IW analyses, from  $k = 1$  to  $k = 30$ , resulted in three MPTs. The topologies of the strict consensus trees obtained from  $k = 1$  to  $k = 6$  are exactly the same, a small change occurs in the topology of strict consensus trees obtained from  $k = 7$  and  $k = 8$  (SOM 4). This change consists of the aggregation of *Nothofagus fusca* (Hooker, 1844) Oersted, 1871 and *Nothofagus solandri* (Hooker, 1844) Oersted, 1871, being the latter highly congruent with the results obtained by Premoli et al. (2011). In all the strict consensus trees resulting from  $k = 9$  to  $k = 30$  *Fuscospora* appears as nested within *Brassospora*, the latter aggregation being contrary to current molecular and morphological evidence.

There is not an optimal criterion to choose any particular value of  $k$  (Goloboff 1993) in the IW analysis. Recently, Goloboff et al. (2018) demonstrated that implied weights outperform other methods such as equally weighted parsimony and even model-based methods. These authors also stated that better results can be obtained when weighting more gently against homoplasy (i.e., using larger values of  $k$ ). This idea seems to be supported by our data when considering the congruence of the results obtained with IW analyses and the current knowledge of *Nothofagus* phylogeny. Below, we discuss the results on the base of the strict consensus of the three MPTs with  $\text{fit} = 5.757$  obtained with  $k = 8$  (Fig. 1). Our chosen hypothesis combines a satisfactory balance between robustness and resolution that is also coherent with current morphological and molecular evidence. In the EW analysis the consensus tree can be improved by pruning *N. alpina* and *N. elongata* Dusén, 1899 as indicated by IterPCR. Our results using the IW analysis recovered the four monophyletic clades corresponding to the four extant subgenera. These are clearly differentiated and close relationships between fossil and extant are observed. We found exclusive synapomorphies supporting all the subgenera of *Nothofagus* except for *Fuscospora* because the character granular epidermal cell walls (25-1) is also present in *N. obliqua* (Mirbel, 1827) Oersted, 1871 (Fig. 1).

The fossil taxa from Southern South America appear related with the subgenera *Lophozonia*, *Fuscospora*, and *Nothofagus* (see dashed lines in Fig. 1). The fossil *N. elongata* is located in the clade corresponding to the subgenus *Lophozonia* (SOM 4) and the species *Nothofagus subferuginea* (Dusén, 1899) Tanai, 1986 is located in the clade of subgenus *Fuscospora*, this happens both on BC and IW analysis (Fig. 1, SOM 4). Finally, the clade of subgenus *Nothofagus* includes the placement of fossils *Nothofagus simplicidens* Dusén, 1899, *Nothofagus variabilis* Dusén, 1899, *Nothofagus serrulata* Dusén, 1899, and *Nothofagus crenulata* Dusén, 1899.

## Discussion

Extant species of the genera *Fagus* and *Nothofagus* have a clear differentiation in their hemispheric distribution, with fossil remains and extant species of *Nothofagus* distributed in the southern hemisphere (Tanai 1986; Read 1990). A combination of fossil leaves and pollen records likely leads to more precise estimation of the evolutionary history of *Nothofagus* (Tanai 1986) and occasionally, leaves are the unique available fossil data of *Nothofagus* together with pollen records (Romero 1986). Variations in venation pattern of leaves within the genus and the relationships between secondary vein termination and leaf margin may be useful in determination of monophyletic clades (Steenis 1953). The species of monophyletic subgenera *Brassospora* (Fig. 1) share the character secondary vein ending at the margin (42-2) and the entire margin character (36-3), which differentiates it from the monophyletic subgenera *Nothofagus* (42-1 and 36-1-0).

The results from our phylogenetic analysis support the hypothesis that fossils of genus *Nothofagus* are closely related to modern species currently distributed in Southern South America (Fig. 1). The intraspecific relationships among extant species of *Nothofagus* (Fig. 1) are in agreement with the results obtained by Hill and Read (1991), Jordan and Hill (1999), Heenan and Smitsen (2013), Vento et al. (2017), and the molecular phylogenetic analysis performed by Setoguchi et al. (1997), Martin and Dowd (1993), Premoli et al. (2011), and Sauquet et al. (2012). Heenan and Smitsen (2013) proposed raising the subgenera *Fuscospora*, *Lophozonia*, and *Nothofagus* to the genus rank and renaming *Brassospora* as *Trisyngyne*. These authors argued that the morphological and molecular differences are sufficient to recognize four clades and that this classification will be more informative than the currently circumscribed *Nothofagus* with four subgenera. Nevertheless, this new classification was criticized for not considering fossil species of *Nothofagus* and how it would complicate the use of the extensive current literature on the genus (Hill et al. 2015). To avoid confusion between the modern taxonomy and the fossil record, the last taxonomic classification made by Heenan and Smitsen (2013) is not used in our work. Setoguchi et al. (1997) constructed a molecular phylogeny using pollen grains from extant species but found several polytomies in the tree within the clade of subgenus *Nothofagus* and the relationship of this clade with subgenus *Brassospora*. In our cladogram (Fig. 1), the strict consensus tree is resolved (probably as a consequence of some shared homoplastic characters), but the support for most of the nodes is low (SOM 4: fig. C2). The classification made by Hill and Read (1991) incorporated into the phylogenetic analysis characters from the cupule, leaf, and cuticle morphology of living *Nothofagus*. The cupule morphology better supports the division of the genus on the basis of pollen types, but uncertainty remains regarding the placement of *Nothofagus betuloides* and *Nothofagus pumilio* (Poeppig and Endlicher, 1838) Krasser, 1896 (Hill and Read 1991). This

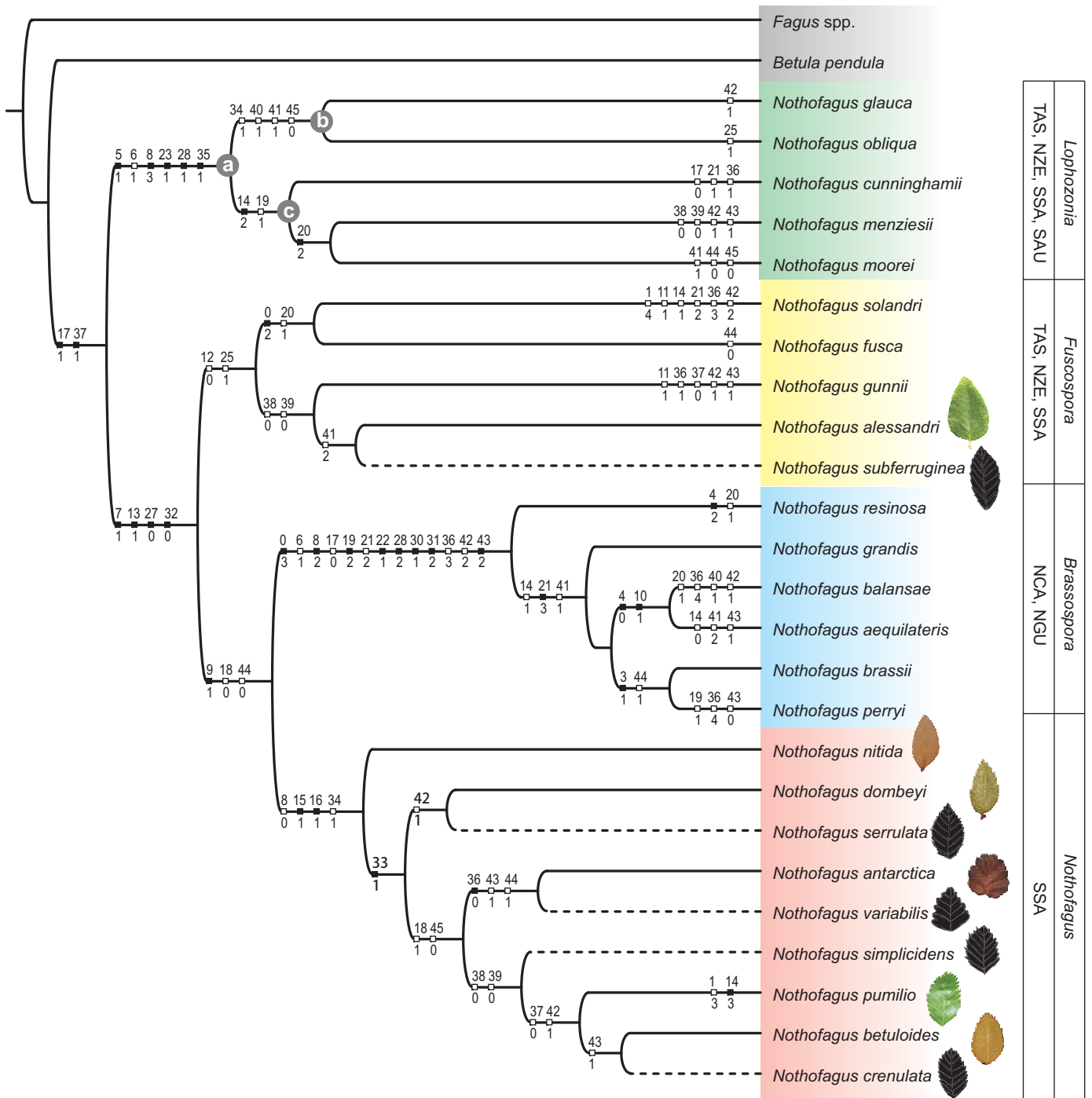


Fig. 1. Strict consensus tree from Implied Weight Analysis (IWA) ( $k = 8$ ). Dashed cladogram branches indicate fossil taxa. Consistency Index (CI): 0.51; Retention Index (RI): 0.78. Shaded circles (a–c) indicate the three possible placements of the two taxa *Nothofagus alpina* and *Nothofagus elongata* before pruning as indicated by IterPCR. Green/brown and black leaves are representatives of living and fossil species, respectively. Abbreviations: NCA, New Caledonia; NGU, New Guinea; NZE, New Zealand; SAU, Southern Australia; SSA, Southern South America; TAS, Tasmania.

issue is resolved in our new analysis where *N. betuloides*, *N. pumilio* and the extinct taxa *N. crenulata* share a common ancestor and belong to the subgenus *Nothofagus* (Fig. 1). According to Hill and Read (1991) leaf architecture gives the least satisfactory classification, since many species bear little resemblance to others in the same genus. The latter authors proposed that the taxonomy of *Nothofagus* leaves is difficult to be resolved based solely on the gross leaf morphology and

venation pattern, and should involve characters of the cupule, pollen grains, and cuticular pattern. For instance, species *N. gunnii* (Hooker, 1844) Oersted, 1871 and *N. pumilio* appear to be very similar on the basis of leaf architecture (Tanai 1986), but the cupule and cuticular morphology of both species are quite distinct (Hill and Read 1991). However, according to the results of our BC analysis (Figs. 1, 2), the inclusion of leaf architecture characters and molecular data,

clearly support the monophyly of the four subgenera when fossil taxa and extant species are considered together in a phylogenetic analysis (SOM 4: figs. B1, B2).

Pollen grains of *Nothofagus* are easily identified due to their characteristic peroblate, stephanocolpate, crassimarginate morphology, with a spinulose exine (Romero 1986). There are three types of grains: “*N. fusca*”, “*N. mensiezii*”, and “*N. brassii*” which are characteristic of each subgenus (Dettman et al. 1990). Living species from Southern South America of subgenus *Lophozonia* has the “*N. mensiezii*” pollen type. In contrast, extant representatives from Southern South America of subgenera *Fuscospora* and *Nothofagus* have “*N. fusca* a” and “*N. fusca* b” pollen type, respectively (Dettman et al. 1990; Hill and Read 1990). “*N. brassii*” type is characteristic of the living species of subgenus *Brassospora* (Dettman et al. 1990; Hill and Read 1991).

The consensus tree (Fig. 1) supports a close relationship between extinct taxa and their modern relatives. The subgenus *Nothofagus* is placed as sister clade to the tropical subgenus *Brassospora*, and the clade, they form is sister to the subgenus *Fuscospora*. These results are similar to the phylogenetic studies by Jordan and Hill (1999), Swenson et al. (2000), and Hill (2001). Sauquet et al. (2012) reported the appearance of *Brassospora* after the Oligocene as it is also revealed by our calibrated tree (Fig. 2). In a preliminary phylogeny that included fossil and extant species from Southern South America, Vento et al. (2017) documented a close relationship between *N. variabilis* and its modern relative *Nothofagus antarctica* (Forster, 1789) Oersted, 1871; *N. elongata* and *N. alpina* and *N. subferruginea* with *N. alessandri* Espinosa, 1928. The fossil taxa *N. variabilis* + *N. serrulata* + *N. simplicidens* + *N. crenulata* are supported as members of the clade that corresponds to the subgenus *Nothofagus* (Fig. 1). The fossil taxon *N. subferruginea* is included in the subgenus *Fuscospora* and a close relationship between this taxon and the modern *N. alessandri* is observed (Fig. 1), what is consistent with earlier works (Tanai 1986; Dutra 1997). The fossil taxa *N. elongata* and its modern relative *N. alpina* are grouped together in the subgenus *Lophozonia* as supported by the scaffold tree in our BC analysis. However, IterPCR indicates that the placement of *N. alpina* and *N. elongata* is unstable for the EW and IW analyses (i.e., they were pruned from the consensus, and not used for support calculations). For EW and IW approaches these taxa have three slightly different possible placements, all within subgenus *Lophozonia* (Fig. 1: a–c; SOM 4: dashed branches). Subgenus *Lophozonia* has been recognized as the most basal clade within Nothofagaceae in majority of studies (Manos 1997; Swenson et al. 2000; Poole 2002; Premoli et al. 2011) except for the cladograms obtained by Hill and Read (1991); Hill and Jordan (1993), and Sauquet et al. (2012). The parasite *Cyttaria* lineage B (34-1) is present in subgenus *Nothofagus* except for *Nothofagus nitida* (Philippi, 1858) Krasser, 1896, which has the synapomorphy: presence of *Cyttaria* lineage A (33-1). The clade *Nothofagus obliqua* + *Nothofagus glauca* (Philippi, 1858) Krasser, 1896 share the

presence of the parasite *Cyttaria* Berkeley, 1842 lineage B (34-1) and the non-exclusive synapomorphies: primary vein curved (40-1), number of secondary veins (41-1) and opposite secondary vein (45-0).

The topology of the IW strict consensus (Fig. 1) shows that fossil and living species of the monophyletic *Nothofagus* clade have the following synapomorphies: annulate pollen aperture thickening (8-0), T-pieces at stomatal poles present (15-1), the stomatal orientation mostly parallel with the midrib (16-1), being the latter two exclusive synapomorphies of subgenus *Nothofagus*. Peterson et al. (2010) suggested a co-evolution between *Nothofagus* and the fungal parasite *Cyttaria* where the phylogeny of *Nothofagaceae* would show that species from Southern Australia and Southern South America are more closely related; however, our analysis resulted in a closer relationship between species from Southern Australia and New Zealand as it was found by other authors (Dettmann et al. 1990; Hill and Jordan 1993; Hill and Read 1991; Manos 1997; Martin and Dowd 1993; Setoguchi et al. 1997; Premoli et al. 2011; Heenan and Smissen 2013). The phylogeny of *Cyttaria* lineage A (33-1) is congruent with the phylogeny of living taxa of the subgenus *Nothofagus*, which might indicate an ancient association and co-evolution of both lineages.

*Nothofagus variabilis* and *N. antarctica* share the leaf type margin (36-0), the apex shape (43-1) and the base morphology (44-1). *Nothofagus serrulata* and *N. dombeyi* (Mirbel, 1827) Oersted, 1871 share the secondary vein ending at the sinus (42-1). *Nothofagus crenulata* and *N. betuloides* (Mirbel, 1827) Oersted, 1871 share the apex morphology (43-1) and they are sisters to *N. pumilio* by sharing the teeth simple (37-0) and the secondary vein ending at the sinus (42-1). Altogether, these taxa share teeth regularly spaced (38-0) and the teeth size (39-0) with the fossil taxa *N. simplicidens*. Dusén (1899) compared *N. crenulata* with the extant *N. cunninghamii* (Hooker, 1844) Oersted, 1871 which is restricted to Australia and Tasmania, while Frenguelli (1941) related it to *N. betuloides*. Tanai (1986) suggested that it is more similar to *N. antarctica* because the margin serrate and the secondary vein end at the tooth.

The clade of subgenus *Fuscospora* contains the fossil *N. subferruginea* and the modern *N. alessandri* that share the synapomorphy: number of secondary veins with more than 12 pairs (41-2), and they share a common ancestor with *Nothofagus gunnii* based on the regular tooth spacing (38-0) and the uniform size of teeth (39-0). A close relationship between *N. gunnii* and *N. pumilio* is proposed by Tanai (1986) on the basis of leaf architecture. A phylogenetic relationship cannot be sustained only on leaf architecture (Hill and Read 1991). Our analysis strongly supports the view of Hill and Read (1991) showing these two taxa in different positions: *N. gunnii* within the subgenus *Fuscospora* and *N. pumilio* within the subgenus *Nothofagus*.

The time-calibrated tree using fossil material from Southern South America (Fig. 2) is an approach to understand the evolutionary history of *Nothofagus*. The lack of fos-

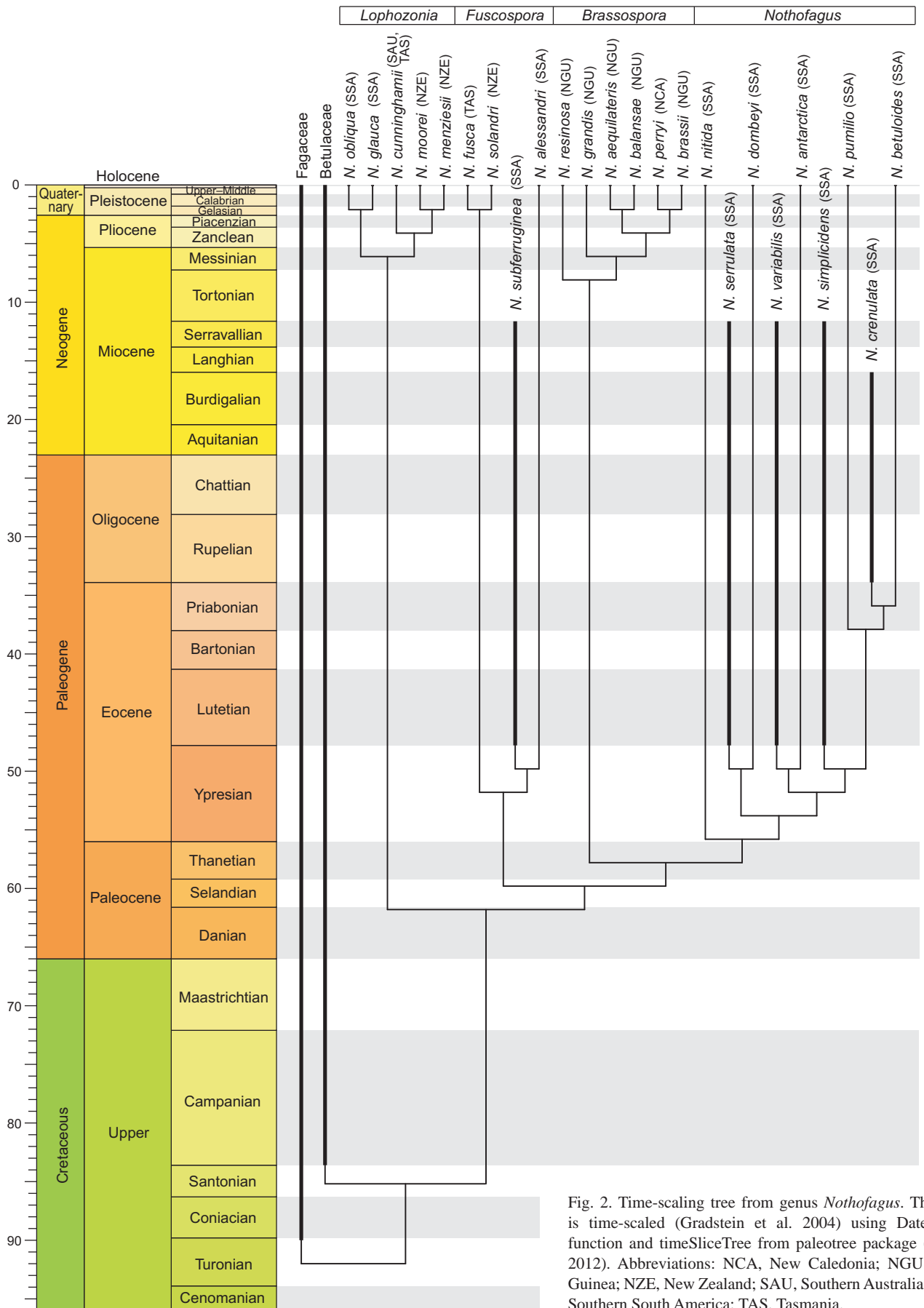


Fig. 2. Time-scaling tree from genus *Nothofagus*. The tree is time-scaled (Gradstein et al. 2004) using DatePhylo function and timeSliceTree from paleotree package (Bapst 2012). Abbreviations: NCA, New Caledonia; NGU, New Guinea; NZE, New Zealand; SAU, Southern Australia; SSA, Southern South America; TAS, Tasmania.

sil information from Antarctica in the phylogenetic analysis (Fig. 2) limits the discussion about the paleobiogeography of this genus and adding this information will certainly constitute an important future contribution. Areas like Southern Australia, New Zealand, and Southern South America together with Antarctica were connected and formed the supercontinent of Gondwana, and an extensive fossil record indicates that *Nothofagus* was a main component of the vegetation there since the Late Cretaceous (Dettmann et al. 1990; Hill 1991; Hill et al. 1996; Swenson et al. 2000; Dutra and Batten 2000; Poole and Cantrill 2002). The origin of *Nothofagus* still remains uncertain. Dutra and Batten (2000) hypothesized that the presence of *Nothofagus* leaf remains in the Antarctic Peninsula earlier than in the other areas of Gondwana, where only pollen grains have been recorded, may indicate the Antarctic Peninsula as a possible centre of evolution and diversification of *Nothofagus* during the Late Cretaceous. Antarctica has a good record of pollen grains and impressions of *Nothofagus* since the Late Cretaceous (Dutra and Batten 2000; Dutra 2004; Hayes et al. 2006; Cantrill and Poole 2012).

The oldest fossil pollen grains were recorded from the Santonian (~85–83 Myr) of the Antarctic Peninsula (Bastos et al. 2013; Reguero et al. 2013), later from the Santonian–Campanian of Southern Australia (Dettman et al. 1990; Dettman 1994) and the Maastrichtian from Southern South America (Archangelsky and Romero 1974). The first mention of fossil leaves of *Nothofagus* in South America was by Dusén (1899), while the oldest record of the leaves from South America has been recently published by Leppe et al. (2016a) from “Las Chinas” Valley (Chilean Patagonia) dated as the lower Maastrichtian (~68.9–71.4 Myr). *Nothofagus* imprints in Antarctica are recorded in the upper Campanian (Dutra 1997; Bastos et al. 2013; Leppe et al. 2016b) and recently also from the lower Campanian by Leppe et al. (2016a). Hayes et al. (2006) mentioned the record of nothofagacean from the Coniacian of James Ross Island. In Argentina, *Dryophyllum australis* Berry, 1937a, with characters similar to Nothofagaceae have been found in the Salamanca Formation (Iglesias 2007) from early to middle Danian (early Paleocene ~65.7–63.5 Myr). Undetermined species of *Nothofagus* are recorded from late Miocene–early Pliocene in the Navidad Formation of Chile (Tanai 1986; Troncoso 1991; Troncoso and Encinas 2006). Fossils of *Nothofagus* are also recorded from the late Pliocene–early Pleistocene of Tasmania (Hill 1991; Hill et al. 1996; Jordan 1999).

The morphology of *Nothofagus* leaves is known to reflect local ecological conditions and climate (Read 1990; Hill 1991; Jordan and Hill 1994; Hinojosa et al. 2016). There are clear differences between environmental and climate requirements of particular species as in the case of the subtropical subgenus *Brassospora* with entire leaf margin and the remaining clades from cooler climate, which are characterized by serrate margin (Troncoso and Encinas 2006).

Living species of the subgenus *Nothofagus* are distributed in a cool temperate climate along 35° S in Southern South

America (Premoli et al. 2011) with some clear differences in their distribution (Veblen et al. 1996). Representatives of this subgenus have leaves with smaller teeth and fewer secondary veins compared with fossil taxa placed into subgenera *Lophozonia* and *Fuscospora* (Romero 1986). Dutra (2004) concluded that bigger leaves suggest warmer conditions. A warm-temperate climate was present from the Late Cretaceous until the beginning of the middle Eocene in the northern Antarctic Peninsula. Smaller leaves are indicative of a deterioration in the climate with cooling conditions during the late Eocene–Oligocene (Dutra 2004; Cantrill and Poole 2012; Reguero et al. 2013). The subgenus *Nothofagus* includes winter deciduous and evergreen species. Extant species inhabiting Southern South America such as *N. nitida*, *N. betuloides*, and *N. dombeyi* are evergreen species whereas *N. antarctica* and *N. pumilio* are deciduous (Hill and Read 1990). It is believed that deciduousness is a primitive character (Hill and Read 1991) and the evergreen habit arose at least three times in the evolution of the genus due to convergent events (Hill and Read 1991; Setoguchi et al. 1997). Currently, species like *N. dombeyi*, *N. pumilio*, and *N. antarctica* thrive at high elevation sites (+1500 m) and species such as *N. betuloides*, *N. obliqua*, and *N. glauca* are distributed from sea level to approximately 1200 m altitude in southern Chile and southwestern Argentina (Veblen et al. 1996). *Nothofagus alessandri*, an endemic species from Chile, grows on slopes exposed to humid conditions (Amigo and Rodríguez 2011). The patterns of distribution along an altitude gradient indicate differences in the ecological niches and climate requirements for living species (Veblen et al. 1996; Cantrill and Poole 2012). The relationship between fossil and living species as *N. pumilio* + *N. serrulata*, *N. antarctica* + *N. variabilis*, and *N. betuloides* + *N. crenulata* can indicate an altitude differentiation from the Eocene–Oligocene (Figs. 1, 2) and until now.

The genus *Nothofagus* appears to be extremely conservative and its fossil record has proven useful for reconstructing the genus past distributions and evolutionary events (Hill 1994, 2001; Hayes et al. 2006).

## Conclusions and future directions

In this work we attempted to merge the information from fossils and extant species of *Nothofagus* from Southern South America into a phylogenetic analysis to refine our understanding of the relationships of this genus. Results of the performed analysis bring new insights on the relationships between modern and fossil species of the genus *Nothofagus* in Southern South America, supporting its monophyly with all four recognized subgenera of *Nothofagus* within single clade. There is a close relationship between fossil taxa and extant species with the former being sister taxa of subgenera *Lophozonia*, *Fuscospora*, and *Nothofagus*. The assignment of fossil *N. elongata* to the subgenus *Lophozonia*, *N. subferuginea* to subgenus *Fuscospora* and *N. serrulata*, *N. varia-*



*bilis*, *N. crenulata*, *N. simplicidens* to subgenus *Nothofagus*, is proposed. Characters such as the venation pattern and the secondary veins ending at the teeth or the sinus and the number of secondary veins are relevant to clarify phylogenetic relationships especially when fossil leaves are available. The fossil record provides evidence for the time of origin and divergence of particular taxa, and it seems that subgenera *Lophozonia* and *Fuscospora* in Southern South America may be older representatives than taxa of the subgenus *Nothofagus*. The climatic conditions for early–middle Eocene were warmer than today. By the late Eocene–early Oligocene the climate probably was similar to the cold climate conditions in southernmost part of Southern South America today. Even though only few morphological characters can be coded from the fossil leaves and most of them can be homoplastic, they still have proven useful in the phylogenetic analysis. Leaf characters have to be considered in the phylogenies especially when no other fossil remains are available. Fossil material from Antarctica has to be included in future phylogenetic analysis for a more accurate interpretation and understanding of the divergence patterns of *Nothofagus* in Southern South America.

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