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# EMERGENCE OF GRASSY HABITATS DURING THE GREENHOUSE–ICEHOUSE SYSTEMS TRANSITION IN THE MIDDLE EOCENE OF SOUTHERN SOUTH AMERICA

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**Abstract.** Phytolith assemblages recovered from the middle Eocene Koluel-Kaike Formation in Central Patagonia, southern South America, confirm the presence of herbaceous components, mainly related to mixed ecosystems or savannahs, and subordinate dry forest communities. This new palaeobotanical information reveals a clear transition between previously described subtropical/tropical woodland-dominated communities from the early Eocene Las Flores Formation, and grass-rich ecosystems from the mid-Eocene Sarmiento Formation. Saddle phytoliths recorded in the Koluel-Kaike Formation would attest the presence of $C_4$ grasses (Chloridoideae) by the middle Eocene of Central Patagonia. These saddle phytoliths is the oldest grassy habits recorded in Central Patagonia, and preceding in at least ~8 Ma the timing proposed by previous contributions. The early appearance of grass-dominated ecosystems in southern South America, would be related to the worldwide greenhouse to icehouse systems transition initiated during the mid-Paleogene.

**Key words.** Phytoliths. Palaeosols. Koluel-Kaike Formation. Palaeoclimate. EECO. Central Patagonia.

**Resumen.** APARICIÓN DE LOS ECOSISTEMAS DOMINADOS POR GRAMÍNEAS DURANTE LA TRANSICIÓN CLIMÁTICA *GREENHOUSE–ICEHOUSE* EN EL EOCENO MEDIO DE PATAGONIA CENTRAL. Asociaciones fitolíticas provenientes de la Formación Koluel-Kaike (Eoceno medio) en Patagonia Central, sur de América del Sur, confirman la presencia de componentes herbáceos, principalmente relacionados a ecosistemas mixto o sabanas, y comunidades arbóreas secas subordinadas. Esta nueva información paleobotánica revela una clara transición entre las comunidades previamente descriptas en las Formación Las Flores (Eoceno inferior), dominadas por componentes leñosos subtropicales/tropicales de tierras bajas, y la sección inferior de la Formación Sarmiento (Eoceno medio), caracterizada por una mayor abundancia de gramíneas. Fitolitos en silla de montar hallados en la Formación Koluel-Kaike indican la presencia de gramíneas $C_4$ (Chloridoideae) en el Eoceno medio de la Patagonia Central. Estos fitolitos del tipo de silla de montar son los registros herbáceos más antiguos de la Patagonia Central y precede al menos ~8 Ma al propuesto por otras contribuciones. La aparición temprana de ecosistemas dominados por gramíneas en el sur de América del Sur, estaría relacionada a la transición climática global, desde sistema *Greenhouse* a *Icehouse*, iniciada a partir del Paleógeno medio.

**Palabras clave.** Fitolitos. Paleosuelos. Formación Koluel-Kaike. Paleoclima. EECO. Patagonia Central.

PREVIOUS studies on phytolith content from early Paleogene successions in Central Patagonia have revealed the presence of forested communities during the early Eocene Las Flores Formation (Brea *et al.*, 2008; Raigemborn *et al.*, 2009), and grass-rich communities in the middle Eocene Gran Barranca Member (lower Sarmiento Formation, Mazzoni, 1979; Zucol *et al.*, 1999, 2004; Andreis, 2008; Zucol *et al.*, 2010a). Vegetation shift occurred in parallel sedimentary, environments and climatic conditions changes. For instance, lacustrine and fluvial sedimentation, under tropical conditions, occurred during the deposition of the Las Flores Formation (Raigemborn *et al.*, 2009, 2010, 2014; Woodburne *et al.*, 2014); and dominant loessic deposition, under semiarid-arid conditions, occurred during the deposition of the Gran Barranca Member, Sarmiento Formation (Mazzoni, 1979; Spalletti and Mazzoni, 1979; Bellosi *et al.*, 2010; Bellosi and González, 2010; Bellosi and Krause, 2014).

In order to better understand the palaeofloristical evolution in Central Patagonia, we document phytolith assemblages from the Koluel-Kaike Formation, a unit bracketed between the underlying Las Flores and the overlying Sarmiento formations. This new information fills the gap in the palaeofloristic record in the San Jorge Basin, Southern South America, between the mid-Ypresian woodland-dominated Las Flores communities, and the late Lutetian, grass-rich Sarmiento ones; and provides key knowledge for understanding the causes of the origin of grasslands in South America, a factor pertinent to regional palaeoecology and the evolution of mammals and feeding strategies (*e.g.*, Strömberg, *et al.* 2013; Dunn *et al.*, 2015). Our aims are to: 1) analyse the floristic composition and changes occurred during the deposition of the Koluel-Kaike Formation, and 2) consider these results in the context of the different hypotheses regarding the emergence of grasslands in South America and their relationship with the global palaeoclimate scenery.

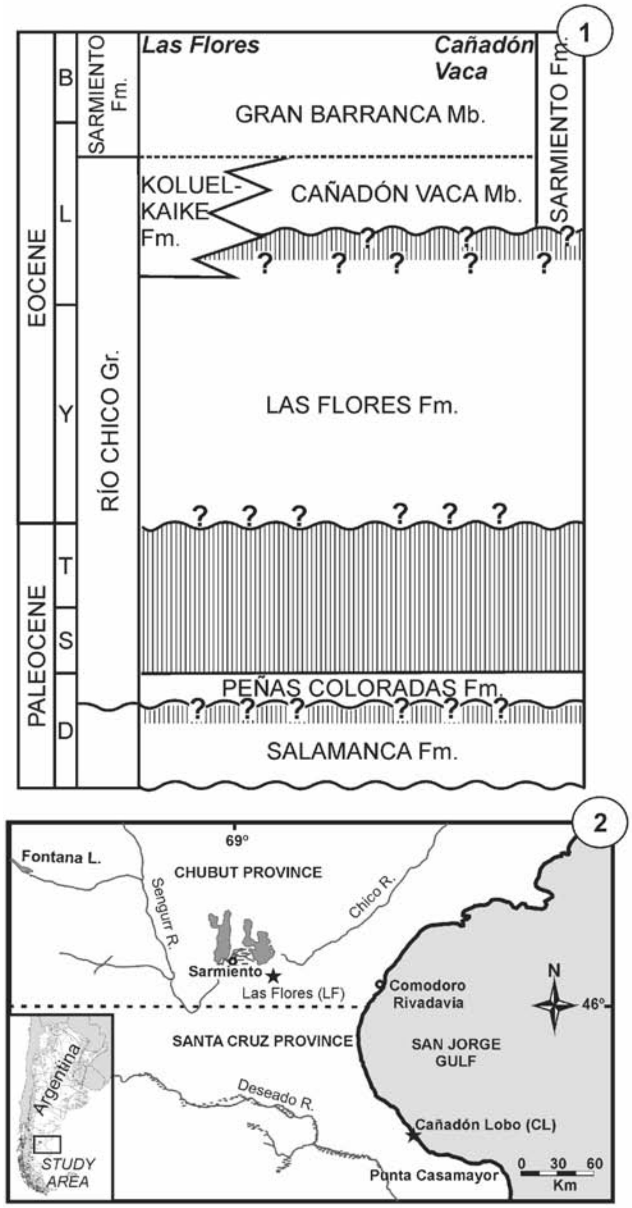
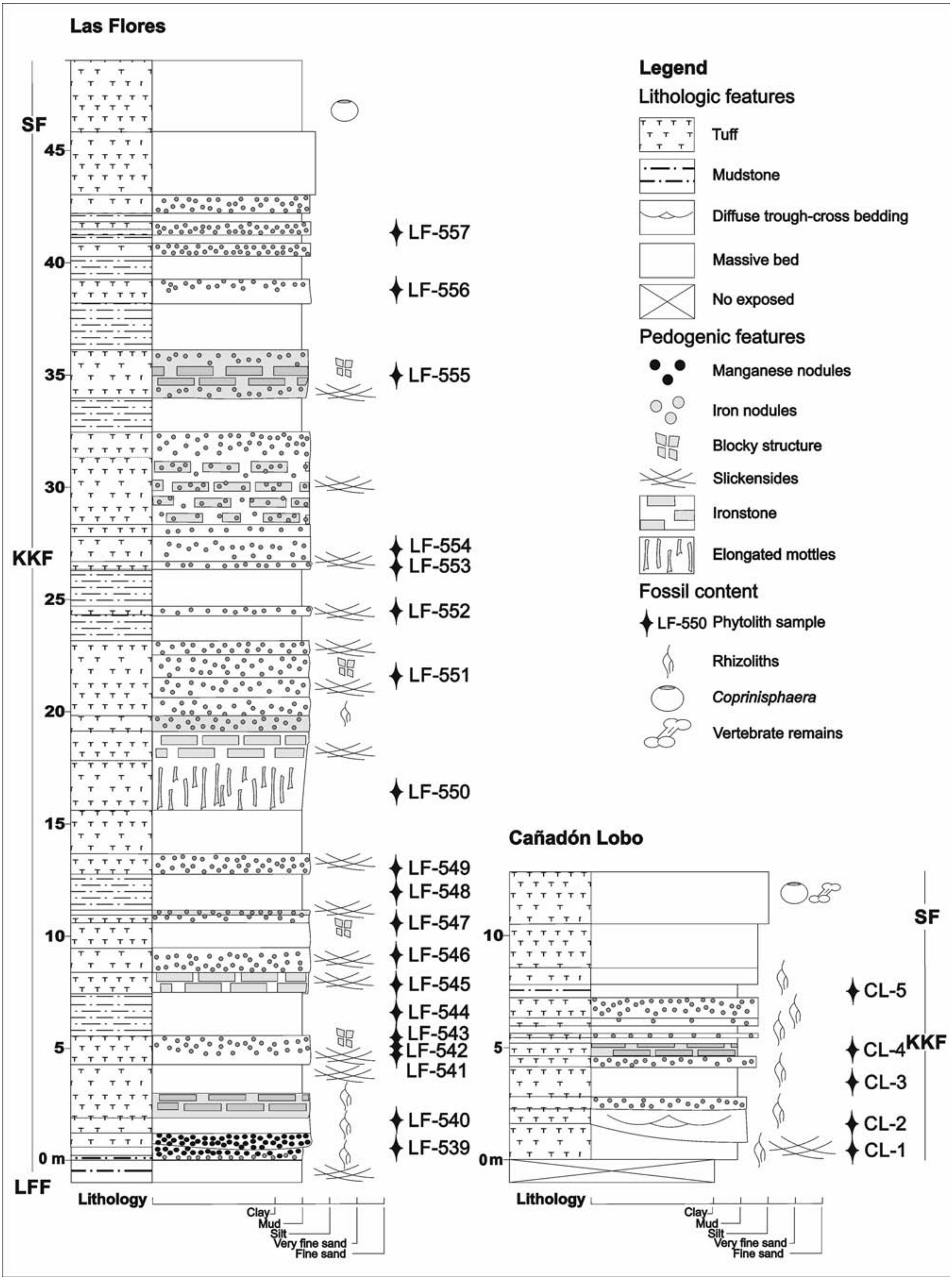


**Figure 1. 1,** Stratigraphic chart for the study area. Modified from Krause *et al.* (2017), and based on Krause and Piña (2012); Clyde *et al.* (2014); and Dunn *et al.* (2013). Stage abbreviations: **D,** Danian; **S,** Selandian; **T,** Thanetian; **Y,** Ypresian; **L,** Lutetian; **B,** Bartonian. **2,** Location of study sites, Las Flores in Chubut Province and Cañadón Lobo in Santa Cruz Province, Central Patagonia, Argentina (San Jorge Basin).

## GEOLOGICAL, SEDIMENTOLOGICAL AND PALAEONTOLOGICAL SETTING

The Koluel-Kaike Formation is a continental unit cropping out in the San Jorge Basin, Central Patagonia, Argentina (Fig. 1.2). In Gran Barranca area the Koluel-Kaike Formation overlies, in probable paraconformity relationship, to the Las Flores Formation (Krause *et al.*, 2017), and underlies in transition to the Gran Barranca Member (lower Sarmiento Formation) (Bellosi *et al.*, 2010; Krause *et al.*, 2010, 2017; Raigemborn *et al.*, 2010). It composes of moderate to highly-silicified, silt-sized, pyroclastic deposits, bentonites, and tuffs, mostly modified to palaeosols (Krause and Bellosi, 2006; Krause *et al.*, 2010; Raigemborn *et al.*, 2010, 2014, 2018). The fossils record of this unit currently restrict to silicified wood, referred to *Taxaceoxylon katuatenkun* Brea, Bellosi *et* Krause (Brea *et al.*, 2009), and trace fossils of cicadas (*Feoichnus challa* Krause *et al.*, 2008a). The temporal span of the middle Las Flores to uppermost Koluel-Kaike succession in the Gran Barranca area was constrained, on the base of magnetostratigraphic and geochronological data, to be ca. 51.4–42.2 Ma (mid Ypresian–late Lutetian) in age, with the Koluel-Kaike being 46.7–42.2 Ma (Lutetian) in age (Krause *et al.*, 2017) (Fig. 1.1).

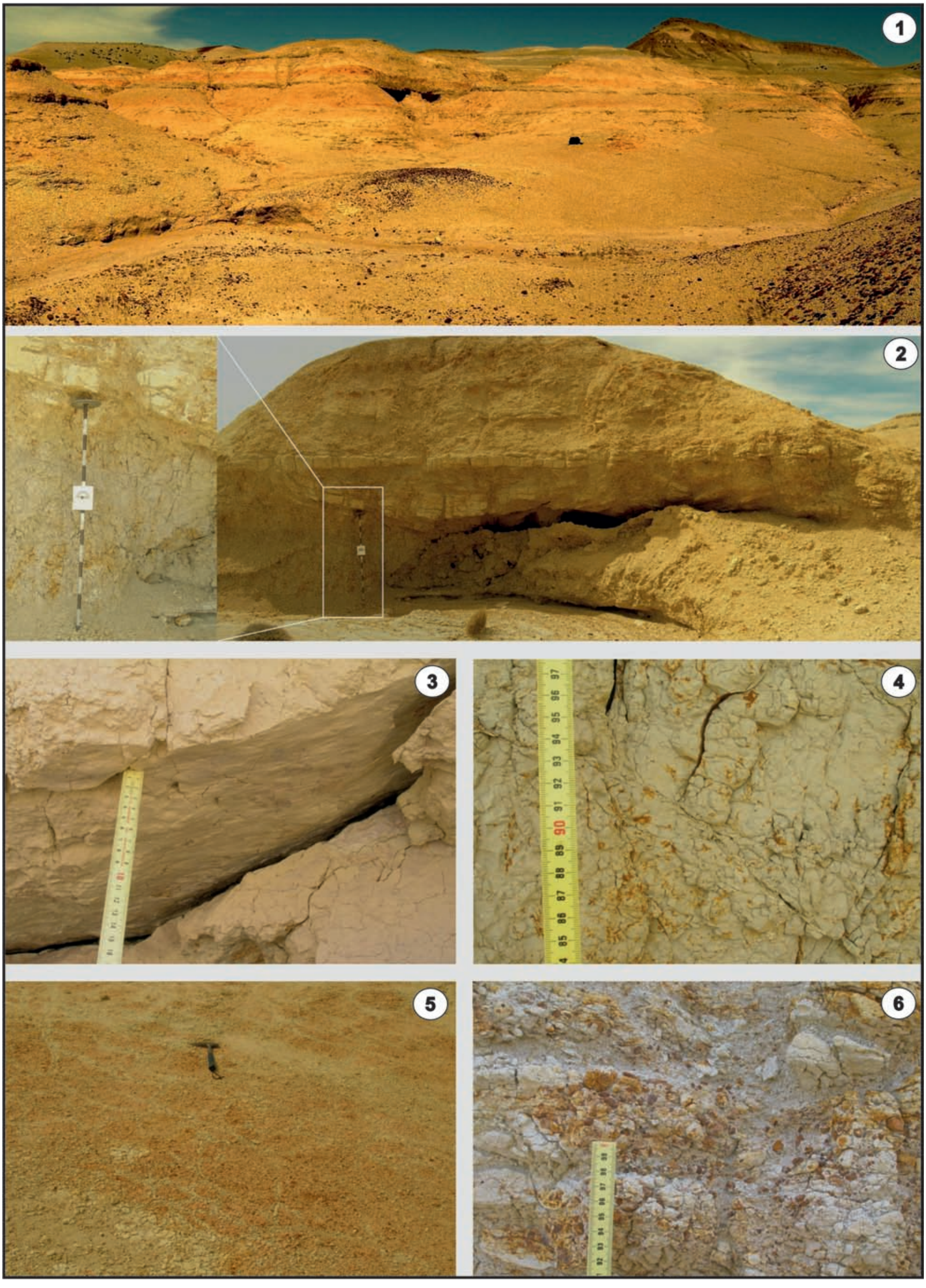
**Figure 2.** Sedimentary sections of the Las Flores (left, modified from Krause *et al.*, 2010) and Cañadón Lobo (right, modified from Raigemborn *et al.*, 2018) localities. Black stars indicate position of studied samples. **LFF,** Las Flores Formation; **KKF,** Koluel-Kaike Formation; **SF,** Sarmiento Formation. The base of the KKF in Cañadón Lobo is not exposed.


Las Flores
SF
KKF
LFF
45
40
35
30
25
20
15
10
5
0 m
LF-557
LF-556
LF-555
LF-554
LF-553
LF-552
LF-551
LF-550
LF-549
LF-548
LF-547
LF-546
LF-545
LF-544
LF-543
LF-542
LF-541
LF-540
LF-539
Lithology
Clay
Mud
Silt
Very fine sand
Fine sand
Legend
Lithologic features
Tuff
Mudstone
Diffuse trough-cross bedding
Massive bed
No exposed
Pedogenic features
Manganese nodules
Iron nodules
Blocky structure
Slickensides
Ironstone
Elongated mottles
Fossil content
LF-550 Phytolith sample
Rhizoliths
*Coprinisphaera*
Vertebrate remains
Cañadón Lobo
SF
KKF
10
5
0m
CL-5
CL-4
CL-3
CL-2
CL-1
Lithology
Clay
Mud
Silt
Very fine sand
Fine sand

For this study, two localities were selected, Las Flores and Cañadón Lobo. The Las Flores locality (LF) (45° 43′ 30″ S; 68° 37′ 22″ W) locates in the eastern edge of Gran Barranca, a classical Cenozoic land-mammal fossil locality (*e.g.*, Ameghino, 1906; Simpson, 1948; Marshall *et al.*, 1983; Madden *et al.*, 2010), ~40 km to the east-southeast of Sarmiento city, south-central Chubut Province (Fig. 1.2). At LF the studied section is ~42 m-thick (Fig. 2), and characterizes by tephric loessic beds, small alluvial channels, and shallow ponds (Krause *et al.*, 2008b, 2010; Raigemborn *et al.*, 2010, 2014). The sedimentation in the Koluel-Kaike Formation would have developed under different climates, with a clear temporal evolution from humid to subhumid-semiarid conditions, as evidenced by palaeosols and clay-mineral information (Krause *et al.*, 2010; Krause, 2012a, b; Raigemborn *et al.*, 2014). Climofunctions applied on palaeo-Ultisols from the lower section, suggest values of Mean Annual Precipitation (MAP) from 1200 to 1300 mm/yr, and Mean Annual Temperature (MAT) ~15° C (Krause *et al.*, 2010), consistent with wet and megathermal conditions. Upward the section, climofunctions applied on weakly-developed palaeosols (*e.g.*, palaeo-Andisols) give values of MAP ~1000 mm/yr and MAT ~12° C Krause *et al.*, 2010), consistent with megathermal–mesic conditions. The change in clay mineralogy throughout the unit, from kaolinite-dominated assemblages, in the lower section, to smectite-dominated associations in the middle–upper ones, also indicates a climatic trend from humid to subhumid conditions, along with pulses of relative stronger seasonality or less intense precipitations periods (Krause *et al.*, 2010; Raigemborn *et al.*, 2014).

The Cañadón Lobo (CL) locality (46° 58′ 24″ S; 66° 50′ 13″ W) places in northern Santa Cruz Province, ~15 km to the northwest of Punta Casamayor, in south border of the San Jorge Basin (Fig. 1.2). At CL the base of the Koluel-Kaike Formation is not exposed. The unit underlies to whitish, massive and tabular beds of the Sarmiento Formation assigned to the Gran Barranca Member, and bearing sparse, fragmentary vertebrate remains from the Casamayoran South American Land Mammal Age (SALMA), fossil dung-beetle brood balls (*Coprinisphaera*) and *Feoichnus* specimens (Pérez *et al.*, 2012; Raigemborn *et al.*, 2018). The contact between the Koluel-Kaike and the Sarmiento formations looks conformable (Pérez *et al.*, 2012), although the absence of around 10–15 m of whitish beds bearing weakly-developed palaeosols recognized in the upper Koluel-Kaike section at LF (Krause *et al.*, 2010), suggests the presence of a paraconformity. The studied section is *ca.* 10 m-thick and mainly composes of massive, silt-sized, pyroclastic deposits contained within tabular and lenticular beds, with minority tabular, massive beds of mudstones (Figs. 2, 3.1–2). Lenticular beds display an erosive base, evidenced by the presence of muddy intraclasts; while tabular beds display sharp bases. Both types of beds lack in recognizable primary, sedimentary structures. Pedogenetic features, among them, iron-rich, reticulate mottles, iron nodules, slickensides, and rhizoliths are common within the tabular pyroclastic and muddy beds. Iron nodules are also present within the lenticular beds (Raigemborn *et al.*, 2018). Based on these features, Krause (2010) interpreted a palaeoenvironment characterized by small fluvial channels, cutting across pyroclastic and muddy floodplains (Fig. 3.2). The presence of iron nodules within the lenticular channel body indicates changes in water content in the original deposit, suggesting a probable ephemeral nature of the channel. On the other side, tabular beds composed of very fine sediments and bearing rhizoliths, slickensides, and iron mottling and concretions, support the presence of pedogenetically modified, floodplain deposits (Krause, 2010; Raigemborn *et al.*, 2018) (Fig. 3.3–4). Palaeosols at CL (Fig. 3.2–6) displaying reddish, reticulate mottles are strongly similar in morphology to those included in the lower Koluel-Kaike Formation at LF (*e.g.*, *Kápenk* pedotype *sensu* Krause *et al.*, 2010), which characterize ironstones levels (Krause *et al.*, 2010) (Fig. 3.5). Since ironstones of the Koluel-Kaike Formation occur typically in the lower

**Figure 3.** The Koluel-Kaike Formation in Cañadón Lobo Locality; **1,** General view showing reddish, indurated, tabular beds, a typical feature of the unit. Thickness of the unit is *ca.* 10 m.; **2,** channel (lenticular whitish bed) and floodplain facies in pyroclastic fine sediments; **3,** slickensides in floodplain deposits; **4,** rhizoliths in pyroclastic mudstones of the floodplain deposits; **5,** reticulated iron mottling (ironstone) in the middle section of the profile; **6,** indurated cornice composed of silicified pyroclastic mudstone and containing abundant ferric nodules. Each division of staff in (B) represents 10 cm. The unit of rule in (3), (4), and (6) is graduated in centimetres. Hammer for scale in (5) is 33 cm long.


1
2
3
4
5
6

section at Las Flores, and are absent in adjacent units (Krause *et al.*, 2010), we correlate the CL section with the lower Koluel-Kaike Formation at LF.

## THE EARLY PALEOGENE OF CENTRAL PATAGONIA: PHYTOLITHS AND OTHERS PALAEOBOTANICAL RECORDS

The early Paleocene–middle Eocene span of time in Gran Barranca and surrounding areas is represented by five formations (Fig. 1.1): Salamanca (estuarine; Danian) (Martínez, 1992; Clyde *et al.*, 2014; Comer *et al.*, 2015), Peñas Coloradas (fluvial; latest Danian) (Raigemborn *et al.*, 2010; Clyde *et al.*, 2014), Las Flores (fluvial, lacustrine; Ypresian) (Raigemborn *et al.*, 2010; Woodburne *et al.*, 2014; Kohn *et al.*, 2015; Krause *et al.*, 2017), Koluel-Kaike (loessic, alluvial; Lutetian) (Krause *et al.*, 2010, 2017; Raigemborn *et al.*, 2010), and lower Sarmiento (Cañadón Vaca and Gran Barranca Members) (loessic, fluvial; late Lutetian–Bartonian) (Bellosi *et al.*, 2010; Ré *et al.*, 2010a, b; Dunn *et al.*, 2013; Bellosi and Krause, 2014).

Phytolith analysis from the Salamanca Formation, proceeding from shallow estuarine and lagoon facies (Zucol *et al.*, 2008) suggesting warm subtropical climate. This interpretation agrees with others palaeobotanical records from the Salamanca Formation, such pollen, leaf compression, permineralized woods and fruits (Berry, 1937; Romero, 1968; Archangelsky, 1973, 1976; Archangelsky and Zamaloa, 1986; Somoza *et al.*, 1995; Brea *et al.*, 2005a, b, 2008; Matheos *et al.*, 2005; Iglesias *et al.*, 2007, Jud *et al.*, 2017; Ruiz *et al.*, 2017, among others).

Phytoliths have not been described for the Peñas Coloradas Formation, but complementary palynomorphs and permineralized woods remains indicate the presence of mixed subtropical to tropical and humid forests (Romero, 1973; Lema *et al.*, 1999; Ruiz *et al.*, 1999; Brea and Zucol, 2006; Raigemborn *et al.*, 2009).

The phytolith assemblages of the Las Flores Formation exhibits high abundance of dicot and palm phytoliths, in association with scarce grasses (Poaceae), sedges, and podostemoid elements, corresponding to subtropical to tropical lowland forests (Brea *et al.*, 2008; Raigemborn *et al.*, 2009). Woody and palm indicators constitute 12.27–39.61% and 8.05–37.19% respectively, with dicots (12.07–34.16%), diagnostic herbs (0–0.92%), non-diagnostic herb/bamboos (8.06–23.09%) and humid herbs (0–4.13%) as understory elements (Brea *et al.*, 2008; Raigemborn *et al.*, 2009).

The phytolith content from the Gran Barranca Member (lower Sarmiento Formation) at Gran Barranca section reveals abundance of palms and up to 15% of herbaceous elements, and a lower proportion of woody plant elements (Zucol *et al.*, 2010a; Strömberg *et al.*, 2013; Dunn *et al.*, 2015; Kohn *et al.*, 2015). Zucol *et al.* (2010a), interpreted this association as the record of savannah palaeocommunities, composed of palms (9.19–38.61%), diagnostic herbs (1.40–18.48%), non-diagnostic herb/bamboo (10.90–35.28%), humid herbs (0–12.38%), dicots (3.57–19.33%), and woody elements (1.39-15.20%), and with a clear increase trend of megathermal components (from $C_4$ grass) upward the unit. Thus, the lower Gran Barranca Member characterizes by the highest abundances of palms (30.19–38.61%), which then decrease into the upper levels to the 12.44–22.50% range. In general, the woody elements have low abundances (1.39–9.74%) in the whole section, with the exception of one sample recorded in the middle-upper section (15.20% in the MMZ9504.0 sample, detailed ID acronyms *sensu* Zucol *et al.*, 2010a). Diagnostic types of grass herbs and non-diagnostic herb/bamboo types respectively increase their abundance from 3.77–6.73% and 10.90–20.00%, in the lower section, to 6.78–18.48% and 14.13–28.57%, in the upper one. This increase relates to the high abundance of panicoid phytoliths. Finally, upward the Gran Barranca Member, the floristic composition recordes an increase in humid herbs, which generally present in low percentages (0–3.43%) throughout the section, rising up to 12.38% in the upper section (sample MMZ9507.5; Zucol *et al.*, 2010a).

However, in spite of similar values of percentage among involved morphotypes obtained for the Gran Barranca Member at type locality obtained by Strömberg *et al.* (2013), Dunn *et al.* (2015), and Kohn *et al.* (2015), these authors do not share the interpretation of a savannah environment. For instance Strömberg *et al.* (2013), on the base of the abundance of palms, suggested an ecological scenery characterized by a closed–humid subtropical forest, an interpretation subsequently modified in Dunn *et al.* (2015). These authors, applying the leaf area index (LAI) proxy, concluded that the declination of the rLAI values, from the middle Eocene to the early Oligocene in the San Jorge Basin, indicating an opening of the landscape from dense vegetation through progressively more open vegetation, that is a nearly grass-free scrubland, an interpretation kept by Kohn *et al.* (2015).

## MATERIALS AND METHODS

Twenty-four sedimentary samples (Fig. 2) were obtained from the Koluel-Kaike Formation for phytolith analysis: 19 from the LF locality (LF539–557), and 5 from the CL locality (CL1–CL5). Nine of these samples (LF541, LF545–546, LF548–549, LF552–555) provided an insufficient phytolith content to obtain a minimal representative sample and thus inadequate for their statistical analysis.

Each sample consisted of 20 grams of sediment, which was processed following a protocol employing various chemical agents to remove soluble salts, organic matter and carbonates (Zucol *et al.*, 2010b). Samples were washed and soluble salts dissolved with distilled water; carbonate material was removed with dilute HCl; organic material was removed by adding $H_2O_2$ followed by deflocculation by Sodium hexametaphosphate. The fractionation was quantified through sieving (coarse fraction) and sedimentation (fine fractions). Four fractions were obtained according to particle diameter: coarse (>250 μm), medium (5–53 μm and 53–250 μm) and fine (<5 μm). Densimetric separation of medium granulometric fractions was performed, in each case, by means of a sodium polytungstate solution (with specific gravity adjusted to 2.345 g/cm$^3$). But for medium fraction (5–250 μm), two densimetric separation were made: one, with the whole fraction (for the counts), and another with both subdivisions (to observe the variations of distribution by size).

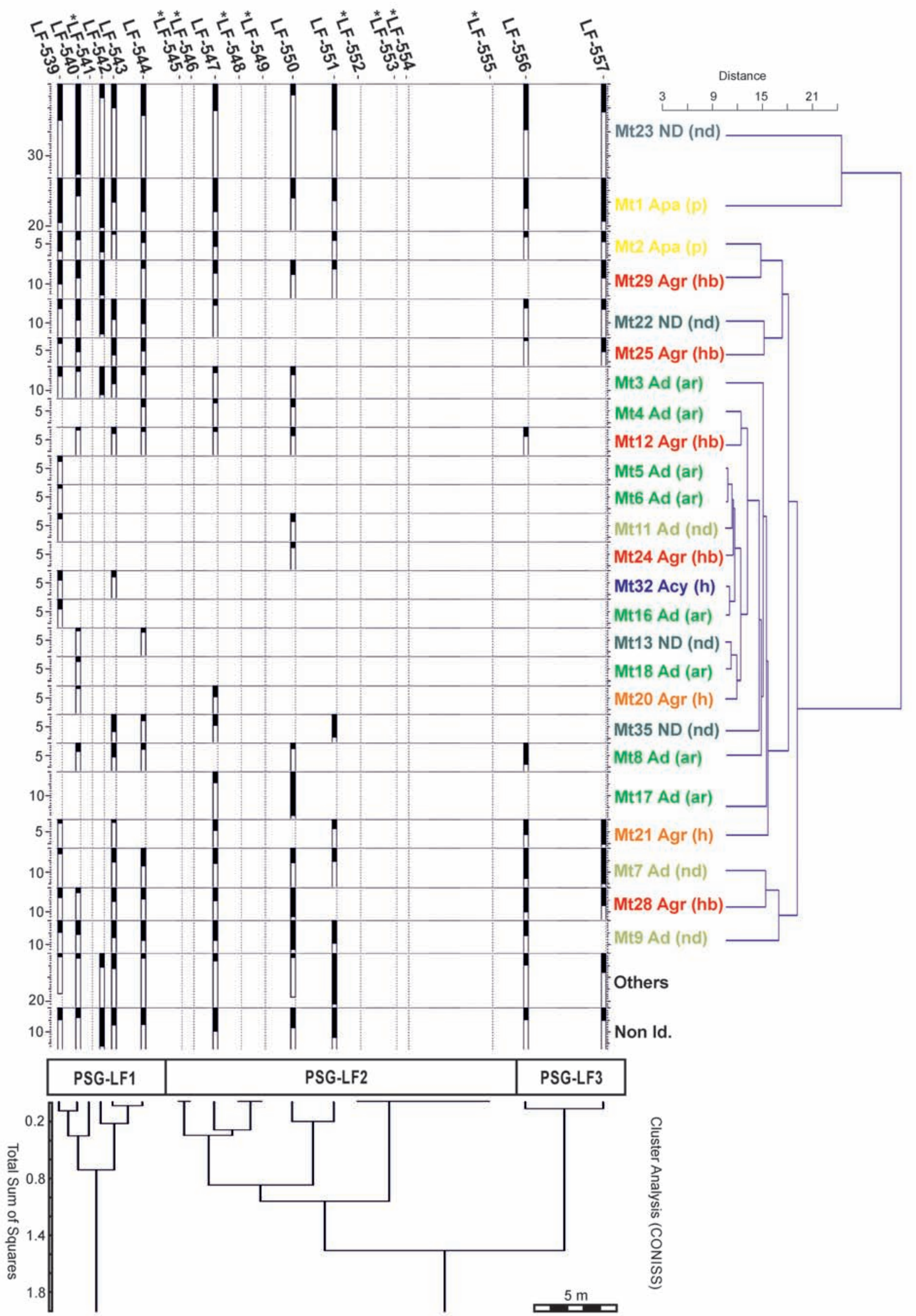
The float fraction were mounted on microscope slides in liquid (oil immersion) and solid (Canada balsam) media; and observed in a Nikon Eclipse E200 light microscope. Microphotographs were taken with a Nikon Coolpix 990 digital camera.

Morphotypes (Mt) were classified based on similar previous studies (Supplementary material Fig. 1; Tab. 1) and their equivalents according to current plants classifications synthesis of phytolith morphotypes in woody (Mercader *et al.*, 2009; Strömberg *et al.*, 2013; Collura and Neumann, 2017) and herbaceous (Barboni and Bremond, 2009; Mercader *et al.*, 2010; Neumann *et al.*, 2016) species (Supplementary material Tab. 2); used for establish life-form categories (Palms, Woody, Marsh or humid herbs, Diagnostic herbs, and Non diagnostic herb/bamboos) and botanical affinity of morphotypes (Supplementary material Fig. 1; Tabs. 1, 2). For the Chrysostomataceae stomatocyst descriptions, the terminology and treatment, Rull and Vegas-Vilarrúbia (2000) and Coradeghini and Vigna (2001) were used.

The minimum representative sample (420 phytoliths/sample) was established by progressive variability counts of random samples (Pearsall, 2000) for obtain the diversity saturation point (DSP *sensu* Rull, 1987) using rarefaction analysis (Birks and Line. 1992). Data processing and its representation in phytolith diagrams (Zucol *et al.*, 2010c) were carried out with the POLPAL Numerical Analysis Program (Walanus and Nalepka, 1999; Nalepka and Walanus, 2003). In order to distinguish patterns in phytolith assemblages, groups of similar composition were established by standard numerical analyses, including constrained single link cluster analysis with square root transformation of data -SQRT- (*sensu* Birks, 1986; Prentice, 1986). Comparison of profile abundances was made applying correspondence analysis using PAST software (Hammer *et al.*, 2001). The abundances (what exceeded the minimum representative sample) of the both analysed profiles from Koluel-Kaike Formation, as well as, from the Las Flores Formation and the Gran Barranca Member (Sarmiento Formation) obtained in previous studies in the region (Brea *et al.*, 2008, Raigemborn *et al.*, 2009, Zucol *et al.*, 2010a), were selected for the correspondence analysis (Basic data matrix in Supplementary material Abundances%). The correspondence analysis routine finds the eigenvalues and eigenvectors for a matrix containing the Chi-squared distances between all data points. The eigenvalues, giving a measure of the similarity accounted for by the corresponding eigenvectors, given in the first four most important eigenvectors.

Sedimentary samples and microscope slides are stored in the Palaeobotanical Laboratory Collection of the Centro de Investigaciones Científicas y Transferencia de Tecnología a la Producción, CICYTTP (CONICET-Provincia de Entre Ríos-UADER), under the acronyms CIDPALBO-MIC 539–557 (LF) and 1868–1872 (CL).

For determining the vegetation palaeocommunity type, Palms phytoliths have been counted as a particular life-form, and then the relative abundance of palm phytoliths were compared to other plant indicators, basically with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Distance
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15
21
Mt23 ND (nd)
Mt1 Apa (p)
Mt2 Apa (p)
Mt29 Agr (hb)
Mt22 ND (nd)
Mt25 Agr (hb)
Mt3 Ad (ar)
Mt4 Ad (ar)
Mt12 Agr (hb)
Mt5 Ad (ar)
Mt6 Ad (ar)
Mt11 Ad (nd)
Mt24 Agr (hb)
Mt32 Acy (h)
Mt16 Ad (ar)
Mt13 ND (nd)
Mt18 Ad (ar)
Mt20 Agr (h)
Mt35 ND (nd)
Mt8 Ad (ar)
Mt17 Ad (ar)
Mt21 Agr (h)
Mt7 Ad (nd)
Mt28 Agr (hb)
Mt9 Ad (nd)
Others
Non Id.
30
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5
10
10
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5
5
5
5
5
10
5
10
10
10
20
10
PSG-LF1
PSG-LF2
PSG-LF3
0.2
0.8
1.4
1.8
Total Sum of Squares
Cluster Analysis (CONISS)
5 m

grasses or trees and shrubs morphotypes presence (Patterer, 2012). This method follows the methodological patterns of Couvreur *et al.* (2011), since palms are one of the most characteristic and ecologically important components of tropical rain forest worldwide, but they are also present in a broad range of environments (explain it, see Supplementary material Palms).

## RESULTS

Analysis of Koluel-Kaike Formation samples permitted demarcation of groups with similar phytolith composition or phytolith sample groups (PSG), thus defining three PSGs at LF (PSG-LF1–3, Fig. 4), and two PSGs at CL (PSG-CL1–2). Broadly, all samples are characterized by a high abundance of prismatic elongate with smooth contours (Mt22 and Mt23), polyhedral bulliform (Mt29, Fig. 5.5), and globular echinate (Mt1 and Mt2, Fig. 6.7), phytoliths. Comparison of phytolith assemblages from LF shows that the groups can be differentiated mainly by the abundance of Mt1, Mt2, Mt7, Mt9, Mt17, Mt28, and Mt29 morphotypes (Fig. 4).

Between grasses indicator phytoliths truncated cones/towers (Mt21), dumbbell (Mt20), and saddle (Mt34) morphotypes were recorded. Phytoliths described within the truncated cone type include smaller or towers (not diagnostic, *sensu* Lu and Liu, 2003), truncated cones themselves (larger towers, principally danthoniods, Kondo *et al.*, 1994), as well as sharp-contour or kernel (described with chloridoid affinity, Babot *et al.*, 2017). Truncated cones phytoliths are the most abundant type and variable in shape, and locate in the lower Koluel-Kaike Formation in both sections CL and LF, and in the middle-upper section at LF, displaying an increase in abundance upward the unit. The dumbbell phytoliths display an irregular contour panicoid-like shape, while saddle phytoliths display short axes. Both dumbbell and saddle restrict to the lower Koluel-Kaike Formation at CL and LF, and to the lower-middle section at LF.

Among the morphotypes assigned to woody plants smooth surface spherical to ellipsoidal globular (Mt3), smooth globular (Mt6), elongate faceted (Mt8), vascular element with simple perforation plates (Mt16), with scaleriform perforation plates (Mt17), and with intervessel pits bordered (Mt18) were observed in different samples. With the exception of elongate faceted phytoliths, which is present throughout the unit, the woody elements restrict to the lower Koluel-Kaike Formation in both LF and CL sections (Fig. 4).

### *Siliceous microfossil records from Las Flores locality*

***PSG-LF1.*** It corresponds to the lower Koluel-Kaike Formation and includes the samples LF539–540 and LF542–544 (Fig. 2). The overall content is characterized by high abundance of globular echinate (Mt1, Mt2), and smooth-surface, spherical to ellipsoidal (Mt3; Fig. 6.7), short and long prismatic elongate with smooth (Mt22 and Mt23) and denticulate (Mt25) contours, laminar puzzle forms (Mt9; Fig. 5.6), polyhedral bulliform (Mt29), pentagonal or hexagonal contour (Mt32), and triangular (Mt35) phytoliths. In less abundance we find isodiametric and elongate faceted phytoliths (Mt7; Fig. 5.3, and Mt8), point-shaped (Mt12; Fig. 5.1), fan-shaped (Mt28; Fig. 5.5), truncated cones (Mt21; Fig. 6. 8) and tracheids with helical thickenings (Mt13 Fig. 6.11) phytoliths, were presented.

The lowermost samples of the PSG (LF539–540) contain vascular elements with simple perforation plates (Mt16) (Fig. 7.4) and vessel with bordered intervessel pits (Mt18), beside others globular smooth (Mt5 and Mt6), hair bases (Mt11, Fig. 5. 2) and dumbbell (Mt20) phytoliths. Particularly the sample LF540 provides the first diagnostic herbs elements. The phytolith assemblage of this sample characterizes by woody (4.58%), dicots (9.92%), palms (11.45%), diagnostic herbs (1.52%), and non-diagnostic herb/bamboo (17.56%) elements.

**Figure 4.** Phytolith diagram of the LF profile, with abundance of principal non–articulated phytolith groups (in percentage). In black: abundance; in white: exaggeration (factor 5) drawing to clearly observe the low abundances. Cluster analysis dendrogram obtained with constrained single link with square root (SQRT) method transformation, showing the observed profile grouped samples (PSG-LF1, PSG-LF2 and PSG-LF3 see reference in text) and graphic illustration of the variability of the three first principal components considered to established this dendrogram. * Samples without silica microremains. Right: abundance according to life–form and botanical affinity (*sensu* Supplementary material, Tab. 1), Palms Apa(p), Woody Ad(ar), Marsh herbs Acy and Apo(h), Diagnostic herbs Agr(h), and Non-diagnostic herb/bamboo Agr(hb) and Non-diagnostic ND(nd). Scale bar= 5 m.

Non-phytolith silica microremains are present only in PSG-LF1, restricted to smooth and spinulose Chrysostomataceae stomatocysts (Fig. 5.4), and smooth and *Ephydatia* type sponge spicules. Conversely, diatoms are scarce.

The PSG-LF1 have the most heterogeneous composition regard PSG-LF2 and PSG-LF3, and differs from them in having: a) a proportionally noticeable high abundance of long smooth contoured elongated (Mt23), and pentagonal or hexagonal contour (Mt32); b) a relative moderate abundance of triangular phytoliths (Mt35); and c) a relative low abundance of truncated cone (Mt21).

***PSG-LF2.*** It corresponds to the middle Koluel-Kaike Forma-

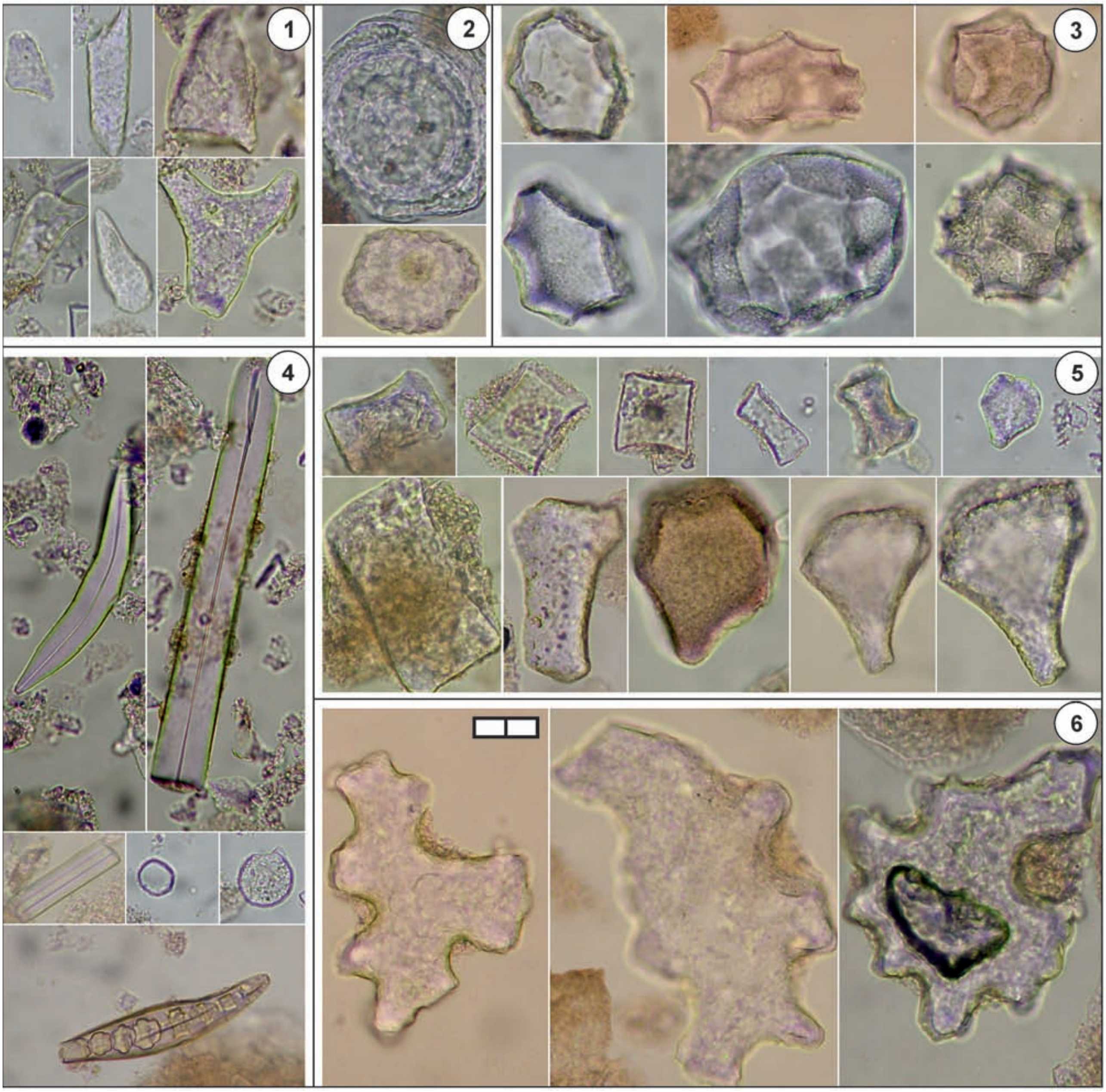


**Figure 5.** Silica microremains and phytoliths from the LF and CL profiles; **1,** point-shaped phytoliths (Mt12); **2,** hair base phytoliths (Mt11); **3,** isodiametric phytoliths with faceted contour (Mt7) and smooth or faceted surface; **4,** various silica microremains such as sponge spicules, Chrysophycean stomatocysts and diatom; **5,** fan-shaped and polyhedral bulliform elements (Mt28 and Mt29, low row) and small polyhedral phytolith (bottom row); **6,** laminar and structured puzzle-shaped phytoliths (Mt9–10). Scale bars in 6 (valid for all)= 20 µm.

tion and includes the samples LF545–555 (Fig. 2). Although this interval displays many barren samples, the almost uniform phytolith composition of samples LF547 and LF551 (Fig. 4) supports the validity of the assemblage. Thus, based on samples with sufficient phytolith for an adequate statistical analysis, the PSG-LF2 is characterizeds the by abundance of echinate and smooth globular (spherical and ellipsoidal, Mt1, Mt2 and Mt3; Fig. 6.7), smooth contoured elongate (Mt23), laminar puzzle (Mt9), fan-shaped (Mt28; Fig. 5.5), polyhedral bulliform (Mt29; Fig. 5.5), isodiametric faceted (Mt7), and triangular (Mt35) phytoliths; together with vascular elements with scalariform perforation plates

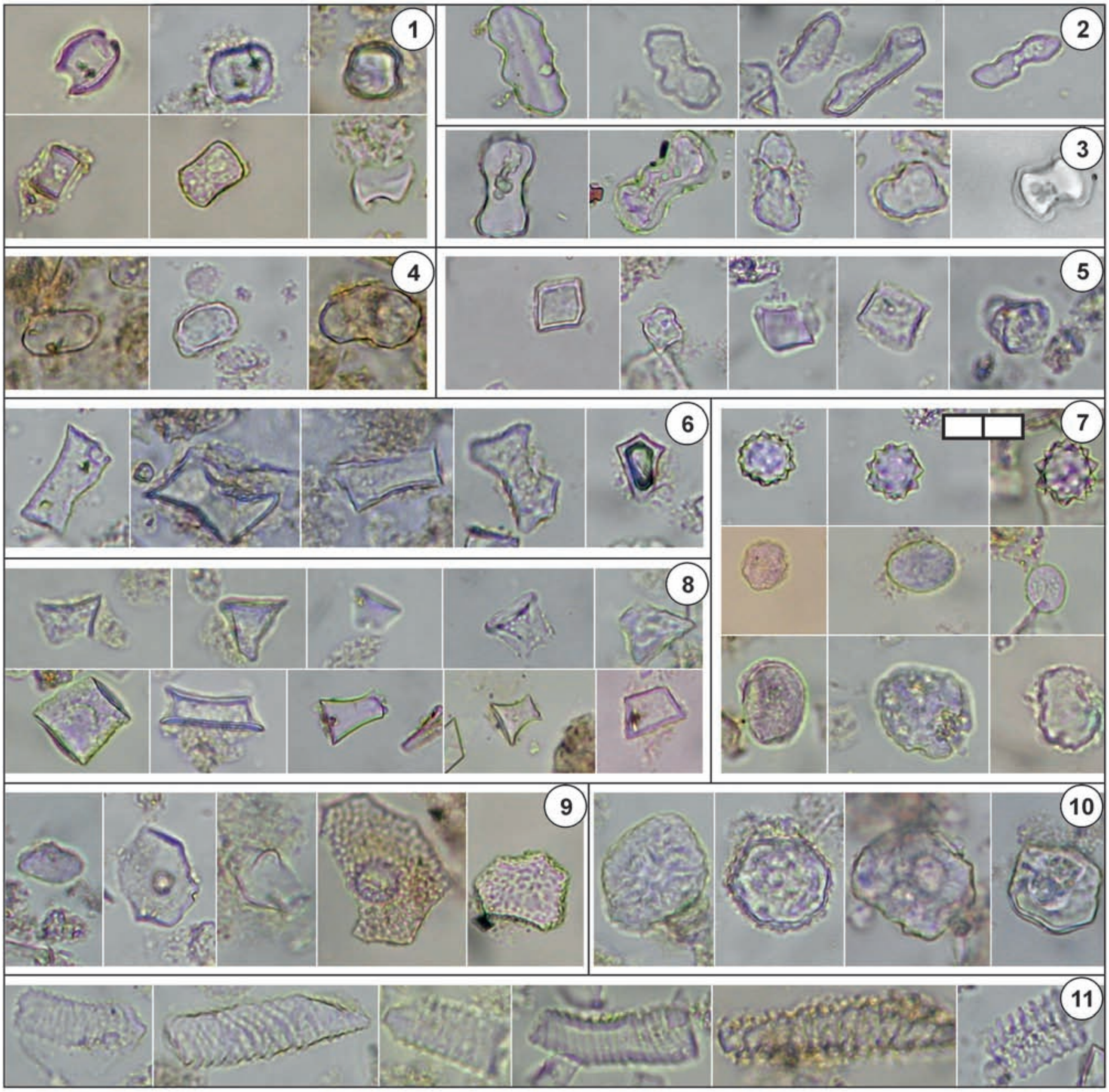


**Figure 6.** Phytoliths from LF and CL profiles; **1,** saddle phytoliths (Mt34); **2,** crenate oblong phytoliths; **3,** dumbbell phytoliths (Mt20); **4,** oblong and rondel phytoliths (Mt30); **5,** square to small cuneiform phytoliths (Mt31); **6,** acute short phytoliths (considered in Other in the phytolith diagrams of Figures 4 and 8); **7,** globular phytoliths, with spinulose, smooth and rugulose surface (Mt1–5); **8,** truncated cones phytoliths (Mt21); **9,** small laminar phytoliths, with circular, pentagonal or hexagonal contours (Mt32); **10,** other conical phytoliths; **11,** small tracheid phytoliths (Mt13). Scale bar in 5 (valid for all)= 20 µm.

(Mt17; Fig. 7.3). Globular spherical rugulose (Mt4; Fig. 6.7), point-shaped (Mt12), elongate faceted (Mt8), and truncated cone (Mt21; Fig. 6.8) phytoliths are also present but in low relative abundance. The PSG-LF2 displays four particularities. The first is the absence of diagnostic herb elements in the sample LF550, in spite of being present in the lower

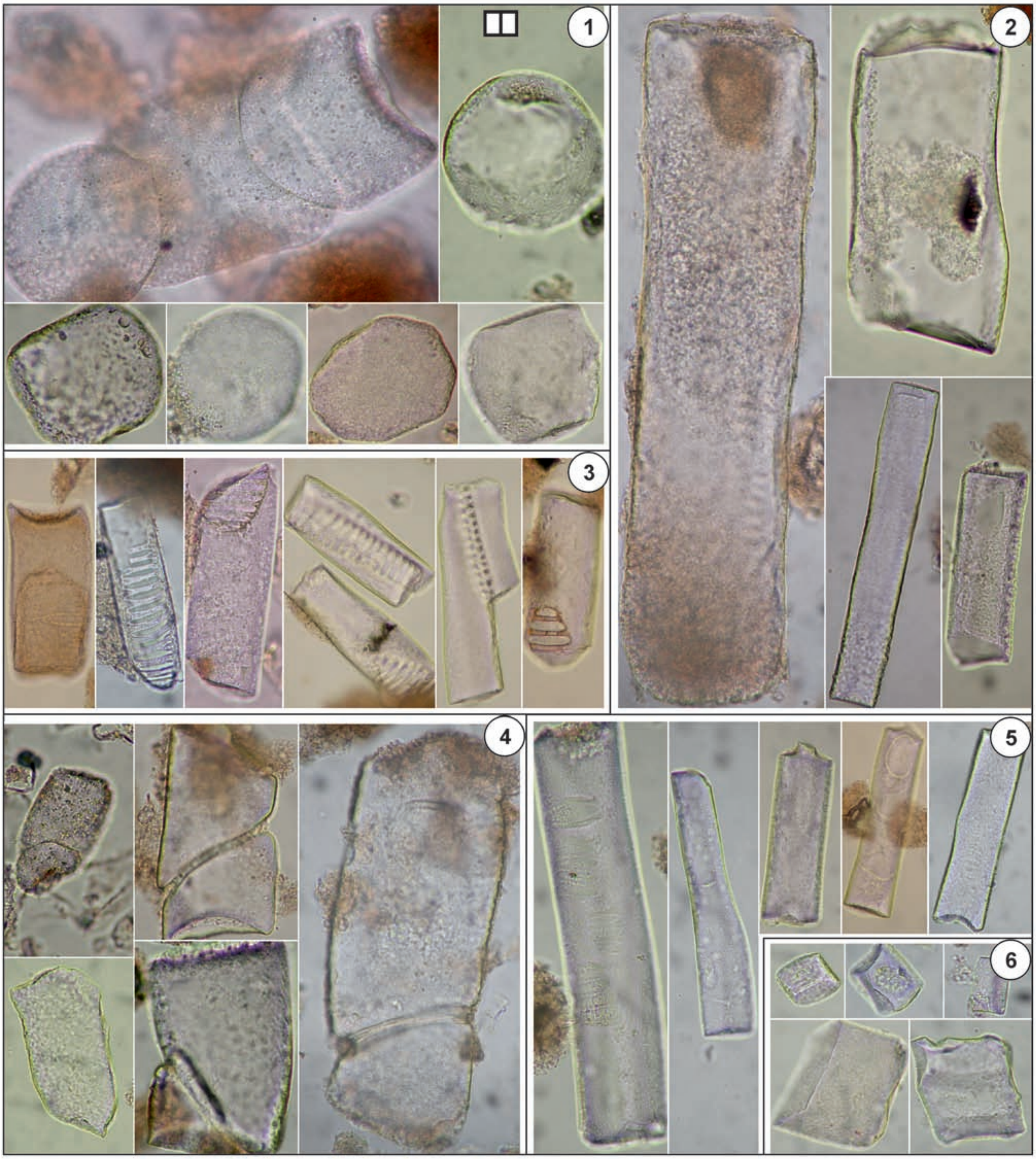


**Figure 7.** Phytolith from the LF and CL profiles; **1,** globular and round polyhedral elements (Mt6); **2,** indeterminated cylindrical phytoliths originated in vascular elements; **3,** cylindrical phytolith originated in vascular element with scaleriform perforation plates (Mt17); **4,** cylindrical phytolith originated in vascular element with simple perforation plates (Mt16); **5,** fibre phytoliths; **6,** single blocky cell phytoliths. Scale bar in 1 (valid for all)= 20 µm.

Koluel-Kaike Formation (*e.g.*, sample LF540). The sample LF550 characterize by woody 27.27%, dicots 25.97%, palms 9.09%, and 11.69% non-diagnostic herb/bamboo elements. The second particularity relates to cases of exclusive morphotypes within samples LF547 and LF550, regard other samples constituting the whole assemblage (Fig. 4). The sample LF547 displays dumbbell (Mt20) and short prismatic elongate with smooth contours (Mt22) phytoliths, which are absent in samples LF550 and LF551. The sample LF550 displays hair bases (Mt11) and elongate with serrate contour (Mt24) elements, which are absent in samples LF547 and LF551. The third particularity is given by the presence of sparse vascular elements with scalariform perforation plates (Mt17) in the sample LF547, morphotype that is highly abundant in the sample LF550, and absent in the sample LF551. The fourth particularity relates to the triangular mophotype (Mt35), abundant in the samples LF547 and LF551 but absent in the sample LF550.

***PSG-LF3.*** It corresponds to the upper Koluel-Kaike Formation (samples LF556 and LF557, Fig. 2). It differs from previous PSG-LF1 and 2 in the marked abundance of globular echinated (Mt1, Mt2), faceted isodiametric (Mt7; Fig. 5.3), prismatic elongated with smooth (Mt22 and Mt23), and denticulate (Mt25) contour, fan-shaped (Mt28), and truncated cone (Mt21) phytoliths. Elongate faceted (Mt8), laminar puzzle (Mt9; Fig. 5.6), and point-shaped (Mt12) are present in the sample LF556, while polyhedral bulliform (Mt29) phytoliths are recorded in the sample LF557.

### *Siliceous microfossil records from Cañadón Lobo locality*

Phytolith assemblages from these samples are strongly uniform, with a noticeable abundance of globular echinated (Mt1), smooth contoured elongate (Mt22 and Mt23), and polyhedral bulliform (Mt29) phytoliths. However, comparisons of phytolith suites enable demarcation informal assemblage groups (Fig. 8), principally defined by the abundance of morphotypes and the exclusive occurrences of a few key phytolith types.

***PSG-CL1.*** It corresponds to the lowermost section of the Koluel-Kaike Formation (samples CL1–CL3) (Fig. 2). It is characterized by a high abundance of elongated with asymmetrical section (Mt26), point-shaped (Mt12), and hair bases (Mt11), besides of those abundant and common morphotypes already mentioned (Fig. 8). Globular smooth and echinated (Mt3, Mt2, Mt5 and Mt6), truncated cone (Mt21; Fig. 6.8), dumbbell (Mt20; Fig. 6.3), square (Mt31; Fig. 6.5), and circular to elliptical (Mt30; Fig. 6.4), bulliform fan-shaped (Mt28), denticulated elongate (Mt25), and tracheids with helical thickenings (Mt13; Fig. 6.11) phytoliths are recorder in low abundance. Beside of the shared phytoliths, other elements are recorded in samples CL1 and CL3. The sample CL1 show globular smooth and faceted (Mt5, Mt7), and saddle (Mt34; Fig. 6.1) phytoliths. The sample CL3 displays vascular elements with simple perforation plates (Mt16), laminar puzzle (Mt9), serrated elongate (Mt24), tracheids with an irregular body and striate ornamentation (Mt14; Fig. 7.8), and laminar with pentagonal or hexagonal contour (Mt32; Fig. 6.9).

***PSG-CL2.*** It corresponds to the lower section of Koluel-Kaike Formation (samples CL4 and CL5) in their upper levels (Fig. 2). Besides the common abundant morphotypes already mentioned, the PSG-CL2 displays abundance of vascular elements with simple perforation plates (Mt16; Fig. 7.4), laminar and structured puzzle (Mt9 and Mt10; Fig. 5.6), fan-shaped and point-shaped (Mt28 and Mt12), and globular echinated with elliptical section (Mt2). Spherical smooth globular smooth and isodiametric faceted (Mt3 and Mt7), tracheids with helical thickenings (Mt13), square (Mt31), elongate with undulate contour (Mt27), dumbbell (Mt20; Fig. 6.3), hair bases (Mt11), and truncated cones (Mt21) complement this assemblage. In the sample CL5 also occur elliptical globular smooth (Mt5), laminar with pentagonal or hexagonal contour (Mt32; Fig. 6.9), and serrate elongate (Mt24) phytoliths; while in the sample CL4, are also recorded globular smooth with greater diameter (Mt6), circular to elliptical (Mt30), saddle (Mt34), and denticulated elongate (Mt25) phytoliths. Diatoms (Fig. 5.4), Chrysostomataceae stomatocysts, microcharcoal and sponge spicules (Fig. 5.4) are rarely observed in all the samples.

The phytolith assemblages (Fig. 8) shows that sample CL3 has the most diverse suite with elements shared with the upper (PSG-CL2) and the lower (PSG-CL1 p.p.) phytolith assemblages, and woody elements mainly represented by silicified vessel elements with simple perforation plates (Mt16) (Fig. 8).

Also, the non-grass herbaceous phytoliths include the prismatic cyperoid types (Mt26, present in PSG-CL1), with a prismatic smooth body and a protuberance or arm forming

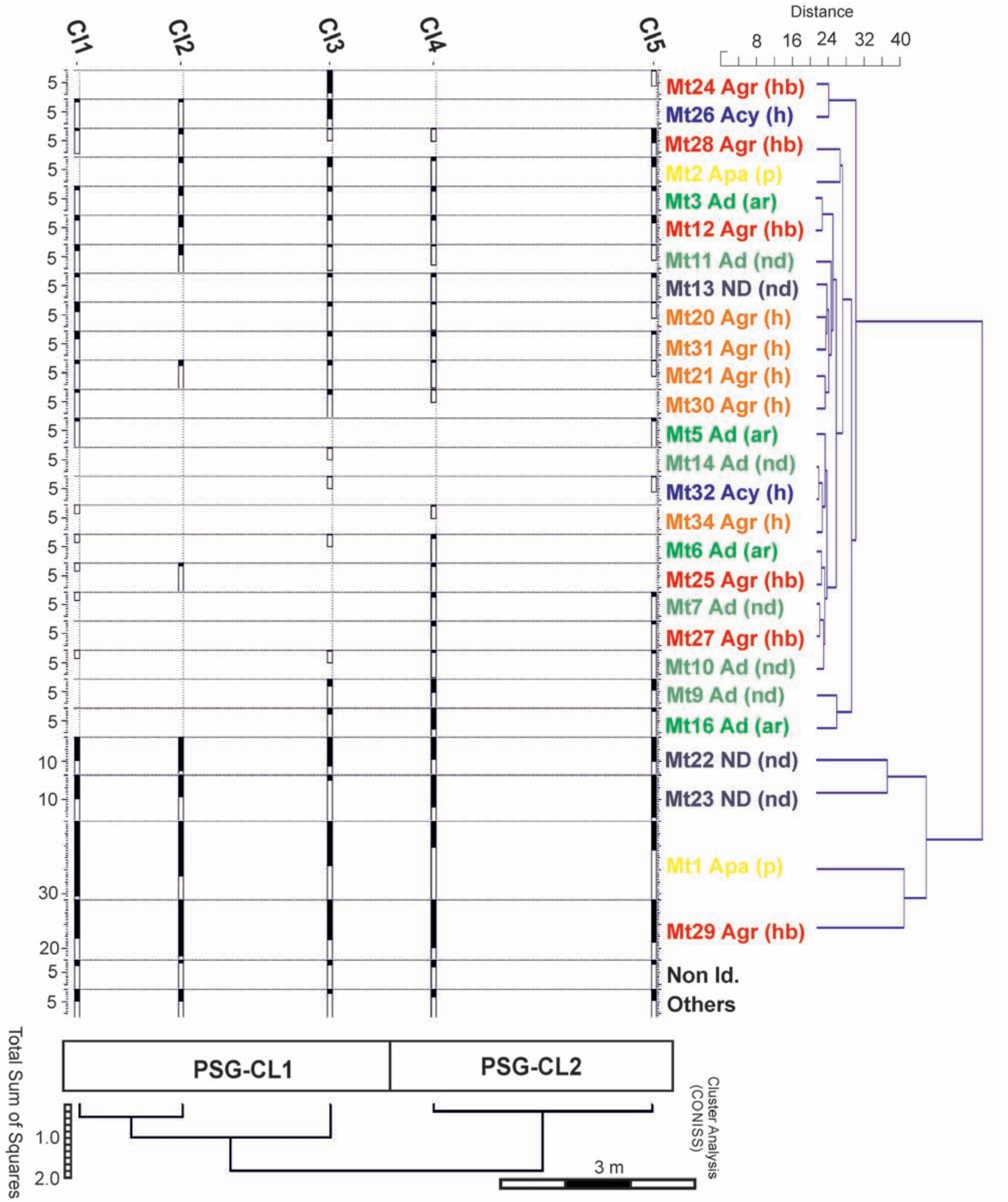


**Figure 8.** Phytolith diagram of the CL profile, with abundance of principal non–articulated phytolith groups (in percentage). In black: abundance; in white: exaggeration (factor 5) drawing to clearly observe the low abundances. Cluster analysis dendrogram obtained with constrained single link with the square root (SQRT) method transformation, showing the observed profile grouped samples (PSG-CL1 and PSG-CL2 see reference in text) and graphic illustration of the variability of the three first principal components considered to establish this dendrogram. Right: abundance according to life–form and botanical affinity (*sensu* Supplementary material, Tab. 1), Palms Apa(p), Woody Ad(ar), Marsh herbs Acy and Apo(h), Diagnostic herbs Agr(h), and Non-diagnostic herb/bamboo Agr(hb) and Non-diagnostic ND(nd). Scale bar= 3 m.

a right angle (Bertoldi de Pomar, 1971; Piperno, 2006). On the other hand, some laminar and conical phytoliths, with pentagonal or hexagonal contour (Mt32, Fig. 6. 9), can considered cyperoid types (Fernández Honaine *et al.*, 2009), turned out to be abundant in PSG-CL1–2 and PSG-LF1.

Among other silica microremains (Fig. 5.4) freshwater sponge spicules (elongate and smooth in contour ones and *Ephydatia* sp. spicule type (*sensu* Ezcurra de Drago, 1975) are recovered. Diatoms with radial and bilateral symmetry occur, but their presence is scarce in all of the analysed samples.

Endogenous siliceous cysts or stomatocysts, produced by seasonal and freshwater algae (Chrysostomataceae) are observed in three principal morphological types in the Koluel-Kaike Formation (according Siver and Vigna, 1997; Coradeghini and Vigna, 2001). The first one is characterised by an unornamented spherical body and pores without collar. This type corresponds to the stomatocysts N° 1 of Coradeghini and Vigna (2001) and probably to the stomatocysts type N° 64 according to Rull and Vegas-Vilarrúbia (2000). The second type has an unornamented, spherical to oval body with two collars around the pores. It corresponds to the stomatocysts type N° 10 (Coradeghini and Vigna, 2001). The third type presented an oval body with spinulose ornamentation which is assigned to the stomatocysts type N° 22 (Coradeghini and Vigna, 2001).

### *Relationships among Las Flores and Cañadón Lobo PSGs*

The comparison of the phytolith composition recorded in the LF and CL PSGs displays cases of noticeable affinity in phytolith composition and cases of exclusive morphotypes. Both sections show the presence of a wide range of spheroid phytoliths (spherical to ellipsoidal, with smooth, echinate, and rugulose ornamentation), faceted elongate and isodiametric phytoliths, and certain elongated elements with rectangular to bulky section, and smooth, serrate or denticulate contour. The dicot-vessel-elements phytoliths are very abundant through the LF profile. The vascular elements with simple perforation plates are present in the lower Koluel-Kaike Formation at both LF and CL sections, whereas vascular elements with scalariform perforation plates are present in PSG-LF2 at LF (Fig. 4).

In general, both PSG-LF1 and PSG-LF2 share around 55% of the involved morphotypes. However, the former is more heterogeneous and denticulate elongate (Mt25), laminar with pentagonal or hexagonal contour (Mt32), triangular (Mt35), smooth globular (Mt5, Mt6), tracheids with helical thickenings (Mt13), simple perforation plates (Mt16), and vessel with bordered intervessel pits (Mt18) are absent in the PSG-LF2.

The PSG-LF1–LF2 and PSG-CL1–2 shares the record of similar relative abundant of spherical smooth globular (Mt3), hair bases (Mt11), and dumbbell (Mt20) phytoliths. All of these morphotypes are absent in the PSG-LF3.

On the other side, the PSG-LF1, PSG-CL1, and PSG-CL2 display a strongly similarity in both composition and abundance of several morphotypes: a) relative highly to very highly abundant globular echinate (Mt1), smooth contoured prismatic elongate (Mt22, and Mt23), laminar with pentagonal or hexagonal contour (Mt32), vascular elements with simple perforation plates (Mt16), and tracheids with helical thickenings (Mt13); and b) relative lowly abundant of truncated cones (Mt21) and some of the globular smooth (Mt5 and Mt6) elements.

Regard exclusive cases, at PSGs level, the PSGs from LF displays triangular (Mt35), globular spherical rugulose (Mt4), faceted elongate (Mt8) phytoliths, and vascular elements with scalariform perforation plates (Mt17) and bordered intervessel pits (Mt18), all of them at CL section. While, structured puzzle forms (Mt10), circular to elliptical (Mt30), square (Mt31) (Fig. 6.4–5), and saddle (Mt34, Fig. 6.1) phytoliths are exclusive of CL section. In addition, elongate with irregular body and striate ornamentation (Mt14, and Mt15) are exclusive of PSG-CL1, and elongate with undulate contour (Mt27) is limited to PSG-CL2.

### *Botanical affinities*

The phytolith content recorded in the Koluel-Kaike Formation displays affinities with woody, palms, humid herbs and grasses elements. Regard the latter, and according to recent grass morphotypes affinities, two main grasses morphotypes groups can be identified (Supplementary material Tab. 1–3): diagnostic (Diag. herbs or PACMAD/BEP types) and non-diagnostic (N.D. herb/bamboo or present in the whole family) phytoliths. Diagnostic grasses morphotypes include those mainly originated in short epidermal cells such as boat-shaped, circular, rondel, dumbbell, saddle, and towers/truncated cones types. Only the three last morphotypes were recorded in the studied sections. Dumbbell phytoliths, recorded in the PSGs LF1, LF2, CL1, and CL2, relate to panicoid (Supplementary material Tab. 2–3), while short-axed saddle phytoliths recorded in PSG-CL1 and PSG-CL2, relate to chloridoid (Supplementary material Tab. 2–3). Truncate cones/towers, recorded throughout the Koluel-Kaike Formation, were considered as non-diagnostic (Supplementary material Tab. 2–3), since towers do not have a subfamily delimitation, but truncated cones relates to danthonoid (Kondo *et al*., 1994) and chloridoid (Babot *et al*., 2017).

Non-diagnostic herb/bamboo morphotypes, include those phytoliths originated in hairs or prickles and bulliform cells (point-shaped (Mt12), fan-shaped (Mt28), and polyhedral bulliform (Mt29)). For the Koluel-Kaike Formation they include too prismatic phytoliths with undulate, serrated and denticulate contour, originated in epidermal long cells.

Other phytoliths with epidermal origins present in these record are the puzzle-piece shaped elements with wavy and undulating walls (Mt9) and hair base and point-shaped ones (Mt11). Silicified anticlinal epidermal lobed cells (or puzzle-piece) are dicot phytolith morphotypes, always considered as characteristic of trees (*e.g.*, Kondo *et al*., 1994), whereas other studies agree that these types may be also produced by shrubs (Carnelli *et al*., 2004; Wallis, 2003) and/or by many herbaceous eudicots (Bozarth, 1992), thus these forms do not allow establish in what kind of dicotyledonous was originated, neither the life form of the plant.

Phytoliths derived from hair bases are common in dicot and can be used for taxa identification (Piperno, 1988). Types found in this study have noticeable affinity with phytoliths of the Boraginaceae family, following Pearsall (2006).

The isodiametric faceted phytoliths (Mt7) are developed in contact with cell walls inside mesophyll cells, or in the intercellular space; while the faceted elongate types (Mt8) originate from silicified terminal tracheids and silicified sclereids of leave mesophylls, where the cells or the intercellular spaces become silicified. Since these phytoliths are absent in herbaceous plants or lianas suggests, they become in a good indicator of woody vegetation in soils and sedimentary environments (Piperno, 1988; Runge, 1999). Faceted phytoliths have been observed in the Magnoliaceae, Annonaceae, Burseraceae and Cucurbitaceae families (Kondo and Peason, 1981; Bozarth, 1987; Piperno, 1988; Runge, 1999). Some smooth surface sclereid types (Fig. 7.5) can present elements of woody species (Mercader *et al*., 2009; Collura and Neumann, 2017); while others types such irregular body with striate to verrucate ornamentation (Mt14 and Mt15) can be assigned to some species of Mimosaceae, a family characterized by trees and shrubs many of which currently can be found in arid to semiarid regions. (*e.g.*, Australian flora, Wallis, 2003). On the other hand, small silicified branched tracheid elements (Mt13 p.p., Fig. 6. 11) with spiral thickenings were considered in this study as angiosperms *sensu lato*, because no longer that establishes its botanical origin.

Other woody elements found in these records are: blocky psilate isolated phytoliths (Fig. 7.6) that Collura and Neumann (2017) assigned such Parenchyma/Cork origin from woody plants. Also, some smooth globular phytoliths (Mt3, Mt5 and Mt6, Fig. 7.1, Globular psilate and Globular psilate large *sensu* Mercader *et al*., 2009) recorded in the PSG-LF1, and all PSG-CL indicates woody plants, as suggested by Mercader *et al*., (2009), who found these morphotypes in African Fabaceae and Euphorbiaceae woody species.

Among small globular phytoliths, three types were herein considered: globular echinate (Mt1 and Mt2), globular smooth (Mt3 and Mt5), and globular folded or granulate (Mt4). Within globular echinate phytoliths can be considerate the typical palms stegmata (between 5–25 µm in diameter, *sensu* Patterer, 2014), and the Bromeliaceae type (between 2.5–5 µm in diameter, *sensu* Benvenuto *et al*., 2015); and a type small and irregular multifaceted must be added of these group. This rugulose and irregularly surface element (spherical irregularly folded *sensu* Thorn, 2001; druses-like, *sensu* Prychid *et al*., 2004, and Chen and Smith, 2013; Costus/Canna [Zingiberales] phytoliths p.p. *sensu* Strömberg *et al*., 2013) occur in three extant monocot families: Marantaceae, Cannaceae and Zingiberaceae from the Zingiberales Order (Piperno, 2006; Prychid *et al*., 2004, Benvenuto *et al*., 2015).

Globular folded and/or granulate were recorded in many different plant families for monocot, eudicots, magnoliids and ANITA-grade (Kondo *et al*., 1994; Piperno, 1988, 2006; Runge, 1999; Mercader *et al*., 2009, Collura and Neumann, 2017), in wood, leaves, fruits and seeds. Meanwhile, some smooth, verrucate and nodular spherical phytoliths (*sensu* Kondo *et al.*, 1994 classes; Bremond *et al.*, 2005, fig. 5A p.p.) have been considered as woody plant indicators.

### *Climatic affinities*

The anatomical characters of the dicot woods can be used not only to establish the specialization lines of the secondary xylem (Frost, 1930a, b, 1931; Carlquist, 1975), but also to reconstruct the climate conditions and the climatic changes in the geologic past (Wheeler and Baas, 1991, 1993; Wiemann *et al.*, 1998; Poole, 2000). The Koluel-Kaike phytoliths derived from vascular tissues are grouped in two ecological categories: EC type 1 and EC type 2. The first involves to the morphotype Mt17 (Fig. 7.3), and includes phytoliths derived from thin, long vessel elements, thin-walled, without helical thickenings and oblique end walls, phytoliths derived from vessel elements with scalariform, transitional and opposite intervessel pits, and some phytoliths derived from vessel elements with scalariform perforation plates containing between 6–8 bars or 12–16 bars (Brea *et al.*, 2008). EC type 2 involves to the morphotypes Mt16 and Mt18 (Fig. 7.4), and includes phytoliths derived from vessel elements with wide diameters and short and oblique end walls, phytoliths with small, opposite to alternate intervessel pits, and phytoliths derived from vessel elements with simple perforation plates (Brea *et al.*, 2008).

The anatomical characters of the EC type 1 are unquestionably primitive features in the evolution of the secondary xylem (Frost, 1930a, 1930b, 1931). Scalariform perforation plates are especially successful for conditions which require low rates of water conduction (Baas, 1976). The abundance of simple perforation plates indicates warm environment, seasonal or permanent drought and/or changes in leaf phenology (deciduous habit) as response to the season changes (Wheeler and Baas, 1991, 1993). Thus, the percentage of genera with scalariform perforation plates in extremely low percentages agree with seasonally arid regions, relative low for tropical lowland rainforests, and high in tropical montane forests and temperate to arctic floras (Van der Graff and Baas, 1974; Carlquist, 1975; Baas, 1976; Wheeler and Baas, 1991).

## DISCUSSION

In the light of the achieved results, the composition of the phytolith assemblages PSG-CL1, PSG-CL2, and PSG-LF1 display a strong affinity, recorded for instance among the samples LF539, LF542, and CL samples (without CL3). This affinity suggests a similar palaeoecological scenery, and provides an additional support to the proposed lithostratigraphic correlation between the Las Flores and Cañadón Lobo sections. Thus, all of those PSG allow to characterize the lower Koluel-Kaike Formation as mainly composed of relative high abundance of woody elements, and fewer herbs. Then, a relative increase in herbs phytoliths is recorded upward the unit. This information allows testing two hypothesis regard the Koluel-Kaike phytoliths: 1) they record a transition from closely arboreal communities to open ones with greater presence of herbaceous elements in the Koluel-Kaike sedimentary sequence, and 2) they provide the oldest record of $C_4$ elements in mixed communities as savannah types for the middle Eocene from Southern South America.

### *The Koluel-Kaike vegetation: an archive from arboreal to herbaceous communities*

Life-form abundances in the Koluel-Kaike Formation is mainly represented by woody (0–27.27%), dicots (0–28.95%), palms (9.09–29.72%), diagnostic herbs (0–10.69%), non-diagnostic herb/bamboos (5–32.47%) and humid herbs (0–8.89%) (Fig. 9.2 and Supplementary material Fig. 2). This composition clearly constitutes a change from the tropical lowland forest vegetation recorded in the Las Flores Formation (Brea *et al.*, 2008; Raigemborn *et al.*, 2009) to a subtropical vegetation described in part of the studied sequence, as well as in the Gran Barranca Member (Zucol *et al.*, 2010a) (Fig. 9.2 and Supplementary material Fig. 2).

First herbaceous indicator appear in the lower Koluel-Kaike Formation. The sample LF540 provides the evidence of an increase in relative abundance of herbaceous elements regard tree ones from the Las Flores Formation to the Koluel-Kaike one. However, the presence of diagnostic herbs is some discontinuous in the middle Koluel-Kaike Formation. For instance, the sample LF550 displays phytolith elements similar to those recorded in the Las Flores Formation assemblages (Brea *et al.*, 2008; Raigemborn *et al.*, 2009) (Figs. 4, 9.5), for instance sharing morphotypes such

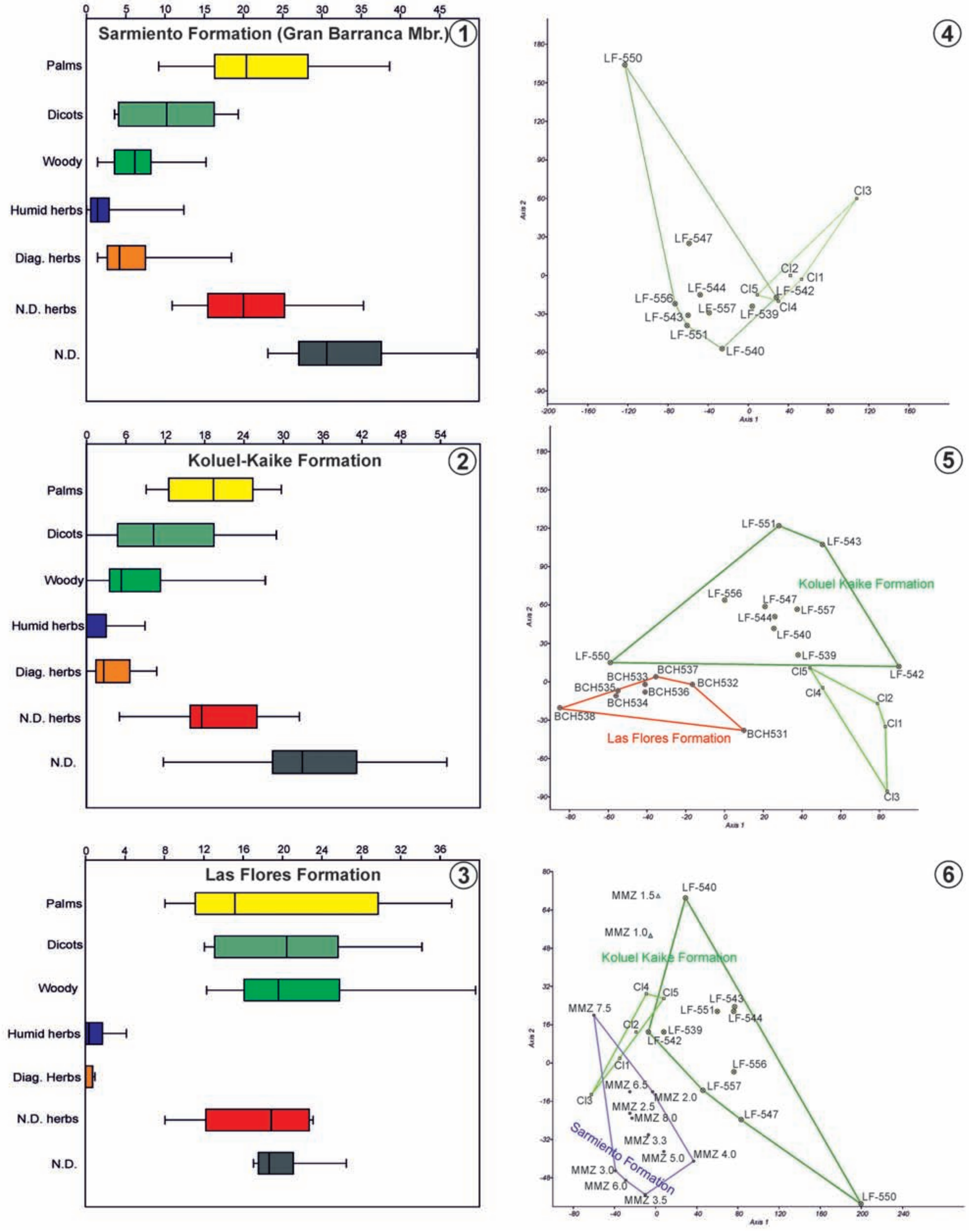


**Figure 9.** Box-plot of abundance of life forms in all analysed samples for each formation; **1,** Sarmiento Formation (Gran Barranca Member); **2,** Koluel-Kaike Formation; **3,** Las Flores Formation. Phytolith assemblages correspondence analysis of: **4,** LF and CL profiles samples; **5,** LF and CL profiles and Las Flores Formation samples (Brea *et al.*, 2008); **6,** LF and CL profiles and Sarmiento Formation (Gran Barranca Member) samples (Zucol *et al.*, 2010a).

as Mt1, Mt2, Mt7, Mt8, Mt9, Mt17, and Mt18, that suggest a relative predominance of arboreal elements. Vascular tissues included in EC type 1 (Mt17) and EC type 2 (Mt16 and Mt18) display a change in the relative abundances between the lower (PSG-LF1) and middle (PSG-LF2) Koluel-Kaike Formation. Simple perforation plates (Mt16 and in less proportion Mt18 elements) predominate in the lower section and decrease in the middle section, where Mt17 become more abundant. High abundance of Mt16 indicates warm and seasonal climate, since Mt16 elements coming from extant wood of South America proceed from subtropical and tropical regions (Wheeler *et al.*, 2007), and simple perforation plates relate to drought periods (Baas, 1976; Wheeler and Baas, 1991, 1993; Baas *et al.*, 2004). On the other hand, Mt17 elements suggest cooler conditions, since such these elements can be present even in artic floras (Van der Graff and Baas, 1974; Carlquist, 1975; Baas, 1976; Wheeler and Baas, 1991). For the case of the early–middle Eocene in Central Patagonia no evidence for freezing conditions is recorded, thus in the analysed sequence the presence of Mt17 indicates just cooler conditions, in agreement with the palaeoclimatic scenery occurred in the San Jorge Basin (Raigemborn *et al.*, 2009; Krause *et al.*, 2010; Raigemborn *et al.*, 2014) (see next section).

Some samples from the Koluel-Kaike Formation also display correspondence with phytolith assemblages recorded in the Gran Barranca Member (Zucol *et al.*, 2010a). The sample LF540 in the lower Koluel-Kaike Formation characterizes by a low abundance of palms, woody, and dicots, but relative high abundance of herbs indicators. This phytolith composition has a strong affinity with the samples MMZ9501.0 and MMZ9501.5 located in the lowermost Gran Barranca Member (Zucol *et al.*, 2010a: fig. 22.3) (Fig. 9.6), suggesting the existence of some linked groups related to the early presence of herbs for the middle Eocene of the San Jorge Basin. Also, the phytolith assemblage of the sample MMZ9107.5 recorded in the upper Gran Barranca Member (Zucol *et al.*, 2010a: fig. 22.3) presents affinity with the lower Koluel-Kaike Formation because the preponderance of marsh herb phytoliths such as prismatic types (Mt26), laminar with pentagonal or hexagonal contour (Mt32) among other phytolith types of humid herbs.

The whole of information by the phytolith assemblages from the Las Flores Formation to the Gran Barranca Member evidences a gradual change in the flora composition, from arboreal to tropical savannahs, with dry seasons and a cooling trend upward the unit. This interpretation find support in palaeoclimatic, palaeoedaphic, and sedimentary reconstructions already developed (*e.g.*, Krause *et al.*, 2010; Raigemborn *et al.*, 2010) (see next section). Regarding the presence of open environment, and particularly savannahs, Dunn *et al.* (2015) also interpret an opening of the landscape from dense vegetation for part of the Paleogene (49.0–32.3 Ma) in the San Jorge Basin. However, based on the scarcity of diagnostic herb elements for the lapse 38.5–38.0 Ma (uppermost Gran Barranca and lowermost Rosado Member, Dunn *et al.*, 2013) they interpret the flora as indicator of a "nonanalog relative free-grass palm shrublands" (Dunn *et al.*, 2015). Without doubts, the difference in these interpretations emerges from two main issues: 1) differences in the selection of recorded elements for the characterization of the palaeohabitat, and 2) difference in the selection of extant habitats for supporting the interpretation, as discussed in next two sub-sections. A third issue, residing in the lack of awareness, regards other palaeoenvironmental information (*e.g.*, paleontological, ichnological, sedimentary studies), and will be addressed in the next section.

### *Palaeohabitat characterization according indicators interpretation*

While Zucol *et al.* (2010a) and Zucol *et al.* (this contribution) interpret the flora considering the whole phytolith assemblage and the relative changes in abundance, Strömberg *et al.* (2013) and Dunn *et al.* (2015) state that when grasses are rare, "traditional" phytoliths analysis cannot resolve habitat openness, thus reducing the potential of traditional phytolith studies for getting palaeoecological information.

To consider just "diagnostic herb" phytolith, leaving "non-diagnostic" herb/bamboos elements aside, can lead to misleading interpretations such as the presence of "palm-rich forest" for the Eocene–Oligocene of the San Jorge Basin (Strömberg *et al.*, 2013).

The application of LAI index afterwards applied by these authors (Dunn *et al.*, 2015) provides a more reliable interpretation, as evidenced in the reinterpretation from fairly closed forest (Strömberg *et al.*, 2013) to open, palm shrubland with a discontinuous canopy habitat by ~38.0–38.5 Ma (Dunn *et al.*, 2015). However, the origin of the discrepancy (relative free-grass palm shrubland vs. tropical savannah), as we note, arises from two differences in the method of analysing the phytolith content, one regarding palms elements and the other regarding to ways of comparisons among palms and grasses elements.

Palms are among the most important and characteristic components of tropical rain forest ecosystems worldwide in terms of species diversity (Couvreur *et al.*, 2011). These authors situate the oldest reliable palm fossils in the Turonian (~93.5Ma), while the Coryphoideae clade (the subtropical or non rain-forest palms) were present to the Cretaceous/Paleocene. In America, extant species grow in different environments such as deserts, xeric environments, swamps, forests, savannahs, and temperate forests. Since phytolith morphotypes do not allow to discriminate particular palm groups, but they constitute significant ecological elements, we consider appropriate to count them as a particular life-form group (Palms). However, to provide a reliable interpretation of the vegetation palaeocommunity type, we consider necessary to compare the relative abundance of palm phytoliths to other plant indicators, basically grasses or trees and shrubs morphotypes presence (Patterer, 2012).

The underestimation of the significance of the non-diagnostic herb elements is also a problem for getting a reliable palaeoecological interpretation. In this sense, we consider that the only presence of diagnostic grass phytoliths, although minority in abundance, is important enough to be considered as indicator of an incipient significant palaeocological change, such as the change from forests to open habitats. However, our interpretation does not only base on the recorded diagnostic grass phytoliths (*e.g.*, saddle, dumbbell), but in the presence of them, their association with abundant non-diagnostic herb-bamboo elements, and the relationship of relative abundances in regard to the arboreal elements. We consider that evaluating the whole phytolith composition within the whole palaeoenvironmental context provides a much more reliable interpretation, rather than to evaluate just selected diagnostic elements. It is true that contributions applying index of phytoliths (*e.g.*, LAI) on extant tree cover (*e.g.*, Bremond *et al.*, 2005) or grasses (*e.g.*, Bremond *et al.*, 2008) consider just diagnostic grass elements for the evaluation, but it is also true that those studies rely on systematic information, which lacks in completeness in the geological record or frequently is absent. The use of the phytoliths indexes (*e.g.*, LAI, D/P: ligneous woody Dicotyledon/Poaceae phytolith index) in sedimentary sequences from the past, undoubtedly provides a good approximation in the discerning magnitude of tree cover or opening habitats, but the final interpretations should be tested with suitable modern analogues.

### *Extant habitats as models for interpretation of palaeohabitats: unsuitable analogues provide unreliable reconstructions*

Dunn *et al.* (2015) provided a reconstruction of LAI for the lapse ~49–11 Ma of middle Patagonia, and linked the obtained results with the known recorded climate and fauna, achieving interesting conclusions regarding the origin of hypsodonty and the probable link with the changing Southern Ocean conditions. However, Dunn *et al.* (2015) recognize an opening of the landscape from ~49–32.2 Ma, spanning the lapse of time and sedimentary sequences involved in this contribution, and they interpret the palaeohabitat as a "non-analog palm shrubland with a discontinuous canopy". The palaeohabitats recorded in the early–middle Eocene sequences of Central Patagonia represent tropical forests (Las Flores Formation and some levels of the lower Koluel-Kaike Formation, *e.g.*, sample LF550), dry forests (most Koluel-Kaike Formation), and tropical savannahs (Gran Barranca Member). Particularly for the Koluel-Kaike Formation, dry forest relates to those levels with first records of grasses. These phytolith assemblages indicate a subtropical forest that underwent intense periods of drought, and have a clear analogue in modern habitats. Seasonally dry tropical forests in South America have been clearly recorded in central Brazil, northern Argentina, western Paraguay and southeastern Bolivia (Pennington *et al.*, 2004), constituting a clear and robust modern analogue for the palaeoflora of the middle Koluel-Kaike Formation, currently distributed in South American transition zone between Neotropical and Andean regions (Morrone, 2004, 2015). The use of modern analogues such as the Costa Rica flora (Dunn *et al.*, 2015), probably denotes a non-analog scenery from the middle Eocene flora of Patagonia, since extant species from Costa Rica have a different evolutionary history (Mesoamerican subregion, *sensu* Echeverry and Morrone, 2013) have transitional composition between Antillean and South American Neotropical subregion and the

Holoartic flora of North America), due the existence of regional geological factors (*e.g.*, the closure of the Panama Isthmus), which makes it a comparative model, but not of the South American lineages (*e.g.*, consider holoartic deciduous species compared to subantarctic species such as the *Nothofagus* ones).

The data described here support the emergence of dry tropical forests in the Koluel-Kaike Formation, recording the transition from tropical forest to herbaceous communities, and provides evidences of the first open canopy vegetation habitats in Central Patagonia, preceding in at least ~8 Ma the timing proposed by previous contributions (Strömberg *et al.*, 2013; Dunn *et al.*, 2015). Moreover, we display that the increase of grassy habitats is gradual rather than sudden, and more important that some grassy elements are related to $C_4$ ones preceding the Eocene/Oligocene Transition (EOT; 33.5–34 Ma; Zachos *et al.*, 2001). These interpretations find support in the current palaeopedogenic, geochemical, mineralogical, sedimentary, phylogenetic, and isotopic framework as shown in the following sections.

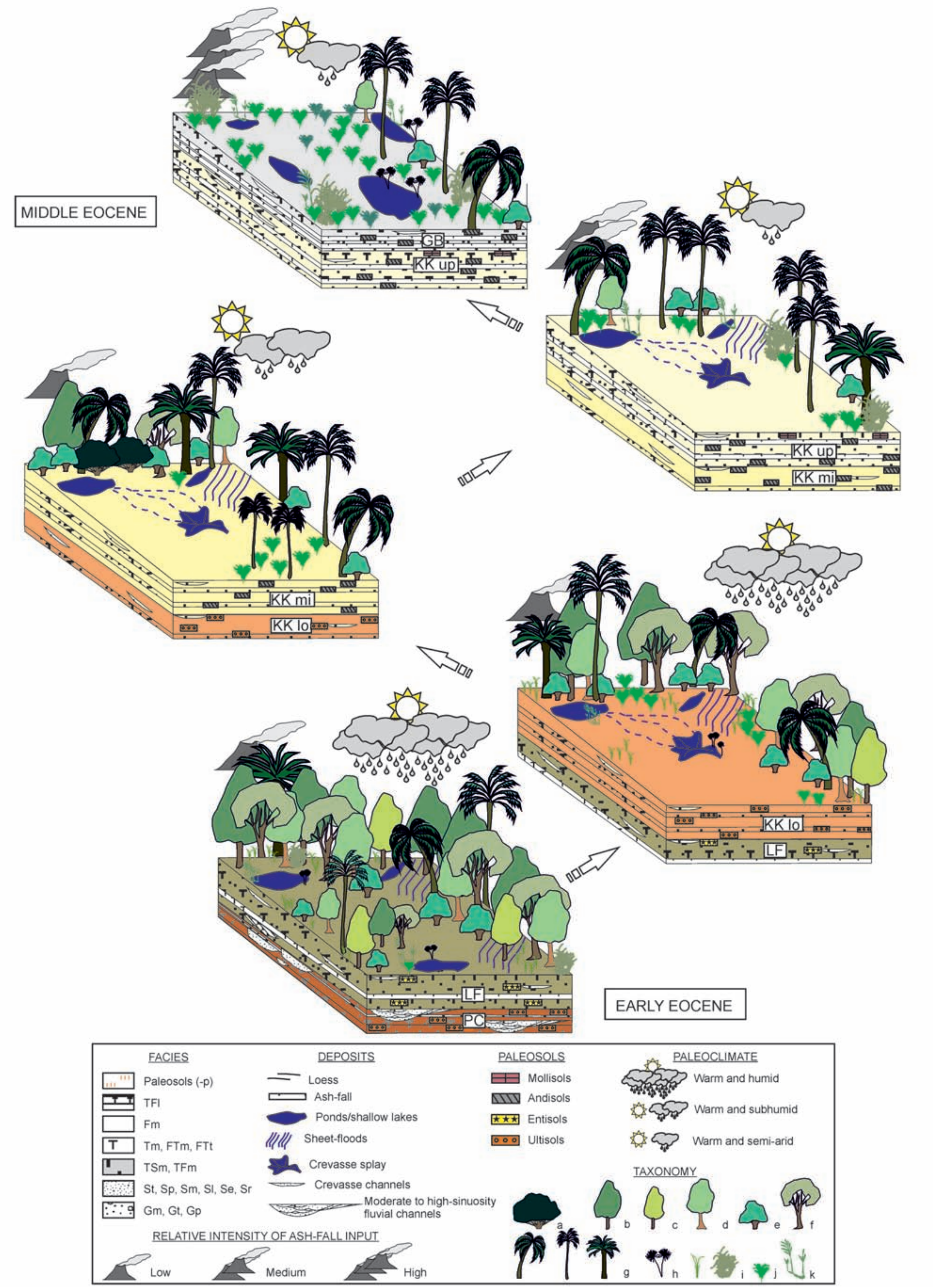
### *Clay mineralogy and palaeosols*

Clay mineralogy studies recorded peaks in kaolinite, along with a decrease in smectite/kaolinite ratios in the middle Las Flores Formation, supporting warm and humid conditions (Raigemborn *et al.*, 2009, 2014), related to the Early Eocene Climatic Optimum (EECO) (Raigemborn *et al.*, 2014; Krause *et al.*, 2017), and congruent with mineralogical records and interpretations from others regions (*e.g.*, Antarctica, Robert and Kennett, 1994; Dingle *et al.*, 1998; Dingle and Lavelle, 2000). Upward the Paleogene sequences, in the upper Las Flores Formation and the middle-upper Koluel-Kaike Formation, a gradual increase in smectite was recorded, but still with peaks in kaolinite in the lower Koluel-Kaike Formation, supporting a palaeoclimatic trend toward warm and subhumid-semiarid conditions, correlated with the transition from the greenhouse to icehouse world (Krause *et al.*, 2010, 2017; Raigemborn *et al.*, 2014, 2018).

On the other side, palaeosols from the Koluel-Kaike Formation (Krause *et al.*, 2010) and Gran Barranca Member (Bellosi *et al.*, 2010; Bellosi and González, 2010) provide evidence of gradual aridification upward the sequence. Palaeosols from the lower-middle Koluel-Kaike Formation characterize by dominance of palaeo-Ultisols, while paleosols from the middle-upper section are characterized by a predominance of palaeo-Andisols (Krause *et al.*, 2010). That study also provides evidence of strong seasonality, evidenced by pedofeatures such as mottling, coatings, and iron/manganese concretions, thus supporting a palaeoclimatic trend from seasonal, humid and warm conditions to subhumid-semiarid ones.

The aridification trend was also recorded in the two lowermost members of the Sarmiento Formation, Cañadón Vaca (~45–42 Ma, Krause *et al.*, 2017) and Gran Barranca (~42–38 Ma, Dunn *et al.*, 2015). The former is at Cañadón Vaca locality (Bellosi and Krause, 2014), placed about 70 km northeast of Las Flores section and correlative to the upper Koluel-Kaike Formation (Krause *et al.*, 2017). The Cañadón Vaca Member displays a suite of palaeosols integrated by Entisols (33%), Andisols (33%), and Alfisols (34%). The Gran Barranca Member, representative of the lower Sarmiento Formation in Las Flores locality characterizes by palaeo-Entisols (54%), palaeo-Andisols (38%), and palaeo-Alfisols (8%). This palaeosol trend agrees with a cooler and drying one from the Koluel-Kaike Formation to the lower Sarmiento Formation (Bellosi and Krause, 2014). Significantly, palaeosols recorded through the Koluel-Kaike-Sarmiento sequence are congruent with soils supporting mixed tropical forests with abundant palms and herbaceous components that only constitute the understory (Lutetian), through herbaceous elements that have occupied forest gaps (middle Lutetian–lower Bartonian) (Krause *et al.*, 2010, 2017) (Fig. 10). Moreover, the palaeoclimatic trend defined on the base of the whole of mineralogical and geochemical data, proceeding from mudstones and palaeosols in the middle Las Flores-lower Sarmiento sequences, constitutes a record of cooler and dryer environments linked to the greenhouse-icehouse transition that started immediately after the EECO, as previously suggested by Krause *et al.* (2010), Raigemborn *et al.* (2014), and recently constrained by Krause *et al.* (2017).

This integrated scheme displays a general decrease in chemical weathering upward the Las Flores-lower Sarmiento sequence, congruent with a primordially general decrease in MAP values, and a transitions from subtropical-tropical to sub-humid and semiarid conditions. However, fluctuations of drier and humid conditions is revealed by


MIDDLE EOCENE
GB
KK up
KK up
KK mi
KK mi
KK lo
KK lo
LF
LF
PC
EARLY EOCENE
FACIES
Paleosols (-p)
TFl
Fm
Tm, FTm, FTt
TSm, TFm
St, Sp, Sm, Sl, Se, Sr
Gm, Gt, Gp
DEPOSITS
Loess
Ash-fall
Ponds/shallow lakes
Sheet-floods
Crevasse splay
Crevasse channels
Moderate to high-sinuosity fluvial channels
PALEOSOLS
Mollisols
Andisols
Entisols
Ultisols
PALEOCLIMATE
Warm and humid
Warm and subhumid
Warm and semi-arid
TAXONOMY
a
b
c
d
e
f
g
h
i
j
k
RELATIVE INTENSITY OF ASH-FALL INPUT
Low
Medium
High

the vertical arrangement of paleosols (Krause *et al.*, 2010; Raigemborn *et al.*, 2018). All data support a gradual transition from arboreal to grassy communities, and explain for instance the phytolith suites as those recorded in the sample LF550, typical of tropical environments, which coincide with the type example of the palaeo-Ultisols recorded in the Koluel-Kaike (*Kapenk* pedotype *sensu* Krause *et al.*, 2010; Raigemborn *et al.*, 2018) (Fig. 2).

### *Sedimentation*

Sedimentary environments of the Koluel-Kaike Formation characterize by loessic and alluvial facies (*e.g.*, Krause *et al.*, 2010; Krause 2012a, b), which combined with palaeopedogenic data support a clear increase in sedimentary rates (Krause 2010, 2012a, b, 2016; Raigemborn *et al.*, 2018). The sediment accumulation in the Cañadon Vaca Member was interpreted as governed by distal, subaerial fallouts of suspended volcanic ash and dust on vegetated plains (tephric loessites), with minor fluvial and lakes (Bellosi and Krause, 2014). Eolian sedimentation would have increase upward the Gran Barranca Member, as evidenced by a broad dominance of tephric loessites originated in subhumid-semiarid rolling plains, with ephemeral ponded areas (Bellosi *et al.*, 2010, Bellosi and Krause, 2014). In that sense, the increase of loessic deposition, can be also engaged with an increase in the sedimentary rate in the lower Sarmiento Formation, since the increase of palaeo-Entisols and Andisols in detriment of palaeo-Alfisols in the Gran Barranca Member, meaning increase in sedimentation inhibiting long periods for palaeosols development.

Moreover, loessite sheets constitute around 65% of the deposits constituting the Sarmiento Formation, and are a significant facial element for the lower members (Bellosi, 2010, Bellosi and Krause, 2014). These kind of facies, early reported by Spalletti and Mazzoni (1979), is congruent with eolian transportation, and accumulation on sub-aerially in arid to subhumid settings (Pye, 1995; Kemp, 2001) during dry/cold periods within a seasonal environment (Kemp, 2001). Thus, the gradual increase in eolian deposits recorded in both Koluel-Kaike and the lower Sarmiento formations is congruent with an increase in dry climates, which favour an expansion of grassy communities. Particularly loesites recorded in the lower Koluel-Kaike (Krause *et al.*, 2010), constitute a non-classical occurrence (volcanic and tropical loess *sensu* Iriondo and Kröhling, 2007), indicating both dry periods within a general tropical-subtropical environment and the first input of eolian sediments in the Eocene of the San Jorge Basin. Significantly the first loessic deposits are located in the lowermost section of the Koluel-Kaike Formation, in the base (horizon C) of the first palaeo-Ultisol recorded, just above pond facies bearing abundant manganese and iron nodules (Krause *et al.*, 2008b; Krause, 2010), and coincident with the position of the sample LF540 bearing the first herbaceous elements (Fig. 2). In this context, loess facies and their gradual increase upward the Koluel-Kaike/lower Sarmiento sequence, support the onset of dry periods characterized by eolian deposition and favouring the emplacement of the first open habitats, gradually increasing from ~46Ma (Krause *et al.*, 2017) to the beginning of the Gran Barranca Member deposition at ~42 Ma.

### *The first open canopy vegetation in Central Patagonia*

Integrative studies linking wildfires, climate, and biotic change, suggest that the first instance of open canopy vegetation occurred by the late Eocene–early Oligocene (~38–33 Ma) in the Sarmiento Formation (Dunn *et al.*, 2015; Selkin *et al.*, 2015). According to our results, forest fragmentation and the appearance of new or more extensive open habitats for forest margin species could be considered previous to the aforementioned. For instance, the only plant

**Figure 10.** Palaeoenvironmental sketches showing the sedimentary environments, the characteristic palaeosols, the intensity of volcanic input, the plant taxonomy, and the palaeoclimate reconstructed in the four main stages of the Las Flores Formation/Koluel-Kaike Formation/Gran Barranca Member. The rolling plain was reconstructed for the Sarmiento Formation following Bellosi and González (2010). **PC,** Peñas Coloradas Formation; **LF,** Las Flores Formation; **KK,** Koluel-Kaike Formation; **GB,** Sarmiento Formation. The colours of each unit correspond to the outcrop colours. Taxonomy: Silhouettes by taxonomic groups: **a,** Taxaceae; **b,** Magnolidae; **c,** Anonaceae; **d,** Chrysobalanaceae; **e,** Mimosoideae; **f,** Burseraceae; **g,** Arecaceae; **h,** Cyperaceae; **i,** Tall grasses; **j,** Short grasses; **k,** Zingiberales.

megafossil (*Taxaceoxylon katuatenkum;* Taxaceae and/or Cephalotaxaceae, Brea *et al.*, 2009) recorded in the middle–upper Koluel-Kaike Formation at La Flores locality (LF548–LF550; Fig. 4) confirms that these families were more widespread in the past than today and were not confined to the Northern Hemisphere. Nowadays, in the Southern Hemisphere, it is found only in New Caledonia as a single species (Jaffré, 1995). The fossil taxon was presumably a component of the forests developed in humid and warm environments of Central Patagonia during the early–middle Eocene (Brea *et al.*, 2009). The Nearest Living Relatives (*Taxus* L., *Pseudotaxus* Cheng, and *Austrotaxus* Compton) are slow-growing and often long-lived trees, evergreen trees of small to moderate size or shrubs that grow in cool-temperate to subtropical climates, distributed in discontinuous patterns and in many cases known populations are particularly small and local (Kubitzki *et al.*, 1990). Extant New Caledonia forests show that *Austrotaxus* is abundant in the understory and often illuminated through the light-gaps in the low and discontinuous canopy (Jaffré, 1995).

On the other hand, and previously noted, open habitats in the form of loessic plains were broadly recorded and described for the Gran Barranca (Spalletti and Mazzoni, 1977, 1979; Mazzoni, 1979; Bellosi *et al.*, 2010; Bellosi and Krause, 2014) and the Cañadón Vaca (Bellosi and Krause, 2014) members. Such sedimentary environments would be been necessary for supporting grassy savannahs with palms or palm-groves and to the ichnological suite recorded in the Sarmiento Formation. The trace fossils content in the Sarmiento Formation characterizes by the high abundance of *Coprinisphaera* (fossil dung beetle brood balls) (Spalletti and Mazzoni, 1979; Bellosi *et al.*, 2010; Bellosi and González, 2010; Sánchez *et al.*, 2010), and constitute a clear example of the *Coprinisphaera* ichnofacies, a strong indicator of open-herbaceous communities (Genise *et al.*, 2000). Moreover, phytoliths extracted from *Coprinisphaera* specimens of the Sarmiento Formation revealed that Sarmiento environments were characterized by high density of grasses (Sánchez *et al.*, 2010).

### *First appearances of $C_4$ elements*

Molecular phylogenies for grasses, calibrated on macrofossil and phytoliths, establish that $C_4$ photosynthesis first originated in the grasses at ~32 Ma (middle Oligocene, Christin *et al.*, 2008; Vicentini *et al.*, 2008). However, also based on phylogenetic analyses that combine molecular genetic data and epidermal and phytolith features across Poaceae, Prasad *et al.* (2011) place the oldest origin of $C_4$ grasses, in the PACMAD-clade (Chloridoideae), around the middle Eocene (~43 Ma). Independently of the age of origin, it is broadly accepted that the former $C_4$ grasses constituted a small fraction of flora in which they developed (Dugas and Retallack, 1993; Cerling, 1999; Sage, 2001; Osborne and Freckleton, 2009). On the other hand, a major expansion of $C_4$ grasslands occurred across Africa, China, Pakistan, South America and North America only during the Late Miocene and Pliocene (2–8 Ma) (Cerling, 1999; Sage, 2001; Osborne, 2011). This expansion have been correlated to the rise of grassland biomes on these continents, and the turnover in the grazing fauna that occurred in these regions at this time (Cerling *et al.*, 1997; MacFadden, 1997; Osborne, 2011).

Although bilobate phytoliths recorded in the Koluel-Kaike Formation does not establish the panicoid presence on it, the mere presence of panicoids would not be indicative the $C_4$ photosynthetic pathway existence (Panicoideae comprise genera that occur in shady, open, and mixed habitats, with $C_3$ and $C_4$ photosynthetic pathways (Morrone *et al.*, 2012), but the presence of chloridoid saddle elements in these sedimentary samples are probably more reliable $C_4$ photosynthetic pathways indicators. Saddle phytoliths have been recorded in both the Koluel-Kaike Formation and the Gran Barranca Member (Zucol *et al.*, 2010a, this contribution), units from Central Patagonia deposited during the Lutetian–Bartonian lapse (Ré *et al.*, 2010a, b; Dunn *et al.*, 2013; Krause *et al.*, 2017). Saddle phytolith type is produced in high proportion by the Chloridoideae grass subfamily (Twiss *et al.*, 1969; Mulholland, 1989; Fredlund and Tieszen, 1994; Kondo *et al.*, 1994), and this subfamily is broadly accepted as representative of $C_4$ grasses adapted to warm and dry climate or dry soil conditions (Tieszen *et al.*, 1979; Livingstone and Clayton, 1980). But how did originate the $C_4$ elements in the middle Eocene of Southern South America?

The origin of grasses $C_4$ lineage and the environmental factors that may have caused its appearance has been broadly discussed (Sage, 2001; Osborne, 2011; Sage *et al.*, 2011 and cites therein). The leading environmental hypothesis based in the reduction of atmospheric $CO_2$ in the late

Oligocene (~32 Ma) increased photorespiration in warm climates, thereby facilitating selection for $CO_2$-concentrating mechanisms such as $C_4$ photosynthesis (Sage, 2001, 2004; Christin *et al.*, 2008, 2014; Vicentini *et al.*, 2008; Edwards *et al.*, 2010; Osborne, 2011). Other factors favouring the appearance of $C_4$ lineage would be related to increasing in aridity and seasonality, creation of high light habitats, increase of fire occurrences, and large animal disturbance (Sage, 2001; Osborne and Freckleton, 2009; Osborne, 2011). Following the leading environmental hypothesis, we note as notorious that all previous studies looking for the link between $C_4$ emergence and declination of atmospheric $CO_2$, focused in the global Cenozoic temperature of Zachos *et al.* (2001), but just in the portion elapsing the Miocene–Pleistocene periods. As Osborne (2011) discusses, the most of the proxy evidences indicate inconclusively the efficiency of the atmospheric $CO_2$ as a unique selection pressure for the evolution of $C_4$ photosynthesis. Considering that concern, we agree with Sage (2001) and Osborne (2011) in that the origin of the $C_4$ flora cannot be viewed simply as a response to any single factor, such as a lower atmospheric $CO_2$, but instead may be a response to a coalition of biological, atmospheric and geological trends.

Thus, to support the hypothesis of the early rise of $C_4$ flora in the lower–middle Eocene of Central Patagonia, we provide assessments regarding the environmental and ecological conditions recorded in the Las Flores-Sarmiento sequence, according to the current regional geological and paleontological knowledge.

### Environmental Preconditions

Low atmospheric $CO_2$ inhibited $C_3$ photosynthesis and contribute to the climatic deterioration that caused arid landscapes to expand (Sage, 2001). The trend of aridification is clearly recorded in the studied sequence through previous mineralogical, geochemical, palaeopedogenic, and palaeobotanical studies as previously developed, beside of the new information here reported.

Different studies provide strong arguments supporting that $C_4$ plants originally occurred at $CO_2$ levels that were intermediate between modern values and those predicted for the mid-Cretaceous (Ehleringer *et al.*, 1997; Sage, 2001), with a subsequent global expansion of $C_4$ plants developed on further reduction in $CO_2$ to near current levels (Cerling *et al.*, 1997; Cerling, 1999; Sage, 2001). The most complete and accepted archive of global Cenozoic temperature based upon foraminiferal oxygen isotopic values, mainly proceeding from Southern Ocean cores (Zachos *et al.*, 2001, 2008), displays that the worldwide early Eocene climate characterized by high concentrations of $pCO_2$ and long-term high temperature (Early Eocene Climatic Optimum [EECO], 51–53 Ma), and that only over the past 34 million years have $CO_2$ concentrations been low, temperatures relatively cool, and the poles glaciated (Zachos *et al.*, 2001, 2008; Francis *et al.*, 2009). The high atmospheric $CO_2$ levels in the middle–late Eocene declined with huge amplitudes and then decreased in several steps during the Oligocene (*e.g.*, Pagani *et al.*, 2005). This decrease in high atmospheric $CO_2$ levels is valid also for Central Patagonia. A recent study based in oxygen isotope compositions of mammal tooth enamel and pedogenic carbonate from the lapse ca. 43–21 Ma in the Sarmiento Formation (Kohn *et al.*, 2015), suggests a trend of aridification from the base of the Koluel-Kaike Formation (c. 45 Ma, c. 1200 mm/yr) to the Cañadón Vaca Member (c. 43 Ma, c. 450 mm/yr), and quasi-constant mean annual precipitation until at least 31 Ma (Puesto Almendra Superior Member), and a moderate temperature drop (3–4°C) across the Eocene/Oligocene transition (EOT).

### Ecological Preconditions

Currently, the major $C_3$ versus $C_4$ interactions on the planet occur between herbaceous $C_4$ grasses and woody $C_3$ dicots in low latitude savannahs (Soares, 1990; Sage, 2001; Sage *et al.*, 2011), a habitat similar as the one proposed by the Gran Barranca Member (Zucol *et al.*, 2010a). This interaction is highly dependent on the occurrence of ecological disturbances, such as large animal activity and fire, since the persistence of $C_4$ grasslands needs regular disturbance events to regulate the invasion and establishment of woody vegetation (Sage, 2001).

Several studies suggest that browsing by large mammals can be an effective control for expansion of $C_3$ flora, since they selectively add pressure on the woody vegetation (Owen-Smith, 1989; Dublin, 1995; Sinclair, 1995; Sage, 2001). Large mammals are not common or directly absent in the early Paleogene of Central Patagonia. The mammal communities for that time in the San Jorge Basin mainly are characterized by small-sized ungulates (*e.g.*, Archaeopithecids, Notopithecids, Notostylopids, Henricosborniids; Simpson, 1967), with records of some medium-sized mammals in the Paleocene (*e.g.*, *Carodnia*; Simpson 1935) and Eocene (*e.g.*, Isotemnids, Trigonostylopids; Simpson 1967). In spite of this, we suggest that the overturning of trophic habits from browsing to grazing could be an additional factor in the onset of a selective pressure on the $C_3$ flora. In this regard, an integrative study developed on Southern South America (below latitude 15° S) (Ortiz-Jaureguizar and Cladera, 2006), displays trophic trends for the region and particularly for Patagonia—many of them from Central Patagonia—as follows: during the early Paleocene, South-Central Patagonian mammal communities were dominated by insectivorous-frugivorous, followed by omnivorous-browsers and insectivorous types (Ortiz-Jaureguizar, 1996; Pascual *et al.*, 1996; Ortiz-Jaureguizar and Cladera, 2006). For Southern America (*sensu* Ortiz-Jaureguizar and Cladera, 2006) mammal communities were dominated by insectivorous, with subordinate browser or omnivorous-browser types. Particularly, browser mammals would have represented 5% (% total number of analyzed genera by Ortiz-Jaureguizar and Cladera, 2006) during the early Paleocene and ~52% during the late Paleocene. During the early Eocene–latest Paleocene land-mammal communities persisted for Southern South America, being dominant browser mammals. However, mixed-feeders and grazer mammals were recorded (only) in Patagonian beds, in low diversity values (Pascual *et al.*, 1996; Ortiz-Jaureguizar and Cladera, 2006). During the middle Eocene land-mammal communities of Southern South America were dominated by browser types, although again low diversity values of mixed-feeders and grazers were present only in Patagonia (Pascual *et al.*, 1996; Croft, 2001). During the late Eocene, land-mammal communities were dominated by browsers, but mixed-feeders and grazers were more diverse in Patagonia. In general, grazers and browsers mammals of Southern South America were respectively represented as follows: 0% and ~65% for the early Eocene; <5% and ~52% for the middle Eocene, and 10%–40% for the late Eocene (Ortiz-Jaureguizar and Cladera, 2006). The expansion of mixed-feeders and grazers recorded during the early Paleogene of Patagonia and Southern South America was suggested as parallel to the expansion of relatively drier climates and plant communities with a major proportion of grasses (Pascual *et al.*, 1996), an interpretation compatible with previous palaeoclimatic inferences (Raigemborn *et al.*, 2009, 2014; Krause *et al.*, 2010; Bellosi and Krause, 2014; Kohn *et al.*, 2015), palaeobotanical studies (Brea *et al.*, 2008; Raigemborn *et al.*, 2009; Zucol *et al.*, 2010a), and our data here reported. Although small- and medium-sized browser mammals probably are not so effective in the control of expansion of arboreal components as, for instance, elephants in nowadays arboreal grasslands (*e.g.*, Dublin, 1995), the turnover from browsing to grazing within a context of close tropical forests during the Paleocene–early Eocene, coupled with the recorded aridification trend, could have been an significant factor in the onset of the opening of habitats.

The fire is a disturbance factor of woody vegetation, promoting more open habitats and, thus, favouring the proliferation and development of grassland, and sustaining the new biome, even in regions with the climatic potential to support forests (Bond *et al.*, 2005; Osborne, 2011). From a combination of the concentration of magnetite and burnt palm phytoliths in loessites from the Eocene–Oligocene Vera Member of the Sarmiento Formation (San Jorge Basin), Selkin *et al.* (2015) recorded intense wildfires during the lapse ~42.0–18.5 Ma (Dunn *et al.*, 2013), and its apparent suppress for ~200 ka shortly after the EOT. Selkin *et al.* (2015) suggest that fire probably played an important role in structuring Eocene–Oligocene landscapes than previously thought, leading to the appearance of open habitats close to the EOT; we agree with the role of fire in this sense. Climates capable of supporting frequent fires were also present during the early–middle Eocene of Central Patagonia as suggested by the record of burnt grass epidermis proceeding from the sedimentary sequence herein presented, and currently under study. Thus, wildfires during the time elapsing the early–middle Eocene, probably were an additional factor promoting the instauration of grassy habitats in the middle Eocene Central Patagonia.

## CONCLUSIONS

The Koluel-Kaike phytolith assemblages constitute the record of the presence of dry forests in the Lutetian of Central Patagonia, attesting the retraction of arboreal communities along with an increase in herbaceous components, principally mixed grass-dominated ecosystems or savannahs, and recording the transition from subtropical/tropical lowland forest recorded in the Las Flores Formation (Ypresian) to the tropical savannahs recorded in the Gran Barranca Member (Lutetian–Bartonian).

Saddle phytoliths recorded in the Koluel-Kaike Formation would attest the presence of $C_4$ grasses (Chloridoideae) by the middle Eocene of Central Patagonia.

The palaeoflora interpreted for the Koluel-Kaike phytolith and its climatic significance find support in sedimentary, mineralogical, geochemical, and palaeopedogenic data. In this context, the Koluel-Kaike contains the oldest record of grassy habitats in Central Patagonia.

The phytolith record here presented, in the context of global climatic changes during the early Paleogene in Patagonia, particularly the existence of noticeable amplitudes in the atmospheric $CO_2$ concentration, along with the presence of fires and first grazers mammals, could suggest the first appearances of $C_4$ elements in mixed communities as savannah types.

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