INTRODUCTION

Cell wall is an ancestral condition in plants, and most of plant cells are enclosed by walls, except some cells concerned with reproductive, or early ontogeny, processes (Kenrick & Crane, 1991; Beck, 2010). Cell wall is modified in different ways as the cell matures, and some of these changes imply extension, thickening, and modification of grosser physical structure. Most xylem cells are highly specialized in conducting water, and for this reason they have anatomical features of interest in physiology, ecology, evolution and systematic of vascular plants (Carlquist, 1975). Walls of secondary xylem cells have many characteristics of systematic and/or evolutionary significance: e.g. tracheid pit arrangement, seriation of tracheid pits, presence of torus or helical thickenings, type of cross-fields, etc. (IAWA committee, 2004; García-Esteban, 2002; Greguss, 1955, 1972). Pits are cavities in the wall of a plant cell where there is no secondary wall and where water
and sap flow. Their arrangement varies through the different species of gymnosperms.

Particularly, pit arrangement is often superficially mentioned in extant gymnosperm (or soft-wood) wood anatomical descriptions. However, the pit arrangement, which includes contiguity (or spacing) of the pits and seriation, is a key character for delimitation of fossil genera and fossil species in gymnosperm fossil woods (Eckhold, 1922; Vogellehner, 1967; Philippe & Bamford, 2008). IAWA committee (2004) code has two characters related to radial pit arrangement, the first one is “predominantly uniseriate” vs. “predominantly biseriate or more” (a discrete character), and the second one is “biseriate or more seriate pitting, alternate” vs. “opposite” (also a discrete character). Neither of the two refers to the contiguity of the pits, and they are both discrete binary characters.

Nicol (1834) first mentioned the division of radial pitting into araucarian and abietinean (without using those terms). Later Kräusel (1917) and Eckhold (1921, 1922) divided fossil wood pit arrangements into three types: araucarian, mixed and abietinean, making emphasis on the mixed type of fossil woods. Mixed (also called generalized or transitional) is a transitional type of pitting between araucarian pitting (which is not exclusive of the Araucariaceae) and currently more common abietinean radial pitting. According to Bamford et al. (2016), this type of pitting is particularly common in Mesozoic woods. Woods with this type of radial pitting (mixed) were grouped into the “Protopinaeae” (=Protopinaceae) by Kräusel (1917) and this definition was followed by different authors (e.g. Eckhold, 1922; Vogellehner, 1967). Recently it was discussed by Bamford et al. (2016) and they recommended not to use this artificial family (Protopinaceae). A fourth type of pit arrangement was described by Müller-Stoll (1951) as xenoxylean, where radial pits are much flattened, more than twice as wide as high, and contiguous with neighbouring pits (Müller Stoll, 1951; Philippe & Bamford, 2008). This division of radial pitting is currently used for delimiting fossil genera (Vogellehner, 1967; Philippe & Bamford, 2008). However, methods for measuring or classifying the radial pits into these three types vary among different authors and they usually assigned the fossil woods to one of these categories without further details (Philippe et al., 2014). The contiguity of the pits plays a key role in these three types of radial pit arrangement.

The seriation of radial pits (uniseriate, biseriate, etc.) is also a character used for fossil species delimitation (e.g. Penhallow, 1907; Pujana et al., 2014, 2015). Usually seriation is given as percentages of uniseriate, biseriate, triseriate, etc. pitting or a vague description is mentioned (“predominantly uniseriate”, “mostly biseriate”, etc.).

In addition, several authors have claimed that it is essential that anatomical characters are quantified (Falcon-Lang & Cantrill, 2000; Poole & Cantrill, 2001) and continuous characters are preferable and more informative for certain statistical or taxonomical analysis rather than discrete characters.

For these reasons, we propose two new indices to measure with precise indications the contiguity and seriation of pitting of gymnosperm woods with circular or hexagonal tracheid pits (not scalariform or transitional pitting).

MATERIAL AND METHODS

Studied extant woods (one slide per species) are from the Xylarium of the Museo Argentino de Ciencias Naturales (acronym BA) and of the Facultad de Ciencias Agrarias y Forestales, UNLP (acronym UNLP). Fossil woods measurements were based on the holotypes of each fossil-species. Fossils are housed in the Museo Provincial Padre Molina (acronym MPM PB), Museo Argentino de Ciencias Naturales (acronym BA Pb) and Facultad de Ciencias Naturales y Museo, UNLP (acronym LPPB). A representation of the different radial pit arrangements was attempted.

Measurements of radial pit contiguity and seriation in different species of both fossil and extant gymnosperm woods were taken (Table 1, Fig. 1-4). For obtaining the two indices, measurements were taken in random different zones (including earlywood and latewood) of the slide (or different zones of the fragment observed with scanning electron microscopy). When it was possible, up to 60 horizontal lines of pits were measured for both indices in each zone. We define a horizontal line of pits as indicated in Fig. 2. In uniseriate rows a horizontal line of pits is a single pit, in biseriate rows are two pits, in triseriate rows are three pits and so on (Fig. 2, 4).

Pits should be counted in random different zones (provided with pits) of the wood to allow the observation of the specimen variation. Figures 2 and 3 show examples of hypothetical typical gymnosperm woods. Note that in those figures the indices were calculated in only one zone of the sample for the purpose of illustrating the methods.
INDICES

Contiguity percentage index (Cp)

The Cp is a percentage (0-100%) that characterizes whether the horizontal lines of pits have contiguous (=in contact or touching) or overlapping with the outer borders of the pits of neighbouring (lower and an upper) horizontal lines (Fig. 2). A Cp with a 0% value indicates that all the horizontal lines of pits are spaced, and a 100% value indicates that all the horizontal lines of pits are contiguous with the neighbouring horizontal lines of pits.

Horizontal lines of pits must be contiguous, flattened or overlapped to be counted positively. Sometimes pits appear to be spaced, if the outer border of the pit is not clearly observed (see Bamford et al., 2016). In Figure 4D-E a typical Araucaria Jussieu wood with contiguous and flattened pits is shown. Note that the pits are apparently not contiguous, but they are even flattened and hexagonal indicating that they are contiguous. In addition, sometimes only the aperture of the pits is observed and it could be confused with spaced pits (Fig. 4C). Contiguous horizontal lines of pits have uniform distances among them (and the distance between the centers of the pits is the diameter of the pit) while in spaced (also called scattered) pits this distance is variable (Bamford et al., 2016).

The first horizontal line of pits next to a ray or at the end or beginning of a tracheid (which could have only lower or upper border in contiguity at most) should not be counted to allow a maximum of 100% contiguous pits (e.g. Fig. 4H). When crassulae (=bars of Sanio) are present between two horizontal lines of pits we consider them spaced (Fig. 4B, F).

Horizontal lines of pits must be measured as in Figure 2, and they should be divided into 3 types: a. Both outer border of pits (upper and lower) separated from other lines of pits. b. Only one outer border, lower or upper, in contact with another line of pits. c. Upper and lower outer borders in contact with the neighbouring horizontal lines of pits. Following the equation in Figure 2, the Cp can be calculated. Note that Cp refers to a single specimen and not to a single tracheid. Consequently, we should count the state of the total horizontal lines of pits in the sample/specimen, and then calculate the Cp.

Seriation index (Si)

The Si index is an average of the seriation of the pits (Fig. 3). It can have a minimum of 1 (all horizontal lines of pits are uniseriate) and a maximum equal to the number of vertical rows (=columns) which a multisieriate pitting tracheid has. Variation in the same tracheid is common; bisieriate pitting portions in mostly uniseriate pitting tracheid and vice versa are commonly found, for this reason we count individual horizontal lines of pits and not tracheids as a whole.

In this index, we also count horizontal lines of pits and not single pits. Horizontal lines of pits are multiplied by one (when uniseriate), by 2 (when biseriate), by 3 (when triseriate) and so on and then a sum of them is calculated. To calculate the Si we finally divide the sum by the total number of horizontal lines we counted (Fig. 3). Si has no units.

Converting previously descriptions of pit seriation is straightforward: for example, to con-
vert a specimen with 68% biseriate lines, 7% triseriate lines and 25% uniseriate lines, $Si = \frac{(1 \times 25 + 2 \times 68 + 3 \times 7)}{100} = 1.82$.

**Notes**

We determined the minimum number of zones to be measured based on successive zone measurements in mature wood of *Podocarpus lambertii* Klotzsch ex Endlicher. The cumulative average is sufficiently stabilized after approximately 8-10 measurements in contiguity percentage index ($Cp$) (Fig. 1A) and after approximately 5 measurements in seriation index ($Si$) (Fig. 1B). Therefore, we suggest that a minimum of 10 zones is preferable to calculate the indices.

Both indices were calculated using the total number of horizontal lines of pits of one sample and not calculating a weighted average of the zones (although this latter can also be carried out if a significant different number of horizontal lines of pits in each zone are measured).

**Other indices**

In fossil wood literature more indices or methods to define radial pitting were used to quantify radial pitting. These can complement the $Cp$ and $Si$:

1) “Coefficient d’écrasement” or pit flattening coefficient measure individual pit flattening, dividing the height by the width of each pit. It is used sporadically (e.g. Serra, 1966; Marguerier & Woltz, 1977; Desplats, 1979; Vozenin-Serra & Grant Mackie, 1996; Torres et al., 1994).

2) “Percentage touching pits” (Falcon-Lang & Cantrill, 2000) the ratio of tracheids with mainly touching, contiguous pitting to those bearing pits usually spaced more than one pit diameter apart. The main difference with $Cp$ is that “percentage touching pits” method gives higher percentages because pits spaced with more than one pit diameter are more difficult to find, particularly in fossil woods. Some cases are not explained in detail, for example, whether biseriate rows should be counted as uniseriate rows.

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**Fig. 2. Hypothetical examples of $Cp$ (contiguity percentage) index measurements and equations.** Note that biseriate (or multiseriate) horizontal lines are counted the same as uniseriate. Dashed lines indicate some horizontal lines of pits.
3) “Contiguity value” (=“pit contiguity”) (Falcon-Lang & Cantrill, 2001) the length of continuous sequences of touching bordered pits on the radial tracheid walls expressed as the range and mean number of pits in each contiguous sequence.

4) Henderson & Falcon-Lang (2011) give the “mean of pit [vertical] rows”, which we supposed is very similar to Si. However, they do not give an explanation of how they calculated it, whether they measured whole tracheids, horizontal lines of pits or individual pits.

5) Philippe et al. (2014) assigned each pit observed to either one of the six types: UD (uniseriate distant); UC (uniseriate contiguous and usually flattened); BA (biseriate alternate); BOR (biseriate opposite round); BOS (biseriate opposite square). This is a very detailed method to describe radial pitting. However, as each type has a percentage, six different characters should be considered.

**DISCUSSION**

Radial pit arrangement is crucial for fossil wood genera/species delimitation. An agreement among authors must be found and followed for delimitation of fossil-genera and terms must have an unambiguous definition. One of the most comprehensive review of fossil wood genera is the article of Philippe & Bamford (2008), which is a reappraisal of most Mesozoic gymnosperm fossil genera. In the key, pit arrangement is particularly important to define the fossil-genera.

Authors can use the indices proposed herein or give a detailed description of the radial pitting, giving quantified descriptions if possible. The indices proposed herein can be included in statistical analysis as continuous characters, which are usually more informative than binary or discrete characters as those proposed by IAWA committee (2004) for pit arrangement.

Measurements of typical extant araucarian pitting (*Araucaria araucana* (Molina) K. Koch and *Araucaria angustifolia* (Bertol.) Kuntze) give results of Cp always higher than 90% (Table 1). In woods of extant Podocarpaceae Cp ranges from 7.8% to 20.7%, in extant Cupressaceae from 0.9% to 4.0% and in extant Pinaceae from 5.3% to 12.5% (Table 1), the three families being usually considered to have abietinean radial pitting. Si exhibits no significant variation and has values close to 1 in most species, except for *Araucaria angustifolia*, which has frequently biseriate pit-
Table 1. Examples of measurements of extant (1-9) and fossil woods (10-15).

| Species                                      | Family/group       | 1 | 2 | 3 | Cp av. [%] | 1 | 2 | 3 | Si av. |
|----------------------------------------------|--------------------|---|---|---|------------|---|---|---|-------|
| 1. Araucaria arauca [BA 1731]                | Araucariaceae      | 97.0 | 91.4 | 91.8 | **93.4** | 1.02 | 1.02 | 1.04 | **1.03** |
| 2. Araucaria angustifolia [BA 1647]          | Araucariaceae      | 98.8 | 97.3 | 97.1 | **97.7** | 1.47 | 1.47 | 1.46 | **1.47** |
| 3. Podocarpus nubigena [FCEyN-F]             | Podocarpaceae      | 16.2 | 26.7 | 19.3 | **20.7** | 1.02 | 1.10 | 1.04 | **1.05** |
| 4. Podocarpus lambertii [LP PIVA]            | Podocarpaceae      | 7.5 | 10.9 | 15.0 | **11.1** | 1.08 | 1.08 | 1.09 | **1.08** |
| 5. Saxegothaea conspicua [FCEyN-G]           | Podocarpaceae      | 8.7 | 9.9 | 4.9 | **7.8** | 1.04 | 1.05 | 1.02 | **1.04** |
| 6. Fitzroya cupressoides [FCEyN-H]           | Cupressaceae       | 3.5 | 6.4 | 2.0 | **4.0** | 1.16 | 1.13 | 1.05 | **1.11** |
| 7. Austrocedrus chilensis [FCEyN-J]          | Cupressaceae       | 1.2 | 0.0 | 1.6 | **0.9** | 1.02 | 1.01 | 1.01 | **1.01** |
| 8. Cedrus deodora [BAw 342]                 | Pinaceae           | 14.3 | 13.5 | 9.6 | **12.5** | 1.01 | 1.01 | 1.01 | **1.01** |
| 9. Pinus radiata [FCEyN-D]                   | Pinaceae           | 5.4 | 7.6 | 3.1 | **5.4** | 1.03 | 1.07 | 1.03 | **1.04** |
| 10. Podocyphloxylon francisciae [BA Pb 14416] | Podocarpaceae      | 77.9 | 69.1 | 71.9 | **73.0** | 1.54 | 1.48 | 1.63 | **1.55** |
| 11. Protopjuniperoxylon ischigualastensis [LPPB 4375] | Cupressaceae/ Protopinaceae | 89.4 | 85.4 | 79.2 | **84.7** | 1.23 | 1.24 | 1.23 | **1.23** |
| 12. Cuproxylon multipunctatus [BA Pb 12902]  | Gymnosperm         | 100.0 | 100.0 | 100.0 | **100.0** | 4.45 | 4.84 | 3.95 | **4.41** |
| 13. Agathoxylon antarcticus [BA Pb 144]      | Araucariaceae      | 96.6 | 93.6 | 92.5 | **94.2** | 1.09 | 1.03 | 1.05 | **1.06** |
| 14. Cupressinoxylon hallei [BA Pb 14429]     | Podocarpaceae/ Cupressaceae | 2.8 | 1.2 | 1.6 | **1.9** | 1.00 | 1.01 | 1.00 | **1.00** |
| 15. Podocarpoxylon sp. [MPM PB 2249]         | Podocarpaceae      | 31.3 | 36.4 | 31.0 | **32.9** | 1.00 | 1.00 | 1.00 | **1.00** |
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