Identification of Conifer Stomata in Pollen Samples from Western North America

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Abstract

Conifer stomata provide important paleoecological information for determining the composition of past plant communities, particularly at the local scale and when plant macrofossils are absent. To aid efforts to identify conifer stomata in fossil pollen samples from western North America, we describe the stomatal morphology of 19 conifer species that occur in the region, with emphasis on species that are present in the conifer-dominated forests along the northwest Pacific coast. We measured 10 morphological traits in a total of 315 stomata from these species. Morphological variability within species and the degree of morphological overlap among species precludes reliable identification to the species level; however, stomatal morphology is relatively consistent within genera and sufficiently unique to permit identification to genus. We used classification and regression trees to identify the critical morphological features for stomata identification and to build classification models. We then used these classification models as the basis for dichotomous identification keys for complete and incomplete conifer stomata. Identification of conifer stomata in fossil pollen samples from western North America should enhance paleoecological records from the region by providing evidence of local conifer presence and potentially clarifying their arrival times. Conifer stomata also provide a possible avenue for increasing taxonomic resolution in some paleoecological records: *Pseudotsuga* and *Larix* as well as members of the Cupressaceae family have indistinguishable pollen morphologies, but our results show that their stomata can be differentiated in most instances.

Keywords: conifer stomata; stomatal morphology; classification and regression tree analysis; identification key; western North America

1. Introduction

The identification of fossil conifer stomata on pollen slides provides useful paleoecological information for reconstructing past vegetation dynamics (MacDonald, 2001). Due to differential pollen production, dispersal and preservation, pollen analysis alone can be insufficient for determining the composition of past plant communities, particularly at the local scale if pollen production is low (Birks and Birks, 2000). Compared to widely dispersed pollen, conifer needles are typically transported only short distances from their source (e.g., Dunwiddie et al., 1987; Parshall, 1999) and thus their presence in peat and lake sediments usually indicates the local presence of conifers. Stomata are liberated from conifer needles during fragmentation and decomposition and their lignified cells are resistant to decay and standard chemical treatments used in pollen analysis. Thus, conifer stomata that are present in pollen samples, as isolated microfossils and in fragments of epidermal tissue, provide evidence of local conifer presence, making them an excellent complement to pollen-based paleoecological studies.

Conifer stomata can also provide greater taxonomic precision than pollen in some cases (Yu, 1997; Lacourse et al., 2012) and have proven useful in estimating the arrival times of conifers (e.g., Hansen, 1995; Hansen and Engstrom, 1996; Froyd, 2005; Lacourse et al., 2005, 2012). Using fossil stomata, a number of studies have shown that conifers were present locally hundreds to thousands of years in advance of increases in conifer pollen that would typically be used to infer local presence as opposed to regional population expansion or long-distance pollen transport (e.g., Clayden et al., 1997; Parshall, 1999; Froyd, 2005; Lacourse et al., 2012; Edwards et al., 2015). Conifer stomata have also been especially valuable in helping to reconstruct vegetation changes at tree line (e.g., Hansen et al., 1996; Pisaric et al., 2003; Wick, 2000; Gervais et al., 2002; Finsinger and Tinner, 2007; Magyari et al., 2012; Li and Li, 2015). However, Leitner and Gajewski (2004) appropriately suggest caution in the interpretation of fossil stomata records, noting that Clayden et al. (1996) and Pisaric et al. (2001) found conifer stomata in modern sediments at lakes situated beyond latitudinal tree line. In both of these studies, the stomata are likely the result of redeposition of older material from eroding peat deposits surrounding the lakes.

Trautmann (1953) was the first to demonstrate that conifer stomata on pollen slides can be identified to genus and developed an identification key for six genera of European conifers. In North America, Hansen (1995) examined the stomata of 11 conifer species and adapted Trautmann's (1953) key to differentiate these taxa, mostly to the genus level, and Yu (1997) documented differences in the morphology of *Thuja occidentalis* stomata compared to those of three *Juniperus* species. Using canonical variate analysis of morphological measurements, Sweeney (2004) built stomata identification keys for six conifer species for use in Scandinavia, although these have been widely used in Europe and elsewhere (e.g., Froyd, 2005; Salonen et al., 2011; Magyari et al., 2012; Mustaphi and Pisaric, 2014). More recent work includes an identification key for conifer stomata in northwest China (Wan et al., 2007) and a species-specific key for *Pinus* stomata in southwest Europe (Álvarez et al., 2014).

Identifying fossil stomata is inherently more difficult in regions with numerous conifer species such as western North America. Hansen's (1995) stomata identification key has been used, in conjunction with reference material, in a number of studies in that region (e.g., Hansen and Engstrom, 1996; Pisaric et al., 2003; Lacourse et al., 2005, 2012; Mustaphi and Pisaric, 2014). However, Hansen's (1995) study on stomatal morphology did not include a number of important conifers that are either widespread in western North America (e.g., *Abies lasiocarpa*, *Pinus ponderosa*, *Pseudotsuga menziesii*, or any species of *Juniperus*) or have distributions that are primarily limited to the conifer-dominated forests of the Pacific coast (e.g., *Abies amabilis*, *A. grandis*, *Picea sitchensis*, *Pinus contorta* var. *contorta*, *Taxus brevifolia*).

Here, we describe the stomatal morphology of 19 conifer species that occur in western North America, with particular attention to species that are common in coastal Alaska, British Columbia, Washington and adjacent regions. We assessed 10 morphological traits in a total of 315 stomata from 64 individuals of these species, and used classification trees and random forest analysis to identify diagnostic morphological criteria for stomata identification. We used the resulting classification models to aid in the production of dichotomous identification keys suitable for conifer stomata in pollen samples from western North America.

The identification keys presented here are designed for identifying stomata from mature needles. Others have shown that stomatal morphology and frequency can vary with leaf ontogeny (e.g., Owens, 1968; Kouwenberg et al., 2004); however, because immature needles are generally smaller and more fragile, their stomata are less likely to be encountered in pollen samples than those from mature needles. As with all identification keys built on modern material, using the keys to identify fossil stomata relies on the assumption that stomatal morphology has been conserved through time. This is a reasonable assumption for late Quaternary fossils, particularly in relation to the long generation times of conifers.

2. Materials and Methods

We obtained mature needles from 19 conifer species that are native to western North America (Table 1). All needles were sampled from voucher specimens housed in the University of Victoria Herbarium (Supplementary Table 1), with the exception of one individual of *Juniperus scopulorum* that was collected by E.C. Grimm from the Black Hills of South Dakota. Needles were sampled from three to five individuals of each species (n = 64 individual plants in total). Botanical nomenclature follows the Flora of North America Editorial Committee (1993).

To isolate stomata, needles were soaked in warm water for 5 min and then chopped into 1–2 mm-long sections. Since the aim was to determine identification criteria for conifer stomata encountered in pollen samples, preparation for light microscopy followed standard palynological techniques (Bennett and Willis, 2001), which consisted of an 8 min treatment in 10% KOH, 3 min in acetolysis, and mounting in 2000 cs silicone oil.

Measurements were made on three to six stomata per individual plant (n = 315 stomata in total) at 630× magnification using a Zeiss M1 AxioImager. We measured the length and width of the upper woody lamellae (UWL) and the width of the polar lamellae or stem (Fig. 1). We also measured guard cell width to account for any differences in how open stomata were and to potentially help guide the identification of incomplete stomata. For each stomate, we assessed the shape of the UWL (circular, oval, or rectangular), which is primarily a function of each stomate's length to width ratio and whether the outer lateral sides of the guard cells are rounded (Fig. 1A) or relatively straight (Fig. 1B). We also noted whether the polar ends of the guard cells

were round (Fig. 1A) or angular (Fig. 1B). To aid differentiation in morphologically similar stomata, we assessed differences in the angle of attachment of the UWL to the polar stem, but in general we found this trait difficult to measure accurately and to lack consistency within most species. Following Hansen (1995), we scored the length of lower woody lamellae (LWL), when present, as either notably longer (~5–10 μm) than the UWL and therefore clearly visible or only slightly longer (~1–2 μm) than the UWL and therefore of almost equal size and barely discernible in surface view. Finally, we counted the number of subsidiary cells present in the Florin ring of 90 additional *Thuja plicata*, *Chamaecyparis nootkatensis*, and *Taxus brevifolia* stomata.

Classification and regression tree (CART) analysis was used to build classification models, which are similar in form to dichotomous identification keys (Breiman et al., 1984). Classification trees consist of binary nodes that identify important splitting variables and threshold values. When splitting variables or thresholds are met, the left tree branch is followed; otherwise, the right branch is followed. Branches ultimately lead to terminal nodes that assign specific classification outcomes, in this case, assigning stomata to species or genus. Total model error is based on misclassification across all terminal nodes. Classification accuracy for individual taxa is based on the number of correctly classified stomata at each terminal node. In an attempt to devise an identification scheme for all 19 conifers, we first built a species-level classification tree. Model inputs included both continuous (UWL length and width, GC width, stem width) and categorical (UWL shape, shape of the polar ends and lateral sides of GC, length of the LWL relative to the UWL, and the presence and type of subsidiary cells) variables. We then built genus-level trees that ultimately provided the foundation for dichotomous identification keys. To produce an identification key for incomplete stomata, i.e., that lack subsidiary cells and LWL, a genus-level tree was built that excluded the presence/type of subsidiary cells and the relative length of the LWL as model inputs. CART analysis was performed using the 'rpart' package (Therneau et al., 2015) in R (R Core Team, 2014). To avoid over-fitting, we pruned the trees using cross-validation (Breiman et al., 1984), although in all cases, pruning did not trim any branch or terminal nodes, indicating that over-fitting was not a problem in our analyses. As a complement to CART analyses, random forest analysis was used as a secondary technique to confirm the main morphological characteristics for differentiating

stomata, using the same model inputs as in the CART analyses. Random forest analysis was conducted using the 'randomForest' package (Liaw and Wiener, 2014) in R.

3. Results and Discussion

3.1 Morphology of Conifer Stomata

The gross morphology of stomata including the overall shape of the UWL, and lateral sides and polar ends of the GC are consistent within each of the 19 conifer species. LWL are present in all species except *Taxus brevifolia*, *Chamaecyparis nootkatensis*, and *Thuja plicata*, which are instead characterized by the presence of four or more raised subsidiary cells that form a Florin ring around the guard cells. However, there is large variability within species and extensive overlap between species in all measured morphological traits (Table 1), precluding the use of mean values for stomata identification. At the level of individual stomata, the length and width of the UWL are positively correlated ($r = 0.76$, $p<0.001$). As would be expected, UWL width and GC width are also positively correlated ($r = 0.80$, $p<0.001$); on average, the width of the UWL is 2.6× the width of one guard cell. In general, the smallest stomata belong to *Larix occidentalis*, *T. brevifolia*, and members of the Cupressaceae family, and the largest stomata belong to *Pinus* spp. and *Picea* spp. Our results are in general agreement with previous studies (Hansen, 1995; Yu, 1997; Sweeney, 2004). We note important differences compared to these studies in morphological descriptions for each genus below.

Abies. *Abies amabilis*, *A. grandis* (Plate I, 1), and *A. lasiocarpa* stomata are rectangular in outline with guard cells that have relatively straight lateral sides and angular polar ends. LWL are 5–10 μm longer than the UWL, making the LWL readily discernible. On average, UWL in *Abies* stomata are 33 μm long and 25 μm wide, and the polar stem is 3 μm wide (Table 1). Stomata of the three *Abies* species are comparable in size and shape to those of *Abies alba* (Sweeney, 2004), but somewhat larger than *A. balsamea* (Hansen, 1995) and smaller than *A. nephrolepsis* (Wan et al., 2007). The stomata of *Abies* spp. in western North America are most readily confused with those of *Larix occidentalis* because of similar morphological traits and overlapping morphometry. Based on our specimens, *Abies* spp. and *L. occidentalis* stomata can only be reliably distinguished when the UWL are relatively large (>35 μm long and >22 μm wide in *Abies*), or small (<26 μm long and <19 μm wide in *L. occidentalis*). Sweeney (2004) also

noted the difficulty in differentiating *A. alba* stomata from those of *Larix sibirica* and ultimately used a difference in the angle at which the UWL meets the polar stem to separate these two species. We did not find any consistent difference in this angle between *L. occidentalis* and the three *Abies* species we examined.

Larix. As in *Abies* spp., the stomata of *Larix occidentalis* (Plate I, 2) are rectangular in outline with guard cells that have relatively straight lateral sides and angular polar ends. LWL are longer than the UWL, typically by 5–6 μm, and often notably wider as well. On average, UWL are 29 μm long and 19 μm wide, and the polar stem tends to be 2–3 μm wide. *Larix occidentalis* stomata are similar to those of *L. decidua* (Trautmann, 1953), *L. sibirica* (Sweeney, 2004; Clayden et al., 1996), and *L. laricina*, although the UWL in *L. occidentalis* are, on average, shorter than in the other three species of *Larix*. Hansen (1995) indicates that the LWL are barely visible in *L. laricina*, which contrasts with clearly visible and relatively long LWL in *L. decidua*, *L. sibirica*, and *L. occidentalis*. In general, *L. occidentalis* stomata cannot be distinguished from those of the three *Abies* spp. we examined, except when the UWL are <26 μm long and <19 μm wide. As noted by Trautmann (1953) and Hansen (1995) for *L. decidua* and *L. laricina*, respectively, *L. occidentalis* stomata appear relatively delicate and transparent compared to the stomata of all other conifers including *Abies*.

Picea. *Picea glauca* (Plate I, 12), *P. mariana*, and *P. sitchensis* have stomata that are, overall, quite similar to each other but distinct from other conifers. *Picea* stomata are oval in outline with guard cells that have rounded lateral sides and polar ends. LWL are only slightly longer than the UWL (<2 μm), making the LWL difficult to discern even at 630× magnification. Across the three *Picea* species, UWL are 40 μm long and 32 μm wide, on average. The polar stems of most *Picea* stomata are relatively wide (~4–6 μm). Hansen (1995) reports similar morphology for *P. glauca* stomata. On average, stomata are longer and wider in our *P. mariana* specimens compared to those in Hansen (1995), although there is overlap between our studies for both dimensions. The stomata of North American spruces are comparable to *Picea abies* (Sweeney, 2004) as well as spruce species in northwestern China (Wan et al., 2007). *Picea* stomata are similar in size and shape to *Tsuga* stomata; however, *Picea* stomata tend to be somewhat larger (Table 1) and are consistently more oval. In surface view, the UWL of *Picea* stomata appear

almost completely attached or flush with the polar stem (Plate I, 12) due to a small angle of attachment (this study; Hansen, 1995; Sweeney, 2004), which is not the case in *Tsuga*, which has UWL that are clearly separated from the polar stem (Plate I, 10 and 11) due to a more obtuse angle of attachment.

Pinus. Pine stomata are rectangular in outline with UWL that are, on average, 42 µm long and 30 µm wide. LWL are 5–10 µm longer than the UWL and therefore clearly visible (Plate I, 6), and polar stems are 4–8 µm wide. The border of the medial lamellae often appears thickened in *Pinus* stomata and was up to 6 µm wide in our specimens. A wide medial lamellae border has also been noted in other pine species (Trautman, 1953; Sweeney, 2004; Álvarez et al., 2014). Based on our results, the stomata of *Pinus albicaulis*, *P. contorta* var. *contorta*, *P. monticola* and *P. ponderosa* are more or less indistinguishable, and the stomata of diploxylon pines (*P. contorta* var. *contorta*, *P. ponderosa*) cannot be differentiated from those of haploxylon pines (*P. albicaulis*, *P. monticola*). In general, the morphological characteristics of the four *Pinus* species are similar to those of a number of other pine species (Sweeney, 2004; Wan et al., 2007, Álvarez et al., 2014), including *Pinus banksiana* (Hansen, 1995), which is found east of the Rocky Mountains in North America. Hansen (1995) reports longer UWL in *Pinus contorta* var. *murrayana* (44–63 µm) compared to our *P. contorta* var. *contorta* specimens (34–50 µm; Table 1).

Pseudotsuga. *Pseudotsuga menziesii* stomata (Plate I, 3) are rectangular in outline and most UWL are 27–33 µm long and 20–26 µm wide. LWL are typically 4–5 µm longer than the UWL. Polar stems are broad (4–6 µm), particularly in relation to the overall size of the stomata. *Pseudotsuga* stomata are similar in overall morphology to *Pinus* stomata, but the UWL and LWL are consistently shorter than in *Pinus* spp. and the border of the medial lamellae is rarely >3 µm wide, allowing these two stomata types to be differentiated. *Pseudotsuga* stomata are similar in size and shape to those of *L. occidentalis* and *Abies* spp., but can be differentiated from those taxa, in most cases, based on a wider polar stem. LWL are also shorter in *P. menziesii* than in *Abies* species, and *Pseudotsuga* stomata are usually more robust in overall appearance compared to the thin, delicate stomata of *Larix* (this study; Trautmann, 1953; Hansen, 1995).

Tsuga. *Tsuga heterophylla* (Plate I, 10) and *T. mertensiana* (Plate I, 11) stomata are similar in morphology and more or less indistinguishable. *Tsuga* stomata range in shape from rectangular to more oval with guard cells having more or less straight lateral sides but rounded polar ends. LWL are only slightly longer than the UWL and barely visible in surface view. This combination of morphological traits makes *Tsuga* stomata intermediate in morphology between *Picea* and most other Pinaceae. On average, UWL in *Tsuga* are 35 μm long and 25 μm wide. Only two *Tsuga* stomata had UWL <30 μm long and <22 μm wide, both of which were *T. heterophylla*. Polar stems are typically 3–4 μm wide and though significantly wider in *T. heterophylla* than *T. mertensiana* ($t = 7.52$, $p<0.0001$), the difference in stem width is only 1.4 μm, on average (Table 1), which is insufficient for consistently differentiating the two *Tsuga* species. The stomatal complex in *Tsuga* is characterized by the presence of four non-lignified subsidiary cells that sit on the lower cuticle surface, i.e., two large subsidiary cells immediately adjacent to and often longer than the guard cells (see Plate I, 10) and two small polar cells that are shared with stomata positioned above and below in stomatal rows. These subsidiary cells are present when stomata remain in sheets of epidermal tissue and are typically absent in stomata that are disassociated completely from epidermal tissue, as is often the case in fossil stomata. Florin (1931) documented these subsidiary cells in *T. mertensiana* and Kouwenberg et al. (2003) noted their presence in *T. heterophylla*. Similar encircling cells are apparently present in the stomatal complexes of other conifers (Florin, 1931), but these were exceptionally clear in our *Tsuga* specimens and not in those of any other conifers. Our results for *T. mertensiana* are in agreement with those of Hansen (1995), in terms of UWL size and shape, relative length of the LWL, and width of the polar stem. However, the stomata of the three *T. heterophylla* individuals we examined bear little resemblance to the stomata of the one *T. heterophylla* individual described by Hansen (1995). There is overlap in the morphological measurements for *T. heterophylla* between our two studies, but in general, our *T. heterophylla* specimens have somewhat longer and narrower UWL, making them more similar to *T. mertensiana* in size and shape. Also, Hansen (1995) reports a stem width of 8 μm for *T. heterophylla*, which is substantially wider than in our *T. heterophylla* specimens. Furthermore, Hansen (1995) describes *T. heterophylla* as having a Florin ring composed of five lignified subsidiary cells bordering the guard cells, a morphology that is typical of *Taxus*, *Chamaecyparis*, and *Thuja* stomata (this study; Hansen, 1995; Sweeney, 2004). However, none of our *T. heterophylla* specimens had a Florin ring of

lignified subsidiary cells, nor did any of the fossil *Tsuga* stomata we identified in Holocene pollen samples from coastal British Columbia (Lacourse et al., 2012). According to Parshall (1999), *Tsuga canadensis* stomata in pollen samples also do not have a Florin ring of lignified subsidiary cells.

Chamaecyparis* and *Thuja. The stomata of *Chamaecyparis nootkatensis* (syn. *Callitropsis nootkatensis*) are oval in outline with UWL that are 31 μm long and 23 μm wide, on average. Only one *C. nootkatensis* had UWL >34 um long and >27 μm wide. Polar stems are 3–4 μm wide. Hansen (1995) reports nearly identical values for this species. *Chamaecyparis* stomata are characterized by a Florin ring, typically consisting of five to eight lignified subsidiary cells that are more oblong than circular and appear in surface view to surround and partially obscure the guard cells. Approximately half (53%) of the 90 *Chamaecyparis* stomata we examined had five to seven subsidiary cells, 4% had eight cells, and 2% had four cells. In the remaining 40%, the cell walls between adjacent subsidiary cells were poorly defined, making the Florin ring appear as one large more or less continuous ring. Hansen (1995) reports that the stomatal complex in *C. nootkatensis* has 6–10 subsidiary cells, but 22% of our specimens had four or five cells and none had more than eight.

Thuja plicata stomata (Plate I, 7 and 8) are circular to oval in outline and are among the smallest of any conifer. UWL are 26 μm long and 22 μm wide, on average, and polar stems are typically 2–3 μm wide (Table 1). Hansen (1995) and Yu (1997) report similar values for *T. plicata* and *Thuja occidentalis*. A Florin ring typically consisting of five to eight lignified subsidiary cells similar in morphology to that of *C. nootkatensis* is present. Of the 90 *Thuja* stomata we examined, 66% had five to seven subsidiary cells, 9% had eight cells, and 2% had either four or nine cells. In 23%, the cell walls between adjacent cells were poorly defined. Hansen (1995) reports that *Thuja* stomata have four to six subsidiary cells, but approximately one-third (29%) of our specimens had seven to nine cells.

We found that *C. nootkatensis* stomata cannot be differentiated from those of *T. plicata* in many instances due to overlapping morphologies. The UWL of *Thuja* stomata are shorter on average, although not more narrow (Table 1), making *Thuja* stomata somewhat more circular in outline

compared to *Chamaecyparis*. Hansen (1995) differentiates *Chamaecyparis* from *Thuja* based on a higher number of lignified subsidiary cells and longer mean UWL length, but our results do not support this distinction. In our specimens, *C. nootkatensis* and *T. plicata* have more or less the same number of subsidiary cells and the length of the UWL overlaps greatly (Table 1). Based on our results as well as Hansen (1995), *Chamaecyparis* and *Thuja* stomata can only be distinguished at the extremes of their size distributions, i.e., when UWL length is >30 μm (cf. *Chamaecyparis*) or <24 μm (cf. *Thuja*).

Juniperus. *Juniperus communis* (Plate I, 9) and *J. scopulorum* stomata are indistinguishable from each other, but have a combination of morphological characteristics that allow them to be readily differentiated from other taxa. Juniper stomata are rectangular in shape with guard cells that have rounded polar ends. LWL are only slightly longer than the UWL (by ~2 μm), and polar stems are narrow (~2 μm). Juniper stomata are smaller than those of most other genera: on average, UWL are 29 μm long and 18 μm wide. These dimensions are more or less in agreement with Sweeney (2004) for *J. communis* and with Yu (1997) for *J. communis*, *J. horizontalis* and *J. virginiana*. UWL are somewhat longer in *J. rigida*, although not wider (Wan et al., 1997). Unlike other Cupressaceae, *Juniperus* stomata in pollen samples do not typically retain their Florin rings (this study; Yu, 1997; Sweeney, 2004). We observed vestigial Florin rings in *J. scopulorum*, but only in a few stomata from two of the individuals we examined. Kvacek (2002) notes that weakly cutinised Florin rings are diagnostic epidermal features of scale-leaf junipers (*Juniperus* sect. *Sabina*); however, in pollen samples, it does not appear possible to differentiate the stomata of needle-leaf (*J. communis*) and scale-leaf (*J. scopulorum*) junipers based on this or other morphological features.

Taxus. *Taxus brevifolia* stomata (Plate I, 4 and 5) are circular to oval with UWL that are 30 μm long and 25 μm wide, on average. Polar stems are wide (4–5 μm) relative to the overall size of the guard cells; only one *T. brevifolia* stomata had a stem <4 μm wide. The Florin ring in *Taxus* consists of four to six subsidiary cells that often completely obscure the guard cells (Plate I, 5). Of the 90 *T. brevifolia* stomata we examined, 67% had four subsidiary cells, 24% had five cells, and 9% had six cells. Subsidiary cells in *Taxus* are strongly lignified and tightly clustered, and are typically circular in shape, although one or more of the cells may be lobate, e.g., the lower

left cell in Plate I, 5. (We refer to this Florin ring morphology as Type 1 in Fig. 2A.) *Taxus brevifolia* stomata are similar to those of *Taxus baccata*, although Sweeney (2004) reports notably longer, although not wider, UWL in *T. baccata*. The surface of the leaf cuticle in *Taxus* is strongly papillose (Ghimire et al., 2014), even on the non-specialized epidermal cells (Plate I, 5), which can be useful in identifying *Taxus* stomata if preserved in sheets of epidermal tissue. Because of their overall size and shape, *Taxus* stomata are most similar to those of *C. nootkatensis* and *Thuja plicata*, but can be differentiated based on their subsidiary cell morphology, slightly thicker polar stem, and densely papillose cuticle. *Taxus brevifolia* stomata are also generally larger than those of *Thuja plicata* and more circular than those of *C. nootkatensis*.

3.2 Classification Trees

CART analyses provide multi-trait classification criteria that allow the stomata of most taxa to be differentiated. The species-level CART (Supplementary Figure 1) places congeneric species into adjacent terminal nodes, highlighting that stomatal morphology is relatively consistent within genera. However, the species-level CART has a high misclassification rate (model error = 38.3%) and even higher cross-validation error (53.3%), indicating that accurate species-level identifications are not possible. Furthermore, the stomata of four species (*Abies lasiocarpa*, *Juniperus scopulorum*, *Picea glauca*, *Pinus contorta* var. *contorta*) are completely misclassified as belonging to other species, albeit usually of the same genus (Supplementary Table 2). For example, all *J. scopulorum* stomata are misclassified as *J. communis*, and all *P. contorta* var. *contorta* stomata are misclassified as belonging to one of the other three species of *Pinus*. In addition, while the species-level CART succeeds in classifying all *Chamaecyparis nootkatensis* stomata as such, 35% of *Thuja plicata* stomata are also classified as *C. nootkatensis*. Similarly, all *Tsuga mertensiana* are classified accurately, but 40% of *T. heterophylla* are misclassified as *T. mertensiana*. Because of the poor classification performance of the species-level model, we grouped congeneric species together as well as *T. plicata* and *C. nootkatensis* and built genus-level classification trees.

The genus-level classification tree (Fig. 2A) is successful in classifying stomata accurately: the misclassification rate is only 8.1% and cross-validation error is 10.7%. At the genus-level,

morphology is relatively stable and sufficiently unique to permit identification to genus in most instances. Classification accuracies for individual genera are generally high: the genus-level tree classifies all genera, with the exception of *Larix* and *Pseudotsuga*, with greater than 89% accuracy (Table 2A). About 47% of *Larix* stomata and 20% of *Pseudotsuga* stomata are misclassified as belonging to *Abies*, reflecting the similar morphology of these taxa. As with the species-level CART, this genus-level model begins by separating genera with LWL that are much longer than the UWL from those lacking this trait, and then uses stem width and the presence of subsidiary cells as secondary criteria (Fig. 2A). Subsequent branches classify stomata based primarily on the size and shape of the UWL and the type of subsidiary cells. The morphological criteria identified by CART as important in classifying stomata to genus are similar to those identified by random forest analysis (Supplementary Table 3), with relative LWL length, stem width, and subsidiary cell type ranked as the three most important traits for distinguishing conifer stomata.

To aid in the identification of incomplete stomata, a genus-level classification tree that excluded the presence/type of subsidiary cells and relative LWL length as model inputs was built (Fig. 2B). This classification model performs reasonably well: total misclassification is 15.6% and cross-validation error is 18.4%. Most genera are classified with 70 to 100% accuracy, but classification accuracy is relatively low for *Larix* and *Pseudotsuga*, with 53% and 35%, respectively, misclassified as *Abies* (Table 2B). Because this model was built without information on subsidiary cells and relative LWL length, it has a fundamentally different structure: it begins by separating genera based on UWL shape (i.e., oval to circular or rectangular) and then uses UWL length and stem width as secondary criteria (Fig. 2B). Random forest analysis (Supplementary Table 3) supports these results: in cases where subsidiary cells and LWL are absent, the most important traits for distinguishing stomata to the genus level are UWL length, stem width and UWL shape.

3.3 Stomata Identification Keys

We used the two genus-level classification trees (Fig. 2) to provide the backbone for two dichotomous identification keys – one that is suited for identifying stomata that are complete (Key A) and another that is designed for identifying stomata that lack LWL and subsidiary cells

(Key B). Classification trees use splits that are based on a single variable at each node, but we have also included additional morphological criteria (e.g., surrogate splitting variables identified by CART analyses) in the identification keys. Furthermore, classification trees are built to categorize the exact cases that are used as model input (i.e., individual stomata in this case) and therefore classification trees cannot consider all potential cases. Thus, although the overall structures of our identification keys mirror the structure of the classification trees, our keys are more conservative in some instances, in order to reflect the overlapping morphological variability present in conifer stomata. For example, *Abies* and *Larix* stomata are grouped together in both identification keys, as are *Thuja* and *Chamaecyparis*, to reflect the fact that the stomata of these genera were indistinguishable in many cases. We provide morphological criteria for separating these genera, where possible, as footnotes to each key. *Tsuga* appears twice in Key A because both LWL and subsidiary cells were present in our *Tsuga* specimens.

Our CART-based stomata identification keys share much in common, in terms of important morphological criteria and overall structure, with other identification keys (Trautmann, 1953; Hansen, 1995; Sweeney, 2004). As in our identification key for complete stomata (Key A), Hansen's (1995) key for North American conifers begins by separating stomata based on whether LWL are readily discernible or whether lignified subsidiary cells are present. This is followed first by relative LWL length and stem width, and then by UWL length and shape to further separate stomata types. Our morphological measurements, classification trees and random forests results confirm these to be important morphological criteria. One noteworthy difference is Hansen's (1995) use of the angle at which the polar stem meets the UWL to help distinguish *Pinus* from *Abies* and *Larix laricina* from *Tsuga mertensiana*, respectively. We did not find consistent differences in this angle between most species, and it only appears once in our key, as one of four morphological criteria to differentiate *Picea* and *Tsuga* stomata. Sweeney's (2004) key for European conifers uses similar dichotomies and morphological criteria as in our key and in Hansen (1995), although ratios of various dimensions are used in place of absolute size in some instances.

Fossil stomata are often incomplete with subsidiary cells and lower woody lamellae only partially preserved or entirely missing. The classification tree for this situation (Fig. 2B) is fairly

successful with a misclassification rate of only 15.6%. The identification key for incomplete stomata (Key B) is inherently more subjective than the key for complete stomata (Key A) because the primary dichotomy is based on the overall shape of the UWL, i.e., whether stomata are oval to circular or rectangular. In some instances, it can be difficult to assess stomatal shape on pollen slides, e.g., if stomata are not lying perfectly flat or are partially obscured, or if the two halves are asymmetrical. Given this as well as the large intraspecific variability and degree of interspecific overlap in the morphology of conifer stomata (Table 1), we recommend that incomplete stomata be given a '-type' designation or a prefatory *cf.* to indicate that identification is uncertain. This is particularly important in regions such as western North America, where there are many different conifers that could potentially contribute stomata to sedimentary archives.

4. Conclusions

Based on our research, species-level identification of conifer stomata is generally not possible; morphological variability within species and the degree of overlap among species precludes reliable identification to the species level. However, stomatal morphology is relatively consistent within genera and sufficiently unique to permit identification to genus. CART analyses provide robust multi-trait classification models for distinguishing the stomata of conifer genera in western North America in most cases. Because both categorical and continuous variables can be included, CART analysis offers a particularly useful statistical approach for identifying important morphological criteria and the resulting classification trees can be easily adapted into dichotomous identification keys. The morphological descriptions and identification keys presented here expand on previous efforts to differentiate conifer stomata in pollen samples, by including more species and more individuals per species. Accordingly, morphological variability within species and genera is better represented than in previous studies based on stomata from only one individual per species. However, in order to confirm the limits of taxonomic differentiation, further study of stomatal morphology with larger sample sizes is needed, especially in taxa such as *Pinus* and *Picea* that have large morphological variability.

The stomata identification keys presented here should aid efforts to differentiate conifer stomata in fossil pollen samples from western North America. In turn, this should strengthen

paleoecological records from the region by providing evidence of local conifer presence and, in some instances, by increasing taxonomic resolution. The identification keys should be used in conjunction with stomata reference material, particularly for visual calibration of subtle differences in shape and subsidiary cell morphology. Given the morphological variability that is present within species and the degree of morphological overlap among species, stomata reference collections should include material from more than one individual per species. Stomatal frequency has been shown to vary spatially and temporally across climatic gradients (Kouwenberg et al., 2003), but whether overall morphology also varies geographically requires further study. To account for any potential regional intraspecific differences in morphology, reference collections should also include individuals from across species ranges. Since the stomatal morphology of congeneric conifers is similar and our morphological measurements and identification keys are in overall agreement with studies from other regions (Trautmann, 1953; Hansen, 1995; Yu, 1997; Sweeney, 2004), the identification keys presented here may also be helpful outside of western North America. However, in that instance, we recommend testing the identification keys against known local reference material prior to using them in paleoecological studies.

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633 **Tables**

634 Table 1: Summary of the morphological measurements of the stomata of each conifer species. *N*
635 = number of stomata/species.

636

637 Table 2: Classification accuracies (%) for classification trees (Fig. 2) that form the bases for the
638 conifer stomata identification keys.

639

640 **Figure Captions**

641 Figure 1: Simplified conifer stomata showing measured morphological features. A. *Picea*-type
642 oval stomata with a wide stem, lower woody lamella (LWL) that is only slightly larger than the
643 upper woody lamella (UWL), and guard cells (GC) with rounded polar ends. B. *Abies*-type
644 rectangular stomata with a narrow stem, LWL that is much longer than the UWL, and GC with
645 angular polar ends. ML = medial lamella. Morphological terminology follows Trautmann (1953),
646 Hansen (1995), and Sweeney (2004).

647

648 Figure 2: Genus-level decision trees for classification of conifer stomata, built with (A) and
649 without (B) information on the lower woody lamellae and subsidiary cells as model inputs.
650 Terminal nodes indicate genus classification. *Tsuga*SC refers to *Tsuga* stomata with non-
651 lignified lateral subsidiary cells. All measured traits are in µm. See Section 3.1 (*Taxus*) for
652 description of Type 1 Florin ring. LWL = lower woody lamellae; UWL = upper woody lamellae.

653

654 Plate I: Surface views (630×) of conifer stomata from reference material prepared using
655 palynological techniques and mounted in silicone oil. 1. *Abies grandis*, 2. *Larix occidentalis*, 3.
656 *Pseudotsuga menziesii*, 4. *Taxus brevifolia* with guard cells in focus, 5. *Taxus brevifolia* with
657 Florin ring of five lignified subsidiary cells in focus, 6. *Pinus contorta* var. *contorta*, 7. *Thuja*
658 *plicata* with guard cells in focus, 8. *Thuja plicata* with Florin ring of six lignified subsidiary cells
659 in focus, 9. *Juniperus communis*, 10. *Tsuga heterophylla* with focus on two large non-lignified
660 lateral subsidiary cells on either side of the guard cells, 11. *Tsuga mertensiana*, 12. *Picea glauca*.
661 Note relative changes in the length of each 20 µm scale bar.

662

663 Key A: Identification Key for Conifer Stomata in Western North America

Key B: Identification Key for Incomplete Conifer Stomata in Western North America

Supplementary Material

Supplementary Table 1: Details on voucher specimens used for morphometry of conifer stomata in this study.

Supplementary Table 2: Classification accuracies (%) for the species-level classification tree (Supplementary Fig. 1). Species abbreviations consist of the first two letters of the genus and the first two letters of the specific epithet e.g., ABAM = *Abies amabilis*. Refer to Table 2 of the main text for a complete list of species.

Supplementary Table 3: Results of random forest analysis: mean Gini decrease and ranked morphological trait importance for the two genus-level stomata classification models. LWL = lower woody lamellae; UWL = upper woody lamellae; SC = subsidiary cell; GC = guard cell.

Supplementary Figure 1: Species-level classification tree for conifer stomata in western North America. Terminal nodes indicate species classification. Species abbreviations at terminal nodes consist of the first two letters of the genus and the first two letters of the specific epithet e.g., LAOC = *Larix occidentalis*. TSHEsc/TSMEsc refer to *Tsuga heterophylla*/*Tsuga mertensiana* with non-lignified lateral subsidiary cells. Refer to Table 2 of the main text for a complete list of species. Note that this tree has high misclassification (38.3%) and cross-validation errors (53.3%). *Abies lasiocarpa*, *Juniperus scopulorum*, *Picea glauca*, and *Pinus contorta* var. *contorta* lack terminal nodes because all stomata of these species are misclassified (see Supplementary Table 2). All measured traits are in µm. See Section 3.1 (*Taxus*) of the main text for description of Type 1 Florin ring. LWL = lower woody lamellae; UWL = upper woody lamellae.

Table 1: Summary of the morphological measurements of the stomata of each conifer species. *N* = number of stomata/species.

Species[a] (No. of individuals examined)	**Upper Woody Lamellae Length (µm)** Mean ± SD	Range	**Upper Woody Lamellae Width (µm)** Mean ± SD	Range	**Guard Cell Width (µm)** Mean ± SD	Range	**Stem Width (µm)** Mean ± SD	Range	***N***
Abies amabilis (3)	32.9 ± 3.4	25.6–36.8	25.1 ± 4.3	19.2–30.4	9.1 ± 1.9	6.4–12.8	2.9 ± 0.8	1.6–4.8	15
Abies grandis (3)	31.7 ± 4.2	27.2–40.8	23.3 ± 2.8	19.2–28.0	9.7 ± 2.4	6.4–14.4	2.7 ± 0.5	1.6–3.2	15
Abies lasiocarpa (3)	34.2 ± 3.5	27.2–40.0	26.8 ± 3.2	21.6–32.8	10.6 ± 1.8	8.0–12.8	3.1 ± 0.7	1.6–4.0	15
Chamaecyparis nootkatensis (4)	30.6 ± 2.9	24.0–35.2	22.8 ± 3.7	16.0–28.0	8.9 ± 1.5	6.4–12.8	3.4 ± 0.4	3.2–4.0	20
Juniperus communis (5)	29.1 ± 2.2	25.6–33.6	17.4 ± 1.8	14.4–19.2	6.4 ± 1.1	4.0–8.8	2.1 ± 0.6	1.6–3.2	25
Juniperus scopulorum (3)	28.1 ± 1.9	24.0–30.4	18.2 ± 2.0	14.4–20.8	7.5 ± 1.6	4.8–11.2	2.0 ± 0.4	1.6–2.4	15
Larix occidentalis (4)	28.8 ± 4.1	20.8–35.2	19.2 ± 2.3	16.0–22.4	7.6 ± 1.3	5.6–10.4	2.8 ± 0.6	1.6–4.0	15
Picea glauca (3)	42.2 ± 4.3	34.4–51.2	33.1 ± 4.2	27.2–42.4	12.4 ± 2.5	8.0–16.0	4.3 ± 1.2	3.2–6.4	15
Picea mariana (3)	40.0 ± 3.6	34.4–46.4	32.3 ± 3.1	28.0–38.4	11.9 ± 1.3	9.6–14.4	4.5 ± 0.9	3.2–6.4	15
Picea sitchensis (3)	36.6 ± 3.4	33.6–43.2	29.6 ± 4.4	24.0 –35.2	11.0 ± 2.7	6.4–14.4	3.8 ± 0.8	3.2–4.8	15
Pinus albicaulis (3)	41.3 ± 3.2	36.8–48.0	32.3 ± 3.8	27.2–38.4	11.5 ± 2.0	8.0–14.4	5.8 ± 0.8	4.0–6.4	15
Pinus contorta var. *contorta* (4)	43.2 ± 4.0	34.4–49.6	30.6 ± 4.2	24.0–38.4	11.8 ± 2.4	7.2–16.0	6.0 ± 0.9	4.8–8.0	20
Pinus monticola (3)	40.1 ± 4.5	33.6–48.0	26.7 ± 3.4	20.0–32.0	10.3 ± 1.8	8.0–12.8	5.5 ± 0.9	4.0–6.4	15
Pinus ponderosa (3)	44.2 ± 6.9	36.8–56.0	28.0 ± 5.1	23.2–37.6	11.7 ± 1.8	9.6–16.0	5.4 ± 0.7	4.8–6.4	15
Pseudotsuga menziesii (4)	30.1 ± 3.3	24.0–35.2	22.9 ± 2.7	19.2–28.8	9.5 ± 1.4	8.0–12.8	4.9 ± 1.0	3.2–6.4	20
Taxus brevifolia (3)	29.6 ± 3.2	24.0–33.6	24.6 ± 2.8	20.8–30.4	9.9 ± 1.9	7.2–14.4	4.2 ± 0.6	3.2–4.8	15
Thuja plicata (4)	25.9 ± 3.2	19.2–30.4	21.5 ± 2.8	16.0–27.2	9.4 ± 1.9	6.4–12.8	2.4 ± 0.7	1.6–3.2	20
Tsuga heterophylla (3)	34.0 ± 3.9	27.2–39.2	24.1 ± 2.7	19.2–30.4	9.4 ± 1.1	8.0–12.0	4.2 ± 0.6	3.2–4.8	15
Tsuga mertensiana (3)	35.7 ± 3.3	30.4–40.0	26.4 ± 2.6	22.4–30.4	9.9 ± 1.2	7.2–11.2	2.8 ± 0.4	2.4–3.2	15

[a] Botanical nomenclature follows the Flora of North America Editorial Committee (1993). *Chamaecyparis nootkatensis* = *Callitropsis nootkatensis*

Table 2: Classification accuracies (%) for classification trees (Fig. 2) that form the bases for the conifer stomata identification keys.

A. Genus-level CART (Fig. 2A) – Total model error: 8.1%

	Abies	***Juniperus***	***Larix***	***Picea***	***Pinus***	***Pseudotsuga***	***Taxus***	***Thuja/ Chamaecyparis***	***Tsuga***	***Tsuga*SC**[a]
Classified As										
Abies	88.9	–	46.7	–	–	20.0	–	–	–	–
Juniperus	–	100	–	–	–	–	–	–	6.7	–
Larix	–	–	46.7	–	–	–	–	–	–	–
Picea	–	–	–	93.3	–	–	–	–	–	–
Pinus	2.2	–	–	–	92.3	5.0	–	–	–	–
Pseudotsuga	8.9	–	6.7	–	7.7	75.0	–	–	–	–
Taxus	–	–	–	–	–	–	100	–	–	–
Thuja/Chamaecyparis	–	–	–	–	–	–	–	100	–	–
Tsuga	–	–	–	6.7	–	–	–	–	93.3	–
*Tsuga*SC	–	–	–	–	–	–	–	–	–	100

B. Genus-level CART with LWL and SC data excluded (Fig. 2B) – Total model error: 15.6%

	Abies	***Juniperus***	***Larix***	***Picea***	***Pinus***	***Pseudotsuga***	***Taxus***	***Thuja/ Chamaecyparis***	***Tsuga***
Classified As									
Abies	97.8	–	53.3	–	4.6	35.0	–	–	3.3
Juniperus	–	100	–	–	–	–	–	–	6.7
Larix	–	–	46.7	–	–	–	–	–	–
Picea	–	–	–	82.2	–	–	–	2.5	–
Pinus	–	–	–	2.2	93.8	5.0	–	–	20.0
Pseudotsuga	2.2	–	–	–	–	55.0	–	–	–
Taxus	–	–	–	4.4	–	–	80.0	15.0	–
Thuja/Chamaecyparis	–	–	–	6.7	–	–	20.0	82.5	–
Tsuga	–	–	–	4.4	1.5	5.0	–	–	70.0

[a] *Tsuga*SC refers to *Tsuga* stomata with lateral and polar non-lignified subsidiary cells (SC).

Figure 1

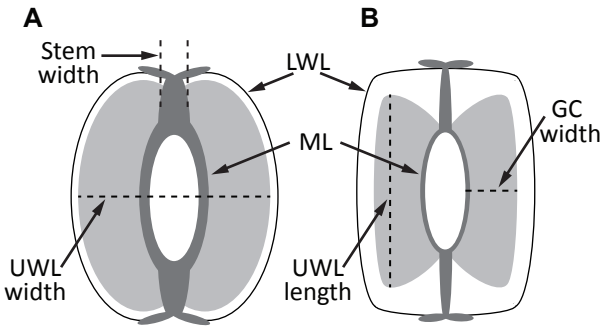
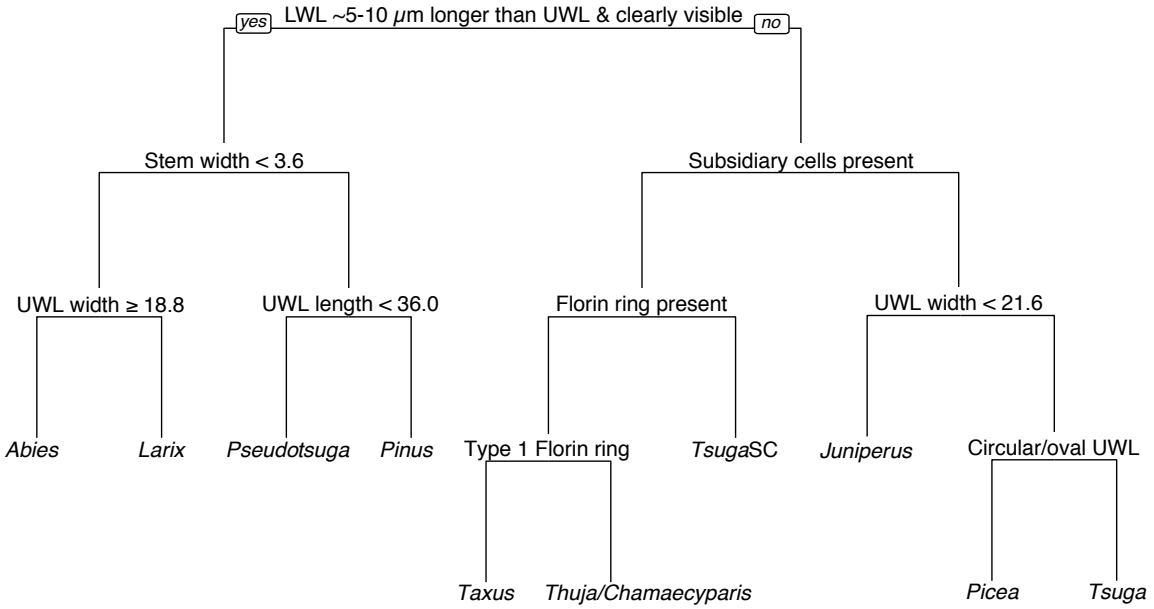
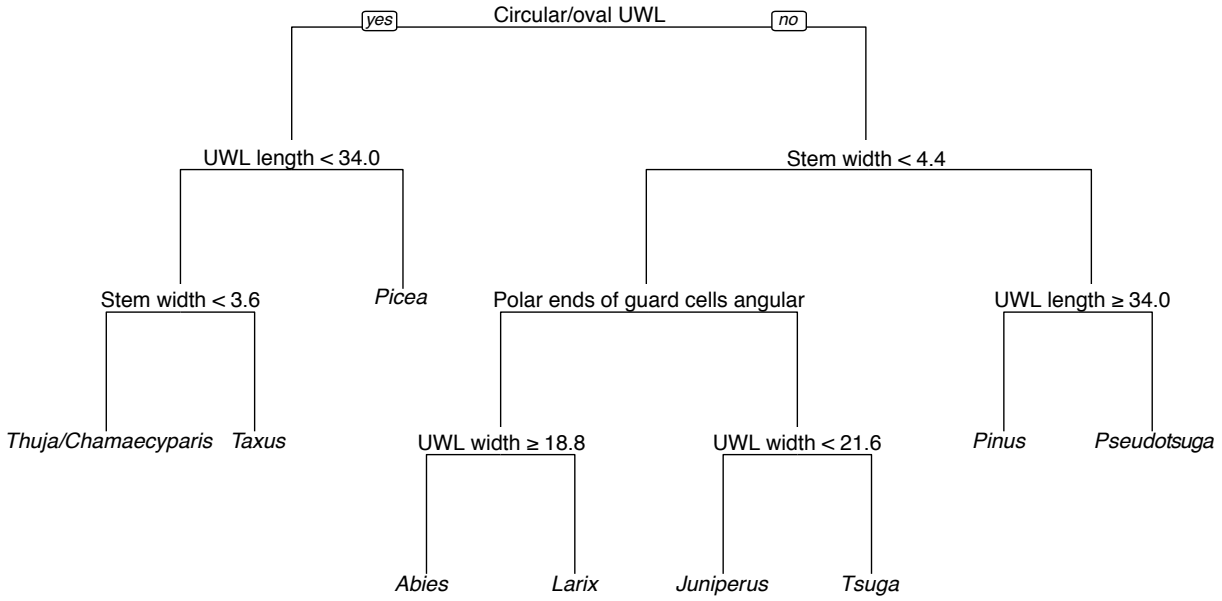


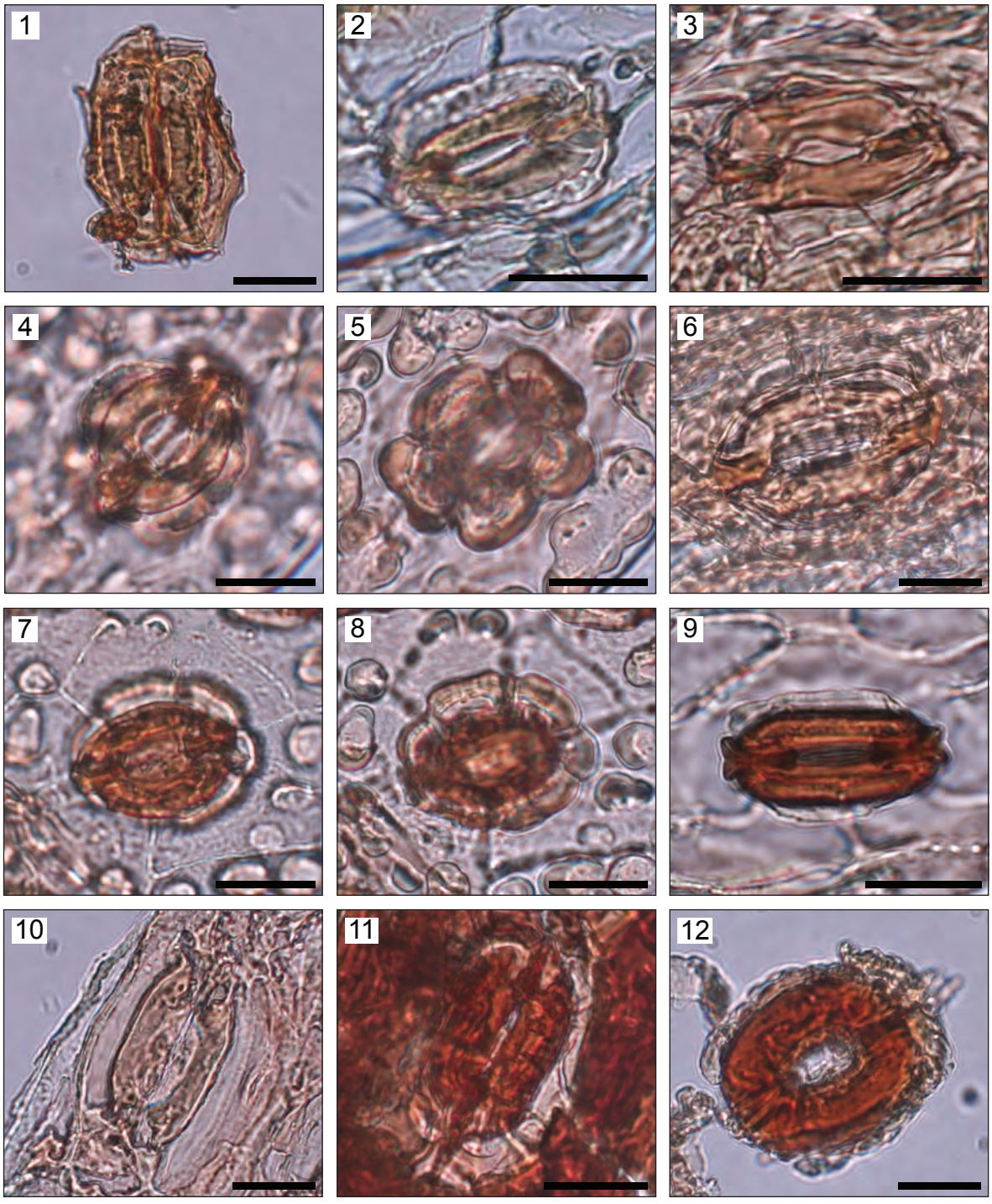
Figure 2

A Genus-level CART (error = 8.1%)



B Genus-level CART with LWL and SC traits excluded (error = 15.6%)

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Key A: Identification Key for Conifer Stomata in Western North America

1a. Lower woody lamellae visible; stomatal complex consists of two guard cells .....…2
1b. Lower woody lamellae not visible; stomatal complex consists of two guard cells and subsidiary epidermal cells or a raised Florin ring composed of four or more lignified cells ……..……7

2a. Lower woody lamellae ~5–10 µm longer than upper woody lamellae and clearly visible at polar ends and beyond lateral sides of guard cells (Fig. 1B) ……3
2b. Lower woody lamellae similar in size to upper woody lamellae and barely visible in surface view (Fig. 1A) ..……..……5

3a. Stem narrow (<4 µm); polar ends of guard cells angular; upper woody lamellae 20–40 µm long (Fig. 1B, Plate I, 1 and 2) ……..*Abies*/*Larix*[1]
3b. Stem wide (>4 µm); polar ends of guard cells angular or rounded; upper woody lamellae 24–56 µm long …..4

4a. Upper woody lamellae >35 µm long and 20–40 µm wide; medial lamellae border often thickened (up to 6 µm wide) (Plate I, 6) ……..*Pinus*
4b. Upper woody lamellae <35 µm long and <29 µm wide; medial lamellae border usually narrow (≤3 µm) (Plate I, 3) ……..…*Pseudotsuga menziesii*

5a. Upper woody lamellae <21 µm wide and 24–34 µm long; stem 1–3 µm wide (Plate I, 9) ..……..….*Juniperus*
5b. Upper woody lamellae >21 µm wide and typically >28 µm long; stem ≥2.5 µm wide ……...…6

6b. Upper woody lamellae oval to circular in outline, typically 34–52 µm long and 24–42 µm wide, and appearing attached to stem at poles due to acute angle of attachment (Fig. 1A, Plate I, 12) ..……...…*Picea*
6a. Upper woody lamellae more rectangular in outline, typically 28–40 µm long and ≤30 µm wide, and clearly separated from stem at poles due to obtuse angle of attachment (Plate I, 11) ……..*Tsuga*

7a. Subsidiary cells consist of two large non-lignified lateral cells (Plate I, 10) and often two smaller polar cells; upper woody lamellae mostly rectangular in outline and typically >28 µm long (Plate I, 11) ..……...*Tsuga*
7b. Subsidiary cells consist of a raised Florin ring of four to eight circular to elongated lignified cells; upper woody lamellae circular to oval in outline and typically <35 µm long ……8

8a. Florin ring of four to six tightly-clustered, lignified subsidiary cells (Plate I, 5) with well-defined cell walls and typically circular in shape, at times lobate; upper woody lamellae 24–34 µm long and 20–30 µm wide, but often obscured by Florin ring; stem typically 4–5 µm wide (Plate I, 4) ……..*Taxus brevifolia*
8b. Florin ring of typically five to eight elongated lignified subsidiary cells (Plate I, 8), often with poorly-defined cell walls between adjacent cells; Florin ring cells appear less dense than in *Taxus* and appear to form a ring surrounding the upper woody lamellae (Plate I, 7); upper woody lamellae 19–35 µm long and 16–28 µm wide; stem 1–4 µm wide ……*Thuja*/*Chamaecyparis*[2]

[1] If upper woody lamellae >22 µm wide and >35 µm long, then cf. *Abies*; if upper woody lamellae <19 µm wide and <26 µm long, then cf. *Larix*. *Abies* stomata are also more robust in overall appearance compared to the thin, delicate stomata typical of *Larix*.

[2] If upper woody lamellae <24 µm long, then cf. *Thuja plicata*; if upper woody lamellae >30 µm long, then cf. *Chamaecyparis nootkatensis*.

Key B: Identification Key for Incomplete Conifer Stomata in Western North America

1a. Upper woody lamellae rectangular in outline (Fig. 1B) ..……...…2
1b. Upper woody lamellae circular to oval in outline (Fig. 1A) ……..…6

2a. Stem wide (>4 µm) ……..……3
2b. Stem narrow (<4 µm) ……...……...…4

3a. Upper woody lamellae >35 µm long and 20–40 µm wide; medial lamellae border often thickened (up to 6 µm wide) (Plate I, 6) ……....*Pinus* type
3b. Upper woody lamellae <35 µm long and <29 µm wide; medial lamellae border usually narrow (≤3 µm) (Plate I, 3) ……*Pseudotsuga menziesii* type

4a. Polar ends of guard cells angular; outer lateral sides of guard cells straight (Fig. 1B, Plate I, 1 and 2) ……...*Abies*/*Larix* type[1]
4b. Polar ends of guard cells rounded (Fig. 1A); outer lateral sides of guard cells straight or rounded ……..…5

5a. Upper woody lamellae <21 µm wide and 24–34 µm long; stem 1–3 µm wide (Plate I, 9) …….*Juniperus* type
5b. Upper woody lamellae >21 µm wide and 28–40 µm long; stem ≥2.5 µm wide (Plate I, 10 and 11) ……*Tsuga* type

6a. Upper woody lamellae >34 µm long, 24–42 wide, and appearing attached to stem at poles due to acute angle of attachment; stem >3 µm wide (Fig. 1A, Plate I, 12) ..……..…*Picea* type
6b. Upper woody lamellae <34 µm long and 16–30 µm wide ……...……...…7

7a. Stem wide (>4 µm); upper woody lamellae 24–34 µm long and 20–30 µm wide (Plate I, 4 and 5) ……..……*Taxus brevifolia* type
7b. Stem narrow (<4 µm); upper woody lamellae 19–34 µm long and 16–28 µm wide (Plate I, 7 and 8) ……...…*Thuja*/*Chamaecyparis* type[2]

[1] If upper woody lamellae >22 µm wide and >35 µm long, then cf. *Abies*; if upper woody lamellae <19 µm wide and <26 µm long, then cf. *Larix*. *Abies* stomata are also more robust in overall appearance compared to the thin, delicate stomata typical of *Larix*.

[2] If upper woody lamellae <24 µm long, then cf. *Thuja plicata*; if upper woody lamellae >30 µm long, then cf. *Chamaecyparis nootkatensis*.

Supplementary Table 1: Details on voucher specimens used for morphometry of conifer stomata in this study.

Species	University of Victoria Herbarium Accession #	Sample Location as Noted on Herbarium Sheet[1]
Abies amabilis	16494	Hair Trigger Lake, Forbidden Plateau (central Vancouver Island, BC)
Abies amabilis	000819	Kitimat (north coastal BC)
Abies amabilis	000812	14 mi west of Port McNeil (northern Vancouver Island, BC)
Abies grandis	000817	North end of Galiano Island (southwestern BC)
Abies grandis	24809	Beaver Lake (southern Vancouver Island, BC)
Abies grandis	000822	Elk River area (central Vancouver Island, BC)
Abies lasiocarpa	000829	Green Mtn., west of Nanaimo (southern Vancouver Island, BC)
Abies lasiocarpa	000836	Mckee Creek Rd., Atlin (northwestern BC)
Abies lasiocarpa	40131	Mountain top of Ogilvie/Wernecke Mtn. (central Yukon)
Chamaecyparis nootkatensis[2]	000726	Green Mtn., west of Nanaimo (southern Vancouver Island, BC)
Chamaecyparis nootkatensis[2]	000734	Twin Peaks, near Port McNeil (northern Vancouver Island, BC)
Chamaecyparis nootkatensis[2]	20291	Slocan Valley (southeastern BC)
Chamaecyparis nootkatensis[2]	26459	Rennel Sound camp, Graham Island (Queen Charlotte Islands, BC)
Juniperus communis	19871	Comox Lake, near Comox (central Vancouver Island, BC)
Juniperus communis	000752	Port McNeil Rd., Port Hardy (northern Vancouver Island, BC)
Juniperus communis	029038	Waterton National Park (southwestern AB)
Juniperus communis	16285	Green Mtn., west of Nanaimo (southern Vancouver Island, BC)
Juniperus communis	029471	McQueen Lake, Kamloops (south-central BC)
Juniperus scopulorum	26146	Near Beaver Point, Salt Spring Island (southwestern BC)
Juniperus scopulorum	25721	10 mi west of Telegraph Creek, Stikine River (northwestern BC)
Juniperus scopulorum[3]	–	Black Hills (western SD)
Larix occidentalis	000844	Yahk (southeastern BC)

Species	University of Victoria Herbarium Accession #	Sample Location as Noted on Herbarium Sheet[1]
Larix occidentalis	000845	Kettle Valley ranger station (south-central BC)
Larix occidentalis	000843	Guichon Creek north branch, near Merritt (south-central BC)
Larix occidentalis	38093	Okanagan Falls (south-central BC)
Picea glauca	34336	Risk Creek, near Alexis Creek (central BC)
Picea glauca	42844	West of Johnson Canyon, Banff National Park (southwestern AB)
Picea glauca	000866	Wheaton Creek, Cassiar District (northwestern BC)
Picea mariana	25757	Thunder Bay District (central ON)
Picea mariana	45851	Fallen Timber Creek, Cochrane (southwestern AB)
Picea mariana	39242	Prince George (central BC)
Picea sitchensis	32528	Upper Carmanagh Valley (southern Vancouver Island, BC)
Picea sitchensis	42839	Guardsman Pass on Haines Rd. (northwestern BC)
Picea sitchensis	19443	Victoria (southern Vancouver Island, BC)
Pinus albicaulis	000903	Mt. Revelstoke (southeastern BC)
Pinus albicaulis	32670	Blackwell Mtn., Manning Provincial Park (south-central BC)
Pinus albicaulis	35680	Skyline Trail, Manning Provincial Park (south-central BC)
Pinus contorta var. *contorta*	26505	Russell Island (southwestern BC)
Pinus contorta var. *contorta*	000888	Trial Island, near Victoria (southern Vancouver Island, BC)
Pinus contorta var. *contorta*	000879	Leechtown (southern Vancouver Island, BC)
Pinus contorta var. *contorta*	000872	Marble Lake, near Jeune Landing (northern Vancouver Island, BC)
Pinus monticola	20285	Allison Pass, Manning Provincial Park (south-central BC)
Pinus monticola	39568	Trail 10, Mt. Revelstoke National Park (southeastern BC)
Pinus monticola	029154	Haley Lake (southern Vancouver Island, BC)
Pinus ponderosa	42195	Kamiak Butte Park in Whitman County (southeastern WA)
Pinus ponderosa	42196	Kamiak Butte Park in Whitman County (southeastern WA)

Species	University of Victoria Herbarium Accession #	Sample Location as Noted on Herbarium Sheet[1]
Pinus ponderosa	000900	Princeton (south-central BC)
Pseudotsuga menziesii	000916	Iron Mine Creek, Quinsam River (central Vancouver Island, BC)
Pseudotsuga menziesii	39569	Trail 10, Mt. Revelstoke National Park (southeastern BC)
Pseudotsuga menziesii	000928	Princeton (south-central BC)
Pseudotsuga menziesii	000911	Victoria (southern Vancouver Island, BC)
Taxus brevifolia	32427	Goldstream Park (southern Vancouver Island, BC)
Taxus brevifolia	39563	Trail 11, Mt. Revelstoke National Park (southeastern BC)
Taxus brevifolia	000981	Near Kamloops (south-central BC)
Thuja plicata	20349	Goldstream Park (southern Vancouver Island, BC)
Thuja plicata	000805	Kitimat (north coastal BC)
Thuja plicata	000798	North end of Galiano Island (southwestern BC)
Thuja plicata	39530	Base of Mt Revelstoke (southeastern BC)
Tsuga heterophylla	029151	Haley Lake (southern Vancouver Island, BC)
Tsuga heterophylla	000956	Winter Harbour (northern Vancouver Island, BC)
Tsuga heterophylla	45038	Durrance Lake, near Victoria (southern Vancouver Island, BC)
Tsuga mertensiana	39948	Summit of Mt. Revelstoke (southeastern BC)
Tsuga mertensiana	39839	Green Mountain (southern Vancouver Island, BC)
Tsuga mertensiana	39245	Mt. Brenton (southern Vancouver Island, BC)

[1] AB (Alberta, Canada), BC (British Columbia, Canada), ON (Ontario, Canada), SD (South Dakota, USA), WA (Washington, USA)
[2] *Chamaecyparis nootkatensis = Callitropsis nootkatensis*
[3] Specimen provided by E.C. Grimm

Supplementary Table 2: Classification accuracies (%) for the species-level classification tree (Supplementary Fig. 1). Species abbreviations consist of the first two letters of the genus and the first two letters of the specific epithet e.g., ABAM = *Abies amabilis*. Refer to Table 2 of the main text for a complete list of species.

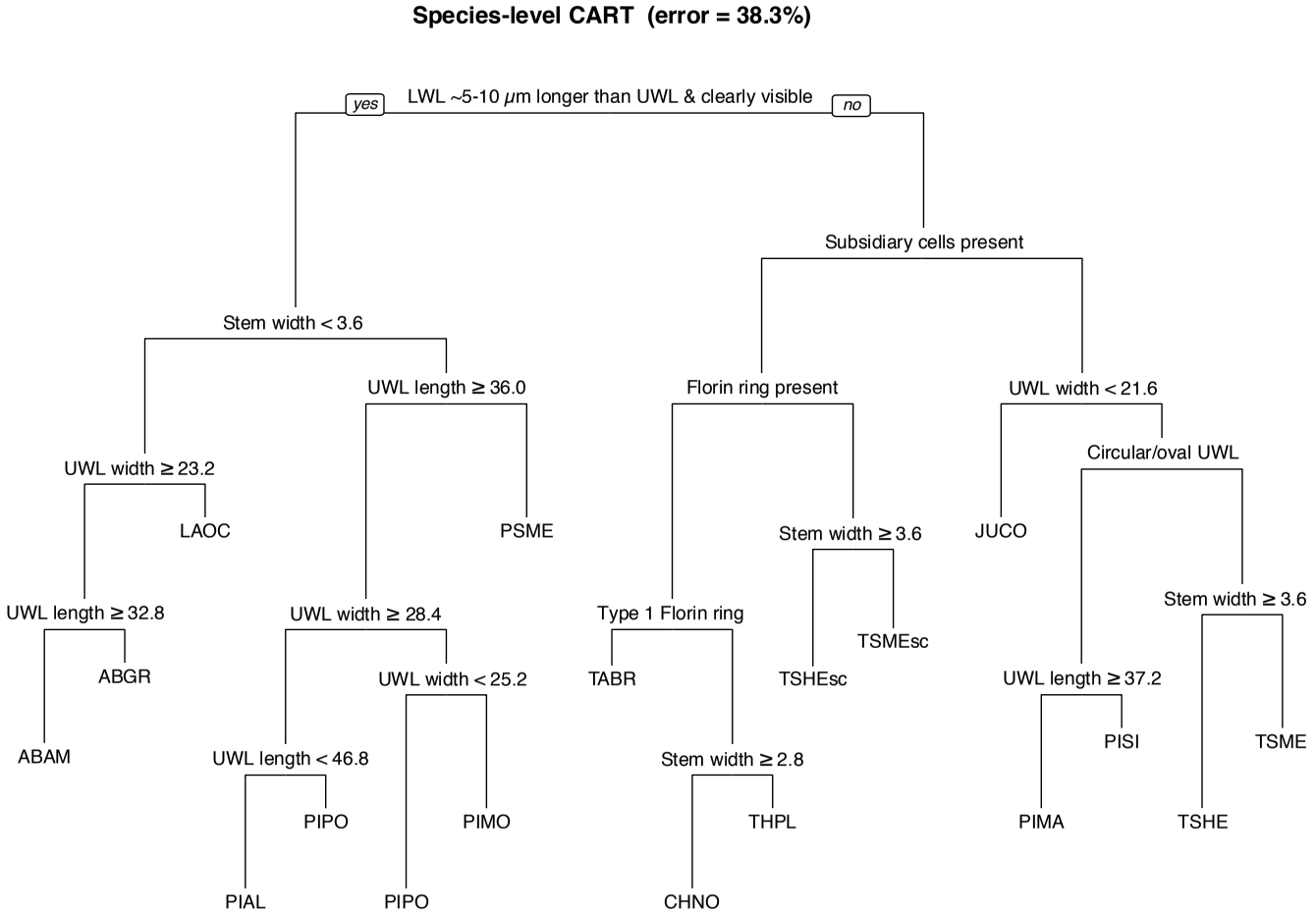
	ABAM	ABGR	ABLA	CHNO	JUCO	JUSC	LAOC	PIGL	PIMA	PISI	PIAL	PICO	PIMO	PIPO	PSME	TABR	THPL	TSHE	TSHEsc	TSME	TSMEsc
Classified As																					
ABAM	**53.3**	13.3	53.3	–	–	–	–	–	–	–	–	–	–	–	5.0	–	–	–	–	–	–
ABGR	–	**40.0**	–	–	–	–	–	–	–	–	–	–	–	–	5.0	–	–	–	–	–	–
ABLA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
CHNO	–	–	–	**100.0**	–	–	–	–	–	–	–	–	–	–	–	–	35.0	–	–	–	–
JUCO	–	–	–	–	**100.0**	100.0	–	–	–	–	–	–	–	–	–	–	–	13.3	–	–	–
JUSC	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
LAOC	40.0	46.7	20.0	–	–	–	**93.3**	–	–	–	–	–	–	–	10.0	–	–	–	–	–	–
PIGL	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PIMA	–	–	–	–	–	–	–	66.7	**80.0**	26.7	–	–	–	–	–	–	–	–	–	–	–
PISI	–	–	–	–	–	–	–	13.3	20.0	**73.3**	–	–	–	–	–	–	–	–	–	–	–
PIAL	–	–	–	–	–	–	–	–	–	–	**86.7**	55.0	33.3	6.7	–	–	–	–	–	–	–
PICO	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PIMO	–	–	–	–	–	–	–	–	–	–	6.7	15.0	**46.7**	20.0	5.0	–	–	–	–	–	–
PIPO	–	–	6.7	–	–	–	–	–	–	–	6.7	25.0	–	**66.7**	–	–	–	–	–	–	–
PSME	6.7	–	20.0	–	–	–	6.7	–	–	–	–	5.0	20.0	6.7	**75.0**	–	–	–	–	–	–
TABR	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**100.0**	–	–	–	–	–
THPL	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**65.0**	–	–	–	–
TSHE	–	–	–	–	–	–	–	20.0	–	–	–	–	–	–	–	–	–	**46.7**	–	6.7	–
TSHEsc[a]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**53.3**	–	–
TSME	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	40.0	–	**93.3**	–
TSMEsc[a]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	46.7	–	**100.0**

[a] TSHEsc and TSMEsc refer to *Tsuga heterophylla* and *Tsuga mertensiana* stomata with non-lignified subsidiary cells (sc).

Supplementary Table 3: Results of random forest analysis: mean Gini decrease and ranked morphological trait importance for the two genus-level stomata classification models. LWL = lower woody lamellae; UWL = upper woody lamellae; GC = guard cell.

	Model with LWL and SC data OOB[a] = 7.57%		**Model without LWL and SC data** OOB[a] = 13.57%	
Morphological Trait	Gini	Rank	Gini	Rank
Length of LWL relative to UWL	38.38	1	–	–
Stem width	36.33	2	41.14	2
Subsidiary cell type	35.61	3	–	–
UWL shape	33.11	4	39.07	3
UWL length	31.57	5	42.08	1
UWL width	29.33	6	36.81	4
Shape of polar ends of GC	13.01	7	30.47	5
GC width	12.68	8	18.02	6
Subsidiary cell presence	12.58	9	–	–
Shape of outer lateral sides of GC	5.11	10	11.31	7

[a] Out-of-bag error rate



Supp. Figure 1: Species-level classification tree for conifer stomata in western North America. Terminal nodes indicate species classification. Species abbreviations at terminal nodes consist of the first two letters of the genus and the first two letters of the specific epithet e.g., LAOC = *Larix occidentalis*. TSHEsc/TSMEsc refer to *Tsuga heterophylla/Tsuga mertensiana* with non-lignified lateral subsidiary cells. Refer to Table 2 of the main text for a complete list of species. Note that this tree has high misclassification (38.3%) and cross-validation errors (53.3%). *Abies lasiocarpa*, *Juniperus scopulorum*, *Picea glauca*, and *Pinus contorta* var. *contorta* lack terminal nodes because all stomata of these species are misclassified (see Supp. Table 2). All measured traits are in µm. See Section 3.1 (*Taxus*) of the main text for description of Type 1 Florin ring. LWL = lower woody lamellae; UWL = upper woody lamellae.