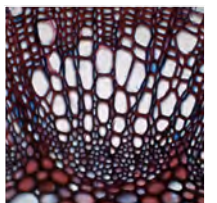
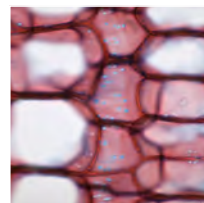
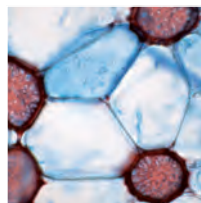
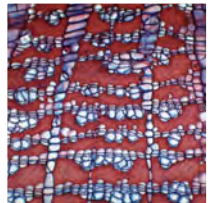
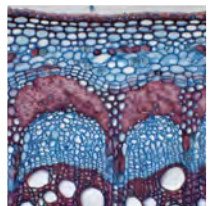


# **Photographic Atlas for the Microscopic Identification of Twigs of Selected Central European Trees and Shrubs**

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**Layout**

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**Cover pictures**

Top: *Rubus fruticosus* L. (Rosaceae), bark, transverse section  
Middle: *Abies alba* MILLER (Pinaceae), twig with bark and needles  
*Tilia cordata* MILLER (Malvaceae), phloem, transverse section  
Bottom: *Tilia platyphyllos* SCOP. (Malvaceae), pith, transverse section  
*Populus nigra* L. (Salicaceae), pith, radial section  
*Frangula alnus* MILLER (Rhamnaceae), primary xylem, transverse section  
Background: *Quercus robur* L. (Fagaceae), two-year-old twig, transverse section

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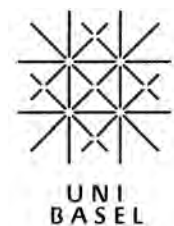
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## Introduction

There are already a number of atlases as well as subject-specific papers on wood anatomy. Atlases of European species were presented by Greguss (1955, 1959), Grosser (1977), Schweingruber (1990a, 1990b), Edlmann *et al.* (1994), Hather (2000) and Akkemik and Yaman (2012), all of which describe mature stem wood. Much less attention was given to bark. Moeller (1882), Holdheide (1951) and Trockenbrodt (1991, 1994) described the barks of Central European wood species, Crivellaro and Schweingruber (2013) wood and bark anatomy of Mediterranean species. The lately published IAWA list of microscopic bark features (Angyalossy *et al.* 2016) is mainly based upon the named older publications and also limited to the presentation of adult bark. Structures of roots have been described in one monograph only (Cutler *et al.* 1987). So far little attention has been given to the anatomy of twigs, although twigs are central in their role as an anatomical link between leaves and stem. Moreover twigs (and not only stem wood) have been used by humankind through all times for the production of artefacts such as baskets, stranded mats, bows and arrows, and combs.

The major goal of this atlas is to characterize twig-specific morphological and anatomical features and to enable the species-specific identification of immature wood, pith and immature bark. The book provides a base for the microscopic identification of twigs from living plants as well as of botanical macro-remains from archaeological and geological deposits. In addition, it offers a base to relate anatomical structures and physiological questions. The youngest anatomical elements in the stem – primary and secondary tissues – represent the first locations where photosynthetic products are structurally deposited in the permanent stem. Therefore young twigs represent the physiological and anatomical link between the non-permanent tissues such as petioles and leaf surfaces and the permanent ones.

The twig atlas addresses itself predominantly to users who are familiar with wood anatomy and have some prior knowledge of wood species. Last but not least we would like to highlight the aesthetic qualities of twig wood anatomy.

The atlas describes one- to four-year-old twigs of 52 central European species. For each species at least five specimens from at least three different locations were examined in compiling the data. The presentation of anatomical characteristics is based on double stained transverse and radial sections. For each species the pith, the primary and secondary xylem and phloem, the cortex and the periderm are portrayed. The nomenclature of the species follows Lauber *et al.* (2012); anatomical terms were established by Evert (2006) and Trockenbrodt (1990).

## Anatomical preparation techniques

All photographs are based on newly prepared microscopic slides following the sampling design and techniques described in Gärtner and Schweingruber (2013). Taxonomically and ecologically clearly characterized fresh long shoots were collected at various locations in Switzerland, Southern Germany and in the Vosges Mountains (France). Before sectioning they were stored in 30% ethanol. Sections of 20–40 µm in thickness were stained for a few minutes with a 1:1 mixture of Safranin/Astra Blue. Dehydration with 96% ethanol, absolute ethanol and xylene followed. Permanent slides were embedded in Canada Balm. Photographs were made in transmitting normal and polarized light with a Leica DFC420 camera through a Leica DM RP stereo microscope. Slides are stored conventionally in preparation boxes and integrated in the slide collection of the Swiss Federal Institute of Forest, Snow and Landscape Research (WSL) at Birmensdorf, Switzerland.

This book is part of the PhD thesis of Petra Zibulski at the University of Basel (Switzerland).

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We are deeply thankful to all people, foundations and organisations who supported us. Without their help this book could never have been realized.

## Conclusions

The anatomical descriptions of 52 wood species reveal some species-specific characteristics of juvenile tissues that differ from the known features of adult wood and which have received little attention so far.

Within the **pith**, the differentiation of pith parenchyma and medullary sheath parenchyma is an important diagnostic feature. The pith parenchyma tissue varies in many aspects and is useful for species diagnosis. Here, one must consider cell size, cell shape, cell wall thickness, the shape and arrangement of pits, the shape of crystals, and the occurrence of ducts. The pith allows species identification even where this is not possible by xylem alone. For instance, *Acer campestre*, *A. platanoides* and *A. pseudoplatanus* may be differentiated by the wall thickness of pith parenchyma cells, *Lonicera nigra*, *L. periclymenum* and *L. xylosteum* by their distinct medullary sheaths.

Juvenile **xylem** is characterized by uniseriate rays and small vessels, ray tracheids are often absent or are not developed in full. There are no species-specific distinguishing features in juvenile xylem and phloem, but distinct genus-specific differences do exist.

Within the juvenile **phloem**, the cell arrangement, the occurrence of crystals or ducts, and the formation of the primary phloem as closed sclereid ring or as sclereid patches represent diagnostic features. Tangential phloem structures may but need not match the number of annual growth rings. Often there are more annual ring-like tangential structures in the phloem than there are annual rings in the xylem.

A specific characteristic of juvenile bark is the presence of **cortex** tissue. Cortex cells are not arranged radially, which clearly distinguishes cortex tissue from phloem and periderm.

The first **periderm** may develop at different locations in the cortex or even in the phloem; this location is species-specific.

We were able to demonstrate clearly that even today it is possible to reveal previously unknown species and genus differences by careful wood anatomical studies.



## **Conifers: feature descriptions**

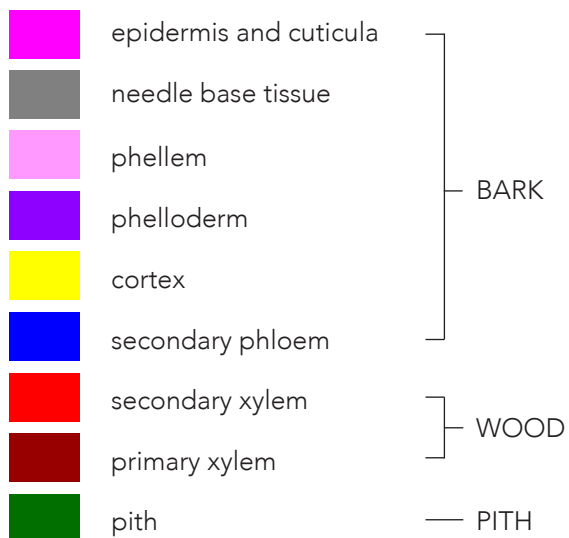
## Introduction

The following part describes all microscopic features mentioned in the descriptions of the conifer species. Most photos are either transverse sections or radial sections. The position of the wood is on transverse sections pith down and bark up, on radial sections pith left and bark right. Exceptions from this rule occur but are self-explaining, e.g. a transverse section showing the whole twig or a radial section showing the whole pith.

### Colour codes

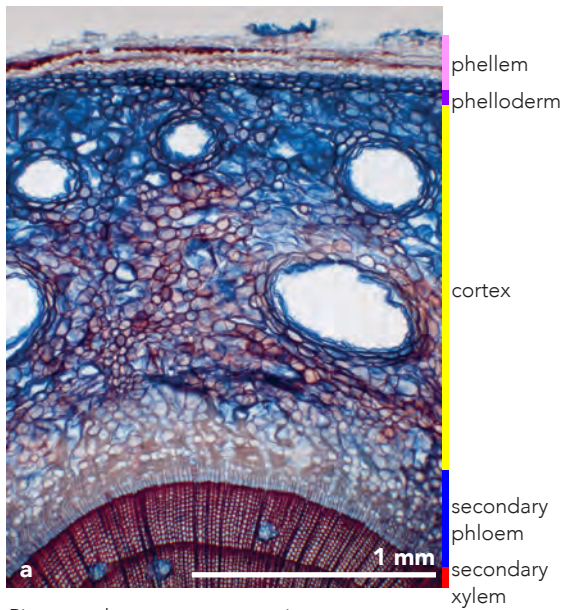
The different tissues are indicated by colour bars, in transverse sections to the right of (a, p. 9), in radial and tangential sections above (b) each photo. On the respective margin of the photos, the borders of the specific tissues correspond exactly with the colour blocks.

Tissues can also be indicated by coloured circles (c). In conifers the tissues normally appear in the following order, from outside in on transverse sections:





## Examples:



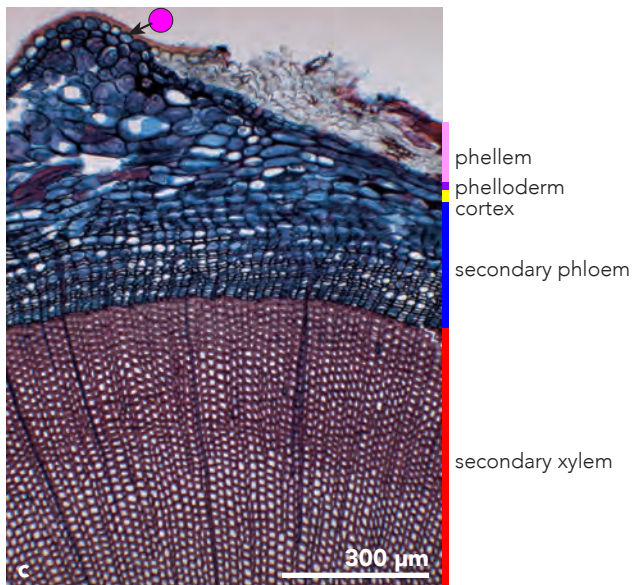
*Pinus cembra*, transverse section

The colour bar helps to understand: this photo shows a transverse section through bark.



*Picea abies*, radial section

In longitudinal sections, the colour bar is above the photo.



*Taxus baccata*, transverse section

The colour bar indicates: this photo shows a transverse section through wood and bark. The pink circle indicates the epidermis, a tissue not present at the right photo margin.



# 1 Pith features

## 1.1 Pith shape

Species-specific pith shapes occur only in well developed internodes. In transverse sections the pith shape is round, roundish or oval (fig. 1), star-shaped (figs. 2, 3), triangular (fig. 4), or rhombic (fig. 5). Because all piths show points where rays emerge, there is a smooth transition between round/roundish/oval and star-shaped pith shapes.

The pith shape varies along the shoot. In tiny shoots it can be very small (fig. 6), and near a nodium the pith shape becomes irregular due to the leaf trace (figs. 6, 7).

## 1.2 Pith cell composition

Different parenchyma cells may occur in the pith. The pith is called homocellular if only normal, unspecialized parenchyma cells of about the same size are present. The pith is called heterocellular if specialized parenchyma cells are present, i.e. some parenchyma cells contain tannins, dark stained substances or crystals, or if unspecialized parenchyma cells of different sizes are present. To decide whether a pith is homocellular or heterocellular, transverse and longitudinal sections have to be observed.

### 1.2.1 Pith homocellular

A homocellular pith consists only of normal, unspecialized parenchyma cells of about the same size (fig. 8).

### 1.2.2 Pith heterocellular

A heterocellular pith includes among the normal parenchyma cells cells with contents (tannins and other dark stained substances, gum, crystals etc.) (fig. 9), or it shows two different sizes of normal parenchyma cells, e.g. species with diaphragms (see 1.3.4).

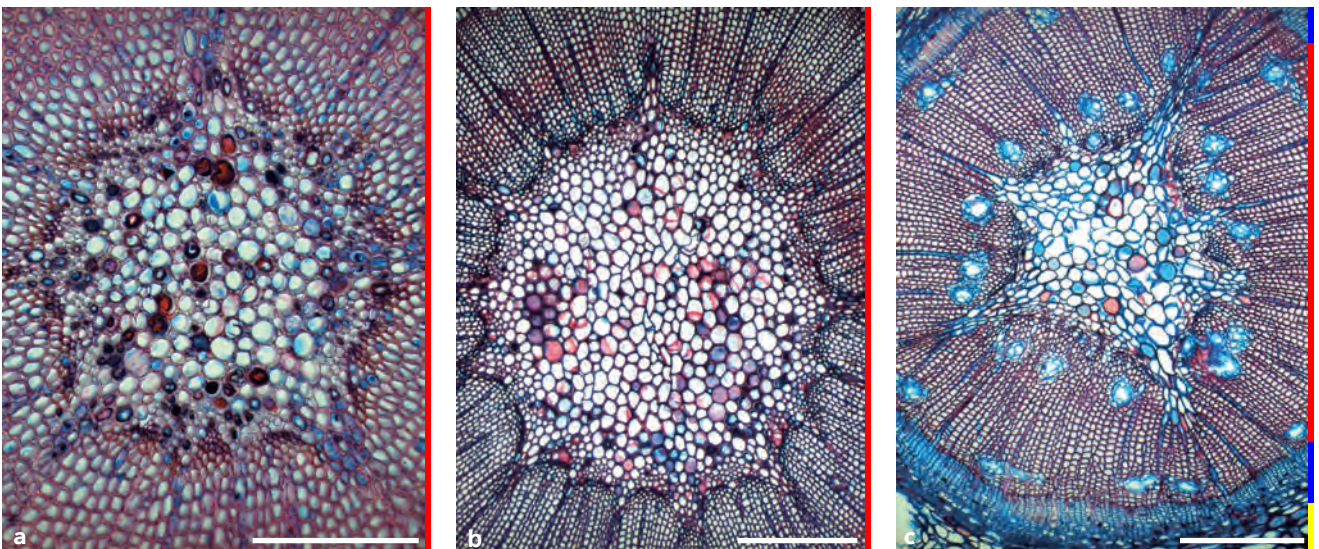


Fig. 1: Pith of *Larix decidua* (1a), *Picea abies* (1b), *Pinus sylvestris* (1c), transverse sections. Round to roundish pith. Scale: 200  $\mu\text{m}$  (1a), 300  $\mu\text{m}$  (1b, 1c).



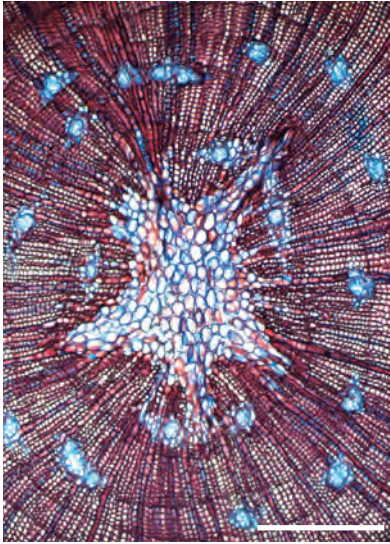


Fig. 2: Pith of *Pinus cembra*, transverse section. Star-shaped pith with five points. Scale: 300  $\mu$ m.

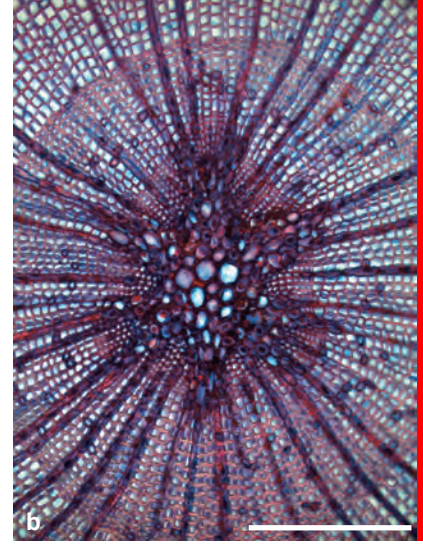
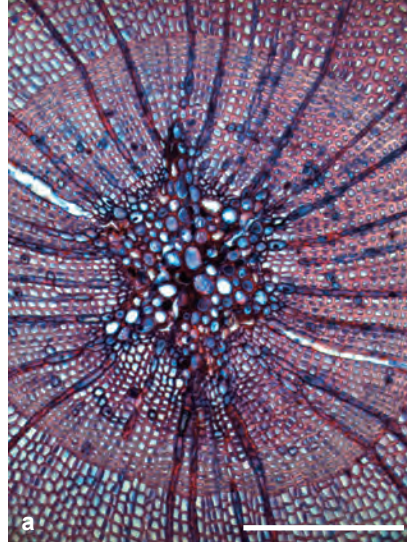


Fig. 3: Pith of *Juniperus communis* (3a, 3b), transverse sections. Star-shaped piths with six points. Scale: 200  $\mu$ m.

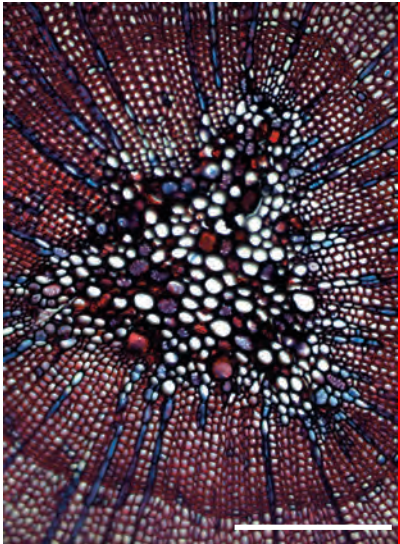


Fig. 4: Pith of *Juniperus communis*, transverse section. Triangular pith. Scale: 200  $\mu$ m.

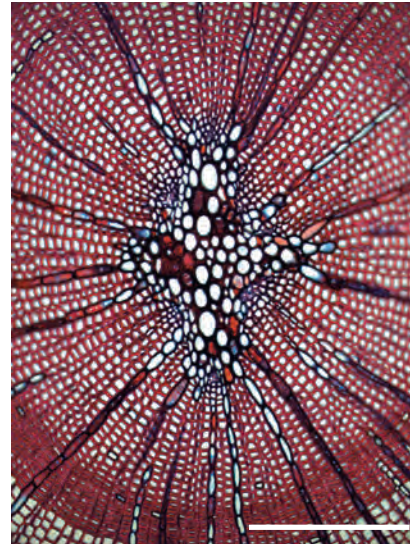


Fig. 5: Pith of *Juniperus sabina*, transverse section. Rhombic pith. Scale: 200  $\mu$ m.

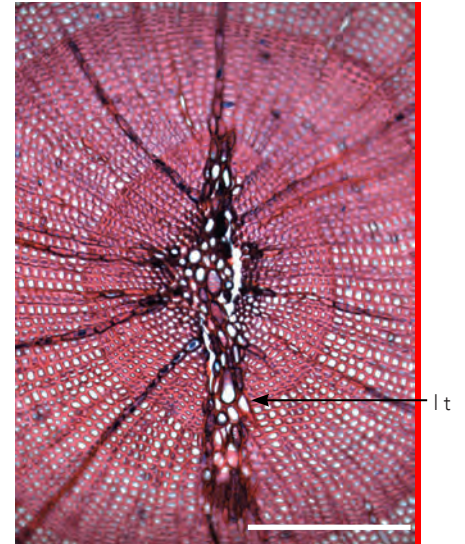


Fig. 6: Pith of *Juniperus sabina*, transverse section. Small pith in a tiny twig, downwards a leaf trace (l t). Scale: 200  $\mu$ m.

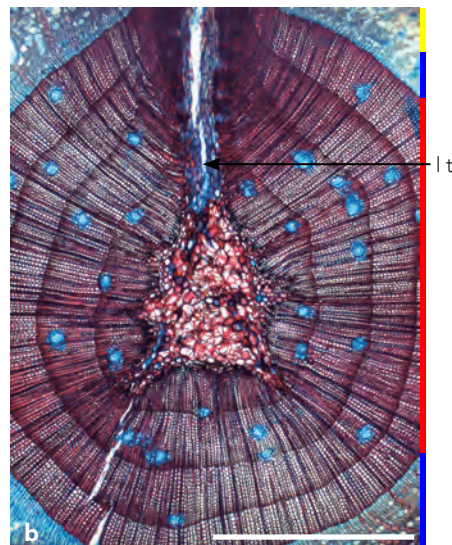
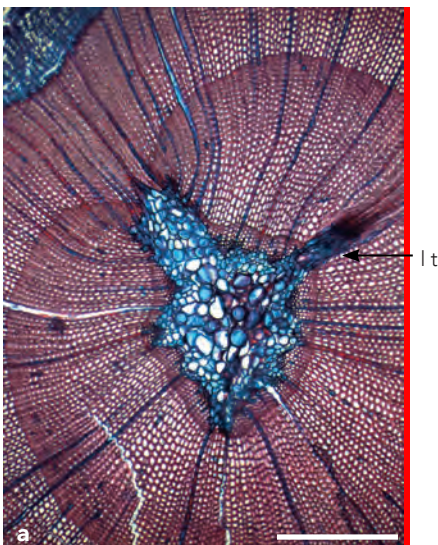


Fig. 7: Pith of *Taxus baccata* (7a) and *Pinus cembra* (7b), transverse sections. Near a nodium the leaf trace (l t) is large. Scale: 300  $\mu$ m (7a), 0.5 mm (7b).



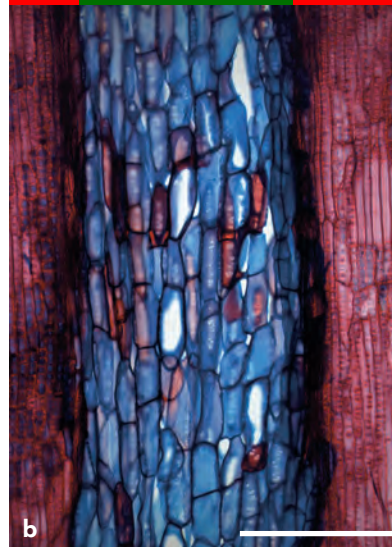
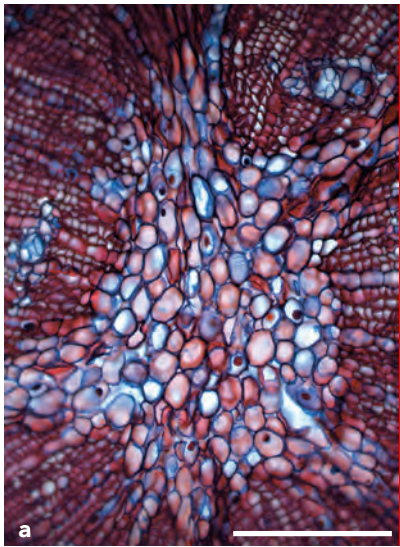


Fig. 8: Pith of *Pinus cembra*, transverse section (8a), radial section (8b). Homocellular pith: only normal parenchyma cells are present. Scale: 200  $\mu$ m.

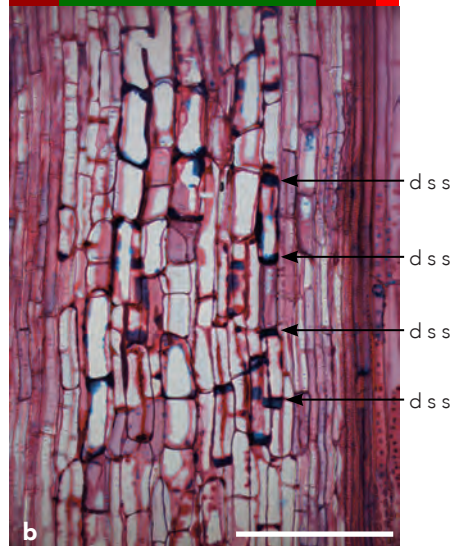
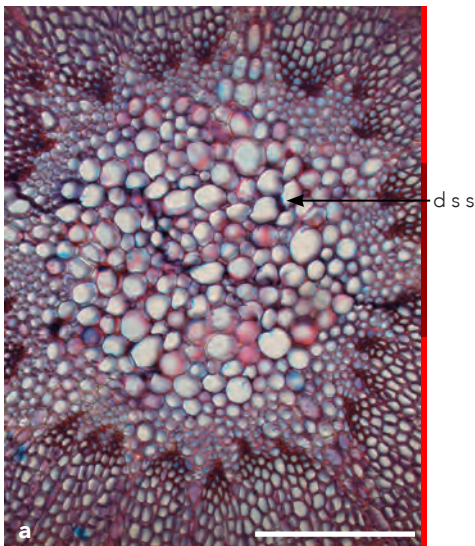


Fig. 9: Pith of *Larix decidua*, transverse section (9a), radial section (9b). Heterocellular pith: among normal parenchyma cells are cells containing dark stained substances (d s s). In this specimen the dark stained substances often concentrate in the axial short edges of a cell (9b). Scale: 200  $\mu$ m.



Fig. 10: Pith of *Larix decidua*, radial section. Pith cells arranged in straight axial rows. Scale: 300  $\mu$ m.

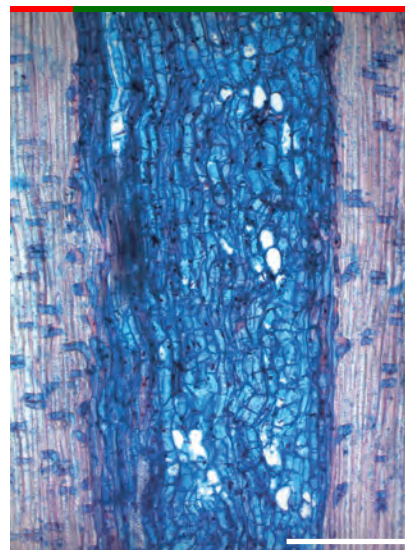


Fig. 11: Pith of *Taxus baccata*, radial section. Pith cells arranged in undulating axial rows. Scale: 300  $\mu$ m.



Fig. 12: Pith of *Pinus mugo*, radial section. Some pith cells arranged in undulating axial rows, others arranged irregularly. Scale: 0.5 mm.



## 1.3 Pith cell arrangement

### 1.3.1 Axial cell arrangement

The axial cell arrangement is expressed in longitudinal sections. When all cells are arranged in axial rows, rows can either be straight (fig. 10) or undulating (fig. 11). In some species not all cells are arranged in axial rows (fig. 12).

### 1.3.2 Localization of specialized parenchyma cells in the pith

In the analyzed species specialized parenchyma cells are rarely found. All such cells contain dark stained substances, occur mainly peripheral (fig. 13) or are equally distributed in the pith (fig. 14).

### 1.3.3 Arrangement of specialized parenchyma cells

Specialized parenchyma cells can be arranged solitary or in axial multiples, i.e. several specialized parenchyma cells follow each other in an axial row (fig. 15).

### 1.3.4 Pith chambered

A chambered or diaphragmed pith is defined by more or less horizontal plates consisting of several layers of axially shortened parenchyma cells (figs. 16, 17). The plates are called diaphragms.

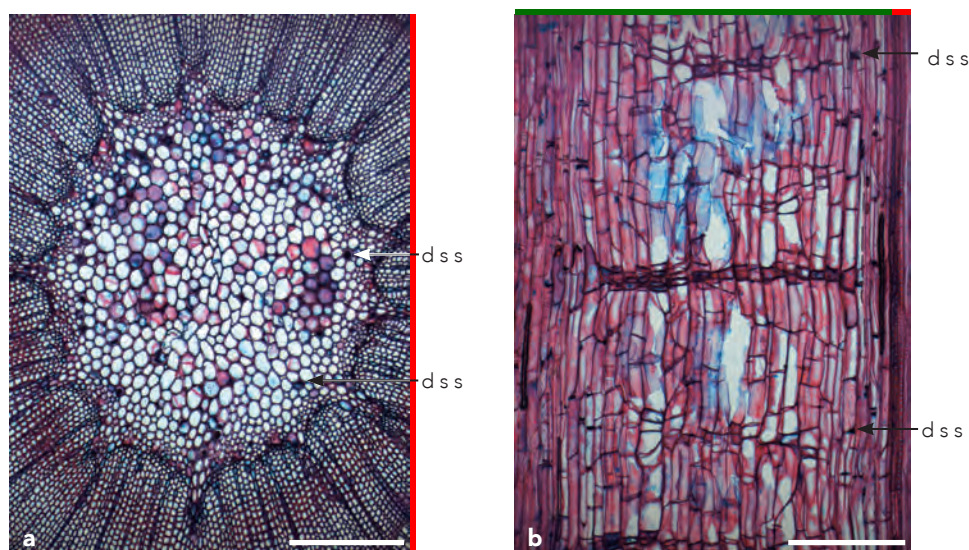


Fig. 13: Pith of *Picea abies*, transverse section (13a), radial section (13b). Specialized parenchyma cells with dark stained substances (d s s) in the axial short edges occur at the periphery of the pith. Scale: 300  $\mu$ m.

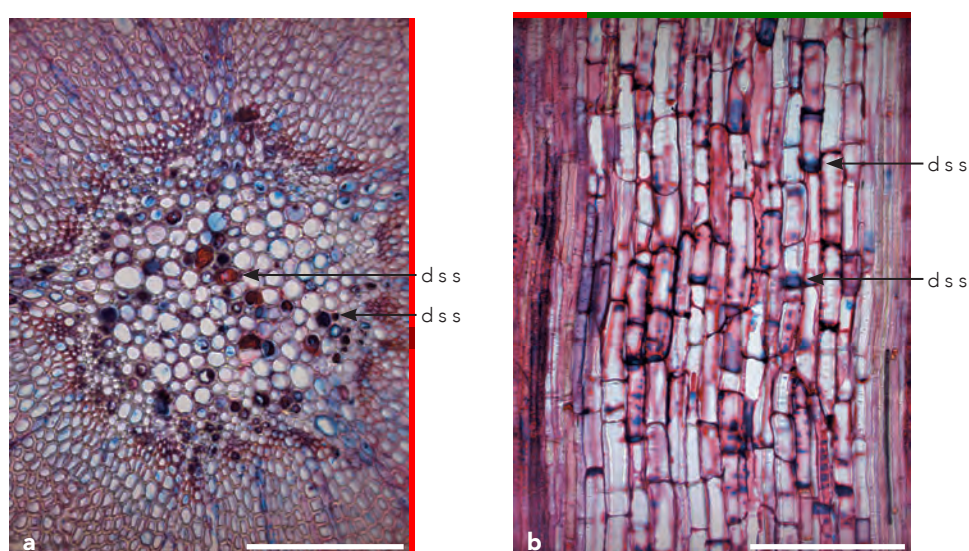


Fig. 14: Pith of *Larix decidua*, transverse section (14a), radial section (14b). Specialized parenchyma cells with dark stained substances (d s s) in the axial short edges are regularly distributed in the pith. Scale: 200  $\mu$ m.



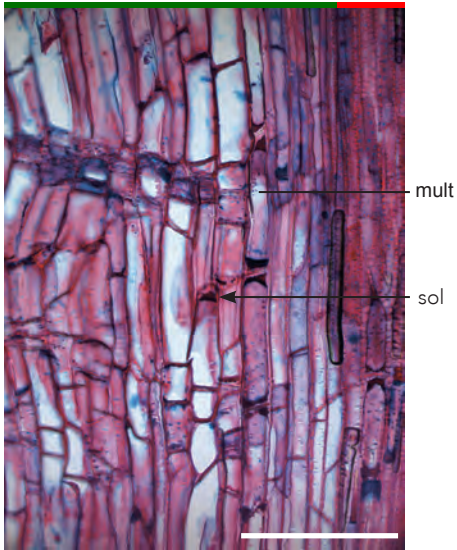


Fig. 15: Pith of *Picea abies*, radial section. Specialized parenchyma cells with dark stained substances in the axial short edges are arranged solitarily (sol) or in axial multiples (mult). Scale: 200  $\mu$ m.

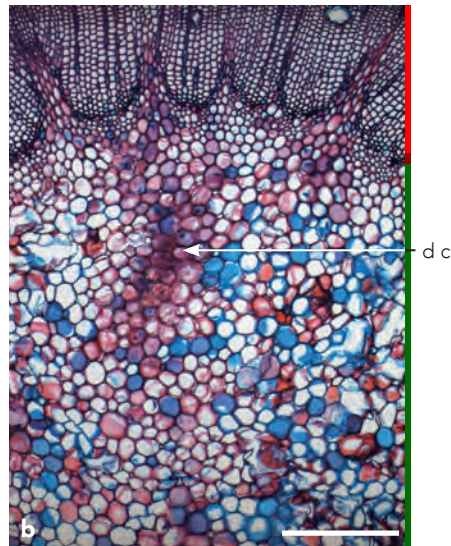
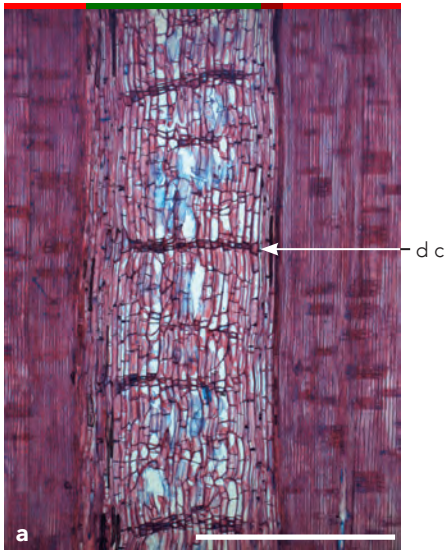


Fig. 16: Pith of *Picea abies*, radial section (16a), transverse section (16b). Chambered pith. Diaphragm cells (d c). Scale: 0.5 mm (16a), 300  $\mu$ m (16b).

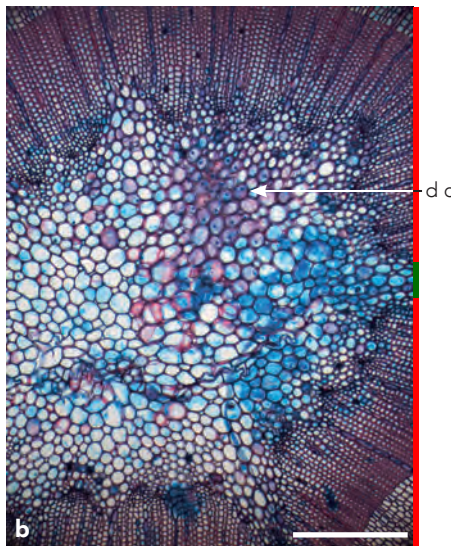
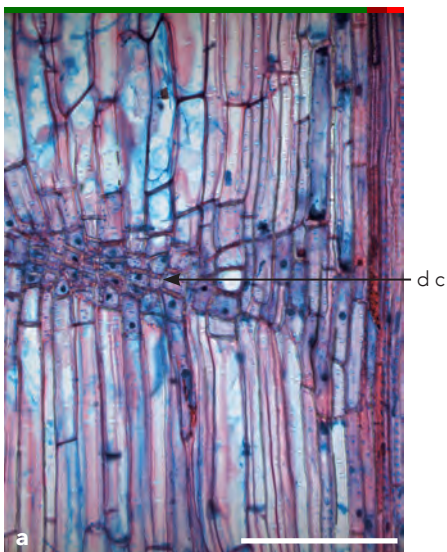


Fig. 17: Pith of *Abies alba*, radial section (17a), transverse section (17b). Chambered pith. Diaphragm cells (d c). Scale: 200  $\mu$ m (17a), 300  $\mu$ m (17b).



## 1.4 Pith parenchyma cells

### 1.4.1 Parenchyma cell shape in transverse section

Most pith cells are round, roundish or oval in transverse section (fig. 18). Angular cells are rarely found (fig. 19). Irregularly shaped cells often occur in *Pinus* (fig. 20).

### 1.4.2 Parenchyma cell shape in radial section

In radial sections most pith cells are rectangular and axially elongated or upright (figs. 21 and 22), some of them may have rounded corners (fig. 21). Radial-

ly elongated or procumbent cells are limited to diaphragms (fig. 22). Irregularly shaped cells often occur in *Pinus* (fig. 23).

### 1.4.3 Size of parenchyma cells

The pith cell diameters may change from the centre to the periphery. The reported cell diameters are determined as follows: on a photo showing the whole pith two straight lines crossing in the pith's centre are drawn (fig. 24) and the maximum diameters of all cells along the lines are measured. This is repeated for three specimens per species. In the species' description the maximum and minimum values are reported. In radial sections, the reported axial dimension of

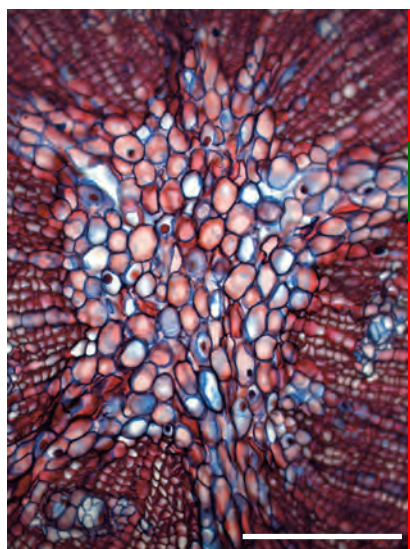


Fig. 18: Pith of *Pinus cembra*, transverse section. Most cells are roundish. Scale: 200  $\mu$ m.

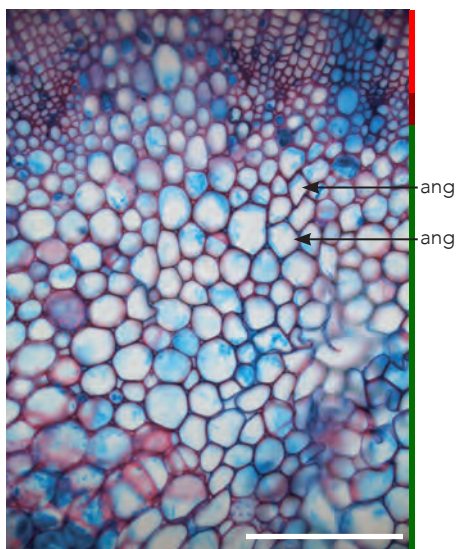


Fig. 19: Pith of *Abies alba*, transverse section. Some pith cells have an angular shape (ang). Scale: 200  $\mu$ m.

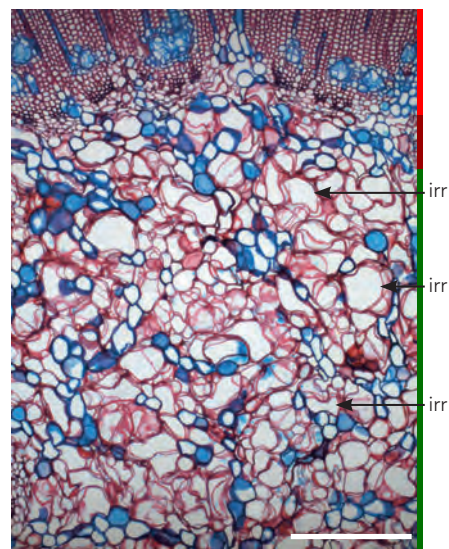


Fig. 20: Pith of *Pinus mugo*, transverse section. Many of the red stained pith cells have an irregular shape (irr). Scale: 300  $\mu$ m.

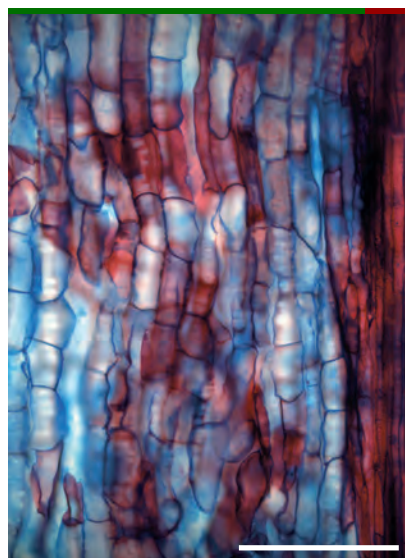


Fig. 21: Pith of *Pinus sylvestris*, radial section. Rectangular, axially elongated pith cells with rounded corners. Scale: 200  $\mu$ m.

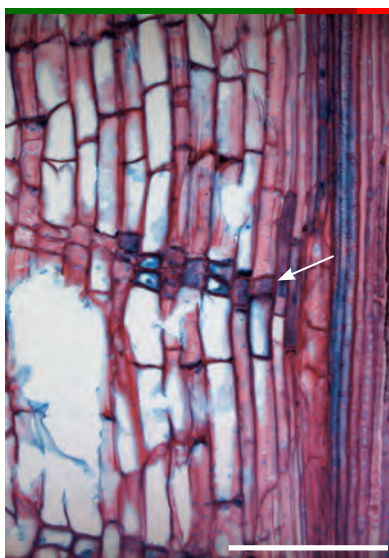


Fig. 22: Pith of *Picea abies*, radial section. Rectangular, axially elongated pith cells. Arrow: radially elongated to square diaphragm cells. Scale: 200  $\mu$ m.

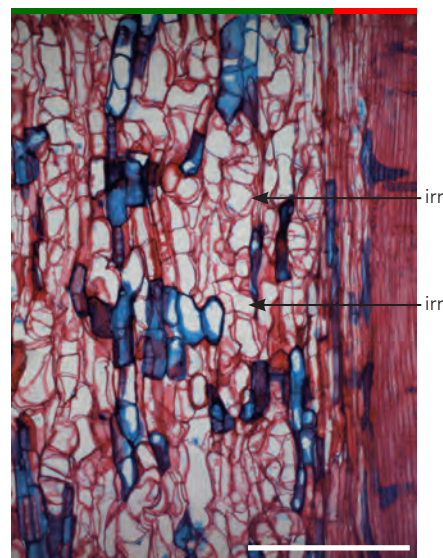


Fig. 23: Pith of *Pinus mugo*, radial section. Irregularly shaped pith cells (irr). Scale: 100  $\mu$ m.



pith cells are also measured in three specimens per species.

The cell wall diameter is measured as double wall diameter from lumen to lumen (fig. 25). In transverse sections the axial cell walls are measured (fig. 25a), in radial sections the transversal cell walls (fig. 25b).

Peripheral pith cells must not be confused with parenchyma cells of primary xylem on the margin of the pith. Peripheral pith cells are larger than parenchyma cells of primary xylem. Primary xylem parenchyma cells are of about the same size as cells of the secondary xylem and are never found in the points of the pith, but always in connection with proto- and metaxylem (see fig. 26 and chapter 2).

#### 1.4.4 Lignification of parenchyma cell walls

To see the lignification of cell walls, the specimens must be stained with both safranine and astrablue. Safranine stains lignified cell walls red, unlignified cell walls get stained blue from astrablue. A red stained pith means that all cell walls are lignified (fig. 27), red and blue cells mixed means that only some cells have lignified walls (fig. 28).

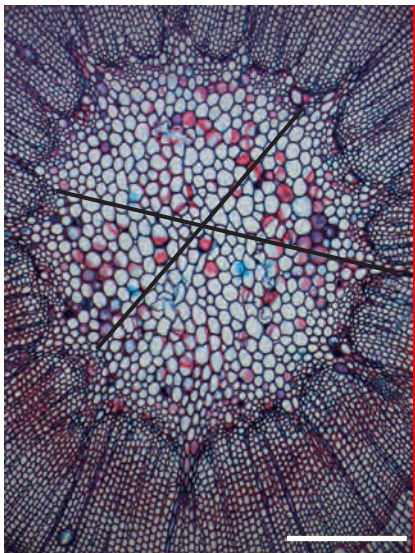


Fig. 24: Pith of *Picea abies*, transverse section. Along the two lines all cells are measured. Scale: 300  $\mu$ m.

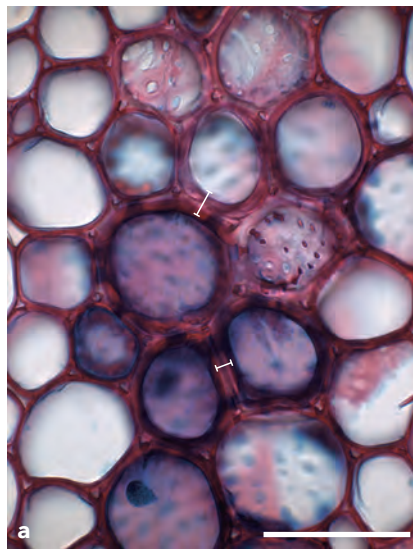


Fig. 25: Pith of *Picea abies*, transverse section (25a) and *Juniperus sabina*, radial section (25b). The double wall diameter is measured from lumen to lumen. Scale: 50  $\mu$ m.

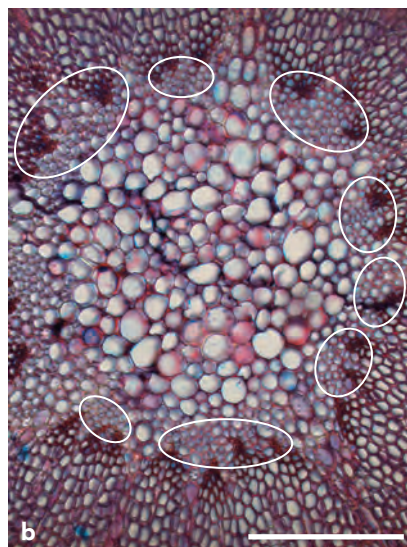
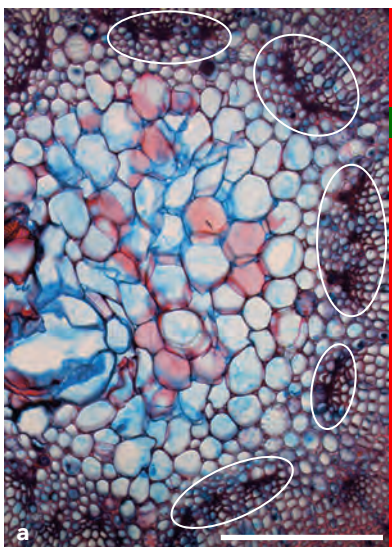


Fig. 26: Pith of *Abies alba* (26a) and *Larix decidua* (26b), transverse sections. The small cells in the ovals are not pith cells, but parenchyma cells of primary xylem. Scale: 200  $\mu$ m.



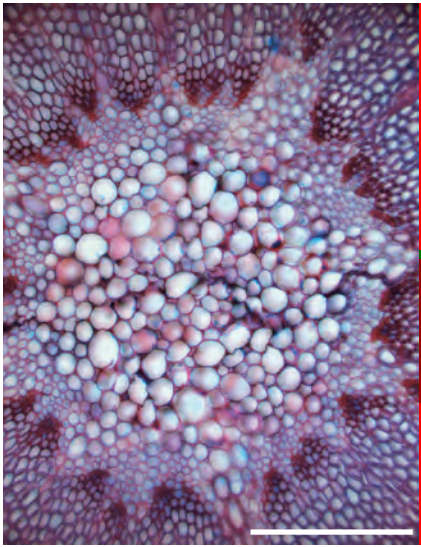


Fig. 27: Pith of *Larix decidua*, transverse section. All cell walls are lignified (red stained). Scale: 200  $\mu\text{m}$ .

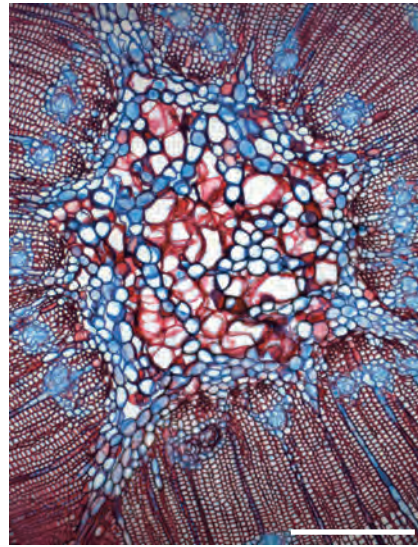


Fig. 28: Pith of *Pinus mugo*, transverse section. Pith with lignified (red stained) and unlignified (blue stained) cell walls. Scale: 300  $\mu\text{m}$ .

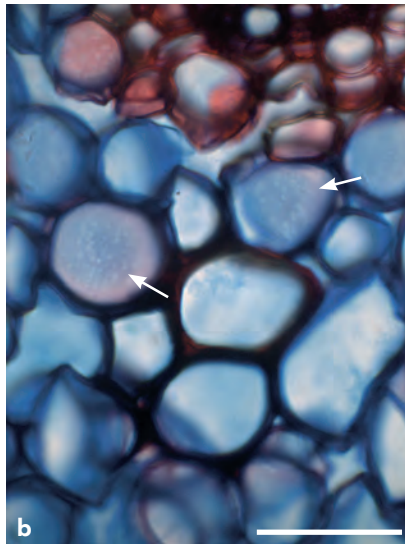
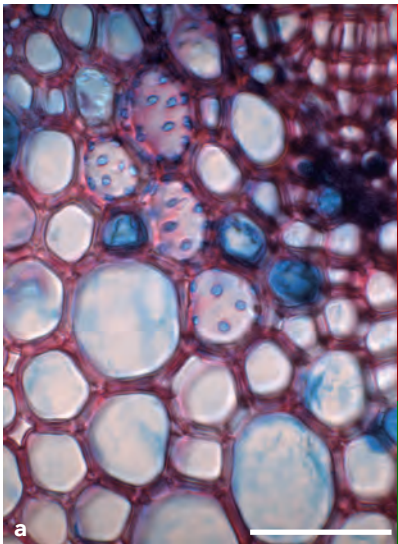


Fig. 29: Pith of *Abies alba* (29a) and *Pinus sylvestris* (29b), transverse sections. Transversal walls with pits. 29b: tiny pits (arrows). Scale: 50  $\mu\text{m}$ .

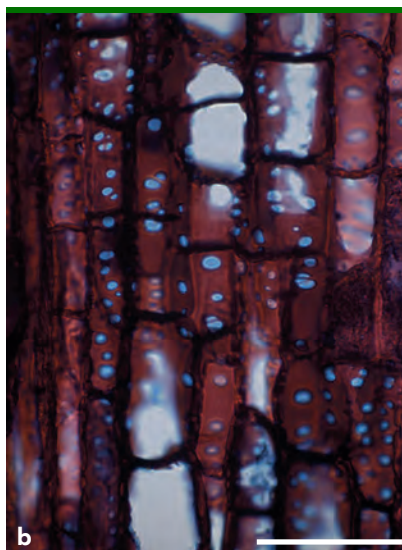
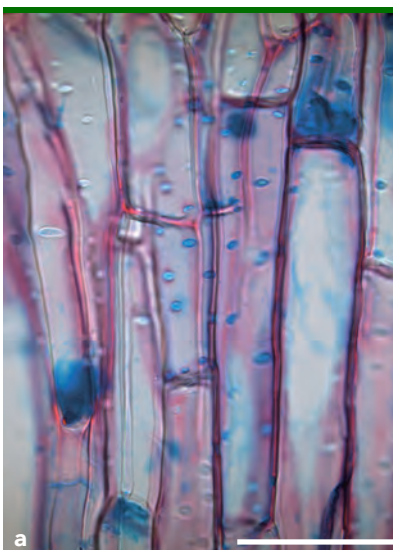


Fig. 30: Pith of *Abies alba* (30a) and *Juniperus communis* (30b), radial sections. Radial walls with pits. Scale: 50  $\mu\text{m}$ .

#### 1.4.5 Pits in parenchyma cell walls

In all observed species the pit shape is round to oval. Pits in transversal cell walls are rather round, in axial cell walls rather oval. The size of a pit is defined by its largest diameter.

In transverse sections some cells show the pits of the transversal walls (fig. 29). In radial sections the pits in radial walls usually are numerous (fig. 30). Some species show in transversal and radial sections the same pits (fig. 29a and 30a), other species show different pit sizes in transversal and radial cell walls (fig. 31) or different pit sizes in the transversal or radial walls of the same specimen (fig. 31a, 32).

#### 1.5 Intercellulars

The size of intercellulars is defined by the number of cells it borders on. A small intercellular borders on three cells, a medium-sized intercellular on four, and a large intercellular on five or more cells (fig. 33). In piths with thick cell walls intercellulars may be absent (fig. 34).

On radial sections additionally large axial intercellulars occur (fig. 35).

#### 1.6 Pith in crossed polarized light

In crossed polarized light (xpl) pith cell walls appear dark (fig. 36), glow faintly (fig. 37) or appear light (fig. 38). In some species there are cells of different lightnesses present (fig. 39, 40).

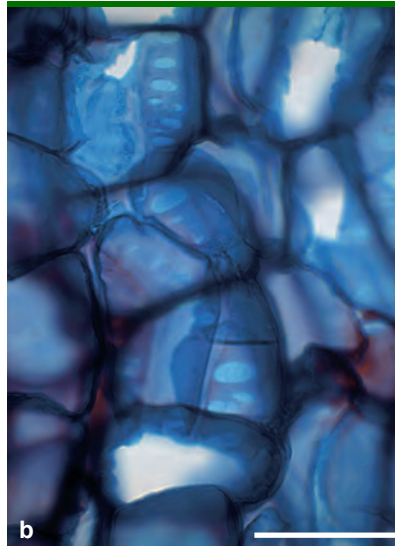
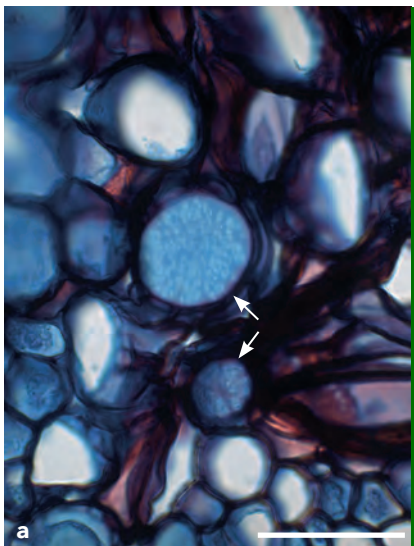


Fig. 31: Pith of *Taxus baccata*, transverse section (31a), radial section (31b). The transverse section shows a cell with tiny pits and a cell with large pits (31a, arrows), the radial section shows large oval pits (31b). Scale: 50  $\mu\text{m}$ .

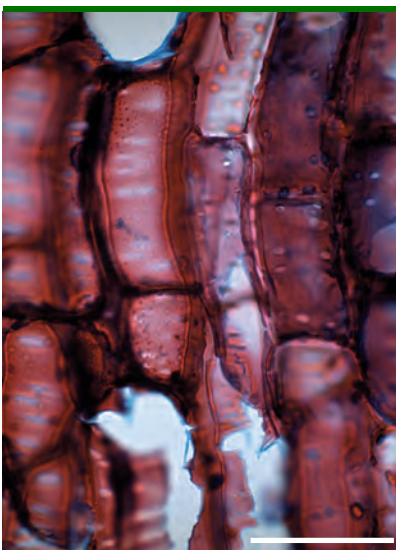


Fig. 32: Pith of *Larix decidua*, radial section. Left side: cells with large oval pits, right side: cells with smaller round pits. Scale: 50  $\mu\text{m}$ .

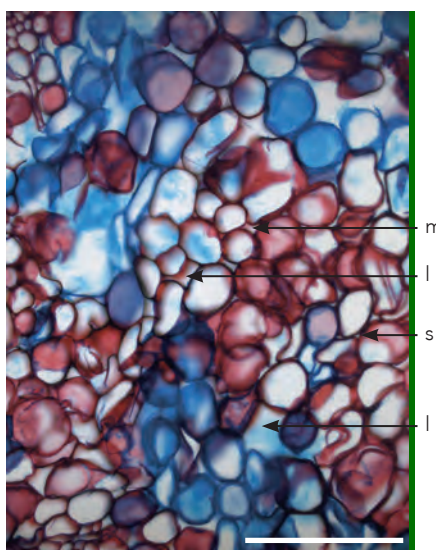


Fig. 33: Pith of *Pinus sylvestris*, transverse section. The pith cells are only in loose connection and show many small (s), medium-sized (m) and large (l) intercellulars. Scale: 200  $\mu\text{m}$ .

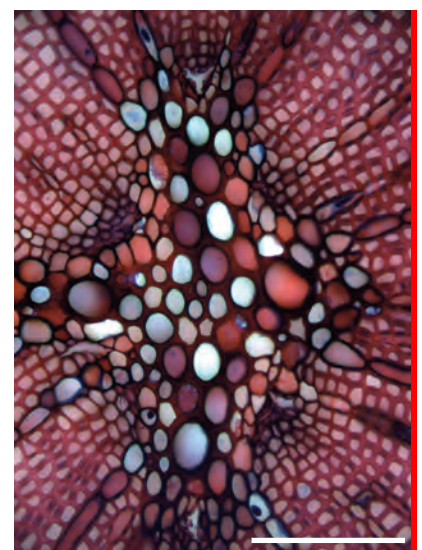


Fig. 34: Pith of *Juniperus sabina*, transverse section. The cell walls of the pith cells are thick and no intercellulars occur. Scale: 100  $\mu\text{m}$ .



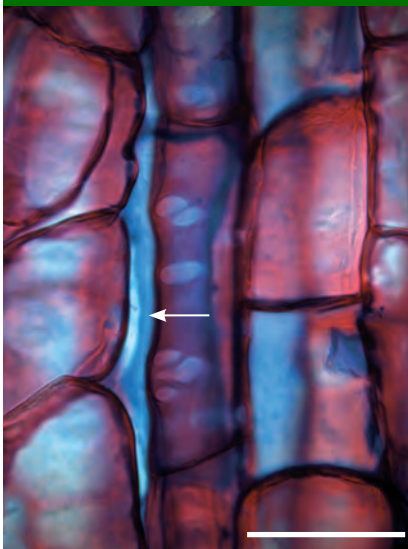


Fig. 35: Pith of *Pinus sylvestris*, radial section. A large axial intercellular (arrow). Scale: 50  $\mu\text{m}$ .

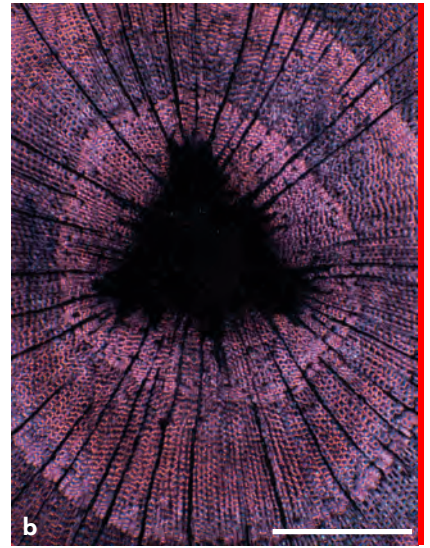
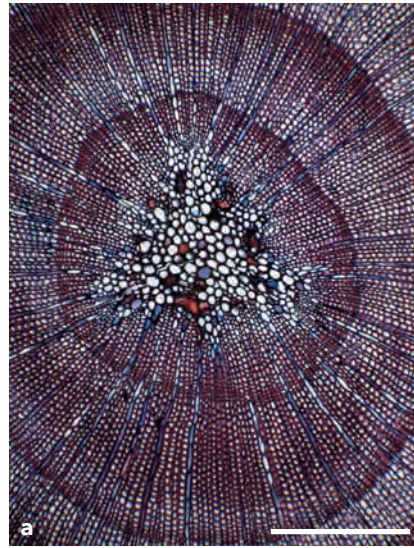


Fig. 36: Pith of *Juniperus communis*, transverse section. 36b = 36a in xpl. Pith cell walls appear dark in crossed polarized light (36b). Scale: 300  $\mu\text{m}$ .

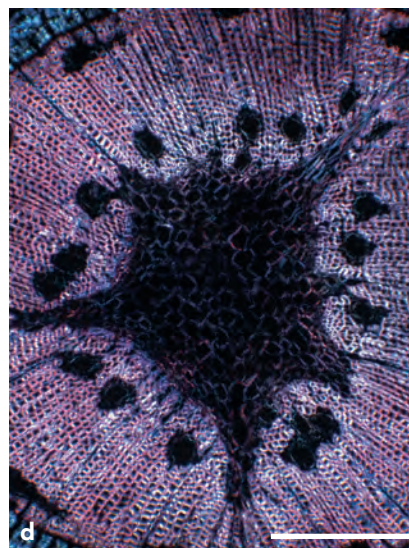
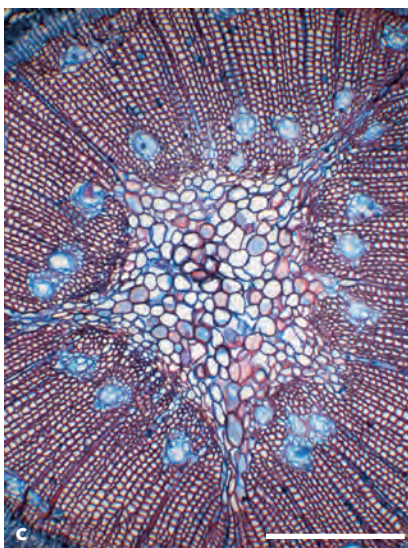
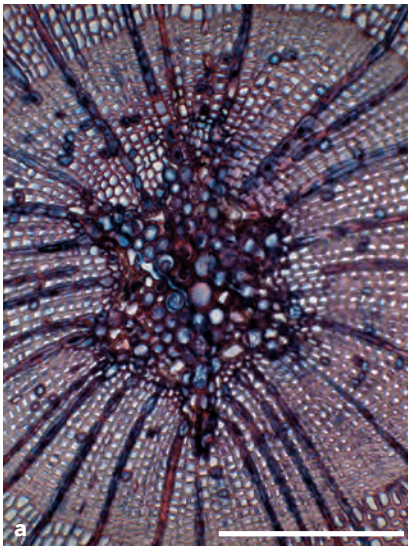


Fig. 37: Pith of *Juniperus communis* (37a, 37b), *Pinus sylvestris* (37c, 37d), transverse sections. 37b = 37a in xpl, 37d = 37c in xpl. Pith cell walls glow faintly in crossed polarized light (37b, 37d). Scale: 200  $\mu\text{m}$  (37a, 37b), 300  $\mu\text{m}$  (37c, 37d).