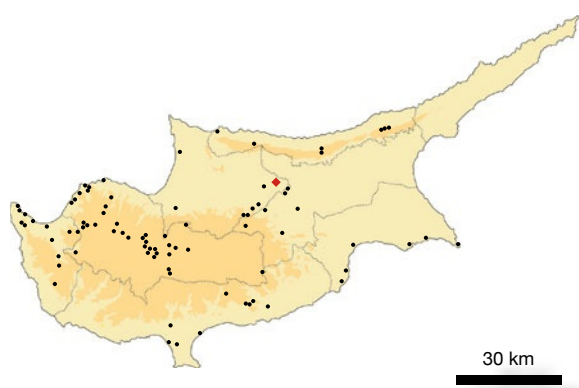


## 2. Materials and Methods

### Origin of the material studied

The extensive sampling of plant material was carried out on Cyprus in 2009 and 2010. We used the range descriptions from the standard references of Cyprus' flora (Meikle 1979, 1985; Tsintides *et al.* 2002) to choose a sampling areas that was representative of the geographic distribution and habitat range for each species. At each selected site we sampled one or two undisturbed individuals. Sampling sites on Cyprus are indicated on the following map.



Sampling sites on Cyprus in black dots. The red square indicates the city of Nicosia.

Rare or protected species were collected in botanical gardens, especially from the arboretum in Athalassa National Forest Park south-east from Nicosia, the capital city of Cyprus. Identification of floral or fruiting material was carried out by the taxonomists of the Department of Forest of the Ministry of Agriculture, Natural Resources and Environment of Cyprus, coauthors of this book. For each plant two samples were taken: one from the main stem and another one from a twig. The stem portion was near the plant's base, and the twig portion was from the top of the stem, or from a branch, selecting 2-4 year old shoot portions and avoiding short shoots. The stem and twig samples were stored in a sealed plastic bag to which several drops of 40% ethanol were added and kept at 3-4°C until they were sectioned. Each plastic bag was identified with a preprinted label that included the plant name, site location and elevation, plant

height, distance from the soil level and stem sampling point, and distance from the apex and twig sampling point.

### Plant material preparation

From each 5-8 cm long sample a 1 cm long disk was cut from the central part, and split longitudinally. Stem disks more than 1,5 cm in diameter were split to obtain a 1 cm<sup>3</sup> sample, and when possible we kept the rays running parallel to one side of the transverse section. In very small samples, the transverse section was cut first. For twig samples special care was taken to preserve the bark and the pith on the same section. Tangential sections were cut from the outermost growth ring, from intermediate and latewood.

The sections were stained with astra blue and safranin, dehydrated with alcohol and xylene, and mounted in Canada balsam (Chaffey 2002; Schweingruber 2007). The cell walls richer in cellulose stained blue and those richer in lignin stained red. Parenchyma cells were usually stained blue and the remaining cells were generally stained red, making it easier to interpret wood patterns in cross-sections.

A transmission-light microscope was used to observe slides. Polarized filters were applied for observing crystal presence, and for examining specific cell wall features. Sections were imaged using a digital camera mounted on the microscope. Magnifications are indicated in  $\mu\text{m}$  (0,001 mm) above a black scale bar in each picture.

### Wood density

Wood density values were calculated for each of the species studied. Wood density was measured on stem sample portions after the bark and the pith were removed. Fresh wood volume was measured using Archimedes' principal by immersing the segment in a beaker containing distilled water on a balance. Once fresh wood volume was measured, the sample was dried at  $103\pm 2^\circ\text{C}$  for 48 hours, allowing us to determine the wood mass. Wood density was calculated on the mass/fresh volume ratio basis ( $\text{g}/\text{cm}^3$ ).