

## **METHODS:**

### **Ascus Structure**

#### **K/I Reaction with Lugol's Iodine**

After Purvis, et al., 1992.

Lugol's iodine = 0.5 g iodine, 1.5 g potassium iodide, 100 ml distilled water.

1. Moisten a healthy looking ascocarp with water for a few minutes.
2. Remove a small portion of the ascocarp including part of the hymenium (preferably a thin section) and place on a glass slide.
3. Add a drop of 10% KOH, and leave for 1-5 minutes (hymenia that have a dense gelatinous matrix require a longer time).
4. Carefully soak up most of the KOH with the edge of a tissue.
5. Add a drop of Lugol's iodine and soak up, being careful to leave the preparation in situ on the slide.
6. Repeat stage 5.
7. Repeat stage 5, but do not soak up; instead, carefully lower a cover slip onto the preparation and observe under the microscope at x 100 or x 400. If there is a bluing of the asci or hymenial gel, the slide is ready to examine more closely. In the case of species in which there is no bluing, the asci or gel will take on a pale yellowish or reddish coloration; all tissues will then be considered K/I-. If there is not bluing, yellowing or reddening, add another drop of Lugol's iodine to the edge of the coverslip and allow the solution to flow over the preparation; this process can be assisted by drawing through the solution by applying a piece of tissue (bibulous paper) to the opposite edge of the coverslip, or by carefully raising the edge of the coverslip with a razor blade. Repeat until a coloration is obtained.
8. Examine preparation at x 400 or preferably x 1000 (oil immersion), make note and sketches. The preparation may need to be lightly tapped to spread out the tissues, but take care as asci are often easily disrupted. If the blue coloration gradually fades away, add another drop of Lugol's iodine to the preparation. Compare results with illustrations.