Protocol for the Determination and Study of Type Specimens

TYPE DETERMINATION

- 1. Enter the bibliographic citation containing the original description into the bibliographic database. Compare this information with that listed in the nomenclator. Note and address immediately any conflicts that occur.
- 2. Scan taxon protologue into computer for posting in the nomenclator. Compare information from the protologue with DATA [D] listed in the nomenclator. Note and address immediately any conflicts that occur.
- 3. Translate protologue and prepare, in outline form, a listing of all specimens that could possibly serve as a type.
- 4. Based upon information from #3, identify all possible herbaria that might hold authentic material of your taxon.
- 5. Survey the loans from these herbaria and pull all authentic specimens. If all specimens are not available, consult with the PI.
- 6. Review all of the authentic collections and determine the status of each, i.e., lectotype, isolectotype, paratype, etc.
- 7. Enter all collection and label data of each of these specimens into the specimen database.
- 8. Enter these above data into your lab data book (xerox 2 copies of each label and tape them into your book one for your original page and the other for your carbon copy).
- 9. Survey all modern treatments of your taxon for possible earlier lectotypfication. Note and address immediately any conflicts that occur.

TYPE STUDY

- 1. Examine your specimen by eye and make notes in your data book, such as the number of packets or sub-packets and the general quality of the specimen.
- 2. Observe the collection with a dissecting microscope and write a description of the general features of the dried specimen in your data book; indicate the number of "plant populations" in the collection, the plant color, the rhizoid color, the presence of capsules, gametangia, tubers, community associates, substrate, etc.
- 3. Study the entire packet contents with a dissecting scope and record any noticeable variability among the plants of the collection. If the vegetative specimens look variable, follow step 4 for each of the proposed separate elements (?different species) for later comparison of character states.
- 4. Carefully segregate out stems with sporophytes and/or gametangia for detailed study. Keep these in subpackets (archival paper or "stamp PAC") labelled with rapidiograph pen.
- 5. Choose 1 (2 if possible) mature intact capsules (depending on how "ample" the material is). If 2 capsules are to be studied, keep each capsule and it's vegetative plant in separate subpackets labelled "a" and "b" so that spores from one capsule can be matched with one vegetative plant and spores of the other capsule(s) can be matched with the other vegetative plant(s). Make the following 2 spore preps:
 - (A.) Prepare an SEM stub for scanning by rubbing a dissecting needle on the stub sticky tape and then touch the stub tape with the spore/elater covered needle. [Non-dehisced capsules are best because there is no possibility of "spore contamination." If only dehisced capsules are available these must be used]. If multiple capsules are used, label "a" or "b" accordingly.
 - 1. With the scanning scope, study at least 10 spores per capsule taking photos of 7 distal and 3 proximal surfaces of randomly chosen spores at x2,000 and of representative elaters at x1,000. Also, high mag shots of both proximal and distal spore faces should be made at x5,000. Make other photos of any observed features of interest such as side views or low mag shots with a representative sample of spores and elaters.
 - 2. In your data book, record the surface marking pattern, and the sublamellar surface ornamentation. Scan the contact prints of all spores and elaters onto the zip disc "FOSS SPORES" and use NIH image to measure spore diameters, the number of lamellae, areolae, projections, or depressions across the spore surface (distal and proximal), and the widths and heights (or depths) of projections.
 - 3. Record all measurements on the Excel spreadsheet and in our data book.
 - 4. In your data book, record the length and width of the elaters; describe the thickening pattern.
 - (B) Prepare a Brite mount of the spores and elaters from each of these capsules and of the inner and outer capsule wall if material is ample. Study these mounts with the compound scope IMMEDIATELY AFTER PREP.

- 1. Record the color and the range of measurements of the spore diameters and the elater band color and elater lengths/widths in your data book.
- 2. Record morphological features visualized with light microscopy (pattern of ornamentation, number of lamellae/areolae, etc. of spores thickening patterns of elaters.
- 3. Prepare photomicrographs of the spores and elaters from these fresh mounts. If multiple capsules are used, label "a" or "b" accordingly so that the actual vegetative plant can be matched with the correct spores/elaters.
- 4. Record and photograph the inner and outer capsule wall thickening patterns.
- Prepare the dried vegetative plant(s) to be studied (preferably the same plant used for sporophyte study) by carefully cleaning it of debris with dissecting needles under a scope. If material is ample, clean 5 - 10 other plants for later measurements. Rehydrate the plants with water. If necessary, boil the plants in water. If necessary, add TWEEN to the water
- 7. Prepare some plants for photography with the CEM dissecting scope, especially fertile plants or plants with gametangia or pseudoperianths. Using water mounts, make habit drawings from these plants with the light microscope. Also prepare drawings of leaf shapes and cells; be sure to record all measurements.
- 8. Use these same plants after the CEM photo session (if ample material) for SEM prep to scan the vegetative characters.
- 9. Vegetative plant study Record general morphological observations: plant width with leaves, leaf stance, leaf spacing, leaf decurrency, leaf contour, leaf shape, leaf apex, leaf margin, etc. following the Excel Spreadsheet of characters.
- 10. Stem sections should be prepared, if ample material, following the above study sequence. Brite mounts should be made of all sections and of all dissections. Measurements and drawings, however, should be made immediately after prep due to possible shrinkage in the Brite Wax.