PROTOCOL FOR THE STUDY OF FIELD COLLECTION

1. Tape a xerographic copy of collection information in the log book along with any other information included in the package. These data should also be entered into the specimen database.

2. Prepare a voucher specimen with archival quality paper and label lightly with a #2 pencil. In addition, prepare a small packet (weigh paper or stamp pac) for the individual gametophyte plants from which sporophytes are harvested (one for Brite wax mount of spores, one for SEM preparation and one for inoculated spore cultures).

3. In the case of multiple collections, work completely through each collection before opening any others to avoid drying.

4. Collection examination:

   A. Carefully evaluate the entire collection. If it is comprised of possible multiple species, separate and prepare voucher packets for each.

   B. Record observations of associated taxa (within the collection) in the log book and database.

   C. Record vegetative morphology (size, stance, seta length, pseudoperianth, color etc.).

   D. Remove a median leaf, make a wet mount, describe the oil bodies (shape and average number per cell). Draw and photograph a typical cell with oil bodies. Record the average oil body and median cell sizes. (**If no sporophytes, proceed to H.**)

   E. Remove a mature, intact sporophyte for axenic culture. Place the gametophyte from which it was harvested in a labeled minipack and place the sporophyte in a covered syracuse dish of distilled water to avoid drying. Place a small piece of paper with an identification number (written in pencil) with the sporophyte.

   F. Make a Brite wax spore mount from a second sporophyte (this capsule can be partially dehisced as long as it is mostly intact). Place the parental gametophyte in a labeled minipack and record the spore size (from a minimum of 10 spores), elater length, number of elater spirals, a brief description of the elaters and capsule wall thickening pattern. Photograph all of the above with the compound microscope, **immediately after prep!**

   G. Prepare a spore stub for SEM observation as follows:

      1. Make sure the stub has been sonicated.

      2. Mark the bottom side of the stub with the identifying number of the specimen and place in the stub holder.

      3. Liberally coat the upper surface of the stub with clear nail polish.

      4. Transfer the sporophyte (can be same condition as e) to the stub while the polish is still tacky (after about 5 minutes).

5. Place the stub/stub holder under the dissecting scope and spread the spores over the stub surface with the tip of a dissection needle (a light tapping of the stub assembly against the stage will spread the spores well). Stubs prepared in this fashion can be sputter-coated after 24 hours.

6. Place the parental gametophyte in the properly labeled minipack.

H. Place a portion of the collection on vermiculite, preferably with green capsules. This will be insurance in case of culture contamination, lack of germination or hopefully obtain additional sporophytes.

I. Once the above steps are complete, place the remainder of the collection, minipacks and any accompanying notes (from the sender) in the voucher packet. Once the vermiculite sample has produced sporophytes, or germination in axenic cultures has occurred, place the remainder of the sample in a mini pack (labeled "vermiculite" with the date of harvest) and include in the original voucher packet.