

of a slight depression in the moss. On removing some of the overlying vegetation, numbers of the larger prothallia were easily obtained. It required, however, careful sorting of the peaty soil with the fingers to secure the younger and more interesting stages. Nearly a week was spent in working over about half the bed, the result being several hundred examples in all stages of development, of the gametophyte and attached sporophyte. Subsequently, in another season, a week was spent on the spot, and all the plants which careful sifting of the soil would yield, were removed. The second harvest amounted to over six hundred specimens, by far the larger number of which, however, were much too old for study. During the same summer, other and older plants were found in rich woods about two miles back of Metis. In the spring of 1896, additional discoveries were made in Foster's Flats, below the Whirlpool, on the Niagara River, and on the east branch of the river Don, a few miles from Toronto. The last mentioned spot proved rich in interesting examples of older stages of the attached sporophyte. Most of these were removed last autumn (1897).

### III.

One of the greatest difficulties in the way of the present research, was the proper preservation of the prothallia. They are singularly impermeable to fixing reagents on account of the thick external cuticle, and must be cut at intervals with a razor, to allow the preserving medium to penetrate. The presence of oil in large quantities in the tissues, also renders aqueous fluids useless, as they scarcely make their way in at all. A saturated solution of picric acid in thirty per cent. alcohol, gave fairly good results; but the best fixation was obtained by using a mixture of three parts of a saturated solution of corrosive sublimate in ninety per cent. alcohol, and one part of saturated solution of picric acid in the same menstruum, diluted with distilled water to reduce the alcohol to thirty per cent. strength. The same reasons which rendered the material hard to preserve, made it difficult to embed. Paraffine was mainly used, and the most satisfying results were obtained by infiltrating with benzole, in a vertical tubular dialyzer with a chamois leather diaphragm, revolved slowly by means of clockwork. It was found that the ordinary type of stationary dialyzer was quite unsuitable for these very delicate objects. When the prothallia in alcohol were placed in the top compartment, and the benzole below, the osmosis was exceedingly slow; and, if the position of the media was reversed, the weight of the benzole carried it through too rapidly, and injurious shrinkage was the result. The continued reversing of the