A HETEROCHRONIC SEQUENCE FOR THE DEVELOPMENT OF PARAPHYSES IN *NECKEROPSIS* SCHIMP. (BRYOPHYTA: NECKERACEAE)

by

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ABSTRACT

Neckeropsis is a genus of tropical distribution with two species in Puerto Rico, *N. disticha* and *N. undulata*, that present leaf-like structures, the ramenta, that grow on the perichaetia after the fertilization of the archegonia. These structures have been identified as ligulate perichaetial leaves or multiseriate paraphyses, but their origins and homology are not clear. A morphological and ontogenetic description was made for *N. disticha* using stereoscope, light microscope, and paraffin and plastic resin infiltration procedures. Transitions between uniseriate and multiseriate paraphyses were documented at different times in the development of the fertilized branch. Nine other species were examined to evaluate the presence and modifications of the ramenta; five species possess the multiseriate paraphyses in different developmental stages. During the development of the fertilized archegonia, the branch presents the transitional stages of the development of paraphyses as a heterochronic sequence similar to the heteroblastic sequence in branch leaves.

RESUMEN

Neckeropsis es un género de distribución trópical con dos especies en Puerto Rico, *N. disticha* y *N. undulata*, que presentan estructuras parecidas a hojas, los ramentos, creciendo en los periquecios luego de la fecundación del arquegonio. Estas estructuras han sido identificadas como hojas periqueciales liguladas o paráfises multiseriados, pero su origen y homología no se han clarificado. La descripción morfológica y ontogenética fue preparada para *N. disticha* usando el estereoscopio, el microscopio de luz y procedimientos de infiltración en parafina y resina plástica. Se documentaron transiciones entre paráfises uniseriados a multiseriados en diferentes etapas del desarrollo de la rama fecundada. Otras nueve especies se examinaron para evaluar la presencia y modificaciones de la ramenta; cinco especies poseen los paráfises multiseriados en varias etapas. Durante el desarollo del arquegonio fecundado, la rama presenta las etapas de transición del desarrollo de los paráfises como una secuencia heterocrónica similar a la secuencia heteroblástica de las hojas de una rama.

To my ancestors...

... and descendants

To my grandmother *mami*, who is the stem and support of my family, who overcame all the difficulties that I could not even imagine. To my mother who inherited her strength, perseverance and compassion, and who has guided me to finding myself.

To my children Gabriela and Adriel, you are the most amazing teachers I have met. When everything seems to be lost, I look at your faces and know that life is beautiful and simple; both of you teach me that I can be all I want.

“ ‘What strength have we... That is the path of despair. Of folly I would say...’
‘Despair or folly?’ said Gandalf. ‘It is not despair, for despair is only for those who see the end beyond all doubt. We do not. It is wisdom to recognize necessity, when all other courses have been weighed, though as folly it may appear to those who cling to false hope.’ ”

J. R. R. Tolkien
The Lord of the Rings
Vol. I

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Elio, this is the achievement of both. I love you. Perseverance brings good fortune!

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INTRODUCTION

Paraphyses are documented in almost all moss groups but studies in this area are limited to describing different types of paraphyses and do not deal with the development and transformations of these structures. The lack of studies about the origin and modifications of the paraphyses can represent a problem when evaluating this character for taxonomic and phylogenetic analysis.

This investigation focuses on determining the homology of leaf-like structures, the ramenta, that appear after fertilization on the reproductive branches of certain species of *Neckeropsis*. These structures were described as paraphyses, but their strong resemblance to leaves has lead to the assumption that they are modified perichaetial leaves (Touw, 1962). Paraphyses are not considered homologous to leaves, and the general definition states that they are cell-filament structures associated with the reproductive organs of bryophytes.

There are two approaches for establishing homology in systematics, by outgroup comparison and by the ontogenetic criterion. Although both methods are valid and play non-overlapping roles (Kitching *et al*, 1998), the ontogenetic approach has been less used, probably because of the absence and difficulty of the data. In bryophytes ontogenetic data are scarce and works in this area are useful and sometimes necessary to define controversial structures and to help understand development mechanisms in plant.

In this study a morphological and ontogenetic description of the ramenta was made and the development and characteristics of the paraphyses were documented in order to resolve the question of the homology of this structure. The overall development of the reproductive branch after fertilization was followed for *Neckeropsis disticha* and

important developmental stages of reproductive branches were studied for other species of *Neckeropsis*.

LITERATURE REVIEW

Reproductive branches of *Neckeropsis*

Neckeropsis (Neckeraceae) is found as terrestrial or epiphytic plants or living on rocks, without ramification or irregularly branched to dendroid with asymmetrical leaves; perichaetia and perigonia are located on small branches on secondary stems. This is a genus of a wide distribution in the tropics, with two species in Puerto Rico. These two species, *N. disticha* and *N. undulata*, are different from the rest of the species in having in the perichaetia a leaf-like structure, the ramenta, as long as or longer than the capsule, and a long pseudopodium that covers part of the seta.

The nature of the ramenta has been controversial because it has been proposed that it consist of elongated perichaetial leaves or modified paraphyses; only one study has briefly described the development of this structure, but it could not clarify the homology. In that study Touw (1962) recognized two types of female gametoecia, each one divided into two subtypes:

> Type **1a** consists of perichaetial leaves that do not increase much in length after fertilization, surrounding the archegonia and the hyaline filiform paraphyses; new filiform paraphyses appear after fertilization and the seta reaches great length. Examples: *N. exserta* and *N. calcicola.*
>
> Type **1b** is the same as **1a** when young; after fertilization inner perichaetial leaves elongate to the maximum length before the complete development of the sporophyte, new filiform paraphyses form, the seta remains short and the capsule is enclosed between the elongated perichaetial leaves. Examples: *N. lepineana*, *N. obtusata, N. madecassa.*

Type **2a** contains hyaline filiform paraphyses, some with a width of two or three cells locally; after fertilization numerous paraphyses grow around the base of the fertilized archegonia, “transitions... are found between filiform and ligulate paraphyses, but it seems that the filiform paraphyses of the young gametoecia are not transformed into ligulate leaves.” The leaf-like paraphyses, most of them inserted in the vaginula, achieve their maximum length in the early stages of the sporophyte. Between the antheridia and the sporophyte the branch axis elongates, resulting in a pseudopodium that contains aborted archegonia and filiform paraphyses. The perichaetial leaves do not elongate significantly, the pseudopodium is as long as the sessile capsule and the ligulate paraphyses reach the mouth of the capsule. Example: *N. disticha* and *N. undulata*.

Type **2b** is very similar to **2a** except for these differences: the gametoecia are unisexual, before fertilization female branches have very short leaf-like paraphyses or none, after fertilization the leaf-like paraphyses are longer than the capsule, the pseudopodium is short or absent and the inner perichaetial leaves elongate greatly. Examples: *N. crinita, N. andamana, N. fimbriata.*

Touw recognized that the first type is common in other *Neckeraceae* but the second type is apparently limited to *Neckeropsis*. He also noted that *N. andamana* “looks like being surrounded by perichaetial leaves, but on closer examination, some of these leaves appear to be paraphyses, inserted in the vaginula and surrounded by a slender whorl of decrepit archegonia”; this illustrates that the distinction between leaves and

paraphyses is not clear. The other species mentioned is *N. nano-disticha,* in which he interpreted the gametoecia as a transition between **2a** and **2b**.

Sastre-De Jesús (1987) used the term ramenta to name the leaf-like structure around the sporophyte of *N. disticha* and *N. undulata*; this structure corresponds to the ligulate paraphyses of the gametoecia 2b described by Touw, but neither he nor Enroth (1993) use the term. Ramenta was apparently first used by Mitten (1869) to describe the inner structures (possibly interpreted as perichaetial leaves) in the perichaetia of *Neckeropsis disticha*.

Development of branches

Neckeropsis is a pleurocarpous moss; therefore, perichaetia are produced on secondary modules on lateral innovations, here referred to as reproductive branches. As described by La Farge-England (1996) fertile branches appear swollen and differentiated from vegetative branches; even juvenile leaves on perichaetia are different from juvenile leaves on vegetative branches. Like other branches, perichaetia present a heteroblastic leaf development where juvenile leaves are defined as the lower leaves on a branch that are produced before the mature leaves (Mishler & De Luna, 1991); young leaves are leaves in development and old leaves are fully developed leaves. In perichaetia the earlier leaves are referred to as outer perichaetial leaves and later juvenile leaves as inner perichaetial leaves.

The pattern of differentiation of the leaves (in the metamer) on a branch is determined by its position at the higher level (the module), following this progression the development of papillae was studied in *Tortula papillosissima* and independent

characters for papillae were identified (Mishler, 1987). Other examples exist of how the ontogenetic sequence presents an important criterion for determining polarity of transformation series in evolutionary studies; heterochrony is the study of “evolutionary change in the timing and rate of development” (Mishler & De Luna, 1991). Following the development of the reproductive branch this study intends to determine the homology of one of its components, the ramenta.

Green (1960) recognizes six stages of the sporophyte development and each one is divided into early and later phases. This classification is useful but modifications have to be made for mosses with immersed capsules, as is the case of many of the species of *Neckeropsis* studied here. The first stage is swollen venter (SV) in which the venter of the archegonium is enlarged and green while the neck is still noticeable and brown. In *Neckeropsis* this stage is evident because the archegonial neck is visible without dissecting the branch. Later in this stage *N. disticha* and other species present leaf-like paraphyses; for this reason the swollen venter stage was extensively studied. The next stage proposed is calyptra in perichaetium (CP), when the calyptra separates and emerges from the perichaetial leaves, following this is the phase calyptra intact (CI) where the seta elongates and later the capsule expands. After these early stages three mature stages are recognized: operculum intact (OI), operculum fallen (OF) and empty and fresh (EF) capsules that have opened recently.

For the purpose of this investigation five stages in the development of the sporophyte were established modified from Green (1960): 1) branches with fertilized archegonia or swollen venter (SV), 2) embryonic sporophyte the phase when the archegonia start to elongate before the separation of the calyptra is CP, 3) young

sporophytes that correspond to early CI where the capsule is not swollen and the seta starts to elongate, 4) immature sporophytes (green capsule with operculum) when the seta achieves its complete length and the capsule expands, and 5) mature sporophytes when the capsule is open. The last two stages are studied to compare the overall development of the paraphyses and the reproductive branches. In addition, a stage of mature fertile branches was established to compare the organs before fertilization.

Structures on the reproductive branches: paraphyses and axillary hairs

Reproductive branches in pleurocarpous mosses are formed by perichaetial leaves (or modified juvenile leaves) that enclose the gametangia. Antheridia and archegonia are produced from the apical cell of the branch, and paraphyses are frequently documented to be intermingled with them. The paraphyses are cell filaments associated with reproductive branches. Some define paraphyses as “filaments occurring side by side with antheridia” (Saito, 1975), others as hyaline uniseriate structures surrounding the archegonia before fertilization (Newton & De Luna, 1999), and others describe paraphyses in both male and female gametoecia (Janzen, 1921).

Other filamentous structures that can be found on the reproductive branches are axillary hairs; these are present in the axils of growing leaves and at branch apices. The difference between paraphyses and axillary hairs is primarily defined by position, and although axillary hairs have been defined for taxonomic purpose, morphological characters have not been pointed out. Saito (1975) stressed the necessity of a careful study of the morphology and origin of paraphyses before re-evaluating them as a taxonomic character.

MATERIALS AND METHODS

Development of the ramenta in *Neckeropsis disticha*

Fresh specimens of *N. disticha* were collected in Mayagüez and Ciales, with reproductive branches or sporophytes in various stages. Voucher specimens were label and deposit in the MAPR Herbarium. Six major stages were identified and collected: reproductive branches (archegonia without fertilization), branches with fertilized archegonia (swollen venter), embryonic sporophytes (before the separation of the calyptra and the vaginula), young sporophytes (capsule not swollen, still have the calyptra), immature sporophytes (green capsule), and mature sporophytes.

Fresh reproductive branches were examined in detail using the stereoscope and light microscope; in addition, permanent slides were prepared using both paraffin and plastic resin infiltration protocols.

Reproductive branches studied with the stereoscope were dissected to remove some of the perichaetial leaves and to expose the receptacle of the branch with the gametangia and paraphyses; specimens were placed on slides and studied under the light microscope. The morphology of the perichaetial leaves and the number of antheridia and archegonia were observed. The position, morphology, length and relative quantity of paraphyses were examined and recorded for each of the stages. The characters taken into account for describing the paraphyses were the thickness of the cellular wall, the relative size and number of the cells in each paraphysis, the color (hyaline, chlorophyllose, red-brown), the width of cells (the lines or series of cells), and the form of the paraphyses (regular, irregular, filiform, ligulate).

On fertilized branches, the receptacle that sustains the gametangia was described and the appearance and development of the pseudopodium was followed in all stages. The elongation of the perichaetial leaves, the position of the gametangia and paraphyses, and the types of paraphyses present were examined from the early-fertilized stages through the maturation of the sporophyte.

Reproductive branches with and without fertilized archegonia and embryonic sporophytes were infiltrated in paraffin (based on Johansen, 1940) and plastic resin (JB-4 Plus Embedding Kit from EMS) to prepare permanent slides. Selected branches were fixed in formalin acetyl alcohol (FAA) for at least 24 hours, then dehydrated through an ethanol dilution series, including concentrations of 65%, 75%, 85%, 95%, and three changes of 100% ethanol (15-20 minutes between changes). To prepare samples for paraffin, serial changes were made from ethanol to tert-butyl alcohol TBA (25% TBA:75% ethanol, 50% TBA:50% ethanol, 75% TBA:25% ethanol), and three changes of 100% TBA. Paraffin infiltration was done gradually in an oven (55ºC) over a period not longer than three hours. Samples were embedded and solidified under refrigeration for 24 hours, then mounted on wood blocks for sectioning. Mounted samples were kept in the refrigerator or in a beaker with iced water.

The samples in paraffin were sectioned longitudinally with a width of 8-11 µm using a rotary microtome; the resulting ribbons were floated on water-flooded slides on a warm plate until dry, stained using the Safranin-Fast Green protocol (Johansen, 1940, modified), and mounted permanently with Permount.

For the plastic resin procedure, samples in 100% alcohol were infiltrated with Solution A of the JB-4 resin in vials completely covered with aluminum foil and on a

rotator. The infiltration was done through 12 changes, with 4 to 6 hours between changes, replacing 1/4th of the total liquid with fresh infiltration solution. Three additional changes were done removing almost all the liquid and replacing with infiltration solution; the first two changes were done waiting 8 hours between these changes and the third was left for 24 hours. The embedding solution was added and samples were kept in the refrigerator for 12 hours to slowly polymerize; later, if solidification was not complete, they were put in a vacuum oven.

Reproductive branches infiltrated in plastic resin were sectioned longitudinally and transversely using an ultramicrotome. The slides were cleaned with 95% ethanol and 10% HCl and prepared on a slide warmer; on each slide 2-3 lines of drops of water were placed and one piece of sample was placed on each drop. The slides were kept on the slide warmer until dry, and then were stained with Toluidine Blue for 5 seconds and washed in water 4-5 times for 15 minutes. Slides were let to dry until the next day and then mounted with Permount.

Slides were examined under a light microscope using bright field microscopy and were microphotographed using a digital camera (Spot Insight Color). Identification of the components of the reproductive branches was made when possible, specifically the antheridia, archegonia (fertilized or not), paraphyses (uniseriate or multiseriate), perichaetial leaves and receptacle.

Description of the reproductive branches of various species of *Neckeropsis*

Neckeropsis species were selected from the list that appears in Brotherus (1925), eliminating non-valid names or synonyms; and including representation of the four types

of female gametoecia described by Touw (1962) and the three sections identified by Brotherus (1925). Species selected were described from the Neotropics (Sastre-De Jesús, 1987), Africa (Enroth, 1993), Asia and the Pacific (Touw, 1962). A loan of non-American species was obtained from to different herbaria through the MAPR Herbarium.

Reproductive branches of each species were studied under the stereoscope and light microscope and microphotographed. Samples were selected for each species: mature perichaetia and/or perigonium, early fertilized or juvenile (when available) and mature sporophyte. The features described for the reproductive branches were the relative number of antheridia or archegonia (or both), the presence or absence and relative number of paraphyses and some features of the leaves surrounding the gametoecia such as the relative length and number of leaves, and the position of the leaves (inner or outer leaves).

On fertilized branches and mature sporophytes the relative length of the perichaetial leaves, the base of the branch and the presence or absence of a pseudopodium and ramenta (or paraphyses) were examined. On mature sporophytes, the receptacle of the reproductive branch was studied; also were documented the length of the seta enclosed within the pseudopodium, the length of the foot of the sporophyte, and the distribution of the gametangia, perichaetial leaves and paraphyses (ramenta) along the elongated receptacle.

In all stages the characters described for the paraphyses were the morphology, the thickness of the cellular wall, the relative size and number of the cells in each paraphysis, the color (hyaline, chlorophyllose, red-brown), the width of cells (the lines or series of cells) and the form of the paraphyses (regular, irregular, filiform, ligulate).

The data were collected for each species and from the studied stages a developmental hypothesis for the paraphyses on the fertilized branch was developed. Later the species were compared to synthesize a general developmental idea for the paraphyses in *Neckeropsis* that can be applied to other bryophytes groups.

Table 1. List of non-American species examined. All specimens are from the Leiden Herbarium (L).

Species	Specimen
N. andamana	*Bartlett 14666* *Williams s.n.*
N. crinita	*Touw 8440*
N. exserta	*Thailand & Touw 8439* *Touw 9439* *Touw 9500* *Touw 10805*
N. fimbriata	*Den Hoed 460* *Touw 8139*
N. lepineana	*Hennipman 5246*
N. nano-disticha	*Weber 1290*
N. nitidula	*Touw 18914* *Touw 19111*
N. obtusata	*Touw s.n.* *Touw 18913* *Touw 19168*

Table 2. List of American species examined. All specimens are deposited in MAPR.

Species	Specimen
N. disticha	*Sastre & Merced 3284* *Sastre 3285*
N. undulata	*Sastre 3283*

RESULTS

Development of the reproductive branch of *N. disticha*

The reproductive branches of *N. disticha* are mostly synoicous, although male branches were also found. The perichaetia consist of 8-10 leaves of which the outer leaves are the early juvenile leaves and the inner are the later juvenile leaves (following Mishler & De Luna, 1991). The inner perichaetial leaves are gradually longer (up to more than twice) than the outer perichaetial leaves (Fig. 1). Before fertilization paraphyses are hyaline and with one line of cells of thin walls; the paraphyses are intermingled with the antheridia and archegonia and inserted at the base of the reproductive branch (Fig. 2-3).

After fertilization new uniseriate paraphyses appear; these are short (2-4 cells) and reddish with thin cell walls (Fig. 4). At a very early stage, the hyaline paraphyses start to divide at the base; both anticlinal and periclinal cell divisions were found. In addition the reproductive branch has new structures growing that appear to be gametangia (Fig. 4b). Following this, numerous hyaline paraphyses were recorded. Later, in the swollen venter stage, longer chlorophyllose paraphyses of 2-5 lines of cells were documented (Fig. 5) and small leaf-like structures were identified as multiseriate paraphyses (Fig. 6); these appeared around the base of the fertilized archegonium. The paraphyses range from uniseriate to two or more cells at some point and to slim multiseriate paraphyses of different width but of thick cell walls (Fig. 7). Some of these multiseriate paraphyses present a narrow base that consists of one or two reddish cells; others present a broad base with several reddish cells. In this stage, paraphyses present great variation and appear as transitional phases (Fig. 8-9); the base remains flat and the perichaetial leaves elongate a little (Fig. 10).

In a transverse section of the branch with fertilized archegonia, various types of paraphyses could be detected (Fig. 11). The distal part of the branch presents the perichaetial leaves enclosing the paraphyses and two fertilized archegonia, one is more developed and the other is smaller and only the neck is visible. The paraphyses are the solitary structures with only one cell, probably uniseriate paraphyses or/and the end of other types of paraphyses. In the next section, paraphyses are numerous and uniseriate; biseriate and multiseriate paraphyses could also be identified. These were organized around the fertilized archegonia, where the multiseriate paraphyses (4-11cells) are located closer and then the biseriate paraphyses; the uniseriate paraphyses are found everywhere on the branch.

After the venter of the fertilized archegonium is swollen it starts to elongate before the formation of the calyptra (Fig. 12); at this stage the ramenta is noticeable and consists of light green multiseriate paraphyses longer than the perichaetial leaves. The uniseriate and biseriate paraphyses are less obvious because they are fewer than in the earlier stages. The axis of the reproductive branch starts to elongate, mainly between the fertilized archegonia and the perichaetial leaves, but the section of the leaves presents a small separation (Fig. 13-14).

In the next stage, when the calyptra separates from the vaginula, the pseudopodium has almost reached its complete length and the ramenta is nearly as long as the sporophyte. Uniseriate paraphyses are visible intermingled with the aborted archegonia along the pseudopodium; none of the biseriate or thinner multiseriate paraphyses (more or less 4-6 lines of cells) could be detected. The seta began to grow and achieved its complete length at the next stage; after that the capsule expands and the

pseudopodium reached its total length. While the sporophyte matures the uniseriate paraphyses become scarcer. At this time the ramenta consists of more than 20 multiseriate paraphyses, generally of 10-15 but up to 23 lines of cells (Fig. 15).

Description of the reproductive branches in *Neckeropsis*

The male reproductive branches of *Neckeropsis* have short perigonial leaves and in some cases the inner leaves were longer than the outer leaves (*N. crinita, N. nano-disticha* and *N. undulata*); all specimens examined contain short hyaline paraphyses (8-12 cells) consisting of only one row of cells with thin to regular cell walls (Fig. 16-17). In *N. fimbriata* and *N. undulata* paraphyses of two (Fig. 18) to three or more lines of cells were observed; also in these two and *N. nano-disticha* a few small leaves (or leaf-like structures) were located inside the inner perigonial leaves; these leaves were as long as or longer than the antheridia (Fig. 19). The male reproductive branch for *N. andamana* was not examined because no material was available.

The female reproductive branches sometimes is different from the male and the difference between the inner and outer perichaetial leaves is more noticeable; both branches consist of about ten ovate leaves (except the perigonium of *N. lepineana* that is more robust). The inner perichaetial leaves are more than twice as long as the outer leaves; in some species the inner perichaetial leaves are more elongated than the rest (*N. andamana* and *N. crinita*). All reproductive branches contain very short uniseriate paraphyses (6-10 cells) (Fig. 20) but shorter paraphyses of 2-4 cells were present in *N. fimbriata, N. andamana* and *N. crinita* (Fig. 21). The last types of paraphyses were hyaline to reddish in color and were not intermingled with the archegonia but located

near the axial part of the inner perichaetial leaves. In contrast to the male reproductive branches, the female branches contain fewer and shorter paraphyses. The small leaf-like structures identified inside the perigonia of some species was also present in the perichaetia of *N. obtusata*, *N. crinita* and *N. nano-disticha* (Fig. 22), but only the last one contains them on both reproductive branches. Other types of paraphyses in *N. nano-disticha* present one and two lines of cells and transitions between these, on these branches fertilized archegonia were not located.

Branches with fertilized archegonium were examined for some species. In *N. lepineana*, *N. obtusata* and *N. nitidula* the perichaetial leaves were considerably more elongated than before fertilization; these species also have multiple hyaline to light green paraphyses of only one row of cells (Fig. 23). The paraphyses of *N. lepineana* were longer (more than three times longer than before fertilization) and of a thicker cell wall than *N. obtusata*, in which the paraphyses were only a little longer than the archegonia. In *N. nitidula* different kinds of paraphyses were identified: short paraphyses of few (8) long cells of thin walls and long paraphyses (>20) of short cells, some of these paraphyses of thin cell walls and other with thick walls (Fig. 24).

Two species in which the leaf-like paraphyses or ramenta were studied and documented in various stages. For *N. andamana* the branch that contains the very early-fertilized archegonium presents a few small hyaline to reddish paraphyses and some new multicellular structures like gametangia (Fig. 25). A later stage, after the separation of the calyptra, already presents the ramenta that looks like ligulate to lanceolate leaves of different widths and not completely developed. The archegonia are located between these structures and the elongated perigonial leaves, and the base that has them does not

elongate much (Fig. 26). The branches with the fertilized archegonia of *N. undulata* are elongated and the ramenta could be seen. On these branches various types of paraphyses are present: new uniseriate paraphyses of thick cell walls, paraphyses with one or two lines of cells and biseriate paraphyses that at the end are multiseriate (Fig. 27). At the stage of the juvenile sporophyte the branch contains a great number of multiseriate paraphyses (ramenta), some were wide as on the mature branches and many others were slim and biseriate; in this stage the ramenta is more heterogeneous and presents many transitions (Fig. 28).

The branches with mature sporophyte were examined in detail; some species have the leaf-like paraphyses, others very elongated leaves and one species has none of these modifications. Another variation among the species studied was the presence and characteristics of the pseudopodium, which consists of an elongation of the reproductive branch axis that enclosed the foot and covers part of the seta. This elongation could occur between the perichaetial leaves or at the end of the branch where the base has the paraphyses and aborted archegonia.

Neckeropsis exserta presents an elongated base that encloses a small portion of the seta; the elongation occur at the end of the branch leaving the barely elongated perichaetial leaves at the lower part of the axis (Fig. 29 a-b). The pseudopodium has the aborted archegonia and antheridia located above the region of the leaves (but not all over the axis) and multiple hyaline paraphyses that reach the lower part of the capsule (>10 cells); these paraphyses consist of only one line of cells of thick walls (Fig. 29 c-d). Other species that present uniseriate hyaline paraphyses are *N. nitidula* (Fig. 30), *N. obtusata* (Fig. 31) and *N. lepineana*; these paraphyses are longer than the archegonia, reaching the

lower part of the capsule, except in *N. lepineana* where paraphyses are much longer (Fig. 32). In these three species the perichaetial leaves are considerably elongated, reaching beyond the mouth of the capsule, and the paraphyses and archegonia are restricted to the upper part of the reproductive axis. The base is hardly elongated in *N. nitidula*, enclosing only the foot, but in *N. lepineana* and *N. obtusata* the base is elongated between the perichaetial leaves, enclosing part of the seta.

The rest of the species examined have the ramenta or leaf-like paraphyses although they varied in the width of the multiseriate paraphyses, the elongation of the perichaetial leaves and the length of the reproductive axis (pseudopodium). *Neckeropsis nano-disticha* has narrow multiseriate paraphyses (5-7 lines of cells at the base) that reach beyond the mouth of the capsule; these numerous paraphyses are light green to yellow and are located intermingled with the old antheridia and remnants of uniseriate paraphyses on the elongated pseudopodium (Fig. 33). The reproductive branch has at the base the perichaetial leaves; some of the inner leaves elongate considerably reaching half the length of the capsule. *Neckeropsis crinita* has also slim multiseriate paraphyses and elongated perichaetial leaves, but a shorter pseudopodium (and seta) that bears the aborted archegonia and short uniseriate paraphyses (Fig. 34). The multiseriate paraphyses of *N. nano-disticha* have thick and smooth cell walls but in *N. crinita* the paraphyses have thick walls with papillae, making the ramenta serrate, and at the base small ramifications (1-2 cells) could be seen (Fig. 34 c-d).

The sporophyte and reproductive branch of *N. undulata* is similar to *N. disticha*; the inner perichaetial leaves elongate after fertilization and the pseudopodium is elongated, holding the old archegonia and paraphyses and enclosing great part of the seta.

The multiseriate paraphyses are wider than in *N. nano-disticha* and *N. crinita* (10-15 lines of cells), but as long as in the other species. In the mature sporophyte most of the uniseriate and biseriate paraphyses are gone and the branch looks less robust than at earlier stages.

The last two species studied (*N. andamana* and *N. fimbriata*) have the wider type of multiseriate paraphyses that look like ligulate to lanceolate leaves located under the vaginula. The reproductive branch presents little increase in length; the elongated perichaetial leaves are located at the base and the small pseudopodium occurs at the end of the branch, bearing the archegonia and remains of uniseriate paraphyses that are distributed over the area within the multiseriate paraphyses. The multiseriate paraphyses or ramenta are restricted to the upper part of the axis and the differentiation from the leaves is evident; the short pseudopodium contains only the foot and the minute seta makes the sporophyte immersed. The ramenta of *N. andamana* have folds near the margins that are serrated and some appear to have costae (also documented by Touw, 1962) but on closer examination this could not be confirmed (Fig. 35). Between the ramenta small (2-3 cells) uniseriate paraphyses were found. The cells of the inner perichaetial leaves are similar to the cells of the multiseriate paraphyses (Fig. 36), but the last ones were wider. The ramenta of *N. fimbriata* are serrated like the perichaetial leaves and before the complete maturation of the sporophyte new multiseriate paraphyses appear (Fig. 37).

DISCUSSION

In *N. disticha* the development of the leaf-like structures, the ramenta, was followed by examination of consecutive growing stages of the reproductive branches. This was done to establish the origin and homology of this structure. Before fertilization paraphyses are short, hyaline and uniseriate; after fertilization these paraphyses start to elongate and broaden, to form irregular paraphyses that at some point are uniseriate and at others biseriate or of three or more lines of cells. During the swollen venter stage until the separation of the calyptra, various types of paraphyses develop; Touw (1962) interpreted these as transitions. Although widening of the paraphyses occurs these do not form part of the ramenta and, like the other slender multiseriate paraphyses that appear later, they are not present at the mature sporophyte stage.

New uniseriate paraphyses appear and these are probably the ones present until the complete development of the sporophyte. The leaf-like structures that start to grow at the early stages seem to be leaves and their development appears to be similar to the cell divisions of leaves, where the apical cell divides in series to form a structure (young leaf) more or less correlated with the form of the apical cell, and like leaves the apical cell is the first that stops dividing and starts to differentiate (Bopp, 1984). But these structures do not develop from a primordium (bud) of a metamer because the apical cell of the reproductive branch stopped growing and the gametangia differentiates from the apical cell (Mishler & De Luna, 1991; La Farge-England, 1996).

These leaf-like structures should be considered multiseriate paraphyses because of the position where they develop and their similarity to the multiseriate paraphyses that develop before them. If the great variations of paraphyses that occur early are transitions,

then a heterochronic sequence is reasonable where the new multiseriate paraphyses are the young paraphyses from a mature stage, while the biseriate and other paraphyses are juvenile forms. The resulting fully developed mature paraphyses form the ramenta. In contrast to the heteroblastic development of leaves on a branch, the developmental sequence of the paraphyses does not remains on the branch and is not visible at the same time because the transitional stages fall. And similar to the heteroblastic development, the sequence of paraphyses is arranged around the fertilized archegonium, leaving the multiseriate paraphyses nearer and the other transitions farther from it.

The reproductive branch elongates mainly between the inner perichaetial leaves and the fertilized arquegonium; this section of the axis is called the pseudopodium and contains the foot and part of the seta. The pseudopodium in Sphagnidae is similar because the elongation occurs between the perichaetial leaves and the fertilized archegonia. It differs in an enlargement (more than elongation) of the distal part of the pseudopodium that encloses the foot of the sporophyte; this enlarged base holds the remaining archegonia. In Andreaeidae the elongation of the pseudopodium is greater and the aborted archegonia remain at the base of the pseudopodium (Roth, 1969). In both Sphagnidae and Andreaeidae, the pseudopodium consists of an extension of the axis of the reproductive branch resulting in a structure like a stem that at the end contains the foot; in contrast the pseudopodium of *Neckeropsis* is an elongation of the axis as a lamina that encloses the foot and part of the seta. According to the description of this character in Newton & De Luna (1999) the pseudopodium of *Neckeropsis* would be the developed vaginula.

For the other species of *Neckeropsis* studied the heterochronic sequence of development for the paraphyses can be applied. The first type of paraphyses to appear are uniseriate and hyaline; these would be considered the earlier juvenile phase of paraphyses, first the paraphyses with thin cell walls and then the thick-walled ones. After this the biseriate and thin multiseriate paraphyses are identified as the late juvenile paraphyses. The mature phases of paraphyses would be the multiseriate leaf-like paraphyses; the early stage is noticeable as the fine long paraphyses in *N. nano-disticha* and *N. crinita*, the next stage would correspond to the wider paraphyses of *N. disticha* and *N. undulata*, and finally the late mature paraphyses would be the ligulate to lanceolate structures in *N. fimbriata* and *N. andamana*. Between these phases numerous transitions are present.

For the species that develop the juvenile but not the mature type of paraphyses, peramorphosis or recapitulation can be used to explain the difference between the species with ramenta. Peramorphosis is when a juvenile descendant has the form of the mature ancestor (Mishler & De Luna, 1991), but this would imply that a close evolutionary relationship exists between *N. nano-disticha* and *N. crinita, N. undulata* and *N. disticha,* and *N. andamana* and *N. fimbriata*; where the first present a plesiomorphic state for this character and the others the apomorphic states.

Neckeropsis nitidula, N. obtusata and *N. lepineana* present only the juvenile types of paraphyses and perichaetial leaves elongate as occure after fertilization as been documented in other pleurocarpous species as modifications involved after fertilization. The growth of the stem apex around the foot and differentiation of the vaginula are present in other taxa (Newton & De Luna, 1999). The elongation of the axis of *N. exserta*

is here identified as a pseudopodium because it is consistent with the definition and the paraphyses are in the juvenile stage. The short paraphyses (2-4 cells) located near the inner perichaetial leaves of *N. fimbriata, N. andamana* and *N. crinita*, could be considered axillary hairs following the definition of Saito (1975); also these filamentous structures do not seem to be part of the heterochronic sequence.

An interesting point is that some non-fertilized reproductive branches have biseriate and multiseriate paraphyses, as is the case of the perigonia of *N. fimbriata, N. nano-disticha* and *N. undulata* and the perichaetia of *N. obtusata, N. crinita* and *N. nano-disticha*. A possible explanation is that the heterochronic development of paraphyses is independent of fertilization but depends on the viable time of the fertile branch. However fertilization prolongs and accelerates the maturation of the branch and in consequence of the paraphyses.

CONCLUSIONS

The homology of the leaf-like structure, the ramenta, was clarified. Different types of paraphyses during the maturation of the fertilized archegonium represent separate stages in the development of the paraphyses. A heterochronic sequence homologous to the heteroblastic development of leaves was identified for paraphyses. The numerous transitions found are phases between juvenile (uniseriate) and mature (multiseriate) paraphyses.

The ramenta consist of the mature multiseriate paraphyses; these present a progression in various species. In *N. nano-disticha* and *N. crinita* the ramenta is thinner and is fully developed at the early mature stage, the paraphyses of *N. undulata* and *N. disticha* are in a middle phase wider than the previous two species. *Neckeropsis andamana* and *N. fimbriata* present the late mature stage with paraphyses that look like slim lanceolate leaves. To avoid confusion the term rameta should be disused.

The pseudopodium in *Neckeropsis* was defined as an elongation of the axis or developed vaginula resulting in a lamina that encloses the foot and part of the seta. In contrast, the pseudopodium in Sphagnidae and Andreaeidae is an extension of the apical part of the reproductive branch as a structure like a stem that at the end contains the foot.

The ontogenetic data provided would be useful for the phylogenetic analysis of the genus *Neckeropsis*, but this information can also be applied to understand paraphyses and other structures in other moss taxa. More ontogenetic studies should be promoted to elucidate other problematic structures.

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Plate I

N. disticha

Figure 1. Perichaetial leaves of *N. disticha* showing the outer perichaetial leaves (opl) and the inner perichaetial leaves (ipl)

Figure 2. Reproductive branch processed in paraffin; the antheridia (ant) are shown in blue inserted on the branch base (b); also shown are the outer perichaetial leaves (opl)

Figure 3. Reproductive branch processed in plastic; inside the uniseriated paraphyses (p), the antheridia (ant) and archegonia (arc)

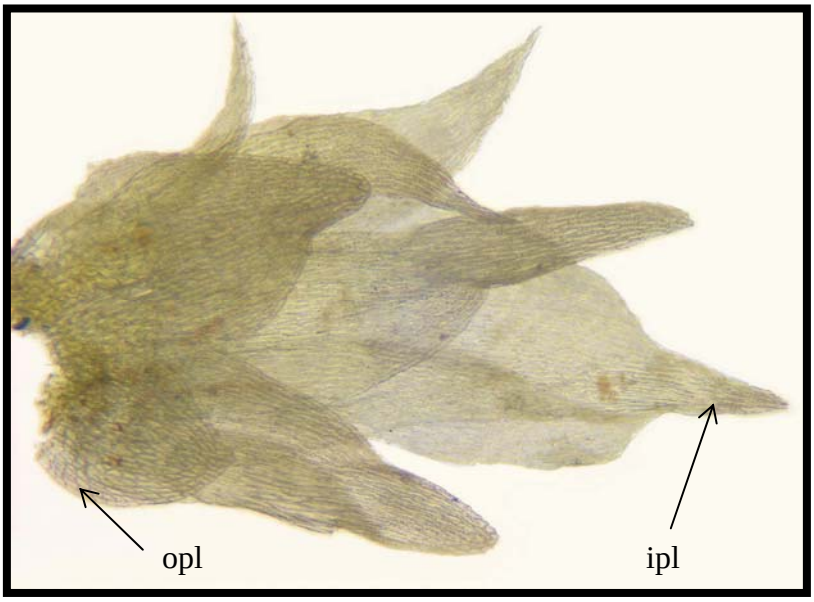
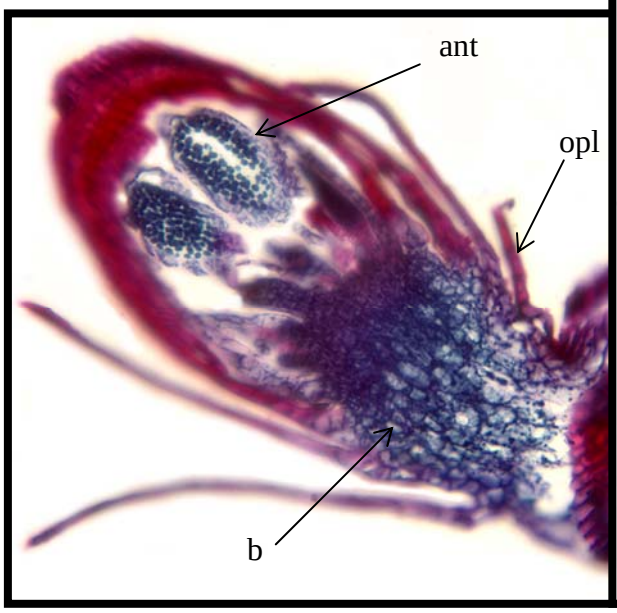


Figure 1



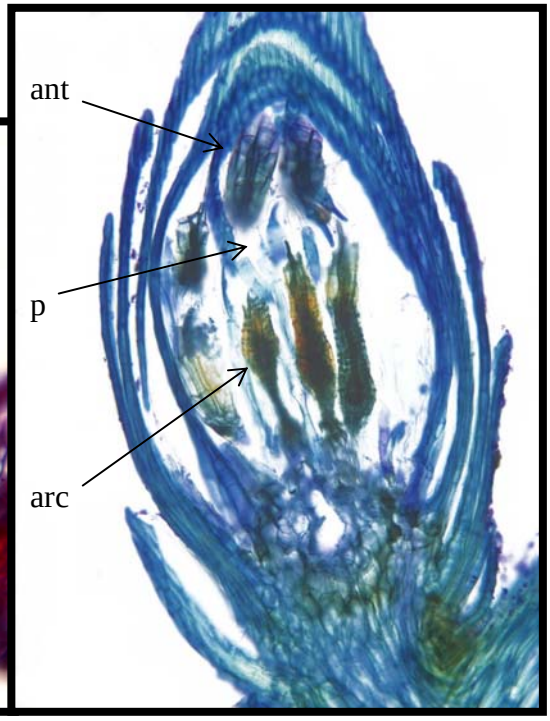


Figure 2 Figure 3

Plate II

N. disticha

Figure 4. Reproductive branch with fertilized archegonium

a) uniseriated paraphyses (p), new paraphyses (np) and new structures (ns) that appears to be gametangia
b) paraphyses with periclinal (pw) and anticlinal (aw) cell wall division

Figure 5. Reproductive branch with fertilized archegonium and chlorophyllose paraphyses: uniseriate (cp), biseriate (bp) and slim multiseriate (smp)

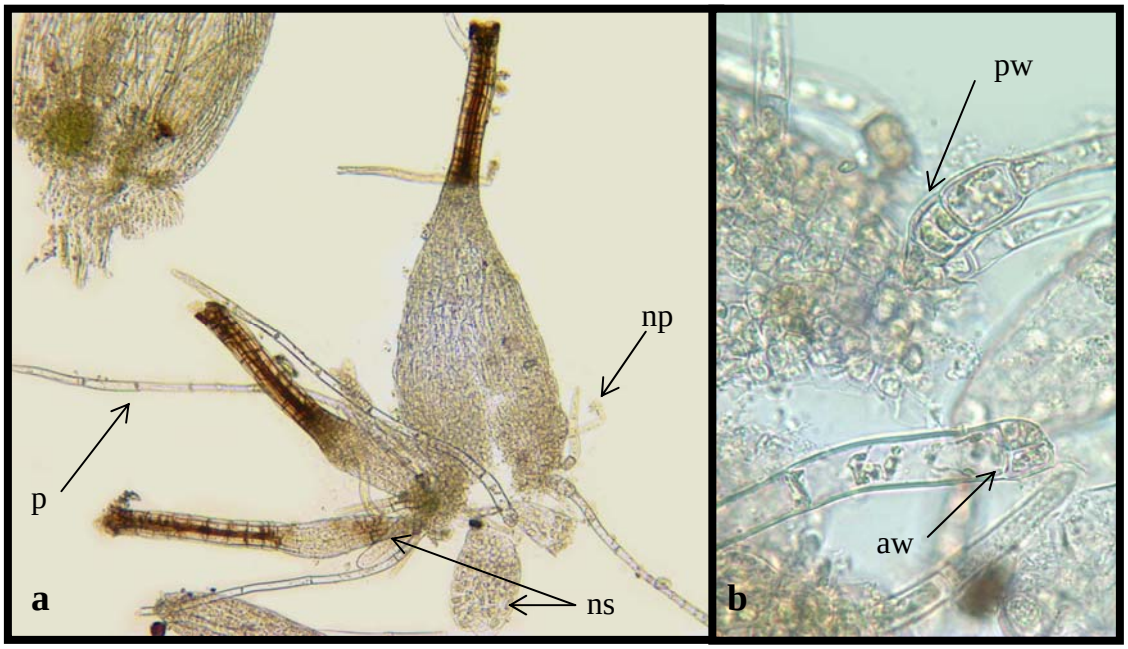


Figure 4

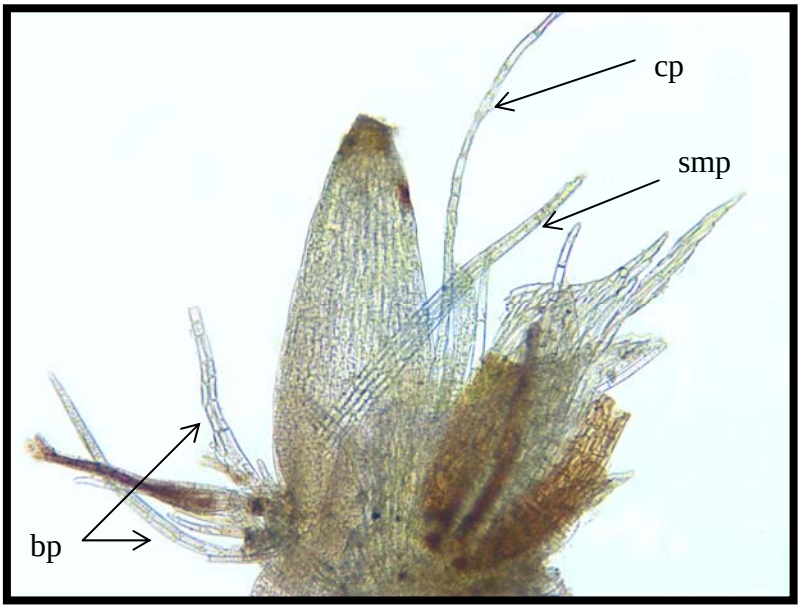


Figure 5

Plate III

N. disticha

Figure 6. Reproductive branch with fertilized archegonium and uniseriate paraphyses (p), new reddish paraphyses (np), biseriate paraphyses (bp), multiseriate paraphyses (mp)

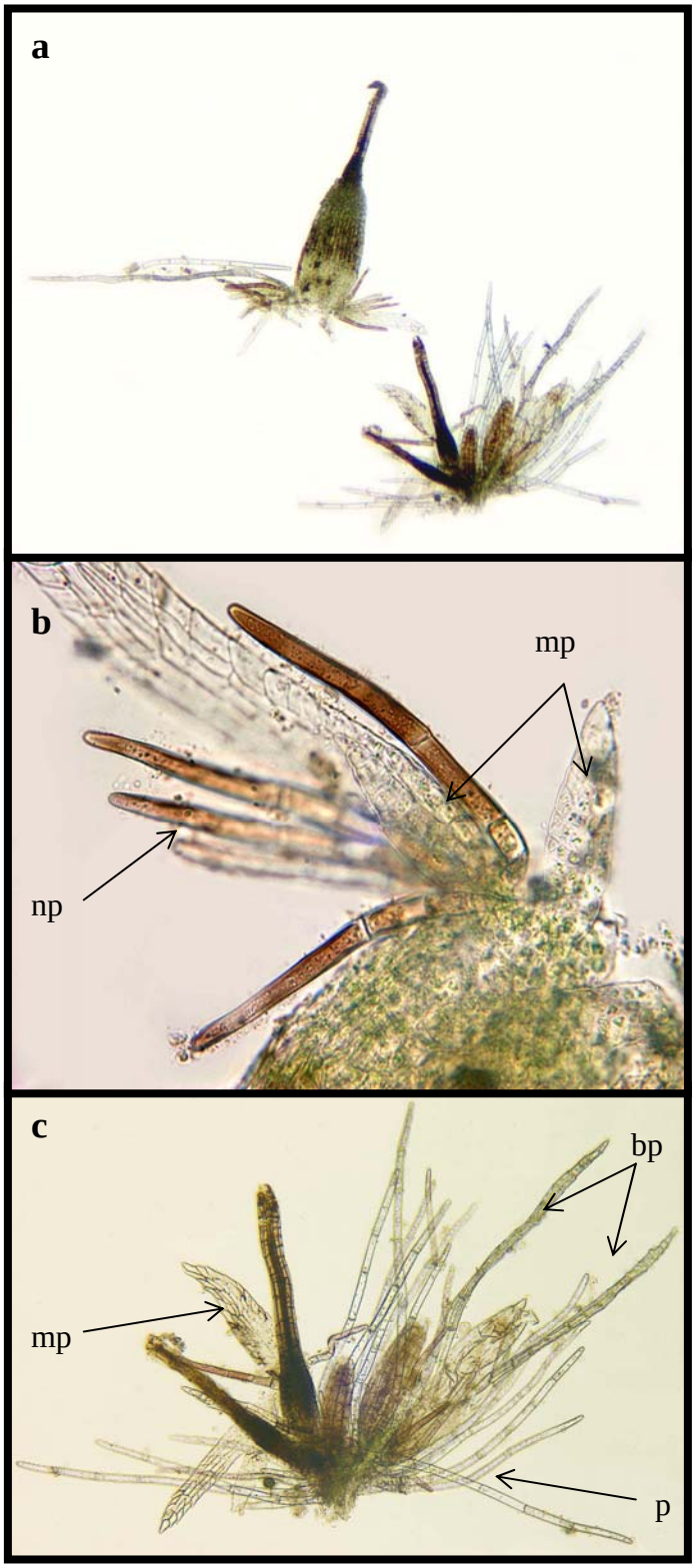


Figure 6

Plate IV

N. disticha

Figure 7. Irregular paraphyses of one to multiple rows of cells

Figure 8. Irregular uniseriate paraphysis with a broadening of various cells at the end

Figure 9. Various types (stages) of paraphyses occurring at the same time

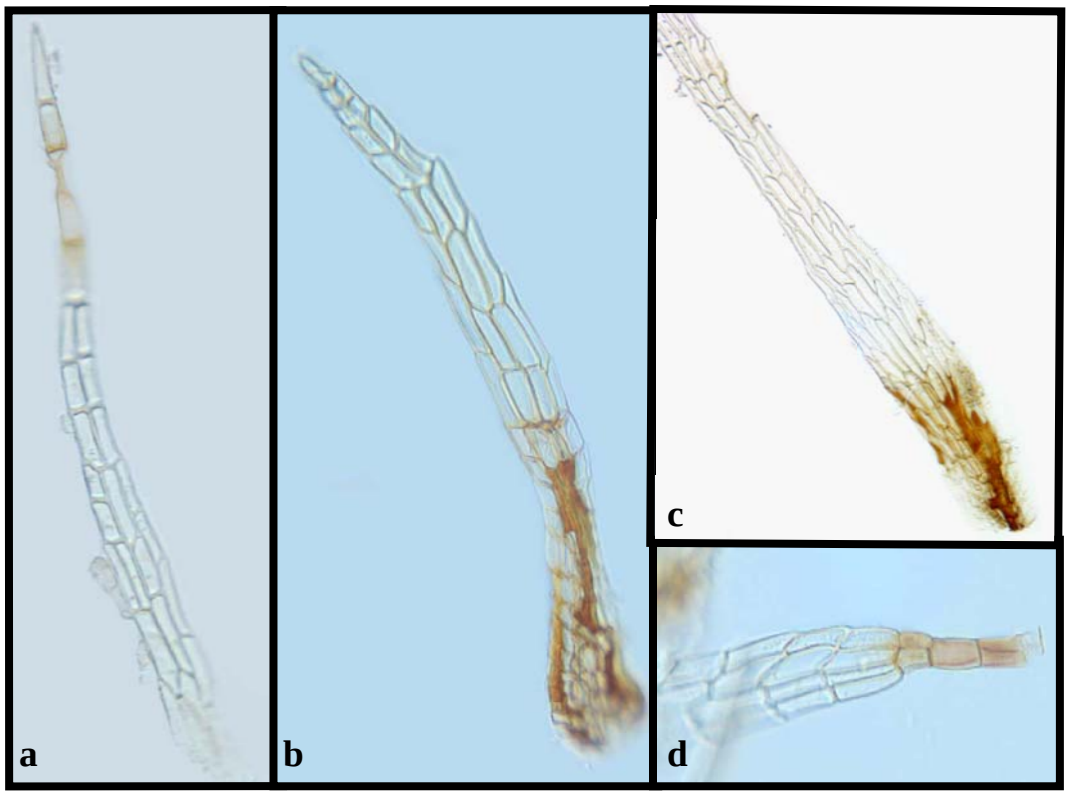
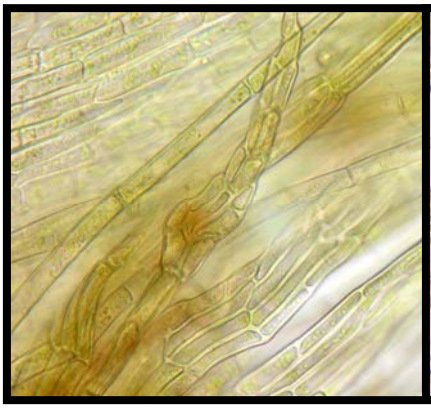


Figure 7



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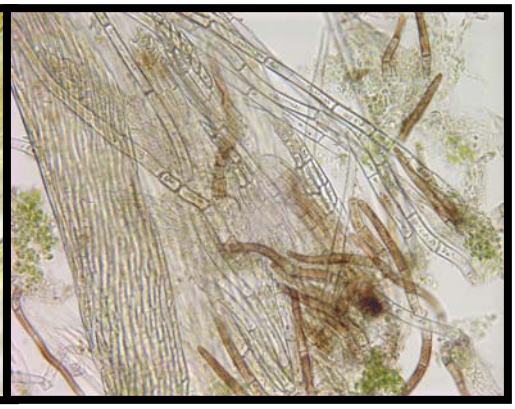


Figure 9

Plate V

N. disticha

Figure 10. Reproductive branch with fertilized archegonium, processed using the plastic resin protocol

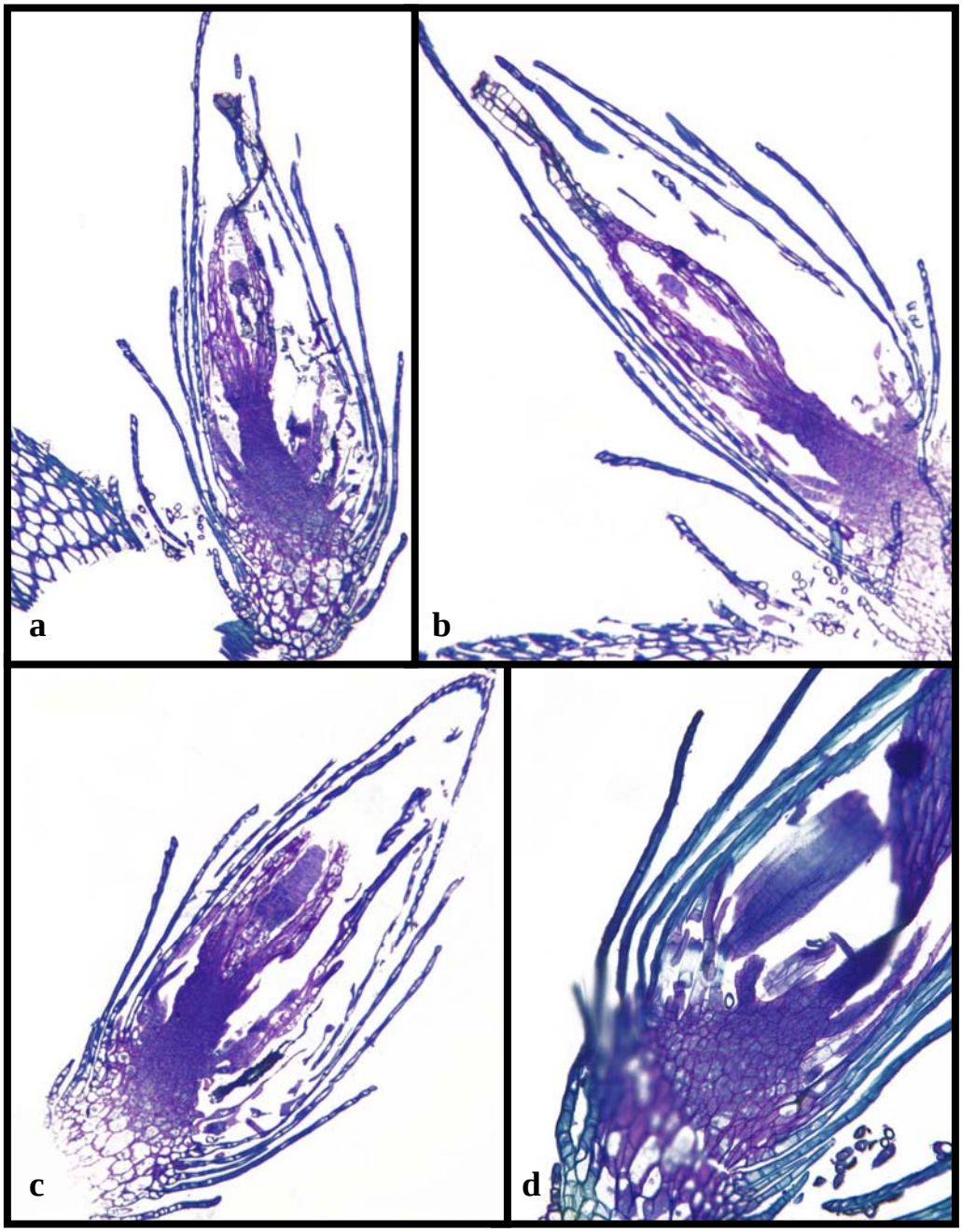


Figure 10

Plate VI

N. disticha

Figure 11. Transverse section of reproductive branch with fertilized archegonia, processed in plastic resin

a) upper section of the branch showing the perichaetial leaves (pl) with two fertilized archegonia (fa), one more developed or dominant (dfa) than the other where only the archegonial neck (an) is visible.
b) a medial section showing the fertilized archegonia (fa) surrounded by the multiseriate paraphyses (mp) and uniseriate paraphyses (p)
c) a lower medial section showing the arrangement of the branch, the perichaetial leaves (pl) and the fertilized archegonia (fa, fda)
d) the lower medial section as in c that on closer examination presents the arrangement of multiseriate (mp) and uniseriate (p) paraphyses
e) the lower medial section with biseriate paraphyses (bp), small multiseriate paraphyses (smp) and uniseriate paraphyses (p)
f) the lower medial section with wider multiseriate paraphyses (mp) of 10 cells near the fertilized archegonium (fa)

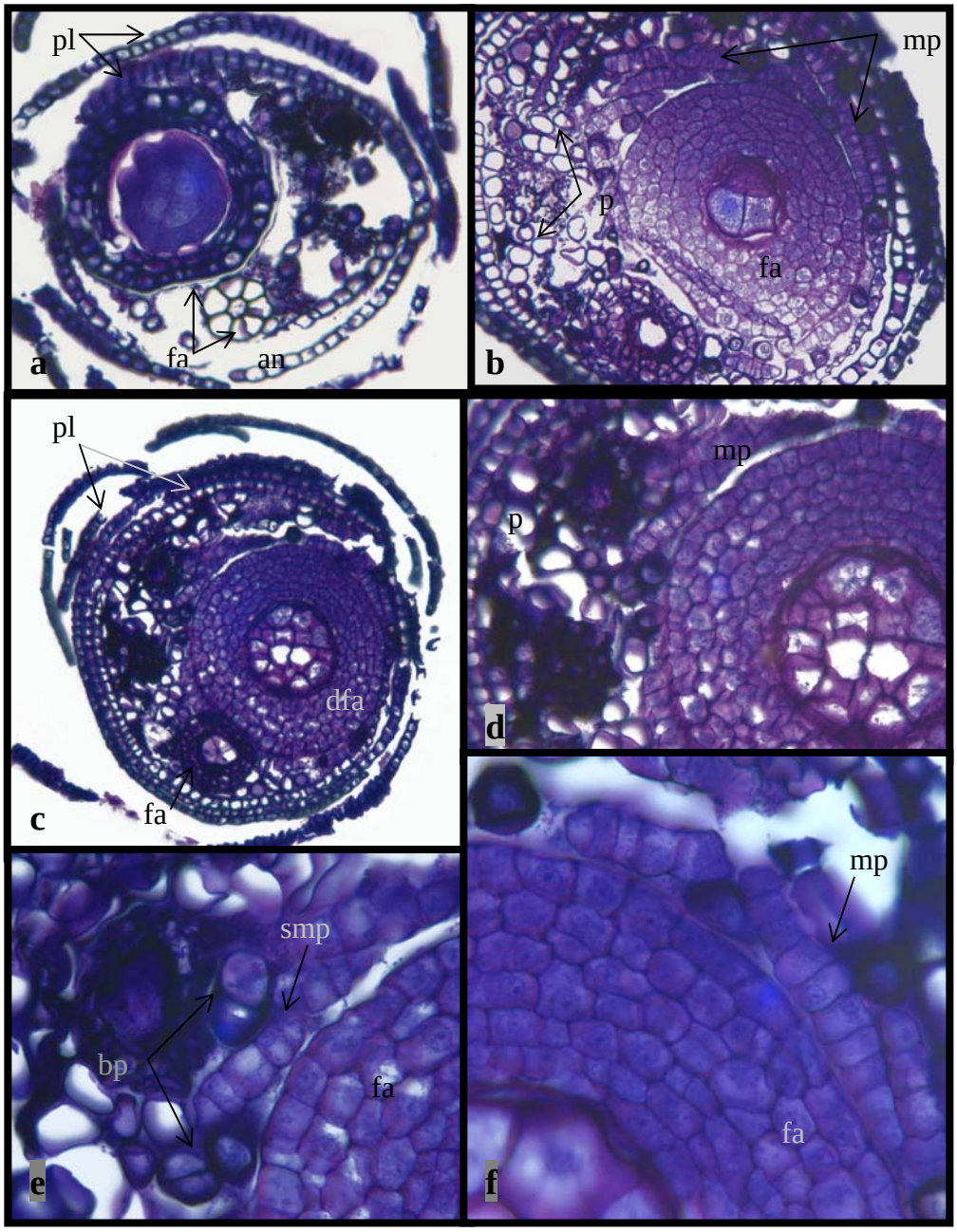


Figure 11

Plate VII

N. disticha

Figure 12. Branch with young sporophyte, in the stage before the separation of the calyptra; processed using the paraffin protocol

a) the young sporophyte (s) showing the pre-calyptra and the pseudopodium (ps) in contrast to the base of the branch (b)
b) the sporophyte foot (sf) inserted in the pseudopodium; the aborted archegonia are located at the base above the perichaetial leaves (pl)
c) a paraphysis (p) located at the pseudopodium

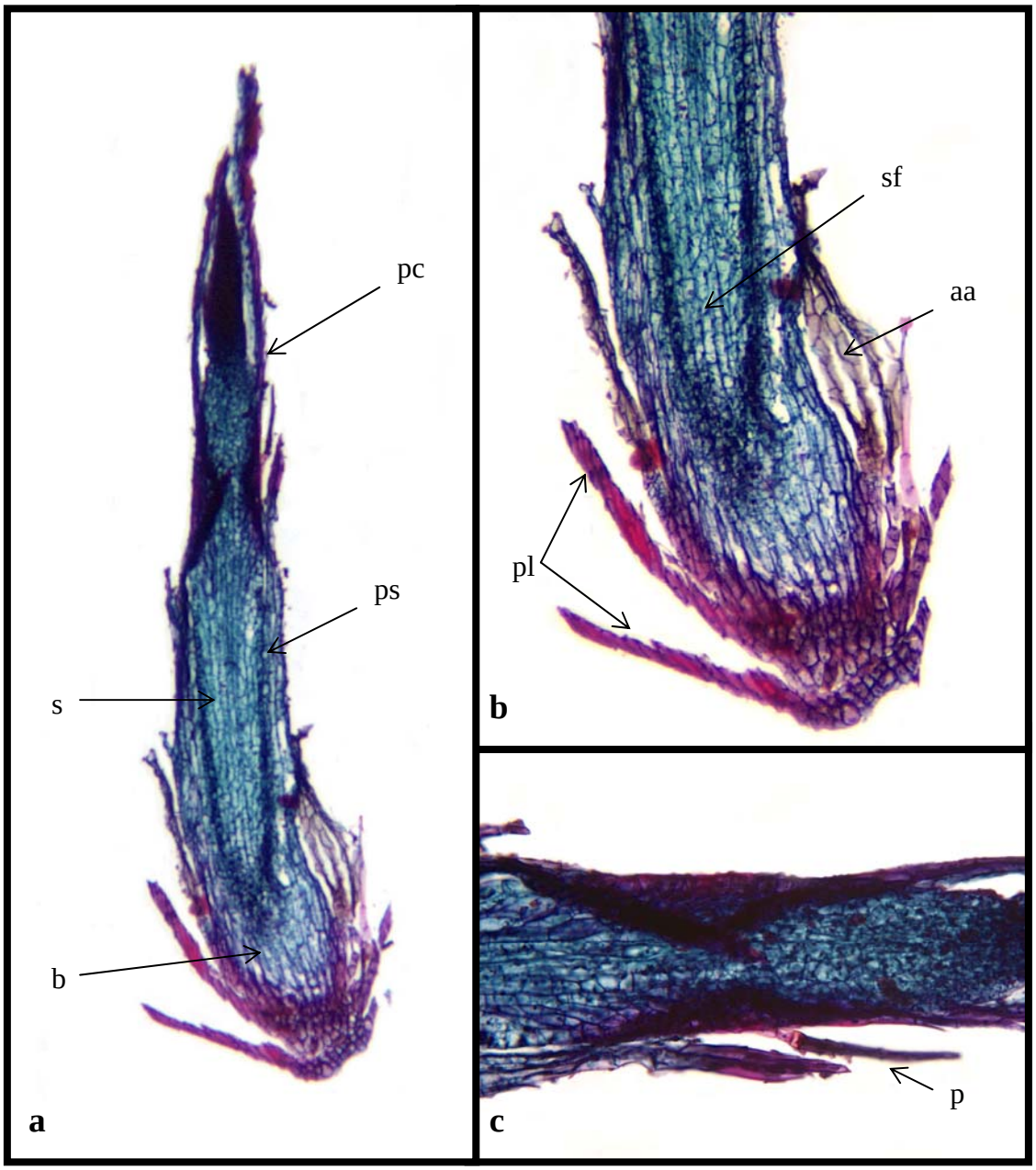


Figure 12

Plate VIII

N. disticha

Figure 13. Branch with young sporophyte, in the stage before the separation of the calyptra; processed using the plastic resin protocol

a) the sporophyte (s) presents the pre-calyptra (pc) and the perichaetial leaves (pl)
b) relative position of the perichaetial leaves (pl) and ramenta (r) and the elongation of the pseudopodium (ps)
c) differentiation between the perichaetial leaves (pl), the ramenta (r) and uniseriate paraphyses (p)

Figure 14. Branch with fertilized archegonium processed using the plastic resin protocol

a) wide section of branch with different types of paraphyses
b) uniseriate (p), biseriate (bp) and multiseriate (mp) paraphyses are present

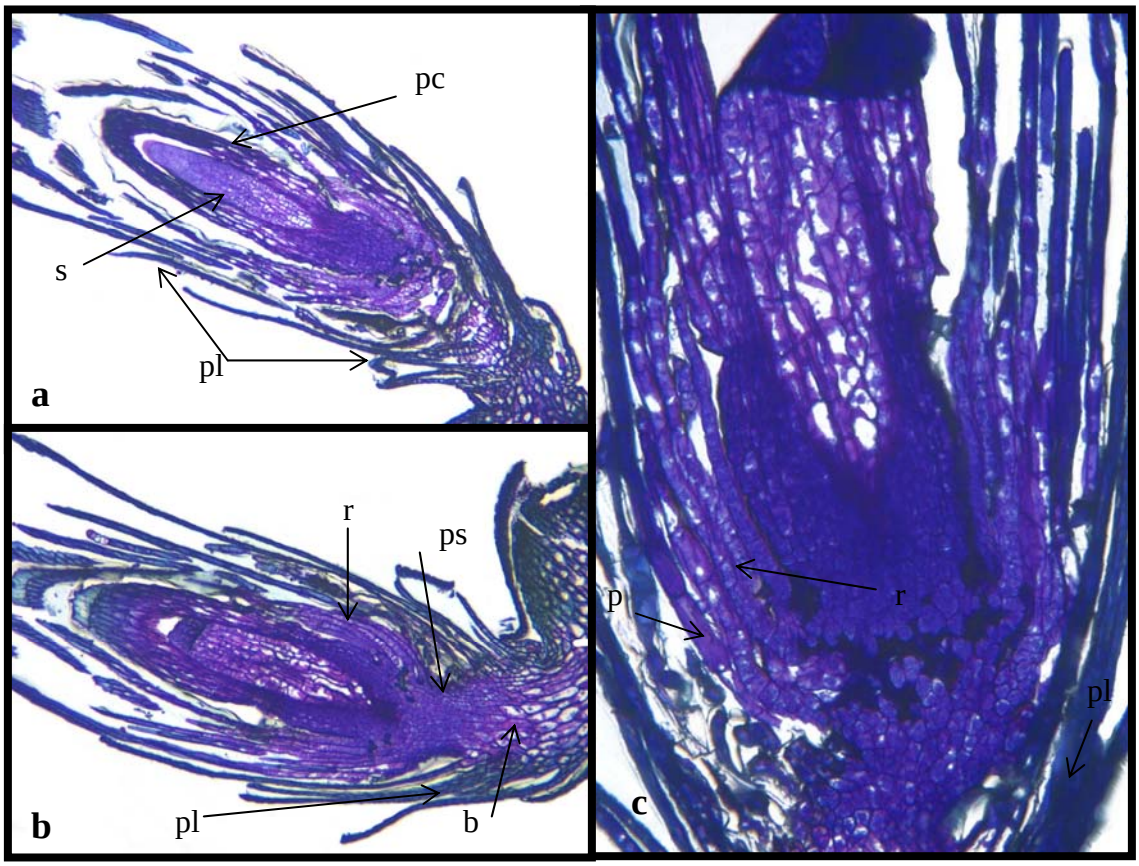


Figure 13

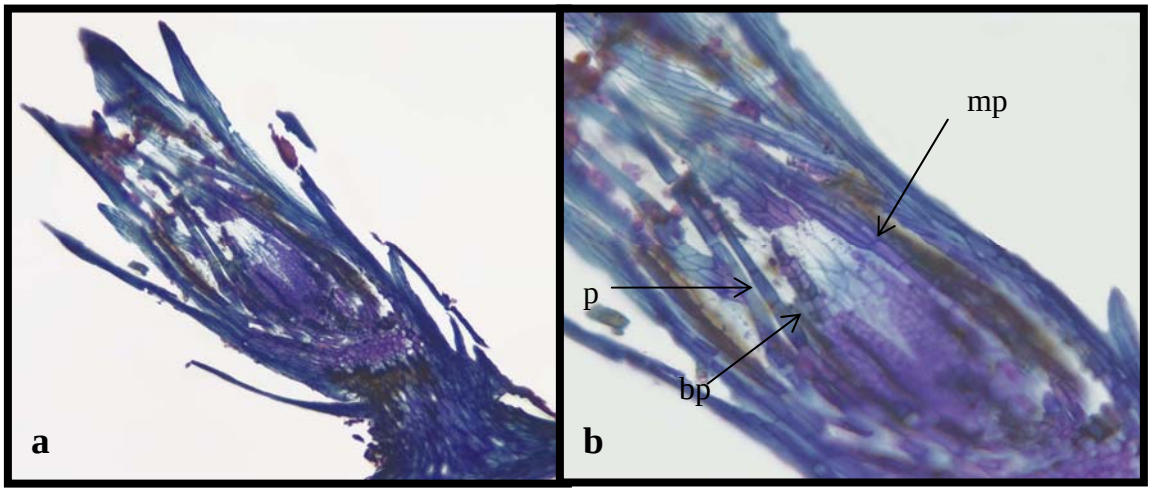


Figure 14

Plate IX

N. disticha

Figure 15. Some of the stages of the sporophyte, examined with the stereoscope

a) young sporophyte after the separation of the calyptra (c); the ramenta (r) are noticeable and a stem leaf (bl)
b) the pseudopodium (ps) is completely elongated
c-e) mature sporophytes
f) the ramenta attached at the end of the pseudopodium

Figure 15

Plate X

Figure 16. Perigonium of *N. exserta* with paraphyses

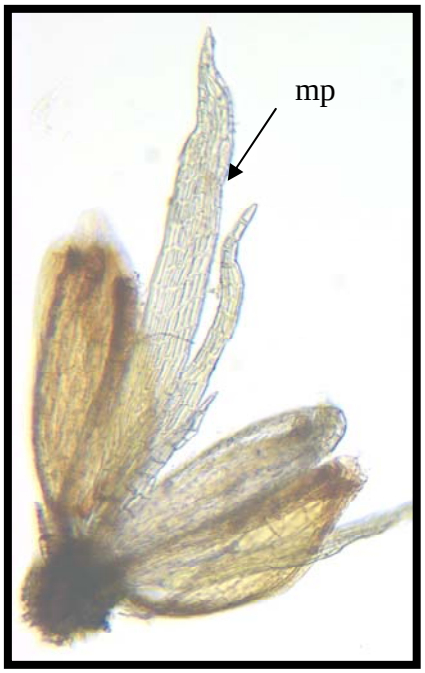
Figure 17. Paraphysis on the perigonium of *N. crinita*

Figure 18. Multiseriate paraphyses (mp) on the perigonium of *N. fimbriata*

Figure 19. Small leaf-like structures (ls) compared to perigonial leaves (pl) on the perigonium of *N. nano-disticha*

Figure 16

Figure 17



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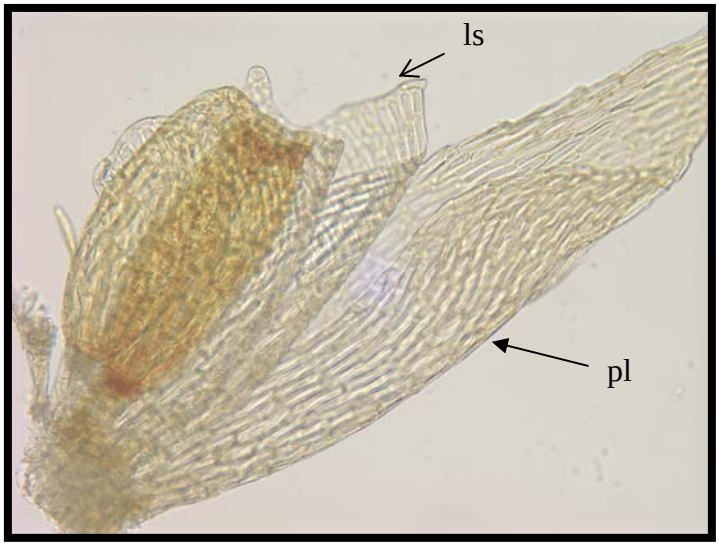

Figure 19

Plate XI

Figure 20. Perichaetia of *N. lepineana* with paraphyses

Figure 21. Shorter reddish paraphyses in perichaetia
a) *N. andamana*
b) *N. fimbriata*

Figure 22. Small leaf-like structures in perichaetia and biseriate paraphyses
a) *N. obtusata*
b) *N. crinita*
c-e) *N. nano-disticha*

Figure 20

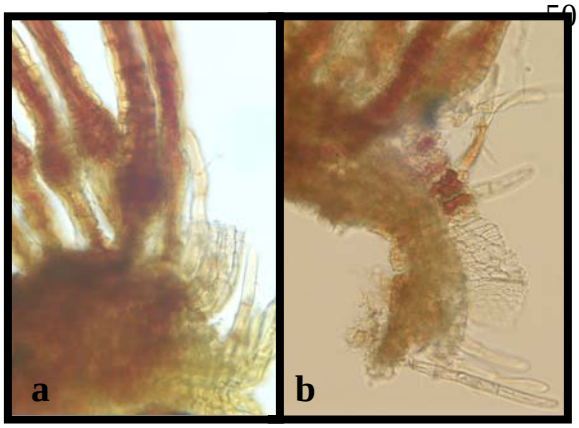

Figure 21

Figure 22

Plate XII

Figure 23. Fertilized archegonia

a) *N. lepineana*
b) *N. obtusata*
c) *N. nitidula*

Figure 24. Different types of paraphyses on fertilized branches of *N. nitidula*

a) paraphyses with thin cell wall
b) paraphyses with thick cell wall

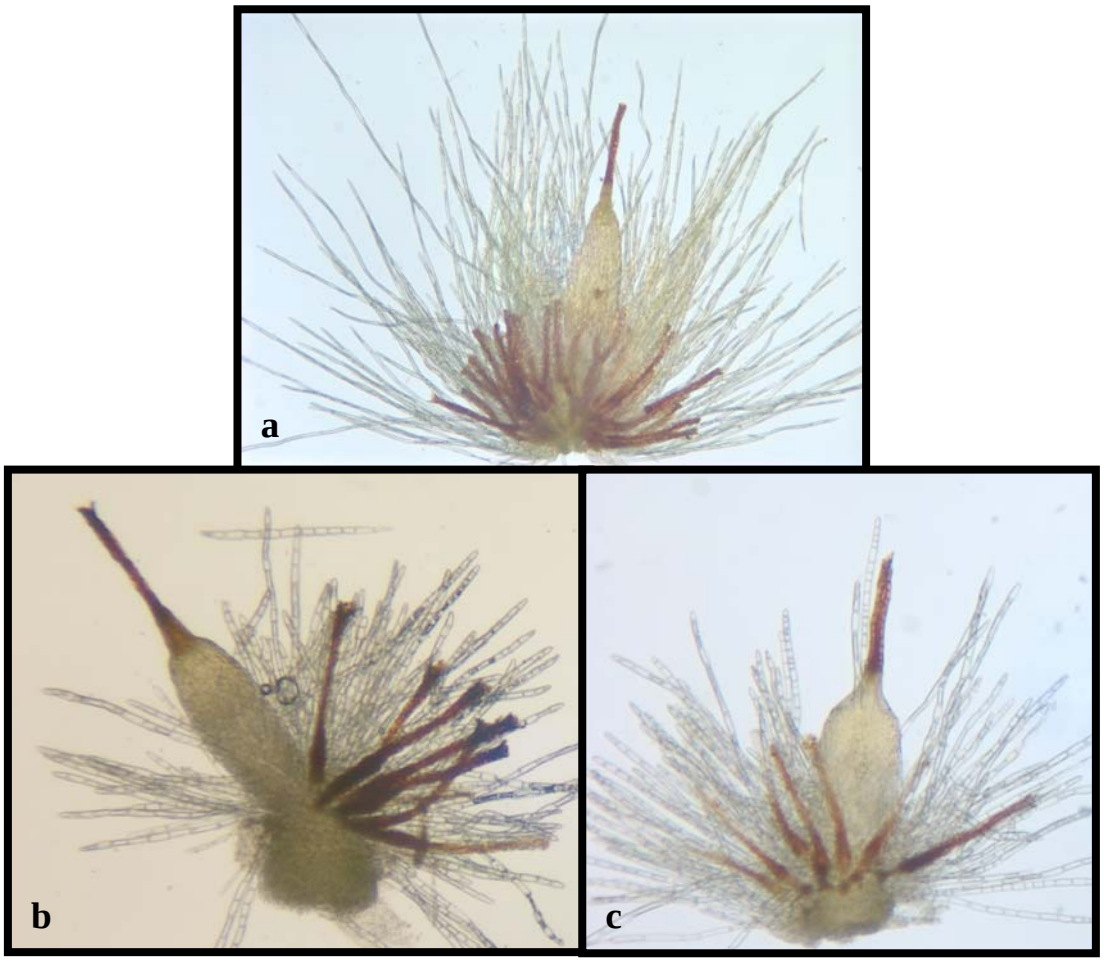

Figure 23

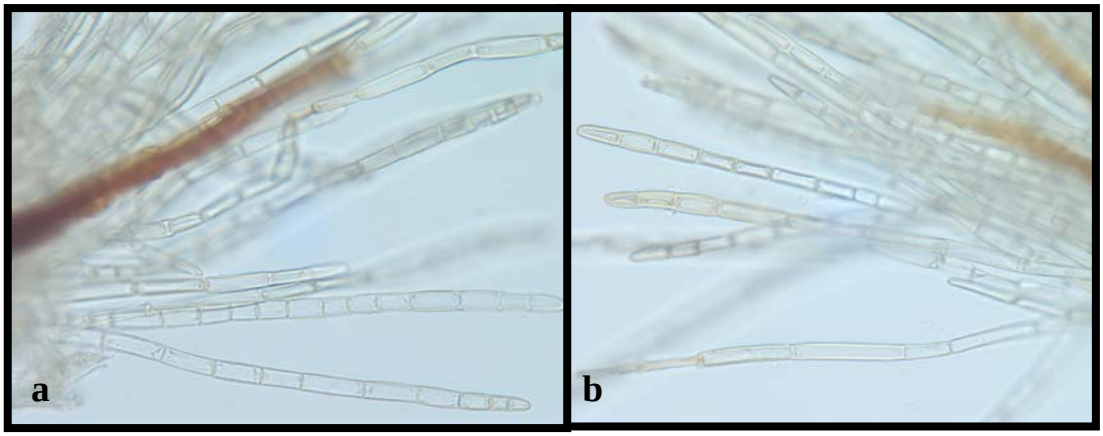

Figure 24

Plate XIII

Figure 25. Fertilized archegonia of *N. andamana*

a) archegonia
b) small paraphysis

Figure 26. Juvenile sporophyte of *N. andamana*

a) stage before the separation of the calyptra
b) perichaetial leaves
c) short paraphysis

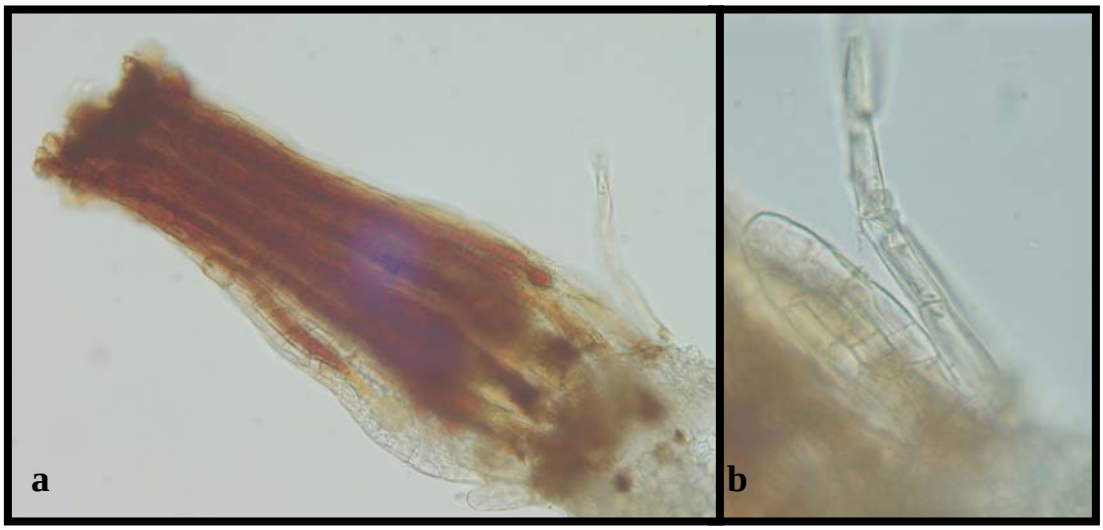

Figure 25

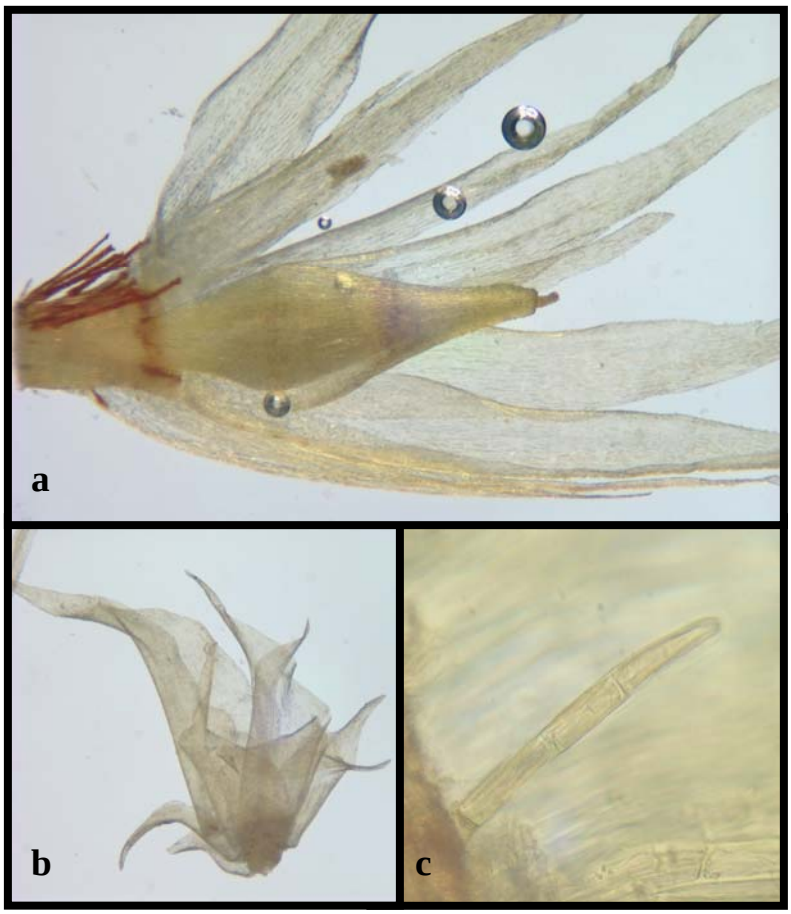

Figure 26

Plate XIV

Figure 27. Sporophyte of *N. undulata* with ramenta on pseudopodium

Figure 28. Branch of *N. undulata* with fertilized archegonium

a) ramenta longer than the fertilized archegonium
b) aborted archegonia and biseriate paraphyses
c) irregular paraphysis

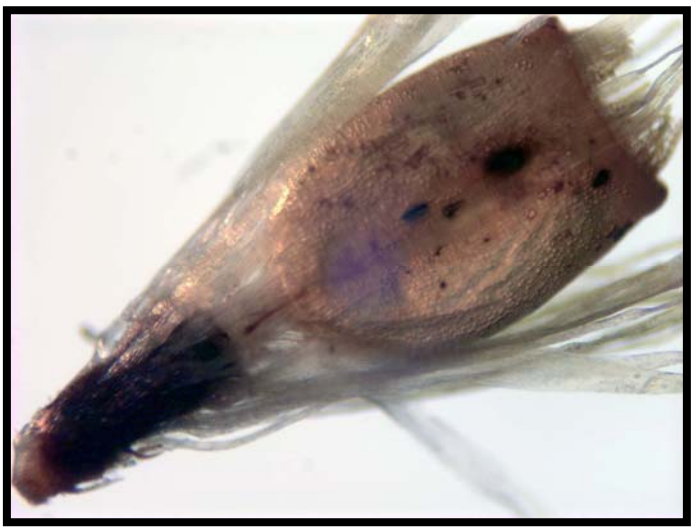

Figure 27

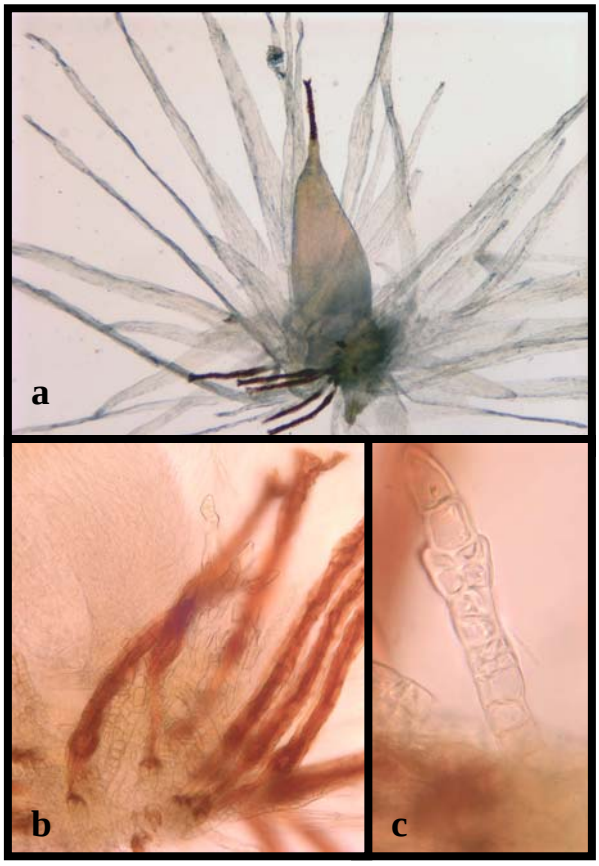

Figure 28

Plate XV

Figure 29. Sporophyte of *N. exserta*

a) seta (se) inserted in the pseudopodium (ps) that bears the paraphyses (p), in the reddish vaginula (v)
b) the sporophyte foot (sf) can be distinguished from the seta
c) the pseudopodium bears the archegonia (arc) and antheridia (ant) at the lower part of the elongated base
d) perichaetial leaves (pl) are different from the long uniseriate paraphyses (p)

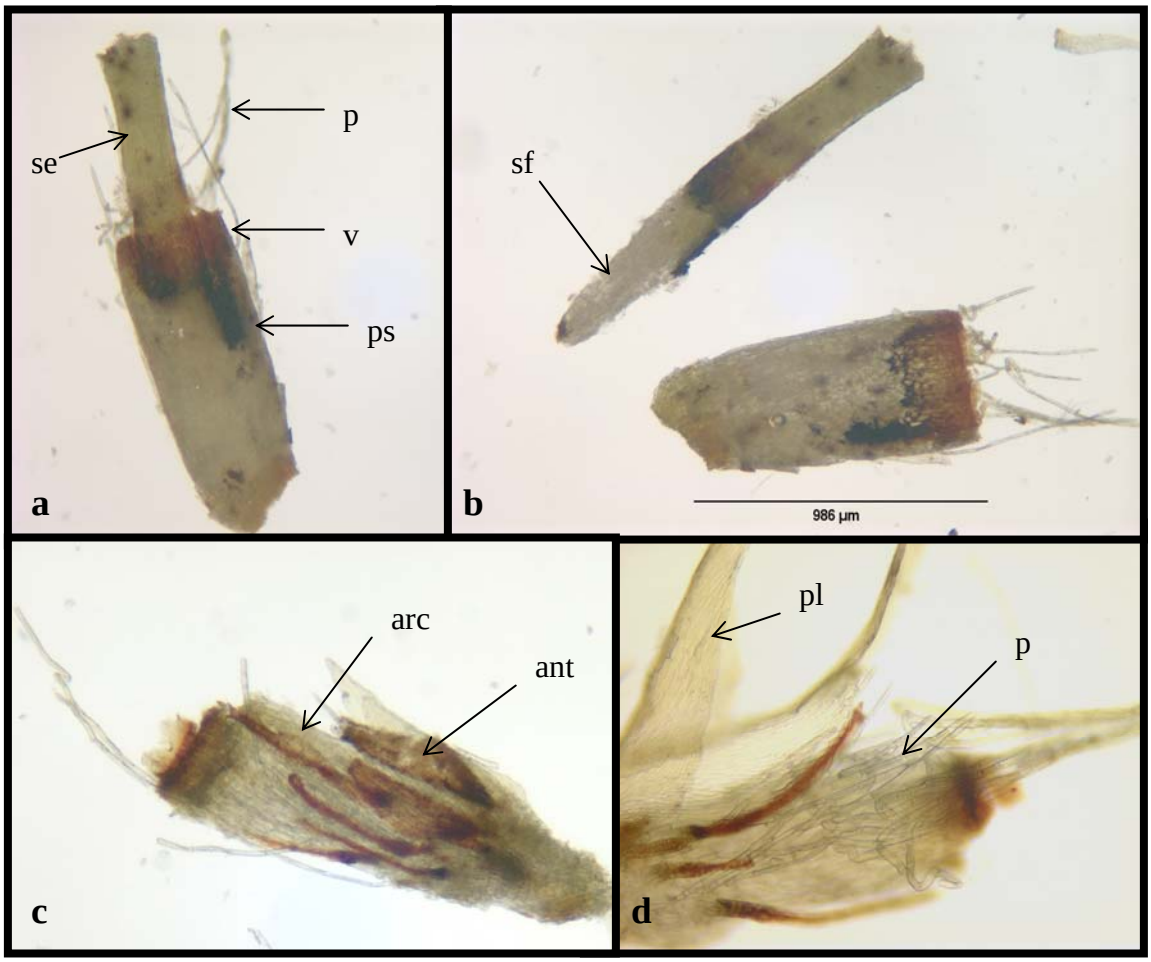

Figure 29

Plate XVI

Figure 30. Sporophyte of *N. nitidula*

a) the inner perichaetial leaves reach the mouth of the capsule
b) sporophyte foot and seta
c) short base with aborted archegonia and paraphyses
d) paraphyses

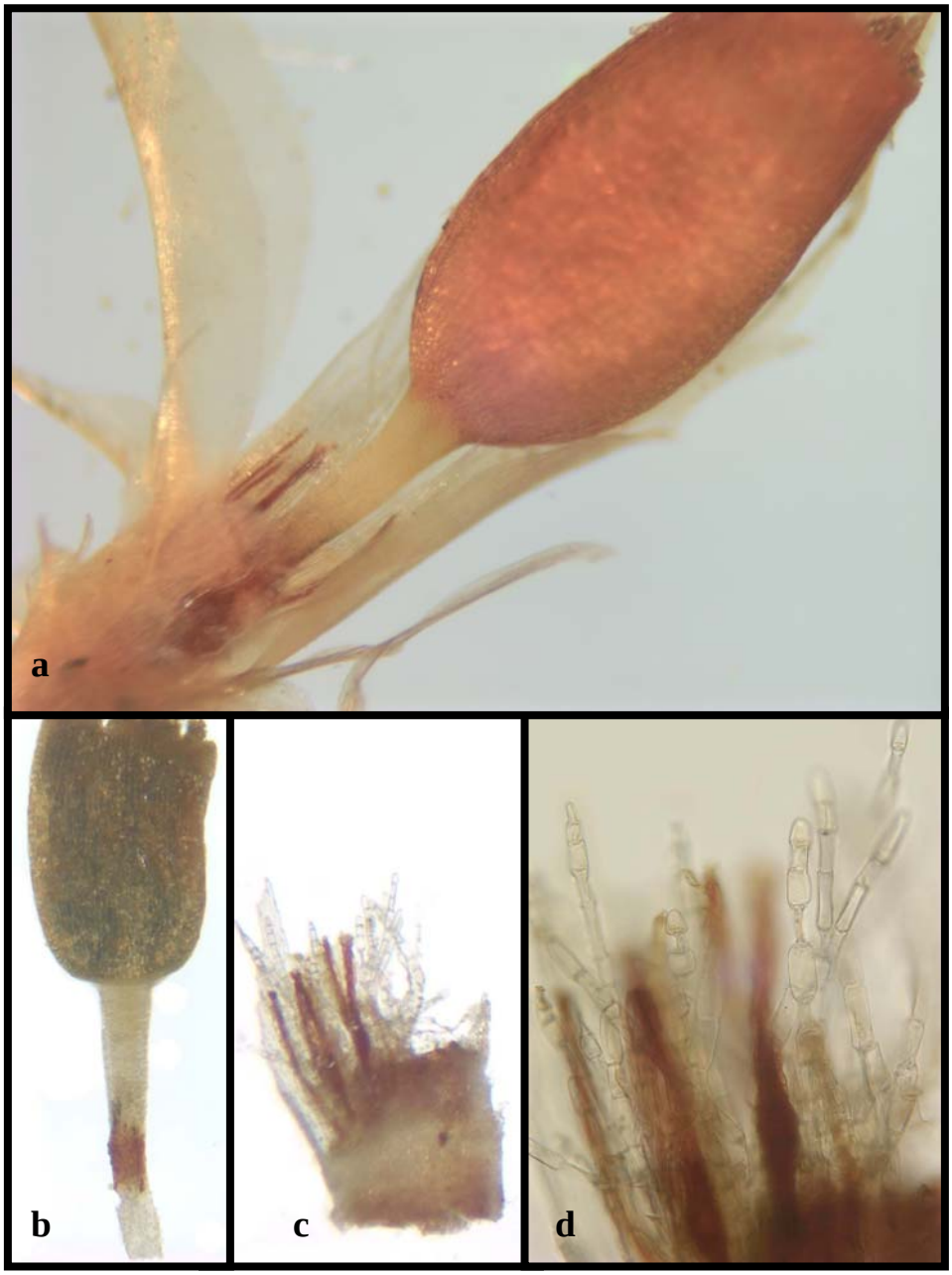

Figure 30

Plates XVII

Figure 31. Sporophyte of *N. obtusata*

a) the elongation of the branch axis occurs in the area between the perichaetial leaves that reach beyond the capsule
b) paraphyses and aborted archegonia located at the upper part of the elongated base (vaginula) and on the calyptra
c) uniseriate paraphyses
d) comparison between leaves and paraphyses

Figure 31

Plate XVIII

Figure 32. Sporophyte of *N. lepineana*

a) pseudopodium and seta
b) end of the pseudopodium with elongated paraphyses and archegonia
c) paraphyses

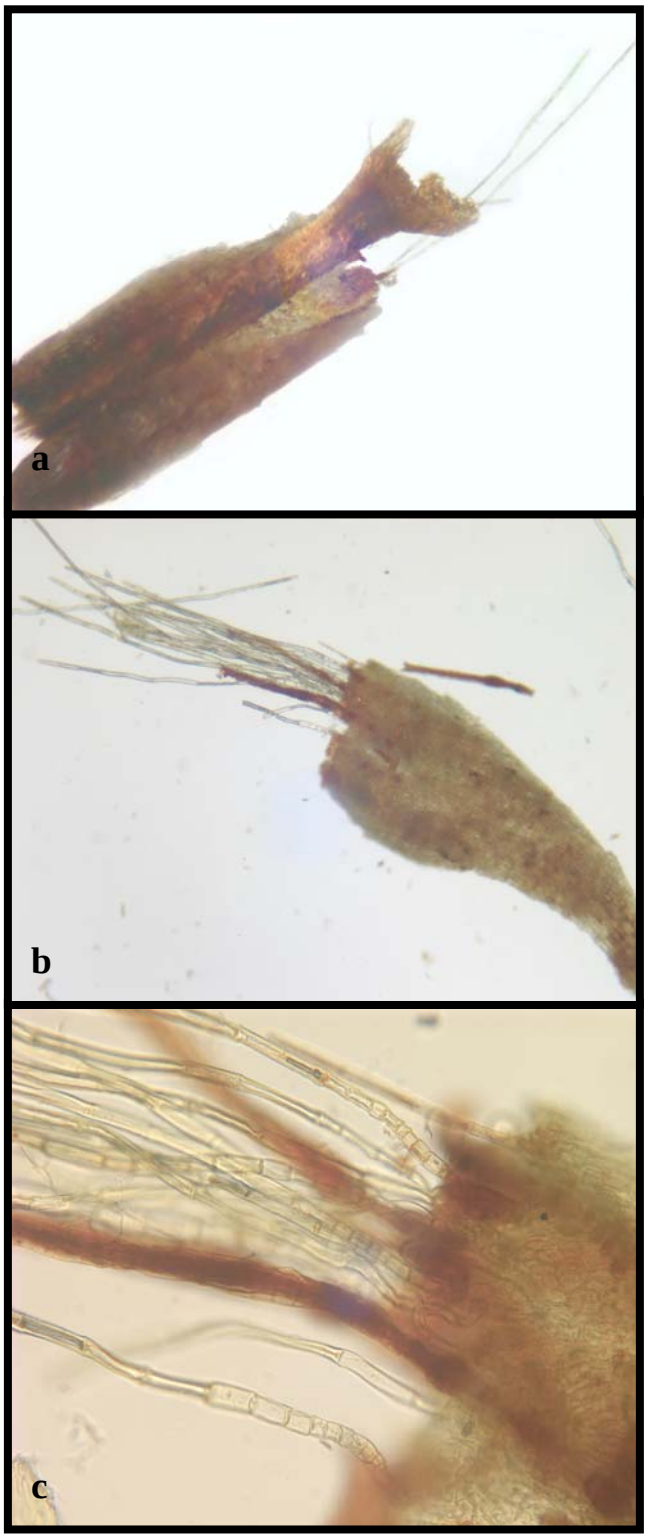

Figure 32

Plate XIX

Figure 33. Sporophyte of *N. nano-disticha*

a) ramenta reach beyond the capsule mouth and elongated perichaetial leaves
b) thin ramenta on pseudopodium
c) base of ramentum

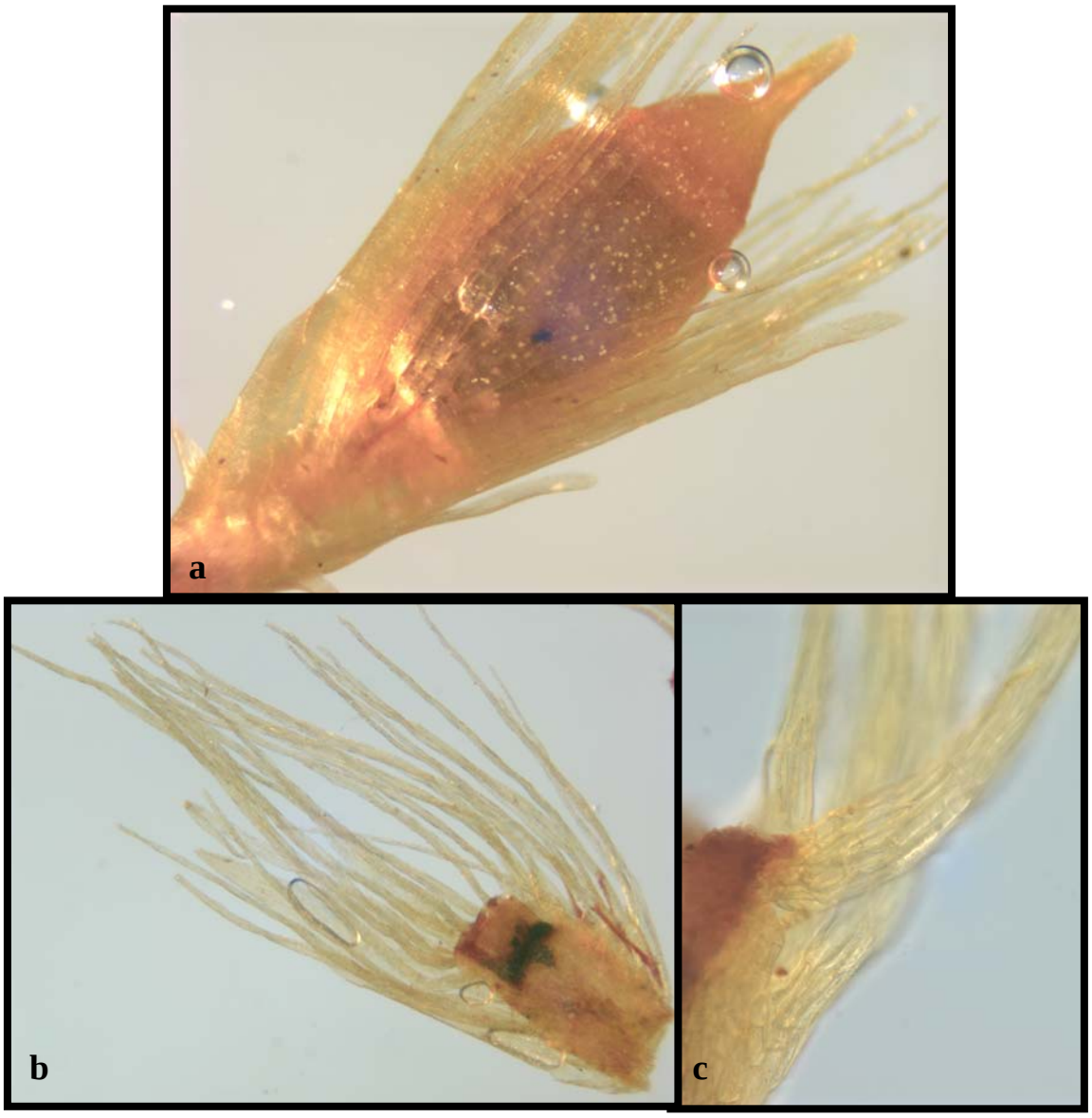

Figure 33

Plate XX

Figure 34. Sporophyte of *N. crinita*

a) ramenta reaching beyond the capsule
b) ramenta inserted on pseudopodium and elongated perichaetial leaves
c) base of ramentum showing small scale-like ramification
d) papillae on cell wall, serrate ramenta

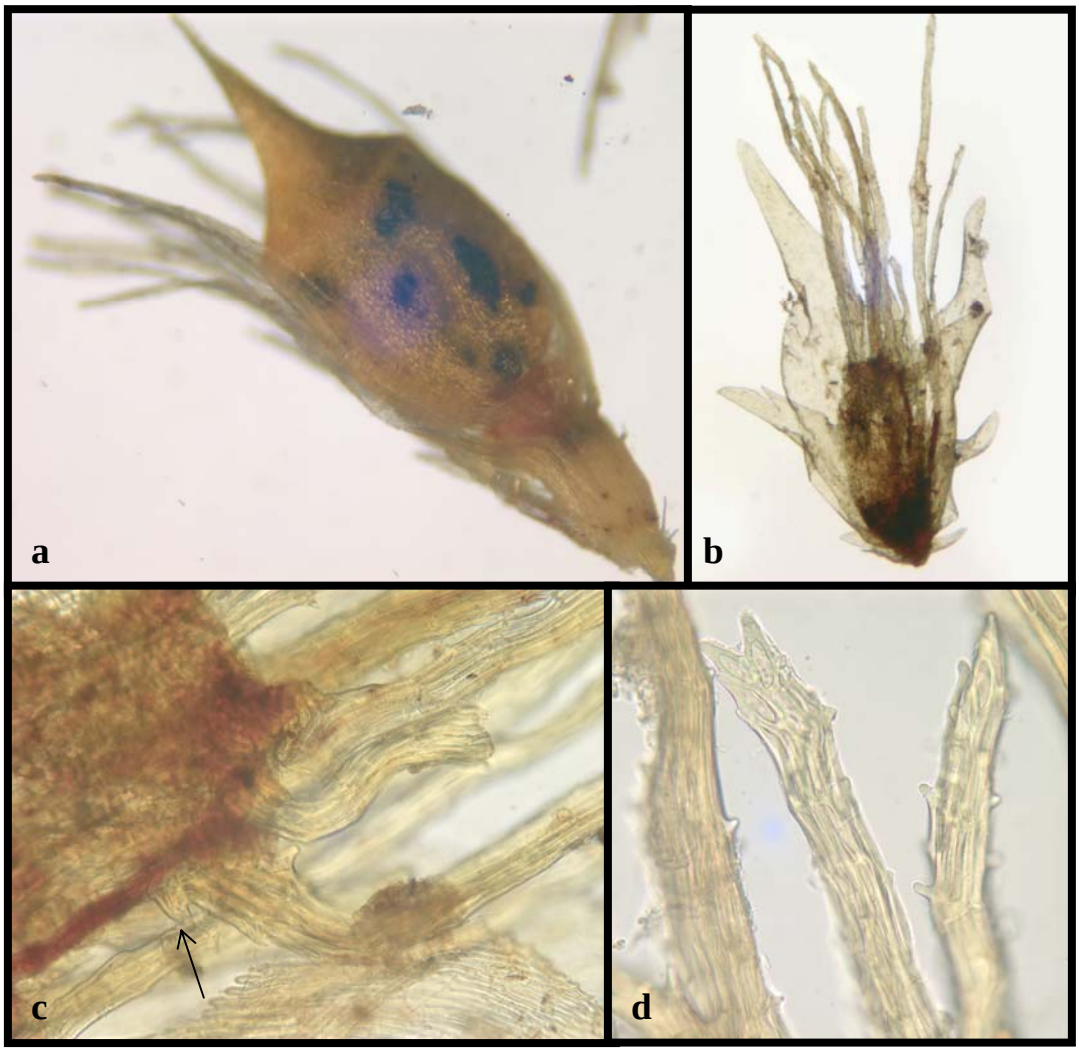

Figure 34

Plate XXI

Figure 35. Young sporophyte of *N. andamana*

a) sporophyte with small base
b) wide ramenta
c) sporophyte with wide ramenta

Figure 36. Cell of *N. andamana*

a) leaf cells
b) ramentum cells

Figure 35

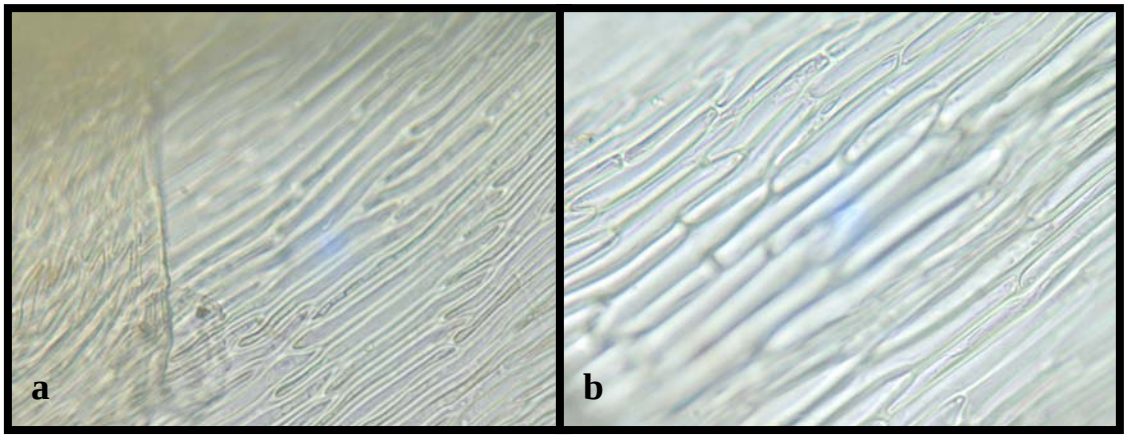

Figure 36

Plate XXII

Figure 37. Ramenta of *N. lepineana*

a) wider ramenta restricted to the upper part of the small base
b) ramentum or multiseriate paraphyses

Figure 37