Order Jubulales W.Frey & Stech
Frullaniaceae Lorch
Frullania Raddi
Lejeuneaceae Casares-Gil.
Subfam. Ptychanthoideae Mizut.
Acrolejeunea (Spruce) Schiffn.; *Caudalejeunea* Steph.; *Frullanoides* Raddi; *Lopholejeunea* (Spruce) Schiffn.; *Marchesinia* Gray; *Ptychanthus* Nees; *Schiffneriolejeunea* Verd.
Subfam. Lejeuneoideae (incl. Cololejeuneoideae Herzog)
Ceratolejeunea (Spruce) Schiffn.; *Cheilolejeunea* (Spruce) Schiffn. (incl. *Leucolejeunea* A.Evans); *Cololejeunea* (Spruce) Schiffn. (incl. *Aphanolejeunea* A.Evans); *Colura* (Dumort.) Dumort.; *Diplasiolejeunea* (Spruce) Schiffn.; *Drepanolejeunea* (Spruce) Schiffn.; *Harpalejeunea* (Spruce) Schiffn.; *Lejeunea* Lib.; *Leptolejeunea* (Spruce) Schiffn.; *Microlejeunea* Steph; *Odontolejeunea* (Spruce) Schiffn.; *Omphalanthus* Lindenb. & Nees; *Prionolejeunea* (Spruce) Schiffn.; *Taxilejeunea* (Spruce) Schiffn.

Subclass Metzgeriidae Barthol.-Began
Order Aneurales W.Frey & Stech
Aneuraceae H.Klinggr.
Aneura Dumort.; *Riccardia* Gray
Order Metzgeriales Chalaud
Metzgeriaceae H.Klinggr.
Metzgeria Raddi

Division Anthocerotophyta Rothm. ex Stotler & Crand.-Stotl.

Class Anthocerotopsida de Bary ex Jancz.
Subclass Anthocerotidae Rosenv.
Order Anthocerotales Limpr. in Cohn
Anthocerotaceae Dumort.
Anthoceros L.
Subclass Notothylatidae Duff, J.C.Villareal, Cargill & Renzaglia
Order Notothyladales Hyvönen & Piippo
Notothyladaceae Müll.Frib. ex Prosk.
Subfam. Phaeocerotoideae Hässel
Phaeoceros Prosk.
Subfam. Notothyladoideae Grolle
Notothylas Sull. ex A.Gray

7. Collection of bryophytes

Bryophyte specimens are invaluable in the study of a local flora as they provide material for comparison. A reference collection will be most helpful for every student of bryology. In order to obtain good specimens, it is necessary to prepare the collecting trip and take sufficient equipment into the field. An overview of bryophyte sampling is also provided by Vanderpoorten *et al.* (2010).

Collection Permits
Permits to collect specimens in protected areas (e.g. National Parks) must be applied for in advance from the Rwanda Development Board (RDB), Conservation and Tourism in Kigali.

Field equipment (Fig. 20)
Essential items are:

- A **hand-lens**, preferably with a magnification ×20. In dense forests where light conditions are low, a ×10 or ×12 hand-lens should also be used.
- **Paper bags** to place each specimen in order to be dried adequately. Plastic bags should not be used as the specimens soon start to become mouldy or etiolated. Polyethylen bags are banned by law in Rwanda.
- A **sharp knife** to remove specimens from soil, rocks or bark.
- **Pencils or water-proof markers** to write locality and habitat details as well as collection number on the bag even under wet conditions in the forest.
- **GPS-equipment** for geographical coordinates and altitude of the locality.
- A small **digital camera** will be very useful for habitat photographs and to take close-ups of the specimens.

What to collect
Bryophyte collections should be of modest quantity as many species are endangered and collecting large amount may seriously deplete the often small populations. However, the specimen should consist of sufficient material to enable identification. Preferably fertile specimens should be collected but these may be rare. If possible pure stands and cushions should be collected, but often intermixed specimens cannot be avoided. Each recognizable species should be packed separately.

Each bag should be labelled with the necessary details and preferably be numbered. As a minimum the following information should be noted: Locality name and geographical coordinates; altitude; microhabitat (e.g. on soil, decaying wood, rock, bark (epiphytic) or on living leaves (epiphyllous); for epiphytic and epiphyllous species, the host (phorophyte) species should be noted); vegetation type; state of habitat (e.g. primary or secondary forest); date.

Epiphyllous bryophytes are collected by gathering the phorophyte leaves, which are lightly pressed in a herbarium press for vascular plants. The bryophyte bags should not be pressed but dried as soon as possible. They can be air-dried in the sun or on a table, or the bags held by pegs on a line, or, under very wet rainforest conditions, dried in an apparatus similar to that described by Frahm & Gradstein (1986). Large cushions can generally be squeezed to remove water before being placed in the paper bag.

Processing the specimens

For herbarium purpose, the collections (when completely dried) should be transferred to new packets folded from a sheet of paper (Fig. 20) and fully labelled. These packets can be stored in metal or wooden cabinets or cardboard shoe boxes. After identification they can be arranged either alphabetically or in systematic order. The bryophyte collection should be stored under dry and well ventilated conditions to avoid infections by fungi or insects.

8. Morphology and characters of Liverworts and Hornworts

Bryophytes comprise three groups of early land plants: Liverworts or hepatics (Marchantiophyta), hornworts (Anthocerotophyta) and mosses (Bryophyta) (Tab. 2).

Table 2: Distinguishing characters of Liverworts, Hornworts and Mosses.

	Liverworts	Hornworts	Mosses
Habit	Foliose (with leaves) or thallose	Thallose	Foliose
Leaves	In 2-3 rows, undivided or lobed, without midrib, apex rounded, if acute then leaf lobed	-	Spirally arranged, rarely in 2-3 rows, always undivided, with or without midrib, apex often acute
Cells	With numerous chloroplasts, pyrenoid lacking, trigones usually present, isodiametric	With 1 (-4) large chloroplasts, with or without pyrenoid, trigones lacking, isodiametric	With numerous chloroplasts, pyrenoid lacking, trigones usually lacking, isodiametric or elongated (prosenchymatous)
Oil bodies	Usually present	Lacking	Lacking
Sporophyte	Surrounded by calyptras and other protective organs (perianth, marsupium, involucrum)	Partially surrounded by involucres, calpyptra lacking	Upper part covered by calyptras, further protective organs lacking
Capsule	Rounded to cylindrical, with colorless fragile seta, or seta lacking, opening at once by (1-) 4 valves, elaters present, columella, stomata and peristome lacking	Cylindrical to elongate, opening from top to bottom by 2 valves, elaters present, columella present, with stomata, peristome lacking	Round to cylindrical, with firm colored seta, opening at once by an operculum or dehiscence irregular, elaters lacking, columella and stomata present, peristome usually present

Life cycle
The life cycle of bryophytes consists of an alteration of two generations: the haploid gametophyte which represents the assimilating green plant, and the diploid sporophyte, “parasitizing” on the gametophyte and consisting of the capsule with seta and foot. The sporophyte is permanently attached to the gametophyte. The gametophyte produces the male (antheridia) and female gametangia (archegonia). The antheridium is usually an ovoid body on a short stalk and produces the biflagellate spermatozoids. These spermatozoids swim chemotactically in a film of water to the archegonium, which is bottle-shaped and consists of a narrow neck and the egg cell. They fuse with the egg cell and produce a diploid zygote, which starts with cell divisions and develops into a basal foot, a seta and the capsule (i.e. the meiosporangium). Meiosis occurs in the spore development out of a diploid spore mother cell which develops into tetrads of 4 spores with usually different ornamentation on outer (distal) and inner (proximal) face. The then haploid spores are dispersed and germinate on humid substrate to a protonema which differentiates into the leafy or thallose gametophyte.

Leafy liverworts

Stems and branches
The stems of leafy liverworts consist of three rows of **merophytes**: two lateral and one ventral. Each merophyte comprises stem tissue and an associated leaf. The leaves of the ventral merophyte are the **underleaves** (amphigastria). Branches either have normal vegetative leaves or are lacking leaves. Branches with scale-like leaves are called **flagellae** (Fig. 21), those without leaves are **stolons**. **Intercalary** branches originate from inner cells of the stem and thus bear a small collar at base. **Terminal** branches originate from a leaf-initial cell or from stem-epidermis, and no collar is formed.

Leaves
Leaves are arranged in three rows: two lateral and one ventral. In *Haplomitrium* (Fig. 22) the leaves of each lateral row are of approximately equal size, while the ventral leaves (underleaves) are normally smaller (Fig. 23) or are sometimes lacking (e.g. in *Cololejeunea*). Leaf position may be incubous, succubous or transverse. Incubous arrangement means that the dorsal leaf margin is nearer to the shoot apex than the ventral margin (Fig. 22). Succubous means that the ventral leaf margin is nearer to the shoot apex than the dorsal margin (Fig. 22). A transverse insertion is perpendicular to the stem (Fig. 22). Leaves may be flat, concave or convex, and variously oriented from the stem. The lamina may be entire, dentate at margin, bilobed, 3-5-lobed or deeply dissected into filiform segments. In *Radula*, *Porella* and the Lejeuneaceae, the leaves are complicate-bilobed (i.e. having a 2-lobed leaf with the lobes folded together longitudinally). Usually the dorsal segment (lobe) is larger than the ventral

segment (lobule), but may be smaller in the Scapaniaceae (e.g. *Diplophyllum*). The lobule (ventral segment) is very diverse in shape, and may be attached to the lobe (dorsal segment) by few stalk-like cells (*Frullania*) (Fig. 23) or attached along most of the ventral side of the lobe (in Lejeuneaceae, Fig. 24). The line of attachment between lobe and lobule is called keel, and the margin of the lobule that is not attached to the lobe is called the free margin. There may be one to several teeth along the free margin. The outermost tooth is called apical tooth (Fig. 24). A tooth between the apical tooth and the stem is the proximal tooth. Sometimes there is a tooth between the apical tooth and the keel called distal tooth (Fig. 25). The apical tooth usually bears a hyaline papilla that may be inserted at the distal or the proximal face of the tooth (Fig. 23). In the carnivorous genus *Colura,* the lobule is strongly involute, so that its free margin is directed towards the keel, and the base of this sac is closed by a moveable valve which is in fact an extension of the apex of the lobule. The moveable valve allows ingress but not egress, and studies have shown that it is part of an apparatus for trapping small protozoa (Barthlott *et al.*, 2000). Underleaves usually differ in size and shape from the lateral leaves (Figs 23, 24). They are highly diverse, ranging from 3-5-lobed to bilobed or entire and rounded.

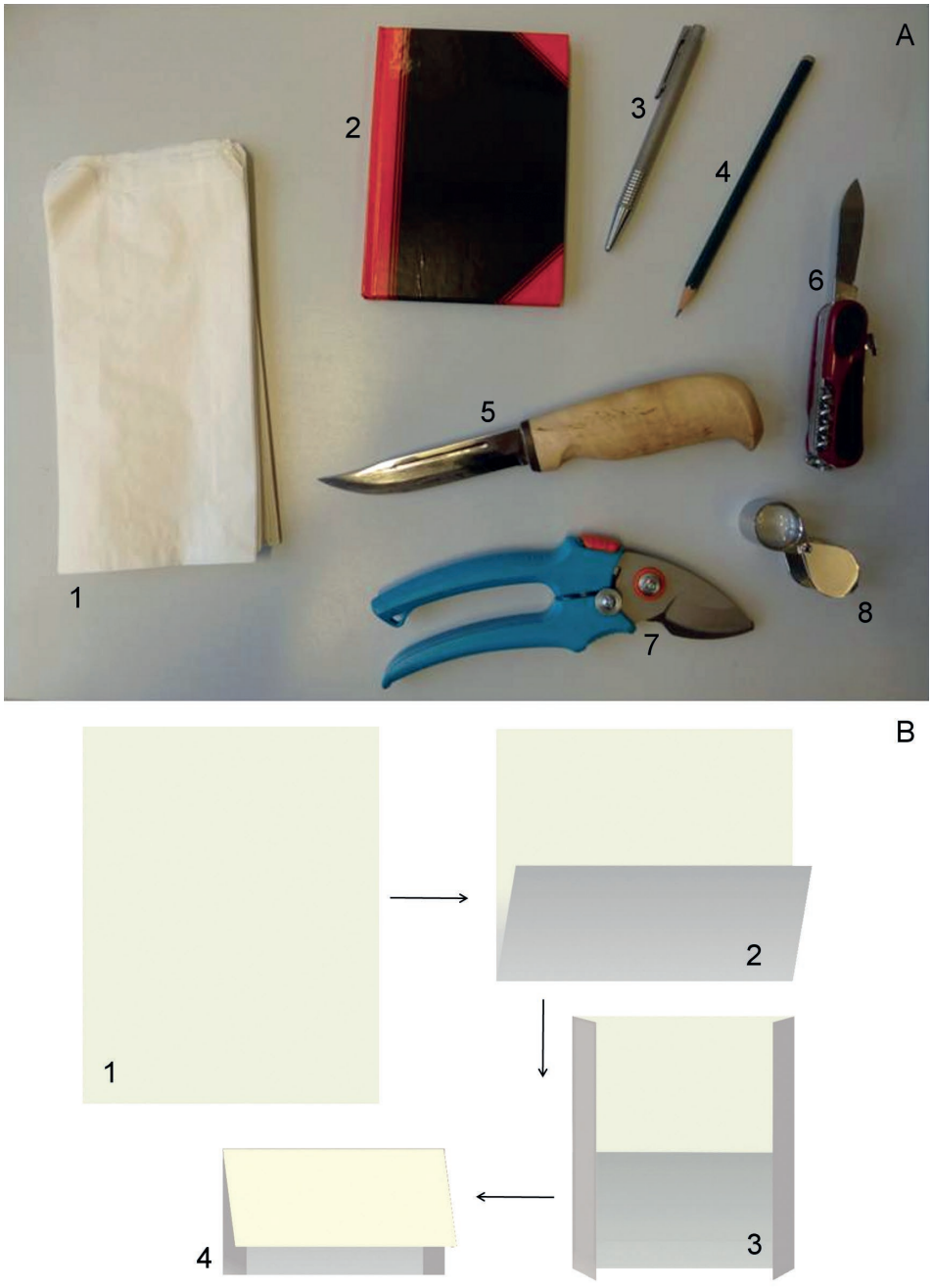

Fig. 20. A. Field equipment for bryophyte collection. 1. Paper bags. 2. Field book. 3. Pen. 4. Pencil. 5. Knife. 6. Pocket-knife. 7. Scissor for branches. 8. Handlens (10 x). **B.** Folding sequence for herbarium packets.

Leaf cells
Leaf cells are isodiametric to long rectangular. Cell walls are uniformly thin or thickened, or bear collenchymatous thickenings. These trigones may be cordate, triangular or nodulose, and may be separate or confluent (Fig. 26, 27). If the cell walls bear thickenings between the trigones these are called intermediate thickenings. In *Herbertus*, *Mnioloma*, *Bazzania* and some *Cololejeunea* spp. the lamina has rows of elongated cells called **vitta** (Fig. 28) which differs from the midrib of a moss in its unistratose structure.

Oil bodies
They are restricted to the liverworts. The number per cell, shape and chemical composition is variable. They consist of aggregated etheric oils easily recognizable by their refractive index which differs from that of the surrounding cell content. Some oil bodies produce a characteristic smell. The oil bodies provide important taxonomic characters but can only be observed in fresh specimens. In most species, they quickly disappear or disintegrate and lose their characteristic structure, some disappearing after only a few days (rarely, in a very few species, they may last for a few months). Kis & Pócs (1997) distinguished 9 types of oil bodies, of which two are mostly observed in ocelli and one in the thallose liverwort *Riccardia*:

- **Massula-Type:** Oil bodies homogeneous or 1-4-segmented, usually numerous and more than 8 per cell (*Porella*, *Cololejeunea*, *Lejeunea eckloniana*) (Fig. 26).
- **Bazzania-Type:** similar to Massula-Type but larger and less than 8 per cell (*Bazzania*, *Ptychanthus*) (Fig. 27).
- **Calypogeia-Type:** Oil bodies coarsely segmented of different globules. Size and number per cell variable (*Calypogeia*, *Frullania*, *Herbertus*, numerous Lejeuneaceae) (Fig. 26, 27).
- **Jungermannia-Type:** Oil bodies finely segmented, of numerous minute globules, finely granular (*Chiloscyphus*, Jungermanniaceae) (Fig. 26, 27).
- **Leucolejeunea-Type:** similar to Jungermannia-Type but very large, only 1-2 per cell (*Cheilolejeunea*) (Fig. 26).
- **Radula-Type:** Oil body 1 or few per cell, roughly granular (*Radula*) (Fig. 27).

In several Lejeuneaceae, e.g. *Diplasiolejeunea*, or *Drepanolejeunea*, specialized cells with one very large oil-body and without chloroplasts occur. They are called **ocelli** (sing. **ocellus**) (Fig. 28). In dried specimens where the oil bodies have already disappeared they can be recognized by their size.

Reproductive organs

Antheridia (male gametangia) are situated in the axils of specialized leaves, i.e.♂ bracts, either on main shoot or on specialized branches. **Archegonia** (♀ gametangia) are protected by bracts or bracteoles. Often they are surrounded by a **perianth** (Fig. 30), a tubular organ originating by fusion of three leaves. The shape of the perianth, its ornamentation (e.g. horns, keels), the number of keels and their position (ventral or dorsal) provide important taxonomic characters. In Lejeuneaceae, the presence or absence of innovations, i.e. branches originating directly below the perianth, and the leaf sequence in the innovations are important taxonomic characters. In **lejeuneoid innovations** (Fig. 29) the first basal leaf is a lateral leaf. In **pycnolejeuneoid innovations** the first basal leaf is an underleaf (fig. 29).

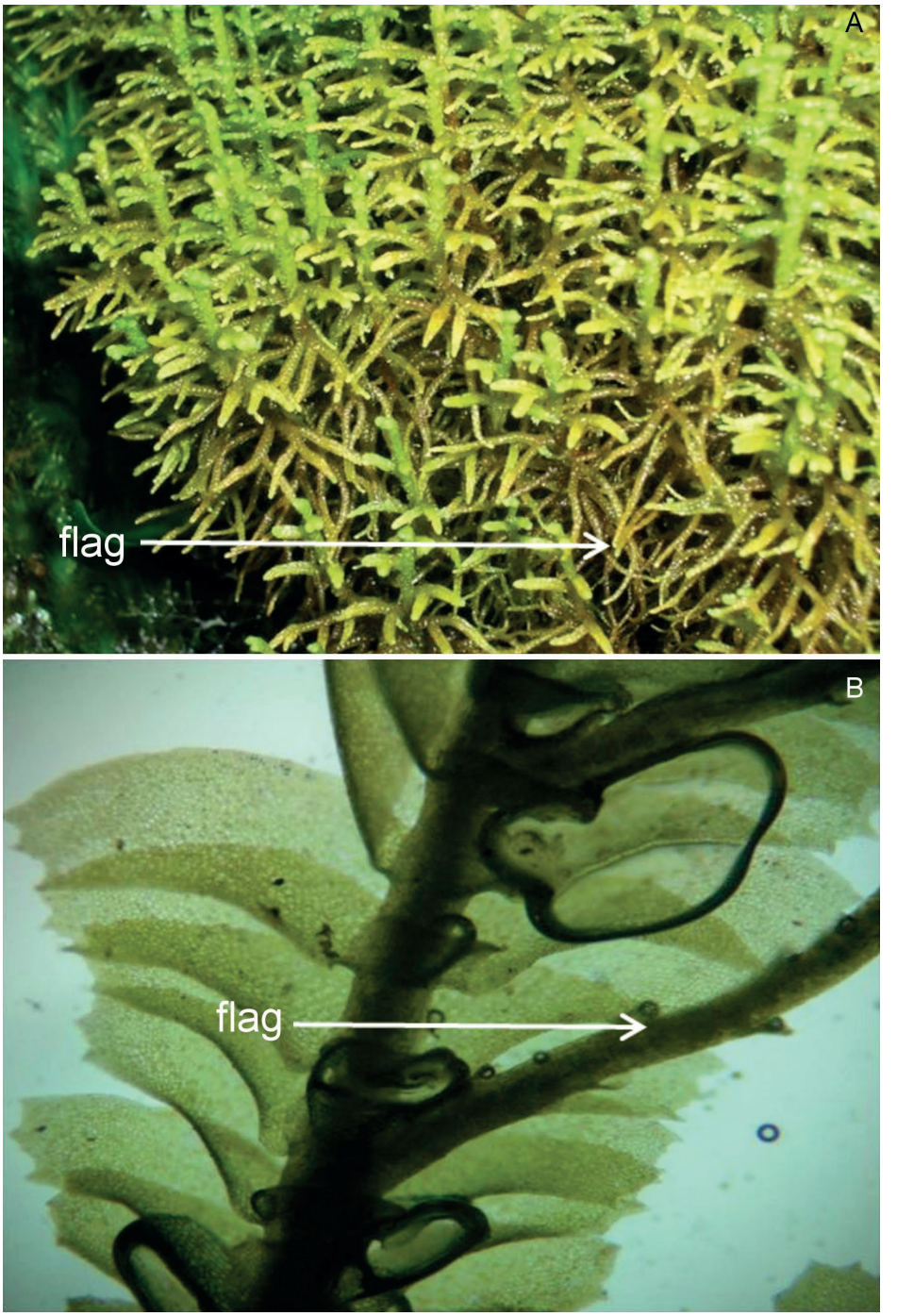

Fig. 21. A. *Mastigophora diclados*. **B.** *Bazzania decrescens*. flag = flagellae.

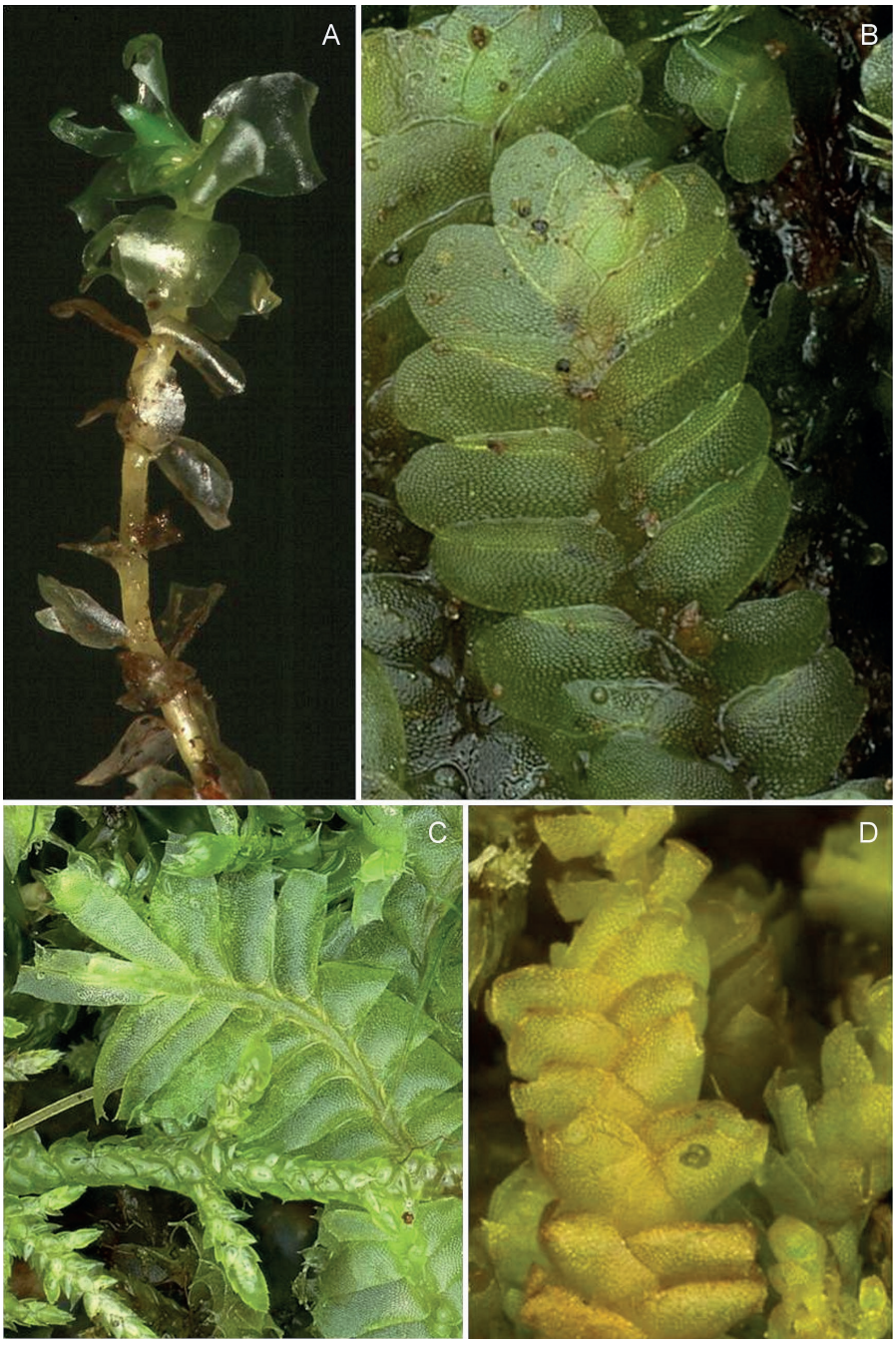

Fig. 22. A. *Haplomitrium blumei*, isophyllous leaves. **B.** *Mnioloma fuscum*, succubous leaves. **C.** *Chiloscyphus martianus*, incubous leaves. **D.** *Isotachis aubertii*, transverse leaves.

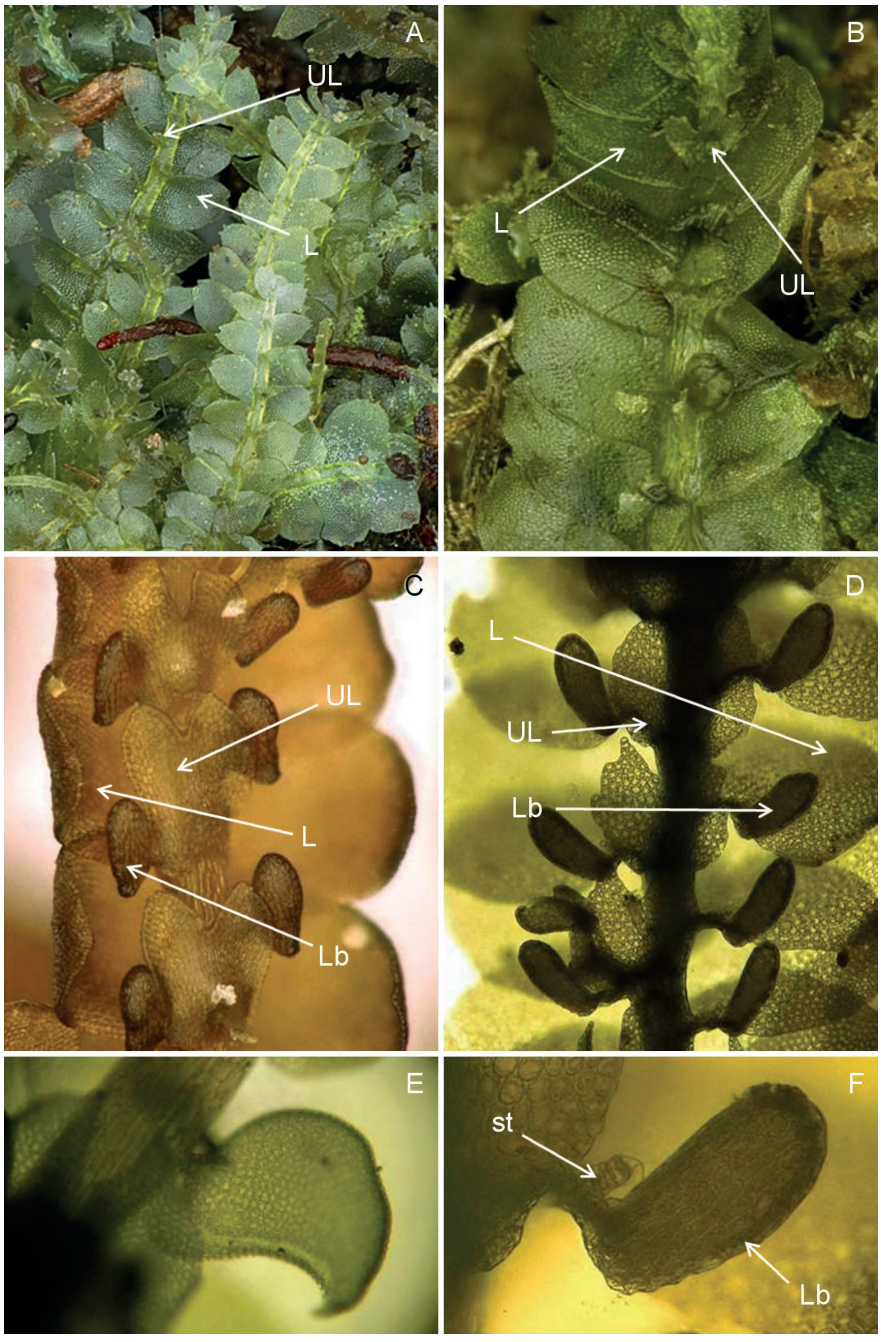

Fig. 23. A. *Calypogeia afrocaerulea*. **B.** *Calypogeia bidentula*. **C.** *Frullania apicalis*. **D, F.** *Frullania lindenbergiana*. **E.** *Frullania ericoides*. L = lateral leaf, UL = underleaf, Lb = lobule, st = stylus.

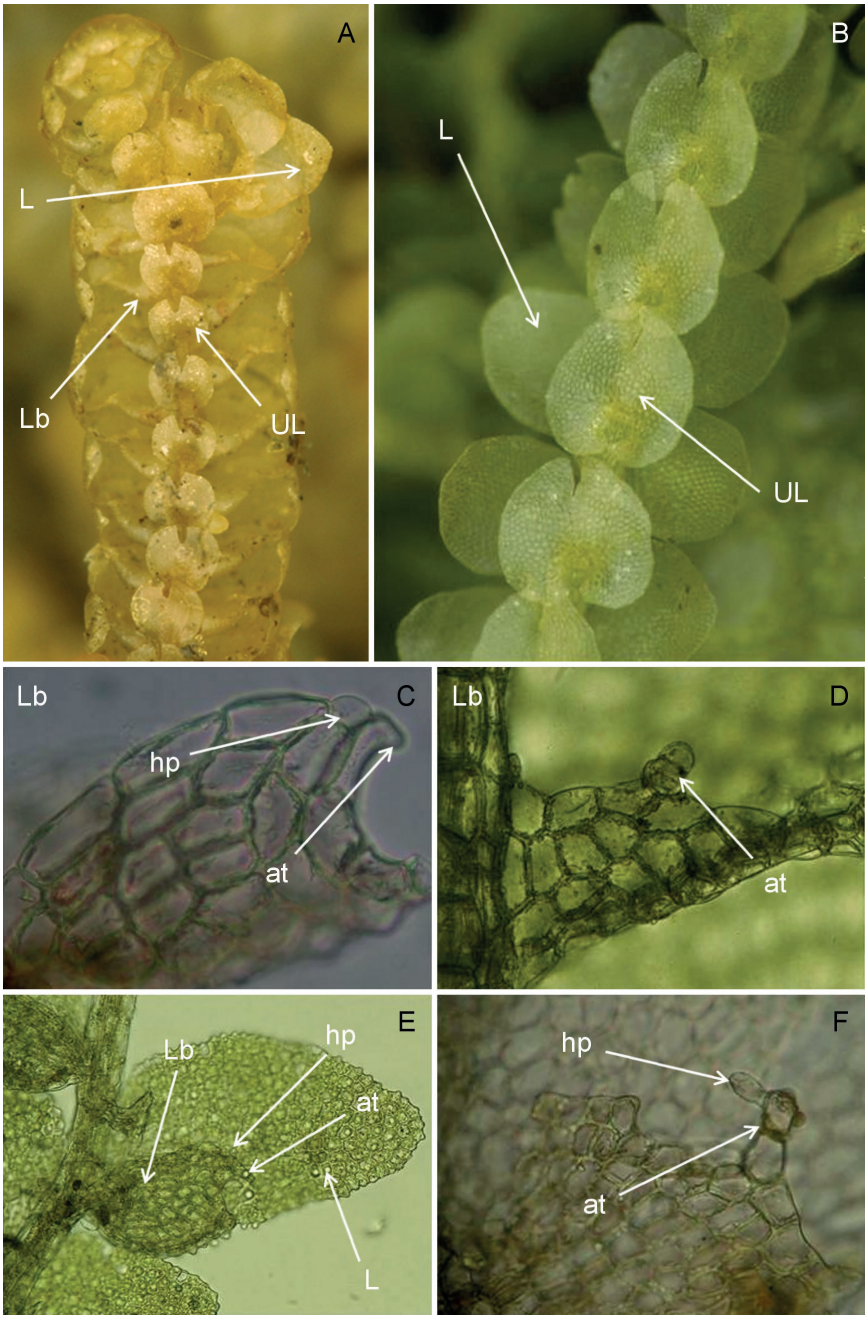

Fig. 24. A. *Cheilolejeunea cordistipula*. **B.** *Lejeunea isophylla*. **C.** *Drepanolejeunea vandenberghenii*, lobule. **D.** *Taxilejeunea conformis*, lobule. **E.** *Cololejeunea platyneura*. **F.** *Cololejeunea pseudo-obliqua*. L = lateral leaf, UL = underleaf, Lb = lobule, at = apical tooth, hp = hyaline papilla.

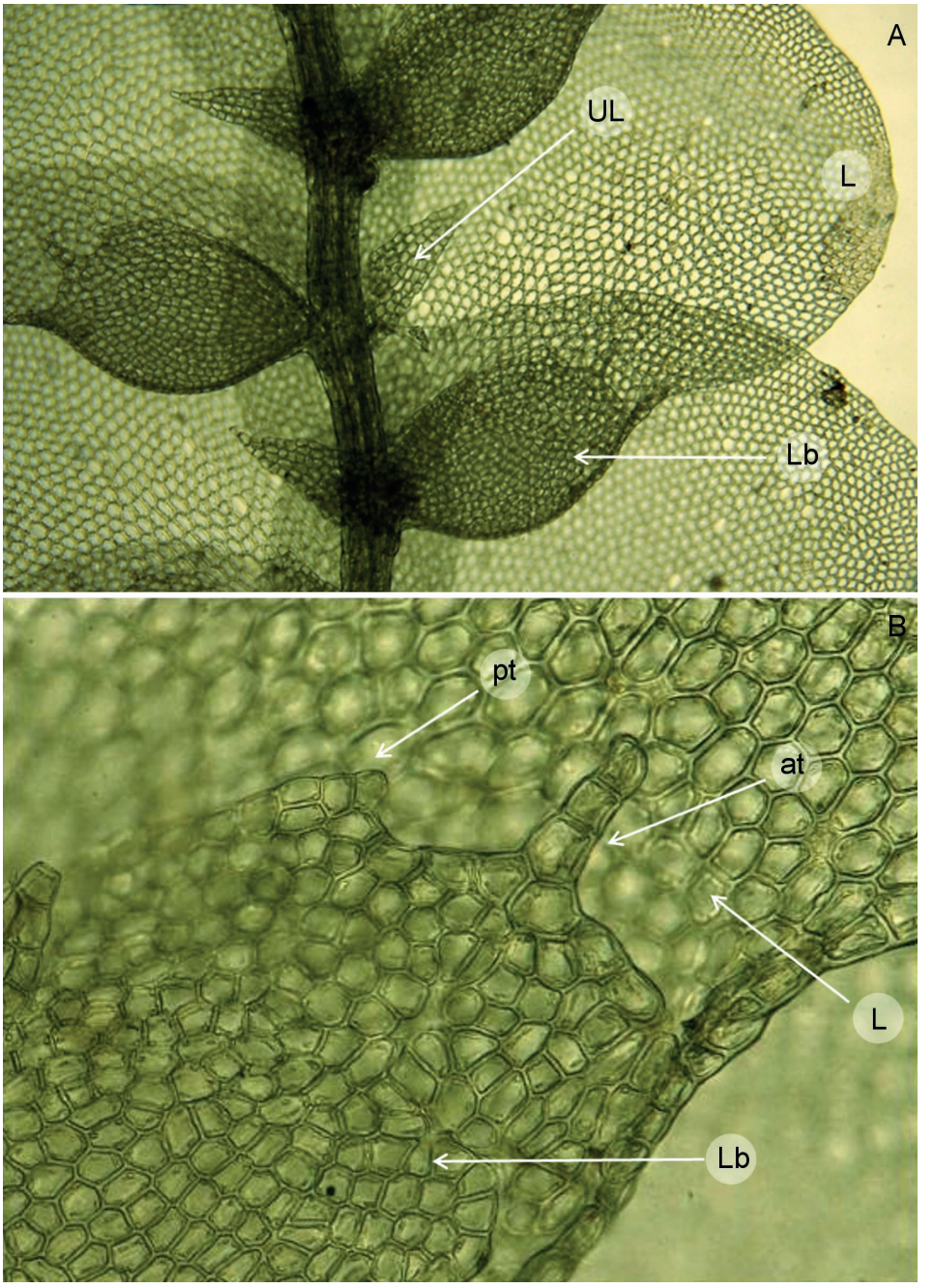

Fig. 25. *Diplasiolejeunea cavifolia*. **A.** Leaves and underleaves. **B.** Detail of lobule. L = lateral leaf, UL = underleaf, Lb = lobule, at = apical tooth, pt = proximal tooth.

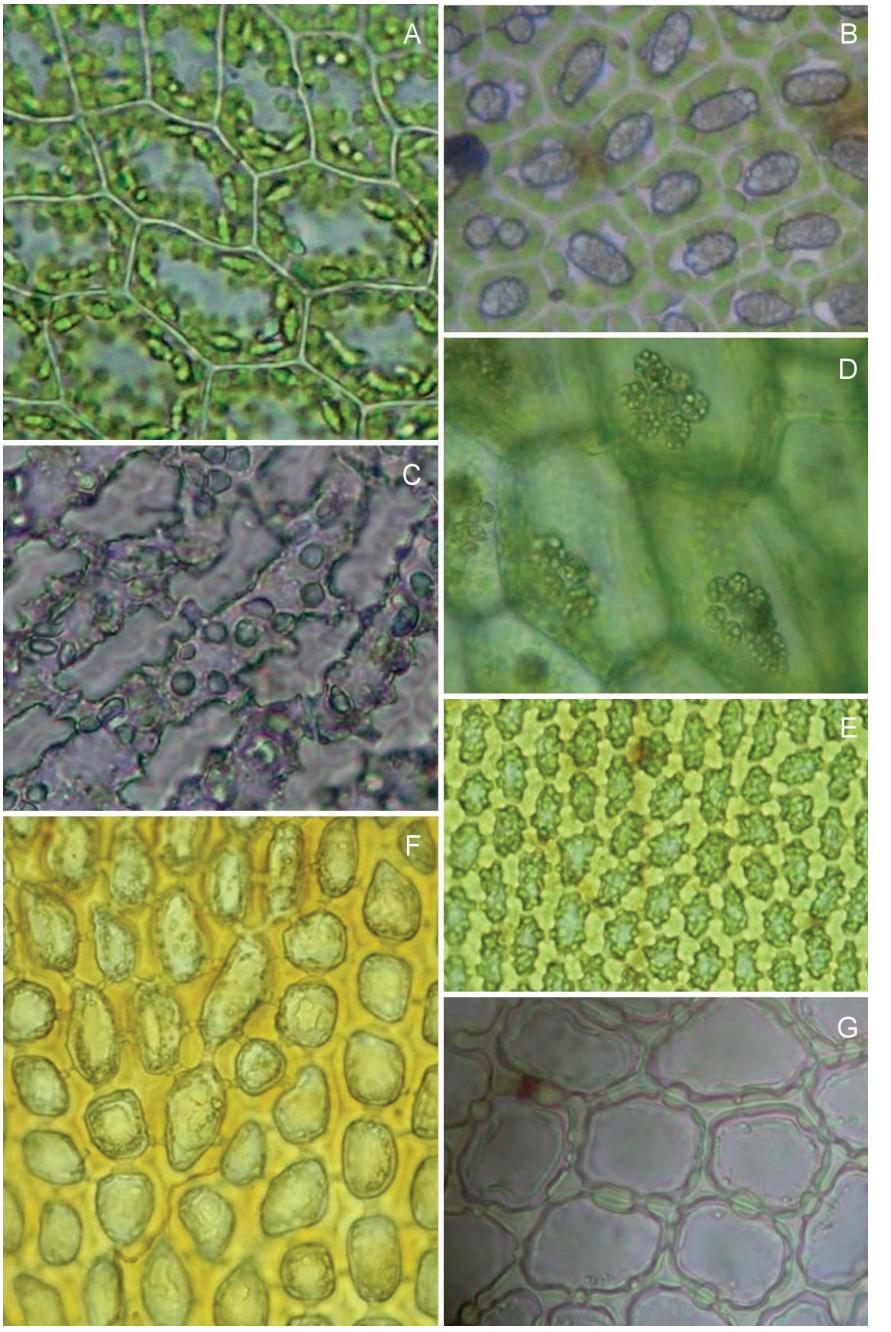

Fig. 26. Oil bodies and cell walls. **A.** *Cololejeunea obtusifolia*. **B.** *Cheilolejeunea xanthocarpa*. **C.** *Herbertus dicranus*. **D.** *Riccardia* sp. **E.** *Plicanthus hirtellus.* **F.** *Mastigophora diclados*. **G.** *Drepanolejeunea vandenberghenii*.

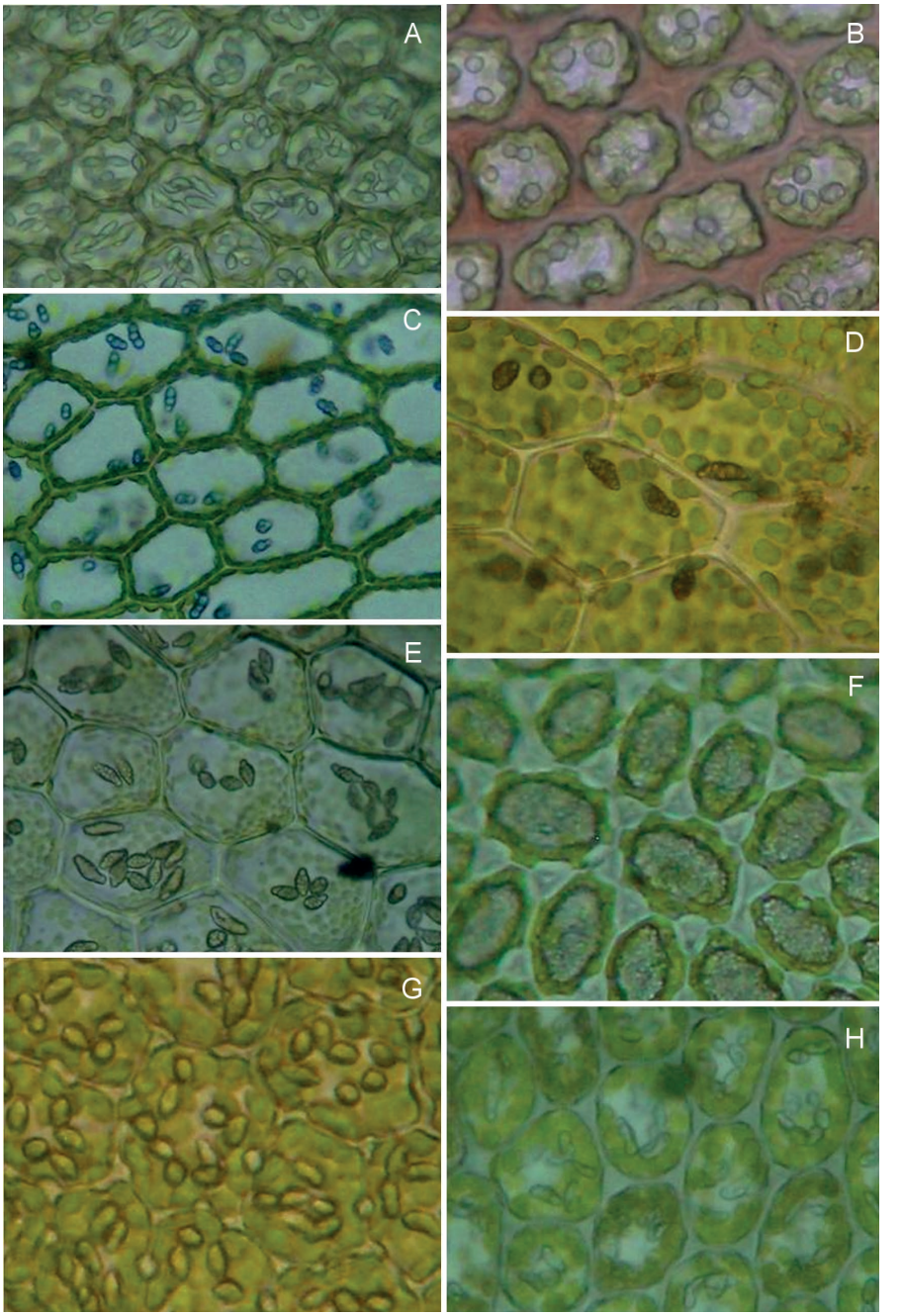

Fig. 27. Oil bodies. **A.** *Acrolejeunea emergens*. **B.** *Anastrophyllum piligerum*. **C.** *Calypogeia afrocaerulea*. **D.** *Calypogeia fissa*. **E.** *Chiloscyphus* sp. **F.** *Radula voluta*. **G.** *Frullania spongiosa*. **H.** *Bazzania decrescens* ssp. *pumila*.

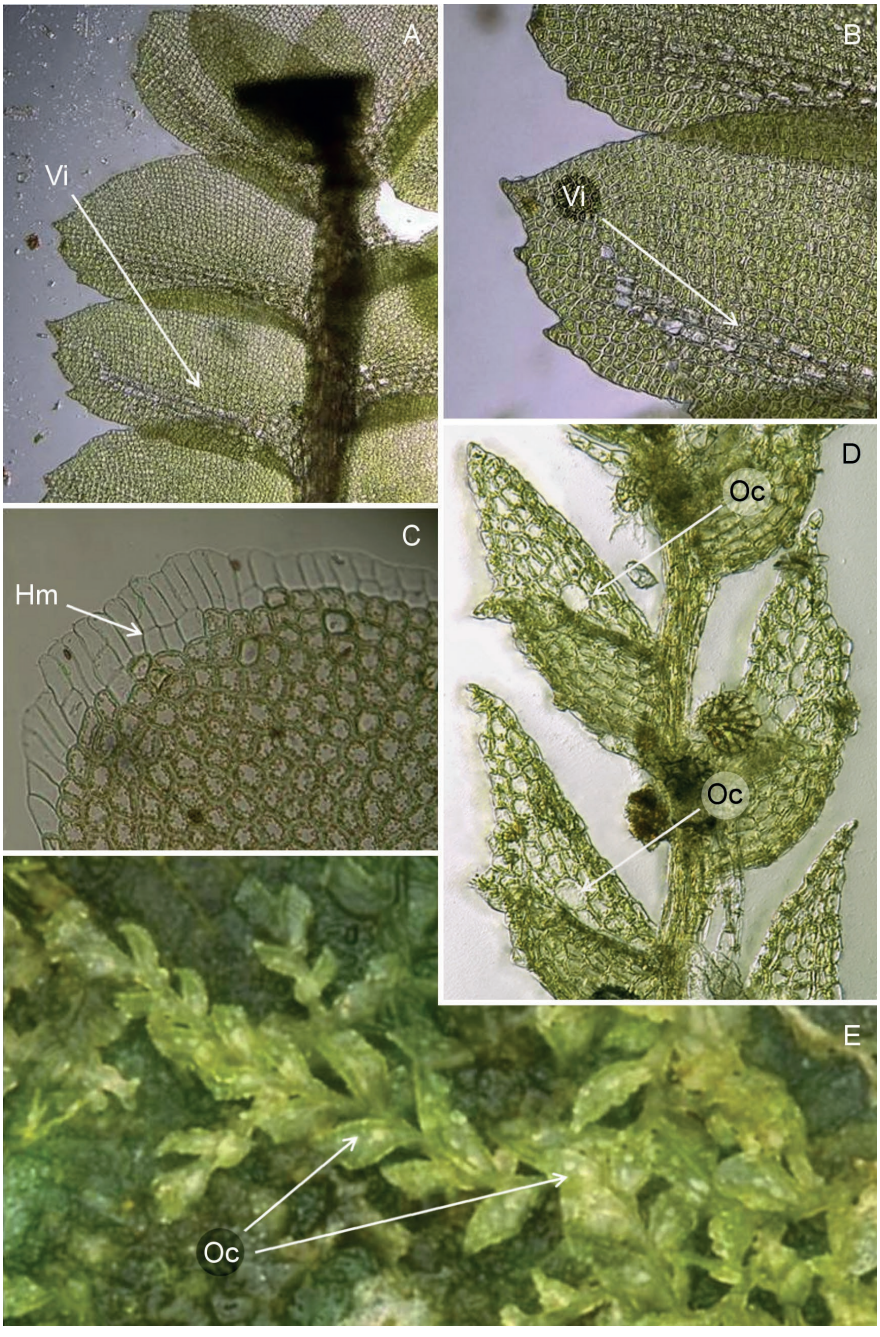

Fig. 28. A-B. *Bazzania nitida*. **C.** *Cololejeunea distalopapillata*. **D.** *Drepanolejeunea ruandensis*. **E.** *Drepanolejeunea cultrella*. Hm = hyaline margin, Oc = ocellus, Vi = Vitta.

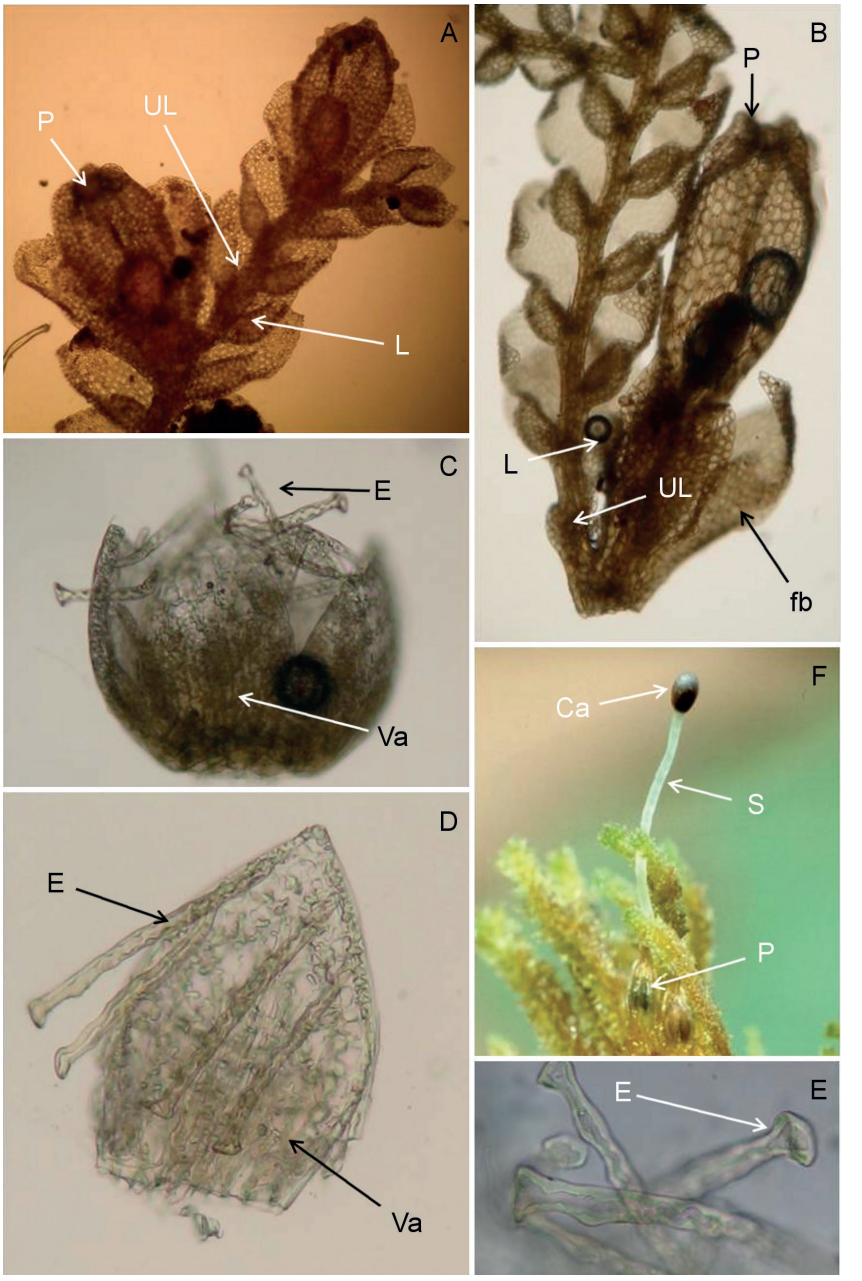

Fig. 29. A. *Harpalejeunea fischeri*, lejeuneoid innovation. **B-E.** *Drepanolejeunea vandenberghenii*. **B.** Pycnolejeuneoid innovation. **C.** Capsule with valves and elaters. **D.** Valve with elaters. **E.** Elaters. **F.** *Plicanthus hirtellus*, perianth and sporophyte. L = Lateral leaf, UL = underleaf. P = perianth, S = seta, Ca = capsule, Va = Valve, E = elater, fb = female bract.

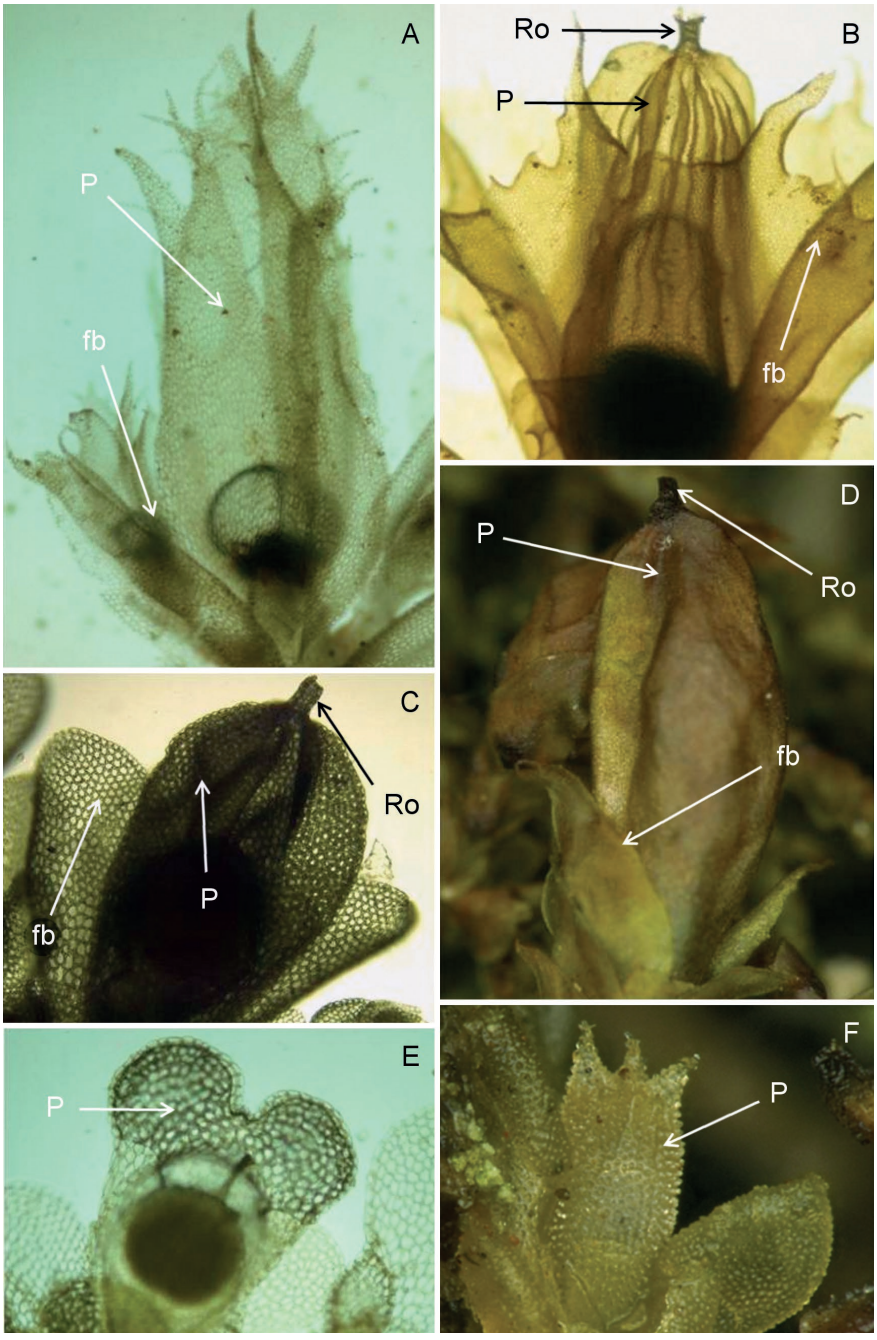

Fig. 30. Perianths. **A.** *Chiloscyphus difformis*. **B.** *Frullania arecae*. **C.** *Lejeunea tabularis*. **D.** *Frullania capensis*. **E.** *Cololejeunea harrisii*. **F.** *Colura berghenii*. P = perianth, fb = female bract, Ro = rostellum.

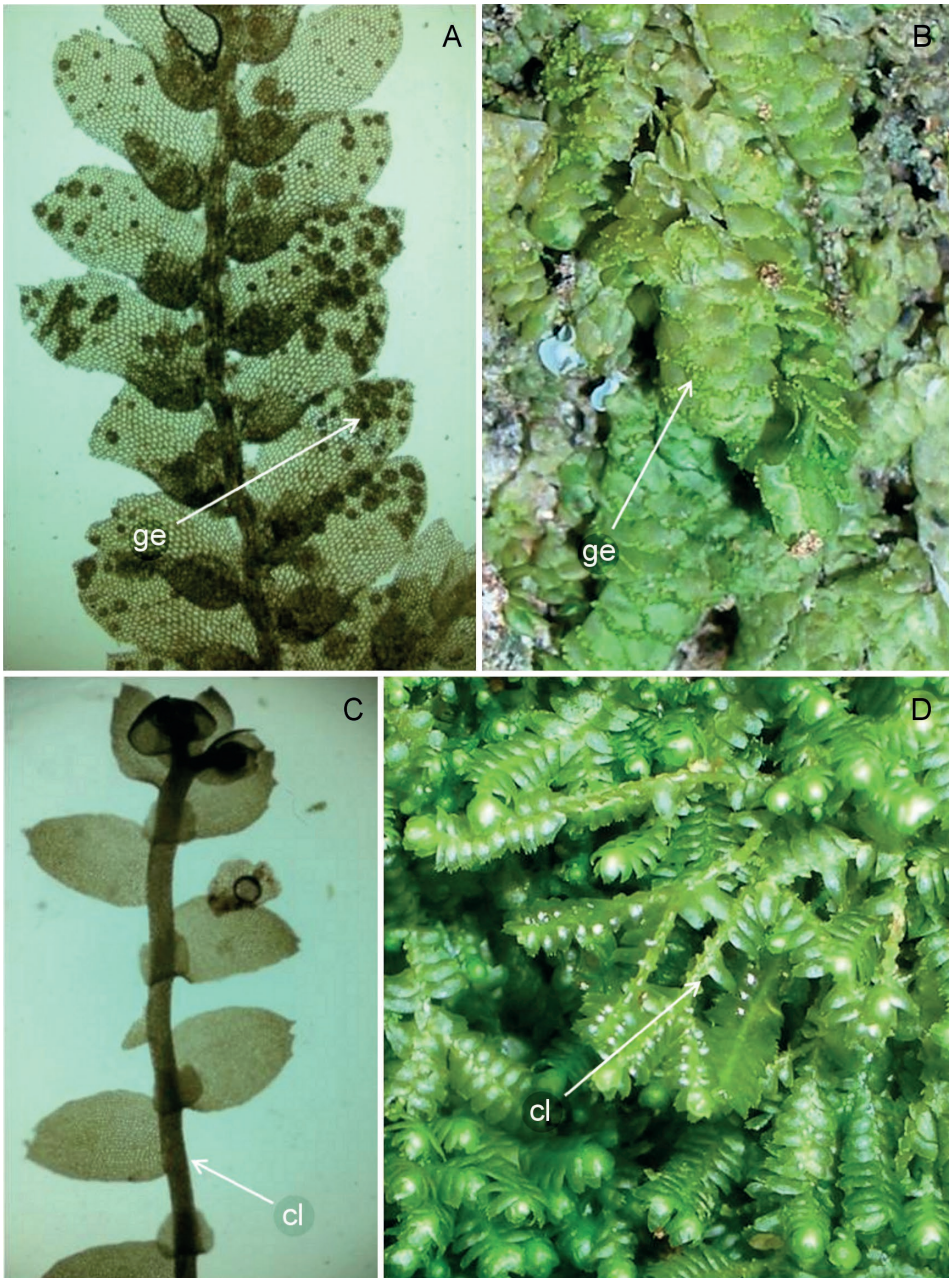

Fig. 31. Vegetative reproduction. **A.** *Cololejeunea malanjae*. **B.** *Radula quadrata*. **C-D.** *Bazzania decrescens* ssp. *pumila*. cl = caducous leaves, ge = gemmae.