

INTRODUCTION

The genus *Prionolejeunea* belongs in the Lejeuneaceae, the most advanced and highly specialized family among the leafy Hepaticae (Mizutani, 1961). This vast family is also the most difficult hepatic group in terms of generic delimitation (Gradstein, 1982). Since the revolutionary generic concept of the Lejeuneaceae was established by Spruce (1884), nearly 100 currently accepted genera with about 2000 species have been described. The difficult taxonomy of the family can be explained by the remarkable morphological diversity of the genera. This variability led to numerous descriptions of new species by many authors, especially by Stephani (1898-1924).

Schuster (1963) attempted to arrange the genera into homogeneous or interrelated groups and emphasized the need for monographic work on the genera. Advances in the classification of the family were provided by new findings on the sporophyte structure and its taxonomical importance by Mizutani (1961). These findings led to a subdivision of Lejeuneaceae into two subfamilies, Ptychanthoideae and Lejeuneoideae, largely based on features of the sporophyte (Mizutani, 1985a; Gradstein, 1994a).

The first modern revisional studies on Lejeuneaceae focused on the ptychantoids, characterized by their rather large plant size. This group has been intensively investigated and is rather well known (Gradstein, 1975, 1977, 1985a, 1985b, 1992a, 1994a, 1994b; Gradstein & Buskes, 1985; Gradstein & van Beek, 1985; Jones, 1970; Kruijt, 1988; Mizutani, 1969, 1979, 1985b, 1986, 1987, 1989, 1993; Stotler & Crandall-Stotler, 1974; Teeuwen, 1989; Thiers & Gradstein, 1989; Vanden Berghen, 1984a, 1984b; van Slageren, 1985; Verdoorn, 1934; etc.). In contrast, the larger subfamily Lejeuneoideae still contains many taxonomic problems, especially because most of its members are very tiny and confusingly variable. Although many genera have been revised or monographed (e.g. Bernecker-Lücking, 1998; Bischler, 1964, 1968a, 1969; Bischler et al., 1963; Dauphin, 2003; Gradstein & Vital, 1975; Grolle, 1966, 1975a, 1975b, 1976, 1977a, 1995; Grolle & Reiner-Drehwald, 1999, 2000; Mizutani, 1961, 1964, 1966, 1967, 1970, 1971, 1972a, 1972b, 1973, 1976; Jovet-Ast, 1953; He, 1999; Piippo, 1986; Reiner-Drehwald, 2000a; Reiner-Drehwald & Goda, 2000; Reiner-Drehwald & Drehwald, 2002; Schäfer-Verwimp, 2004; Schuster & Schäfer-Verwimp, 1995; Tixier, 1973), a considerable number are poorly known. Recent works have demonstrated that the circumscriptions of some genera of the Lejeuneoideae were based on ambiguous characters justifying their synonymy (e.g. Grolle et al., 2001; Grolle &

Zhu, 2000; Ilkiu-Borges, 2005) or were herogeneous and necessitated the description of new genera (e.g. He & Grolle, 2001).

The genus *Prionolejeunea* has never been revised and the status of the majority of its species is unclear. Moreover, there are little means to identify them, in spite of the publication of taxonomical treatment of individual species and species groups by various authors (see next chapter). The situation was well characterized by Schuster (1992), who stated that the taxonomy of this genus is chaotic.

The present study aims at revising the species of *Prionolejeunea* based on study of the type specimens and additional collections, clarifying the generic delimitation and circumscription of the species, as well as testing the monophyly of the genus and trying to reconstruct the phylogeny of the group based on morphological and molecular data. It aims at filling a gap in the literature and providing an identification key to the currently accepted species in the genus.

HISTORICAL SURVEY

The name *Priono-Lejeunea* was introduced by Spruce (1884) for one of his 39 subgenera of *Lejeunea* Lib. Because of its divided underleaves *Priono-lejeunea* belonged to “Schizostipae” (in contrast to the “Holostipae”, with entire underleaves). The subgenus *Priono-lejeunea* comprised 11 species (2 previously described ones and 9 new species) and was recognized by leaves with acute apex and denticulate margins, gynoecea with or without innovation, and flattened, 2-keeled perianths with dentate margins. Spruce (1884) provided information on the habitat and detailed descriptions of the species, showing a rather clear understanding of the group. In subsequent works, Stephani (1890) and Spruce (1895) added further species to the subgenus *Priono-Lejeunea*, although Stephani (loc. cit.) was not clear concerning the status of the group classifying it interchangeably as genus or sub-genus and thus producing many invalid names.

Together with other subgenera established by Spruce (1884), Schiffner (1893) raised *Priono-Lejeunea* Spruce to generic rank as *Prionolejeunea* (Spruce) Schiffn. comprising 5 species. Later on, Stephani (1896, 1897) and Schiffner (1897) added 9 species to the genus. Nevertheless, the greatest contribution to the study of *Prionolejeunea* was made by Evans (1904), who demonstrated the Afro-American distribution of the genus and provided a clear description of its morphology. Evans described five species of which four were new to science, and three further species were

considered new synonyms [*Prionolejeunea vulcanica* (Spruce) Schiffn. = *P. aemula* (Gottsche) A.Evans, *P. bicristata* Steph. = *P. decora* (Taylor) Steph., and *Lejeunea* “*Priono-Lejeunea*” *leptocardia* Spruce = *Cyclolejeunea accedens* (Gottsche) A.Evans]. The author stressed the importance of characters derived from the leaves, lobules, underleaves, sexual branches and perianths, pointed out the great variation in the shape of leaf apex and the leaf margin denticulation in the species and warned for the use of these characters at the species level. He also established new characters of *Prionolejeunea*, such as the presence of a large cell at each underleaf base and the proximal position of the hyaline papilla.

In the “Species Hepaticarum”, Stephani (1913) recognized 60 species of *Prionolejeunea* worldwide, arranging them by region into “Africa” (7 species), “America tropica” (48 species) and “Asia tropica” (5 species). The list of taxa included all the species previously published under the genus, as well as two species synonymized by Evans (1904): *P. vulcanica* and *P. bicristata*. Many species were new combinations of taxa described by Stephani earlier authors (Gottsche et al., 1845; Gottsche, 1864; Hampe & Gottsche, 1852; Mitten, 1861, 1886; Montagne, 1845; Spruce, 1884, 1895; Taylor, 1846) under *Lejeunea*, and 21 species were newly described.

Pearson (1922) described two new species from Congo, *P. uncatifolia* and *P. corbisieri*, and Stephani (1923) added *P. aberrans*, *P. madagascariensis* and *P. pyriflora* from Africa, and *P. erosodentata* and *P. galliotii* from South America. In the next almost thirty years, Herzog (1927, 1937, 1947, 1951, 1955) published new taxa in *Prionolejeunea*, describing six new species and a new form from the Neotropics, one new species from Cameroon, and one from Taiwan (Herzog & Nogushi, 1955). In the meanwhile, Vanden Berghen (1948) choose *P. microdonta* (Gottsche) Steph. as the type of the genus and included *P. principensis* Vand. Bergh. from Africa (Vanden Berghen, 1960). Grolle (1963) transferred the neotropical *Lejeunea transparens* Corda to *Prionolejeunea* and Robinson (1967) described a new species from Colombia. The last valid species included in *Prionolejeunea* before this revision was *P. pellucida* (Herzog) R.M.Schust. from Brazil (Schuster, 1999). Schuster (1992) invalidly introduced 10 new species names in *Prionolejeunea*, the material of which could not be examined during this revision. In total, the number of species and infrageneric taxa in *Prionolejeunea* includes 116 names.

In recent years several new synonyms have been found by different authors, contributing to the knowledge of the morphological variability in *Prionolejeunea*, eliminating misidentification and clearing the boundary between the genus and related ones. Four of the six species reported from Asia were transferred to other genera of Lejeuneaceae, such as *Drepanolejeunea* (Spruce) Schiffn. (Grolle, 1979), *Lejeunea* (Grolle & Piippo, 1984; Grolle 1987a) and *Otolejeunea* Grolle & Tixier (Grolle, 1985). From the 14 African species only *P. grata* (Gottsche) Schiffn. and *P. principensis* Vanden Bergh. remained in *Prionolejeunea*, the other species were synonymized with *Cheilolejeunea* (Spruce) Schiffn., (Grolle, 1977b), *Drepanolejeunea* (Grolle, 1979; Widdington & Grolle, 1996), *Lejeunea* (Grolle, 1981; Jones & Harrington, 1983; Vanden Berghen, 1952) or with *Prionolejeunea grata* (Grolle 1978, Vanden Berghen, 1952).

From 56 valid species and 1 form reported from the Neotropics, many have been placed in other genera or were synonymized. Two species were referred to new genera, *Echinocolea asperrima* (Spruce) R.M.Schust. (Schuster, 1963) and *Pictolejeunea picta* (Gottsche ex Steph.) Grolle (Grolle, 1977a) which also included *Prionolejeunea cuatrecasii* Robins. as a synonym. Seven neotropical species were transferred to *Cyclolejeunea* A.Evans (Grolle, 1984, 1987a, 1991), *Lejeunea* (Grolle, 1988a; Grolle & Reiner-Drehwald, 1999), *Lepidolejeunea* R.M.Schust. (Piippo, 1986) or *Xylolejeunea* He, X.-L. & Grolle (He & Grolle, 2001), and four were identified as synonyms of other *Prionolejeunea* species (Grolle, 1987b, 1991; Gradstein & Costa, 2003). The genus *Echinocolea* R.M.Schust. merged into *Lejeunea*, and *Prionolejeunea glauca* Steph. and *P. immersa* Steph. were regarded as synonyms of *Lejeunea subspathulata* Spruce (Ilkiu-Borges, 2005). Thus, the genus *Prionolejeunea* remained with 40 valid species and 1 form in the Neotropics. Recently, *P. grollei* Ilkiu-Borges & Schäfer-Verwimp was newly described (Ilkiu-Borges & Schäfer-Verwimp, 2005).

Advanced studies on poorly known characters such as stem anatomy and morphology, branching system, oil bodies and sporophytes, not only improved the delimitation of subfamilies and tribes in Lejeuneaceae, but also that of the genera. Bischler (1966) studied the stem anatomy of the Lejeuneaceae and described this character in 12 species of *Prionolejeunea*. Stem morphology was also studied by Crandall (1969), including *P. aemula*. Mizutani (1970) distinguished different types of gynoecial innovation in Lejeuneaceae, which were renamed by Grolle (1980), adding an

important character for distinguishing between genera. The latter author included *Prionolejeunea* in his studies, classifying its innovation branches as *Lejeunea*-type, which was later confirmed by Thiers (1986) in describing the branching system of this genus. Detailed information on the oil bodies of *P. aemula* were first published by Schuster & Hattori (1954). Later on, Schuster (1992) described cell characters such as cell shape, cell size, cell walls, intermediate thickenings, pseudocelli and oil bodies in 16 “species” (the majority of them invalid) and 1 “phenotype” of *Prionolejeunea*. This author considered asexual reproduction, cell features and oil bodies characters as giving the best clues to determine species and described 10 new species based almost wholly on cell features. Characters of the sporophyte of *Prionolejeunea* and other Lejeuneaceae were studied by Weis (2001), providing significant and new taxonomic information and reinforcing the placement of the genus in the Lejeuneoideae. Some of the sporophyte characters have been shown to be important for the generic delimitation of *Prionolejeunea* (Ilkiu-Borges, 2005).

The relationships of *Prionolejeunea* with other genera of Lejeuneaceae were dealt with by Spruce (1884), Evans (1904), Schuster (1963, 1992, 2001) and Gradstein et al. (2001) who reached similar conclusions. The latter authors indicated that the genus was probably closest related to *Cyclolejeunea* or *Echinocolea*.

A cladistic analysis of relationships among 69 genera of Lejeuneaceae was carried out by Gradstein et al. (2003a), using a morphological data matrix of 50 gametophytic and sporophytic characters. In the strict (equal weighting) consensus tree the phylogenetic position of *Prionolejeunea* was unresolved. However, in both majority rule consensus trees based on equal or successive weighting, *Prionolejeunea* was resolved as sister to *Cyclolejeunea* (bootstrap support 100%); the relationship between *Prionolejeunea* and *Echinocolea* was not supported. Ilkiu-Borges (2005) subsequently showed that *Echinocolea* is a synonym of *Lejeunea*.

MORPHOLOGY AND ADAPTATION

MEROPHYTES

The merophytes originate from three cutting faces of the apical cell resulting in two lateral merophytes, which include the lateral parts of the stem and leaves, and one ventral merophyte which includes the ventral part of the stem, underleaves and rhizoids (Douin, 1925). In *Prionolejeunea*, the ventral merophyte is always two stem cells wide.

STEM ANATOMY

Stem anatomy in *Prionolejeunea* was first studied by Bischler (1966), who compared the stem of twelve species of the genus and showed that they presented the typical pattern of the subfamily Lejeuneoideae as defined by Evans (1935). The stems in *Prionolejeunea* possess seven rows of epidermal cells (2 ventral and 2-3 lateral) surrounding a variable number (5-26) of smaller medullary cells. The number of epidermal cells is constant; additional cells observed occasionally in cross section are assumed to belong to the lobule or are leaf brace cells (e.g. Fig. 63S). In transverse section the epidermal cells are large and short, while the medullary cells are thin and long (Fig. 1B, C).

Based on anatomical characters of the stem, Bischler (1966) separated the family Lejeuneaceae into three groups. Variability at the infrageneric level was considered to be insufficient for the recognition of subdivisions. Stem anatomy in *Prionolejeunea* was rather variable regarding the number and relative size of cells, wall thickness, pigmentation and trigone size. *Prionolejeunea* fell into group 1, which was characterized by 7 cortical cells, (3-)4-48 medullary cells, ventral merophyte of 2 cells, relative size between cortical and medullary cells of 1-10/1, thin to thick-walled cells, colourless to pigmented cell walls, and indistinct to large or nodulose trigones. This group contained both species with bifid and undivided underleaves, supporting the notion (Mizutani, 1961; Schuster, 1963) that a correlation between stem anatomy (complex, simple) and underleaves shape (bifid, undivided) as assumed by Evans (1935) is lacking. Yet, the evolutionary hypothesis proposed by the latter author of a general trend of reduction of medullary cells within Lejeuneoideae was confirmed with slight modifications by Bischler (1966). The latter trend is also observed in *Prionolejeunea* in which smaller plants have fewer medullary cells, an exact correlation between plant size and number of medullary cells could not be established, however. It is possible to use the number of medullary cells to separate very large species of *Prionolejeunea* from the smaller ones (e.g. *P. limpida* versus *P. recurvula*), however it is not suitable for distinguishing between species of rather similar plant size. For example, *P. muricatoserrulata* (0.2-0.6 mm wide) has 7-10 medullary cells and *P. recurvifolia* (0.5-0.9 mm wide) ca. 5 medullary cells. In fact, the number of medullary cells highly varies within a plant (e.g. 5-15 in the type specimen of *P. meissneri*) and within a species, even in the ones which are morphologically constant such as *P. galliotii* (7-17 medullary cells).

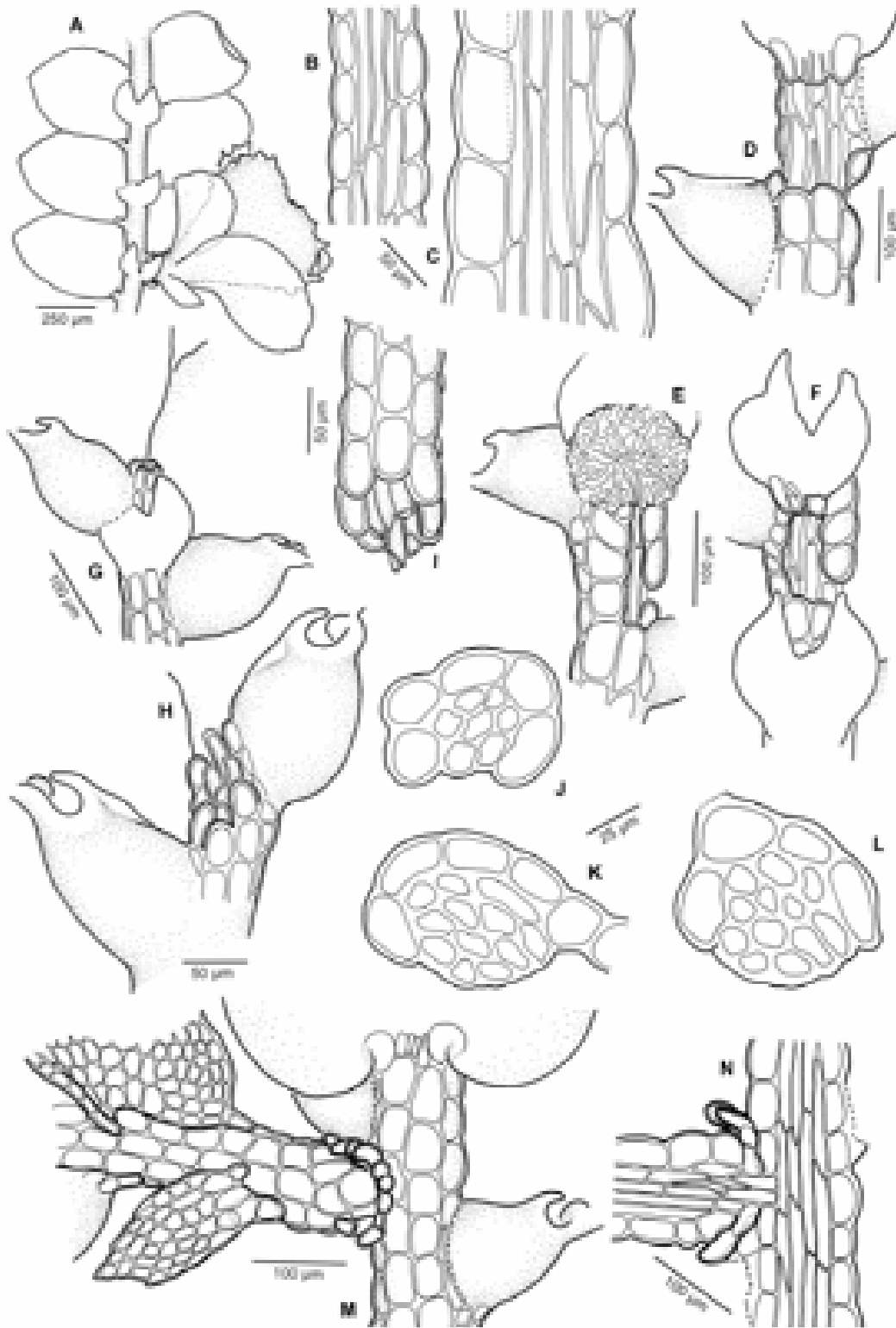


FIG. 1. **A.** Contrary branch, fertile. **B-C.** Transversal section of stem. **D-F.** Stem fragmentation, initial stage. **G-H.** Apex of a stem after fragmentation. **I.** Base of a stem after fragmentation. **J-L.** Cross section of stems in the initial stage of a stem fragmentation. **M.** Base of a contrary branch. **N.** Cross section of a contrary branch. (A from *Benjamins 418*; B, H from *Degelius s.n.*; C from *Bischler 440/a*; D, F, G, K, L from *Duss 1199*; E, J from isotype of *P. principensis*; I from the holotype of *P. circinulata*; M, N from *Gradstein 8964*.).

BRANCHING

Two types of branches are found in *Prionolejeunea*: gyrothecal *Lejeunea*-type branches and athecal *Radula*-type branches (innovations only). According to Crandall (1969), who studied numerous species from different genera of the Lejeuneaceae, including *Prionolejeunea aemula*, the *Lejeunea*-type branch develops from a ventral basiscopic cortical cell below the leaf insertion and is characterized by the presence of a collar derived from leaf brace cells.

Some species of *Prionolejeunea* are able to develop “contrary” branches (new observation). These are vegetative or fertile branches that do not turn around after developing, but arise with their ventral side upward as opposed to the main shoot (Fig. 1A, M, N; 45D, E). Contrary branches were occasionally found in *P. galliotii*, *P. limpida*, *P. magnistipula*, *P. principensis* and *P. scaberula*. Their taxonomic significance remains unclear and requires study of a larger amount of specimens. Also the ecological importance of contrary branches remains unknown.

Microphyllous (not caducous) branches were observed in *P. trachyodes* only. Fulford et al. (1970) reported microphyllous branches in *P. aemula* and observed that the entire branch was readily detached and dispersed. The latter branches are cladia and are discussed under Vegetative Reproduction. Innovations are described under Gynoeceia.

LEAVES

The leaf in *Prionolejeunea* has the typical kind of insertion found in Lejeuneaceae (Schuster, 1963). It is divided into a large dorsal lobe and a small ventral lobe, which are attached to the stem along a J-shaped insertion line. Three hyaline papillae are usually found at the leaves, as in other Lejeuneaceae (Gradstein, 1975, 1994a) except in very old plants where they are usually broken due to their fragility. One papilla is situated at the dorsal leaf insertion, attached between the stem and the upper base of the lobe. A second papilla representing the stylus is found at the lobule insertion between the end of the free margin of the lobule and the stem, and the third one is proximal to the first lobular tooth. The leaf position is hardly different in dry and moist conditions and varies from distant to imbricate, being suberect to obliquely spreading as in *P. schlimiana* or widely spreading as in *P. limpida* and *P. principensis*. The leaf lobe can be plane or convex and highly variable in shape (e.g. orbicular, suborbicular, oval,