



**Taxonomical revision of *Lopholejeunea* (Spruce) Schiffn. (Lejeuneaceae,
Hepaticae) in Africa**

Synopsis of doctoral thesis

ANDREA SASS-GYARMATI

Gödöllő

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Doctoral School

Name: Doctoral School of of Biological Sciences

Discipline: Biology

Leader: DR. ZOLTÁN NAGY, DSc
Institute leading University Professor
SZIU, Agricultural and Environmental Sciences
Institute of Botany and Ecophysiology

Supervisors: PROF DR. SÁNDOR ORBÁN, DSc
University Professor
EKC, Department of Botany and Ecology

DR. TAMÁS PÓCS, MHAS
Professor Emeritus
EKC, Department of Botany and Ecology

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Confirmation of
Head of Doctoral School

.....
Confirmation of Supervisors

.....
Confirmation of Supervisors

BACKGROUND AND RESEARCH OBJECTIVES

My study is related to the actual questions on the current subject of global biodiversity, as the relentless deforestation of extant rainforests which leads to a great loss to such habitats of tropical liverworts; loss of biodiversity, both at species and at population level. Species are disappearing before they have actually been discovered and described for the science. The aim of my dissertation was the taxonomical revision of the genus *Lopholejeunea* (fam. *Lejeuneaceae*) in Africa – a liverwort genus widely distributed in the tropics. Earlier literature data was available for scientists but the only synthetic work describing the African *Lopholejeunea* species was published more than thirty years ago (Vanden Berghen, 1984). This work contains detailed descriptions and geographical distribution of the previously known African species. Since then, as results of intensive fieldwork, several new species have been described from this region, many new localities have been discovered during our work and complete monograph of the American, Asian and Australian species were published (Gradstein 1994, Zhu & Gradstein 2005, Thiers & Gradstein 1989), which contain numerous nomenclatural changes.

Aims of the study:

- 1. identifying collections of our intensive fieldwork and materials sent by other collectors from the East African region (and at smaller extent west African material)
- 2. detailed study of the type material for:
 - a. establishment of the new synonymy and
 - b. the more accurate species distribution
- 3. comparative diagnosis of the species
- 4. new determinative key of the *Lopholejeunea* species
- 5. further objective were to answer specific taxonomical questions formulated during identifying material as:
 - a. the reinterpretation of the sections of the gen. *Lopholejeunea*, and
 - b. describing new species as well
- 6. gathering all information on literature concerning species habitat and distribution
- 7. upgrading floristic knowledge regarding tropical Africa and Indian ocean islands

As a summary of the objectives: I have compiled scientific work, which greatly facilitates researchers on the study of *Lopholejeunea* species, with a taxonomic key that easily identifies species distributed in Africa, a detailed description that integrates the literature and field knowledge.

MATERIAL AND METHODS

Examined material: short presentation of the genus *Lopholejeunea*

Lopholejeunea (Spruce) Schiffner is the largest genus of Lejeuneaceae subfam. Ptychanthoideae. The genus is well characterized by the usually blackish pigmented plants, unlobed underleaves, stems with enlarged cortical cells, usually four ventral merophyte cells, isodiametrical leaf cell and *Massula* type homogeneous oil bodies. Perianth two to five-keeled, gynoeical innovations usually absent, perianth keels toothed to laciniate, non-articulate seta and 30 elaters per capsule.

Tools and methods

Methods: description of *Lopholejeunea* genus and species

a. Literature work:

All existing papers of the *Lopholejeunea* species distributed in Africa were collected and the information critically analysed based on my experience. Diagnosis given by various authors were compared with one another and unified. The given information combined with my own results can be seen in specific parts of the “Results” chapter.

b. Herbaria work, analysis of dried scientific material:

During my work firstly I have examined *Lopholejeunea* specimens collected mainly by professor Tamás Pócs from Africa and Indian Ocean islands deposited in the herbarium of the Eszterházy Károly College (EGR). and several other materials sent as exchange. Additionally I supplemented the data with my own collections from Tanzania (2004) and Reunion Island (1996). I have identified numerous specimens sent by other collectors and from other herbaria.

In total I have examined 3000 dried *Lopholejeunea* specimens deposited in the Herbaria of Eszterházy Károly College (EGR), the Belgian National Herbaria (BR), the National History Museum from London (BM), Herbarium of Geneva (G), Herbarium of the University of Dresda (DR) and the Field Museum of Chicago (F). Type specimens sent on loan from the relevant international herbaria were also examined and marked with (!) key. The study area covers all African biogeographical regions. Geographical and distributional data was taken from labels, as well as from the relevant literature and my own field experience. All data on herbaria labels reads as: species name, auctor name, collection locality, name of collector, collecting data and GPS coordinates (if available). At the enumeration of the representative specimens all data are published in original language without any changes. All studied representative specimens are enumerated after detailed species descriptions arranged in alphabetical order of counties or regions.

c. Morphological and anatomical analysis:

Macro morphological analysis as branch length and width of the stem, type of vegetative branches were observed under Leica MZ 16 microscope (magnification: 12-60x) in dry condition of the specimens. Micro morphological analysis as stem structure, shape and size of leaf cells, wall thickness, oil bodies, leaf lobe and lobule connections, under leaf shape and insertion line on the stem and perianth characters were examined under Olympus CX 40 light microscope (magnification: 40-100-400-600x) after specimens were fully moistened with tap water. In case of the new species I have made own drawings with aid of DP-12 photo capturer installed on the Olympus CX 40 light microscope. In every other case I have used illustrations that were already published. In my other publications related to this topic, scanning electron microscope (SEM) images made with SEM Hitachi S-2360 microscope at the Semmelweis University were also used. Measurements always represent the complete observed morphological variation of the plants.

RESULTS

Results based on literature: phylogenetics of the genus *Lopholejeunea* and relationships with other genera

All authors agreed that *Lopholejeunea* is a member of the subfamily Ptychanthoideae, characterized mainly by the ptychanthoid-type capsule and seta consisting of 16 outer rows and 4 inner rows of cells (Van Slageren 1985, Gradstein 1994). Several attempts were in the past to classify the *Lopholejeunea* genera (Schuster 1963, Bischler 1965, Gradstein 1975, 1994, Weis 2001). Cladistical analysis of the phylogenetic tree based on 49 morphological (18 sporophyte and 31 gametophyte) characters shows that genus *Lopholejeunea* is the member of Acrolejeuneinae clade and *Caudalejeunea* (Steph.) Schiffn. is the closest relative (Gradstein et al. 2003). This analysis correlates well with earlier study (Gradstein 1994) which evaluates fewer characters and less number of species. Combined molecular analysis reveal a robust core group of 7-8 liverwort genera in the Ptychanthoideae subfamily including *Lopholejeunea* and several smaller clade (Wilson et al. 2007, Gradstein 2013). Further studies are needed to clarify whether the Ptychanthoideae are a monophyletic group or instead it forms an assemblage of several smaller clades.

Taxonomical treatment based on literature and my own data:

Subgenera and sections

Thiers (1983) firstly suggested division of *Lopholejeunea* genera into subgenera and proposed subgen. *Pteryganthus* for the *Lopholejeunea sphaerophora* (Lehm. et Lindenb.) Steph. species collected from Mauritius island. Sass-Gyarmati (2001) described *Lopholejeunea leioptera* Gyarmati species collected from Madagascar which fits well into this subgenera. In his treatment on the *Lopholejeunea* species from Africa Vanden Berghen (1984) accepted three subgenera (subg. *Laciniatae*, subg. *Lopholejeunea* and subg. *Pteryganthus*) and two sections (sect. *Muellerianae* and sect. *Lopholejeunea*) based mostly on the perianth morphology and characters. He proposed new subgenera *Laciniatae* Vanden Berghen based on the five keeled perianth. As it is shown in this study gynoecia characters in the *Lopholejeunea* genus are very variable even at species level and it can be used carefully for infrageneric taxonomical division. Earlier reduction of the sections to synonyms was done by Zhu & Gradstein (2005). For systematic classification I considered both the perianth and morphological characters of leaves.

***Lopholejeunea* sections in Africa**

Lopholejeunea species distributed in Africa and the Indian Ocean islands are classified into two subgenera: subgen. *Lopholejeunea* and subgen. *Pteryganthus*. Subgen. *Lopholejeunea* divides into another two sections: sect. *Lopholejeunea* with pantropical distribution characterized by connection of leaf lobule to the lobe across 2-8 cells. This section is represented by three species and a variety in Africa: *Lopholejeunea subfusca* (Nees) Schiffn., *L. subfusca* (Nees) Schiffn. var. *elongata* Vanden Berghen, *L. borbonica* Gottsche ex Steph., *L. lepidoscypha* Kiaer & Pearson.

Lopholejeunea sect. *Eulophae* also pantropical distribution includes those African species where leaf lobules are connected to leaf lobe across only one cell. This section summarizes six species in Africa: *Lopholejeunea eulopha* (Tayl.) Schiffn., *L. nigricans* (Lindb.) Schiffn., *L. revoluta* Jones, *L. laciniata* Jones, *L. jonesii* Vanden Berghen és *L. minima* Vanden Berghen.

Vanden Berghen (1984) gives a detailed description and illustration of species which are member of subgen. *Pteryganthus* and gives a determination key. Originally this subgenera comprises five species. Later, another two species was added to this subgenera: *Lopholejeunea erugata* Thiers (Thiers, 1984) and *L. leioptera* Gyarmati (Sass-Gyarmati, 2001). In this study *Lopholejeunea tixieriana* Vanden Berghen is treated as a new synonym of *L. onraedtii* Vanden Berghen. According this subgen. *Pteryganthus* includes six species and is a typical endemic group of the Indian Ocean islands.

In this study, 15 species and a variety is accepted from Africa and Indian Ocean islands as five species proved to be synonymous. All species could be identified from the new determination key and illustrations. Beside the known distributional data (Vanden Berghen 1984, Thiers 1984, Jones 1957) I increased with 229 new data and I give several new details to the known species distribution.

New scientific results

1. African species are classified in two subgenera (subg. *Lopholejeunea*, subg. *Pteryganthus*) and two sections (sect. *Lopholejeunea*, sect. *Eulophae*). Based on literature, fieldwork and examination of 3000 herbarium specimens I have not been able to find other species belonging into other subgenera.
2. I have made a new determination key for the *Lopholejeunea* species distributed in Africa and the Indian Ocean Islands.
3. During this work, I gathered and standardized diagnoses of *Lopholejeunea* species distributed in tropical Africa, so they can easily be compared to each other. For each of these species, I have indicated the type specimen of the herbaria where it is being preserved, as well as detailed species descriptions, additional illustrations, species distribution, habitat characteristics, reference literature and the important and representative information from the examined herbarium specimens.
4. I have compiled an updated synonym list of *Lopholejeunea* species distributed in Africa.
5. I described *Lopholejeunea leioptera* (Sass-Gyarmati, 2001) as a new species to science from Madagascar, and based on particular perianth characteristics, it was classified in the subgenus *Pteryganthus*.

I found that five species are synonymous with an earlier described species:

6. With the permission of M. T. Herzog the protologue of *Lopholejeunea obtusilacera* Herz. species was published by Vanden Berghen (1950) but when describing he mentions that this species could be a morphological form of *L. subfusca* (Nees) Schiffn. During the examination of the holotype, I considered *Lopholejeunea obtusilacera* Herz. as a synonym of *L. subfusca* (Nees) Schiffn., based on attachment of the leaf lobule apex to the leaf lobe and the perianth characters.
7. Vanden Berghen (1984) described *Lopholejeunea quinquecarinata* Vanden Berghen species based on Tanzanian specimens (East-Africa) characterized by the presence of the dorsal keel of the perianth, however the presence or absence of dorsal keel is a variable character of *L. nigricans* (Lindb.) Schiffn., varying even within populations, so *L. quinquecarinata* Vanden Berghen is considered a new synonym of *L. nigricans* (Lindb.) Schiffn.

8. Description of *Lopholejeunea multilacera* Steph. was based on the presence of dorsal keel of the perianth and other overestimated characters such as the relatively large underleaves and the pointed leaf tips. As we have seen in the previous case – neither the presence/absence of dorsal keel of the perianth, nor the larger underleaves and pointed leaves are not sufficient to separate new species, therefore *Lopholejeunea multilacera* Steph. species is considered here as a new synonym of *L. nigricans* (Lindb.) Schiffn.
9. *Lopholejeunea paramultilacera* Vanden Berghen is known only from the collecting site of the type specimen and in the description Vanden Berghen considered it as an etiolate form of *L. multilacera* Steph. It differs from *L. multilacera* Steph. based on the smaller and distant underleaves, the ventrally slightly concave leaf lobe and the missing dorsal keel of the perianth. As *Lopholejeunea nigricans* (Lindb.) Schiffn. is the most variable species within the genus and the differences outlined above are not sufficient to maintain *L. paramultilacera* Vanden Berghen as a separate species. Therefore, it fits well in the boundaries of *L. nigricans* (Lindb.) Schiffn. and I consider it as a new synonym.
10. *Lopholejeunea tixieriana* Vanden Berghen and *L. onraedtii* Vanden Berghen are considered as two different phenotypes of the same species. Size of the perianth beak and the grade of integrity or teethness of the perianth keels are not sufficient to maintain them as separate species. For this reasons I consider *Lopholejeunea tixieriana* Vanden Berghen as a synonym of *L. onraedtii* Vanden Berghen.
11. Occurrence of *Lopholejeunea eulopha* (Taylor) Schiffn. on the island of Principe which is considered new distribution even though is considered pantropical species it hasn't been indicated yet from West Africa. Also occurrence of *Lopholejeunea laciniata* EW Jones in Kenya is a new distributional data.
12. *Lopholejeunea sphaerophora* (Lehm. et Lindenb.) Steph. species was known only from Mauritius island for a long time, so specimens collected from Madagascar represent also a new distributional data.

CONCLUSIONS AND PROPOSALS

The Indian Ocean islands have a very high diversity and very rich in endemic taxa as well. This is probably due to the gondwanian origin of part of the islands and isolation from the continent. For this reasons Indian Ocean islands are considered one of the diversity centres of the genus *Lopholejeunea* and the secondary centre of evolution. Endemic species are represented in a very high percent (62,5%), a total of ten species proved to be endemic in Madagascar, Seychelles-islands and to Reunion island.

More intensive fieldwork is necessary to refine the conservation status for species indicated as rare in my study. Additionally, further discovery of new species can be expected, and distributional data of certain species can be extended.

I wish to extend my examinations to the Pacific archipelago, which is considered also as a biodiversity centre and two new species were described from this region: *Lopholejeunea pocsii* Gyarmati (Sass-Gyarmati, 2005) and *Lopholejeunea vojtkoana* Gyarmati (Sass-Gyarmati, 2008). The genus *Lopholejeunea* shows a typical example of the insular speciation and evolution and therefore further investigation of the island's flora is definitely a promising objective.

Furthermore I would like to supplement and refine the morphology-based approach with advanced molecular genetic studies. Within this context it can be established what could be the informative characters and good indicators of the evolution or if it is just a case of mere homoplasy.

SCIENTIFIC PUBLICATIONS IN THE SUBJECT OF THE DISSERTATION

Publications in English with IF:

- Sass-Gyarmati A. & Pócs T. (2014). *Acanthocoleus elgonensis* Gyarmati et Pócs, sp. nov. from Mount Elgon (Uganda). *Cryptogamie/Bryologie* **35 (2)**: 119–125.
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- Sass-Gyarmati A. (2005). *Lopholejeunea pocsii* Gyarmati (*Lejeuneaceae*, subfam. Ptychanthoideae), a new species of subgenus *Pholianthus* B. Thiers & Gradstein from the Fiji-Islands. *Cryptogamie/Bryologie* **26 (4)**: 403–410.
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- Heinrichs J., Kettunen E., Lee GE., Pócs T., Ragazzi E., Renner MAM., Rikkinen J., Sass-Gyarmati A., Schäfer-Verwimp A., Scheben A., Solórzano Kraemer MM., Svojtka M., Schmidt A. (2015). Diverse Lejeuneaceae (Marchantiophyta) from a species-rich taphocoenosis in Miocene Mexican amber, with a short survey of liverworts in amber. *Review of Palaeobotany and Palynology* (in press).
- Ahonen I., Sass-Gyarmati A. & Pócs T. (2005). Molecular, morphological, and taxonomic evaluation of the *Ptychanthus striatus* (Lejeuneaceae, Marchantiophyta) complex. *Acta Botanica Hungarica*. **47** (3-4): 225–246.
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