

***Cheilolejeunea lamyi* sp. nov., a fossil Lejeuneaceae from Miocene Dominican amber**

Jochen HEINRICHS^a*, Alfons SCHÄFER-VERWIMP^b,
Matthew A. M. RENNER^c & Kathrin FELDBERG^a

^aLudwig Maximilian University, Faculty of Biology, Department of Biology
and Geobio-Center, Menzinger Straße 67, 80638 Munich, Germany

^bMittlere Letten 11, 88634 Herdwangen-Schönach, Germany

^cRoyal Botanic Gardens and Domain Trust, Mrs Macquaries Road, Sydney,
NSW 2000, Australia

Abstract – An inclusion in Miocene Dominican amber is described as *Cheilolejeunea lamyi*, sp. nov. The fossil resembles the extant *Cheilolejeunea rigidula* but differs in the partly subacute leaf lobes, often apiculate underleaf lobes, and the slightly more acute lobes of the bracteole. The two other *Cheilolejeunea* species in Dominican amber, *Cheilolejeunea antiqua* and *C. suzannensis*, have entire rather than bifid underleaves.

Epiphyte / *Hymenaea* resin / Jungermannioipsida / Lejeuneaceae / liverwort / Neogene / Porellales

INTRODUCTION

Fifteen to twenty million years old Dominican amber (Itturalde-Vinent & McPhee, 1996) is the most important source of fossils of the largely epiphytic liverwort family Lejeuneaceae (Wilson *et al.*, 2007). Representatives of 17 extant genera of Lejeuneaceae are known from this Miocene amber forest (Lee *et al.*, 2015), a tropical lowland forest with the resin-exudating trees belonging to the legume genus *Hymenaea* L. (Poinar, 1991; Penney, 2010).

So far, two representatives of *Cheilolejeunea* (Spruce) Steph. have been reported from Dominican amber, the common *Cheilolejeunea antiqua* (Grolle) W.Ye & R.L.Zhu (Grolle, 1990; Kaasalainen *et al.*, 2017) and *Cheilejeunea suzannensis* (Grolle) Grolle & R.L.Zhu, a rare fossil species known only from the type. Both species were originally assigned to other genera [*Leucolejeunea antiqua* Grolle (Grolle, 1983); *Cyrtolejeunea suzannensis* Grolle (Grolle, 1984)] yet molecular phylogenies suggested a synonymy of *Leucolejeunea* A.Evans and *Cyrtolejeunea* A.Evans with *Cheilolejeunea* (Schäfer-Verwimp *et al.*, 2014; Ye *et al.*, 2015).

* Corresponding author: jheinrichs@lmu.de

Revisionary studies of amber inclusions of liverworts yielded a third representative of *Cheilolejeunea* in a piece of Dominican amber kept in the Museum of Natural History Stuttgart. This fossil was assigned to the extant species *Cheilolejeunea rigidula* (Nees ex Mont.) R.M.Schust. by the late Riclef Grolle (identification slip from 1988) but the identification was obviously never published. Our examination pointed to morphological differences of the fossil and extant material of *C. rigidula*, and we therefore name it in honour of Denis Lamy on the occasion of his 70th birthday.

MATERIALS & METHODS

Specimen SMNS Do-4263-B-1 consists of a triangular piece of amber with a length of 15 mm and a maximum width of 8 mm that was ground flat and embedded in synthetic resin. It has a diameter of ca. 2 mm and includes a single liverwort growing on a fragment of bark. The fossil was examined with a Leica M50 dissection microscope and a Carl Zeiss AxioScope A1 compound microscope using incident and transmitted light simultaneously. Images were captured with a Canon EOS 60D digital camera attached to the compound microscope. To enhance the depiction of three-dimensional structures, figures have been prepared as photomicrographic composites, digitally stacked from up to 33 focal planes with the software package HeliconFocus 6.7 (Figs 1-8). Several Dominican amber fossils of *Cheilolejeunea antiqua* were studied using the same methodology. Specimen SMNS Do-4263-B-1 was compared with descriptions in the botanical literature and other liverwort fossils from the American Museum of Natural History New York, the Museum für Naturkunde at Berlin, the Stuttgart State Museum of Natural History, the Geoscientific Collections of the Georg August University Göttingen, the Geological-Palaeontological Museum of the University of Hamburg, the Bavarian State Collection for Palaeontology, and the National Museum of Natural History of the Smithsonian Institution, and with herbarium material of extant liverworts from the private collection of Alfons Schäfer-Verwimp and the Bavarian State Collection for Botany (M).

SYSTEMATIC PALAEOBOTANY

Cheilolejeunea lamyi Heinrichs, Schäf.-Verw.,
M.A.M.Renner & Feldberg, **sp. nov.**

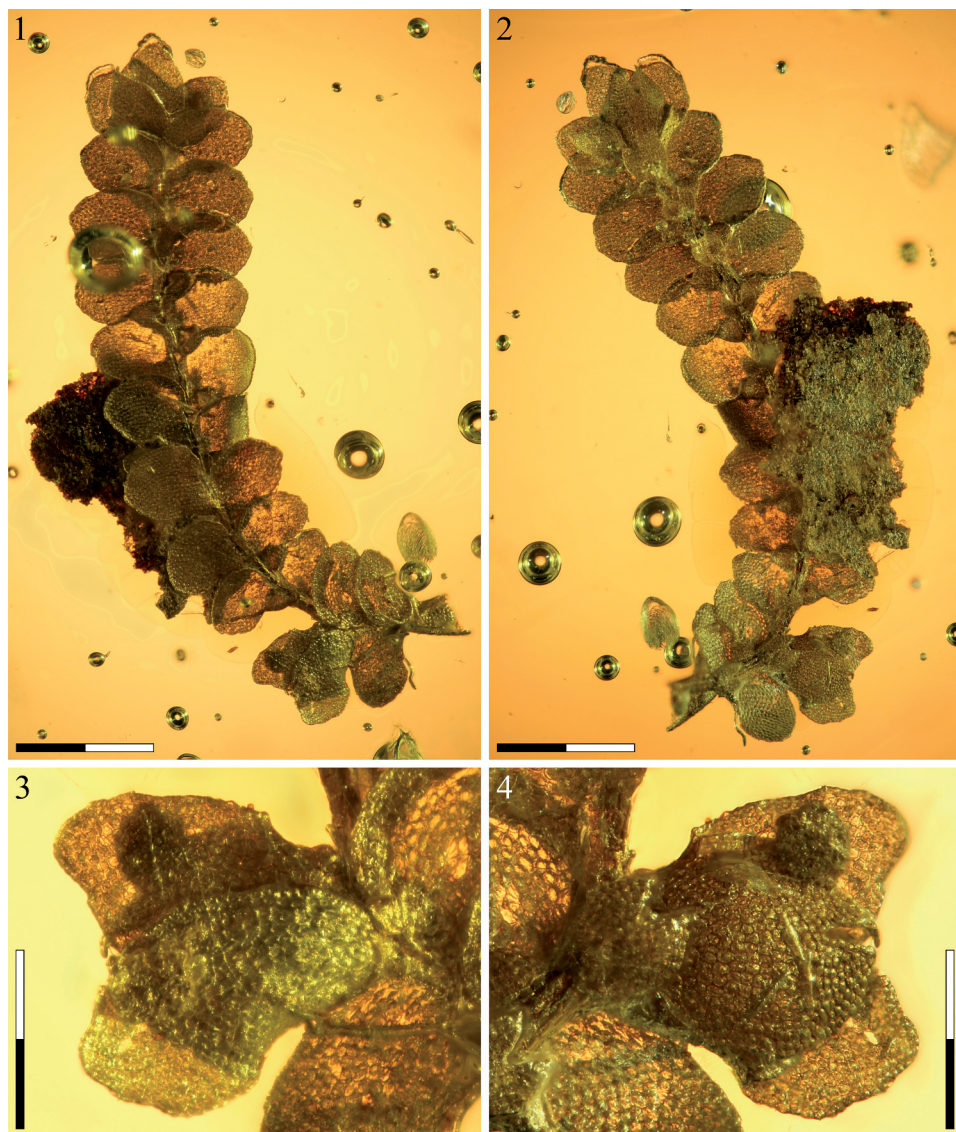
Figs 1-8

Holotype: Liverwort amber inclusion SMNS Do-4263-B-1

Syninclusions: fragments of bark

Repository: The specimen is deposited in the amber collection of the Museum of Natural History Stuttgart (SMNS).

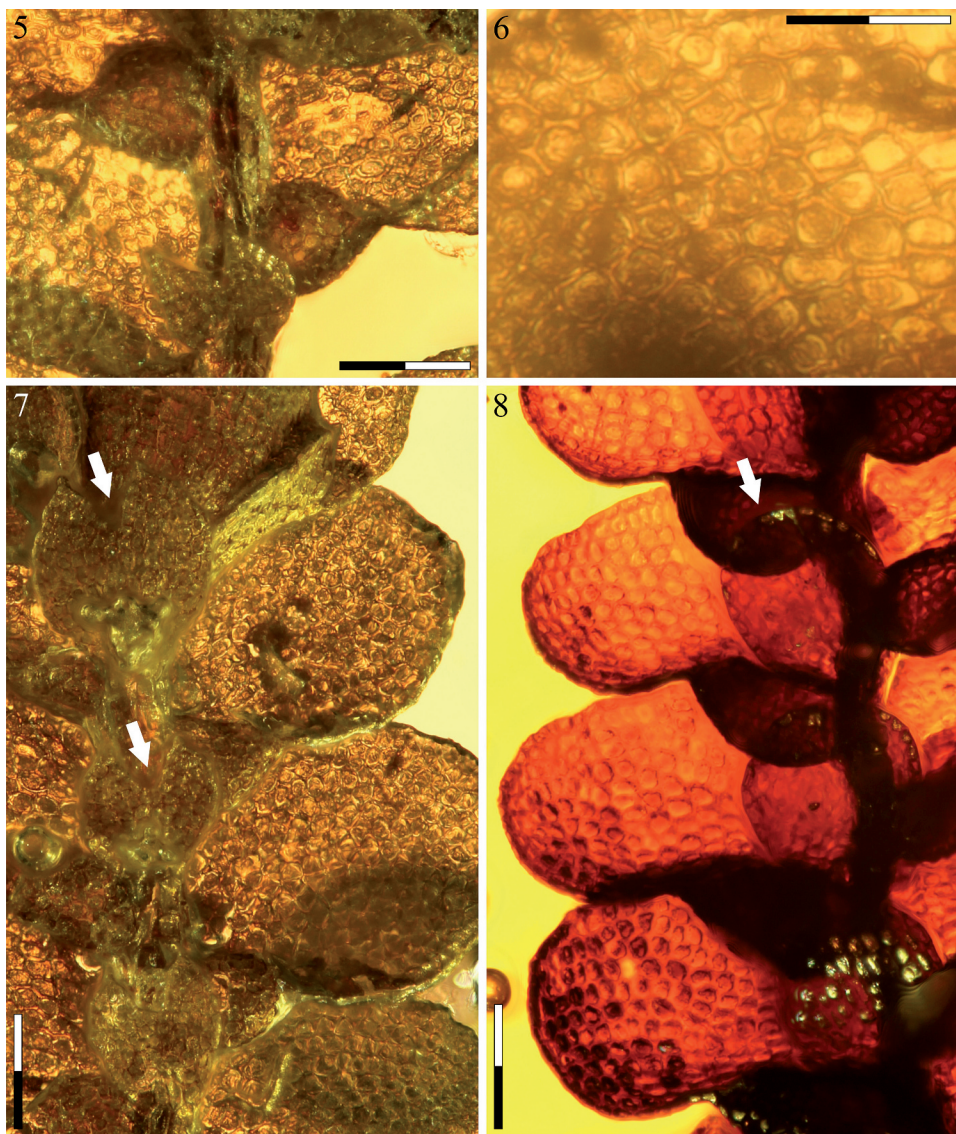
Etymology: The species is named in honor of Denis Lamy, editor of the journal *Cryptogamie*, *Bryologie* for many years.



Figs 1-4. Holotype of *Cheilolejeunea lamyi*, sp. nov. (SMNS Do-4263-B-1) growing on a piece of bark. 1. Specimen in dorsal view. 2. Specimen in ventral view. 3. Female gynoeceium in dorsal view, perianth not preserved. 4. The same in ventral view. Scale bars 1, 2 = 0.5 mm; 3, 4 = 0.2 mm.

Specific diagnosis: Liverwort differing from the extant *Cheilolejeunea rigidula* in the partly subacute, slightly elongate leaf lobes, often apiculate underleaf lobes, and the slightly more acute lobes of the bracteole.

Description: A single pale-brown female shoot with a length of 2.5 mm and a diameter of 0.5-0.67 mm; stem brown, 0.5-0.6 mm in diameter, regularly slightly curved, with somewhat enlarged epidermal cells and a two cells wide ventral



Figs 5-8. Holotypes of *Cheilolejeunea lamyi*, sp. nov. (5-7) and *Cheilolejeunea antiqua* (8). **5.** Close-up of bifid underleaf and leaf lobules. **6.** Leaf lobe cells with distinctly thickened walls. **7.** Portion of shoot in ventral view, note the bifid underleaves with apiculate lobe margins (arrows). **8.** Portion of shoot in ventral view with entire-margined underleaves (arrow). Scale bars 5, 7, 8 = 0.1 mm; 6 = 0.05 mm.

merophyte, epidermal cells in top view short-rectangular, *ca* (20-)25-35 × 15-20 µm, walls thin to slightly thickened; rhizoids in bundles at underleaf base. Leaves contiguous, wide-spreading; leaf lobes suborbicular to ovate, plane, 0.24-0.31 × 0.21-0.27 mm, margin entire, apex rounded or subacute, dorsal margin arched, ventral margin slightly arched to straight. Cells in leaf centre ± isodiametric,

ca 18-25(-30) μm in diameter, towards leaf base overall slightly more elongate and slightly larger, near leaf apex overall slightly smaller, sometimes only 11-15 μm in diameter, walls thin, trigones medium sized to large, subnodulose to nodulose and often confluent, intermediate thickenings lacking; cuticle smooth; ocelli absent. Lobules uniformly developed, strongly inflated, ovoid, 1/4-1/3 of leaf length, apical tooth short, blunt (visible only in a few leaves). Underleaves distant, \pm appressed to the stem, \pm oval, 0.11-0.13 \times 0.11-0.13 mm, bifid to 1/3-1/2 of length, sinus U- or V-shaped, lateral margins entire, lobes sometimes apiculate, apical cell blunt. Dioicous (?). Gynoecia cladogynous, appearing lateral through single innovation, one gynoecium situated near base of shoot, a juvenile one near apex; female bracts larger than leaves, lobes ca 0.37 mm long \times 0.22 mm wide, apex rounded, somewhat narrowed near lobule insertion, these not well visible. Bracteole larger than underleaves, obovate, ca 0.30-0.32 mm long \times 0.22 mm wide, bifid to c. 1/3 of length, lobes erect to somewhat incurved, acute. Perianth not preserved.

DISCUSSION

DNA sequence based divergence time estimates provide evidence for a Paleogene origin of the *Cheilolejeunea* crown group (Feldberg *et al.*, 2014; Laenen *et al.*, 2014) and thus do not conflict with the assignment of the Miocene fossil to this genus. The pantropical genus *Cheilolejeunea* in the circumscription of Ye *et al.* (2015) is characterized by 1-5 large, coarsely, rarely finely segmented oil bodies per leaf cell, presence or absence of ocelli, spores with numerous rosettes, a pale green to brownish color, thin stems with a 2-4 cells wide merophyte and usually enlarged epidermis cells, leaf lobules with 1-2 teeth and a hyaline papilla at the distal base of the second tooth, bifid or entire underleaves, gynoecia without or with 1-2 lejeuneoid or pycnolejeuneoid innovations, and an inflated perianth. The characteristics of the free lobule margin are important for the identification of *Cheilolejeunea* (Bastos, 2010; Renner, 2012). Unfortunately the preservation of the leaf lobules of the liverwort fossil SMNS Do-4263-B-1 does not allow an investigation of the hyaline papilla; most other diagnostic characters are also missing. However, two cells wide ventral merophytes and bifid underleaves support an assignment to tribe Lejeuneae (Gradstein, 2013), of which *Lejeunea* Lib. and *Cheilolejeunea* are the most credible candidates to accommodate the fossil. Four fossil species of *Lejeunea* are known from Dominican amber, all of which have thin-walled leaf cells without or with very small trigones (Kaasalainen *et al.*, 2017; Lee *et al.*, 2017). The distinct, often subconfluent or confluent trigones are characteristic for many species of *Cheilolejeunea* including the extant *C. rigidula* (Schuster, 1980; Gradstein & Ilkiu-Borges, 2009). Considering available evidence, especially the wide overlap of the fossils morphology with that of the extant *C. rigidula*, we feel that an assignment to *Cheilolejeunea* is the best possible solution.

Cheilolejeunea lamyi differs from *C. rigidula* only slightly in leaf shape and the shape of the underleaf and bracteole apices. We need to consider possible morphological changes during the life time of a species, i.e. the time interval from its origin to the next speciation event (Willmann, 1985). However, acknowledging the incomplete preservation of character states, the numerous examples of morphologically cryptic or near cryptic speciation in Lejeuneaceae (Renner *et al.*, 2013), and the observed morphological disparity, we prefer to treat the Miocene

inclusion as a fossil species rather than an early representative of the extant *C. rigidula* with slightly deviant morphology. *Cheilejeunea lamyi* can be separated from Dominican amber species of *Lejeunea* by its rather strong, often somewhat confluent trigones, and from Miocene fossil *Ceratolejeunea* species by a lack of ocelli with a larger size than the surrounding cells (Heinrichs *et al.*, 2014). The two other Dominican amber species of *Cheilejeunea*, *C. antiqua* and *C. suzannensis* have entire rather than bifid underleaves (Fig. 8).

Acknowledgements. We thank the curators of the above-mentioned scientific collections for loans of amber fossils of liverworts and access to herbarium material, especially Günter Bechly and Karin Wolf-Schwenninger (Museum of Natural History Stuttgart) who granted a loan of the holotype of *Cheilejeunea lamyi*.

REFERENCES

- BASTOS C.J.P., 2010 — The position of the hyaline papilla and the genus concept of *Cheilejeunea* (Spruce) Schiffn. (Lejeuneaceae). *Revista brasileira de botânica* 32: 379-380.
- FELDBERG K., SCHNEIDER H., STADLER T., SCHÄFER-VERWIMP A., SCHMIDT A.R. & HEINRICHS J., 2014 — Epiphytic leafy liverworts diversified in angiosperm-dominated forests. *Scientific reports* 4: 5974.
- GRADSTEIN S.R. & ILKIU-BORGES A.L., 2009 — Guide to the plants of Central French Guiana Part 4. Liverworts and hornworts. *Memoirs of the New York Botanical Garden* 76: i-iv, 1-140.
- GROLLE R., 1983 — *Leucolejeunea antiqua* n. sp., das erste Lebermoos aus Dominikanischem Bernstein. *Stuttgarter Beiträge zur Naturkunde Serie B* 96: 1-9.
- GROLLE R., 1984 — *Cyrtolejeunea suzannensis* spec. nov. ein weiteres fossiles Lebermoos in dominikanischem Bernstein. *Cryptogamie Bryologie-Lichénologie* 5: 27-32.
- GROLLE R., 1990 — *Leucolejeunea antiqua* (ein Lebermoos in dominikanischem Bernstein) erstmals mit Gynözium, Perianth und Andrözium. *Nova Hedwigia* 50: 473-479.
- HEINRICHS J., SCHÄFER-VERWIMP A., BOXBERGER J., FELDBERG K., KRAEMER M.M. & SCHMIDT A.R., 2014 — A fossil species of *Ceratolejeunea* (Lejeuneaceae, Porellales) preserved in Miocene Mexican amber. *The bryologist* 117: 10-14.
- ITURRALDE-VINENT M.A. & MACPHEE R.D.E., 1996 — Age and paleogeographical origin of Dominican amber. *Science* 273: 1850-1852.
- KAASALAINEN U., HEINRICHS J., RENNER M.A.M., HEDENÄS L., SCHÄFER-VERWIMP A., LEE G.E., IGNATOV M.S., RIKKINEN J. & SCHMIDT A.R., 2017 — A Caribbean epiphyte community preserved in Miocene Dominican amber. *Earth and environmental science transactions of the royal society of Edinburgh* 107, doi.org/10.1017/S175569101700010X, published on line 19 Dec. 2017.
- LAENEN B., SHAW B., SCHNEIDER H., GOFFINET B., PARADIS E., DÉSAMORÉ A., HEINRICHS J., VILLARREAL J.C., GRADSTEIN S.R., MCDANIEL S.F., LONG D.G., FORREST L.L., HOLLINGSWORTH M.L., CRANDALL-STOTLER B., DAVIS E.C., ENGEL J., VON KONRAT M., COOPER E.D., PATIÑO J., COX C.J., VANDERPOORTEN A. & SHAW A.J., 2014 — Extant diversity of bryophytes emerged from successive post-Mesozoic diversification bursts. *Nature communications* 5: 6134.
- LEE G.E., BECHTELER J., SCHÄFER-VERWIMP A. & HEINRICHS J., 2015 — *Microlejeunea miocenota* sp. nov., the first fossil of a subcosmopolitan genus of leafy liverworts. *Review of palaeobotany and palynology* 222: 16-21.
- LEE G.E., KOLBERG L., BECHTELER J., SCHÄFER-VERWIMP A., RENNER M.A.M., SCHMIDT A.R. & HEINRICHS J., 2017 — The leafy liverwort genus *Lejeunea* (Porellales, Jungermanniopsida) in Miocene Dominican amber. *Review of palaeobotany and palynology* 238: 144-150.
- PENNEY D., 2010 — Dominican Amber. In: Penney D. (Ed.), Biodiversity of fossils in amber from the major world deposits, Manchester, Siri Scientific Press, pp. 22-41.
- POINAR G.O. JR., 1991 — *Hymenaea protera* sp. n. (Leguminosae, Caesalpinioideae) from Dominican amber has African affinities. *Experientia* 47: 1075-1082.

- RENNER M.A.M., 2012 — Further insight into lobule teeth homology in Lejeuneaceae subf. Lejeuneoideae from *Cheilolejeunea oscilla*, a new species from Australia. *The bryologist* 115: 536-556.
- RENNER M.A.M., BROWN E.A. & WARDLE G.M., 2013 — Averaging v. outlier removal. Decrypting variance among cryptic *Lejeunea* species (Lejeuneaceae: Jungermanniopsida) using geometric morphometrics. *Australian systematic botany* 26: 13-30.
- SCHÄFER-VERWIMP A., FELDBERG K., DONG S., VAN MELICK H., PERALTA D.F., SCHMIDT A.R., SCHNEIDER H. & HEINRICHS J., 2014 — Towards a monophyletic classification of Lejeuneaceae III: the systematic position of *Leirolejeunea*. *Phytotaxa* 170: 187-193.
- SCHUSTER R.M., 1980 — *The Hepaticae and Anthocerotae of North America, IV*. New York, Columbia University Press.
- WILLMANN R., 1985 — Die Art in Raum und Zeit. Das Artkonzept in der Biologie und Palaeontologie. Berlin, Parey.
- WILSON R., HEINRICHS J., HENTSCHEL J., GRADSTEIN S.R. & SCHNEIDER H., 2007 — Steady diversification of derived liverworts under Tertiary climatic fluctuations. *Biology letters* 3: 566-569.
- YE W., GRADSTEIN S.R., SHAW A.J., SHAW B., HO B.C., SCHÄFER-VERWIMP A., PÓCS T., HEINRICHS J. & ZHU R.-L., 2015 — Phylogeny and classification of Lejeuneaceae subtribe Cheilolejeuneinae (Marchantiophyta) based on nuclear and plastid molecular markers. *Cryptogamie Bryologie* 36: 313-333.