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Transfer of the Miocene *Lejeunea palaeomexicana* Grolle to *Ceratolejeunea*

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Abstract – *Lejeunea palaeomexicana* was described in 1984 based on an inclusion of a sterile leafy liverwort in a piece of Miocene Mexican amber. A reinvestigation of the type specimen provided evidence for a basal group of ocelli in the leaf lobes. Ocelli are unknown among extant representatives of *Lejeunea* but occur in several other genera of Lejeuneaceae. Based on the brown colour of the cell walls, the asymmetrical leaf lobes, the two cells wide ventral merophyte and the position of the ocelli, we propose a transfer of *Lejeunea palaeomexicana* to *Ceratolejeunea*.

Amber / Cenozoic / fossil / Lejeuneaceae / Porellales

INTRODUCTION

With some 1.700 species (He & Zhu, 2011) Lejeuneaceae is the largest family in the Porellales, a largely epiphytic clade of the leafy liverworts (Heinrichs *et al.*, 2005; Feldberg *et al.*, 2014). Lejeuneaceae are characterized by complicate bilobed, incubous leaves with a small ventral lobule attached along a keel, the presence of underleaves and/or rhizoid tufts, *Lejeunea*-type branching, the absence of reddish pigmentation, and the existence of only one archegonium per perianth (Ahonen *et al.*, 2003; Wilson *et al.*, 2004). Their classification into genera is notoriously difficult and subject to controversy (Gradstein *et al.*, 2003). Accordingly, numerous species of Lejeuneaceae have been assigned to more than one genus based on deviating valuations of the morphological evidence (Heinrichs *et al.*, 2013). However, molecular phylogenies revised the morphology-based supraspecific classification of Lejeuneaceae and supported a reduction of Lejeuneaceae genera from some 90 to 68 (Gradstein, 2013). Molecular phylogenies also allowed to reconstruct the distribution of character states (Sukkharak *et al.*, 2011; Dong *et al.*, 2012; Heinrichs *et al.*, 2015) and refine the morphological circumscription of genera (Heinrichs *et al.*, 2012; Wang *et al.*, 2014). Towards this end, Dong *et al.* (2013)

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demonstrated the importance of ocelli – specialised gametophyte cells containing only a single large oil body (He & Piippo, 1999) – for the systematics of Lejeuneaceae tribe Lejeuneeae. These authors also provided evidence for the consistent absence of ocelli in *Lejeunea*.

Lejeuneaceae are not only common in extant tropical forests but also have a comprehensive fossil record. Lejeuneaceae have been reported as amber inclusions in Miocene Dominican and Mexican amber (Grolle, 1984; Gradstein, 1993), Oligocene Bitterfeld and Eocene Baltic amber (Grolle & Meister, 2004), as well as Eocene Rovno amber (Mamontov *et al*., 2013). Furthermore, Yang & Wu (2010) proposed an affiliation of the Jurassic compression fossil *Sinolejeunea yimaensis* Yang & Wu to Lejeuneaceae.

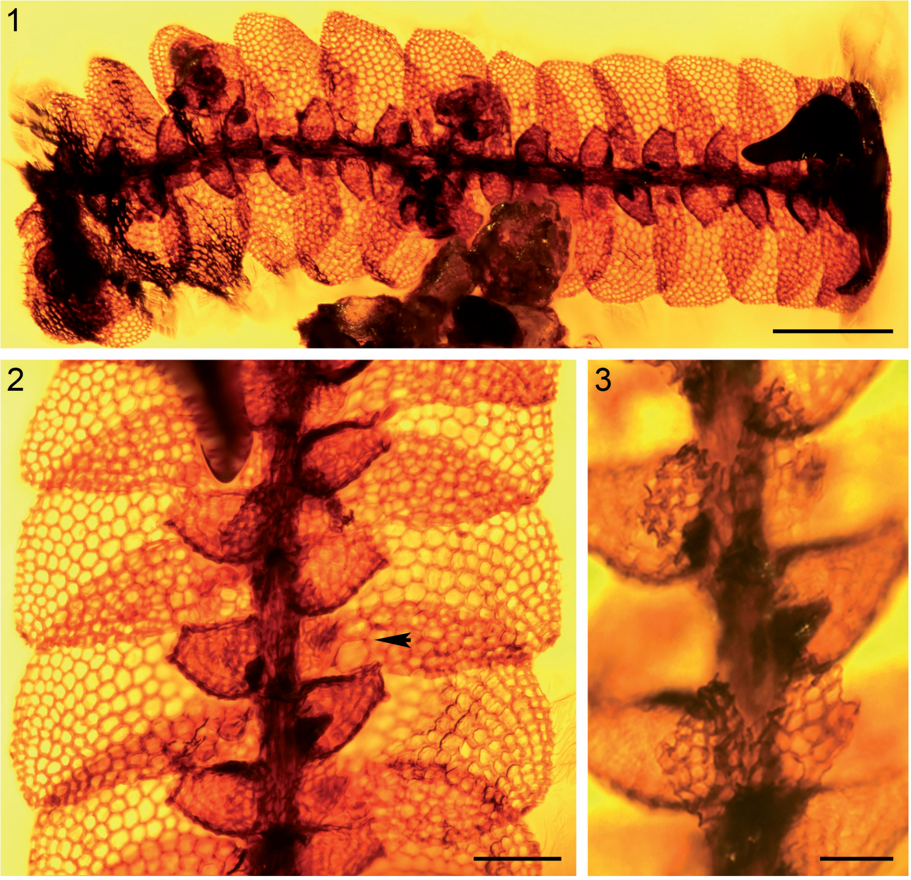
The first liverwort from Mexican amber was described by Grolle (1984) based on a single gametophyte fragment kept in the Museum of Natural History at Stuttgart (SMNS), Germany. This author proposed the name *Lejeunea palaeomexicana* Grolle for the fossil, thereby noting that 1-2 supra-basal ocelli appear to be present in the lobes of the fossil. *Lejeunea* as circumscribed by Dong *et al.* (2013) and Heinrichs *et al.* (2013) lacks ocelli, and consequently the systematic position of the Mexican fossil has been questioned (Heinrichs *et al.*, 2014). Based on a thorough re-investigation of the holotype of *Lejeunea palaeomexicana*, we confirm the presence of ocelli in the leaf lobes, and, consequently, transfer the taxon to *Ceratolejeunea*.

MATERIALS AND METHODS

Mexican amber originates from trees of the Fabaceae genus *Hymenaea* L. and is early to middle Miocene in age (15-20 Mya; Solórzano Kraemer, 2007, 2010). Specimen SMNS Mx-119-D-1 consists of a 1.4 mm thick amber platelet that is embedded in polyester resin. It contains the holotype of *Lejeunea palaeomexicana* and some detritus. The specimen was covered with a drop of water and a cover slip for investigation, and studied using a Leica M50 dissection microscope and a Carl Zeiss AxioScope A1 compound microscope, the latter equipped with a Canon 60D digital camera. Incident and transmitted light were used simultaneously. The images accompanying this contribution represent digitally stacked photomicrographic composites consisting of 50 (Fig. 1), 18 (Fig. 2) and two (Fig. 3) focal planes obtained by using the software package HeliconFocus 6.0. Drawings of the fossil (Figs 4-7) were produced using a Leica DM1000 microscope equipped with a drawing tube.

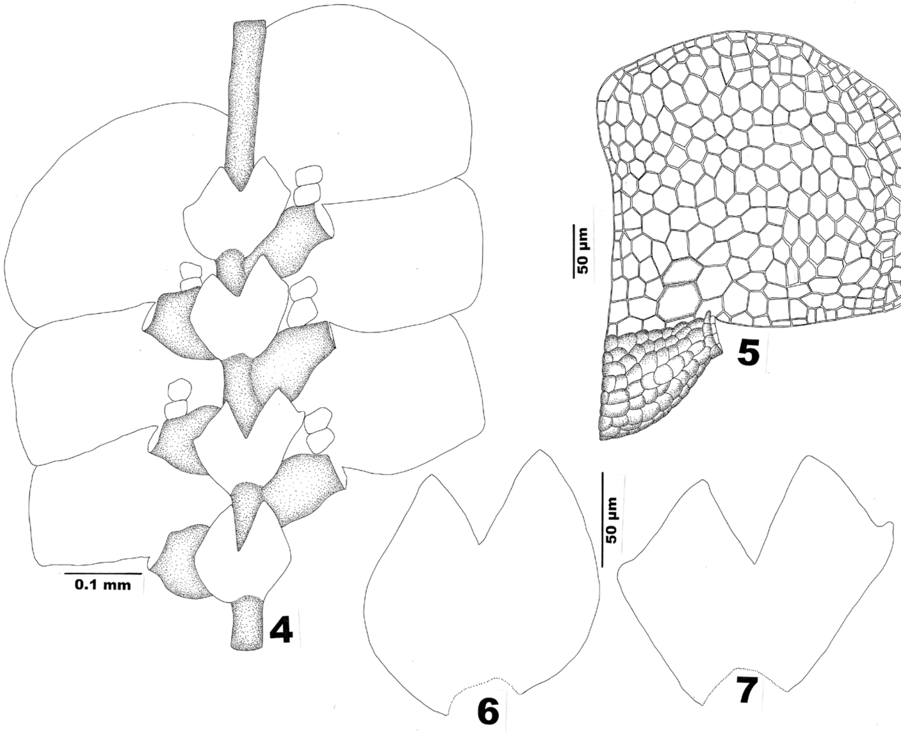
RESULTS AND DISCUSSION

The holotype of *Lejeunea palaeomexicana* (Figs 1-3) occurs as a brown, sterile gametophyte with asymmetrical leaf lobes which possess a +/– straight free ventral margin. The lobe cells are thin-walled and brown. Several of the lobes in the upper sector of the shoot display a group of 2-3 somewhat inflated cells that are somewhat larger than the surrounding cells and correspond to a type of ocelli that



Figs 1-3. Holotype of *Ceratolejeunea palaeomexicana* (SMNS Mx-119-D-1). **1.** Sterile gametophyte with four branches in ventral view (scale bar = 200 µm). **2.** Close up of the upper portion of the main shoot in ventral view. The arrowhead points to a group of two suprabasal ocelli (scale bar = 100 µm). **3.** Portion of stem with two bifid underleaves (scale bar = 50 µm).

occurs frequently in extant *Ceratolejeunea*, a genus that is also characterized by a brownish colour of the gametophytic shoots, occasional occurrence of utriculi (inrolled lobules forming a water sac, Crandall, 1969), two cells wide ventral merophytes, pycnolejeuneoid innovations, and inflated perianths with horn-like projections (Dauphin, 2003; Pócs & Chantanaorrapint, 2015). A definite recognition of ocelli in fossil liverworts is hampered by the rare preservation of oil bodies, which to date have been reported exclusively from the Miocene *Bazzania oleosa* Grolle (Grolle & Braune, 1988); however, inflated cells that differ from the surrounding leaf cells in size (Fig. 2) are indicative of ocelli (Heinrichs *et al.*, 2015) and argue against affinities of the fossil to *Lejeunea*. Distinctly asymmetrical leaves with a straight ventral margin are also not typical of *Lejeunea* but are known to occur in several species of *Ceratolejeuna* (Dauphin, 2003). Based on our interpretation of the basal leaf cells, the asymmetrical leaf lobes, the two cells wide ventral merophyte and the brown colour of the cell walls, we propose a transfer of *Lejeunea palaeomexicana* to *Ceratolejeunea* (see below).



Figs 4-7. Reconstruction of *Ceratolejeunea palaeomexicana* based on the holotype. **4.** Portion of main shoot in ventral view. **5.** Leaf in ventral view. **6, 7.** Underleaves.

The classification of incompletely preserved fossils remains a challenging task. The fossil discussed here does not show several of the key characters typically used to substantiate affinities to *Ceratolejeunea*, especially the perianths with horn-like projections and utriculi. Utriculi are not known in all extant *Ceratolejeunea* species and are also not formed consistently in all individuals of a species (Dauphin, 2003). As a result, the absence of these structures does not contradict placement of the fossil in *Ceratolejeunea*. *Ceratolejeunea palaeomexicana* differs from all other *Ceratolejeunea* species in the straight free ventral lobe margin that ends at the level of the apical lobule tooth to form a 40-85° angle towards the lobule keel (Figs 1-4). Similar lobules are known in several *Lepidolejeunea* species (Piippo, 1986), however, *Lepidolejeunea* species have leaves with scattered ocelli rather than leaves with a basal group of ocelli. Ocelli that are considerably larger than the surrounding leaf cells are also unknown in *Lepidolejeunea*.

Extant *Ceratolejeunea* has a pantropical distribution with a center of diversity in humid tropical American forests (24 regional species, Pócs & Chantanaorrapint, 2015). *Ceratolejeunea palaeomexicana* is the second species of this genus observed in Mexican amber. The other species, *Ceratolejeuna antiqua* Heinrichs & Schäf.-Verw. differs from *C. palaeomexicana* by its relatively larger lobes and a curved ventral lobe margin that is not forming a right angle with the stem (Heinrichs *et al.*, 2014). Gradstein (1993) reported a third fossil from coveal amber of the Dominican Republic resembling the extant *Ceratolejeunea plumula* (Spruce) Steph. [currently considered a synonym of *C. coarina* (Gottsche) Schiffner (Dauphin, 2003: 37)]. All these fossils provide evidence of a diverse genus *Ceratolejeunea* in the early to middle Miocene of tropical America.

Amber inclusions have been used to calibrate molecular clocks and play important roles in divergence time analyses of liverworts (e.g., Wilson *et al.*, 2007; Cooper *et al.*, 2012; Feldberg *et al.*, 2014; Laenen *et al.*, 2014; Sun *et al.*, 2014). Based on our results, *Lejeunea palaeomexicana* must no longer be used as an age constraint for this genus. However, the transfer of the fossil to *Ceratolejeunea* does not challenge earlier divergence time reconstructions relying on Grolle's (1984) classification since a *Lejeunea* fossil is known from coveal Dominican amber (Reiner-Drehwald *et al.*, 2012).

Taxonomic treatment

Ceratolejeunea palaeomexicana (Grolle) G.E. Lee, Schäf.-Verw., A.R. Schmidt & Heinrichs, ***comb. nov.*** Basionym: *Lejeunea palaeomexicana* Grolle, *Stuttgarter Beitr. Naturk., Ser. B.* 108: 2, 1984. Type: MEXICO. Miocene amber inclusion [holotype SMNS inv. no. Mx-119-D-1!] (Figs 1-3).

Description [see also the description in Grolle (1984), in German]. Sterile gametophyte, ca. 2 mm long and 625-675 µm wide, with four 180-250 µm wide and up to ca. 0.45 mm long branches, each branch with 3-4 pairs of leaves. Stem straight, 40-50 µm in diameter, with a two cells wide ventral merophyte. Leaves imbricate, incubously inserted, widely spreading, ovate, asymmetrical, 325-350 × 250-275 µm, margins entire, apex narrowly rounded to subacute, dorsal margin strongly arched, free ventral margin +/– straight or slightly curved outwards, near the apical lobule tooth abruptly decurrent towards the lobule keel, thereby forming a 40-85° angle. Leaf cell walls thin, brownish, trigones absent or small, triangular, intermediate thickenings missing, cuticle smooth; marginal leaf cells quadrate to rectangular, ca. 10-20 × 8-14 µm, cells ca. in upper third of leaf irregularly elliptical-polygonal, some wider than long, ca. 12-25 µm in diameter, median and basal leaf cells isodiametrical to elliptical-polygonal, ca. 20-30(-35) × 15-25(-35) µm, 2(-3) of them forming an indistinct supra-basal group of somewhat swollen, thin-walled cells likely representing ocelli, the largest with a diameter of ca. 45 µm. Lobules ovoid to subovoid, 0.3-0.4 the leaf lobe length, free margin involute, apical tooth short, unicellular. Underleaves remote, suborbicular, 1 per leaf pair, plane, ca. 125-150 µm wide and 100-125 µm long, ca. 3 × the stem width, ca. 50% bifid, sinus V-shaped, lobes broadly triangular, ending in a one cell long, uniseriate tip, margins entire, sometimes with a blunt angle on the outer margins. Vegetative reproduction and utriculi not observed.

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