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HEPATICS FROM ROVNO AMBER (UKRAINE), 5. *CEPHALOZIELLA NADEZHDAE* SP. NOV.

ПЕЧЕНОЧНИКИ ИЗ РОВЕНСКОГО ЯНТАРЯ (УКРАИНА), 5. *CEPHALOZIELLA NADEZHDAE* SP. NOV.

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Abstract

Light microcscopy and laser scanning confocal microscopy were used to investigate a sterile inclusion of a leafy liverwort in a piece of Late Eocene Rovno amber. The fossil is described as *Cephaloziella nadezhdae* sp. nov. The tiny liverwort resembles extant species of *C.* subg. *Schizophyllum* but differs by its small leaf cells. Leaf cells with a diameter of ca. 5–8 µm are known from the extant East Asian species *C. microphylla*; however, this species differs from the fossil in leaf shape and insertion, as well as the presence of acute conical mamillae. *Cephaloziella nadezhdae* is the first fossil representative of this subcosmopolitan genus.

Резюме

Стерильный листостебельный печеночник в ровенском янтаре позднеэоценового возраста исследован с помощью световой и конфокальной микроскопии и описан как *Cephaloziella nadezhdae* sp. nov. Это очень мелкое растение напоминает современные виды из подрода *Schizophyllum* рода *Cephaloziella,* но отличается от них более мелкими клетками пластинки листа. Столь же мелкие клетки диаметром 5–8 µm известны у восточноазиатского вида *C. microphylla*, который отличается от описываемого вида формой листа и косым его прикреплением, а также наличием острых конических мамилл. *Cephaloziella nadezhdae* является первым ископаемым представителем этого космополитного рода.

KEYWORDS: fossil, *Cephaloziella*, liverwort, Marchantiophyta, Ukraine, Rovno, Cenozoic, Late Eocene, amber

INTRODUCTION

Liverworts from Late Eocene Baltic amber have been studied since the 19th century (Grolle & Meister, 2004), while inclusions in the roughly contemporary Ukrainian Rovno amber came into focus of attention only recently (Konstantinova *et al.,* 2012). Both ambers are rich in inclusions of the liverwort genus *Frullania* Raddi. However, Rovno amber yielded a few liverwort genera that have not yet been observed in Baltic amber, namely *Acrolejeunea* (Spruce) Schiffn. and *Anastrophyllum* (Spruce) Steph. (Mamontov *et al.*, 2013, 2014). Here, we describe another fossil liverwort that has so far been observed exclusively in Rovno amber.

MATERIALS AND METHODS

Rovno amber is considered to be of late Eocene origin. Details on the amber deposit and the age reconstruction are summarized in Perkovsky *et al.* (2003, 2007, 2010). The investigated amber piece is part of the Rovno amber collection of the Schmalhausen Institute of Zoology in Kiev (SIZK). After primary preparation the original amber piece had a weight of 1.15 gram. At first it was studied with a compound light miscoscope Olympus BX61 and a stereomicroscope Olympus SZX16, the latter equipped with an Infinity 4 digital camera. The micrograph presented in Fig. 2 was obtained from several optical sections and composed using the software package HeliconFocus 4.50 (Kozub *et al.*, 2008) for a better

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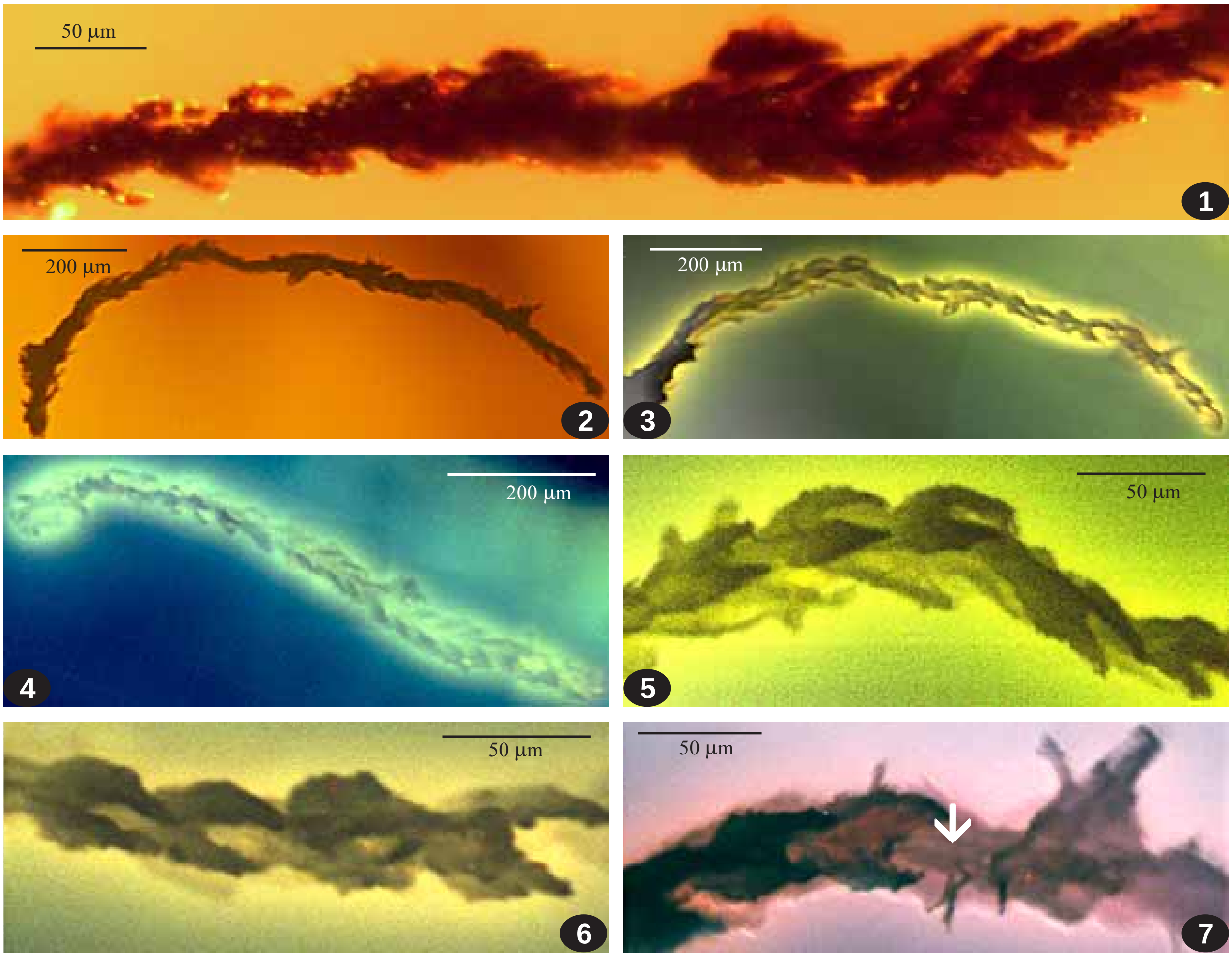


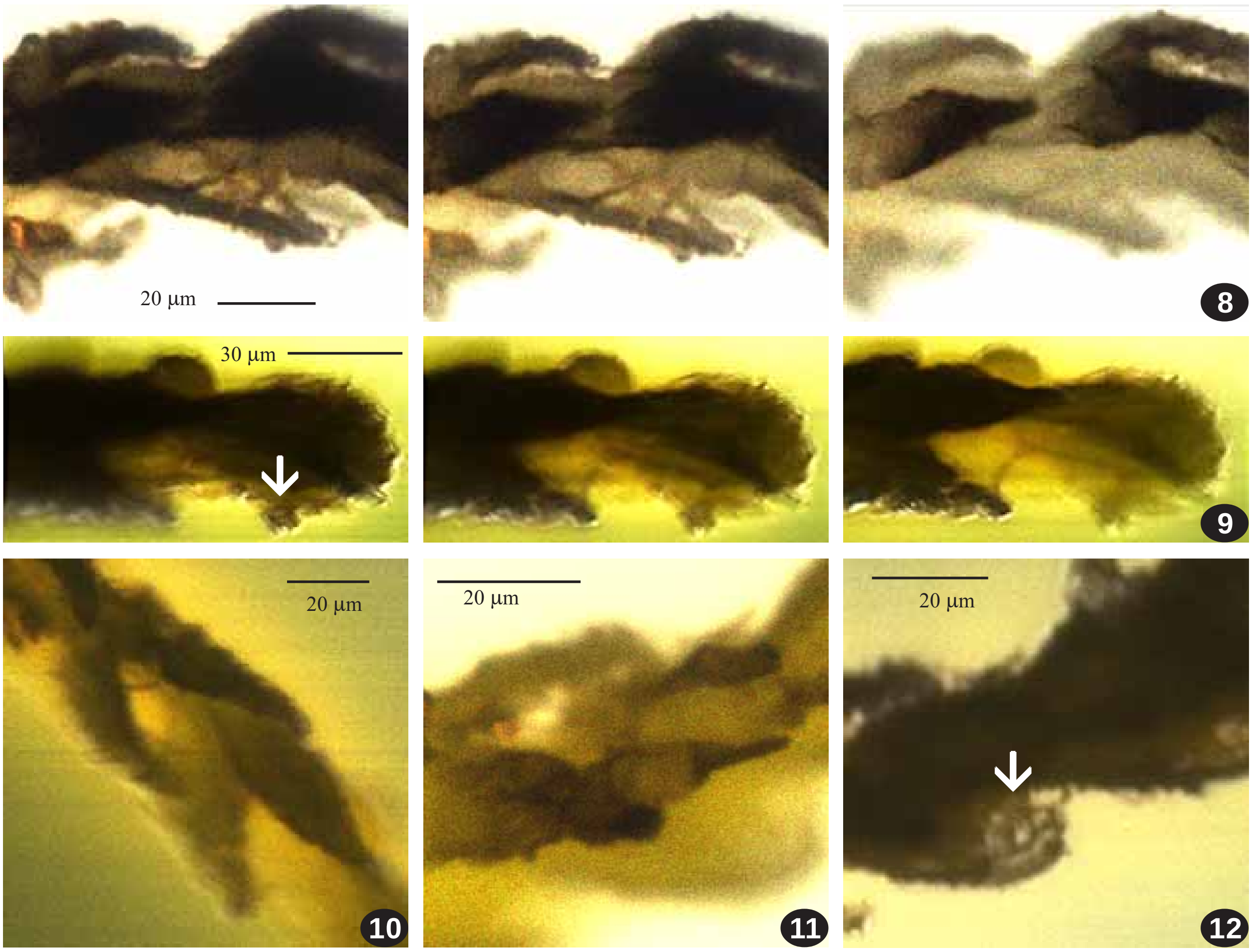
Figs. 1-7. *Cephaloziella nadezhdae* sp. nov. (holotype, 1-2 light microscopy, 3-7 laser scanning confocal microscopy): 1-3: habit in lateral view; 4: habit in ventral view; 5-7: portions of shoot in lateral view, showing mamillose abaxial leaf surfaces and dentate leaves.

illustration of the three-dimensional inclusion. However, the strong light scattering in the amber piece disabled an investigation of cellular structures using light microscopy. Thus, the liverwort was studied using a laser scanning confocal microscope Olympus FV1000 equipped with an objective with maginification 10x. Three lasers were used (wavelengths 405, 473 and 567 nm). Although the fossil itself gave no reasonable emission, the material in the surrounding area appeared shining. Several optical sections were combined using the Z-stack method. This approach allowed us to investigate some morphological details of the fossil including cell shape and diameter. Subsequently the original piece was separated into several segments in order to reduce light scattering during light microscopy investigation. The segment with the liverwort inclusion was studied using the 40x objectives of the Olympus FV1000 and SZX16 microscopes. Due to the curved surface of the amber, the ventral side of the fossil could only be studied with the 10x objective. Light microscopy was still insufficient to study cellular details of the fossil, while laser scanning confocal microscopy resulted in images showing at least some cellular structures, although the thin amber segment provided less bright fluorescence. Hence, some pictures from the non-segmented amber piece (10x objective) show more details than the images taken from the thin amber-segment (40x objective).

Although confocal microscopy usually allows combining individual pictures with the Z-stacking method, this option could not be applied to the fossil at hand due to its limited own fluorescence. Thus, three selected focal planes are presented in Figs. 8, 9, 13, 14.

Taxonomy

The studied inclusion consists of a single minute, unbranched sterile shoot with deeply bilobed, succubous, almost transversely inserted, basally ciliately toothed leaves with apiculate to acute lobes and subisodiametric lobe cells with a diameter of 5-8 µm. Similar character state combinations are known from several extant liverwort genera or sections including *Eremonotus* Pearson, *Odontoschisma* sect. *Iwatsukia* (N. Kitag.) Gradst., S.C. Aranda & Vanderp., *Sphenolobopsis* R.M. Schust. & N. Kitag., and *Cephaloziella* (Spruce) Schiffn. s.l. (incl. *Dichiton* Mont., *Evansia* Douin & Schiffn., *Prionolobus* (Spruce) Schiffn. and *Cylindrocolea* R.M. Schust.).



Figs. 8-12. *Cephaloziella nadezhdae* sp. nov. (holotype, laser scanning confocal microscopy): 8: part of shoot in dorso-lateral view; 9: uppermost, mamillose leaves with apical cluster of gemmae and adjoining angulate gemmae (arrow); 10: two leaves from upper shoot sector; 11: portion of shoot with two unequally lobed leaves; 12: portion of shoot, arrow points to visible leaf cells.

Extant members of *Odontoschisma* sect. *Iwatsukia* differ from the amber inclusion by their larger size, presence of geotropic, flagelliform axes, and conspicuous underleaves. The toothed leaves and cellular projections on the abaxial leaf surface of the fossil (Figs. 5, 6, 8, 13, 15) allow the exclusion of *Eremonotus* and *Sphenolobopsis*. These structures are more typical of *Cephaloziella* s.str. (Cephaloziellaceae), whereas the *Cephaloziella* segregate *Cylindrocolea* has oblique rather than transversely inserted leaves (Schuster, 2002).

Cephaloziella leaves are usually transversely inserted and almost plane or slightly concave, although in some species the leaves are strongly concave. Furthermore, the leaves of many species of *Cephaloziella* are armed abaxially and/or marginally by mamillae and uni- to multicellular outgrowths. Some leaves of the fossil have marginal teeth, and low, knob-like mamillae as well as multicellular, ridge-like outgrowths on the abaxial leaf surface (Figs. 13, 14, 17).

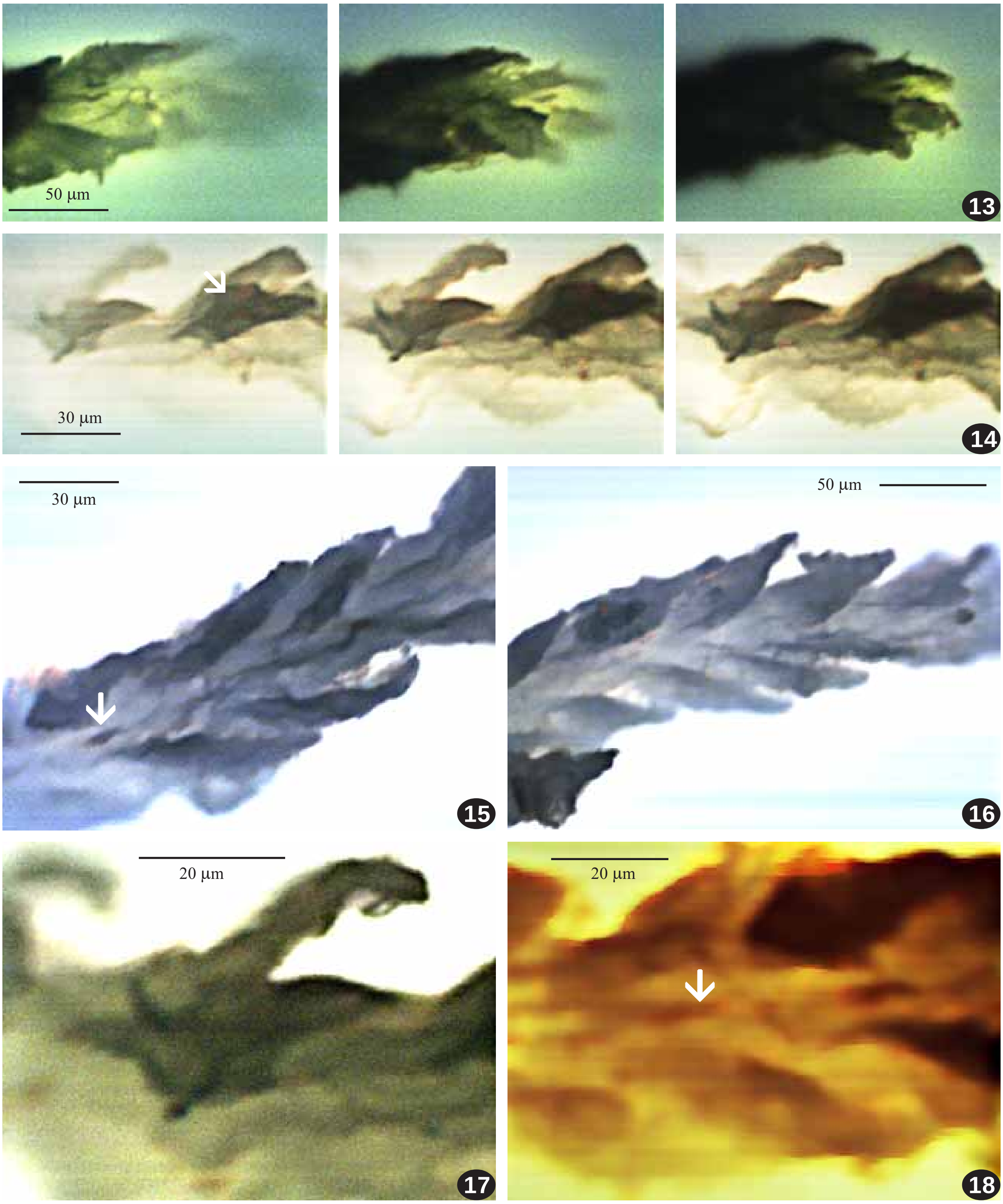
Cephaloziella leaf cells are usually 10-20 µm wide, however, *C. microphylla* (Steph.) Douin. (=*C. hunanensis* W.E. Nicholson) has small leaf cells with a diameter of only 4-6 µm (Handel-Mazzetti, 1930), resembling those of the fossil in question. Species of *Cephaloziella* often produce elliptical or quadrate to subquadrate, smooth to angulate gemmae on the shoot tips, and angular gemmae are likely present on the shoot tip of the fossil (Fig. 9). In summary, we consider morphological evidence sufficient to assign the fossil to *Cephaloziella* s.str., and describe it as an extinct species, *C. nadezhdae*.

Extant species of Cephaloziellaceae typically grow on rotten wood, soil and rock, but a few taxa in tropical and subtropical forests occur on bark (Schuster, 2002). Considering the presence of the liverwort inclusion in a piece of fossil tree resin, it is not unlikely that a lignicolous species is at hand. The relatively large size of the original amber piece provides some evidence for its origin from a trunk base.

Genus **Cephaloziella** (Spruce) Schiffn. (extant, family Cephaloziellaceae)

Description (for characters seen in the amber inclusion).

Plants minute, creeping or ascending, irregularly and usually rather distantly branched; geotropic stoloniform axes usually absent. Stem delicate, usually less than 100 µm in diameter. Leaves remote, alternate, succubously-



Figs. 13-18. *Cephaloziella nadezhdae* sp. nov. (holotype in ventral view, laser scanning confocal microscopy): 13: shoot apex with toothed leaves; 14: portion of shoot, visible cell outlines in diatal part of lobes arrowed; 15, 16: portions of shoot, with unclear structures (arrowed); 17: leaf base, close up from Fig. 14; 18 portion of shoot with unclear structures (arrowed).

transverse inserted, almost flat to concave or conduplicate, 50-85% bifid, lobes usually divergent, often narrowly triangular, entire or toothed, usually 2–11 cells wide at base, abaxially smooth or with outgrowths. Leaf cells firm, mostly thick-walled, quadrate to rectangular, (4–) 8–13(–20) μm in diameter, smooth or papillose, sometimes with mamillose projections on the abaxial leaf side. Underleaves lacking or small, lanceolate to ovate, rarely bilobed. Gemmae 2-celled, produced in chains from margins of more or less reduced leaves of ascending axes.

Type species: *Cephaloziella divaricata* (Sm.) Schiffn.

Cephaloziella is a subcosmopolitan genus including about 90 species (Söderström et al., 2015). So far only a single fossil taxon has been described. *Cephaloziella dimorpha* (Casp.) Grolle was based on fossils from Baltic amber; however, this taxon was later transferred to *Cylindrocolea* (Grolle & Meister, 2004).

Cephaloziella nadezhdae Mamontov, Heinrichs & Váňa, sp. nov. Figs. 1-18.

ETYMOLOGY: named in honour of Nadezhda A. Konstantinova, outstanding Russian hepaticologist.

HOLOTYPE: Klesov (Pugach quarry). Rovno amber. Late Eocene. SIZK-K-24755-F. Syninclusions: Aranei (exuvium of Theridiidae), Symphypleona.

Description: Sterile shoot fragment 1.2 mm long and up to 50 µm wide. Stem 15–25 µm in diameter; cortical cells short-rectangular, 8–11×6–8 µm. Leaves somewhat ovarlapping, transversely inserted, succubous by presence of dorsally oriented lobes, not or somewhat decurrent along stem (Figs. 1, 8, 14-18), flat to concave, 23–40×40–60 µm, erect to erect spreading, at places subimbricate, elliptical, almost entire-margined in lower third of shoot, in upper two thirds of shoot with irregular, obtuse marginal teeth (Figs. 5-7, 13, 14, 17). Abaxial leaf face sometimes with low knob-like projections (Figs. 5, 6, 8) and ridge-like multicellular outgrowths below the sinus (Fig. 7). Leaves bilobed to 0.5–0.85 of its length, sinus acute or rounded (Figs. 5, 7, 8); lobes almost equal in leaves from lower half of shoot but slightly to clearly unequal in leaves from upper shoot sectors (Figs. 6, 7, 9, 10), narrow to broadly triangular (Figs. 7, 9), apparently 2–3 cells wide at base, straight to contiguous, incurved to stem at places (Figs. 8, 10-15, 17), apex acuminate to acute, ending in a one cells long uniseriate tip, apical cell triangular. Leaf cells hardly visible, apparently subisodiametric, near leaf apices obviously somewhat elongated, those at base of lobes 5–8 µm wide, walls thin to incrassate, without distinct nodulose trigones. Gemmae angulate, 9.7×7.7 µm; in clusters at shoot apex, subtended by an uppermost leaf (Fig. 11).

Differentiation: The only fossil species assigned to Cephaloziellaceae, *Cylindrocolea dimorpha* (Casp.) Grolle, is known from Baltic and Bitterfeld amber (Grolle & Meister, 2004). It differs from *Cephaloziella nadezhdae* in 1) the larger size of its shoots being 120–470 µm wide and 3–10 mm long vs. 50 µm wide and 1.2 mm long, 2) a clearly succubous leaf insertion vs. subtransverse to indistinctly succubous leaf insertion, 3) leaves bilobed only to 0.2–0.33 (–0.5) vs. divided to 0.5–0.85 of leaf length, and 4) larger leaf cells with a diameter of 14–18 µm vs. 5–8 µm wide. *Cephaloziella nadezhdae* superficially resembles a number of extant *Cephaloziella* species including the northern Holarctic *C. arctogena* (R.M. Schust.) Konstant., *C. uncinata* R.M. Schust., and especially representatives of *C.* subg. *Schizophyllum,* namely the Holarctic *C. elachista* (J.B. Jack) Schiffn. and *C. spinigera* (Lindb.) Jørg., and the New Zealand *C. pulcherrima* R.M.Schust. However, these species differ from *C. nadezhdae* in having much larger cells of both stem and leaves, and by ellipsoidal to citron-shaped gemmae. Small leaf cells were observed in *C. microphylla* (Steph.) Douin (Handel-Mazzetti, 1930). However the latter species differs from *C. nadezhdae* by 1) the abaxial leaf surface having high conical mamillae and cellular outgrowths, 2) regularly dentate leaf margins with 1-celled acute teeth, 3) mostly broadly triangular leaf lobes, and 4) deeply concave to folded leaves with reflexed margins. Angulate gemmae are present in *C.* subg. *Evansia* (Douin & Schiffn.) R.M. Schust. and *C.* subg. *Prionolobus* (Spruce) Müll. Frib., however, the extant species of these subgenera differ from *C. nadezhdae* by much larger shoots and deviating leaves (Schuster, 2002).

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