ON THE SYSTEMATIC POSITION OF LEPTODICTYUM MIZUSHIMAE (BRYOPHYTA)

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Abstract

Leptodictyum mizushimae (Sakurai) Kanda (= Hygrohypnum mizushimae Sakurai), a rare East Asiatic species, known from Japan, Kuril Islands and Kamchatka, is studied by three molecular markers, including nuclear ITS, chloroplastic rps4 and mitochondrial nad5, and all of them indicate its affinity with Climaciaceae, despite lacking morphological similarity: plants are not dendroid and stem lacks any paraphyllia-like structures. A new genus, Limnohypnum is described to accomodate this species. Morphological description and illustrations are provided.

KEYWORDS: mosses, taxonomy, molecular phylogenetics, Limnohypnum gen. nov., Climaciaceae, East Asia, morphology

INTRODUCTION

Hygrohypnum mizushimae Sakurai has been described from Honsu in Japan (Sakurai, 1952), and later transferred to the genus Leptodictyum by Kanda (1975). This species is so rare, that it is absent in the “Illustrated moss flora of Japan” by Noguchi (1991), who included in the book only species checked by himself, and likely he failed to obtain any specimen for personal study.

Morphologically this species resembles neither Hygrohypnum nor Leptodictyum. It is a large and quite conspicuous plant looking like a big Plagiothecium, although with more extensive branching and a moderately long costa in the leaves. Unlike most Leptodictyum species, L. mizushimae (Sakurai) Kanda does not commonly occur with sporophytes, and its stem and branch leaves are much more shortly acute, in contrast to long acuminate leaves of L. riparium (Hedw.) Warnst. and other species of the genus described from East Asia (Kanda, 1975).

The genus Hygrohypnum appeared to be an unnatural assemblage in the recent molecular studies (Oliván et al. 2007; Ignatov et al., 2007), and it has been split into several genera. However, most species of all these genera have a double costa. Three species with the single costa provide exceptions: Hygrohypnella polaris, (Lindb.) ignatov & Ignatova, which differs in well-defined hyalodermis, and Hygrohypnum luridum (Hedw.) Jenn. and Platthyphnum smithii (Sw.) Ochyra, which are relatively small plants, with rather rigid and concave leaves, quite unlike Leptodictium mizushimae. The confusing habit forced us to check the systematic position of the latter species with molecular phylogenetic methods, which in many cases already solved long-standing problems of systematic placements of moss genera. The results of the molecular phylogenetic studies have rectified familial placements of Anacamptodon (Vanderpoorten et al., 2002), Clasmatodon (Buck et al., 2000), Struckia (Pedersen & Hedenäs, 2002), Sasaokaea (Ignatov & Milyutina, 2010), Bissetia, Homaliadelphus and Myiabea (Olsson et al., 2009), Lecuraea and Pyiatricia (Gardiner et al., 2005), Habrodon (Budyakova et al., 2003), Acrocladium (Tangney et al., 2010) and at least a few tens of other moss genera.

MATERIAL AND METHODS

The species has been recently collected in Kuril Islands in Shikotan (Bakalin et al., 2008) and in Kamchatka (Chernyadjeva, 2012). These two specimens were compared with the set of pleurocarpous mosses used for previous molecular phylogenetic analyses. The ITS set included mostly samples used in analyses of Gardiner et al. (2005) and Ignatov

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Fig. 1. MP strict consensus tree based on ITS sequences. Bootstrap support >50 calculated for 2000 iterations in TNT is shown below branches. Clade with Leptodictyum (Limnohypnum) mizushimae and Clima- ciaceae is boxed.
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Fig. 2. MP strict consensus tree based on nad5 mitochondrial sequences. Clade with Leptodictyum (Limnohypnum) mizushimae and Climaciaceae is boxed.

Fig. 3. MP strict consensus tree based on rps4 chloroplastic sequences. Clade with Leptodictyum (Limnohypnum) mizushimae and Climaciaceae is boxed.
et al. (2007), with some subsequent addition from a limited analysis of Sasaokaecae by Ignatov & Milyutina (2010).

A preliminary analysis of ITS placed the species in a strongly unexpected position, near Climacium, thus additional analyses using the chloroplast rps4 and mitochondrial nad5 were conducted, using GenBank data, mostly from the analysis of Huttunen et al. (2012), where a significant block of data was taken from Cox et al. (2010). This dataset made possible an analysis of a broader set of families, which was difficult with ITS as the alignment included too many gaps. New sequence data are given in Appendix 1.

We analysed the ITS, rps4 and nad5 datasets separately, as a putative hybrid origin and recombination were suspected because of known cases in pleurocarpous mosses (Ignatov & Milyutina, 2011).

The protocols of PCR and sequencing were standard (e.g., Gardiner et al., 2005), and the TNT program was used (Goloboff et al., 2003). Bootstrap support was calculated based on 2000 iterations.

RESULTS OF MOLECULAR PHYLOGENETIC ANALYSIS

The phylogenetic analysis of ITS sequences resulted in a moderately resolved tree, which is not discussed here in detail as its topology is almost identical to those that has been discussed already several times for analyses where ITS has been used (Gardiner et al., 2005; Ignatov et al., 2007, Huttunen et al., 2012). The bootstrap tree is composed of a basal grade formed by four small clades of the Plagiotheciaceae (Herzogiella, Isoterygiopsis, Myurella, Orthothecium, Plagiothecium, Platycladix), Habrobotontaceae (Habroboton), Leucodontaceae (Leucodon), Pterigynandraceae plus Hypnaceae (Pterigynandrum, Hypnum, Eurohypnum) and then the terminal clade represents a polytomy, where most species are grouped in small clades, formed by species of one genus or one family. Bootstrap support was found for clades of Amblystegiaceae (98) and Calliergonaceae (100) with several genera. Many families represented by 2 genera were also supported, e.g., Leskea+Haplocladium of Leskeaceae (73); Taxiphyllum+Glossadelphus of Taxiphylaceae (98), Leptodon+Neckera of Neckeraceae (98). The clade formed by Leptodictyum mizushimae (shown in the tree as Limnohypnum), Climacium americanum and Pleuroziopsis rutherenia received bootstrap support 77, i.e., close to the value 83 obtained for the subclade Pleuroziopsis+Climacium, and 100 was found for two specimens of Leptodictyum mizushimae from Kamchatka and Shikotan Island (ca. 1000 km apart). This clade is nested in a bigger clade with the species of Hylocomiaceae (Loeskeobryum, Rhytididales, Neodolichomitra) and Antitrichiaceae (Antitrichia), i.e., having a topology similar to that in previous analyses, although without support.

Two trees built by ITS1 and ITS2 separately (not shown) have low support (calculated in TNT for 1000 iterations). However, in both of them the clade composed of Pleuroziopsis+Climacium+Limnohypnum was found.

The chloroplast rps4 and mitochondrial nad5 regions are less variable, and branches in their trees have only very low support (not shown). Figures 2 & 3 present strict consensus trees for them (both built in TNT, with maximal precision and accuracy).

The tree by rps4 in Fig. 2 displayed a large polytomy, with a number of small clades combining species of the families Anomodontaceae (Anomodon and Haplophyllum), Fontinalaceae (Fontinalis and Dicrhyllum), tropical Hypnaceae (Ectropothecium and Vescularia), Calliergonaceae (Calliergon and Warnstorfia), Taxiphylaceae (Taxiphyllum and Schwetscheckopsis), Sematoplylaceae (Acroporium, Sematophyllum, Trichosteleum), Neckeraeaceae (Forrestroemia, Leptodon, Neckera/Exsertotheca), Entodontaceae (Entodon, Erythrodontum, Mesodon), Hylocomiaceae (Rhytididales, Loeskeobryum, Meteoriella, Mittenothamnium), but Hydrocomium splendens grouped with the two Brachytheciaceae genera Brachythecium and Clasmatodon, Lembophylaceae (Camptochaete, Lembophyllum, Rigodium, Weymouthia), Plagiotheciaceae (Myurella, Orthothecium, Platydictya), Amblystegiaceae (Amblystegium, Campyliadelphus, Cratoneuron, Drepannoncladus, Palustriella, Vittia). One clade combined species that were found in the analysis by Huttunen at al. (2012) as members of basal Hypnalean families (Orthorrhynchium, Pseudoryphaea, Rhizofabronia, Trachyloma). Leptodictyum mizushimae was found in the same clade as with ITS, with Pleuroziopsis and Climacium, but the clade was not grouped with Hylocomiaceae.

The nad5 tree differs from the rps4 one in a larger number of species/genera which do not group with any other one. However, species from individual families formed clades, e.g., Amblystegiaceae, Anomodontaceae, Brachytheciaceae, Calliergonaceae, Entodontaceae, Hypnaceae, Neckeraeaceae, Lembophylaceae, Plagiotheciaceae + Rhizofabroniaceae, and Sematophyllaceae (Fig. 3). The clade joining Pleuroziopsis+Climacium+Limnohypnum appeared in this analysis as well.

Summing up, all three genome regions independently provide an evidence that Leptodictyum mizushimae is not a member of Amblystegiaceae, but closely related to Climacium and Pleuroziopsis. This ‘impossible’ placement will be discussed below, but in any case, it is clear that the moss has to be segregated in its own genus.

TAXONOMIC TREATMENT

Limnohypnum Ignatov & Czernyadjeva, gen.nov.

Plants robust. Stem prostrate or floating, irregularly pinnately branched, subcomplanately foliate, with central strand, hyalodermin absent, sclerodermin 2-3-stratose, only slightly differentiated. Proximal branch leaves broadly triangular. Axillary hairs 5(-6)-celled, with 3(-4) elongate upper cells. Rhizoids below leaf insertion, reddish-brownish, smooth. Stem leaves erecto-patent, ovate-lanceolate, gradually tapered to apex or shortly and indistinctly acuminate, decurrent; margin plane, subentire; costa single or rarely with lateral spurs, reaching...
Fig. 4. *Limnohypnum mizushimae* (Sak.) Ignatov & Czernyadjeva (from: Russia, Kuril Islands, Shikotan, Bakalin #K-54-29-07, MHA): 1, 2 – habit, dry; 3 – axillary hairs; 4-6 – leaves; 7-8 – stem transverse section; 9 – proximal branch leaves. Scale bars: 2 cm for 2; 5 mm for 1; 1 mm for 4-6; 100 μm for 3, 9-10.
0.6-0.75(-0.9) leaf length, rather thin, 3-4-stratose, ending without tooth; lamina cells elongate, at and below mid-leaf 3-7:1, at 3/4 leaf length narrower, thin-walled; basal cells broader, forming lax areolation across the base, indistinctly enlarged in alars. Gametangia and sporophytes unknown.

Type species of the genus: Limnohypnum mizushimae (Sak.) Ignatov & Czernyadjeva.

Ethymology: Limne – lake, often applies for various inland water-bodies; Hypnum – genus of pleurocarps, commonly used in sense of an indefinite ‘hypnalean’ moss. Limnohypnum mizushimae (Sak.) Ignatov & Czernyadjeva, comb. nov.


Plants in very loose tufts, pale-green, yellowish-green to golden, slightly glossy. Stem 5-8 cm, remotely irregularly pinnately branched, subcomplanately to complanately foliate, with weak central strand 5-7 cells across, sclerodermis 2-3-stratose, its cells moderately thick-walled; branches 3-10(-40) mm; proximal branch leaves broadly triangular, the first having 4 o’clock position; axillary hairs 5(-6)-celled, to 300 μm long, with two brownish proximal cells and 3 upper cells to 18 μm wide. Stem leaves 2.5-4.0×0.9-1.9 μm, broadest at 1/3–1/4 leaf length, gradually tapered to apex or slightly and indistinctly acuminate, ovate-lanceolate, slightly to conspicuously asymmetric, decurrent; margin plane, subintire; costa single or, rarely, with lateral spurs, reaching 0.6-0.75(-0.9) leaf length, rather slender, 40-80(-100) μm wide at base, 3-4-stratose, without tooth at its end; laminal cells thin-walled, in distal part linear, 90-130×8-12 μm (8-12:1), in median part elongate, 65-100×8-14 μm (5-9:1), towards margins longer, to 120-180 μm long, in many leaves along the margin in 1-2 rows very long and narrow, 160-240×5-7 μm; cells just below apex shorter, 3-7:1, basal cells broader, rectangular, forming lax areolation across base, indistinctly to distinctly enlarged in alars, cells in decurrencies rectangular to round, inflated. Branch leaves usually smaller than stem leaves. Gametangia and sporophytes unknown.

Variation. Japanese specimens are larger than Russian ones, to 15 cm long, leaves 5.5-5.5×2.5 mm and lamina cells 100-125×15-18 μm. Such a difference is rather common for moss sizes between the northern limit and more southern areas, in this case likely accentuated because the only two Russian collections do not represent optimally developed plants (cf. Kanda, 1975).

Ecology. In Kamchatka, the species has been found in as individual shoots among Calliergon richardsonii and Warnstorfia exannulata, in a flooded hollow of a sedge mire with thermal springs in Paratun’ka River Valley.
The species composition in this place include: Carex cryptocarpa C.A. Mey (projective cover 60%), Carex vesicata Meinsh. (10%), Calamagrostis purpurea (Trin.)Trin. (10%), Equisetum fluviatile L. (5%), Comarum palustre L. (1%), Warnstorfia examnulata (Bruch et al.) Loeske (3%), Calliergon richardsonii (Mitt.) Kindb. (1%), Sphagnum riparium Ångstr. (1%), Sphagnum subsecundum Nees (1%).

According to Bakalin et al. (2009), in Shikotan the species grew on very wet soil among Phragmites australis on low lake bank.

**Distribution.** The species occurs in Hokkaido and Honshu, and until recently it had been considered as an endemic of Japan (Kanda, 1975). Despite a considerable distance of more than 2000 km between the localities in Kamchatka (52°N) and Honshu (36°N), such a distribution pattern is not unique, and can likely be explained by dispersal with the migratory birds. Campylopus umbellatus and Entodon flavescens provide additional examples of southern species found in Kamchatka, disjunct from localities in Japan (Ignatov & Samkova, 2006; Czernyadjeva, 2012). Noticeably, both of them occur in Kamchatka near thermal springs, like Limnohypnum mizushimae.

**Specimens examined:** Kuril Islands, Shikotan, Bakalin K-54-29-07 (VLA, MHA); Kamchatka, Termalny, 7.VIII.2001, Czernyadjeva (LE, MHA).

**Discussion.**

All previous authors kept Limnohypnum mizushimae, either in Leptodictyum or Hygrohypnum, in the family Amblystegiaceae (Sakurai, 1934; Kanda, 1975; Iwatsuki, 2004). The gametophyte morphology of Limnohypnum does not contradict Amblystegiaceae in its current circumscription, but this family is peculiar mostly in perichaetal and sporophytic characters (plicate perichaetal leaves, long arcuate capsules, long-pored stomata), which are not useful for the present discussion, as sporophytes are unknown in this genus. A certain difference in proximal branch leaves is briefly discussed below. Traditionally, the family Amblystegiaceae has been a “waste-basket” for various hygrophilous groups, thus the species ecology has often been crucial for this familial placement. Mostly or solely for ecological reasons, the Amblystegiaceae included the genera Platyhypnidium (now included in Rhynchosetigium, Brachytheciaceae, cf. Hutten & Ignatov, 2010), Donrichardia (now in Brachytheciaceae, cf. Hutten & Ignatov, 2004), Sasaokaea (now in Leskeaceae, Ignatov & Milyutina, 2010), genera of the Calliergonaceae, i.e., Calliergon, Loeskiyhnun, Straminergon, Warnstorfia (Vanderpoorten et al., 2002).

ITS sequence data (Fig. 1) placed Limnohypnum in a clade with the Climaciaceae, which is nested in a larger clade with the Antitrichiaceae and Hylcomiaceae, families unrelated to the Amblystegiaceae according to previous analyses (Tsubota et al. 2004; Cox et al., 2010; Huttenen et al., 2012). Ignatov et al. (2007) showed that the Amblystegiaceae, on the one hand, and the Climaciaceae, Antitrichiaceae and Hylcomiaceae, on the other, belong to two major different lineages, found earlier in the cladistic analysis of morphological characters by Hedenäis (1989, 1995, 1997). Contrary to the Amblystegiaceae, the three latter families belong to a group called by Hedenäis ‘taxa with Brachythécium-like capsules’. They are characterized, among other things, by eplicate perichaetal leaves, relatively short capsules and round-pored stomata. Although sporophytes of Limnohypnum are unknown, we may predict that it has the latter character states.

The new familial position requires a discussion, as the Climaciaceae is one of the best delimited families of pleurocarps, immediately recognized by habit, representing the only clearly dendroid plants in the boreal zone of the Northern Hemisphere, while almost none of their character states occur in Limnohypnum.

According to most classifications, the family Climaciaceae includes two genera, Climacium and Pleuroziopsis (Brotherus, 1925; Frey & Stech, 2009; Goffinet, 2009; Iwatsuki, 2004; Ignatov et al. 2006). Ireland (1968) suggested to separate Pleuroziopsis in its own family, but Norris & Ignatov (2000) disagreed, finding a specific stem structure in these genera as likely homologous. A doubt regarding the position of Pleuroziopsidaceae close to Climacium was expressed by Hedenäis (1995), who included it in the Hylcomiaceae. The recent molecular phylogenetic analyses based on different gene regions, e.g., nuclear ITS and chloroplast trnL-F (Ignatov et al., 2007) or chloroplast rps4, mithochondrial nad5 and part of nuclear 26S RNA (Cox, 2010) found them closely related, although some analyses did not join them, as in the rbcl-based analysis of Tsubota et al. (2004). All the analyses with nuclear ITS showed Climaciaceae related to the Hylcomiaceae and Antitrichia (e.g., Gardiner et al., 2005; Huttenen et al., 2012). The latter genus was thus segregated in its own family (Ignatov & Ignatov, 2004), removed from the Luedodontaceae, which was also supported by the arrangement of the proximal branch leaves (Spirina & Ignatov, 2010; Ignatov & Spirina, 2013).

The tree topologies obtained here indicate that Limnohypnum is related to Climaciaceum and Pleuroziopsid, and rps4 even indicates that it is more closely related to Climaciaceum, than to Pleuroziopsid. There are likely five possible solutions for how to reflect the current tree topologies in the classification: 1) separate Limnohypnum in its own family, distinct from the Climaciaceae (with Climaciaceum and Pleuroziopsid); 2) accept three genera, Limnohypnum, Climaciaceum and Pleuroziopsid in three families of their own; 3) expand the circumscription of the Climaciaceae, to include the third genus Limnohypnum; 4) include Limnohypnum, Climaciaceum and Pleuroziopsid (and then also Antitrichia) in Hylcomiaceae, reducing Climaciaceae (and then likely also Antitrichiaceae) into synonymy of Hylcomiaceae; 5) leave Hylcomiaceae paraphyletic, accepting Climaciaceae as a separate family, but place Limnohypnum in the Hylcomiaceae, considering it as a transitional step from Hylcomiaceae to Climaciaceae.
Advantages and disadvantages of each solution can be evaluated from different point of views, such as practical classification and ease of building keys, strictly following cladistic rules, or a balance between molecular and morphological approaches, with variable weighting of different data sets.

The idea of a separate family will allow avoiding a very indefinite circumscription of Climaciaceae, but it will indicate nothing about their relationship.

Expansion of Hylolomiacineae may have a certain basis, as this family includes both plants with paraphyllia, e.g., Hylolomium, Hylolomiastrum, Loesoeobryum, and some without them, e.g., Rhytiadiaphus and Neodolichomitra. However, inclusion of additional genera will make the circumscription of the family even more vague, and the interesting fact of the close relationship between Limnophyllum, Climaciaceum and Pleuroziopsis will be neglected.

Thus we suggest acceptance of these tree genera in one family, the Climaciaceae, despite that the key characters of the family will almost disappear. Thus the rule that aquatic habitat strongly modifies moss morphology, making them superficially very different from the most closely related terrestrial ancestors will obtain additional support.

Similarly, the transition from terrestrial to epiphytic growth of pleurocarps often results in strong changes (cf. Hedenäs, 2012; Huttunen et al., 2004, 2008, 2013; Ignatov et al., 2012; Vanderpoorten et al., 2002). Naturally, a numerous striking familial displacement happened due to investigations of these facts: Anacampseton (was Fabroniaceae, now Amblystegiaceae), Clasmatodon (was Fabroniaceae, now Brachytheciaceae), Struckia (was Fabroniaceae, now Plagiotheciaceae), and many other ones.

The transition from terrestrial to epiphytic growth affects mostly sporophytic characters (Hedenäs, 2012; Huttunen et al., 2004), although common trends to relatively smaller plants, with shorter and more thick-walled cells occur in many groups like Leskeaceae, Taxiphyllyaceae, Brachytheciaceae, and Amblystegiaceae. Hedenäs (2012) found that the short double costa significantly correlates with the epiphytism in pleurocarpous mosses.

Morphological traits associated with the change of environmental preference from terrestrial to aquatic include larger plant size, a tendency to hyalodermis development, enlarged alar cells, longer leaf decurrencies and longer axillary hairs. These traits are not as many and also not so specific to aquatic plants, being present in some terrestrial lineages as well, as, for example, in Plagiotheciaceum.

The sharp differentiation between clearly dendroid Climaciaceum and Pleuroziopsis and not dendroid at all in Limnophyllum may appear less impossible considering one recently studied case in the genus Kindbergia (Brachytheciaceae). This genus includes 4-8 species, and one of them, the Japanese endemic K. arbuscula, is very distinct in its dendroid plant architecture. At the same time, molecularly it has been found not to differ from the widespread K. praelonga by Hedenäs (2010), who suggested to consider such abrupt change as a result of mutation in development regulation, where shoot differentiation was redirected along another path. A similar, but much older case may be present in the evolution of Climaciaceae. Concerning paraphyllia, abundantly present in both Climaciaceum and Pleuroziopsis, in the related family of Hylocomiaceae, the stronger the sympodial branches with a ‘stipe’ zone at base of the sympodial innovations, the better developed are the paraphyllia: cf. Hylolomium, Hylolomiastrum and Loesoeobryum versus Rhytiadiaphus. Pleuroziump and Neodolichomitra.

Summing up, Limnophyllum is yet another case where molecular phylogenetic inference indicates an ‘unbelievably’ different position, akin to what was found in the genera Leptodon (Sotiaux et al., 2009) and Pinnatella (Enroth et al., 2010). However, it also points at the ability of pleurocarps for a relatively fast morphological evolution, especially in connection with change of environment.

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LITERATURE CITED


