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Genus *Aneura* (*Marchantiophyta*) in Slovenia and new potential lineages for Europe

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Abstract: The liverwort genus *Aneura* (*Aneuraceae*) has been the focus of many studies aimed at revealing the hidden cryptic diversity within the *A. pinguis* complex. In the last few years, the knowledge of its genetic heterogeneity and geographical distributions of the alleged species has notably increased. Genetic markers can be used to identify distinct taxa, resulting in a greater understanding of the diversification processes in this genus. This study investigated taxa of the genus *Aneura* present in Slovenia and shed light on the diversity of liverworts in the south-central part of Europe. The particular aim of this work was to use molecular methods to identify cryptic taxa and compile an up-to-date reference list of Slovenian bryophyte species with notes on their ecology. Using a standardized methodology outlined in several related studies, three plastid genetic regions (*mat*K, *trn*H-*psb*A, *trn*L-*trn*F) and the complete nuclear ITS sequence dataset were analysed for 55 *Aneura* samples from different geographic regions in the country. Phylogenetic position of the specimens studied was investigated using distance and maximum likelihood methods. The genus *Aneura* is genetically separated into roughly 20 clades corresponding to proposed cryptic species, nine of which were confirmed for Slovenia. These were the clades A, B, C, E, G, M (previously regarded as *A. maxima* in Europe) and *A. pinguis* s. str. Additionally, two new lineages for Europe were discovered and discussed. Some accessions grouped closely with a North American taxon *A. sharpii* and a Southeast-Asian clade, which was first discovered in Thailand, but does not have a formal name. Several previously recorded European distinct phyletic lineages were detected in clades A, B, C and E. The monophyletic status of taxon B is unclear, as sequences from *A. pellioides* and accessions from Thailand were grouped within the confines of clade B. Furthermore, 20 morphological traits were selected for detailed study. Results from a morphological analysis indicate that the species differ somewhat in thallus width, thallus thickness, number of cells in the central part of the thallus, length of dorsal epidermal cells, the presence of a translucent margin and some other traits in the case of specific taxa. There were, however, many overlapping features among the clades examined. Ecological preferences in terms of suitable habitat characteristics are provided, narrowing the knowledge gap between the perceived ecological niches that these taxa occupy. The present paper outlines the taxonomic diversity and distribution of the genus *Aneura* in Slovenia and provides suggestions for further work.

Keywords: *Aneura pinguis*, *Aneura sharpii*, cryptic species, DNA barcoding, morphometry, ecological preferences, Europe, liverwort, Slovenia

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Introduction

The diverse simple thalloid liverwort genus *Aneura* Dumort, which belongs to the family *Aneuraceae* H. Klinggr., has a worldwide distribution (Söderström et al. 2016). The most prominent representative species is *Aneura pinguis* (L.) Dumort., first described by Linnaeus under the name *Jungermannia pinguis* L. (Linnaeus 1753). In the past few decades, there has been an accumulation of considerable evidence indicating that the genetic diversity of *A. pinguis* is very high (Bączkiewicz & Buczkowska 2005, Wachowiak et al. 2007). This led to the recognition of many evolutionary distinct lineages, which are considered cryptic or pseudocryptic species in *A. pinguis* s. lat. (Bączkiewicz et al. 2017). At the time of writing this paper, several of the newly identified taxa were not formally described, and thus were given distinct labels according to different authors. Morphological studies have, for the most part, not yet provided reliable distinguishing features between all new putative species (Buczkowska et al. 2005, Buczkowska et al. 2006, Anantaprayoon et al. 2023). However, there are biochemical (Wawrzyniak et al. 2021) and genetic markers (Bączkiewicz et al. 2008, Bączkiewicz & Buczkowska 2016, Buczkowska et al. 2016, Bączkiewicz et al. 2017) that can help with the identification process of cryptic diversity in this genus. Also, complete mitochondrial and chloroplast genomes are available for some of the taxa (Myszczyński et al. 2017).

Besides genetic differences, the species also differ in their ecology, especially in the habitats in which they thrive, suggesting different ecological preferences (Bączkiewicz et al. 2016, Bączkiewicz et al. 2017). For example, certain clades are known to grow only in bogs, while others thrive on old decaying wood in forests, on damp rocks or muddy clay soil. This observation further supports the hypothesis of evolutionary distinct lineages within the genus *Aneura*. Along with the Polish authors (Bączkiewicz et al. 2017), this genus is of interest to other researchers (e.g. Wickett & Goffinet 2008, Preußing et al. 2010, Reeb et al. 2018, Anantaprayoon et al. 2023, Long et al. 2023, Söderström et al. 2023, Gospodinov & Rayna Natcheva 2024), who have obtained DNA sequence data from different geographic locations that is available from public databases, fostering new and comparative studies. However, most research focusing on the diversification in the genus *Aneura* is geographically limited, underscoring the gap in knowledge of this genus in most of its distribution.

Long et al. (2023) provide a lectotype and epitype of *A. pinguis* s. str. and clarify which phyletic lineage corresponds to the type species. This is cryptic species F according to Bączkiewicz et al. (2017), which has an affinity for base-rich substrates, and thus grows in places such as damp calcareous meadows, dune slacks and limestone quarries (Long et al. 2023). The global checklist of hornworts and liverworts (Söderström et al. 2016) includes around 50 currently accepted species for the genus *Aneura*. Four of these are thought to occur in Europe: *A. latissima* Spruce, *A. maxima* (Schiffn.) Steph., *A. mirabilis* (Malmb.) Wickett et Goffinet and *A. pinguis* s. lat. (Hodgetts et al. 2020), but the existence of two, namely *A. maxima* and *A. latissima*, in Europe is questionable. Currently available data indicates that both occur on other continents, which makes them unlikely to be present in Europe (Hodgetts et al. 2020, Paton 2022). This is also supported by Söderström et al. (2023), where the authors re-examined the concept of *A. maxima* and other similar *Pellia*-like *Aneura* species. *Aneura maxima* was thought to thrive in most European countries, but new evidence suggests that it is likely to be widespread only in

Southeast Asia, Australia and the Pacific (Söderström et al. 2023). Although currently known distribution of these species is limited to other continents, this does not constitute definitive evidence that these taxa cannot occur naturally in Europe. Moreover, the present state of knowledge regarding the plant identified as *A. latissima* in Europe remains incomplete (Hodgetts et al. 2020).

The cryptic diversity of the genus is still only partially understood, with each new study offering interesting new insights into this matter. Authors from Thailand (Anantaprayoon et al. 2023) report several putative species; at least two of them are confirmed for Thailand and cannot be distinguished based only on morphology. The study also highlights the vastly different outcomes when comparing different species delimitation methods using molecular data. Which lineages should be regarded as separate species is still a matter of debate. Along with several delimitation methods, the constructed datasets can also lead to incongruencies among studies. For instance, Polish authors (Bączkiewicz et al. 2017) obtained a list of 13–18 candidate species when using ABGD analysis based on both individual and combined genetic loci. On the other hand, Anantaprayoon et al. (2023) report either four, 28 or 45 putative species using delimitation methods that rely on ultrametric trees (GMYC, bPTP) and sequence-based methods (ASAP, ABGD) using four genetic loci. Differences in methodology, therefore, hinder straightforward comparison.

In the latest published checklist of liverwort and hornwort species in Slovenia (Martinčič 2024), the taxon *A. pinguis* is listed as the only species in this genus. In recent surveys, a number of specimens were collected from various locations in Slovenia, which, according to their habitat preferences, may correspond to other putative taxa. Several of them are morphologically closer to species with a thin, translucent thallus margin, such as *A. maxima*, as described in some identification keys (Schumacker & Váňa 2005, Frey et al. 2006, Casas et al. 2009, Hugonnot & Chavoutier 2021). To better understand the ecology and diversity of liverworts in Slovenia, we investigated which taxa of the genus *Aneura* are present in this part of Europe. The main research aims of this study were: (i) to determine the underlying diversity of the genus *Aneura* in Slovenia using standardized DNA barcoding methods, (ii) to provide information on the morphology of the specimens studied, and (iii) determine the link between different taxa and their habitat preferences.

Material and methods

Field work

Systematic sampling of *Aneura* specimens was done in different parts of Slovenia. Known geographic localities were obtained from the database BioPortal (2024) and from voluntary observations made in previous years. As many microhabitats as possible were studied and the habitat of the collected plants was categorized. The fresh material was placed in labelled plastic bags or petri dishes, later cleaned of adhering substrate and dried with silica gel (Kemika). Three samples from the Triglav National Park were collected with a permit for sampling in the restricted area of the National Park (No. 35609-2/2023-2).

The plant material used in this study consisted of 55 *Aneura* samples, with 42 accessions representing newly collected thalli and 13 herbarium specimens from the Herbarium LJU. The localities sampled are listed in Supplementary Table S1. Voucher specimens are stored in the Herbarium LJU (Department of Biology, Biotechnical Faculty, University of Ljubljana) and as DNA isolates.

DNA extraction, PCR and sequencing

Total genomic DNA was extracted from newly collected and herbarium specimens using NucleoSpin Plant II kit (Macherey-Nagel) following the manufacturer's protocol. The concentration and quality of the isolated DNA were evaluated spectrophotometrically using a NanoDrop 1000 (Thermo Scientific).

For PCR amplification, four genetic regions were chosen, in accord with previous studies (Bączkiewicz et al. 2017, Anantaprayoon et al. 2023, Long et al. 2023, Söderström et al. 2023), namely *mat*K*,* *trn*H-*psb*A*,* *trn*L-*trn*F and the complete nuclear ITS region (ITS1-5.8S-ITS2). For the standard primer sequences used in this survey see Supplementary Table S2. PCR amplification was carried out in a Biometra Thermal Cycler (Analytik Jena). Each reaction contained 25 μl of the following final concentrations of reagents: 1× One Taq reaction buffer, 300 μM dNTPs, 0.5 μM of the appropriate reverse and forward primers, 0.625 U One Taq DNA polymerase (New England BioLabs) and 3 μl 10× diluted template DNA (initial concentration in the range of 10–200 ng/μl). Mili-Q water was used to reach the final volume of the reaction tube. The cycling protocols were as follows: initial denaturation for 3 min at 94 °C, followed by 35 cycles of 30 sec at 94 °C, 30 sec at 41 °C (*mat*K), 49 °C (*trn*H-*psb*A), 50 °C (*trn*L-*trn*F) or 46 °C (ITS), 1 min 68 °C and final elongation for 5 min at 68 °C. PCR products were evaluated using gel electrophoresis on a 1% agarose gel. In cases where the reaction was unsuccessful, the procedure was repeated with the template DNA of a higher dilution factor.

The amplified genetic regions were sent for purification and bidirectional Sanger sequencing (Macrogen, Amsterdam) using the same pairs of primers.

Phylogenetic inference

Sequence data was assembled, trimmed and manually edited in Geneious Prime version 2024.0.7 (Biomatters Ltd., https://www.geneious.com). Summary information about each genetic locus produced in this study was obtained using the software MEGA version 11.0.13 (Tamura et al. 2021). Sequences were uploaded on to the BOLD database under the project code ANESL. Accession number for each specimen is listed in Supplementary Table S1. Relevant DNA barcodes were downloaded from public databases (BOLD, GenBank) to which newly produced sequences were added and later used in the analyses.

A total of 232 newly assembled sequences of high or acceptable quality, along with available sequence data from other authors (Supplementary Table S3) for each of the four loci were aligned using the program MAFFT version 1.5.0 (Katoh & Standley 2013) under the G-INS-i algorithm. The alignments were visually checked for ambiguous sites. Phylogenetic analysis consisted of two parts. First, the data was explored using a split network to inspect possible obscure and conflicting signals in the dataset before constructing a maximum likelihood phylogeny.

The NeighborNet algorithm (Bryant & Huson 2023) implemented in the software SplitsTree App version 6.3.34 (Huson & Bryant 2006) was used to generate a split network. Preliminary analyses showed little disagreement between networks for the nuclear ITS region and the plastid loci, respectively (Supplementary Fig. S1). Thus, the final analysis was done with a concatenated dataset of the four loci. To avoid possible artefacts, accessions with only one or a few available sequences per voucher specimen were removed prior to the final analysis. Accessions with extremely long branches were also excluded. The distance matrix was calculated for up to 112 accessions using HKY 85 (Hasegawa et al. 1985) distances with the parameter options set to be calculated from the alignment. The Ts/Tv ratio and percentage of invariable sites were calculated in MEGA and SplitsTree. The substitution model was determined with ModelFinder (Kalyaanamoorthy et al. 2017) to be the best-fitting model for the combined dataset.

According to Preußing et al. (2010), Rabeau et al. (2017) and Anantaprayoon et al. (2024), the genera *Lobatiriccardia* (Mizut. et S.Hatt.) Furuki and *Riccardia* Gray are all nested within the *Aneuraceae*, with the former being a sister clade to *Aneura*. Therefore, three accessions of *Lobatiriccardia* spp. and *Riccardia* spp. were chosen as an outgroup for the following phylogenetic inference. The dataset consisted of an alignment for 135 accessions representing each genetic locus, which were subsequently concatenated into a supermatrix in Geneious Prime. To test the applicability of different partitioning schemes, PartitionFinder 2 (Lanfear et al. 2017) was used, which implements the PhyML program by default (Guindon et al. 2010). The search algorithm was set to greedy (Lanfear et al. 2012). Afterwards, a maximum likelihood partition analysis was conducted using IQ-Tree web server (Trifinopoulos et al. 2016), employing the results obtained with PartitionFinder 2. Best-fitting model of evolution for each partition was determined automatically with ModelFinder according to the Bayesian information criterion (BIC); for more information see Supplementary Table S4. Maximum likelihood analysis was performed under an edge-linked partition model, a total of 5,000 ultrafast bootstrap pseudoreplicates (Minh et al. 2013) and a random seed of 206741. The recovered phylogeny was displayed in TreeGraph 2 (Stöver & Müller 2010) and Geneious Prime. Inkscape version 1.3.2. (Inkscape Project 2023, https://inkscape.org) was used to visually edit the final trees and networks. Genetic distances between the Slovenian accessions were calculated in SplitsTree using the HKY 85 model for all new sequences combined.

Clade nomenclature

To avoid confusion with previous studies, we followed the system of molecular operational taxonomic units (MOTUs) or proposed cryptic species by the one letter code used by Bączkiewicz et al. (2017). Clade F is regarded as *A. pinguis* s. str. (Long et al. 2023). Since the resolved status of *A. maxima* in Europe (Söderström et al. 2023), the taxon referred to as *Aneura* sp. 1 by the authors and the taxon *A. maxima* as mentioned by Bączkiewicz et al. (2017) is regarded here as clade M. The proposed taxa discovered in Southeast Asia by Anantaprayoon et al. (2023) are given provisional names such as Thai 1, Thai 2 and Thai 3, hence referring to three major genetically delimited clades mainly based on ASAP and ABGD analyses, and clade Thai 4 given under the GMYC model, conducted by the authors. We chose only the newly added accessions from Thailand to be included in clades Thai 1 and Thai 4, and retained the remaining clade labels as given by Bączkiewicz et al. (2017). The two potentially new lineages that are grouped together with *A. sharpii* Inoue et N. G. Mill. and one of the clades discovered in Southeast Asia, are labelled *Aneura* aff. *sharpii* and *Aneura* aff. Thai 2, implying their close evolutionary relationship to these taxa.

Morphological analysis and light microscopy

Dried plant material was rehydrated and subsequently used for morphometry. All 55 voucher specimens were investigated using light microscopy. A total of 20 morphological and anatomical traits were assessed for each specimen, including 16 quantitative and four qualitative characters. This assay comprised the dorsal and ventral sides of the gametophyte, along with cross-sections and oil bodies when present. The choice of morphological features was largely based on findings of previous studies (Buczkowska et al. 2006, Anantaprayoon et al. 2023), with the inclusion of some novel characters (see Supplementary Table S5 for a compiled list and description of each diagnostic character included in the study). Continuous and discrete characters were assessed in the first ~10 mm from the apex. Detailed inspections of the thallus were performed using a stereomicroscope and an Axio Imager M2 (Zeiss) microscope. All measurements were conducted by the same beholder to minimize observer bias.

Multiple replicate measurements were recorded per specimen, from which summary statistics were calculated in software R version 4.4.1 (R Core Team 2024). Significant differences between taxa were evaluated using the non-parametric Kruskal–Wallis test for numerical data using the function kruskal.test in the package stats, and the P-values were adjusted using the Benjamini–Hochberg correction to control for the false discovery rate, and the chi-squared test for categorical variables, for which the command chisq.test from the package stats was applied to a constructed contingency table. A P-value of less than 0.05 was considered to reflect significant differences between the groups compared. Dimensionality reduction of the numeric morphometric dataset was accomplished with principal component analysis (PCA) using the package factoextra (Kassambara & Mundt 2020) implemented in R. Prior to analysis, all continuous and discrete numerical variables were standardized and only variables with robust and unambigious measurements were used. To assess differences in multivariate space, a PERMANOVA was carried out using the adonis2 function in the vegan package (Oksanen et al. 2024), relying on a Euclidean distance matrix. To evaluate the assumption of homogeneity of group variances, a test for homogeneity of multivariate dispersion was done using betadisper (package vegan), followed by an ANOVA on the distances to resulting group centroids.

Habitat preferences

To gain a clearer understanding of the ecological niches of the different *Aneura* lineages, a list of microhabitats was compiled of where the plants were growing at the time of the field survey and sampling. Data from the herbarium specimens was also included (see Supplementary Table S1 for the description of each site sampled). Specimens growing in close proximity to one another can occupy different microhabitats at the same site; hence, microhabitats can vary greatly, even for the same species. Therefore, some of the microhabitats were grouped into habitat categories that combine the substrate the thalli were growing on or the overall habitat, e.g. carbonate rocks, humic soil, swamps and mires. The variation in microhabitats and the affinity of the plants for them is discussed. The frequencies of each species occurring in a specific habitat were graphically presented using the package ggpubr (Kassambara 2025) in software R. To further analyse the data, a constructed contingency table was applied in the correspondence analysis using the package factoextra (Kassambara & Mundt 2020) implemented in R. Habitat categories and associated taxa were treated as categorical variables. In addition, a map created in QGIS version 3.28.3 (https://qgis.org) is provided, which depicts the geographic distribution of the Slovenian samples.

Results

Examining the DNA barcodes used

For most samples, high-quality DNA was obtained, followed by a final 100% PCR amplification rate of the four genetic loci. A total of 235 new assembled sequences were obtained from 55 specimens belonging to the genus *Aneura* and three *Riccardia* specimens. Summary statistics for the four genetic loci of the Slovenian *Aneura* samples is given in Table 1. The alignment of the nuclear ITS1-5.8S-ITS2 region consisted of 792 sites, which included 615 conserved and 177 variable sites, of which 169 were parsimony informative. With that, the ITS region has the highest percentage of parsimony informative sites (21.3%) in the DNA barcodes studied. The length of the alignment for the plastid loci was 808, 816, 524 sites for *mat*K, *trn*H-*psb*A and *trn*L-*trn*F, respectively. Of them, the most parsimony informative marker is the gene *mat*K (16.1%), encoding the enzyme maturase K, whereas *trn*H-*psb*A and *trn*L-*trn*F are more conserved regions, showing lower nucleotide variation between the taxa studied.

Table 1. Characteristics of alignments for each genetic locus of the Slovenian *Aneura* specimens.

	ITS	*mat*K	*trn*H-*psb*A	*trn*L-*trn*F
sequence length (mean)	780	808	787	520
alignment length	792	808	816	524
conserved sites	615	673	707	453
variable sites	177	135	107	70
parsimony informative	169	130	107	67
parsimony informative (%)	21.3	16.1	13.1	12.8
singletons	8	5	0	3

Identification of taxa and genetic divergence

Genetic diversity in the *Aneura* complex was investigated using distance and maximum likelihood methods. Both approaches revealed comparable topology, congruent in the depiction of multiple distinct clades (Figs 1 and 2). Two major monophyletic groups were apparent, one containing clades A, D, E and *A. maxima*, while the other major group holds clades B, C, G, H, I, M, *A. pinguis* s. str., *A. mirabilis*, *A. sharpii, A. pellioides* (Horik.) Inoue and the three Southeast-Asian entities Thai 1, Thai 2 and Thai 3. NeighborNet split networks for each genetic locus are depicted in Supplementary Fig. S1, which reveal a stable topology across the four utilized barcodes. Therefore, the plastid and nuclear datasets were combined in the proceeding analysis. The resulting split network of the concatenated dataset is depicted in Fig. 1, uncovering a complex phylogenetic structure in this genus. Longer edges are interpreted as greater genetic distance. The accessions group into distinct, tight clusters, indicating strong genetic cohesion within each lineage. The network not only describes well-established groups but also shows the

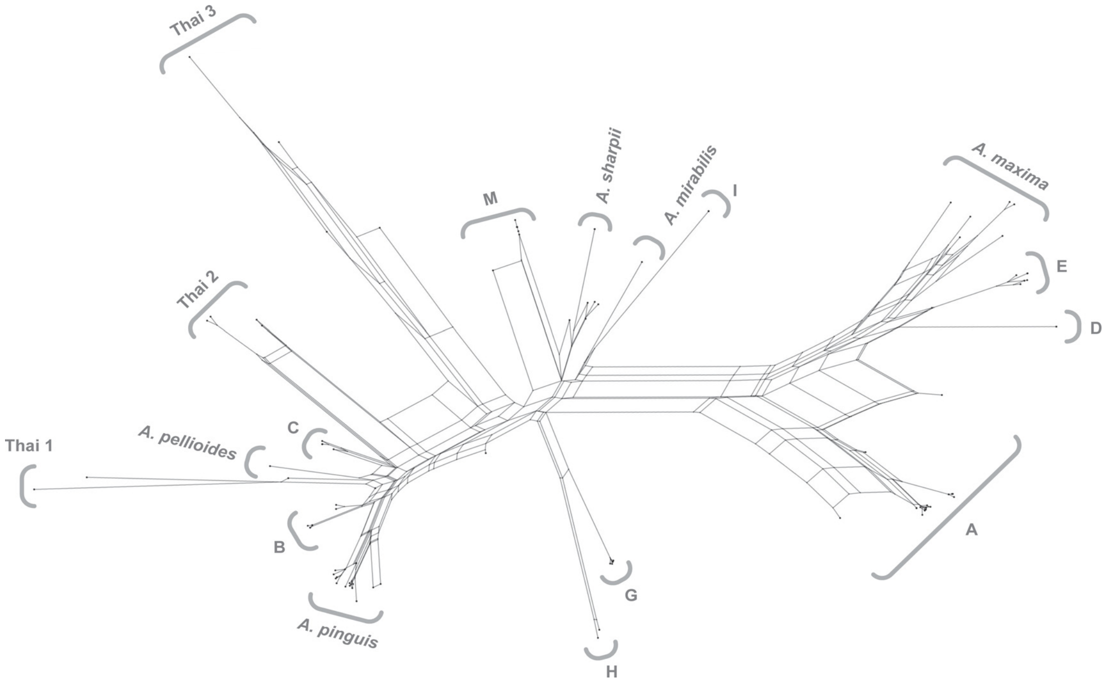

Fig. 1. An unrooted split network based on the combined nuclear and plastid dataset (ITS, *mat*K, *trn*H-*psb*A and *trn*L-*trn*F) was generated for 112 specimens, representing phylogenetic information as groupings or splits between taxa. The diagram was constructed using the NeighborNet method, based on distances calculated from a concatenated multiple sequence alignment under the HKY85 model. The following MOTUs, aff. *sharpii*, aff. Thai 2 and lineage B_1, are incorporated under their respective closely related taxon label.

apparent interclade heterogeneity of taxon A and *A. maxima*. Note the different number of accessions for these taxa compared to other groups. Reticulations in the centre of the diagram may indicate deep and conflicting phylogenetic signals. The terminal groups, however, are evidently separated from each other, suggesting clear evolutionary divergence among the taxa studied.

The maximum likelihood analysis recovered a tree with the highest log likelihood of –19,655. A consensus tree depicted as a cladogram inferred numerous MOTUs, roughly 20 or more candidate species (Fig. 2). For a phylogram with information on branch lengths, see Supplementary Fig. S2. Bootstrap support values for most of the clades are in the believable or high range (80–100%), with the branch leading to clade C having the lowest support value (72%). Formally established taxon named J (represented here with two accessions from Japan; POZW 40511 and POZW 40544) as well as accessions from Thailand under the given name Thai 4 (NA 29, NA 30, SCOS 1570, SCOS 1373) and a sample from Ecuador (STU Preussing) are nested in *A. maxima*. The monophyletic origin of taxon B is in this analysis shown to be questionable as accessions of both *A. pellioides* and clade Thai 1 are nested in what is currently recognized as clade B. The distinctly evolving lineage B_1 (represented here by three accessions; POZW 42019, POZW 40261 and NY Lan., Bak., Maks. 1) is probably more closely related to *A. pellioides* and clade Thai 1 than to other phyletic lineages in taxon B.

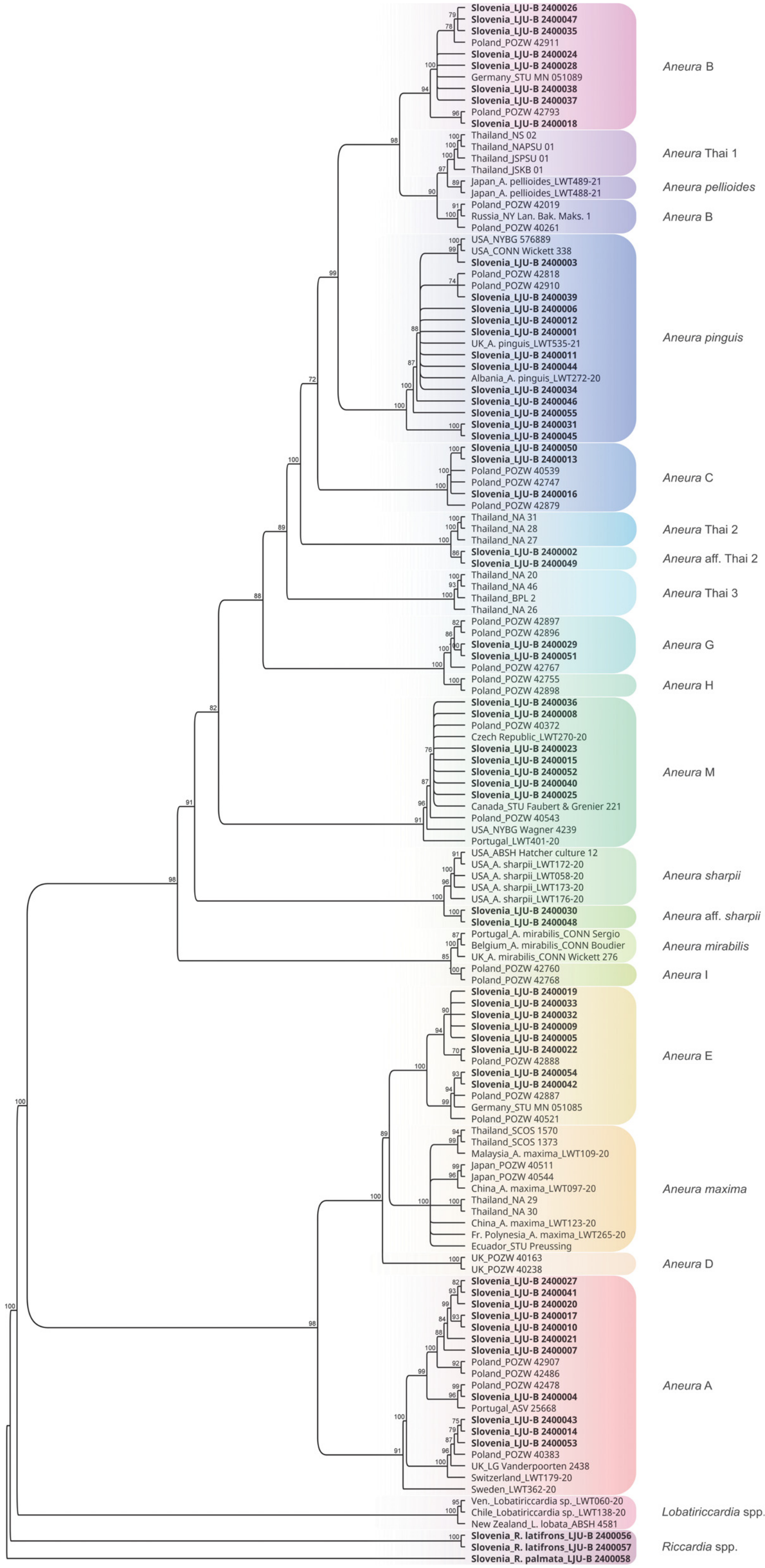

Fig. 2. Recovered maximum likelihood (ML) four-gene phylogeny of the genus *Aneura*, also including *Lobatiriccardia* and *Riccardia* as an outgroup. The tree depicted presents a consensus tree of a partitioned analysis and has a log-likelihood of –19,655. Terminals in bold represent accessions for which new sequences were obtained in this study. ML ultrafast bootstrap values are represented by numbers besides nodes. Only branches above a threshold of 70% are shown.

Samples from Slovenia clustered in both analyses with the following taxa: A, B, C, E, G, M and *A. pinguis* s. str. However, four Slovenian specimens were not assigned to any of the recognized European lineages but instead formed a well-supported clade with the North American taxon *A. sharpii* (LJU-B 2400030, LJU-B 2400048) and the Asian clade *Aneura* sp. Thai 2 (LJU-B 2400002, LJU-B 2400049). Both are part of the big clade containing *A. pinguis* s. str., although the sister position of *A. sharpii* s. lat. to the group of remaining clades is not fully supported. The phylogenetic positions of the four specimens that were identified as thus far undiscovered lineages present in Europe have plausible support values, hence most likely increasing the number of known species in this genus. They form separate lineages that are presumably closely related to *A. sharpii* and the Southeast-Asian clade Thai 2.

The combined dataset of recently acquired sequence data indicates marked differences in average genetic divergence among the local lineages studied (Table 2). The lowest genetic divergence was recorded for species pairs *A. pinguis* s. str. and B (1.7%) and higher divergences were observed when comparing taxon E with clade aff. Thai 2 (9.6%) and *A. pinguis* s. str. (8.6%). The distance between aff. Thai 2 and clade A is almost the same (8.3%). Intraspecific sequence diversity is at least 10× lower than the interspecific genetic variation, with lineage A having the highest (0.73%) and *A. pinguis* s. str. the second highest (0.46%) intraspecific distance. This intraspecific variation is also reflected in the split network and maximum likelihood analysis (Figs 1 and 2), which depict how different accessions form distinct lineages within the frame of a particular taxon. The lowest genetic differences were recorded in specimens assigned to clades G, aff. *sharpii* and aff. Thai 2 (< 0.017%). It is noteworthy, however, that the results are highly biased by the different number of samples studied. These findings highlight the importance of examining genetic variation in local Slovenian populations and the need for further similar studies.

Table 2. Average genetic distances (%) for the combined genetic regions (ITS, *mat*K, *trn*H-*psb*A and *trn*L-*trn*F) in the Slovenian accessions of the genus *Aneura* under the HKY 85 substitution model. In reference to the paraphyletic nature of taxon B, only Slovenian accessions were used in the computation of distances.

	A	B	C	E	G	M	aff. Thai 2	aff. *sharpii*
B	7.31							
C	6.96	2.00						
E	5.38	8.09	7.90					
G	6.37	4.50	4.38	7.04				
M	6.18	4.93	4.51	6.40	4.27			
aff. Thai 2	8.32	3.70	3.51	9.64	5.86	6.41		
aff. *sharpii*	6.25	4.87	4.45	6.45	4.01	3.31	6.08	
A. pinguis	7.55	1.71	2.28	8.61	4.88	5.17	3.82	5.19

Notes on morphological discernment

The rehydrated thalli showed an overall structure comparable to that of living plants. Thus, the rehydrated herbarium specimens likely retain the gross morphology of the intact fresh material. A frequent concern, however, was the accurate evaluation of the number of cells forming the delicate unistratose margin of some species, as it was often damaged or torn off by handling herbarium material. Summary statistics with graphical representations of the morphological data are incorporated in Supplementary Figs S3 and S4.

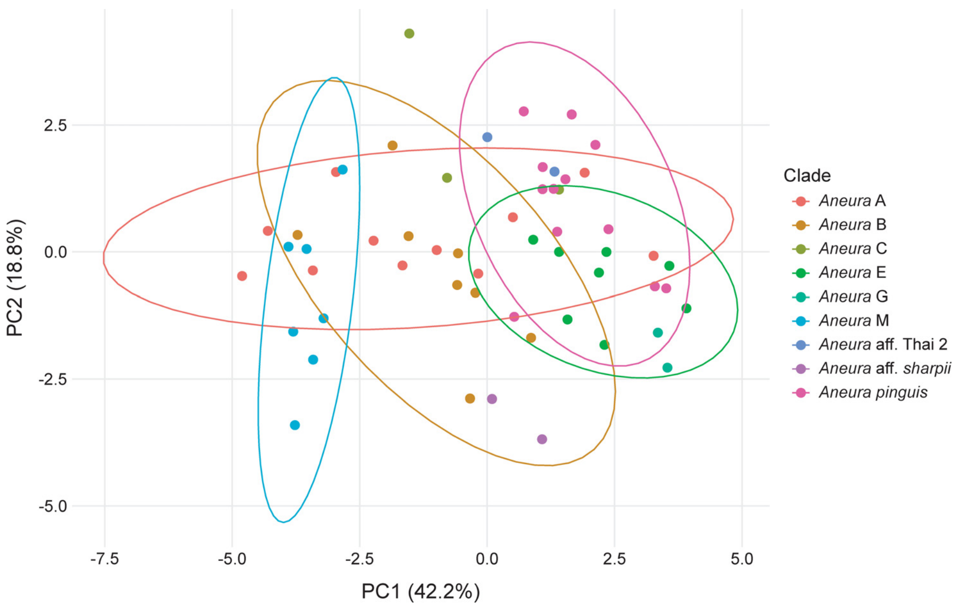

Fig. 3. Principal component analysis (PCA) of 14 quantitative morphological and anatomical traits in nine genetically delimited clades. The first two principal components (PC1 and PC2) together account for 61% of the variance. Individuals are colour-coded by taxon kinship. PC1 is primarily associated with thallus width, while PC2 reflects height-related traits. Ellipses represent 90% confidence intervals for each group in PCA space. Clustering patterns with little overlap indicate some morphological separation between the clades studied, while a substantial degree of similarity remains within the chosen character set.

The most pronounced differences between the taxa studied were recorded for the following quantitative traits: thallus width, dorsal/ventral epidermal cell length, thallus thickness and the number of cell layers in the middle part of the thallus. The broadest thallus was documented for taxon M (mean±SD = 6.1±0.8 mm), followed by taxon B (5.3±1.6 mm), while the narrowest thallus was observed in specimens corresponding to lineage G (1.4±0.5 mm). Taxon M is one of the lineages with the largest gametophytes and has values in the upper limits for many of the measured characters, whereas taxon G can be considered the smallest of the *Aneura* species studied. Measurements for other taxa often fall between these two with a high degree of overlap. There is also a small difference between particular lineages in the length and height of the median thallus cells in cross-sections and the number of cell rows in the bistratose part of the margin, with taxa M and B having the greatest range. In spite of this, recorded differences for many traits are very small (few µm) and the disparity between species decreases with the addition of more samples.

Principal component analysis (PCA) was performed on 14 quantitative traits (oil body diameter and the number of unistratose marginal cells were excluded) in order to explore the pattern in morphological variation among the taxa studied (Fig. 3). The first two principal components accounted for a substantial percentage of the total variance explained. PC1 (42.2%) was mainly associated with both macroscopic and microscopic traits incorporating the overall thallus broadness. These included thallus width, inner cell height, ventral/dorsal epidermal cell width and marginal cell width, indicating that PC1 represents an axis of general gametophyte width. PC2 (18.8%) captured variation in proportion and height-related traits, that is thallus thickness, the number of cell layers in the central part and dorsal epidermal cell height/length. When plotted in two-dimensional space, taxa grouped into somewhat distinct clusters, with clade M clearly separated along PC1 from clades E, G, aff. Thai 2 and *A. pinguis* s. str., whereas these lineages partially overlap with clades A and B, suggesting some degree of convergence in their morphology. The PERMANOVA test revealed significant differences in the morphological and anatomical features of the taxa studied ($R^2 = 0.478$, $P = 0.001$), indicating that taxon affiliation accounted for nearly half of the recorded variance in the dataset. However, the assumption of homogeneity of multivariate dispersion was not met ($P = 0.0026$; Supplementary Fig. S5), revealing that some of the recorded group differences may be partially due to unequal within-group variability. These results show significant morphological cohesion for at least some of the lineages studied, while also highlighting the general similarity of the gametophyte thallus in several lineages of *Aneura*.

Analysis of four qualitative traits (Supplementary Fig. S4) revealed that the state of the translucent or opaque thallus margin and the presence or absence of reduced thread-like lateral branches could be used to differentiate between some clades. Taxa with a translucent margin are G, M and aff. *sharpii*, while *A. pinguis* s. str., E, aff. Thai 2 and possibly taxon C typically have opaque margins. Both states were recorded in clades A and B. Attenuate thread-like lobes were most pronounced in taxon G and aff. *sharpii*, which is distinguished from the former by the undulated margin. A faint brown tinge was frequently observed in the rhizoids, possibly due to the drying process. Interesting to note was the phenotype of the samples assigned to clade aff. Thai 2. These specimens had a noteworthy thick and leathery thallus texture. In cross-section, the thalli appeared to be flat on the ventral side and convex on the dorsal side. No other individuals examined displayed this shape.

Even though the study was conducted on herbarium samples, oil bodies were recorded multiple times. Their diameter was in the range of 4.2–12.7 µm for all taxa. Oil bodies were of heterogeneous shape and consisted of small circular or ellipsoidal granules, which could also differ within a clade.

Notes on ecology and geographic distribution

Data about the different *Aneura* lineages were obtained from field collections and herbarium specimens, encompassing a broad spectrum of habitats. Fig. 4 illustrates some of the variation in ecological preferences of the specimens studied.

Descriptive data on microhabitats was further evaluated and combined into habitat categories. Currently, we present eight habitats, or rather habitat groups, namely carbonate rocks, carbonate fen, humic rocky soil, clay soil, silicate rocks, decaying wood, swamp forest and mire. The preferences of taxa for these habitats are shown in Fig. 5 (for a complete list of microhabitats, see Supplementary Table S1). The most frequently recorded habitat was carbonate rocks, which includes small gravel-sized rocks and large boulders, usually at shaded sites with water seeping over them. All clades recorded in Slovenia display an affinity for somewhat humid habitats, although, dependent on the species, the site can vary from slightly moist to wet. Taxa A, E and *A. pinguis* s. str. had the widest tolerance of different substrates, despite being restricted to limestone or

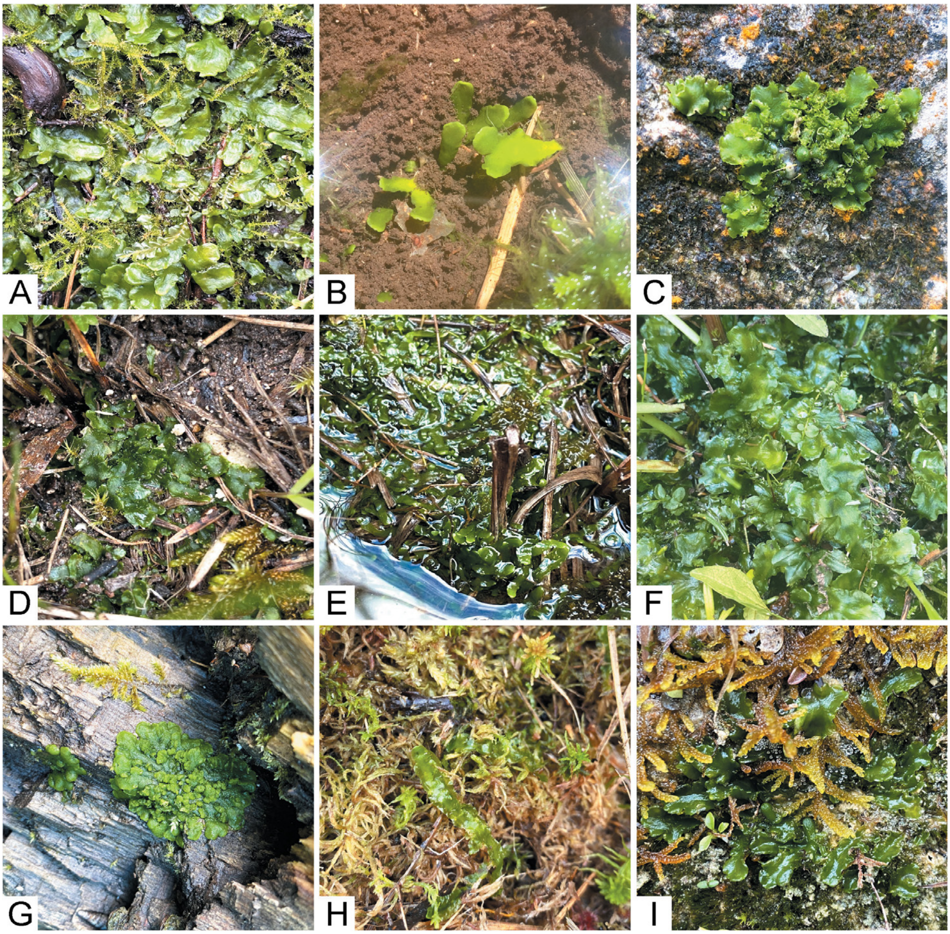

Fig. 4. Different taxa of the genus *Aneura* grow in a variety of habitats. (A) taxon A growing on gravel and sandy soil among pleurocarpous mosses; (B) taxon B submerged in water in a mire; (C) taxon C occupying the surface of bare shaded limestone; (D) taxon E on humic sandy soil next to a fen; (E) taxon G growing in small hollows on a raised peat bog; (F) taxon M next to a gravel forest road with water seeping over it; (G) taxon aff. Thai 2 firmly attached to a fallen decaying log; (H) *A.* aff. *sharpii* unfolding among *Sphagnum* mosses on a transitional mire; and (I) *A. pinguis* s. str. on damp carbonate rocks.

other types of carbonate bedrock, whereas clades B, G and M had an affinity for very wet environments, as they were seen growing submerged in water, in raised peat bogs and other types of mires or swamps.

The newly discovered European lineage related to *A. sharpii* was also recorded in mires. The two specimens were detected growing in shallow depressions on old decaying wood, hollows under old trees and among *Sphagnum* mosses in transitional mires. The accompanying vegetation growing around the thalli consisted, at both locations, of the following species: *Sphagnum capillifolium* (Ehrh.) Hedw., *Sphagnum palustre* L., *Dicranum scoparium* Hedw., *Campyliadelphus chrysophyllus* (Brid.) R. S. Chopra, *Pseudotaxiphyllum elegans* (Brid.) Z. Iwats., *Thuidium delicatulum* (Hedw.) Schimp., *Hypnum*

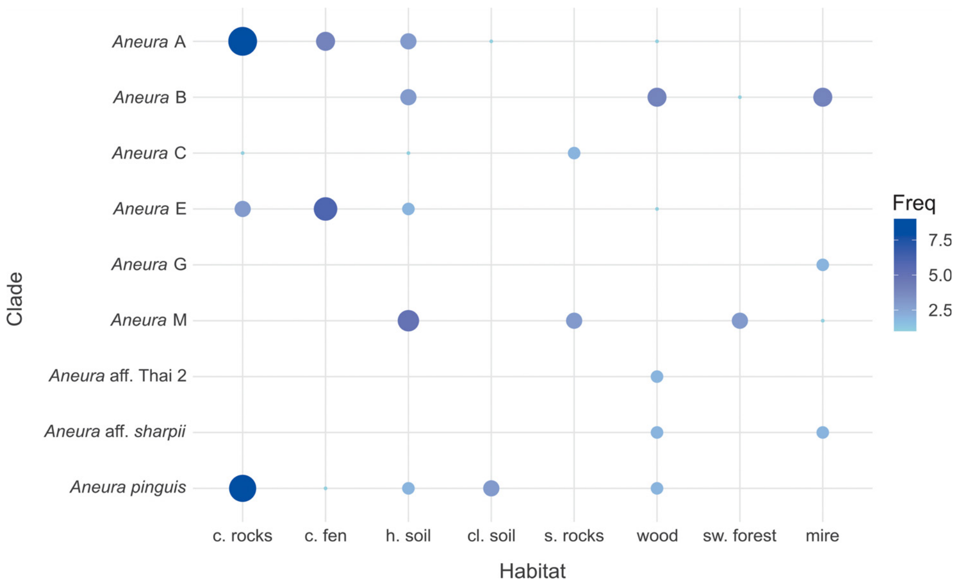

Fig. 5. Habitat affinities of the proposed species in the genus *Aneura* recorded in Slovenia. Habitat categories: c. rocks – carbonate rocks, c. fen – carbonate fen, h. soil – humic rocky soil, cl. soil – clay soil, s. rocks – silicate rocks, sw. forest – swamp forest. The size of the blue circle corresponds to the number of observations in each category. Note: at the same site, many individual thalli of the same taxon could grow in different microhabitats, thus the size of the circle does not represent the number of samples.

cupressiforme Hedw. var. *cupressiforme*, *Calypogeia arguta* Nees et Mont., *Pinus sylvestris* L., *Molinia caerulea* (L.) Moench, *Agrostis canina* L., *Frangula alnus* Mill., *Lysimachia vulgaris* L., *Potentilla erecta* (L.) Raeusch. and *Rubus* sp. The samples of clade aff. Thai 2 were collected at two localities in a montane beech forest on fallen, damp, decaying trunks lying across forest streams. The accompanying species on the logs were *Hypnum cupressiforme* var. *cupressiforme* and *Brachythecium rutabulum* (Hedw.) Schimp.

To further inspect the habitat characteristics of the taxa studied, a correspondence analysis was done using the frequencies at which each lineage was recorded in a given habitat. Results of chi-squared test ($P < 0.0001$) signify a high degree of association between specific taxa and implemented habitat categories, thereby confirming highly significant differences in their habitat preferences.

The relationship between categorical variables is visualized in Fig. 6. More than 70% of total inertia was accounted for by the first two dimensions. Taxa such as A, E and *A. pinguis* s. str. are grouped closely together, exhibiting an affinity for carbonate-rich microhabitats, thus suggesting similarities in their ecological niches. The positions of taxa C and M indicate that their affinities for particular habitats are to some extent similar, with both growing on silicate rocks or on humic, sandy and gravelly soils, while taxon M shows higher association with swampy, flooded forests. Clades B, G and aff. *sharpii* are all associated with mires, which often include raised peat bogs. While accessions affiliated with Thai lineage 2 were in this survey only recorded on old moist wood. The occurrence of different cryptic taxa in highly diverse microhabitats demonstrates differences in the ecological niches they occupy.

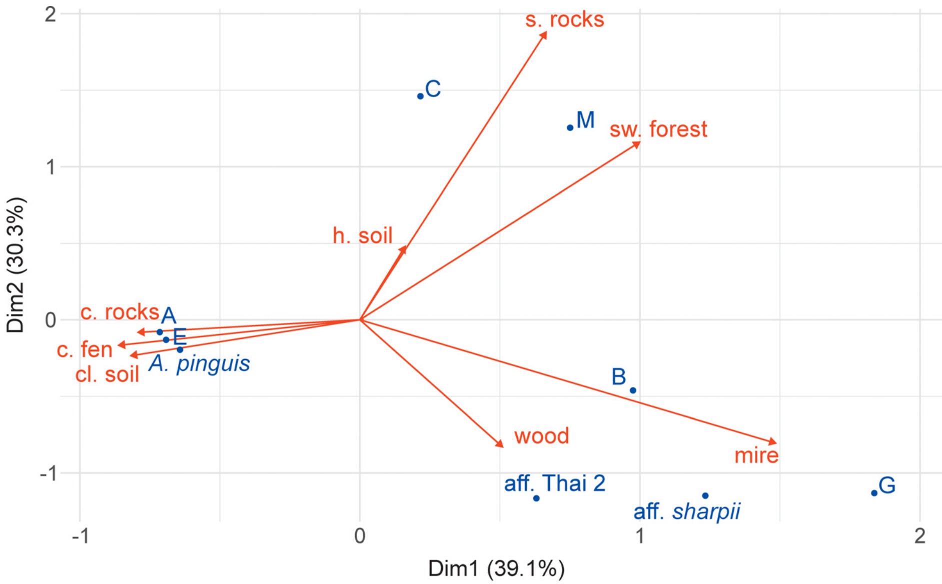

Fig. 6. Biplot of the correspondence analysis highlighting the effect of different habitats on the distribution of the studied *Aneura* taxa in two-dimensional space. Habitat categories: c. rocks – carbonate rocks, c. fen – carbonate fen, h. soil – humic rocky soil, cl. soil – clay soil, s. rocks – silicate rocks, sw. forest – swamp forest.

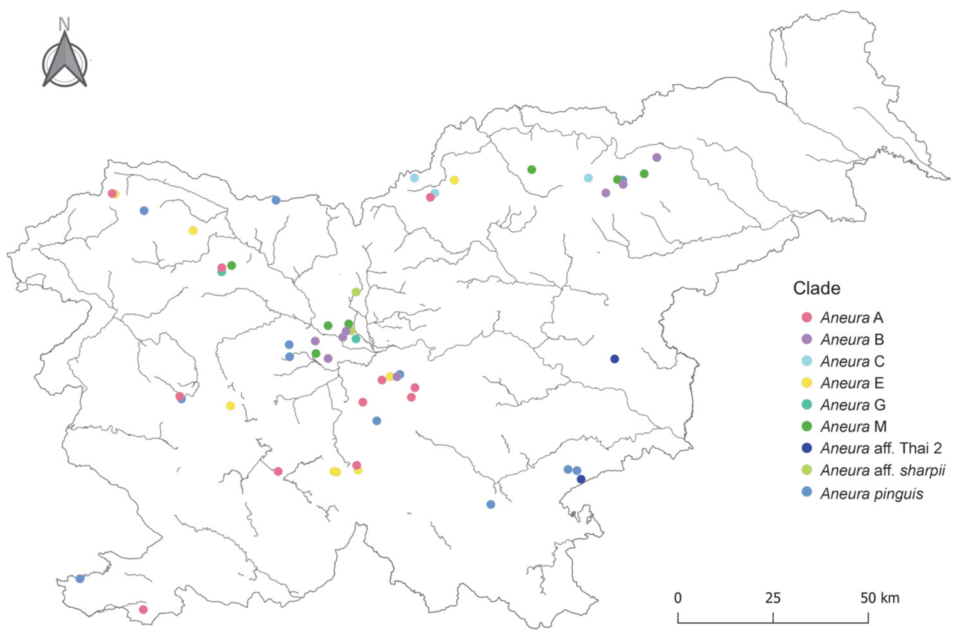

Fig. 7. Distribution map depicting the current localities of the specimens in the genus *Aneura* examined in Slovenia.

The most widespread and common taxa in Slovenia are taxon A and *A. pinguis* s. str., here represented by 11 and 12 samples, respectively (Fig. 7). Both have an affinity for base-rich substrates, ranging from the lowlands to high mountains. As indicated by the field survey (Fig. 5), taxon A is often found in more humid microhabitats, e.g. gravel or sand besides streams with water seeping over them or in fens on carbonate bedrock. The clades for which only two samples each were collected, considered to be rare in the area studied, are the lineage G found in peat bogs, aff. *sharpii* and the taxon aff. Thai 2.

Discussion

Cryptic species diversity is a widespread biological phenomenon found throughout the tree of life and is receiving increasing attention through the use of molecular methods (Bickford et al. 2007). Bączkiewicz et al. (2017) laid the groundwork for the application of molecular barcoding for investigating cryptic diversity in the liverwort genus *Aneura*. Other authors (e.g. Anantaprayoon et al. 2023, Long et al. 2023, Söderström et al. 2023, Gospodinov & Rayna Natcheva 2024) increased the global understanding of this genus. What was once viewed as a monophyletic taxon *A. pinguis*, is now split into several distinct evolutionary units, referred to as cryptic species, with seemingly no tangible morphological distinguishing traits. This concept should be applied cautiously and only as a temporary formalization of taxonomic groups, for which a sturdy morphological framework is not yet established (Korshunova et al. 2017). Nevertheless, according to divergence time estimates, the currently recognized cryptic species in the genus *Aneura* began to diversify around 60 million years ago (Anantaprayoon et al. 2024), coinciding with major paleoclimatic and geological events (Vajda & Bercovici 2014, Kaiho et al. 2016). The relatively long evolutionary history might encourage the search for stable diagnostic characters and the formal description of these taxa.

In this survey, the hidden genetic, morphological and ecological diversity of the genus *Aneura* in Slovenia was studied. Implementing molecular techniques for studying biodiversity has the potential to reveal new and intriguing outcomes. Thus, methods like DNA barcoding are widely utilized in biodiversity and taxonomic projects (Gostel & Kress 2022, Alam et al. 2024, Letsiou et al. 2024). The results of this study confirmed the existence of what is commonly referred to as cryptic variation in the genus *Aneura* in Slovenia. The presence of nine established clades was discovered. Two lineages were new to Europe, i.e. *A.* aff. *sharpii* and a lineage that is affiliated with the clade named Thai 2, as they are linked to taxa currently only reported from North America (Söderström et al. 2023) and Thailand (Anantaprayoon et al. 2023), respectively. Providing that these two molecular taxonomic units correspond to the two closely related species, their transcontinental disjunct distribution would not be surprising, in light of the well-established theory stating that bryophytes typically have broad distributions and low endemism, which is likely to be due to their ability of long-distance dispersal (Vanderpoorten & Goffinet 2009, Patiño & Vanderpoorten 2018, Fichant et al. 2023). Shaw (2001) discusses that cryptic species of bryophytes frequently have broadly overlapping geographic ranges across multiple continents, although often with distinct ecological attributes. Whether these intercontinental geographic patterns in liverwort distributions were mainly shaped by ancient vicariance or recent dispersal is still a matter of debate

(Vanderpoorten et al. 2010). However, many studies report narrower distributions for resolved species complexes than previously thought (e.g. Caparrós et al. 2016, Vigalondo et al. 2019), as is also the case for liverworts (Feldberg et al. 2006, Dong et al. 2012). Despite this, it is still seen as a general trait of bryophytes.

The results on selected barcoding loci, genetic divergences and tree topology are largely comparable with previous reports on European lineages of *Aneura* (Bączkiewicz et al. 2017). Taking into consideration different assumptions made for the substitution rates, the interspecific and intraspecific genetic variation of the Slovenian species (Table 2) is proportionally similar to other populations at a wider geographical scale. Close matches were also revealed in the general phylogeny (Fig. 2). The taxonomic status of the proposed species is still a work in progress as different species delimitation models give incongruent results (Anantaprayoon et al. 2023). It is likely that species boundaries, predicted by the GMYC model, which was the focus of the authors' work, are too broad, as clade 1 based on the former approach evidently consists of many lineages (Figs 1 and 2), which could represent putative species. Although more in-depth studies are needed to clarify this matter, the present findings provide preliminary evidence supporting this conclusion.

Many of the distinctly evolving units are reported here in clades A, B, C and E (Bączkiewicz et al. 2017). All three phyletic lineages of clade A are hereafter confirmed also for this part of Europe. Lineages in clade B remain partly unresolved, although there are at least two lineages present in Slovenia, namely B_2 and B_3. Phyletic lineage B_1 is a sister group to the clade containing *A. pellioides* and Thai 1. The taxonomic status of these entities needs further study. For clade C, the presence of C_1 is confirmed and for the clade E, the existence of both previously reported lineages E_1 and E_2 is determined, plus some unresolved ones. Whether these evolutionary units represent separate species remains unresolved.

A distinct phenotype is considered a key component of valid taxonomic status. Previous studies on the morphology of the genus *Aneura* did not study all of the lineages currently recognized, as they only compared three or two genetic clusters (Buczkowska et al. 2006, Anantaprayoon et al. 2023). The concept of the *A. pinguis* complex and its global distribution is yet to be fully resolved, posing challenges for accurate and reliable morphological delimitation. Such uncertainty may obscure the conclusions drawn from related studies. Moreover, the number of morphological and anatomical features available to choose from in these simple thalloid liverworts can be quite limited. The absence of clear morphological differentiation of all genetically detected lineages is a major obstacle to understanding this genus; nonetheless, the data presented (Fig. 3) revealed some degree of morphological segregation. Notwithstanding earlier assumptions, the proposed genetic entities might not be as cryptic as previously thought. This is especially so if one considers the diverse ecological attributes of these taxa. Buczkowska et al. (2006) report some pivotal points in the morphological differentiation of clades A, B and C, which were recognized at that time. The significant differences reported were most marked for the dorsal epidermal cells, the thickness and number of cells in the thallus cross-section, the size of inner central cells and the thallus width. Furthermore, the authors report differences between male and female plants. The results of this paper (see Supplementary Figs S3 and S4) are strikingly similar to the expected amount of biological variation. Most notable deviations are reported for taxon B. According to Buczkowska et al. (2006), the Polish populations are characterized by narrower thalli (on average 2.7 mm as opposed to 5.3±1.6 mm), smaller cells at the thallus margin (36.7 µm vs. 51.2±7.4 µm) and smaller dorsal epidermal cells (59.7 µm vs. 78.1±8.0 µm). The possible paraphyletic state of this lineage (Fig. 2) might be the reason for these discrepancies. Substantial differences were also deducted for taxon C, where the thallus thickness of Polish populations is about 322.1 µm and Slovenian populations 566.1±100.3 µm, which is consistent with a lower number of cells in the midrib (7.3 as opposed to 15.3±0.9).

Based on the findings presented, it is reasonable to propose that the two new lineages related to *A. sharpii* and Thai 2 be recognized as potential new taxa, therefore increasing the number of liverwort species in the European bryoflora, although additional candidate species may also be present. While little is known about the clade Thai 2 or other similar looking entities in Thailand (Anantaprayoon et al. 2023), *A. sharpii* is a formally described taxon, and thus there is more descriptive information for it. It was discovered in 1985 by H. Inoue and N. G. Miller from the eastern United States (Inoue & Miller 1985) and later validated using molecular methods (Söderström et al. 2023). Besides looking superficially very similar to other species with a thin, translucent unistratose margin, for instance, *A. maxima*, it is characterized by some key diagnostic morphological features, which are mentioned in the original species description by Inoue & Miller (1985) and also emphasized by Söderström et al. (2023). These include the often irregularly to more or less regularly lobed thallus margin and smaller number, i.e. (10–)14–25, of globose, subelliptical to fusiform and nearly homogenously shaped oil bodies. Current results confirm the reports on the morphology of the thallus margin and possibly that of the oil bodies for the two accessions from Slovenia (Fig. 8). Although probably not a unique trait for this species, it provides indications of some morphological differentiation in this genus. Results of the morphological analysis (Supplementary Figs S3 and S4.) are mostly in accord with the original species description of *A. sharpii*, except for thallus width and dorsal/ventral epidermal cell height, which are slightly less than the reported range. The two Slovenian specimens are generally small plants, with the thallus about 3.3±1.1 mm wide, rather than having the main axes 4–8 mm wide. They also develop attenuate, thread-like branches. The epidermal cells are shorter, averaging 25.7±0.7 µm on the dorsal side and 21.3±2.8 µm on the ventral side, instead of 30–40 µm (Inoue & Miller 1985). In one case, a greater number of cells in a row forming the unistratose margin (> 20 rather than the typical 8–15) was observed. After close examination of the oil bodies, it is concluded that their shape ranges from globose to fusiform spheres. However, they were more often composed of small, flattened, globular and elliptical granules, giving them a heterogeneous appearance. This observation is in partial accord with the description provided by Inoue & Miller (1985). Moreover, the habitat of the two newly discovered specimens assigned to the clade aff. *sharpii* does not fully align with the original species description, which states that the species grows in loose colonies over organic debris, among mosses and sometimes on damp rocks. This includes sites, such as wet cliffs, calcareous wetlands, wet humus, fens and damp hollows beneath trees and shrubs at the edge of rich fens. The two Slovenian specimens do not exhibit an affinity for base-rich sites (Fig. 6).

Whether the two new lineages in fact represent the species *A. sharpii* and clade Thai lineage 2, or separate but closely related entities of former groups is still a matter of discussion. Genetic distances in the outlined datasets indicate very high nucleotide similarity as the average uncorrelated p-distance between haplotypes of LJU-B 2400030, LJU-B

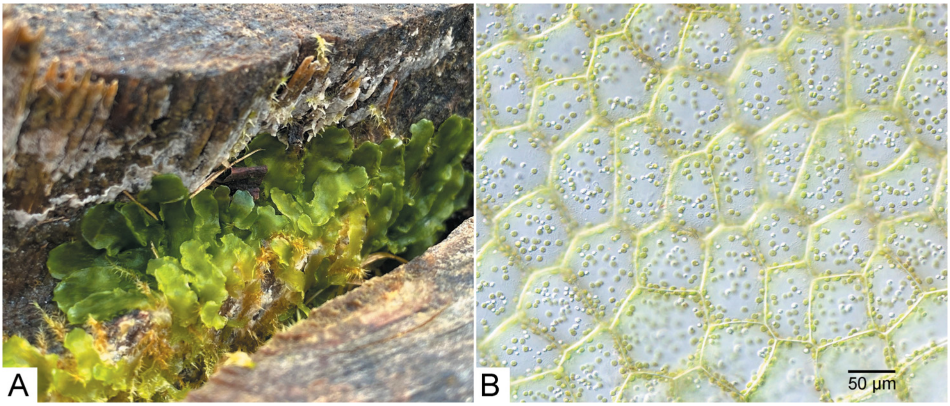

Fig. 8. (A) The candidate species closely related to *Aneura sharpii* growing on decaying wood with slightly lobed thallus margins, (B) upper epidermal cells of the same specimen with nearly homogenous oil bodies observed using DIC microscopy.

2400048 and *A. sharpii* is 1.004%, and 1.604% for LJU-B 2400002, LJU-B 2400049 and other Thai 2 accessions. However, the interspecific divergence was far greater than the intraspecific variation in the clade comprising *A. sharpii* s. lat. The dataset is quite limited, and further aspects should be considered in order to resolve this matter. Nevertheless, it seems likely that the aff. *sharpii* accessions represent a distinct candidate species, similar to the closely related *A. sharpii*, but set apart by their narrower thalli, formation of attenuate lobes, small epidermal cells and possibly by developing a wide unistratose margin.

Clades not detected in Slovenia during this survey (Fig. 2) include D, H, I, Thai 1, Thai 3, *A. maxima*, *A. pellioides* and the peculiar mycoheterotroph *A. mirabilis*. It is apparent that the number of samples studied, limited distribution ranges, specific ecological requirements, habitat availability and overall rarity account for the absence of these species in Slovenia. The presence of *A. maxima* in Europe has raised much controversy in the past (Hodgetts et al. 2020). The current study found no evidence for its occurrence in the area studied. This taxon occurs throughout the southern hemisphere, including areas in East Asia (Söderström et al. 2023). Using sequences from other authors (Preußing et al. 2010, Bączkiewicz et al. 2017, Anantaprayoon et al. 2023, Söderström et al. 2023) produced a heterogeneous clade together with *A. maxima*. Apart from China, Malaysia and French Polynesia, it also occurs in Japan, Thailand and Ecuador.

Along with genetic variation, substantial differences in ecological preferences were deducted (Figs 5 and 6), which provided additional evidence of the validity of the species studied. The data presented here shows some incongruencies with previous findings in terms of suitable habitats of the taxa studied. For instance, Bączkiewicz et al. (2016, 2017) report that taxon B grows mainly on clay soil or humus, while the current study recorded it submerged in peat bogs or swamps, on humic soil and decaying wood beside forest streams. The genetic heterogeneity of the clade comprising taxon B, Thai 1 and *A. pellioides* could account for the differences in habitat preferences at a wide geographical scale.

Conclusions

This study presents which taxa of the simple thalloid liverworts genus *Aneura* are present in Slovenia. The DNA barcodes employed were further verified, and both genetic divergence and the phylogenetic placement of the studied clades were discussed. In summary, seven of the already recorded taxa were detected for the first time in south-central Europe, plus two additional lineages which are thought to be new candidate species. The clades studied all share many common morphological features, although high-ranking morphological groups could be distinguished, based on thallus width and thickness, number of cells in the central part, length of dorsal epidermal cells, the translucent margin and the presence of small attenuate lateral lobes. Habitat heterogeneity of the associated taxa could be the main reason for cryptic speciation within this genus. The integration of ecological attributes and distinctive morphological traits appears to support the reliable identification of at least a subset of the taxa studied. Still, many questions remain unanswered, and some new ones emerge. Unforeseen detections of two new lineages in Europe are just one example of the current limited understanding of the biology of the genus *Aneura*. Thus, studying the diversity and ecology of liverworts can be important, not only for understanding their specific ecological requirements, but also for narrowing the knowledge gap between bryophytes and vascular plants. Integrative taxonomic studies are needed to better understand the status of proposed species, particularly the clade B, Thai 1 and *A. pellioides* complex. New tools coupled with traditional cladistic approaches and ecological characteristics could result in a renewed and stable understanding of the species concepts within the genus *Aneura*.

Supplementary materials

Fig. S1. Phylogenetic networks of the four genetic loci used in the present study (ITS, *mat*K, *trn*H-*psb*A, *trn*L-*trn*F).
Fig. S2. Phylogram of the examined *Aneura* accessions with annotated clades, providing information on branch lengths.
Fig. S3. Morphological analysis: graphical representations of results (quantitative data).
Fig. S4. Morphological analysis: graphical representations of results (qualitative data).
Fig. S5. Boxplot of distances to group centroids.
Table S1. Plant material, including collection details and BOLD accession codes for specimens gathered in this study.
Table S2. Primer sequences used in the present study.
Table S3. Collection details and BOLD/GenBank accession codes of the compiled sequences list used in the phylogenetic analyses.
Table S4. Partitions and substitution models used in maximum likelihood partition analysis.
Table S5. Morphological analysis; list of the morphological and anatomical characters examined.

Supplementary materials are available at https://www.preslia.cz.

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Rod *Aneura* (*Marchantiophyta*) ve Slovinsku a nové potenciální linie pro Evropu

Rod játrovek *Aneura* (*Aneuraceae*) byl předmětem mnoha studií zaměřených na odhalení kryptické diverzity uvnitř komplexu *A. pinguis*. V posledních letech se výrazně rozšířily poznatky o jeho genetické heterogenitě a geografickém rozšíření předpokládaných druhů. Genetické markery lze využít k identifikaci odlišných taxonů, což přispívá k lepšímu pochopení procesů diverzifikace v tomto rodu. Tato studie se zabývala taxony rodu *Aneura* vyskytujícími se ve Slovinsku a přispívá k poznání diverzity játrovek v jižní části střední Evropy. Hlavním cílem této práce bylo využít molekulární metody k identifikaci kryptických taxonů a sestavit aktuální referenční seznam slovinských mechorostů s poznámkami o jejich ekologii. Při použití standardizované metodiky popsané v několika souvisejících studiích byly analyzovány tři úseky plastidové DNA (*mat*K, *trn*H-*psb*A, *trn*L-*trn*F) a sekvence jaderné ribozomální oblasti ITS u 55 vzorků *Aneura* z různých geografických částí země. Fylogenetická pozice zkoumaných exemplářů byla hodnocena pomocí metod založených na vzdálenosti a maximální věrohodnosti. Rod *Aneura* je geneticky rozdělen přibližně do 20 kladů odpovídajících navrhovaným kryptickým druhům, z nichž devět bylo potvrzeno pro Slovinsko. Jednalo se o klady A, B, C, E, G, M (dříve v Evropě považovaný za *A. maxima*) a *A. pinguis* s. str. Kromě toho byly objeveny a popsány dvě nové linie pro Evropu. Některé sekvence se seskupily v blízkosti severoamerického taxonu *A. sharpii* a jihovýchodoasijského kladu, který byl poprvé zaznamenán v Thajsku, ale dosud nemá formální jméno. Několik dříve zaznamenaných evropských fyletických linií patří do kladů A, B, C a E. Monofyletický status taxonu B je nejasný, protože sekvence z *A. pellioides* a některé thajské vzorky byly zařazeny do kladu B. Dále bylo studováno 20 morfologických znaků. Výsledky morfologické analýzy naznačují, že se druhy částečně liší šířkou a tloušťkou stélky, počtem buněk v její střední části, délkou dorzálních buněk epidermis, přítomností průsvitného okraje a některé taxony i dalšími znaky. Byly však zaznamenány i četné překryvy ve variačním rozpětí znaků mezi zkoumanými klady. Pro jednotlivé taxony jsou uvedeny charakteristiky obývaných stanovišť. Předkládaná práce shrnuje taxonomickou diverzitu a rozšíření rodu *Aneura* ve Slovinsku a poskytuje doporučení pro další výzkum.

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