



Scopus Indexed Journal

Waldökologie, Landschaftsforschung und Naturschutz – Forest Ecology, Landscape Research and Nature Conservation

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Morphological and genetic heterogeneity in relict populations of *Pinus rotundata* in Northern Bavaria

Morphologische und genetische Heterogenität in Reliktpopulationen der Moorspirke (*Pinus rotundata*) in Nordbayern

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Abstract

The bog pine, *Pinus rotundata* Link is a putative hybrid species derived from *Pinus uncinata* mainly distributed in the western Alps and Pyrenees and *Pinus mugo*, an eastern European species. It has a scattered and relictual distribution in Bavaria, found in bogs in the pre-alpine region and eastern Bavarian mountains, among them in north-eastern Bavaria the Fichtel Mountains (German: Fichtelgebirge) and the Upper Palatinate Forest (German: Oberpfälzer Wald). In this study, we investigated the genetics and cone morphology of 11 northern Bavarian populations using a chloroplast barcode marker (*trnL-F*) and measuring taxonomic relevant cone characters such as the size of cones and apophyse depth and width. We identified two different genetic variants, mostly occurring sympatrically, differing by one single nucleotide polymorphism (SNP) (G, T) in *trnL-F*. The genetic variants partly overlap in cone morphology. However, the G-type exhibited stronger hook-shaped apophyses, resembling *Pinus uncinata*, while the T-type was closer to *P. mugo*. Distribution models revealed a relationship between genotype frequencies and space, with closer populations being more similar in frequency distributions of genotypes than distant populations. Moreover, the G-type (*P. uncinata* like plants) was more frequent in areas with harsher climates (higher elevation, shorter vegetation time) whereas the *P. mugo* like plants dominated at low elevation. Considering the old age and isolation of the relict populations, the polymorphic population structure consisting of at least two distinguishable subtypes is remarkable. Reproductive barriers not detected so far may be still effective within the populations. The rare T-type with *P. mugo* like cone morphology could be interpreted as a novelty pointing to some degree to hybrid speciation by transgression.

Keywords: *Pinus mugo* agg., haplotype heterogeneity, genetic plasticity, polymorphism, ecological niche, speciation

Zusammenfassung

Die Moorspirke, *Pinus rotundata* Link, ist eine mutmaßliche Hybridart aus der Haken-Kiefer *Pinus uncinata*, die hauptsächlich in den Westalpen und Pyrenäen verbreitet ist, und der Latsche *Pinus mugo*, einer osteuropäischen Art. In Bayern kommt sie zerstreut und als Relikt in Mooren des Voralpenlandes und der ostbayerischen Gebirge vor, darunter im Fichtelgebirge und im Oberpfälzer Wald. Mittels eines Chloroplasten-Barcodemarkers (*trnL-F*) und anhand der Zapfenmorphologie wurde in dieser Studie die genetische und morphologische Variabilität der Moorspirke in 11 nordbayerischen Populationen untersucht. Taxonomisch relevante Zapfenmerkmale wie die Größe der Zapfen sowie Tiefe und Breite der Apophyse wurden vermessen und mit genetischen Unterschieden korreliert. Wir identifizierten zwei verschiedene genetische Varianten, die meist sympatrisch auftreten und sich durch einen einzigen Nukleotid-Polymorphismus (SNP) (G, T) im Marker *trnL-F* unterscheiden. Die genetischen Varianten überschneiden sich teilweise in der Zapfenmorphologie. Der G-Typ wies jedoch stärker hakenförmige Zapfenschuppen auf, die an *Pinus uncinata* erinnerten, während der T-Typ näher an *P. mugo*-Zapfen lag. Verbreitungsmodelle ergaben, dass näher aneinander gelegene Populationen sich in der Häufigkeitsverteilung der Genotypen ähnlicher waren als weit voneinander entfernte Populationen. Außerdem war der G-Typ (*P. uncinata*-ähnliche Pflanzen) in Gebieten mit rauherem Klima (höhere Lage, kürzere Vegetationszeit) häufiger, während die *P. mugo*-ähnlichen Pflanzen in niederen Lagen dominierten. In Anbetracht der langen geographischen Isolierung der Reliktpopulationen ist die unterschiedliche Populationsstruktur, die aus mindestens zwei unterscheidbaren Subtypen besteht, bemerkenswert. Möglicherweise sind innerhalb der Populationen noch Fortpflanzungsbarrieren wirksam, die bisher nicht entdeckt wurden. Der seltene T-Typ mit einer *P. mugo* ähnlichen

Zapfenmorphologie könnte als neuer Subtyp interpretiert werden, der auf eine hybridogene Artbildung durch Transgression hinweist.

Schlüsselwörter: *Pinus mugo* agg., genetische Plastizität, Polymorphismus, ökologische Nische, Artbildung, Haplotyp-Heterogenität

1 Introduction

Ecology

Peat bogs with *Pinus rotundata* Link (*Pinus mugo* subsp. *rotundata* (Link) A.E.Murray) are among the most threatened ecosystems in Europe, e.g. due to the lowering of the groundwater table by wells in the drainage area, the lasting effects of former direct drainage systems in peatlands and climate change (GRZYBOWSKI & GLIŃSKA-LEWCZUK 2020, UNAR et al. 2012). *P. rotundata* forms the climax community of the strongly threatened bog vegetation in Central European mountains with a subcontinental climate, i.e. in the Northern Alps, the Black forest, the Fichtel Mountains, Ore Mountains, the Bavarian Forest, as well as in Lower Austria or Eastern European bogs and mountains. In eastern Europe as in Poland there is a closely related or even identical taxon, called *P. uliginosa*, which shows the same ecology as *P. rotundata* (NEUHÄUSL et al. 1969). As a tree species, the taxon has a key role in bog ecology, i.e. the trees improve the conditions for *Sphagnum* mosses by producing shade and protecting from direct radiation. Due to their ecological importance, mountain pine bog forests consisting of *Pinus rotundata* are listed as a priority habitat type (*) in Annex I of the Habitats Directive and protected as a subtype (code *91D3) of the bog forest habitat type (*91D0) under the Natura 2000 network in the EU.

Evolutionary and taxonomical aspects

The evolutionary origin of the bog pine plays a key role in understanding its ecological function. It shows intermediate characters between *P. mugo*, which is found in the Alps, Apennines, Balkans, and Greece, and *P. uncinata*, which is found in the Pyrenees, Massif Central, and western Alps (CHRISTENSEN 1987a; b; PRUS-GŁOWACKI et al. 1998; DZIALUK et al. 2012). Some authors (CHRISTENSEN 1987a; b) consider it to be a hybrid between *P. mugo* and *P. uncinata*, others treat it as synonym of *P. uncinata* (BUSINSKÝ & KIRSCHNER 2010). However, HAMERNÍK & MUSIL (2007) point out that their hybrid origin has not yet been proven. Moreover, it is still unproven whether the similar eastern European *P. uliginosa* is identical with *P. rotundata* (BORATYŃSKA & BORATYŃSKI 2007) or not (i.e. ŁABISZAK & WACHOWIAK 2021); for *P. uliginosa* many genetic studies were published so far (i.e. ŁABISZAK et al. 2021; LEWANDOWSKI, BORATYŃSKI, MEJNARTOWICZ 2000), but not for *P. rotundata*, and it is unclear whether the results also apply to the latter taxon. Nevertheless, *P. rotundata* shows intermediate characteristics of the parents, i.e. the cones are intermediate in size and hook size (CHRISTENSEN 1987a; b). Mostly upright growing, it can also occur as a shrub like *P. mugo* (SCHMIDT 2011). Despite numerous publications on the morphological and genetic delimitation of the *P. mugo* aggregate, including *P. uncinata* Ramond ex DC., *P. mugo* Turra, *P. uliginosa* G.E. Neumann, and *P. rotundata* (CHRISTENSEN 1987; PRUS-GŁOWACKI et al. 1998; WACHOWIAK & PRUS-GŁOWACKI 2009; DZIALUK et al. 2013; v. LEININGEN 1906), as with many putative hybridogenic species, the taxonomy of

this species remains difficult, and its taxonomic position has changed considerably over the years. Currently, *P. rotundata* Link is regarded by many authors, including CHRISTENSEN (1987a) and HAMERNÍK & MUSIL (2007), as a synonym of *P. uncinata* subsp. *uliginosa* (G.E. Neumann ex Wimm.) Businský. However, BUSINSKÝ & KIRSCHNER (2007) argue that the species is not identical to *P. uliginosa* and point out that the type specimen of *P. rotundata* originates from the Plansee in Austria and is close to *P. uncinata*. Nevertheless, recent publications use the name *P. rotundata* for Bavarian bog pines (ŁABISZAK et al. 2021b). *P. rotundata* has become the standard name for the taxon in Germany, thus pointing out that it is not identical with *P. uncinata* (JÄGER 2011). The name *P. rotundata* is used also in this publication.

A preliminary study at the cone morphology of *P. rotundata* in the Fichtel mountains revealed a strong variability of sizes, shapes and hook intensities of cones, leading to some doubts about the identity of the trees. Similarly, GOLDE (2000) detected a polymorphic cone morphology in *P. rotundata* in the Ore Mountains, and MARCYSIAK & BORATYŃSKI (2007) found variation in *P. uliginosa*. These cone characteristics are considered to be important for understanding the complex of *P. mugo* agg. (MARCYSIAK & BORATYŃSKI 2007; MAIER 1993). MARCYSIAK & BORATYŃSKI (2007) note that the cones of *P. uliginosa* in the Sudetes and Tatra are variable, and that the length/depth ratio clearly differentiates *P. uliginosa* from *P. uncinata*, which has the lowest value of this feature among the taxa compared, at about 1.5. They also mention that CHRISTENSEN (1987b) found a value of 0.66 for *P. mugo*.

As a presumed hybrid taxon, the question arises as to where the contact zone between the parents *P. mugo* and

P. uncinata was located and during what period and how often mixing took place. Charcoal fossils (WILLIS & VAN ANDEL 2004) have shown that the *P. mugo* complex occurred in the Czech Republic during the Ice Age. The exact age and geographic origin of *P. rotundata* is yet unknown and there may be old hybrids and younger ones (as in the Southern Alps where both parents still grow together). The northern Bavarian moors may have served as relict refugia for *P. mugo* agg. during the Ice Age and may represent continuous populations since that time. It cannot be excluded that these refuges were home to more than one taxon, as the populations are known for their variability (MARCYSIAK & BORATYŃSKI 2007). Species of *P. mugo* agg. could have mixed in sympatry and formed hybrids (compare Heuertz et al., 2010; ŁABISZAK & WACHOWIAK 2021a).

Bogs with *P. rotundata* occur scattered in northern Bavaria under different climatic conditions. There are currently two pine bog areas: one in the High Fichtel Mountains, a mountainous region at an altitude of approx. 700 metres, and one in the Upper Palatinate Highlands, a colline region at an elevation of approx. 400 metres a.s.l. (RÖSLER 1994; GEIGER 1994; LUTZ 1956; Fig. 1). Both clusters differ significantly in terms of climatic parameters such as precipitation and length of the growing season, which could correlate with the traits and morphological differences of the bog pines in these different clusters. Additionally, due to genetic drift and isolation by distance also geographical proximity may play a role.

As *P. rotundata* is believed to be a hybrid between *P. uncinata* with large cones and conspicuous hooks on the apophyses, and *P. mugo* with small cones without hooks (CHRISTENSEN 1987 a,b), cone size and apophyse features are of particular interest. In a hybrid species, these characters may

be intermediate or segregate. In this study, we determined the cone morphology of the individuals of Northern Bavarian populations, including the size of cones, shape of the apophyse hooks, and their depth (referred to as apophyse depth according to MARCYSIAK & BORATYŃSKI 2007).

Barcoding markers, such as the trnC/f spacer, have been successfully and regularly used for taxonomic studies in many plant families (KRESS et al. 2005). We investigated the northern Bavarian populations of *P. rotundata* by using the trnC/f spacer, a chloroplast marker that is parentally inherited and has been used for barcoding pine species (CELIŃSKI et al. 2007). To compare the morphological and genetic characters, samples from other regions (such as the Pyrenees and Austria) and published barcoding markers were used.

Here we ask the following questions:

- (1) Is there genetic and morphological variation within the populations of *P. rotundata* in northern Bavaria and is there a relationship between morphological and genetic variation?
- (2) Are there differences between the two geographically separated subclusters analysed here and are they influenced by genetic drift (geographical proximity) and environmental factors?

2 Material and Methods

2.1 Sampled populations

Populations were sampled in the subareas High Fichtel Mountains (HF) and Upper Palatinate Highlands (OH). One

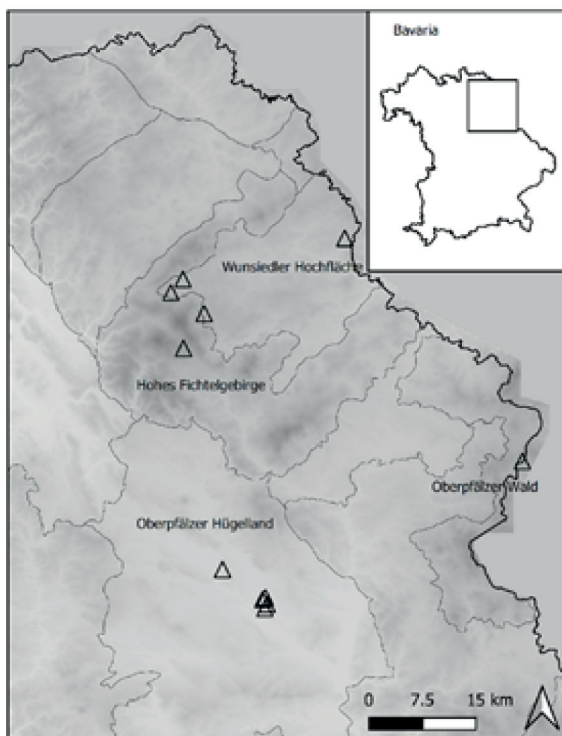


Fig. 1: Overview of the populations sampled for morphological and genetic investigations.

Abb. 1: Übersichtskarte der Populationen, die für morphologische und genetische Untersuchungen beprobt wurden.

population „Häusellohe“ belongs to the Selb-Wunsiedel Highland and another to the Upper Palatinate Forest. The altitude of these areas varies between 410 and 750 m a.s.l.

We used sequences published in GenBank by CELIŃSKI et al. (2017) as well as self-collected samples from the bogs Kreuzbauer Moor and Große Heide in Austria and samples from the Pyrenees belonging to *P. uncinata* for comparison.

The coordinates and elevation of the sampled populations is given in Table 1.

2.2 Cone morphology

To conduct morphological analyses, we collected and analyzed one to three cones per tree. In 15 % of the samples, only one cone per tree was collected, as there were only a few fruits on the tree. The following cone characters were measured: height, width, and maximal diameter. For the apophyse characters, we measured the height, length, and depth of the apophyses (Fig. 2), as well as the proportion of the median depth to the median width. We standardized the measurements of the apophyse characters by using the apophyse of the third or fourth basal row of the seed scale. In cases where the cones were asymmetric, we chose the outer side of the cone distal to the tree stem, where the apophyse was of maximal depth, for measurement.

2.3 Genetic analyses

DNA of fresh or frozen (-20°C) needles of *Pinus* sp. was isolated and the trnL-trnF intron (TABERLET et al. 1991) was amplified using primers c and f and sequenced following the standard procedures described in LIEDE-SCHUMANN et al. (2014). The sequences were compared with sequences from other studies (i.e. CELIŃSKI et al. 2017) and GenBank accessions. After successful amplification and processing, samples were sent to the GATC-Biotech laboratory (Kons-
tanz, Germany) for LIGHTRUNTM-Sanger sequencing to

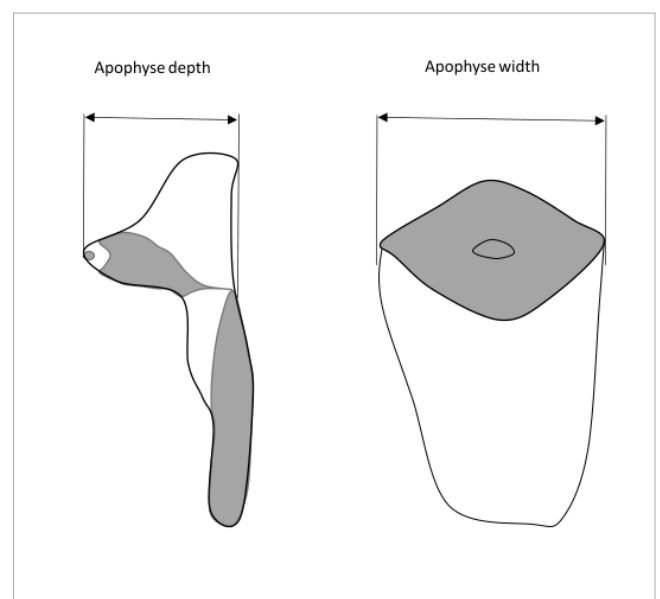


Fig. 2: Schematic overview of morphological characters measured of apophyse depth and width.

Abb. 2: Schematische Zeichnung der gemessenen Tiefe und Breite der Apophyse als morphologische Merkmale.

Tab. 1: Number of samples and their coordinates, names of the populations and sub-areas to which they belong, and the elevation of the populations.

Tab. 1: Anzahl und Koordinaten der beprobten Populationen sowie Fundgebiet und Höhe des Vorkommens.

Population	samples	x	y	subarea	Elevation (mean)	Data sampled (m=morphology, g=genetic, lm=model)
Voitsumra	5	50,09027	11,83977	High Fichtel Mountains	690	m,g, lm
Fichtelsee	5	50,01964	11,86003	High Fichtel Mountains	690	m,g, lm
Rupertsgrün	7	50,10663	11,86414	High Fichtel Mountains	690	m,g, lm
Meierhof	5	50,06252	11,90305	High Fichtel Mountains	690	m,g, lm
Häusellohe	5	50,14985	12,18393	High Fichtel Mountains	690	m,g, lm
Gscheibte Loh	8	49,68864	11,99852	High Fichtel Mountains	690	m,g, lm
Hirsch nord	5	49,70245	12,000407	Upper Palatinate Highlands	411	m,g, lm
Stürzer Loh	3	49,69382	12,003119	Upper Palatinate Highlands	410	m,g, lm
Hirschberg	3	49,70014	11,995074	Upper Palatinate Highlands	410	m,g, lm
Moos	5	49,73992	11,919507	Upper Palatinate Highlands	415	m,g, lm
Griesbach	7	49,85970	12,510775	Upper Palatinate Forest	710	m,g, lm
DNA- reference samples						
Kreuzbauer Moor	2	48,01037	13,43151	Austria		g
Große Heide (Austria)	4	48,56791	14,75907	Austria		g
Pyrennees Andorra	3	-	-	Andorra		g
Pyrennees France	8	42,84252	0,20660	France		g

digitize base sequences using the same primer combination. Sequence analysis was performed using the Geneious program (R10.2.3, Biomatters, Auckland, New Zealand). Corresponding alignments and consensus sequences were made from the respective primer combinations, which were subsequently used to identify the samples.

2.4 Climate niche analyses

We conducted a principal component analysis (PCA) to investigate the distribution of genetic types among the climatic parameters. However, due to the high multicollinearity of these parameters, we used a generalized linear model (GLM) with a reduced set of parameters, which included precipitation, vegetation length, frost days, elevation, and longitude.

To determine the best model, we tested all combinations of the above-mentioned parameters and used AIC for

model selection. To assess the model's goodness-of-fit, we calculated Tjur's R^2 , which is commonly used for logistic regressions as an adjustment measure. Tjur's R^2 calculates the mean predicted value for each category of the dependent variable, representing the mean predicted value for all samples where the response variable is 0 and the mean predicted value for all samples where the response variable is 1. It then takes the absolute difference between these means. Tjur's R^2 ranges from 0 to 1, with a value of 1 indicating perfect separation between the predicted values of the response variable.

To analyze significant morphological differences between the two genetic groups, we conducted independent-sample t-tests on measured cone characteristics. In addition, we investigated the ecological niches of the genotypes by incorporating climate parameters for each population provided by the German Weather Service (DWD). The dataset included the 30-year mean for the years 1990–2021 of annual

precipitation (mm), vegetation length, number of frost days, elevation, latitude, and longitude. To determine vegetation length, we calculated the number of days between the beginning and end of the vegetation period, which is indicated by the start of *Forsythia* flowering and the leaf fall of pedunculate oak, respectively (DWD 2018). Inferring relationships between genotype (G or T) and climate, we fitted a binomial GLM on the binary response variable (genotype G), with significant parameters precipitation, vegetation length and longitude. This model was selected due to the lowest AIC value by stepwise model selection with different combinations of parameters (dredge-function in R, package “MuMIn”, BURNHAM & ANDERSEN 2002). We tested for overdispersion with the package “DHARMa” (HARTIG 2022) due to slight increase in residual deviance (47.602 on 38 degrees of freedom) with a non-significant result (dispersion = 1.054, p-value = 0.712).

3 Results

In the northern Bavarian populations, we found two different varieties of copies of the trnC/f region, being different by one base. According to the reference sequences (CELIŃSKI et al. 2017) the single nucleotide polymorphism is at forward (5'–3') base position 100. The percentage distribution of the genetic varieties, called genotype GT G and GT T is shown in table 2.

Figure 3 shows the phylogenetic tree including reference sequences of the Pyrenees, Lower Austria and GenBank reference sequences. The *P. mugo* aggregate is sister to *P. sylvestris* samples and forms 3 groups. A *P. uncinata* individual from the Pyrenees with shrublike growth is standing alone at the base of the *P. mugo* aggregate. The rest of the

samples including *P. uncinata* from the Pyrenees is separated into two groups.

Cone morphology

The two-sample t-test showed that the two genotypes G and T differed in the mean depth of the cone apophysis, which means that the G variety tended to have deeper and longer „hooks“ than the T genotype (df = 41.727, p-value = 0.074). The difference in ratio between apophyse depth and width was not significant (df = 41.908, p-value = 0.1197), but the values for the *P. uncinata* like cones of genotype G with were higher, whereas the cones of genotype T resemble more the cones of *P. mugo* showing no or almost no hooks (Fig. 7). A difference between the height of the cones between the genotypes was not revealed (df = 32.383, p-value = 0.5925), see discussion.

Figure 4 shows a comparison between the frequency distribution of the ratio depth/width of the apophyse between the genetic groups. The frequency overlaps, but differences are significant at the 0,5-significance level (Kolmogorov Smirnov D = 0,29, p = 0,3), meaning that among the cones of genotype G with longer hooks are more prevalent.

Looking at all data, independently of the genotype and taxon, we found a highly significant linearity between cone size and apophyse depth, respectively apophyse length ($R = 0,51$, $p < 0,0004$). The higher the cone, the more conspicuous are the apophyses (Fig. 5).

Looking only at genotype G, the linearity between median cone height and apophyse depth is also significant ($R = 0,5$, $p < 0,05$, Fig. 6, left picture). If only the genetic variety T

Tab. 2: Genetic varieties of Trnf/c region and their frequency in the investigated populations.

Tab. 2: Genetische Varianten der Trnf/c-Region und deren Häufigkeitsverteilung in den untersuchten Populationen.

	N	elevation	GT G [%]	GT T [%]
Hohe Heide (Austria)	4	940	50	50
Voitsumra	5	640	60	40
Fichtelsee	5	750	100	
Ruppertsgrün	6	700	50	50
Meierhof	8	670	75	25
Häusellohe	5	575	75	25
Gscheibte Loh	6	410	33	67
Hirschberg nord	4	410	50	50
Stürzer Loh	3	410	67	31
Hirschberg	2	410	100	
Moos	5	415	20	80
Grießbach	8	710	80	20
Ammergebirge	3	1750	100	
Kreuzbachmoor (Austria)	1	522		100
Pyrenees (<i>P. uncinata</i> , Spain)	3	1500	67	33
Reference mugo/uncinate/rotundata GenBank accessions MF193391 -MF193397 from CELINSKY 2017 a,b, botanical Garden	6		100	

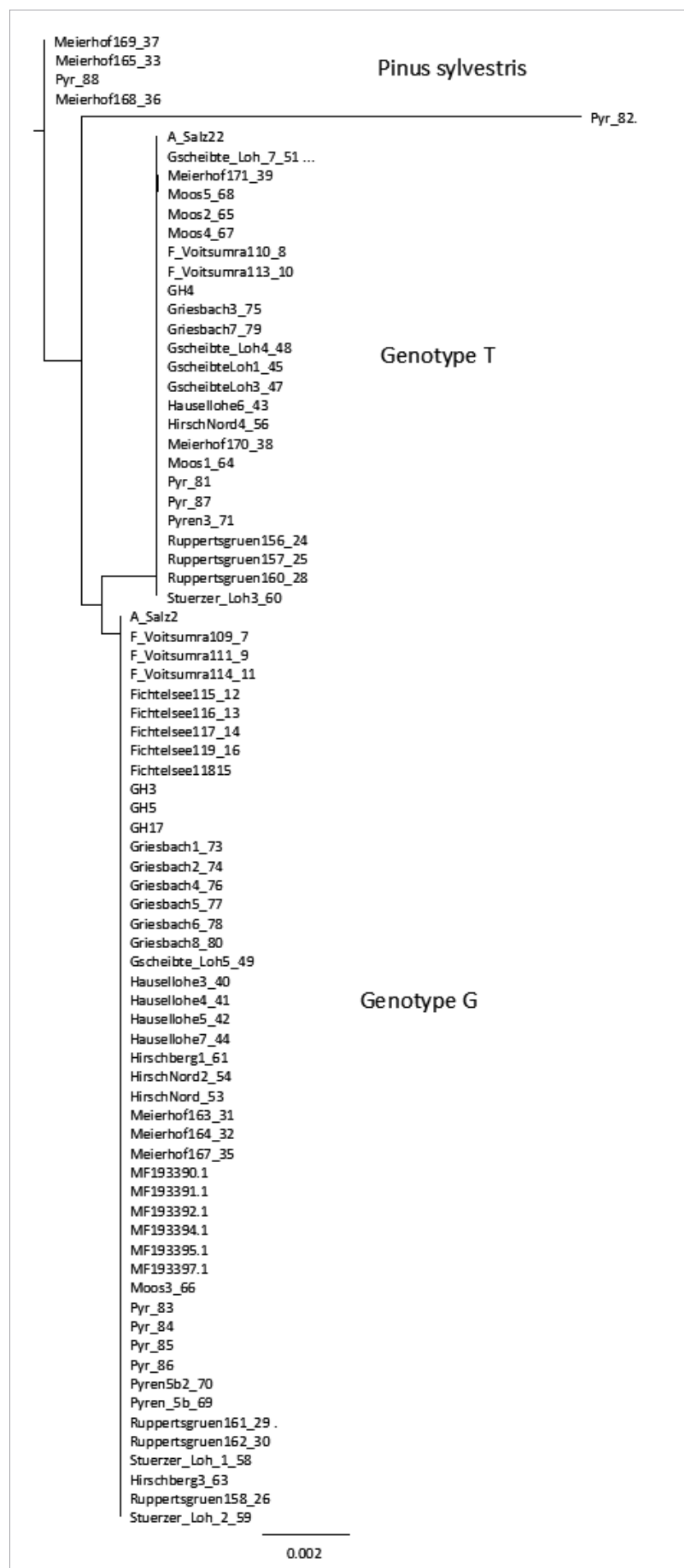


Fig. 3: Neighbour-joining tree based on Nei's genetic distance of *P. sylvestris* samples and references from *GenBank* (CELIŃSKI et al. 2017).

Abb. 3: Phylogenetisches Baumdiagramm basierend auf der genetischen Distanz der *Pinus*- Proben und Referenzen aus der *GenBank* (CELIŃSKI et al. 2017).

($n = 17$) is considered, the linear relationship is much less clear, as only the two highest values (Gscheibte Loh 7, Voitsumra 113) are responsible for the linearity. When eliminating the outlier Gscheibte Loh 7 in apophyse depth, the linearity was not significant ($R = 0,4$, $p = 0,1$).

Frequency of genetic types in the sub-areas of the study area

The High Fichtel Mountains (HF) and Upper Palatinate Highlands (OH) differed in the percentage of genetic types within populations. The mean percentage of the G-type was with 71 % in the subarea HF much higher than in OH (about 50 %, Tab. 2).

The individual genotype based PCA is displayed in Figure 8, with the first axis explaining 69.9 % of variance and the second 29.9 % (together 90,5 %). The genotypes overlap mostly in their climatic niche. However, slight shift of the G type to the lower left suggests a weak difference in the climatic preferences or tolerances of the genotypes.

The frequency distribution of the genotype G was significantly explained by longitude, vegetation length and mean precipitation (tab. 4). Overall, the model only achieved a weak to moderate fit with a Tjur's $R^2 = 0.178$. The genotype G decreased with longitude (estimate -21.6), showing a strong influence of the nearby location of the populations with higher G frequencies, located mainly in HF. The genotype G decreases also to the southern direction of the study area in OH. However, vegetation duration and precipitation are also negatively correlated with the frequency of genotype G, which means that it occurs more frequently with shorter vegetation duration and lower precipitation, while mean annual precipitation (mm) explains the occurrence of genotype G only to a lesser extent (estimated value -0.054). The parameters precipitation and vegetation length

Tab. 3: Apophyse and cone character length of 44 investigated samples for the different genotypes G and T.**Tab. 3:** Mittlere Apophysentiefe und Zapfenhöhe von 44 untersuchten Proben für die verschiedenen Genotypen G und T.

character	n	Genetic Group G	Genetic group T
Median depth of apophyse	44	3,61±0.797 mm	3,24±0.539 mm
Ratio median apophyse depth/median apophyse width	44	0,459±0.115	0,414 ±0.0748
Height of cone	44	34.4±4.68 mm	33.5±5.01 mm

. significant with p-value <0.1

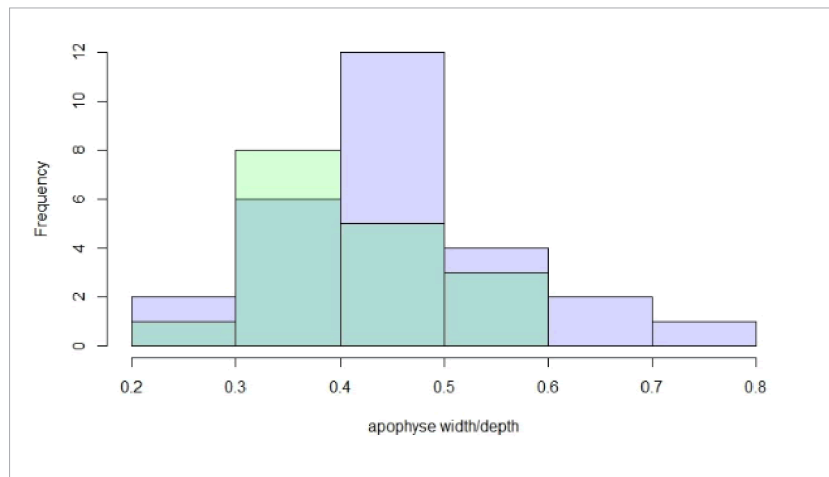
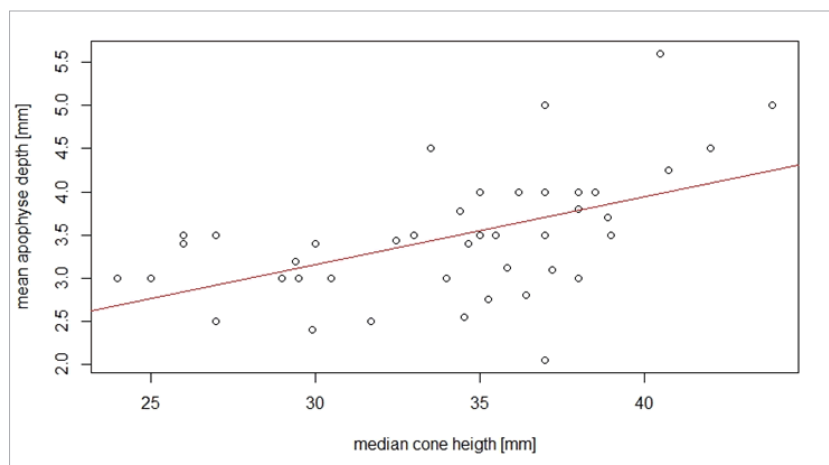
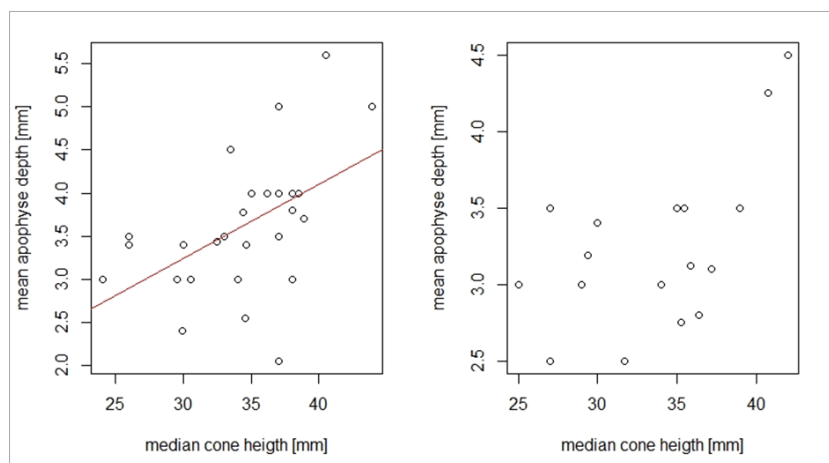
**Fig. 4:** Frequency distribution of the ratio depth/width of the apophyse of one cone depending on the genetic group (green = group with base T, blue = group with base G).**Abb. 4:** Häufigkeitsverteilung des Verhältnisses Tiefe/Breite der Apophyse eines Zapfens in Abhängigkeit der genetischen Gruppe (grün = Gruppe mit Base T, blau = Gruppe mit Base G).**Fig. 5:** Relationship between cone size and apophyse depth.**Abb. 5:** Verhältnis zwischen Zapfenhöhe und Apophysentiefe.**Fig. 6:** Median cone height (mm) (x-axis) and mean apophyse depth (mm) in genetic type G (left) with significant linear relationship ($R = 0,5$, $p < 0,05$) and without a significant relationship in genetic type T (right).**Abb. 6:** Mittlere Zapfenhöhe (mm) (x-Achse) und mittlere Apophysentiefe (mm) beim genetischen Typ G (links) mit signifikanter linearer Beziehung ($R = 0,5$, $p < 0,05$) und ohne signifikante Beziehung beim genetischen Typ T (rechts).



Fig. 7: Cone types, left typical for the T-type, right typical for the G-type.

Abb. 7: Typische Zapfenformen für den T-Typ (links) und den G-Typ (rechts).

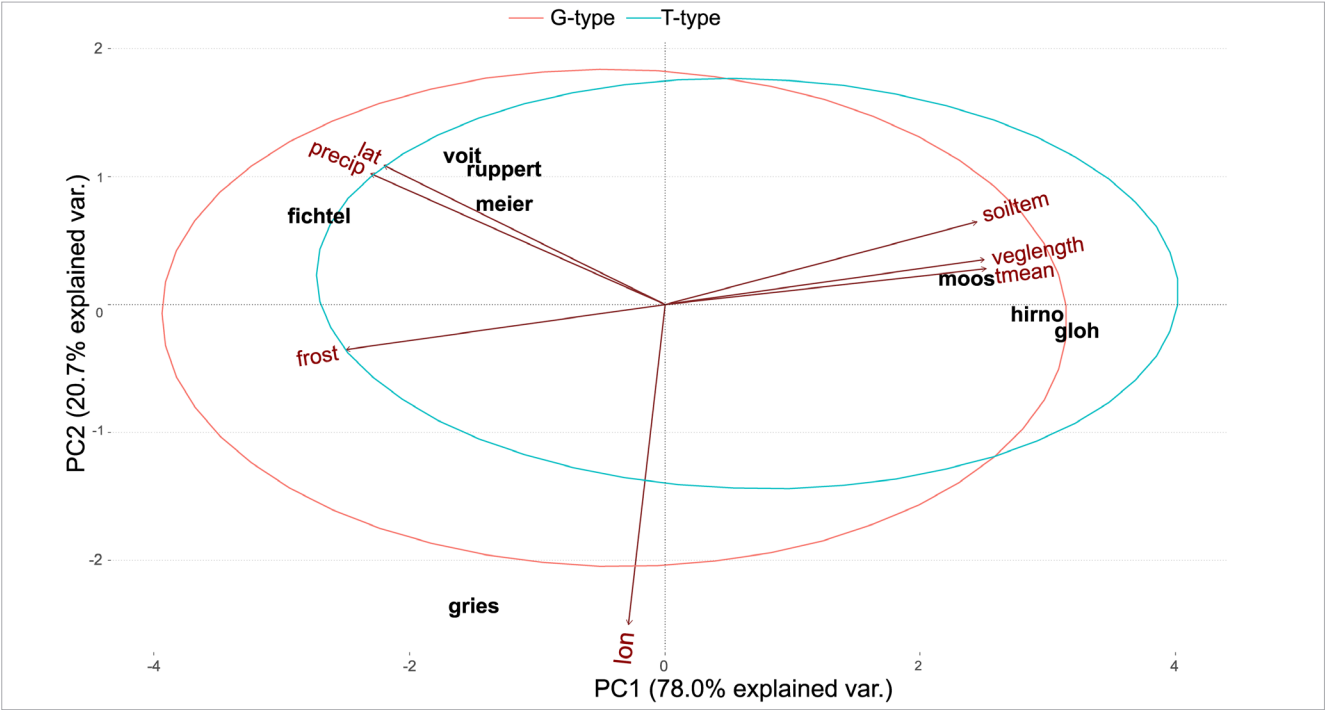


Fig. 8: Principal component analysis (PCA) based on individual genotypes (SNP's) G (red) and T (blue). The first axis comprises temperature-related parameters such as the number of frost days, the length of vegetation, the average annual temperature and the soil temperature based on data from the German Weather Service (DWD). Longitude contributes to the second axis. Precipitation and latitude contribute to both axes. Ellipses summarize the dispersion of the point cloud of each genotype. Abbreviations of the population localities according to table 2.

Abb. 8: Hauptkomponentenanalyse (PCA) auf Basis der einzelnen Genotypen (SNP's) G (rot) und T (blau). Die erste Achse umfasst temperaturbezogene Parameter wie die Anzahl der Frosttage, die Vegetationsdauer, die durchschnittliche Jahrestemperatur und die Bodentemperatur auf der Grundlage von Daten des Deutschen Wetterdienstes (DWD). Die zweite Achse wird hauptsächlich durch den Längengrad gebildet. Niederschlag und Breitengrad tragen zu beiden Achsen bei. Die farbigen Ellipsen fassen die Streuung der Punktwolken der beiden Genotypen zusammen. Abkürzungen der Populationsstandorte gemäß Tabelle 2.

Tab. 4: Best generalized linear model with climatic parameters accounting for the prevalence of the G genotype based on lowest AIC value (55.602) and predefined set of variables (see methods section). All p-values significant at a 0.05-significance level. Tjur's $R^2 = 0.178$.

Tab. 4: Bestes allgemeines lineares Modell mit Klimaparametern, die die Prävalenz des Genotyps G berücksichtigen, basierend auf dem niedrigsten AIC-Wert (55,602) und ausgewählten Variablen (siehe Abschnitt Methoden). Alle p-Werte sind signifikant auf dem 5 Prozent Signifikanzniveau. Tjur's $R^2 = 0,178$.

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	536.5377	253.96498	2.113	0.0346*
precipitation	-0.05403	0.02547	-2.121	0.0339*
vegetation length	-1.09873	0.53955	-2.036	0.0417*
longitude	-21.64266	9.96778	-2.171	0.0299*

are negatively correlated (Pearson correlation coefficient = -0.84, p-value = 3.679e-12). Higher precipitation and shorter vegetation periods are particularly evident in the mountainous regions, i.e. the Fichtelgebirge, while lower precipitation and longer vegetation periods apply to the lower altitudes in the Upper Palatinate. The fact that these parameters both in turn negatively influence the occurrence of the genotype G indicates a shift of the ecological adaptation of the species, i.e. a narrowing of the climatic niche. Genotype G is therefore not only genetically but also ecologically slightly different.

4 Discussion

In the Northern Bavarian populations of *P. rotundata*, we found two distinct genetic varieties for the TrnL-F intron: the T-type and the G-type. Comparison with GenBank sequences published by CELIŃSKI et al. (2017) revealed that the T-type has not been represented in previous GenBank accessions, which included sequences from *P. rotundata*, *P. mugo*, *P. uliginosa* and *P. uncinata*. We found the T-type in two bogs in Austria (Hohe Heide and Niederbauernmoor) as well as in *P. uncinata* from the Pyrenees. This suggests that the current number of sequences in GenBank is too small to accurately represent the genetic variability of this chloroplast marker. I.e., a sample of *P. uncinata* from the Pyrenees is placed as a basal sister group to the *P. mugo* clades (Fig. 3) being of particular interest because it differs in 4 SNPs from all other individuals investigated.

The T-type is a clearly differentiated genetic variety that is distinct from *P. uliginosa*, which shows only the G-variety in GenBank accessions (CELIŃSKI et al. 2017).

The cone morphology of the T-type of the Northern Bavarian samples is similar to *P. mugo*, as it lacks or has short apophyse hooks (measured here as apophyse depth) and slightly smaller cones (Fig. 7). As in MARCYSIAK AND BORATYŃSKI (2007), we used the relation between width and depth of the apophyse to characterize the hook shape of the cones. However, we reduced the number of cone characters measured, as the genotypes were sufficiently differentiated with this smaller set. We did not measure cone symmetry due to the complicated methodology involved. However, we observed remarkable differences in symmetry between the genotypes, as the T-type had almost symmetric cones, while the G-types typically have asymmetric cones. The cones of both genotypes do not differ significantly in height (Tab. 4). The frequency distribution showed that there is an overlap in hook size per apophyse width between the two genetic varieties; but the G-type exceeds the T-type in the ratio apophyse depth/width at least with a considerable number of individuals with much longer apophyses (Fig. 4). This suggests partial genetic linkage between genotype and morphotype. Additionally, we found an overall positive correlation between hook size and cone height, which was significant in the samples with the G-type, but less significant in those with the T-type (see Fig. 7). Large cones with particularly large hooks are typical characteristics of *P. uncinata* (CHRISTENSEN 1987a, b). Therefore, the weak correlation of these characteristics in the T-type is another special feature of the T-type. This T-type with *P. mugo*-like cones is unique in northern Bavaria and has not yet been recorded. The mixed populations are to our best knowledge not derived from recent afforestation with plants of different origin; there is no such record in the botanical literature.

There is also a relationship between the frequency of the genotypes in the populations and climate, including precipitation, vegetation length, and longitude (see GLM-results). The G-type is more frequent in harsher climates and at higher elevations (areas HF, Griesbach), while the T-type is more common in milder climates (subarea OH). This may indicate that the G-type is better adapted to colder climates, although genetic drift cannot be ruled out due to the scattered relict distribution. The best model according to AIC also included longitude, which serves as a proxy for distance of the populations and carries climate and soil information.

Niche differentiation, as proposed by GRANT (1981), is the primary driver for hybrid establishment. In the case of the *P. rotundata* populations of Northern Bavaria, the recombinant hybrid speciation exhibits unique features. Some individuals have small *P. mugo*-like cones but erect stems, which is a novel combination, mainly found in the T-type. Conversely, others show characters typical or similar to *P. uncinata*, differing only in cone size. The presence of parental-like plants in this relict and scattered hybrid zone is unusual since recombinational speciation should result in a fusion of parental traits.

Hybridization may still be ongoing, and transgression due to recombinant speciation could also occur (HEUERTZ et al., 2010, ŁABISZAK & WACHOWIAK 2021a). The T-type with cones similar to *P. mugo* could be a candidate for recombinant speciation, as the T-variant in *P. mugo* chloroplast has not yet been reported. However, two geno- and morphotypes are still genetically and morphologically present. This means that in the recent *P. rotundata* populations, we found hints of conflicting processes, starting recombinational speciation leading to homogenization of characters (compared to ZABOROWSKA et al. 2019) and still effective recombinational barriers. Further studies are needed to identify possible genetic barriers such as different flowering times and somatic isolation, which would help to clarify the mechanism of recombinant speciation. The pattern found in the northern Bavarian populations with ecological differentiation of two types underlines the importance of extending the seed harvest for cultivation of *P. rotundata* and include trees with the T-type to protect the total genetic reservoir of the species in this region.

Another aspect of the high phylogenetic plasticity and incomplete homogeneity is the resistance to environmental changes. According to the insurance hypothesis (YACHI & LOREAU 1999), these populations could be rather resistant to environmental changes due to their plasticity, provided that the changes are not too fast, which unfortunately is the case with climate warming. Modern theories such as the insurance hypothesis could present an interesting aspect for further research on the genetic structure and evolution of the genus *Pinus*, which is urgently needed to develop consistent and efficient protection measures.

While our study provides some insights into the taxonomy of the studied populations, a broader study design is necessary for a better taxonomic concept. Recent research by ŁABISZAK et al. (2019, 2021) made important contributions to this topic. They taxonomically included Bavarian *P. rotundata* populations from the northern Alps in *P. uliginosa*, but those plants did not cluster with the rest of the *P. uliginosa* populations investigated (ŁABISZAK et al., 2019). Overall, a differentiation in two genetic groups is also found in *P. uliginosa* (ŁABISZAK et al. 2021).

Our findings suggest the need for a closer taxonomic concept that distinguishes erect bog pines with *P. mugo*-like cones from those with hook-shaped cones. Our results also confirm the genetic significance of cone morphology, as noted in the investigations and distribution maps of CHRISTENSEN (1987a, b). The bog pine populations of Northern Bavaria may have been important refuges (ŁABISZAK et al. 2019) from which bog pines repopulated the pre-alpine bogs and slopes after the last glacial maximum and formed a secondary contact zone with immigrants from western and southern refugia. Understanding migratory paths and the origin of current populations might help emphasize their ecological importance and reinforce protection measures to preserve populations and relict habitats.

Statements and Declarations

Conflict of interest

The authors declare no competing interests.

Acknowledgements

We thank the Bayerischen Staatsforsten for financial support, Viktor Klaus and Manuel Steinbauer for helpful comments on the manuscript.

Funding

This work was supported by the Bayerische Staatsforsten.

Data availability

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Declarations

The authors have no relevant financial or non-financial interests to disclose.

Literature

- BORATYŃSKA, K., BORATYŃSKI, A. (2007): Taxonomic differences among closely related pines *P. sylvestris* *P. mugo*, *P. uncinata*, *P. rotundata* and *P. uliginosa* as revealed in needle sclerenchyma cells. *Flora* **202**: 555-569. <https://doi.org/10.1016/j.flora.2006.11.004>.
- BUSINSKY, R., KIRSCHNER, J. (2006): Nomenclatural notes on the *P. mugo* complex in Central Europe. *Phyton* **46**: 129-139.
- BUERKLE, C.A., MORRIS, R.J., ASMUSSEN, M.A., RIESEBERG, L.H. (2000): The likelihood of homoploid hybrid speciation. *Heredity* **84**: 441-451. <https://doi.org/10.1046/j.1365-2540.2000.00680.x>.
- BURNHAM, K.P., ANDERSON, D.R. (2002): Model selection and multimodel inference: a practical information-theoretic approach. 2nd edn., Springer, New York.
- CELIŃSKI, K., KIJAK, H., WOJNICKA-PÓŁTORAK, A., BUCZKOWSKA-CHMIELEWSKA, K., SOKOŁOWSKA, J., CHUDZIŃSKA, E. (2017): Effectiveness of the DNA barcoding approach for closely related conifers discrimination: A case study of the *P. mugo* complex. *C. R. Biol.* **340**: 339-348. <https://doi.org/10.1016/j.crv.2017.06.002>.
- CHRISTENSEN, K.I. (1987a): Taxonomic revision of the *P. mugo* complex and *P. rhaetica* (*P. mugo sylvestris*) (Pinaceae). *Nord. J. Bot.* **7**: 383-408. <https://doi.org/10.1111/j.1756-1051.1987.tb00958.x>.
- CHRISTENSEN, K.I. (1987b): A morphometric study of the *P. mugo* Turra complex and its natural hybridization with *P. sylvestris* L. (Pinaceae). *Feddes. Repert.* **98**: 623-635.
- DZIALUK, A., BORATYŃSKI, A., BORATYŃSKA, K., BURCZYK, J. (2012): Geographic patterns of genetic diversity of *Pinus mugo* (Pinaceae) in Central European mountains. *Dendrobiology* **68**: 31-41.
- DWD Climate Data Center (CDC) Multi-annual grids of the begin of the vegetation period in Germany, version 0.x, 01.02.2022.
- DWD Climate Data Center (CDC) Multi-annual grids of the end of the vegetation period in Germany, version 0.x, 01.02.2022.
- DWD Climate Data Center (CDC) Multi-annual means of grids of air temperature (2m) over Germany 1981–2010, version v1.0.
- DWD Climate Data Center (CDC) Multi-annual grids of number of frost days over Germany, version v1.0.
- DWD Climate Data Center (CDC) Multi-annual grids of precipitation height over Germany 1981–2010, version v1.0.
- DWD Climate Data Center (CDC) Multi-annual grids of soil temperature in 5 cm depth under uncovered soil, version 0.x, 01.02.2022.
- DZIALUK, A., MUCHEWICZ, E., BORATYŃSKI, A., MONTERRAT, J.M., BORATYŃSKA, K., BURCZYK, J. (2009): Genetic variation of *P. uncinata* (Pinaceae) in the Pyrenees determined with cpSSR markers. *Plant. Syst. Evol.* **277**: 197-205. <https://doi.org/10.1007/s00606-008-0123-y>.
- FEULNER, M., MÖSELER, B.M., NEZADAL, W. (2001): Introgression und morphologische Variabilität bei der Blauen Himmelsleiter *Polemonium caeruleum* L. in Nordbayern Deutschland. *Feddes. Repert.* **112**: 231-246. <https://doi.org/10.1002/fedr20011120306>.
- GEIGER, R. (1994): Vorkommen und Vergesellschaftung der Moorspirke (*Pinus mugo* ssp. *rotundata*) in Nordostbayern. – unpubl. Master thesis Univ. Bayreuth, pp. 76.
- GOLDE, A. (2000): Die Moorpöpopulationen der Berg-Kiefer (*P. mugo* agg.) in Sachsen. *Mitt. Deutsch. Dendrol. Ges.* **85**: 23-38.
- GRANT, V. (1981): Plant Speciation. Columbia University Press, New York.
- GRZYBOWSKI, M., GLIŃSKA-LEWCZUK, K. (2020): The principal threats to the peatlands habitats, in the continental bioregion of Central Europe – a case study of peatland conservation in Poland. *Journal for Nature Conservation*, **53**, 125778. doi:10.1016/j.jnc.2019.125778.
- HAMERNÍK, J., MUSIL, I. (2007): The *P. mugo* complex – its structuring and general overview of the used nomenclature. *Journal of Forest Science* **53**: 253-266.
- HARTIG, F. (2022) DHARMA: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.4.5. <http://florianhartig.github.io/DHARMA/>.
- HEUERTZ, M., TEUFEL, J., GONZÁLEZ-MARTÍNEZ, S.C., SOTO, A., FADY, B., ALÍA, R., VENDRAMIN, G.G. (2010): Geography determines genetic relationships between species of mountain pine (*P. mugo* complex) in western Europe. *J. Biogeogr.* **37**: 541-556. <https://doi.org/10.1111/j.1365-2699.2009.02223.x>.

- ILHAM, M., MUKARROMAH, S.R., RAKASHIWI, G.A., INDIATI, D.T., YOKU, B.F., PURNAMA, P.R., WHAYUNDI, D.K. (2022): Morpho-anatomical characterization and DNA barcoding of *Achillea millefolium* L. Biodiversitas Journal of Biological Diversity, **23**(4). doi:10.13057/biodiv/d230430.
- JÄGER, E.J. (Ed.) (2011): Rothmaler Exkursionsflora von Deutschland. Gefäßpflanzen: Grundband. – 20. Auflage, Spektrum Akademischer Verlag, Heidelberg.
- KLAUS, W. (1978): On the taxonomic significance of tectum sculpture characters in alpine *P.* species. Grana **17**: 161-166. <https://doi.org/10.1080/00173137809431961>.
- KRESS, W.J., WURDACK, K.J., ZIMMER, E.A., WEIGT, L.A., JANZEN, D.H. (2005): Use of DNA barcodes to identify flowering plants. PNAS **102**: 8369-8374. <https://doi.org/10.1073/pnas.0503123102>.
- ŁABISZAK, B., WACHOWIAK, W. (2021a): Molecular signatures of reticulate evolution within the complex of European pine taxa. Forests **12**(4): 489.
- ŁABISZAK, B., ZABOROWSKA, J., WACHOWIAK, W. (2019): Patterns of mtDNA variation reveal complex evolutionary history of relict and endangered peat bog pine (*P. uliginosa*). AoB Plants **11**: plz015. <https://doi.org/10.1093/aobpla/plz015>.
- ŁABISZAK, B., ZABOROWSKA, J., WÓJKIEWICZ, B., WACHOWIAK, W. (2021b): Molecular and paleo-climatic data uncover the impact of an ancient bottleneck on the demographic history and contemporary genetic structure of endangered *P. uliginosa*. J. Syst. Evol. **59**: 596-610. <https://doi.org/10.1111/jse.12573>.
- LAURANSON-BROYER, J., KRZAKOWA, M., LEBRETON, PH. (1997): Reconnaissance chimiosystématique et biométrique du pin de tourbière *P. uliginosa* (Neumann). Compt. Rend. Hebd. Séances Acad. Sci. Paris, Sér. 3, Sci. Vie. **320**: 557-565.
- LEWANDOWSKI, A., BORATYŃSKI, A., MEJNARTOWICZ, L. (2000): Allozyme investigations on the genetic differentiation between closely related pines – *P. sylvestris*, *P. mugo*, *P. uncinata* and *P. uliginosa* (Pinaceae). Pl. Syst. Evol. **221**: 15-24.
- LIEDE-SCHUMANN, S., NIKOLAUS, M., SOARES E SILVA, U.C., RAPINI, A., MANGELSDORFF, R.D., MEVE, U. (2014): Phylogenetics and biogeography of the genus *Metastelma* (Apocynaceae-Asclepiadoideae-Asclepiadeae: Metastelmatinae). Syst. Bot. **39**: 594-612. <https://doi.org/10.1600/036364414X680708>.
- LUTZ, J.L. (1956): Spirkenmoore in Bayern. – Ber. Bayer. Bot. Ges. **31**: 58-69.
- MAIER, J. (1993): Zapfenuntersuchungen bei *Pinus mugo* Turra. – Mitt. Dtsch. Dendrol. Ges. **81**: 5-12.
- MARCYSIAK, K., BORATYŃSKI, A. (2007): Contribution to the taxonomy of *P. uncinata* (Pinaceae) based on cone characters. Plant. Syst. Evol. **264**: 57-73. <https://doi.org/10.1007/s00606-006-0501-22>.
- NEUHÄUSL, R. (1969): Systematisch-soziologische Stellung der baumreichen Hochmoorgesellschaften Europas. Vegetatio Acta Geobot. **18**: 104-121. <https://doi.org/10.1007/BF00332833>.
- PRUS-GŁOWACKI, W., BUJAS, E., RATYŃSK, H. (1998): Taxonomic position of *P. uliginosa* Neumann as related to other taxa of *P. mugo* complex. Acta Soc. Bot. Pol. **67**: 269-275. <https://doi.org/10.5586/asbp.1998.035>.
- RIESEBERG, L.H., ARCHER, M.A., WAYNE, R.K. (1999): Transgressive segregation adaptation and speciation. Heredity **83**: 363-372. <https://doi.org/10.1038/sj.hdy.6886170>.
- RÖSLER, R. (1994): Der Spirkenbestand des Naturwaldreservates „Gscheibteloh“ in der Oberpfalz. – Forst und Holz **49**: 36-40.
- SCHMIDT, P.A. (2011): *P. mugo* agg In: ROLOFF, A. et al: Enzyklopädie der Holzgewächse **58** Erg. Lfg. 06/11 WILEY-VCH, Weinheim. S 1-32.
- TRUJILLO-ARGUETA, S., DEL CASTILLO, R.F., VELASCO-MURGUÍA, A. (2022): Testing the effectiveness of rbcLa DNA-barcoding for species discrimination in tropical montane cloud forest vascular plants (Oaxaca, Mexico) using BLAST, genetic distance, and tree-based methods. PeerJ. **10**: e13771 <https://doi.org/10.7717/peerj.13771>.
- UNAR, P., JANIĆ, D., SOUČEK, J., VRŠKA, T., ADAM, D., KRA, K., HORT, L. (2012): The *P. rotundata* Link Bog forests on mined peat bogs – is the conservation of undisturbed edge an effective tool for its protection? Polish Journal of Ecology **60** (4): 707-715.
- V. LEININGEN, W. (1906) Beschreibung von Mooren in der Umgegend von Schongau. – Naturw. Zeitschr. F. Land- und Forstwirtschaft. **4** (6): 1-33.
- WACHOWIAK, W., PRUS-GŁOWACKI, W. (2009): Different patterns of genetic structure of relict and isolated populations of endangered peat-bog pine (*Pinus uliginosa* Neumann). – J. Appl. Genet. **50** (4): 329-339.
- WILLIS, K.J., VAN ANDEL, T.H. (2004): Trees or no trees? The environments of central and eastern Europe during the last glaciation. Quaternary Science Reviews **23**: 2369-2387.
- YACHI, S., LOREAU, M. (1999): Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. PNAS **96**: 1463-1468. <https://doi.org/10.1073/pnas.96.4.1463>.
- ZABOROWSKA, J., ŁABISZAK, B., WACHOWIAK, W. (2020): Population history of European mountain pines *P. mugo* and *P. uncinata* revealed by mitochondrial DNA markers. J. Syst. Evol. **58**: 474-486. <https://doi.org/10.1111/jse.12520>.

submitted: 25.09.2023

reviewed: 17.10.2023

accepted: 15.11.2023

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Appendix / Anhang

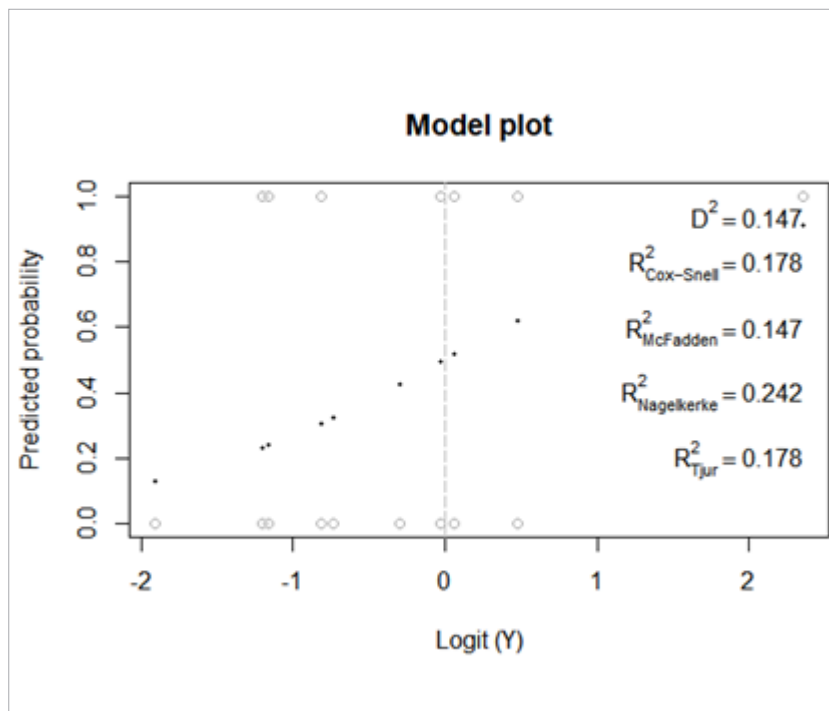


Fig. A1: Model plot of the GLM, genotype ~ vegetation length + precipitation + longitude.

Abb. A1: Modellplot des GLM, Genotyp ~ Vegetationslänge + Niederschlag + Längengrad..

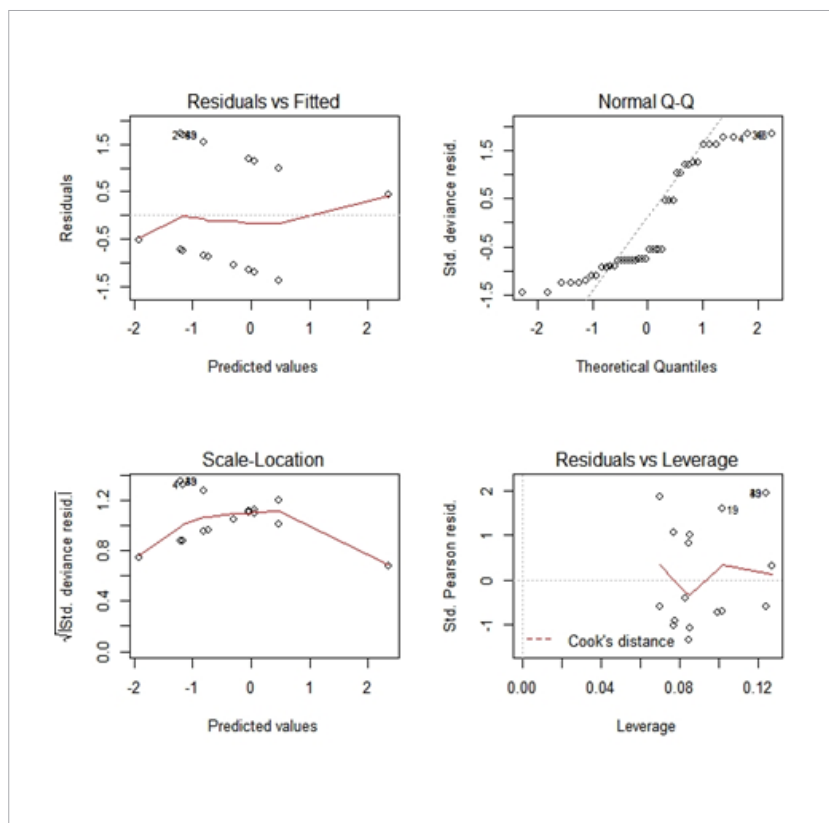


Fig. A2: Diagnostics of the GLM.

Abb. A2: Diagnostik des GLM.