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Distribution and dispersal ecology of *Lobaria pulmonaria* in the largest primeval beech forest of Europe

Olga Nadyeina · Lyudmyla Dymytrova · Anna Naumovych · Sergyi Postoyalkin · Christoph Scheidegger

Abstract Occupancy and density of the epiphytic lichen *L. pulmonaria* were studied in the mountains of Uholka–Shyrokyi Luh (Ukraine), which include the largest primeval beech forest in Europe. The lichen occupancy was assessed on 314 plots laid out on a systematic grid. Additional data on population density were collected from 483 trees growing both, on and between these plots. The trees harbouring *L. pulmonaria* were distributed very sparsely within Uholka–Shyrokyi Luh, and occupy nearly 10 % of the studied perimeter. The generalized linear models showed that area of occupancy of *L. pulmonaria* was significantly influenced by altitude and canopy cover, whereas the species' density was explained by habitat types and slope exposition. Population density is higher at the timberline than in the interior forest or on lowland meadows. We found a bimodal altitudinal distribution of *L. pulmonaria*, with maxima below and above 900 m a.s.l., where it prefers forest stands with loose or scattered canopy. The preferred position of *L. pulmonaria* on host tree trunks depends on stand density and allows the species to get the necessary level of insolation also in shaded sites where it grows higher up on the trunk than in open stands. While *L. pulmonaria* occupied trees with various diameters, juvenile individuals are more frequent on small trees, but mature lichen individuals are predominantly found on trees of average or large sizes. Fertile individuals require specific environmental conditions, which are available at intermediate altitudes, related with sheltered light, and horizontal terraces on slopes with eastern exposition. In general, the primeval beech forest of Uholka–Shyrokyi Luh harbours a high percentage of juvenile thalli of *L. pulmonaria*, which lack vegetative propagules. Mature individuals have a

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low frequency of fruit bodies and reproduce mainly with vegetative diaspores. We interpret this as an indication of a currently growing population of *L. pulmonaria* within the area. We hypothesize that transboundary air pollution has decreased the lichens' population frequency and density and has altered the ratio of developmental stages in *L. pulmonaria* during earlier decades.

Keywords Carpathians · Epiphytic lichen · Topographic and forest-stand variables · Juvenile · Mature · Fertile

Introduction

Habitat degradation and loss, fragmentation and environmental pollution are amongst the most important causes of a dramatic decline of biodiversity worldwide (Groom et al. 2006). Nature reserves and integrative conservation instruments (Bollmann and Braunisch 2013) provide a comprehensive framework for biodiversity conservation in forest ecosystems, where spatio-temporal habitat dynamics and species characteristics interact with and relate to species persistence (Van Teeffelen et al. 2012). Integrative conservation instruments such as structural retention, old-growth stand protection or ecological process areas provide a minimum habitat quality for the majority of generalist species, do however not provide long-term existing niches for many habitat specialist, particularly species that depend on extended habitat tradition, e.g., lichens, fungi, insects (Bollmann and Braunisch 2013; Scheidegger et al. 2010). Nature reserves provide habitat to specialist species but natural dynamics is likely to change special habitats such as old-growth structures over time (Bruun et al. 2006). This may lead to the disappearance of recolonization sources of old-growth dependent species in relatively small protected areas and consequently extinction becomes the dominant population process unless nature reserves are considerably larger than the largest disturbance patch size, including rare patches (Pickett and Thompson 1978). As more and more of the earth is modified by humans, the mismatches in scale between present nature reserves and the natural dynamics of ecosystems become more pronounced (Bengtsson et al. 2003).

Epiphytic lichens are among the most suitable indicator taxa of forest biodiversity (Hansson 1997) and under certain climatic conditions many species appear to require continuity of old trees (Kuusinen 1994, 1996; Nilsson et al. 1995; Rose 1976; Tibell 1992). However, species that depend on old-growth forests in one region may be less specific under more favourable climatic conditions. Therefore the general utility of lichens as indicators of woodland ecological continuity has recently been questioned (Whittet and Ellis 2013). Although these species often require minimal habitat size, e.g., a single tree, to grow and survive for many decades but for the maintenance of viable populations, most of these species depend on extended old-growth structures such as veteran trees, and extended habitat tradition that includes a spatio-temporal continuum of old-growth forest structures at a small spatial scale. However, in small protected areas old-growth forest dependent lichen populations may be extremely small and thus face an increased risk of local extinction if the reserve is small compared to the minimum dynamic area (Pickett and Thompson 1978). Increased extinction frequency in small habitat patches could thus decrease the indicator value of species because of false negatives in small reserves. In forest key habitats in southern Finland 20 % of the populations tree colonizing lichen species disappeared within <10 years because their only host tree had fallen, a snag had

further decomposed, or for an unknown reason (Pykälä 2004). Habitat requirements, abundance and density of possible indicators of forest ecological continuity have therefore to be studied in large protected areas where the requirements for a colonisation/extinction equilibrium are likely to be fulfilled.

Lobaria pulmonaria is an epiphytic lichen, frequently used as indicator species to identify forest of high conservation priority (Scheidegger and Werth 2009) and woodland ecological continuity questioned (Whittet and Ellis 2013). This species prefers to grow in primeval or old-growth forests in Central Europe, although it is also known from wooded pastures and chestnut orchards in other regions with a more oceanic climate (Rose 1976, 1992; Gauslaa 1994; Jüriado et al. 2012). In spite of the large Holarctic distribution area (Yoshimura 1971), the species is threatened in many European countries (Scheidegger and Werth 2009). *Lobaria pulmonaria* is known to be sensitive to anthropogenic influence, including air pollution (Rose 1976) and forest management (Rose 1988). Furthermore, abundance, spatial and temporal connectivity of mature and veteran trees, i.e., habitat network dynamics of forest landscapes with old-growth characteristics, may explain the regional distribution and dispersal pattern of the epiphytic lichen *L. pulmonaria* (Rose 1992; Otalora et al. 2011; Kalwij et al. 2005). Although the species distribution and abundance has frequently been studied in fragmented or partially managed forest landscapes (Fritz et al. 2009; Nascimbene et al. 2013; Otalora et al. 2011), the species natural distribution pattern is largely unknown from reference ecosystems such as large continuous primeval forest areas.

The Uholka–Shyrokyi Luh Mountains (Eastern Carpathians, Ukraine) comprise more than 10,000 ha and are considered the largest primeval beech forest of Europe (Brändli and Dowhanytsch 2003). This area may be regarded as a reference natural ecosystem for the biodiversity assessment, because no anthropogenic or large-scale natural disturbances have been reported during the last centuries (Brändli and Dowhanytsch 2003; Commarmot et al. 2005; Trotsiuk et al. 2012). Data on air pollution of this area is lacking, however it is remote enough from current pollutant sources. The primeval forest of Uholka–Shyrokyi Luh consists of almost pure beech stands (Brändli and Dowhanytsch 2003), which is one of the most suitable host-tree species for *L. pulmonaria* in continental Europe (Mikryukov 2011; Scheidegger et al. 2012). Thus management disturbances and host-tree impact on the distribution and dispersal characteristics of the *L. pulmonaria* can be excluded. Alternatively, the focus can be on which topographic and forest-stand factors influence the distribution and dispersal success of the lichen.

In this study we aimed to investigate the distribution, density and reproductive structure of *L. pulmonaria* in the primeval beech forest landscape of Uholka–Shyrokyi Luh, and to analyze the characteristics of the species' populations in different parts of the reserve. We hypothesized that *L. pulmonaria* occurs frequently and forms dense populations with a high proportion of fertile individuals in the largest primeval, uneven-aged beech forest of Europe. We also wanted to study (1) which environmental factors shape the occupancy and density of *L. pulmonaria* in the study region, and (2) which factors explain the density of juvenile and mature vegetatively reproducing, and fertile individuals of *L. pulmonaria*, respectively.

Materials and methods

Study species

Lobaria pulmonaria is lichen-forming ascomycete, which forms an obligate mutualistic symbiosis with the green alga *Dictyochloropsis reticulata*. It can reproduce clonally and

sexually (Dal Grande et al. 2012; Singh et al. 2012). Clonal spread is achieved by symbiotic propagules such as soredia, isidioid soredia or thallus fragments (Scheidegger and Goward 2002). The soredia in *L. pulmonaria* may develop in "isidioid soredia", when an outer layer of hyphae form a cortex above aggregates of soredia (Büdel and Scheidegger 2008). Heterothallic, sexual reproduction of the fungal symbiont by ascospores is limited to a changing percentage of individuals, which is hypothesized to depend on the availability of mating types (Zoller et al. 1999; Singh et al. 2012), environmental quality (Edman et al. 2008) and developmental stage of the lichen thallus (Denison 2003). Lichen thalli without soralia and isidioid soredia were defined here as *juveniles* (Honegger and Scherrer 2008; Jüriado et al. 2011), while thalli with isidioid soredia were considered as *mature*, and with fruit bodies as *fertile*.

The biology of *L. pulmonaria* varies depending on part of its distribution range, which contrast in oceanic and continental regions (Coxson et al. 2003; Høistad and Gjerde 2011; Jüriado et al. 2012; Werth et al. 2006). Vegetative dispersal is dominating in this species at the local scale up to 30 m (Walser 2004), however sexual ascospores are essential for long-distance dispersal (Werth et al. 2006, 2007). Local abundance of this lichen and variation in its generative or vegetative stages are an important population pattern and indicator of individual vitality (Öckinger and Nilsson 2010).

Study area

The study was carried out in the Uholka–Shyrokyi Luh Massif of the Carpathian Biosphere Reserve, which is situated in the south-western part of Ukraine between 48°18′ N and 23°42′ E (Fig. 1a). The mountain slopes are covered by almost pure beech forests (97.3 % of *Fagus sylvatica*) within two contiguous areas Uholka and Shyrokyi Luh. These forests occupy an area of 10,383 ha, from which 8,500 ha (88 %) are considered primeval forests (Brändli and Dowhanytsch 2003).

The landscape ranges from 400 and 1,500 m a.s.l., with an anthropogenically influenced timberline at around 1,140 m a.s.l. The slopes have different aspects and inclinations, ranging from horizontal terraces to steep slopes up to 100 % inclination, with a mean inclination of approx. 50 % (Commarmot et al. 2013). South-facing and less steep slopes at lower altitudes are frequent in the Uholka area, while the Shyrokyi Luh area is more varied (Commarmot et al. 2013). The climate is temperate with a mean annual temperature of +8 °C and an annual precipitation of 1,130 mm, measured in Uholka at altitude of 430 m (Bursak 1997; Commarmot et al. 2013). In the Shyrokyi Luh, the annual temperatures are slightly lower than in the Uholka (Brändli and Dowhanytsch 2003).

The beech forest stands are characterized by an uneven-aged and multi-layered structure. The canopy is mostly closed, making forest floristically homogeneous (Bursak 1997; Commarmot et al. 2005), with little local differentiation of the epiphytic lichens (Dymytrova et al. 2014). The mean age of beech trees in the upper canopy cored in the Uholka part is over 200 years, with a maximum of 550 years identified by age estimation methods (Trotsiuk et al. 2012). There are no relations between size of the tree and its age in the Uholka–Shyrokyi Luh beech forest (Commarmot et al. 2013).

Sampling design and variables measured

Selection of spatial scale for analyses

During 2009–2011 we studied the occupancy of *Lobaria pulmonaria* on 314 forest-inventory plots of 0.05 ha each within a perimeter of 10,282 ha. Clusters of two sample
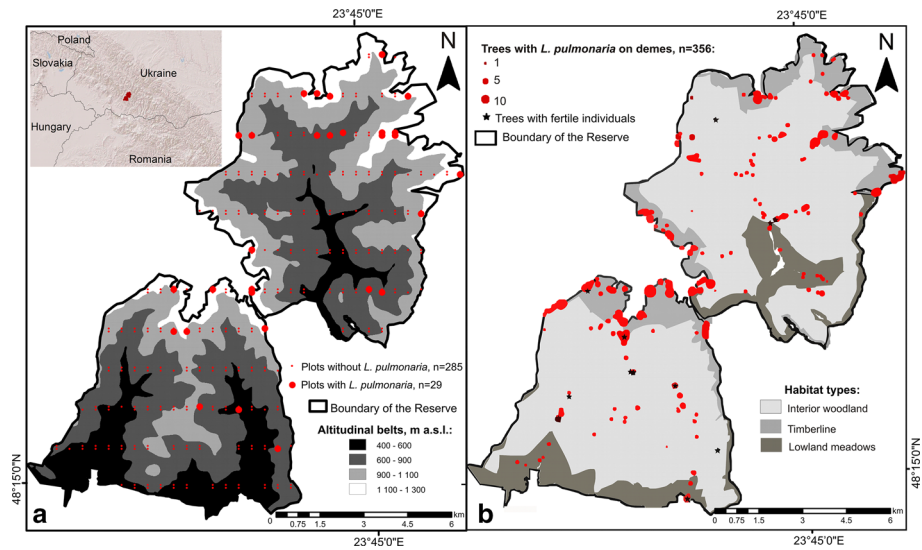
Fig. 1 **a** Location of Uholka–Shyrokyi Luh in Europe, the area of primeval beech forest is shown with altitudinal belts. The forest inventory plots established are shown as *red dots* (*n* = 314), depending on presence of trees with *L. pulmonaria* on plot. **b** Main habitat types within reserve are shown as gradation of *grey*. The demes harbouring trees with *L. pulmonaria* are shown by *red dots* (*n* = 356), depending on density of trees with *L. pulmonaria* per deme. The trees with fertile individuals marked with *asterisk* (*n* = 14). (Color figure online)

plots each were arranged on a systematic grid of 445 m × 1,235 m with a distance of 100 m between the plots of one cluster (Fig. 1a). This dense net of plots (Fig. 1a) covered the total altitudinal range and all topographic elements within the perimeter of the inventory (Commarmot et al. 2013). We also assessed the whole forest area for *L. pulmonaria* occupancy and density outside the inventory plots. This search for *L. pulmonaria* over the entire study perimeter allowed an intensive screening of the whole area, covering the plots and the area in between, and thus a near complete sampling of the trees with *L. pulmonaria*. The trees harbouring this lichen species were scattered over the entire forest massif. We measured coordinates of each tree with *L. pulmonaria* with a GPSmap 62st (GARMIN, Bucher and Walt, St.-Blaise, Switzerland). To assess the most effective *deme* (Werth et al. 2006) size for density analysis, we made a preliminary test (Fig. 2).

Trees with *L. pulmonaria* were plotted in ArcGis.10.1 (ESRI, http://www.esri.com/software/arcgis), and then the functions *Grid Index Features* and *Spatial Join* were used to group trees with lichen into demes with different sizes (0.05, 0.1, 0.5, 1, 5, 10, 25, 50 and 100 ha), as implemented in ArcGis.10.1. Generalized linear models (GLMs) were used to analyze mean number of trees with *L. pulmonaria* per demes of different sizes. This analysis show that number of trees with *L. pulmonaria* per deme increased on one tree, when enlarging a deme on each 2 ha (Fig. 2a). Demes up to 1 ha had no significant effect on number of trees with *L. pulmonaria* (Fig. 2a, b) and we therefore selected 0.05 ha (square with 22.4 m side) as preferred size to study occupancy and density of *L. pulmonaria* in the primeval beech forest. The scale of 0.05 ha was also used in intensive forest inventory of Uholka–Shyrokyi Luh area (Commarmot et al. 2013), because spatial autocorrelation within such clusters found to be very small (Commarmot et al. 2013; Lanz 2011). Additionally, the
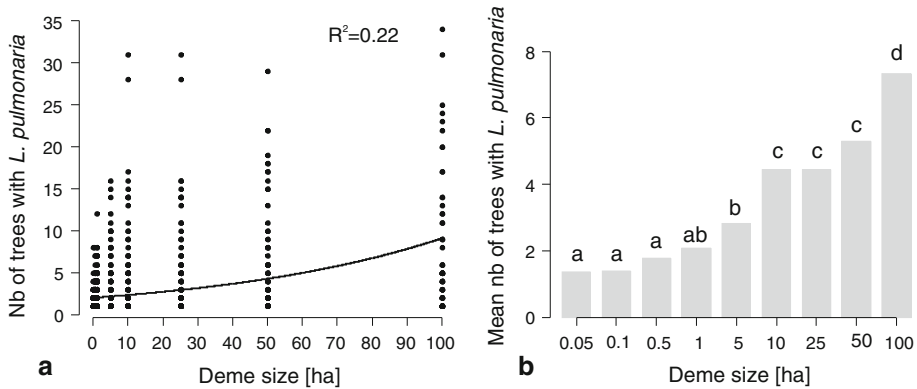
Fig. 2 Number of trees with *L. pulmonaria* per deme depends on deme size in the primeval beech forest of Uholka–Shyrokyi Luh. **a** Scatterplot and GLM fit *line* MacFadden's pseudo $R^2 = 0.22$, $p \leq 0.001$. **b** Barplot showing mean number of trees with *L. pulmonaria* on the plots of different size. The *letters above bars* show differences in the level of means based on Tukey–Kramer HSD *t*-test

distance up to 30 m is considered as a limit for vegetative dispersal of *L. pulmonaria* (Werth et al. 2006), and genetic diversity within such a small clusters supposed to be high in an old-growth forests (Scheidegger et al. 2012).

Taking into account result of our preliminary analysis, all the following analyses were performed on a scale of 0.05 ha within perimeter 10,282 ha.

Occupancy

Occupancy, or probability of occurrence, was understood as presence/absence of trees with of *L. pulmonaria* on the 314 forest inventory plots (Commarmot et al. 2013). They cover the entire forest reserve area with an altitudinal gradient from 458 to 1,269 m a.s.l. The degree of crown cover in all layers of the stand varied between 15 and 100 % with a mean of 76 %. The average tree diameter at breast height per plot varied between 11 and 114 cm with a mean of 35 cm, however the highest density of trees is in the smallest diameter class, with a second small peak in the mid-diameter range (Commarmot et al. 2013). A total of 222 plots (71 %) were situated in the core zone of the reserve, while 72 plots (23 %) were located in the buffer zone, 16 plots (5 %) within the regulated protection zone, and 4 plots (1 %) belonged to the anthropogenic landscapes (Commarmot et al. 2013). In order to study the distributional pattern of *L. pulmonaria* in the different parts of the primeval forest we distinguished three habitat types, which were related to functional zoning of the reserve: (1) *timberline* forest stands, close to subalpine meadows (pooled buffer and anthropogenic landscapes zones at the forest margin above 900 m), *lowland meadows* (pooled buffer and anthropogenic landscapes zones along the rivers or streams below 900 m), and *interior woodland* stands (include continuous forest within core zone of the reserve and zone of regulated protection; Fig. 1b). The buffer and anthropogenic landscape zones are traditionally used as cattle pasturing, what affected tree growth along the timberline nearly at 1,140 m a.s.l. (Brändli and Dowhanytsch 2003). The regulated protection zone (Brändli and Dowhanytsch 2003) comprises 50 m width buffers on both sides of the main roads through the core zone, where e.g., cutting of trees fallen across the paths is allowed. The topographic and forest-stand variables were measured during the

Table 1 List of variables used for *L. pulmonaria* occupancy study (314 systematically arranged plots)

Variables	Types
Topographic	
X and *Y* coordinates (UTM)	Continuous
Altitude a.s.l. (m)	Continuous
Slope aspect (gon)	Continuous
Slope inclination (%)	Continuous
Relief [flat area, foot slope (depressions), middle slope, hilltop]	Nominal
Forest-stand	
Canopy cover, degree of crown cover in the upper, medium and lower layers of the stand (%)	Continuous
Canopy closure in upper layer (closed, loose, scattered)	Ordinal
Size of gap above sample plot center (categories in m^2: from totally open to close)	Ordinal
Stem density (per ha on the plot)	Continuous
Mean tree diameter (cm)	Continuous
Deadwood (m^3/ha)	Continuous
Response	
Trees with *L. pulmonaria* (presence/absence)	Binomial

inventory of the primeval beech forest structure, while slope aspect and inclination of the plots were extracted from a digital elevation model of the Carpathian Biosphere Reserve (Table 1; Commarmot et al. 2013).

Density

The *density* of *L. pulmonaria* was understood as number of trees with *L. pulmonaria* per deme (percent of trees with lichen per total number of trees), and was evaluated for 483 trees belonging to 356 demes. The lichen thalli (interpreted here as individuals) were collected up to 5–6 m on the trees using an avalanche probe (Black Diamond, Reinach, Switzerland) and along the whole trunk on lying trees. The following parameters were assessed in the field or extracted from the digital elevation model (Commarmot et al. 2013) for each tree with *L. pulmonaria*: coordinates, altitude, tree parameters (species, diameter at breast level, and vitality), relief characteristics (valley, slope or ridge; slope aspect and inclination), and habitat types (Table 2). Host tree vitality was classified as standing trees (living or dead) and lying trees (deadwood).

Developmental stages of L. pulmonaria

Developmental stages of *L. pulmonaria* were expressed as number of juvenile, mature or fertile individuals per deme (percent of those stages per total number of specimens; Table 2). Those stages were assessed for 356 demes, as for lichen density evaluation, but take into account 1,520 lichen individuals assessed. In average three thallus fragments (considered here as individuals) per each tree were assessed, preferably from different aspects of the trunk. The following parameters were measured at the individuals level: height and aspect on the trunk, developmental stage (juvenile, mature, fertile), and we

Table 2 List of variables used for the study of density the of trees with *L. pulmonaria*, and its different developmental stages (356 demes, 483 trees, 1,520 individuals of *L. pulmonaria*)

Variables	Types
Topographic	
X and Y coordinates (UTM)	Continuous
Altitude a.s.l. (m)	Continuous
Slope aspect (°)	Continuous
Slope inclination (%)	Continuous
Relief (valley, slope, ridge)	Nominal
Distance from the stream or river (m)	Continuous
Forest-stand	
Host tree diameter (cm)	Continuous
Host tree vitality (dead lying, dead standing, recently fallen, living standing)	Nominal
Habitat type (interior woodland, timberline, lowland meadow)	Nominal
Lichen characteristics	
Sample height on the trunk (m)	Continuous
Sample exposition on the trunk (°)	Continuous
Fertility (fertile, sterile)	Nominal
Thallus developmental stage (juvenile, sorediate, mature)	Nominal
Plectocarpon lichenum infection (present, absent)	Nominal
Responses	
Trees with *Lobaria pulmonaria* per deme (%)	Continuous
Juvenile individuals per deme (%)	Continuous
Mature individuals per deme (%)	Continuous
Fertile individuals per deme (%)	Continuous

Lichen characteristics were fixed for each of 1,520 *L. pulmonaria* individuals

visually inspected for an infection with the host-specific fungal parasite *Plectocarpon lichenum* (Table 2).

Statistical analyses

Statistical analyses were performed with *R* (R Development Core Team 2011) and considered significant when $p < 0.05$. The distances of trees with lichen from the nearest stream were square-root-transformed. The host tree diameters were log-transformed. The slope aspect was arcsine-transformed and the exposition expressed as Eastness and Northness (Roberts 1986). The relationships between the presence or absence of *L. pulmonaria* on forest inventory plots with topographic and forest-stand factors were tested with GLMs using a "binomial" model family. Density of *L. pulmonaria*, and its developmental stages per deme, as response to topographic and forest-stand factors, were tested with GLM using "gaussian" or "poisson" model families. The best models were selected on the basis of Akaike information criterion (1974) using "both" directions as implemented in *mass* package. This approach allows to drop out multicollinear variables with high information inflation factor (VIF). Densities of plots with and without *L. pulmonaria* were visualized with sm.density.compare function in *sm* package, and density of total trees with *L. pulmonaria* per deme with density function in *mixtools* package.

Analyses of variance ANOVA, with following Tukey–Kramer HSD t-tests, were applied to examine relations between continuous responses and categorical variables, i.e., number of trees with *L. pulmonaria* per deme with different spatial scales, number of trees with *L. pulmonaria* per deme with habitat types, number of individuals of different developmental stages with categories of tree vitality.

All maps were drawn in ArcGis.10.1, which was used also to calculate the distances of the trees with lichen from the nearest stream.

Results

Occupancy of *L. pulmonaria*

We found trees with *L. pulmonaria* on 29 (9.29 %) of the total 314 forest-inventory plots (Table 3; Fig. 1a). Within these plots we found a total of 42 trees of *Fagus sylvatica* with lichen.

The occupancy of *L. pulmonaria* in the Uholka–Shyrokyi Luh primeval forest area significantly depended on altitude above sea level (Table 4; Fig. 3a, b) and canopy cover (Table 4; Fig. 3c, d).

We found that the altitudinal distribution of the plots with *L. pulmonaria* have a bimodal pattern, whereas the plots without lichen showed an unimodal distribution (Fig. 3b, d). The plots without *L. pulmonaria* were distributed over a wide altitudinal range (458–1,269 m a.s.l.), with a mean at 850 m a.s.l., while plots with *L. pulmonaria* were concentrated around 765 and 1,170 m, respectively (Fig. 3b). Analogously, the mean canopy cover on the forest inventory plots without *L. pulmonaria* was 75.5 %, while 72.4 % of the plots with *L. pulmonaria* were concentrated in loose stands and 26.3 % in scattered stands (Fig. 3d).

Density of *L. pulmonaria*

We analyzed 483 trees carrying a total of 1,520 *L. pulmonaria* individuals, which were scattered on 356 demes (Table 3; Fig. 1b) between 392 and 1,307 m a.s.l. Trees with *L. pulmonaria* were equally distributed among the two forest massifs ($\chi^2 = 2.7$, $p = 0.1^{ns}$, Table 3). However the number of individuals was slightly higher in Shyrokyi Luh area ($\chi^2 = 5.6$, $p = 0.018^*$, Table 3). *Lobaria pulmonaria* was growing predominantly on 471 trees of *Fagus sylvatica* and only occasionally on *Acer pseudoplatanus* (12 trees) in the Uholka–Shyrokyi Luh primeval beech forest.

Table 3 Frequency of *L. pulmonaria* in the primeval beech forest of Uholka–Shyrokyi Luh

Part of the area	Nb of forest inventory plots with *L. pulmonaria*/total	Nb of demes	Nb of trees	Nb of individuals
Uholka	8/145	158	224	715
Shyrokyi Luh	21/169	198	259	805
Interior woodland	19/230	122	151	449
Timberland	8/42	189	282	159
Lowland meadows	2/42	45	50	912
Total	29/314	356	483	1,520

Table 4 Best fitting general linear model (GLM), which explain the occupancy of *L. pulmonaria* as a function of topographic and forest-stand variables collected on 314 forest inventory plots in the primeval beech forest of Uholka–Shyrokyi Luh

Variables	Estimates	SE	z
$R^2 = 0.23$			
Intercept	−5.36	1.6067	−3.34***
Altitude a.s.l.	0.006	0.0013	4.94***
Canopy cover	−0.04	0.0138	−2.97**

R^2 MacFadden's pseudo R^2, z Wald's statistic

Significant variables are indicated with *** ($p \leq 0.001$) and ** ($p \leq 0.01$)

The total altitudinal distribution of *L. pulmonaria* individuals showed a clear bimodal pattern with one group being concentrated around 690 m a.s.l. and the other around 1,188 m a.s.l. (Fig. 4a). The majority of trees harbouring *L. pulmonaria* (nearly 80 %) were above 900 m a.s.l. Another part of the trees with *L. pulmonaria* was found very scattered in the lowlands below 900 m (Fig. 4a).

The density of trees with *L. pulmonaria* on 356 demes found to be related to altitude a.s.l., habitat types and slope aspect (Table 5; Fig. 4). The density increases with altitude and was highest at the timberline with scattered tree stands, where it reached 8 trees/per
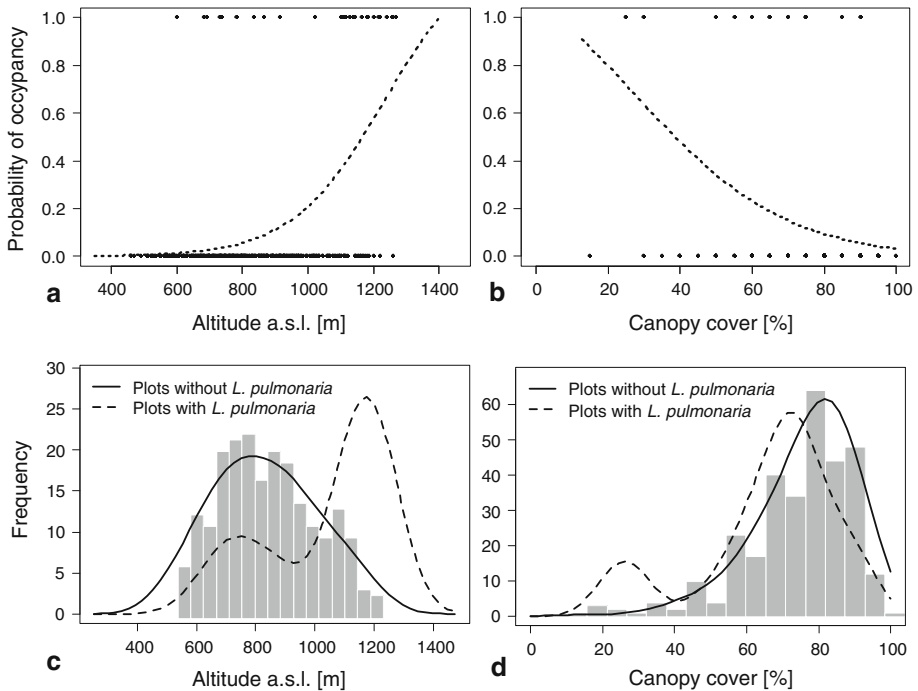

Fig. 3 Distribution of variables, which explain presence of *L. pulmonaria* on the studied plots ($n = 314$) in the Uholka–Shyrokyi Luh: **a**, **c** altitude a.s.l., **b**, **d** canopy cover. Compared densities show unimodal distribution for the plots without *L. pulmonaria* (**c**, **d**, *solid line*), and bimodal for the plots with *L. pulmonaria* (**c**, **d**, *dashed line*). *Grey* histogram show total distribution of plots
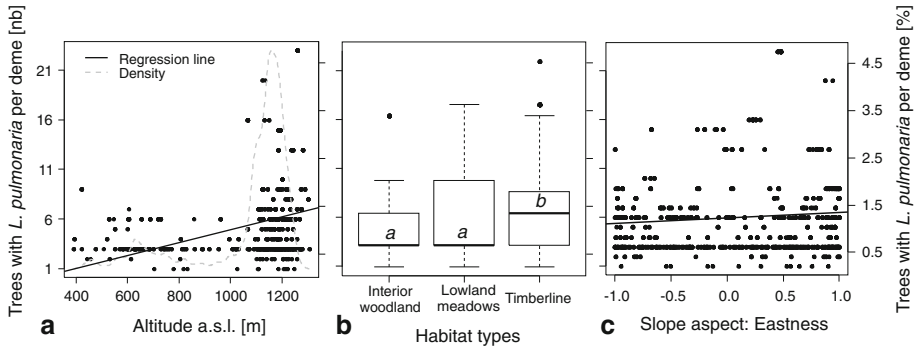
Fig. 4 Distribution of variables, which explain density of *L. pulmonaria* on the studied demess (*n* = 356) in the Uholka–Shyrokyi Luh: **a** altitude a.s.l., **b** habitat types, and **c** slope aspect. *Solid lines* show linear regression trends. The *dashed density line* show bimodal altitudinal distribution of trees with *L. pulmonaria*. Boxplots show mean number of trees with *L. pulmonaria* in different habitat types. The *letters above the mean line* show differences in the level of means based on Tukey–Kramer HSD *t*-test

deme or 1.5 % of the total trees which were colonized with *L. pulmonaria* (Fig. 4a, b). Trees with *L. pulmonaria* reached a higher density at the eastern slopes of the mountains (Fig. 4c).

The mean height where *L. pulmonaria* was growing at the trunk of the host tree was 2.6 m, and this value was not different between two forest massifs of Uholka and Shyrokyi Luh ($\chi^2 = 0.03$, $p = 0.8^{ns}$) and mean height was negatively correlated with altitude a.s.l. (Fig. 5a). The mean height of *L. pulmonaria* on the trunk also varied among different habitat types within the reserve and was typically growing at 4 m within interior woodland, at 3.5 m in the lowland meadows, and at 2 m at the timberline (Fig. 5b).
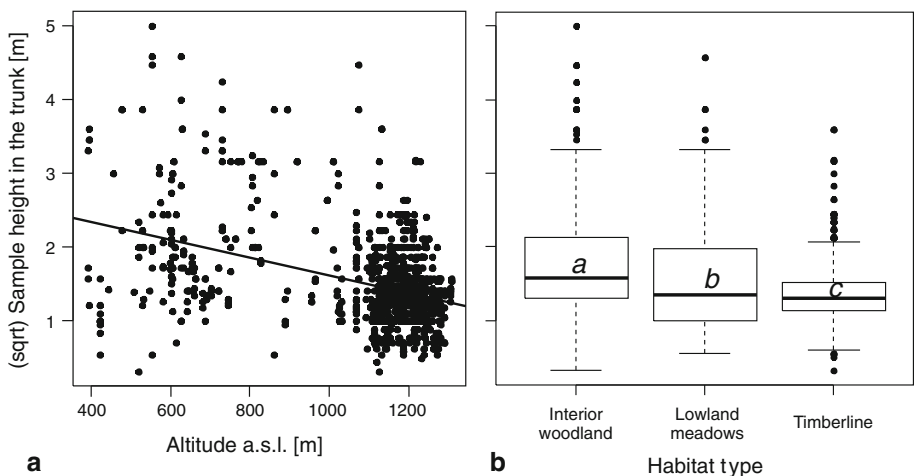

Fig. 5 Variation of sample height on the trunk with altitude. **a** Negative Spearman's rank correlation $R = -0.43^{***}$. **b** Boxplot showing mean sample height on the trunk in different habitat types. The *letters above mean line* show differences in the level of means based on Tukey–Kramer HSD *t*-test

Fig. 6 Differences in frequency
of *L. pulmonaria* within thallus
developmental stages
(*n* = 1,520)
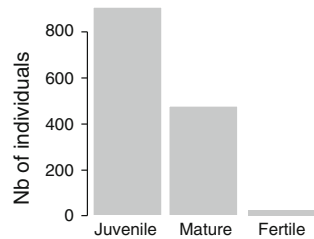
Developmental stages of *L. pulmonaria*

Most thalli were lacking any vegetative propagula (911 individuals or 59.86 %) or *juvenile*, 134 individuals (8.80 %) were sorediate and another 480 thalli developed isidioid soredia (31.54 %), and considered as *mature* in our analysis (Fig. 6). We found only 39 *fertile* individuals of *L. pulmonaria* (2.56 % of total) in the entire study area; they were concentrated on 14 trees (11 trees of *Fagus sylvatica* and three trees of *Acer pseudoplatanus*; Fig. 1b, 6). The fruit bodies were found on individuals, which developed isidioid soredia (15 specimens, 38.5 %), soredia (11 specimens, 28.2 %), but on 13 fertile specimens (33.3 %) no vegetative propagules appeared.

The individuals of different developmental stages had similar or opposite trends with different environmental variables (Table 5; Fig. 7). The density of trees with *L. pulmonaria* per deme positively correlated with all developmental stages, and all developmental stages were more common on eastern slopes of the mountains (Fig. 7a, b). Altitude a.s.l. positively affected the density of juvenile and mature individuals, but negatively influenced the density of fertile individuals (Fig. 7c). Fertile individuals were concentrated on terraces, while the densities of the other developmental stages were not influenced by inclination (Fig. 7e). Juvenile individuals were more abundant on trees of smaller diameters, and mature preferred trees with bigger sizes, while this factor was not significant for fertile individuals (Fig. 7d). Juvenile individuals occurred equally on trees of different vitality, while mature individuals were more frequent on dead standing trees (Table 5; Fig. 7f). Fertile individuals were always found on living trees.

Fertile individuals were equally distributed among the two forest massifs (Fig. 1b) and their presence correlated with a high density of *L. pulmonaria* per deme and was concentrated at intermediate elevations, eastern aspects and horizontal terraces (Table 5; Fig. 7a–d).

Discussion

This is the first assessment of *Lobaria pulmonaria* populations in the largest primeval beech forest in Europe, based on a non-stratified plot network of over 10,000 ha (Commarmot et al. 2013). Contrary to our expectations of high frequency and population density of *L. pulmonaria* in a primeval beech forest, *L. pulmonaria* was found on less than 10 % of the plots, and a high density of trees with *L. pulmonaria* was only found at the timberline. Instead, we found that the distribution of *L. pulmonaria* in this primeval beech forest had a bimodal pattern related to altitude a.s.l. and canopy openness. Also contrary to our expectations the developmental stages were dominated by juvenile, non-reproducing individuals and sexual reproduction was found to be extremely rare in the study area.

Table 5 Best fitting general linear models (GLM), which explain the density of *L. pulmonaria* and its dispersal strategies success per deme in terms of the topographic and forest-stand variables

Parameter/*response/dependent variables*	Estimates	SE	*t*
Density. *Response* trees with *Lobaria pulmonaria* per deme (%)			
$R^2 = 0.09$, $F = 33.4$***			
Intercept	0.4998	0.1352	3.70***
Habitat (timberline)	0.3444	0.0678	5.07***
Slope aspect (Eastness)	0.1337	0.0333	4.01***
Altitude a.s.l.	0.0005	0.0001	3.28**
Juvenile individuals. *Response* trees with juvenile individuals per deme (%)			
$R^2 = 0.54$, $F = 542.7$***			
Intercept	0.1897	0.0186	10.18***
Nb of trees with *L. pulmonaria* per deme	0.0334	0.0009	38.18***
Altitude a.s.l.	0.0001	0.0000	4.07***
Host tree diameter	−0.0005	0.0002	−3.18**
Mature individuals. *Response* trees with mature individuals per deme (%)			
$R^2 = 0.29$, $F = 91.85$***			
Intercept	0.0448	0.0308	1.46
Nb of trees with *L. pulmonaria* per deme	0.0257	0.0011	22.04***
Host tree vitality (dead standing)	0.0887	0.0244	3.63***
Host tree diameter	0.0007	0.0002	3.31***
Slope aspect (Eastness)	−0.0179	0.0073	−2.47*
Fertile individuals. *Response* trees with fertile individuals per deme (%)			
$R^2 = 0.23$, $F = 101.20$***			
Intercept	0.0922	0.0174	5.29***
Nb of trees with *L. pulmonaria* per deme	0.0134	0.0008	16.56***
Slope aspect (Eastness)	0.0285	0.0049	5.83***
Altitude a.s.l.	−0.0001	0.0000	−5.70***
Slope inclination (%)	−0.0027	0.0004	−7.15***

Data includes 483 trees with *L. pulmonaria* found on and/or between forest inventory plots in the primeval beech forest of Uholka–Shyrokyi Luh

R^2 determination coefficient, *t* *t*-statistic

Significant variables are indicated with *** ($p \leq 0.001$), ** ($p \leq 0.01$) and * ($p \leq 0.05$)

The bimodal distribution of *Lobaria pulmonaria*

We showed that altitude is the main factor determining the bimodal distribution of *L. pulmonaria* in the Uholka–Shyrokyi Luh primeval forest (Tables 4, 5; Figs. 3a, b, 4a). A bimodal altitudinal distribution for *L. pulmonaria* has been documented in other parts of Ukrainian Carpathians (Kondratyuk et al. 1998), Switzerland (Stofer et al. 2008) and southeastern Europe (Scheidegger et al. 2012).

The decreasing air temperature and increasing solar/UV-radiation with altitude are generally known climatic trend (Barry 1981; Körner 2007). The solar radiation varies little along the relatively short altitudinal gradient in our study region, and altitude not influences homogeneous forest structure within the primeval beech forest of Uholka–Shyrokyi Luh (Commarmot et al. 2013). Nevertheless, the probability to found trees with
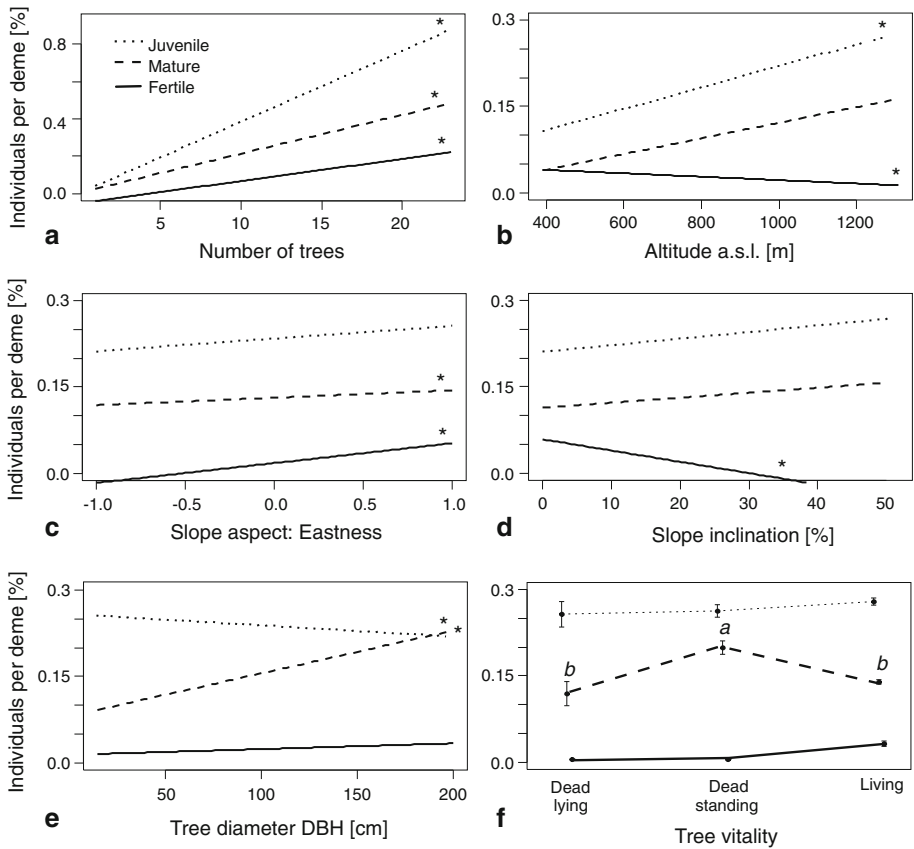
Fig. 7 The trends of variables, which explain density of *L. pulmonaria* per deme in a primeval beech forest of Uholka–Shyrokyi Luh: **a–e** linear regressions showing influence of number of trees per deme, altitude a.s.l., slope aspect and inclination, and host tree diameter. **f** Level of means between trees of different vitality. The *letters above* mean show differences in the level of means based on Tukey–Kramer HSD *t*-test. Developmental stages shown with different *lines*: juvenile with *dotted*, mature with *dashed*, and fertile with *solid line*. Developmental stages, which influence significant effect of tested variable, are shown with *asterisks*

L. pulmonaria is very low within forest stands with closed canopy along the entire altitudinal gradient, but the species was more frequently found between stands with loose or scattered canopy at higher altitudes (Fig. 3).

The lichen was most abundant along the timberline, than in the lowland meadows and interior woodland stands (Fig. 4b). A similar trend was found in the study region for species richness of epiphytic lichens within forest stands of different canopy closure and elevations (Dymytrova et al. 2014). Thus we conclude that both, altitude and canopy closure explain the bimodal distributional pattern of *L. pulmonaria* occupancy and density in the primeval beech forest. In addition to temperature gradients and light availability, non-linear gradients in air humidity among these habitats are hypothesized to explain the different lichen occupancy/density. Mountain ridges at timberline in the forest of Uholka–Shyrokyi Luh have a frequently fluctuating air humidity while the bottom of the slopes or valleys have more constant, relatively high humidity.

Habitat shift under dense canopy

Most of the lichen individuals grow on trees of small or mid-diameter classes, as most of the trees in the Uholka–Shyrokyi Luh lays in this diameter range (Commarmot et al. 2013). We showed that tree diameter did not affect the presence or total density of *L. pulmonaria* (Tables 4, 5), but it is essential for the density of juvenile and mature individuals (Table 4; Fig. 7). The host trees of smaller diameters of are often lacking microstructural diversity such as bark crevices and bryophyte patches, which may facilitate high lichen abundance. Similar trends of occupancy on trees with various diameters, including small, has been found for the sites of *L. pulmonaria* with optimal climate and habitat conditions (Carlsson and Nilsson 2009; Hakulinen 1964; James et al. 1977; Wolseley 1991). However many studies stated that old/large trees are more suitable for *L. pulmonaria* (Gu et al. 2001; Gustafsson et al. 1992; Mikhailova et al. 2005; Öckinger et al. 2005).

We found that *L. pulmonaria* prefers relatively open stands in the generally dense stands of the primeval beech forest of Uholka–Shyrokyi Luh (Fig. 3b, d). In lowland demes the lichen grows relatively high up at the tree trunk (Fig. 5), where more light is available and air humidity is still relatively high because of the topographic situation at the bottom of valleys. Obviously, the difference in microclimatic parameters, which are co-dependent with altitude, did not affect the beech forest stands which showed an uneven-aged structure at small spatial scales (Commarmot et al. 2005; Trotsiuk et al. 2012). However, the architecture of forest stands affects local temperature, solar radiation and air humidity, which may differ strongly from standard meteorological data (Körner 2007). The epiphytic lichen *L. pulmonaria* tends to grow either in habitats with loose or scattered canopy (Fig. 3b, d) where it grows at relatively low parts of the trunks, however the species' light requirements require a relatively high position at host tree trunk (Fig. 5) in stands with a higher canopy closure as it is generally found at lower altitudes in the study region. Low light availability at sites with *L. pulmonaria* has been reported to be limiting the establishment of diaspores (Hilmo et al. 2011; Jüriado et al. 2011). Under otherwise favourable climatic conditions the species is known to grow best in habitats with relatively high light availability (Jüriado et al. 2012; Pannewitz et al. 2003).

Our data revealed habitat shift (Schoener 1975) of *L. pulmonaria* between more open stands at highland timberline, where the mean height at the trunk was found at 2 m, and under more dense canopy in the interior woodland the preferred height at the trunk was 4 m (Fig. 5b). This habitat shift enables *L. pulmonaria* to select suitable habitats where light availability and humidity are the main limiting factors. Thus, *L. pulmonaria* can profit from higher air humidity close to the ground in the places where light is available down to the lower part of the trunk. In contrary, *L. pulmonaria* has to colonize higher parts of the trunk in places where light availability is limited. However, this is only possible under topographic situations where air humidity is high enough to allow for prolonged physiological activity, i.e., along the bottom of the valleys, but not slopes. The habitat shift described for *L. pulmonaria* corroborates the law of relative habitat constancy (Breckle 2002), which has previously been confirmed for various lichen species and their habitats, including xerothermic saxicolous species (Poelt 1987; Scheidegger 1991).

Dispersal strategies

We found mature individuals tending to grow on slopes of eastern exposition (Fig. 7b), what also typical for total density of trees with *L. pulmonaria* in the massive (Fig. 4c). Mature individuals prefer trees with large diameter (Fig. 7d), which are often dead

standing (Fig. 7f). This reflects long-lasting growth and local spread of *L. pulmonaria* thalli on "veteran" trees. Mature individuals are effective for vegetative dispersal, which is considering as a dominating strategy for dispersal of *L. pulmonaria* (Öckinger et al. 2005; Widmer et al. 2012).

Our analysis showed that fertile individuals were more frequent on eastern slopes and flat terraces, always on living trees and distributed in intermediate altitudes, mean at 850 m, and occurred mainly in the interior woodland stands (Figs. 1b, 7). This altitude correspond to canopy cover of 75.5 % (data of forest inventory), which is higher compared to *L. pulmonaria* demes growing between loose canopy (72.4 %) and scattered canopy (26.3 %). This may indicate that the formation of apothecia requires sheltered places in the forest under the climatic conditions of the study region. In other regions with more humid climate, e.g., at the west coast of Scotland (Rose 1976, 1992), apothecia can also be formed on isolated trees where the lichen is exposed to considerably higher light availability.

Furthermore, the formation of apothecia in the heterothallic species *L. pulmonaria* (Singh et al. 2012; Zoller et al. 1999) depends on the availability of suitable mates belonging to either of two mating types and on optimal growth conditions, which is often reflected by a high abundance (Mikryukov et al. 2010; Scheidegger 1995). In our study region apothecia were produced by thalli belonging to different developmental stages, including thalli without vegetative propagules, which are usually interpreted either as juvenile or suppressed (Honegger and Scherrer 2008).

Vegetative dispersal capacity has been reported as the most important factor in limiting the local distribution of *L. pulmonaria* (Öckinger et al. 2005), and the distance of 30 m is optimal for the vegetative propagation of *L. pulmonaria* (Werth et al. 2006). However, the trees with *L. pulmonaria* are scattered in the studied area at higher distances (Fig. 1b). Furthermore, relatively low percentage of mature and fertile individuals make local dispersal even more complicated. We suggest the *L. pulmonaria* supports dispersal within populations in a close relation to natural gap dynamic of the forest, i.e., fallen trees are a vector for lichen, which allow moving for a new host trees up to 30 m and more.

Low frequency of reproducing individuals

The juvenile thalli without any vegetative propagules (59.86 %) dominated in the studied populations of *L. pulmonaria* in the Uholka–Shyrokyi Luh primeval beech forest, while less thalli were mature, i.e., formed soredia or isidioid soredia (40.34 %; Fig. 6). This contrasts with studies, where the prevailing developmental stage of lichen was characterized as the reproduction by vegetative symbiotic propagules (Scheidegger 1995; Scheidegger et al. 2012). Sorediate individuals have been reported as dominated in populations (Mikryukov et al. 2010), while sterile thalli without vegetative propagules are of a comparatively short-living, transitional nature (Scheidegger et al. 1997; Scheidegger and Goward 2002).

Considerably higher levels of fertility are known from other regions, even not old-growth. That is, up to 25 % of fertile thalli were indicated for the lichen population along the eastern cost of North America (Denison 2003), 30 % for SW Finland (Carlsson and Nilsson 2009), 32 % from the NE of the Altai Mountains (Mikryukov 2011), 36 % for wooded meadows of Estonia (Jüriado et al. 2012). Following, we also expected higher presence of fertile thalli in the studied primeval forest. However, the only nearly 3 % of total thalli harboured fruit bodies in Uholka–Shyrokyi Luh (Figs. 1b, 6).

Thus we interpret the high percentage of sterile thalli, a relatively small average size of individuals and the low frequency of fertile thalli as a signature of a growing population of

L. pulmonaria within the primeval beech forest of Uholka–Shyrokyi Luh. We conclude that in this specific primeval beech forest under a continental, relatively dry climate harbours a very low density and abundance of *L. pulmonaria*. Increased population density of *L. pulmonaria* may occur next to a gap where light availability can support the growth during several decades until the next cohort of trees creates a light limited environment for the epiphytic lichen and bryophyte community at the trunks. Several cohorts of beech trees were however found to harbour *L. pulmonaria* within the same area (Fig. 7e), thus enabling a vast temporal connectivity at a small spatial scale. Additional evidence of spatio-temporal connectivity is the presence of the lichenicolous fungus *Plectocarpon lichenum* in several populations. Because this is a host-specific parasymbiont on *L. pulmonaria* its availability indicates the continuous presence of this lichen species (Ertz et al. 2005).

There is, however, an alternative hypothesis to explain the low frequency of reproducing individuals. Transboundary air pollution has possibly decreased the population frequency, abundance and portion of developmental stages during former decades. The decline in fertile specimens and total vitality has earlier been demonstrated for *L. pulmonaria* relating with acid rain (Öckinger et al. 2005) and ozone (Scheidegger and Schroeter 1995). Thus, the populations could also have suffered under elevated levels of air pollutants, including acid rain that was higher before 1990s (Oulehle et al. 2010; Šebesta et al. 2011; Vestreng et al. 2007). The pollution by acid rains and heavy metals are known from the surrounding regions of the Uholka–Shyrokyi Luh, as Ivano-Frankivsk and Chernivtsi regions in Ukrainian Carpathians (Shparyk and Parpan 2004), Romanian Transilvania (Lacatusu et al. 2001) and Romanian part of Eastern Carpathians (Donisa et al. 2000). The accumulation of heavy metals and radioactive elements were reported for lichens growing in Romanian Carpathians (Bartok 1988; Purvis et al. 1999). Accounting north-eastern crossroads for the air pollutant in upper troposphere (Lelieveld et al. 2002), it is likely that some environmental pollution also influenced our study area, especially because *L. pulmonaria* prefer eastern slopes (Fig. 7c). We showed that juvenile individuals tended to grow more densely at higher altitudes and at trees with small diameter (Fig. 7b, e). This may indicate substantial colonization of younger trees and spread to higher altitudes in the studied area. A possible explanation is that the highest elevations have been most affected by transboundary air pollution (Eilers et al. 1994). This could explain, why regeneration of *L. pulmonaria* is now more pronounced at high altitudes after a substantial decrease of transboundary air pollutants.

The decline of the lichens of *Lobarion* community was reported from the western part of Transcarpathians (Kondratyuk et al. 1998) and authors hypothesized possible relations with long-range atmospheric pollution during pre-1980s. Thus, transboundary air pollution may be suggested as a possible explanation for the untypical ratio of *L. pulmonaria* developmental stages observed in the study area. Recently this negative effect has decreased (Vestreng et al. 2007) and allows the lichen's regeneration either by vegetative dispersal or by regenerations of remnants of lobes. Therefore the low frequency and abundance of the lichen together with a low frequency of reproducing individuals is the consequence of elevated levels of air pollution during past decades. The high portion of trees that are colonized with juvenile thalli only can be considered an indication of a growing population since air quality has improved during the last 15 years or so. The high stand-structural diversity of the primeval forest landscape has obviously provided enough safe sites for the survival of *L. pulmonaria* over the entire area. This contrasts with other areas where the species went locally extinct because of air pollution (Wirth 1995). Because detailed data on air quality inside the primeval forest area are not available, we suggest that the

environmental pollution hypothesis has to be tested in the framework of a long-term monitoring of the *L. pulmonaria* population and its developmental stages in the primeval beech forest of Uholka–Shyrokyi Luh.

Conclusions

This is the first analysis of the occupancy and density of *Lobaria pulmonaria*, including its developmental stages in the largest primeval beech forest in Europe using a non-stratified sampling design of over 10,000 ha. The total 483 trees harbouring *L. pulmonaria* were distributed very scattered within Uholka–Shyrokyi Luh, and occupied nearly 10 % of studied perimeter. The GLMs showed that altitude and canopy cover influenced the presence of *L. pulmonaria* in the primeval beech forest, while the density of its populations was mostly affected by habitat type and slope aspect. We showed a bimodal distribution of *L. pulmonaria* which is frequent both below and above elevations of 900 m, between beech forest stands with loose and scattered canopy in the generally dense canopy of the primeval beech forest of Uholka–Shyrokyi Luh. The habitat shift enables *L. pulmonaria* to survive also in dense forest canopy by climbing high up along a host tree trunk to get the necessary level of insolation. In general, *L. pulmonaria* occupied trees with various diameters, but juvenile individuals are more frequent on small trees, while mature individuals were found on trees of average or large sizes. Fertile individuals require specific environmental conditions such as intermediate altitudes, favorable light conditions, and flat terraces in eastern expositions of the slopes. Overall, the primeval beech forest of Uholka–Shyrokyi Luh harbours a high percentage of juvenile thalli of *L. pulmonaria* without any vegetative propagules and a low frequency of fruit bodies. We interpret this as an indication of a growing population of *L. pulmonaria* within the area. In earlier decades transboundary air pollution has likely decreased the lichens' population occupancy and density.

The pronounced distribution pattern of *L. pulmonaria* within the largest European primeval beech forest, described here, has consequences for the understanding of lichens as indicator species of woodland ecological continuity (Whittet and Ellis 2013). A significant association of a putative indicator species with old-growth forest patches can only be expected within a narrowly defined ecological niche of the indicator species. Given the relatively dry regional climatic conditions of Eastern Europe the niche of *L. pulmonaria* in the primeval forest is limited to the bottom of valleys with continuously high relative air humidity and zones at higher altitude. Furthermore, indicators of ecological continuity are often rare species even within their niche and they show a patchy distribution pattern. Interactions between climatic conditions, habitat preferences and occurrence of indicator species can only be well-understood in large primeval forest reserves. Reserves, like the Uholka–Shyrokyi Luh primeval forest, are thus unique reference ecosystems to study complex interactions between primeval forest lichens and their environment.

Conflict of interest There is no conflict of interest declared.

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