Lichen speciation is sparked by a substrate requirement shift and reproduction mode differentiation

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Summary

We show that obligate lignicoles in lichenized *Micarea* are predominately asexual whereas most facultative lignicoles reproduce sexually. Our 3 loci phylogenetic analyses (ITS, mtSSU, *Mcm*7) together with ancestral state reconstruction show that the shift in reproduction mode has evolved independently several times within the group and that facultative and obligate lignicoles are sister species. The analyses support the assumption that the ancestor of these species was a facultative lignicole. We hypotezise that a shift in substrate requirement from bark to wood leads to differentiation in reproduction mode and becomes a driver of speciation. This is the first example of lichenized fungi where reproduction mode is linked to substrate requirement. This is also the first example where such a linkage is demonstrated to spark lichen speciation. Our main hypothesis is that obligate species on dead wood need to colonize new suitable substrata relatively fast and asexual reproduction is more effective a strategy for successful colonization. Our main hypothesis for explaining the discovered phenomenon involves the species' life cycle: species on decaying wood face a significant challenge because the ecological properties of their substratum change relatively fast. When this happens, species need to colonize new suitable substrata. This may set a time limit, where asexual reproduction is a faster and more effective strategy for successful colonization.

Introduction

Despite increased knowledge on lichen diversity, the factors influencing species richness and speciation are still largely unknown. Existing studies have mostly focused on extrinsic factors and found that diversification events are usually correlated with climatic changes such as climatic cooling events during the Tertiary (Printzen & Lumbsch 2000), aridification during the Oligocene–Miocene transition (Kraichak et al. 2015) and Pleistocene glacial cycles (Leavitt et al. 2013). Only few studies on lichens have considered extrinsic environmental factors and intrinsic lineage-specific traits jointly: Innovations in secondary chemistry (= extracellular products) together with a shift in substrate requirement were found to trigger adaptive radiation in the lichen family Teloschistaceae (Gaya et al. 2015). Increased nitrogen availability after acquisition of cyanobacterial symbionts led to an adaptive radiation in *Placopsis* (L.) Linds (Schneider et al. 2016). Green-algal or cyanobacterial symbiont interactions through time and space may influence diversification in the genus *Sticta* (Schreb.) Ach. (Widhelm et al. 2018). Interplay between intrinsic traits related to reproduction and extrinsic traits related to ecological opportunities are often correlated with shifts in species diversification in other

organisms (Vamosi & Vamosi 2011, Wagner et al. 2012, Karunarathne et al. 2018, Nakov et al. 2018) but to our knowledge, this has never been examined in lichenized fungi.

Lichenized fungi have developed diverse reproduction strategies. Many have the ability to reproduce both sexually (ascospores) and asexually (conidia, thallus fragments i.e. soredia, isidia, goniocysts), while some are either sexual or asexual (Tripp 2016, Tripp & Lendemer 2018). Diverse reproduction strategies are at least partly related to lichen symbiosis: asexual reproduction via thallus fragments ensures the continuation of symbiosis (Bowler & Rundell 1975), whereas successful sexual reproduction via ascospores requires that the germinating mycelium makes contact with a compatible free-living photobiont before the lichen thallus can be developed (Buschbom & Mueller 2006). One exception is the asexual propagules produced by the fungal partner, called conidia: they do not contain the photobiont, so they need to find a suitable one from the new substratum they land on. However, both modes of asexual reproduction consume less energy than sexual reproduction and do not rely on the availability of suitable mating partners. Therefore, asexual lichen lineages have been suggested to be more efficient and rapid at colonizing newly exposed substrates (Poelt 1963, Stofer et al. 2006, Ludwig et al. 2017). Recent studies on lichens have shown that asexual lineages are long-lived evolutionarily and can give rise to sexual lineages (i.e. are not evolutionary dead ends), suggesting that asexual lineages may represent a source for evolutionary innovation (see Tripp 2016).

The microlichen genus *Micarea* is an excellent model for studying the effects of reproductive traits and environmental factors on speciation. The genus is widespread worldwide and has lately received much scientific interest, resulting in over 20 new species descriptions (Czarnota 2007; Czarnota & Guzow-Krzemińska 2010; Sérusiaux et al. 2010; van den Boom et al. 2017; Guzow-Krzemińska et al. 2016 & 2019; Kantvilas & Coppins 2019; Launis & Myllys 2019; Launis et al. 2019a, b; van den Boom et al. 2020; Kantelinen et al. 2021a). *Micarea* shows intricate variation in substrate requirements and reproduction modes (Coppins 2009). Certain species are generalists able to grow on various substrata, while some are specialized and living in strict microhabitats (Coppins 1983, Czarnota 2007, Launis & Myllys 2014, Myllys & Launis 2018). A wide range of sexual and asexual propagules is found in *Micarea* (Fig.1), including ascospores, three types of conidia (micro-, meso-, and macroconidia), and thallus fragments called goniocysts that likely act as asexual propagules including both symbiotic partners. Some of the species are predominately sexual, while some frequently lack sexual fruiting structures but bear numerous pycnidia where asexual conidia are produced. The actual roles of the three types of conidia present are not thoroughly known, but Coppins (1983) suggested that mesoconidia act as asexual propagules, based on an observation that several species frequently occur without apothecia but have numerous mesoconidia-producing pycnidia.

Micarea represents one of the most important microlichen genera occurring on dead wood (Spribille et al. 2008). Of the 27 European species in the *M. prasina* group (a monophyletic "core group" including the type species), 17 are facultative lignicoles, five are obligate lignicoles, and four have never been found on dead wood (Czarnota 2007; Spribille et al. 2008; Guzow-Kremínska et al. 2016; Launis et al. 2019a, b; Launis & Myllys 2019; van den Boom et al. 2020). Of the facultative lignicoles, some species are often encountered on dead wood while others rarely occupy the substratum. Furthermore, some of the obligate lignicoles are rare and have very narrow ecological amplitudes,

occuring only on wood of specific decay stages (Czarnota 2007; Myllys & Launis 2018; Launis & Myllys 2019).

In this study, the aim is to examine the reproduction modes and evolution of substrate preferences in the *M. prasina* group and how these features affect speciation. The focus is especially on asexual mesopycnidia and presence/absence of sexual apothecia.
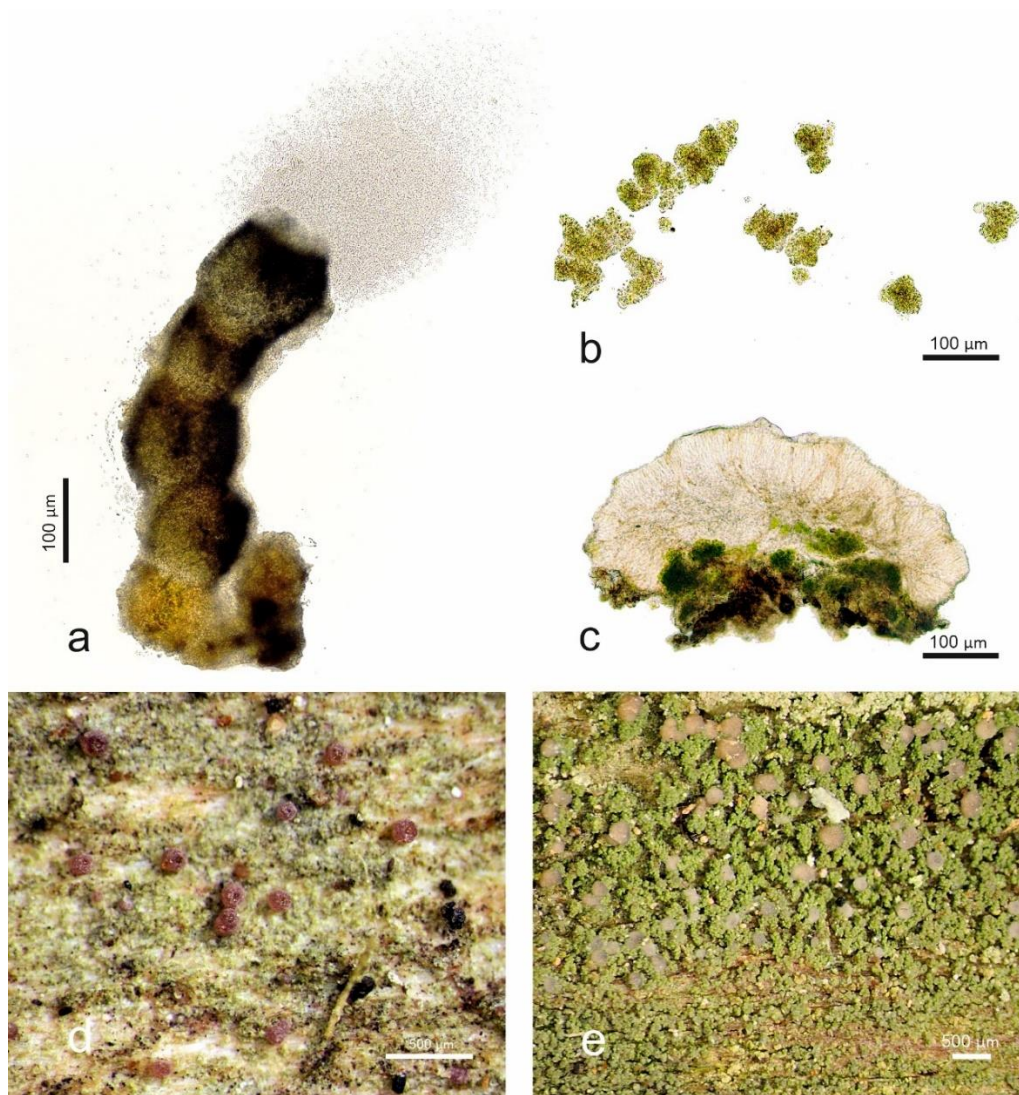

Fig. 1 Sexual and asexual structures and reproduction strategies in the *Micarea prasina* group. a.) A pycnidium of *Micarea fennica* extruding asexual mesoconidia (Kantelinen 3220 holotype, H). Mesoconidia are small, likely easily carried by wind and insects and allow long-distance dispersal, b.) Thallus goniocysts of *M. hedlundii* including a mycobiont and a photobiont (Kantelinen 67119, H). Goniocysts are asexual vegetative structures that are relatively big and therefore probably more effective on short distance colonization, c.) Apothecial section of *M. microareolata* (Pykälä 47787, H). Sexual ascospores developed in apothecia are small, likely easily carried by wind and insects, and their development requires a mating partner and more energy than asexual diaspores, d.) Pycnidia and thallus of *M. tomentosa* (Kantelinen 29151, H), e.) Apothecia and thallus of *M. prasina* (Kantelinen 229106, H). Photos A. Kantelinen.

Results

Altogether 516 herbarium specimens were studied. Each specimen was identified to species level, and the reproduction mode and substrate was recorded (Appendix). Our results confirm previous findings that obligate and facultative preference for dead wood are species-specific traits (e.g. Coppins 1983; Czarnota 2007; Launis et al. 2019a&b).

Representative specimens of each taxa were selected for phylogenetic reconstruction (Table 1). The analyses included 3 loci (ITS, *Mcm*7, mtSSU) and consisted of 110 sequences and of 1655 characters. The topologies of the Bayesian and maximum likelihood analyses did not show any strongly supported conflicts, and therefore only the tree obtained from the Bayesian analysis is shown (Fig. 2).
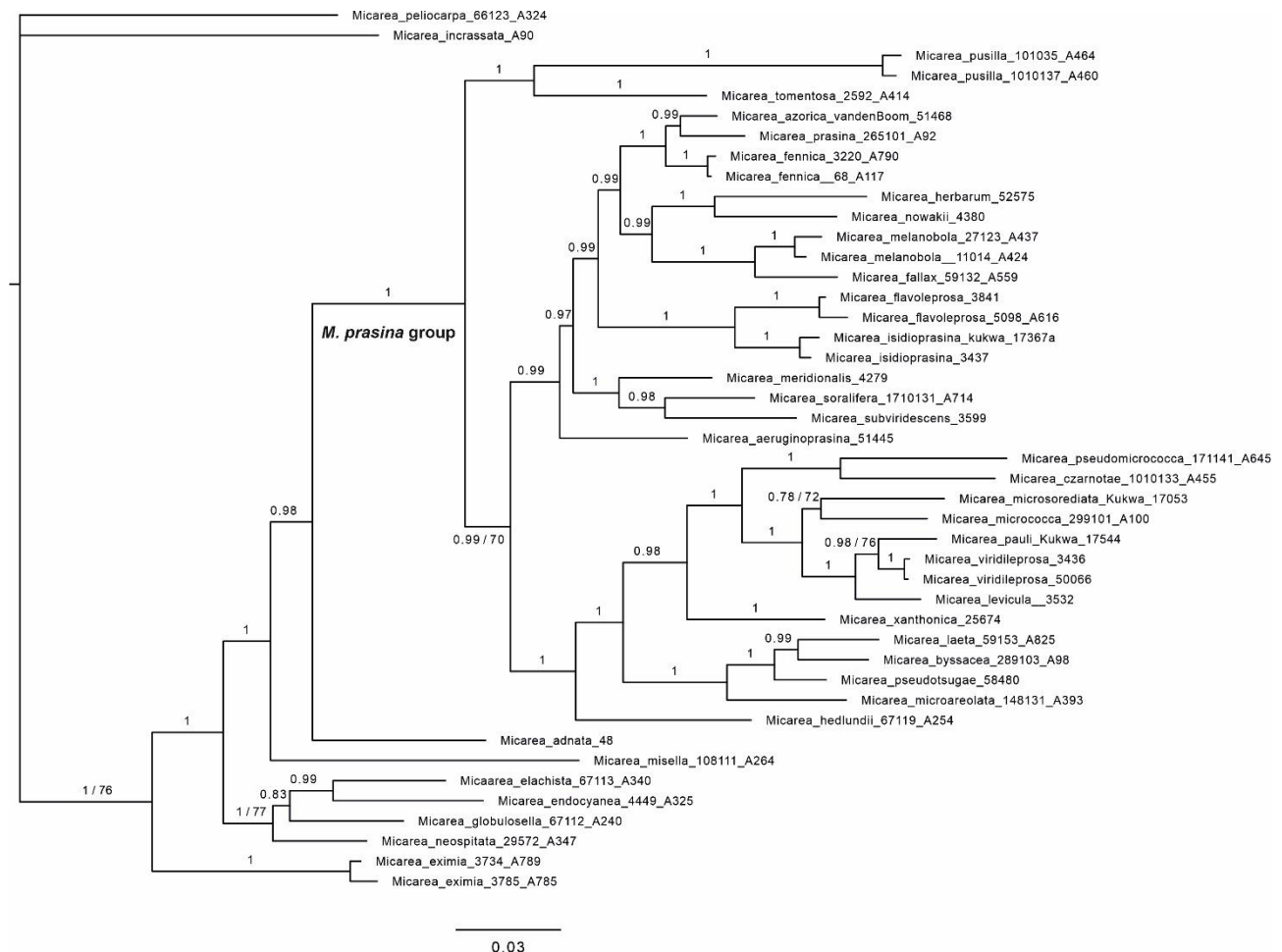

Fig. 2 Bayesian tree based on concatenated sequences of ITS, mtSSU and *Mcm7*. Bayesian posterior probabilities are indicated above the nearest branches. Maximum likelihood values are marked if less than 80.

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Our results show that obligate lignicoles occupying mid- to late decay stages are predominately asexual while most facultative lignicoles reproduce sexually. Our ancestral state reconstruction shows that the shift in reproduction mode has evolved independently several times within the group and that facultative and obligate lignicoles are sister species (Fig. 3). Furthermore, the ancestral state reconstructions support the ancestor of these species being a facultative lignicole. The correlation between reproductive mode and substrate preference is statistically significant (Table 2).

With strong support values, our phylogenetic reconstruction shows that lignicolous substratum preference has evolved several times independently within the group. Three out of the five obligate lignicoles are nested in the *M. prasina* -complex, i.e. *M. fennica*, *M. flavoleprosa,* and *M. nowakii*. *Micarea hedlundii* is resolved to be a separate lineage as a sister of the *M. byssacea* and *M. micrococca* complexes. *Micarea tomentosa* is resolved as a sister of the *M. byssacea*, *M. micrococca,* and *M. prasina* complexes. The 18 facultative lignicoles in our dataset are found in several lineages within the phylogeny.
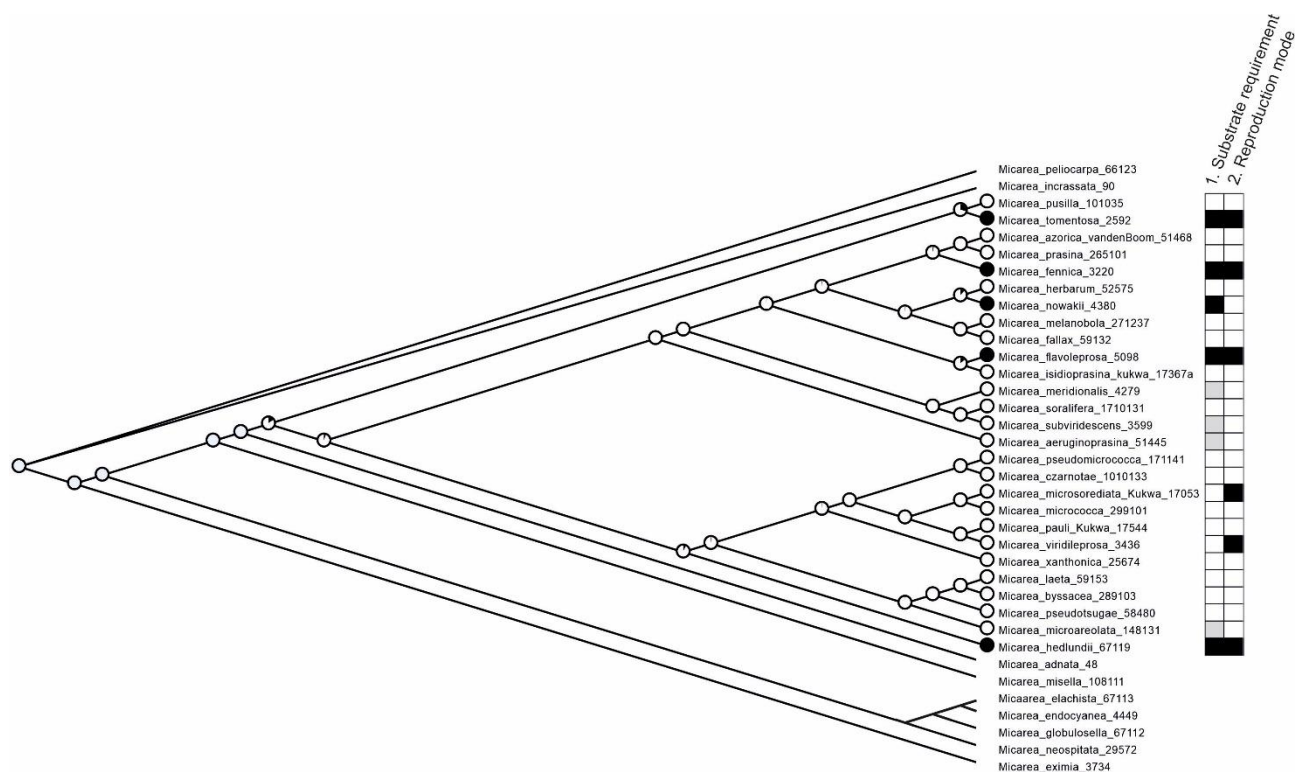

Fig. 3 A maximum likelihood phylogram depicting ancestral character state reconstruction of the evolution of obligate lignicoles. Individuals of the same species were pruned and collapsed at the branches of the corresponding nodes. Pies represent probabilities of each ancestor being in two potential states for obligate lignicole (yes = black, no = white). In addition, substratum requirement and reproduction mode are mapped with black, grey and white boxes at the tips of the tree as follows: 1. Substratum requirement: black = obligate lignicole; white = facultative lignicole; grey = never found on dead wood. 2. Reproduction mode: black = predominately asexual; white = predominately sexual.

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Discussion

Here, we report the *Micarea prasina* group as a first example of lichenized fungi where reproduction mode is linked to substrate requirement (Table 2 and 3). This is also the first example where such a linkage is demonstrated to be a driver of lichen speciation. Our study reveals that the shift in reproduction mode has evolved independently several times within the group and that facultative and obligate lignicoles are sister species (Fig. 3). Before our study, intrinsic and extrinsic factors involved in lichen speciation have rarely been studied, and relationships between lichen substrate requirements and reproduction modes have not been deeply understood (but see Spribille et al. 2008; Resl et al. 2018).

Many species in the *M. prasina* group are important colonizers of dead wood (eg. Czarnota 2007). Spribille et al. (2008) concluded that most of the obligate lichen species growing on dead wood are sexually reproducing crustose lichens. Contrary to this view, our study shows that *Micarea* species occupying bark are predominantly sexual in their reproduction mode, whereas species restricted to dead wood reproduce asexually.

Several interpretations are possible for explaining our results. Our main hypothesis involves the species' life cycle. Species on decaying wood face a significant challenge as their substratum gradually changes and inevitably vanishes. When this happens, species need to colonize new suitable substrata. This may set a time limit, where asexual reproduction via mesoconidia, or with goniocysts acting as diaspores, is a faster and more effective strategy for successful colonization. In previous studies, asexual lichen lineages have been suggested to be more efficient and rapid at colonizing newly exposed substrates (Poelt 1963, Stofer et al. 2006, Ludwig et al. 2017). The complete decay of a log or stump can, however, take decades depending on the position of the tree (Stokland et al. 2012), and being restricted to an ephemeral substrate only leads to selection towards asexual reproduction if the generation time is long enough to effectively limit reproduction. Our results and literature show that many obligate lignicoles are restricted to certain decay stages (Coppins 1983; Czarnota 2007; Launis & Myllys 2019; Launis et al. 2019b), which shortens the time frame they have for growth and reproduction (Russell et al. 2013; Russell et al. 2014). *Micarea fennica*, *M. flavoleprosa*, *M. hedlundii*, and *M. tomentosa* mostly occur on late decay stages. *M. nowakii*, on the other hand, occupies hard wood in well-lit habitats. It is the only obligate lignicole in our data set that is predominately sexual (with additional mesoconidia nearly always present). This observation of *M. nowakii* can be explained by our hypothesis: selection would not work against sexual reproduction because the decay process is slower in the niche it favors and the time limit for growth and reproduction is not as strictly limited.

The background of the speciation process suggested above is unknown, but a possible population genetic scenario is that generalist species experience lower fitness on wood than on bark, and selection favors genotypes that are better adapted to wood. These adaptations to wood could have made the species less competitive on bark, ultimately leading to exclusive lignicoles.

In addition to our main hypothesis, the results could be explained by three alternative hypotheses, although we consider them as less likely. **Our first alternative hypothesis** is ecological: facultative lignicoles could experience lower fitness and produce less offspring on wood than on bark because of some inherent wood properties. Perhaps wood suppresses the formation of viable ascospores in

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Micarea (even if apothecia may still be produced) and being less dependent on ascospores for reproduction would lead to selection favoring asexual reproduction. Although logical, we do not have any evidence to support this theory. **Our second alternative hypothesis** is dead wood rarity: species in the *M. prasina* group could be heterothallic, and dead wood rarity in space and time could lead to fragmented and geographically isolated populations – Zoller *et al.* (1999 b) showed that such a situation could hinder the possibility for finding a mating partner, and sexual reproduction would therefore become unnecessary and rare. Plasticity in the reproduction of heterothallic species has previously been recorded in several lichen genera (Honegger et al. 2004; Honegger & Zippler 2007; Ament-Velásquez et al. 2021). However, despite this possibly explaining the prevalence of asexual reproduction in obligate lignicoles, it only holds true if populations on wood have already lost their capacity to live on bark. In other words, the development of asexual reproduction is only possible after speciation has already occurred. Substrate specialization could still lead to asexual reproduction but not be a driver of speciation. To date, we have no evidence to support this theory, e.g. *Micarea* specimens collected in areas with high dead wood densities are not more frequently sexual. In addition, the mating systems of *Micarea* have not been examined. **Our third alternative hypothesis** is closely connected to the previous one: studies (e.g. Ament-Velásquez et al. 2021) have shown that lichens can reproduce asexually near the margins of their natural distribution while remaining sexual in central areas. This is usually because genetic diversity decreases towards margins making it difficult for heterothallic species to find mating partners. In theory, the obligate lignicoles in our data set could represent marginal populations of sexually reproducing species. So far, we have no evidence to support this theory either. We also consider it illogical that distribution would only affect obligate lignicoles, while facultative lignicoles remain sexual.

Our data includes two species that are predominately asexual but are not obligate lignicoles. *Micarea microsorediata* and *M. viridileprosa* occur on bark, wood, and on terrestrial substrates such as mosses. They rarely develop apothecia, and pycnidia have never been found from the latter. *Micarea microsorediata* mostly occurs in microhabitats where only few other lichen species co-exist, namely asexual *Lepraria* (Guzow-Krzemińska et al. 2019). *Micarea viridileprosa*, on the other hand, develops widely spreading thallus that consists of small granules called goniocysts, and it appears to be an opportunistic species that is occasionally found on ephemeral substrates and growing over mosses and other lichens (Czarnota 2007; van den Boom & Coppins 2001). Based on our results, *Micarea* species that are not obligate lignicoles are mostly sexual in their reproduction mode. However, the results on *M. microsorediata* and *M. viridileprosa* indicate that species occupying demanding low-light microhabitats or those that have opportunistic lifestyles benefit from asexual reproduction. Ecological strategies, niche requirements, and reproduction mode often correlate in other organisms (e.g. Simon et al. 2002; Silvertown 2008; Gomez-Mestre et al. 2012; Dańko et al. 2020).

Conclusions
Our results show that asexual reproduction is an evolutionary reaction on substrate specialization. Based on our preliminary results the relationship between asexual reproduction mode and wood-inhabiting lifestyle appears to exist beyond the *M. prasina* group. For example, *M. anterior*, *M. misella,* and the taxa in the *M. nigella* group are mostly found on dead wood and are predominately

asexual (Coppins 1983, Czarnota 2007, Myllys & Launis 2018). Future large-scale phylogenetic analyses will clarify how widespread this phenomenon is.

In general, asexual reproduction in lichens is often regarded as a deficient version of sexual reproduction, but we encourage instead to view it more as a gained ability and an advantageous strategy.

Methods

Taxon sampling

Altogether 516 *Micarea* specimens were studied in the herbarium collections of FR, GPR, H, UPS and few specimens were also studied in LE, O and Hb Malíček (Appendix). We included all available specimens of relevant species into our data set. The studied specimens are collected from bark and dead wood by several collectors between 1940 and 2019. Older specimens from the 1800s and early 1900s were excluded from our study because asexually reproducing lichens were rarely collected at the time. Our data set includes *Micarea* specimens from the best known and widely collected areas in the world, i.e. Fennoscandia (Finland, Norway, Sweden) and Central Europe (Belarus, Czech Republic, the Netherlands, Germany, Poland, western Russia).

Reproduction structures (apothecia/mesopycnidia) and substratum (bark/dead wood/other) were recorded for each specimen (Appendix). The predominant reproduction mode and substrate requirement (facultative/obligate lignicole) were then calculated for each species using percentages (Table 3). Species with over 94% occurrence on dead wood were regarded as obligate lignicoles, *M. tomentosa* being the only such species with less than 100% occurrence on dead wood (one specimen from Poland is collected from decaying bark).

Relevant literature on species ecology was also studied (Coppins 1983; Coppins & Tønsberg 2001; Czarnota 2007; Czarnota & Guzow-Krzemińska 2010; van den Boom et al. 2017; Guzow-Krzemińska et al. 2016 & 2019; Myllys & Launis 2018; Konoreva et al. 2019; Launis & Myllys 2019; Launis et al. 2019a, b; van den Boom et al. 2020; Kantelinen et al. 2021b; Weber et al. 2021), and our results are in line with it. Some of the specimens studied by previous authors are not included in our data set, however, because substrate and reproduction mode for the specimen is rarely reported.

Morphology and chemistry

Each specimen in the data set was carefully studied and identified. Specimens were initially studied using dissecting microscopes. Anatomical features were then examined on hand-cut apothecial sections and squash preparations mounted in water using compound microscopes. Ascospore dimensions and other anatomical measurements were made in water and in potassium hydroxide (K). Chemical spot tests were performed under a compound microscope using sodium hypochlorite (C) and 10% (K) (Orange et al. 2010). Pigments were defined following Coppins (1983), Meyer & Printzen (2000), and Czarnota (2007). Some specimens were further studied using thin-layer chromatography (solvent C) following Culberson & Kristinsson (1970) and Orange et al. (2010). The

crystalline granules of selected specimens were investigated using compound microscopes with polarization lenses.

DNA extraction, polymerase chain reaction, and DNA sequencing

Selected specimens were sequenced and compared to sequences in GenBank to confirm species identifications. However, the majority of sequences have already been prepared for our previous studies (e.g. Launis et al. 2019b; Kantelinen et al. 2021a). Genomic DNA was extracted from 1–3 apothecia of specimens stored for a maximum of one year, using the DNeasy Blood & Tissue Kit (Qiagen, Maryland, USA) following the protocol described by Myllys et al. (2011). Polymerase chain reactions (PCRs) were prepared using PuReTaq Ready-To- Go PCR Beads (GE Healthcare, Chicago, Illinois, USA). Each 25-µl reaction volume contained 19 µl distilled water (dH2O), 1 µl of each primer (10 µM), and 4 µl extracted DNA. The primers listed below were used for PCR amplification and sequencing. For the ITS region, PCR was run under the following conditions: initial denaturation for 5 min at 95 °C followed by five cycles of 30 s at 95 °C (denaturation), 30 s at 58 °C (annealing), and 1 min at 72 °C (extension); for the remaining 40 cycles, the annealing temperature was decreased to 56 °C; the PCR program ended with a final extension for 7 min at 72 °C. The primers used were ITS1-LM (Myllys et al. 1999) and ITS4 (White et al. 1990). For the mtSSU gene, PCR was run under the following conditions: initial denaturation for 10 min at 95 °C followed by six cycles of 1 min at 95 °C (denaturation), 1 min at 62 °C (annealing), and 1 min 45 s at 72 °C (extension); for the remaining 35 cycles, the annealing temperature was decreased to 56 °C; the PCR program ended with a final extension of 10 min at 72 °C. The primers used were mrSSU1 and mrSSU3R (Zoller et al. 1999). For the *Mcm*7 gene, PCR was run under two different conditions depending on the primers selected. For the first protocol, initial denaturation for 10 min at 94 °C was followed by 38 cycles of 45 s at 94 °C (denaturation), 50 s at 55 °C (annealing), and 1 min at 72 °C (extension), with the PCR program ending with a final extension for 5 min at 72 °C. The primers used were MCM7_AL1r and MCM7_AL2f (Launis & Myllys 2019). The second protocol used an initial denaturation for 10 min at 94 °C, followed by 38 cycles of 45 s at 94 °C (denaturation), 50 s at 56 °C (annealing), and 1 min at 72 °C (extension); the PCR program ended with a final extension for 5 min at 72 °C. The primers used were x.Mcm7.f (Leavitt et al. 2011) and Mcm7.1348R (Schmitt et al. 2009). PCR products were cleaned and sequenced by Macrogen Inc. (Amsterdam, The Netherlands; www.macrogen.com).

Phylogenetic analyses

Phylogenies comprising 29 ITS, 44 mtSSU, and 37 *Mcm*7 sequences were first aligned separately with MUSCLE v.3.8.31 (Edgar 2004) using the European Molecular Biology Laboratory, European Bioinformatics Institute's (EMBL-EBI) freely available web server (http://www.ebi.ac.uk/Tools/msa/muscle/). The single gene trees did not show any strongly supported conflicts according to the approach of Kauff & Lutzoni (2002) (with threshold bootstrap values ≥ 75%), and the three data sets were combined into a concatenated matrix in PhyDE® (Phylogenetic Data Editor, http:// www.phyde.de/index.html). Based on our previous studies (Launis et al. 2019a, b) and our preliminary phylogenetic reconstruction, *Micarea incrassata* Hedl. and *M. peliocarpa* (Anzi) Coppins & R. Sant. were used as outgroups. The hypervariable region at the end of the mtSSU and the ambiguously aligned region at the end of the ITS2 were removed from the analyses. The concatenated data set, including 44 terminals, was subjected to Bayesian inference using MrBayes (v. 3.2.7a) (Ronquist & Huelsenbeck 2003) and to maximum likelihood (ML) analysis using RAxML

8.1.15 (Stamatakis 2014). For the Bayesian analysis, substitution models were selected by having the MCMC procedure sample across models (Huelsenbeck et al. 2004). The convergence of the four parallel runs was checked after 500 000 generations using Tracer (v. 1.5)(Rambaut et al. 2018) and graphed using FigTree (v. 1.4.4). For the ML analysis, the combined data set was assigned to seven partitions: ITS1, 5.8S, ITS2, mtSSU, and each of the three codon positions of *Mcm*7. An independent GTR + G model was used for each subset, and branch lengths were assumed to be proportional across subsets. Node support was estimated with 1000 bootstrap replicates using the rapid bootstrap algorithm. The alignments are available from the Dryad Digital Repository (https://doi.org/xx).

Ancestral state reconstruction
A binary matrix was prepared with character states given for each taxon (obligate lignicole: yes/no). Reconstructions were made with Mesquite 3.40 (Maddison & Maddison 2018) using parsimony and maximum likelihood methods.

In addition, substratum requirement and reproduction mode were studied by mapping states at the tips of the tree based on calculations in Table 3. Substratum requirement was mapped as: 1. obligate lignicole; 2. facultative lignicole; 3. neither. Predominant reproduction mode was mapped as: 1. asexual, i.e. mesopycnidia present and often numerous, apothecia rare or absent; 2. sexual, i.e. apothecia present and often abundant, mesopycnidia sometimes present (in some cases mesopycnidia may be present but invisible).

Statistical test
A Fisher´s Exact test was performed for our data to test if the association between species´ reproduction modes and substrate preferences are significant across the studied species. The test statistic value is 0.0114, and the result is significant at $p < .05$ (Table 2).

Contributions

A.K. conceived the study and designed it. Analyses were carried out by A.K. with input from C.P., P.P. and L.M. C.P facilitated access to the Germain specimens and provided important context for their use. A.K. led the interpretation of the data and writing of the manuscript, with valuable input from C.P., L.M., and P.P.

Ethics declarations

The authors declare no competing interests.

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Table 1 List of *Micarea* specimens used in the phylogenetic analyses with locality, voucher information and GenBank accession numbers.

Taxon	Locality	Voucher information, sequence ID	ITS	mtSSU	*Mcm7*
M. adnata	Japan	*Andersen* 48 (BG)	AY756468	AY567751	—
M. aeruginoprasina	Portugal, Azores	*van den Boom* 51445 (LG), 3973	—	MK562024	MN105888
M. azorica	Portugal, Azores	*van den Boom* 51468 (LG), 3977	—	MK562026	MN105891
M. byssacea	Finland	*Launis* 289103 (H), A98	MG521562	MG707768	MG692527
M. czarnotae	Finland	*Launis* 1010133 (H), A455	MG521557	MG707760	MG692517
M. elachista	Finland	*Launis* 67113 (H), A340	MG521548	MG707745	—
M. endocyanea	USA, Maine	*Kantelinen* 4449 (H), A325	MT981601	MT982135	MT981445
M. eximia	Finland	*Kantelinen* 3785 (H), A785	MT981600	MT982134	MT981444
M. eximia	Finland	*Kantelinen* 3734 (H), A789	MT981599	MT982133	MT981443
M. fallax	Finland	*Launis* 59132 (H), A559	MK454942	MK454759	MK456617
M. fennica	Finland	*Launis* 3220 (H), A790	MK517712	MK517716	MK520931
M. fennica	Finland	*Launis* 68 (H), A117	MK517711	MK517715	MK520930
M. flavoleprosa	France	*Sérusiaux* s.n. (LG), 3841	—	MK454754	MK456613
M. flavoleprosa	Czech Republic	*Malíček* 5098 (H), A616	—	MK454756	MK456615
M. globulosella	Finland	*Launis* 67112 (H), A240	MG521546	MG707743	MG692507
M. hedlundii	Finland	*Launis* 67119 (H), A254	MG521551	MG707749	MG692512
M. herbarum	Netherlands	*P.* & *G. van den Boom* 52575 (hb. van den Boom), LG DNA 4236	—	KX459349	MG692513
M. incrassata	Finland	*Kantelinen* 90 (H), A90	MT981598	MT982132	MT981442

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M. isidioprasina	France	*Sérusiaux* s.n. (LG), 3437	MN095788	KX459362	MN105894
M. isidioprasina	Poland	*Kukwa* 17367a & *Łubek* (UGDA)	MN095789	MK562016	MN105897
M. laeta	Finland	*Launis* 59153 (H), A825	MG521565	MG707771	MG692530
M. levicula	France, Réunion	*Sérusiaux* s.n. (LG), 3532	—	MK562019	MN105900
M. melanobola	Finland	*Launis* 27123 (H), A437	MK454946	MK454770	MK456625
M. melanobola	Finland	*Launis* 11014 (H), A424	MK454950	MK454774	MK456630
M. meridionalis	Portugal	*van den Boom* s.n. (LG), 4279	—	KX459353	MN105901
M. microareolata	Sweden	*Launis* 148131 (H), A393	MG521558	MG707762	MG692518
M. micrococca	Finland	*Launis* 299101 (H), A100	MG521552	MG707753	MG692514
M. microsorediata	Poland	*Kukwa* 17053 (UGDA)	MN095791	MK562012	MN105906
M. misella	Finland	*Launis* 108111 (H), A264	MG521545	MG707742	MG692506
M. neostipitata	USA, North Carolina	*Lendemer* 29572 (H), A347	—	MT982136	—
M. nowakii	Romania	*Sérusiaux* s.n. (LG), 4380	—	KX459359	MN105908
M. pauli	Poland	*Kukwa* 17544 & *Łubek* (UGDA)	MN095795	MK562010	MN105913
M. peliocarpa	USA, Maine	*Launis* 66123 (H), A324	MG521544	MG707741	MG692505
M. prasina	Finland	*Launis* 265101 (H), A92	MG521549	MG707747	MG692510
M. pseudomicrococca	Scotland	*Launis* 171141 (H), A645	MG521556	MG707758	MG692516
M. pseudotsugae		*van den Boom 58480 UGDA*	—	MN547361	—
M. pusilla	Finland	*Launis* 1010137 (H), A460	MK454941	MK454752	MK456611
M. pusilla	Finland	*Launis* 101035 (H), A464	—	MK454753	MK456612

M. soralifera	Poland	*Kukwa* 13001 & *Łubek* (UGDA)	KT119887	KT119886	MN105917
M. subviridescens	Scotland	*Czarnota* 3599 (GPN)	—	EF453666	—
M. tomentosa	Finland	*Kantelinen* 2592 (H), A414	—	MT982138	MT981447
M. viridileprosa	Poland	*Czarnota* 3436 (GPN)	—	EF453671	—
M. viridileprosa	Netherlands	*P.* & *B. van den Boom*, 50066 (hb. van den Boom), LG DNA 3493	—	KX459366	MN105918
M. xanthonica	USA	*Tønsberg* 25674 (BG)	—	AY756454	—

Table 2 Significance of the association between species´ reproduction modes and substrate preferences studied by Fisher Exact Test. The test statistic value is 0.0114, and the result is significant at p < .05.

	Sexual	Asexual	Marginal Rows Totals
Obligate lignicole	1	4	5
Facultative lignicole	14	2	16
Marginal Column Totals	15	6	21 (grand total)

Table 3 Number of studied specimens, their reproduction mode and substrate requirement. Five species are recorded based on literature: *M. herbarum* and *M. meridionalis* (van den Boom 2017), *M. subviridescens* (van den Boom 2003 & NBN Atlas online records from herbarium E), *M. viridileprosa* (van den Boom & Coppins 2001) and *M. xanthonica* (Coppins & Tønsberg 2001).

Species	No of studied specimens	No of speciemens with apothecia	No of speciemens with pycnidia	No of specimens on dead wood	No of specimens on bark	No of specimens on other substrata	% of specimens on dead wood	Substrate preference and predominant mode of reproduction
Micarea aeruginoprasina	4	4	rarely visible	0	4	0	0	Never on dead wood, sexual
Micarea azorica	4	4	rarely visible	0	4	0	0	Never on dead wood, sexual
Micarea byssacea	92	92	rarely visible	10	82	0	10, 9	Facultative lignicole, sexual
Micarea czarnotae	10	10	10	2	8	0	20	Facultative lignicole, sexual
Micarea fallax	52	52	rarely visible	46	7	0	86,8	Facultative lignicole, sexual
Micarea fennica	4	0	4	4	0	0	100	Obligate lignicole, asexual
Micarea flavoleprosa	9	1	5	9	0	0	100	Obligate lignicole, asexual
Micarea hedlundii	53	5	53	53	0	0	100	Obligate lignicole, asexual
Micarea isidioprasina	10	7 (few)	rarely visible	6	2	2	60	Facultative lignicole, sexual
Micarea laeta	19	19	rarely visible	2	17	0	10,53	Facultative lignicole, sexual
Micarea melanobola	24	24	rarely visible	9	15	0	37,5	Facultative lignicole, sexual
Micarea microareolata	9	9	rarely visible	0	9	0	0	Never on dead wood, sexual
Micarea micrococca	10	10	rarely visible	5	5	0	50	Facultative lignicole, sexual
Micarea microsorediata	29	4 (few)	none	7	22	0	24,1	Facultative lignicole, asexual
Micarea nowakii	17	17	14	17	0	0	100	Obligate lignicole, sexual
Micarea pauli	9	6	rarely visible	2	7	0	22,2	Facultative lignicole, sexual
Micarea prasina	43	43	rarely visible	38	5	0	88,37	Facultative lignicole, sexual
Micarea pseudomicrococca	23	23	rarely visible	14	9	0	60,86	Facultative lignicole, sexual
Micarea pseudotsugae	4	4	rarely visible	1	3	0	25	Facultative lignicole, sexual
Micarea pusilla	17	17	rarely visible	14	3	0	82,35	Facultative lignicole, sexual
Micarea soralifera	63	40	none	54	9	0	85,71	Facultative lignicole, sexual
Micarea tomentosa	17	5	17	16	1	0	94,12	Obligate lignicole, asexual
Micarea viridileprosa	29	Ap. rare	very rare	10	11	8	34,48	Facultative lignicole, asexual
Micarea xanthonica	41	20	not seen	not known	not known	not known	not known	Facultative lignicole, sexual