A candidate gene cluster for the bioactive natural product

gyrophoric acid in lichen-forming fungi

3

4 Garima Singh^{1,2,*}, Anjuli Calchera¹, Dominik Merges¹, Jürgen Otte¹, Imke Schmitt^{1,2,3},

5 Francesco Dal Grande^{1,2}

6 ¹ Senckenberg Biodiversity and Climate Research Centre (SBiK-F), 60325 Frankfurt am

7 Main, Germany * e-mail: garima.singh@senckenberg.de

8 ² LOEWE Center for Translational Biodiversity Genomics (TBG), 60325 Frankfurt am Main,

9 Germany

10 ³ Institute of Ecology, Diversity and Evolution, Goethe University, 60325 Frankfurt am Main,

11 Germany

12

13

Key words

15 Biosynthetic genes, depsides, fungi, genome mining, long-read sequencing, microbial

16 biotechnology, PKS phylogeny, Secondary metabolites, *Umbilicaria*

17

Abstract

19 Natural products of lichen-forming fungi are structurally diverse and have a variety of

20 medicinal properties. Despite this, they have limited implementation in industry, because the

21 corresponding genes remain unknown for most of the natural products. Here we implement a

long-read sequencing and bioinformatic approach to identify the biosynthetic gene cluster of the bioactive natural product gyrophoric acid (GA). Using 15 high-quality genomes representing nine GA-producing species of the lichen-forming fungal genus *Umbilicaria*, we identify the most likely GA cluster and investigate cluster gene organization and composition across the nine species. Our results show that GA clusters are promiscuous within *Umbilicaria*, with only three genes that are conserved across species, including the PKS gene. In addition, our results suggest that the same cluster codes for different but structurally similar NPs, i.e., GA, umbilicatic acid and hiascic acid, bringing new evidence that lichen metabolite diversity is also generated through regulatory mechanisms at the molecular level. Ours is the first study to identify the most likely GA cluster, and thus provides essential information to open new avenues for biotechnological approaches to producing and modifying GA and similar lichen-derived compounds. We show that bioinformatics approaches are useful in linking genes and potentially associated natural products. Genome analyses help unlocking the pharmaceutical potential of organisms such as lichens, which are biosynthetically diverse but slow growing, and difficult to cultivate due to their symbiotic nature.

Importance

The implementation of natural products in the pharmaceutical industry relies on the possibility of modifying the natural product (NP) pathway to optimize yields and pharmacological effects. Characterization of genes and pathways underlying natural product biosynthesis is a major bottleneck for the use of natural products in the pharmaceutical industry. Genome mining is a promising and relatively cost- and time-effective approach to exploit unexplored NP resources for drug discovery. In this study, we identify the most likely

gene cluster for the lichen-forming fungal depside gyrophoric acid in nine *Umbilicaria* species. This compound shows cytotoxic and antiproliferative properties against several cancer cell lines, and is also a broad-spectrum antimicrobial agent. We identify the putative GA cluster from nine *Umbilicaria* species. This information paves the way for generating GA analogs with modified properties by selective activation/deactivation of genes.

Introduction

Natural products (NPs) and their derivatives/analogues constitute about 70% of modern medicines (1, 2). NPs alone, however, i.e., unmodified molecules as produced by organisms themselves in nature, constitute only a small portion of this. The vast majority, about 60-65%, are derivatives and analogues of naturally-occurring substances, synthesized through biotechnology or synthetic approaches (2, 3). The use of NPs in the pharmaceutical industry relies on the ability to modify NP pathways in order to optimize yields and pharmacological effects. Culture-dependent approaches to identifying/producing NPs are labor-intensive and time-consuming, and not successful for every organism (4, 5). As a result, the biosynthetic potential of many biosynthetically prolific organisms remains untapped. Information on the genetic background and mechanisms of NP synthesis may thus contribute to fast-track NP-based drug discovery (2, 6).

Lichens, symbiotic organisms composed of fungal and photosynthetic partners (green algae or cyanobacteria, or both at the same time) (7–9), are a treasure chest of NPs (10–12). So far, about 1,000 NPs with great structural and functional diversity have been reported from lichen-forming fungi (LFF), and about 300-400 have been screened for bioactivity (11). However, the genetic background of more than 97% of lichen NPs is unknown (13–16).

Lichen compounds have great pharmacological potential, encompassing antimicrobial, antiproliferative, cytotoxic and antioxidant properties (11, 17–20). However, there are various major bottlenecks for using lichen NPs in the pharmaceutical industry, including low yield in nature, slow growth, tedious isolation/culturing methods, and a lack of understanding of their genetic background. Targeted genome mining approaches integrate the latest DNA sequencing technologies with computational advancements and large, publicly-available databases of pre-identified BGCs to characterize genes coding for NPs (1, 21, 22). This approach combines genome mining with the expected genetics of the NP to narrow down the candidate biosynthetic genes.

In-silico approaches for linking natural products with their respective biosynthetic gene clusters (BGCs) – genomic clusters of biosynthetic-related genes typically found in fungi (23–25) – are becoming more common in LFF due to the increased availability of genomic resources and databases (1, 21, 22), improvement of detection software and genome mining tools, stabilizing PKS phylogenies, and information gained from recent successes in the heterologous expression of *PKSs* from LFF (13, 14). For instance, the clade “Group I, PKS 16” from Kim et al (13) is associated with the biosynthesis of orsellinic acid derivatives (orcinol depsides and depsidones) such as lecanoric acid (14), grayanic acid (15), physodic acid and olivetoric acid (16), whereas the clade “Group IX, PKS23” from Kim et al (13) is associated with the biosynthesis of methylated orsellinic acid derivatives (β -orcinol depsides and depsidones) such as atranorin. The cluster linked to usnic acid biosynthesis is also fairly well understood (26, 27) and corresponds to “Group VI, PKS8” from Kim et al (11).

Here, we combine high-throughput long-read sequencing with a comparative genomics approach to identify the putative cluster(s) linked to the synthesis of gyrophoric acid (GA). GA is an NP produced by several LFF species, with a broad spectrum of

bioactivity (pharmacological properties such as anticancer and antimicrobial activity and industrially-relevant properties such as usage as dyes (19, 28–30)), for which the molecular mechanism and genetics of synthesis remain unknown. Identification of the GA gene cluster would facilitate its production via biotechnology to optimize the yield as well as to generate GA analogs with the desired pharmaceutical effect. For this study, we chose nine species of GA producers belonging to the lichen-forming fungal genus *Umbilicaria* (Table 1). GA is the most characteristic compound of this genus, and is found at high concentrations in all the chosen species (28, 31–33). It is a depside containing three orsellinic acid-type rings joined together by ester bonds (Fig. 1). Apart from GA, several other structurally-related depsides such as umbilicaric acid, lecanoric acid and hiascic acid (Fig. 1) have also been reported from *Umbilicaria*, but these usually constitute a minor fraction (<10%) of the total NPs detected via HPLC (Fig. 1).

In the present study, we assembled highly contiguous long-read-based genomes of LFF of the genus *Umbilicaria*, identified the biosynthetic gene clusters of all the species and singled out candidate genes linked to GA biosynthesis.

Materials and methods

Sampling and dataset

We collected samples of the following eight *Umbilicaria* species: *U. deusta*, *U. freyi*, *U. grisea*, *U. subpolyphylla*, *U. hispanica*, *U. phaea*, *U. pustulata*, and *U. spodochoa* for genome sequencing (voucher information in Supplementary Table S1). When possible, we sequenced two samples of the same species collected in different climatic zones. This was done to take into account possible intra-specific variation in BGC content as recently shown

in Singh et al. (34). The genome of *U. muhlenbergii* was downloaded from the JGI database.

In addition, we sampled *Dermatocarpon miniatum* as a control, as it does not produce

depsides/depsidones.

DNA extraction, library preparation and genome sequencing

Lichen thalli were thoroughly washed with sterile water, and checked under the

stereomicroscope for the presence of possible contamination and other lichen thalli. DNA was

extracted from all the samples using a CTAB-based method (35) as presented in (36).

SMRTbell libraries were constructed according to the manufacturer's instructions of

the SMRTbell Express Prep Kit v. 2.0 following the Low DNA Input Protocol (Pacific

Biosciences, Menlo Park, CA). Total input for samples was approximately 170-800 ng.

Ligation with T-overhang SMRTbell adapters was performed at 20°C for 1 h or overnight.

Following ligation, the libraries were purified with a 0,45 x or 0,8 x AMPure PB bead clean

up step. The subsequent size selection step to remove SMRTbell templates <3 kb was

performed with 2,2 x of a 40% (v/ v) AMPure PB bead working solution.

SMRT sequencing was performed on the Sequel System II with the Sequel II

Sequencing Kit 2.0 using the continuous long read (CLR) mode or the circular consensus

sequencing (CCS) mode, 30 h movie time with no pre-extension and Software SMRTLINK

8.0. Each metagenomic library was sequenced on one SMART cell at the Medical Center

Nijmegen (the Netherlands), or at MPI Dresden.

Genome assembly and annotation

The continuous long reads (i.e. CLR reads) from the PacBio Sequel II CLR run were first processed into highly accurate consensus sequences (i.e. HiFi reads) using PacBio tool CCS v5.0.0 with default parameters (<https://ccs.how>). HiFi reads were then assembled into contigs using the assembler metaFlye v2.7 (37). The resulting contigs were scaffolded with LRScaf v1.1.12 (github.com/shingocat/lrscaf, (38)). The scaffolds were then taxonomically binned to extract Ascomyocota reads with blastx using DIAMOND (--more-sensitive --frameshift 15 --range-culling) on a custom database and following the MEGAN6 Community Edition pipeline (39). All scaffolds assigned to Ascomycota were extracted as to represent the *Umbilicaria* spp. Assembly statistics such as number of contigs, total length and N50 were accessed with Assemblathon v2 (40) (Table 1). The completeness of the received mycobiont bins (i.e. the fungal genomes) was estimated using Benchmarking Universal Single-Copy Orthologs (BUSCO) analysis in BUSCO v4 (41).

Identification and Annotations of Biosynthetic Gene Clusters

Functional annotation of genomes, including genes, proteins and BGC prediction (antiSMASH (antibiotics & SM Analysis Shell, v5.0)) was performed with scripts implemented in the funannotate pipeline (42, 43). First, the genomes were masked for repetitive elements, and then the gene prediction was performed using BUSCO2 to train Augustus and self-training GeneMark-ES (41, 44). Functional annotation was done with InterProScan (45), egg-NOG-mapper (46, 47) and BUSCO (41) with ascomycota_db models. Secreted proteins were predicted using SignalP (48) as implemented in the funannotate 'annotate' command.

Selecting candidate gene clusters linked to GA biosynthesis

We used the following criteria to select the candidate gene cluster associated with GA synthesis in *Umbilicaria*: 1) it must contain a NR-PKS (as some of the structural features of a NP can be directly inferred from the domain architecture of the *PKS*: *PKSs* without reducing domains (*NR-PKSs*) are linked to non-reduced compounds such as gyrophoric acid, olivetoric acid (16), physodic acid (16) and grayanic acid (15)), 2) it must be present in all the *Umbilicaria* genomes, as all the species have GA as the major secondary metabolite (33), and 3) it must be closely related to the *PKSs* involved in the synthesis of orsellinic acid-based compounds (15, 16), because orsellinic acid units constitute the building blocks of GA.

Phylogenetic analyses

We extracted the amino acid sequences of all the NR-PKS from the BGCs predicted by the antiSMASH for all the *Umbilicaria* species and *Dermatocarpon miniatum* (Supplementary Table S2). Additionally, the NR-PKS sequences of the following species were downloaded from the previous publications and public databases: *Cladonia borealis*, *C. grayi*, *C. macilenta*, *C. metacorallifera*, *C. rangiferina*, *C. uncialis*, *Pseudevernia furfuracea*, *Stereocaulon alpinum* and *Umbilicaria muhlenbergii*. The final dataset contains amino acid sequences of 229 NR-PKSs from 18 species belonging to four LFF genera. The sequences were aligned using MAFFT as implemented in Geneious v5.4 (49, 50). Gaps were treated as missing data. The maximum likelihood search was performed on the aligned sequences with RAxML-HPG BlackBox v8.1.11 (51) on the Cipres Scientific gateway (52). Phylogenetic trees were visualized using iTOL (53).

BGC clustering and novel BGCs: BiG-SCAPE and CORASON

We used BiG-SCAPE and CORASON (54) to identify the gene cluster networks and infer evolutionary relationships among clusters of interest among different *Umbilicaria* spp. BiG-SCAPE utilizes antiSMASH (42) and MIBiG databases (55) for inferring BGC sequence similarity networks, whereas CORASON employs a phylogenomic approach to infer evolutionary relationships between the clusters. BiG-SCAPE v1.0.1 was run in --auto mode, to identify BGC families using antiSMASH output files (.gbk) as input. Networks were generated using similarity thresholds of 0.25. The most likely GA cluster from all the *Umbilicaria* spp. was examined for conservation and variation among different *Umbilicaria* species using CORASON pipeline. The antiSMASH .gbk files of the corresponding clusters, based on phylogenetic grouping, were used as input. The most-likely GA cluster from *U. deusta* was used as reference to fish out the most closely-related clusters from the other *Umbilicaria* spp.

Results

Genome sequencing, assembly and annotation

The genome quality stats and assembly reports of all the genomes generated for this study are presented in Table 1.

Phylogenetic analysis

To search for PKS genes involved in the synthesis of GA, we performed a phylogenetic analysis by incorporating our sequences into the most comprehensive PKS dataset currently available (Supplementary Table S2) (13, 16). NR-PKSs have been categorized into nine

groups based on protein sequence similarity and PKS domain architecture (13). We identified a total of 110 NR-PKSs that were present in 15 *Umbilicaria* genomes (12 NR-PKSs on average per species). Four NR-PKSs were common to all species: PKS15, PKS16, PKS20 and a novel PKS clade (forming a monophyletic, supported clade to PKS33, Fig. 2). Only one NR-PKS per species formed a supported monophyletic clade with PKS16 (Group I, i.e., orsellinic acid, depside and depsidone NR-PKSs) (Fig. 2). The most-likely NR-PKS for the depsidone grayanic acid and the depsides olivetoric and physodic acid fall within this PKS clade.

Gyrophoric acid cluster

The cluster most likely associated with GA is the cluster containing PKS16 (Fig. 2), as 1) it is present in all *Umbilicaria* spp., 2) it contains an *NR-PKS* and 3) it forms a monophyletic group with the clade “Group I, PKS 16” from Kim et al (11). This cluster contains about 11-15 genes, including the core biosynthetic gene *NR-PKS* and a *cyt P450* (Fig. 3). The other genes code for unidentified proteins. The *U. deusta* PKS16 cluster is presented as an example of GA cluster (Fig. 3). The *PKS* is present in all *Umbilicaria* species investigated and displays high homology across species.

BGC clustering: BiG-SCAPE and CORASON

BGC sequence similarity networks group gene clusters at multiple hierarchical levels. This analysis implements a ‘glocal’ alignment mode that accurately groups both complete and fragmented BGCs. The BGCs forming a supported monophyletic clade to PKS16 (Group I) were then analyzed for conservation across species using CORASON. The CORASON

analysis showed that only three genes on the cluster were shared among the studied *Umbilicaria* species: the core *PKS* and the two genes of unknown function/proteins adjacent to the core gene (Fig. 4).

Discussion

Gyrophoric acid *PKS*

We found only one *PKS* per species forming a supported monophyletic clade to *PKS16* (Group I, i.e., orsellinic acid and depside/depsidone *PKSs*) (Fig. 2). These are the most likely GA *PKSs*.

The BGC associated with the biosynthesis of the following lichen depsides and depsidones have been identified so far: atranorin (13), lecanoric- (14), grayanic- (15), olivetoric- (16) and physodic acid (16). All these studies demonstrate that the *PKS* alone is capable of synthesizing the backbone depside, whereas modifications such as methylation and oxidation are made by enzymes coded by other genes in the cluster after the release of the depside from the *PKS*. For instance, the synthesis of atranorin involves at least three genes present within the atranorin cluster, but the core depside is coded only by the *PKS* (13). The other two genes, a carboxyl methylase (O-methyltransferase) and a *cyt P450*, methylate the carboxyl group and oxidize the methyl group (into -CHO), respectively, to produce the final product atranorin (Fig. 1). As GA does not have side chain modifications (Fig. 1) we propose that the *PKS* alone is involved in GA synthesis.

The depside *PKSs* identified so far code for didepsides, i.e. they contain two phenolic rings joined with an ester bond, for example grayanic acid, atranorin, physodic acid and olivetoric acid (13, 15, 16). Ours is the first study to identify the most-likely *PKS* associated

with a tridepside synthesis, i.e., three phenolic rings joined with two ester bonds. Our study suggests that the *PKS*s coding for a didepside and a tridepside differ only in the length of the sequence of the SAT domain. A tridepside *PKS* contains longer SAT coding sequence than the didepside *PKS*. The number of ACP and PT domains is the same between the two.

GA cluster in *Umbilicaria* spp.

The most-likely GA cluster contains about 11-15 genes in different *Umbilicaria* spp. (Fig. 3, 4). Interestingly, only three genes are common to all analyzed species, the *PKS* and two genes of unknown function (with low sequence similarity to known genes) upstream and downstream of the *PKS I* (Fig. 4). This suggests that these three genes form an integral part of the GA cluster, whereas the other genes are facultative among GA producers. Differences among the clusters synthesizing the same compound have been reported before, and have been associated with species-specific BGC regulation or modifications to the depside released by the *PKS* (26, 56).

The most-likely GA cluster also contains a *cyt P450* (Fig. 3, 4), which has been associated with depsidone production or oxidation of an acyl chain (13, 15, 16). However, the location and orientation of the *cyt P450* in the putative *Umbilicaria* GA cluster is different from a typical depsidone cluster (Fig. 3) (15). In the GA cluster, the *cyt P450* is not located next to and has the same orientation as the *PKS*, whereas in a depsidone cluster, *cyt P450* lies next to the *PKS*, in opposite orientation. Such organization is suggestive of genes being regulated and co-expressed by the same promoter (15). This is the case for the depsidone grayanic acid synthesis (in *Cladonia grayi*), which involves the synthesis of the depside intermediate by *PKS* followed by oxidation of the released depside into depsidone (15). The *PKS* and *cyt P450* form the integral part of depsidone synthesis (57) whereas the depside is

coded by the *PKS* alone, with the exception of the side chain modifications (13, 14). Therefore, despite being part of the GA cluster, the *cyt P450* does not seem to be involved in GA synthesis or in the synthesis of umbilicaric- and/or lecanoric acid reported from *Umbilicaria* spp. analyzed in this study. The synthesis of hiascic acid however would require the hydroxylation of a methyl group by *cyt P450* enzyme after the depside is released from the *PKS* (Fig. 1, the OH group in bold in hiascic acid). The lower proportion of hiascic acid as compared to GA could be because the *cyt P450* may not be co-expressed with the *PKS*.

Even though the functions of most of the genes identified in the present study are unknown, our study provides novel insights into GA cluster composition and organization across different species (Fig. 4). This information is crucial in order to open the way for future genetic manipulation of the GA biosynthetic pathway that may be aimed at increasing structural diversity and/or yield of the products, as well as in order to generate analogs with novel properties.

One cluster, different compounds

Variation in cluster composition reflects the potential to produce diverse NPs. Apart from GA, other depsides related in structure to GA, i.e., lecanoric-, umbilicaric- and hiascic acid (Fig. 1) are often reported in *Umbilicaria* spp. as minor metabolites (31). Interestingly, we found only one orcinol-depside *PKS* in *Umbilicaria* spp (Fig. 2). This strongly indicates that all the *Umbilicaria* depsides are coded by the same *PKS* cluster. One cluster coding for different, structurally-related compounds has also been reported previously (16, 56, 58). For instance, in the case of the antifungal drug caspofungin acetate, a semisynthetic derivative of the NP pneumocandins from the fungus *Glarea lozoyensis*, selective inactivation of different genes in this biosynthetic gene cluster generates 13 different analogues, some of them with elevated

antifungal activity relative to the original compound and its semisynthetic derivative (59). Similarly, the aspyridone biosynthetic cluster from *Aspergillus nidulans* produces eight different compounds in a heterologous host (58). These studies show that a single PKS cluster is capable of producing different compounds depending upon which genes are co-expressed and on the available starters. In lichens, a single *PKS* has been associated with the synthesis of olivetoric and physodic acid (16) and the same *PKS* has been shown to be involved in the synthesis of lecanoric acid in a heterologous host (14). We propose that the same PKS cluster is most likely involved in the synthesis of GA, umbilicaric- (an additional methyl group, Fig. 1), hiassic- (additional hydroxyl group, Fig. 1), and lecanoric acid (didepside with no side chains, Fig. 1) in *Umbilicaria*. It is possible, however, that in nature only GA is synthesized in members of the genus *Umbilicaria*, and the co-occurring minor compound lecanoric acid is a hydrolysis product of GA (57).

Interestingly, although umbilicaric acid is reported from some *Umbilicaria* species (*U. grisea*, *U. freyi*, *U. mühlenbergii* and *U. subpolyphylla* (31, 61)), O-methyltransferase (OMT) was not identified in the depside-related BGC of any *Umbilicaria* species (Fig. 3). OMT would be required for the methylation of oxygen to produce umbilicaric acid (Fig. 1). Its absence from depside-related BGCs suggests that an external OMT, e.g. from other BGCs, might be involved in the production of umbilicaric acid in *Umbilicaria*. This could explain the lower amounts of umbilicaric acid as compared to GA found in these species (31). In contrast, when the O-methylated compound is the major secondary metabolite, as in the case of grayanic acid and atranorin, OMT is an integral part of the BGC and is co-expressed along with the other crucial genes for grayanic acid production, i.e., the *PKS* and *cyt P450* (13, 15).

322

323 **Future perspectives**

324 Advances in long-read sequencing and in computational approaches to genome mining not
 325 only enable linking biosynthetic genes to NPs but also provide an overview of the entire gene
 326 cluster composition and organization. Ours is the first study to identify the most-likely GA
 327 cluster, which is essential for opening up avenues for biotechnological approaches to
 328 producing and modifying this compound and possibly other lichen compounds. In particular,
 329 this information can be applied to generate novel NP analogs with improved pharmacological
 330 properties via synthetic biology, biotechnology and combinatorial biosynthesis approaches.
 331 This paves the way to an entirely new horizon for utilizing these understudied taxa for
 332 pharmacological industry and drug discovery.

333

334 **Acknowledgements**

335 We thank Prof. Daniele Armaleo (Duke University) for his inputs on the domain composition
 336 of didepsides and tridepsides.

337

338 **Figure legends**

339 **Figure 1** Chemical structures and nomenclature. Structure of a lichen depside, atranorin, GA
 340 and other depsides produced by *Umbilicaria* spp.

341 **Figure 2** NR-PKS phylogeny of lichen-forming fungi. This is a maximum-likelihood tree
 342 based on amino acid sequences of NR-PKSs from nine *Umbilicaria* spp., six *Cladonia* spp.,
 343 *Dermatocarpon miniatum*, *Stereocaulon alpinum* and *Pseudevernia furfuracea*. Branches in

bold indicate bootstrap support >70%. Green color clades represent the PKSs common to all nine *Umbilicaria* spp. used in this study. PKS groups are based on Kim et al. (13).

Figure 3 Gyrophoric acid cluster from *Umbilicaria deusta* as predicted by antiSMASH.

Colored boxes indicate genes. Genes in grey represent genes coding for unknown proteins

Figure 4 CORASON-based PKS phylogeny to elucidate evolutionary relationships and

cluster organization of GA cluster in *Umbilicaria* spp. The bar plot below depicts the

percentage of *Umbilicaria* species in which a particular gene is present.

Table 1: Genome quality and annotation statistics

Supplementary Tables

1. Voucher information table of the *Umbilicaria* samples collected for this study.
2. Dataset used for the phylogenetic analysis, along with the amino acid sequences of the PKS

References

1. Newman DJ, Cragg GM. 2016. Natural products as sources of new drugs from 1981 to 2014. J Nat Prod. American Chemical Society.
2. Newman DJ, Cragg GM. 2020. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J Nat Prod. American Chemical Society.
3. Newman DJ, Cragg GM. 2007. Natural products as sources of new drugs over the last 25 years. J Nat Prod.
4. Xu W, Klumbys E, Ang EL, Zhao H. 2020. Emerging molecular biology tools and strategies for engineering natural product biosynthesis. Metab Eng Commun. Elsevier

- 367 B.V.
- 368 5. Palazzotto E, Tong Y, Lee SY, Weber T. 2019. Synthetic biology and metabolic
369 engineering of actinomycetes for natural product discovery. *Biotechnol Adv.* Elsevier
370 Inc.
- 371 6. Atanasov AG, Zotchev SB, Dirsch VM, Orhan IE, Banach M, Rollinger JM, Barreca
372 D, Weckwerth W, Bauer R, Bayer EA, Majeed M, Bishayee A, Bochkov V, Bonn GK,
373 Braidy N, Bucar F, Cifuentes A, D'Onofrio G, Bodkin M, Diederich M, Dinkova-
374 Kostova AT, Efferth T, El Bairi K, Arkells N, Fan TP, Fiebich BL, Freissmuth M,
375 Georgiev MI, Gibbons S, Godfrey KM, Gruber CW, Heer J, Huber LA, Ibanez E,
376 Kijjoo A, Kiss AK, Lu A, Macias FA, Miller MJS, Mocan A, Müller R, Nicoletti F,
377 Perry G, Pittalà V, Rastrelli L, Ristow M, Russo GL, Silva AS, Schuster D, Sheridan
378 H, Skalicka-Woźniak K, Skaltsounis L, Sobarzo-Sánchez E, Bredt DS, Stuppner H,
379 Sureda A, Tzvetkov NT, Vacca RA, Aggarwal BB, Battino M, Giampieri F, Wink M,
380 Wolfender JL, Xiao J, Yeung AWK, Lizard G, Popp MA, Heinrich M, Berindan-
381 Neagoe I, Stadler M, Daglia M, Verpoorte R, Supuran CT. 2021. Natural products in
382 drug discovery: advances and opportunities. *Nat Rev Drug Discov.* Nature Research.
- 383 7. Hawksworth DL, Honegger R. 1994. The lichen thallus: a symbiotic phenotype of
384 nutritionally specialized fungi and its response to gall producers., p. 77–98. *In*
385 Williams, MAJ (ed.), *Plant Galls: Organisms, Interactions, Populations*. Clarendon
386 Press, Oxford.
- 387 8. Ahmadjian V. 1993. *The Lichen Symbiosis*. John Wiley and Sons., New York.
- 388 9. Ahmadjian V. 1982. Algal/fungal symbioses., p. 179–233. *In* Round, FE Chapman, DJ
389 (ed.), *Progress in Phycological Research*. Elsevier Biomedical Press, Amsterdam.
- 390 10. Calchera A, Dal Grande F, Bode HB, Schmitt I. 2019. Biosynthetic gene content of the

- “perfume lichens” *Evernia prunastri* and *Pseudevernia furfuracea*. *Molecules* 24:203.
11. Goga M, Elečko J, Marcinčinová M, Ručová D, Bačkorová M, Bačkor M. 2020. Lichen metabolites: an overview of some secondary metabolites and their biological potential, p. 175–209. *In* .
12. Boustie J, Grube M. 2005. Lichens—a promising source of bioactive secondary metabolites. *Plant Genet Resour* 3:273–287.
13. Kim W, Liu R, Woo S, Kang K Bin, Park H, Yu YH, Ha H-H, Oh S-Y, Yang JH, Kim H, Yun S-H, Hur J-S. 2021. Linking a gene cluster to atranorin, a major cortical substance of lichens, through genetic dereplication and heterologous expression. *MBio* e0111121.
14. Kealey JT, Craig JP, Barr PJ. 2021. Identification of a lichen depside polyketide synthase gene by heterologous expression in *Saccharomyces cerevisiae*. *Metab Eng Commun* e00172.
15. Armaleo D, Sun X, Culberson C. 2011. Insights from the first putative biosynthetic gene cluster for a lichen depside and depsidone. *Mycologia* 103:741–754.
16. Singh G, Armaleo D, Dal Grande F, Schmitt I. 2021. Depside and depsidone synthesis in lichenized fungi comes into focus through a genome-wide comparison of the olivetoric acid and physodic acid chemotypes of *Pseudevernia furfuracea*. *Biomolecules* 11:1445.
17. Barbero M, Artuso E, Prandi C. 2017. Fungal anticancer metabolites: synthesis towards drug discovery. *Curr Med Chem* 25:141–185.
18. Stanojković T. 2015. Investigations of lichen secondary metabolites with potential anticancer activity, p. 127–146. *In* Lichen Secondary Metabolites: Bioactive Properties and Pharmaceutical Potential. Springer International Publishing.

19. Ingelfinger R, Henke M, Roser L, Ulshöfer T, Calchera A, Singh G, Parnham MJ, Geisslinger G, Fürst R, Schmitt I, Schiffmann S. 2020. Unraveling the pharmacological potential of lichen extracts in the context of cancer and inflammation with a broad screening approach. *Front Pharmacol* 11:1322.
20. Molnár K, Farkas E. 2010. Current results on biological activities of lichen secondary metabolites: A review. *Zeitschrift für Naturforsch - Sect C J Biosci*. Verlag der Zeitschrift für Naturforschung.
21. Van Santen JA, Kautsar SA, Medema MH, Linington RG. 2021. Microbial natural product databases: Moving forward in the multi-omics era. *Nat Prod Rep*. Royal Society of Chemistry.
22. Bachmann BO, Van Lanen SG, Baltz RH. 2014. Microbial genome mining for accelerated natural products discovery: Is a renaissance in the making? *J Ind Microbiol Biotechnol*. *J Ind Microbiol Biotechnol*.
23. Hoffmeister D, Keller NP. 2007. Natural products of filamentous fungi: Enzymes, genes, and their regulation. *Nat Prod Rep*.
24. Keller NP. 2019. Fungal secondary metabolism: regulation, function and drug discovery. *Nat Rev Microbiol*. Nature Publishing Group.
25. Brakhage AA. 2013. Regulation of fungal secondary metabolism. *Nat Rev Microbiol*.
26. Pizarro D, Divakar PK, Grewe F, Crespo A, Dal Grande F, Lumbsch HT. 2020. Genome-wide analysis of biosynthetic gene cluster reveals correlated gene loss with absence of usnic acid in lichen-forming fungi. *Genome Biol Evol* 12:1858–1868.
27. Abdel-Hameed M, Bertrand RL, Piercey-Normore MD, Sorensen JL. 2016. Putative identification of the usnic acid biosynthetic gene cluster by de novo whole-genome sequencing of a lichen-forming fungus. *Fungal Biol* 120:306–316.

- 439 28. Nguyen K-H, Chollet-Krugler M, Gouault N, Tomasi S. 2013. UV-protectant
440 metabolites from lichens and their symbiotic partners. *Nat Prod Rep* 30:1490.
- 441 29. Lohezic-Le Devehat F, Legouin B, Couteau C, Boustie J, Coiffard L. 2013. Lichenic
442 extracts and metabolites as UV filters. *J Photochem Photobiol B Biol* 120:17–28.
- 443 30. Buçukoglu TZ, Albayrak S, Halici MG, Tay T. 2013. Antimicrobial and antioxidant
444 activities of extracts and lichen acids obtained from some *Umbilicaria* species from
445 central Anatolia, Turkey. *J Food Process Preserv* 37:1103–1110.
- 446 31. Posner B, Feige GB, Huneck S. 1992. Studies on the chemistry of the lichen genus
447 *Umbilicaria* hoffm. *Zeitschrift fur Naturforsch - Sect C J Biosci* 47:1–9.
- 448 32. Narui T, Culberson CF, Culberson WL, Johnson A, Shibata S. 1996. A contribution to
449 the chemistry of the lichen family umbilicariaceae (Ascomycotina). *Bryologist* 99:199–
450 211.
- 451 33. Davydov EA, Peršoh D, Rambold G. 2017. Umbilicariaceae (lichenized Ascomycota)
452 – Trait evolution and a new generic concept. *Taxon* 66:1282–1303.
- 453 34. Singh G, Calchera A, Schulz M, Drechsler M, Bode HB, Schmitt I, Dal Grande F.
454 2021. Climate-specific biosynthetic gene clusters in populations of a lichen-forming
455 fungus. *Environ Microbiol* 00:1462-2920.15605.
- 456 35. Rubio-Piña JA, Zapata-Pérez O. 2011. Isolation of total RNA from tissues rich in
457 polyphenols and polysaccharides of mangrove plants. *Electron J Biotechnol* 14.
- 458 36. Merges D, Dal Grande F, Greve C, Otte J, Schmitt I. 2021. Virus diversity in
459 metagenomes of a lichen symbiosis (*Umbilicaria phaea*): complete viral genomes,
460 putative hosts and elevational distributions. *Environ Microbiol* 23:6637–6650.
- 461 37. Kolmogorov M, Yuan J, Lin Y, Pevzner PA. 2019. Assembly of long, error-prone
462 reads using repeat graphs. *Nat Biotechnol* 37:540–546.

38. Qin M, Wu S, Li A, Zhao F, Feng H, Ding L, Ruan J. 2019. LRScaf: Improving draft genomes using long noisy reads. *BMC Genomics* 20:955.
39. Huson DH, Beier S, Flade I, Górska A, El-Hadidi M, Mitra S, Ruscheweyh H-J, Tappu R. 2016. MEGAN Community Edition - Interactive exploration and analysis of large-scale microbiome sequencing data. *PLOS Comput Biol* 12:e1004957.
40. Bradnam KR, Fass JN, Alexandrov A, Baranay P, Bechner M, Birol I, Boisvert S, Chapman JA, Chapuis G, Chikhi R, Chitsaz H, Chou W-C, Corbeil J, Del Fabbro C, Docking TR, Durbin R, Earl D, Emrich S, Fedotov P, Fonseca NA, Ganapathy G, Gibbs RA, Gnerre S, Godzaridis É, Goldstein S, Haimel M, Hall G, Haussler D, Hiatt JB, Ho IY, Howard J, Hunt M, Jackman SD, Jaffe DB, Jarvis ED, Jiang H, Kazakov S, Kersey PJ, Kitzman JO, Knight JR, Koren S, Lam T-W, Lavenier D, Laviolette F, Li Y, Li Z, Liu B, Liu Y, Luo R, MacCallum I, MacManes MD, Maillet N, Melnikov S, Naquin D, Ning Z, Otto TD, Paten B, Paulo OS, Phillippy AM, Pina-Martins F, Place M, Przybylski D, Qin X, Qu C, Ribeiro FJ, Richards S, Rokhsar DS, Ruby JG, Scalabrin S, Schatz MC, Schwartz DC, Sergushichev A, Sharpe T, Shaw TI, Shendure J, Shi Y, Simpson JT, Song H, Tsarev F, Vezzi F, Vicedomini R, Vieira BM, Wang J, Worley KC, Yin S, Yiu S-M, Yuan J, Zhang G, Zhang H, Zhou S, Korf IF. 2013. Assemblathon 2: evaluating de novo methods of genome assembly in three vertebrate species. *Gigascience* 2:10.
41. Simão FA, Waterhouse RM, Ioannidis P, Kriventseva E V., Zdobnov EM. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31:3210–3212.
42. Blin K, Shaw S, Steinke K, Villebro R, Ziemert N, Lee SY, Medema MH, Weber T. 2019. antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline.

- Nucleic Acids Res 47:W81–W87.
43. Palmer J, Stajich J. 2019. Funannotate v1.7.4. Zenodo
<https://doi.org/https://doi.org/10.5281/ZENODO.2604804>.
44. Borodovsky M, Lomsadze A. 2011. Eukaryotic gene prediction using GeneMark.hmm-E and GeneMark-ES. Curr Protoc Bioinforma CHAPTER:Unit-4.610.
45. Quevillon E, Silventoinen V, Pillai S, Harte N, Mulder N, Apweiler R, Lopez R. 2005. InterProScan: Protein domains identifier. Nucleic Acids Res 33:W116.
46. Huerta-Cepas J, Szklarczyk D, Heller D, Hernández-Plaza A, Forslund SK, Cook H, Mende DR, Letunic I, Rattei T, Jensen LJ, Von Mering C, Bork P. 2019. EggNOG 5.0: A hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. Nucleic Acids Res 47:D309–D314.
47. Huerta-Cepas J, Forslund K, Coelho LP, Szklarczyk D, Jensen LJ, Von Mering C, Bork P. 2017. Fast genome-wide functional annotation through orthology assignment by eggNOG-mapper. Mol Biol Evol 34:2115–2122.
48. Almagro Armenteros JJ, Tsirigos KD, Sønderby CK, Petersen TN, Winther O, Brunak S, von Heijne G, Nielsen H. 2019. SignalP 5.0 improves signal peptide predictions using deep neural networks. Nat Biotechnol 37:420–423.
49. Katoh K, Kuma K, Toh H, Miyata T. 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Res 33:511–8.
50. Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Thierer T, Wilson A. 2011. Geneious. 5.4.
51. Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–90.

- 511 52. Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for
512 inference of large phylogenetic trees, p. 1–8. *In* Proceedings of the Gateway
513 Computing Environments Workshop (GCE) New Orleans.
- 514 53. Letunic I, Bork P. 2021. Interactive tree of life (iTOL) v5: An online tool for
515 phylogenetic tree display and annotation. *Nucleic Acids Res* 49:W293–W296.
- 516 54. Navarro-Muñoz JC, Selem-Mojica N, Mullowney MW, Kautsar SA, Tryon JH,
517 Parkinson EI, De Los Santos ELC, Yeong M, Cruz-Morales P, Abubucker S, Roeters
518 A, Lokhorst W, Fernandez-Guerra A, Cappelini LTD, Goering AW, Thomson RJ,
519 Metcalf WW, Kelleher NL, Barona-Gomez F, Medema MH. 2020. A computational
520 framework to explore large-scale biosynthetic diversity. *Nat Chem Biol* 16:60–68.
- 521 55. Kautsar SA, Blin K, Shaw S, Navarro-Muñoz JC, Terlouw BR, Van Der Hooft JJJ,
522 Van Santen JA, Tracanna V, Suarez Duran HG, Pascal Andreu V, Selem-Mojica N,
523 Alanjary M, Robinson SL, Lund G, Epstein SC, Sisto AC, Charkoudian LK, Collemare
524 J, Linington RG, Weber T, Medema MH. 2020. MIBiG 2.0: A repository for
525 biosynthetic gene clusters of known function. *Nucleic Acids Res* 48:D454–D458.
- 526 56. Martinet L, Naômé A, Deflandre B, Maciejewska M, Tellatin D, Tenconi E,
527 Smargiasso N, De Pauw E, Van Wezel GP, Rigali S. 2019. A single biosynthetic gene
528 cluster is responsible for the production of bagremycin antibiotics and ferroverdin iron
529 chelators. *MBio* 10:e01230-19.
- 530 57. Armaleo D. 1995. Factors affecting depside and depsidone biosynthesis in a cultured
531 lichen fungus. *Crypt Bot* 5:14–21.
- 532 58. Wasil Z, Pahirulzaman KAK, Butts C, Simpson TJ, Lazarus CM, Cox RJ. 2013. One
533 pathway, many compounds: Heterologous expression of a fungal biosynthetic pathway
534 reveals its intrinsic potential for diversity. *Chem Sci* 4:3845–3856.

59. Chen L, Yue Q, Zhang X, Xiang M, Wang C, Li S, Che Y, Ortiz-López FJ, Bills GF, Liu X, An Z. 2013. Genomics-driven discovery of the pneumocandin biosynthetic gene cluster in the fungus *Glarea lozoyensis*. BMC Genomics 14.
60. Leuckert C. 1985. Probleme der Flechten-Chemotaxonomie -- Stoffkombinationen und ihre taxonomische Wertung. Problems of lichen chemotaxonomy -- patterns of substances and their taxonomical valuation. Ber Dtsch Bot Ges 98:401–408.
61. Davydov EA, Blum OB, Kashevarov GP, Grakhov VP. 2019. Umbilicaria subpolyphylla Oxner: The correct name for U. iberica Sancho & Krzewicka and its bipolar distribution pattern. Lichenologist. Cambridge University Press.
2. Newman DJ, Cragg GM. 2020. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J Nat Prod. American Chemical Society.
3. Newman DJ, Cragg GM. 2007. Natural products as sources of new drugs over the last 25 years. J Nat Prod.
4. Atanasov AG, Zotchev SB, Dirsch VM, Orhan IE, Banach M, Rollinger JM, Barreca D, Weckwerth W, Bauer R, Bayer EA, Majeed M, Bishayee A, Bochkov V, Bonn GK, Braidy N, Bucar F, Cifuentes A, D’Onofrio G, Bodkin M, Diederich M, Dinkova-Kostova AT, Efferth T, El Bairi K, Arkells N, Fan TP, Fiebich BL, Freissmuth M, Georgiev MI, Gibbons S, Godfrey KM, Gruber CW, Heer J, Huber LA, Ibanez E, Kijjoo A, Kiss AK, Lu A, Macias FA, Miller MJS, Mocan A, Müller R, Nicoletti F, Perry G, Pittalà V, Rastrelli L, Ristow M, Russo GL, Silva AS, Schuster D, Sheridan H, Skalicka-Woźniak K, Skaltsounis L, Sobarzo-Sánchez E, Bredt DS, Stuppner H, Sureda A, Tzvetkov NT, Vacca RA, Aggarwal BB, Battino M, Giampieri F, Wink M, Wolfender JL, Xiao J, Yeung AWK, Lizard G, Popp MA, Heinrich M, Berindan-

- Neagoe I, Stadler M, Daglia M, Verpoorte R, Supuran CT. 2021. Natural products in drug discovery: advances and opportunities. *Nat Rev Drug Discov. Nature Research.*
5. Hawksworth DL, Honegger R. 1994. The lichen thallus: a symbiotic phenotype of nutritionally specialized fungi and its response to gall producers., p. 77–98. *In* Williams, MAJ (ed.), *Plant Galls: Organisms, Interactions, Populations*. Clarendon Press, Oxford.
6. Ahmadjian V. 1993. *The Lichen Symbiosis*. John Wiley and Sons., New York.
7. Ahmadjian V. 1982. Algal/fungal symbioses., p. 179–233. *In* Round, FE Chapman, DJ (ed.), *Progress in Phycological Research*. Elsevier Biomedical Press, Amsterdam.
8. Calchera A, Dal Grande F, Bode HB, Schmitt I. 2019. Biosynthetic gene content of the “perfume lichens” *Evernia prunastri* and *Pseudevernia furfuracea*. *Molecules* 24:203.
9. Goga M, Elečko J, Marcinčinová M, Ručová D, Bačkorová M, Bačkor M. 2020. Lichen metabolites: an overview of some secondary metabolites and their biological potential, p. 175–209. *In* .
10. Boustie J, Grube M. 2005. Lichens—a promising source of bioactive secondary metabolites. *Plant Genet Resour* 3:273–287.
11. Kim W, Liu R, Woo S, Kang K Bin, Park H, Yu YH, Ha H-H, Oh S-Y, Yang JH, Kim H, Yun S-H, Hur J-S. 2021. Linking a gene cluster to atranorin, a major cortical substance of lichens, through genetic dereplication and heterologous expression. *MBio* e0111121.
12. Kealey JT, Craig JP, Barr PJ. 2021. Identification of a lichen depside polyketide synthase gene by heterologous expression in *Saccharomyces cerevisiae*. *Metab Eng Commun* e00172.
13. Armaleo D, Sun X, Culberson C. 2011. Insights from the first putative biosynthetic

- gene cluster for a lichen depside and depsidone. *Mycologia* 103:741–754.
14. Singh G, Armaleo D, Dal Grande F, Schmitt I. 2021. Depside and depsidone synthesis in lichenized fungi comes into focus through a genome-wide comparison of the olivetoric acid and physodic acid chemotypes of *Pseudevernia furfuracea*. *Biomolecules* 11:1445.
15. Barbero M, Artuso E, Prandi C. 2017. Fungal anticancer metabolites: synthesis towards drug discovery. *Curr Med Chem* 25:141–185.
16. Stanojković T. 2015. Investigations of lichen secondary metabolites with potential anticancer activity, p. 127–146. *In* Lichen Secondary Metabolites: Bioactive Properties and Pharmaceutical Potential. Springer International Publishing.
17. Ingelfinger R, Henke M, Roser L, Ulshöfer T, Calchera A, Singh G, Parnham MJ, Geisslinger G, Fürst R, Schmitt I, Schiffmann S. 2020. Unraveling the pharmacological potential of lichen extracts in the context of cancer and inflammation with a broad screening approach. *Front Pharmacol* 11:1322.
18. Molnár K, Farkas E. 2010. Current results on biological activities of lichen secondary metabolites: A review. *Zeitschrift fur Naturforsch - Sect C J Biosci*. Verlag der Zeitschrift fur Naturforschung.
19. Van Santen JA, Kautsar SA, Medema MH, Linington RG. 2021. Microbial natural product databases: Moving forward in the multi-omics era. *Nat Prod Rep*. Royal Society of Chemistry.
20. Bachmann BO, Van Lanen SG, Baltz RH. 2014. Microbial genome mining for accelerated natural products discovery: Is a renaissance in the making? *J Ind Microbiol Biotechnol*. *J Ind Microbiol Biotechnol*.
21. Pizarro D, Divakar PK, Grewe F, Crespo A, Dal Grande F, Lumbsch HT. 2020.

- Genome-wide analysis of biosynthetic gene cluster reveals correlated gene loss with
absence of usnic acid in lichen-forming fungi. *Genome Biol Evol* 12:1858–1868.
22. Abdel-Hameed M, Bertrand RL, Piercey-Normore MD, Sorensen JL. 2016. Putative
identification of the usnic acid biosynthetic gene cluster by de novo whole-genome
sequencing of a lichen-forming fungus. *Fungal Biol* 120:306–316.
23. Nguyen K-H, Chollet-Krugler M, Gouault N, Tomasi S. 2013. UV-protectant
metabolites from lichens and their symbiotic partners. *Nat Prod Rep* 30:1490.
24. Lohezic-Le Devehat F, Legouin B, Couteau C, Boustie J, Coiffard L. 2013. Lichenic
extracts and metabolites as UV filters. *J Photochem Photobiol B Biol* 120:17–28.
25. Buçukoglu TZ, Albayrak S, Halici MG, Tay T. 2013. Antimicrobial and antioxidant
activities of extracts and lichen acids obtained from some *Umbilicaria* species from
central Anatolia, Turkey. *J Food Process Preserv* 37:1103–1110.
26. Posner B, Feige GB, Huneck S. 1992. Studies on the chemistry of the lichen genus
Umbilicaria hoffm. *Zeitschrift fur Naturforsch - Sect C J Biosci* 47:1–9.
27. Narui T, Culberson CF, Culberson WL, Johnson A, Shibata S. 1996. A contribution to
the chemistry of the lichen family umbilicariaceae (Ascomycotina). *Bryologist* 99:199–
211.
28. Davydov EA, Peršoh D, Rambold G. 2017. Umbilicariaceae (lichenized Ascomycota)
– Trait evolution and a new generic concept. *Taxon* 66:1282–1303.
29. Singh G, Calchera A, Schulz M, Drechsler M, Bode HB, Schmitt I, Dal Grande F.
2021. Climate-specific biosynthetic gene clusters in populations of a lichen-forming
fungus. *Environ Microbiol* 00:1462-2920.15605.
30. Rubio-Piña JA, Zapata-Pérez O. 2011. Isolation of total RNA from tissues rich in
polyphenols and polysaccharides of mangrove plants. *Electron J Biotechnol* 14.

31. Merges D, Dal Grande F, Greve C, Otte J, Schmitt I. 2021. Virus diversity in metagenomes of a lichen symbiosis (*Umbilicaria phaea*): complete viral genomes, putative hosts and elevational distributions. *Environ Microbiol* 23:6637–6650.
32. Kolmogorov M, Yuan J, Lin Y, Pevzner PA. 2019. Assembly of long, error-prone reads using repeat graphs. *Nat Biotechnol* 37:540–546.
33. Qin M, Wu S, Li A, Zhao F, Feng H, Ding L, Ruan J. 2019. LRScaf: Improving draft genomes using long noisy reads. *BMC Genomics* 20:955.
34. Huson DH, Beier S, Flade I, Górská A, El-Hadidi M, Mitra S, Ruscheweyh H-J, Tappu R. 2016. MEGAN Community Edition - Interactive exploration and analysis of large-scale microbiome sequencing data. *PLOS Comput Biol* 12:e1004957.
35. Bradnam KR, Fass JN, Alexandrov A, Baranay P, Bechner M, Birol I, Boisvert S, Chapman JA, Chapuis G, Chikhi R, Chitsaz H, Chou W-C, Corbeil J, Del Fabbro C, Docking TR, Durbin R, Earl D, Emrich S, Fedotov P, Fonseca NA, Ganapathy G, Gibbs RA, Gnerre S, Godzaridis É, Goldstein S, Haimel M, Hall G, Haussler D, Hiatt JB, Ho IY, Howard J, Hunt M, Jackman SD, Jaffe DB, Jarvis ED, Jiang H, Kazakov S, Kersey PJ, Kitzman JO, Knight JR, Koren S, Lam T-W, Lavenier D, Laviolette F, Li Y, Li Z, Liu B, Liu Y, Luo R, MacCallum I, MacManes MD, Maillet N, Melnikov S, Naquin D, Ning Z, Otto TD, Paten B, Paulo OS, Phillippy AM, Pina-Martins F, Place M, Przybylski D, Qin X, Qu C, Ribeiro FJ, Richards S, Rokhsar DS, Ruby JG, Scalabrin S, Schatz MC, Schwartz DC, Sergushichev A, Sharpe T, Shaw TI, Shendure J, Shi Y, Simpson JT, Song H, Tsarev F, Vezzi F, Vicedomini R, Vieira BM, Wang J, Worley KC, Yin S, Yiu S-M, Yuan J, Zhang G, Zhang H, Zhou S, Korf IF. 2013. Assemblathon 2: evaluating de novo methods of genome assembly in three vertebrate species. *Gigascience* 2:10.

36. Simão FA, Waterhouse RM, Ioannidis P, Kriventseva E V., Zdobnov EM. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31:3210–3212.
37. Blin K, Shaw S, Steinke K, Villebro R, Ziemert N, Lee SY, Medema MH, Weber T. 2019. antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. *Nucleic Acids Res* 47:W81–W87.
38. Palmer J, Stajich J. 2019. Funannotate v1.7.4. Zenodo <https://doi.org/https://doi.org/10.5281/ZENODO.2604804>.
39. Borodovsky M, Lomsadze A. 2011. Eukaryotic gene prediction using GeneMark.hmm-E and GeneMark-ES. *Curr Protoc Bioinforma* CHAPTER:Unit-4.610.
40. Quevillon E, Silventoinen V, Pillai S, Harte N, Mulder N, Apweiler R, Lopez R. 2005. InterProScan: Protein domains identifier. *Nucleic Acids Res* 33:W116.
41. Huerta-Cepas J, Szklarczyk D, Heller D, Hernández-Plaza A, Forslund SK, Cook H, Mende DR, Letunic I, Rattei T, Jensen LJ, Von Mering C, Bork P. 2019. EggNOG 5.0: A hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. *Nucleic Acids Res* 47:D309–D314.
42. Huerta-Cepas J, Forslund K, Coelho LP, Szklarczyk D, Jensen LJ, Von Mering C, Bork P. 2017. Fast genome-wide functional annotation through orthology assignment by eggNOG-mapper. *Mol Biol Evol* 34:2115–2122.
43. Almagro Armenteros JJ, Tsirigos KD, Sønderby CK, Petersen TN, Winther O, Brunak S, von Heijne G, Nielsen H. 2019. SignalP 5.0 improves signal peptide predictions using deep neural networks. *Nat Biotechnol* 37:420–423.
44. Katoh K, Kuma K, Toh H, Miyata T. 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Res* 33:511–8.

45. Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Thierer T, Wilson A. 2011. Geneious. 5.4.
46. Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22:2688–90.
47. Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees, p. 1–8. *In* Proceedings of the Gateway Computing Environments Workshop (GCE) New Orleans.
48. Letunic I, Bork P. 2021. Interactive tree of life (iTOL) v5: An online tool for phylogenetic tree display and annotation. *Nucleic Acids Res* 49:W293–W296.
49. Navarro-Muñoz JC, Selem-Mojica N, Mullowney MW, Kautsar SA, Tryon JH, Parkinson EI, De Los Santos ELC, Yeong M, Cruz-Morales P, Abubucker S, Roeters A, Lokhorst W, Fernandez-Guerra A, Cappelini LTD, Goering AW, Thomson RJ, Metcalf WW, Kelleher NL, Barona-Gomez F, Medema MH. 2020. A computational framework to explore large-scale biosynthetic diversity. *Nat Chem Biol* 16:60–68.
50. Kautsar SA, Blin K, Shaw S, Navarro-Muñoz JC, Terlouw BR, Van Der Hooft JJJ, Van Santen JA, Tracanna V, Suarez Duran HG, Pascal Andreu V, Selem-Mojica N, Alanjary M, Robinson SL, Lund G, Epstein SC, Sisto AC, Charkoudian LK, Collemare J, Linington RG, Weber T, Medema MH. 2020. MIBiG 2.0: A repository for biosynthetic gene clusters of known function. *Nucleic Acids Res* 48:D454–D458.
51. Martinet L, Naômé A, Deflandre B, Maciejewska M, Tellatin D, Tenconi E, Smargiasso N, De Pauw E, Van Wezel GP, Rigali S. 2019. A single biosynthetic gene cluster is responsible for the production of bagremycin antibiotics and ferroverdin iron chelators. *MBio* 10:e01230-19.

52. Armaleo D. 1995. Factors affecting depside and depsidone biosynthesis in a cultured lichen fungus. *Crypt Bot* 5:14–21.
53. Wasil Z, Pahirulzaman KAK, Butts C, Simpson TJ, Lazarus CM, Cox RJ. 2013. One pathway, many compounds: Heterologous expression of a fungal biosynthetic pathway reveals its intrinsic potential for diversity. *Chem Sci* 4:3845–3856.
54. Chen L, Yue Q, Zhang X, Xiang M, Wang C, Li S, Che Y, Ortiz-López FJ, Bills GF, Liu X, An Z. 2013. Genomics-driven discovery of the pneumocandin biosynthetic gene cluster in the fungus *Glarea lozoyensis*. *BMC Genomics* 14.
55. Chen L, Li Y, Yue Q, Lokszejn A, Yokoyama K, Felix EA, Liu X, Zhang N, An Z, Bills GF. 2016. Engineering of new pneumocandin side-chain analogues from *Glarea lozoyensis* by mutasynthesis and evaluation of their antifungal activity. *ACS Chem Biol* 11:2724–2733.
56. Li Y, Chen L, Yue Q, Liu X, An Z, Bills GF. 2015. Genetic manipulation of the pneumocandin biosynthetic pathway for generation of analogues and evaluation of their antifungal activity. *ACS Chem Biol* 10:1702–1710.
57. Leuckert C. 1985. Probleme der Flechten-Chemotaxonomie -- Stoffkombinationen und ihre taxonomische Wertung. Problems of lichen chemotaxonomy -- patterns of substances and their taxonomical valuation. *Ber Dtsch Bot Ges* 98:401–408.
58. Davydov EA, Blum OB, Kashevarov GP, Grakhov VP. 2019. Umbilicaria subpolyphylla Oxner: The correct name for U. iberica Sancho & Krzewicka and its bipolar distribution pattern. *Lichenologist*. Cambridge University Press.
2. Newman DJ, Cragg GM. 2020. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *J Nat Prod*. American Chemical Society.

3. Newman DJ, Cragg GM. 2007. Natural products as sources of new drugs over the last 25 years. *J Nat Prod*.
4. Atanasov AG, Zotchev SB, Dirsch VM, Orhan IE, Banach M, Rollinger JM, Barreca D, Weckwerth W, Bauer R, Bayer EA, Majeed M, Bishayee A, Bochkov V, Bonn GK, Braidy N, Bucar F, Cifuentes A, D'Onofrio G, Bodkin M, Diederich M, Dinkova-Kostova AT, Efferth T, El Bairi K, Arkells N, Fan TP, Fiebich BL, Freissmuth M, Georgiev MI, Gibbons S, Godfrey KM, Gruber CW, Heer J, Huber LA, Ibanez E, Kijjoo A, Kiss AK, Lu A, Macias FA, Miller MJS, Mocan A, Müller R, Nicoletti F, Perry G, Pittalà V, Rastrelli L, Ristow M, Russo GL, Silva AS, Schuster D, Sheridan H, Skalicka-Woźniak K, Skaltsounis L, Sobarzo-Sánchez E, Bredt DS, Stuppner H, Sureda A, Tzvetkov NT, Vacca RA, Aggarwal BB, Battino M, Giampieri F, Wink M, Wolfender JL, Xiao J, Yeung AWK, Lizard G, Popp MA, Heinrich M, Berindan-Neagoe I, Stadler M, Daglia M, Verpoorte R, Supuran CT. 2021. Natural products in drug discovery: advances and opportunities. *Nat Rev Drug Discov*. Nature Research.
5. Hawksworth DL, Honegger R. 1994. The lichen thallus: a symbiotic phenotype of nutritionally specialized fungi and its response to gall producers., p. 77–98. *In* Williams, MAJ (ed.), *Plant Galls: Organisms, Interactions, Populations*. Clarendon Press, Oxford.
6. Ahmadjian V. 1993. *The Lichen Symbiosis*. John Wiley and Sons., New York.
7. Ahmadjian V. 1982. Algal/fungal symbioses., p. 179–233. *In* Round, FE Chapman, DJ (ed.), *Progress in Phycological Research*. Elsevier Biomedical Press, Amsterdam.
8. Calchera A, Dal Grande F, Bode HB, Schmitt I. 2019. Biosynthetic gene content of the “perfume lichens” *Evernia prunastri* and *Pseudevernia furfuracea*. *Molecules* 24:203.
9. Goga M, Elečko J, Marcinčinová M, Ručová D, Bačkorová M, Bačkor M. 2020.

- 751 Lichen metabolites: an overview of some secondary metabolites and their biological
752 potential, p. 175–209. *In* .
- 753 10. Boustie J, Grube M. 2005. Lichens—a promising source of bioactive secondary
754 metabolites. *Plant Genet Resour* 3:273–287.
- 755 11. Kim W, Liu R, Woo S, Kang K Bin, Park H, Yu YH, Ha H-H, Oh S-Y, Yang JH, Kim
756 H, Yun S-H, Hur J-S. 2021. Linking a gene cluster to atranorin, a major cortical
757 substance of lichens, through genetic dereplication and heterologous expression. *MBio*
758 e0111121.
- 759 12. Kealey JT, Craig JP, Barr PJ. 2021. Identification of a lichen depside polyketide
760 synthase gene by heterologous expression in *Saccharomyces cerevisiae*. *Metab Eng*
761 *Commun* e00172.
- 762 13. Armaleo D, Sun X, Culberson C. 2011. Insights from the first putative biosynthetic
763 gene cluster for a lichen depside and depsidone. *Mycologia* 103:741–754.
- 764 14. Singh G, Armaleo D, Dal Grande F, Schmitt I. 2021. Depside and depsidone synthesis
765 in lichenized fungi comes into focus through a genome-wide comparison of the
766 olivetoric acid and physodic acid chemotypes of *Pseudevernia furfuracea*.
767 *Biomolecules* 11:1445.
- 768 15. Barbero M, Artuso E, Prandi C. 2017. Fungal anticancer metabolites: synthesis towards
769 drug discovery. *Curr Med Chem* 25:141–185.
- 770 16. Stanojković T. 2015. Investigations of lichen secondary metabolites with potential
771 anticancer activity, p. 127–146. *In* *Lichen Secondary Metabolites: Bioactive Properties*
772 *and Pharmaceutical Potential*. Springer International Publishing.
- 773 17. Ingelfinger R, Henke M, Roser L, Ulshöfer T, Calchera A, Singh G, Parnham MJ,
774 Geisslinger G, Fürst R, Schmitt I, Schiffmann S. 2020. Unraveling the pharmacological

- potential of lichen extracts in the context of cancer and inflammation with a broad screening approach. *Front Pharmacol* 11:1322.
18. Molnár K, Farkas E. 2010. Current results on biological activities of lichen secondary metabolites: A review. *Zeitschrift fur Naturforsch - Sect C J Biosci*. Verlag der Zeitschrift fur Naturforschung.
19. Van Santen JA, Kautsar SA, Medema MH, Linington RG. 2021. Microbial natural product databases: Moving forward in the multi-omics era. *Nat Prod Rep*. Royal Society of Chemistry.
20. Bachmann BO, Van Lanen SG, Baltz RH. 2014. Microbial genome mining for accelerated natural products discovery: Is a renaissance in the making? *J Ind Microbiol Biotechnol*. *J Ind Microbiol Biotechnol*.
21. Pizarro D, Divakar PK, Grewe F, Crespo A, Dal Grande F, Lumbsch HT. 2020. Genome-wide analysis of biosynthetic gene cluster reveals correlated gene loss with absence of usnic acid in lichen-forming fungi. *Genome Biol Evol* 12:1858–1868.
22. Abdel-Hameed M, Bertrand RL, Piercey-Normore MD, Sorensen JL. 2016. Putative identification of the usnic acid biosynthetic gene cluster by de novo whole-genome sequencing of a lichen-forming fungus. *Fungal Biol* 120:306–316.
23. Nguyen K-H, Chollet-Krugler M, Gouault N, Tomasi S. 2013. UV-protectant metabolites from lichens and their symbiotic partners. *Nat Prod Rep* 30:1490.
24. Lohezic-Le Devehat F, Legouin B, Couteau C, Boustie J, Coiffard L. 2013. Lichenic extracts and metabolites as UV filters. *J Photochem Photobiol B Biol* 120:17–28.
25. Buçukoglu TZ, Albayrak S, Halici MG, Tay T. 2013. Antimicrobial and antioxidant activities of extracts and lichen acids obtained from some *Umbilicaria* species from central Anatolia, Turkey. *J Food Process Preserv* 37:1103–1110.

- 799 26. Posner B, Feige GB, Huneck S. 1992. Studies on the chemistry of the lichen genus
800 *Umbilicaria* hoffm. Zeitschrift fur Naturforsch - Sect C J Biosci 47:1–9.
- 801 27. Narui T, Culberson CF, Culberson WL, Johnson A, Shibata S. 1996. A contribution to
802 the chemistry of the lichen family umbilicariaceae (Ascomycotina). Bryologist 99:199–
803 211.
- 804 28. Davydov EA, Peršoh D, Rambold G. 2017. Umbilicariaceae (lichenized Ascomycota)
805 – Trait evolution and a new generic concept. Taxon 66:1282–1303.
- 806 29. Singh G, Calchera A, Schulz M, Drechsler M, Bode HB, Schmitt I, Dal Grande F.
807 2021. Climate-specific biosynthetic gene clusters in populations of a lichen-forming
808 fungus. Environ Microbiol 00:1462-2920.15605.
- 809 30. Rubio-Piña JA, Zapata-Pérez O. 2011. Isolation of total RNA from tissues rich in
810 polyphenols and polysaccharides of mangrove plants. Electron J Biotechnol 14.
- 811 31. Merges D, Dal Grande F, Greve C, Otte J, Schmitt I. 2021. Virus diversity in
812 metagenomes of a lichen symbiosis (*Umbilicaria phaea*): complete viral genomes,
813 putative hosts and elevational distributions. Environ Microbiol 23:6637–6650.
- 814 32. Kolmogorov M, Yuan J, Lin Y, Pevzner PA. 2019. Assembly of long, error-prone
815 reads using repeat graphs. Nat Biotechnol 37:540–546.
- 816 33. Qin M, Wu S, Li A, Zhao F, Feng H, Ding L, Ruan J. 2019. LRScaf: Improving draft
817 genomes using long noisy reads. BMC Genomics 20:955.
- 818 34. Huson DH, Beier S, Flade I, Górska A, El-Hadidi M, Mitra S, Ruscheweyh H-J, Tappu
819 R. 2016. MEGAN Community Edition - Interactive exploration and analysis of large-
820 scale microbiome sequencing data. PLOS Comput Biol 12:e1004957.
- 821 35. Bradnam KR, Fass JN, Alexandrov A, Baranay P, Bechner M, Birol I, Boisvert S,
822 Chapman JA, Chapuis G, Chikhi R, Chitsaz H, Chou W-C, Corbeil J, Del Fabbro C,

- Docking TR, Durbin R, Earl D, Emrich S, Fedotov P, Fonseca NA, Ganapathy G, Gibbs RA, Gnerre S, Godzaridis É, Goldstein S, Haimel M, Hall G, Haussler D, Hiatt JB, Ho IY, Howard J, Hunt M, Jackman SD, Jaffe DB, Jarvis ED, Jiang H, Kazakov S, Kersey PJ, Kitzman JO, Knight JR, Koren S, Lam T-W, Lavenier D, Laviolette F, Li Y, Li Z, Liu B, Liu Y, Luo R, MacCallum I, MacManes MD, Maillet N, Melnikov S, Naquin D, Ning Z, Otto TD, Paten B, Paulo OS, Phillippy AM, Pina-Martins F, Place M, Przybylski D, Qin X, Qu C, Ribeiro FJ, Richards S, Rokhsar DS, Ruby JG, Scalabrin S, Schatz MC, Schwartz DC, Sergushichev A, Sharpe T, Shaw TI, Shendure J, Shi Y, Simpson JT, Song H, Tsarev F, Vezzi F, Vicedomini R, Vieira BM, Wang J, Worley KC, Yin S, Yiu S-M, Yuan J, Zhang G, Zhang H, Zhou S, Korf IF. 2013. Assemblathon 2: evaluating de novo methods of genome assembly in three vertebrate species. *Gigascience* 2:10.
36. Simão FA, Waterhouse RM, Ioannidis P, Kriventseva E V., Zdobnov EM. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31:3210–3212.
37. Blin K, Shaw S, Steinke K, Villebro R, Ziemert N, Lee SY, Medema MH, Weber T. 2019. antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. *Nucleic Acids Res* 47:W81–W87.
38. Palmer J, Stajich J. 2019. Funannotate v1.7.4. Zenodo <https://doi.org/https://doi.org/10.5281/ZENODO.2604804>.
39. Borodovsky M, Lomsadze A. 2011. Eukaryotic gene prediction using GeneMark.hmm-E and GeneMark-ES. *Curr Protoc Bioinforma* CHAPTER:Unit-4.610.
40. Quevillon E, Silventoinen V, Pillai S, Harte N, Mulder N, Apweiler R, Lopez R. 2005. InterProScan: Protein domains identifier. *Nucleic Acids Res* 33:W116.

- 847 41. Huerta-Cepas J, Szklarczyk D, Heller D, Hernández-Plaza A, Forslund SK, Cook H,
848 Mende DR, Letunic I, Rattei T, Jensen LJ, Von Mering C, Bork P. 2019. EggNOG 5.0:
849 A hierarchical, functionally and phylogenetically annotated orthology resource based
850 on 5090 organisms and 2502 viruses. *Nucleic Acids Res* 47:D309–D314.
- 851 42. Huerta-Cepas J, Forslund K, Coelho LP, Szklarczyk D, Jensen LJ, Von Mering C,
852 Bork P. 2017. Fast genome-wide functional annotation through orthology assignment
853 by eggNOG-mapper. *Mol Biol Evol* 34:2115–2122.
- 854 43. Almagro Armenteros JJ, Tsirigos KD, Sønderby CK, Petersen TN, Winther O, Brunak
855 S, von Heijne G, Nielsen H. 2019. SignalP 5.0 improves signal peptide predictions
856 using deep neural networks. *Nat Biotechnol* 37:420–423.
- 857 44. Katoh K, Kuma K, Toh H, Miyata T. 2005. MAFFT version 5: improvement in
858 accuracy of multiple sequence alignment. *Nucleic Acids Res* 33:511–8.
- 859 45. Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J,
860 Kearse M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Thierer T, Wilson A.
861 2011. Geneious. 5.4.
- 862 46. Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic
863 analyses with thousands of taxa and mixed models. *Bioinformatics* 22:2688–90.
- 864 47. Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for
865 inference of large phylogenetic trees, p. 1–8. *In* Proceedings of the Gateway
866 Computing Environments Workshop (GCE) New Orlean.
- 867 48. Letunic I, Bork P. 2021. Interactive tree of life (iTOL) v5: An online tool for
868 phylogenetic tree display and annotation. *Nucleic Acids Res* 49:W293–W296.
- 869 49. Navarro-Muñoz JC, Selem-Mojica N, Mullowney MW, Kautsar SA, Tryon JH,
870 Parkinson EI, De Los Santos ELC, Yeong M, Cruz-Morales P, Abubucker S, Roeters

- 871 A, Lokhorst W, Fernandez-Guerra A, Cappelini LTD, Goering AW, Thomson RJ,
872 Metcalf WW, Kelleher NL, Barona-Gomez F, Medema MH. 2020. A computational
873 framework to explore large-scale biosynthetic diversity. *Nat Chem Biol* 16:60–68.
- 874 50. Kautsar SA, Blin K, Shaw S, Navarro-Muñoz JC, Terlouw BR, Van Der Hooft JJJ,
875 Van Santen JA, Tracanna V, Suarez Duran HG, Pascal Andreu V, Selem-Mojica N,
876 Alanjary M, Robinson SL, Lund G, Epstein SC, Sisto AC, Charkoudian LK, Collemare
877 J, Linington RG, Weber T, Medema MH. 2020. MIBiG 2.0: A repository for
878 biosynthetic gene clusters of known function. *Nucleic Acids Res* 48:D454–D458.
- 879 51. Martinet L, Naômé A, Deflandre B, Maciejewska M, Tellatin D, Tenconi E,
880 Smargiasso N, De Pauw E, Van Wezel GP, Rigali S. 2019. A single biosynthetic gene
881 cluster is responsible for the production of bagremycin antibiotics and ferroverdin iron
882 chelators. *MBio* 10:e01230-19.
- 883 52. Armaleo D. 1995. Factors affecting depside and depsidone biosynthesis in a cultured
884 lichen fungus. *Crypt Bot* 5:14–21.
- 885 53. Wasil Z, Pahirulzaman KAK, Butts C, Simpson TJ, Lazarus CM, Cox RJ. 2013. One
886 pathway, many compounds: Heterologous expression of a fungal biosynthetic pathway
887 reveals its intrinsic potential for diversity. *Chem Sci* 4:3845–3856.
- 888 54. Chen L, Yue Q, Zhang X, Xiang M, Wang C, Li S, Che Y, Ortiz-López FJ, Bills GF,
889 Liu X, An Z. 2013. Genomics-driven discovery of the pneumocandin biosynthetic gene
890 cluster in the fungus *Glarea lozoyensis*. *BMC Genomics* 14.
- 891 55. Chen L, Li Y, Yue Q, Lokszejn A, Yokoyama K, Felix EA, Liu X, Zhang N, An Z,
892 Bills GF. 2016. Engineering of new pneumocandin side-chain analogues from *Glarea*
893 *lozoyensis* by mutasynthesis and evaluation of their antifungal activity. *ACS Chem*
894 *Biol* 11:2724–2733.

56. Li Y, Chen L, Yue Q, Liu X, An Z, Bills GF. 2015. Genetic manipulation of the pneumocandin biosynthetic pathway for generation of analogues and evaluation of their antifungal activity. ACS Chem Biol 10:1702–1710.
57. Leuckert C. 1985. Probleme der Flechten-Chemotaxonomie -- Stoffkombinationen und ihre taxonomische Wertung. Problems of lichen chemotaxonomy -- patterns of substances and their taxonomical valuation. Ber Dtsch Bot Ges 98:401–408.

Table 1 Genome quality and annotation statistics

Species	TBG number	ccs HiFi yield	# scaffolds	N50	Completeness	assembly size (Mb)	# Genes	# Proteins
<i>Umbilicaria freyi</i>	TBG_2329	47.39%	113	2575640	95.7	53.4	10,156	10,065
<i>Umbilicaria freyi</i>	TBG_2330	46.41%	54	2043163	85.9	50	8,848	8,773
<i>Umbilicaria deusta</i>	TBG_2334	47.86%	47	1669916	97.6	41.6	8,949	8,857
<i>Umbilicaria deusta</i>	TBG_2335	43.54%	42	1865302	90.2	37.4	8,194	8,049
<i>Umbilicaria hispanica</i>	TBG_2322	38.71%	130	3125324	96.8	43.4	9,111	9,021
<i>Umbilicaria hispanica</i>	TBG_2337	54.22%	60	4226768	97.3	41.9	8,781	8,696
<i>Umbilicaria pustulata</i>	TBG_2333	33%	26	2620629	97.3	37.7	9,569	9,503
<i>Umbilicaria pustulata</i>	TBG_2345	32.26%	31	2364512	96.8	35.7	8,790	8,740
<i>Umbilicaria spodochoa</i>	TBG_2434	34.20%	139	993216	97.0	40.9	8,791	8,705
<i>Umbilicaria spodochoa</i>	TBG_2435	40.93%	97	1249424	97.1	40.1	8,612	8,507
<i>Umbilicaria subpolyphylla</i>	TBG_2323	41.14%	190	1544375	99.6	58.2	16,993	16,852
<i>Umbilicaria subpolyphylla</i>	TBG_2324	33.68%	42	1514392	97.6	33.7	8,556	8,410
<i>Umbilicaria grisea</i>	TBG_2336	42.54%	40	1822796	96.9	44.43	NA	NA
<i>Dermatocarpon miniatum</i>	TBG_2326	29.36%	28	5077191	98.1	63.5	9,273	9,189
<i>Dermatocarpon miniatum</i>	TBG_2331	26.28%	22	4245366	98.4	49.8	7,938	7,871

902

Figure 1

Figure 2

Figure 3

Figure 4