

DNA-barcoding of fungi in Austria – state of the art of the HSRM project at the University of Vienna

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A considerable number of sequences of Austrian fungi can be found in publicly accessible databases, such as GenBank and UNITE, but without being specially marked as or meeting the quality requirements of DNA-barcodes. To improve this situation, a three-year higher education structure funding project (HSRM) is currently underway within the framework of the DNA barcoding initiative ABOL with the aim of establishing barcoding pipelines at Austrian universities. From 2017-2019 the University of Vienna will contribute ITS sequences of macromycetes to the project. In the first year about 2000 barcodes of about 500 species could be achieved. Both the checklist of the macromycetes of Austria and the fungarium WU-Mykologicum at the university are central resources. Exemplarily, some results in the genus *Cortinarius* are briefly presented, such as new records of species for the area or rediscovery of red list species, clarification of incorrect determinations and uncertain interpretations of species. Barcoding of fungi is now successfully established at the University of Vienna and will bring further interesting results in the next few years.

KRISAI-GREILHUBER I., 2018: DNA-Barcoding von Pilzen in Österreich – State of the Art des HSRM-Projekts an der Universität Wien.

Es gibt zwar einiges an Sequenzen österreichischer Pilzbelege in öffentlich zugänglichen Datenbanken, wie GenBank und UNITE, jedoch ohne speziell als DNA-Barcodes gekennzeichnet zu sein bzw. die Anforderungen an die Qualität eines genetischen Barcodes zu erfüllen. Um diese Situation zu verbessern läuft derzeit im Rahmen der Barcode-Initiative ABOL ein dreijähriges Hochschulraumstrukturmittel-Projekt mit dem Ziel der Etablierung von Barcoding Pipelines an österreichischen Universitäten. An der teilnehmenden Universität Wien werden dazu von 2017-2019 ITS-Sequenzen von Makromyzeten generiert. Im ersten Jahr konnten etwa 2000 Barcodes von ca. 500 Arten erstellt werden. Dabei sind sowohl die Checkliste der Pilze Österreichs als auch das an der Uni zur Verfügung stehende Fungarium WU-Mykologicum zentrale Ressourcen. Beispielfhaft werden einige Resultate in der Gattung *Cortinarius* kurz vorgestellt, wie neue Artnachweise für das Gebiet oder Wiederauffinden von Rote-Liste-Arten, Klärungen von Fehlbestimmungen und unsicheren Artauffassungen. Barcoding von Pilzen konnte erfolgreich an der Universität Wien etabliert werden und wird in den nächsten Jahren weitere interessante Ergebnisse bringen.

Keywords: ABOL, Austrian Barcode of Life, fungarium, fungi, ITS, *Cortinarius*.

Introduction

Fungal species, typically outnumber vascular plant species, e.g. in temperate forest ecosystems. Despite their enormous importance in ecological respect they are often ignored and still rarely used, e.g. in defining conservation priorities. To prove this fact WINKLBAUER (2018) simply performed a google search term analysis, which is correlated with public interest in a topic. Out of queries for the terms animals, plants, bacteria, and fungi, the lowest values were related to fungi (Fig. 1).

Initial situation

A recent checklist (DÄMON & KRISAI-GREILHUBER 2017) documented more than 4500 macromycete species for Austria whose data was sufficient for critical assessment of bio-

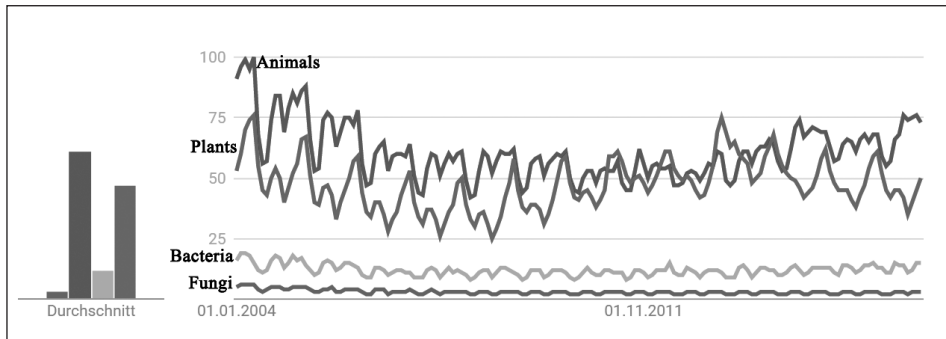


Fig. 1: Number of queries for the terms animals, plants, bacteria, fungi. Source: www.google.com/trends, 29.03.2017, 19h30 (after WINKLBAUER 2018). - Abb. 1: Anzahl der Suchanfragen für die Begriffe Tiere, Pflanzen, Bakterien, Pilze. Quelle: www.google.com/trends, 29.03.2017, 19h30 (nach WINKLBAUER 2018).

diversity risks. As in other groups of organisms, many problems are associated with the identification of fungi. A lot of fungi are still morphologically identified. In December 2017, there were only few barcodes of Austrian fungal specimens in BOLD: 718 Oomycota (private, not yet released), 21 Basidiomycota, 24 Ascomycota, no other groups. Of course, there are also Austrian sequences deposited in diverse public databases, e.g. GenBank or UNITE, but they are not specifically labelled as DNA barcodes. Many *Cortinarius* sequences from IB are deposited in the UNITE database. At the moment a project at the University of Innsbruck runs on DNA Barcoding of the Mycological Collection IB (<http://www.mycologytyrol.org/>). Some Austrian downy mildews (traditionally dealt with by mycologists, although they now are stramenopiles) have been barcoded by CHOI et al. (2015). Concerning applied aspects barcoding of downy mildews of poppy (VOGLMAYR et al. 2014) offers a direct benefit for crop protection in Austria. Several groups of the Ascomycetes were investigated molecular genetically and also barcoded within the scope of other projects, e.g. publications by JAKLITSCH and VOGLMAYR deserve to be mentioned (e.g. DOILOM et al. 2013, FRIEBES et al. 2016, JAKLITSCH 2009, 2011, JAKLITSCH et al. 2013, 2014, 2015, ROBIDEAU et al. 2011, VOGLMAYR, H. & JAKLITSCH 2011, 2014, 2017, VOGLMAYR et al. 2012, 2016). For some genera, where ITS does not resolve enough, secondary barcodes have to be used (XU 2016).

An important improvement – ABOL and the HRSM-project

To improve the situation of having only few barcodes available, not only for fungi but also for other groups of animals (especially invertebrates) and plants, ABOL – an interdisciplinary research network – was launched. In addition to a base financing by the Ministry of Science, individual projects are being applied for. One of these projects that have been approved is the Hochschulraumstrukturmittel (HRSM) project, which is a collaboration of several Austrian universities, led by the University of Graz. The project aims at building university DNA barcoding pipelines for ABOL. As part of this project, barcodes will also be generated at the University of Vienna during the years 2017-2019.

Barcoding of macromycetes started in May 2017. The basis currently used for it is the fungarium WU-Mycologicum, supplemented with freshly collected material. Specimens

from other herbaria all over Austria will be added in the next years. Until April 2018 approx. 2000 sequences have been produced, meaning barcodes for ca. 500 fungal taxa from Austria.

A glance at the first results

To give a first impression of these results some examples from the genus *Cortinarius* are presented. *Cortinarius* is the largest genus of ectomycorrhizal fungi with 4.700 described taxa worldwide (LIMATAINEN et al. 2014), 450 of which occur in Austria. They are characterized by a cob-web like partial veil, the cortina, and brown, ornamented spores and are traditionally placed in several subgenera; but the infrageneric hierarchy proved to be artificial.

Barcoding in the genus is used for confirmation of known taxa, revealing of cryptic species and misidentifications, detection of new species, lumping of species and aims at easy identification of all of these taxa in future.

A specimen determined as *Cortinarius arvinaceus*, which is a taxonomically unclear species, was revealed as a common species, *C. lividoochraceus* s. auct. p.p. and also *C. elatior* s. auct. p.p.. But also these two old names are problematic to interpret and there is a search going on for the oldest name with type sequence in order to come to a stable interpretation (BALINT DIMA, pers. comm.). Another specimen, namely WU 8183, collected in 1989, turned out to belong to *C. alticaudus*, which was described in 2008 from France with *Pinus* as symbiont. A recently made collection looked similar to *C. vernus* and was in fact *C. nolaneiformis*. This species was described by Velenovsky as *Hydrocybe* and recently recombined into *Cortinarius* by DIMA et al. (2014). It is known from several European countries, but can be easily confused with similar species, such as *C. uraceomajalis* or *C. vernus*.

The ITS sequence of WU 7994 from 1989, formerly identified as *C. paracephalixus* matched with the type of *C. rioussetiae*. The latter is considered as synonymous to the former by the Nordic *Cortinarius* School, but is treated separately by French authors (CAMPO 2015).

The rare and red-listed species - IUCN-category endangered - *C. aureopulverulentus*, *C. populinus* and *C. xanthochlorus* could be reconfirmed.

Two other specimens, identified as the doubtful species *C. pseudodiabolicus* and *C. pseudherpeticus* had matches with *C. riederi* and thus have to be included in this species.

Cortinarius scaurocaninus could be recorded with barcoding, and we do not know of any other record for this species in Austria (KRISAI-GREILHUBER et al. 2017). The same is true for *C. alboamarescens*, recently described as a new species of the *C. vibratilis* complex, in 2015, and known from Denmark, Finland, France, Sweden, England (KIBBY et al. 2016), and now also Austria. *Cortinarius balteaticlavatus* was described as a new species of the *C. balteatus* alliance in 2014 (LIMATAINEN et al. 2014) from Finland and is now also confirmed for Austria from Carinthia (WU 28720).

The specimen WU 29938, morphologically identified by *Cortinarius* specialists as *C. bayeri* proved to be *C. floccopus*. WU 38030 could be confirmed as *C. parasuaveolens* with a 100 % match. The species was described as *C. sodagnitus* var. *parasuaveolens* and elevated to species level in the year 2000 by BIDAUD, MOËNNE-LOCCOZ and REUMAUX. Another



Fig. 2: *Cortinarius boreocyanites* (above) and *C. violaceorubens* (below) from the *C. cyanites* alliance, discovered as new for Austria by DNA barcoding. – Abb. 2: *Cortinarius boreocyanites* (oben) und *C. violaceorubens* (unten) aus der *C. cyanites* Gruppe, für Österreich durch DNA barcoding neu entdeckt.

specimen, WU 31271, had a type match with *C. aurantiobasalis*, which was described from France and is a species forming symbiosis with deciduous and coniferous trees.

Species in the *Cortinarius cyanites* alliance are very rare. This group was recently studied by LIMATAINEN et al. (2014) and now comprises three species. We so far sequenced three specimens, two were revealed as *C. boreicyanites* and one as *C. violaceorubens* (Fig. 2). *Cortinarius boreicyanites* is a boreal species described by LIMATAINEN et al. (2014) and is known from Sweden, Finland, Scotland and now Austria. *Cortinarius violaceorubens* is known from Finland, France, Sweden, Germany and now Austria. Also the morphologically difficult group around *C. cephalixus* is best supplemented by sequences. Within this group we could confirm *C. cinctipes*, now called *C. pseudocephalixus*. *Cortinarius infestans*, described by Moëgne-Loccoz from France on calcareous soil in mixed forest, and also known from Norway from a *Tilia/Corylus* forest, is now confirmed for Austria.

The list of *Cortinarius* species, which could in addition to morphological characters be confirmed molecular genetically as present in Austria, is much longer. Here I just continue to mention some species, revealed by barcoding, which most likely seem to be new additions to the Austrian Funga, namely *C. collocandoides*, *C. flavoaurantians*, *C. infucatus*, *C. lilacinovelatus*, *C. luteobrunnescens*, *C. piceidisjungendus*, *C. pseudofallax*, *C. rapaceoides*, *C. uraceonemoralis*, and *C. vacciniophilus* (= *C. pseudonaevosus*). There is also evidence of new species records for Austria in other genera. Details would go beyond the scope of this opinion paper and will be presented elsewhere.

Conclusio

- Barcoding of fungi has been successfully established at the University of Vienna, all the generated barcodes will be deposited in the ABOL-database and thereby also in BOLD;
- Continuation of barcoding to better characterize the Austrian high species biodiversity is very important;
- Consideration of mycological data in conservation issues would be an important step forward;
- Funding for further research is urgently necessary.

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