BARCODING QUARANTINE FUNGI: LESSONS FROM THE EUROPEAN QBOL PROJECT AND Q-BANK DATABASE

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Phytosanitary risk represents one of the biggest constraints on global import and export of agricultural produce (see for example Haack et al. 2014). This risk has been translated into quarantine legislation by numerous countries of the world, with some countries being more restrictive and others being less so depending on the danger of a foreign introduction to local agriculture. The European Union also has such legislation (COUNCIL DIRECTIVE 2000/29/EC) in place to regulate quarantine pests. Cooperation in plant protection between 50 member states is coordinated by EPPO (European and Mediterranean Plant Protection Organization; http://www.eppo.int). The EPPO quarantine list contains 373 entries and is divided into A1 (currently absent from the EPPO region; 214 entries) and A2 (locally present but controlled in the EPPO region; 159 entries) lists.

Breakdown of number of entries on the EPPO lists per organism group.

	Bacteria and phytoplasmas	Fungi	Parasitic plants	Insects and mites	Nematodes	Viruses and virus-like organisms	Total
A1 list	17	36	12	121	5	23	*214*
A2 list	26	28	11	61	11	22	*159*
Total	*43*	*64*	*23*	*182*	*16*	*45*	***373***

Communication of the identity of fungal pests is hampered by the fact that individual species are often named for their particular morphs ("dual nomenclature") and therefore, for example, a sexual morph could be listed under its asexual name on a quarantine list and as such potentially escape interception at a customs or quarantine authority inspection. A recent example includes the confusion surrounding the application of *Uredo rangelii* ("myrtle rust") or *Puccinia psidii* ("Eucalyptus rust"); where both names refer to a genetically identical or highly similar fungus but of which the second is a serious quarantine organism in many countries (Carnegie & Cooper, 2011). The dual nomenclatural system was abandoned at the International Botanical Congress in Melbourne (Hawksworth et al. 2011, Wingfield et al. 2012) and the process to decide on a single name for any given fungus is currently progressing (e.g. Crous et al. 2014).

In 2009, the EU 7th Framework Program financed the Quarantine Barcoding of Life (QBOL; Bonants et al. 2010) project for three years to develop a DNA barcode-based diagnostic tool for selected quarantine species. The project consortium had 20 partners (universities, research institutes and phytosanitary organisations; see http://www.qbol.org) sharing their expertise in the field of DNA barcoding of arthropods, bacteria, fungi, nematodes, phytoplasmas and viruses. Quarantine species were selected from the EU Council Directive and EPPO lists based on the availability of specimens and/or taxonomic expertise. Vouchered specimens or strains of included species were sequenced for informative genes (vs. complete genomes for viruses) and selected gDNA are maintained in a DNA bank. The Work Package Fungi targeted species from the genera *Ceratocystis*, *Pseudocercospora* (Crous et al. 2013), *Melampsora*, *Monilinia*, *Mycosphaerella* (Quaedvlieg et al. 2012), *Puccinia*, *Septoria* (Quaedvlieg et al. 2013; Verkley et al. 2013) and *Thecaphora* to supplement data on *Colletotrichum* (e.g. Damm et al. 2012, 2013), *Phoma* and allied genera (Aveskamp et al. 2010; de Gruyter et al. 2013) and *Phytophthora* already present in Q-bank (http://www.q-bank.eu; Bonants et al. 2013). The obtained results were disseminated by training at global mandated labs and obtained sequences and associated metadata were placed in the Q-bank database.

The internally transcribed spacer (ITS) regions of the nrDNA operon are used as primary barcode to confirm the taxonomic identity of all strains and to evaluate the resolution of this commonly used region in a given genus or species complex. The ITS locus has been formally proposed as barcode for *Fungi* (Schoch et al. 2012). The locus is in general easy to amplify with universal primers, are present in multiple copies, the locus size (approximately 500 bp) is ideal for routine Sanger sequencing, numerous reference sequences are already available in public database such as NCBI's GenBank and it works well for species identification in many genera. However, for some genera such as *Cercospora* (Groenewald et al. 2013), *Colletotrichum* (Cannon et al. 2012), *Pseudocercospora* (Crous et al. 2013), and *Septoria* (Verkley et al. 2004; Quaedvlieg et al. 2013), this locus has limited resolution for species identification. Additional house-keeping genes, such as translation elongation factor 1-alpha, beta-tubulin, calmodulin etc., are used as secondary, or sometimes even tertiary, barcodes to identify species with poor ITS resolution. Of importance for all loci is that a sufficiently large "barcode gap" exists for the selected locus to serve as proper barcode (Hebert et al. 2003).

The Q-bank database (http://www.q-bank.eu) is a reference database aimed at, for example, national plant protection organizations, general

inspection bodies, and private laboratories. The website contains databases focused on species of quarantine importance to Europe and their closest relatives and includes regulated plant pests of bacteria, fungi, insects, nematodes, phytoplasmas, and viruses, as well as invasive plants. Curators with taxonomic, phytosanitary and diagnostic expertise are responsible for the contents of the databases. Voluntary contributions of missing data or organisms by third-party experts are possible and guidelines for this are provided on the website. The Q-bank Fungi database contains mainly DNA sequence data of more than 800 species (represented by more than 2,800 strains or specimens) that are of relevance to phytopathology and includes species of the genera *Colletotrichum*, *Ceratocystis*, *Melampsora*, *Monilinia*, *Mycosphaerella*, *Phoma*, *Phyllosticta*, *Puccinia*, *Stenocarpella* and *Phaeocytostroma*, *Thecaphora*, *Verticillium*, and the Oomycete genus *Phytophthora*. Entries are linked to other databases such as EPPO, MycoBank, GenBank and culture collection websites. An identification of an unknown sequence can be performed against all sequences in Q-bank or, in cases where prior information about the source of the sequence is available, against a specific organism group. Once a sequence has a significant similarity to one of the fungal groups in Q-bank, a focused simultaneous multilocus identification is possible within that group. Detailed methodologies for obtaining the required sequence(s) and a molecular decision scheme for the included organisms of quarantine importance to Europe are available on the website. The biggest advantages of the Q-bank database are (i) the robust simultaneous identification based on sequence data from multiple loci, (ii) the hyperlinks to vouchered specimens, EPPO information sheets, and MycoBank for taxonomic information, and (iii) the fully-customizable search queries which allow a user to get the most out of the database, at both strain and species level.

The QBOL project and Q-bank database have taught us some interesting lessons in the past five years and also provide some challenges for the future. One of the biggest surprises during the QBOL project was that it was quite challenging to obtain sufficient material for some of the quarantine species, making it almost impossible to determine the intraspecific variation. This highlights the need to place good specimens in herbaria and cultures in public culture collections. Another challenge was to find a primary or secondary barcode that could work for all included genera. This proved to be nearly impossible, therefore the ITS was used as primary barcode and, through a molecular decision scheme, the choice of a secondary barcode is proposed based on the outcome of the ITS identification. The secondary barcode locus, and in some cases even the primers for this locus, are dependent on the genus in question. One of the biggest challenges of the Q-bank database is general maintenance, such as having correct species names in a time of constantly changing taxonomy (including the move towards a single name for a fungus) and even having the correct species name linked to a specific specimen or culture (due to cryptic species complexes being unraveled by molecular studies). Another challenge is community involvement; currently the curators are actively involved in making data available through the Q-bank portal, but ideally experts on the included fungal genera should become motivated to make their data available pro-actively to the database.

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