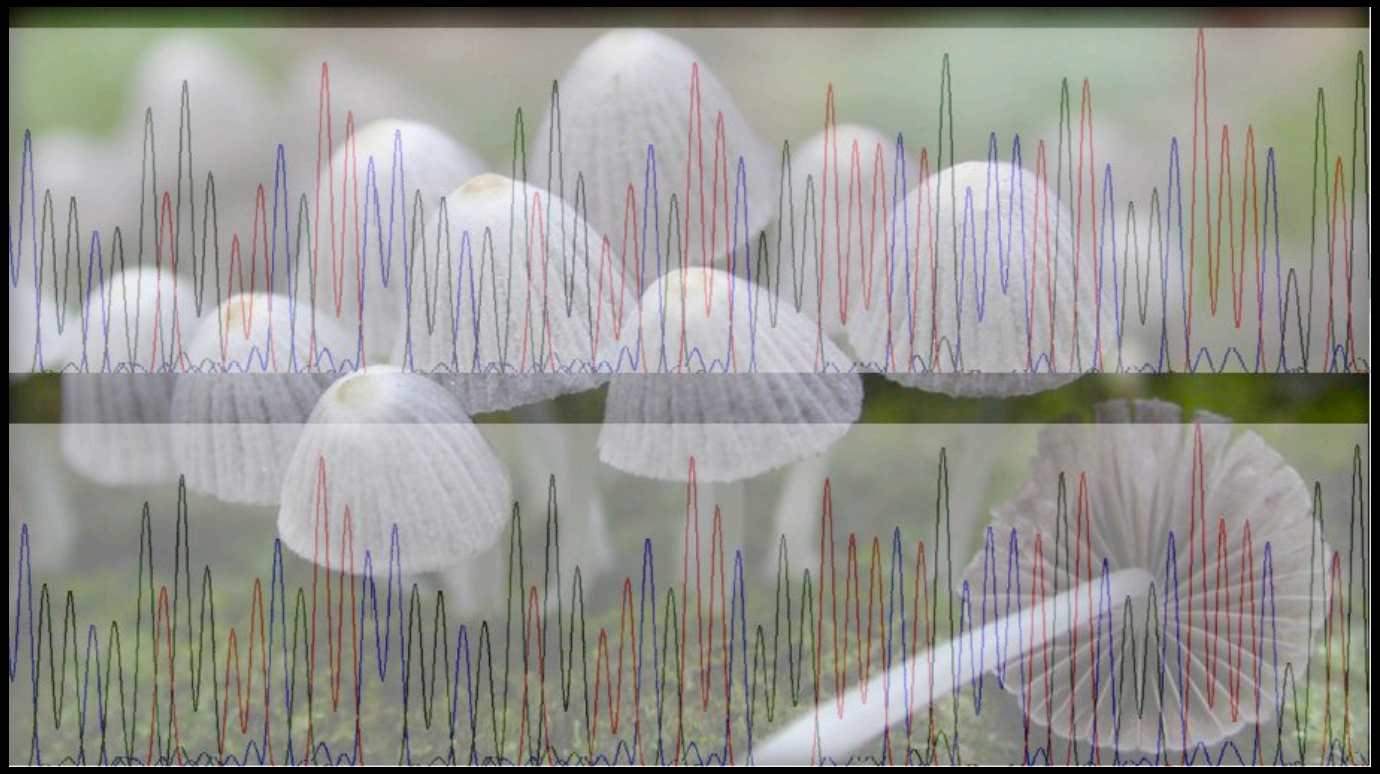
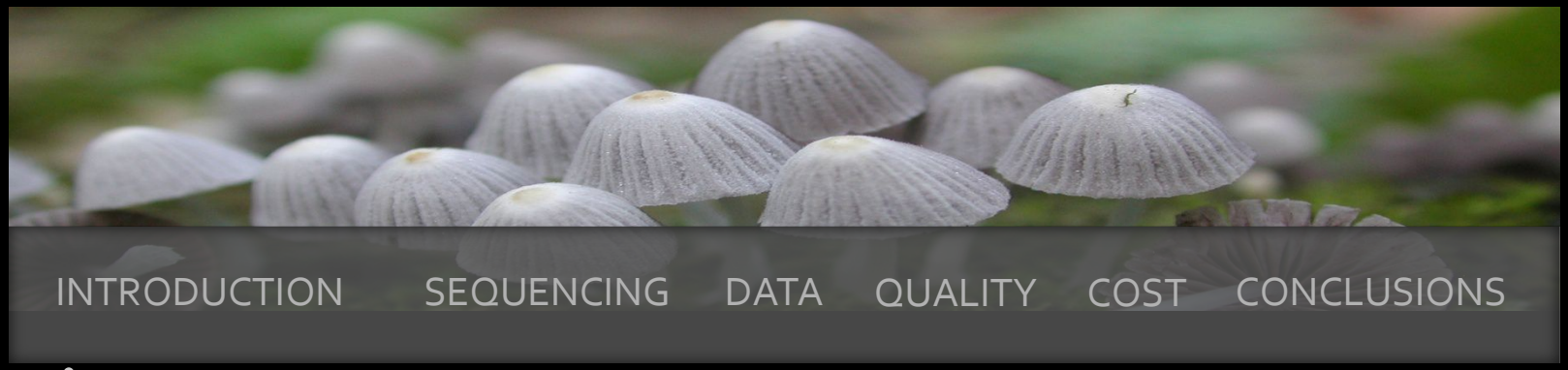


LARGE-SCALE BARCODING OF FUNGAL COLLECTIONS

Todd W. Osmundson & Matteo M. Garbelotto
University of California, Berkeley, CA, USA



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Venice:
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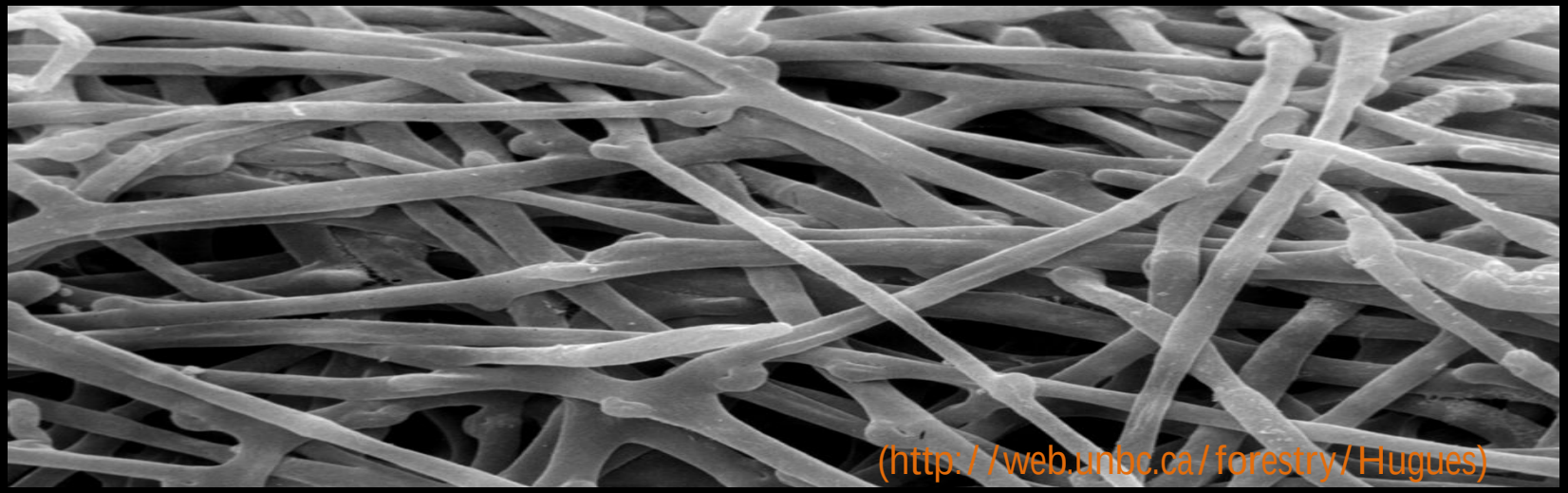
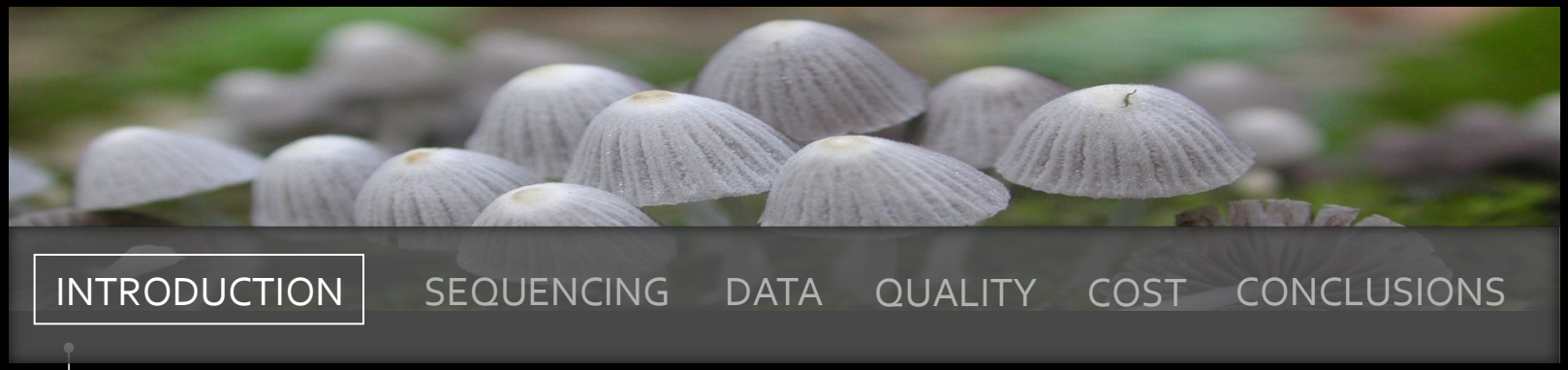
Lydia Baker

Moorea:
Sarah Bergemann
Neil Davies
Chris Meyer
Lydia Smith
Lydia Baker
Gordon & Betty Moore Foundation

- Fungi are diverse and poorly-known

  - Most are cryptic over the majority of their life cycle



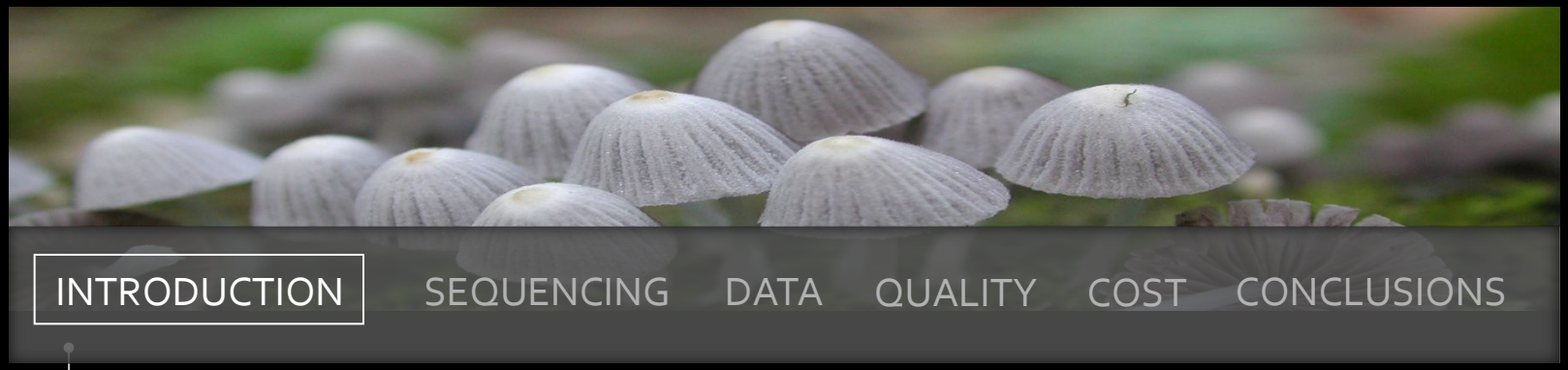


Unknowns:

- Diversity of life cycle stages (e.g., endophyte to pathogen transitions)
- Host ranges
- Geographic ranges / biogeography
- Community ecology

DNA Barcoding is a useful and important tool for identifying fungi and thereby understanding these factors…

… However, utility dependent upon existence of comprehensive sequence databases and methods for confidently assigning taxonomic identities via sequence comparisons.



Environmental DNA sequencing has generated large numbers of "insufficiently identified" sequences in INSD; will increase with high-throughput sequencing

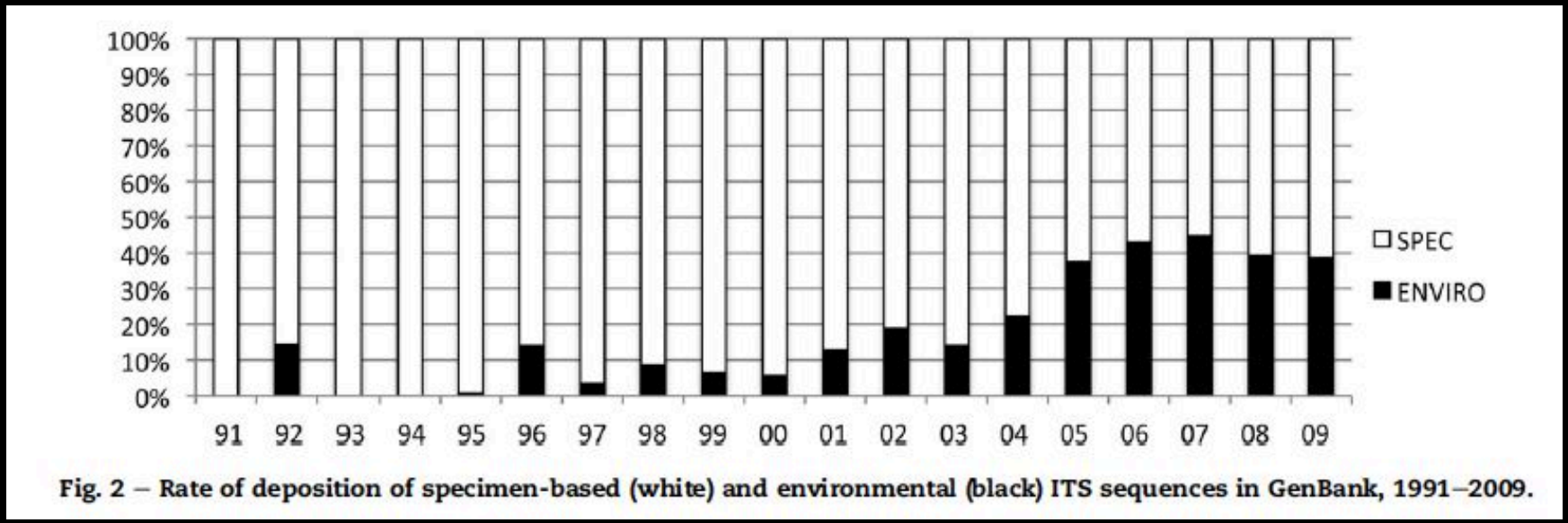
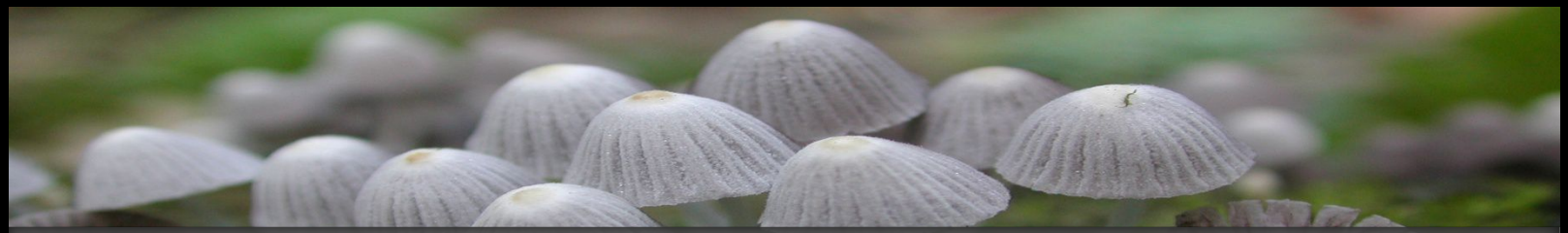


Fig. 2 – Rate of deposition of specimen-based (white) and environmental (black) ITS sequences in GenBank, 1991–2009.

Hibbett et al. 2011, *Fungal Biol. Rev.*



Causes:

- Many identified species not sequenced
- Unknown taxa

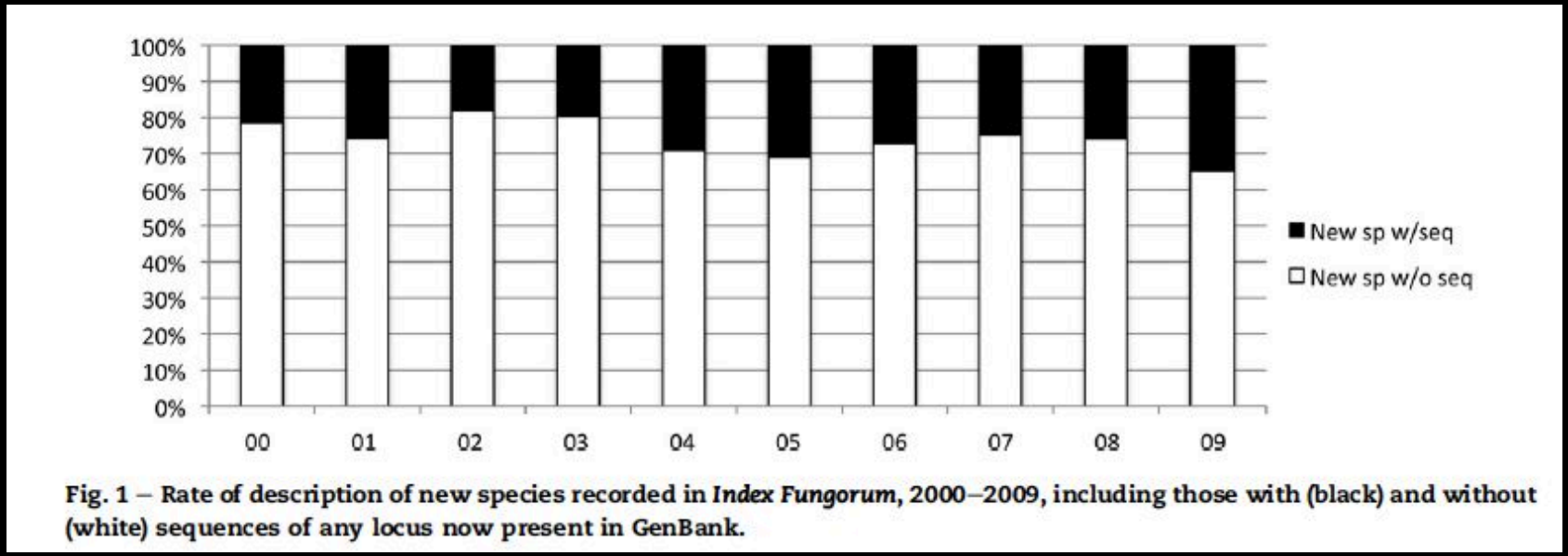
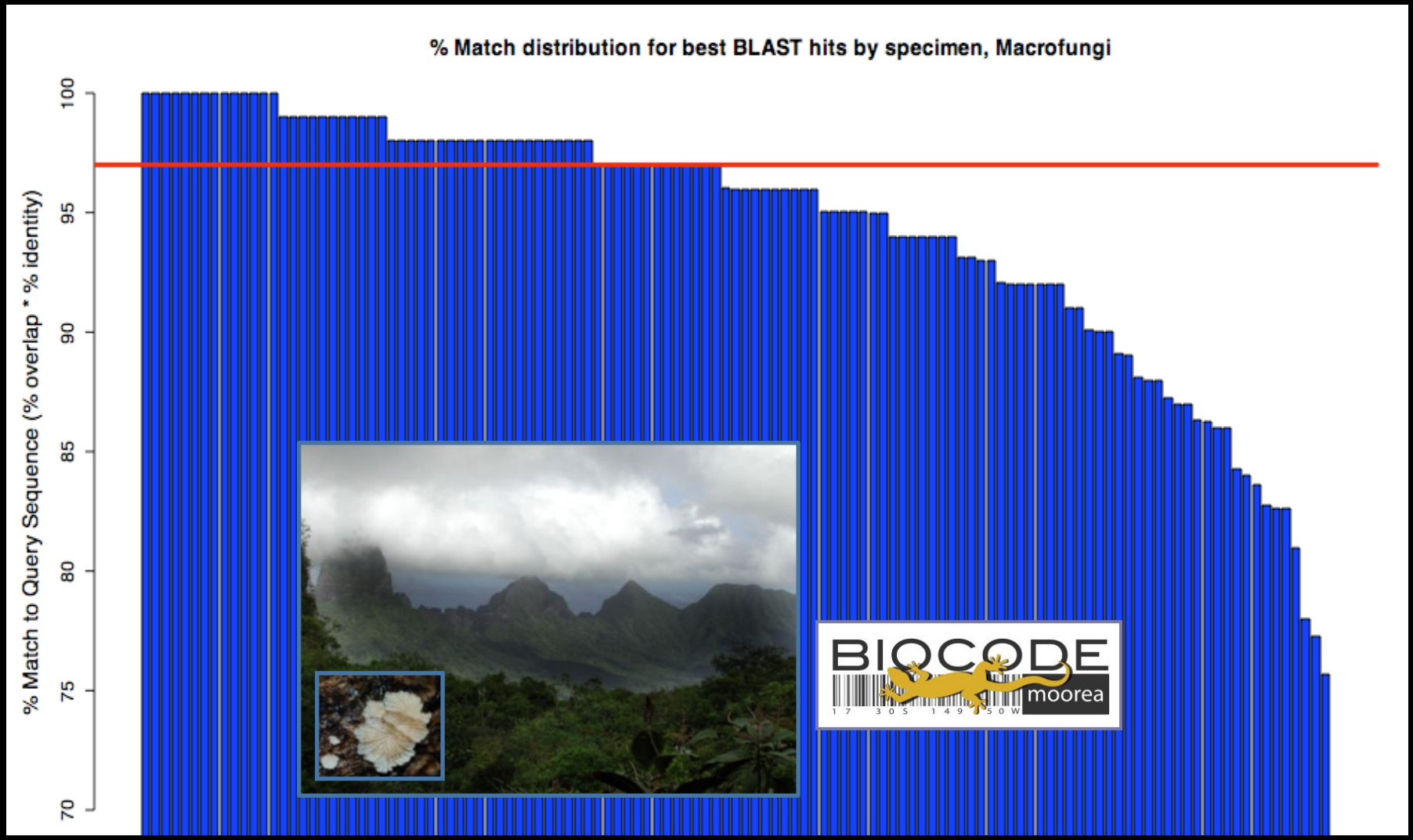
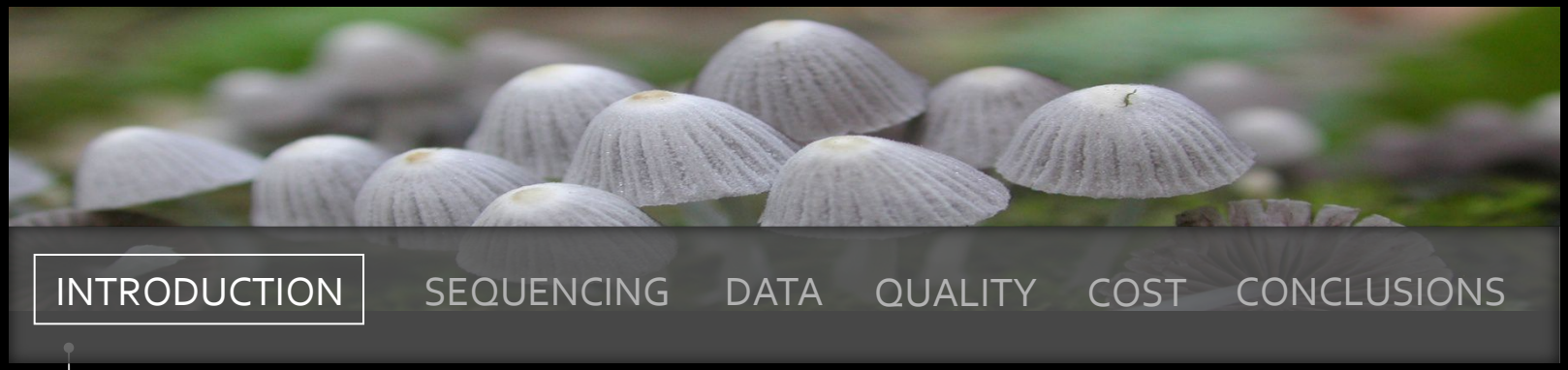


Fig. 1 – Rate of description of new species recorded in *Index Fungorum*, 2000–2009, including those with (black) and without (white) sequences of any locus now present in GenBank.

Hibbett et al. 2011, *Fungal Biol. Rev.*

Moorea: 62% exhibit < 97% similarity to GenBank sequences; 23% exhibit best BLAST match to environmental sequence (cultured or uncultured)

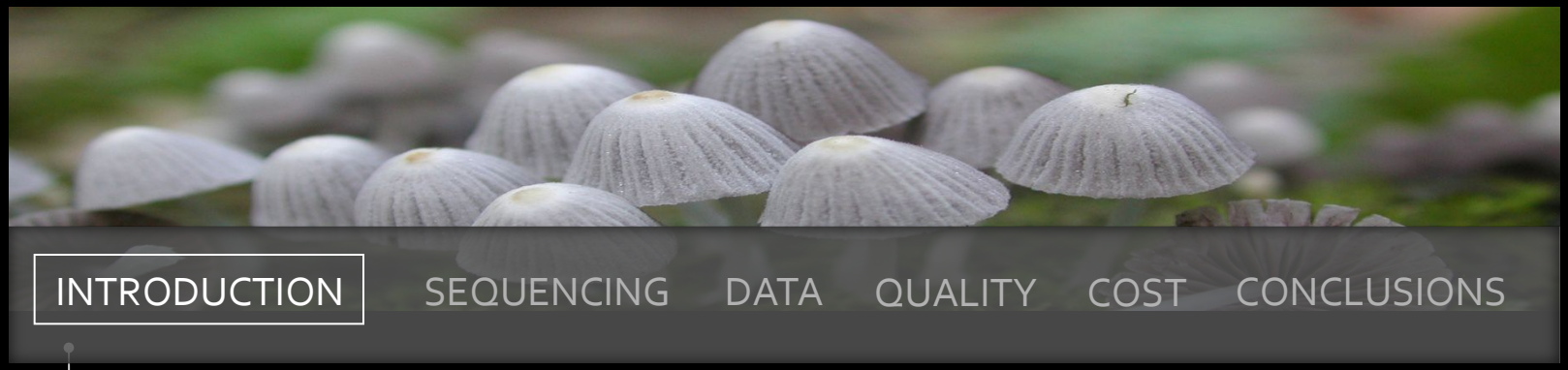




- Having a sequence first can be useful
  - Jones et al. 2011, *Nature*; Cryptomycota
  - Nilsson et al. 2011, *Cladistics*; phylogenetic utility
- Naming sequence OTUs (MOTUs) can provide a common language

2 Charges:

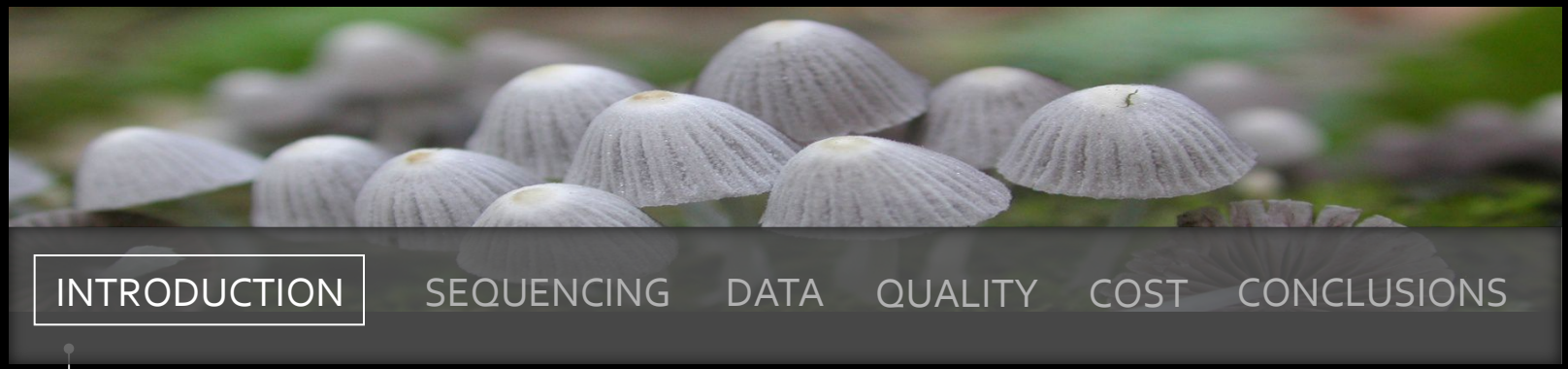
- Sequencing known taxa
- Gaining biological understanding for sequences that do not yet correspond to a "known" taxon (community ecology; sequence-generated autecological studies; phylogenetic understanding)



Large-scale barcoding projects can aid in closing the sequencing gap.
2 recent projects:

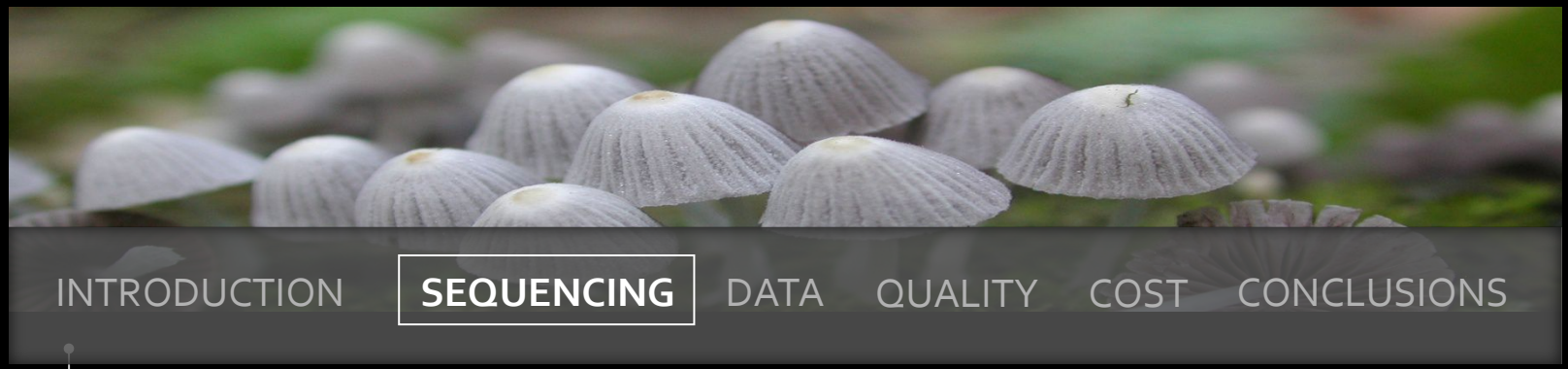
Barcoding the Venice Museum of Natural History Fungal Collection (Institutional)

The Moorea Biocode Project (Geographic)



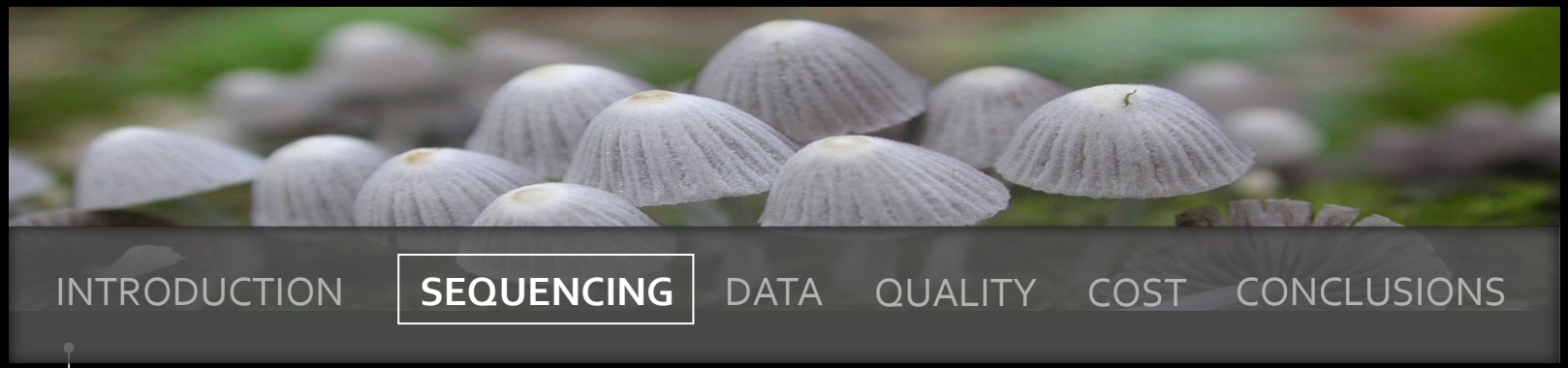
Questions:

1. How can we streamline the sequencing process?
2. How can we streamline and address issues in data management?
3. How can we improve quality control?
4. How can we pay for it?



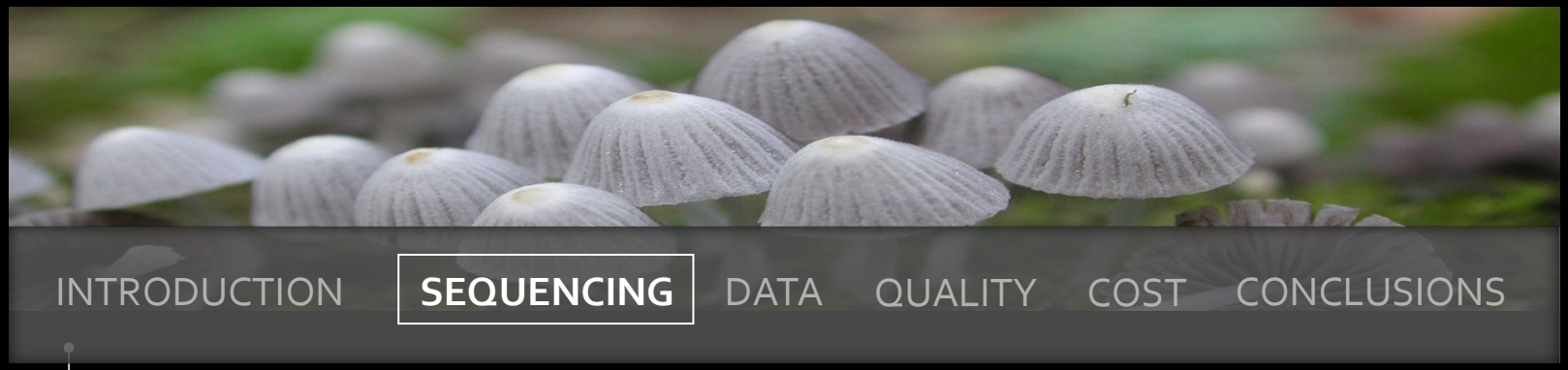
How can we streamline the sequencing process?

- (A.) Rapid extraction techniques (Osmundson et al., in review)
  - NaOH works for most applications (ITS, multicopy microsatellites); ROSE better for *Phytophthora ramorum* detection in tanoak leaves
  - Extract dilutions viable for at least 3 years
  - Improved sequence success from contaminated sporocarps



How can we streamline the sequencing process?

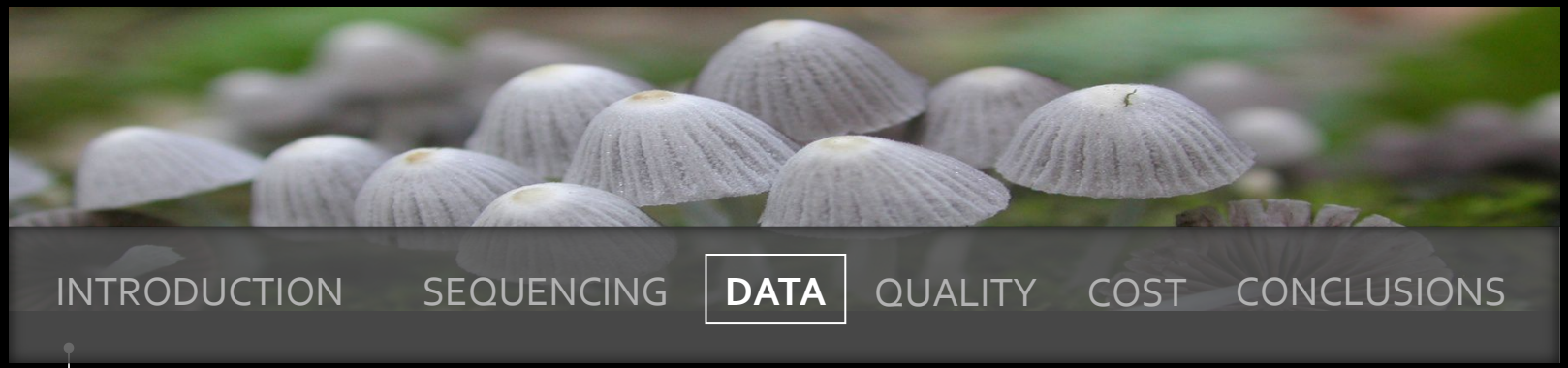
- (B.) Triage: What are our community priorities?
- (C.) Centralized sequencing facilities
  - Cost reduction
  - Uniform methodologies
  - High throughput
  - Centralized data management expertise



How can we streamline the sequencing process?

- (D.) High-throughput methodologies
  - Limits: read lengths, number of multiplex tags
  - Ion Torrent chip $99; ½ the cost of a Sanger plate; how many specimens can we fit on it?
  - Multi-locus barcodes are coming
- (E.) Mini-barcodes
- (F.) Volunteer lab help (e.g., local mycophiles)

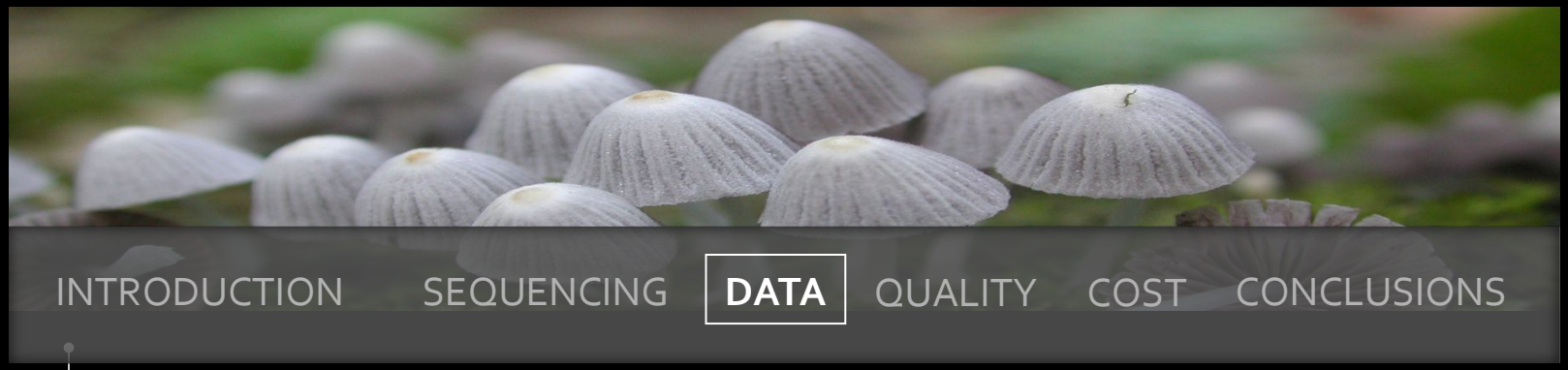
	Complete	ITS1	ITS2
Complete	1		
ITS1	0.7317	1	
ITS2	0.6430	0.5433	1



How can we streamline and address issues in data management?

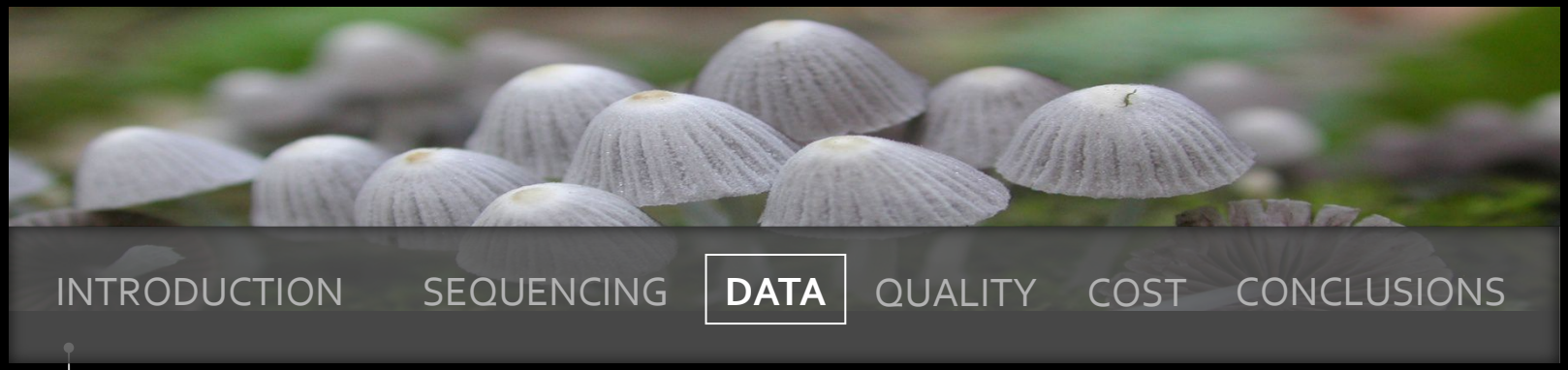
2 components:

- Sequence data
  - Venice: brute force approach to contig editing, BLAST verification, etc.
  - GenBank submission: tbl2asn; perl
  - Moorea Biocode workbench: track lab workflows and run batch BLAST searches
  - Automation:
    - Feature annotation tables
    - Error checking/contig assembly
    - BLAST verification
    - Sequence submission
    - Taxon naming (eliminating “back and forth”)



How can we streamline and address issues in data management?

- Metadata
  - Venice: source annotation tables prepared by hand
  - Moorea Biocode workbench: FIMS + LIMS → GenBank



Certainly, more metadata can go into GB record than usually does, but still many additional types:

- Taxonomic (Mycobank, Index Fungorum)
- Range (GBIF, Mushroom Observer)
- Photographs (MO, Encyclopedia of Life, Wikipedia)
- Rarity and conservation status
- Identification (Pacific NW Key Council, Fusarium ID)

Data linkage – output of one program converted to input of another ("data permaculture")

A field guide for the (early) 21st century: Static → Dynamic

Hygrocybe noelokelani
Desjardin & Hemmes

Cap 10–30 mm broad, convex expanding to plano-convex, striate, glutinous to viscid, glabrous, not hygrophanous, deep pink fading to pinkish white. Gills adnate to shallowly subdecurrent in age, distant, broad, pinkish white. Stem 25–40 x 2–5 mm, cylindrical, equal, glutinous to viscid, glabrous, yellow to yellowish white. Spore deposit white. Edibility: unknown.

This spectacularly pink *Hygrocybe*, described from Hawai'i, is initially covered by a thick jellylike layer in wet conditions that becomes a thin viscid (sticky) layer as the mushroom matures. It grows on moss-covered substrates and is allied with *H. laeta* from the northern hemisphere and *H. graminicolor* from New Zealand. The stem is also covered with a thick jellylike coating. The species name refers to the pink rose of Maui. Is: HA, KA, MO.

Note the jellylike coating on the stem of *Hygrocybe noelokelani*. These specimens were growing in a kipuka along the Saddle Road on the Big Island.

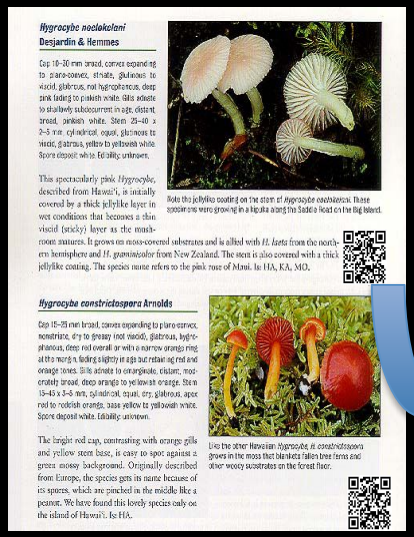
***Hygrocybe constrictospora* Arnolds**

Cap 15–25 mm broad, convex expanding to plano-convex, nonstriate, dry to greasy (not viscid), glabrous, hygrophanous, deep red overall or with a narrow orange ring at the margin, fading slightly in age but retaining red and orange tones. Gills adnate to emarginate, distant, moderately broad, deep orange to yellowish orange. Stem 15–45 x 3–5 mm, cylindrical, equal, dry, glabrous, apex red to reddish orange, base yellow to yellowish white. Spore deposit white. Edibility: unknown.

The bright red cap, contrasting with orange gills and yellow stem base, is easy to spot against a green mossy background. Originally described from Europe, the species gets its name because of its spores, which are pinched in the middle like a peanut. We have found this lovely species only on the island of Hawai'i. Is: HA.

Like the other Hawaiian *Hygrocybe*, *H. constrictospora* grows in the moss that blankets fallen tree ferns and other woody substrates on the forest floor.

A field guide for the (early) 21st century: Static → Dynamic



Hygrocybe noelokelani
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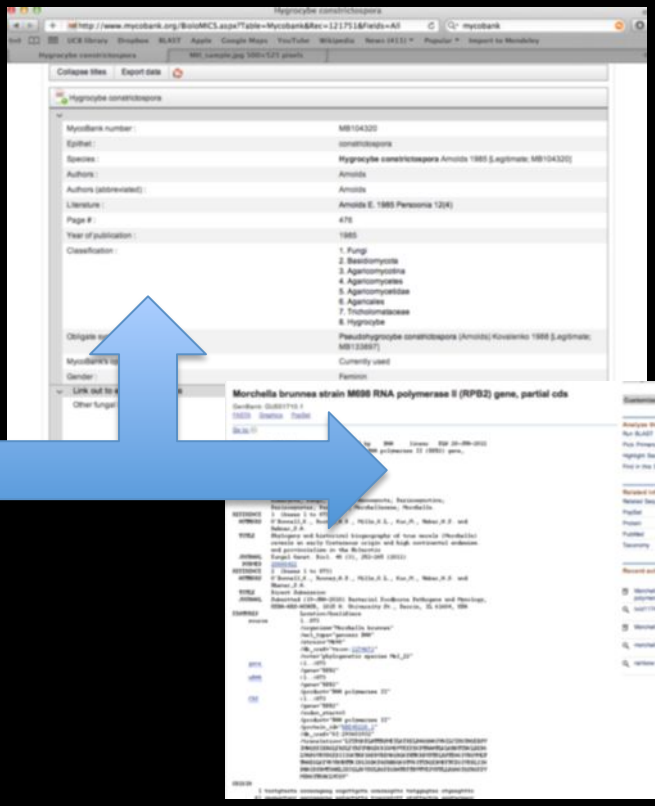
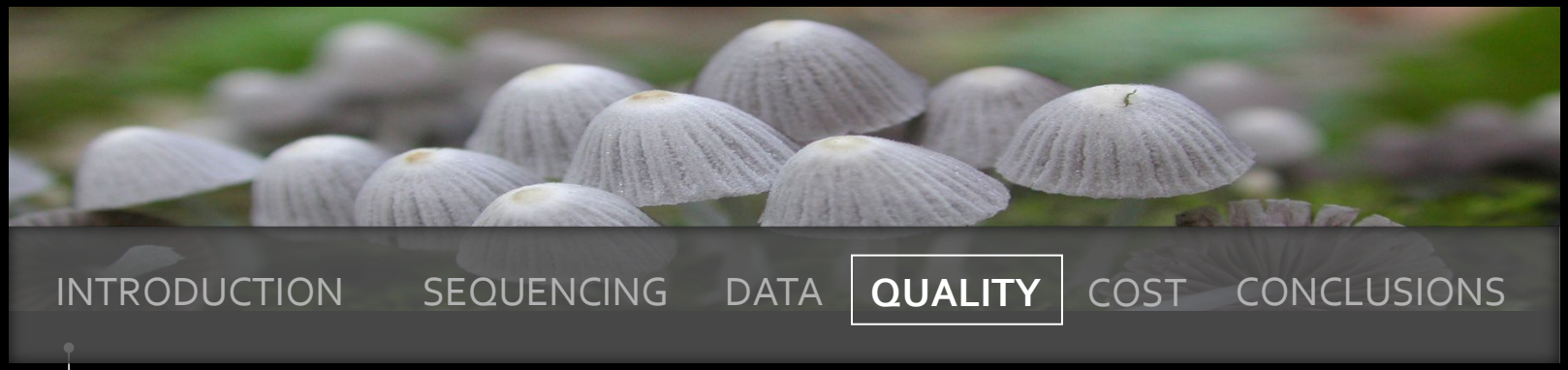
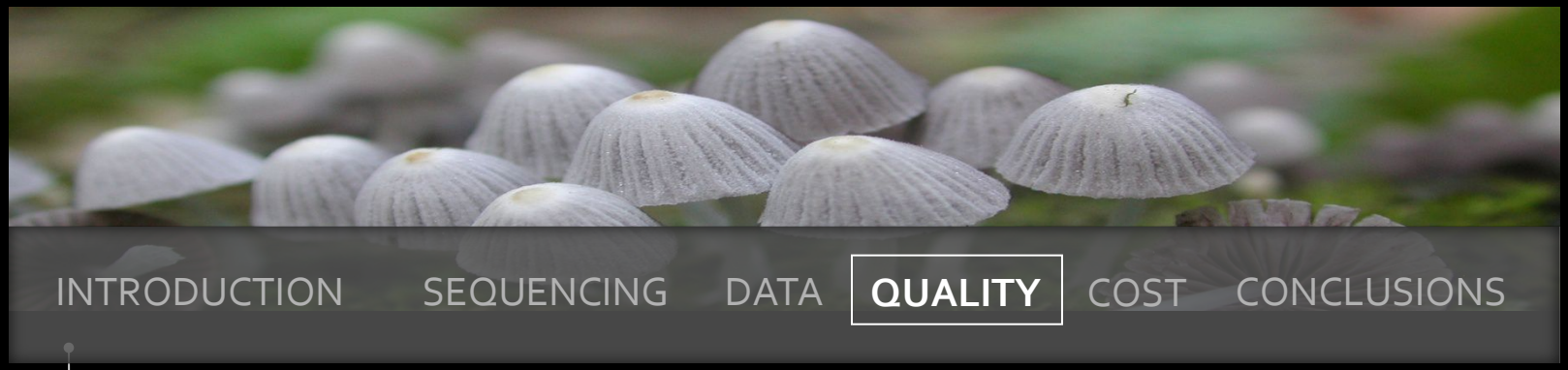


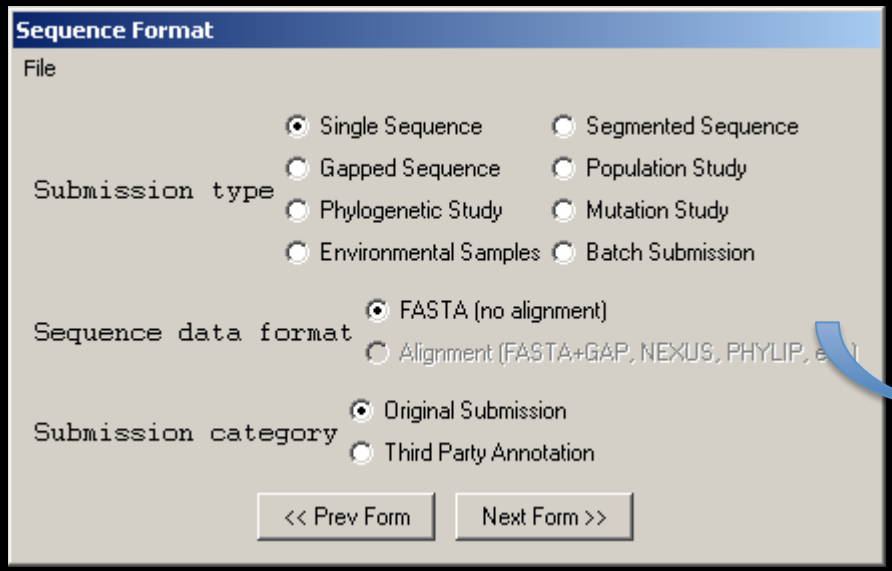


How can we improve quality control?

- Lab-level (chimeras, contaminants, etc.)
- Taxonomic (is the ID correct?)
  - No easy answers
  - An *iterative*, *collaborative* process
    - Collaborations with taxonomic specialists: important specimens get sequenced, and are well identified (e.g., Vellinga, Halling & Hibbett, Barge, Jarvis, Wenck-Reilly, Smith), and sometimes extra lab help!



- Taxonomic (continued)
  - 3rd-party annotations of sequence records



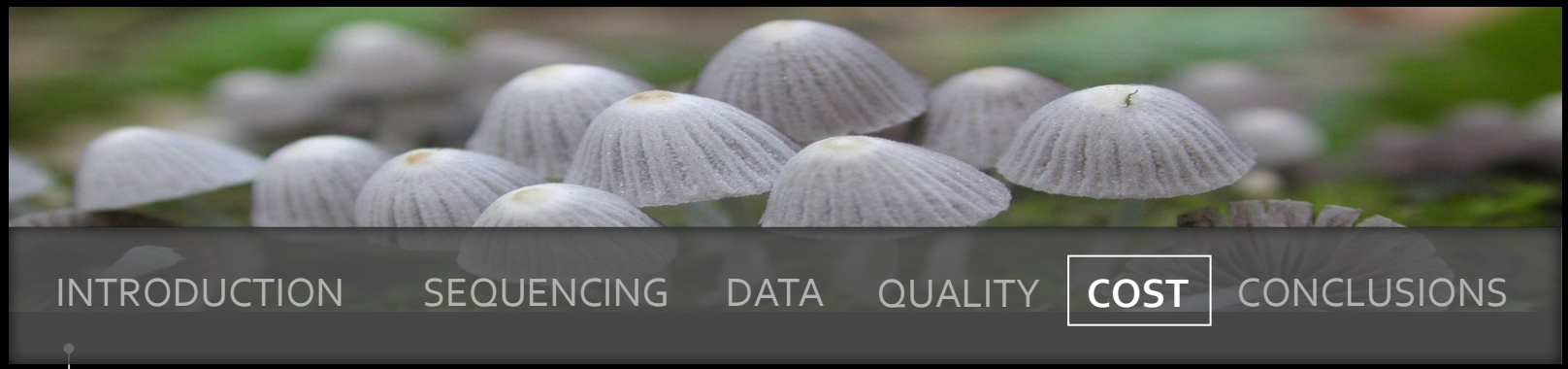
  - GenBank note: “Name taken from herbarium label; specimen not examined”

How can we pay for it?

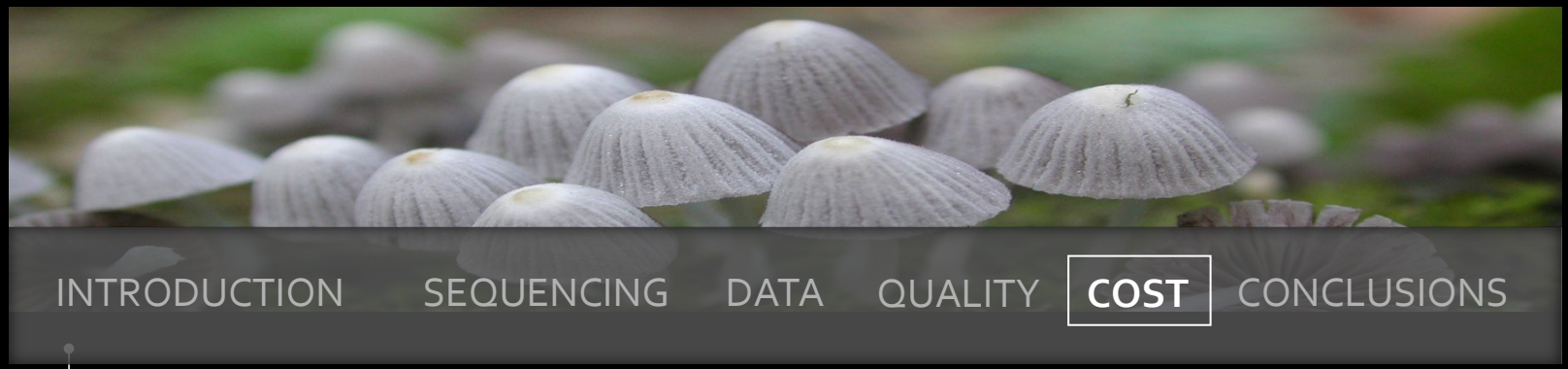
- We have the advantage of less animosity between "taxonomic" and "molecular" contingents, due to cryptic manifestations and paucity of morphological characters
- Presenting a unified front; making the case for how essential molecular data are to much of the work that we do; the Deep Hypha model

How can we pay for it?

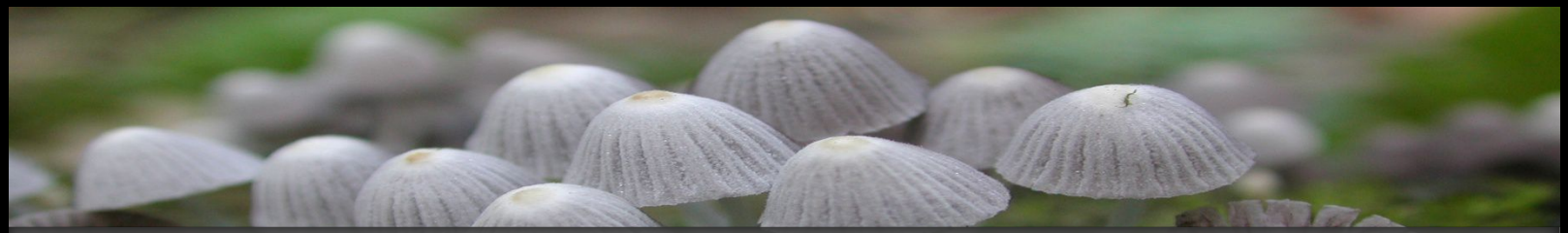
Public goods and the "free rider problem"



- Who are the end-users of fungal biodiversity data?
  - Taxonomists
  - Ecologists
  - Mushroom clubs
  - Commercial harvesters
  - Land managers



- Potential funding sources
  - Request grant funding for barcoding component in ecological and taxonomic studies
  - Mushroom clubs
  - Publishers of field guides (sequence data as a "value-added" component)
  - Small grants from parks, etc. (e.g., Pt. Reyes)
  - Rent capture on mushroom harvests
  - PI research gift funds



A mycoflora-scale barcode database is a big job, but it can be done.

Main elements:

1. Methodological advances (sequencing, data management, IT, bioinformatics)
2. Collaboration (systematists, ecologists, mushroom clubs, knowledgeable "amateurs," bioinformaticians, etc.)
3. Unified front for requesting funding and doing the work