From Barcoding to Biodiversity:

What Are We Learning About Fungi?

Mary Ann Hawke PhD
Scripps Institution of Oceanography

San Diego Mycological Society
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Thanks!

- SDMS Board
- Collectors
- Volunteers
- Exhibitors
- Mesa College

Outline

Describe recent advances in research on fungi

Explain how they are relevant to San Diego and the SDMS!

Background/Context

- Importance of biodiversity
- Citizen science review

Barcoding

- What is it?
- Why should we care?
- How to get involved!







Biodiversity

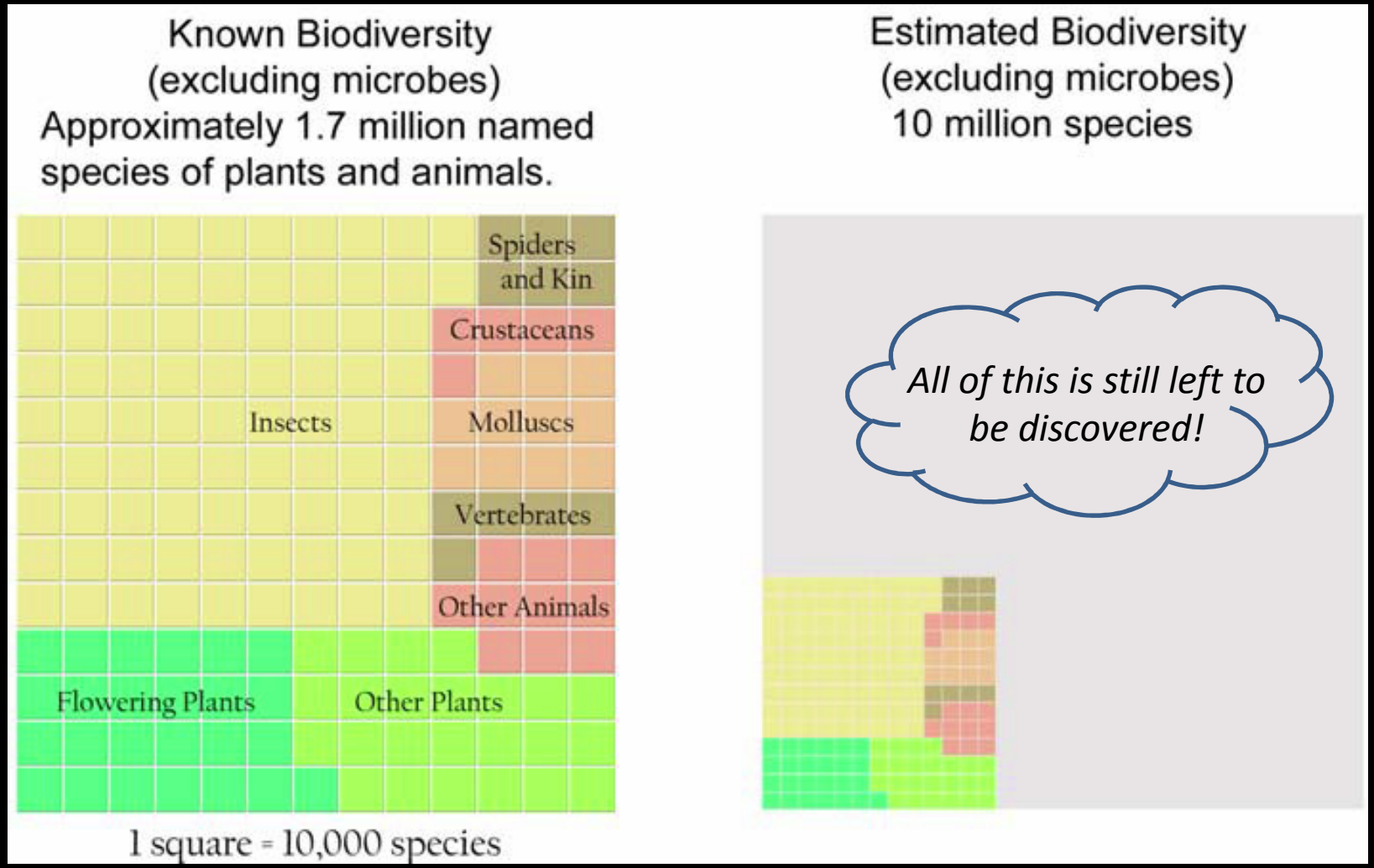


Why is Biodiversity Important?

"It is reckless to suppose that biodiversity can be diminished indefinitely without threatening humanity itself."

E.O. Wilson (Harvard University biologist)

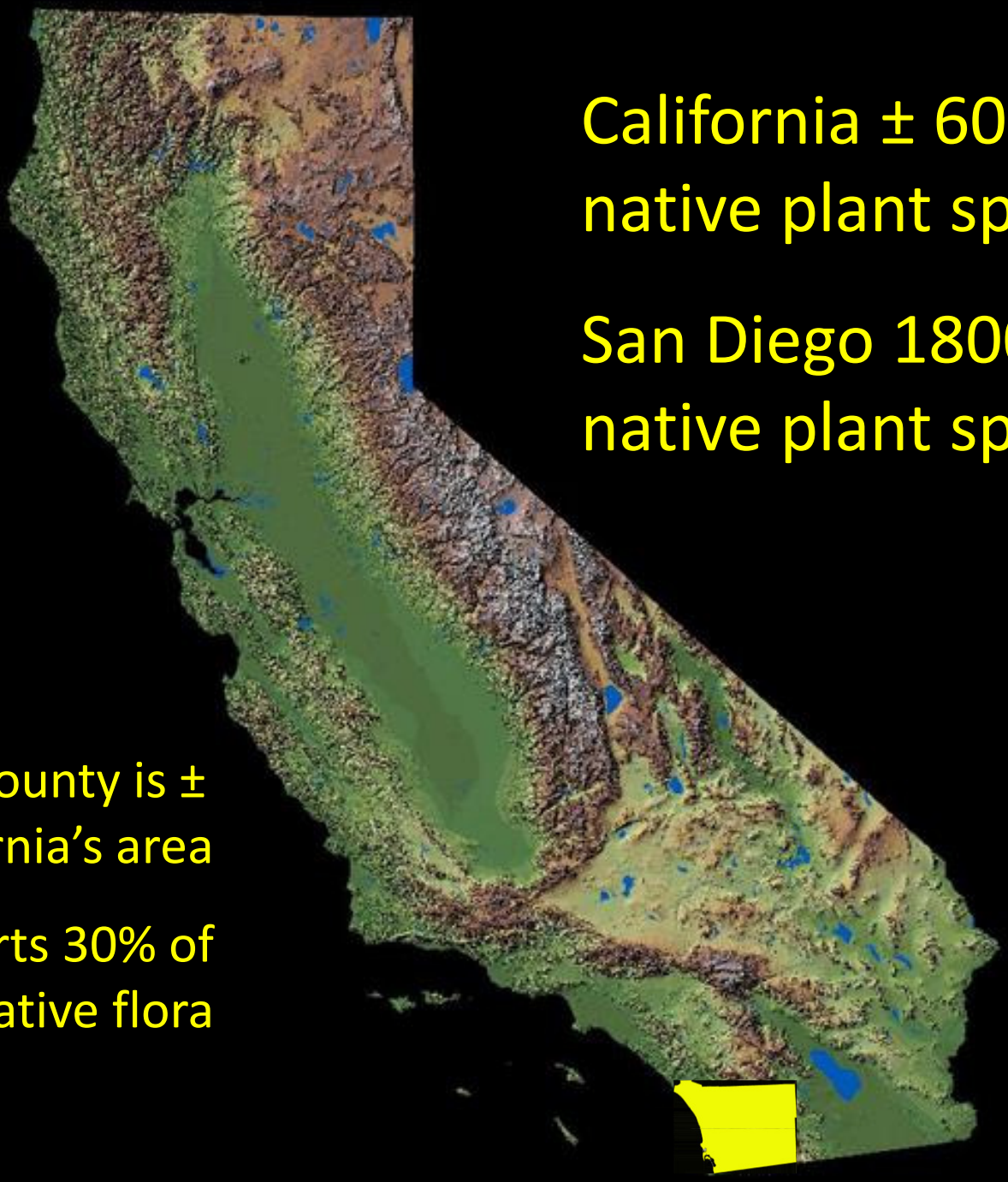
Biodiversity Knowledge Gap



This County is an international “hotspot” of biodiversity and has greater floristic diversity than some entire states!





California ± 6000
native plant species

San Diego 1800+
native plant species

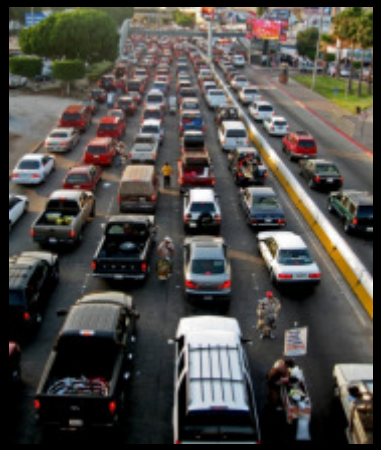
San Diego County is ±
4% of California's area

But it supports 30% of
the state's native flora

A Diversity of Lifestyles

Coastal Sage Scrub	drought-deciduous shrubs
Chaparral	drought tolerant shrubs
Riparian	scrub to closed-canopy forest deciduous and evergreen
Oak Woodlands	mostly evergreen
Desert	drought-deciduous shrubs, spiny succulents, spring annuals
Vernal pools	shallow seasonal wetlands
Grasslands	perennial bunch grasses, annual grasses (native and not), forbs

San Diego's biodiversity is threatened from urban sprawl and other human–caused stresses



Key Unanswered Questions

- What areas of the County have the greatest diversity?
- Are there new species yet to be discovered in the county?
- Where should we be conserving land?
- How can we provide decision-makers with sound, scientifically based information?

Citizen Science

- Contributes to the understanding of key scientific concepts
- Builds interest in scientific activities
- Develops science-related skills
- Improves understanding of local conservation issues

Benefits

Get Outdoors

Inquiry

Observe

Record data

Analyze

Science

PPSR – Public Participation in Scientific Research

Citizen Science Central - Cornell

Citizenscience.org - Citizen science, volunteer monitoring... this site supports initiatives where the public is involved in scientific research.

San Diego Citizen Science Network

Encourages citizen science, volunteer monitoring, participatory action in San Diego. Find them on Facebook!


DNA Barcoding:
What is it?
Why should we care?

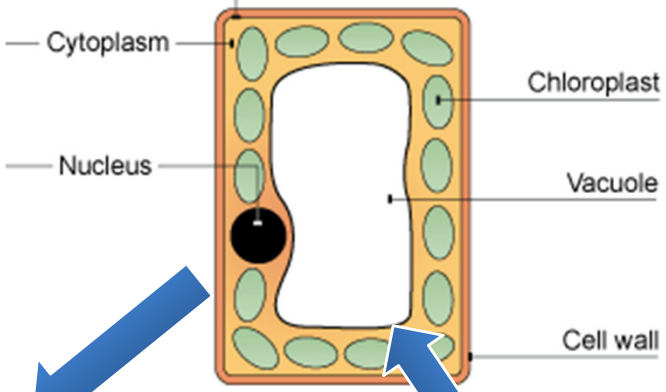
What if Life in Nature Came With a Barcode?

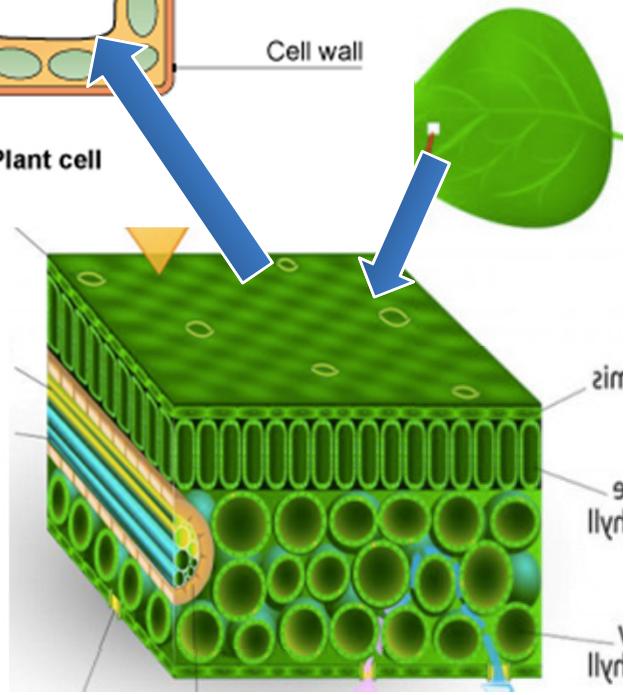
It Does!

No, Not This Way!

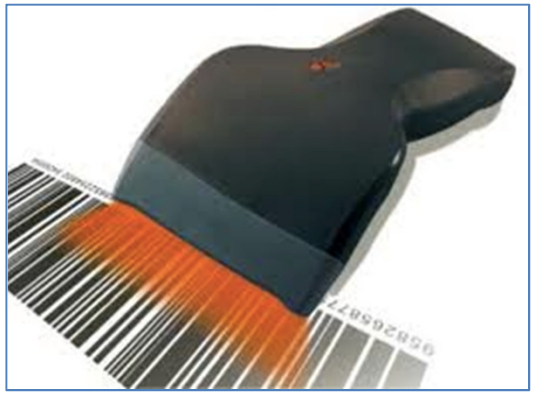
DNA is Nature's Barcode

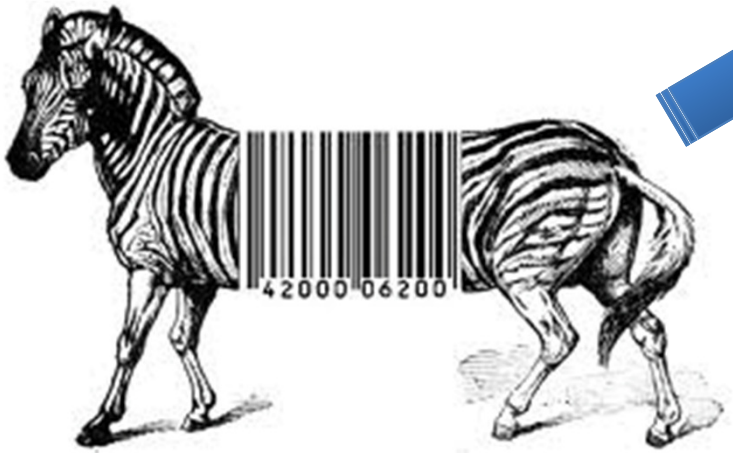
EQUALS





Reading Nature's Barcode





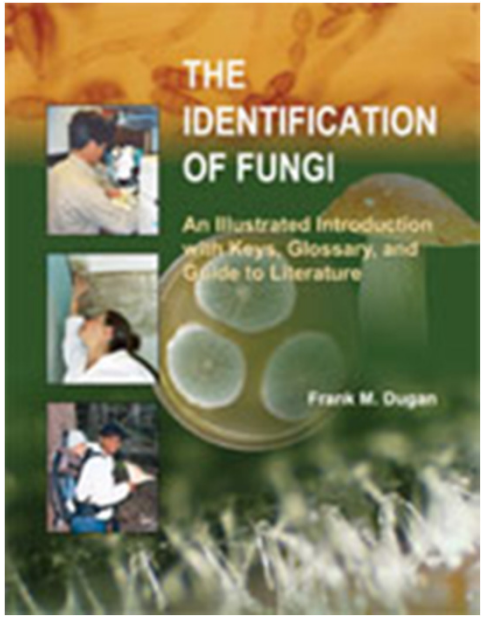
CCTATACCTAATCTTCGGAGCATGAG…

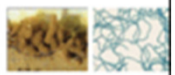
BARCODE
Of LIFE

"It's a Zebra"

Why Barcode?

Morphological identification can be difficult!



1. a. Fruiting body plasmodiocarpous	go to 4	
1. b. Fruiting body sporangiate	go to 2	
2. a. Sporocarps globose	*Arcyria globosa*	
2. b. Sporocarps cylindrical	go to 3	
3. a. Capillitial net rather wide-meshed	*Arcyria affinis*	
3. b. Capillitial net rather dense	*Arcyria denundata*	

Identification

- How do we go about documenting ~1.5 million species of fungi, when 90–95% of them remain undescribed?
- Traditional methods of identification usually require trained experts and add only ~1000 new species a year.

Barcoding

Barcoding creates

Barcoding creates a sea change!

Specimen

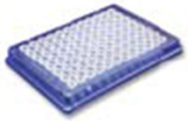
Collection Data !!

Tissue Sample

Photograph !!

HOW?

Extract DNA



PCR Amplify

Sequence

DNA Barcoding

Barcoding rapidly identifies species using short, standard genetic markers in an organism's DNA.

These DNA sequences show few differences *within* a species, but large differences *between* species.

- For <u>animals</u>, the most commonly used barcode region is a segment of ~600 base pairs of the ***mitochondrial*** gene cytochrome oxidase I (COI)
- For land <u>plants</u>, a 2-marker system of ***chloroplast*** genes (rbcL and matK) was adopted.

- For <u>fungi</u>, the internal transcribed spacer (ITS) region of the nuclear ***ribosomal*** RNA is used instead.

Barcode of Life

www.barcodeoflife.org

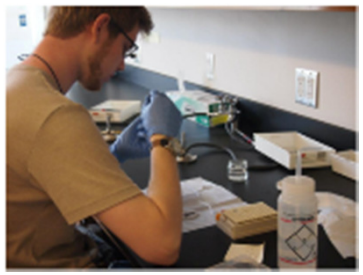
www.ibol.org

Goal: the construction of an enormous, online, freely available sequence database.

The Barcode of Life Datasystems (BOLD) serves DNA barcode data online, with more than 2.7 million barcode sequences representing about 370,000 species.

The growth of national and regional networks as well as taxon and ecosystem campaigns has driven much of the progress.

The launch of the **International Barcode of Life (iBOL)** at the U of Guelph (Canada) in 2010 increased the speed of barcode data generation and reduced costs.



It will transform the way we look at biodiversity and conservation.

international BARCODE OF LIFE

Why Barcode?

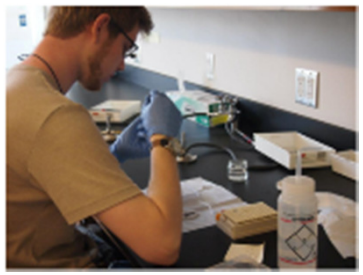
www.ibol.org

Much biological research depends on species diagnoses, yet taxonomic expertise is collapsing.

The best prospect is to employ DNA sequences as taxon “barcodes”.

DNA barcoding has grown into a globally accepted technology with enormous potential because it advances the following:

- Identification of different life stages, e.g. seeds and seedlings
- Identification of small fragments of material
- Forensics
- Verification of herbal medicines/foodstuffs
- Biosecurity and trade in controlled species
- Inventory and ecological surveys

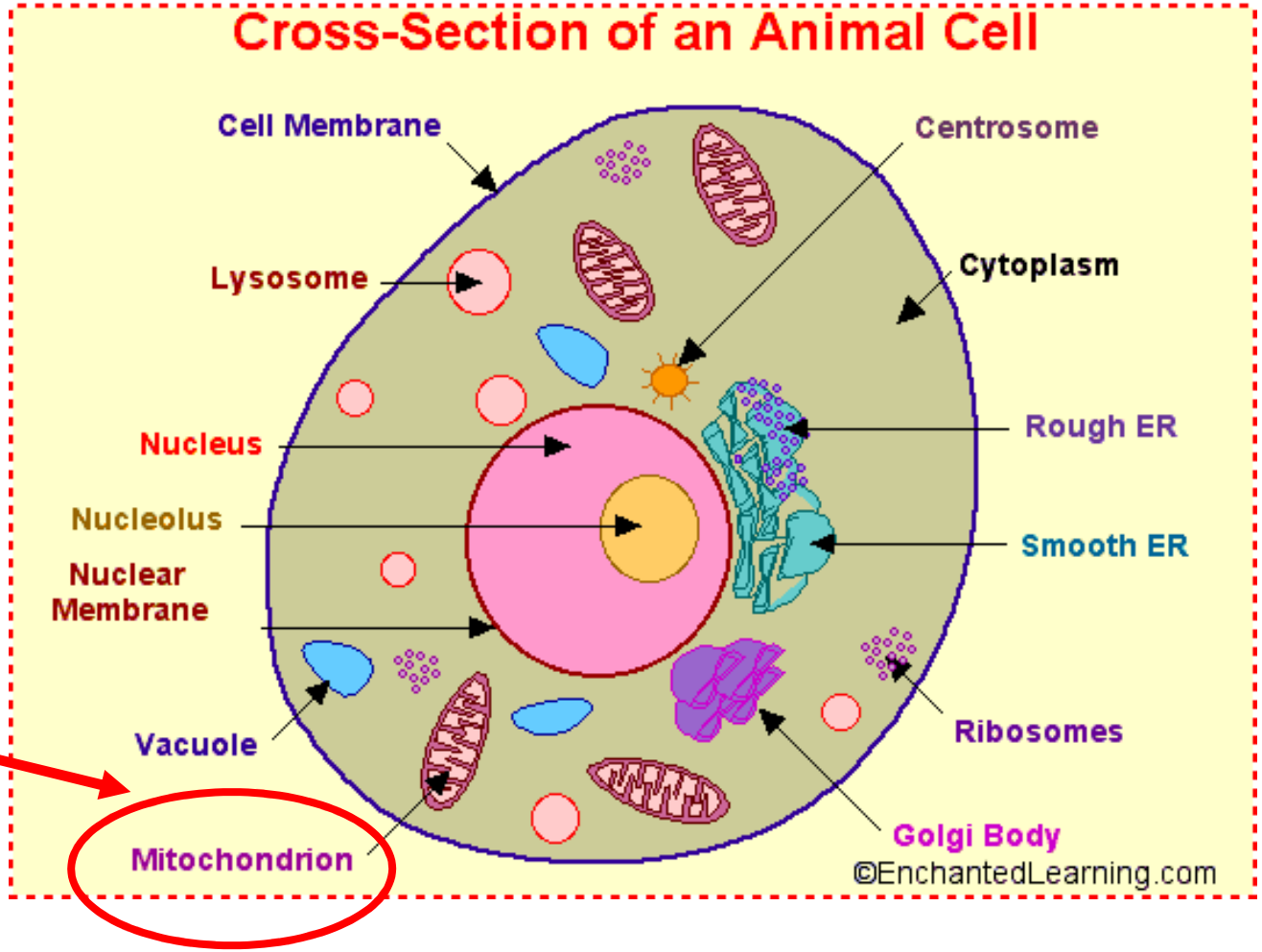


It will transform the way we look at biodiversity and conservation.

What is Barcoding?
(Alert: The Science Bit!)

Animals - cytochrome c oxidase subunit 1 (CO1) in *mitochondria* is sequenced.

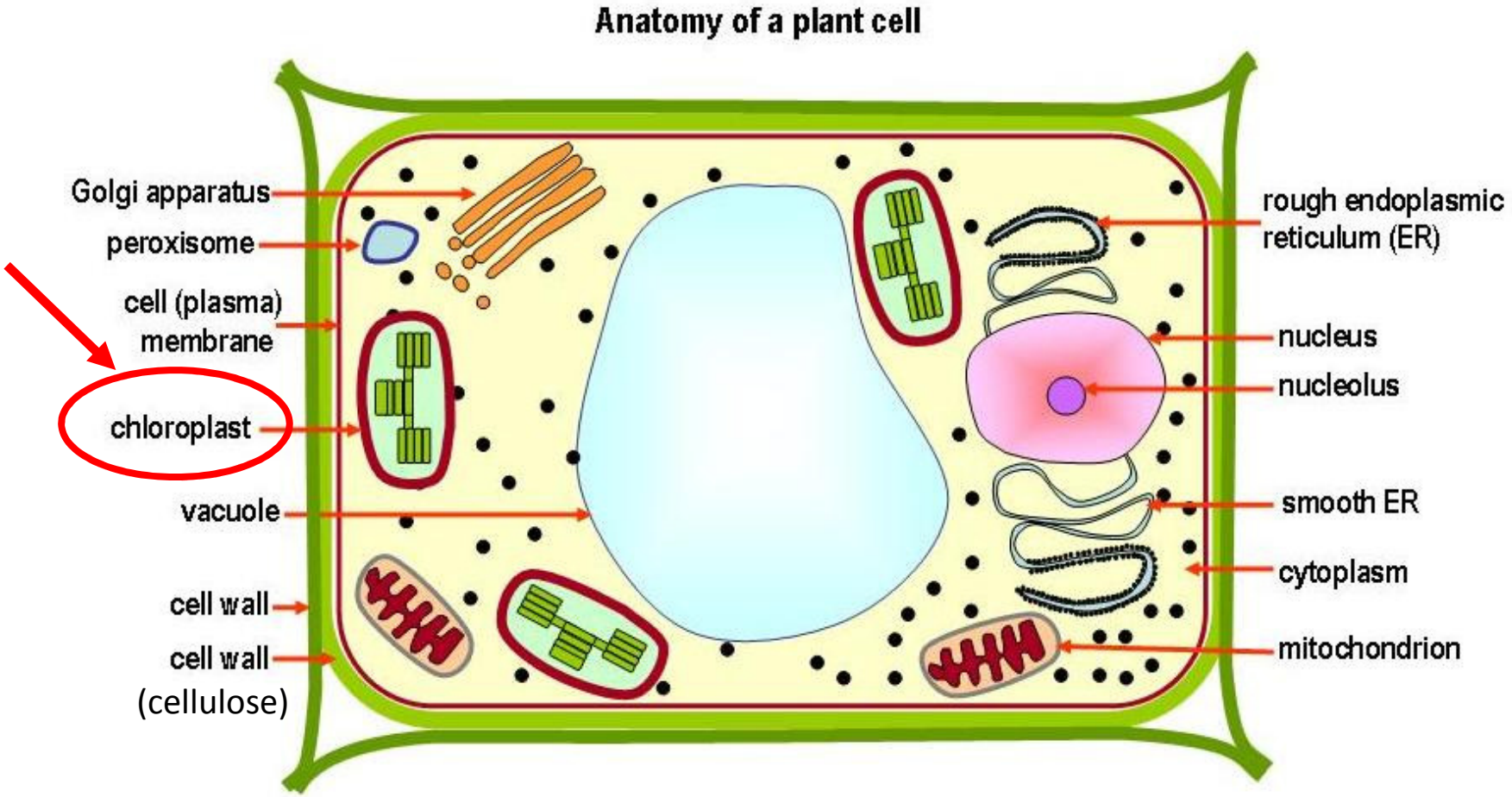
The CO1 gene codes for a protein that has an essential role in cellular respiration. (Respiration is how the cell generates energy, by breaking down food molecules, like glucose, to release carbon dioxide and water.)



Why do mitochondria have their own DNA?
Mitochondria used to be independent organisms and probably evolved to work together with other cells in a symbiotic relationship. Over time, they've evolved to depend completely on their hosts.

Plants – 2 *chloroplast* genes (rbcL and matK) are sequenced*.

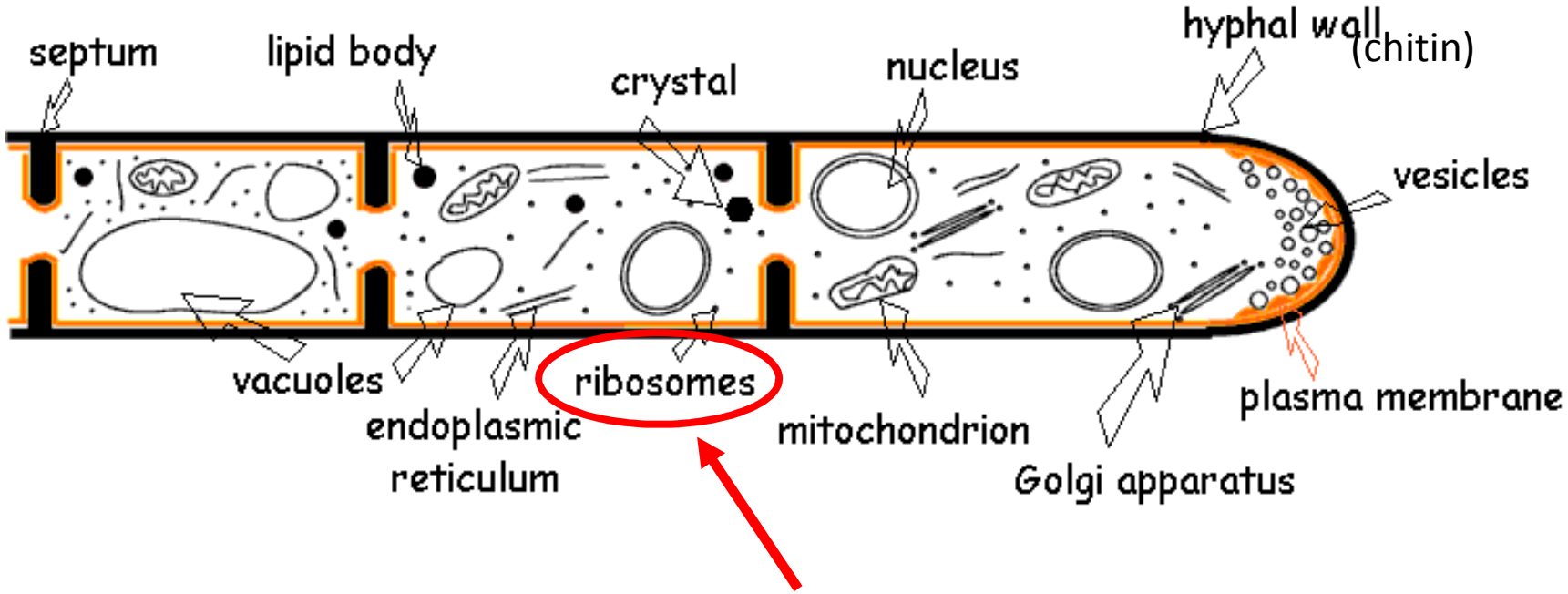
rbcL codes for an enzyme (RuBisCO) involved in carbon fixation, and matK (maturase K) codes for a protein



*Mitochondrial DNA in land plants is problematic because its prone to rearrangements and incorporation of foreign DNA, so chloroplast DNA is used instead.

Fungi – ITS region of the RNA in ribosomes are sequenced.

The internal transcribed spacer (ITS) region of nuclear ribosomal RNA has the highest probability of successful identification for the broadest range of fungi



Ribosomes are complex molecular “machines” inside all cells that manufacture proteins

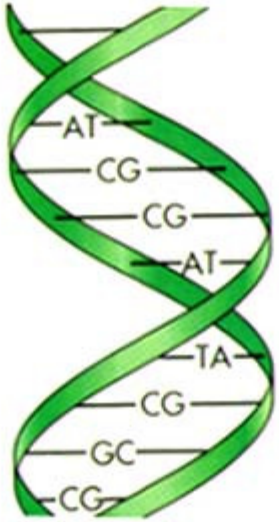
DNA Sequencing

Determining the precise order of the 4 bases (A T C G)

DNA "Double Helix"

Bases

A = Adenosine
T = Thymine
C = Cytosine
G = Guanine



8 base pairs

CCTATACCTAATCTTCGGAGCATGAG…

Typical barcode is ~600 base pairs long

Examples of Barcoding Projects

Royal Botanic Gardens (Kew Gardens) in London England has an active mycology program and a “Fungarium” 0f 1.2 M specimens dating back to 1845.

DNA barcoding has expanded the number of known British species of “waxcaps” (Hygrocybe) from 50 to almost 100 in 3 yrs of work!

Two new species have been described from the parrot waxcap complex, including one with royal purple fruiting bodies.

It was named *Gliophorus reginae* to commemorate the Queen’s Diamond Jubilee.

Ainsworth A, Cannon P, Dentinger B (2013) DNA barcoding and morphological studies reveal two new species of waxcap mushrooms (Hygrophoraceae) in Britain. MycoKeys 7 : 45–62

Examples of Barcoding Projects

Revolutionising the Fungarium - a Genomic Treasure Trove?

A DNA sequencing breakthrough used samples from Kew's Fungarium to show that genetic information can be accessed from even very old samples.

This represents the promise of significant discoveries which may have profound impacts on all our lives - just as the discoveries of powerful medicines like penicillin and cyclosporine have done before.

Kew's Fungarium alone may contain more than 50 million Mb (50 million million!) of genomic data – a treasure trove of potential discoveries!

The historical fungus (*Phytophthora infestans*) that caused the major blights of potato in the 19th century (leading to the infamous Irish Potato Famine) has been shown to be a distinct lineage that is now extinct, thanks to barcoding old specimens

Yoshida et al. 2013. The rise and fall of the *Phytophthora infestans* lineage that triggered the Irish potato famine. eLife. Article DOI: http://dx.doi.org/10.7554/eLife.00731

How is this relevant?

Herbarium Collections at SDNHM

Total = 70 specimens; 34 genera; 32 taxa

Most common taxa:

Agaricus – 8
- *A. sp.*
- *A. bernardii*

Amanita – 4
- *A. sp.*
- *A. ocreata*
- *A. rubescens*
- *A. velosa*

Boletus – 3
- *B. sp.*
- *B. flaviporus*
- *B. dryophilus*

Helvella – 2
- *H. compressa*
- *H. lacunosa*

Naematoloma – 2
- *N. fasciculare*
- *N. aurantiaca*

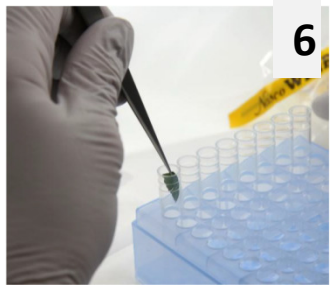
Other genera:

Astraeus
Chlorophyllum
Clavatia
Clitocybe
Coprinus
Cortinarius
Entoloma
Exidia
Geastrum
Ganoderma
Lactarius
Laetiporus
Lepiota
Morchella
Omphalotus
Panaeolus
Phellinus
Polyporus
Psathyrella
Rhizopogon
Rhodocollybia
Scleroderma
Stereum
Suillus
Trametes
Tricholoma
Tulostoma
Volvariella

Most from 2009
1 in 2004
12 in 2010
16 in 2014

SDMS Barcoding Project

1. Mushroom collecting foray – specimens and data collected
2. Mushrooms brought in for display at Fungus Fair or monthly meetings
3. Experts identify specimens
4. Specimens photographed
5. Data entered into computer and uploaded to internet
6. Small tissue samples removed and placed in ethanol for storage
7. Sample tubes shipped to University of Guelph in Canada for DNA barcoding
8. Mushrooms dried, and deposited in collection at San Diego Natural History Museum



Data Slips – Please Use Them!

SDMSA16

Tremella mesenterica

Collector	Date	*Genus*	*species*
Cindy Trubovitz	2-9-14	Witches butter	

Location/GPS	Terrain
Penasquitos Canyon trail	slope facing N S E W

Description of Vegetation/Habitat

on decaying oak bark under oak tree

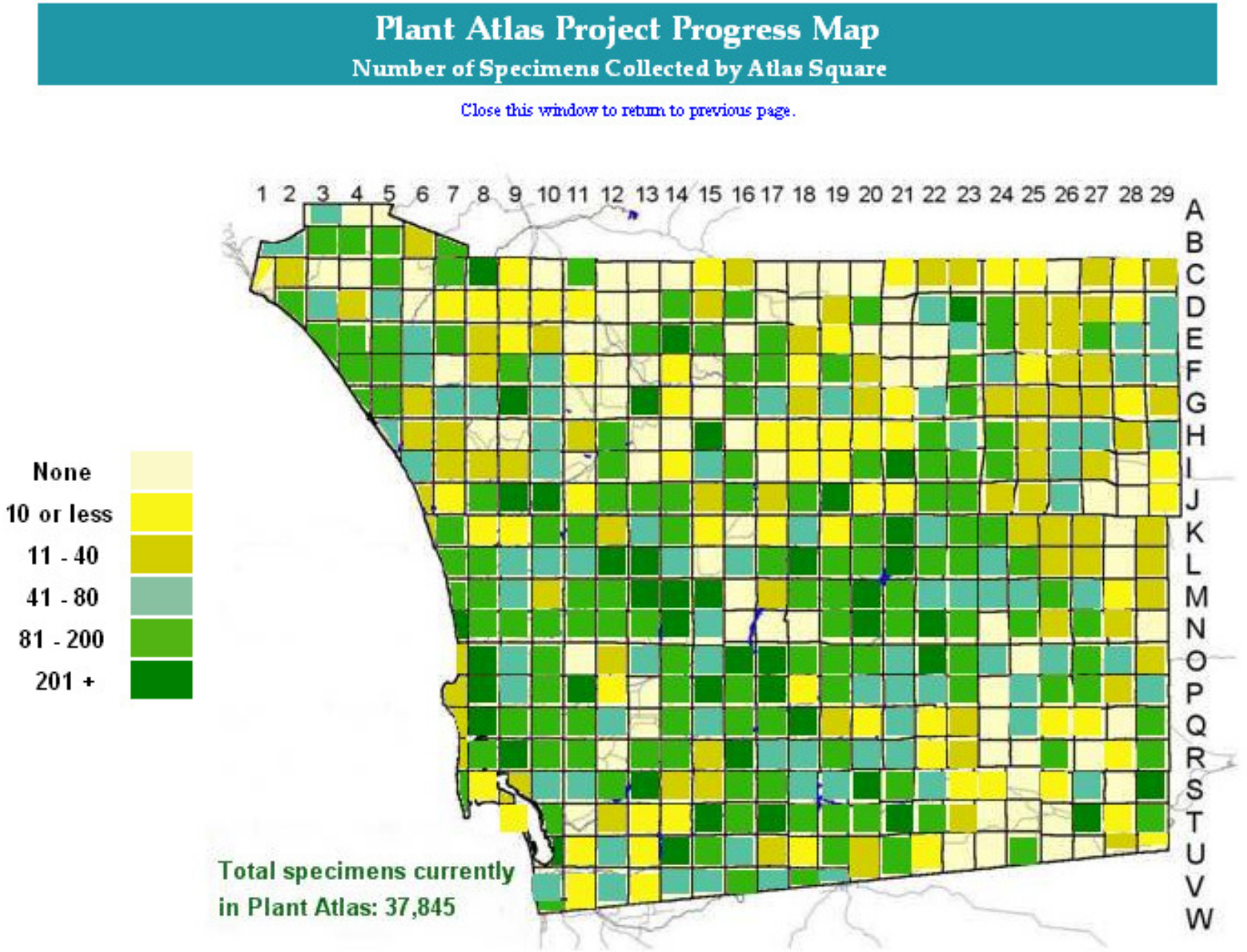
Substrate			
	wood	fungus	dung
soil	duff	insect	other

Description of Specimen

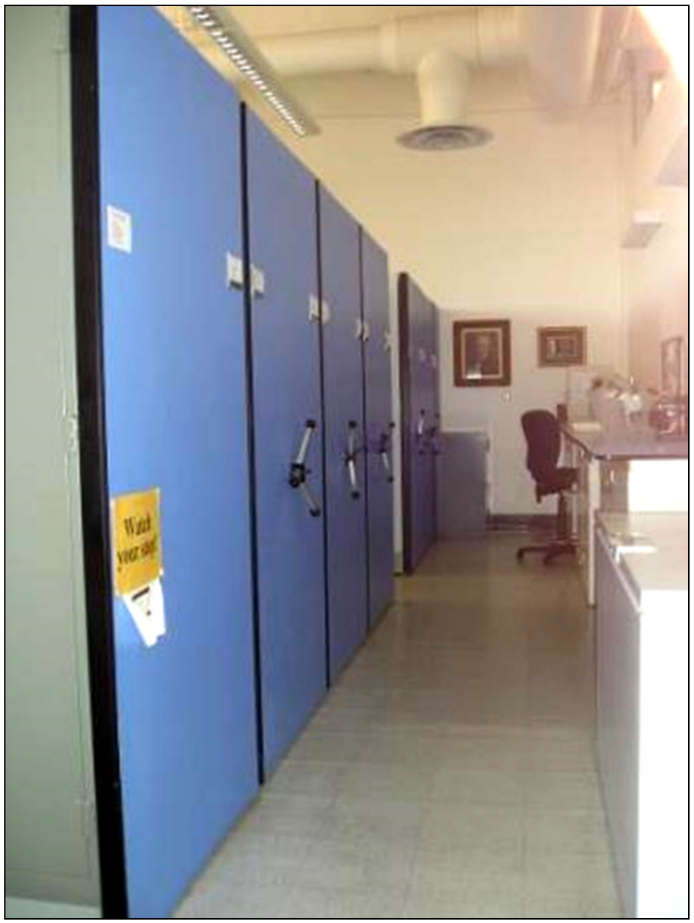
Yellow jelly fungus

Spore Print Description	Taste
	Odor

Photo	Mycologist ID
Comments on back	Voucher #

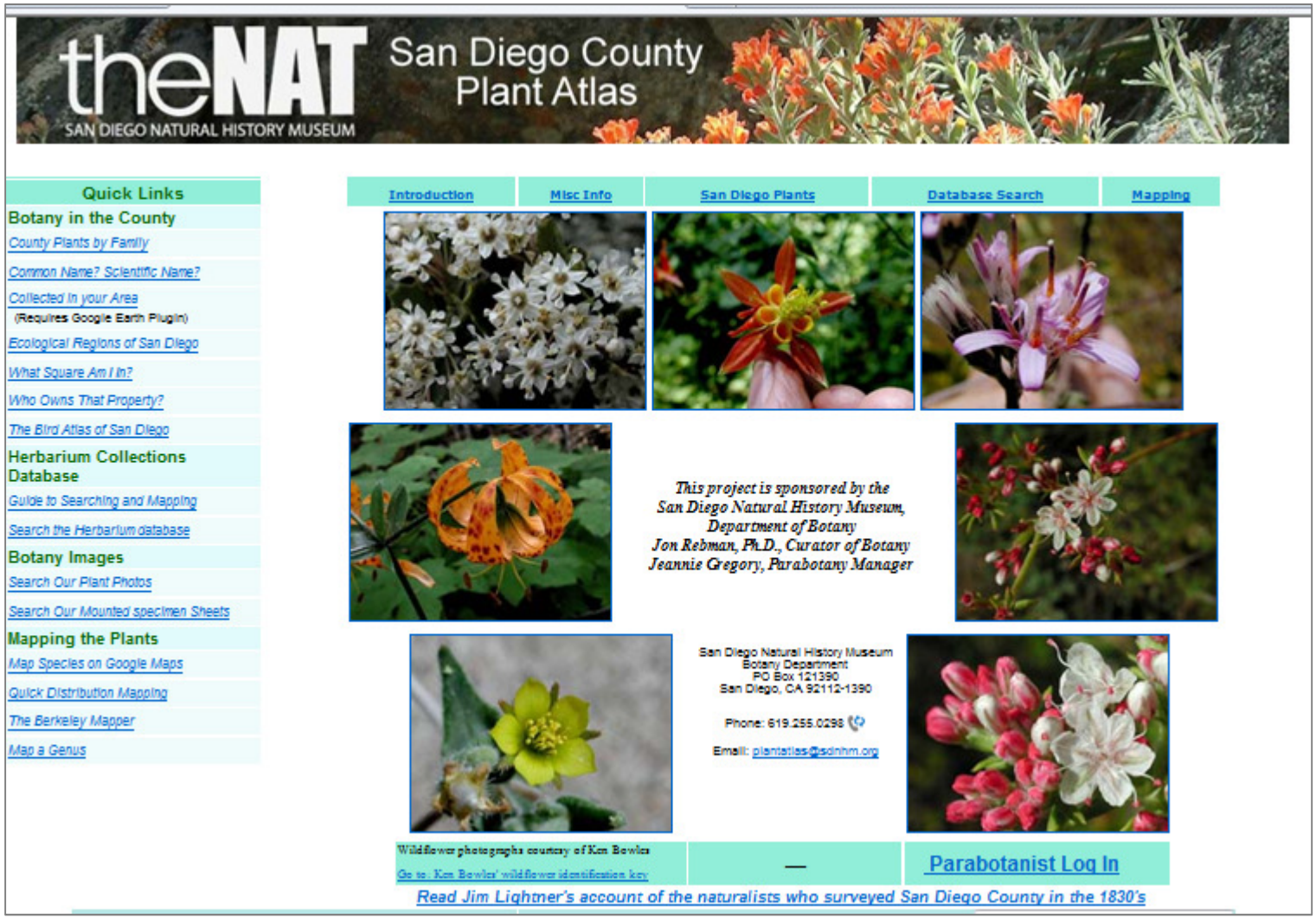


Grid squares adopted



Specimens are Archived in the Herbarium at SD Natural History Museum

sdplantatlas.org



Get Involved!

The SDMS is undertaking this pilot citizen science project to scientifically document mushrooms collected in San Diego County.

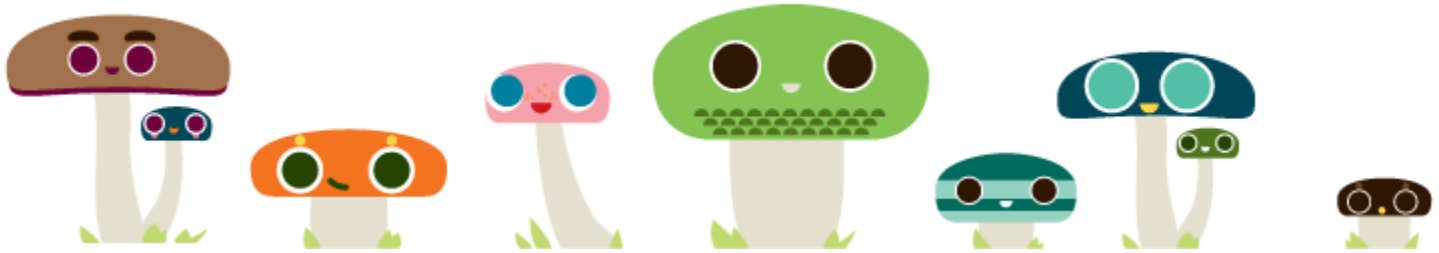
How You Can Help!

Funding is needed to help pay for:

- The curation of the specimens by the herbarium at the *San Diego Natural History Museum*
- The lab work required to do the DNA barcoding (the *Biodiversity Institute of Ontario* at the University of Guelph in Canada is doing that work).

Contact SDMS member Dr. Mary Ann Hawke (mhawke@ucsd.edu) if you (or your organization/business) wishes to donate to support this project.

Support the **San Diego Mycological Society** by becoming a member – join today for the small annual fee of $20



Thank You!

SAMS#16 Tremella mesenterica

Collector	Date	Genus	species
Cindy Wabbit	2-9-14	Witches butter	
Location/GPS		Terrain	
Tenaya Lakes Canyon Trail		slope facing	N S E W
Description of Vegetation/Habitat: on decaying oak bark under oak tree			
Substrate: soil, wood, duff		fungus, insect	dung, other
Description of Specimen: Yellow jelly fungus			
Spore Print Description			Taste, Odor
Photo		Mycologist ID	
Comments on back		Voucher #