

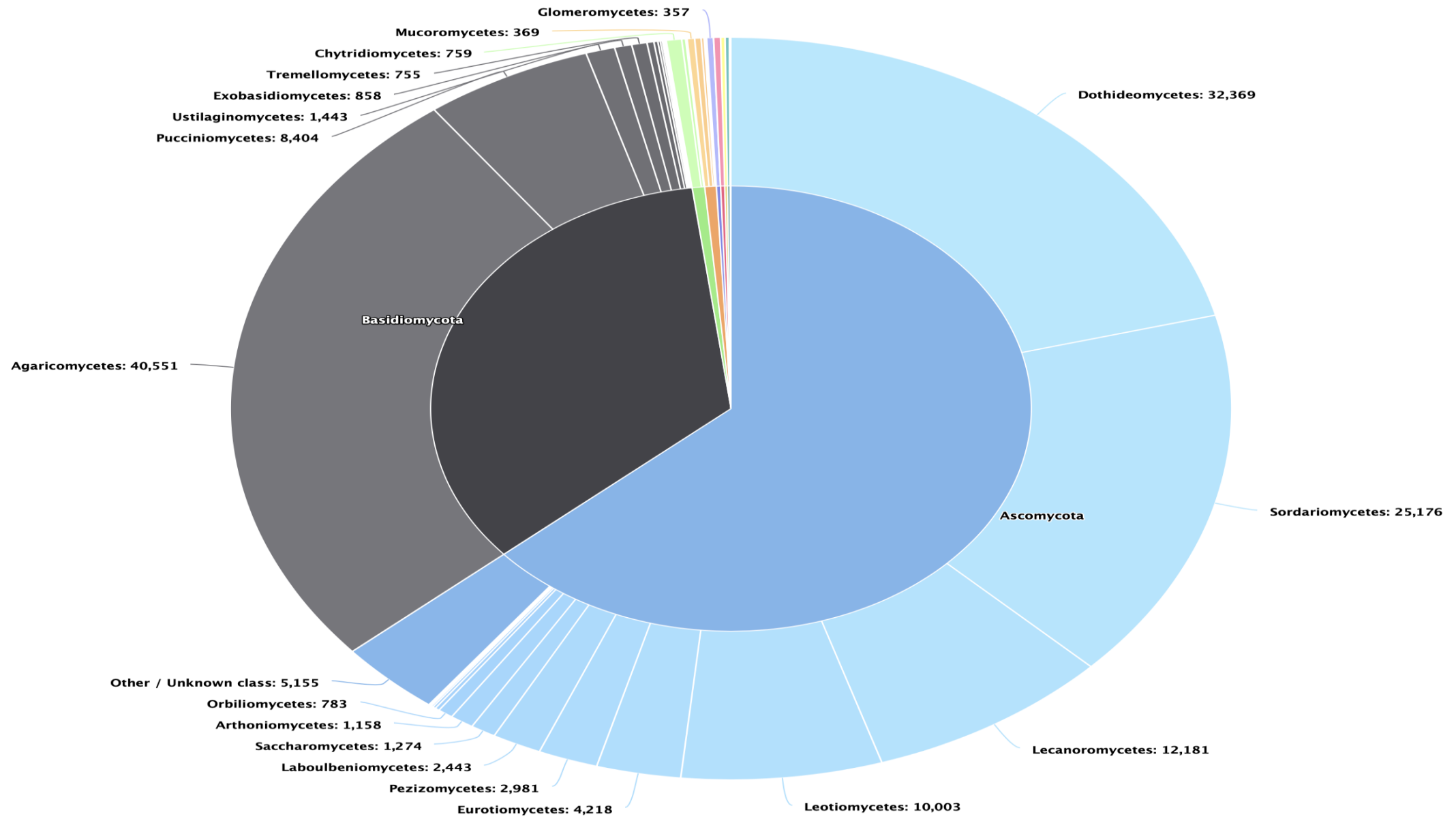


# Fungal Identification by Polyphasic Techniques

Bassem A. Balbool  
Lecturer of Microbiology  
MSA University  
Egypt

# Numbers of fungi !

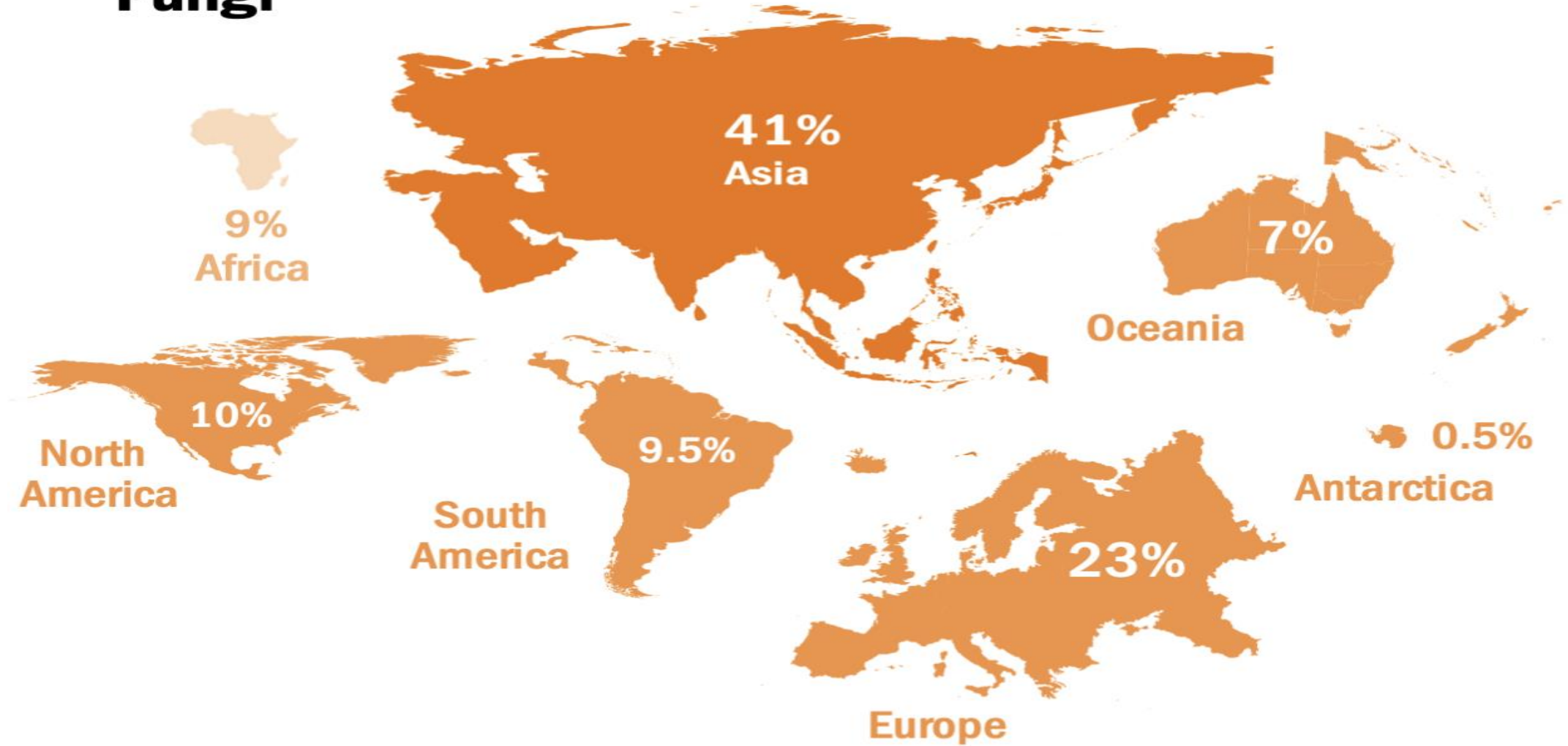
- D.L. Hawksworth in 1991 estimated that there were roughly 1.5 million species.
- Recently scientists estimate the fungal species numbers ranging from 2.2 -3.8 millions (based on host association),
- 11.7-13.2 million species using high-throughput sequencing (HTS).
- The number of new species descriptions added per year currently averages around 2,000 — an increase over the last decade that shows no sign of saturation.
- However, only around 150,000 fungal species have been described to date.



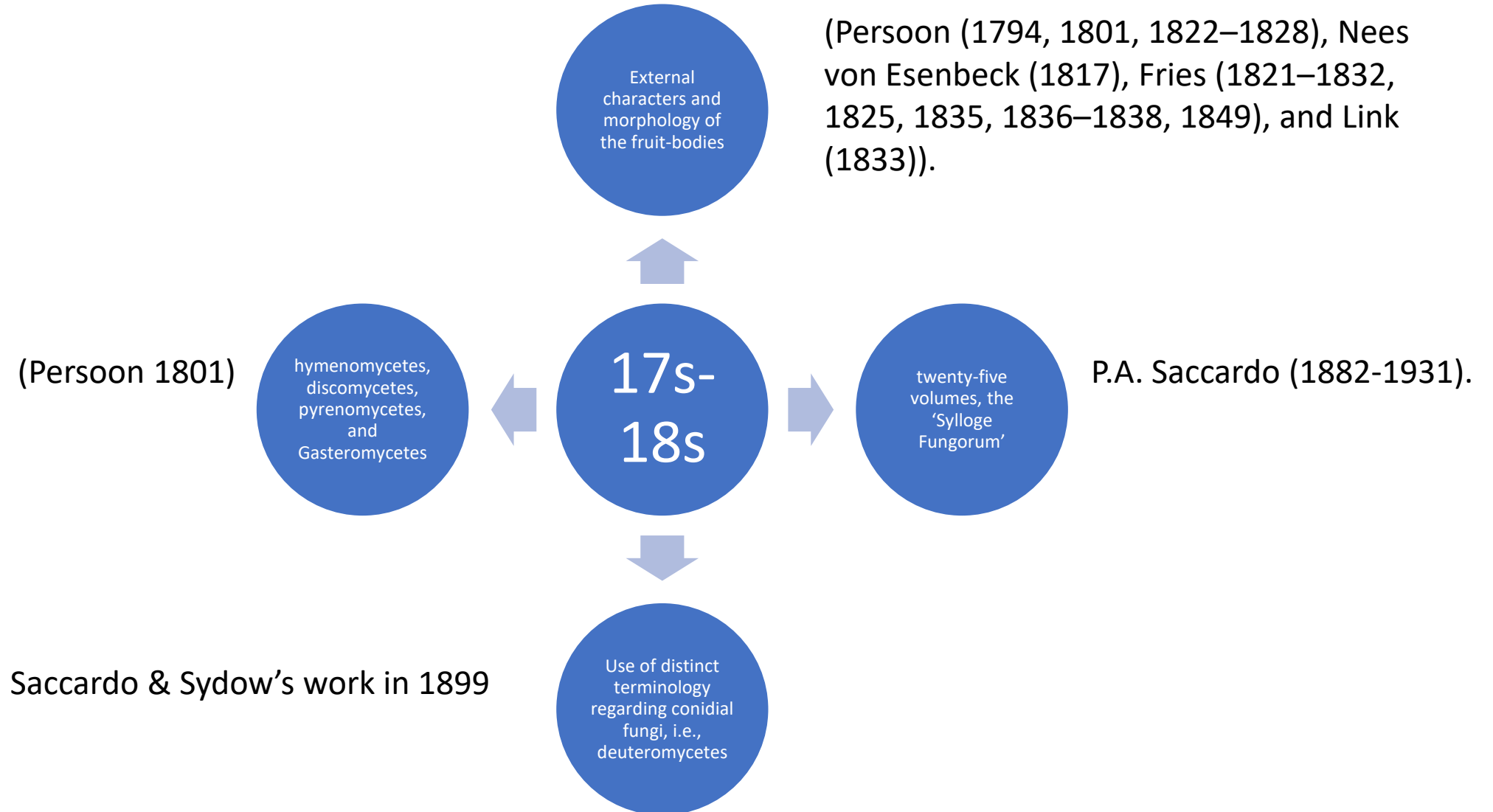
[Fungi in catalogue of Life Checklist \(2023-02-07\)](http://www.speciesfungorum.org)  
<http://www.speciesfungorum.org>

The proportion of species from each continent named as new to science in 2019

## Fungi



# History of fungal classification

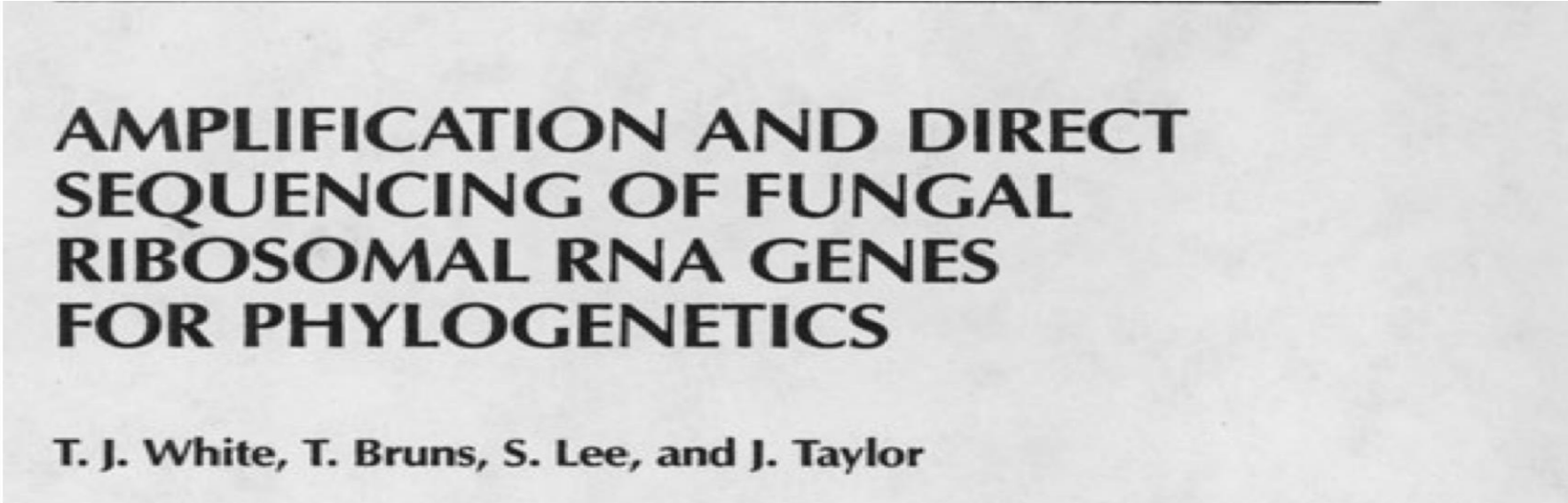


# History of fungal classification

- Ainsworth (1966) and Moore (1971) introduced, Deuteromycotina and Deuteromycota.
- Series Systema Ascomycetum (Eriksson 1982, Eriksson & Hawksworth 1998).
- All these efforts have focused on sexually typified genera and their classification.

# History of fungal classification

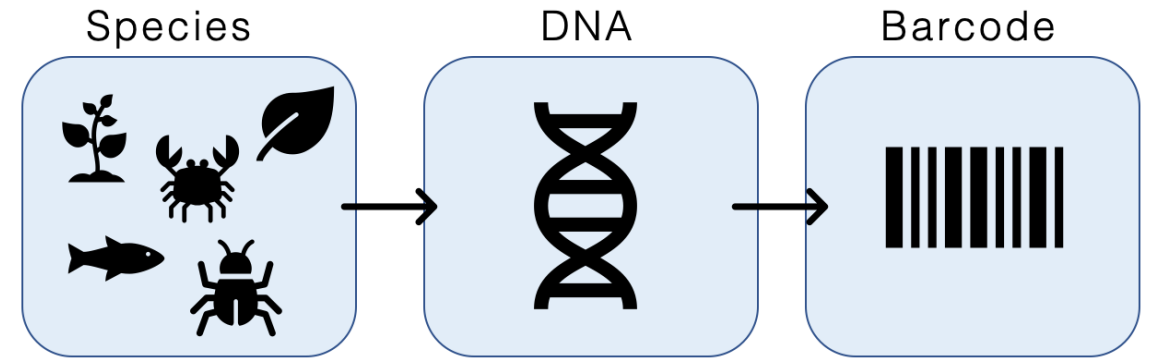
- White et al. (1990),
- With these implementations, the dual nomenclature (e.g., Saccardo 1904) was abandoned and the 'one fungus, one name' concept for pleomorphic taxa was implemented (McNeill et al. 2011).



**AMPLIFICATION AND DIRECT  
SEQUENCING OF FUNGAL  
RIBOSOMAL RNA GENES  
FOR PHYLOGENETICS**

**T. J. White, T. Bruns, S. Lee, and J. Taylor**

# Fungal barcoding



**Table 1.** Selected top-performing loci and primers as primary and secondary fungal DNA barcodes based on Schoch et al. (2012) and Stielow et al. (2015).

Locus	Primer name	Oligo nucleotides (5'→3')	Reference
ITS7	ITS1	TCCGTAGGTGAACCTGCGG	White et al. 1990
	ITS4	TCCTCCGCTTATTGATATGC	White et al. 1990
ITS-1	ITS1	TCCGTAGGTGAACCTGCGG	White et al. 1990
	ITS2	GCTGCCGTTCTTCATCGATGC	White et al. 1990
ITS-2	ITS3	GCATCGAATGAAGAACGCAGC	White et al. 1990
	ITS4	TCCTCCGCTTATTGATATGC	White et al. 1990
nLSU	LROR	ACCCGCTGAACCTTAAGC	<a href="http://sites.biology.duke.edu/fungi/mycolab/primers.htm">sites.biology.duke.edu/fungi/mycolab/primers.htm</a>
	LR5	TCCCTGAGGGAAACTTCG	<a href="http://sites.biology.duke.edu/fungi/mycolab/primers.htm">sites.biology.duke.edu/fungi/mycolab/primers.htm</a>
TEF1α	EF1-1018F	GAYTTCATCAAGAACATGAT	Stielow et al. 2015
	EF1-1620R	GACGTTGAADCCGACGTTGTC	Stielow et al. 2015
60S L10	60S-506F	GHGACAAGCGTTTCTCNGG	Stielow et al. 2015
	60S-908R	CTTVAVYTGGAACITGATGGT	Stielow et al. 2015
PGK	PGK-533F	GTYGAYTTC AAYGTYCC	Stielow et al. 2015
	PGK-533R	ACACCDGGDGGRCCGTTCCA	Stielow et al. 2015
TOP1	TOP1-501F	ACTGCCAAGGTTTTCCGTACHTACAACGC	Stielow et al. 2015
	TOP1-501R	CCAGTCCTCGTCAACWGACTTRATRGCCCA	Stielow et al. 2015
LNS2	LNS2-468F	GGCCATGTGCTGAACATGATCGGHCGWGAYTGGAC	Stielow et al. 2015
	LNS2-468R	CGGTTGCCRAAKCCRGCATAGAAKGG	
TEF3	EF3-3185F	TCYGGWGGHTGGAAGATGAAG	Stielow et al. 2015
	EF3-3538R	YTTGGTCTTGACACCNTC	Stielow et al. 2015

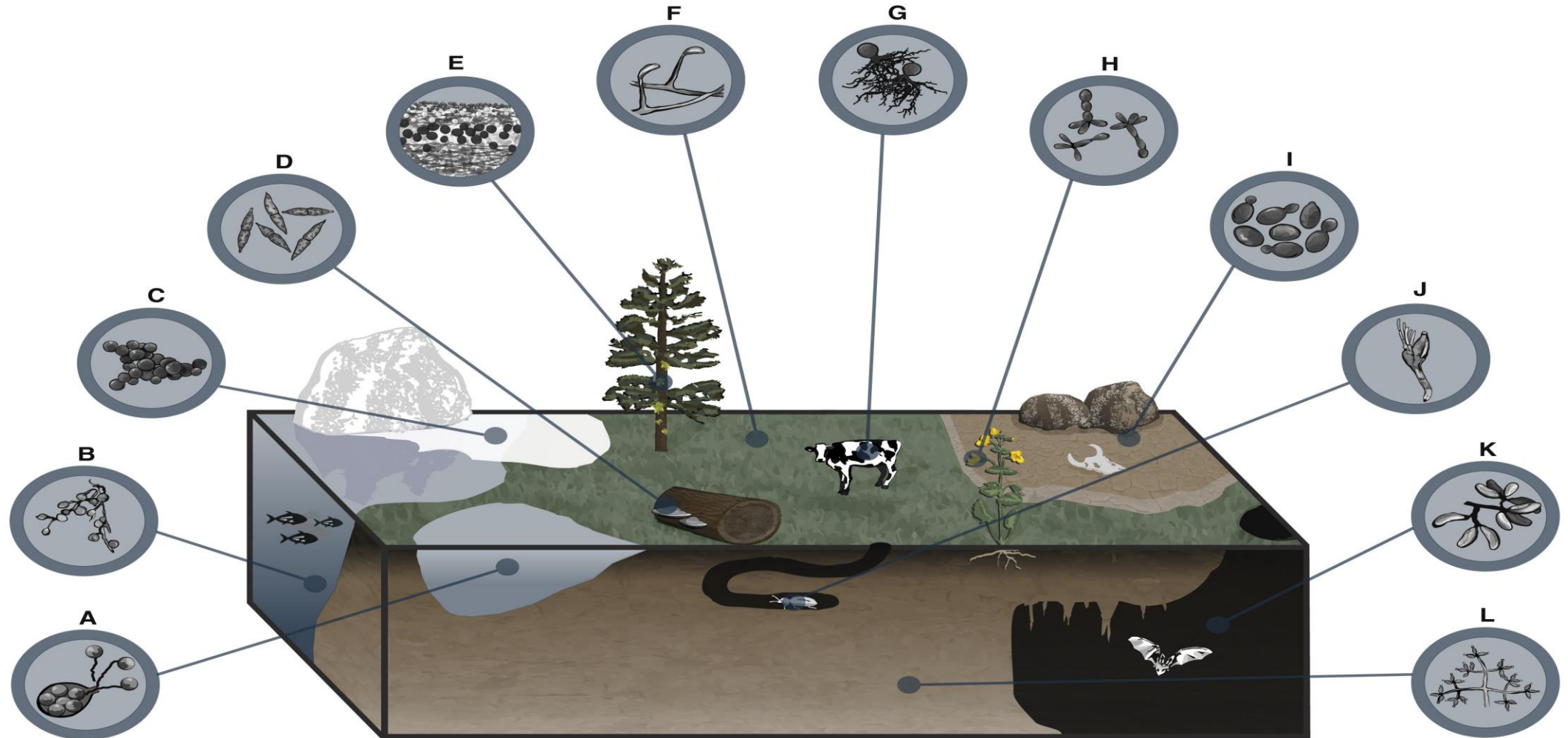




# Morphology in the molecular era of fungal classification

- Morphological characterization of newly described fungal taxa is still needed (Hyde et al. 2010).
- The shape descriptions should be made with examination in the living state (Baral 1992, Dominguez de Toledo 1994 and Kirk et al. 2008).
- Image analysis is a novel, high-throughput method that allows the automated recording and evaluation of microscopic images (Posch et al. 2012).
- Colony colours can be determined by the 'RGB profiling' procedure (Puchkov 2016).
- Details hardly visible on photographs should be illustrated with line drawings, SEM, or different focus /illumination settings of a light microscope.

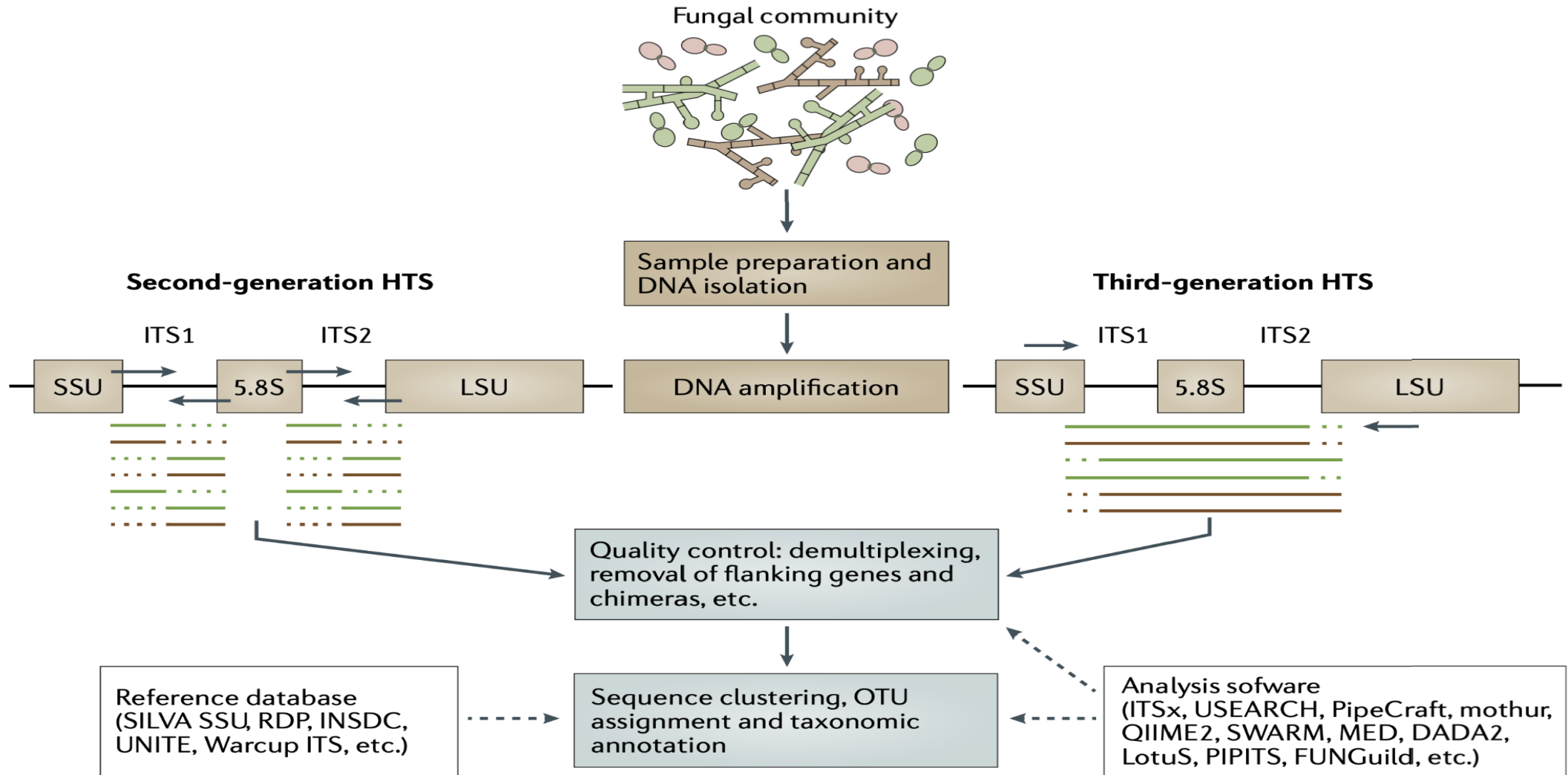
# The use of ecology to improve fungal classification



# Why fungal diversity

- Given the essential importance of fungi for ecosystem functioning, it is necessary to investigate fungal diversity using the most diverse methods and empirical demonstrations of the world fungal diversity and to address their roles in the different ecosystems (Hawksworth 1991).

# Fungal metabarcoding



# Biodiversity

```
graph LR; Biodiversity --> alpha["α-Diversity"]; Biodiversity --> beta["β-diversity"]; Biodiversity --> gamma["γ-diversity"]; Biodiversity --> E["E-diversity"];
```

**α-Diversity**

Sometimes called within-habitat diversity, is the diversity in a patch

**β-diversity**

Describes the contribution of multiple habitats to overall diversity of a site.

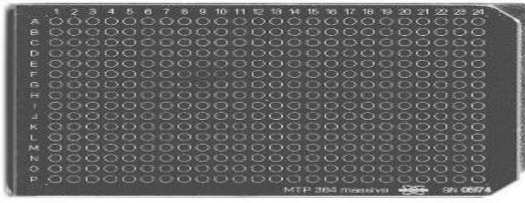
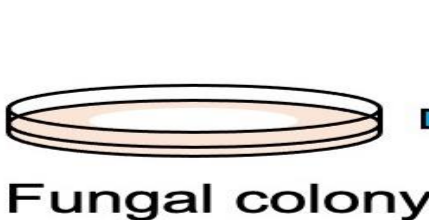
**γ-diversity**

Represents the number of species within a region or landscape

**E-diversity**

Is the diversity of a large biogeographic region, such as a biome.

# MALDI-tof MS

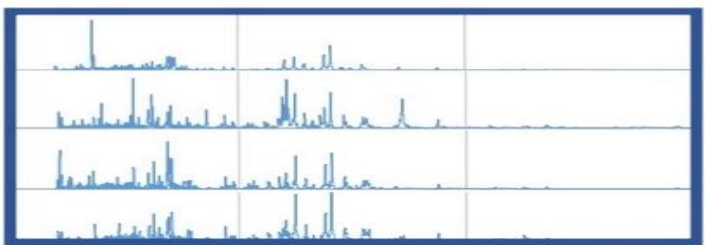


Protein extraction

MALDI target



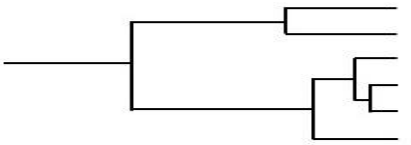
MALDI-TOF MS



Mass spectrum pattern



Identification  
Typing  
Classification



# Other approaches

- The use of whole genome data for classification
- The use of chemical profiles to improve fungal classification
- The use of physiology to improve fungal classification

# Classical techniques for conservation of fungi

## Fungariums

- Processing fungal specimens
- Specimen maintenance
- Record keeping
- Herbarium activities
- Electronic specimen catalogues



# Thank you !

## Contacts

[bassembalbool@gmail.com](mailto:bassembalbool@gmail.com)

[bbalbool@msa.edu.eg](mailto:bbalbool@msa.edu.eg)

