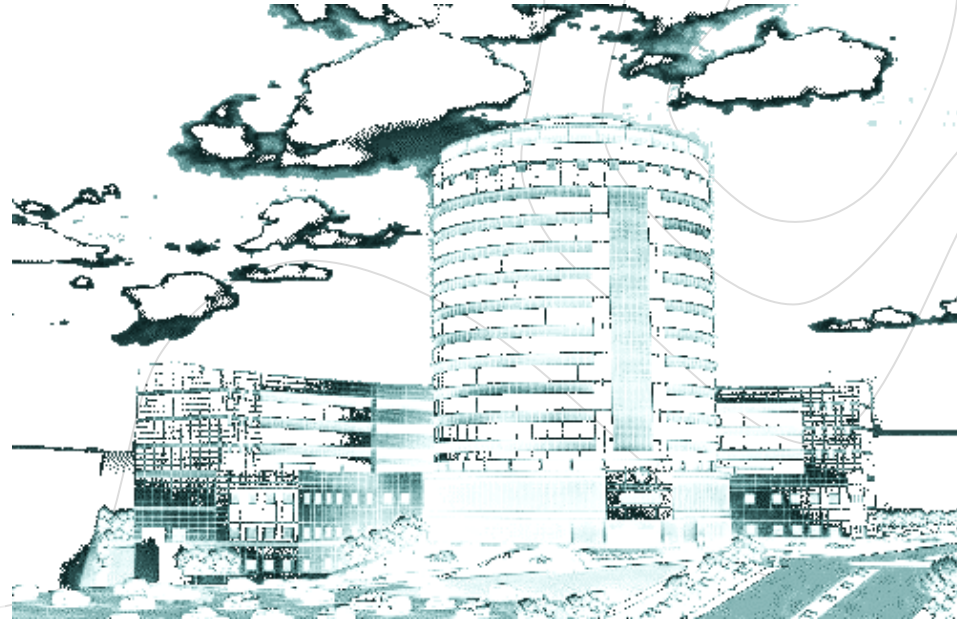


Prof.Dr.Çağrı Ergin
Pamukkale Üniversitesi Tıp Fakültesi
Tıbbi Mikrobiyoloji AD - Denizli



**8.Ulusal Tanısal ve Moleküler
Mikrobiyoloji Kongresi
Haziran 2014**



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TOM GAULD

- Taksonomi
- Neler, neden değişiyor ?
- Tıbbi mikolojiyi bekleyenler
- Yarına bir bakış

Biological identifications through DNA barcodes

Paul D. N. Hebert*, Alina Cywinska, Shelley L. Ball
and Jeremy R. deWaard

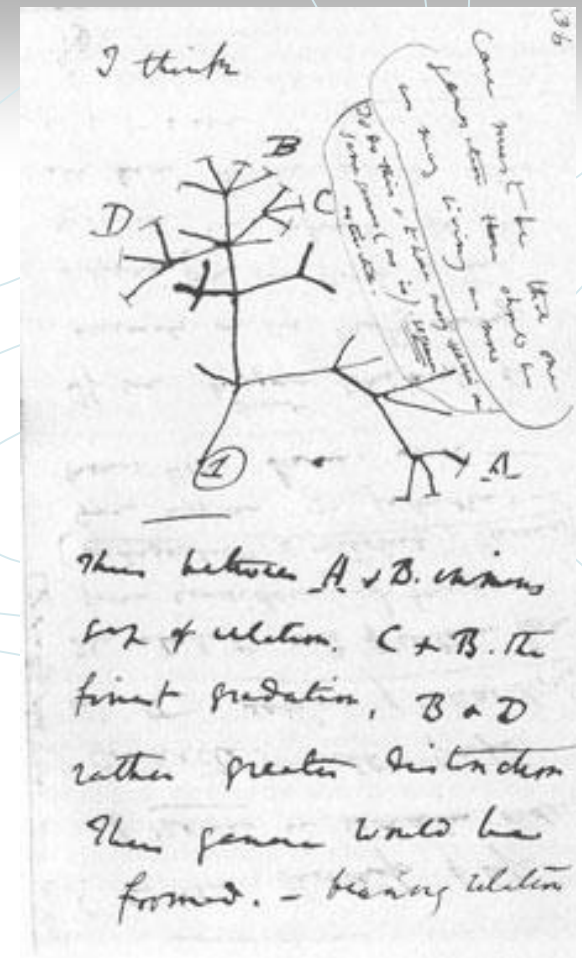
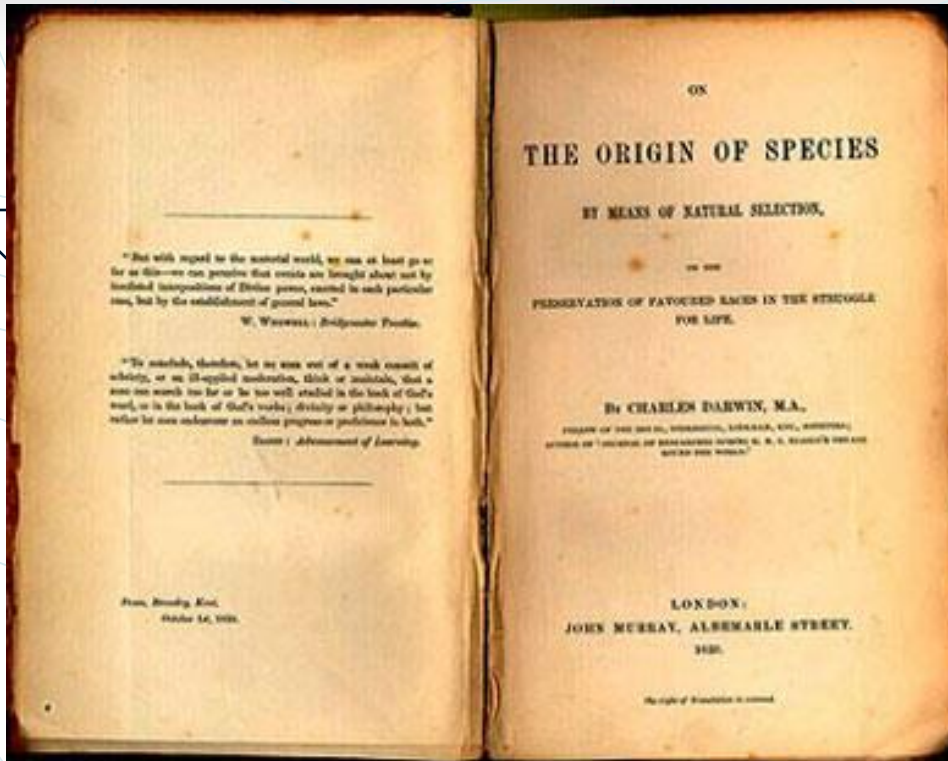
Department of Zoology, University of Guelph, Guelph, Ontario N1G 2W1, Canada

Although much biological research depends upon species diagnoses, taxonomic expertise is collapsing. We are convinced that the sole prospect for a sustainable identification capability lies in the construction of systems that employ DNA sequences as taxon 'barcodes'. We establish that the mitochondrial gene cytochrome *c* oxidase I (COI) can serve as the core of a global bioidentification system for animals. First, we demonstrate that COI profiles, derived from the low-density sampling of higher taxonomic categories, ordinarily assign newly analysed taxa to the appropriate phylum or order. Second, we demonstrate that species-level assignments can be obtained by creating comprehensive COI profiles. A model COI profile, based upon the analysis of a single individual from each of 200 closely allied species of lepidopterans, was 100% successful in correctly identifying subsequent specimens. When fully developed, a COI identification system will provide a reliable, cost-effective and accessible solution to the current problem of species identification. Its assembly will also generate important new insights into the diversification of life and the rules of molecular evolution.

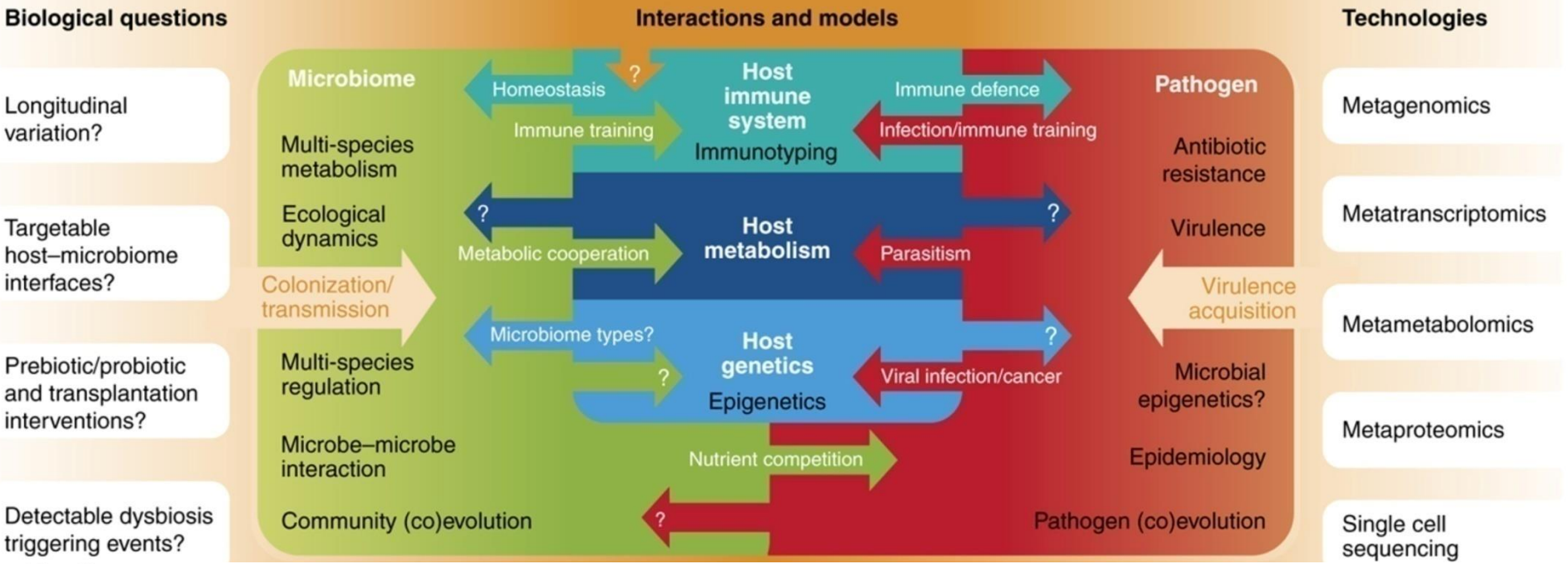
Appl Microbiol Biotechnol, 2014 Apr;98(8):3425–36. doi: 10.1007/s00253-014-5550-9. Epub 2014 Feb 13.

Keywords: molecular taxonomy; mitochondria DNA barcoding to map the microbial communities: current advances and future directions.

Chakraborty C¹, Doss CG, Patra BC, Bandopadhyay S.



- Morfoloji temelli yöntemler nereye kadar?
- ** Hızlı ** Güvenilir ** Kabul edilebilir
- ** Tekrarlanabilir ** Ucuz



Technologies

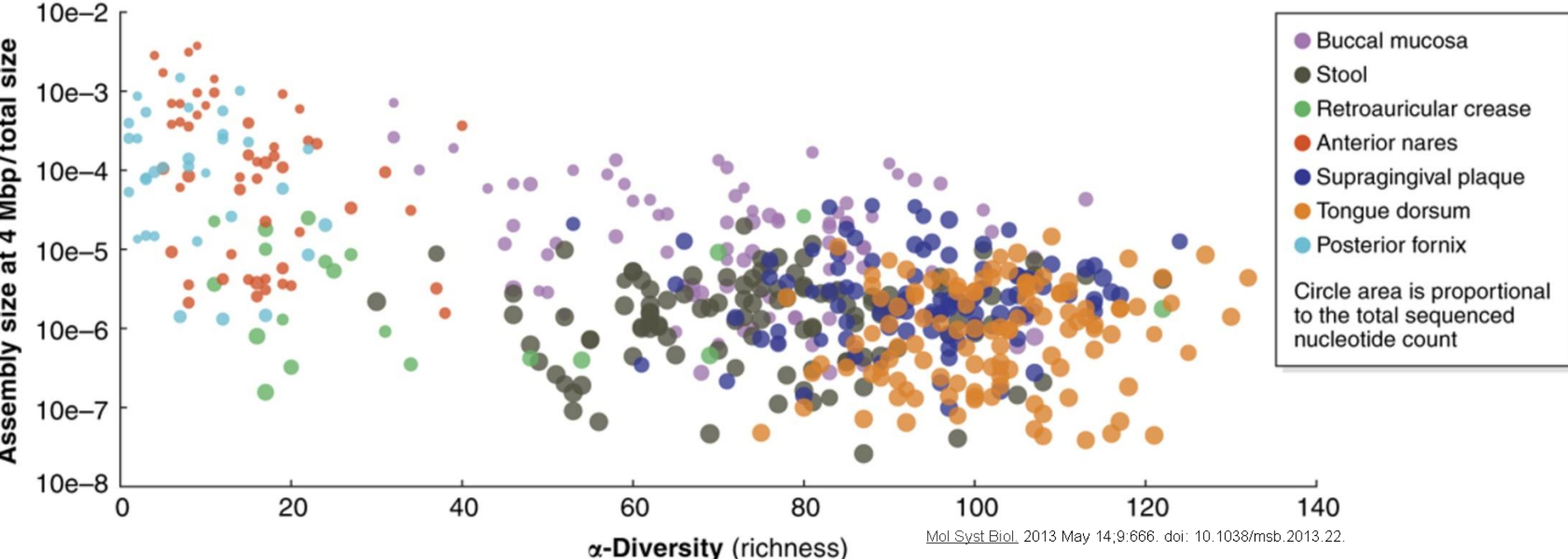
Metagenomics

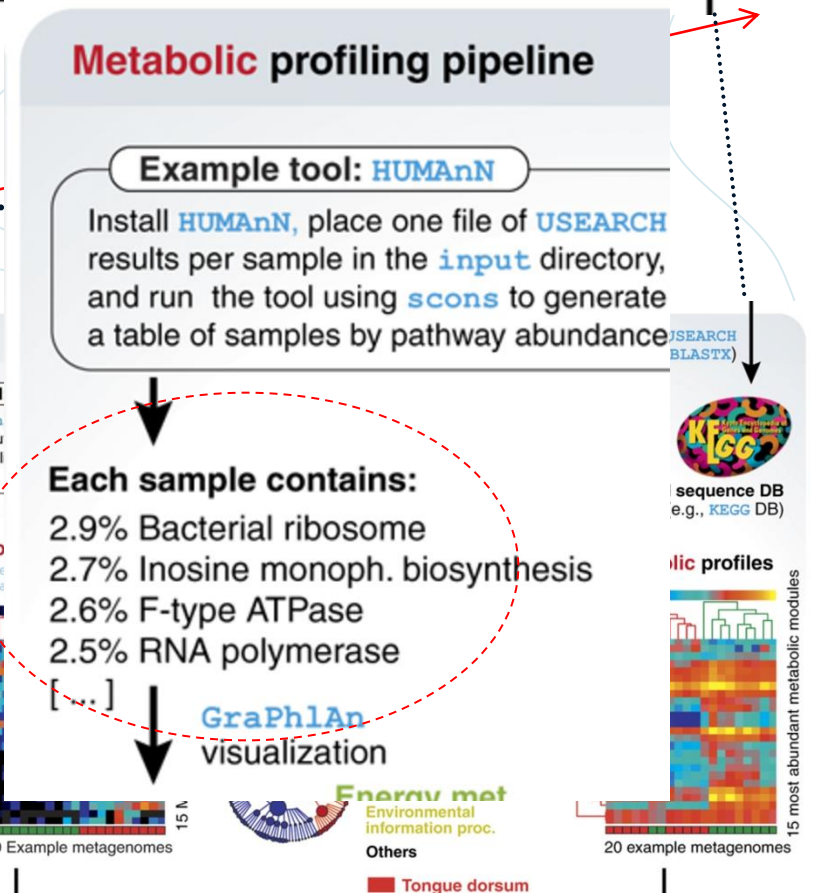
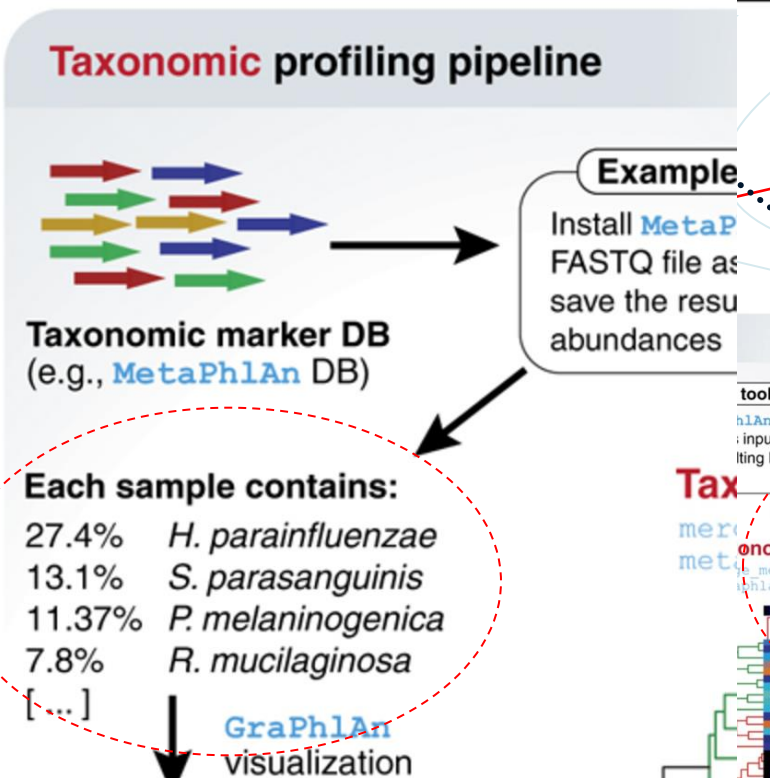
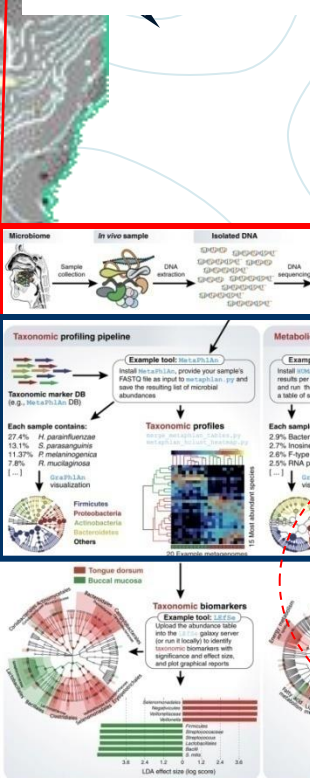
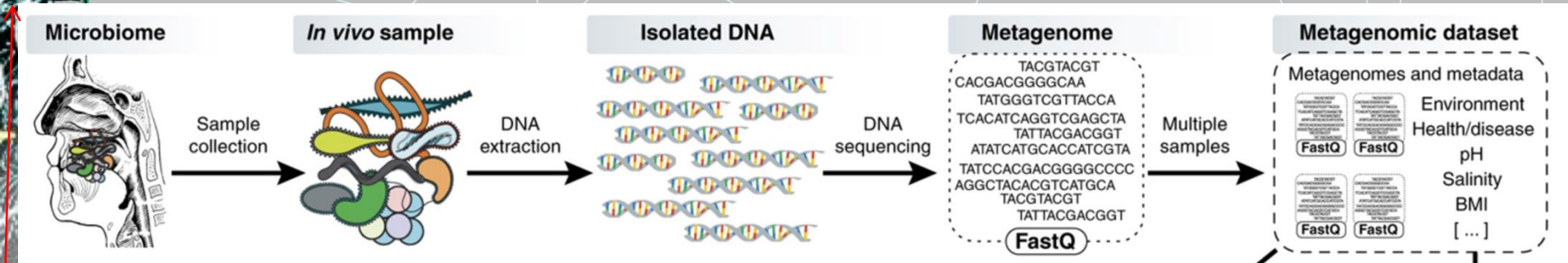
Metatranscriptomics

Metametabolomics

Metaproteomics

Single cell sequencing





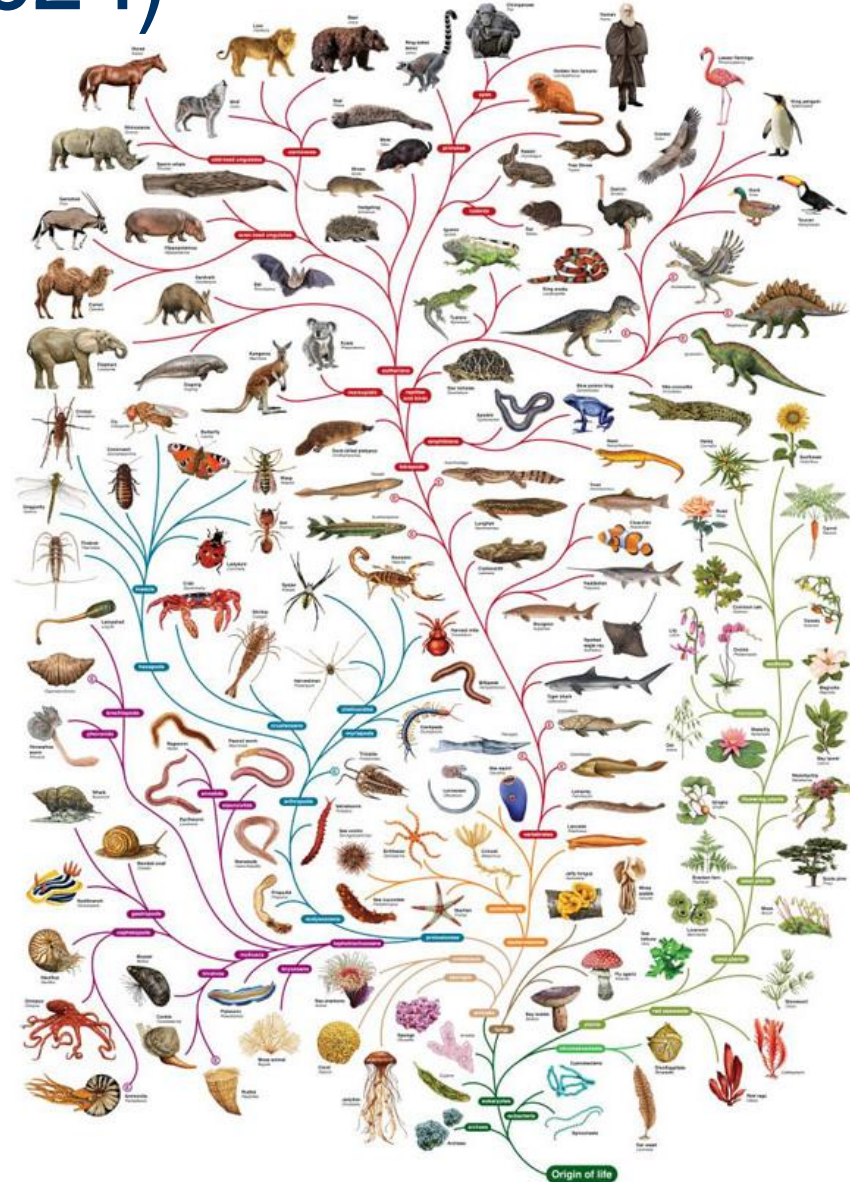
Mol Syst Biol. 2013 May 14;9:666. doi: 10.1038/msb.2013.22.

Computational meta'omics for microbial community studies.

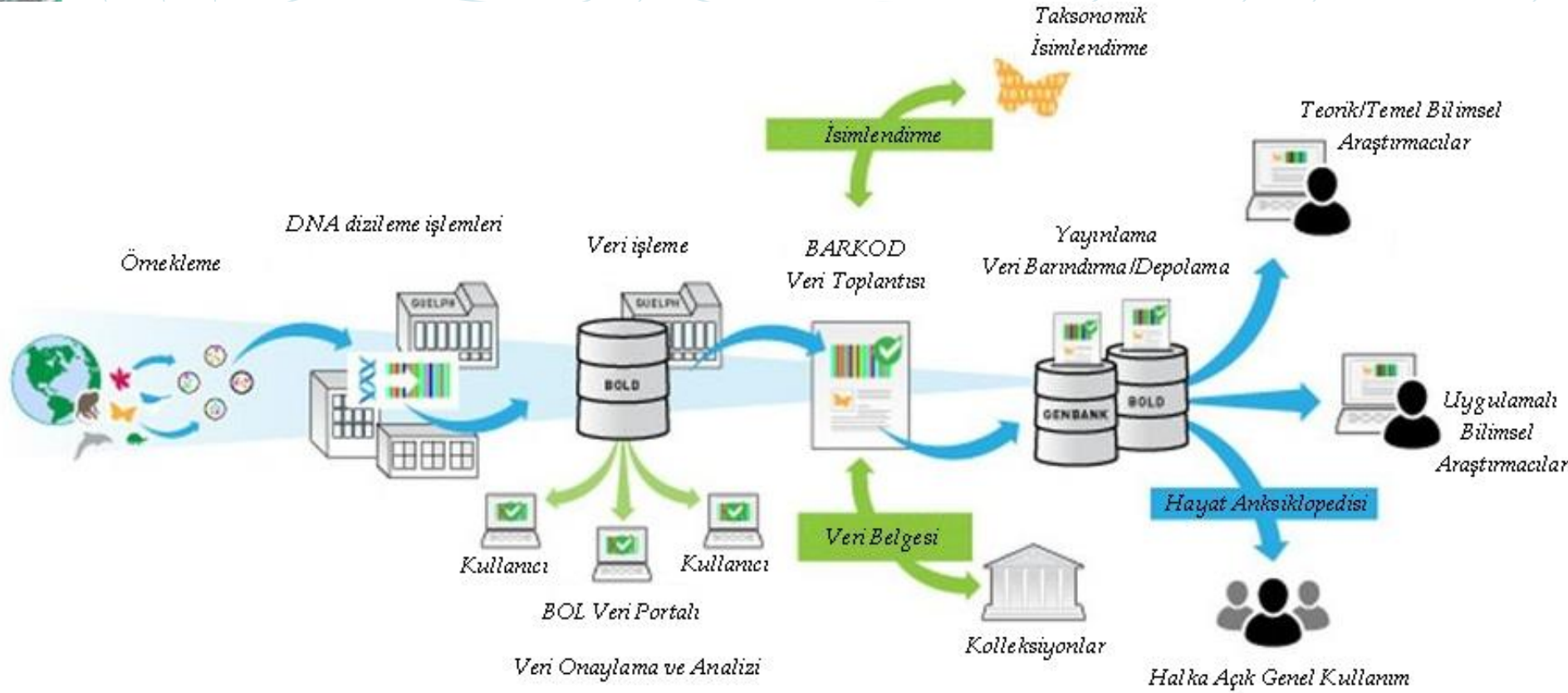
Segata N¹, Boernigen D, Tickle TL, Morgan XC, Garrett WS, Huttenhower C.

Basit bir öneri (hipotez !)

- Organizmaların genomlarının küçük parçalarındaki DNA dizisi farklılıkları vardır.
- Bu farklılıklar bir canlının tür seviyesinde tanımlanmasını sağlayacak biyolojik barkodlardır.



! BARKOD kullanımı



Tek gen bölgesi (!!)



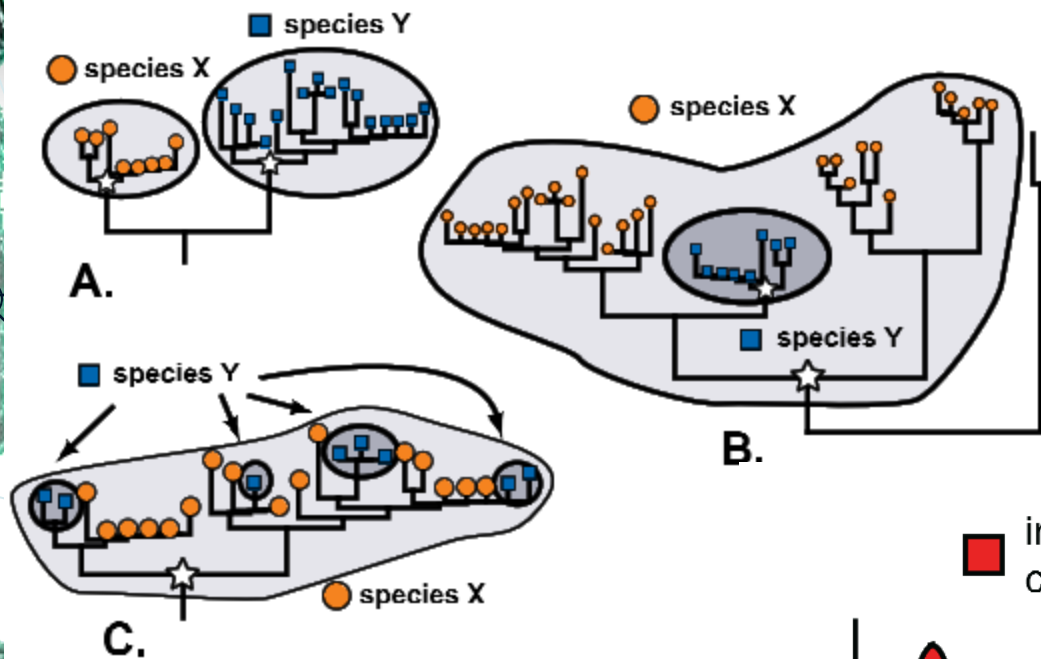
- Tür seviyesinde belirgin bir genetik varyasyon ve ayırım gücüne sahip olmalı
- Geniş bir taksonomik ölçekten canlılar için uygun evrensel primerler ile çoğaltılabilen, korunmuş uç bölgelerine sahip olmalı
- DNA ekstraksiyonu ve PCR sırasında sorun yaratmayacak kısa dizi uzunluğuna sahip olmalı



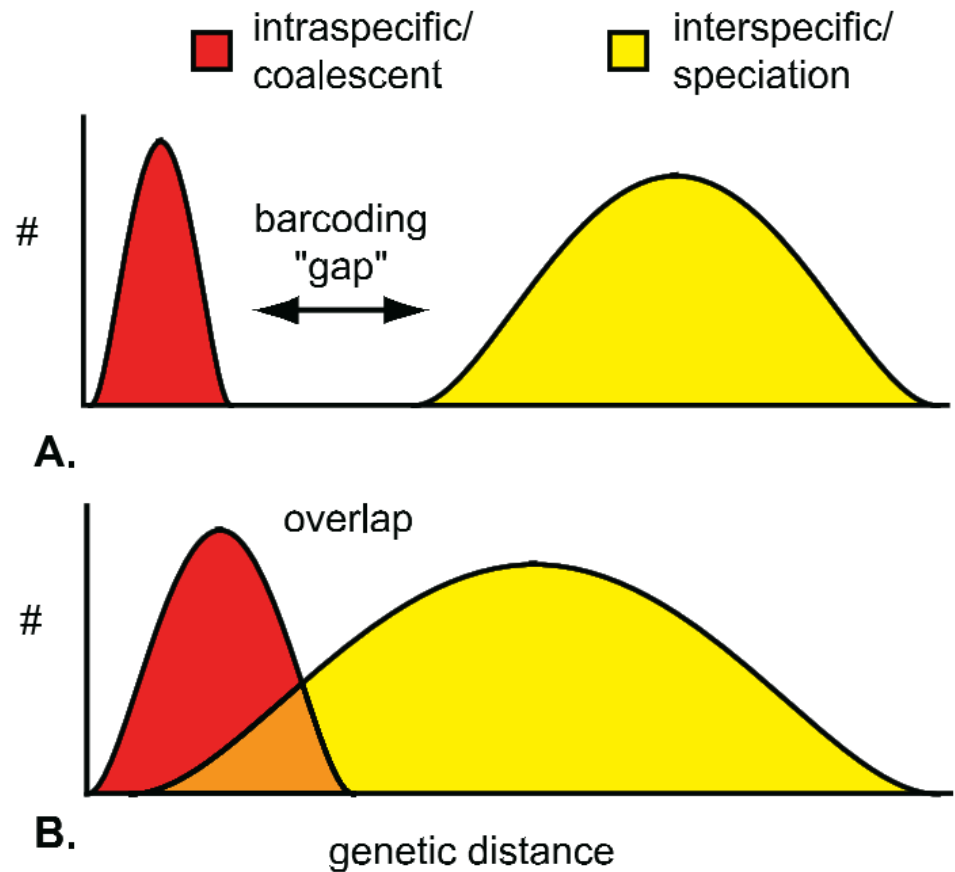
COI (mitokondriyal sitokrom oksidaz – altünite 1) geni

- rbcL*, *matK* ----- Bitkiler

ITS ----- Mantarlar



A. Ayır dallarda monofiletik
B. Parafiletik
C. Polifiletik



Analizde “*ad-hoc*” yöntem - Yazılımlar



➤ Dallandırma yöntemleri

- Bilinmeyen barkodun DNA barkod ağacına yerleştirme
- Parsimony, Neighbor joining, Bayesian Inference

➤ Benzerlik yöntemleri

- Bilinmeyen barkod DNA barkodları arasında hangisine benzer ?
- BLAST, NN, TaxonDNA

➤ Tanısal (Karakter temelli) yöntemler

- Bilinmeyen barkodun yapıları var/yok olarak eşleştirilir
- DNA-BAR, BLOG, CAOS, BRONX, PTIGS-IdIt, Linker

Sorun: Sadece insan için ...

- 
- 
- Kazanılmış idiopatik sideroblastik anemi....
 - Mitokondriyal kompleks-IV eksikliği ...
 - Tekrarlayan myoglobulinemi ...
 - Kolorektal kanser ...

NCBI-Gene

- Sağırılık; sendrom dışı tasnif, sensorinöral
- Juvenil nöropati, ensefalopati, laktik asidoz, inme

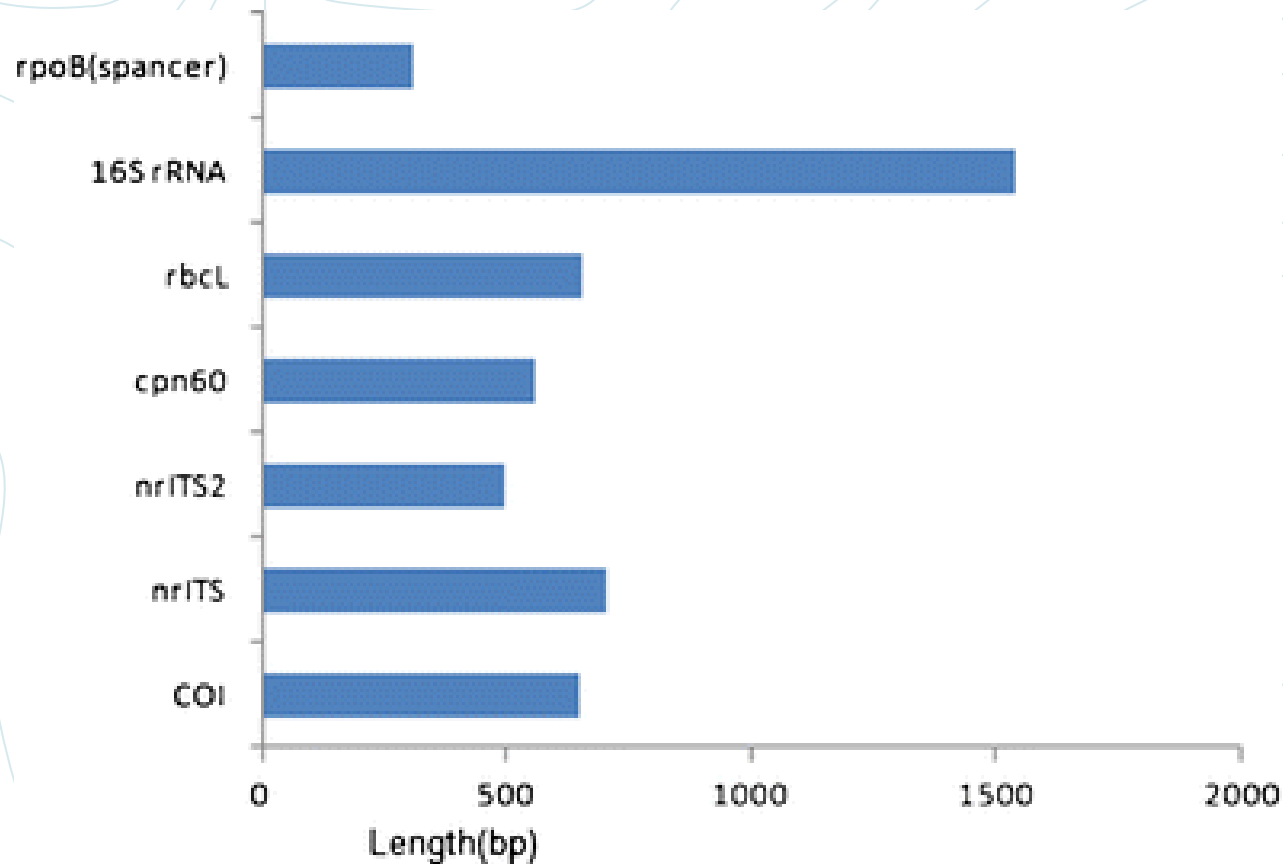
DNA barkodlama ne değildir ?

- 
- DNA taksonomisi değildir; tek bir gen yeterli olmaz.
 - Barkod kümeleri filogenetik ağaç için yeterli değildir.
 - Barkodlama sadece COI değildir.
 - Taksonomide moleküller yeni değildir, ancak geniş ölçekleme ve standardizasyon **yeni**dir.

Kısıtlılıklar

- 
- Test edilen bölge farklı barkodlar ile “overlap”
 - Veritabanı karmaşık
 - Aynı tür için farklı DNA barkodlar
 - Hangi bölge uygun ?
 - Tür olarak aynı gruba dahil, ancak hayat döngülerinde farklılıklar var
 - Karışık ? Flora ?

Mikrobiyolojide durum nedir ?



[Appl Microbiol Biotechnol](#). 2014 Apr;98(8):3425-36. doi: 10.1007/s00253-014-5550-9. Epub 2014 Feb 13.

DNA barcoding to map the microbial communities: current advances and future directions.

[Chakraborty C¹](#), [Doss CG](#), [Patra BC](#), [Bandopadhyay S](#).

Viruslar için

- Moleküler dinamizm doğal bir süreç
 - İnsan enterovirus
 - Mavidil virus
 - Avian influenza

Analyst, 2010 Feb;135(2):337-42. doi: 10.1039/b916821b. Epub 2009 Dec 15.

Detection of avian influenza virus by fluorescent DNA barcode-based immunoassay with sensitivity comparable to PCR.

Cao C¹, Dhumpa R, Bang DD, Ghavifekr Z, Høgberg J, Wolff A.

2010

J Virol Methods, 2011 Dec;178(1-2):225-8. doi: 10.1016/j.jviromet.2011.05.014. Epub 2011 May 17.

Nanoparticle-based bio-barcode assay for the detection of bluetongue virus.

Yin HQ¹, Jia MX, Shi LJ, Yang S, Zhang LY, Zhang QM, Wang SQ, Li G, Zhang JG.

2011

Bakteriler için ...

→ *COI*

- Solunum zinciri son enzimi

➤ *rpoB*

- Bakterial RNA polimerazın β -altünitesinin kodlanması

➤ *16S rRNA*

- Nükleer DNA ve “housekeeping” genler
- 16S rDNA ile ilişkili kodlanan genler

➤ *cpn60 (GroEL, Hsp60)*

- Geniş barkodlama alanı

[Annu Rev Biophys Biomol Struct.](#) 1998;27:329-56.

Cytochrome c oxidase: structure and spectroscopy.

[Michel H¹](#), [Behr J.](#), [Harrenqa A.](#), [Kannt A.](#)

1998

[Cell.](#) 2001 Mar 23;104(6):901-12.

Structural mechanism for rifampicin inhibition of bacterial rna polymerase.

[Campbell EA¹](#), [Korzhova N.](#), [Mustaev A.](#), [Murakami K.](#), [Nair S.](#), [Goldfarb A.](#), [Darst SA.](#)

2001

[J Clin Microbiol.](#) 2007 Sep;45(9):2761-4. Epub 2007 Jul 11.

16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls.

[Janda JM¹](#), [Abbott SL.](#)

2007

[Clin Microbiol Infect.](#) 2008 Oct;14(10):908-34. doi: 10.1111/j.1469-0691.2008.02070.x.

Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories.

[Woo PC¹](#), [Lau SK.](#), [Teng JL.](#), [Tse H.](#), [Yuen KY.](#)

2008

[PLoS One.](#) 2012;7(11):e49755. doi: 10.1371/journal.pone.0049755. Epub 2012 Nov 26.

The chaperonin-60 universal target is a barcode for bacteria that enables de novo assembly of metagenomic sequence data.

[Links MG¹](#), [Dumonceaux TJ.](#), [Hemmingsen SM.](#), [Hill JE.](#)

2012

Protozoalar için ...

➤ 18S *rRNA*

- Protozoal Küçük subünite (SSU)

[J Anim Sci](#), 2003 Mar;81(3):812-5.

Technical note: Specific PCR amplification of protozoal 18S rDNA sequences from DNA extracted from ruminal samples of cows.

[Karnati SK¹](#), [Yu Z](#), [Sylvester JT](#), [Dehority BA](#), [Morrison M](#), [Firkins JL](#).

2003

➤ COI

- Amiplerin barkodlanması

[Protist](#), 2012 May;163(3):415-34. doi: 10.1016/j.protis.2011.10.003. Epub 2011 Nov 30.

COI barcoding of *Nebelid testate amoebae* (Amoebozoa: Arcellinida): extensive cryptic diversity and redefinition of the *Hyalospheniidae* Schultze.

[Kosakyan A¹](#), [Heger TJ](#), [Leander BS](#), [Todorov M](#), [Mitchell EA](#), [Lara E](#).

2012

➤ 28S *rRNA*

- Piroplasma barkodlama

[Acta Trop](#), 2012 Oct;124(1):92-7. doi: 10.1016/j.actatropica.2012.07.001. Epub 2012 Jul 14.

A DNA barcode for Piroplasma.

2012

[Gou H¹](#), [Guan G](#), [Liu A](#), [Ma M](#), [Xu Z](#), [Liu Z](#), [Ren Q](#), [Li Y](#), [Yang J](#), [Chen Z](#), [Yin H](#), [Luo J](#).

➤ ITS

- Piroplasmalar için ITS2 bölgesi

[Proc Natl Acad Sci U S A](#), 2012 Apr 17;109(16):6241-6. doi: 10.1073/pnas.1117018109. Epub 2012 Mar 27.

Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi.

[Schoch CL¹](#), [Seifert KA](#), [Huhndorf S](#), [Robert V](#), [Spouge JL](#), [Levesque CA](#), [Chen W](#); [Fungal Barcoding Consortium](#); [Fungal Barcoding Consortium Author List](#).

2012

Mantarlar için ... - I

- 
- 
- Multi-takson bir yapılanma !!!
 - Tıbbi mikoloji alanında tür düzeyinde tanı !!!
 - Birçok mantar henüz tanımlanmamış durumda...
 - Mikroskopik fenotiplemenin koleksiyonu ???
 - COI --- uygun değil
 - Günden güne değişim (NCBI – takip)

Mantarlar için ... - II

➤ *COI*

- Grup I intron içeren bölge

[Proc Natl Acad Sci U S A](#), 1998 Nov 24;95(24):14244-9.

Explosive invasion of plant mitochondria by a group I intron.

[Cho Y¹](#), [Qiu YL](#), [Kuhlman P](#), [Palmer JD](#).

1998

➤ *RPB*

- Ribozomal polimeraz 1 ve 2

[Mol Ecol Resour](#), 2009 May;9 Suppl s1:83-9. doi: 10.1111/j.1755-0998.2009.02635.x.

Progress towards DNA barcoding of fungi.

[Seifert KA](#).

2009

➤ *ITS*

- Ribozomal sistron bölgesi

[Proc Natl Acad Sci U S A](#), 2012 Apr 17;109(16):6241-6. doi: 10.1073/pnas.1117018109. Epub 2012 Mar 27.

Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi.

[Schoch CL¹](#), [Seifert KA](#), [Huhndorf S](#), [Robert V](#), [Spouge JL](#), [Levesque CA](#), [Chen W](#); [Fungal Barcoding Consortium](#); [Fungal Barcoding Consortium Author List](#).

2012

➤ *LSU*

- 28S LSU rRNA bölgesi vs...

[Appl Microbiol Biotechnol](#), 2010 Jun;87(1):99-108. doi: 10.1007/s00253-010-2585-4. Epub 2010 Apr 20.

Current state and perspectives of fungal DNA barcoding and rapid identification procedures.

[Begerow D¹](#), [Nilsson H](#), [Unterseher M](#), [Maier W](#).

2010

➤ *SSU*

- 16S SSU rDNA bölgesi vs...

[Appl Microbiol Biotechnol](#), 2010 Jun;87(1):99-108. doi: 10.1007/s00253-010-2585-4. Epub 2010 Apr 20.

Current state and perspectives of fungal DNA barcoding and rapid identification procedures.

[Begerow D¹](#), [Nilsson H](#), [Unterseher M](#), [Maier W](#).

2010

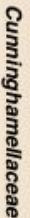
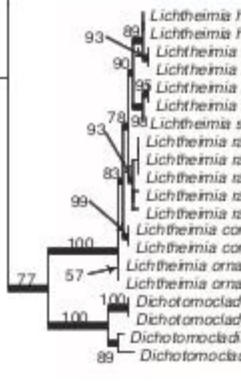
Mantarlar için ... - III



Persoonia, 2013 Jun;30:11-47. doi: 10.3767/003158513X665070. Epub 2013 Mar 4.

DNA barcoding in Mucorales: an inventory of biodiversity.

Walther G¹, Pawłowska J, Alastruey-Izquierdo A, Wrzosek M, Rodríguez-Tudela JL, Dolatabadi S, Chakrabarti A, de Hoog GS.



Walther G¹, Pawłowska J, Alastruey-Izquierdo A, Wrzosek M, Rodriguez-Tudela JL, Dolatabadi S, Chakrabarti A, de Hoog GS.



Mikrobiyol Bul. 2014 Apr;48(2):292-9.

[Evaluation of a new medium, eggplant (*Solanum melongena*) agar as a screening medium for *Cryptococcus neoformans* in environmental samples].

[Article in Turkish]

Sengul M¹, Ergin C, Kartal T.

Mantarlar için... - I

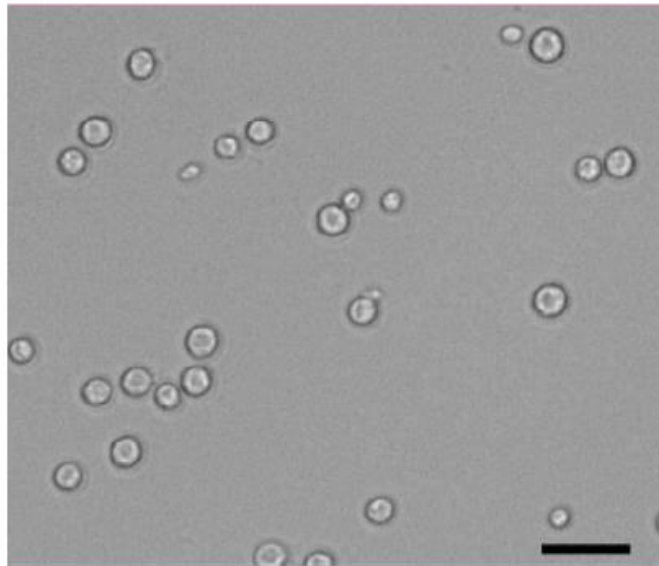
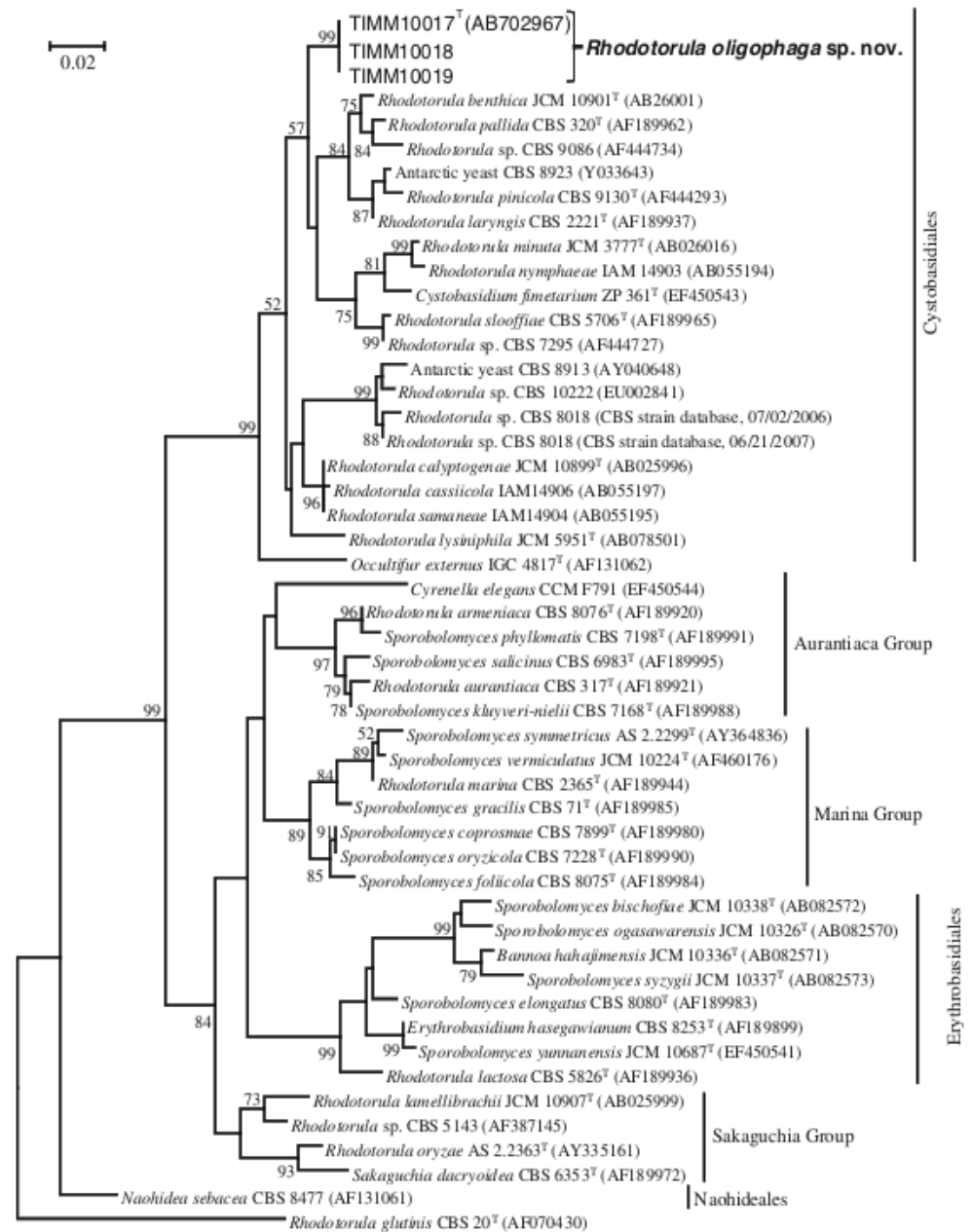


Fig. 1 *Cryptococcus lacticolor* TIMM 10013^T. Growth on 5 % malt extract agar after 7 days at 17 °C. Scale bar = 10 µm



Supplementary Table 4. Similarity comparison of ITS 1+2 between *Cryptococcus lacticolor* sp. nov. TIMM10013^T and closely related species.

	TIMM 10013 ^T	CBS 7227 ^T	CBS 8290 ^T	ATCC 18803	CBS 139 ^T	IMUFRJ 51996	U2	HBZ 194	CBS 7810	RJB2846-8	ATCC 201820	CCJ 1192	CCJ 1418
TIMM 10013 ^T (AB375774)	—	78.5	77.4	77.0	77.0	77.8	77.7	82.9	74.9	83.7	75.6	75.6	75.6
<i>Bullera pseudoalba</i> CBS 7227 ^T (AF444399)	78.5	—	82.9	89.3	89.0	89.6	86.8	81.2	75.1	80.7	79.6	79.4	79.1
<i>Bullera unica</i> CBS 8290 ^T (AF444441)	77.4	82.9	—	81.7	80.8	82.4	78.8	78.7	73.8	78.2	77.6	77.4	77.2
<i>Cryptococcus laurentii</i> ATCC 18803 (EU266564)	77.0	89.3	81.7	—	100.0	96.7	94.5	80.0	76.5	80.3	80.1	79.1	78.8
<i>Cryptococcus laurentii</i> CBS 139 ^T (AF410468)	77.0	89.0	80.8	100.0	—	97.8	94.5	80.0	76.5	79.4	78.3	78.3	77.7
<i>Cryptococcus laurentii</i> IMUFRJ 51996 (FN428921)	77.8	89.6	82.4	96.7	97.8	—	94.9	79.7	76.3	79.7	78.8	79.0	78.7
<i>Cryptococcus laurentii</i> U2 (GU943490)	77.7	86.8	78.8	94.5	94.5	94.9	—	81.9	75.9	80.5	76.4	76.4	76.0
<i>Tremella indecorata</i> HBZ 194 (AF042432)	82.9	81.2	78.7	80.0	80.0	79.7	81.9	—	74.7	86.2	74.2	74.2	73.5
<i>Tremella moriformis</i> CBS 7810 (AF444331)	74.9	75.1	73.8	76.5	76.5	76.3	75.9	74.7	—	73.6	84.3	84.3	83.3
<i>Tremella moriformis</i> RJB2846-8 (AF042426)	83.7	80.7	78.2	80.3	79.4	79.7	80.5	86.2	73.6	—	75.4	75.2	74.8
<i>Tremella nivalis</i> ATCC 201820 (DQ680073)	75.6	79.6	77.6	80.1	78.3	78.8	76.4	74.2	84.3	75.4	—	99.8	98.5
<i>Tremella nivalis</i> CCJ1192 (AF042414)	75.6	79.4	77.4	79.1	78.3	79.0	76.4	74.2	84.3	75.2	99.8	—	98.3
<i>Tremella nivalis</i> CCJ1418 (AF042419)	75.6	79.1	77.2	78.8	77.7	78.7	76.0	73.5	83.3	74.8	98.5	98.3	—

Supplementary Table 5. Similarity comparison of D1/D2 LSU between *Cryptococcus lacticolor* sp. nov. TIMM10013^T and closely related species.

	TIMM 10013 ^T	CBS 7227 ^T	CBS 8290 ^T	CBS 139 ^T	CRUB 1395	IMUFRJ 51996	U2	HBZ 194	CBS 7810	MW 335	RJB2846-8	CCJ 1192	CCJ 1418	GY15L04
TIMM 10013 ^T (AB375775)	—	93.2	94.9	95.0	94.5	95.2	92.7	94.7	94.4	92.5	95.8	91.4	88.4	90.8
<i>Bullera pseudoalba</i> CBS 7227 ^T (AF075504)	93.2	—	95.7	96.7	96.1	96.7	94.3	94.4	92.2	91.5	92.9	94.3	87.2	89.2
<i>Bullera unica</i> CBS 8290 ^T (AF075524)	94.9	95.7	—	94.9	95.0	95.7	94.3	94.7	93.3	91.1	94.2	89.5	86.7	88.7
<i>Cryptococcus laurentii</i> CBS 139 ^T (AF075469)	95.0	96.7	94.9	—	99.7	99.8	97.0	95.8	93.3	92.2	93.2	90.1	87.5	89.5
<i>Cryptococcus laurentii</i> CRUB 1395 (EF585173)	94.5	96.1	95.0	99.7	—	99.5	96.6	95.4	93.2	91.7	92.8	89.4	87.2	88.9
<i>Cryptococcus laurentii</i> IMUFRJ 51996 (FN428921)	95.2	96.7	95.7	99.8	99.5	—	97.2	95.8	93.2	92.4	93.3	90.1	87.4	89.6
<i>Cryptococcus laurentii</i> U2 (GU943503)	92.7	94.3	94.3	97.0	96.6	97.2	—	94.0	90.8	90.2	91.5	87.1	84.4	87.4
<i>Tremella indecorata</i> HBZ 194 (AF042250)	94.7	94.4	94.7	95.8	95.4	95.8	94.0	—	93.3	92.6	95.2	90.0	87.5	89.5
<i>Tremella moriformis</i> CBS 7810 (AF075493)	94.4	92.2	93.3	93.3	93.2	93.2	90.8	93.3	—	93.7	92.8	92.7	89.9	92.1
<i>Tremella moriformis</i> MW 335 (AF042269)	92.5	91.5	91.1	92.2	91.7	92.4	90.2	92.6	93.7	—	91.9	91.5	88.9	91.0
<i>Tremella moriformis</i> RJB2846-8 (AF042244)	95.8	92.9	94.2	93.2	92.8	93.3	91.5	95.2	92.8	91.9	—	89.6	87.0	89.0
<i>Tremella nivalis</i> CCJ 1192 (AF042232)	91.4	94.3	89.5	90.1	89.4	90.1	87.1	90.0	92.7	91.5	89.6	—	96.8	99.7
<i>Tremella nivalis</i> CCJ 1418 (AF042237)	88.4	87.2	86.7	87.5	87.2	87.4	84.4	87.5	89.9	88.9	87.0	96.8	—	97.1
<i>Tremella nivalis</i> GY15L04 (FJ527111)	90.8	89.2	88.7	89.5	89.6	89.6	87.4	89.5	92.1	91.0	89.0	99.7	97.1	—

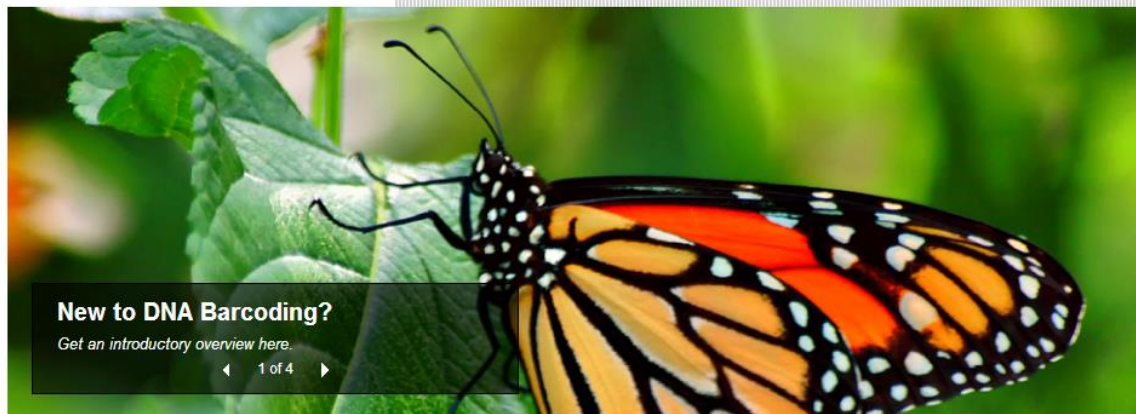
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Identifying Species with DNA Barcoding

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PUBLICATIONS

OBSERVATIONS ON AN IRRUPTION EVENT OF THE MOTH ACHAEA CATOCALOIDES (LEPIDOPTERA: EREBIDAE) AT KAKAMEGA FOREST, KENYA

Martins, D.J.; Miller, S.E.; Cords, M.; Hirschauer, M.T. and Goodale, C.B., 103(1): 31–38, Apr 6 2014, *Journal of East African Natural History*

UPCOMING EVENTS

There are no upcoming events. Revisit [past events here](#).

[VIEW ALL](#)

RECENT ACTIVITY

Nothing to see here.

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The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy

Guillou L, Bachar D, Audic S, Bass D, Berney C, Bittner L, Boutte C, Burgaud G, de Vargas C, Decelle J, Del Campo J, Dolan JR, Dunthorn M, Edvardsen B, Holzmann M, Kooistra WH, Lara E, Le Bescot N, Logares R, Mahé F, Massana R, Montresor M, Morard R, Not F, Pawlowski J, Probert I, Sauvadet AL, Siano R, Stoeck T, Vaulot D, Zimmermann P, Christen R., 40 (21), Nov 27 2012, *Nucleic Acids Research*

The Chaperonin-60 Universal Target Is a Barcode for Bacteria That Enables De Novo Assembly of Metagenomic Sequence Data

Links MG, Dumonceaux TJ, Hemmingsen SM, Hill JE, 7(11):e49755, Nov 26 2012, *PLoS One*

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CONSORTIUM FOR THE BARCODE OF LIFE

NCBI



BOLD
SYSTEMS

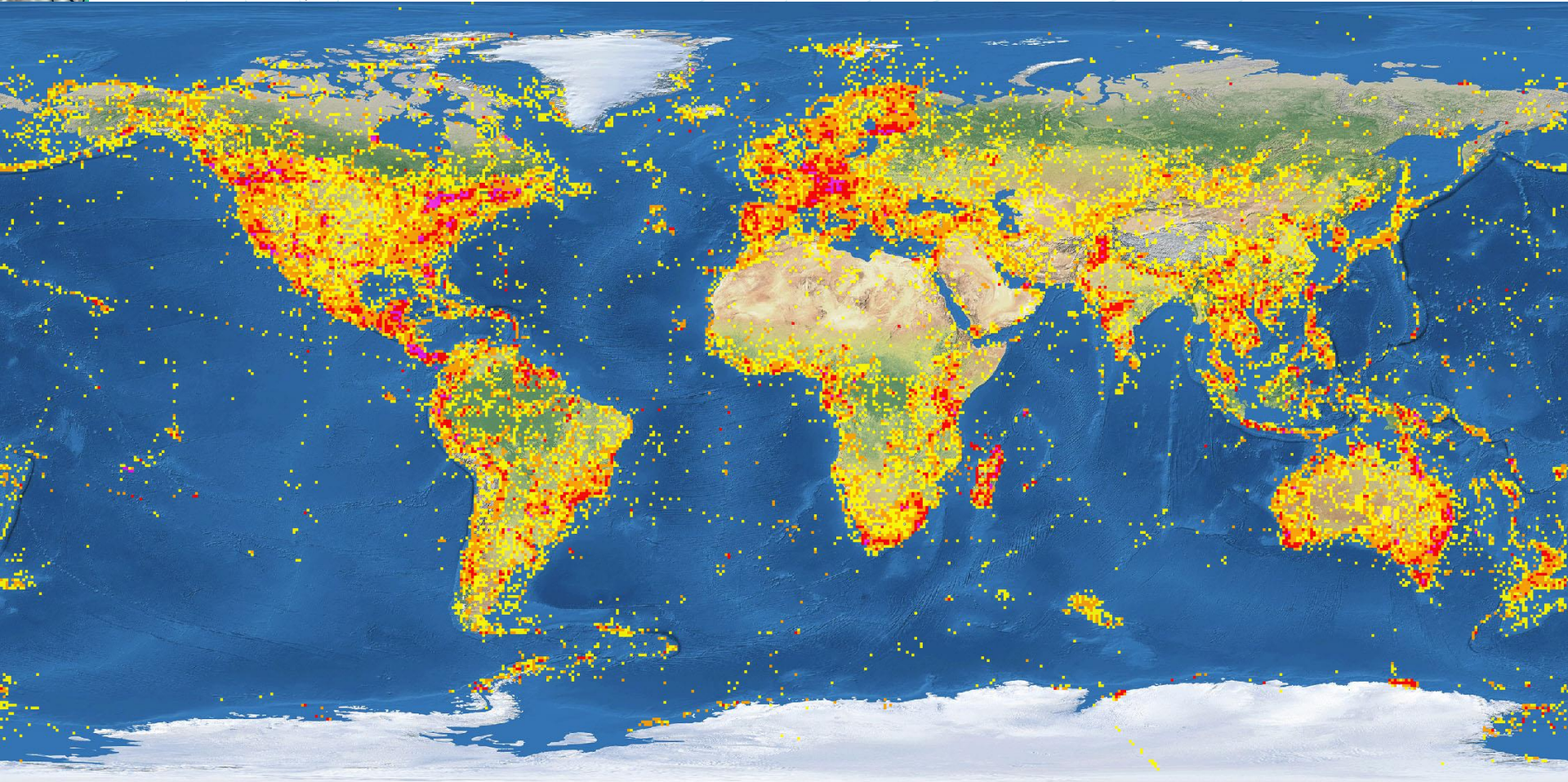


Canadian
Barcode of Life
Network

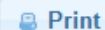


Encyclopedia of Life

CBOL veritabanı nasıl oluřtu?



Identification Request

**Animal Identification**
[COI]**Fungal Identification**
[ITS]**Plant Identification**
[rbcL & matK]

The BOLD Identification System (IDS) for ITS is the default identification tool for Fungal barcodes and accepts sequences from the Internal Transcribed Spacer Region and returns a species-level identification when possible. Further validation with independent genetic markers will be desirable in some forensic applications. The BLAST algorithm is employed in place of the standard BOLD identification engine for ITS sequences.

There are very few ITS records on BOLD so most queries will likely not return a successful match.

Search Database:

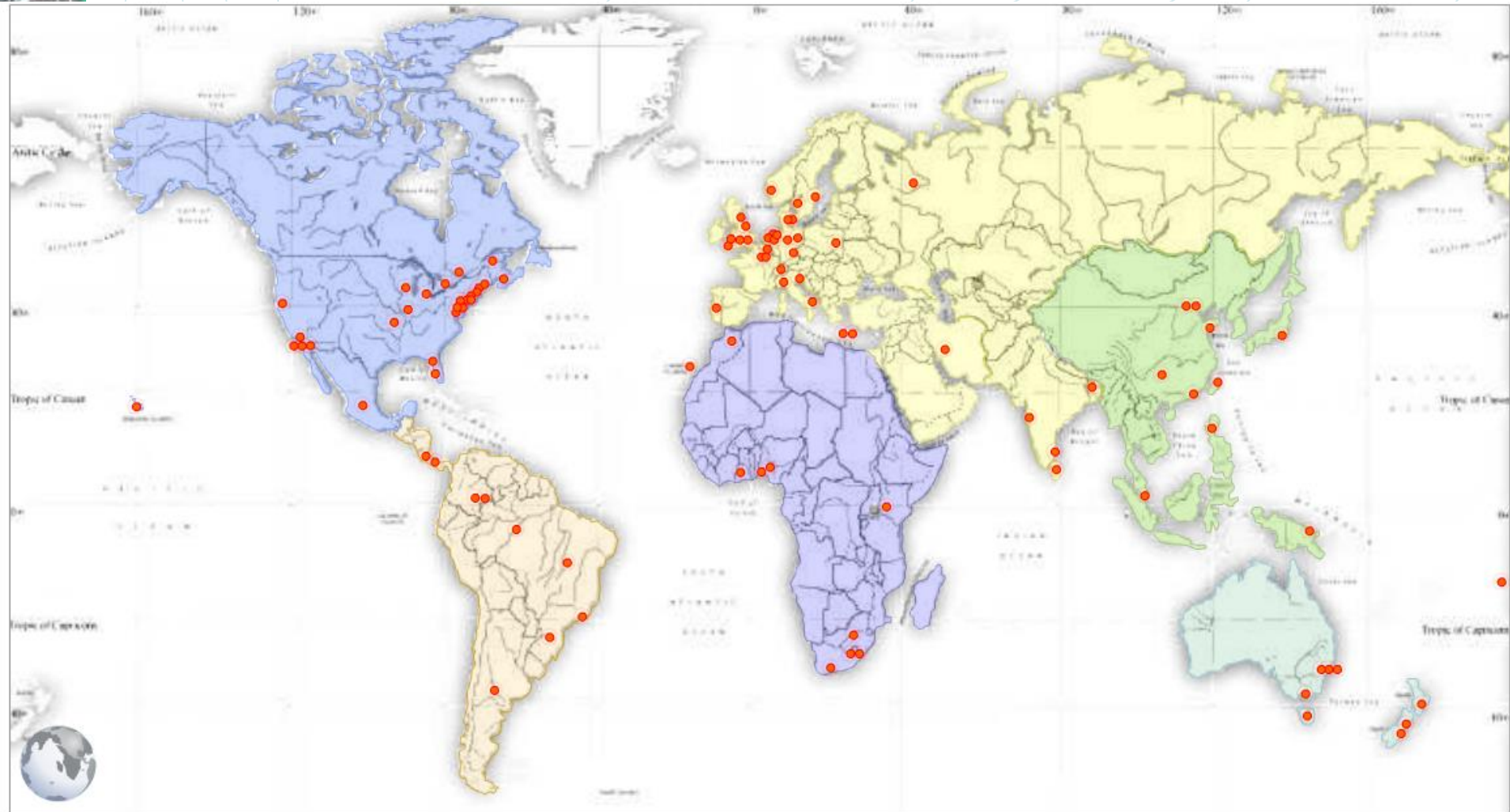
☒ **ITS Sequences**

Every ITS barcode record on BOLD with a minimum sequence length of 100bp (warning: unvalidated database that includes records without species level identification). This includes many species represented by only one or two specimens as well as all species with interim taxonomy. This search only returns a list of the nearest matches and does not provide a probability of placement to a taxon.

Enter sequences in fasta format:

Submit

CBOL üyelikleri



Producing Barcode Data: 2010?

Barcode data anywhere, instantly



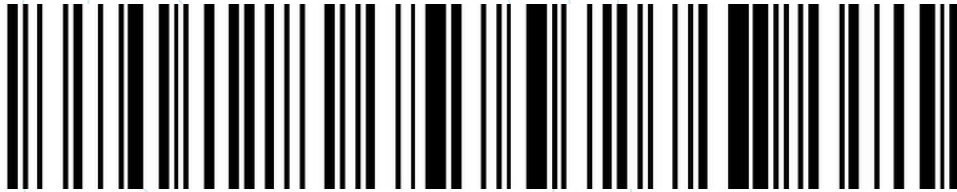
- Data in seconds to minutes
- Pennies per sample
- Link to reference database
- A taxonomic GPS
- Usable by non-specialists

Korean Research Institute for Bioscience and
Biotechnology - **23 January 2007**



8. Ulusal Tanısal ve Moleküler Mikrobiyoloji Kongresi

DNA Barkodlama



*Pamukkale Üniversitesi
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Mikrobiyoloji AD*

Dr. Çağrı Ergin

