Large-scale phylogenetic analyses elucidate the evolutionary affiliations of two novel microbial eukaryotes, *Tsukubamonas globosa* and *Palipitomonas bilix*

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Our goal is

A well-resolved global eukaryotic phylogeny

- Model the evolutions of traits in eukaryotic cells
  - ✓ Mitochondria, plastids, other bacterial endosymbionts, translation systems, etc.
- *Novel microbial eukaryotes*, which have not been observed (or studied in detail)
  - ✓ *lots of them* in environments
  - ✓ Find & isolate
  - ✓ Cultivate
  - ✓ Characterize
  - ✓ Generate large-scale sequence data
  - ✓ Determine the phylogenetic position

Next-generation sequencing

'Phylogenomic' analysis

How we find, characterize, and analyze novel eukaryotes

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① Sampling

Culturing

② Microscopic observation

Small-scale DNA sequencing

Small subunit rRNA gene

Next-generation sequencing

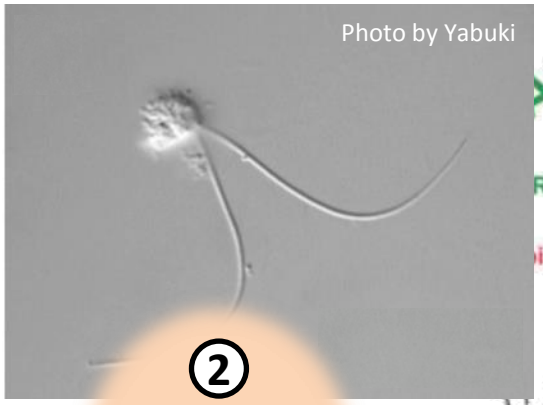
③

④

Large-scale transcriptomic data
And/or genome data

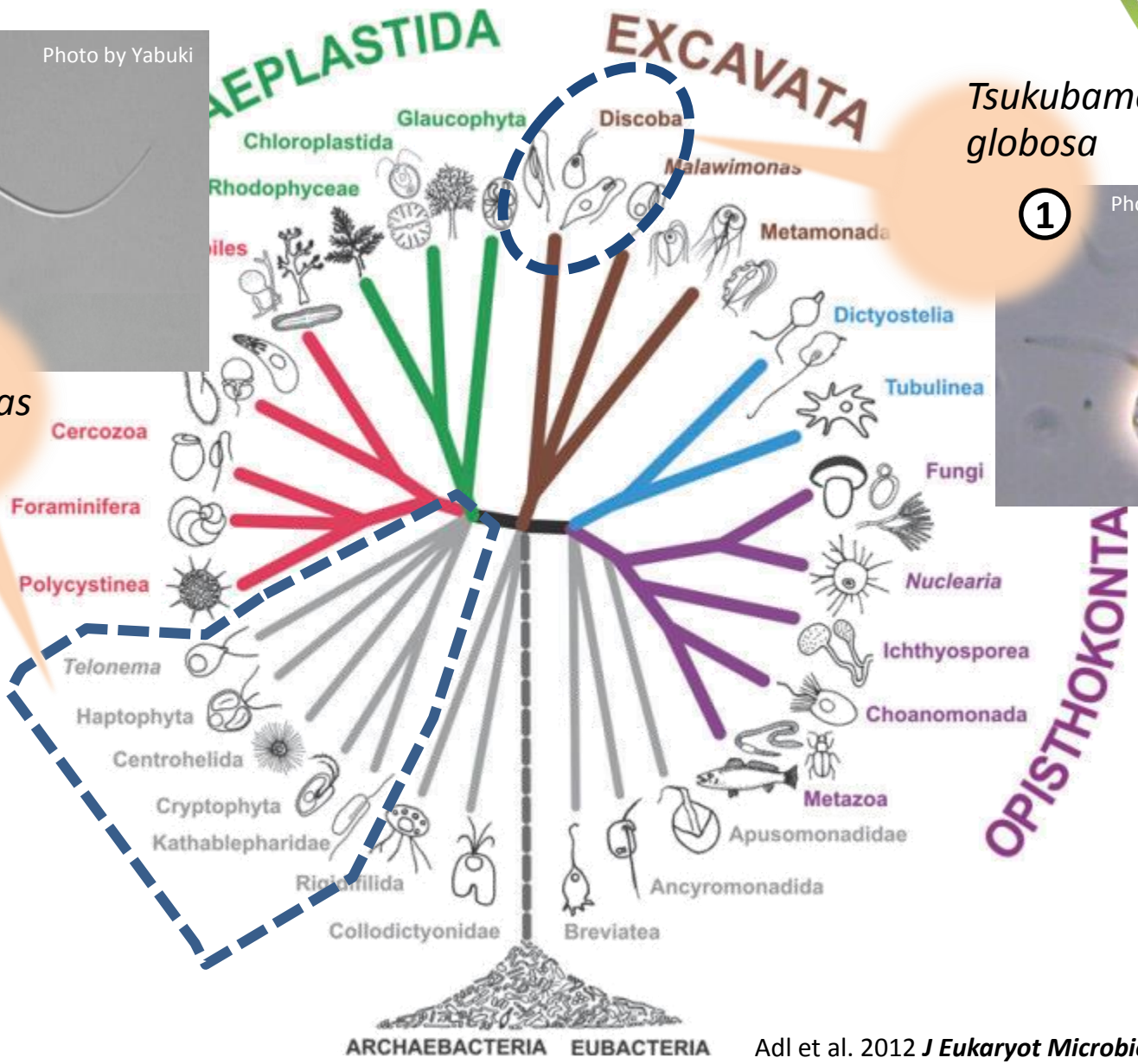
Phylogenomic analyses

Two novel eukaryotes



② *Palpitomonas bilix*

Tsukubamonas globosa ①

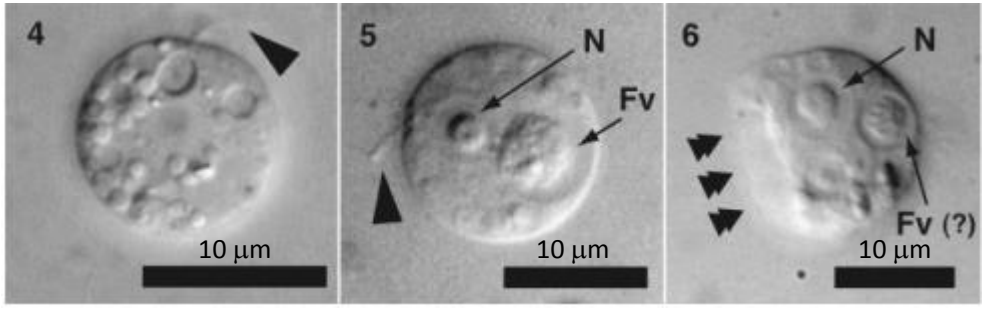


Adl et al. 2012 ***J Eukaryot Microbiol*** 59:429-493

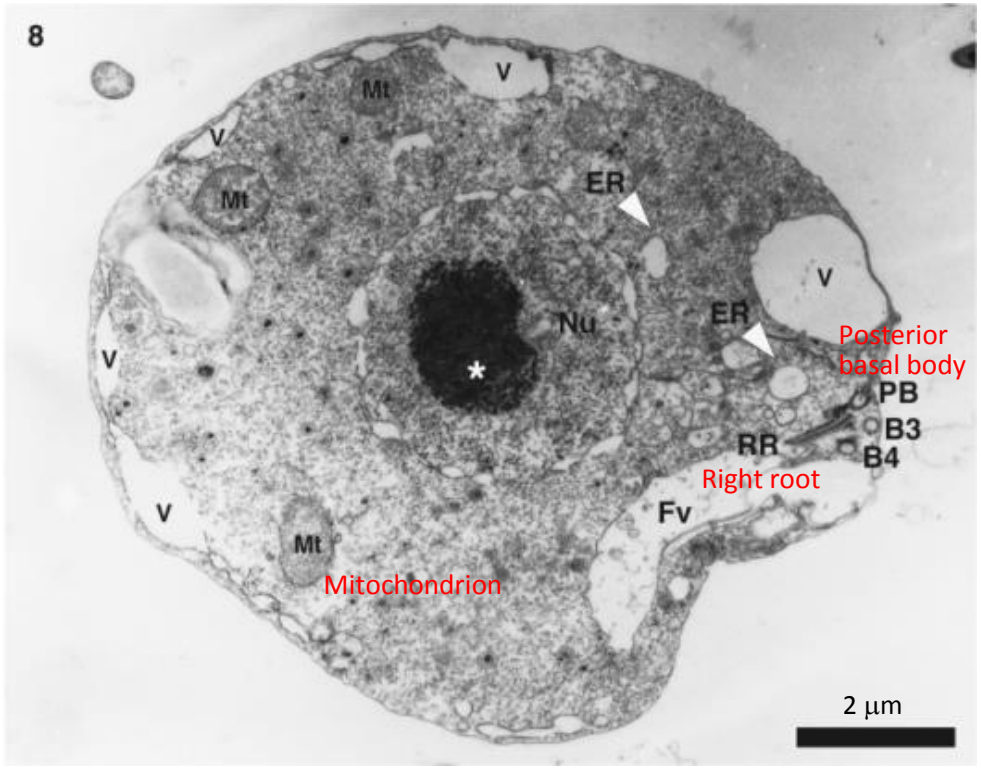
University of Tsukuba: Homo of *Tsukubamonas globosa*

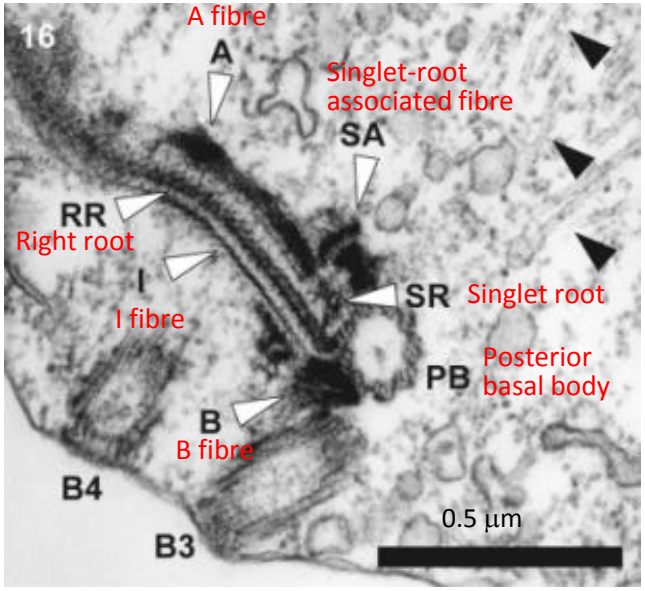
- Isolated from Hyoutaro-pond
- Maintained in UR-YT medium at 20°C since October 2002

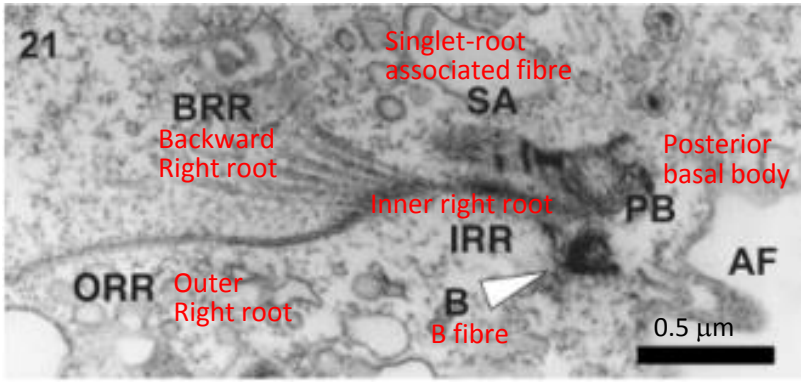
Tsukubamonas: How it looks like

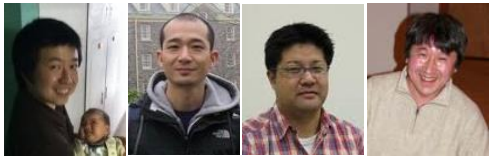


N, Nucleus; Fv, Food vacuole









Yabuki et al. 2011 ***J Eukaryot Microbiol*** 58:319-331

Tsukubamonas: Excavate-like flagellar apparatus

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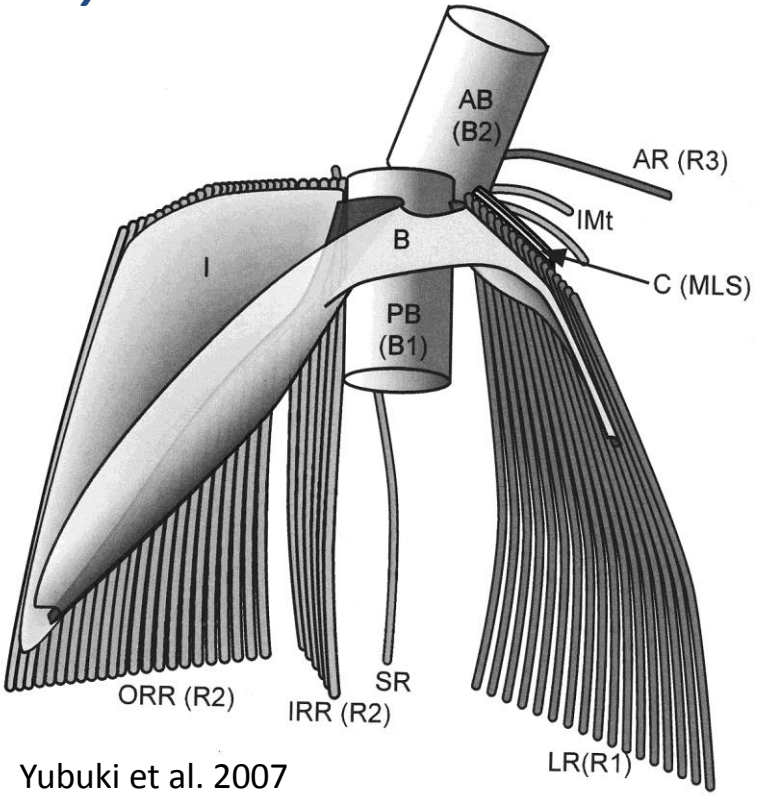
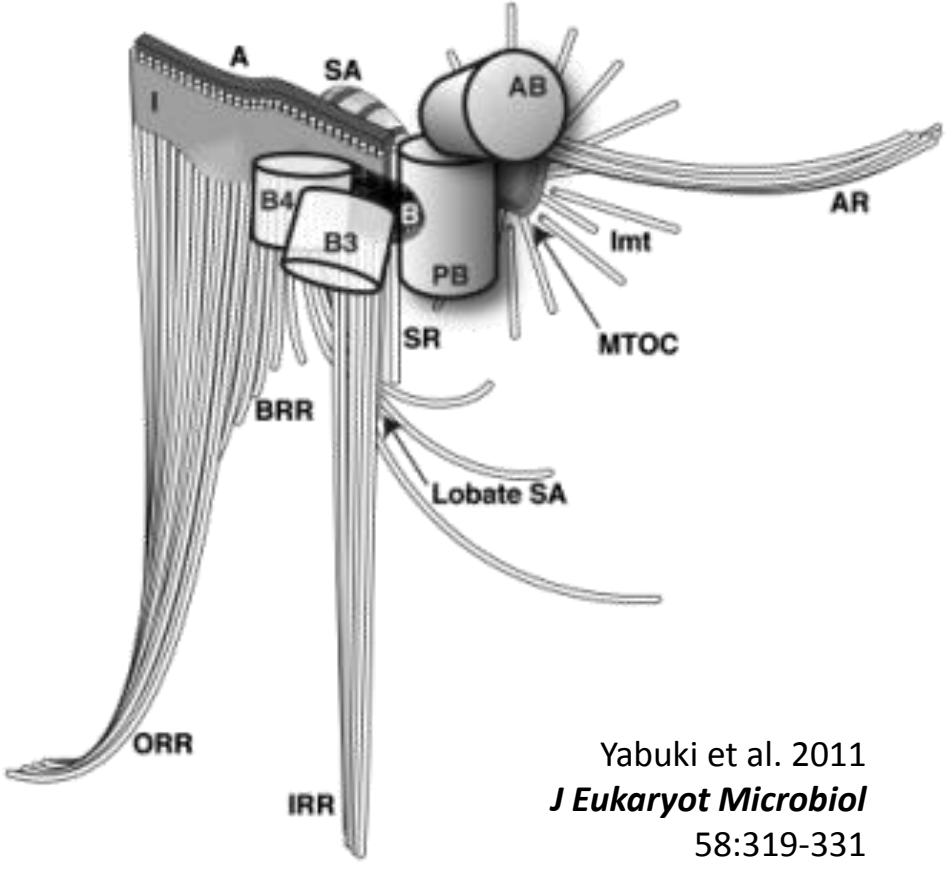
Dysnectes brevis



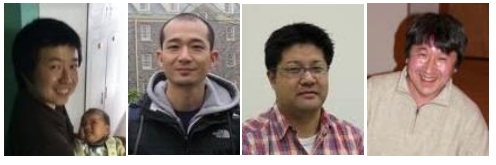
Yubuki et al. 2007
J Eukaryot Microbiol
54:191-200

Tsukubamonas globosa

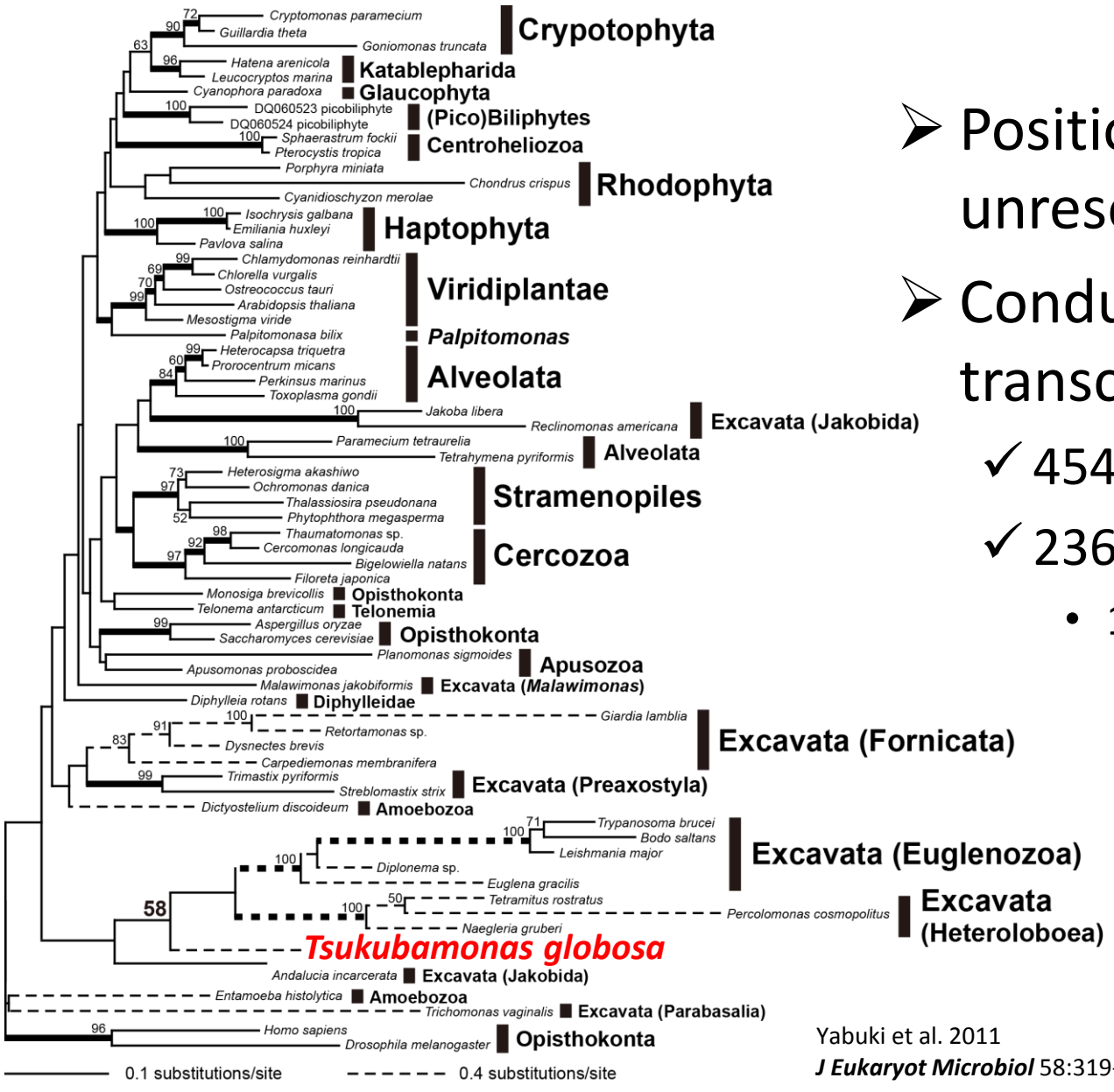


Yabuki et al. 2011
J Eukaryot Microbiol
58:319-331

➢ Is *Tg* a member of Excavata?



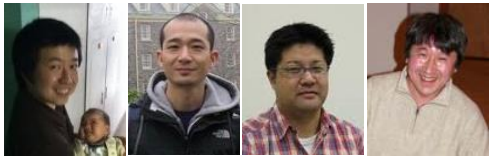
Tsukubamonas: SSU rRNA phylogeny



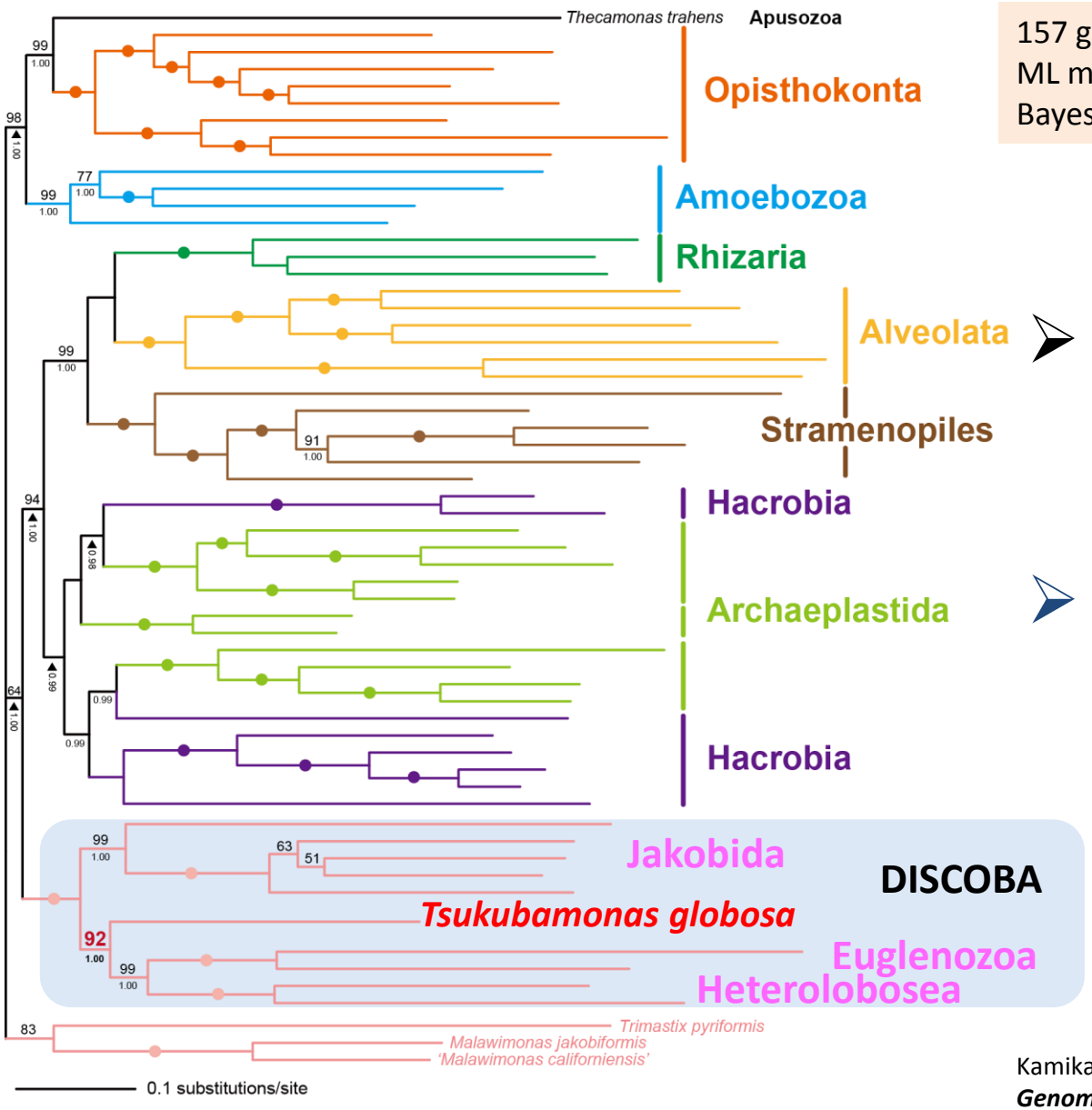
- Position of *Tg* was unresolved
- Conducted a transcriptmic analysis
  - ✓ 454 Titanium sequencing
  - ✓ 236,871 reads
    - 12,694 large contigs

ML & Bayesian methods
GTR + Γ model
1,347 nuc positions

Yabuki et al. 2011
J Eukaryot Microbiol 58:319-331



Tsukubamonas: phylogenomic analyses



157 genes, 41,372 aa positions
ML method, LG + Γ + F model
Bayesian method, CAT + Γ model

- *Tg* is basal to euglenozoans + heteroloboseans
- *Tg* is a novel member of Discoba

Kamikawa et al. 2014
Genome Biol Evol 58:319-331

Members of Discoba have large mt genomes

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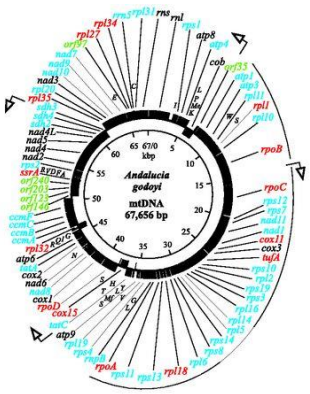
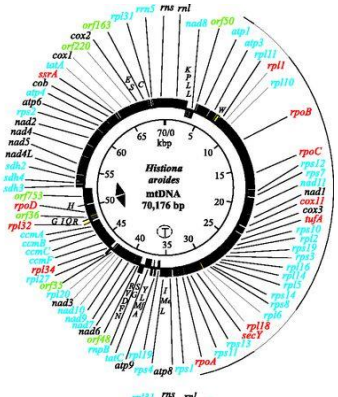
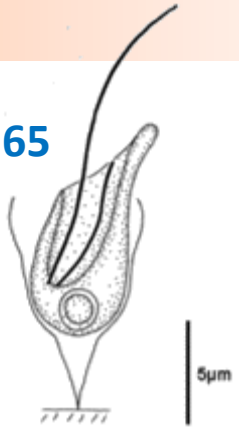
66

Andalucia godoyi
67 Kbp





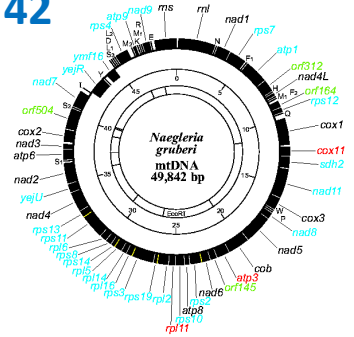
65



Histiona aroides
70 Kbp

Naegleria gruberi
50 Kbp

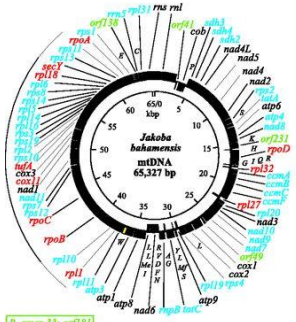
42

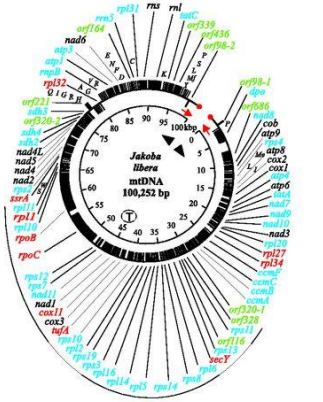


http://megasun.bch.umontreal.ca/ogmp

64

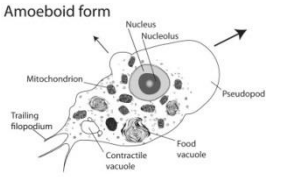
Jakoba bahamensis
65 Kbp





60

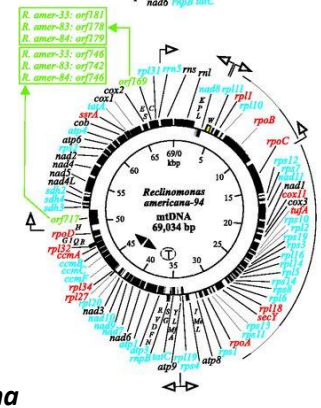
Jakoba libra
100 Kbp

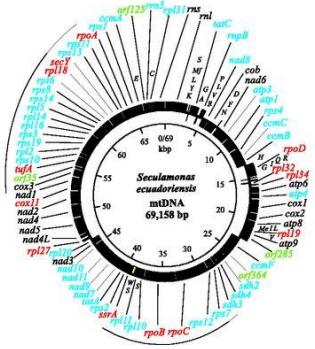


Fritz-Laylin et al. 2010 ***Res Microbiol*** 162:162:607-618

65

Reclinomonas americana
69 Kbp





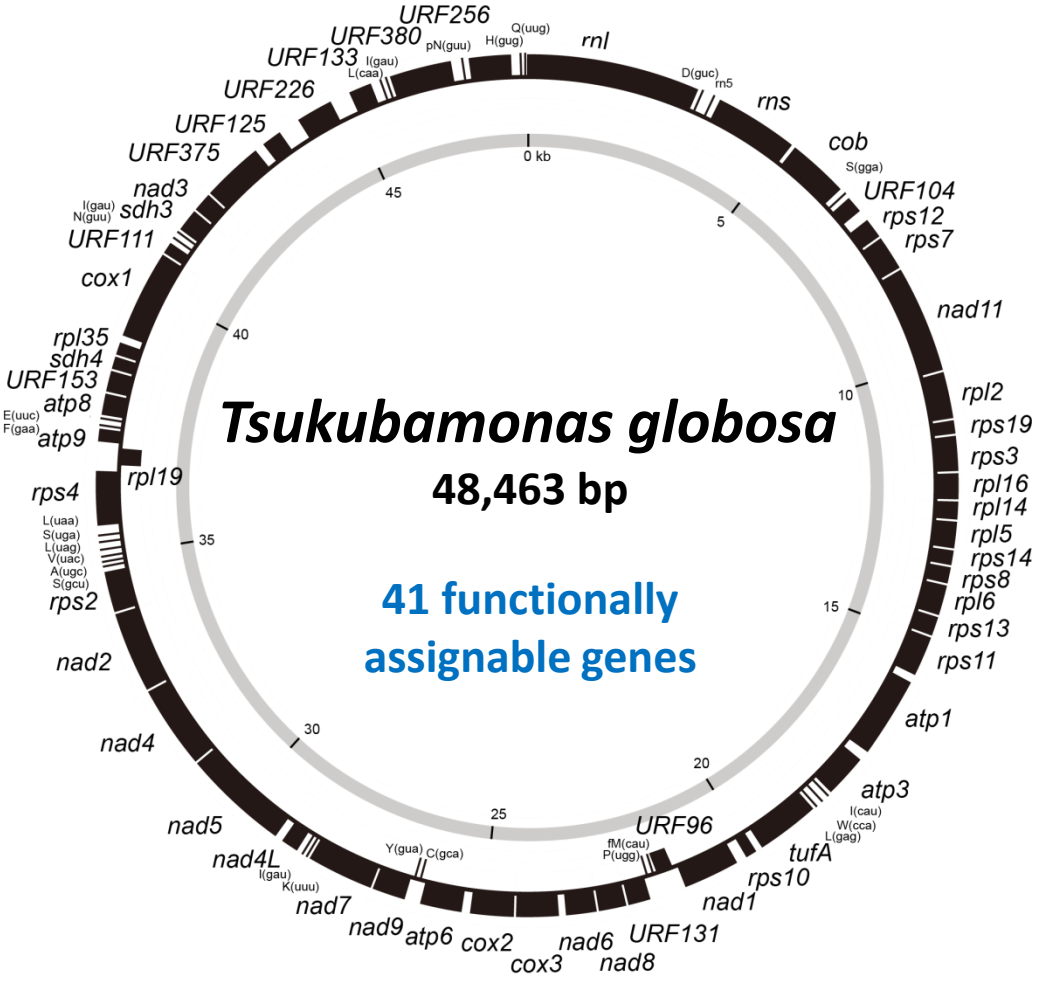
65

Seculamonas ecuadoriensis
69 Kbp

Burger et al. 2013 ***Genome Biol Evol*** 5:418-438

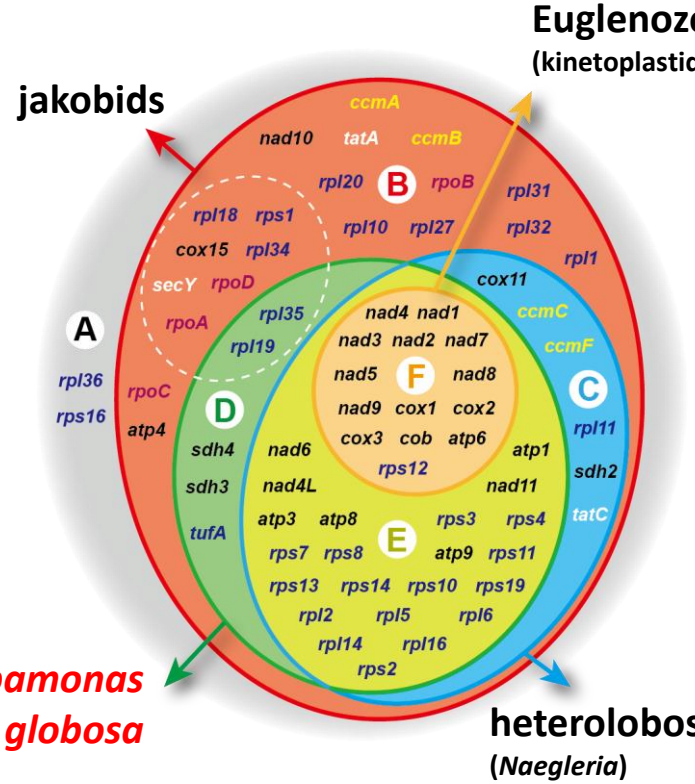
*All line-drawings were taken from ToL website (tolweb.org)

Tsukubamonas: mt genome



Kamikawa et al. 2014
Genome Biol Evol 58:319-331

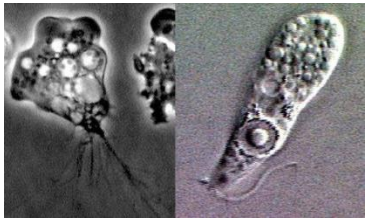
- Amplified the mt genome by rolling circle amplification
- 454 sequenced the amplified DNA



Tsukubamonas: Summary

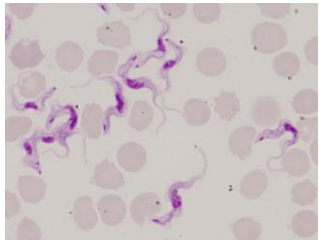
- *Tg* is a new member of Discoba
- *Tg* is important to infer the gene content evolution in discobid mt genomes
- *Tg* can be important to infer the evolution of life-style
  - ✓ Heteroloboseans are free-living
  - ✓ Euglenozoans do 'everything'
    - Kinetoplastids are *parasites*
    - Euglenids are *phototrophs*
    - Diplonemids are *free-living*

Naegleria



Images were taken from Wikipedia

Trypanosoma



ToL website (tolweb.org)

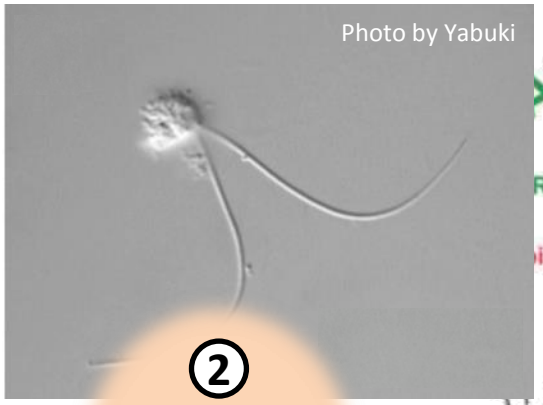
Euglena

http://www.fcps.edu/islandcreekes/ecology/euglena.htm

Diplonema

Image taken from Micro*scope

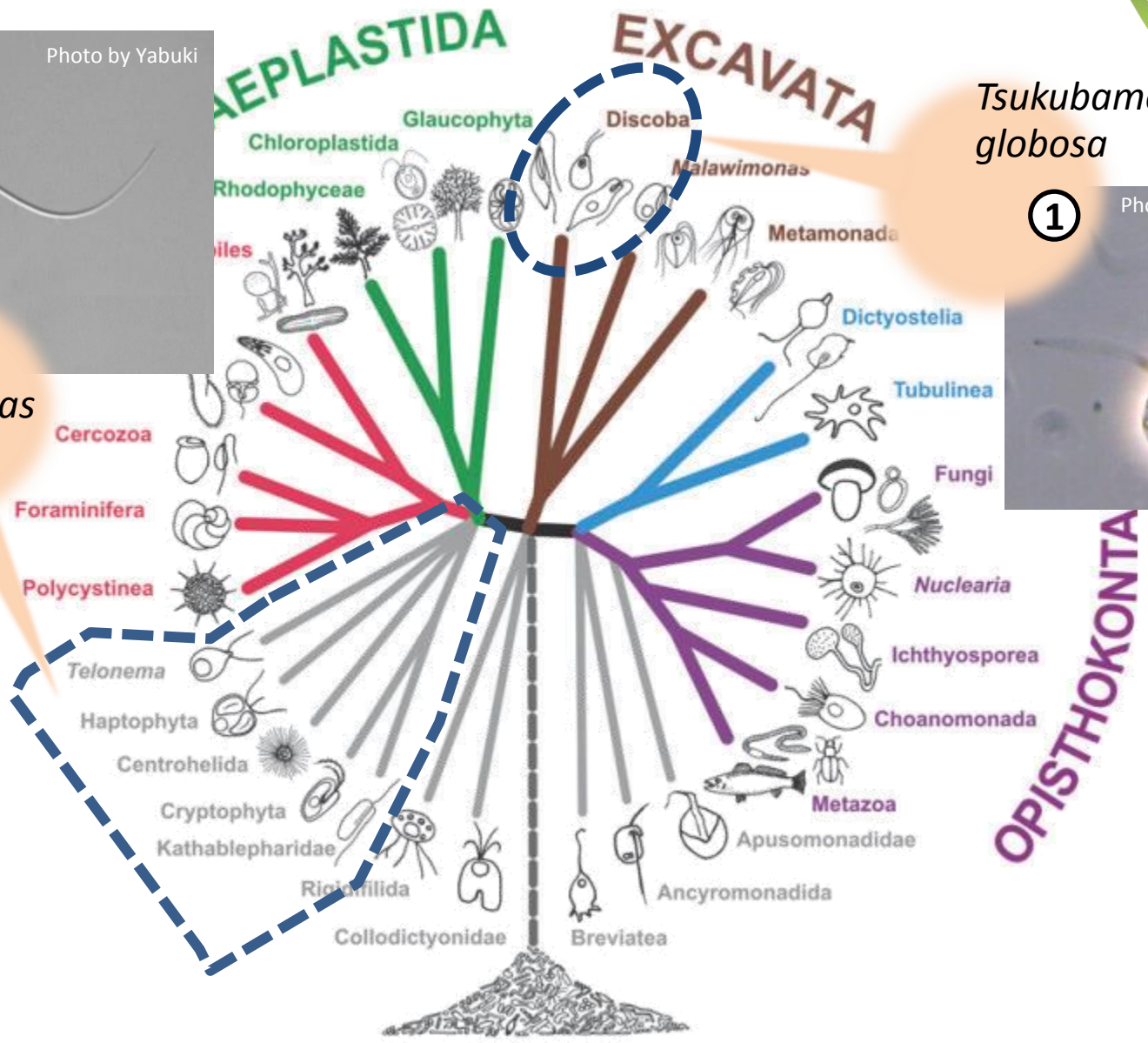
Palpitomonas bilix



② *Palpitomonas bilix*

Tsukubamonas globosa

① Photo by N. Yubuki

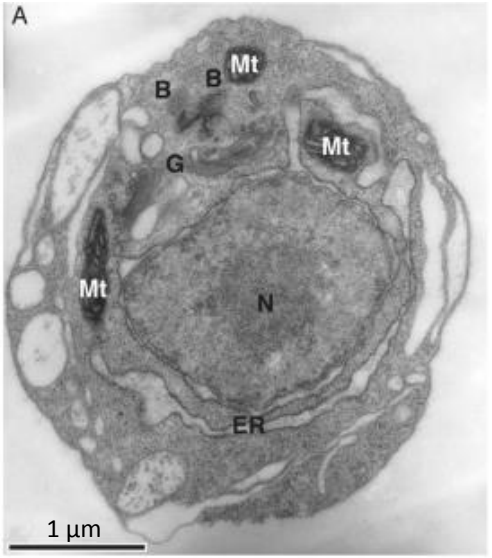


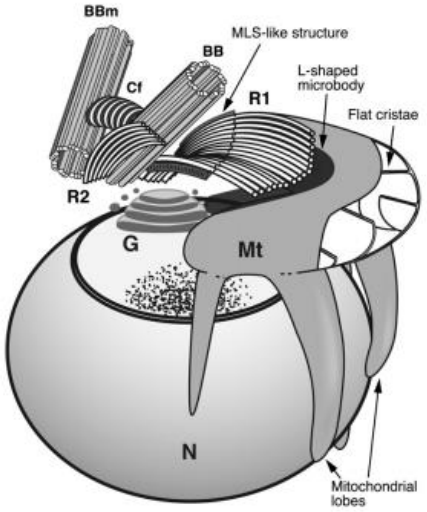
Adl et al. 2012 ***J Eukaryot Microbiol*** 59:429-493

Palpitomonas bilix: Yabuki, Inagaki, Ishida 2010 Protist 161:523-38

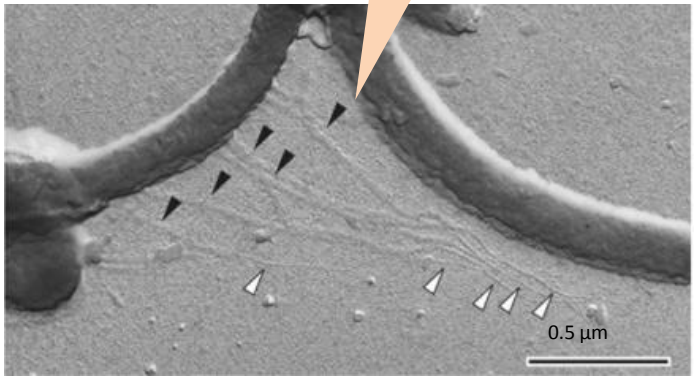
- Isolated from seawater sampled on Macharchar island, on July 2006
- Maintained in EMS medium at 20°C

Yabuki et al. 2010
Protist 161:523-538

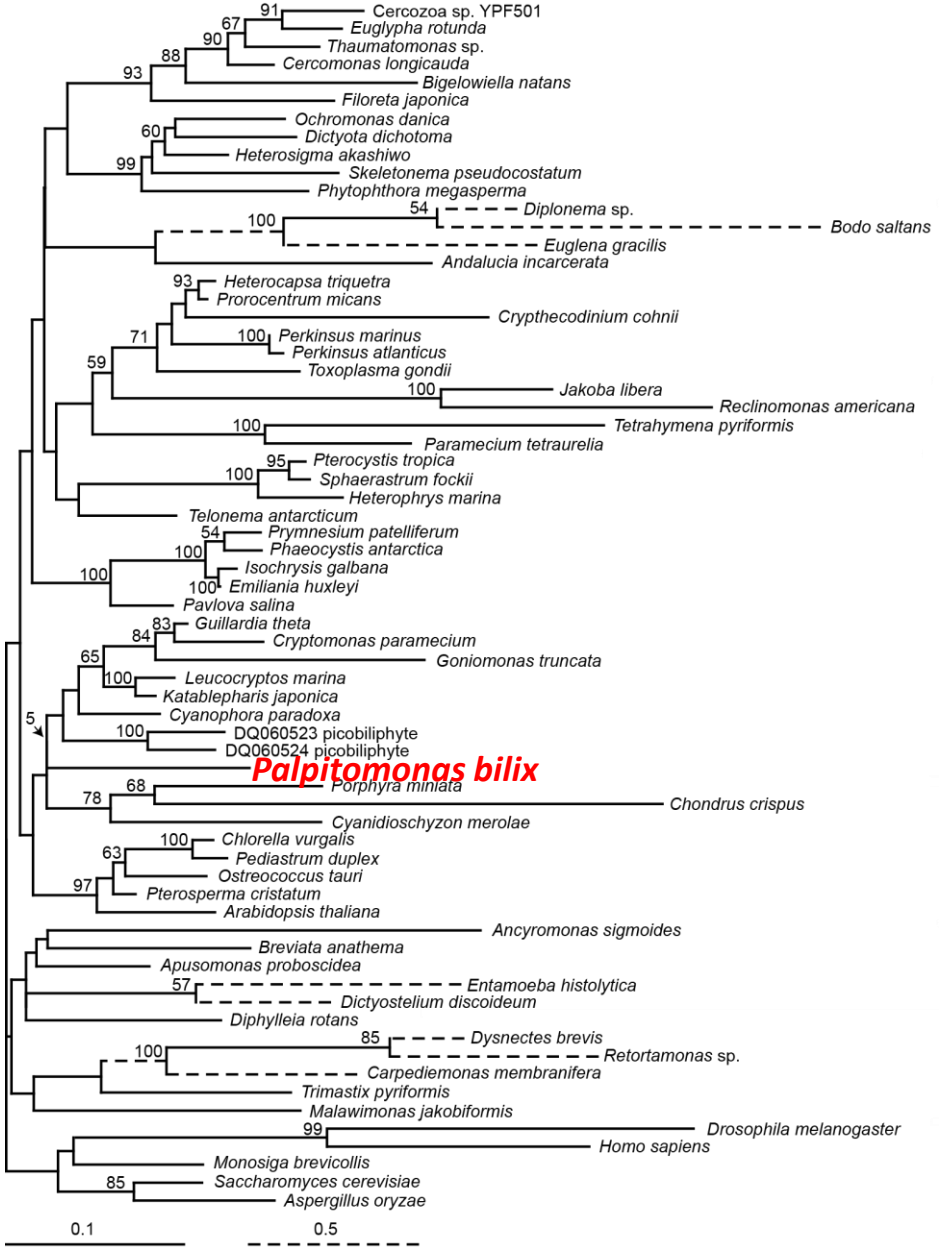




Is *Pb* a relative of cryptophytes?



Palpitomonas: SSU rRNA phylogeny

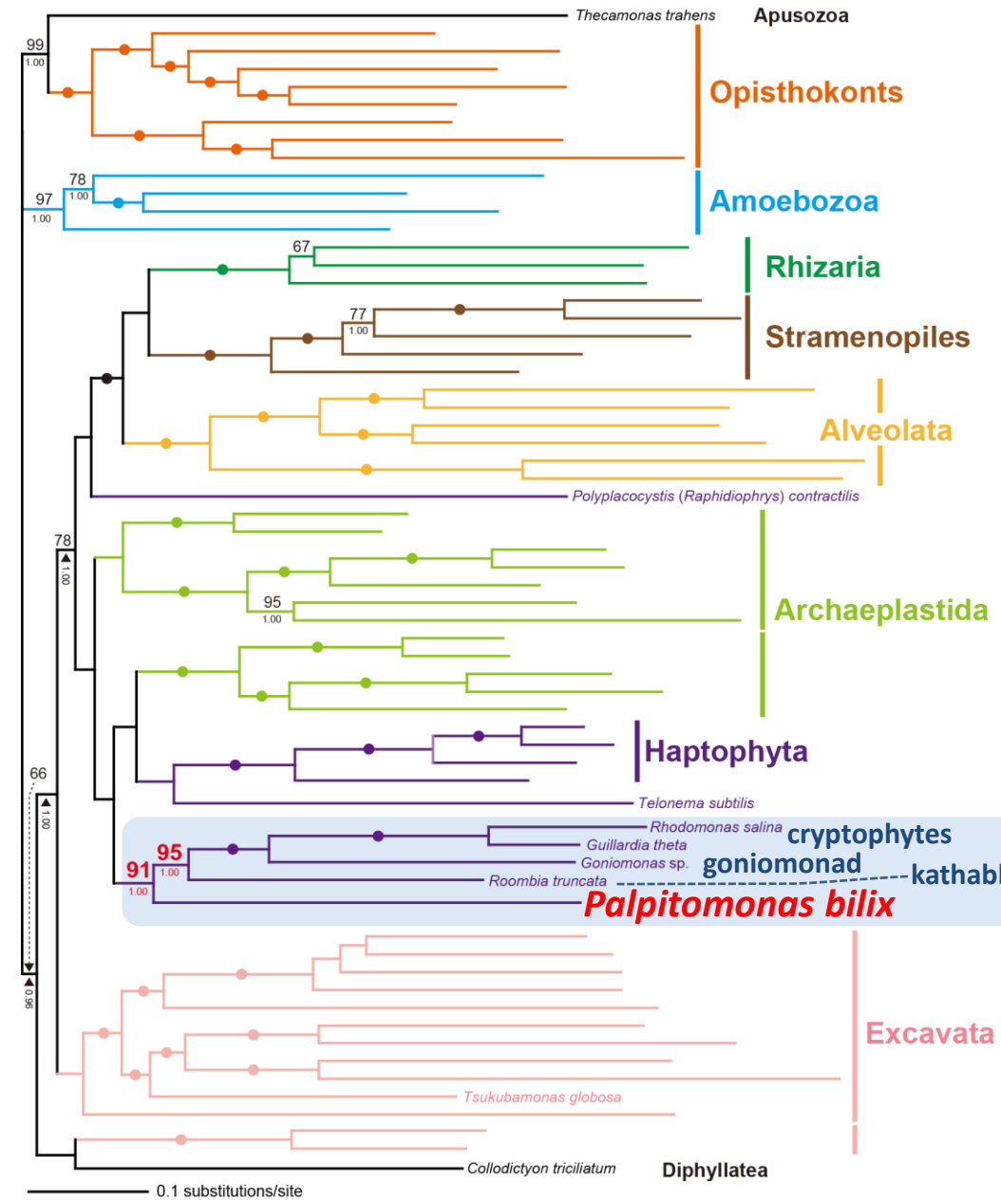


- Position of *Pb* was unresolved
- Conducted a transcriptmic analysis
  - ✓ 454 Titanium sequencing
  - ✓ 104,136 reads
    - 8,586 large contigs

ML & Bayesian methods
GTR + Γ model
1,335 nuc positions

Yabuki et al. 2010
Protist 161:523-538

Palpitomonas: Phylogenomic analysis



157 genes, 41,372 aa positions
ML method, LG4X model
Bayesian method, CAT + Γ model

- *Pb* is a basal to a clade of kathablepharids, goniomonads and cryptophytes
- *Pb* is a novel member of Cryptista

Yabuki & Kamikawa et al. 2014
Sci Rep in revision.

Palpitomonas: Summary

- *Pb* is a basal lineage of Cryptista
- Diversity of cryptophytes and their relatives has been underestimated
  - ✓ Will continue surveying potential cryptist members

Image taken from Micro*scope

Photo by Yabuki

Photo by Yabuki

Photo by Yoshida

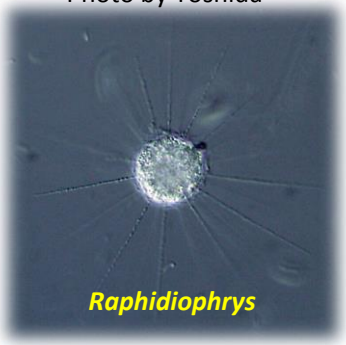


Photo by Nakayama

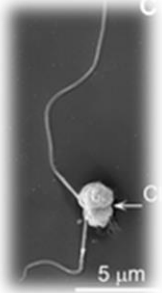
Image taken from Micro*scope

Photo by Shiratori

Rappemonads


Kim et al. (2011)

Picomonas judraskeda

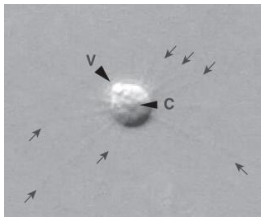


Seenivasan et al. (2013)

Future works

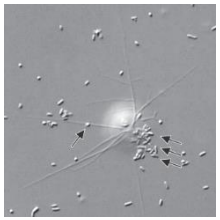
- Subject culture strains for novel eukaryotes to next-generation sequencing and phylogenomic analyses

Microheliella maris



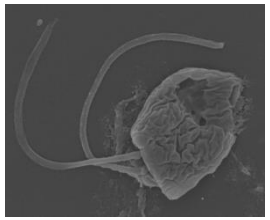
Yabuki et al. 2011
Protist 163:356-388

Rigifila ramosa



Yabuki et al. 2013
Protist 164:75-88

SRT149



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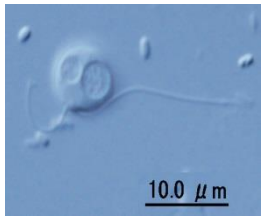
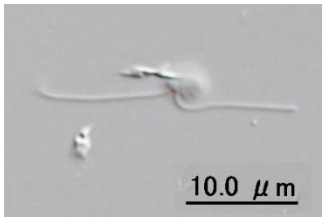


SRT312



- Continue surveying environments for more novel eukaryotes

A well-resolved global eukaryotic phylogeny

Acknowledgements

科研費 KAKENHI

JAPAN SOCIETY FOR THE PROMOTION OF SCIENCE · FOUNDED 1932 ·

JSPS grants

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- 2340513 & 2347038 (Hashimoto)

文部科学省

MATPËШKA

Grant-in-Aid for Scientific Research on Innovative Areas from the MEXT

Matryoshka-type evolution

(No. 3308)