

**Phylogenomic analyses unveiled
multiple endosymbioses of
pedinophycean green algae
in distantly related dinoflagellates**

Our research interests

Eukaryotic phylogeny

- Precise diversity of eukaryotes
 - Search for novel protists in the natural environments
- Phylogenomic analyses
 - Subject large-scale multigene alignments to phylogenetic analyses
 - Assess the relationship among the major groups (i.e. ancient splits) in the tree of eukaryotes

Origins & evolutions of bacterium-derived organelles

- Detailed process of organellogenesis
 - Mitochondria and plastids
 - Obligate bacterial endosymbionts
- Endosymbiosis-derived genome evolution
 - Endosymbiotic gene transfer
 - Reduction of endosymbiont genomes

An example of A phylogenetic tree

- Based on the difference in amino acid/DNA sequences
- External nodes represent extant organisms (sequences)
- Internal nodes are hypothetical ancestor (ancestral sequences)
- Branch length between nodes represents the number of substitutions

Sequence data for phylogeny

```
Human      mvlspadktnvkaawgkvgahageygaealermflsfpttktyfphf-dlshgsaqvkgh
dog        mvlspadktnikstwdkigghagdyggealdrftqsfpttktyfphf-dlspgsaqvka
chicken    mvlsaadknnvkgiftkiaghaeeygaetlermfettyptktyfphf-dlshgsa
carp       mslsdkaavkglwakispkaddigaealgrmltvypqtktyfahwadlspgsgpvk
* **  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
```

```
human      gkkvadaltnavahvddmpnalsalsdlhahklrvdpvnfkllshc11vtlaahlpaeft
dog        gkkvadalttavahliddlpalsalsdlhayklrvdpvnfkllshc11vtlachhpteft
chicken    gkkvvaalieaanhiddiagtlsklsdlhahklrvdpvnfkllgqcf11vvaihpaalt
carp       gkvimgavgdavskiddlvvglaalse1hafklrvdpanfkilahnvi1vigmlypgdfp
**      *  *  **  *  **  ***  *****  ***  *  *  *
```

```
human      pavhasldkflasvstvltskyr
dog        pavhasldkfftavstvltskyr
chicken    pevhasldkflcavgtvltakyr
carp       pevhm1svdkffqnlalalsekyr
* ** *  ***  *  ***
```

*identical residues among the four organisms

- Amino acid residues vary among the four sequences
 - Substitutions occurred during the evolution of vertebrates

Maximum-likelihood method for phylogeny

Xh : time reversible Markov process, independent evolution for each branch

Based on the formula of Chapmann-Kolmogorov,

$f(x_1, x_2, x_3, x_4 | \theta)$ ← probability for getting data of h 'th site
with a given $P_{ij}(t)$ and a given tree topology

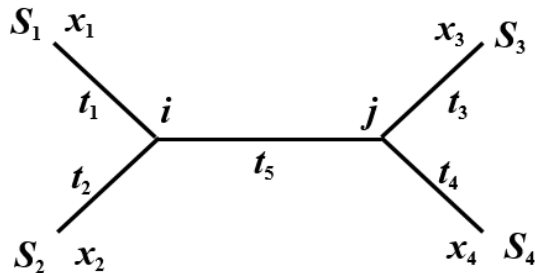
$$= \sum_i \sum_j P\{X_0 = i\} P\{X_{t_1} = x_1, X_{t_2} = x_2, X_{t_5} = j | X_0 = i\}$$

$$\times P\{X_{t_5+t_3} = x_3, X_{t_5+t_4} = x_4 | X_{t_1} = x_1, X_{t_2} = x_2, X_{t_5} = j, X_0 = i\}$$

$$= \sum_i \left\{ \pi_i P_{ix_1}(t_1) P_{ix_2}(t_2) \sum_j P_{ij}(t_5) P_{jx_3}(t_3) P_{jx_4}(t_4) \right\}$$

Transition rate matrix Q
 \Rightarrow Transition probability matrix
 $P_{ij}(t) = e^{tQ}$

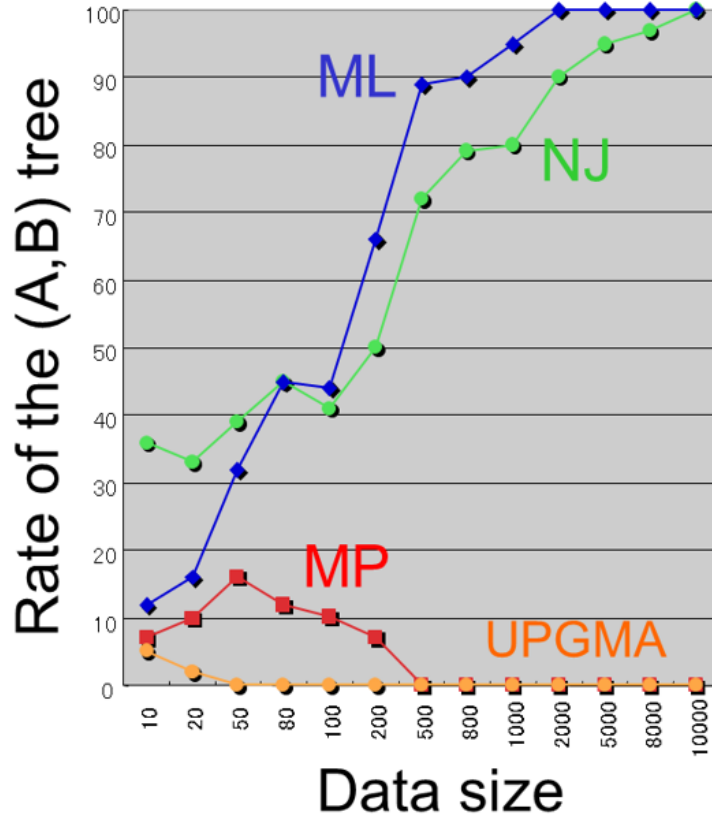
π_i : composition of the base (amino acid) i



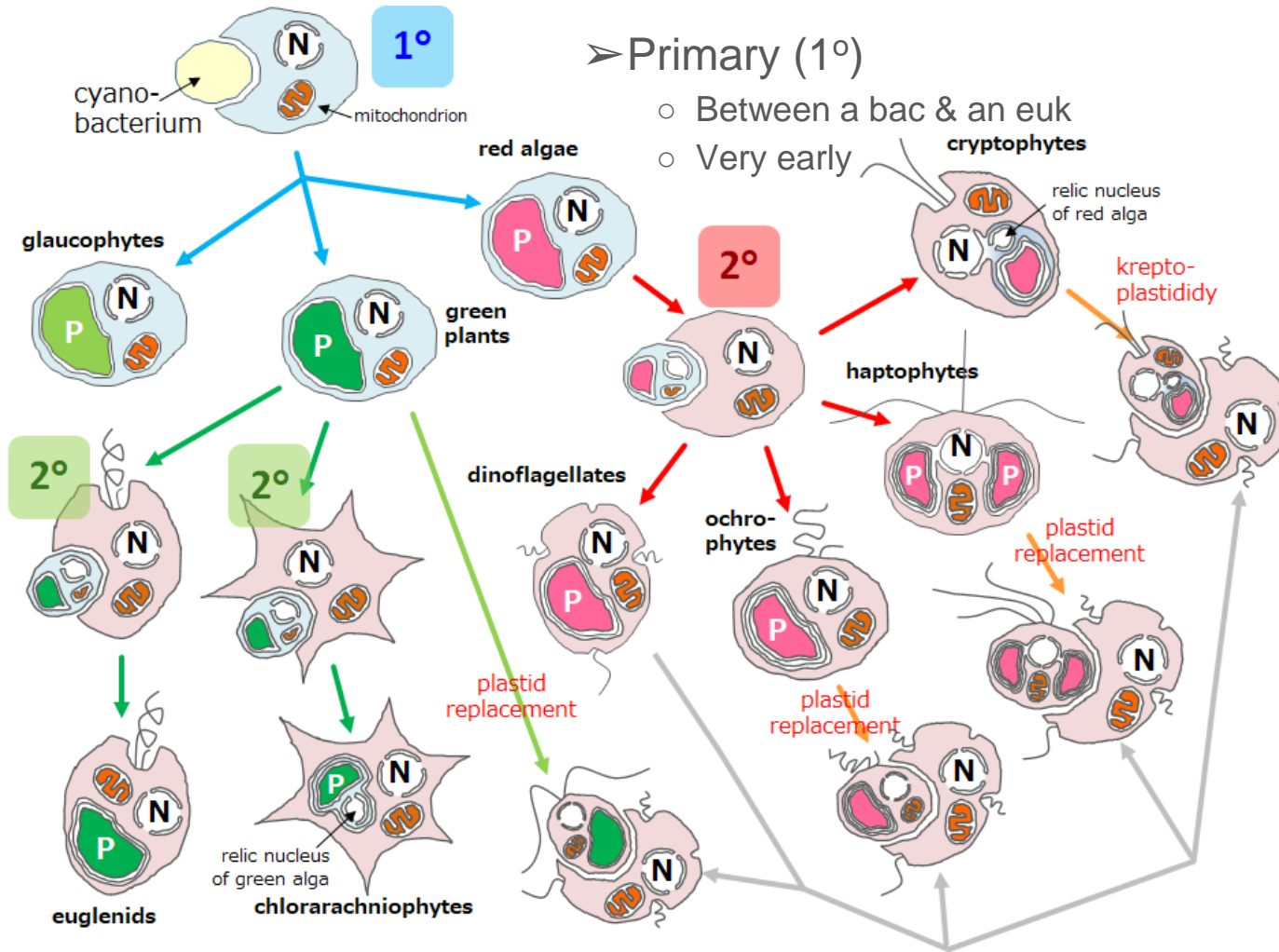
On internal nodes (ancestral species),
all possibilities for the base (amino acid)
 i or j are summed over
In case of base, i or $j = A, C, G, T$

S_1, \dots, S_4 : extant species
 x_1, \dots, x_4 : sequence data of h 'th site
for extant species
 i, j : sequence data for ancestral species
 t_1, \dots, t_5 : branch lengths

Importance of data size in phylogeny



- Larger the data, higher the accuracy
 - *Only* in the ideal conditions
- A large amount of sequence data is available
 - Genome data
 - Transcriptome data
- Technology is rapidly growing
 - We can generate sequence data quickly & reasonable price



➤ Primary (1°)

- Between a bac & an euk
- Very early

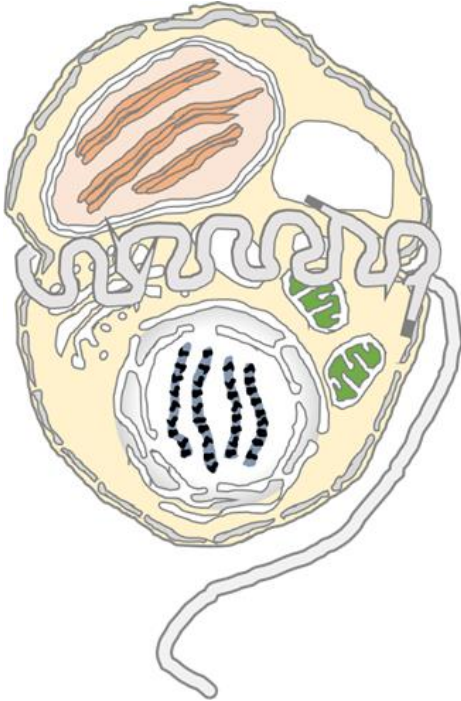
➤ Secondary (2°)

- Between two euks
- Fairly early but later than primary
- Multiple times

➤ Plastid replacements

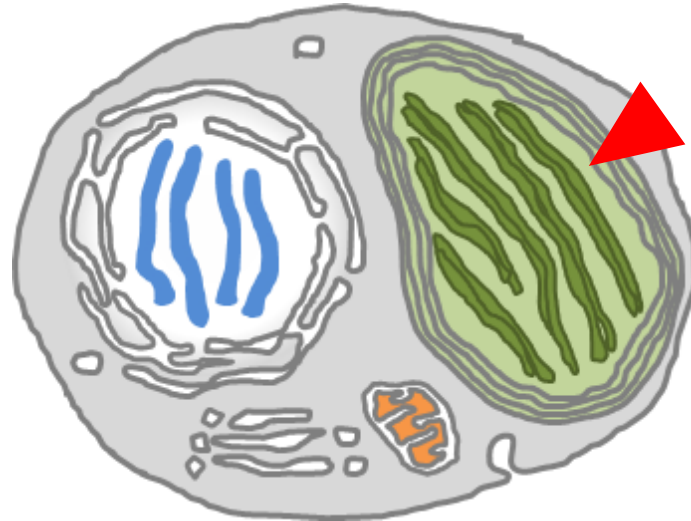
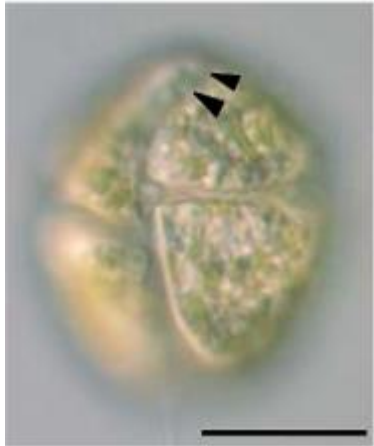
- Within dinoflagellates
- Quite recent
- Multiple time

Dinoflagellates are uni-cellular algae



- Marine & fresh water
 - One of the major primary producers
- Photosynthetic, heterotrophic or parasitic
- Plastids containing a unique carotenoid, *peridinin*
- Red alga-derived plastids
 - Several lineages have non-canonical plastids containing no peridinin

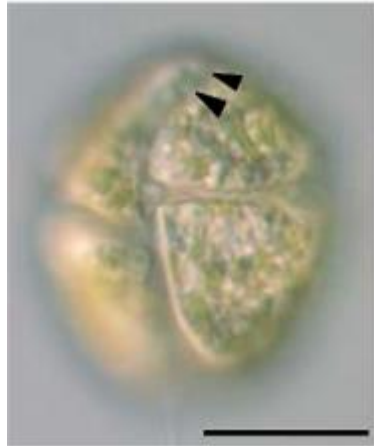
Lepidodinium chlorophorum has a non-canonical, green-colored plastid



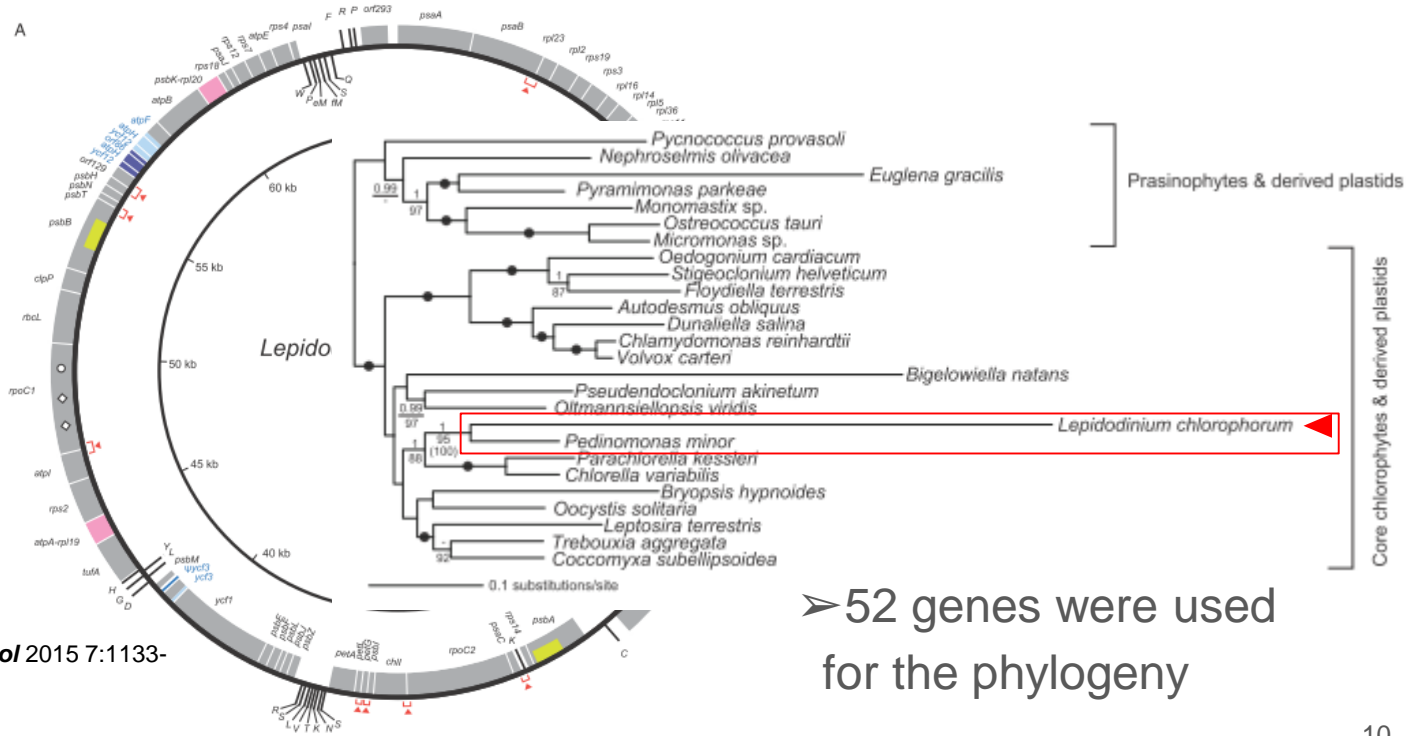
- Plastids possess their own genome
- Phylogeny based on the plastid gene(s) indicates the origin of the plastid

Takishita et al. *Gene* 2008 410:26-36.

Lepidodinium chlorophorum has a pedinophyte-derived plastid



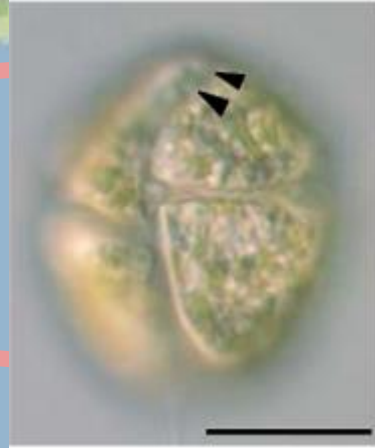
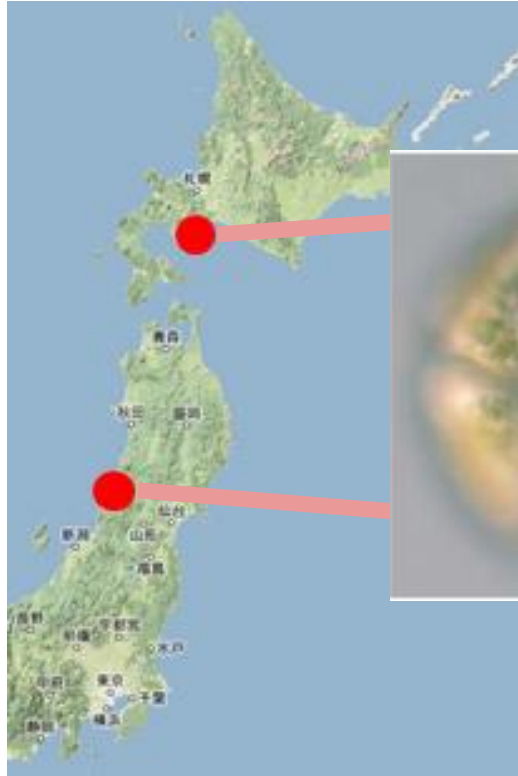
Takishita et al. *Gene* 2008 410:26-36.



➤ 52 genes were used for the phylogeny

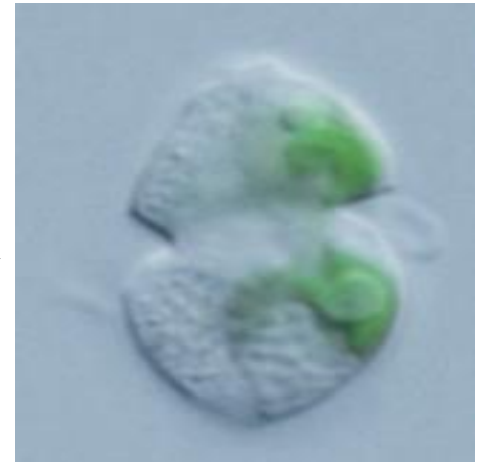
Kamikawa et al. *Genome Biol Evol* 2015 7:1133-1140.

Two green-colored dinoflagellates were isolated



◀ **MRD-151**

Green algal plastid
containing Chl *a+b*

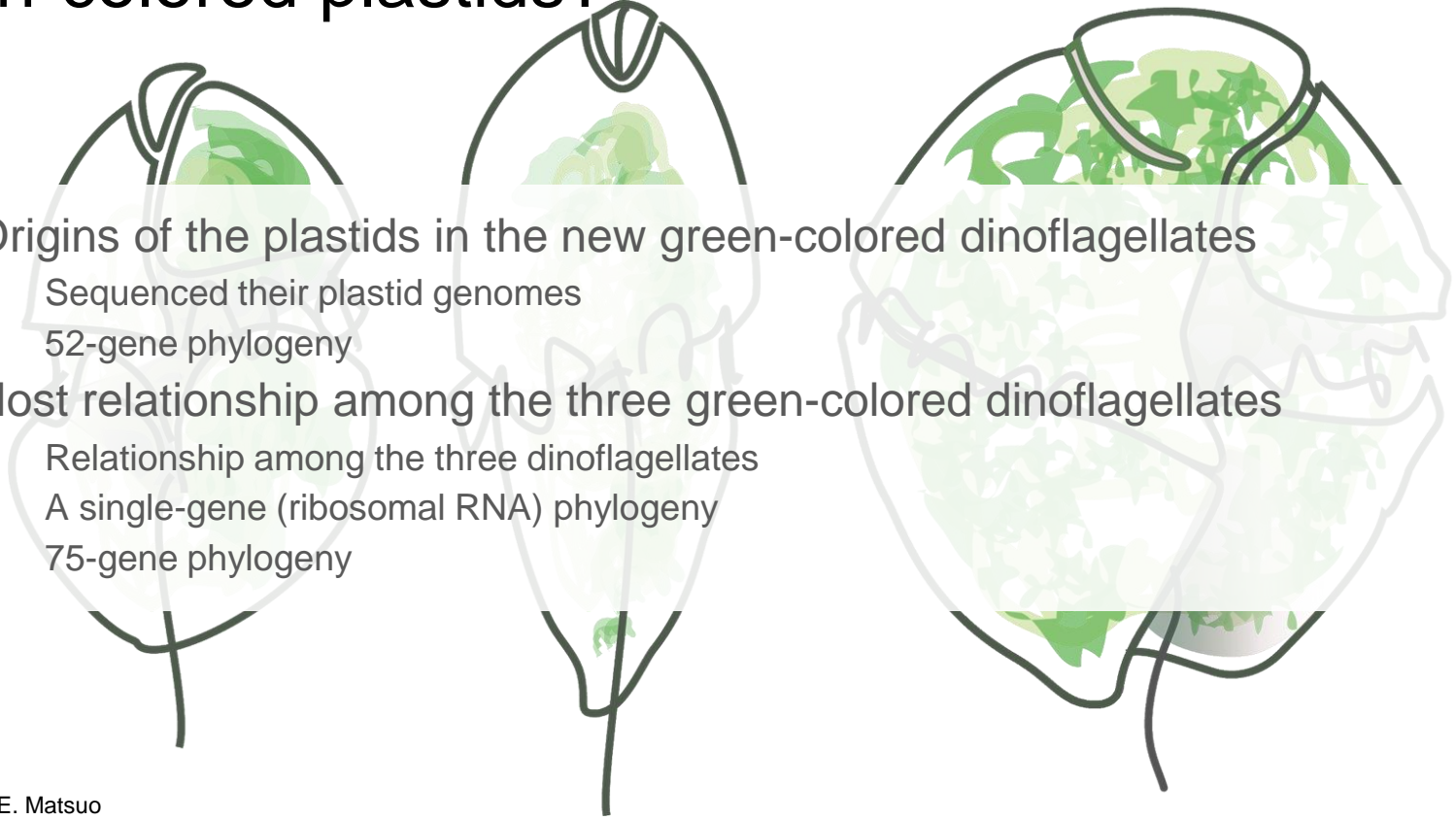


TRD-132 ▶

Green algal plastid
containing Chl *a+b*

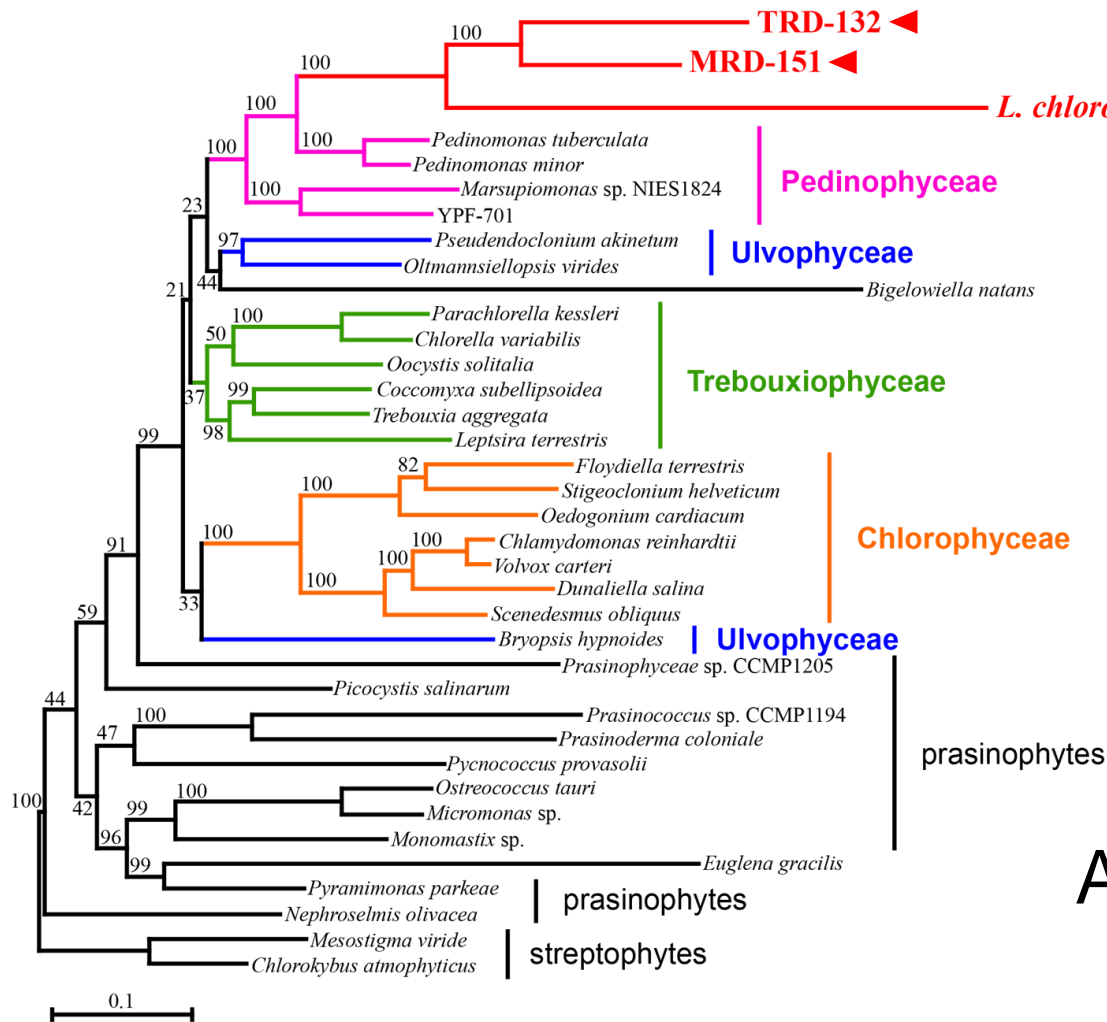
How did the three dinoflagellates establish the green-colored plastids?

- Origins of the plastids in the new green-colored dinoflagellates
 - Sequenced their plastid genomes
 - 52-gene phylogeny
- Host relationship among the three green-colored dinoflagellates
 - Relationship among the three dinoflagellates
 - A single-gene (ribosomal RNA) phylogeny
 - 75-gene phylogeny



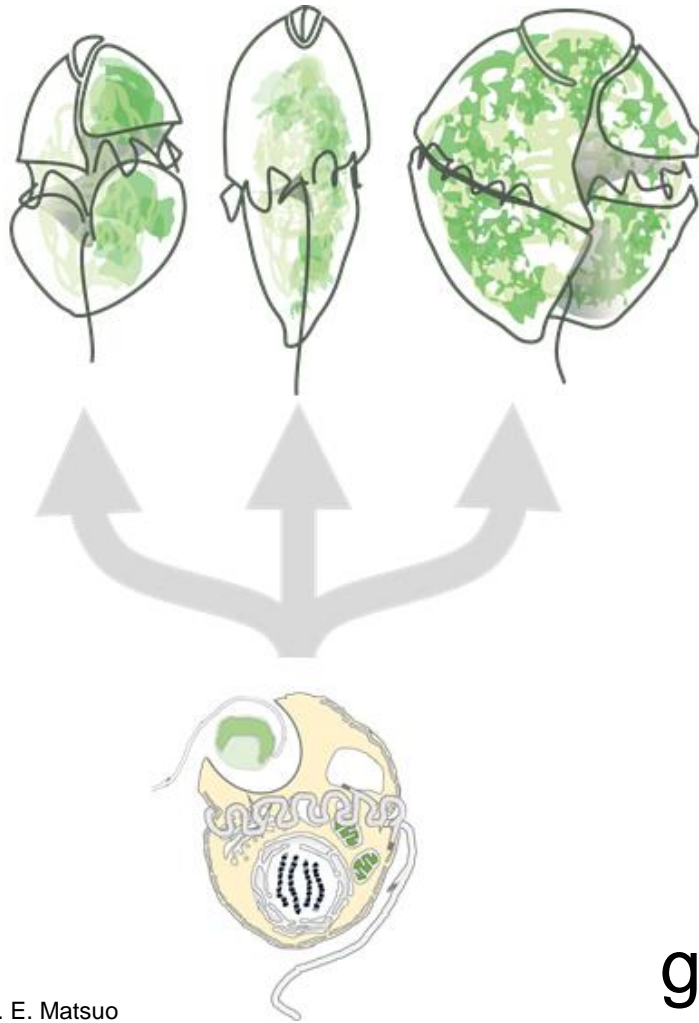
Plastid genomes of MRD & TRD

- Analyzed together with another green-colored dinoflagellate *Lepidodinium chlorophorum*



- Three plastids grouped together
- Showed a specific affinity to a small group of green algae, Pedinophyceae

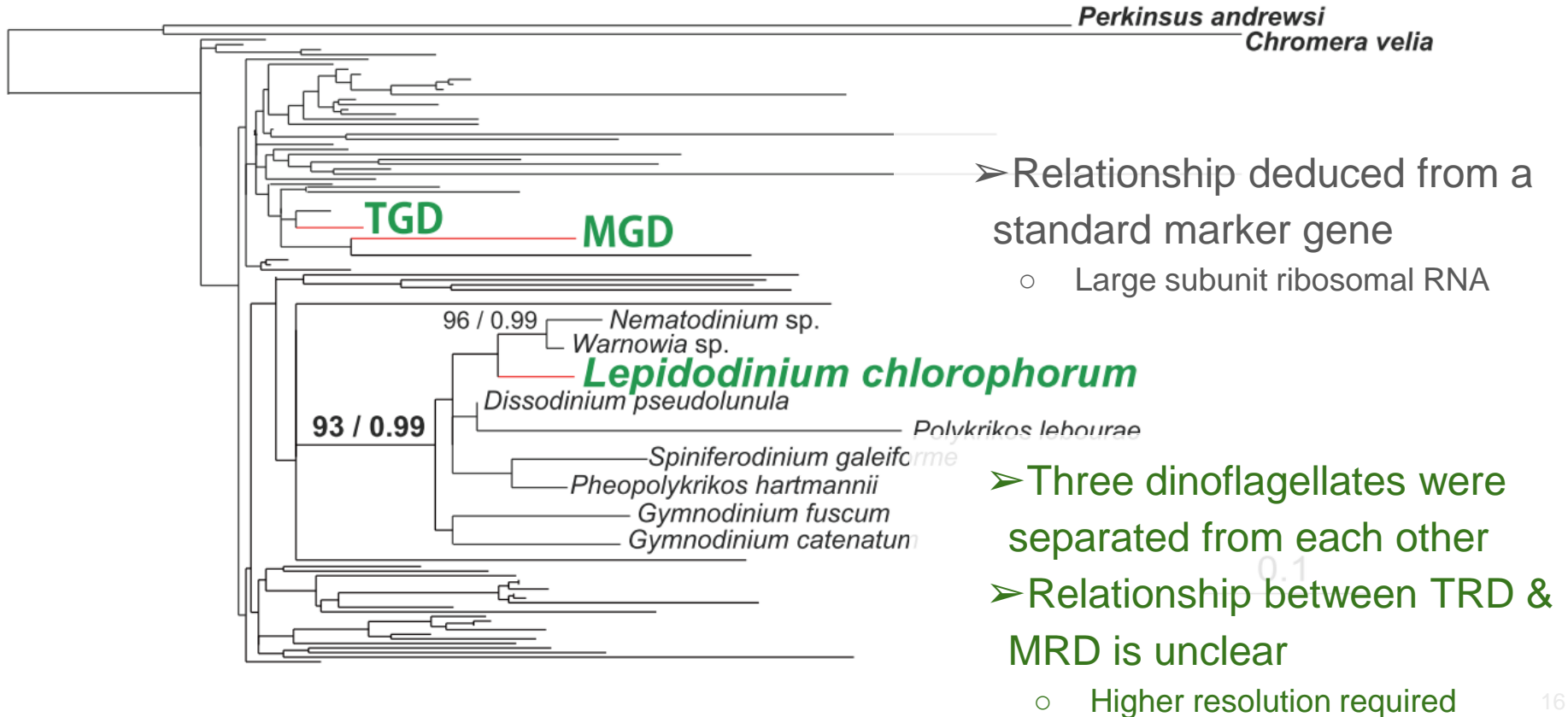
A single origin of green-colored plastids



- Three plastids grouped together
- Showed a specific affinity to a small group of green algae, Pedinophyceae
- Three dinoflagellates were evolved from a single dinoflagellate with a pedinophyte-derived plastid

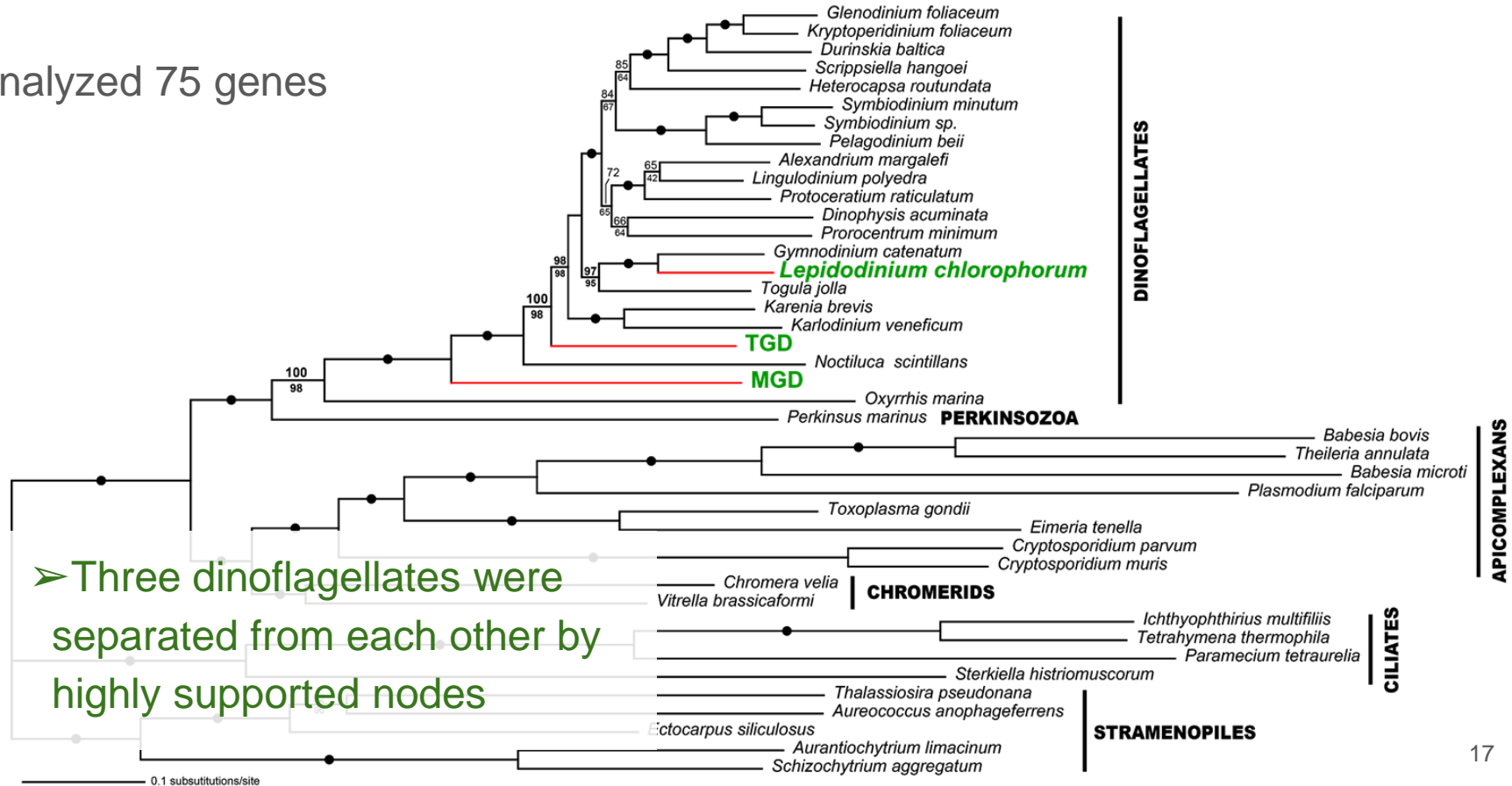
A single origin of green-colored dinoflagellates?

Host relationship among of *Lc*, MRD & TRD

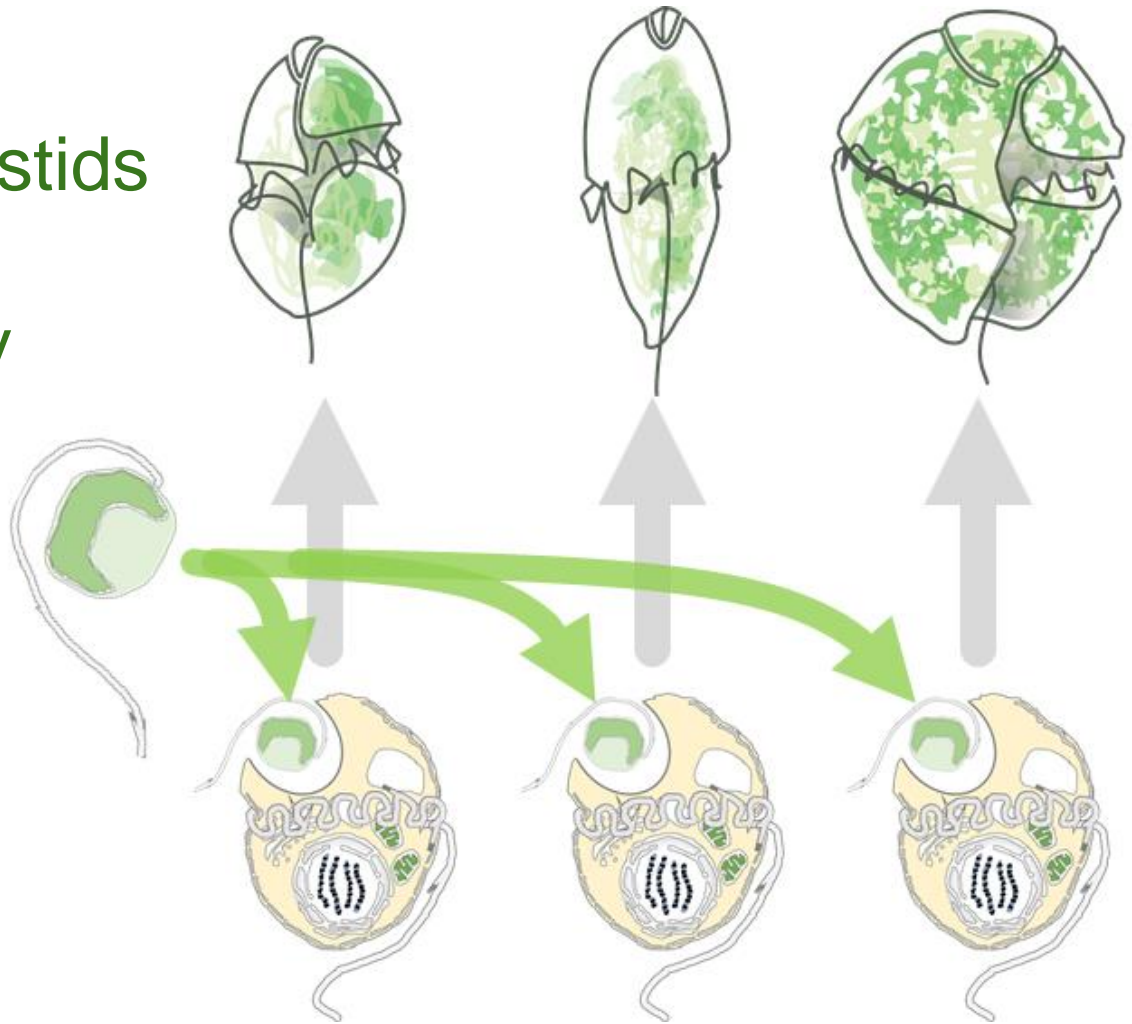


Host relationship among of *Lc*, MRD & TRD

➤ Analyzed 75 genes



Lc, MRD & TRD
established their plastids
separately from the
same alga or closely
related green algae



Conclusion & perspective

- *Lepidodinium*, MRD & TRD possess pedinophyte-derived plastids
- The three pedinophyte-derived plastids were established independently
- Additional cases of independently established, pedinophyte-derived plastids probably exist among dinoflagellates

Acknowledgements



University of Tsukuba

Center for Computational Sciences

筑波大学 計算科学研究センター



Yamagata
University



University
of Tokyo



Kyoto University