

Which gene region is useful for classification of land snails?

~The building of the phylogenetic tree; centered on three land snails around Kobe~

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<Background>

- Conventional criteria for the classification of land snails by phenotype isn't concrete.
- 66th year student of Kobe High School made the phylogenetic tree based on the mitochondrial COI gene. And in this phylogenetic tree, two species belong to the same clade, even though there are big difference in phenotype.



COI gene is not proper to study relations between species in land snails?

<Objects>

- Making of the phylogenetic tree of the land snails of both genetic region, the mitochondrial COI gene and the mitochondrial 16S rRNA gene, and suggesting the method to analyze the genealogy of species correctly
- Revealing the genetic relationship of each land snails

<Method>

- We extracted DNA from land snail's soft body, and amplified COI and the mitochondrial 16S genes by PCR.

- After mitochondrial DNA sequence, we built a phylogenetic tree by neighbor joining method and maximum parsimony method based on the data we got. About COI, we used 39 analyzable samples and 87 samples from the study of 66th student of kobe high school, and about 16S, 32 samples analyzable and base sequence of Euhadra amaliae quoted from Genbank.

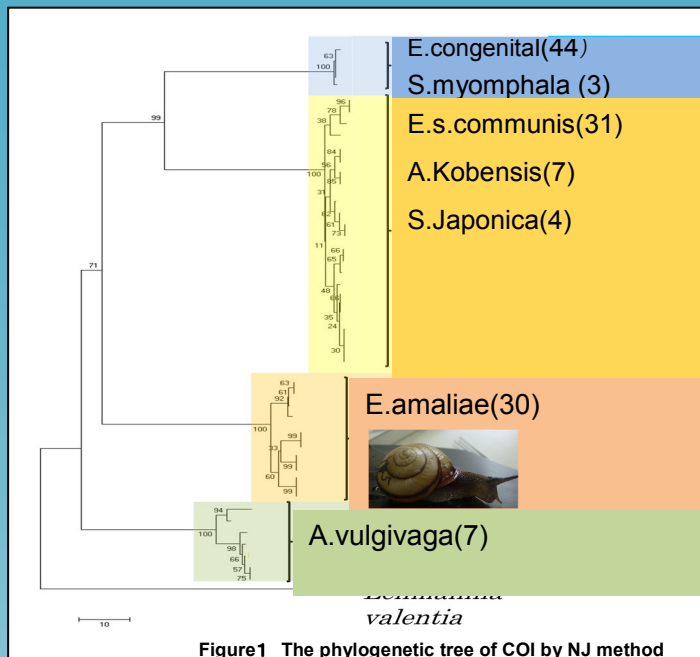


Figure1 The phylogenetic tree of COI by NJ method

Figure The number of replacement in COI -16s gene in the species

| | Congenital | communis | amaliae | vulgivaga | Kobensis | japonica |
|------------------|------------|----------|---------|-----------|----------|----------|
| COI (bases) | 2 | 22 | 25 | 46 | 11 | 6 |
| COI (amino acid) | 1 | 1 | 3 | 3 | 3 | 0 |
| 16S (bases) | 6 | 29 | — | 13 | 50 | 74 |

Figure The number of replacement between species in amino acid encoded by COI(Top right corner),16s gene.(The lower left).

| | Congenital | communis | amaliae | vulgivaga | Kobensis | japonica |
|------------|------------|----------|---------|-----------|----------|----------|
| Congenital | | 8 | 11 | 21 | 10 | 7 |
| communis | 112 | | 9 | 20 | 4 | 1 |
| amaliae | 144 | 153 | | 21 | 11 | 8 |
| vulgivaga | 194 | 199 | 186 | | 21 | 19 |
| Kobensis | 239 | 241 | 240 | 209 | | 3 |
| japonica | 266 | 264 | 273 | 239 | 272 | |

< Consideration about the credibility of the phylogenetic tree >

COI...This genetic region has good keeping and encords amino acids.

→We could not get correct data in comparison of species with different family and genus. It was because it substitutes very limited region.

But in the case of analyzing the genetic relationship of species they are closely related, this region is suitable to get correct data.

16S...It substitutes easily.

→It has recorded many genetic variations. So the phylogenetic tree of 16S did not show that some species belong to the same clade when there are big differences in phenotype among them.

It is using both regions that is effective way to analyze the genetic relationship of these land snails above the level of genus.

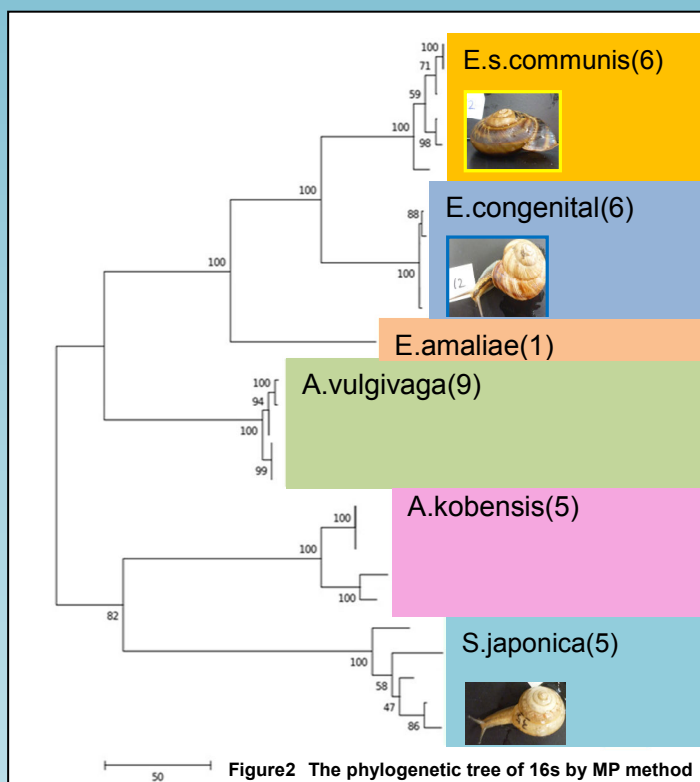


Figure2 The phylogenetic tree of 16s by MP method

<Discussion about three species around Kobe>

《Family Bradybaenidae Genus Aegista》

• Vulgivaga

They related to Euhadra more closely than Kobensis from heredity view point.

• Kobensis

They divided earliest in the Bradybaenidae we studied (This idea is standed by their outside forms such as their thick shells, small bodies.)

《Family Bradybaenidae Genus Euhadra》

• Congenital

The number of replacement in Base sequence in them is smallest in 6 species.

They divided recently.



Vulgivaga



Kobensis

<Books for references>

- Hirotaka Nishi and Teiji Sota et al. 2005“Phylogeographic Study of the Land Snail *Euhadra* in Chugoku District Based on Analysis of Mitochondrial DNA Sequences”