

YSLME Gene pool workshop (May 14-15, 2007)

# Species identification and genetic diversity in Yellow Sea marine animals by DNA barcode and oligonucleotide chip analysis

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# Barcode of life

- **Barcoding** is a standardized approach to identifying animals and plants by minimal sequences of DNA
- For barcoding, standardization should help construction of a comprehensive reference library of DNA sequences and development of economical technologies for species identification.
- It will help many people quickly and cheaply recognize known species and retrieve information about them, and will speed discovery of the millions of species yet to be named.
- It will provide vital new tools for appreciating and managing Earth's immense and changing biodiversity.

## Applications of Fish Barcode

- Identification of fish, fillets, fins, and fragments (*marketing, substitutions, quota and bycatch management*).
- Identification of processed product e.g. canned fish, dried fish, mixtures (*substitutions*).
- Identification of threatened, endangered and protected species (*conservation*).
- Identification of fish eggs and fish larvae (*ecosystem research, direct and indirect fisheries management*).
- Identification of prey items in stomach contents (*food webs and ecosystem research*).
- Identification of historical, archived and museum material (*taxonomy*).
- Identification of new species and possible fusions, insights into phylogenetic relationships (*fish biology, evolution*).
- Possible production of DNA microarrays from the sequence data

# Skates and Rays

## Introduction

**Skates** are cartilaginous fishes belonging to the **family Rajidae**. They are carnivorous, feeding mostly on smaller fish and crustaceans. They have **flat pectoral fins**, two dorsal fins and spineless tail.

They are **benthic** (bottom-dwelling) and are found throughout the world.

They are oviparous fishes, laying **eggs in a horny case**. It is thought that egg-laying in skates is an evolutionary reversal, that is, **skates are descended from ovoviviparous ancestors**.



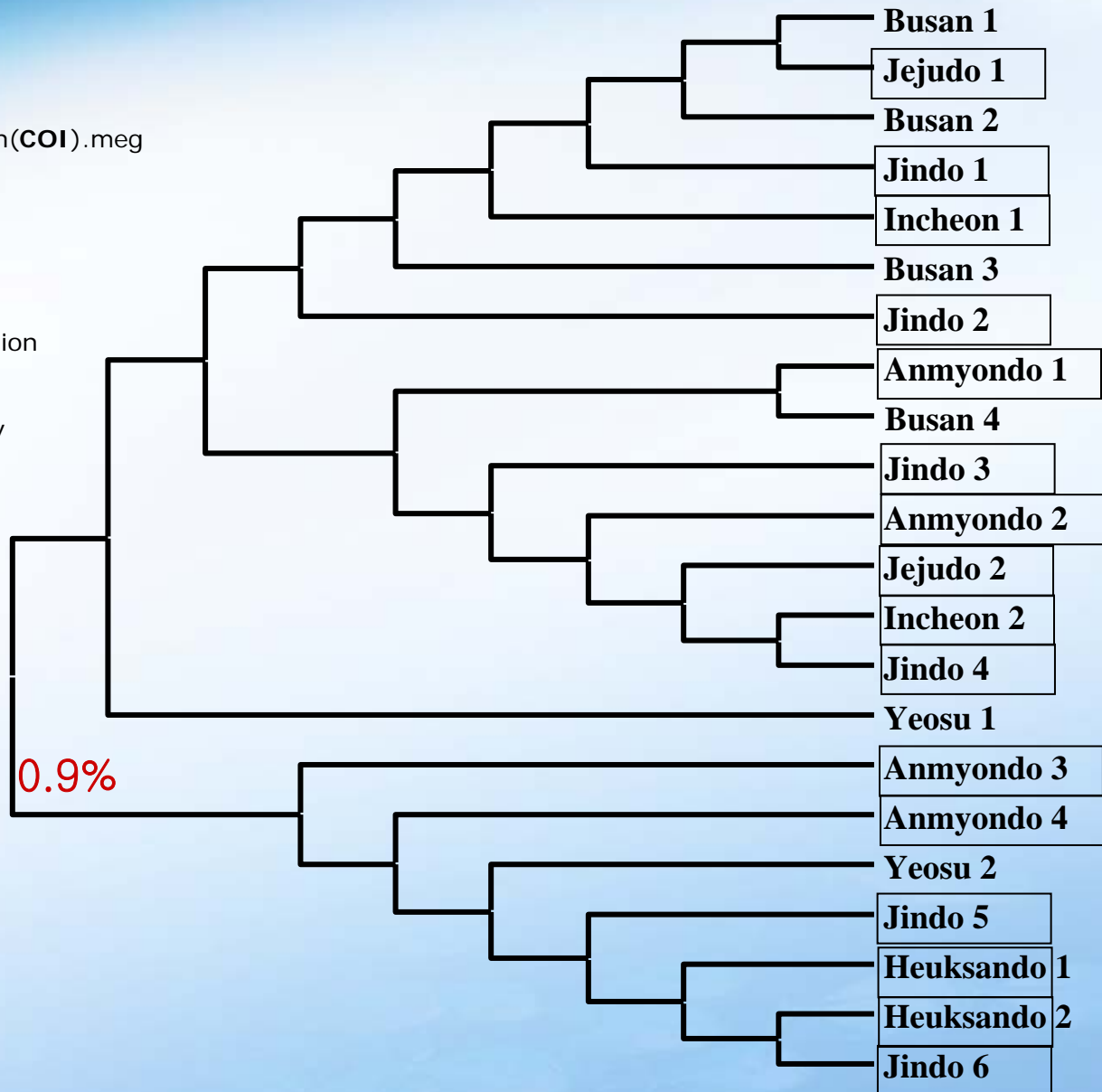
# In Korea

- Imported skate and rays amount 12,000ton/year (= 20 million \$) from China, Japan, USA, Canada, Brazil, Argentina, Chile, Uruguay, Russia, Indonesia, Vietnam, New Zealand, Spain, Italia, Portugal----
- Customs problems: differential duties between skates and rays.



# Okamejei kenojei, COI Phylogenetic Tree

No. of Taxa : **22**  
 Data File : 가오리-흉어\rayCOI\F0ken(COI).meg  
 Data Type : Nucleotide  
 Analysis : Phylogeny reconstruction  
 Tree Inference :  
 Method : **Neighbor-Joining**  
 Phylogeny Test and options : None  
 Include Sites :  
 Gaps/Missing Data : Complete Deletion  
 Substitution Model :  
 Model : Nucleotide: Tamura-Nei  
 Substitutions to Include : d: Ts + Tv  
 Pattern among Lineages : Same  
 Rates among sites : Uniform rates  
 No. of Sites : **655**



# The Variable sites and haplotype of COI gene fragment of skate *Okamejei kenojei* from the Yellow Sea and East Sea/Japan Sea.

## Variable site

	Variable site															
	2	4	7	7	8	2	2	2	2	2	3	3	4	4	6	
	7	2	2	3	4	3	6	7	8	9	5	8	3	7	4	
	7	2	2	3	4	7	1	0	2	7	4	7	6	0	8	
• FOken050308-1	A	A	A	C	G	A	G	C	T	A	G	G	C	A	G	
• FOken050308-2	.	G	.	.	A	.	.	.	.	.	.	.	.	.	.	
• FOken050308-3	.	.	.	T	.	.	A	.	.	.	.	.	A	.	.	
• FOken050308-4	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	
• FOken050317-1	G	.	G	.	.	.	.	.	.	.	.	.	.	.	.	
• FOken050317-2	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	
• FOken050317-3	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	
• FOken050317-4	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	
• FOken050326-1	.	G	.	.	A	.	.	.	.	.	.	.	.	.	.	
• FOken050326-2	.	G	.	.	A	.	.	.	.	.	.	.	.	.	.	
• FOken050402-1	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	
• FOken050402-2	.	.	.	.	.	C	.	.	.	.	A	.	.	.	.	
• FOken050404-1	.	G	.	.	A	.	.	.	.	C	.	.	.	.	.	
• FOken050404-2	.	.	.	.	.	.	.	.	.	.	A	.	.	G	.	
• FOken050513-1	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	
• FOken050513-2	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	
• FOken050513-3	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	
• FOken050513-4	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	
• FOken050513-5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
• FOken050513-6	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	
• FOken050513-1	.	.	.	.	.	.	A	A	.	.	.	A	.	.	.	
• FOken050513-2	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	

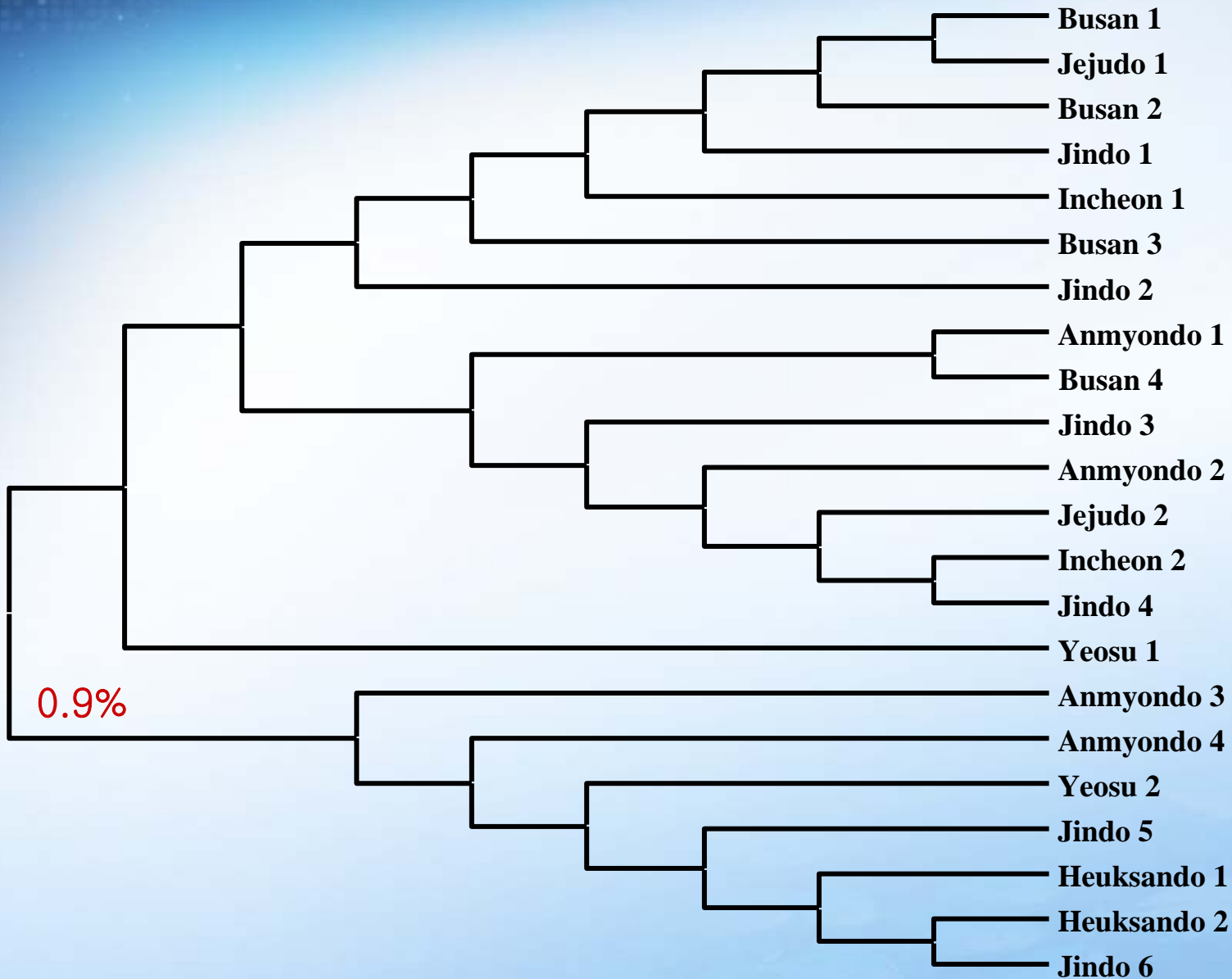
# Okamejei kenojei, COI Pairwise Distance

	[ 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21]
[ 1]																					
[ 2]	0.003																				
[ 3]	0.005	0.008																			
[ 4]	0.002	0.005	0.006																		
[ 5]	0.003	0.006	0.008	0.005																	
[ 6]	0.002	0.002	0.006	0.003	0.005																
[ 7]	0.002	0.005	0.006	0.000	0.005	0.003															
[ 8]	0.003	0.003	0.008	0.005	0.006	0.002	0.005														
[ 9]	0.003	0.000	0.008	0.005	0.006	0.002	0.005	0.003													
[10]	0.003	0.000	0.008	0.005	0.006	0.002	0.005	0.003	0.000												
[11]	0.002	0.005	0.003	0.003	0.005	0.003	0.003	0.005	0.005	0.005											
[12]	0.003	0.006	0.008	0.002	0.006	0.005	0.002	0.006	0.006	0.006	0.005										
[13]	0.005	0.002	0.009	0.006	0.008	0.003	0.006	0.005	0.002	0.002	0.006	0.008									
[14]	0.003	0.006	0.008	0.002	0.006	0.005	0.002	0.006	0.006	0.006	0.005	0.003	0.008								
[15]	0.002	0.005	0.003	0.003	0.005	0.003	0.003	0.005	0.005	0.005	0.000	0.005	0.006	0.005							
[16]	0.002	0.005	0.003	0.003	0.005	0.003	0.003	0.005	0.005	0.005	0.000	0.005	0.006	0.005	0.000						
[17]	0.002	0.005	0.003	0.003	0.005	0.003	0.003	0.005	0.005	0.005	0.000	0.005	0.006	0.005	0.000	0.000					
[18]	0.002	0.005	0.006	0.003	0.005	0.003	0.003	0.005	0.005	0.005	0.003	0.005	0.006	0.005	0.003	0.003	0.003				
[19]	0.000	0.003	0.005	0.002	0.003	0.002	0.002	0.003	0.003	0.003	0.002	0.003	0.005	0.003	0.002	0.002	0.002	0.002			
[20]	0.003	0.003	0.008	0.005	0.006	0.002	0.005	0.003	0.003	0.003	0.005	0.006	0.005	0.006	0.005	0.005	0.005	0.005	0.005	0.003	
[21]	0.005	0.008	0.006	0.006	0.008	0.006	0.006	0.008	0.008	0.008	0.003	0.008	0.009	0.008	0.003	0.003	0.003	0.006	0.005	0.008	
[22]	0.002	0.005	0.006	0.000	0.005	0.003	0.000	0.005	0.005	0.005	0.003	0.002	0.006	0.002	0.003	0.003	0.003	0.003	0.002	0.005	0.006

Max. variation: 0.009



Okamejei kenojei, COI Phylogenetic Tree



## Okamejei kenojei, Control region Phylogenetic Tree

No. of Taxa : 13

Data File : D가오리-홍어 ray CTR FOken(ctr).meg

Data Type : Nucleotide

Analysis : Phylogeny reconstruction

Tree Inference : =====

Method : **Neighbor-Joining**

Phylogeny Test and options : None

Include Sites : =====

Gaps/Missing Data : Complete Deletion

Substitution Model : =====

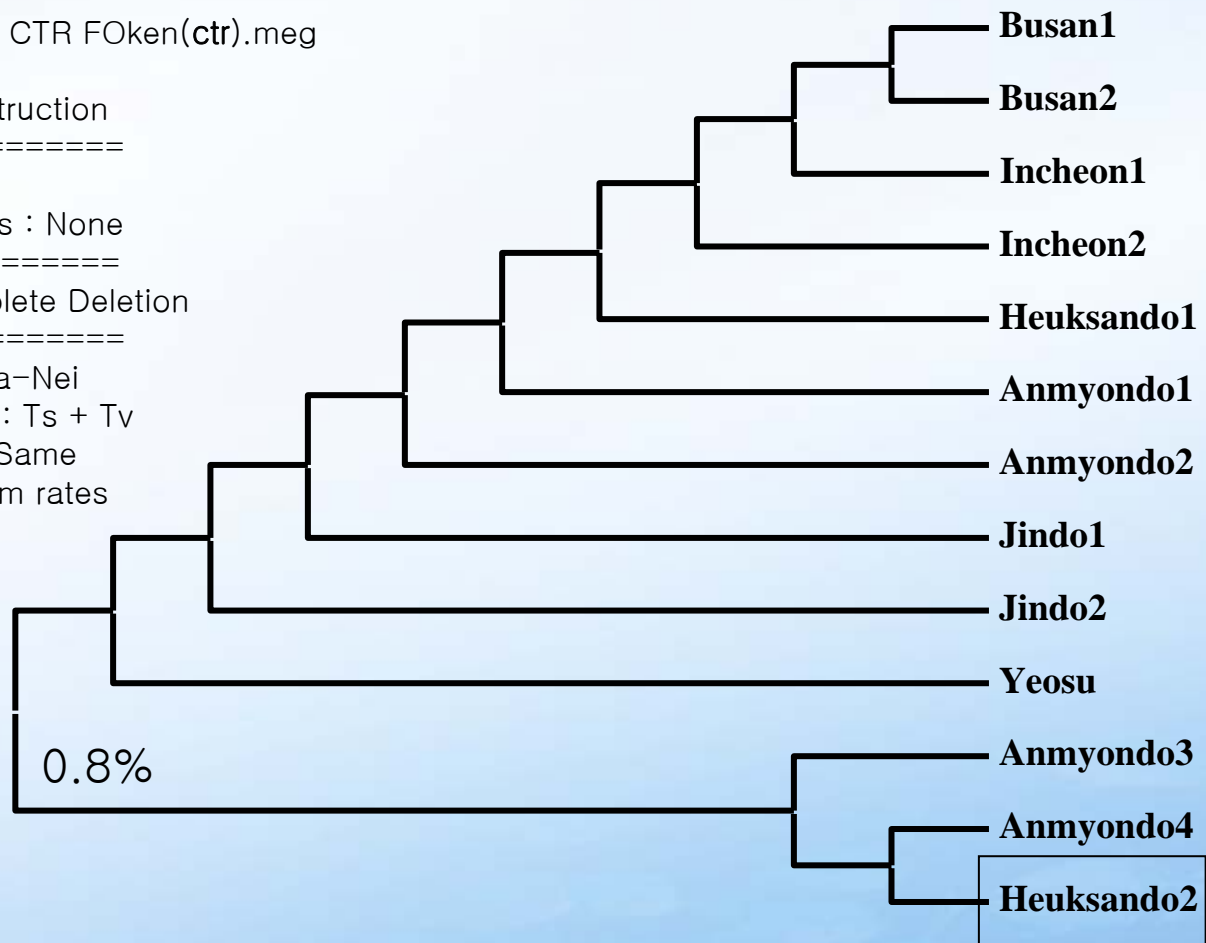
Model : Nucleotide: Tamura-Nei

Substitutions to Include : d: Ts + Tv

Pattern among Lineages : Same

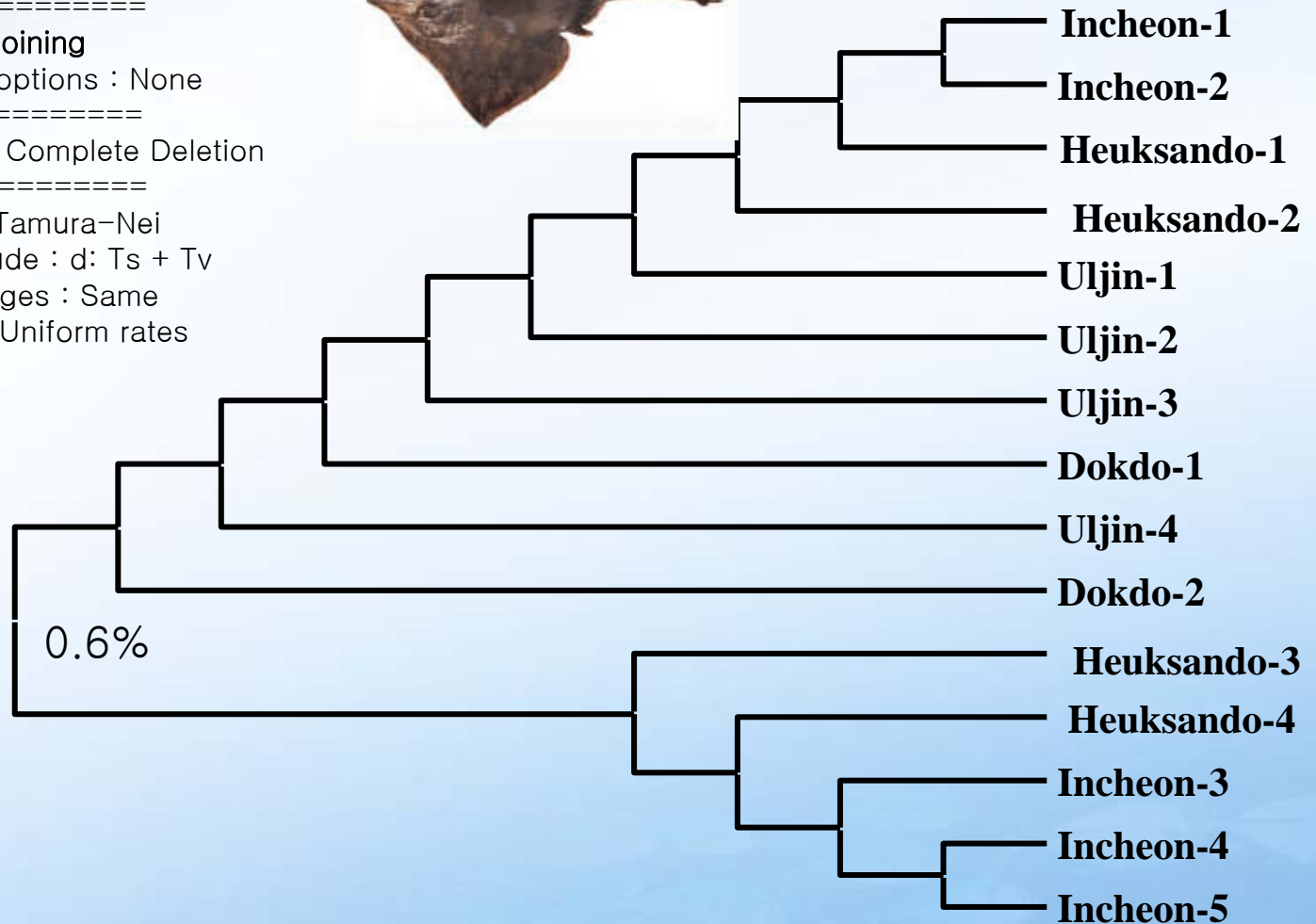
Rates among sites : Uniform rates

No. of Sites : 607

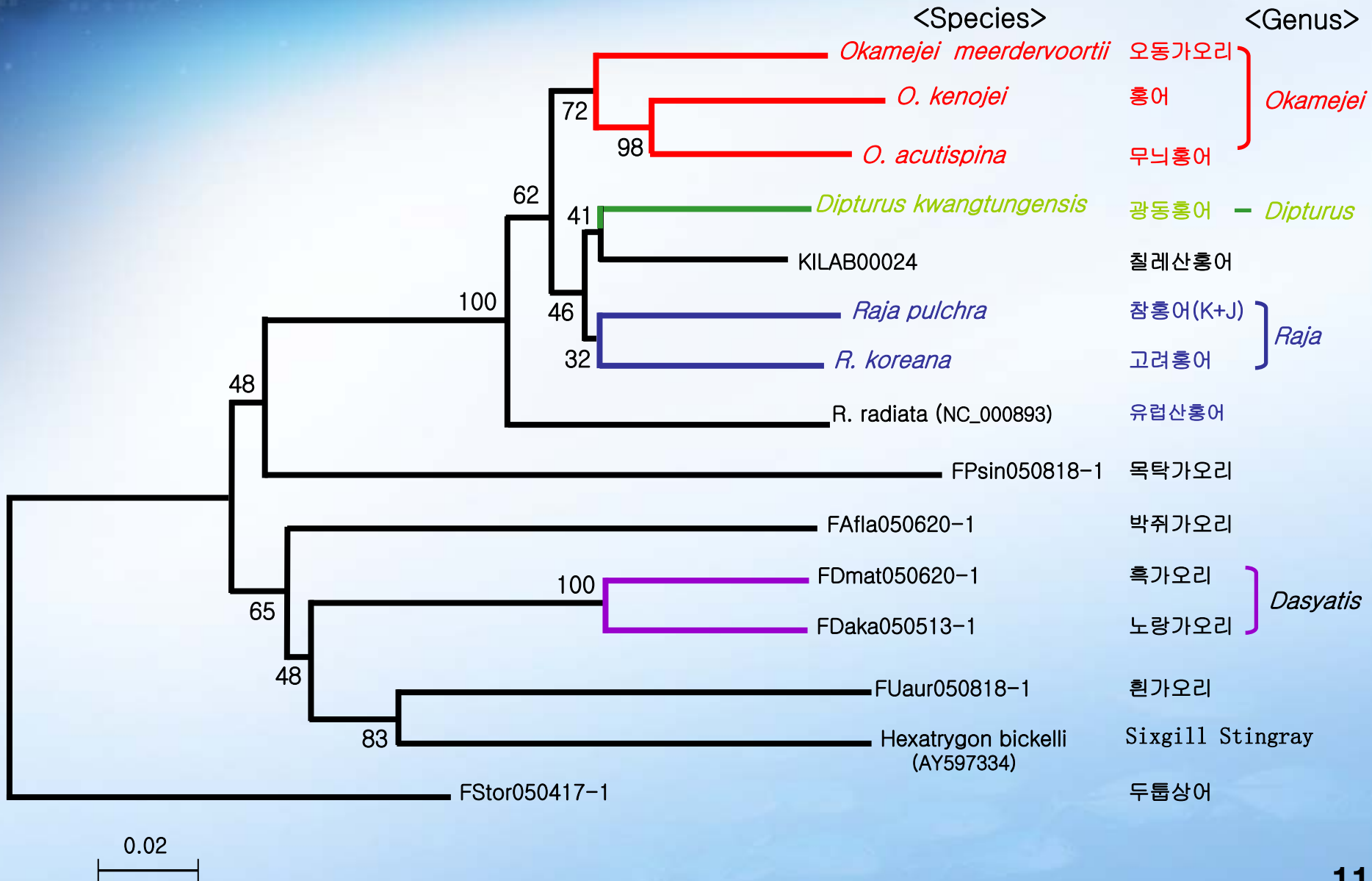


# Raja pulchra, COI Phylogenetic Tree

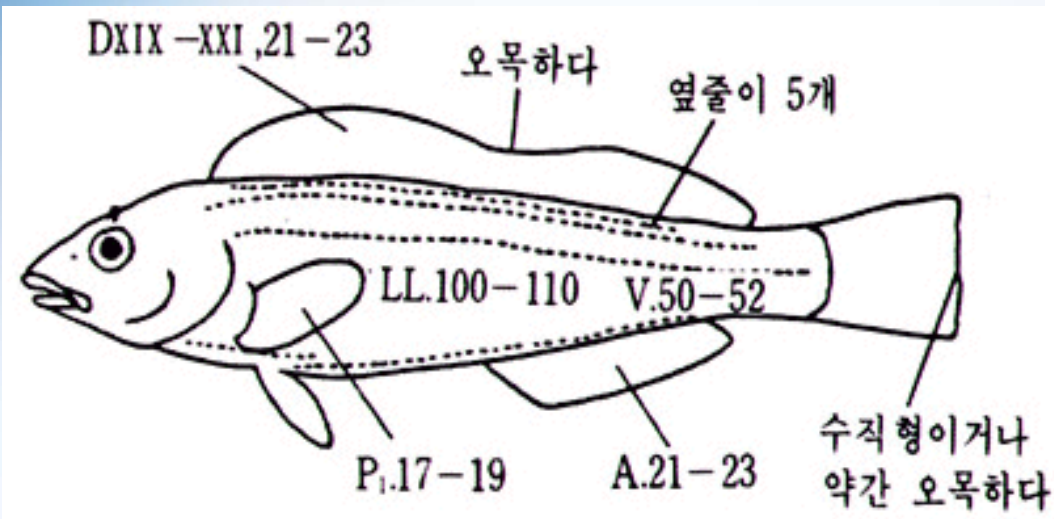
No. of Taxa : 15  
 Data File : rayCOWFRpul(COI).meg  
 Data Type : Nucleotide  
 Analysis : Phylogeny reconstruction  
 Tree Inference : =====  
 Method : **Neighbor-Joining**  
 Phylogeny Test and options : None  
 Include Sites : =====  
 Gaps/Missing Data : Complete Deletion  
 Substitution Model : =====  
 Model : Nucleotide: Tamura-Nei  
 Substitutions to Include : d: Ts + Tv  
 Pattern among Lineages : Same  
 Rates among sites : Uniform rates  
 No. of Sites : 655



# Phylogenetic tree of Korean skates and rays (COI)



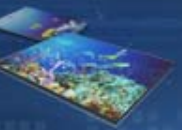
# 쭈노래미 Rock trout (*Hexagrammos otakii*)



(*H. Otakii*)

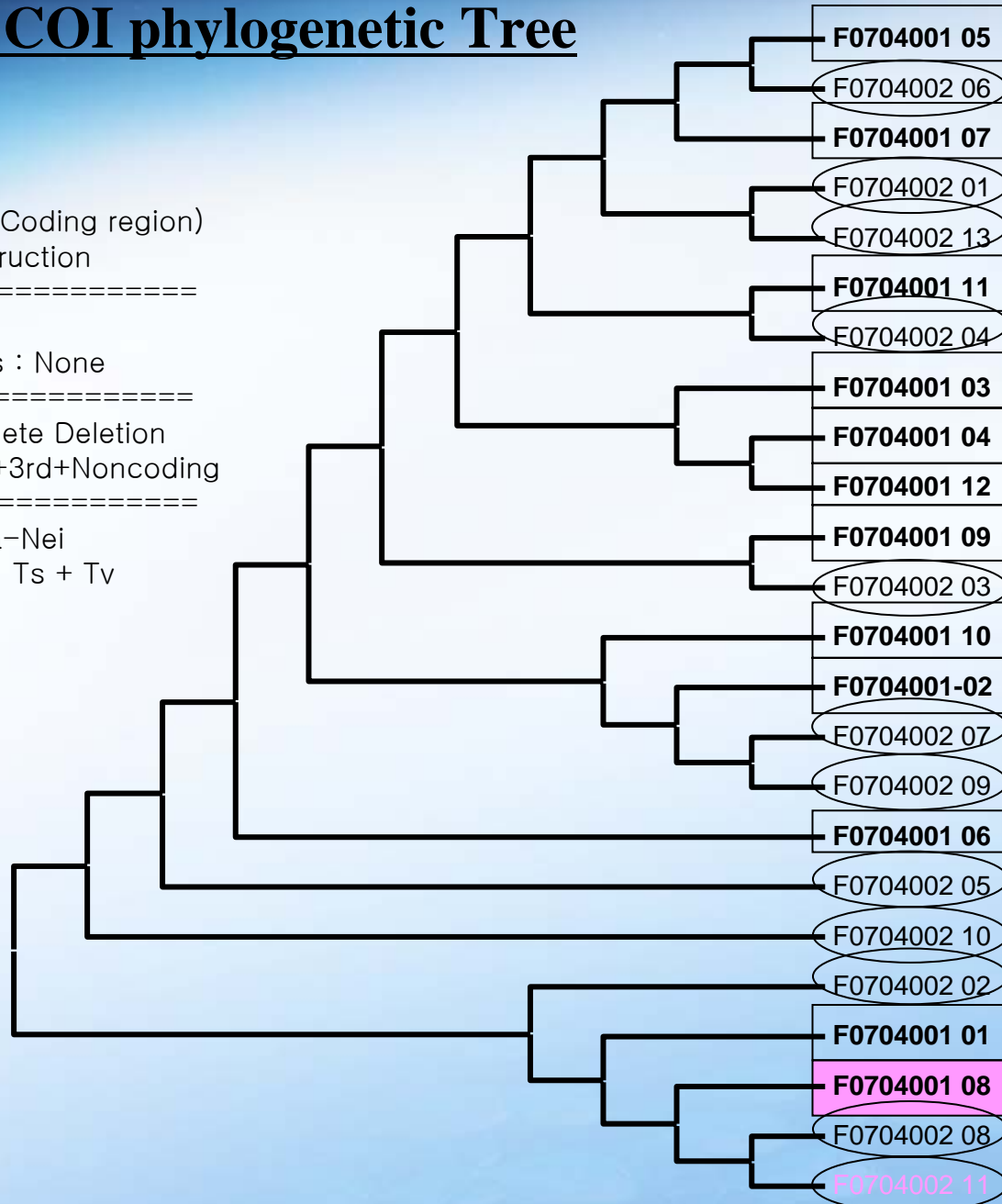


노래미 Spotty belly greenling (*H. agrammus*)



# Rock trout COI phylogenetic Tree

No. of Taxa : 24  
 Data Type : Nucleotide (COI Coding region)  
 Analysis : Phylogeny reconstruction  
 Tree Inference : =====  
 Method : **Neighbor-Joining**  
 Phylogeny Test and options : None  
 Include Sites : =====  
 Gaps/Missing Data : Complete Deletion  
 Codon Positions : 1st+2nd+3rd+Noncoding  
 Substitution Model : =====  
 Model : Nucleotide: Tamura-Nei  
 Substitutions to Include : d: Ts + Tv  
 No. of Sites : 697



Yellow Sea

East/Japan Sea

# Pairwise Distance

Yellow Sea samples

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	
[ 1]																								
[ 2]	0.007																							
[ 3]	0.006	0.001																						
[ 4]	0.007	0.003	0.001																					
[ 5]	0.006	0.001	0.000	0.001																				
[ 6]	0.006	0.001	0.000	0.001	0.000																			
[ 7]	0.006	0.001	0.000	0.001	0.000	0.000																		
[ 8]	0.004	0.009	0.007	0.009	0.007	0.007	0.007																	
[ 9]	0.007	0.003	0.001	0.003	0.001	0.001	0.001	0.009																
[10]	0.007	0.003	0.001	0.003	0.001	0.001	0.001	0.009	0.003															
[11]	0.007	0.003	0.001	0.003	0.001	0.001	0.001	0.009	0.003	0.003														
[12]	0.007	0.003	0.001	0.003	0.001	0.001	0.001	0.009	0.003	0.003	0.003													
[13]	0.006	0.001	0.000	0.001	0.000	0.000	0.000	0.007	0.001	0.001	0.001	0.001												
[14]	0.001	0.006	0.004	0.006	0.004	0.004	0.004	0.003	0.006	0.006	0.006	0.006	0.004											
[15]	0.006	0.001	0.000	0.001	0.000	0.000	0.000	0.007	0.001	0.001	0.001	0.001	0.000	0.004										
[16]	0.006	0.001	0.000	0.001	0.000	0.000	0.000	0.007	0.001	0.001	0.001	0.001	0.000	0.004	0.000									
[17]	0.006	0.001	0.000	0.001	0.000	0.000	0.000	0.007	0.001	0.001	0.001	0.001	0.000	0.004	0.000	0.000								
[18]	0.006	0.001	0.000	0.001	0.000	0.000	0.000	0.007	0.001	0.001	0.001	0.001	0.000	0.004	0.000	0.000	0.000							
[19]	0.007	0.000	0.001	0.003	0.001	0.001	0.001	0.009	0.003	0.003	0.003	0.003	0.001	0.006	0.001	0.001	0.001	0.001						
[20]	0.003	0.007	0.006	0.007	0.006	0.006	0.006	0.004	0.007	0.007	0.007	0.007	0.006	0.001	0.006	0.006	0.006	0.006	0.006					
[21]	0.007	0.000	0.001	0.003	0.001	0.001	0.001	0.009	0.003	0.003	0.003	0.003	0.001	0.006	0.001	0.001	0.001	0.001	0.001	0.000				
[22]	0.006	0.001	0.000	0.001	0.000	0.000	0.000	0.007	0.001	0.001	0.001	0.001	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.001	0.006			
[23]	0.004	0.009	0.007	0.009	0.007	0.007	0.007	0.006	0.009	0.009	0.009	0.009	0.007	0.003	0.007	0.007	0.007	0.007	0.007	0.009	0.001	0.009	0.007	
[24]	0.007	0.003	0.001	0.003	0.001	0.001	0.001	0.009	0.003	0.003	0.003	0.003	0.001	0.006	0.001	0.001	0.001	0.001	0.001	0.003	0.007	0.003	0.001	0.009

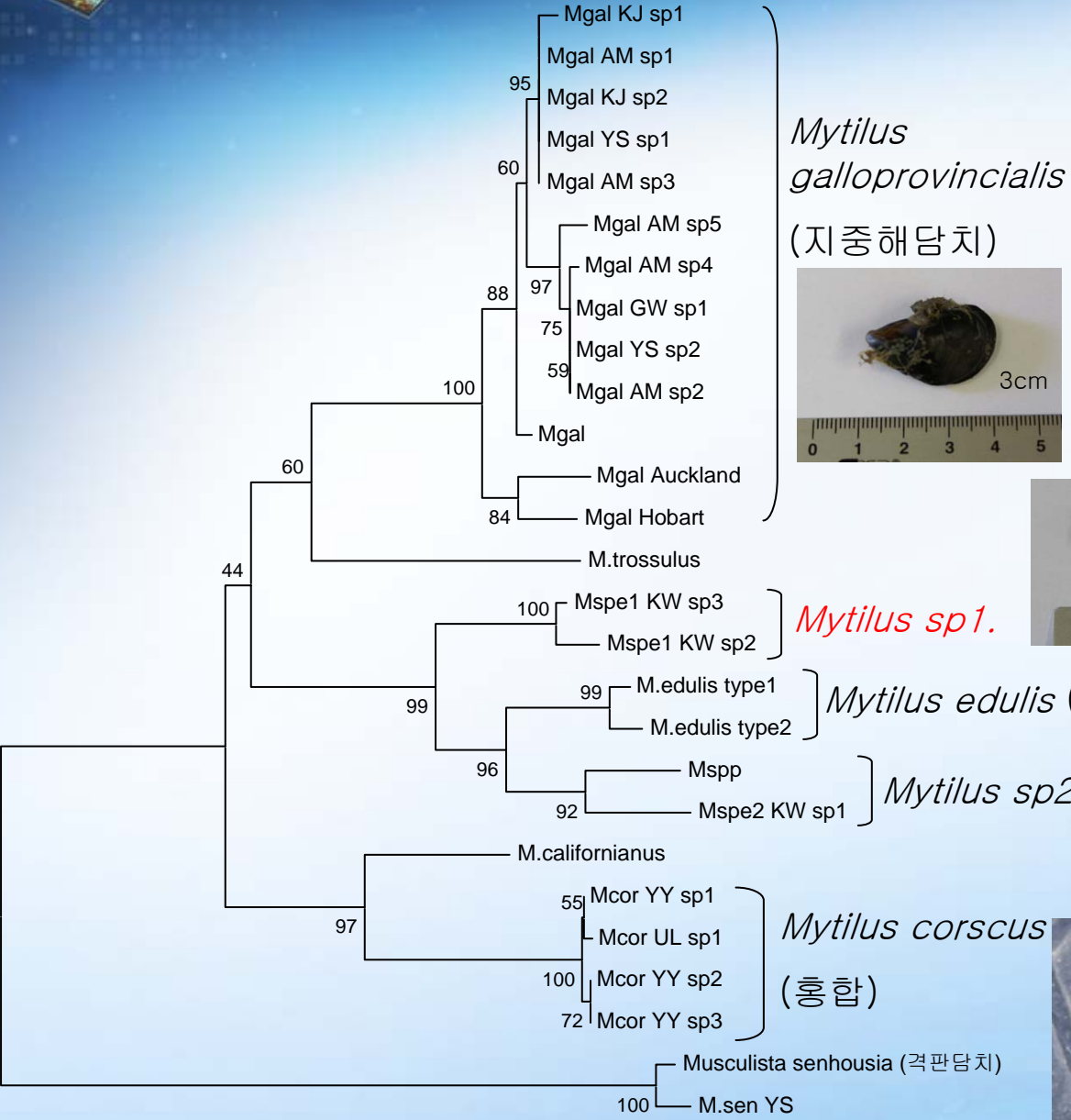
Tamura – Nei method

Max. variation: 0.009

Min. variation: 0.000

Max. COI sequence divergence: 0.9 %

# Diversity of mytilus species in Korea Sea Waters



Phylogenetic tree reconstructed with CO1 sequences by NJ method in MEGA 3.1. 1000 times bootstrap was applied. Mgal denotes *Mytilus galloprovincialis*, and Mcor denotes *Mytilus corscus*. KJ, AM, YS, GW, YY, and UL represent Kijang, Anmyeondo, Yeosu, Ganwoldo, Yangyang, and Uleungdo, respectively. Mspe1, Mspe2, M.spp are unidentified species. Two *Musculista senhousia* are used as an outgroup.

*Mytilus galloprovincialis*

(지중해담치)



*Mytilus sp1.*

*Mytilus edulis* (진주담치)



*Mytilus sp2.*

*Mytilus corscus*

(홍합)



0.05





## Diversity of mytilus species in Korea Sea Waters

Haplotype diversity among each species

1. *Mytilus galloprovincialis*: 10 haplotypes are found in Korea Sea Waters. The difference of each haplotype ranges from 0.6 ~ 3.1%. Haplotypes in Korea Sea Waters are different from that in other regions, such as Auckland and Hobart by 1.2~6.8%.

2. *Mytilus coruscus*: Three haplotypes are found in Korea Sea Water. CO1 sequence from Uleung Island doesn't show distinct difference from Yangyang. The sequence differences of each haplotype are from 0.3% to 0.6%.

3. *Mytilus sp1*: Two haplotypes are exist, and are different by 1.9%. The sequence differences from *Mytilus edulis* range from 11.8% to 19.1%.

4. *Mytilus sp2*: A CO1 sequence from Korea Sea Water is different from that from NCBI with unidentified status by 6.5%. They may be different species.

# Diversity of Sponge from Jeju Island



*Callyspongia sp.*



*Spirastrella sp. 1*



*Cliona sp. 1*



*Cliona sp. 2*

# Diversity of Sponge from Jeju Island



*lotrochota sp.*



*Suberites sp.*

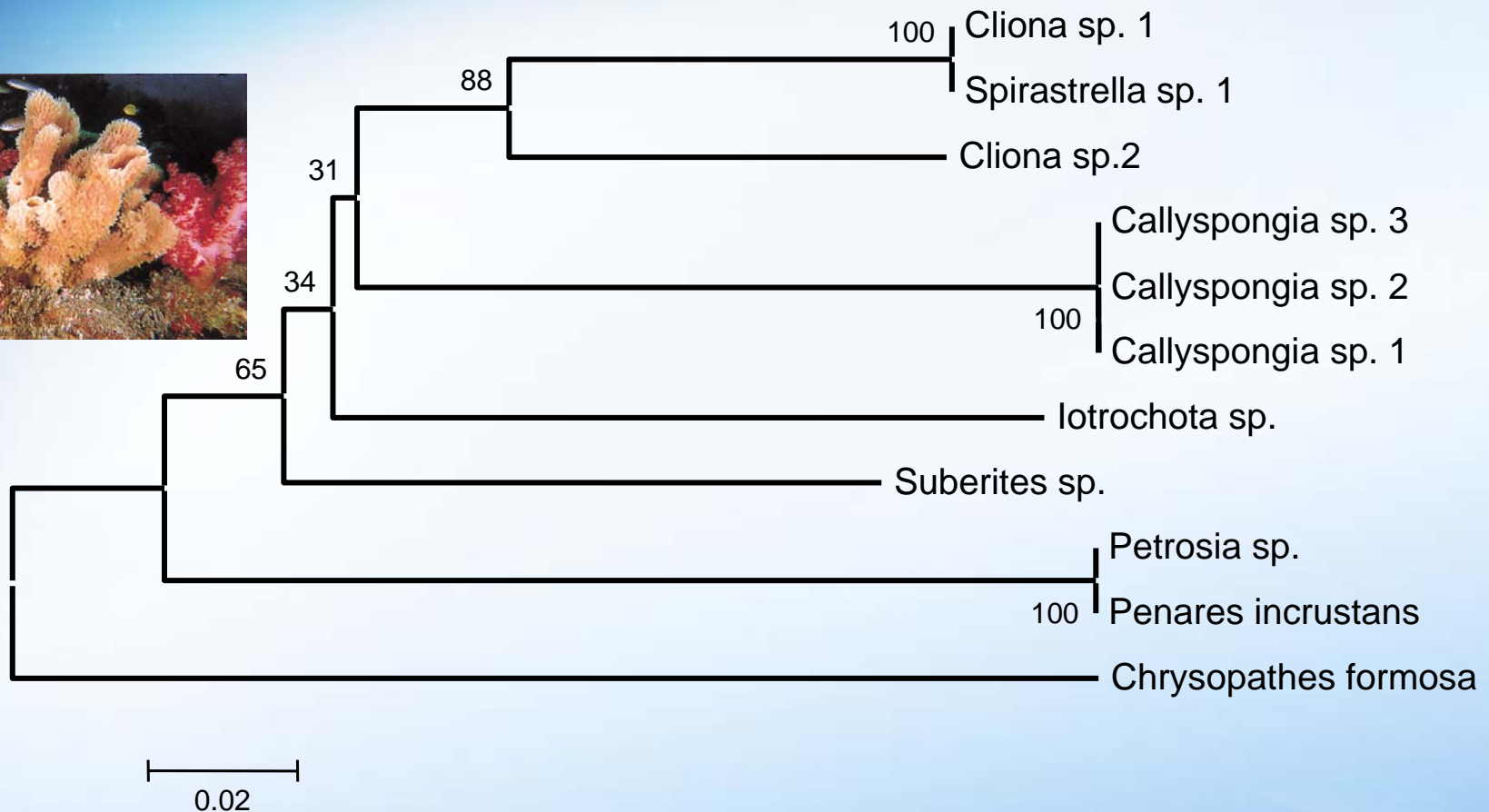


*Petrosia sp.*



*Penares incrustans*

# Diversity of Sponge from Jeju Island



Phylogenetic tree reconstructed with CO1 sequence of Sponge by NJ method in MEGA 3.1. 1000 times bootstrapping was applied. Classification by morphology is not consistent with that by genetic marker, CO1 sequences.

## Molecular Approach

Skate and Stingray species are distinctive in their single nucleotide polymorphisms (SNPs) of mtDNA COI region sequences.

## Solution : Genetic Analysis

- Genetic biomarker:

Using Nucleotide sequence differences in the genes of mtDNA, COI (650bp)

Primer set

Raja -COI-F: 5'-TCA GCC ATC TTA CCT GTG GC-3'

Raja -COI-R1: 5'-GGG TGT CCG AAG AAT CAG AA-3'

rF110-133 : 5'-ATT TAT AAT GTC ATT GTT ACA GCC-3'

rR460-437 : 5'-(A/G)AT AAT TGT GGT GAT GAA GTT AAT-3'

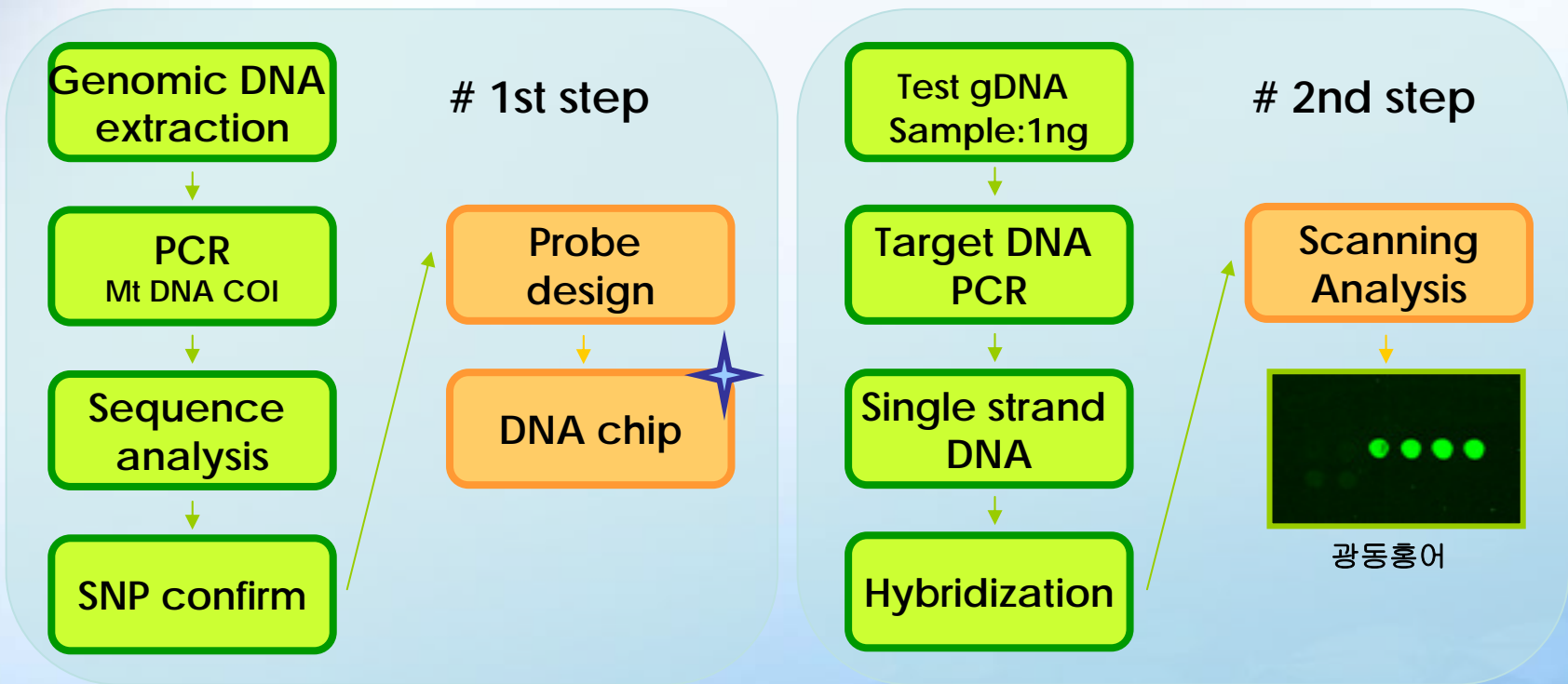
- Examining method: **[DNA Chip]**

Hybridization with specific primer probes

# Materials and Methods



Morphologically Identified samples



# Materials and Methods

## Probe Decision

- length (17~25 nucleotides)
- Specificity
- Melting temperature
- Secondary structure stability

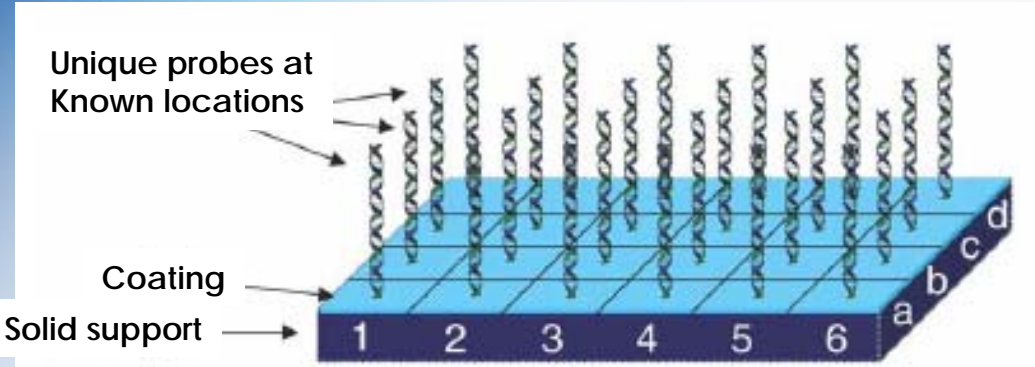
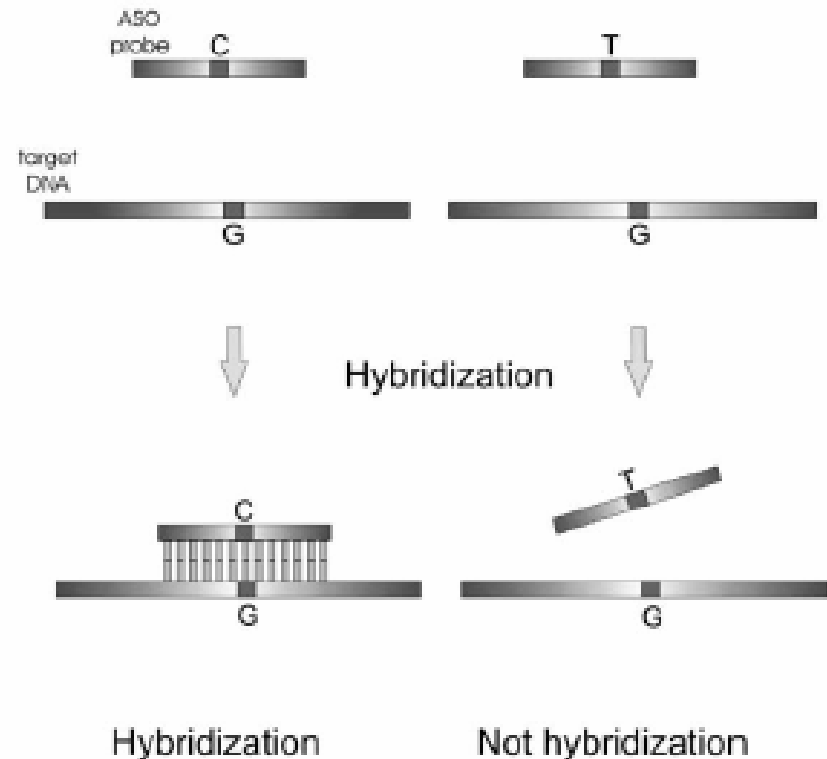


Fig. 3 Simplified pictorial representation of a spotted, DNA microarray. Unique nucleic acid probes are immobilized at distinct locations (i.e. position 1-a) on a coated support. Note that the image is an oversimplified example, as a DNA microarray can have a much higher density (>500 probes/cm<sup>2</sup>) and many copies of a unique probe are found at each location.

## ALLELIC SPECIFIC HYBRIDIZATION



# Primer Design

	101	Forward Primer				150
<b>Rajidae</b>	FOKEN050308-1	GATGATCAGA	TTTATAATGT	CATTGTTACA	GCCACGCCT	TAGTAATAAT
<b>(Family)</b>	FOKEN050402-1	.....	.....	.....	.....	.....
	FOKEN050317-1	.....	.....	.....	.....	.....
	FOKEN050513-1	.....	.....	.....	.....	.....
	FOACU050513-1	.C.....	.....	.....	.....	.C.....
	FOACU050513-2	.C.....	.....	.....	.....	.C.....
	FOME050915-2	....C.....	.....	.....	.....	.T....G..
	FDKWA050511-1	.....	.....	.....	.....	.T.....
	FDKWA050721-1	.....	.....	.....	.....	.T.....
	FRPUL050402-1	.C....A.	.....	.....	.T..C	.....G..
	FRPUL050417-1	.C....A.	.....	.....	.T..C	.....G..
	FRKOR050818-1	.C.....	.....	.....	.T....	.T.....
	FRKOR050818-2	.C.....	.....	.....	.T....	.T.....
<b>stingray</b>	FDMAT050620-1	....C..A.	.C..C.....	A....C..C	.....	.C.....
	FDMAT050818-1	....C..A.	.C..C.....	A....C..C	.....	.C.....
	FAFLA050620-1	....C....	.C.....	A..C..C..G	....T....	.T.....
	FPSIN050818-1	.....A.	.C.....	T.....C	....T..T.	.T..T....
	FUAUR050818-1	.....	....C....	G..C..C..	....T....	.C.....
	FDACA050513-1	....C..A.	.C.....	A....C	.....	.C.....
	FSTOR050417-1	.....	.....	A..C..A..	....T....	.T.....



# Primer Design

**Rajidae  
(Family)**

**stingray**

	410			Reverse Primer	460
FOKEN050308-1	GCAGGCATCT	CATCTATCCT	AGCCTCC	<b>ATT AACTTCATCA CCACAATTAT</b>	
FOKEN050402-1	.....	.....	.....	.....	.....
FOKEN050317-1	.....	.....	.....	.....	.....
FOKEN050513-1	.....	.....	.....	.....	.....
FOACU050513-1	.....	.....	C.....	.....	.....
FOACU050513-2	.....	.....	C.....	.....	.....
FOME050915-2	.....	.....	.....	.....	.....
FDKWA050511-1	.....T..T.	.....T..	G.....	.....	.....
FDKWA050721-1	.....T..T.	.....T..	G.....	.....	.....
FRPUL050402-1	.....TG.T.	.....	G.....	.....	.....
FRPUL050417-1	.....TG.T.	.....	G.....	.....	.....
FRKOR050818-1	.....T..T.	.....T..	G.....	.....	.....
FRKOR050818-2	.....T..T.	.....T..	G.....	.....	.....
FDMAT050620-1	..C..TG.T.	.C.....	..A.....	.....T..T..A.....	
FDMAT050818-1	..C..TG.T.	.C.....	..A.....	.....T..T..A.....	
FAFLA050620-1	.....GG.T.	.T..A.....	G..A..T..C	.....T.....C..	
FPSIN050818-1	..T..AG.T.	.C..C..T..	.....A...	..T..T..T..T..T..C..	
FUAUR050818-1	.....GG.T.	.C..C.....	T..A..A...	.....T.....T.....	
FDACA050513-1	..C..T..T.	.C.....	G..A.....	.....T...A.....	
FSTOR050417-1	..T.....T.	....A..TT.	.....A...	.....T..T..A..T.....	

# Materials and Methods

## PCR

### Components

Template	>1ng(1ul)
Taq	0.1ul
10Xbuffer	2ul
dNTP(2.0mM)	1ul
Primer	1ul(each)
D.W	13.9ul
<b>Total</b>	<b>20ul</b>

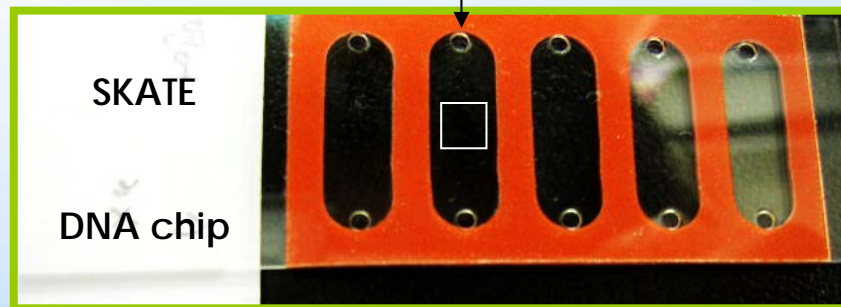
## Cycle

95°C(15sec) - 50°C(45sec) - 72°C(1min) (35cycles)

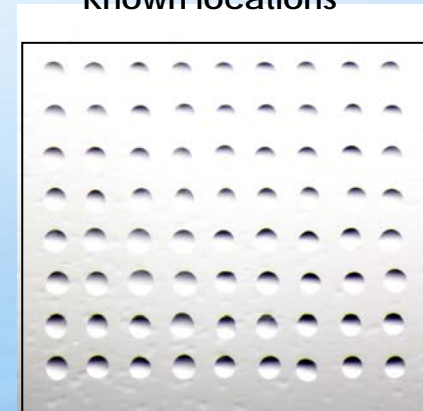
72°C(5min)

8°C

Hybridization solution + PCR product (SS) mixture



Unique probes at  
Known locations

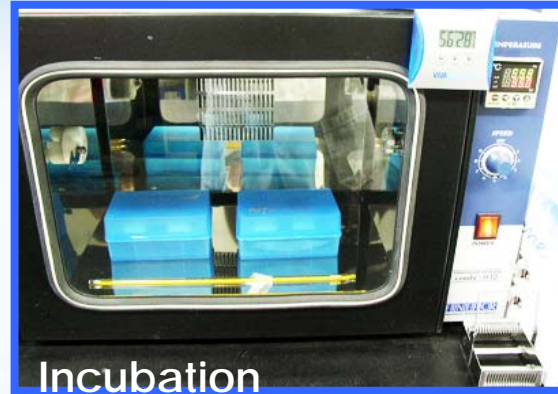


# Design of species-specific probes

		151			200	
Skate (Family Rajidae)	홍어	<b>FOken050308-1</b>	CTTTTTTATG	GTTATACCAA	TTA-TAATCG	<b>CGGGTTCGG TAACTGACTT</b>
		FOken050402-1	.....	.....	-.....	.....
		FOken050317-1	.....	.....	-.....	.....
		FOken050513-1	.....	.....	-.....	.....
	무늬홍어	<b>FOacu050513-1</b>	.....	.C.....	..G....	<b>T..A..T.. ..T..G..C</b>
		FOacu050513-2	.....	.C.....	..G....	T..A..T.. ..T..G..C
		FOmee050915-2	.....	.A.....	-.....	..A..T.. ..T....C
	광동홍어	<b>FDkwa050511-1</b>	.....	.A.....	-.....	<b>A..A..T.. ..T....C</b>
		FDkwa050721-1	.....	.A.....	-.....	A..A..T.. ..T....C
	참홍어	<b>FRpul050402-1</b>	.....	.A..C.....	.C-.....	<b>G.....T.. C..T....C</b>
	FRpul050417-1	.....	.A..C.....	.C-.....	G.....T.. C..T....C	
	FRkor050818-1	.....	.C.....	-.....	G.....T.. C..T....	
	FRkor050818-2	.....	.C.....	-.....	G.....T.. C..T....	
ray		FDmat050620-1	T..C..C..A	..A.....	.C-.....	A..A..T.. ..T....A
		FDmat050818-1	T..C..C..A	..A.....	.C-.....	A..A..T.. ..T....A
		FAfla050620-1	.....	.A.....	.C-.....	T.....T.. A.....G...
		FPsin050818-1	.....	.A.....	.C-.....	.....T...T.G
		FUaur050818-1	T....C...	.....C.	.C-.....	T..T..... ..C
		FDaka050513-1	T..C..C...	..A.....	-.....	A..A..... ..T....A
		FStor050417-1	T..C....A	.....G..G	.A-.....	T.....T.. A.....T.A

# Materials and Methods

## Hybridization



Incubate at 55°C for 1hr.  
Hybridization Solution  
(3x SSC, 0.3% SDS)

Washing  
1x SSC  
0.1% SDS ->  
3min



Drying Slide  
30sec.



Scanning  
Image Analyzing

# Scanning Image Analyzing - *Okamejei kenojei*

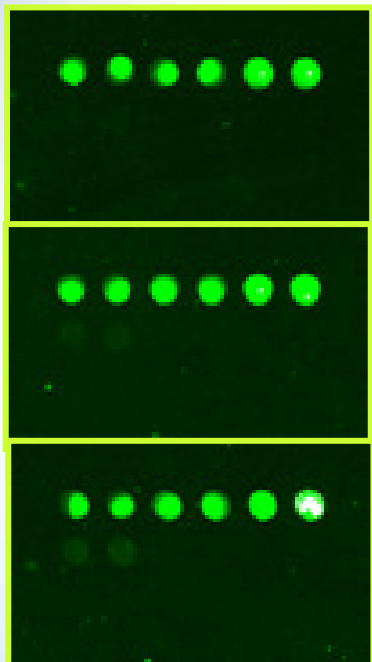
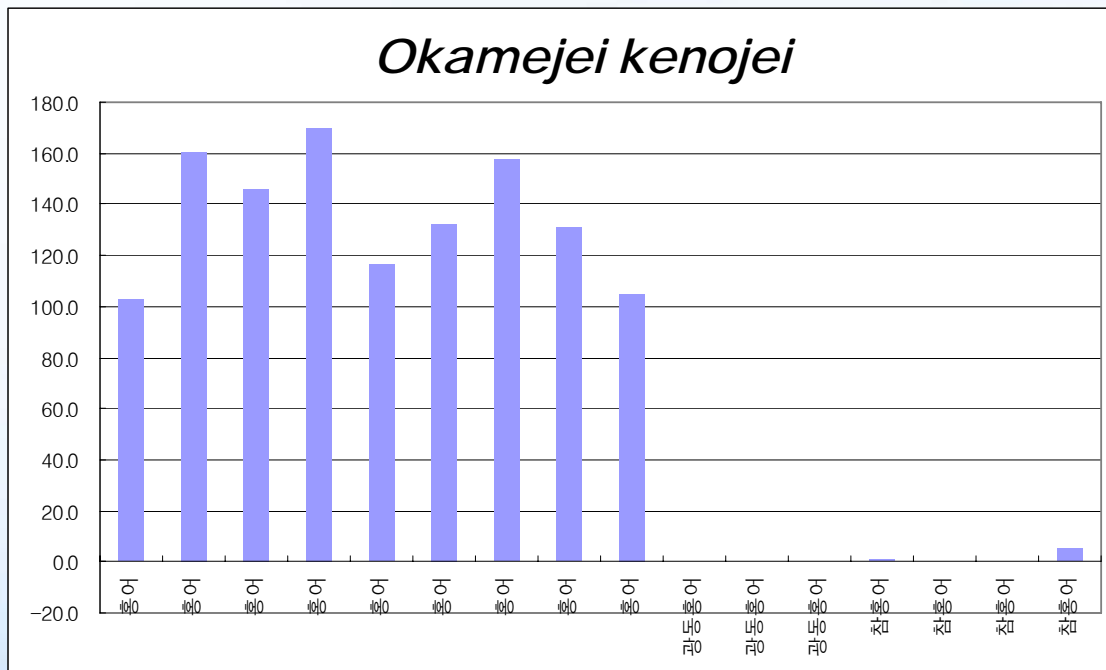
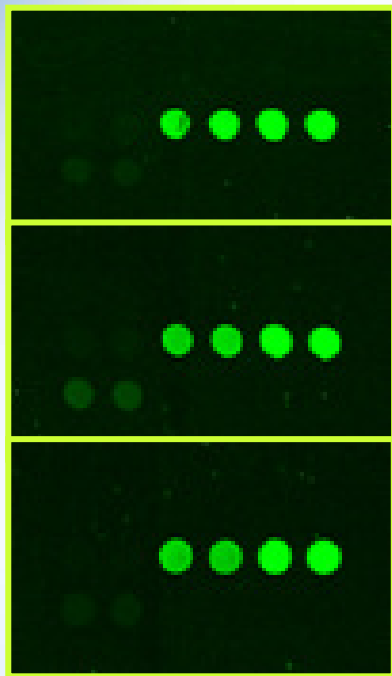


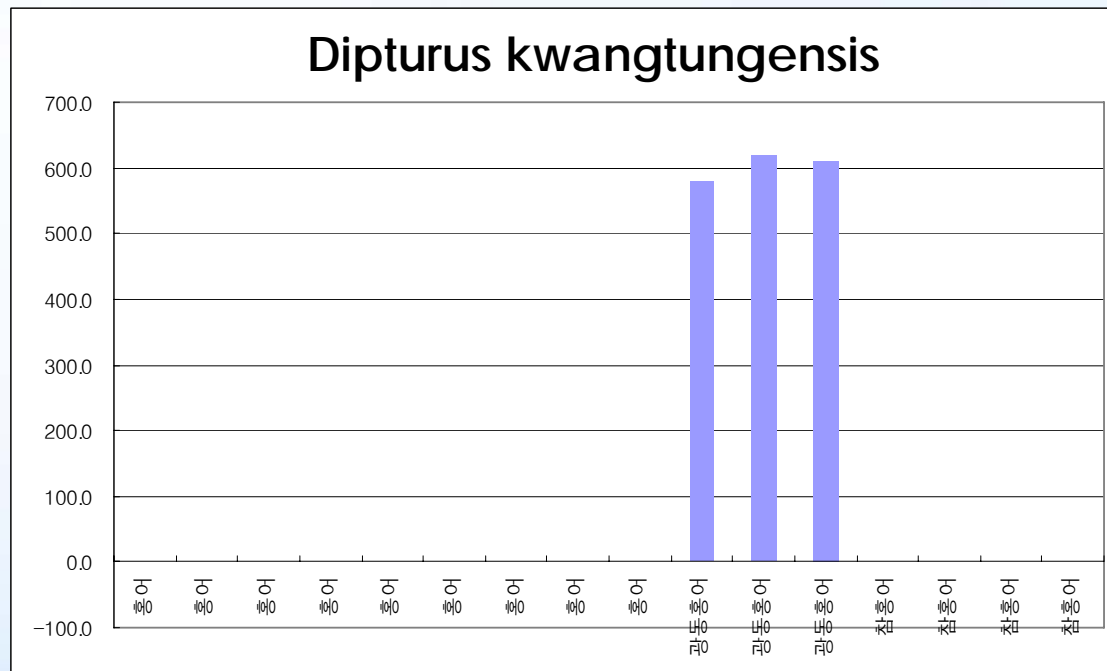
Figure 1



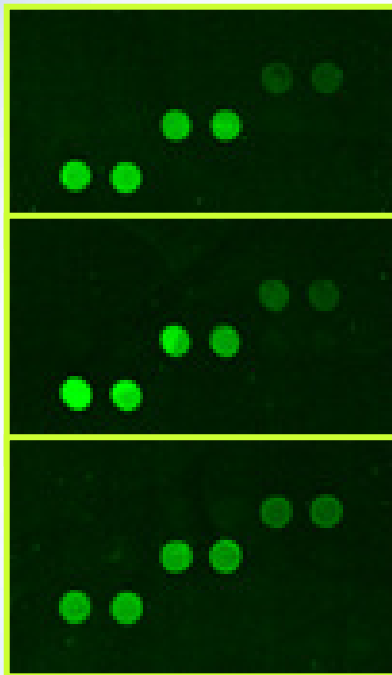
# Scanning Image Analyzing – *Dipturus kwangtungensis*



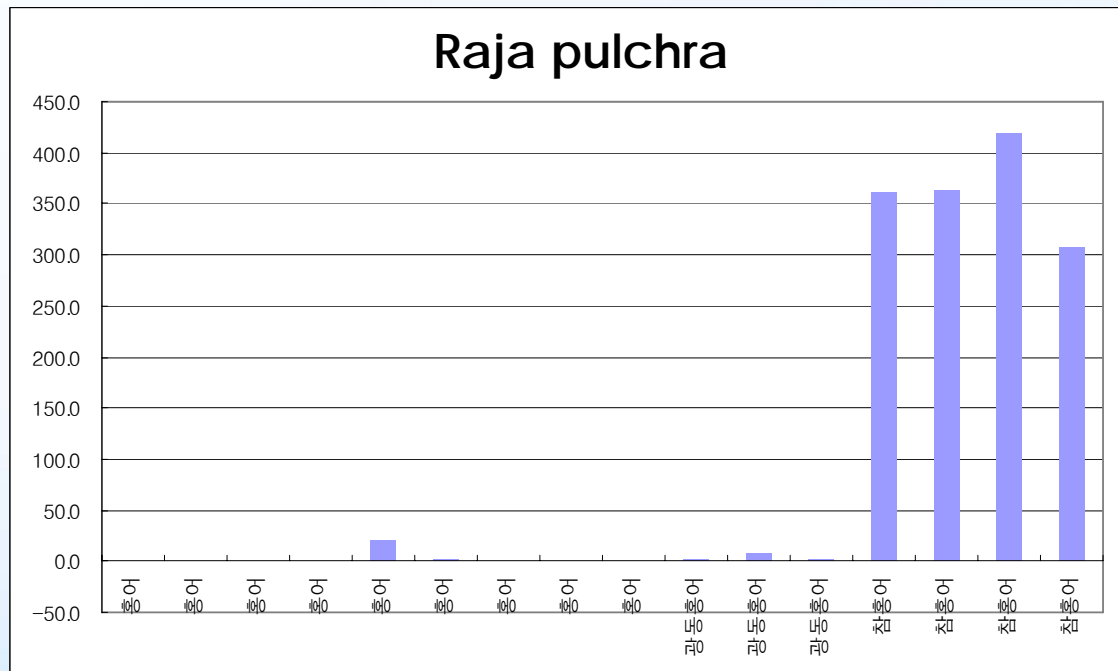
광동홍어

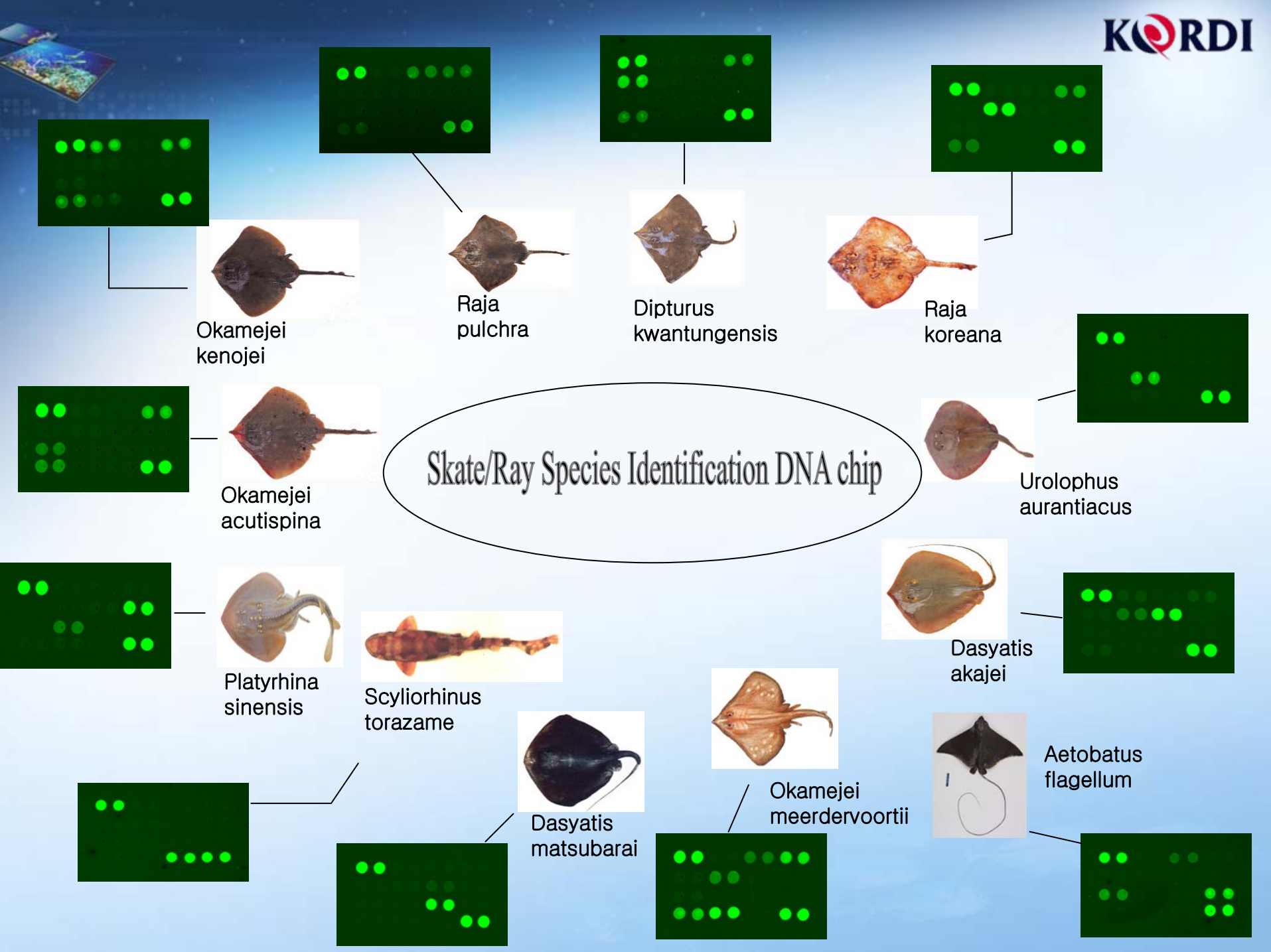


# Scanning Image Analyzing - *Raja pulchra*



참홍어





Okamejei kenojei

Raja pulchra

Dipturus kwantungensis

Raja koreana

Okamejei acutispina

Urolophus aurantiacus

Platyrrhina sinensis

Scyliorhinus torazame

Dasyatis akajei

Dasyatis matsubarai

Okamejei meerdervoortii

Aetobatus flagellum



## Summary of DNA chip analysis

- ✓ For skate and ray species, mtDNA COI sequences were analyzed and compared.
- ✓ Using species-specific sequences, DNA probes were designed and spotted on a DNA chip.
- ✓ To identify an unknown sample, target DNA was amplified and hybridized with probes on the DNA chip.
- ✓ A distinct DNA chip pattern tells the identification of the unknown sample.



# Thank you for your attention!

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