



Comparisons between two butterflyfish hybrid groups at Christmas Island, within the Indian and Pacific Ocean suture zone

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Australian Government

Department of Sustainability, Environment, Water, Population and Communities

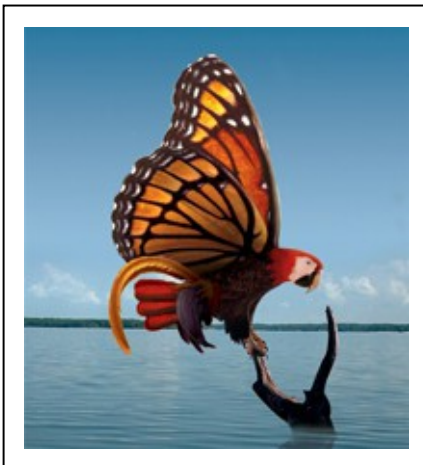


Centre for
Sustainable
Tropical Fisheries
& Aquaculture



Natural hybridisation

- crossing of genetically distinct clusters, production of viable hybrids
- > 25% of plants, > 10% animals
- important for evolution



- occurs clustered in narrow geographic locations: suture zones (Avice 2000)
- common in corals and reef fishes (Gardner 1997, Willis et al 2006)

Natural hybridisation in reef fishes

over 80 species of reef fishes hybridise
causes and consequences unknown

terrestrial- and freshwater-derived theory:

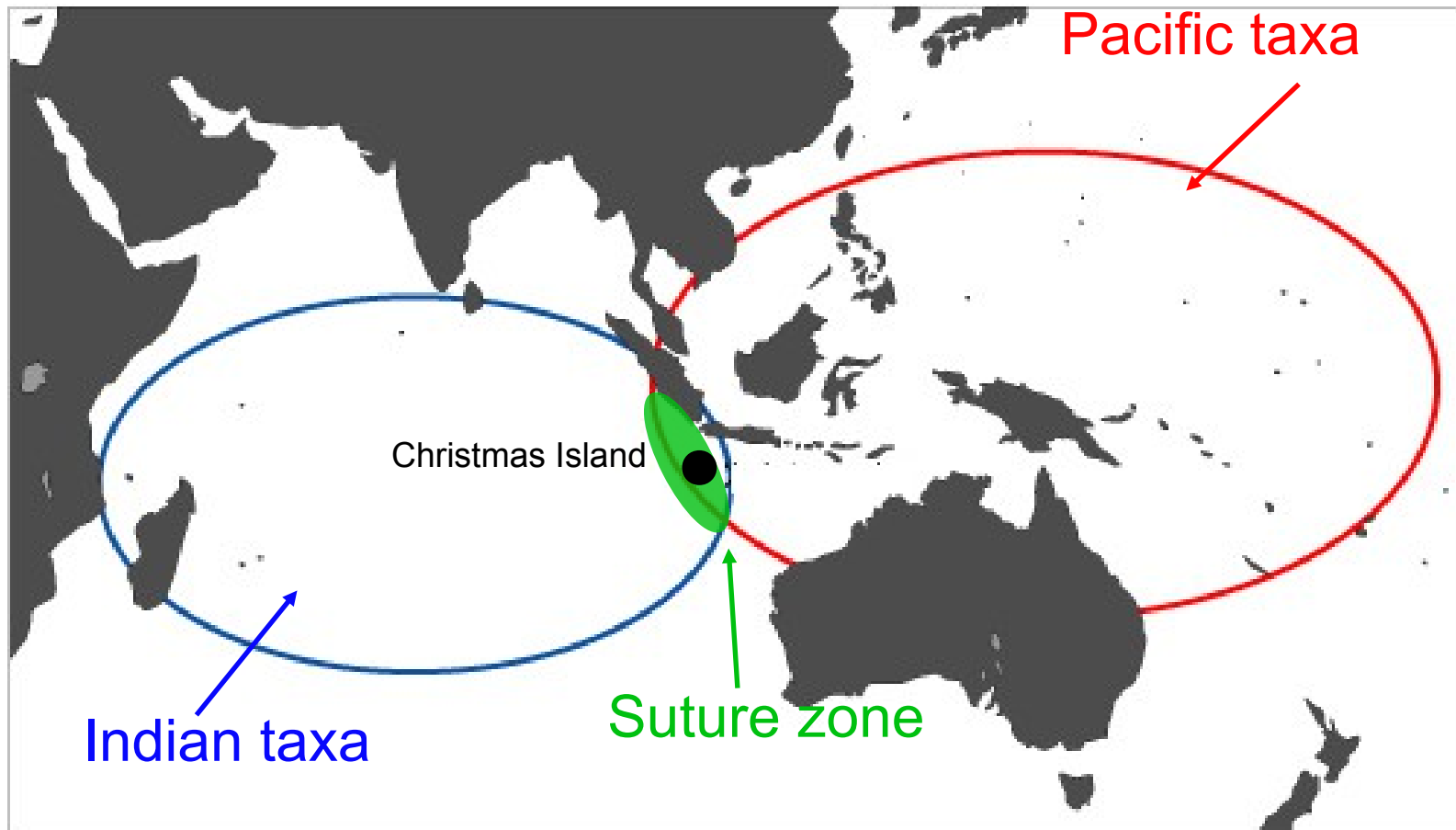
overlapping ecology; rarity of parental species (Grant and Grant 2002, Mallet 2005)

genetic distance influences outcomes (Smith et al. 2003, Taylor et al. 2006)



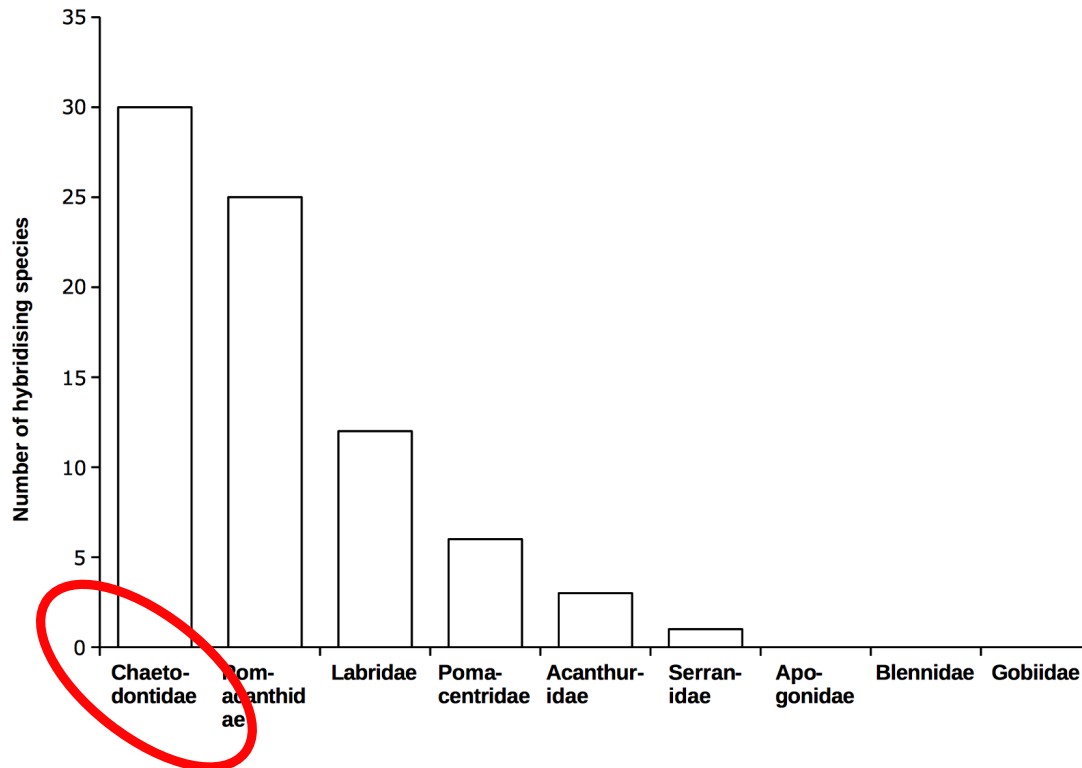
Indo-Pacific biogeographic border: reef fish suture zone

- recently identified as reef fish suture zone
- 16 hybrids reported, involving 25 species across 7 families (Hobbs et al. 2009)
- comparative hybridisation studies in reef fishes



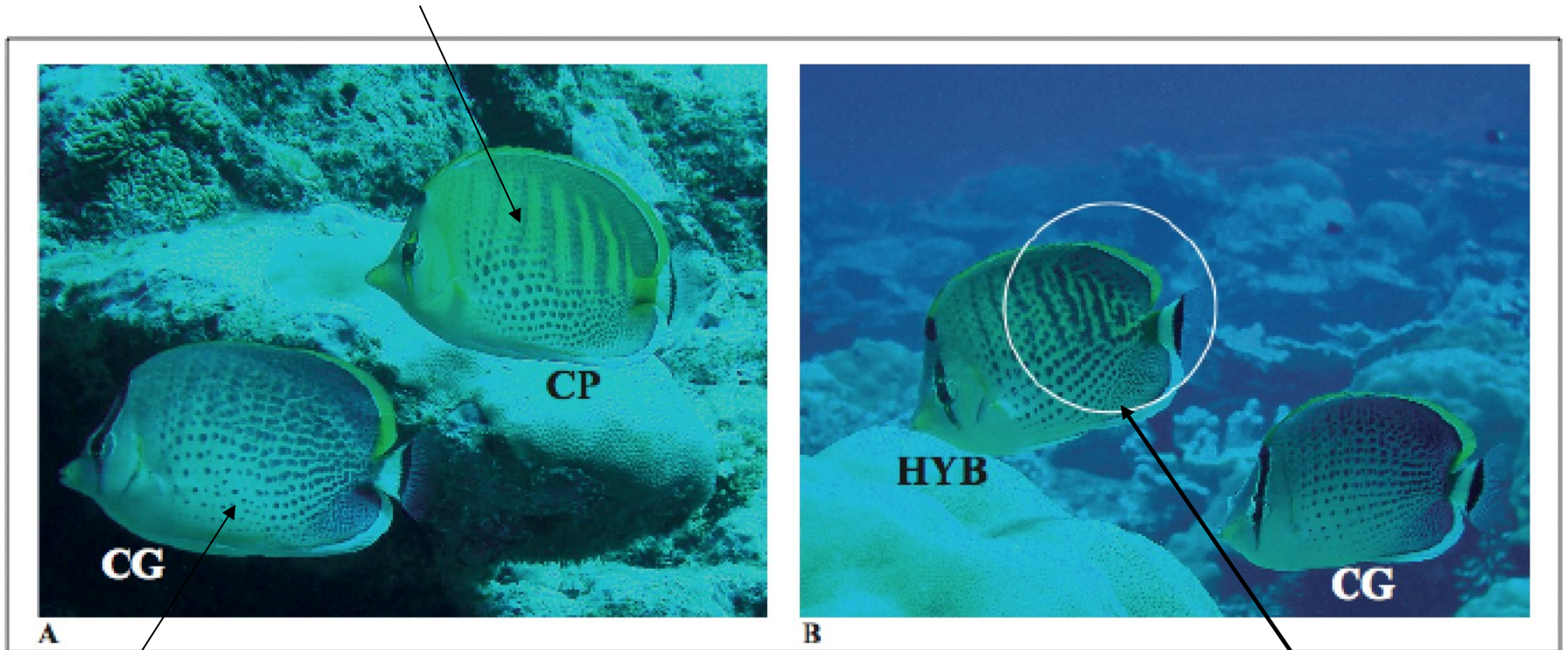
Study species: butterflyfishes

- most reported hybrids than any other reef fish family
- over 40/120 species found to hybridise, >30% (cf other taxa)
- hybrids intermediate colouration, easy to identify



Study species: butterflyfishes

Chaetodon punctatofasciatus (CP) – Pacific Ocean

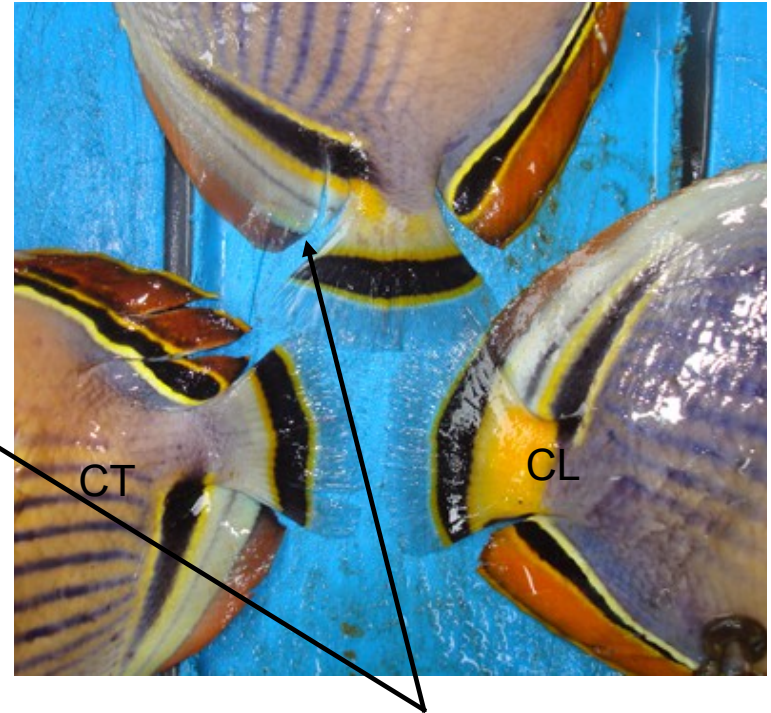
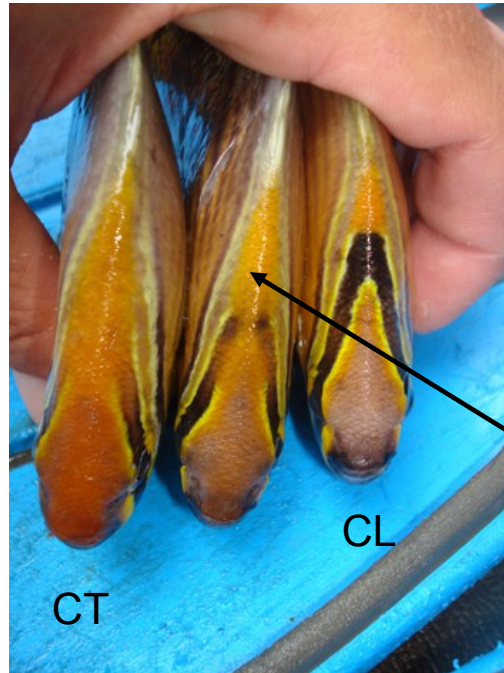


Chaetodon guttatissimus (CG) – Indian Ocean

Hybrid

Study species: butterflyfishes

Chaetodon lunulatus (CL) - Pacific Ocean



Chaetodon trifasciatus (CT) - Indian Ocean

Hybrid

Aims

Compare ecological drivers of hybridisation

- **habitat use** (Underwater Visual Census- UVC)
- **diet** (3 minute observations)
- **abundance** (UVC)
- **mate choice** (UVC)

Compare genetics of hybridisation

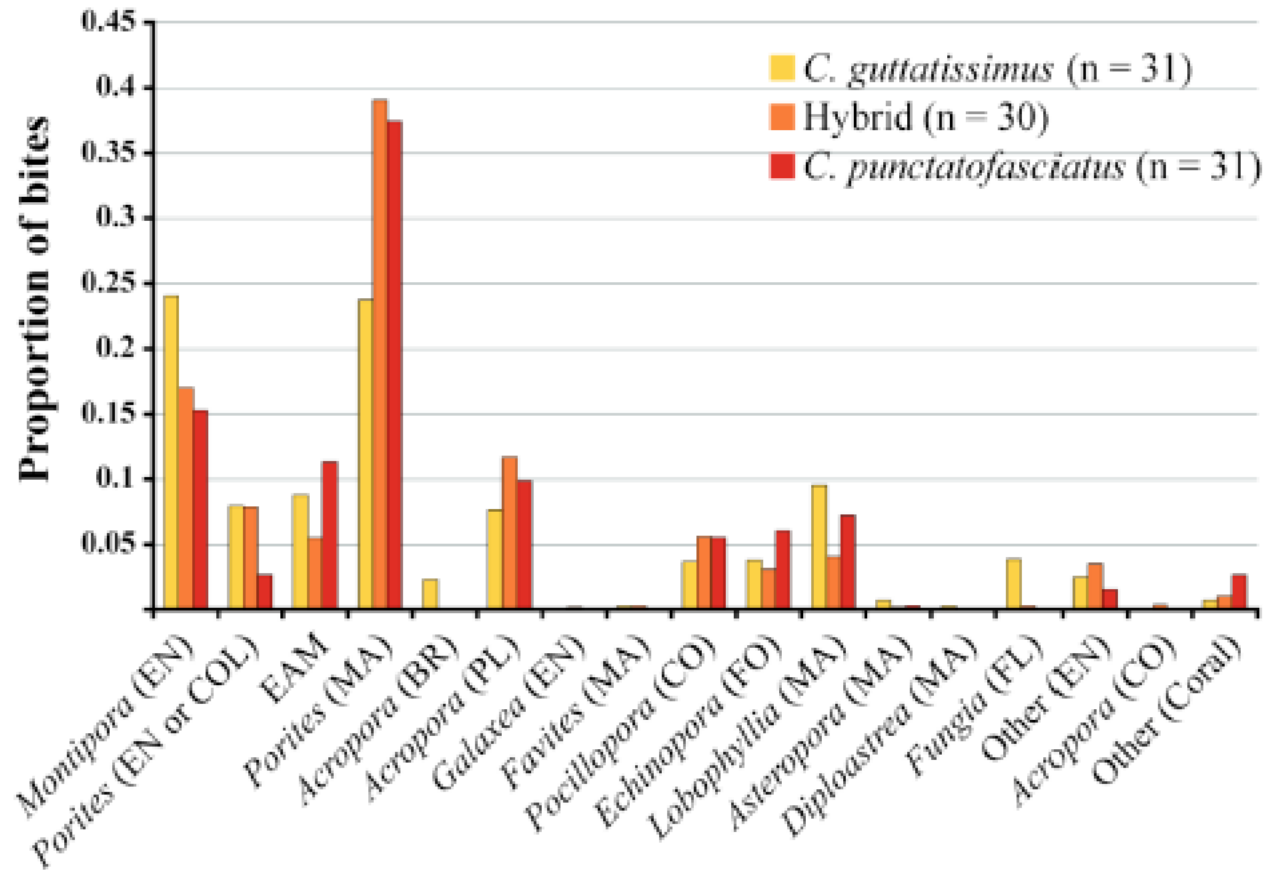
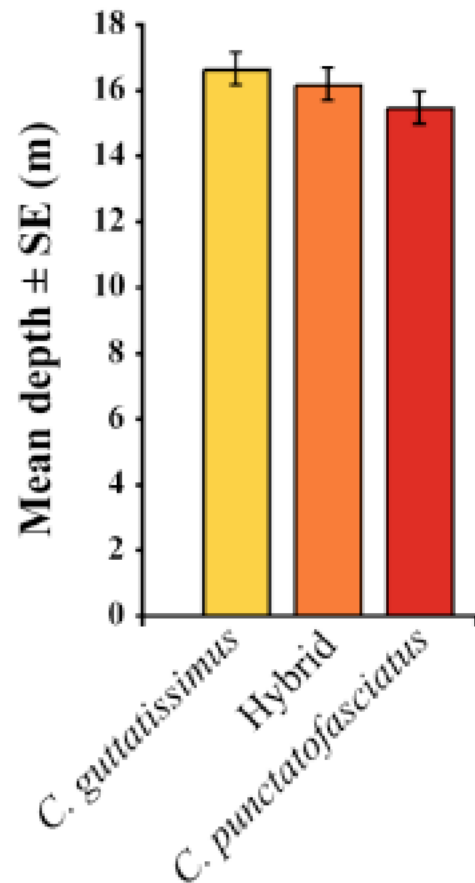
- **hybrid status**
- **directionality**
- **introgression**

Check theoretical consistency



Depth distribution and diet:

C. guttatissimus x *C. punctatofasciatus*



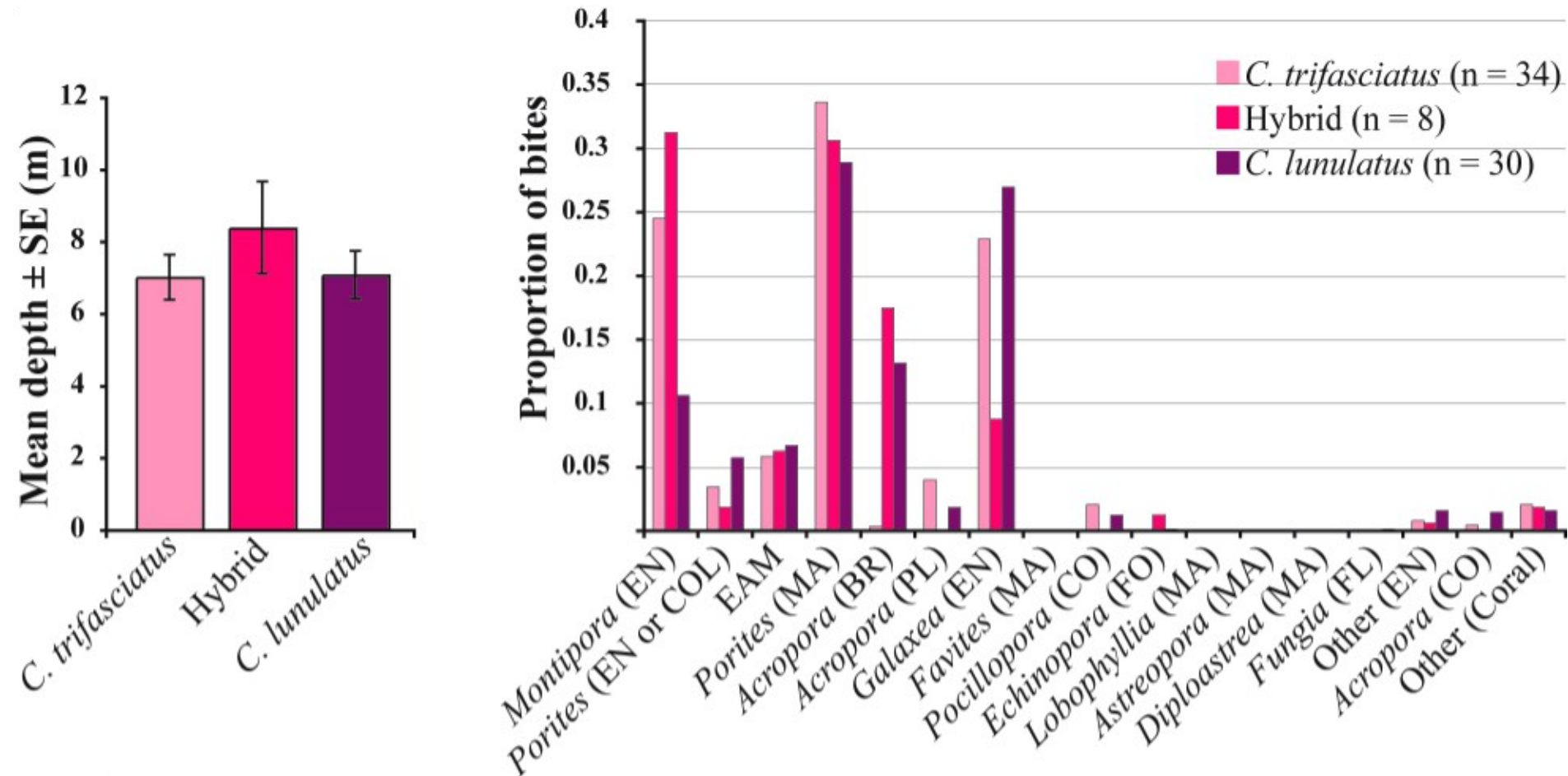
ANOVA, df = 1, F = 3.137, p = 0.08

MANOVA, Pillai's Trace = 0.511, hypothesis df = 34, p = 0.085



Depth distribution and diet:

C. trifasciatus x *C. lunulatus*



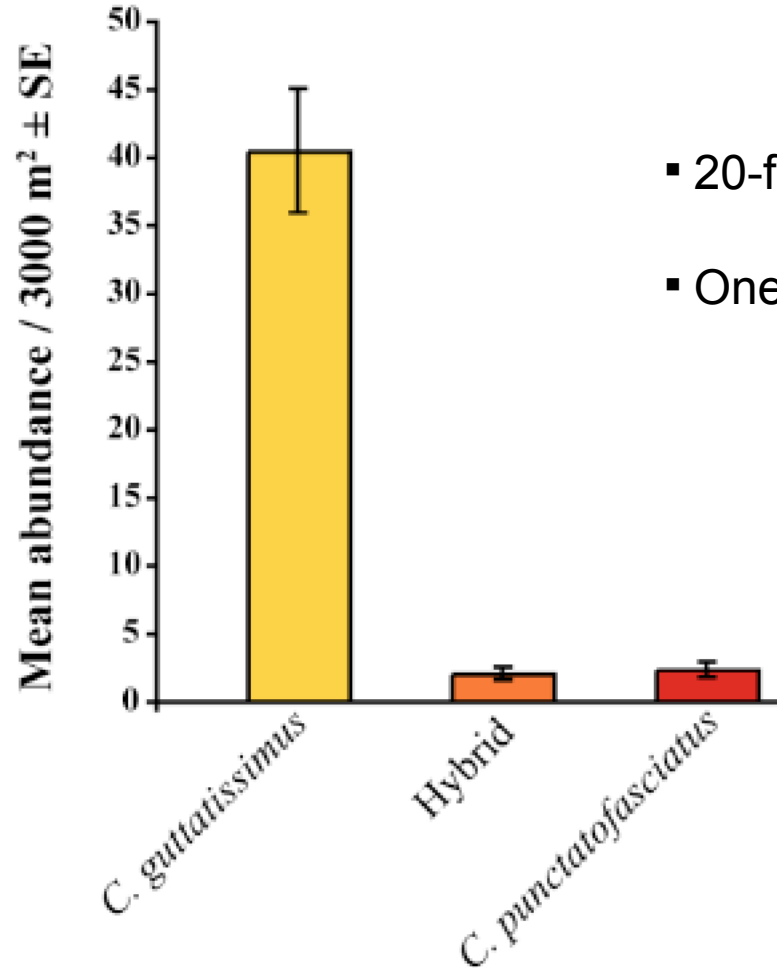
ANOVA, df=2, F=0.489, p=0.615

MANOVA, Pillai's Trace=0.715, hypothesis df=32, p=0.062



Abundance:

C. guttatissimus x *C. punctatofasciatus*



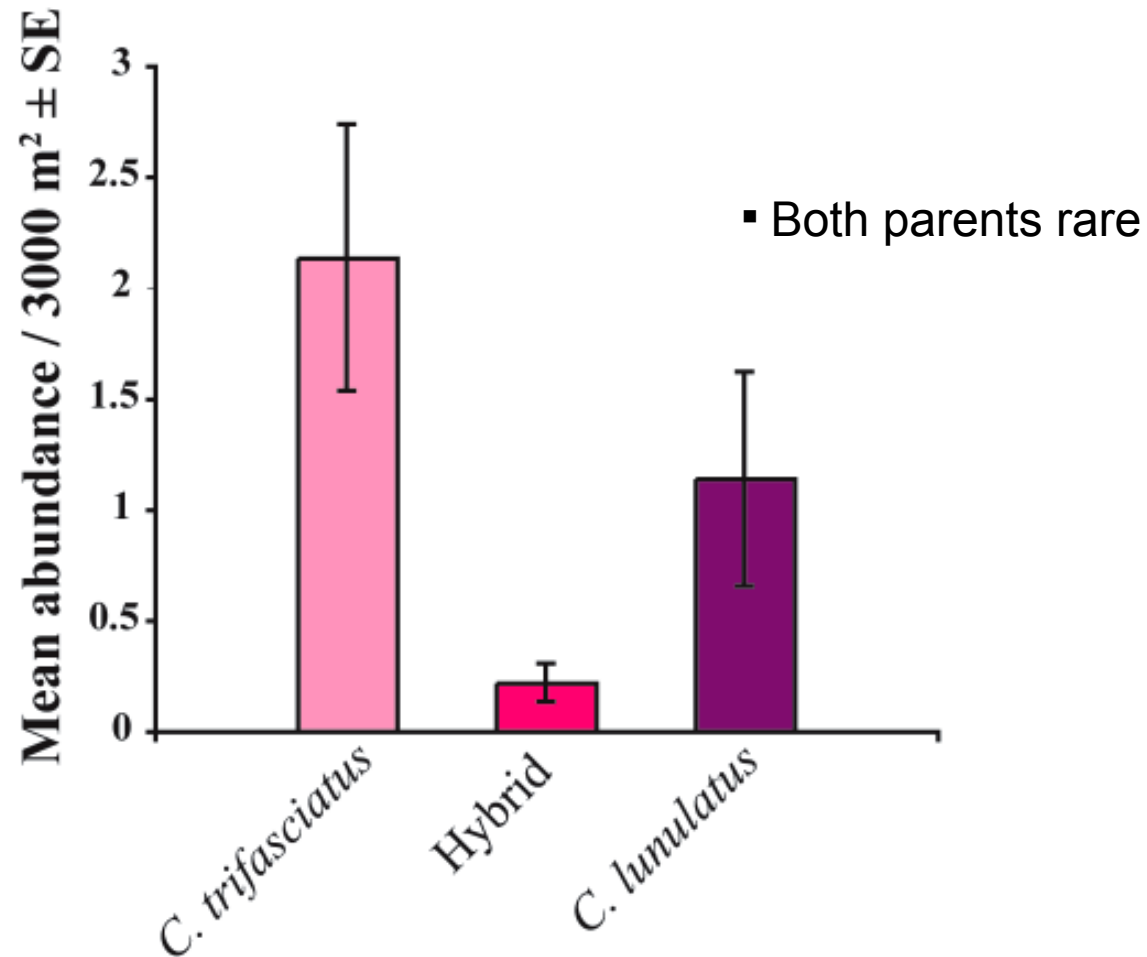
- 20-fold abundance disparity
- One parent rare

t-test, $t = 8.325$, $df = 13$, $p < 0.0001$



Abundance:

C. trifasciatus x *C. lunulatus*

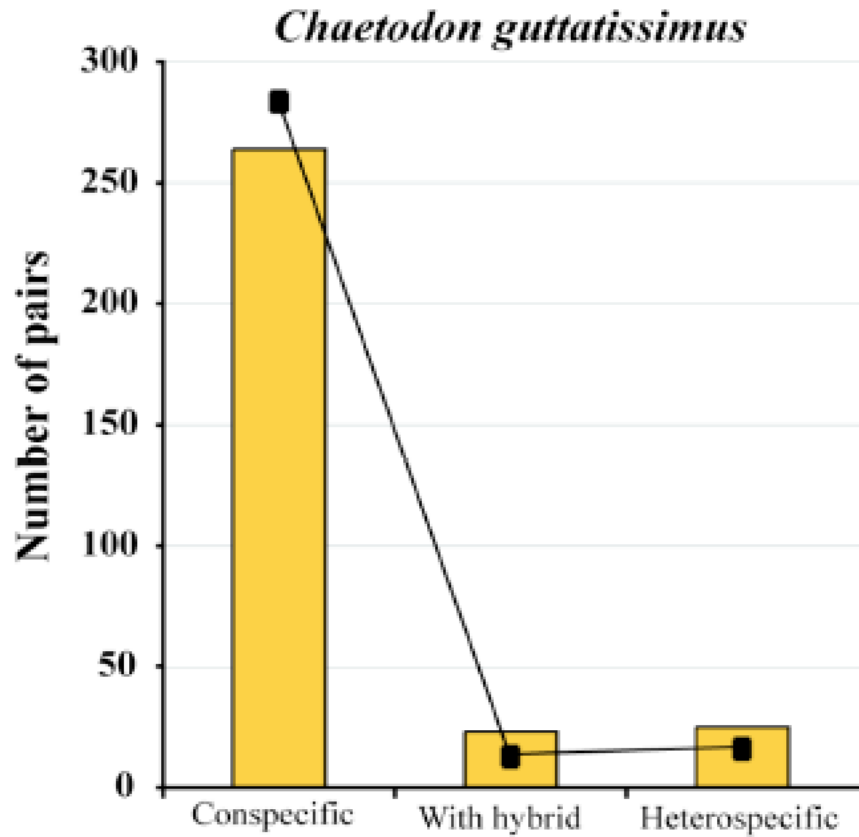


t-test, $t = 1.285$, $df = 38$, $p = 0.207$

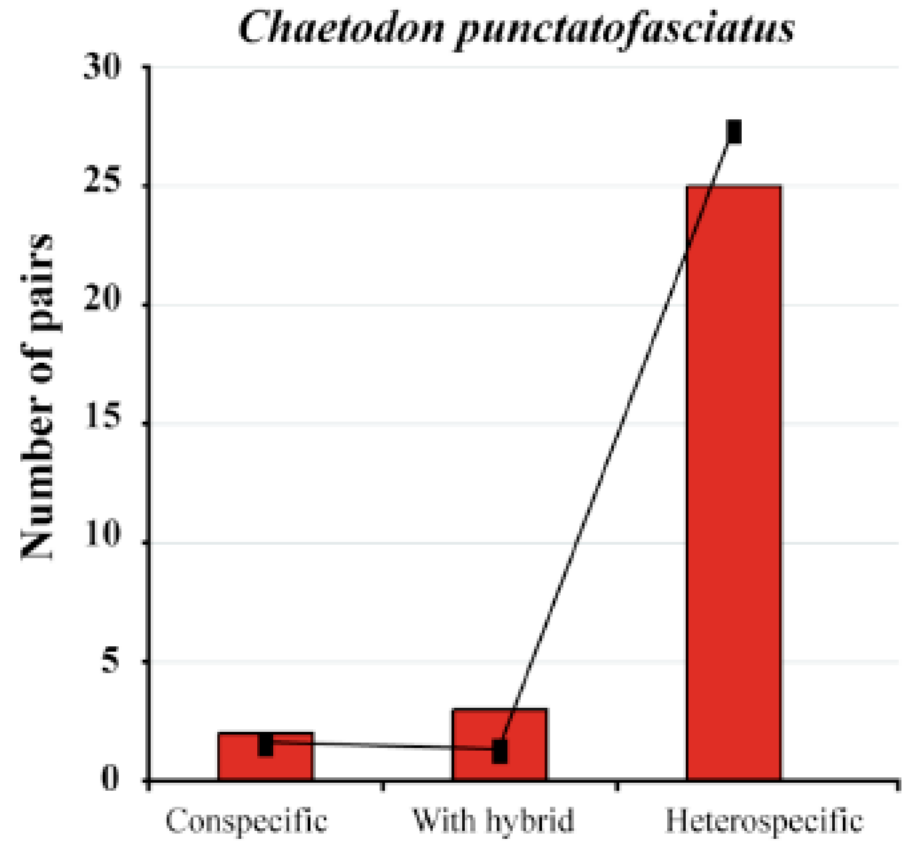


Pairing behaviour:

C. guttatissimus x *C. punctatofasciatus*



$\chi^2 = 14.91$, $df = 2$, $p < 0.001$

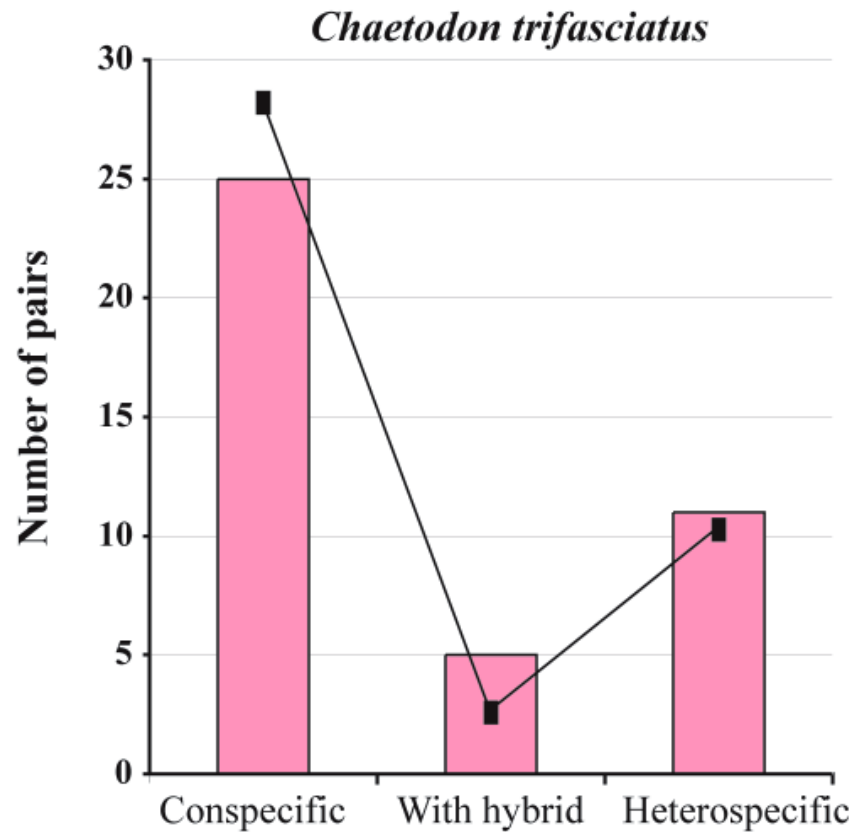
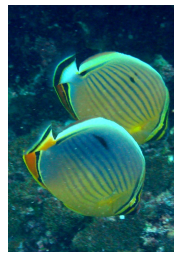


$\chi^2 = 2.89$, $df = 2$, $p = 0.24$

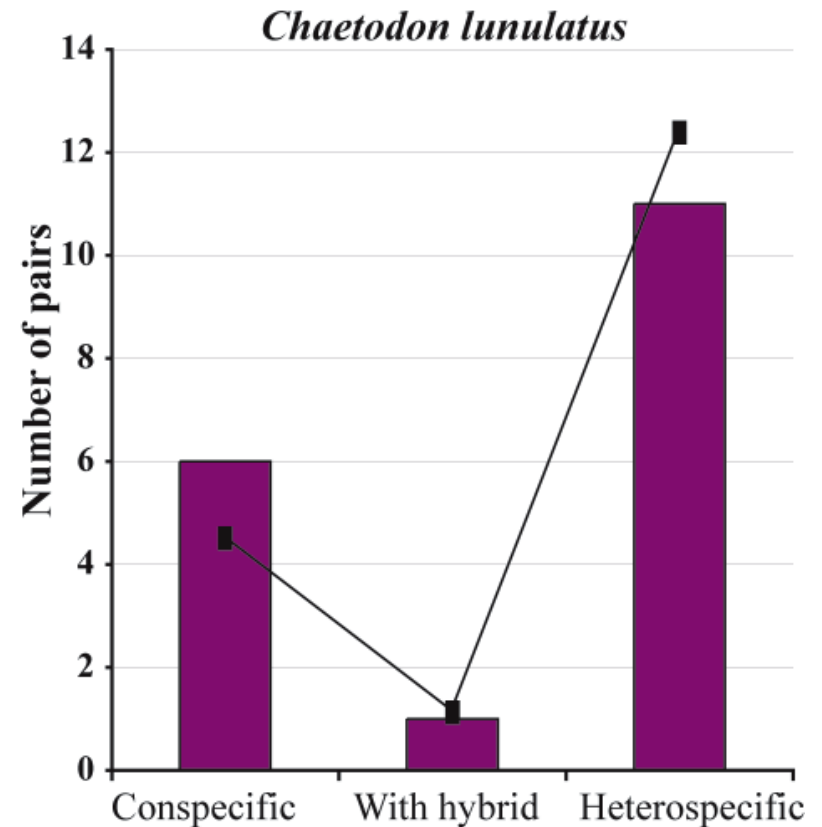


Pairing behaviour:

C. trifasciatus x *C. lunulatus*

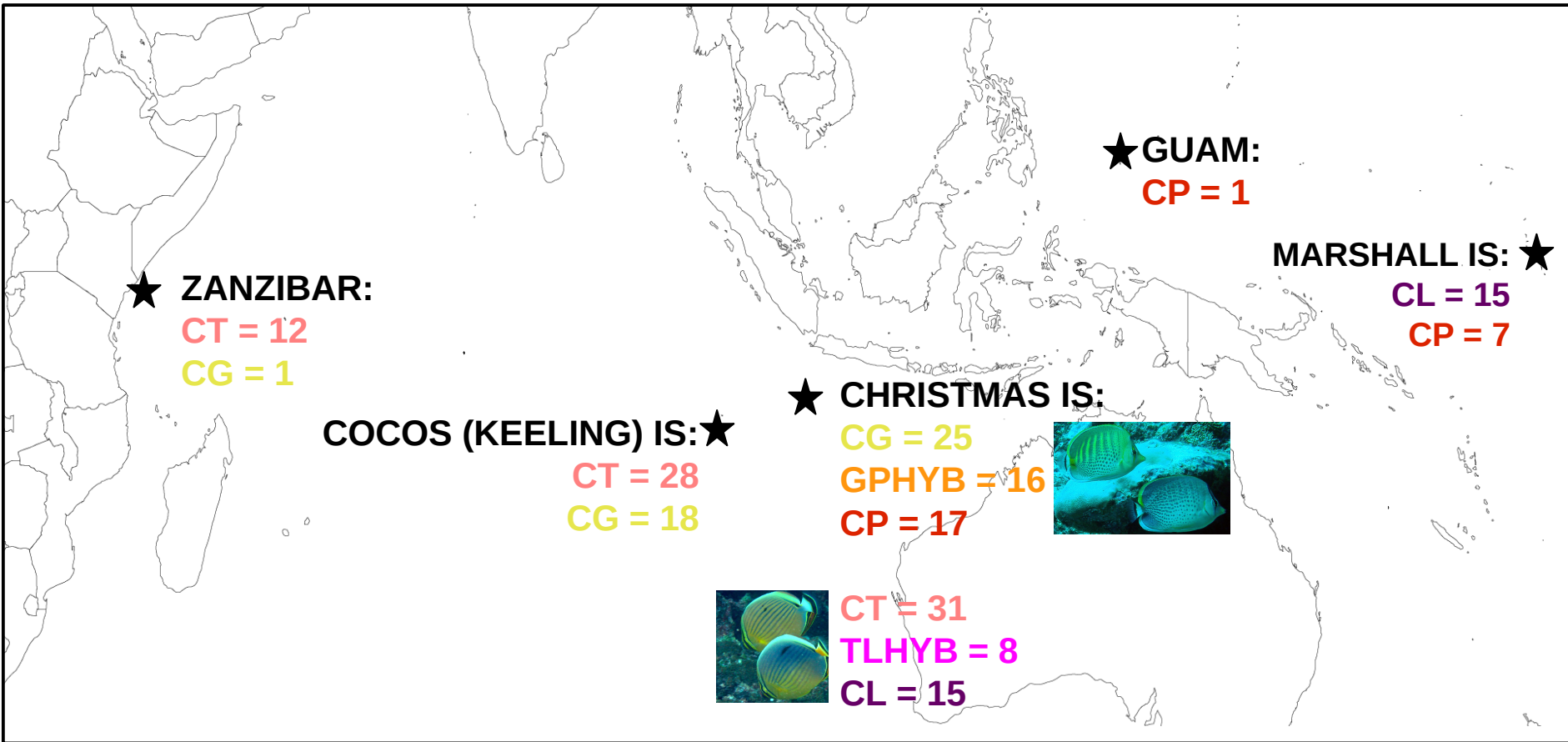


$\chi^2=2.73$, $df=2$, $p = 0.26$



$\chi^2=0.67$, $df=2$, $p = 0.71$

Molecular genetics: methodology



Mitochondrial DNA:

Partial *cyt b* (McMillan and Palumbi 1995)

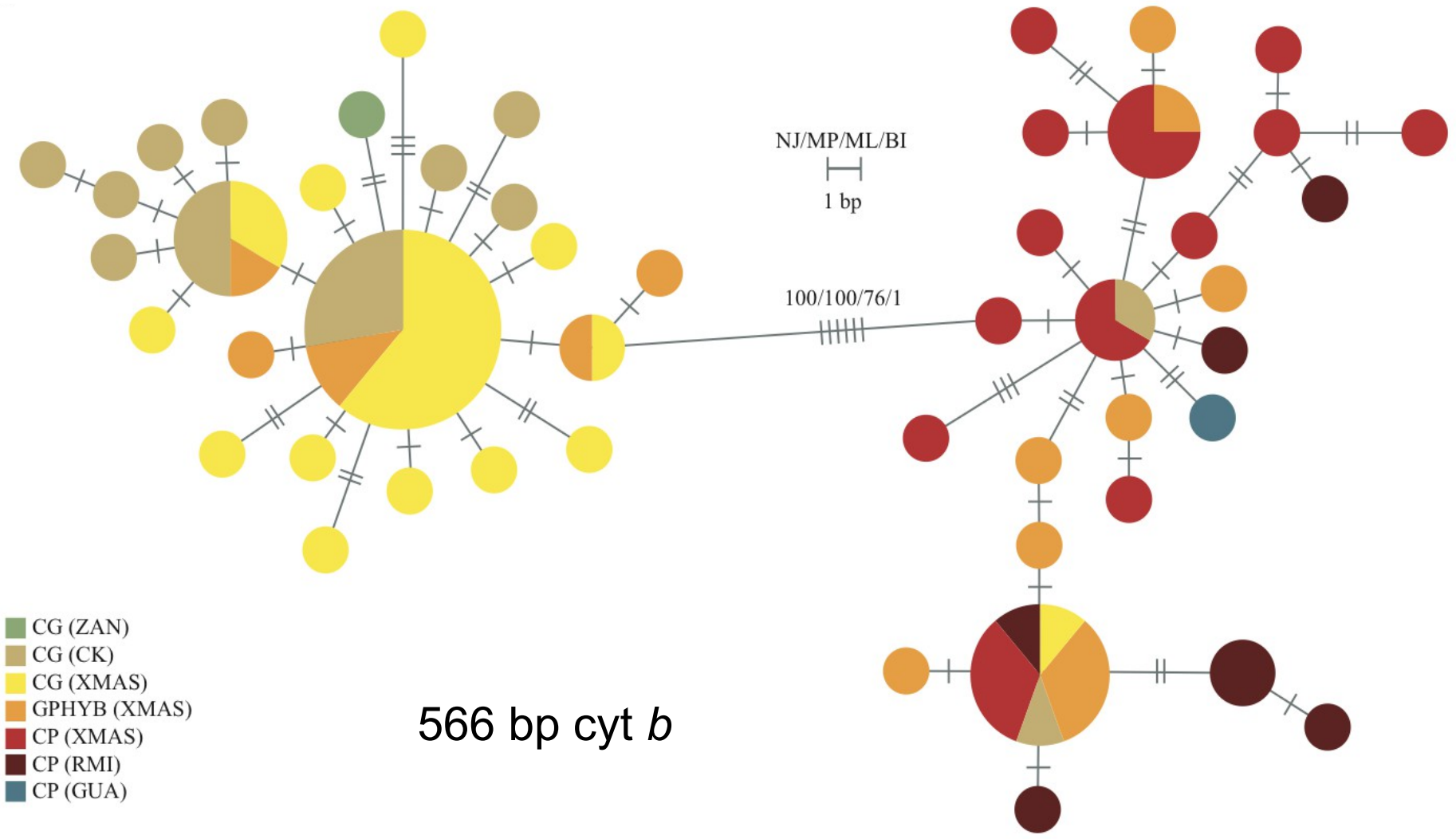
Nuclear DNA:

12 msat markers for *C. lunulatus*; 20 msat markers for *C. punctatofasciatus*
(Lawton et al. 2009) (Montanari et al. 2013)



mtDNA:

C. guttatissimus x *C. punctatofasciatus*

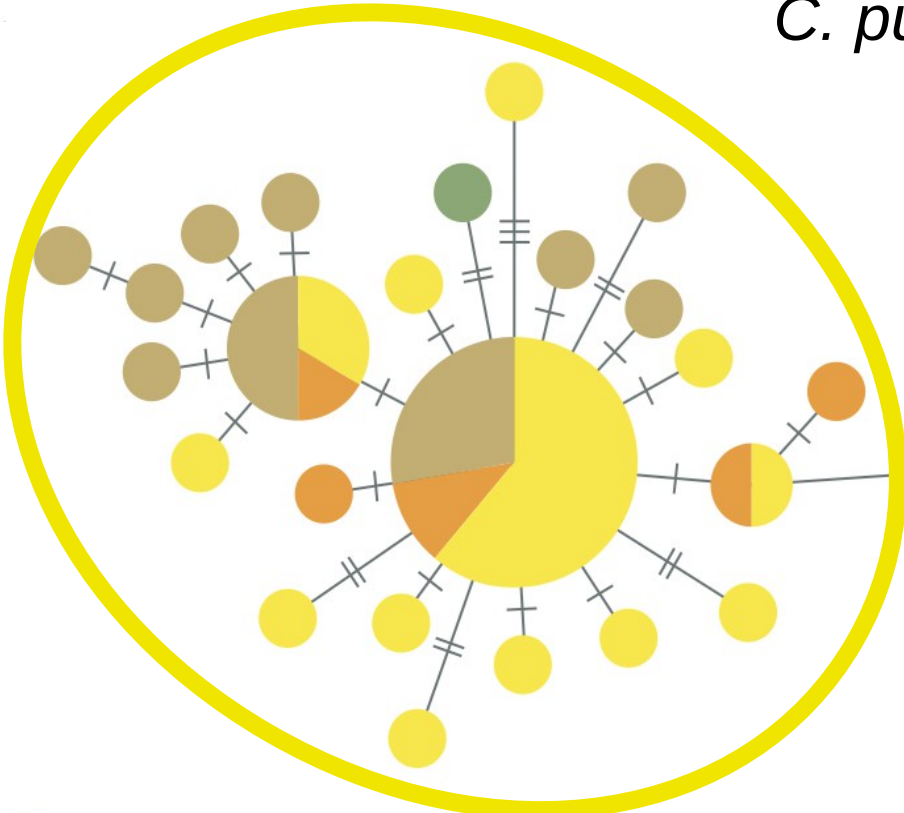




mtDNA:

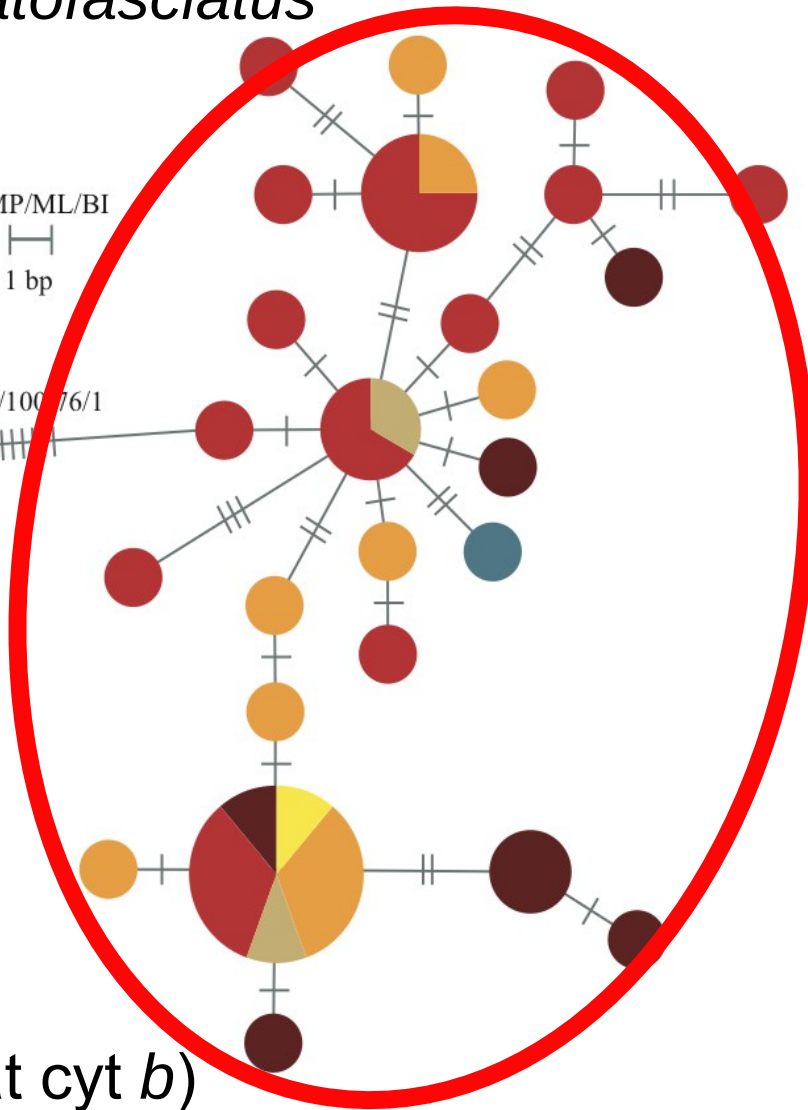
C. guttatissimus x *C. punctatofasciatus*

C. punctatofasciatus



NJ/MP/ML/BI
1 bp

100/100/76/1



C. guttatissimus

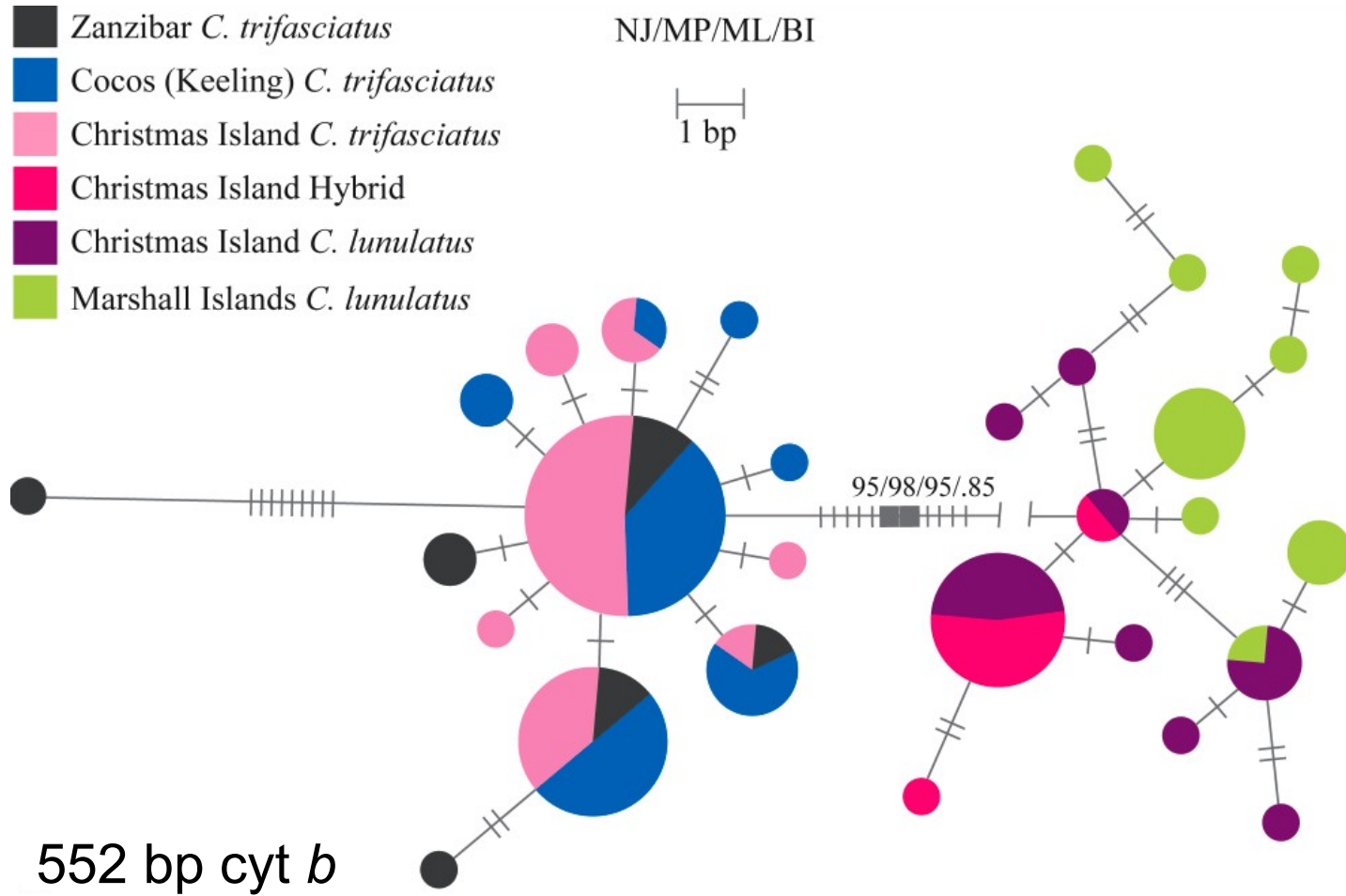
- CG (ZAN)
- CG (CK)
- CG (XMAS)
- GPHYB (XMAS)
- CP (XMAS)
- CP (RMI)
- CP (GUA)

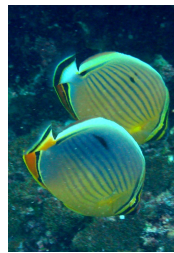
Clear parental clades (1% at *cyt b*)



mtDNA:

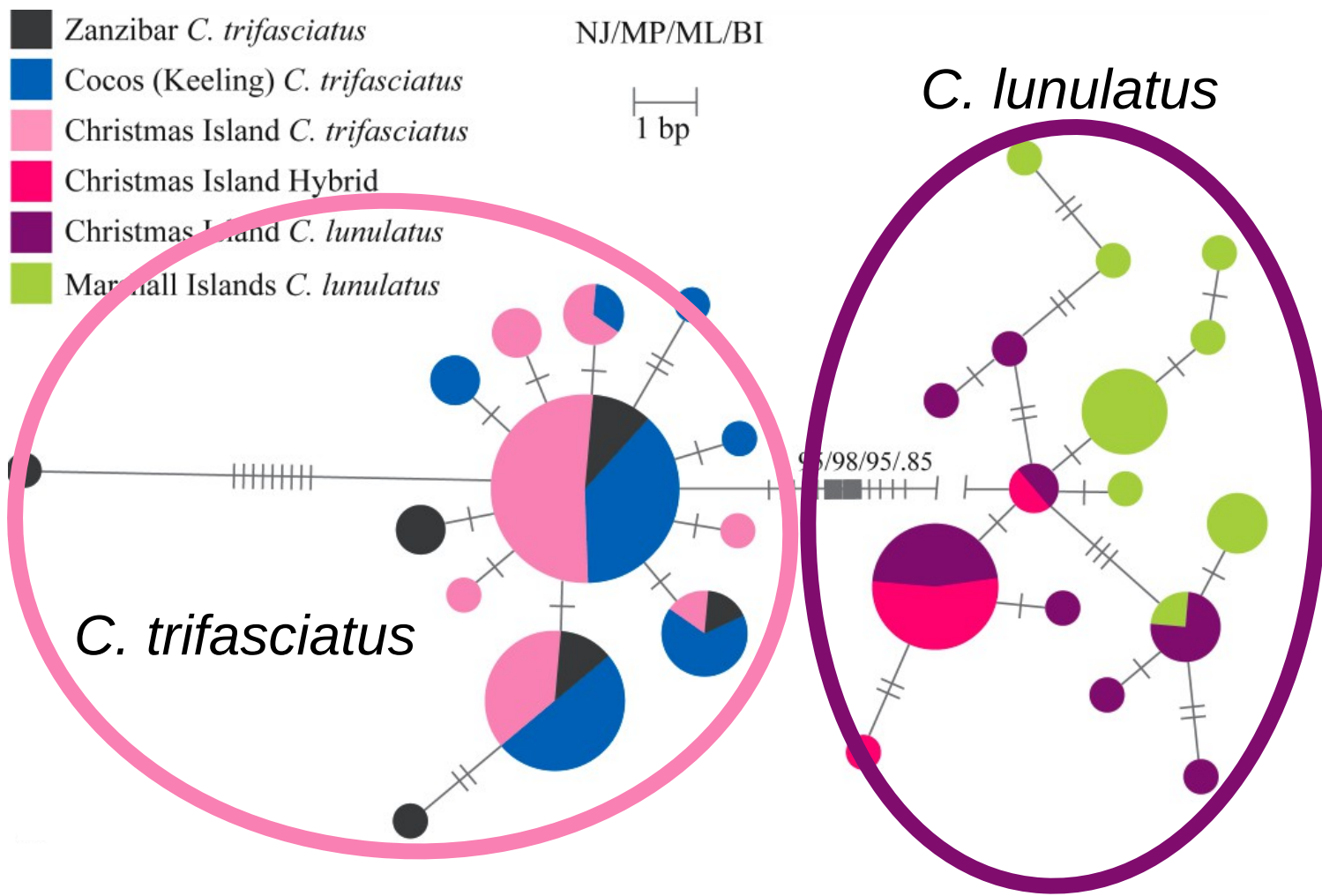
C. trifasciatus x *C. lunulatus*





mtDNA:

C. trifasciatus x *C. lunulatus*

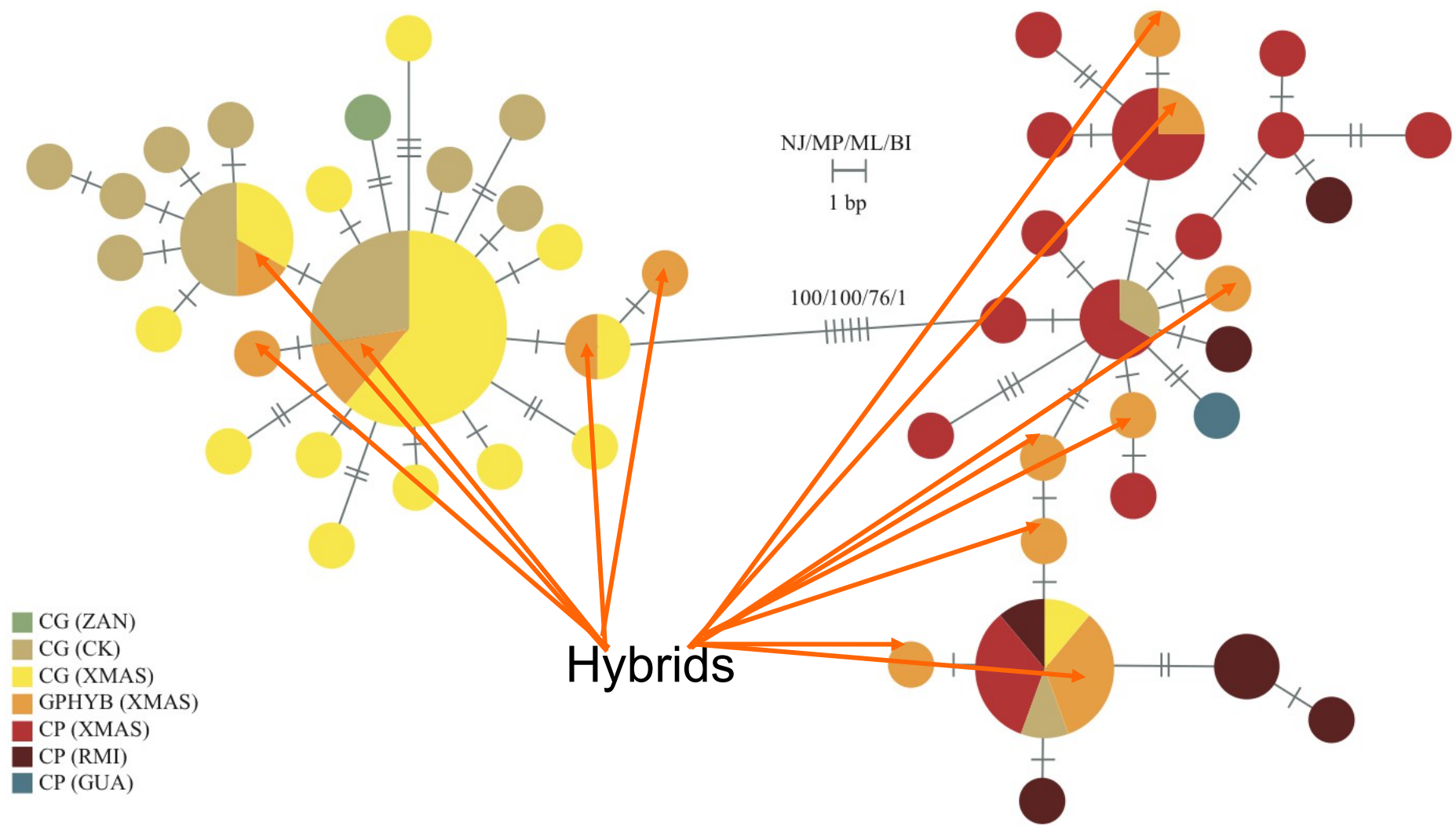


Clear parental clades (5% at *cyt b*)



mtDNA:

C. guttatissimus x *C. punctatofasciatus*

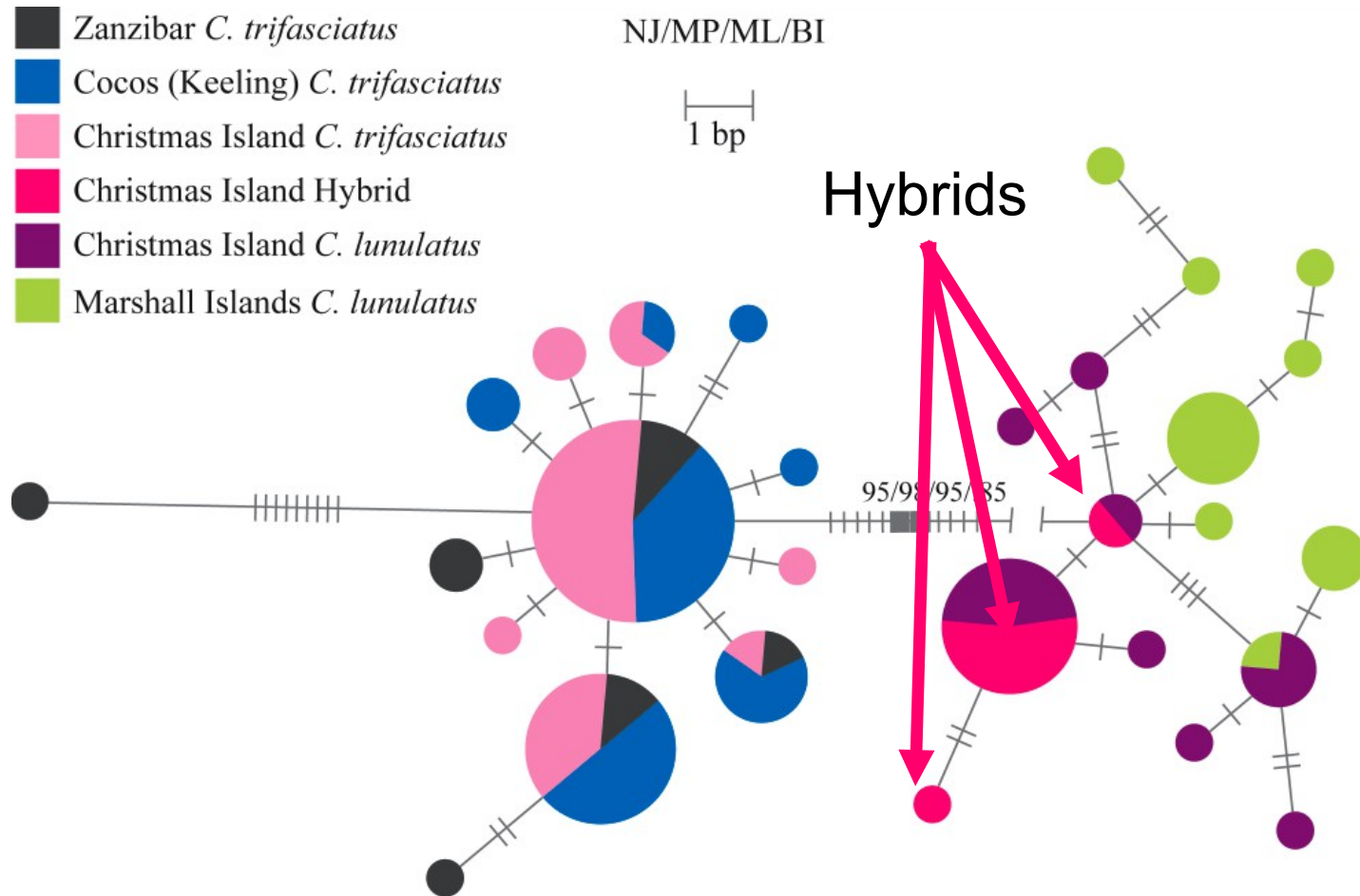


Hybrids in both clades: bidirectional maternity



mtDNA:

C. trifasciatus x *C. lunulatus*

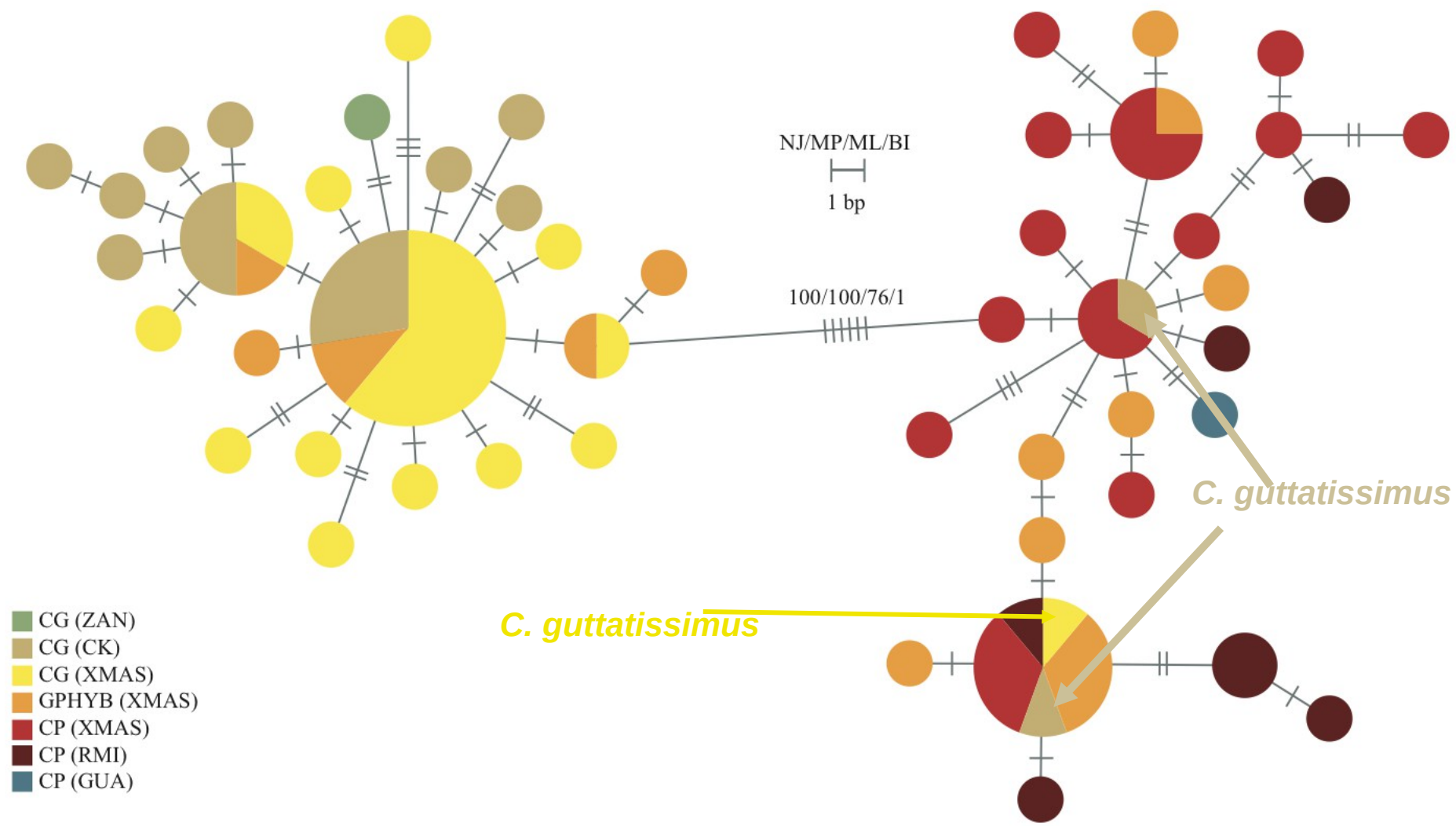


Hybrids in one clade only: unidirectional maternity

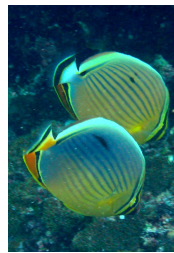


mtDNA:

C. guttatissimus x *C. punctatofasciatus*

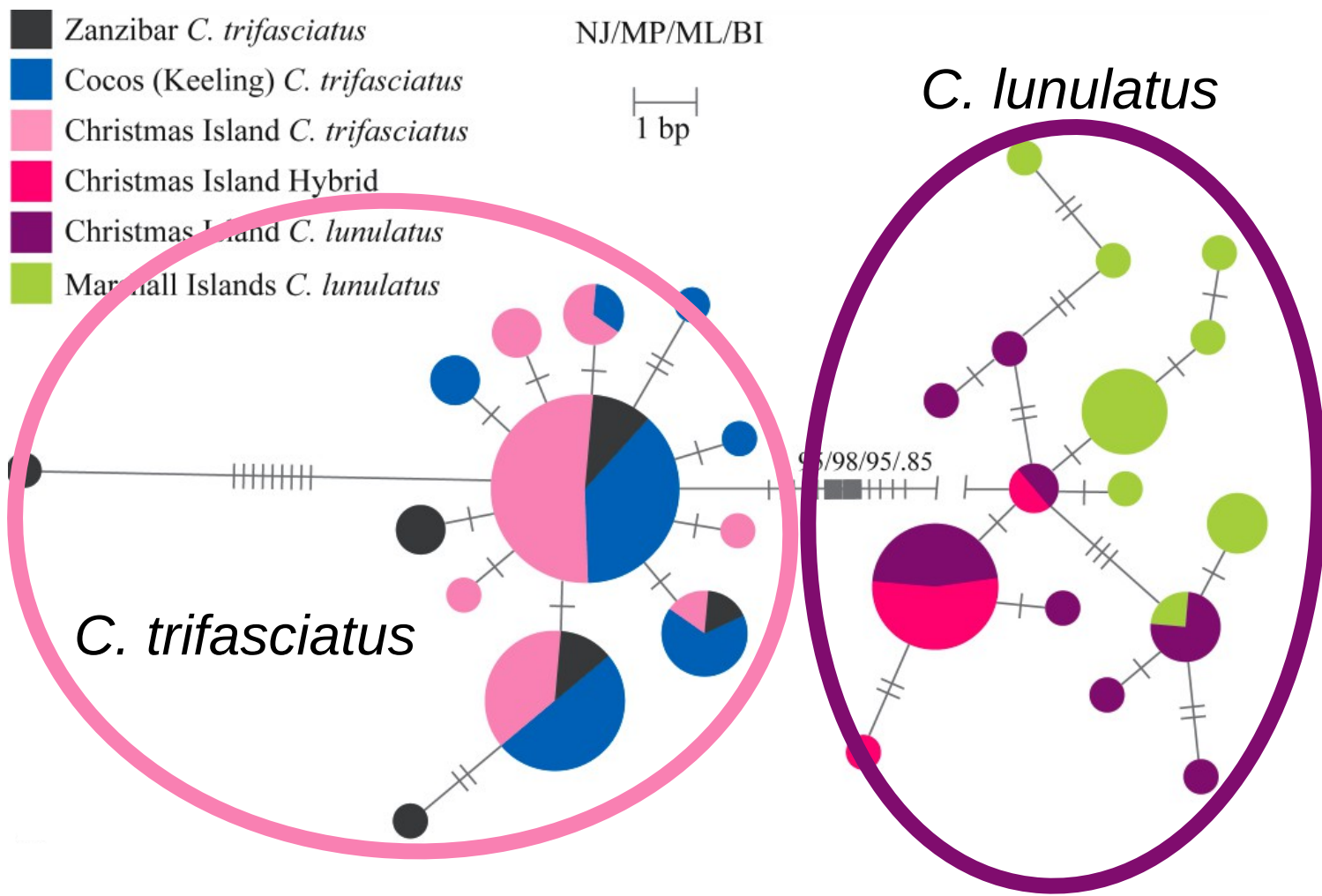


Evidence of introgression: in and out of hybrid zone



mtDNA:

C. trifasciatus x *C. lunulatus*

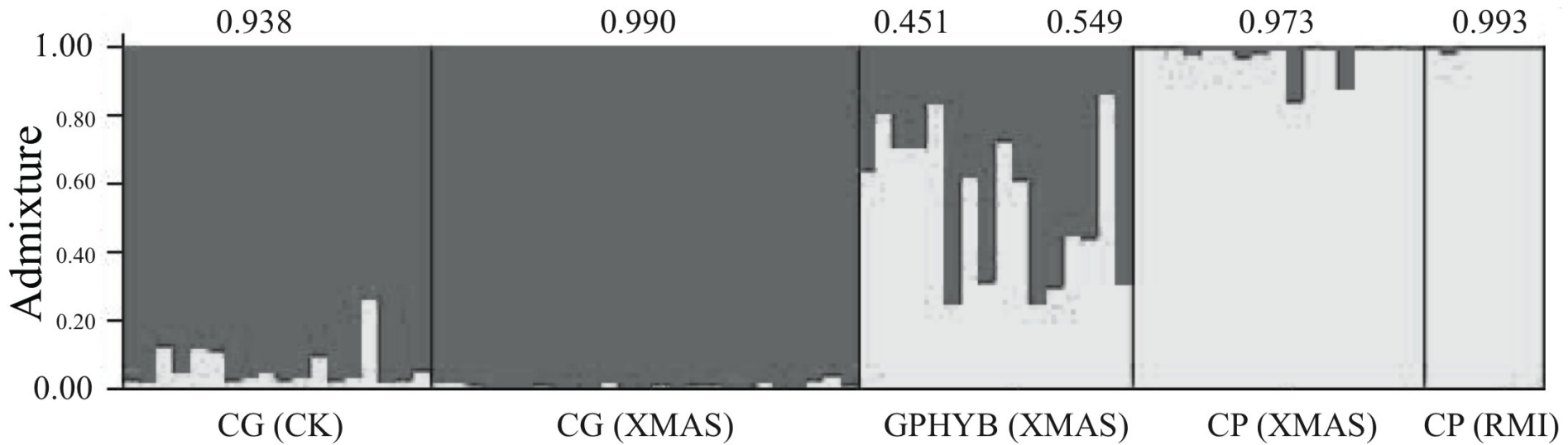


No evidence of introgression



Nuclear msatDNA:

C. guttatissimus x *C. punctatofasciatus*

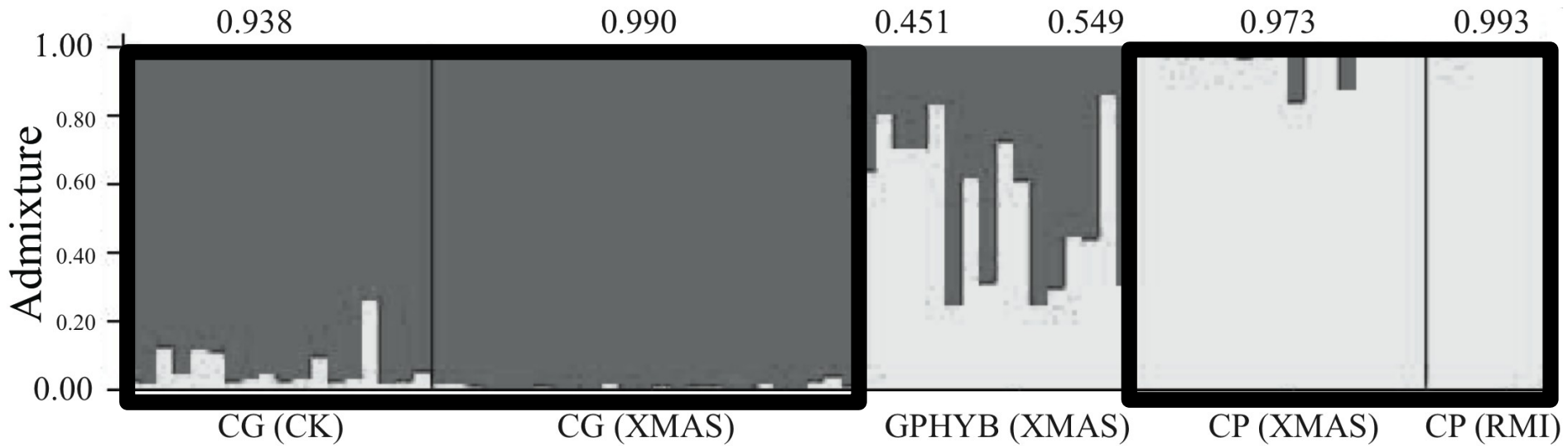


- STRUCTURE: (Q plot)
- 20 msat loci
- k = 2 clusters



Nuclear msatDNA:

C. guttatissimus x *C. punctatofasciatus*

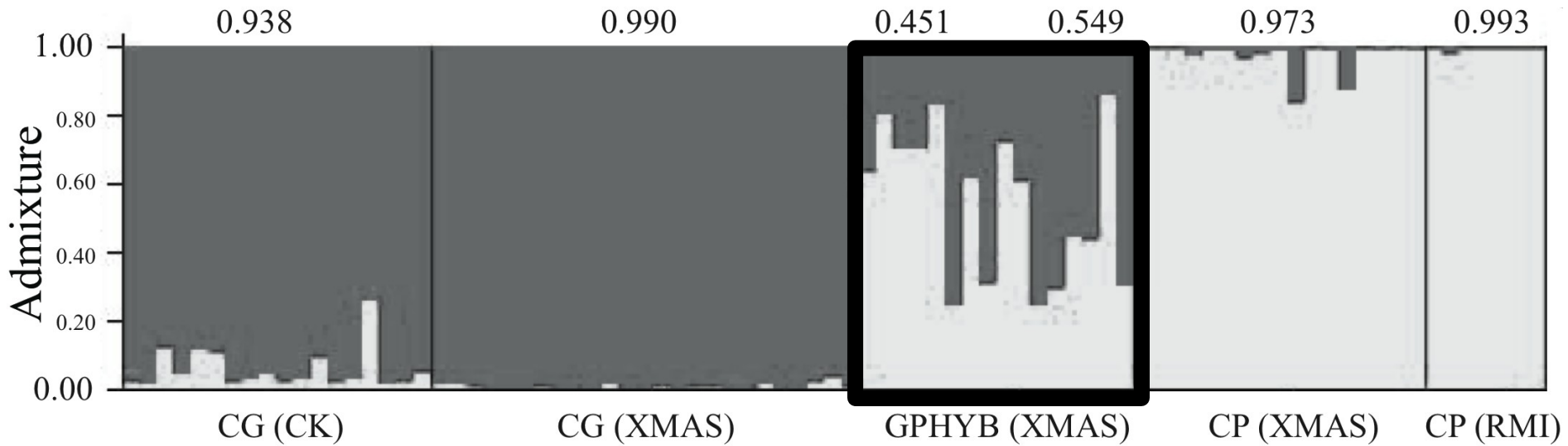


- 2 clusters correspond to parental species
- species-level: little admixture (< 6.2%)

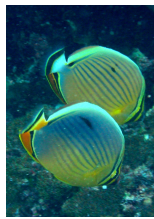


Nuclear msatDNA:

C. guttatissimus x *C. punctatofasciatus*

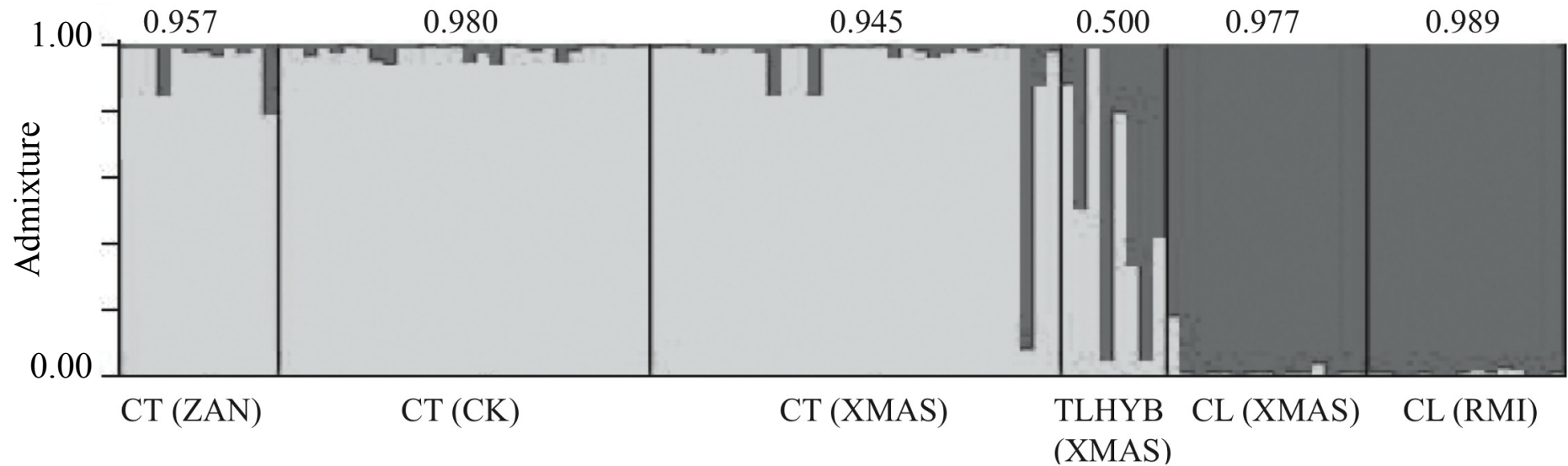


- hybrids belong to either cluster
- overall, a mix between parental clusters
- not enough power to discriminate classes

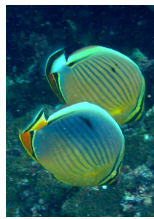


Nuclear msatDNA:

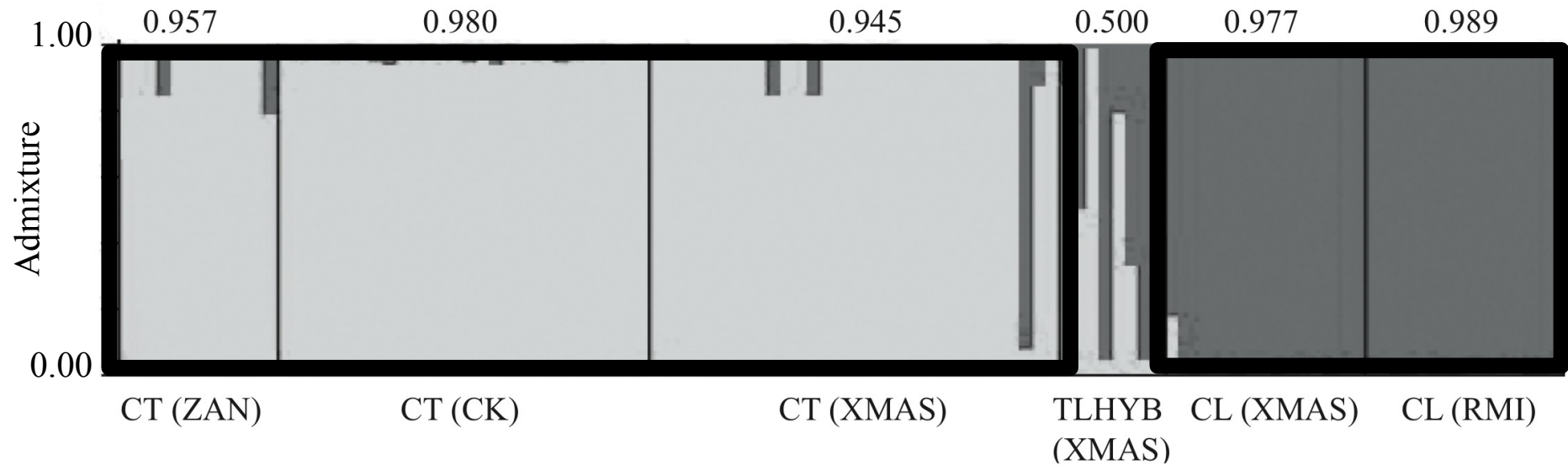
C. trifasciatus x *C. lunulatus*



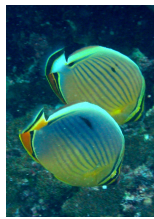
- STRUCTURE: (Q plot)
- 12 msat loci
- $k = 2$ clusters



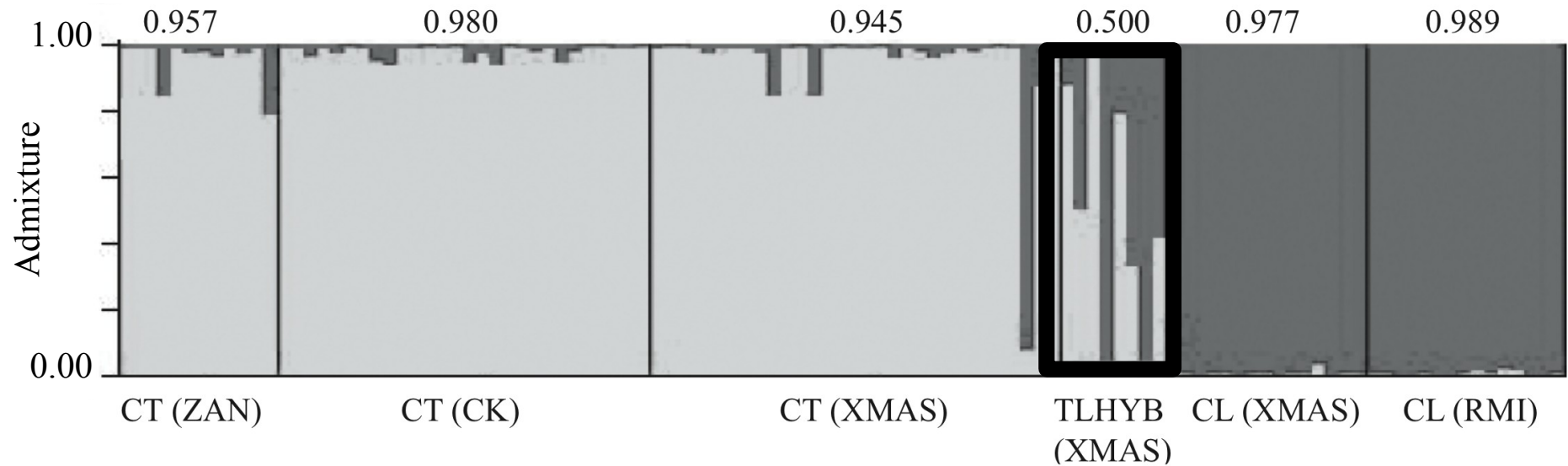
Nuclear msatDNA: *C. trifasciatus* x *C. lunulatus*



- 2 clusters correspond to parental species
- species-level: little admixture (< 5.5%)



Nuclear msatDNA: *C. trifasciatus* x *C. lunulatus*

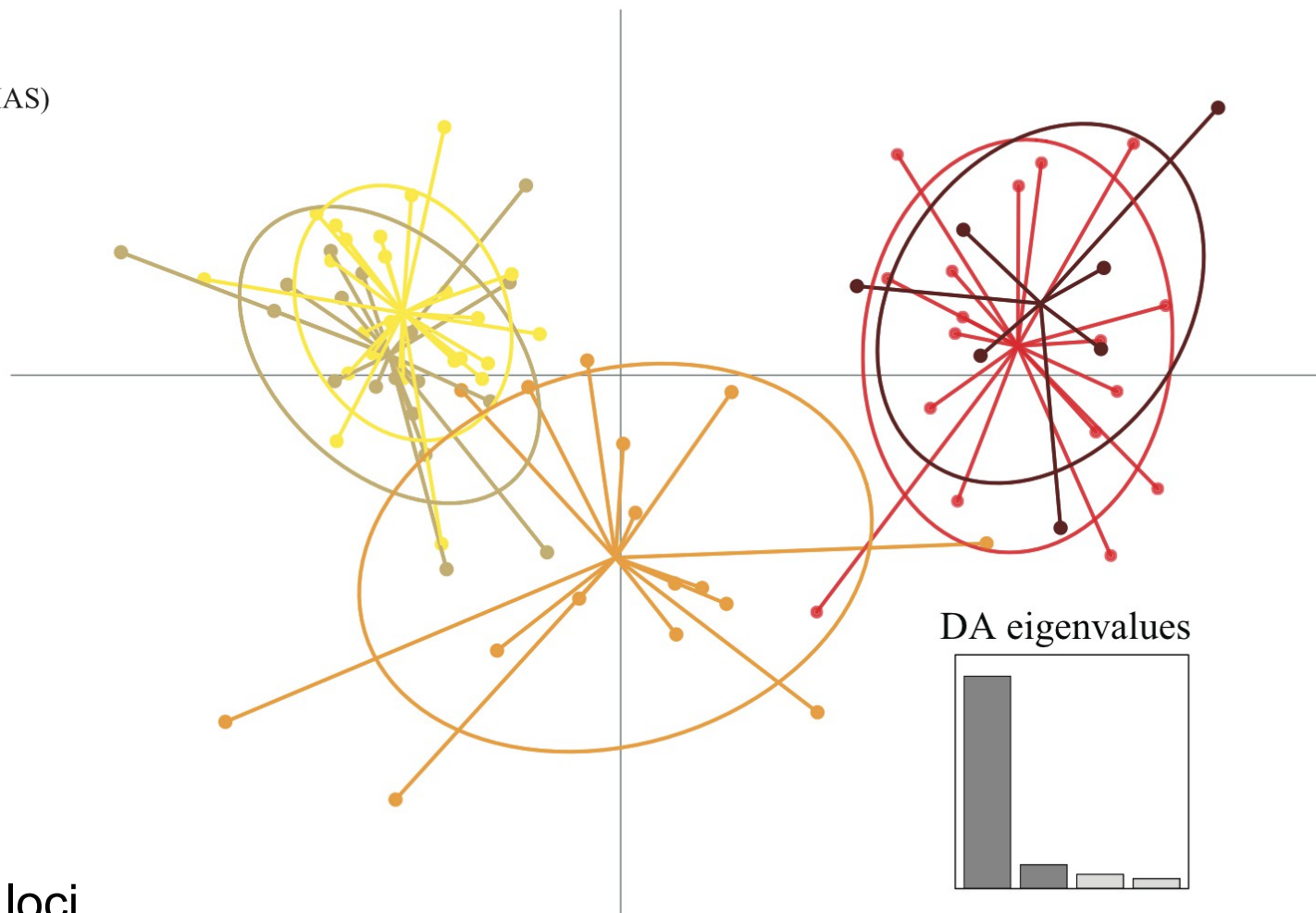


- hybrids belong to either cluster
- 50% admixture
- cannot distinguish classes



Nuclear msatDNA:

C. guttatissimus x *C. punctatofasciatus*



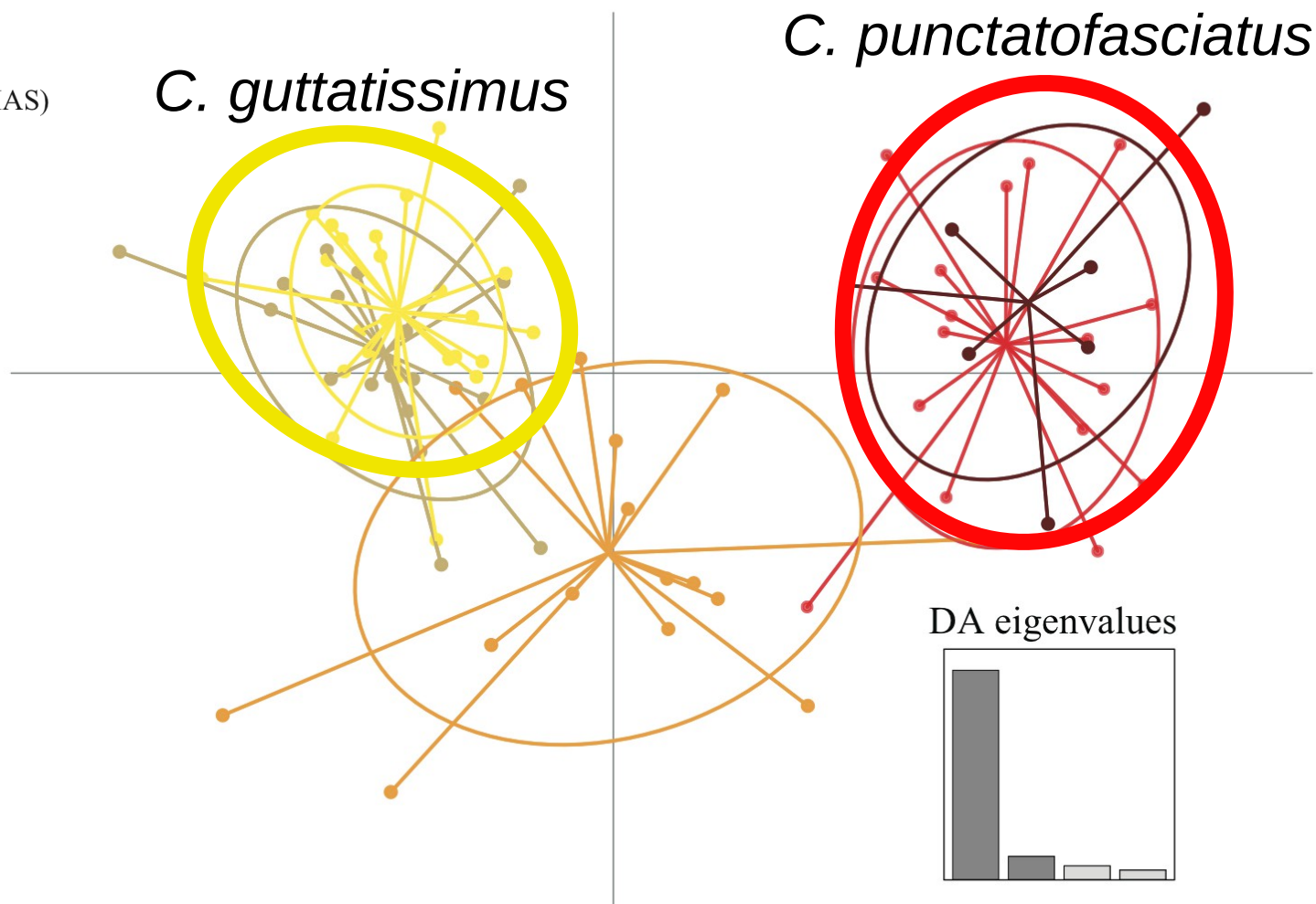
DAPC:

- 20 msat loci
- 27 PCs: 65% genetic variability retained



Nuclear msatDNA:

C. guttatissimus x *C. punctatofasciatus*

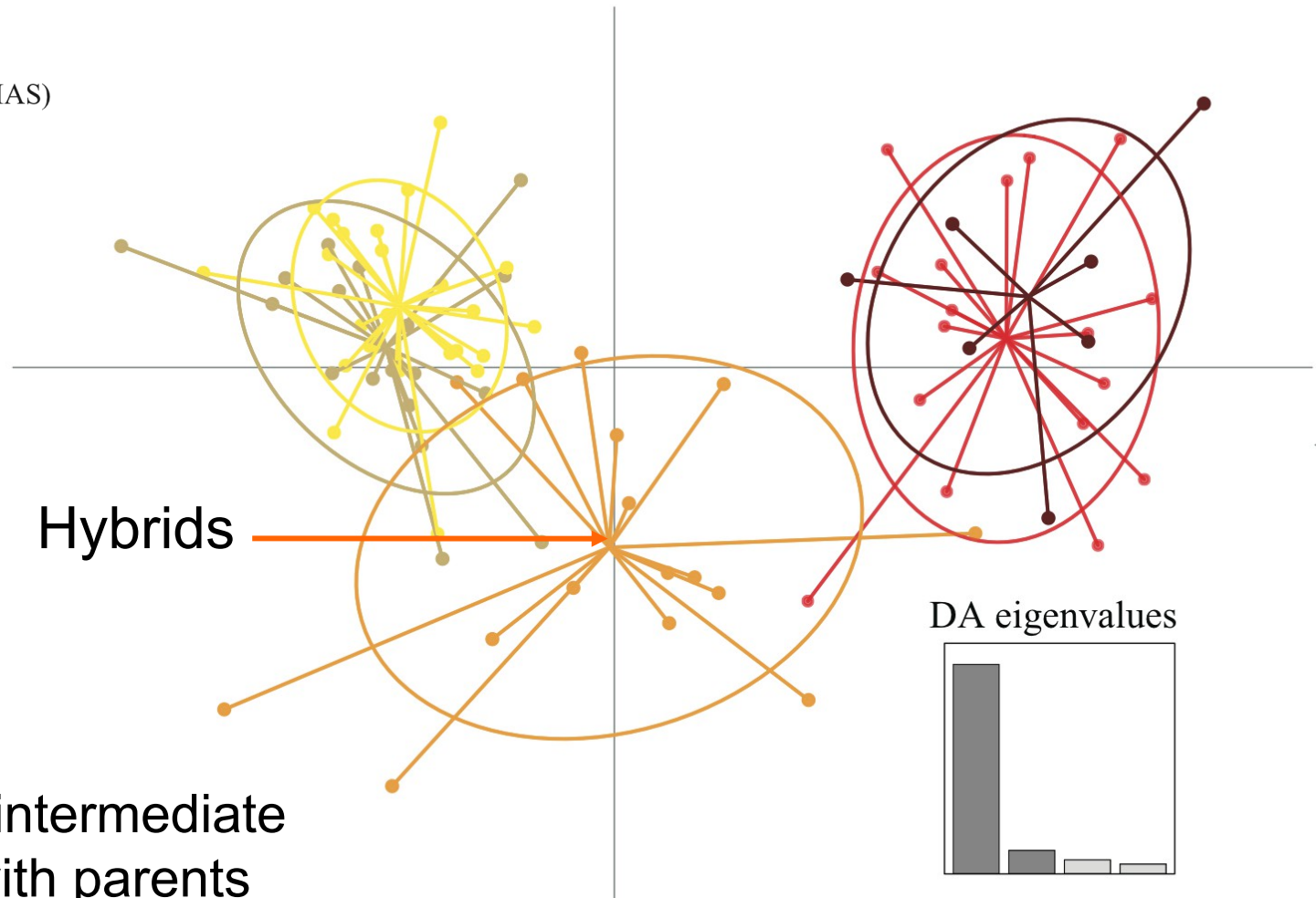


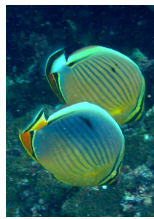
▪ parental species distinct



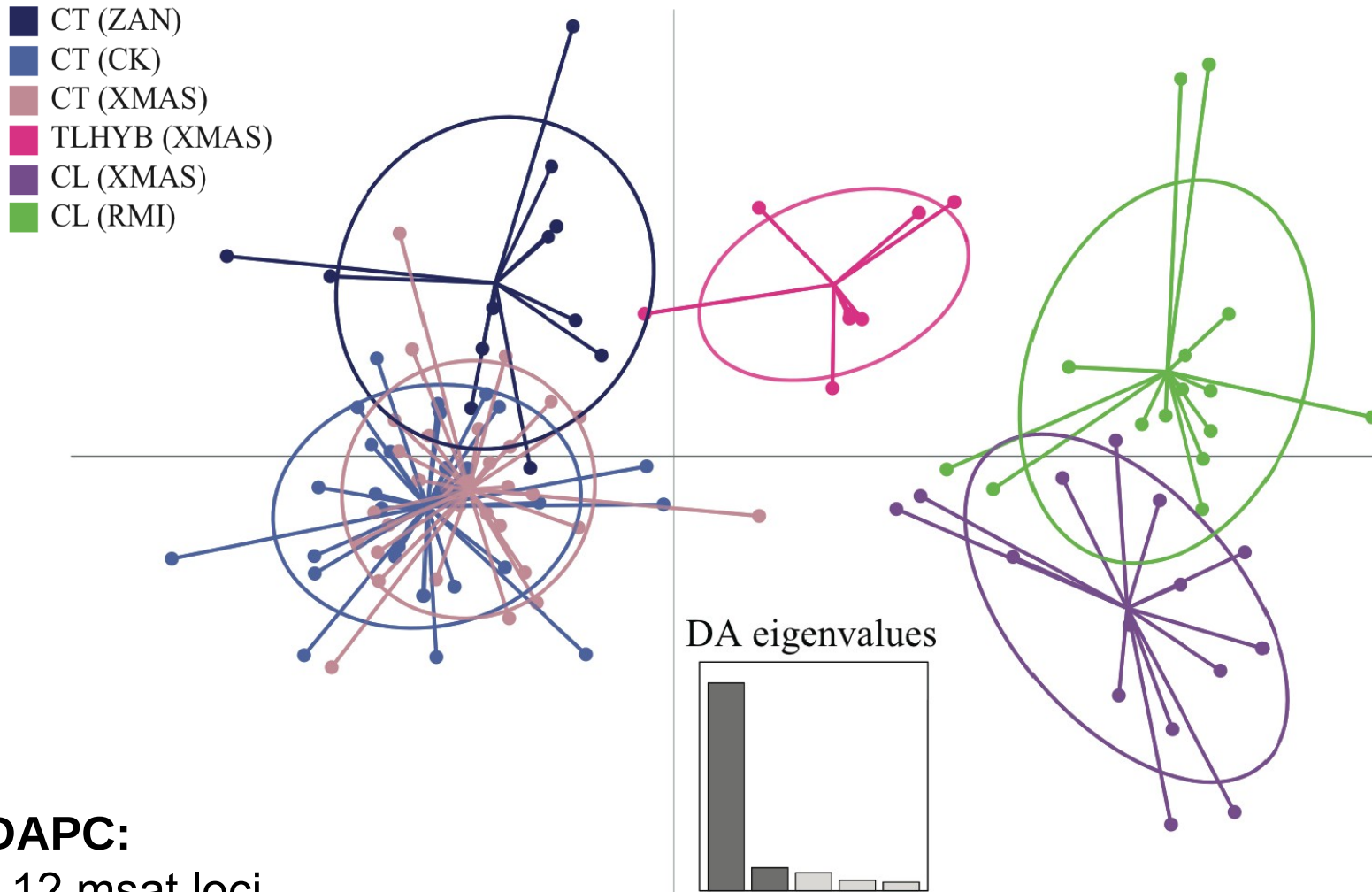
Nuclear msatDNA:

C. guttatissimus x *C. punctatofasciatus*



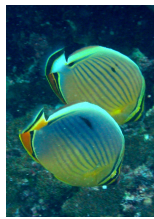


Nuclear msatDNA: *C. trifasciatus* x *C. lunulatus*

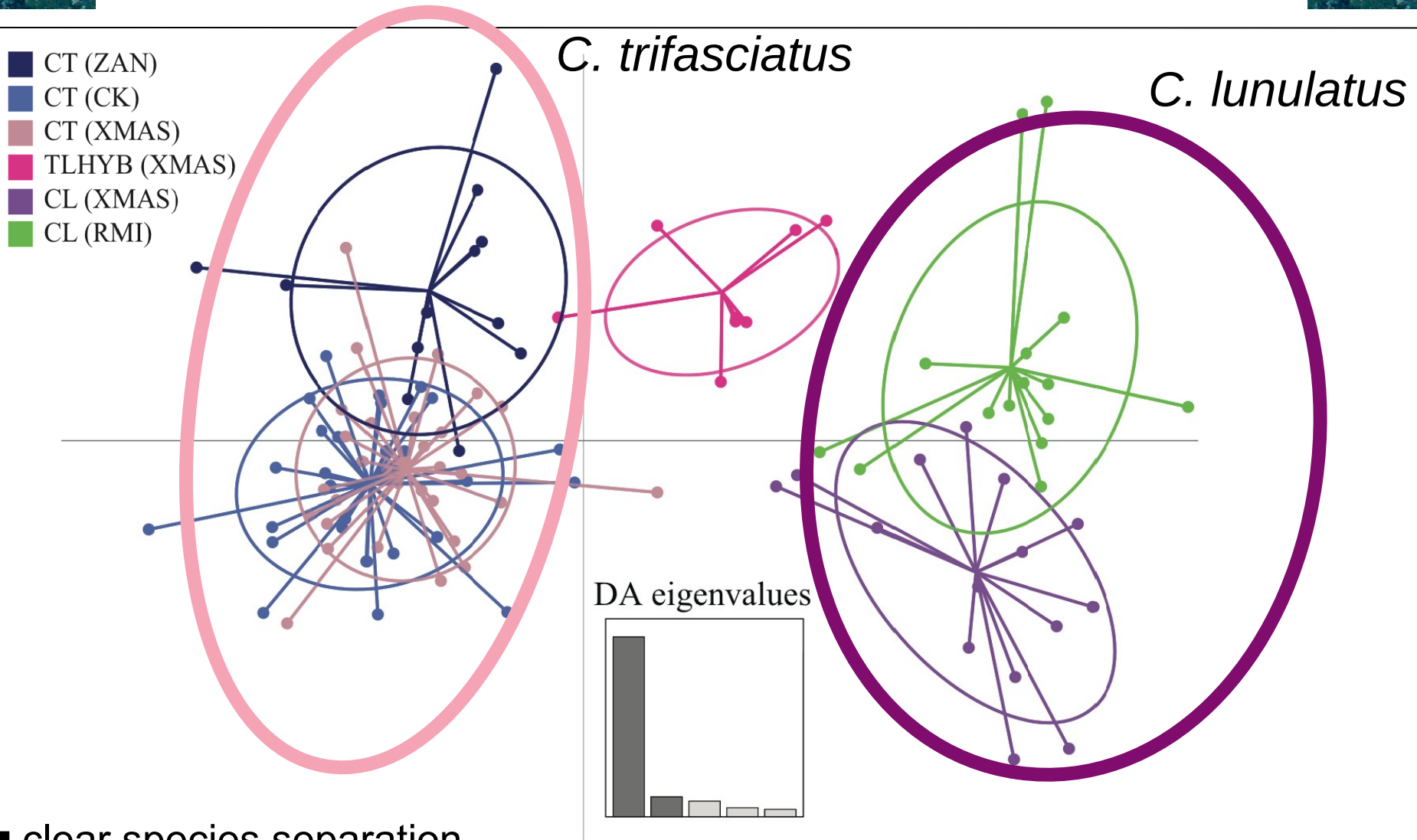


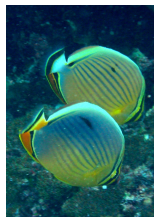
DAPC:

- 12 msat loci
- 36 PCs: 69% genetic variability retained

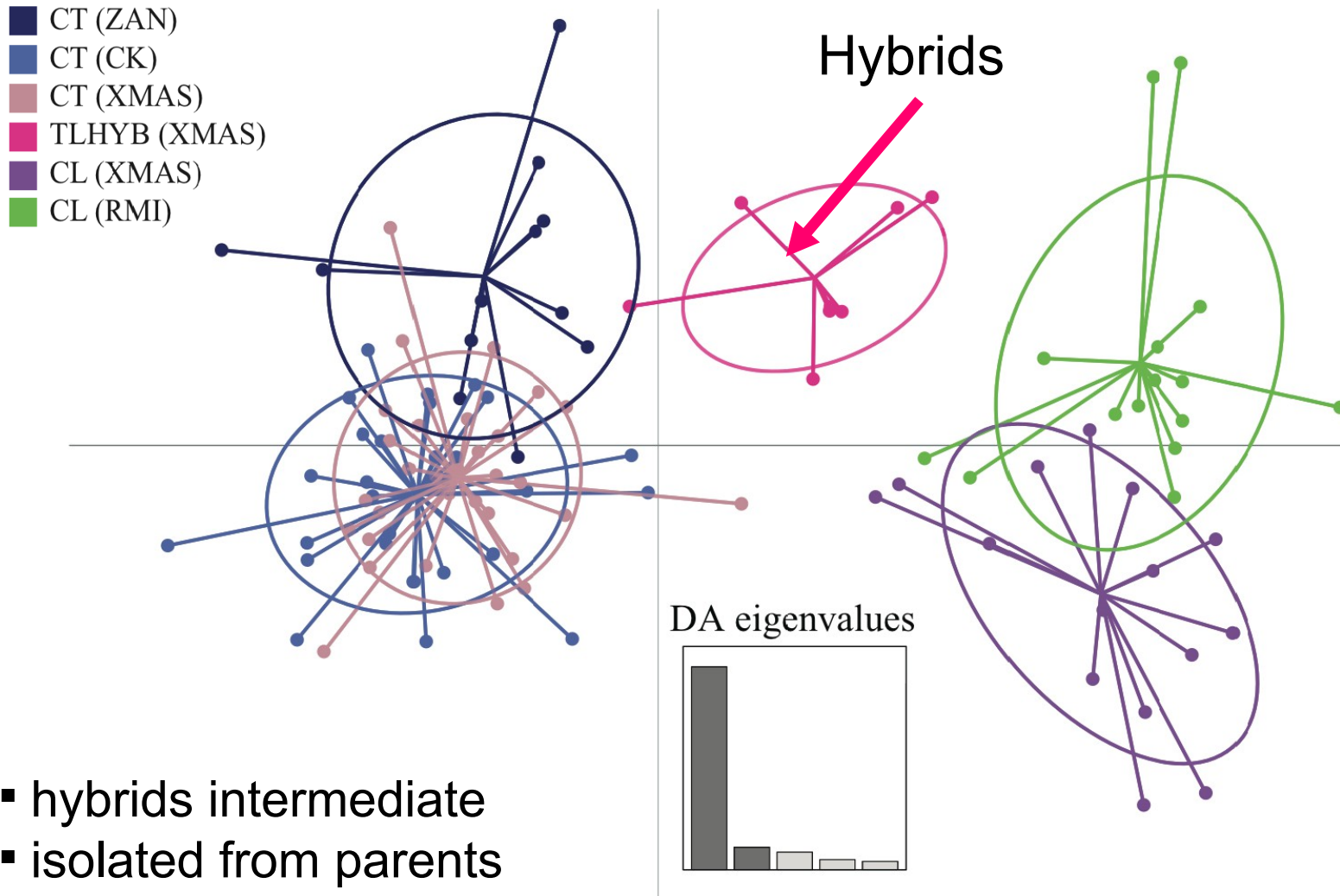


Nuclear msatDNA: *C. trifasciatus* x *C. lunulatus*





Nuclear msatDNA: *C. trifasciatus* x *C. lunulatus*



Conclusions

Similar ecological frameworks

- niche overlap
- rarity of parents
- non-assortative mating



Differences in genetics

- maternal inheritance
- introgression
- hybrid classes
- barriers to backcrossing



=> Hybridisation influenced by genetic break



Another mixed pair from Christmas Island?

Population	n	nh	<i>h</i>	π
Cocos Is. <i>C. guttatissimus</i>	18	14	0.95	0.007
Christmas Is. <i>C. guttatissimus</i>	25	19	0.93	0.004
Christmas Is. Hybrid	16	15	0.99	0.011
Christmas Is. <i>C. punctatofasciatus</i>	18	14	0.97	0.006
Marshall Is. <i>C. punctatofasciatus</i>	7	7	1	0.008
Population	n	nh	<i>h</i>	π
Zanzibar <i>C. trifasciatus</i>	10	6	0.89	0.007
Cocos Is. <i>C. trifasciatus</i>	28	10	0.82	0.002
Christmas Is. <i>C. trifasciatus</i>	28	8	0.71	0.001
Christmas Is. Hybrid	9	4	0.58	0.001
Christmas Is. <i>C. lunulatus</i>	15	8	0.83	0.005

Diversity metrics comparable between 2 hybridising groups