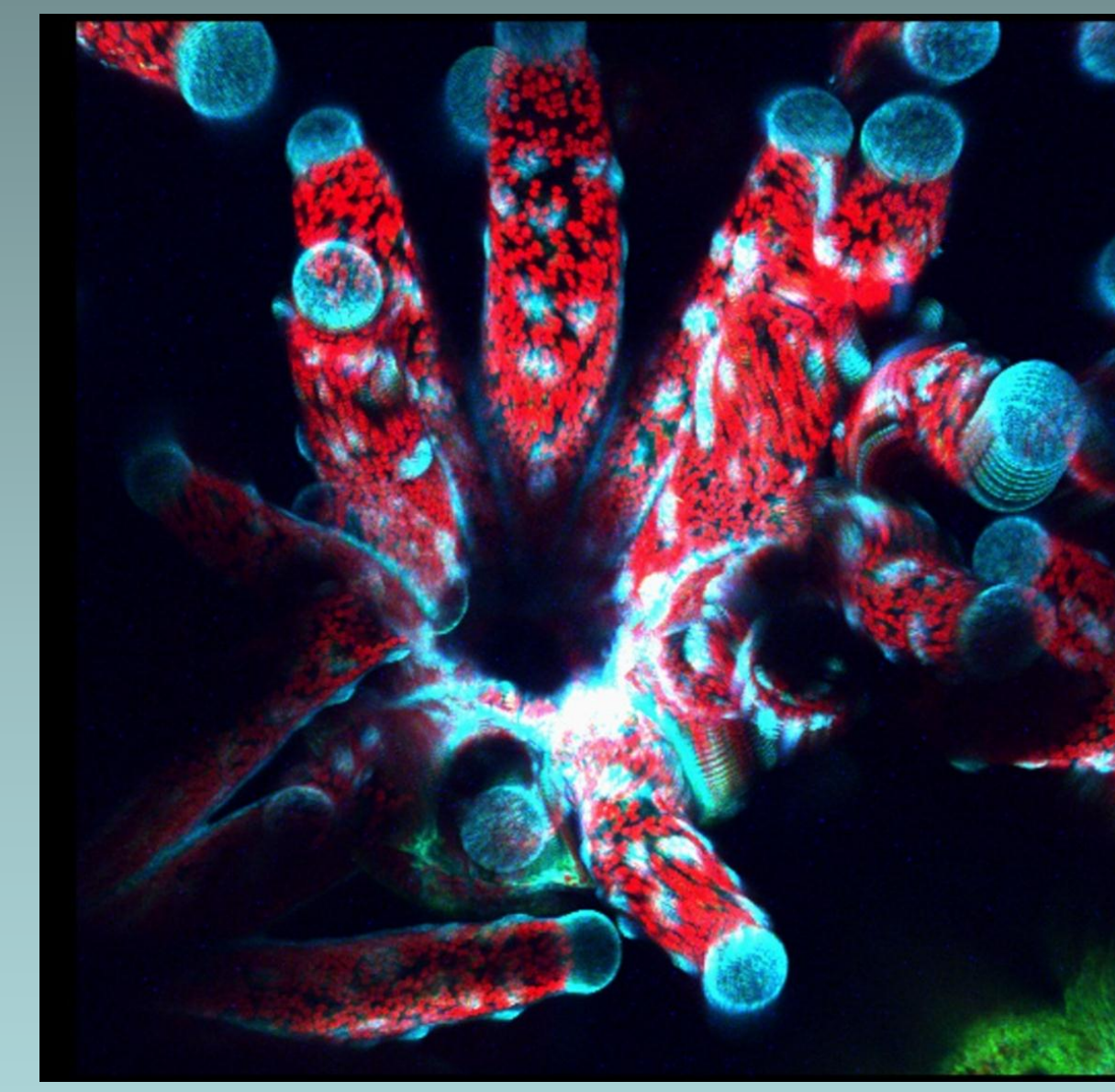




Barcoding corals in the genus *Pocillopora*: geographically restricted cryptic diversity



Living *Pocillopora damicornis* colony autofluorescing under a laser confocal microscope
Image credit: Zac Forsman & Chris Farrar

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ABSTRACT

BACKGROUND:

Identifying coral species boundaries is challenging particularly in *Pocillopora*, a coral genus known to be highly phenotypically plastic.

METHODS:

In this study we barcode 98 samples from Moorea with the mitochondrial ORF marker, and construct a haplotype network of all available ORF sequences.

RESULTS:

Species names based on colony morphology vary wildly within and among mitochondrial haplotypes, while SEM images of skeletal microstructure agree more with genetic data. Several unique haplotypes are geographically restricted.

CONCLUSIONS:

Phenotypic polymorphism and plasticity are likely to be underestimated in this genus. Locally restricted haplotypes may represent cryptic endemic species. Range-wide surveys and quantitative morphological work is needed to determine species boundaries in this important group of organisms.

BACKGROUND

Plasticity

- ▶ *Pocillopora* is known to be highly phenotypically plastic (e.g. Figure 1)

Species Boundaries

- ▶ Hybridization has been suspected to play an important role in the evolution and biodiversity of reef building corals, however; recent studies have shown patterns more consistent with polymorphism and plasticity (e.g. Flot et al. 2008, Forsman et al. 2009; Forsman et al. 2010; Pinzon & LaJeunesse 2011).

Barcoding

- ▶ Mitochondrial markers evolve unusually slowly in corals, however Flot & Tillier (2007) discovered a rapidly evolving mitochondrial gene of unknown function 'ORF' that is unique to *Pocillopora*. Subsequent studies have shown that the ORF region has far higher resolution but is generally concordant with other markers (Flot et al. 2008, Souter 2010; Pinzon & LaJeunesse 2011).
- ▶ Here we barcode 98 corals from Moorea and place them in context with available taxonomic and geographic data.

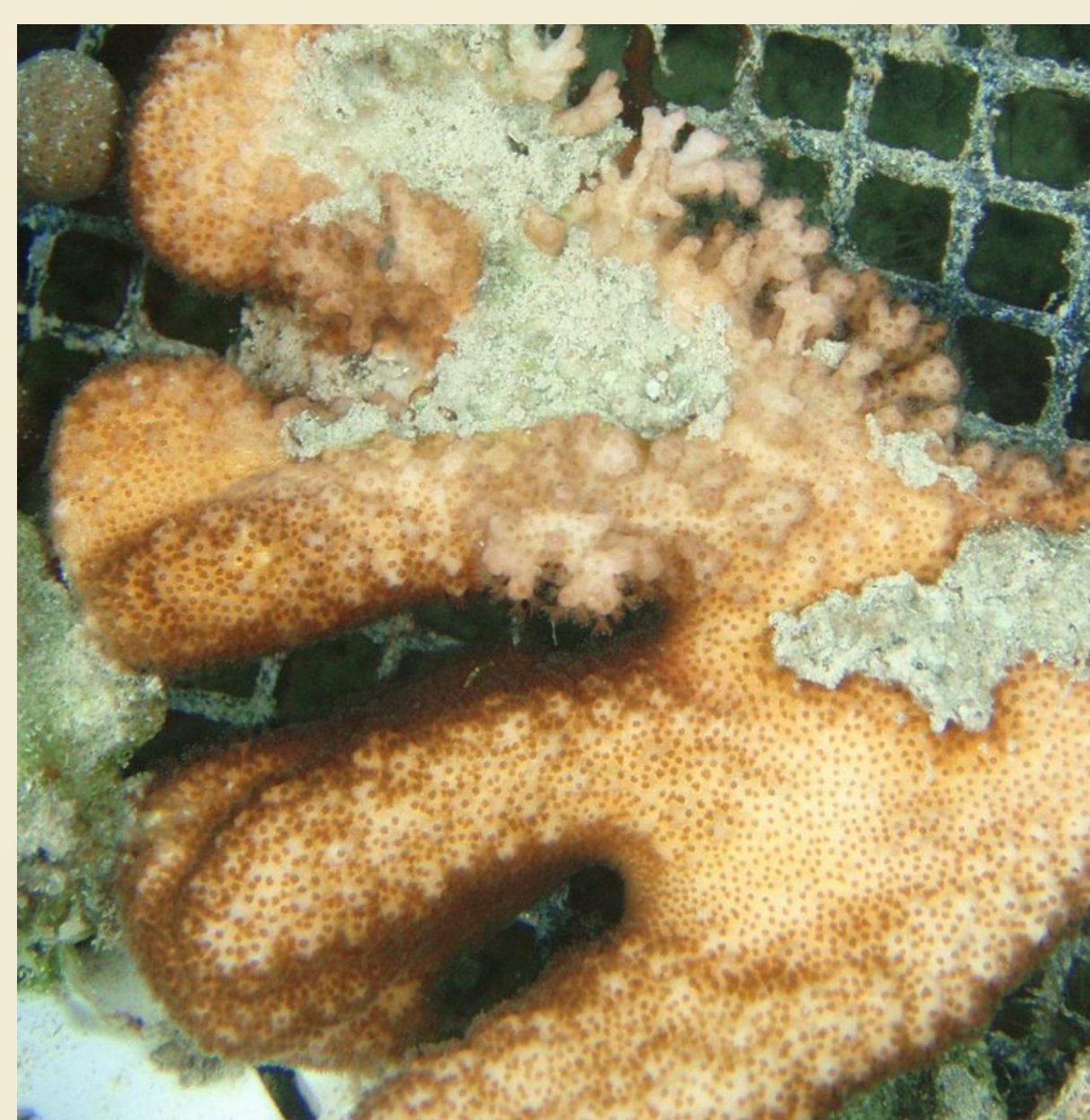


Figure 1 | An example of phenotypic plasticity in *Pocillopora*: A colony identified in the field as *P. meandrina* begins to grow with *P. damicornis*-like morphology after growing for several months in a low-flow environment.

METHODS

- ▶ 2-5 cm tissue samples (n = 98) putatively identified as *P. c.f. meandrina* were collected from Moorea and sequenced using the mitochondrial ORF primers (Flot and Tillier 2007; Fatp6.1 TTTGGGSAATTCGTTAGCAG; RORF SCCAATATGTAAACASCATGTA)
- ▶ Additional samples (n = 59) were collected with photo vouchers and SEM images (Marti-Puig 2011; Marti-Puig et al. in Prep)
- ▶ These samples were compared with a Blastn search of the National Center for Biological Information (NCBI) database, yielding 457 additional mtORF sequences for comparison. A 219 bp unambiguous sequence alignment was constructed.
- ▶ The program Network and Network Publisher were used to draw haplotype networks color coded to phenotype (species names) or geography.

RESULTS

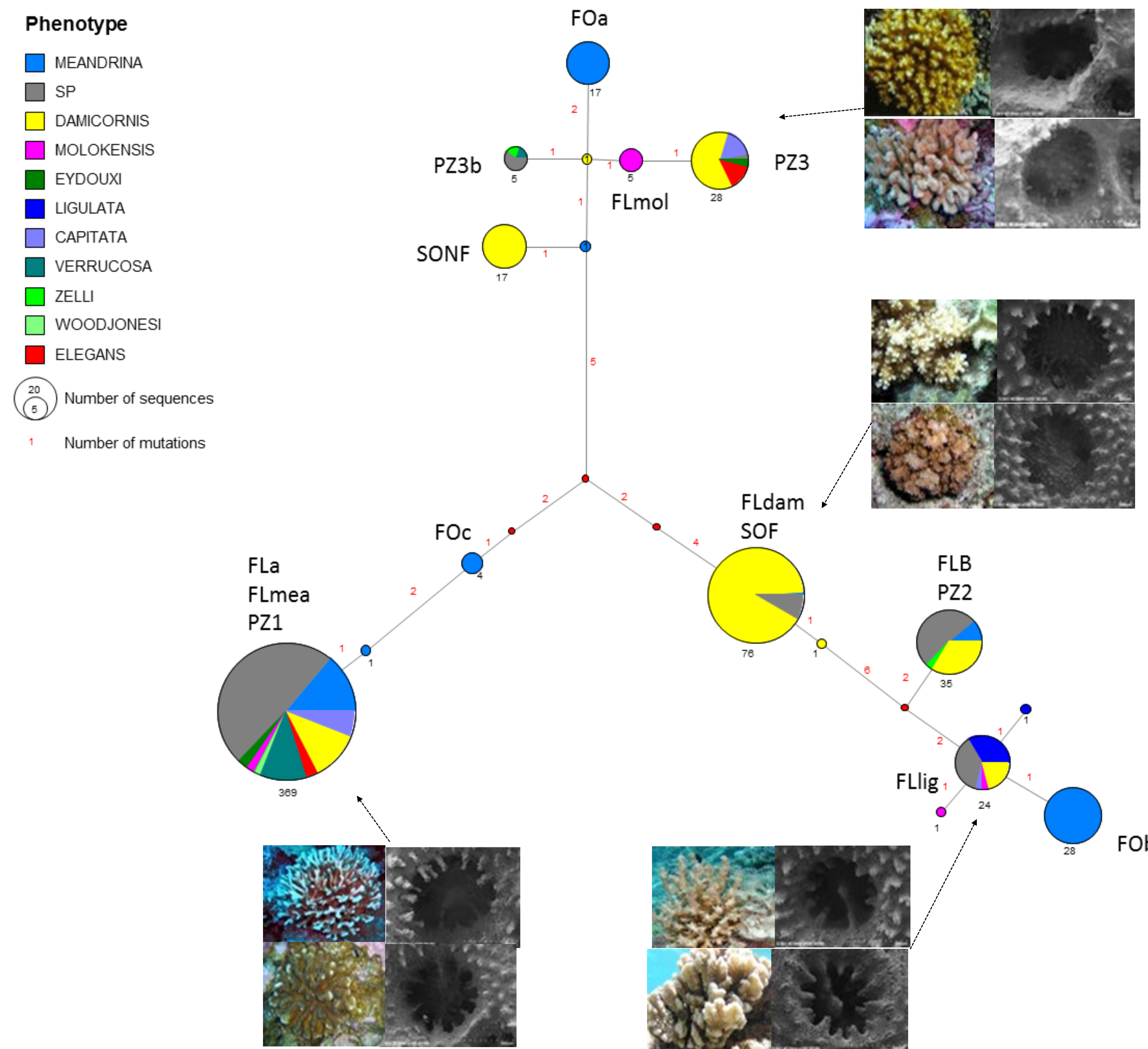


Figure 2 | Species names and colony morphology show high variation within and between mitochondrial haplotypes, while micromorphology is less variable within haplotypes and more different between haplotypes.

Abbreviations:
Forsman et al. (this study): FOa (*P.c.f. meandrina*), FOb (*P.c.f. meandrina*), FOC (*P.c.f. meandrina*)
Flot et al. 2008: FLlig (*P. ligulata*), FLdam (*P. damicornis*), FLmol (*P. molokensis*)
Flot et al. 2010: FLa (*Pocillopora* sp.), FLb (*Pocillopora* sp.)
Souter 2010: SOF (*P. damicornis* "Fixed" type), SONF (*P. damicornis* "Not Fixed" type)
Pinzón & LaJeunesse 2010: PZ1 (Type 1; *Pocillopora* spp.), PZ2 (Type 2; *Pocillopora* spp.), PZ3 (Type 3; *Pocillopora* spp.)

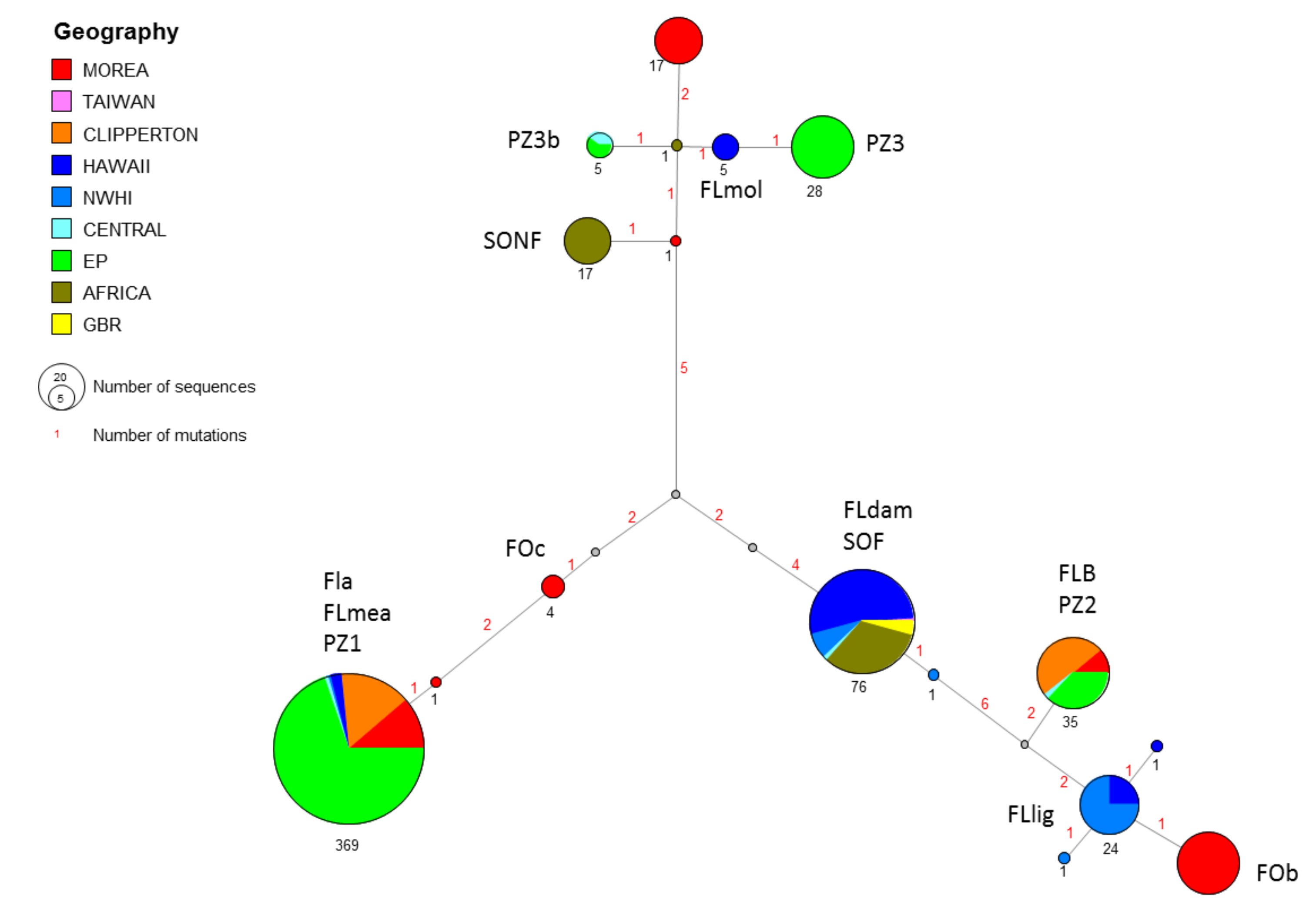


Figure 3 | The geographic distribution of mitochondrial haplotypes reveals cryptic diversity that is geographically restricted. Unique haplotypes occur in Hawaii (FLlig) and Moorea (FOa, FOb., FOC).

Abbreviations:
Forsman et al. (this study): FOa (*P.c.f. meandrina*), FOb (*P.c.f. meandrina*), FOC (*P.c.f. meandrina*)
Flot et al. 2008: FLlig (*P. ligulata*), FLdam (*P. damicornis*), FLmol (*P. molokensis*)
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Souter 2010: SOF (*P. damicornis* "Fixed" type), SONF (*P. damicornis* "Not Fixed" type)
Pinzón & LaJeunesse 2010: PZ1 (Type 1; *Pocillopora* spp.), PZ2 (Type 2; *Pocillopora* spp.), PZ3 (Type 3; *Pocillopora* spp.)

DISCUSSION

- ▶ Colony-level morphology, and nominal taxonomy, are highly variable within most haplotypes. Misidentification may partially explain this pattern, however *in-situ* voucher photos show a wide range of variation within haplotypes (Fig. 2).
 - SONF (*P. damicornis* Ftype), SONF/FLdam (*P. damicornis* NF type), FOa & FOb (*P.c.f. meandrina*) are exceptions to this trend, however; these studies focused on sampling a single morphospecies, but yielded several cryptic genetic groups (Fig. 2).
- ▶ Micromorphology from SEM images is less variable within haplotypes, and more distinct between haplotypes (Fig. 2).
 - FLlig (*P. ligulata*), and FOb (*P.c.f. meandrina*) show discrete differences: clearly developed septa and collumella (Fig. 2).
- ▶ Some haplotypes are geographically restricted, while others occur over an enormous geographic range. The restricted haplotypes indicate that gene flow is extremely limited, and may represent isolated cryptic species.
 - Restricted: FOa, FOb, FOC, PZ3, FLlig, SONF (Fig. 3)
 - Large range: FLdam/SOF, FLa/FLmea/PZ1 (Fig. 3)
- ▶ Moorea has very high genetic diversity, even though only one morphospecies was targeted for collection.
 - Moorea also has a high proportion of unique haplotypes that have not yet been found elsewhere (Fig. 3).

CONCLUSIONS

- ▶ Colony-level morphology is likely to be unreliable for taxonomy in this group, while micromorphology shows some discrete characters and agreement with mitochondrial groups.
- ▶ Moorea has high levels of genetic diversity that is unique from the Central Pacific, indicating restricted gene flow and possibly cryptic species.

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