



Slide 1: Title



## Slide 2 – Aeolidiidae Biology

- Opisthobranchia - Order Nudibranchia - Suborder Aeolidina
  - Family Aeolidiidae (**Aeolida papillosa**)
  - Family Facelinidae (**Hermisenda crassicornis**)
  - Family Flabellinidae (**Flabellina trilineata**)
- Most benthic (= bottom dwellers)
- Found in all the world's oceans in either a cosmopolitan, circumtropical or localized Distribution from Alaska to Mexico
- Food source is usually also their home
- All are carnivorous and diet encompasses all major marine animal phyla except Echinodermata - most aeolids eat sea anemones, most outgroups eat a less specific diet including corals, hydroids and other nudibranchs
- Color derived from food is common for camouflage - often show aposematic coloration; some color also due to algal symbionts or zooxanthelle
- Use of discharged nematocysts (=stinging cells) in defense - obtained by feeding on cnidarians, hydroids and sea anemones; stored in specialized sacs at tip of ceras

# Taxonomic Issues

- Parallelism
- Convergent Evolution
- Morphology varies

## Slide 3 – Taxonomic Issues

- A great deal of parallelism has occurred within the opisthobranchs: Species are so closely related that organisms appear nearly the same
  - Convergent evolution is also an issue: Organisms not closely related (not monophyletic), independently evolve similar traits as a result of having to adapt to similar environments or ecological niches ([http://en.wikipedia.org/wiki/Convergent\\_evolution](http://en.wikipedia.org/wiki/Convergent_evolution)).
  - Certain morphological traits show as much variability for a single characteristic within a single species as between species: What becomes important is choosing the **RIGHT** characteristics to analyze
    - Can lead people to question the structure of certain genera – ex. With *Spurilla* and *Berghia* or *Aeolidiella* with *Spurilla*
      - Many species have been moved to different genera 2 to 3 times over the course of 50 years: Ex. *Aeolidiella chromosoma* once belonged to *Berghia* and what I refer to as *Aeolidiella indica* was moved to *Anteaeolidiella* in 2001
  - Lots of species relationships are based on inferences about morphology but interestingly, no one has created a phylogenetic tree of the group
- **MY GOAL!!**

# Nudibranch Morphology



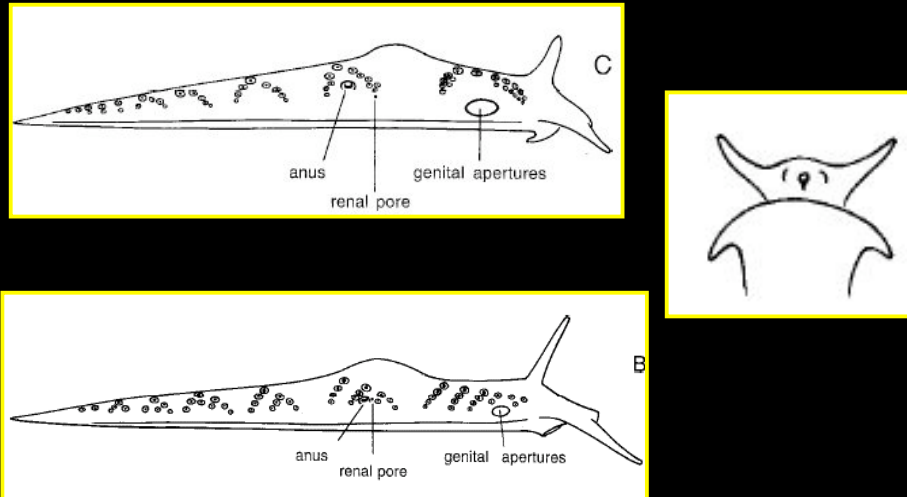
Seaslugforum.net

- Ceratal Arrangement
- Foot Corners
- Anus Location
- Genital Aperture
- Rhinophores
- Radula
- Jaws

## Slide 4 – Nudibranch Morphology

- Respiratory organs on dorsal surface are either a circle of gills or thin-walled papillae (= **cerata**)
  - Cerata arranged in bilaterally symmetrical patterns along each side of the body
  - Arrangement reflects arrangement of the internal ducts from the stomach
- Anus in Aeolidiidae are **cleioproctic**:
- Head has a cephalic shield
  - In more derived forms, posterior corners form chemosensory tentacles called **rhinophores**
- Have a muscular pharynx which possesses two jaws and a **radula**
  - In advanced forms only have one row of teeth in radula

# External Morphologies

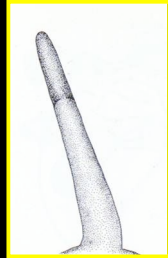


Miller 2001

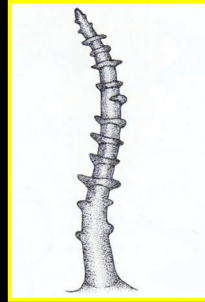
## Slide 5 – External Morphologies

- Cerata are arranged in rows or arches
- Can see slits where the oral glands exit
- Anus is either **acleioproctic**, **cleioproctic**,
- Sea slugs are hermaphroditic
  - Genital opening either has a hermaphroditic pore that then branches to penis and vagina or two separate entrances on the surface

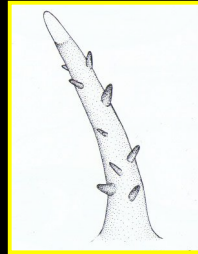
## Rhinophore Morphologies



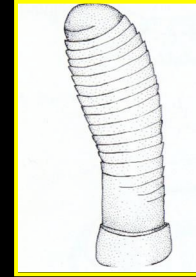
**Smooth**



**Annulate**



**Papillate**



**Lamellate**

Gosliner 1987

### **Slide 6 – Radular Morphologies**

- Teeth are either pectinate or cuspidate
- Pectinate teeth vary in shape – arches and heart-shaped
- The cusp can be pronounced or extremely deep

# Radular Morphologies

## Pectinate



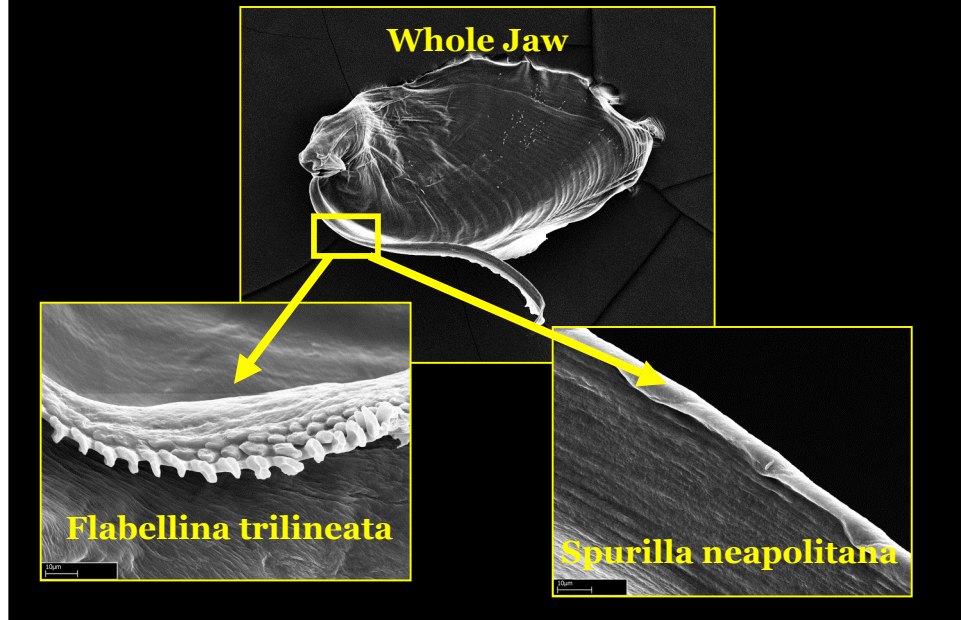
## Cuspidate



### Slide 7 – Rhinophore Morphologies

- Teeth are either pectinate or cuspidate
- Pectinate teeth vary in shape – arches and heart-shaped
- The cusp can be pronounced or extremely deep

# Jaw Morphologies



## Slide 8 – Jaw Morphologies

- The jaw has a masticatory edge
  - Either smooth or denticled
  - Varying types of denticles – smooth or nodules
  - Varying number of rows of denticles



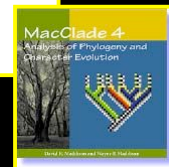
## Questions

- How are species within the family Aeolidiidae related?
- Are morphological characteristics effective in creating a phylogeny of the Aeolidiidae?

Slide 9 – Questions

## Methods

- **Assessing 15 species**
- **Morphology**
  - **Dissecting Microscope**
  - **SEM**
  - **MacClade**
  - **PAUP**
- **Molecular**
  - **Genes: 16S and CO1**
  - **MacClade**
  - **Mr. Bayes**



### Slide 10 – Methods

- Morphological trees based on 21 characters (excluded foot corners, shape of receptaculum seminis, twists to ampulla and twists to penis)
  - Made a data matrix of characters in MacClade
  - Generated strict consensus trees in PAUP
- Using CO1 (a mitochondrial gene) and 16S (a ribosomal gene)
- rRNA—stem data recovers deeper nodes and loop data is useful for lower level comparisons
- CO1 is “not a reliable molecular marker for ancient divergences” – to resolve families and genera
- Aligned sequences in MacClade
- Mr. Bayes – does Bayesian analysis on sequences. Bayesian analysis makes inferences based on a model of evolution and seeks the best set of trees

## **Morphological Results**

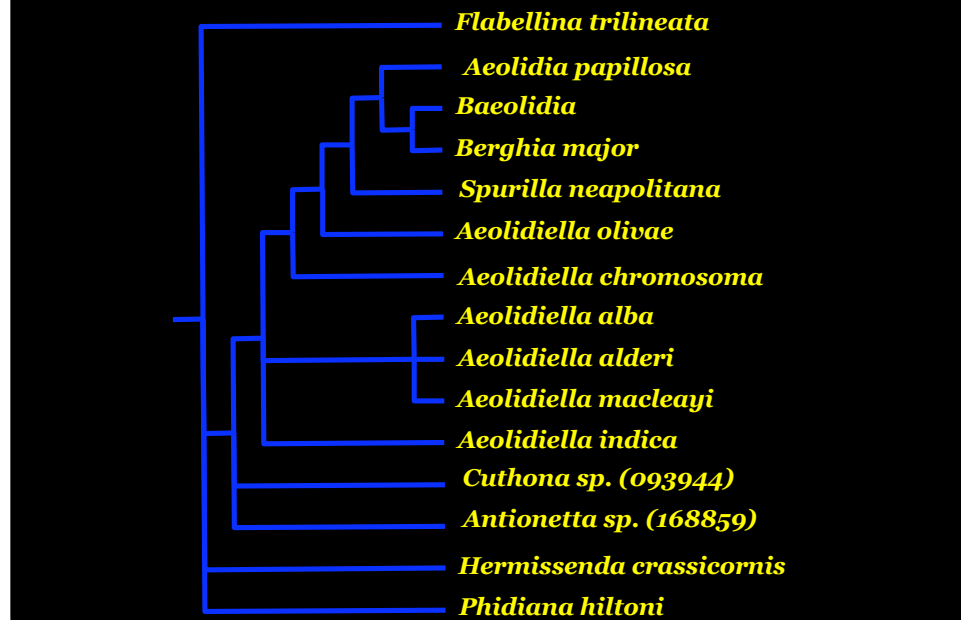
- **Monophyletic Aeolidiidae**
- **Presence of Oral Glands**
- **Shape of Pectinate Teeth**
- **Rhinophores**



### **Slide 11 – Morphological Results**

- Monophyletic Aeolidiidae
- Several characters provided good phylogenetic signal – presence of oral glands, rhinophores, shape of pectinate teeth, rachidian teeth type and masticatory edge

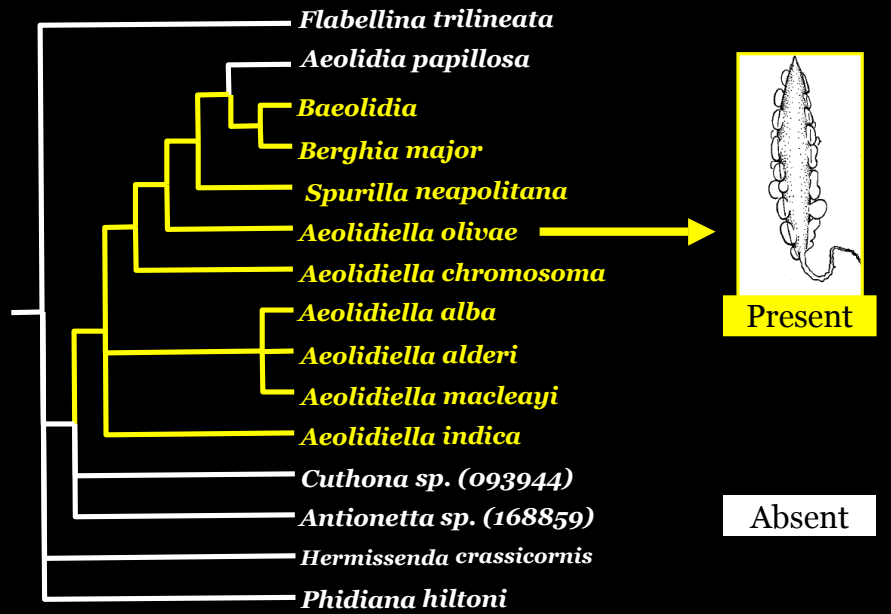
## Morphological Tree



### Slide 12 – Morphology Tree

- *Flabellina trilineata* is used as an outgroup
  - *Hermisenda crassicornis* and *Phidiana hiltoni* are part of the family
  - Flabellinidae (sister to Aeolidiidae)
  - While clade isn't fully resolved, the Aeolidiidae are shown as monophyletic
  - Presence of oral glands, cuspidate teeth, smooth masticatory edge and lack of bursa copulatrix consistently define clade
- *Berghia* and *Spurilla* are removed enough that they can't be considered that same; as is the case with *Aeolidiella* and *Spurilla*
- Believed *Cuthona sp.* and *Antionetta sp.* to be *Aeolidiella sp.* but the tree reveals that they fall outside of the tree (an instance of misidentification based on external morphology)

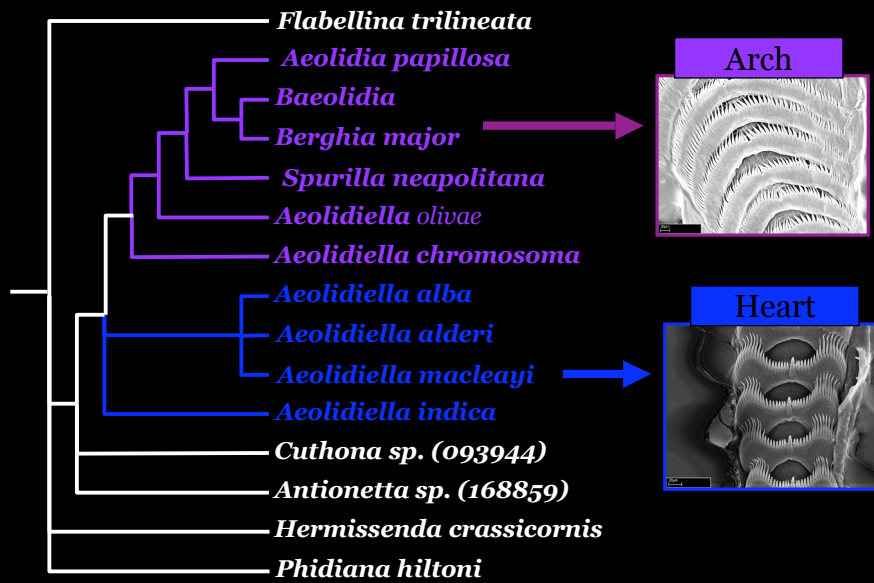
# Presence of Oral Glands



## Slide 13 – Presence of Oral Glands

- Oral glands are a characteristic specific to the clade Aeolidiidae (minus *Aeolidia papillosa*)

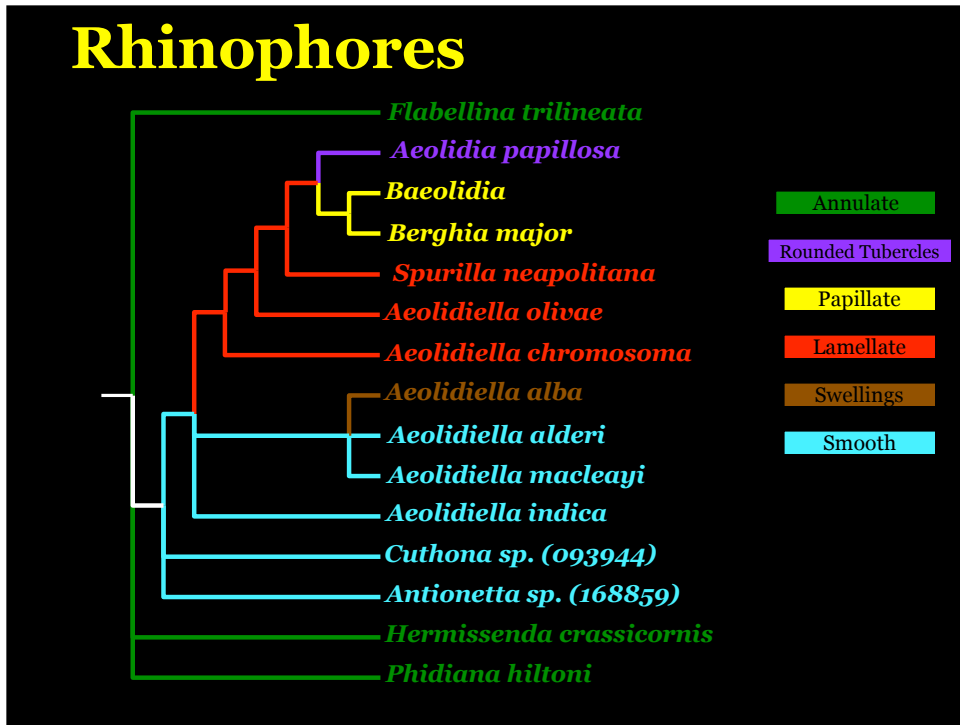
# Shape of Pectinate Teeth



## Slide 14 – Shape of Pectinate Teeth

- 2 subgroups within the clade Aeolidiidae based on radular tooth shape

# Rhinophores



## Slide 15 – Rhinophores

- Within the clade, smooth rhinophores are shown to be a more basal characteristic while papillate are shown to be the most derived
  - Papillate rhinophores support grouping of *Baeolidia* and *Berghia*

## Molecular Results

- **Aeolidiidae are NOT monophyletic**
- **Genera *Spurilla* and *Berghia* are not synonymous**
- **Disproves general belief that *A. papillosa* is a basal taxon**

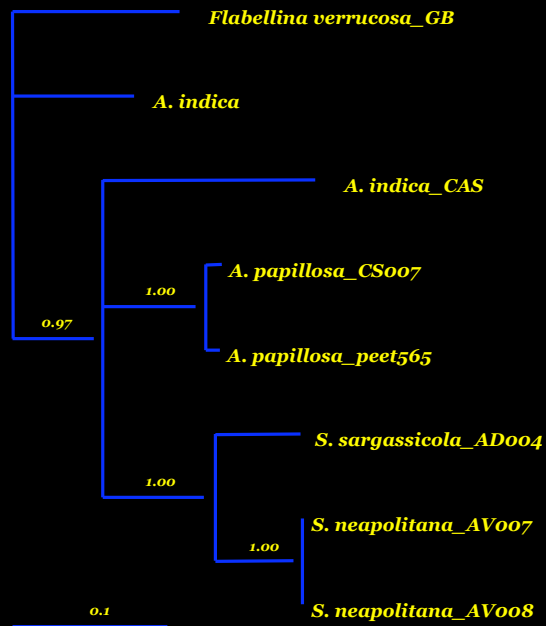


### Slide 16 – Molecular Results

- Aeolidiidae are not monophyletic
- *Berghia* and *Spurilla* are not synonymous genera
- *Aeolidia papillosa* is basal

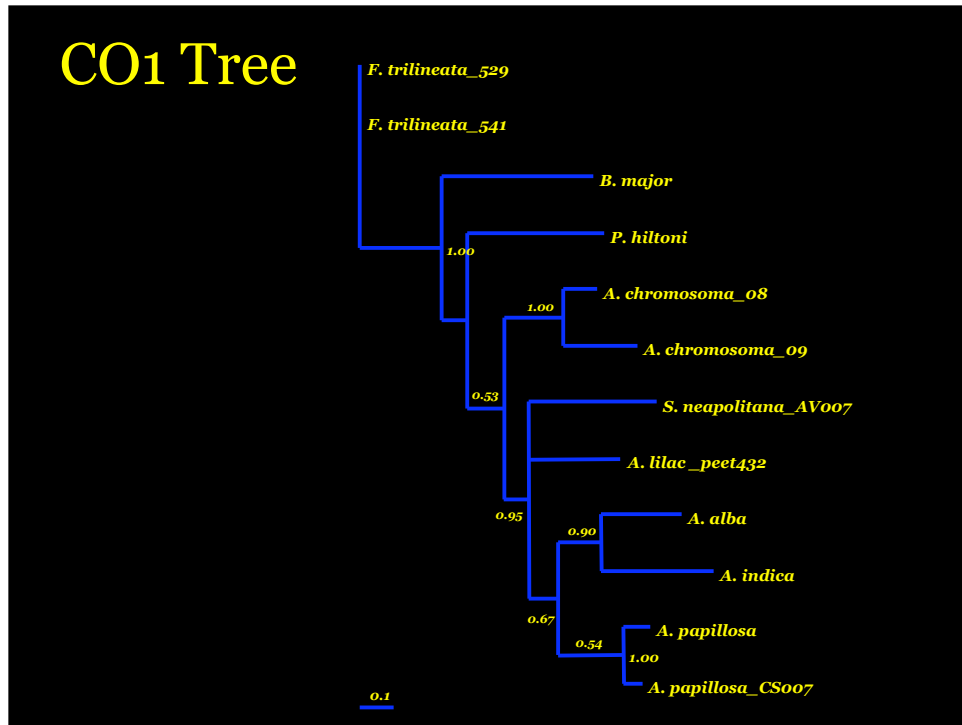


## 16S Tree



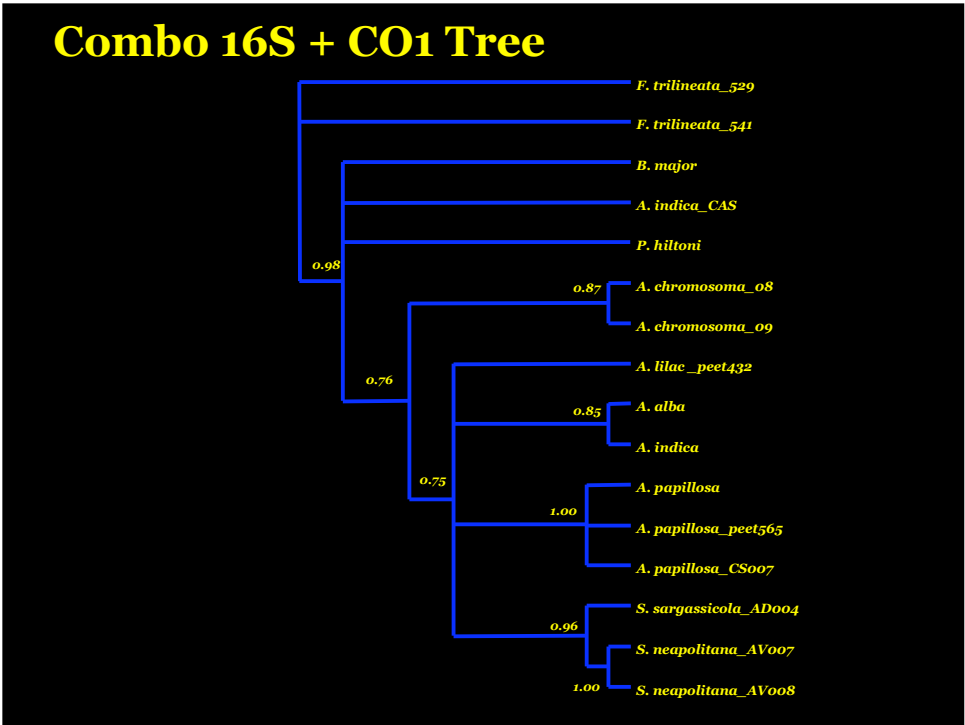
### Slide 17 – 16S Tree

- Tree has the fewest taxon – had trouble getting them to amplify
  - Got some Hyla and even some human
- Flabellina verrucosa is used as the outgroup
- Aeolidiidae is not show to be monophyletic
  - Unresolved with respect to one of the A. indica
    - May reveal that Eastern and Western Pacific populations are in fact separate species
- A. indica remains one of the most basal aeolids
- The genera Aeolidia and Spurilla are most derived



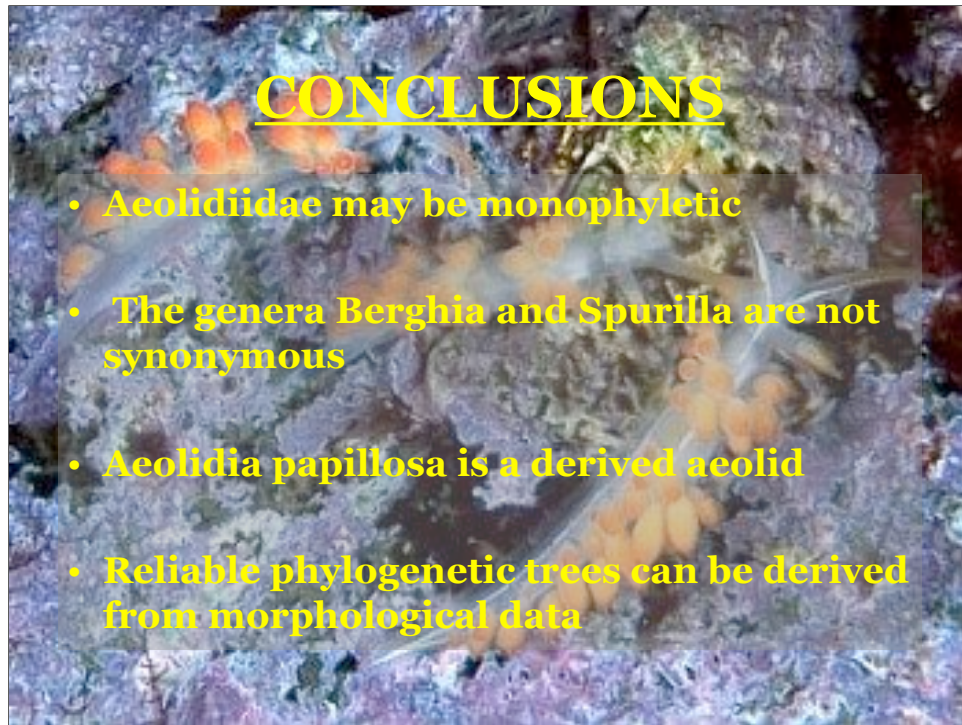
## Slide 18 – CO1 Tree

- Supports a non-monophyletic Aeolidiidae
  - A. lilac groups within clade
  - Berghia falls outside the clade and Phidiana hiltoni groups within
    - Phidiana hiltoni is a species in the sister family Facelinidae
- A. indica which was shown as basal in 16S and Morphological tree, is shown as more derived
- A. alba which was more basal comes out more derived
- Berghia is shown as basal – different than morphological data
- Spurilla and Berghia don't group together, as shown with morphological data



**Slide 19 – Combined 16S + CO1 Trees**

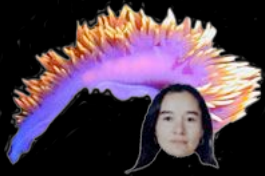
- Supports a non-monophyletic Aeolidiidae
  - Due to a basal polytomy



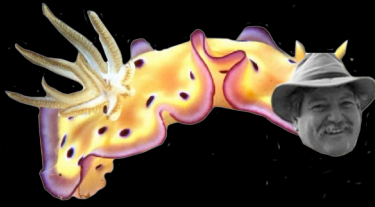
**Slide 20 – Conclusions**

- Monophyletic Aeolidiidae is a possibility
- While in general morphological data is reconfirmed by molecular data, inconsistencies do exist
  - Can see if more taxon sampling will help certain genera to stabilize within the phylogenetic trees

# Many Thanks to...



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**Slide 21: Acknowledgments**