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).

• 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 Antaeolidiella in 2001

• Lots of species relationships are based on inferences about morphology but surprisingly, no one has created a phylogenetic tree of the group

– MY GOAL!!
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
Slide 5 – External Morphologies

- Cerata are arranged in rows or arches
- Can see slits where the oral glands exit
- Anus is either acleiproctic, 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
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
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
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 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

• 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)
Slide 13 – Presence of Oral Glands

- Oral glands are a characteristic specific to the clade Aeolidiidae (minus Aeolidia papillosa)
Slide 14 – Shape of Pectinate Teeth

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

• Aeolidiidae may be monophyletic

• The genera Berghia and Spurilla are not synonymous

• Aeolidia papillosa is a derived aeolid

• Reliable phylogenetic trees can be derived from morphological data

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
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