

SFI CONTRIBUTES TO GLOBAL SYNGNATHID SURVEY

From when we began our conservation work on the inshore fish in 2003 it became apparent that little was known about their taxonomy and that there would be many undescribed species. There had been no significant work done in this area since the mid 80's. We were soon finding species that were very distinct, but other inshore fish looked pretty similar but begged the question. Are you a different species?

Besides species that look distinctly different from others some species look very similar to others. This can be through species mimicking each other, convergent evolution where animals tend to adopt the same shape, size etc. when they fill the same ecological niche, or through us humans not being able to pick up differences by the species being small and not having dramatic differences.

An example we are working on now is a small pipefish Kevin Smith discovered that lays three times the number of eggs than the species it looks much like, which is very common and was described in 1856. Normal features used to distinguish fish species all appear similar in the two species; these include the number of rays in fins, and other features like body ring counts,.

The relatives of the seadragons, the pipefish, have quite a few species in closely related groups that look very similar. One way that is often used to tell species apart is the use of DNA molecules. As a species evolves from another its DNA, the chemical code that compiles a species, increasingly varies from the parent species. So more than about 2% difference means you can be certain that the two species are different. The study of the relationship between two species is called molecular phylogeny (ancestry).

Molecular phylogeny can also be used to create a tree showing the ancestry of different species. This can go back 40 million years to find by what family pathways that syngnathids including seadragons evolved. At each stage in the family tree each, species (blue gum), cluster of closely related species (gum trees), or more distant groups (trees, plants) are called 'clades'.

Until the last couple of decades this used to be done by using appearance. Feathers on birds, hair on mammals etc. However, as we know appearance can be deceptive and unless backed by a fossil record, or barriers being produced like island formation, it is difficult to know when the clades diverged.

If we know when the clades diverged we can then tell how old species are, and perhaps relate the different levels of clades to events such as previous climate or the distribution of continents. We can also separate forces of evolution, such as environmental, social and behavioral, reproductive, and morphological.

To some extent molecular phylogeny can tell us when clades diverged. This is because there are sections of the DNA that change their structure over time. By looking at the amount of difference between two species we can tell how long ago they diverged. For instance humans diverged from chimpanzees six million years ago.

The Seadragon Foundation and its component the Inshore Fish Group have been supporting two independent molecular phylogeny projects including syngnathids from South Australia. One is a detailed study of the unique, and primarily South Australian, monkey tailed group (*Stigmatopora* species), and the other the ancestry of all syngnathids. From these projects our understanding of the species and their relationships will undergo a sea change. These projects will also provide a firm foundation based on the most reliable techniques for powerful new studies to support syngnathid conservation.

Monkeytail pipefish project

The Inshore Fish Group began work with DNA when an apparently new species of pipefish was

found in the South Australian Museum collection in 2003. A Dr Michael Dawson from the contacted me by email and asked for specimens from the *Stigmatopora* genera.

The *Stigmatopora* genera are a group of closely related species. Each species has a generic name and a specific name. A group of ancient pipe fish with only three previously known species all in southern Australia or New Zealand is the *Stigmatopora* genera. For instance the two species common in South Australia are the widebody pipefish *Stigmatopora nigra* and the spotted pipefish *Stigmatopora argus*.

The *Stigmatopora* are particularly interesting to us because they are only found in southern Australia and New Zealand, they are a group as ancient as the seahorses or seadragons, they are one of the few types of syngnathids that have prehensile tails, and they are the most common type close inshore. As people become more familiar with the *Stigmatopora* - and their prehensile tails always coiling and grasping – they are becoming known as the monkey tailed pipefish.

We were in luck this new species from the museum appeared to be a monkey tail and we had a chance to sort out our uncertain South Australian species. Incredibly the new species that Kevin Smith found also appeared to belong to a *Stigmatopora*. Could we have doubled the number of *Stigmatopora* known from southern Australia?

We almost immediately had confirmation from Michael that the pipefish found in the museum was certainly a new species and we have described it in the Journal of Museum Victoria. We named it after the South Australian gulfs, the Southern Gulfs Pipefish. The study of the rest of the group is just being finalised now, and so we are unsure about the second species. Michael and his team are in the final stages of lab work and preparing their papers. These will tell the story of the species, evolution and relationship of the unique *Stigmatopora*.

This research is confirming that South Australia supports the highest diversity of *Stigmatopora*. South Australia is becoming recognised as a syngnathid center of biodiversity, supporting both the unique seadragons and *Stigmatopora*. Both the new species, and the possible new species are found close inshore. The new named species has very limited distribution in the gulfs. The finding of such biologically important species close inshore near population centers shows just how little is known of the inshore marine biodiversity in South Australia. It also shows the vulnerability of these species without groups like the Seadragon Foundation to research and protect them based on scientific knowledge.

Ancestry of all syngnathids

The Seadragon Foundation is also proud to support a project to produce a complete phylogenetic tree of the syngnathids from their first ancestors 40 million years ago. We were contacted early in 2007 about assisting with the provision of samples for this project from Graham Short (beautiful images in Inshore Fish Group website www.ifg.bioteck.org photo index).

Graham is working with Dr Healy Hamilton from the Californian Academy of Sciences and they are using a number of molecular techniques. Graham and Healy were searching the world for a collection of tissues from all the possible species in the syngnathid family tree. That is no mean task as these are more than several hundred possible species and some of these are very rare. To create a general tree you do not need every species, which makes things a bit easier.

A direct benefit to the conservation of syngnathids of this project will be its ability to separate similar looking species. Of course we gave Graham tissue samples from all the species we could, provided contacts and information, and very importantly from specimens that we thought might be new species.

Excited by our syngnathid work and discoveries Graham came for a diving trip and Kevin Smith showed him many of our top spots. Graham was impressed by the variety and number of syngnathids he observed. Shortly after Graham and Healy then came out to South Australia to do some direct field work on syngnathids. We decided to sample the north coast of Kangaroo Island, as it was a region where we had some previous knowledge, but had not directly investigated.

Using hand nets in the shallows this three day trip greatly increased our knowledge of syngnathids in the area, and provided samples of some pipefish we wanted tested. We also found a rare goby of conservation significance.

Graham, Healy and their research team are progressing well. We look forward to the discoveries about syngnathid evolution and ancestry, and finding the taxonomic status of the uncertain pipefish specimens they are processing. Both these are very important to conservation. The ancestral tree will tell us the status of the species or clades in biodiversity terms. Knowing the range and distribution of different species is critical to their individual conservation.

The syngnathids have been traditionally considered to have evolved from the Sticklebacks (Gasteroformes). Sticklebacks are found in Eurasia and North America, and have nests with eggs. They share many common features with the seadragons and other syngnathids.

Below is a segment from an email from Graham, 18th September 2007.

“ At this point we have extracted DNA and performed PCR for four mitochondrial and one nuclear gene fragment for many syngnathid genera including pipefish, seadragon, seahorse, seahorse, and also coronet fish, trumpet fish, snipefish, and out groups including different genera of sticklebacks . This represents a huge amount of work and we are still receiving and have yet to receive other genera of pipefish, so it's all very exciting. The next three weeks will be focused on creating phylogenetic trees from the gene sequences and analyzing the data to look at patterns in morphology and geography.

A recent paper by Miya Makaki on the phylogeny of Gasteroformes based on whole mitochondrial DNA sequences states that Gasteroformes and Syngnathiformes are far apart with respect to phylogenetic distance but they were unable to resolve some branches because they didn't use nuclear gene data which evolve more slowly than their mitochondrial counterparts, so our data should help resolve this issue.”