The tale of two fish: A case study on vicariance and allopatric speciation

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Ernst Mayr (1947) proposed that the first step towards the formation of new species is an interruption of gene flow and splitting of a population. Volcanic eruptions, the separation of continents, and the formation of rivers and mountain ranges are all geological events that could split a population. Over time, the two partitioned populations are exposed to different ecological conditions and selection pressures that push them in different evolutionary directions, potentially forming two species from the original one. Thus, it is a vicariance event - the disruption of gene flow in a population due to geologic change – is the precursor to allopatric speciation. One approach to testing Mayr's model of allopatric speciation in the real world is seeking out cases in which the origin of a species mirrors the timeline of documented geological events.

Figure 1..Photograph of Taal Lake surrounding Taal Island, Philippines. Source www.taalvolcano.com

In the Philippines there is a volcano surrounded by a lake, surrounded by a volcano, surrounded by a lake on an island in the Pacific. The central volcano is called Taal Volcano and with its 30 plus eruptions since the 16th century, it has transformed the landscape of the region including the putative formation of Taal Lake (Figure 1). Historically Taal 'Lagoon' was connected to the adjacent South China Sea, known from records of ocean sailing ships that would trade with towns and missionaries along the lagoon's shores (Hargrove 1991). However, in 1754 a series of violent volcanic eruptions rocked the area and largely closed the connection between the lake and sea. Nowadays the water in Taal Lake is freshwater and although there is no longer a navigable waterway to the South China Sea, there is lingering evidence of the lake's marine history.

Endemic to Taal Lake is the only freshwater representative of the Clupeiformes genus *Sardinella*, the species *Sardinella tawilis*. Tawilis, as it is locally called, is the most valued commercial fishery of Taal Lake and a Filipino delicacy (Mutia et al. 2001). The importance of *S. tawilis* has prompted many studies on its biology, ecology, and even evolutionary origins. Several studies had proposed that *S. tawilis* shared a

common ancestor with the marine *S. albella* that occurs in the nearby South China Sea (Samonte et al. 2000, 2009), however, the limited use of specimens and diagnostic features leaves room for speculation. Indeed, a later study suggested *S. albella* could not be the sister species of *S. tawilis* and rather the true origin was 'waiting to be discovered' (Quilang et al. 2011).

Answer questions 1-3 using the below table of morphological features, meristics, and coloration of *Sardinella* species in the region (adapted from Willette et al. 2011).

Traits	*S. tawilis*	*S. albella*	*S. gibbosa*	*S. lemuru*	*S. hualiensis*
Body length (mm)	100-110	100-140	120-170	150-230	100-125
Lower gill rakers	61-74	41-68	45-59	77-188	51-66
Pelvic fin rays	8	8	8	9	8
Enlarged last two anal fin rays	Yes	Yes	Yes	Yes	Yes
Scales with overlapping striae	Yes	No	No	No	Yes
Black tips of caudal fin	Yes	No	Yes	No	Yes
Golden midline & spot behind gill	No	No	Yes	No	No
Historically reported in the Philippines?	Yes	Yes	Yes	No	No

1. Using the table above, identify and list two differences between *S. albella* and *S. tawilis*?
   Sardinella albella and *S. tawilis* differ in the following two diagnostic features: Scales with overlapping striae, and black tips on caudal fins.

2. How many traits do *S. tawilis* and *S. hualiensis* have in common? How many traits do *S. tawilis* and *S. lemuru* share? Using the number of shared traits between *Sardinella* species, which species is most similar to *S. tawilis*?
   S. tawilis and *S. hualiensis* share 7 traits, and only differ in where they were reported historically. *S. tawilis* and *S. lemuru* share two traits. *S. hualiensis* is most similar to *S. tawilis*.

3. Which trait is un-informative for distinguishing *Sardinella* included in the Table?
   The trait of Enlarged last two anal fin rays is un-informative because all fish share this trait. It does not distinguish any Sardinella from another.

Six species of *Sardinella* have geographic distributions inclusive of the Philippine archipelago, with the Taiwan sardinella *S. hualiensis* reported in fish catches only recently in 2010 (Willette et al. 2011). This recent discovery of *S. hualiensis* in the Philippines and the high level of similarity between it and *S. tawilis* (Figure 2) allow for the testable hypotheses: a) Is the marine *S. hualiensis* the sister species of *S. tawilis*? and if so, b) Is the divergence of the two species the result of a vicariance event associated Taal Lake's volcanic history?

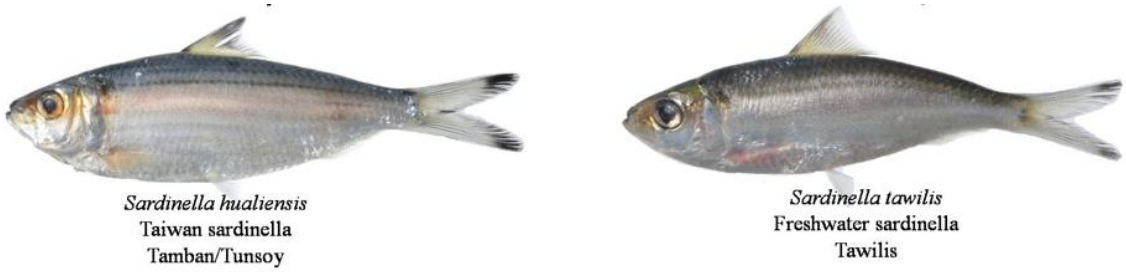


Figure 2. Photographs of *S. hualiensis* and *S. tawilis* to illustrate color and morphological similarity.

Using morphological and meristic data of ten sardines from three locations – *S. hualiensis* from Taiwan, *S. hualiensis* from the Philippines, and *S. tawilis* from the Taal Lake – researchers contrasted and compared the fish using a Bray-Curtis dissimilarity index, illustrated in Figure 3 dendrogram.

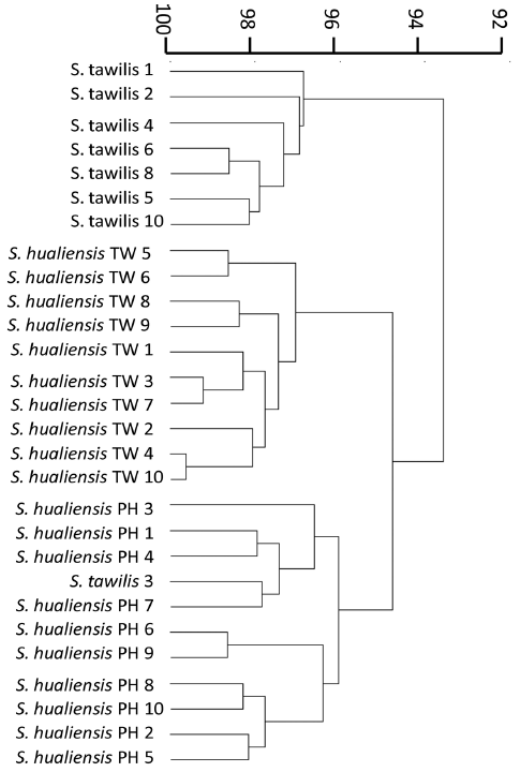


Figure 3. Dendrogram based on similarity of morphological and meristic features of *S. hualiensis* from Taiwan (TW), *S. hualiensis* from the Philippines (PH), and *S. tawilis.* Percent similarity indicated on horizontal axis.

Please answer questions 4-6 using Figure 3:

4. What do fish in the same clade (branch group) on the dendrogram tell you about their relationship to 'clade-mates' versus fish in other clades?

The closer two fish are on the dendrogram, the more similar or related they are.

5. Is *S. hualiensis* TW 5 more closely related to TW 10 or to *S. tawilis* 10? Why?

TW 5 is more closely related to TW 10. They're within the same clade, *S. tawilis* groups in a distinct and separate clade.

6. This dendrogram is based morphological and meristic data. Suggest an explanation for the location of *S. tawilis* 3.

Multiple answers are acceptable; for example, *S. tawilis* 3 may have morphological features (namely length & gill rakers) closer to those of the marine Sh PH. Sh are larger so this could have been a large S. tawilis.

Now that you've assessed the relationship between the three populations of fish using morphological and meristic data, examine the below two trees based on molecular genetics data regions and please answer questions 7 - 9.

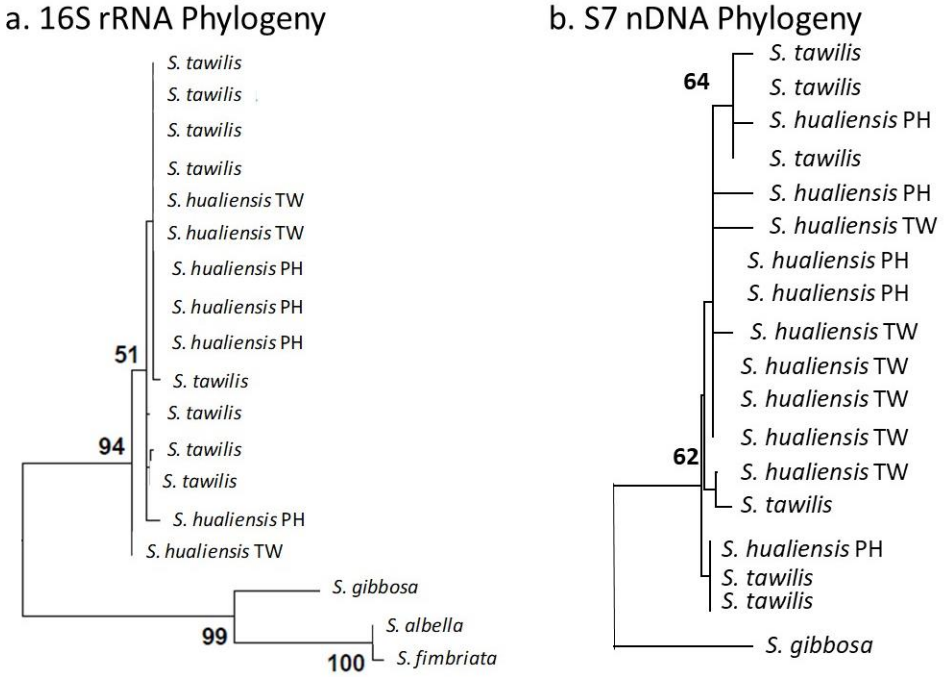


Figure 4. Inferred phylogenetic relationship of *S. hualiensis* TW, *S. hualiensis* PH, *S. tawilis*, and other Sardine species (outgroups) based on (a) 16S rRNA and (b) S7 nDNA sequence data. Bootstrap probability from 1000 tree iterations is show at branch nodes for values > 40% (adapted from Willette et al. 2014).

7. In each phylogeny and using a bootstrap probability cut-off of 90%, draw a circle around all fish in the same clade.
   No circles drawn in S7, 3 circles drawn for 16S. On around all S tawilis & S. hualiensis in a single group, one around S. gibbosa, one around S. albella/S. fimbriata.
8. What do fish in the same clade (branch group) on the phylogeny tell you about their relationship to 'clade-mates' versus fish in other clades?
   The closer two fish are on the phylogeny, the more genetically similar or related they are.
9. Briefly describe how 16S rRNA and S7 nDNA phylogenies are similar or differ.
   Both trees group all S. tawilis and S. hualiensis in a single clade, distinct from other Sardinella species (S. gibbosa, S. albella, S. fimbriata). They differ in that the bootstrap value of the single St/Sh clade in S7 is low, giving less confidence in the grouping.

10. How many groups are distinguishable among the *S. hualiensis* TW, *S. hualiensis* PH, and *S. tawilis*? Would you call this group/these groups distinct species? How do these results compare to your answer in question 5 above?
    There is a single clade in both phylogenetic trees that contain all *S. tawilis* and *S. hualiensis*. Based on the Phylogenetic Species Definition – they would constitute a single species. These result differ from #5, here there is 1 clade, in #5 there are three clades.

Phylogenic trees, such as the 16S rRNA and S7 nDNA above, infer the relatedness among individuals using molecular genetics data. To examine the relationship between populations or species, researchers can pool individuals of a population to create a species tree**.** By including a mutation rate for a particular gene region, a species tree can also provide an estimate of when population or species lineages diverged.

Using Cyt *b* gene region sequence data from *S. tawilis* and *S. hualiensis* TW and PH, and using an established mutation rate of 2%/million years and a hypothetical faster rate of 10%/million years, Willette et al. (2014) generated the below species tree to estimate when the three geographically distinct species diverged genetically.

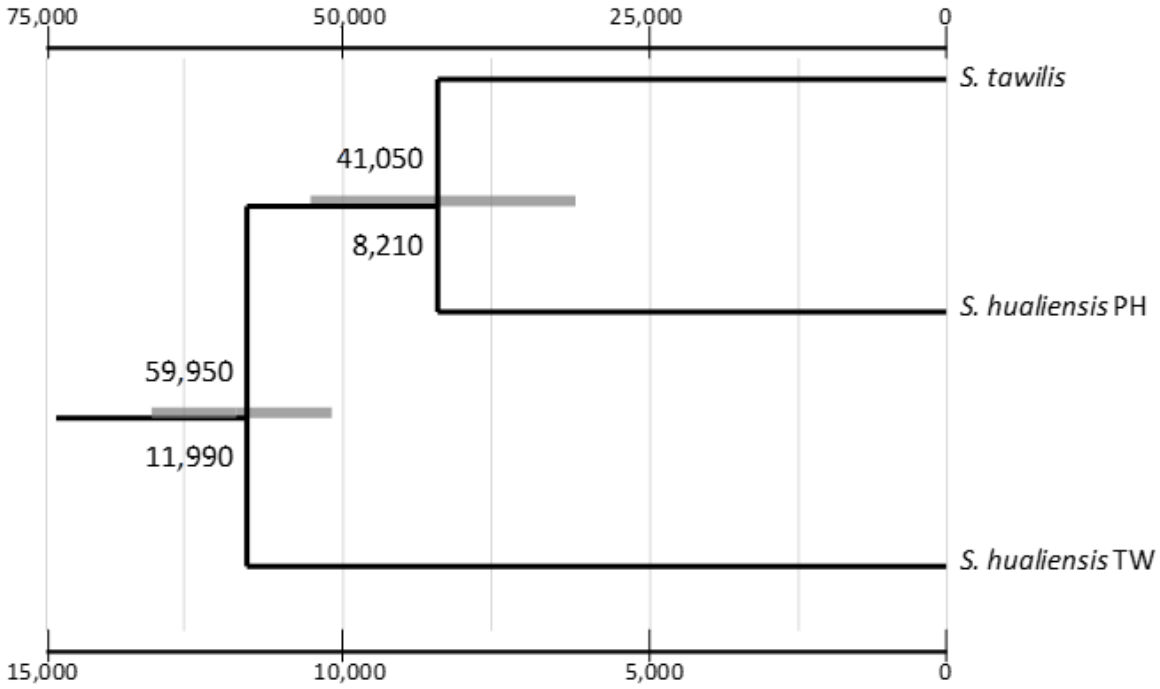


Figure 5. Species tree for three sardine groups using Cyt *b* sequence data. Estimated time of divergence for the 2%/million years is shown above the node and for the 10%/million years is shown below the node. Grey error bars represent 95% HPD intervals of the divergence; top scale bar shows 75,000 years ago to present for the 2% rate, and bottom scale bar shows 15,000 years ago to present for the 10% rate. Adapted from Willette et al. 2014.

Using the species tree (Figure 5), please answer questions 10 – 13.

11. Which group of fish split earliest from the others? Which two groups of fish are most closely related?

Sardinella hualiensis from Taiwan split earliest. *Sardinella tawilis* and *S. hualiensis* from the Philippines are most closely related on this species tree.

12. Based on the species tree that estimates lineage divergence time, when did *S. tawilis* and *S. hualiensis* last share a common ancestor?

41,050 years ago using the 2%/My rate, 8,210 years ago using the 10% rate.

13. Does the estimated time of divergence between *S. tawilis* and *S. hualiensis* support the hypothesis that the volcanic activity that formed Taal Lake was also the vicariance event that preceded allopatric speciation between *S. tawilis* and *S. hualiensis*? Provide evidence from the figures to support your answer.

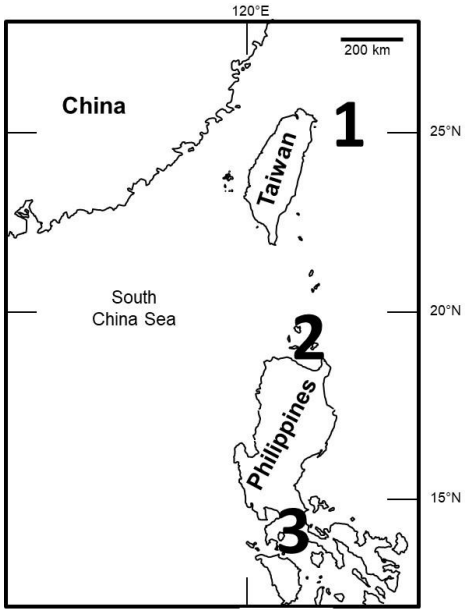
    No. Taal Lake formed in 1754, which is very recent compared to the 8,000-41,000 years ago the lineages diverged based on the species tree.

14. Based on divergence time estimates from the species tree, in which geological epoch did the divergence between *S. tawilis* and *S. hualiensis* occur? What global phenomenon was characteristic of this epoch that may have also acted as a vicariance event?

    The divergence between all three fish groups occurred during the P**leistocene epoch**, specifically during the late Pleistocene. This time was characterized with the last global **ice age**. Because ice ages result in lower sea levels, it is possible this last Ice Age may be the vicariance event that prompted allopatric speciation between the sardines.

15. Given the physiological and geographic barriers that exist between the marine *S. hualiensis* in Taiwan (indicated by '1' on map), marine *S. hualiensis* from the northern Philippines ('2' on map), and the freshwater *S. tawilis* in Taal Lake (indicated by '3' on map), how plausible is genetic exchange between the fish populations? Would you consider them one or two species? Why?

    Based on the Biological Species Definition by Ernst Mayr, two groups of individuals that cannot interbreed are not the same species. The species are reproductively isolated due to the geographic distances between them (several hundred kilometers on based on the map) and physiological/environmental differences of *S. tawilis* living in a freshwater (and volcanic) land-locked lake and *S. hualiensis* occurring in the marine environment.



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*Taal Lake photograph from www.taalvolcano.com

**Sardinella tawilis* and *S. hualiensis* photographs from D.A. Willette

*All figures (except photographs) adapted from data and publication by Willette et al. 2014