TO BE OR NOT TO BE THE SAME SPECIES?

Interpreting the significance of colour patterns in the sea slug genus Nakamigawaia

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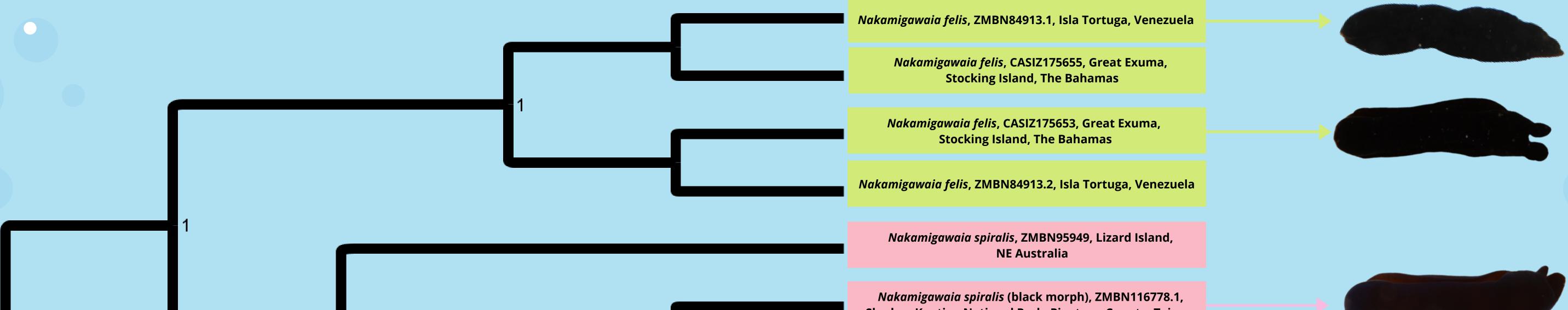
BACKGROUND, GOALS & METHODS

Nakamigawaia is a small genus of marine sea snails in the family Aglajidae with two described species, *N. spiralis* known from the western Pacific Ocean and *N. felis* restricted to the Caribbean region in the western Atlantic Ocean. These sea slugs are small (approx. 0.5 cm) with a uniform all-black body. They are commonly found in tropical shallow waters in sandy habitats [1].

In 2017, during an expedition carried out by the University Museum of Bergen (UM/UiB) to Taiwan, black specimens of *Nakamigawaia* with white dots scattered over the dorsum were collected. This raised the question whether these white-dotted specimens were conspecific with *N. spiralis* or could be an undescribed species.

The aim of the project is to determine whether the white-dotted colour morph of *Nakamigawaia* found in Taiwan represents a new species or is part of the natural variability of *N. spiralis*.

DNA was extracted from specimens of *Nakamigawaia* from the Caribbean, Taiwan, and Japan. Three gene markers (the mitochondrial COI and 16S rRNA and the nuclear 28S rRNA) were amplified and sequenced. Additional sequences were obtained from GenBank. A Bayesian molecular phylogeny was infer using the programme MrBayes [2]. Sequences were edited in Geneious [3], alignments performed with Muscle [4], and best fit models of evolution estimated with JModelTest [5]. Uncorrected *p*-distances between and within species and colour morphs were calculated with Mega [6].



Shadao, Kenting National Park, Pingtung County, Taiwan

Nakamigawaia spiralis, ZMBN95960, Lizard Island, NE Australia

Nakamigawaia spiralis (white-dotted morph), ZMBN116777.1, Shadao, Kenting National Park, Pingtung County, Taiwan

Nakamigawaia spiralis (black morph), ZMBN116778.2, Shadao, Kenting National Park, Pingtung County, Taiwan

Nakamigawaia spiralis (white-dotted morph), ZMBN116777.2

Philinopsis speciosa, ZMBN95997, Kyoda Beach, Okinawa, Japan

0.6 Figure 1: Bayesian molecular phylogeny for the genus *Nakamigawaia* based on the mitochondrial gene marker COI. Figures on nodes refer to posterior probabilities. Yellow stars refer to the white-dotted morph of *Nakamigawaia spiralis*.



Figure 2: *Nakamigawaia spiralis* black morph (Kenting, Taiwan; ZMBN116778).



Figure 3: Nakamigawaia spiralis white-dotted morph (Kenting, Taiwan; ZMBN116777).

RESULTS & CONCLUSIONS:

- The COI Bayesian tree shows two well differentiated clades with maximum support; one with all Caribbean specimens (*N. felis*) and the other with western Pacific specimens (*N. spiralis*).
- No phylogenetic and genetic differentiation was detected between white-dotted and black morphs of *N. spiralis*.
- The COI uncorrected genetic *p*-distance between white-dotted and black morphs of *N. spiralis* was estimated at 0–0.33%.
- The COI uncorrected genetic *p*-distance between *N. spiralis* and *N. felis* was 19.77–20.10%.
- The genus *Nakamigawaia* includes two species: *N. spiralis* and *N. felis*.
- The white-dotted and black morphs are part of the natural variability of *N. spiralis*.

Sources:

[1] Zamora-Silva, A. & Malaquias, MAE. 2018. Zool. J. Linn. Soc., 183: 1–51. [2] Huelsenbeck, J.P. & Ronquist, F. 2001. Bioinformatics, 17: 754–755. [3] Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. & Drummond, A. 2012. Bioinformatics, 28: 1647–1649. [4] Edgar, R.C. 2004. Nucleic Acids Research, 32: 1792–7. [5] Guindon, S. & Gascuel, O. 2003. Systematic Biology, 52: 696–704. [6] Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamaura, K. 2018. Molecular Biology and Evolution, 35: 1547–1549.

