RESEARCH NOTE
Discovery of the only living population of *Pupoidopsis hawaiensis* (Gastropoda: Pupillidae) in the last 50 years

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Abstract: *Pupoidopsis hawaiensis* Pilsbry & C.M. Cooke, 1921, and its monospecific genus, is the only representative of Pupillidae in tropical Pacific Islands. Although most of native species from that region are single island endemics, *P. hawaiensis* has been recorded from Hawaii to the Tuamotus. However, it has not been recorded alive since 1965. Here we report the only modern living population of that species from a single site in Anaa (Tuamotu archipelago). A single COI haplotype and a single 28S allele support the hypothesis of self-fertilization. The species should be listed as Critically Endangered, and included in the list of protected species of French Polynesia. An Action Plan for its conservation should be undertaken, with protection status given to its last known stronghold in Anaa.

Key words: upraised coral reefs, Tuamotu, Anaa, conservation

Pupoidopsis hawaiensis Pilsbry & C.M. Cooke, 1921
(Pupillidae, Pupoidinae) is noteworthy because this species, the sole representative of the genus, is endemic to the Pacifi c Islands and has historically been reported with a wide distribution in this area, while most other native species are single island endemics. It is also the unique indigenous representative of the Pupillidae in tropical Pacifi c islands. First described as an extinct genus and species from Oahu, Molokai, and Maui in the Hawaiian islands (Pilsbry 1920- 1921), it is now known from Hawaii, Line islands (Kiribati) and Tuamotu archipelago (French Polynesia). It has only been found alive four times: in Kiritimati – Christmas (Line islands, Kiribati) in 1924 (Anonymous 1926; Cooke and Neal 1928) and 1965 (Christensen 2013) and in Tabuaeran - Fanning (Line islands, Kiribati) and Hao (Tuamotu) in 1934 (Christensen 2013).
All collectors report the species as belonging to the lowland fauna, either on high islands or atolls. The heavy human impact on lowland habitats is thought to have been the cause of extirpation of *P. hawaiensis* from its former distribution (Gargominy and Meyer 2012).

Here we report the presence of *P. hawaiensis* from Anaa (Tuamotu) for the fi rst time, and document the only currently known living population of this species.

MATERIALS AND METHODS

A malacological survey of Anaa (Tuamotu Islands, French Polynesia) was conducted January 4 - 18, 2018, on behalf of the NGO Pu Tahi Haga no Ganaa and funded by BEST 2.0 (Gargominy *et al.* 2018). Data were collected from 40 localities, representing a total of 676 lots, 22,000 specimens and 38 taxa. Specimens are deposited in the collection of the Museum national d' Histoire naturelle (Paris, France), under numbers MNHN-IM-2018-46, -71, -80, -129, -141, -302, -333, -491, -527, -562, -584, -624 and MNHN-IM-2013-76001 to 76005.
Five specimens of *P. hawaiensis* were prepared for molecular analyses (MNHN-IM-2013-76001 to 76005). Total genomic DNA was extracted using the Qiagen DNA Mini Kit and following the manufacturer's protocol. Two standard genes fragments were selected for PCR amplifi cation: the mitochondrial cytochrome oxidase I (COI, amplified with primers LCO1490 and HCO2198, Folmer *et al.* 1994) and the nuclear large subunit rRNA gene (28S, amplifi ed with primers C1 and D2, Jovelin and Justine 2001). Sequences were compared to other Pupillidae sequences available in Genbank. We assembled two datasets: the COI (655bp) and the 28S (784bp) datasets, including 10 and nine other Genbank Pupillidae sequences, respectively. We used Valloniidae sequences as outgroup. Both datasets were analysed under the maximum likelihood criterion, using RAxML v. 7.0.3 (Stamatakis 2006), selecting a GTR+Γ+I model and random starting tree, with empirical base frequencies

and estimated α-shape parameters and GTR-rates. Nodal support was estimated using 100 bootstrap replicates.

RESULTS

Pupoidopsis hawaiensis shells were collected in one fourth of the collecting sites (11 stations out of 40). These stations are distributed all over the island (Fig. 1), but the species was recorded in greater abundance on the south-western part of the island (Otepipi, Okuku, Hapuku-Putuahara and Tekahora islets). *Pupoidopsis hawaiensis* was found alive at a single station on the motu Otepipi (Figs. 1, 2, 3). The area was no more than 100 square meters. It was quite abundant, with up to ten individuals fixed under a single limestone pebble a dozen centimeters in diameter. It was only found on the ground under rocks. At this site, the vegetation was dominated by the native trees and shrubs *Allophylus rhomboidalis* (Nadeaud) Rdlk., *Cyclophyllum barbatum* (G.Forst.) N.Hallé & J.Florence, *Glochidion wilderi* J.Florence, *Guettarda speciosa* L., *Pisonia grandis* R .Br., *Planchonella tahitensis* (Nadeaud) Pierre ex Dubard, *Premna serratifoli a* L., *Sophora tomentosa* L., *Timonius unifl orus* (Banks ex C.F.Gaertn.) Govaerts, *Xylosma suaveolens* (J.R.Forst. & G.Forst.) G.Forst. The understory was very scarce on this rocky station, with native ferns *Asplenium polyodon* G.Forst. and *Microsorum grossum* (Langsd. & Fisch.) S.B.Andrews.

Among the fi ve sequenced specimens, we recovered a single COI haplotype and a single 28S allele (GenBank accession numbers MT457074-MT457078 and MT457405- MT457409, respectively). The phylogenetic reconstruction places *Pupoidopsis hawaiensis* in the Pupillidae (Fig. 4). In the COI tree *Pupoidopsis* is sister to *Pupoides albilabris*, while in the 28S tree it is basal to a clade containing *Pupoides albilabris*, *Pupilla* and *Gastrocopta*, albeit with no bootstrap support.

DISCUSSION

Although the phylogenetic reconstruction confi rms that *Pupoidopsis hawaiensis* belong to the Pupilloidea, the limited number of published sequences available in Genbank prevent us to clarify its place within the family. A better resolution requires a larger, multigene dataset and the inclusion of the type species of *Pupoides* (*Bulimus nitidulus* L. Pfeiffer, 1839, type locality in Cuba).

The fact that among the five sequenced specimens, we recovered a single COI haplotype and a single 28S allele is congruent with Cooke and Neal (1928) who observed that most specimens are female by lacking penis, and we suggest that the species could reproduce by self-fecundation. This probable self-fertilization, allowing colonization of a new island with a single individual, is congruent with the exceptionally wide distribution of the species within the Pacific.

Anaa is an uplifted atoll, i.e. typically composed of lowland habitats either on sand or on rocky calcareous substrate. Preferred habitat for *P. hawaiensis* in this island is *feo*, which corresponds to areas of upraised limestone (coral reef) up to few meters high, or areas neighbouring *feo* with great density of limestone pebbles on the ground. *Feo* represent areas where vegetation is in the best state of conservation on this atoll, as these areas were not converted to coconut plantations due to the rocky substrate. This relatively good state of conservation of *feo* probably accounts for the fact that *P. hawaiensis* still survives there. However, we are unable to explain why the species has been extirpated everywhere in Anaa except in a single place on Otepipi. No rat predation on this species is recorded, but it is worth noting that just as elsewhere in Anaa, Otepipi harbours two rat species (*Rattus exulans* and *R. rattus*).

As a comparison, the land snail *Mautodontha daedalea* (A.A. Gould, 1846), endemic to Makatea and Anaa atolls in the Tuamotu, was found alive on fi ve stations in Anaa (with 12 additional stations where shells only were found). Despite the fact it belongs to the Endodontidae, a family which was much impacted by habitat perturbations (Solem 1976 ; Abdou and Bouchet 2000 ; Zimmermann *et al.* 2009 ; Sartori *et al.* 2013 ; 2014), it is still more represented than *P. hawaiensis*. *Pupoidopsis hawaiensis* may still survive in Anaa in other stations, but the contrast between the station on Otepipi, where live individuals were abundant, and other stations were only empty shells were found suggests that if it is still extant somewhere else, its abundance is much lower than it used to be. We hypothesize that it has disappeared from 1 0 out of 11 stations, i.e. a reduction of more than 90% of its former range on this single atoll.

As stated by Christensen (2013), some islands in Kiribati and Tuamotu may still support living populations of *P. hawaiensis*, as it is now proved for Anaa. However, the species must be highly threatened, since it is extinct in most of its known former range (Kiribati, Hawaiian archipelago – Christensen (2013)) and close to extinction in Anaa. As for most invertebrates which are under-studied, we cannot rule out the fact that it is still extant somewhere else. However, it has never been found in French Polynesia, where extensive collections have been done (for instance, Society islands, Austral islands, Gambier islands, Makatea – e.g. Gargominy 2008, Christensen and Kahn 2017, Sartori *et al*., 2013, 2014, Abdou and Bouchet 2000). Additional surveys should be

undertaken in neighboring islands (especially Niau, another uplifted atoll) to fi nd the species. *Pupoidospis hawaiensis* is currently classifi ed as data defi - cient DD in the IUCN Red List (Cowie and Pokryszko 1996). We propose that it should be listed as Critically Endangered B1,B2ab (i,ii,iii,iv,v) (only one living popula tion on a single atoll and general decline of extent of occurrence, area of occupancy, quality of habitat, number of locations, number of mature individuals). We recommend that it should be included in the list of protected species of French Polynesia (Anonymous 2018), and an Action Plan for its conservation should be undertaken. The *feo* of Otepipi could also be given a protection status, for instance through the general management plan (PGA) of the Anaa municipality. *Feo* also provide strong protection against natural disasters as barriers to the ocean.

ACKNOWLEDGMENTS

We thank the European Commission which founded part of this work through BEST 2.0 project 1264 "Sauvegarde de la fl ore menacée de l'atoll de Anaa et défi nition de zones prioritaires de conservation basées sur la flore et la malacofaune sur les atolls soulevés de Anaa et Niau aux Tuamotu". We thank also Maima Matai and Mare Paeahi who helped for malacological collections in the field. Frédéric Alain Jacq prepared the map of Figure 1. This work was supported by 'Service de Systématique Moléculaire' (UMS2700 Acquisition et Analyse de Données pour l'Histoire naturelle, MNHN). We also thank two anonymous reviewers for helpful comments on the manuscript.

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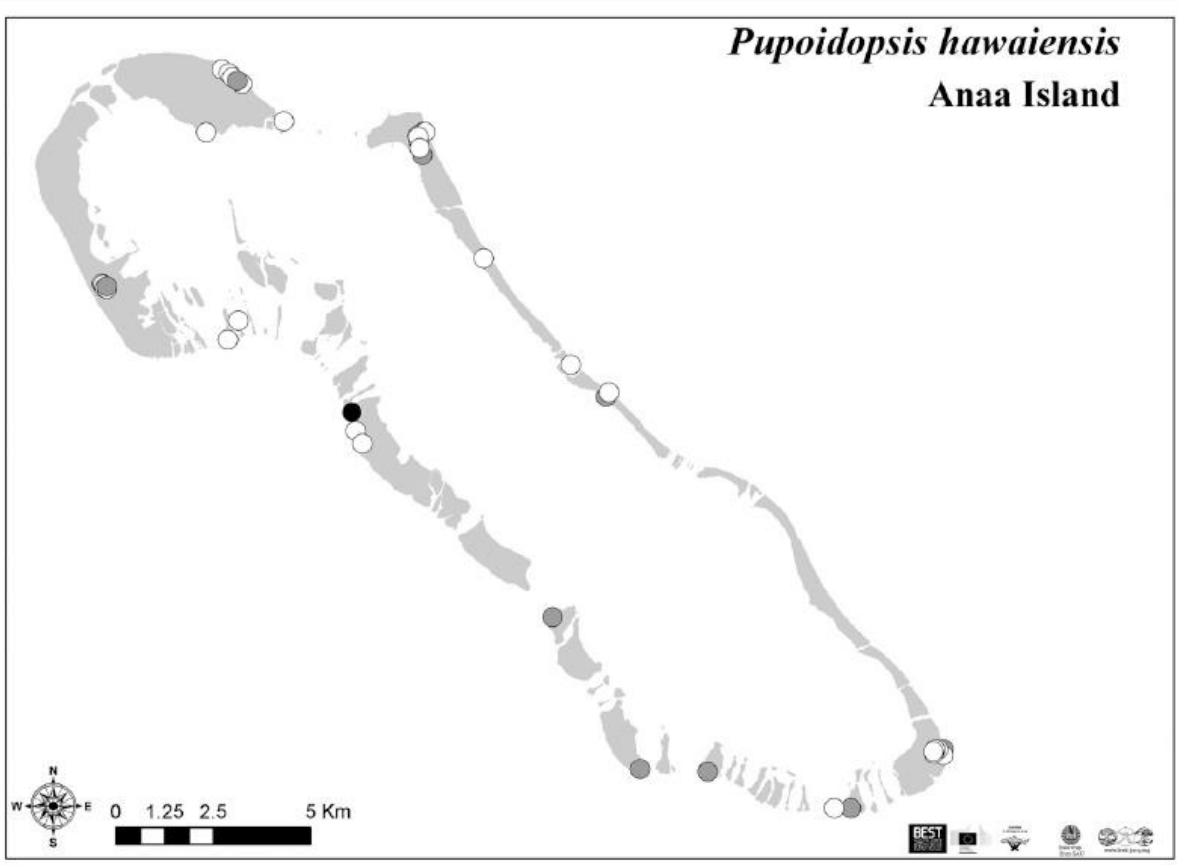

Figure 1. Map showing the locations where *Pupoidopsis hawaiensis* was found. Black circles: living population, grey circles: shells only, white circles: absent.

Figure 2. Live *Pupoidopsis hawaiensis* in place under a stone on the ground.

Figure 3. Live *Pupoidopsis hawaiensis*.

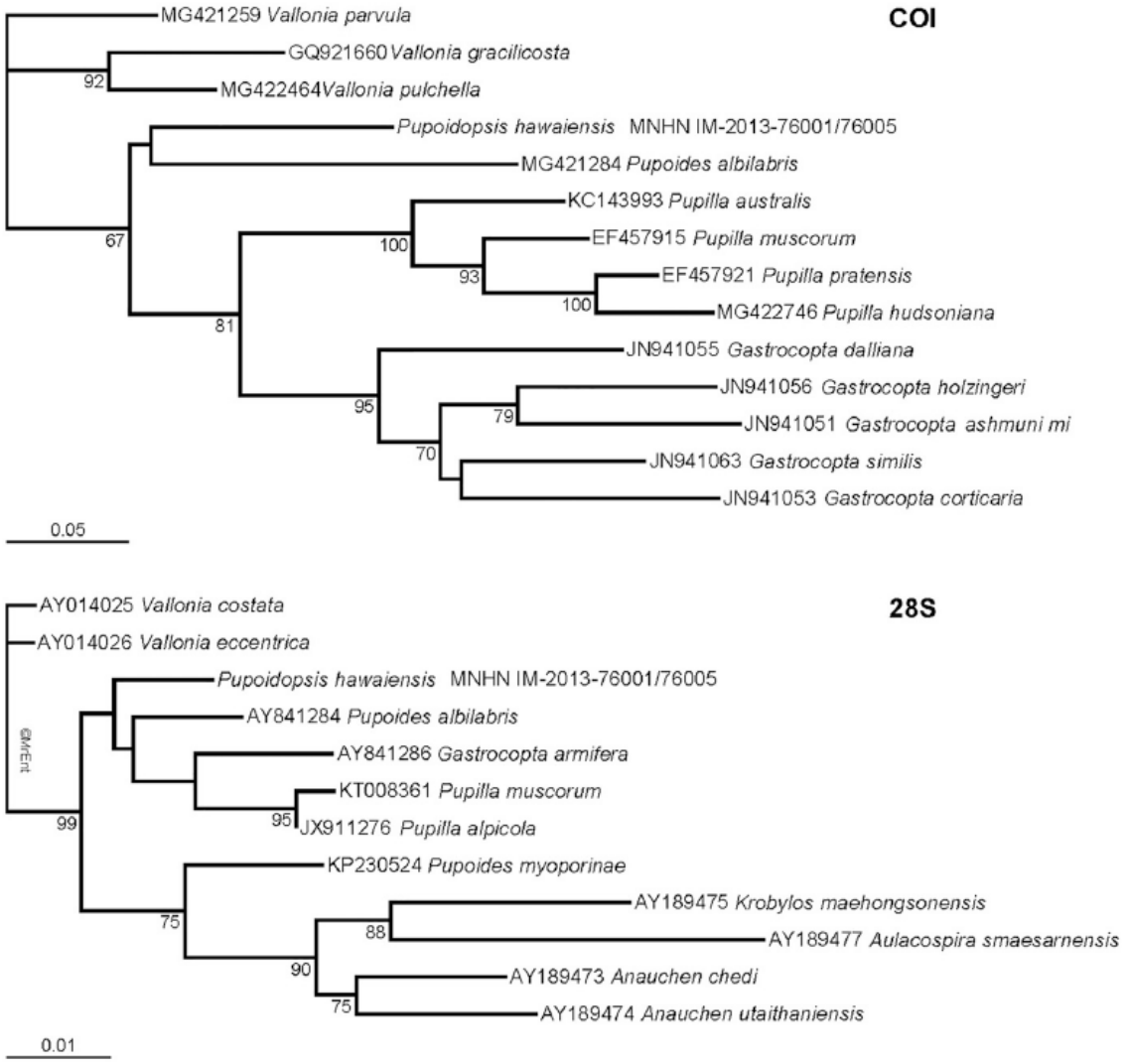

Figure 4. Maximum-likelihood trees based on COI (top) and 28S sequences (bottom). The bootstrap support values (>50%) are indicated at the node.