

ORIGINS OF SOFTSHELL TURTLES IN HAWAII WITH IMPLICATIONS FOR
CONSERVATION

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ABSTRACT

Genetic approaches, along with ecological and natural history data, can help address the status of non-native species, particularly in taxonomically problematic introductions to clarify their identities and geographic origins. We used these approaches to investigate non-native softshell turtles (family: Trionychidae) in the Hawaiian Islands: *Palea steindachneri* and *Pelodiscus sinensis*. In light of multiple knowledge gaps surrounding these introduced populations and native range conservation concerns, we clarified their range and distribution as well as their genetic status in Hawaii. Field surveys indicate that the current range of *P. steindachneri* is limited to Kauai whereas *Pelodiscus* is limited to Oahu. Our genetic study revealed two species of the *Pelodiscus* complex present (*P. sinensis sensu stricto* and *P. maackii*) and low genetic diversity within *P. steindachneri*. Historical and genetic data indicate that Hawaiian populations of *P. sinensis* and *P. steindachneri* originate from China and *P. maackii* originates from Japan.

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INTRODUCTION

Non-native species have the potential to induce large-scale ecological changes that threaten the biodiversity of native communities in a multitude of ways including competition, habitat alteration, hybridization, predation, and the transmission of pathogens and parasites (Vitousek et al. 1997, Wilcove et al. 1998, Gurevitch and Padilla 2004). These effects are of particular importance in island ecosystems where non-native species tend to exhibit amplified negative impacts relative to continental range expansions (Mack et al. 2000, Sax et al. 2002, Sax and Gaines 2008), with a local biota that is itself often endemic and at a higher risk for extinction (Steadman 2006). Although conspicuous invasive taxa receive the most scientific attention, they comprise only a small proportion of introductions (Williamson and Fitter 1996), leaving the majority of non-native species and their potential impacts relatively unknown.

While ecological and natural history data have conventionally influenced conservation policies (Simberloff 2003), advances in genetic analyses can further illuminate the dynamics and consequences of biological introductions. The application of molecular approaches to introduced populations can reveal hybridization and introgression (Holsbeek et al. 2008, Parham et al. 2013, Gering et al. 2015), elucidate geographic origin (Jousson et al. 2000, Johnson et al. 2011, Siler et al. 2014), infer population demography (Thibault et al. 2009, Kalinowski et al. 2010; Purcell et al. 2012), and elucidate the presence of cryptic species (May and Marsden 1992, Holland et al. 2004, Stepien and Tumeo 2006). Genetic approaches are particularly helpful when unraveling introduction events that involve taxonomically problematic, sympatric and morphologically indistinguishable species, a situation particularly common to aquatic introductions (see Booth et al. 2007 and Geller et al. 2010 for recent reviews). A lack of fundamental knowledge, such as the taxonomic identities and geographic origins of invaders, obstructs the ability to understand or predict the severity of the impact of introduced species and hinders the development of effective management plans (Byers et al. 2002).

Here we report on the non-native populations of softshell turtles (family: Trionychidae) in the Hawaiian Islands. Softshell turtles were introduced multiple times to Hawaii starting in the late 1800s, with a brief halt during WWII (1939-1945) and then resuming until the Department of Agriculture formally prohibited importation in 1999 (Brock 1947, McKeown and Webb 1982). Two species are currently established on Oahu and Kauai. These turtles were

initially imported under the name '*Pelodiscus sinensis*', although the later discovery of introduced *Palea steindachneri* (Sienbenrock 1906) populations indicated that two species were actually being imported under a single name (Webb 1980). Although these two species are largely morphologically similar, adults can be distinguished by the presence of coarse tubercles at the base of the neck on *P. steindachneri* whereas *P. sinensis* lacks these. Hatchlings of *P. steindachneri* have a pale yellow neck stripe extending from behind the eye, whereas *P. sinensis* hatchlings have orange plastrons with black spots (Ernst and Barbour 1989). More recently, the monotypic *Pelodiscus sinensis* (Wiegmann 1835) has been shown to comprise a complex of four morphologically cryptic putative species-level lineages: *P. sinensis*, *P. maackii* (Brant 1857), *P. parviformis* (Tang 1997), and *P. axenaria* (Zhou et al. 1991) (Stuckas and Fritz 2011, Yang et al. 2011). Therefore, this revised understanding of diversity and taxonomy in *P. sinensis sensu lato* necessitates an identification of which or how many of these four species are established in the Hawaiian Islands.

The initial ambiguity regarding which species were being imported is unsurprising given that *P. steindachneri* and the *Pelodiscus* species complex share broad morphological similarities and co-occur in much of their native ranges across Asia. *Palea steindachneri* is native to Laos, Vietnam, and southeastern China, including the islands of Hong Kong and Hainan (Ernst and Barbour 1989). The *Pelodiscus* complex occurs natively across a broad region of eastern Asia: *P. sinensis sensu stricto* occurs in central and eastern China, Taiwan, Vietnam, and Japan; *P. maackii* occurs in northern China, the Korean Peninsula, Japan, and southeastern Russia; lastly, *P. parviformis* and *P. axenaria* occur sympatrically in Guangxi, China (Fritz and Havaš 2007).

Softshell turtle populations in Hawaii had historically been presumed to have been initially imported and released by Chinese immigrants who brought them to the islands as a food source (Brock 1947). While this is plausible, no data or documentation has yet been brought to bear on the geographic origin of the naturalized populations. Hawaii experienced immigration from both China and Japan beginning in 1852 and in 1885, respectively. The overall number of Japanese immigrants that had arrived in Hawaii by 1924 was at least three-fold the number of Chinese immigrants (Ichihashi 1932, Strong 1934, Lind 1955). Given that a species of *Pelodiscus* is likely native to Japan (Suzuki and Hikida 2014) and that the first large-scale softshell farm appeared in Japan in 1866 (Mitsukuri 1906), preceding its counterpart in China by

at least a century (Shi and Parham 2001, Shi et al. 2004), it is also plausible that populations in Japan were a source for Hawaii populations.

In their native ranges, wild populations of both *Pelodiscus* and *Palea* have been depleted by high levels of exploitation for food and medicinal uses, compounded by habitat degradation and pollution (van Dijk et al. 2000). *Palea steindachneri* is listed as ‘endangered’ on the International Union for Conservation of Nature (IUCN) Red List and appears on the Convention on International Trade in Endangered Species (CITES) Appendix II indicating that trade of these taxa must be controlled in order to avoid further threats to their survival (CITES 2014, IUCN 2014). Therefore the presence of *P. steindachneri* in Hawaii is significant precisely because the native range populations are imperiled. This population is one of only two remaining viable global populations (Asian Turtle Trade Working Group 2000), the other being Mauritius, and thus is an important assurance colony that could prove critical for long-term survival of the species. *Pelodiscus sinensis sensu lato* is listed as ‘vulnerable’ by the IUCN and although the newly revised species designations have not yet been evaluated by the IUCN, they appear on the CITES Appendix II (CITES 2014). Currently both *Pelodiscus* and *P. steindachneri* are raised in farms, although the vast majority of these efforts focus on raising *Pelodiscus* (approximately 98% of the farm raised populations; Shi et al. 2008). These farming efforts form the basis of a multibillion dollar turtle trade in China, making *Pelodiscus* the most economically important chelonian in the world (Shi et al. 2008). Rather than alleviating the pressure due to commercial demand on wild populations, farming generates further stress by creating issues such as the necessity of sustained harvest of wild populations to supplement breeding stock in farms, enabling interspecific and intergeneric hybridization (Parham et al 2001, Parham and Shi 2001), and increasing the market availability which in turn feeds demand (Shi et al. 2007). The cultural demand for softshell turtles, enhanced by large-scale farming, makes conservation efforts in the native range particularly challenging.

McKeown and Webb (1982) provided a thorough description of the distribution and abundance of these species on Oahu and Kauai, and described *P. steindachneri* as the more abundant of the two species with populations on both Oahu and Kauai, whereas *Pelodiscus* was limited to streams in Kapaa on Kauai. In light of the extended importation and inter-island transport of these species in subsequent decades, recent taxonomic revisions within *P. sinensis sensu lato*, ongoing global conservation concerns, and unknown ecosystem impacts in Hawaii,

this work is now in need of a comprehensive update. Here we studied the naturalized softshell populations of Hawaii in order to clarify the contemporary distribution of these species as well as their genetic status. We conducted extensive field surveying and trapping efforts and found the current distribution differed dramatically from the previously reported distribution (McKeown and Web 1982). Our results suggest the current range of *P. steindachneri* to be essentially limited to Kauai whereas the range of *Pelodiscus* is limited to Oahu. Our molecular systematic results revealed that two species within the *Pelodiscus* complex are established on Oahu, and the genetic diversity of *P. steindachneri* is small. This work provides a fundamental clarification of the taxonomic status of introduced softshell turtles in Hawaii and highlights the importance of continued survey and monitoring efforts with the aim of developing management strategies to protect these threatened lineages and to learn more about ecological interactions with native Hawaiian fauna.

MATERIALS AND METHODS

Survey and capture methodology

Sampling sites on Oahu and Kauai were chosen based on the previously reported range of softshells (McKeown and Webb 1982), previous trapping success, and information from local residents (Figure 1). When possible, we further surveyed at freshwater bodies beyond the historic range in an attempt to locate additional populations. Following an initial visual survey of the locality, we utilized turtle traps that were baited with a combination of sardines, anchovy paste, chicken liver, and checked at 24-hour intervals. Sampling efforts along the eastern coast of Kauai were conducted for six day periods in 2007, 2008, 2009, 2011, 2013, and 2014. Sampling efforts on Oahu focused on the southeastern half of the island and were conducted for 37 days in 2011, 14 days in 2013 and 15 days in 2014. Captured individuals were released following data collection. In addition to sampling wild populations, we surveyed pet store and food markets on Oahu for the availability of softshell turtles in 2014. We further inspected specimens of *Pelodiscus* and *P. steindachneri* from the collection housed at the Bernice Pauahi Bishop Museum in Honolulu to verify previous taxonomic identifications and supplement our locality data.

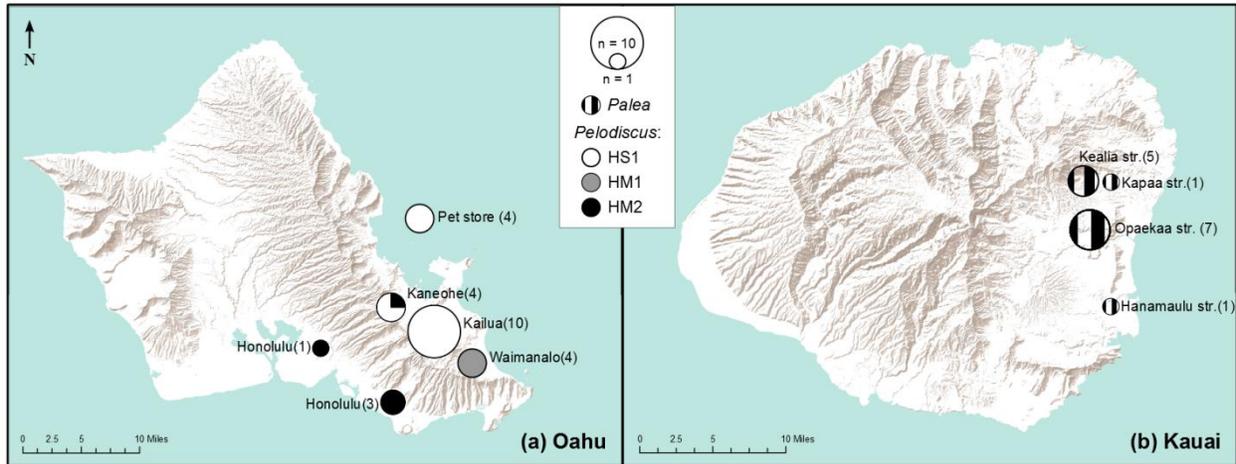


Figure 1. Maps of Oahu and Kauai showing sampling localities. Sites on Oahu are indicated with the relative haplotype frequencies found at each site, with pet store samples offset to avoid implying a geographical association. Sites on Kauai are indicated with the number of individuals captured at each site. Sample sizes are given in parentheses.

Tissue sample collection

Twenty six tissue samples of *Pelodiscus* spp. were collected from five localities on the island of Oahu, Hawaii. Of these, 21 samples were obtained from wild-caught specimens, four from a pet store in Honolulu, and one loaned from the Bishop Museum. All available tissue samples of *Palea steindachneri* were used, consisting of six samples from two localities on the island of Kauai, Hawaii. See Figure 1 for sampling localities in Hawaii and Table 1 for details of all individuals sampled. For individuals of both *Pelodiscus* and *Palea*, tissue samples were taken from the webbing of the feet or skin of the shell and placed in 95% ethanol until processing.

Table 1. Details of *Palea steindachneri* and *Pelodiscus* samples collected from Hawaii. Nuclear C-mos gene was not sequenced from all samples.

Specimen ID	Taxon	Haplotypes		Locality
		mtDNA	C-mos	
TNE2806	<i>Palea steindachneri</i>	-	-	Kauai: Kealia Stream
TNE2805	<i>Palea steindachneri</i>	-	-	Kauai: Kealia Stream
TNE4060	<i>Palea steindachneri</i>	-	-	Kauai: Kapaa Stream
TNE4065	<i>Palea steindachneri</i>	-	-	Kauai: Kealia Stream
TNE4036	<i>Palea steindachneri</i>	-	-	Kauai: Kealia Stream
TNE4039	<i>Palea steindachneri</i>	-	-	Kauai: Kealia Stream
TNE4029	<i>Pelodiscus sinensis</i>	HS1	Cmos4	Oahu: Kaneohe: Hoomaluhia Bot Gar
TNE4019	<i>Pelodiscus sinensis</i>	HS1	Cmos1	Oahu: Kaneohe: Hoomaluhia Bot Gar
BPBM31603	<i>Pelodiscus sinensis</i>	HS1	-	Oahu: Kaneohe: 45-567 Pahia Rd
TNE4042	<i>Pelodiscus sinensis</i>	HS1	Cmos1	Oahu: Kailua: Maunawili Stream
TNE4030	<i>Pelodiscus sinensis</i>	HS1	Cmos5	Oahu: Kailua: Maunawili Stream
TNE4074	<i>Pelodiscus sinensis</i>	HS1	Cmos5	Oahu: Kailua: Maunawili Stream
TNE4020	<i>Pelodiscus sinensis</i>	HS1	Cmos6	Oahu: Kailua: Maunawili Stream
TNE4028	<i>Pelodiscus sinensis</i>	HS1	Cmos2	Oahu: Kailua: Maunawili Stream
TNE4070	<i>Pelodiscus sinensis</i>	HS1	Cmos5	Oahu: Kailua: Maunawili Stream
TNE4067	<i>Pelodiscus sinensis</i>	HS1	Cmos5	Oahu: Kailua: Maunawili Stream
TNE4027	<i>Pelodiscus sinensis</i>	HS1	Cmos5	Oahu: Kailua: Maunawili Stream
TNE4026	<i>Pelodiscus sinensis</i>	HS1	Cmos3	Oahu: Kailua: Maunawili Stream
TNE4072	<i>Pelodiscus sinensis</i>	HS1	Cmos7	Oahu: Kailua: Maunawili Stream
RCT632	<i>Pelodiscus sinensis</i>	HS1	-	Oahu: Kalihi Pet Center
RCT633	<i>Pelodiscus sinensis</i>	HS1	-	Oahu: Kalihi Pet Center
RCT634	<i>Pelodiscus sinensis</i>	HS1	-	Oahu: Kalihi Pet Center
RCT635	<i>Pelodiscus sinensis</i>	HS1	-	Oahu: Kalihi Pet Center
TNE4043	<i>Pelodiscus maackii</i>	HM1	Cmos4	Oahu: Waimanalo: Waimanalo Stream
TNE4016	<i>Pelodiscus maackii</i>	HM1	Cmos2	Oahu: Waimanalo: Waimanalo Stream
TNE4057	<i>Pelodiscus maackii</i>	HM1	Cmos1	Oahu: Waimanalo: Waimanalo Stream
TNE4012	<i>Pelodiscus maackii</i>	HM1	Cmos4	Oahu: Waimanalo: Waimanalo Stream
RCT631	<i>Pelodiscus maackii</i>	HM2	Cmos1	Oahu: Honolulu: Honolulu Country Club
RCT614	<i>Pelodiscus maackii</i>	HM2	Cmos4	Oahu: Honolulu: Palolo Stream
RCT615	<i>Pelodiscus maackii</i>	HM2	Cmos3	Oahu: Honolulu: Palolo Stream
RCT616	<i>Pelodiscus maackii</i>	HM2	Cmos3	Oahu: Honolulu: Palolo Stream
TNE4046	<i>Pelodiscus maackii</i>	HM2	Cmos7	Oahu: Kaneohe: Hoomaluhia Bot Gar

DNA extraction, amplification and sequencing

Genomic DNA was extracted using either the Qiagen DNeasy Blood and Tissue Kit or a salt extraction protocol (Sambrook and Russell 2001). We sequenced one nuclear and three mitochondrial markers. Our nuclear marker is the C-mos gene and mitochondrial markers consist of the 12S rRNA gene, NADH dehydrogenase subunit 4 (ND4), and cytochrome b (Cyt b). These markers were selected because they have been shown to be informative for separation of phylogenetic relationships within the genus *Pelodiscus* (Fritz et al. 2010) and relationships between chelonian terminal taxa in general (Weisrock and Janzen 2000, Honda et al. 2002, Feldman and Parham 2004, Le et al. 2006). MangoTaq-mediated 25 μ l PCR amplifications were performed with an initial denaturation of 5 min at 94°C, followed by 35 cycles of denaturation (94°C for 30 sec), annealing (45 sec at 40-65°C), and extension (72°C for 60 sec) with a final extension period of 72°C for 5 min. See Table 2 for marker-specific primers and annealing temperatures. PCR products were purified with ExoSAP-IT (Affymetrix) and sequenced in both directions on an ABI 3130XL 16-capillary array Genetic Analyzer.

Table 2. Primers used for amplification and sequencing of mitochondrial and nuclear markers, and the associated model of sequence evolution selected via MrModelTest.

Marker	Model		Primers	Annealing	Primer sources
	Pelodiscus	Palea			
12S rRNA	GTR + G	HKY + I	L1091, H1478	65°C	Kocher et al. 1989
ND4	GTR + G	GTR + G	ND4-672, H- Leu	54°C	Engstrom et al. 2004, Stuart and Parham 2004
Cyt b	GTR + G	GTR + I	Cytb-G, mt-f- na, mt-C2, mt- E-rev2	40-44°C	Spinks et al. 2004, Fritz et al. 2006
C-mos	-	-	Cmos1, Cmos3	56°C	Le et al. 2006

Phylogenetic and haplotype network analyses

Sequences were edited and aligned in Geneious v6.1.7 (Kearse et al. 2012). Additional sequences of *Pelodiscus* spp. and *Palea steindachneri* representing the native range were downloaded from GenBank and aligned with our data according to their respective taxon (Table 3). In total, we utilized 89 additional sequences of *Pelodiscus* and four of *P. steindachneri*, including the sequenced mitochondrial (AY687385) genomes of *P. sinensis*, *P. maackii* (AY962573), and *Palea steindachneri* (FJ541030). A sequencing error in positions 292-293 of AY962573 was corrected as noted by Fritz et al. (2010). GenBank sequences were excluded from the concatenated datasets unless it was clear that they were derived from the same individual. We selected two other closely related softshell turtles, *Apalone spinifera* (JF966197) and *Rafetus euphraticus* (FM999033, AY259604, AY259554), and either *Palea steindachneri* (FJ541030) or *Pelodiscus sinensis* (AY687385) as outgroups. Our concatenated mitochondrial DNA (mtDNA) data set for *Pelodiscus* was composed of up to 2,394 base pairs (bp) for 47 individuals and the matrix was nearly complete with 2.4% missing data. For *Palea steindachneri*, our concatenated mtDNA data set was composed of up to 2,252 bp for 10 individuals with 0.4% missing data.

We used the Akaike Information Criterion (AIC) as implemented in MrModelTest v2.3 (Nylander 2004) to select a model of molecular evolution for each dataset (Table 3) and performed Bayesian phylogenetic analyses in MrBayes v3.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003) for the concatenated mtDNA data set and each individual gene. Bayesian analyses consisted of two independent analyses each comprising four incrementally heated chains run for 10 million generations and sampled every 1000 generations with the first 25% of samples discarded as burn-in. We assessed convergence by checking that the average standard deviation of split frequencies approached zero and verified that the runs had reached stationarity by checking that the potential scale reduction factor approached one. In addition, we examined the Markov chain Monte Carlo (MCMC) output in Tracer and AWTY (Wilgenbusch et al. 2004, Rambaut and Drummond 2007, Nylander et al. 2008) to ensure that all chains were sampling from the same target distribution.

In addition to phylogenetic trees, haplotype networks were also constructed. At the population level, the persistence of intraspecific ancestral haplotypes and the possibility of

recombination events in nuclear markers violate assumptions in phylogenetic methods. These events may result in reticulate relationships that are imperfectly represented by bifurcating trees (Posada and Crandall 2001). To address the possibility of these issues, haplotype networks were constructed in PopART (<http://popart.otago.ac.nz>) implementing the statistical parsimony algorithms of TCS v1.21 (Clement et al. 2000). GenBank sequence fragments of short length were excluded from haplotype analyses to minimize the amount of missing data. For the nuclear marker, we additionally used PHASE v2.1 (Stephens et al. 2001, Stephens and Donnelly 2003) to reconstruct haplotype data before producing a parsimony network using TCS v.121 in PopART.

Table 3. GenBank sequences of *Palea steindachneri* and *Pelodiscus* used for comparison.

Accession #	Marker	Taxon	Locality	Reference
AF043414	12S rRNA	<i>Palea steindachneri</i>	China: Unknown: Market	Wu et al. 1999
AY259552	Cyt b	<i>Palea steindachneri</i>	China-Vietnam border	Engstrom et al. 2004
FJ541030	Genome	<i>Palea steindachneri</i>	China: Unknown	Li 2010 (unpub)
AY259602	ND4	<i>Palea steindachneri</i>	China-Vietnam border	Engstrom et al. 2004
AY743421	12S rRNA	<i>Pelodiscus axenaria</i>	China: Unknown	Chen et al. 2005
HQ116616	12S rRNA	<i>Pelodiscus axenaria</i>	China: Quanzhou County	Yang et al. 2011
HQ116617	12S rRNA	<i>Pelodiscus axenaria</i>	China: Quanzhou County	Yang et al. 2011
HQ116618	12S rRNA	<i>Pelodiscus axenaria</i>	China: Quanzhou County	Yang et al. 2011
HQ116619	12S rRNA	<i>Pelodiscus axenaria</i>	China: Quanzhou County	Yang et al. 2011
HQ116620	12S rRNA	<i>Pelodiscus axenaria</i>	China: Quanzhou County	Yang et al. 2011
AY583693	Cyt b	<i>Pelodiscus axenaria</i>	China: Hunan: Market and Breeding Farm	Chen et al. 2006
HQ116592	Cyt b	<i>Pelodiscus axenaria</i>	China: Quanzhou County	Yang et al. 2011
HQ116593	Cyt b	<i>Pelodiscus axenaria</i>	China: Quanzhou County	Yang et al. 2011
HQ116594	Cyt b	<i>Pelodiscus axenaria</i>	China: Quanzhou County	Yang et al. 2011
HQ116595	Cyt b	<i>Pelodiscus axenaria</i>	China: Quanzhou County	Yang et al. 2011
HQ116596	Cyt b	<i>Pelodiscus axenaria</i>	China: Quanzhou County	Yang et al. 2011
HQ116584	ND4	<i>Pelodiscus axenaria</i>	China: Quanzhou County	Yang et al. 2011
HQ116585	ND4	<i>Pelodiscus axenaria</i>	China: Quanzhou County	Yang et al. 2011
HQ116586	ND4	<i>Pelodiscus axenaria</i>	China: Quanzhou County	Yang et al. 2011
HQ116587	ND4	<i>Pelodiscus axenaria</i>	China: Quanzhou County	Yang et al. 2011
HQ116588	ND4	<i>Pelodiscus axenaria</i>	China: Quanzhou County	Yang et al. 2011
FM999003	12S rRNA	<i>Pelodiscus maackii</i>	Russia: Lake Khanka	Fritz et al. 2010
JQ815362	12S rRNA	<i>Pelodiscus maackii</i>	Korea: Daegu Dalseong-gun	Jeong et al. 2013
JQ837982	12S rRNA	<i>Pelodiscus maackii</i>	Korea: Daegu Dalseong-gun	Jeong et al. 2013
JQ837983	12S rRNA	<i>Pelodiscus maackii</i>	Korea: Daegu Dalseong-gun	Jeong et al. 2013
AB904720	Cyt b	<i>Pelodiscus maackii</i>	Japan: Wild and Farm Bred	Suzuki and Hikida 2014
AB904721	Cyt b	<i>Pelodiscus maackii</i>	Japan	Suzuki and Hikida 2014
AB904722	Cyt b	<i>Pelodiscus maackii</i>	Japan: Wild and Farm Bred	Suzuki and Hikida 2014
AB904723	Cyt b	<i>Pelodiscus maackii</i>	Japan	Suzuki and Hikida 2014
AB904724	Cyt b	<i>Pelodiscus maackii</i>	Japan	Suzuki and Hikida 2014
AB904725	Cyt b	<i>Pelodiscus maackii</i>	Japan	Suzuki and Hikida 2014
FM999011	Cyt b	<i>Pelodiscus maackii</i>	Russia: Lake Khanka	Fritz et al. 2010
AY962573	Genome	<i>Pelodiscus maackii</i>	Korea: Daegu: Breeding Farm	Jung et al. 2006
FM999019	ND4	<i>Pelodiscus maackii</i>	Russia: Lake Khanka	Fritz et al. 2010

Table 3. (Continued) GenBank sequences of *Palea steindachneri* and *Pelodiscus* used for comparison.

AY743420	12S rRNA	<i>Pelodiscus parviformis</i>	China: Unknown	Chen et al. 2005
HQ116621	12S rRNA	<i>Pelodiscus parviformis</i>	China: Quanzhou County	Yang et al. 2011
HQ116622	12S rRNA	<i>Pelodiscus parviformis</i>	China: Quanzhou County	Yang et al. 2011
HQ116623	12S rRNA	<i>Pelodiscus parviformis</i>	China: Quanzhou County	Yang et al. 2011
AY259553	Cyt b	<i>Pelodiscus parviformis</i>	China: Unknown: Market	Engstrom et al. 2004
AY583692	Cyt b	<i>Pelodiscus parviformis</i>	China: Hunan: Market and Breeding Farm	Chen et al. 2006
HQ116597	Cyt b	<i>Pelodiscus parviformis</i>	China: Quanzhou County	Yang et al. 2011
HQ116598	Cyt b	<i>Pelodiscus parviformis</i>	China: Quanzhou County	Yang et al. 2011
HQ116599	Cyt b	<i>Pelodiscus parviformis</i>	China: Quanzhou County	Yang et al. 2011
AY259603	ND4	<i>Pelodiscus parviformis</i>	China: Unknown: Market	Engstrom et al. 2004
HQ116589	ND4	<i>Pelodiscus parviformis</i>	China: Quanzhou County	Yang et al. 2011
HQ116590	ND4	<i>Pelodiscus parviformis</i>	China: Quanzhou County	Yang et al. 2011
HQ116591	ND4	<i>Pelodiscus parviformis</i>	China: Quanzhou County	Yang et al. 2011
AB090017	12S rRNA	<i>Pelodiscus sinensis</i>	Japan: Pet Trade	Honda et al. 2002
AF043413	12S rRNA	<i>Pelodiscus sinensis</i>	China: Unknown: Market	Wu et al. 1999
FM999004	12S rRNA	<i>Pelodiscus sinensis</i>	China: Hong Kong: Market	Fritz et al. 2010
FM999005	12S rRNA	<i>Pelodiscus sinensis</i>	China: Hanzhong: Market	Fritz et al. 2010
FM999006	12S rRNA	<i>Pelodiscus sinensis</i>	China: Hong Kong: Market	Fritz et al. 2010
FM999007	12S rRNA	<i>Pelodiscus sinensis</i>	China: Suzhou: Market	Fritz et al. 2010
FM999008	12S rRNA	<i>Pelodiscus sinensis</i>	China: Guangyuan: Market	Fritz et al. 2010
FM999009	12S rRNA	<i>Pelodiscus sinensis</i>	Vietnam: Phong Nha-Ke Bang Reserve	Fritz et al. 2010
FM999010	12S rRNA	<i>Pelodiscus sinensis</i>	Vietnam: Khánh Hòa: vicinity of Nha Trang	Fritz et al. 2010
JQ688040	12S rRNA	<i>Pelodiscus sinensis</i>	China: Breeding Farm	Xu et al. 2012
JQ688041	12S rRNA	<i>Pelodiscus sinensis</i>	China: Breeding Farm	Xu et al. 2012
JQ688042	12S rRNA	<i>Pelodiscus sinensis</i>	China: Breeding Farm	Xu et al. 2012
AB904726	Cyt b	<i>Pelodiscus sinensis</i>	Japan	Suzuki and Hikida 2014
AB904727	Cyt b	<i>Pelodiscus sinensis</i>	Japan	Suzuki and Hikida 2014
AB904728	Cyt b	<i>Pelodiscus sinensis</i>	Japan	Suzuki and Hikida 2014
AB904729	Cyt b	<i>Pelodiscus sinensis</i>	Japan	Suzuki and Hikida 2014
FM999012	Cyt b	<i>Pelodiscus sinensis</i>	China: Hong Kong: Market	Fritz et al. 2010

Table 3. (Continued) GenBank sequences of *Palea steindachneri* and *Pelodiscus* used for comparison.

FM999013	Cyt b	<i>Pelodiscus sinensis</i>	China: Shaanxi: Hanzhong: Market	Fritz et al. 2010
FM999014	Cyt b	<i>Pelodiscus sinensis</i>	China: Hong Kong: Market	Fritz et al. 2010
FM999015	Cyt b	<i>Pelodiscus sinensis</i>	China: Jiangsu: Suzhou: Market	Fritz et al. 2010
FM999016	Cyt b	<i>Pelodiscus sinensis</i>	China: Sichuan: Guangyuan: Market	Fritz et al. 2010
FM999017	Cyt b	<i>Pelodiscus sinensis</i>	Vietnam: Phong Nha-Ke Bang Reserve	Fritz et al. 2010
FM999018	Cyt b	<i>Pelodiscus sinensis</i>	Vietnam: Khánh Hòa: vicinity of Nha Trang	Fritz et al. 2010
FR851459	Cyt b	<i>Pelodiscus sinensis</i>	China: Macao: Tiger River	Stuckas and Fritz 2011
JQ837977	Cyt b	<i>Pelodiscus sinensis</i>	Korea: Daegu Dalseong-gun	Jeong et al. 2013
JQ837978	Cyt b	<i>Pelodiscus sinensis</i>	Korea: Daegu Dalseong-gun	Jeong et al. 2013
JQ837979	Cyt b	<i>Pelodiscus sinensis</i>	Korea: Daegu Dalseong-gun	Jeong et al. 2013
AY687385	Genome	<i>Pelodiscus sinensis</i>	China: Unknown: Market	Peng et al. 2005
FM999020	ND4	<i>Pelodiscus sinensis</i>	China: Hong Kong: Market	Fritz et al. 2010
FM999021	ND4	<i>Pelodiscus sinensis</i>	China: Shaanxi: Hanzhong: Market	Fritz et al. 2010
FM999022	ND4	<i>Pelodiscus sinensis</i>	China: Hong Kong: Market	Fritz et al. 2010
FM999023	ND4	<i>Pelodiscus sinensis</i>	China: Jiangsu: Suzhou: Market	Fritz et al. 2010
FM999024	ND4	<i>Pelodiscus sinensis</i>	China: Sichuan: Guangyuan: Market	Fritz et al. 2010
FM999025	ND4	<i>Pelodiscus sinensis</i>	Vietnam: Phong Nha-Ke Bang Reserve	Fritz et al. 2010
FM999026	ND4	<i>Pelodiscus sinensis</i>	Vietnam: Khánh Hòa: vicinity of Nha Trang	Fritz et al. 2010
KF652200	ND4	<i>Pelodiscus</i> spp.	China: Breeding Farm	Zhang et al. 2014
KF652201	ND4	<i>Pelodiscus</i> spp.	China: Breeding Farm	Zhang et al. 2014
KF652202	ND4	<i>Pelodiscus</i> spp.	China: Breeding Farm	Zhang et al. 2014
KF652203	ND4	<i>Pelodiscus</i> spp.	China: Breeding Farm	Zhang et al. 2014
KF835471	ND4	<i>Pelodiscus</i> spp.	China: Breeding Farm	Zhang et al. 2014
KF835472	ND4	<i>Pelodiscus</i> spp.	China: Breeding Farm	Zhang et al. 2014
KF835473	ND4	<i>Pelodiscus</i> spp.	China: Breeding Farm	Zhang et al. 2014
KF835474	ND4	<i>Pelodiscus</i> spp.	China: Breeding Farm	Zhang et al. 2014
KF835475	ND4	<i>Pelodiscus</i> spp.	China: Breeding Farm	Zhang et al. 2014
KF835476	ND4	<i>Pelodiscus</i> spp.	China: Breeding Farm	Zhang et al. 2014
KF835477	ND4	<i>Pelodiscus</i> spp.	China: Breeding Farm	Zhang et al. 2014
KF835478	ND4	<i>Pelodiscus</i> spp.	China: Breeding Farm	Zhang et al. 2014

RESULTS

Distribution on Oahu and Kauai

Trapping efforts on Kauai resulted in the capture of a total of five individuals of *Palea steindachneri* in 2007 from Opaekaa and Hanamaulu Streams, two individuals in 2008 from Opaekaa Stream (with two recaptures), two individuals in 2009 from Kapaa and Opaekaa Streams, six individuals in 2011 from Kealia and Kapaa Streams, and none in 2013 and 2014. Trapping efforts on Oahu in 2011 resulted in the capture of a total of 18 individuals of *Pelodiscus* spp. from Hoomaluhia Botanical Garden, Maunawili Stream, and Waimanalo Stream. One individual was captured from Honolulu Country Club in 2013, and three individuals were captured from Palolo Stream in 2014. Additional surveys of food markets and pet stores on Oahu in 2014 revealed the presence of live *Pelodiscus* individuals for sale (Figure 1, Table 1).

Phylogenetic analyses

Palea steindachneri.—The majority-rule consensus of the posterior distribution of trees from the Bayesian analysis of our concatenated mtDNA data set for *Palea steindachneri* grouped our samples from Kauai with the mitochondrial genome (FJ541030) to form a monophyletic clade with strong support [Bayesian posterior probability (PP) = 1] (Figure 2). Our six samples are genetically undifferentiated from each other but differed from the genome by 2 bp. In addition, our samples differed from market specimens from China by a single bp and further analyses of individual gene trees showed that our samples formed monophyletic clades with these specimens (AF043414, AY259552, AY259602) with strong support values (PP = 1) (Figure 3). Because all sequences were identical, further haplotype analyses were not conducted on this dataset.

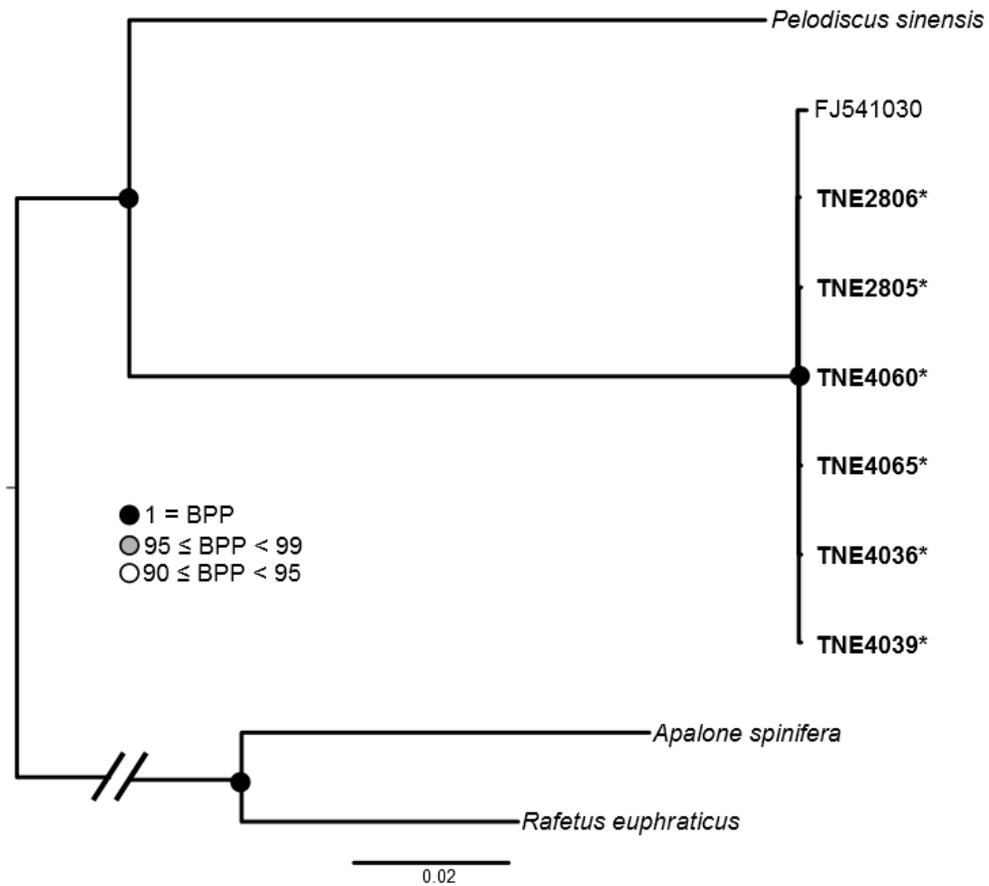


Figure 2. Majority-rule consensus of the posterior distribution of trees from the Bayesian analysis of the concatenated mtDNA dataset of *Palea steindachneri* (10 individuals, 2252 bp), partitioned by gene. Bayesian posterior probabilities (BPP) > 0.90 as indicated. Our samples are highlighted in bold with an asterisk. The outgroup branch is not drawn to scale.

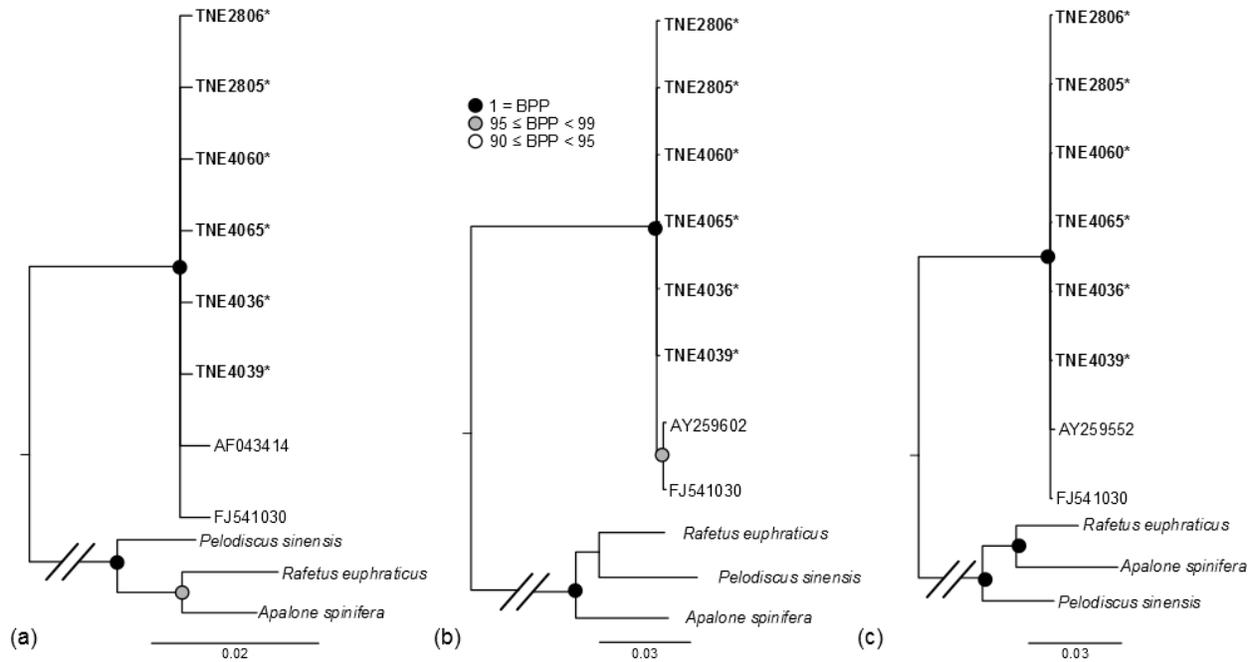


Figure 3. Bayesian trees of individual genes for *Palea steindachneri* and their models of evolution:

- (a) 12S rRNA (11 individuals, 396 bp), estimated under HKY + I.
- (b) ND4 (11 individuals, 714 bp), estimated under GTR + G.
- (c) Cyt b (11 individuals, 1142 bp), estimated under GTR + I.

Pelodiscus spp.—Although the monophyly of *Pelodiscus* as a genus was well-supported in previous studies, relationships within it had previously remained largely unresolved. Bayesian and maximum likelihood analyses of the Cyt b and ND4 region have consistently placed *P. axenaria* as the most distinct lineage but had produced conflicting topologies for the relationships among *P. maackii*, *P. sinensis*, and *P. parviformis* (Stuckas and Fritz 2011, Yang et al. 2011, Suzuki and Hikida 2014). Nodes with low support from these analyses can be collapsed, essentially transforming the three species into a polytomy. Using our comparatively more extensive sampling of genes and individuals, we reconstructed a molecular phylogeny for *Pelodiscus* to resolve this polytomy. The majority-rule consensus of the posterior distribution of trees from the Bayesian analysis of the concatenated mtDNA data set for *Pelodiscus* was generally well-resolved and supported, with 10/17 nodes strongly supported (PP = 1) and 6/17 nodes well-supported (PP > 0.90) (Figure 4). We recovered four major monophyletic clades which showed *P. maackii* and *P. parviformis* as reciprocally monophyletic and sister to *P. sinensis*, with *P. axenaria* as the successive sister species.

From our sampling in Hawaii, we recovered two distinct mitochondrial clades that corresponded to *P. maackii* and *P. sinensis*, confirming the presence of these two distinct species on Oahu. Samples from Kailua, Kaneohe, and a pet store formed a strongly supported clade (PP = 0.99) with previously published sequences from market specimens of *P. sinensis* from China (MTD5559, MTD43357). *P. sinensis* as a species was strongly supported with a PP of 1.0 for 6/7 nodes and a PP of 0.99 for the last node. All samples from Honolulu and Waimanalo and one individual from Kaneohe formed a clade (PP = 1) with two previously published sequences of *P. maackii* from Korea (AY962573) and Russia (MTD4236). Our samples of *P. maackii* are further subdivided by a weakly supported bifurcation (PP = 0.57) into two clades comprising samples from Waimanalo and Korea (AY962573) in one clade with Honolulu and one sample from Kaneohe in the sister clade (Figure 4).

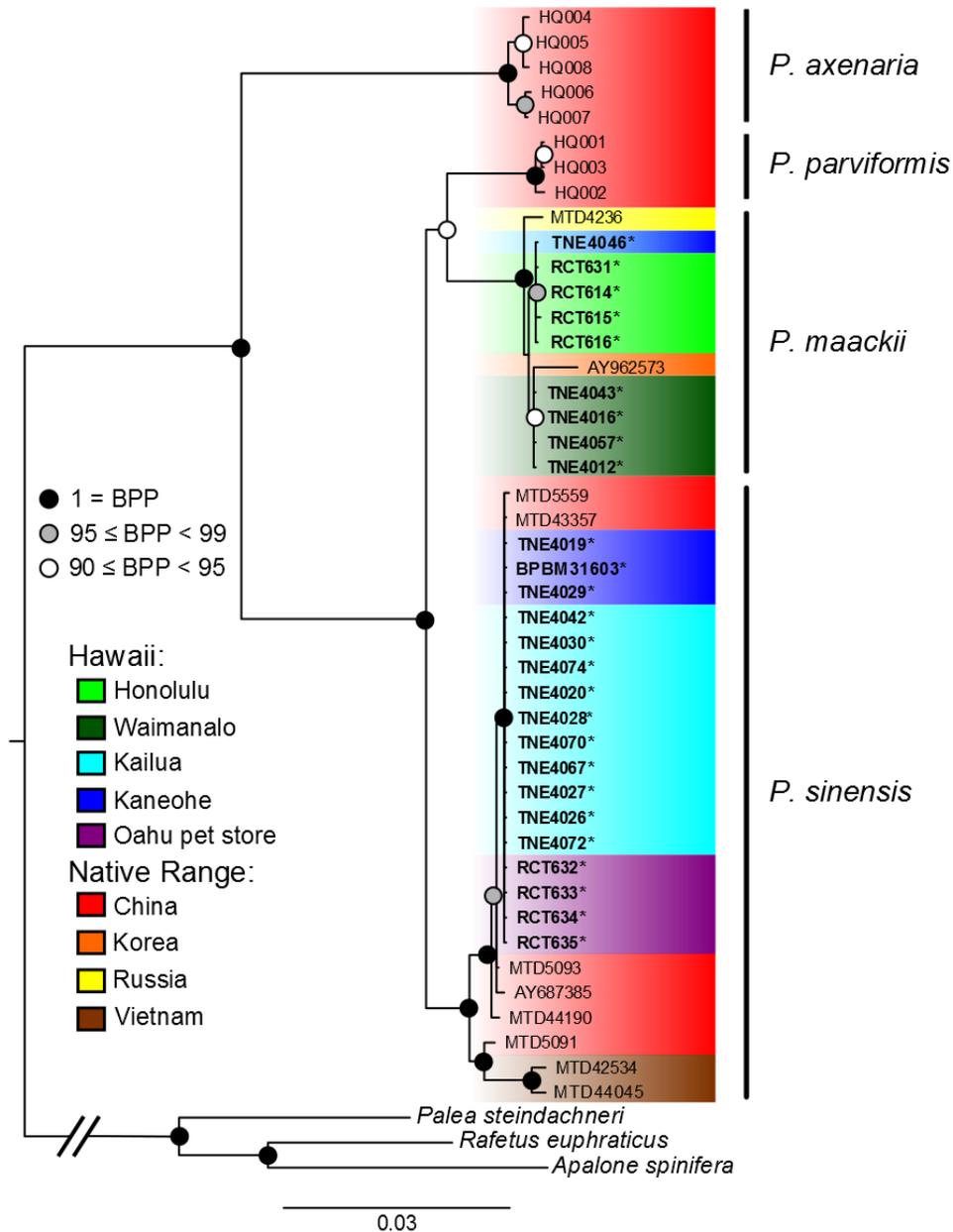


Figure 4. Majority-rule consensus of the posterior distribution of trees from the Bayesian analysis of the concatenated mtDNA dataset for the genus *Pelodiscus* (47 individuals, 2394 bp), estimated under the GTR + G model of sequence evolution. Bayesian posterior probabilities (BPP) > 0.90 as indicated. Our samples are highlighted in bold with an asterisk. Geographic origin of the specimen is indicated in color and species assignment is shown on the right. The outgroup branch is not drawn to scale.

Bayesian analysis of the individual gene trees allowed for additional comparison with GenBank sequences that were excluded from the concatenated data set and revealed more detailed relationships between specimens from Hawaii and those from the native range. The Cyt b gene tree was topologically identical to the concatenated dataset and showed that our samples of *P. sinensis* formed a clade with a wild-caught specimen from Japan (AB904727). Additionally, our samples of *P. maackii* formed a clade with wild-caught and farm-bred specimens from Japan (AB904722, AB904725, AB904724, AB904720, AB904723) (Figure 5). The gene tree for ND4 was topologically identical to that of Stuckas and Fritz (2011) and included sequences from four main breeding strains currently available from farms in China, not previously analyzed in the context of the four species of *Pelodiscus*: Taihu Lake (TL), Taiwan (TW), Japan (JP) and Yellow River (YR), with the name indicating the original habitat. The TW strain was polyphyletic and appeared in clades of both *P. sinensis* and *P. maackii* (KF652203, KF835477). The clade consisting of our samples of *P. maackii* from Waimanalo included the JP strain (KF652202, KF835473). The YR strain formed a clade within *P. maackii* and the TL strain formed a monophyletic clade within *P. sinensis*, but neither grouped directly with our samples (Figure 6). Compared to the other two gene regions, the 12S rRNA gene tree provided less resolution and showed *P. sinensis* as a paraphyletic group and combined the two sister clades in *P. maackii* into one clade. It additionally grouped our samples of *P. sinensis* and *P. maackii* with market specimens from China (AF0434413, JQ688040, JQ688041) and further grouped our samples of *P. maackii* with wild-caught specimens from Korea (JQ815362, JQ837982, JQ837983) (Figure 7).

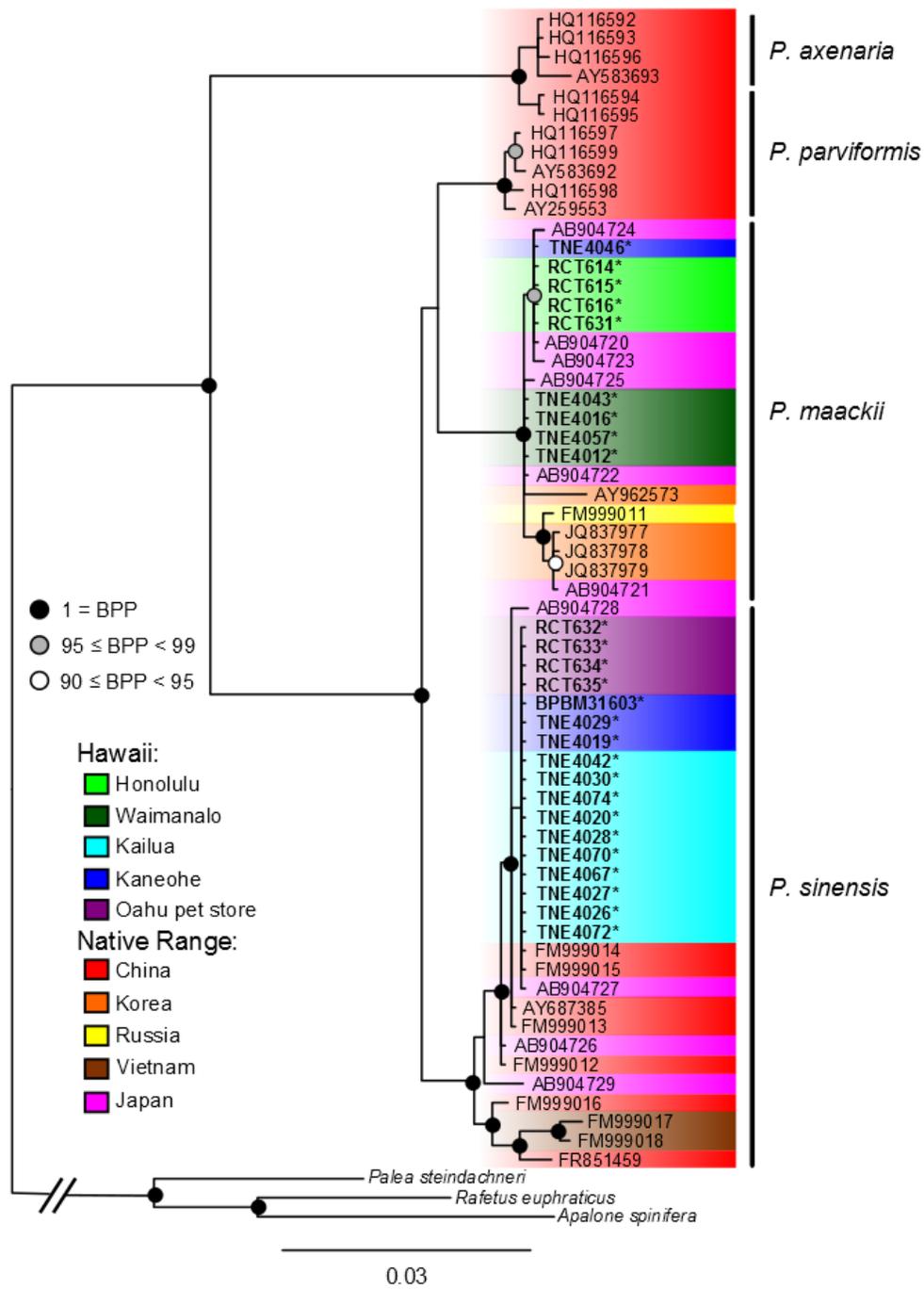


Figure 5. Bayesian tree of the Cyt b region for *Pelodiscus* (64 individuals, 1172 bp) estimated under the GTR + G model of evolution.

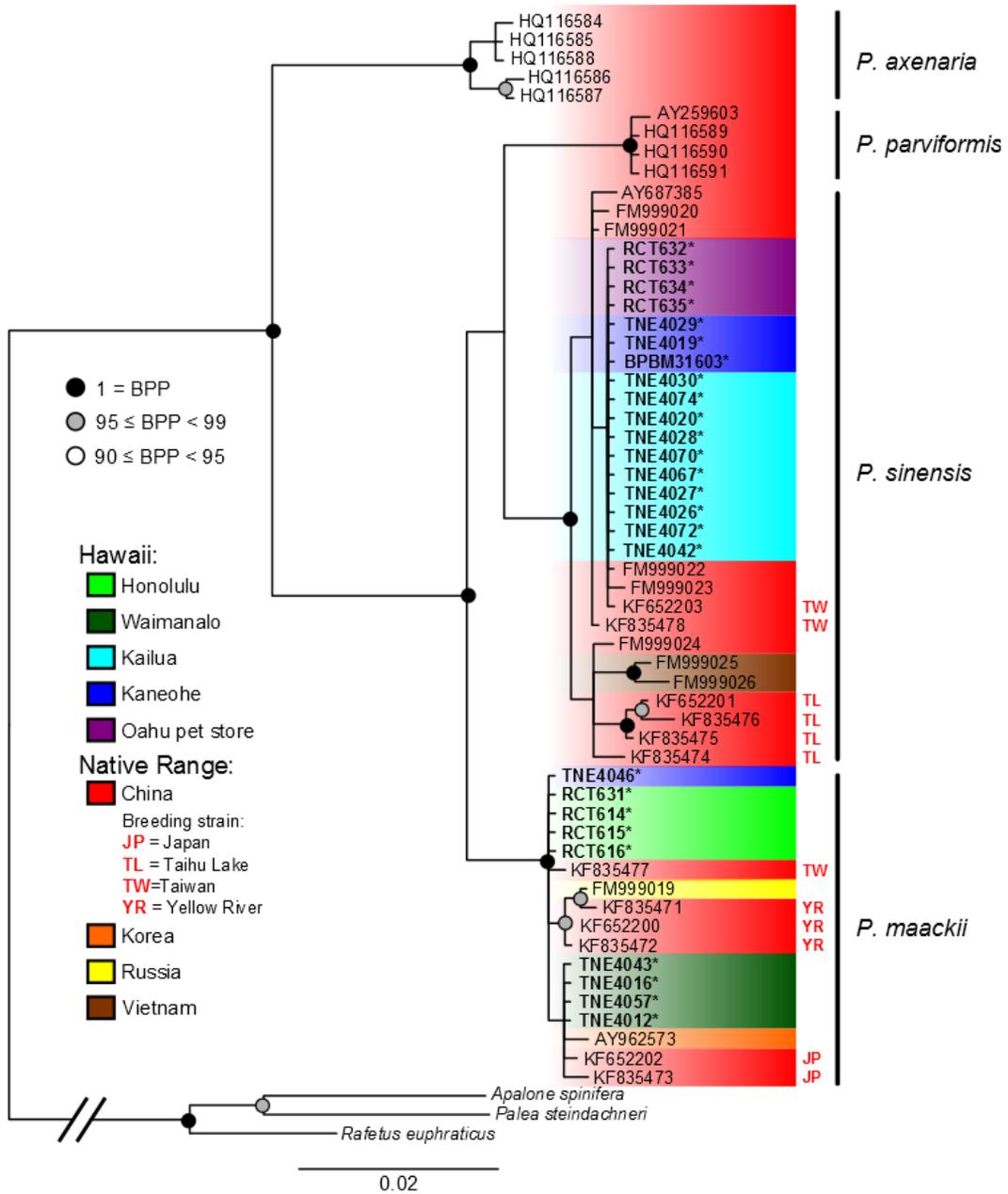


Figure 6. Bayesian tree of the ND4 region for *Pelodiscus* (60 individuals, 826 bp) estimated under the GTR + G model of evolution.

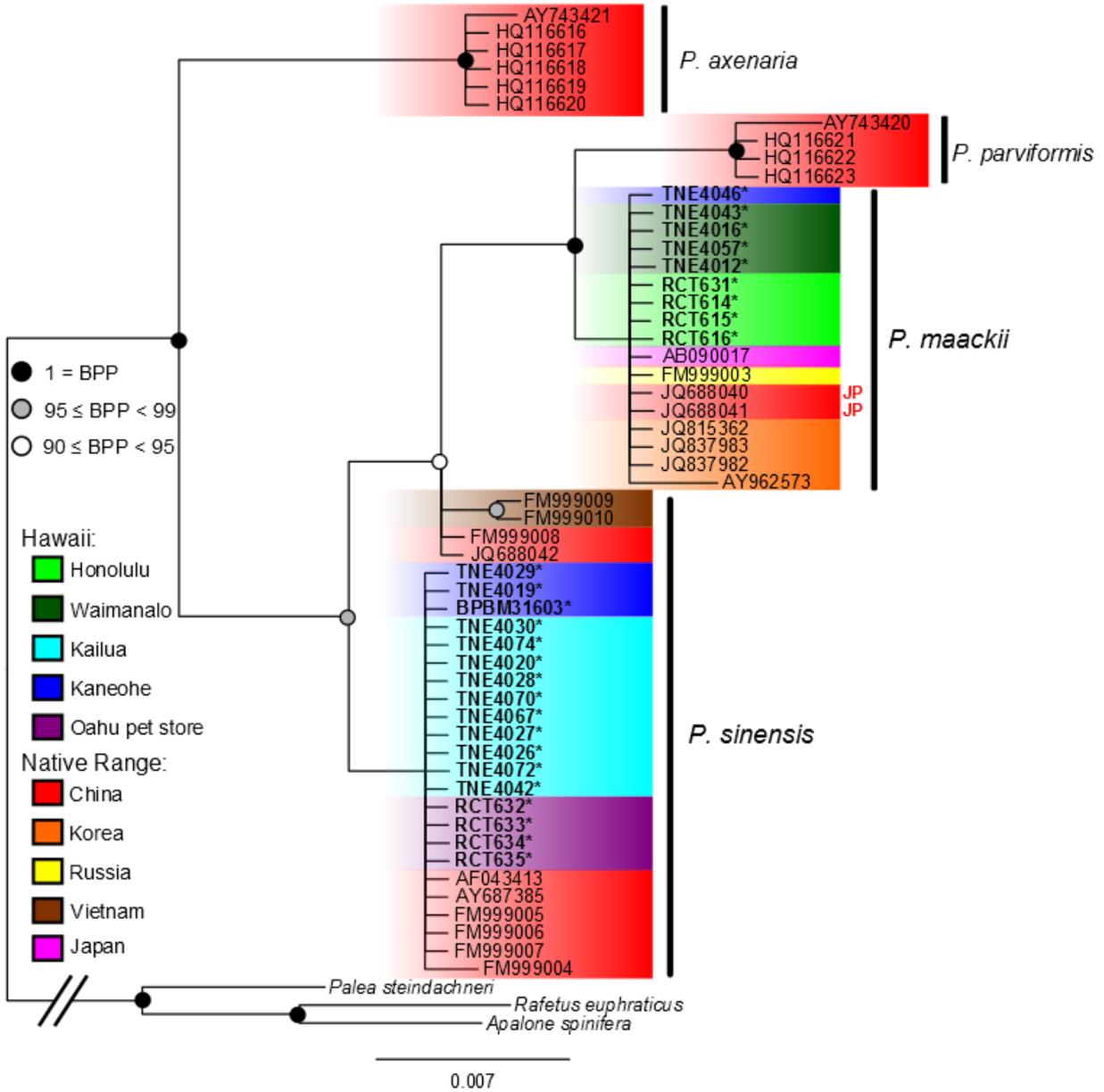


Figure 7. Bayesian tree of the 12S rRNA region for *Pelodiscus* (57 individuals, 396 bp) estimated under the GTR + G model of evolution.

Haplotype analyses of Pelodiscus spp.

Statistical parsimony networks of mitochondrial genes recovered four distinct clades corresponding to the four species seen in the phylogenetic trees and three haplotypes from our samples which we refer to as HS1, HM1, and HM2 (Figure 8). HS1 corresponds to individuals representing *P. sinensis* while HM1 and HM2 correspond to individuals representing *P. maackii*. While all differed in the Cyt b and ND4 regions, HM1 and HM2 were identical in the 12S rRNA region (Figures 8, 9, and 10). HS1 was recovered in all samples from Kailua, Kaneohe, and a pet store, with the exception of one individual from Kaneohe that was identified as HM2. Haplotype HS1 also included market specimens from China (MTD43357, MTD5559), wild-caught specimens from Japan (AB904727), and a specimen of the TW breeding strain (KF652203) (Figures 8 and 9). HS1 was a tip haplotype and differed from the genome (AY687385) and a market specimen from China (FM999013) by one mutational step in the Cyt b region. Haplotypes representing *P. sinensis* were connected by a maximum of 32 steps (Figure 8). Haplotype HM1 was found in all samples from Waimanalo, while HM2 was found in all samples from Honolulu and one sample from Kaneohe. Both HM1 and HM2 haplotypes included wild-caught and farm-bred specimens from Japan (AB904720, AB904722, AB904723) and differed from each other by one mutational step. HM1 and HM2 also differed from the previously published mitochondrial genome of *P. maackii* (AY962573) by 9 and 10 steps, respectively. Additionally, both were separated from specimens of the JP breeding strain by up to two mutational steps (Figure 9). Haplotypes representing *P. maackii* were connected by a maximum of 18 steps (Figures 8).

Haplotype analyses of nDNA sequences of *P. sinensis* and *P. maackii* provide less resolution and do not recover the groupings suggested by mtDNA analyses (Figure 11). This incongruence could suggest that the substitution rate for this gene was not sufficient to reflect evolutionarily recent lineage diversification events shown in the mtDNA (Hudson and Coyne 2002), particularly for chelonians which are characteristically marked with long generation times. Our samples yielded seven haplotypes in the nuclear C-mos gene, which we named Cmos1-Cmos7 (Figure 11). There was no clear geographical pattern as all sites contained a mixture of at least three nuclear haplotypes.

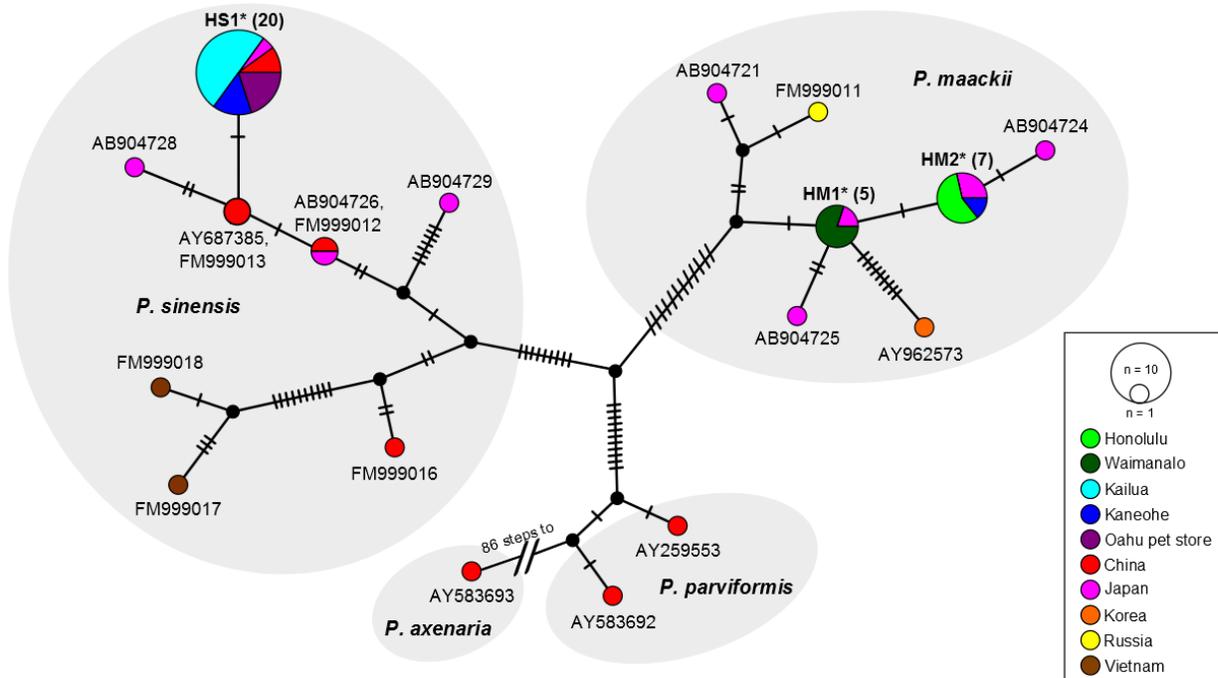


Figure 8. TCS parsimony network of *Pelodiscus* haplotypes constructed from the Cyt b region. Circle size corresponds to frequency of haplotypes, open circles symbolize known haplotypes, dots are hypothetical missing haplotypes, and each hash indicates one mutational step. Accession numbers for GenBank sequences shown. Geographic origin is indicated in color. Haplotypes found from our samples are indicated in bold with an asterisk and numbers for each are given in parentheses.

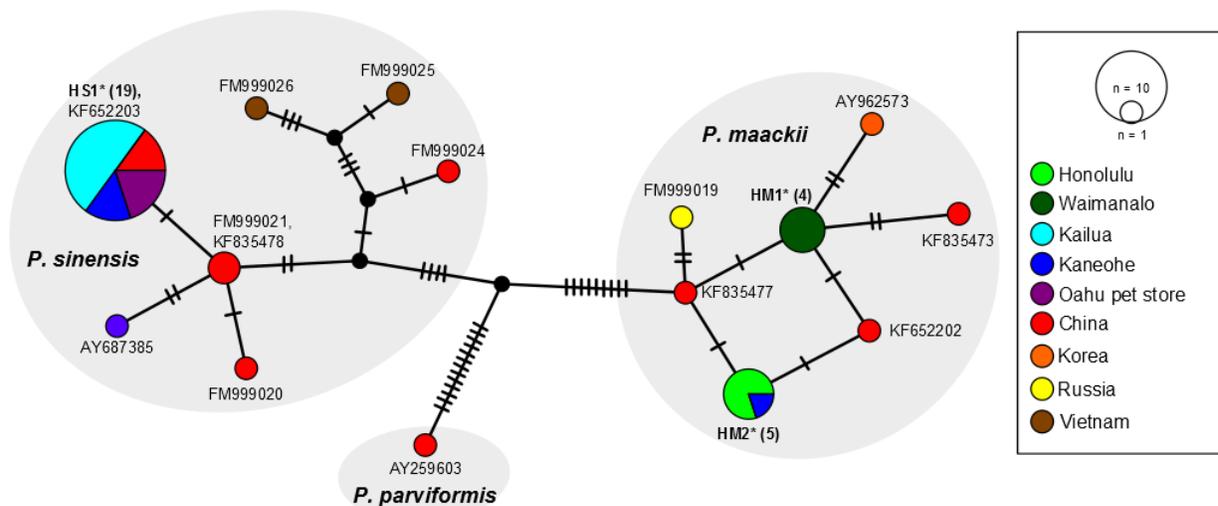


Figure 9. Parsimony network of *Pelodiscus* haplotypes constructed from the ND4 region. Excludes sequences from the TL and YR breeding strains to reduce unnecessary noise and HQ116584-HQ116590 due to short length.

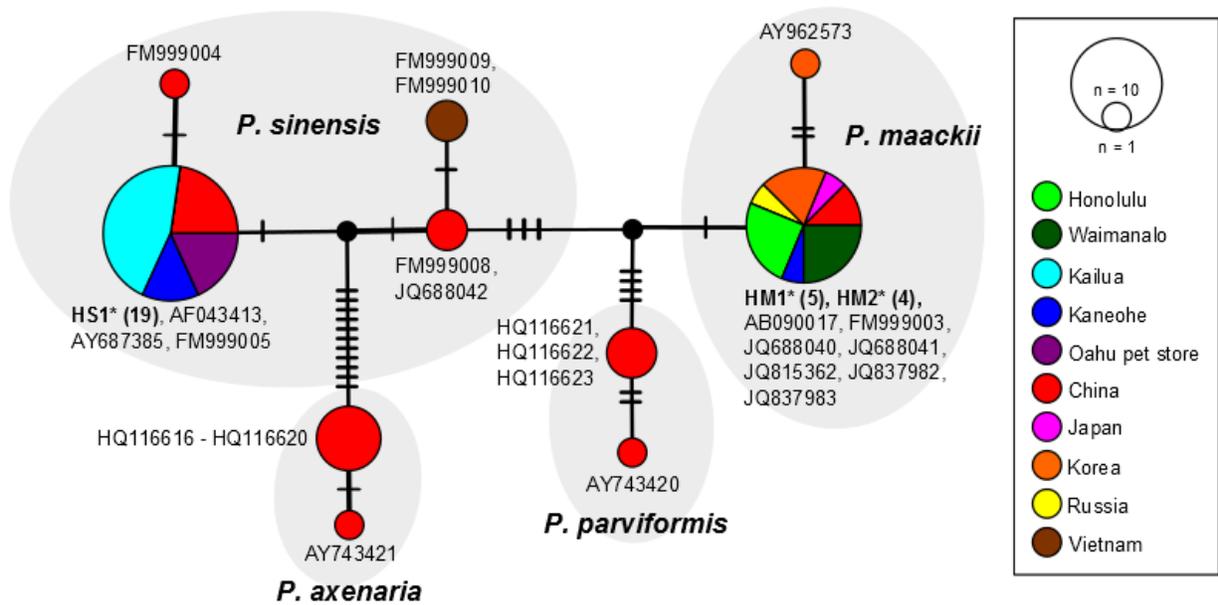


Figure 10. Parsimony network of *Pelodiscus* haplotypes constructed from the 12S rRNA region.

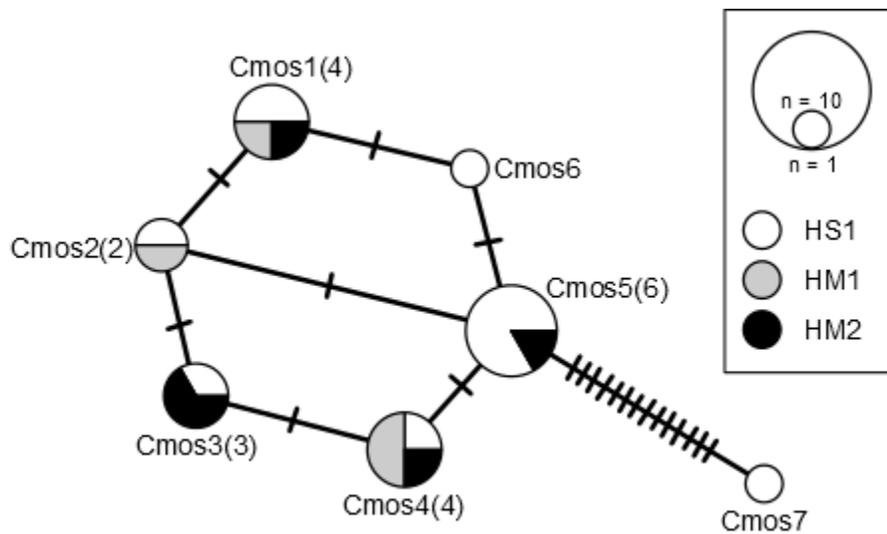


Figure 11. Parsimony network for nuclear C-mos haplotypes found in *Pelodiscus* samples from Hawaii. Shading of slices indicates the haplotype as inferred from the mtDNA genealogy. Numbers for each haplotype are given in parentheses.

DISCUSSION

Distribution and geographic structure of species

This study has shed new light on populations of softshell turtles in the Hawaiian Islands by revealing that they are composed of three distinct species that showed geographic structure: *Pelodiscus maackii*, *Pelodiscus sinensis*, and *Palea steindachneri*. The results of our trapping and survey efforts from 2007-2014 suggest that *P. steindachneri* only occurs on Kauai whereas *P. sinensis* and *P. maackii* occur only on Oahu (Figure 1). This is in contrast to a previous documentation of softshell turtle distributions (McKeown and Webb 1982) where *P. steindachneri* was thought to be widespread, occurring on Oahu and Kauai, whereas *Pelodiscus* was limited to a single locality on Kauai (McKeown and Webb 1982). The current range of *P. steindachneri* on Kauai reflects the historical range and extends along the eastern coast from Hanamaulu Stream in Lihue upwards towards Kealia Stream in Kapaa with probable populations in the northern streams from Kilauea to Hanalei. Along the northern coast, softshell turtles of unverified identity have been reported in Hanalei National Wildlife Refuge within the past 15 years (pers. comm., Shannon Smith), and have been documented from Kalihiwai Stream in 2004 and Pukaele reservoir in 2007 (pers. comm., Nicolai Barca). The absence of verified *Pelodiscus* individuals despite extensive field efforts suggests that the species may no longer be present on Kauai, which was confirmed by Don Heacock (Kauai District Aquatic Biologist).

On the other hand, sampling of wild populations as well as market and pet store specimens on Oahu indicates that there are multiple populations of *Pelodiscus* but *P. steindachneri* may not occur. The current range of *Pelodiscus* on Oahu appears to encompass streams, marshes, and channelized drainages around Honolulu (from Salt Lake to Manoa Valley), Waimanalo, Kailua, and Kaneohe. The two species of *Pelodiscus* that we identify here are geographically structured with *P. sinensis* (HS1 haplotype) found only from Kailua and Kaneohe sites, and within *P. maackii* the HM1 haplotype was found only from Waimanalo while the HM2 haplotype was primarily found from Honolulu sites. The presence of a single HM2 haplotype from a site in Kaneohe is an outlier that may represent a single translocation at some time in the past, or the presence of a small undetected population of *P. maackii* in the area. Samples from the pet store were identified as *P. sinensis* (HS1) and although the seller refrained from disclosing details of their origin, it was indicated that they were obtained from a small-scale farming operation on Oahu. A survey of Chinatown markets in Honolulu revealed live

Pelodiscus specimens for sale which could feasibly have originated from this same farm or from wild-populations in streams. We also encountered people searching for turtles to sell to Chinatown or on the internet during stream surveys on Oahu. We were unable to obtain tissue samples from market specimens so it is unclear if they represented *P. sinensis* or *P. maackii*. There is the possibility that a greater sampling of *Pelodiscus* populations may reveal undetected lineages and haplotypes.

Due to the gap in data collection between McKeown and Webb (1982) and our present study, it is difficult to ascertain what factors have shaped the present day distribution of these species in Hawaii. Anecdotal reports from local residents are difficult to interpret due to the likelihood of species misidentifications. Thus, specimens from the Bishop Museum serve as an important source of verifiable data. This collection contains just one specimen of *P. steindachneri* received from the Honolulu Zoo in 1980, but it documents the first specimen of *Pelodiscus* on Oahu in 1992 followed by five additional specimens collected through 2008. This collection also documents the first occurrence of softshell turtles on Maui with a single *Pelodiscus* from Wailea in 2000 (Kraus 2002) and one *P. steindachneri* from Kihei in 2005. Anecdotal evidence suggests that they arrived on Maui prior to 2000 with probable populations in the southern and central regions of the island (Kraus 2002, Radford 2011). Additionally, the Smithsonian Institute National Museum of Natural History collection contains two specimens of *P. steindachneri* that were collected from Kaneohe on Oahu in 1992 and 1993.

The red eared slider, *Trachemys scripta elegans*, is the only other species of freshwater turtle that has established reproducing populations in the Hawaiian Islands. Introduced via the pet trade in 1980, it was initially reported to occur on Kauai and Oahu, but the only reproducing population was located in Kawai Nui marsh on Oahu (Devick 1991, McKeown 1996). The Bishop Museum records contain three specimens from Kailua and Honolulu collected in 1999-2001. *Trachemys scripta* was first recorded on Maui in 2002 (Kraus and Duvall 2004). Radford (2011) and our survey results indicate that their numbers have increased and there are now reproducing populations on Oahu, Kauai, and Maui. They occur sympatrically with softshell turtles and appear to be relatively more abundant.

Inferences from genetic analyses

The presence of *P. maackii* on Oahu suggests that there have been introductions of softshell turtles originating from populations in Japan. Hawaii haplotypes HM1 and HM2 were found in wild-caught and farm-bred samples of *P. maackii* from Japan and not in any other native range samples (Figure 8). They were also similar to the JP breeding strain, a farm-bred lineage present in Chinese turtle farms that purportedly originates from Japan (Figure 9; Zhang et al. 2014). Although it is unclear whether or not the softshell populations in Japan are indigenous (Stejneger 1907, Nakamura and Ueno 1963, Fritz et al. 2010, Suzuki and Hikida 2014), it is established that *Pelodiscus* has occurred in Japan since at least the early 1800s (Temminck and Schlegel 1835). The predominant species of softshell turtle in Japan is *P. maackii* with reports of more sparsely distributed populations of *P. sinensis* (Suzuki and Hikida 2014). Although our haplotype HS1 was found in wild-caught samples from Japan, it was also found in market specimens from China (Figure 8), which along with their limited distribution in Japan, is consistent with the suggestion that *P. sinensis* was introduced to mainland Japan at a later date via the food trade (Hasegawa 2011).

The presence of *P. sinensis* and *P. steindachneri* in Hawaii are likely due to introductions from China, as inferred from both historical and genetic data. Early introductions (late 1800s to 1980) were prior to the establishment of large-scale farming in China, so softshell turtles introduced to Hawaii from China were likely wild-caught. Although these could hypothetically have been either *P. sinensis* or *P. steindachneri*, the native range of *P. steindachneri* includes southeastern China and encompasses Guangzhou, Guangdong province in southeastern China, the geographic source of late 19th century Chinese immigrants (Reece 1914), whereas the native range of *P. sinensis sensu stricto* is in central and eastern China. This may provide an explanation for the initial abundance of *P. steindachneri* compared to the rarity of *P. sinensis* as reported in 1982 (McKeown and Webb). The later years of introduction (1980s to 1999), coincide with the appearance of large-scale farms in China that consist predominantly of *P. sinensis*. It is likely that importation from these farms influenced the shifting composition of softshell turtle populations on Oahu towards an increasing abundance of *P. sinensis*. Additionally, imported softshell turtles almost always arrived through Honolulu (McKeown and Webb 1982) which could be a factor contributing to the increasing abundance of *Pelodiscus* on Oahu and not on Kauai where *P. steindachneri* occurs.

Our results showed that samples of *P. sinensis* from Hawaii were genetically identical to current market specimens from China, which suggests that farm-bred individuals were imported to Hawaii (Figure 4). The comparison of the few available GenBank sequences of *P. steindachneri* to our data suggest that populations in Hawaii are nearly genetically identical to specimens from China (Figures 2 and 3), although small sample sizes from both native and introduced populations prevent further clarification. However, given that some of these sequences are from market specimens and that *P. steindachneri* is present but rare in turtle farms in China (approximately 1-2%; Shi and Parham 2000), it is possible that there were further introductions of farm-raised *P. steindachneri*. One difficulty in working with threatened taxa like *Pelodiscus* and *P. steindachneri* is that genetic data from wild-caught specimens from the native range is often difficult to obtain. Thus, nearly all Chinese *P. sinensis* and *P. steindachneri* mtDNA sequences were from market specimens.

Conservation and management implications

Here we have shown that two of the Hawaiian Islands, Oahu and Kauai, contain introduced populations of three species of softshell turtles. Interestingly, each of these species is exploited and of high conservation concern in their native ranges in Asia. In terms of management of these populations, two alternative scenarios exist and consideration should be given to both. In the first case, these introduced species are an aquatic predator whose impacts on the native fauna are uncharacterized (Layhee et al. 2014). When considering the potential for ecosystem damage caused by non-native predatory herpetofauna in Hawaii (Holland et al. 2010, Chiaverano and Holland 2014), the precautionary principle would incline us to propose eradication of the softshell turtles from Hawaii. However, a primary issue to consider is habitat overlap and in this regard, interactions between softshell turtles and native fauna may be minimal. The native freshwater species of Hawaii, comprise five amphidromous fishes (four of which are endemic), two amphidromous crustaceans, two endemic amphidromous neritid snails, and four endemic freshwater lymnaeid snails (one occurs in Kauai and is listed as ‘endangered’, IUCN 2014). These species prefer the biotic and hydrologic characteristics of undeveloped waterways (Brasher et al. 2006) whereas the turtles inhabit highly disturbed lowland waterways dominated by the presence of other introduced species. Although, the amphidromous life cycle of native species necessitates migration through lower elevation freshwater habitat to access the

coastal ocean, and as pristine sites become increasingly rare due to development pressures, interactions with among native taxa and softshell turtles, as well as other non-native aquatic taxa, may increase.

On the other hand, softshell turtle populations in Hawaii can be viewed as harboring potential to serve as assurance colonies for the declining native populations. Although chelonians lineages are threatened globally, the lineages native to Asia in particular are at increased risk for extinction (van Dijk et al. 2000). The survival of these taxa is intrinsically linked to reducing the cultural demand for food and medicinal uses and until this challenge is addressed, ex-situ conservation strategies like the establishment of assurance colonies aim to maintain the species and maximize future conservation actions for the recovery of wild populations. To maximize the success of these colonies and future translocations and reintroductions, insights into the genetic architecture of both the colonies and wild populations are essential to preserving biodiversity as a whole and enhancing long-term persistence of populations (Fong et al. 2007, Spinks and Shaffer 2007, Groombridge et al. 2012). Given these goals, the conservation value of Hawaii populations may be somewhat dampened by their genetic similarity to those sampled from Chinese markets. However, market specimens do not necessarily equate to farm-bred because individuals are still heavily harvested from wild populations to supplement farm breeding stocks and for direct sale at markets to fill a cultural demand for wild-caught game (Shi et al. 2007). In addition, due to the sampling bias towards market specimens, a more comprehensive dataset of wild-caught samples spanning the native range would be necessary to align our populations to geographic source regions.

Furthermore, population sizes of all three species of softshell turtles in Hawaii may be in decline. Although the abundance of softshell turtles is difficult to assess using visual surveys because these species lack the strong basking habit that many other turtle species exhibit, our trapping efforts from 2007-2014 yielded fewer captures in later years. Given that import of these taxa has ceased but they remain in demand as a food item with no regulations surrounding the collection and sale of individuals, decreased population levels could be due to continued exploitation in Hawaii. The market price in Hawaii for softshell turtles, adjusting for inflation to buying power of 2014, averaged \$90 per pound during WWII, \$64 per pound in 1947 (Brock 1947), and \$98 per pound in the 1950s (Ernst and Barbour 1972). In 2014, live *Pelodiscus*

individuals in Honolulu's Chinatown were available for \$40 per pound and hatchlings sold for \$29 per individual in pet stores. The absence of governmental regulation on collection stems from their classification as an 'injurious species' in the state of Hawaii.

Future work should focus on addressing the tension of their dual status as highly threatened taxa in the native range and potentially introduced aquatic predators in Hawaii. In particular, several major points should continue to be addressed with regards to these species: 1) continued surveying to detect additional populations and improve our estimates of range and abundance, 2) a precise comparison of the genetic diversity in Hawaii with the native range to preserve overall biodiversity for the possibility of future reintroductions, especially for the more imperiled and understudied *P. steindachneri*, 3) ecological analyses in the context of the native biota in order to develop management decisions that take into consideration the complex interplay of conservation concerns for Asian softshell turtles and native Hawaiian fauna, and the relationship between them.

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