Endangered seahorses for dinner? phyC DNA sequence data identifies seahorses for sale in NYC Chinatown.

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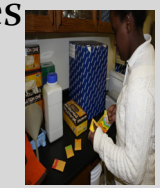
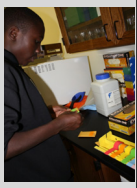




ABSTRACT

Seahorses (genus *Hippocampus*) are endangered species, but up to now there has not been any conclusive way of telling which of the species being sold in different areas in the world are threatened or endangered. We decided to investigate the seahorse species sold in New York City's Chinatown to determine whether the species sold there are among those listed on the International Union for Conservation of Nature (IUCN) Red list. Sequences of the Cytochrome B gene were generated for each sample that was purchased. The species identified were *Hippocampus trimaculatus, Hippocampus ingens,* and *Hippocampus algiricus. Hippocampus trimaculatus* and *Hippocampus ingens* are vulnerable to extinction according to the IUCN and *Hippocampus algiricus* is listed as "data deficient." The data from this research shows that *Hippocampus algiricus* is traded internationally and thus may be listed as threatened in the future. Further, this research shows that the rules for distributing seahorses internationally should be more strictly enforced.

INTRODUCTION

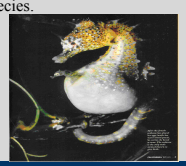
Seahorses are species of the genus *Hippocampus* and in the family Syngnathidae. They are vertebrate fishes that are known for their unusual equine shape. Seahorses are very unique creatures because unlike other fishes, they are monogamous and mate for life. Seahorses are among the only animal species on earth in which the male bears the unborn young. This adds to their uniqueness. They are found in shallow tropical and temperate waters throughout the world. Because of the shape of their bodies, seahorses are bad swimmers and can easily drown in rough seas. Seahorses swim by propelling themselves, using a small fin on their back that flaps up to 35 times per second (National Geographic). They also "anchor themselves with their prehensile tails to sea grasses and corals, using their elongated snouts to suck in plankton and small crustaceans that drift by" (National Geographic). Seahorses are known to be uncontrolled eaters, they can consume 3,000 or more brine shrimp a day because they eat continuously (National Geographic).

Seahorses are widely cultivated and traded for usage as a medicinal ingredient, mostly in China. They are used medicinally by preparing it into a soup cooked with other ingredients (Chen, 2009). The Chinese traditional use of seahorses is to treat respiratory illnesses such as asthma (idrc.ca), male virility (I. Shen, pers. comm.), and urinary incontinence (seahorse-austrailia.com.au). Many countries outside of Asia, including the United States, participate in seahorse trade for medicinal or commercial purposes. 24.5 million seahorses are traded annually (Saarman, 2007). Many seahorse species are threatened by extinction in their natural habitats.

Seahorses are very rare species, they are listed as vulnerable by the Convention on International Trade in Endangered Species (CITES). This is an organization to make sure that the trade of wild animals and plants does not negatively affect their survival. Our research is to sequence the cytochrome b (Cytb) gene of seahorses purchased in Chinatown New York to identify the species. This research is important because, once the species of the seahorses are found it can be determined whether these seahorses are being threatened. After receiving the results of our research it will be contributed to the Seahorse Sleuth website. Seahorse Sleuth is a project similar to ours, but conducted in Chinatown California. We predict that our research will show that seahorses sold in Chinatown are ones considered vulnerable to extinction.

METHODS & MATERIALS

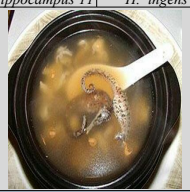
Different seahorse species purchased from Chinatown New York were labeled and bagged with the name of the store it was purchased from. A bag of silica gel was placed in each ziploc bag with the seahorses. DNA extraction were done to the seahorses. To be able to extract DNA, the seahorse tail must be "powdered" from each species. The seahorses were powered by cutting of a piece of the tail and crushing it using a blade. PCR Amplification was performed using primers (Forward: shf2 5'- TTG CAA CCG CAT TTT CTT CAG-3' Reverse: shr2 5'- CGG AAG GTG AGT CCT CGT TG-3') to obtain the sequence of DNA of species. Gel Electrophoresis was performed for the DNA samples. This is a technique used to confirm the success of PCR by size and brightness of the DNA band. PCR purification procedure was then performed to separate PCR products from the leftover components of the PCR reaction – it is most important to separate primers and dNTP's that would unfavorably affect the sequencing reactions. The purified PCR was sent to Macrogen to be sequenced. Once the sequences were sent back, they were edited. The sequences were then BLAST searched on Genbank, to determine the different seahorse species.



DATA

Seahorse Number	Species	Family	Date Collected	Location
1	Undefined	*Syngnathidae*	10/5/2009	Chinatown, NYC
2	Undefined	*Syngnathidae*	10/5/2009	Chinatown, NYC
3	Undefined	*Syngnathidae*	10/5/2009	Chinatown, NYC
4	*H. ingens*	*Syngnathidae*	10/5/2009	Chinatown, NYC
5	*H. ingens*	*Syngnathidae*	10/22/2009	Chinatown, NYC
6	*H. algiricus*	*Syngnathidae*	10/22/2009	Chinatown, NYC
7	*H. algiricus*	*Syngnathidae*	10/22/2009	Chinatown, NYC
8	*H. trimaculatus*	*Syngnathidae*	10/22/2009	Chinatown, NYC
9	*H. trimaculatus*	*Syngnathidae*	10/22/2009	Chinatown, NYC
10	*H. ingens*	*Syngnathidae*	10/22/2009	Chinatown, NYC
11	*H. ingens*	*Syngnathidae*	10/22/2009	Chinatown, NYC

Samples	BLAST ID'ed Species	GenBank match number	% Match
Hippocampus 4	*H. ingens*	AF192673.1	99%
Hippocampus 5	*H. ingens*	AF192673.1	100%
Hippocampus 6	*H. algiricus*	AF192644.1	99%
Hippocampus 7	*H. algiricus*	AF192644.1	99%
Hippocampus 8	*H. trimaculatus*	AF192702.1	99%
Hippocampus 9	*H. trimaculatus*	EU179929.1	99%
Hippocampus 10	*H. ingens*	AF192673.1	100%
Hippocampus 11	*H. ingens*	AF192674.1	99%



RESULTS

Samples one, two, and three were identified by an expert as seadragons (M. Duggan, pers. comm.). Cytochrome B DNA sequences were not obtained for these three samples because the primers used for seahorses did not work for those species of seadragons. The percent match and the names of the species were acquired by BLAST searching the DNA sequence of each species on GenBank, which then compared the sequences to those of others and found the ones closely related by percent matches. The highest percent matches were the ones recorded as identifications. Seahorse sample four, five, ten, and eleven, were identified through GenBank, as *Hippocampus ingens* with a percent match of 99% and 100% (Table 2). Seahorse six and seven were identified as *Hippocampus algiricus* with a percent match of 99% each. Seahorse eight and nine were identified as *Hippocampus trimaculatus* each with a percent of 99%.

Our data show that eight of the eleven samples purchased are on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species. The status of *Hippocampus trimaculatus* according to IUCN are vulnerable. "*H. trimaculatus* is listed as Vulnerable (VU A4cd) based on inferred declines of at least 30% caused by targeted catch, incidental capture, and habitat degradation." *Hippocampus ingens* are "listed as Vulnerable (VU A4cd) based on inferred declines of at least 30% caused by targeted catch, incidental capture and habitat degradation" and *Hippocampus algiricus* are "Data Deficient."(International Union for Conservation of Nature)

http://deepcreative.files.wordpress.com/2008/07/28-seahorse.jpg

http://lapencetweedy.com/blog/wp-content/uploads/2009/11/ch_dd4_375x281.jpg

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DISCUSSION

The International Union for Conservation of Nature (IUCN) Redlist determines plant and animal species that are threatened or endangered. Cytochrome B (cytB) DNA sequences identified the species of the entire seahorse samples purchased in New York City's Chinatown and each is covered by the IUCN. The results identified two of the species as *Hippocampus trimaculatus* and *Hippocampus ingens*. According to the IUCN, both of these species are vulnerable to extinction because of "targeted catch, incidental capture and habitat degradation," traditional medicine, and decorative use. This has resulted in a decrease of at least 30% in the populations of these two species (Vincent and Perry, in prep.).

The third species identified by our research, *Hippocampus algiricus*, according to IUCN is "data deficient," meaning that not enough is known about the biology of and threats to this species. The IUCN states that imports of West African seahorses have been reported in Hong Kong. These West African countries include Gambia, Guinea, Nigeria, Senegal, and Togo. The IUCN further states that the *Hipocampus algiricus* seahorse species is distributed in that region, and thus *Hippocampus algiricus* may potentially be involved in the international trade, but it is not known for sure. The research conducted in this project determines definitively that *Hippocampus algiricus* is involved in the international trade of seahorses. The results show that two of the samples purchased in New York City were *Hippocampus algiricus*, with a DNA match of 99% to known cytB sequences of the species.

The Convention on International Trade in Endangered Species (CITES) is an organization that makes sure the trade of wild animals and plants does not negatively affect their survival. The entire genus *Hippocampus* is listed in Appendix II of CITES. This means that commercial trade of these species is permitted, but is strictly regulated and every seahorse must be issued with its own CITES certificate in order for sale to take place. According to the Endangered Species Act (ESA), "all seahorse (*Hippocampus* sp.) must be separately declared at the species level on the declaration form and must be accompanied by the required valid CITES permit or certificate and any imports or exports that occur on or after May 15, 2004, are accompanied by valid CITES documents." The U.S. Customs and Border Protection is the government agency responsible for the control of all imports and exports including all *Hippocampus* species in the United States. CITES oversees the international seahorse trade while the U.S. Customs and Border Protection, the U.S. Department of Agriculture (specifically the Animal and Plant Health Inspection Service, APHIS), and the Fish and Wildlife Service monitor the trade within the U.S. borders. The results from our research shows that there could potentially be a problem in the way the species are being monitored. The seahorses purchased in this project are in fact endangered species, but they are still being sold in the city without a CITES certificate.

Therefore, further research must take place to verify whether or not these stores have CITES certificates. Additionally, this research shows that the trade and sale of seahorses should be more strictly monitored. We plan to use our data to help get a threatened species greater legal global conservation protection. We plan to expand this research by collecting more seahorses or possibly different organisms found in Chinatown and elsewhere in New York City. We also plan to publish our research in a scientific journal, hoping that scientists may consider our research and help us in our cause.