The Banbury Springs Lanx (*Lanx* n sp.: Lymnaeidae) was listed as an endangered species by the U.S. Fish and Wildlife Service in 1992. The primary reasons for listing were threats associated with habitat modification and loss and deteriorating water quality from pollution, runoff and diversion of water for irrigation and aquaculture (Myler et. al., 2006).

The aim of the present study is to determine the taxonomic status of the Banbury Springs Lanx using anatomical, morphological and genetic characters. In addition, it is also to determine the levels of differentiation, if any, between the four known populations and what significance if any this has for the conservation of the Banbury Springs Lanx.

Material

Specimens were collected from all four known populations of the Banbury Springs Lanx and were preserved in 95% ethanol or frozen. Representative material of all named species of *Lanx* and *Fisherola* were examined to help determine the generic and species relationships of the Banbury Springs Lanx and included the type species of *Lanx* Clessin, 1880 (*Ancylus patelloides* (Lea, 1856), *Walkerola* Hannibal, 1912 (*Lanx klamathensis* Hannibal, 1912) and *Fisherola* Hannibal, 1912 (*Fisherola lancides* Hannibal, 1912).

Methods

Dissection

Specimens for dissection and molecular analyses were either preserved in ethanol or frozen. Dissections were carried out using a stereomicroscope fitted with a camera lucida. The dissections were pinned out in a small dish with a black background and dissected under water. One individual from each of the four known populations of the Banbury Springs Lanx was dissected. The largest specimen from each lot was chosen for dissection.

On removing the reproductive and digestive systems from the posterior part of the animal about half of the posterior portion of the foot was removed for genetic analysis. Where possible both the anatomy and the DNA sequence data are from the same individual.

DNA sequencing

DNA was extracted by taking a tissue clip or the whole animal and placing it in about 500 µl CTAB (hexadecyltrimethylammoniumbromide) extraction buffer (Winnepenninckx *et al*., 1993) with 10 µl proteinase K. It was placed on a rotator table in a 37°C oven and allowed to digest (overnight to a few days). Then 500 µl of 24:1 chloroform: isoamyl alcohol was added. The samples were gently mixed by inverting for about 2 minutes and then centrifuged for about 5 minutes. The upper layer was transferred to a new tube with 400 µl cold isopropyl alcohol and then placed in a freezer a few hours to overnight. The tubes were centrifuged and the isopropyl removed. The DNA was washed with 200 µl 70% ethanol and dried. It was then dissolved in 20-60 µl TE.

Polymerase Chain Reaction (PCR) was used to amplify an 859 base pair region of the 28S nuclear gene. PCR used 2 µl of sample DNA, usually from a 1/10 dilution but sometimes 1/100 or undiluted worked better. The primers used, D23F and D6R were from Park and Ó'Foighil (2000). PCR used 2 minutes denaturing at 95ºC; 40 cycles of 95ºC, 30 seconds; 47ºC, 30 seconds, 72ºC, 90 seconds; 10 minutes extension at 72ºC and then holding at 4ºC. The product was cleaned and then gel purified using Qiagen Qiaquick and gel extraction kits. The concentration of the cleaned product was then measured using a Nannodrop spectrometer. BigDye sequencing reactions (10 µl total volume) were used. Sequencing reactions used 2 minutes denaturing at 92ºC, followed by 40 cycles with 92ºC, 15 seconds; two degrees below the annealing temperature, 15 seconds; 65ºC, 4 minutes. A final 10 minutes extension at 72ºC preceded holding at 4ºC. The product was cleaned with Qiagen DyeEx kits, dried and run on the ABI 3100 automated sequencer and both strands were sequenced. Outgroups included other lymnaeids and other freshwater basommatophorans.