



RESEARCH NOTE

PRESENCE OR ABSENCE OF CARINAE IN CLOSELY RELATED POPULATIONS OF *LEPTOXIS AMPLA* (ANTHONY, 1855) (GASTROPODA: CERITHIOIDEA: PLEUROCERIDAE) IS NOT THE RESULT OF ECOPHENOTYPIC PLASTICITY

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Ecophenotypic plasticity, or nonheritable variation of the phenotype with local conditions, has been invoked in order to partly explain conchological differences within freshwater snail species as responses to fluvial conditions and stream size (Adams, 1915; Lam & Calow, 1988; Minton, Norwood & Hayes, 2008; Minton *et al.*, 2011), river substrate (Urabe, 1998, 2000) and presence or absence of predators (Holomuzki & Biggs, 2006; Hoverman & Relyea, 2007; Lakowitz, Brönmark & Nyström, 2008). In the freshwater gastropod family Pleuroceridae, Dillon (2011) and Dillon & Robinson (2011) used similarities in allozyme loci to conclude that conchological differences (e.g. presence or absence of carinae, slenderness or robustness of shells) in multiple species of *Pleurocera* and *Elimia* were attributable to intraspecific ecophenotypic plasticity. However, no analysis of shell morphology was performed by Dillon (2011) or Dillon & Robinson (2011) to test for either an ecophenotypic or genetic component of the observed variation.

In order to understand the effects of ecophenotypic plasticity on the presence or absence of carinae on the shell of one species of Pleuroceridae, we cultured two populations of *Leptoxis ampla* (Anthony, 1855), one with a carinate shell morphology and one with a smooth shell morphology (Fig. 1). In culture, these snails were exposed to uniform environmental conditions. Therefore, observed morphological differences among juveniles raised from egg to adult were not affected by the environment. Culturing methods are ideal for analysing variation in shell morphology in the Pleuroceridae because not only can environmental conditions be controlled, but complications arising from translocation experiments (such as transfer of disease vectors and the potential for mating with native populations) are avoided. Although culturing methods have not previously been used for studying ecophenotypic plasticity in Pleuroceridae, they have been successfully applied to other freshwater gastropod families such as Planorbidae (Hoverman & Relyea, 2007) and Lymnaeidae (Lam & Calow, 1988; Lakowitz *et al.*, 2008).

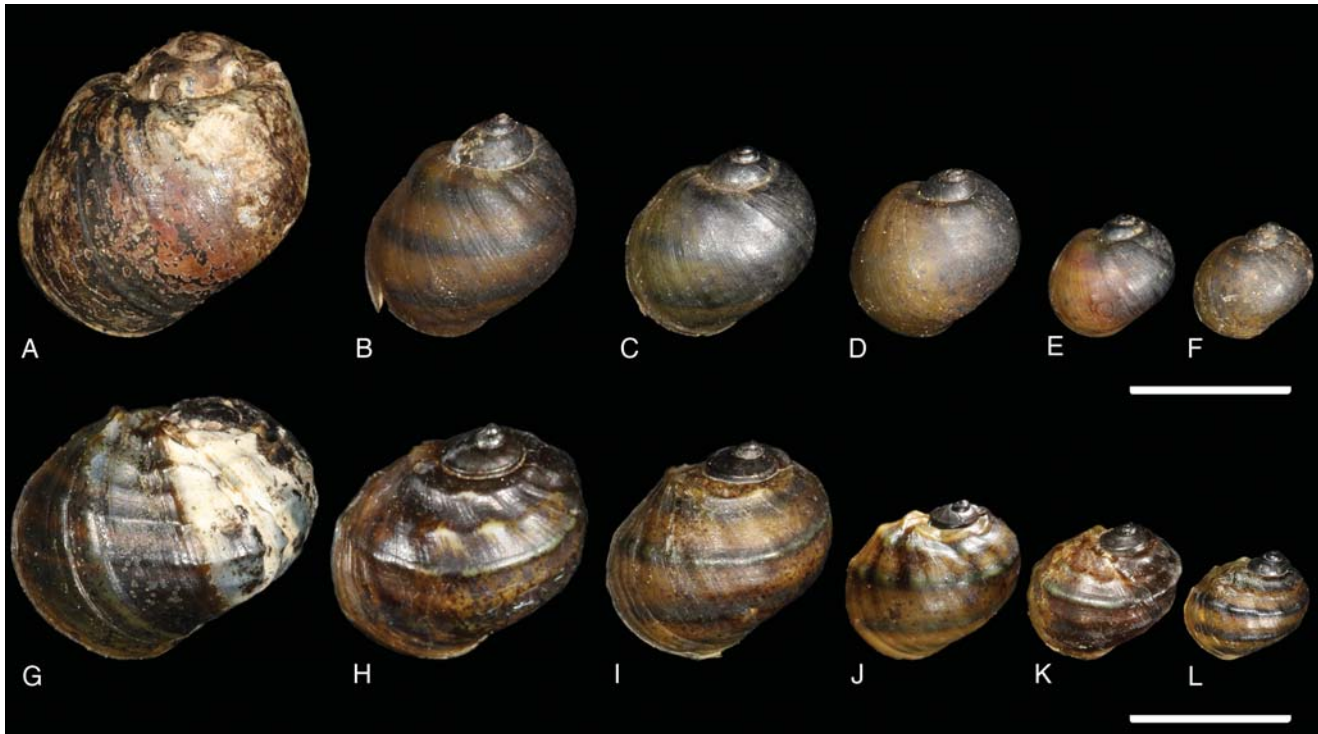
*Leptoxis ampla* is a federally threatened pleurocerid restricted to the upper Cahaba River and certain tributaries in central Alabama. Throughout most of its range the shell morphology is smooth (Fig. 1), but one population from the lower Little Cahaba River in Bibb County, AL, USA, possesses a distinct

shell phenotype characterized by 3–4 carinae on the upper part of each whorl (Fig. 1). Based on analyses of *L. ampla* populations cultured in a uniform environment we show that the presence or absence of this discrete character is the result of heritable genetic differences rather than ecophenotypic plasticity.

The carinate form of *L. ampla* from the Little Cahaba River was described as *L. mimica* (Goodrich, 1922) based on the presence of its distinctive carinae (Fig. 1). This was later synonymized with *L. ampla* (Fig. 1), because of similarities in overall shell morphology (Burch & Tottenham, 1980). Populations above and below the confluence of the Little Cahaba River and Cahaba River possess a smooth shell morphology. The carinate form was historically found in both the Little Cahaba River and Six Mile Creek in Bibb County, AL, USA (Goodrich, 1922), but recent survey work indicates that it is now restricted to the lower Little Cahaba River (unpublished ADCNR survey data). The morphological pattern seen in *L. ampla* is not an example of clinal variation as in, for example, *Lithasia geniculata* (Minton *et al.*, 2008) or *Io fluviatilis* (Adams, 1915), since there is no intergradation in morphology and the carinate form is restricted to a single tributary near the centre of the species' historical range.

In mid-March 2010, we collected 45 adult individuals of *L. ampla* from the Cahaba River with smooth shells and 45 individuals from the Little Cahaba River with carinate shells and placed them into artificial culture systems at the Alabama Aquatic Biodiversity Center (AABC) in Marion, AL, USA (see Table 1 for locality information). No differences in body pigmentation or shape of egg clutches were observed between populations. Fifteen snails (three replicates per population) were placed in 20-l acrylic tanks equipped with 0.84-cm bulkhead fittings allowing constant exchange of well water acclimated to ambient outdoor temperature (*c.* 8 l/h). Each 20-l culture tank had water from a shared source, but independent aeration and drainage. A 15 l/min powerhead attached to each tank lid created a constant flow regime. Females in all treatments attached eggs to tank walls in concentric clutches from late March to early June 2010.

Within 90 d of hatching, cultured juveniles from each population showed the same phenotype as their parental population



**Figure 1.** Growth series of *Leptoxis ampla* showing the typical smooth form found throughout most of the species' range (A–F) and the carinate form found in the Little Cahaba River (G–L). A, G. Wild-type adults. B–F, H–L. Juveniles grown in culture. Individuals B and H are *c.* 7 months old with each smaller snail (C–F, I–L) decreasing in age by *c.* 1 month. All juveniles possess the shell phenotype of their parents. Scale bars = 5 mm.

**Table 1.** Locality information, GenBank accession numbers and registration numbers of specimen vouchers (National Museum of Natural History, Smithsonian Institution, USNM) for COI sequences of *Leptoxis ampla*.

Shell form	GenBank	Locality	USNM reg. no.	Degrees N	Degrees W
Smooth	JQ307001	Cahaba River, Bibb Co., AL, USA	1156983 1156984	33.07994	87.06680
Carinate	JQ307002	Little Cahaba River, Bibb Co., AL, USA	1156981 1156982	33.05373	87.06021

and this was retained after a year in culture (Fig. 1). Throughout the culture period, all individuals from the Little Cahaba River population possessed carinae. The number of carinae differed among juveniles, as also seen in the wild. No Cahaba River juveniles developed carinae. Variation was observed in the banding patterns on shells from both populations, but this is also seen in the wild. Clearly, ecophenotypic plasticity cannot explain the presence or absence of carinae, because water quality, substrate, absence of predators and flow conditions were identical in each tank.

Two individuals from each population were sequenced for the rapidly evolving cytochrome *c* oxidase I (COI) mitochondrial gene (Table 1). PCR followed the protocol of Graf & O'Foighil (2000), using the primers of Folmer *et al.* (1994); sequencing was done on an ABI 3100 automatic sequencer. Less than 1% sequence divergence (uncorrected p-distance) was found between populations and both individuals from the same population had identical haplotypes. Similarities in

egg-laying strategies, soft body pigmentation and mitochondrial DNA are consistent with both populations belonging to a single species. Additional studies with faster-evolving molecular markers (e.g. microsatellites or amplified fragment length polymorphisms) are needed to assess gene flow between populations and determine whether *L. ampla* from the lower Little Cahaba River represents a discrete Evolutionarily Significant Unit (ESU).

Our findings indicate that differences in shell morphology between *L. ampla* from the Cahaba River and *L. ampla* from the Little Cahaba River are heritable and genetically controlled. It is unclear what selective advantages, if any, each shell form provides to the populations studied. Future studies should analyse whether the carinate form provides protection from predators, as the spiny-shelled form does for *Potamopyrgus antipodarum* (Holomuzki & Biggs, 2006). It is also possible that the carinate form has arisen through genetic drift rather than natural selection. This is the first documentation in Pleuroceridae that a qualitative difference in shell morphology between populations of the same species is not the result of ecophenotypic plasticity.

The applicability of our findings for interpreting shell morphological variation in other pleurocerids is yet to be tested. Experiments are needed to assess whether clinal variation in *Io fluviatilis* (Adams, 1915) and *Lithastis geniculata* (Minton *et al.*, 2008) and morphological differences in other pleurocerids are the result of ecophenotypic plasticity (Dillon, 2011), are genetically controlled or are the result of a combination of the two mechanisms. Meanwhile, we suggest that ecophenotypic plasticity should not be the null hypothesis to explain shell differences in the Pleuroceridae. In light of our findings, taxonomic changes should not be based on untested hypotheses of ecophenotypic plasticity (e.g. Dillon, 2011; Dillon & Robinson, 2011).

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