A NON-OBLIGATE ASSOCIATION BETWEEN THE RED ALGA, *BOLDIA,* AND PLEUROCERID SNAILS

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ABSTRACT

An unusual freshwater red alga, *Boldia,* inhabits certain streams of the Southern Appalachians often growing upon the shells of three species of pleurocerid snails. While this alga-snail association is not obligate in all habitats, evidence suggests a long-term evolution of a protocooperative or commensalistic type bound to the seasonal reproductive cycles, development, and survival of the two organisms in simlar riffle habitats of Appalachian streams. The relatively high manganese content of both the snail periostracum and rocks to which *Boldia* was found attached, as well as other environmental features (i.e., photoperiod, water chemistry, and flow regime) suggest explanations for the habitat requirements and present distribution of this red alga.

Boldia is an unusual freshwater red algal genus which frequently develops on the shells of three species of snails, *Leptoxis (Mudalia) dilatata, L. cranata,* and *Oxytrema laqueata.*[1] During a three-year seasonal investigation of the systematics, distribution, and ecology of *Boldia* (Howard, 1977), the association of *Boldia* with various pleurocerid snails was often observed (Figure 1). While no obligate form of symbiosis was observed, it seemed unlikely that this polymorphic red alga would be able to maintain a population in streams without the snail association. Apparently, the alga-snail association is beneficial to one, if not both, organisms, and the association probably had a long evolutionary history within streams of the southern Appalachian Mountains.

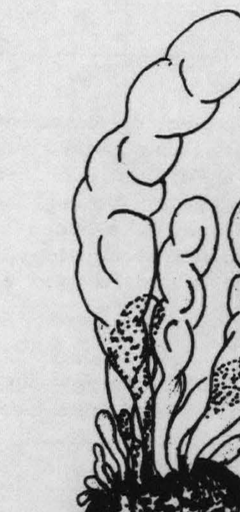


Figure 1. Ink drawing of *Boldia erythrosiphon* growing attached to *Leptoxis (Mudalia)* (bar-1 cm).

Presented here is a review of the current knowledge of this *Boldia* - pleurocerid snail association, including ideas on its origin and development. For brevity, relatively few methods which have been detailed in Howard (1977) are described in this paper. Other special methods will be noted in the results and discussion.

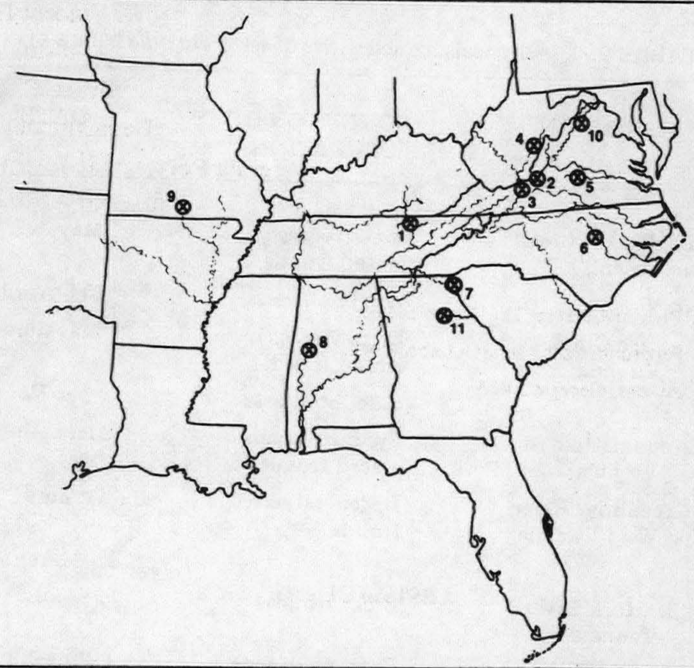
1 The nomenclature of the Pleuroceridae is confusing. Van der Schalie (pers. comm.) refers to the two genera associated with Boldia habitats as Nitocris and Goniobasis; Rosewater (pers. comm.) identified these as Mudalia and Oxytrema, while the Philadelphia Academy uses the names Mudalia and Goniobasis. We shall use Leptoxis (Mudalia) Rafinesque (1819) and Oxytrema Rafinesque (1819) respectively, based on the rule of strict priority as discussed by Parodiz (1956).

Habitat Description

Twelve known habitats from which *Boldia* has been collected since its first discovery in Big Walker Creek, Giles County, Virginia in 1958 (Herndon, 1964) are depicted in Figure 2. The North River, Tuscaloosa County, Alabama habitat has since been destroyed by impoundment. The Rain Bow Springs, Missouri habitat was discovered by Rufus Thompson (pers. comm.) and has not been visited during these investigations.

Figure 2. Map of southeastern U.S. showing locations of *Boldia* habitats and approximate % of observed fronds attached to snails (?% = insufficient collections).

(1) Clearfork R., Scott County, Tenn. (100%);
(2) Craig Cr. (80%) and Johns Cr. (10%), Craig County, Va.;
(3) Big Walker Cr., Giles County, Va. (75%);
(4) Greenbrier R., Greenbrier County, W. Va. (50%);
(5) Appomattox R., Buckingham - Prince Edward County Line, Va. (25%);
(6) Lower Barton Cr., Wake County, N.C. (5%);
(7) Ramsey Cr., Oconee County, S.C. (0%);
(8) North R., Tuscaloose County, Ala. (?%, site destroyed by inundation, not collected in this study);
(9) North Fork R., Texas County, Missouri (?%, not collected in this study);
(10) Passage Creek, Shenandoah County, Va. (5%);
(11) Cloud's Creek, Oglethorpe - Madison County Line, Georgia (5%).



The 12 habitats represent unglaciated streams which flow over rocks of lower Cambrian to Pennsylvanian age. While the surficial geology of these habitats often differed, a common feature was high calcium and magnesium content of the rocks. Analyses included routine water chemistry, plasma emission spectroscopy, and neutron activation analysis. The habitats showed widely variable $CaCO_3$ alkalinty, pH ranges of 7.0 - 8.5, and universal presence of Na, Ni, Sn, Al, Mn, Be, and Cu. Numerous streams lacking *Boldia* had either slightly acidic pH or lower levels of Ca, Mg, Na and Cu, with no detectable Ni. The *Boldia* streams were rich in aluminum, an element found to be abundant in the algal thallus by X-ray energy dispersive analysis (Table 1).

TABLE 1. X-ray energy dispersive analysis of *Boldia* and associated stream substrates showing the dominant, subdominant and trace elements detected in order of abundance.

SAMPLE	LOCATION	ELEMENT COMPOSITION	
		Dominant Element	Subdominant & Trace Elements
B. erythrosiphon	Lower Barton Creek North Carolina	S	Ca, Fe, Al, Si, Mg, P, K, Cl
B. erythrosiphon	Ramsey Creek South Carolina	S	Ca, Al, Si, Fe, Mg, P, K
Leptoxis (Mudalia) (periostracum)	Big Walker Creek Virginia	Mn	Ca, Si, Mg, Al, S, P, K, Na, Cl
Leptoxis (Mudalia) (inner surface of shell)	Big Walker Creek Virginia	Ca	Al, Si
Quartz (surface coating)	Appomattox River Virginia	Mn	Fe, Al, Si, Ca, S, K, Cl, Na
Mica-Schist (surface coating)	Lower Barton Creek North Carolina	Mn	Fe, Al, Si, Ca, S, K, Ti

Water temperatures varied seasonally among the habitats (Table 2) and values above 25 C were generally associated with growth cessation and degradation of *Boldia* thalli. Lower Barton Creek represented an exception to the seasonal development of the alga in that water temperatures were less extreme and *Boldia*, although reduced in abundance, was collected throughout the year.

TABLE 2. Physical characteristics of select *Bolodia* habitats (1975-1977).

LOCATION	CONDITION OF POPULATION	DATE	TEMPERATURE (C°)	pH	DISSOLVED OXYGEN (ppm)
Big Walker Creek Virginia	Erect to degenerated fronds	May-July	21.0-23.0	8.3-8.5	13.0
	Basal holdfasts	Feb-April	11.5-21.0	7.9-8.2	13.0
Passage Creek Virginia	Erect to degenerated fronds	May-July	17.0-22.5	8.0	14.0
	Basal holdfasts	Oct-Nov		7.1-7.2	
Appomattox River[1] Virginia	Erect to degenerated fronds	March-July	12.5-24.0	7.0-7.3	10.0-13.0
Greenbriar River West Virginia	Degenerated fronds	July	24.0-26.0	7.8	13.0
		March	9.5	7.2	
Clearfork River Tennessee	Erect fronds	July	22.0		15.0
Ramsey Creek South Carolina	Erect to degenerated fronds	June-July	18.5-22.0	7.1-7.4	13.0
	Basal holdfasts	March	8.0-11.0	7.1	
Lower Barton Creek North Carolina	Erect fronds	Feb-April	13.0-16.5	7.1-7.5	13.0
	Erect fronds	June	22		13.0
	Erect fronds	September	17.5		
Total Range			8.0-26.0	7.0-8.5	13.0-15.0

[1]Includes data from Dickson & Abbott, 1976.

Figure 2 presents the percentage of mature *Boldia* thalli attached to snails within the particular habitat, based on visual estimates. The alga-snail association was predominant in Clearford River (100 percent) and Big Walker Creek (75 percent), while in Lower Barton Creek and Passage Creek snails were rarely found with attached algal thalli. Of interest was the observation that *Boldia* in Craig Creek, Craig County, Virginia grew predominantly (80 percent) on smails with most of the remaining population attaching to relatively sparcely distributed rocks always having a black mineral coating. In contrast, nearby John's Creek contained numerous black mineral-coated rocks, upon whihc 90 percent of the resident algal population grew. X-ray energy dispersive analyses revealed that both the rock coatings and the periostracum of *Leptoxis (Mudalia) dilalata* contained manganese as the major elemental component.

Seasonal development of *Boldia* fronds occusrred in all streams esxcept in Lower Barton Creek, North Carolina, where fronds were collected on a year-round basis (Figure 3). Table 3 summarized the parallel development of alga and snail which seems to involve the attachment of monospores to the shells of young snails; *Leptoxis (Mudalia) dilatata* represents the predominant species. An account by Flint (1970) of the

TABLE 3. Seasonal development of snail and alga.

APPROXIMATE TIME OF YEAR	*LEPTOXIS (MUDALIA)**	*BOLDIA*
October-November	Mating Peak	---
March	Oviposit Peak	---
April-June	Young Snails	Frond Production Commences
June - July	Immature Snails	Peak Monospore Production
July-August	Immature Snails	*Boldia* Frond Degeneration
October-November	Mating Peak	*Boldia* Basal System Developing
April-July	Immature Snails	*Boldia* Frond Developing

*Approximate times based on our observations and taken from Van Cleave (1932) and Dazo (1965) for *Pleurocera acuta* and *Goniobasis livescens.*

Boldia holdfast dissolving a hole in the shell of the associated snail *(Viviparus),* followed by development of snail larvae within the algal thallus was never observed during these investigations. To our knowledge, the holdfast develops only on the manganese-rich periostracum of the three pleurocerid snail species mentioned earlier, not *Viviparus.* Furthermore, snails inside *Boldia* saccate thalli have never been observed during these investigations.

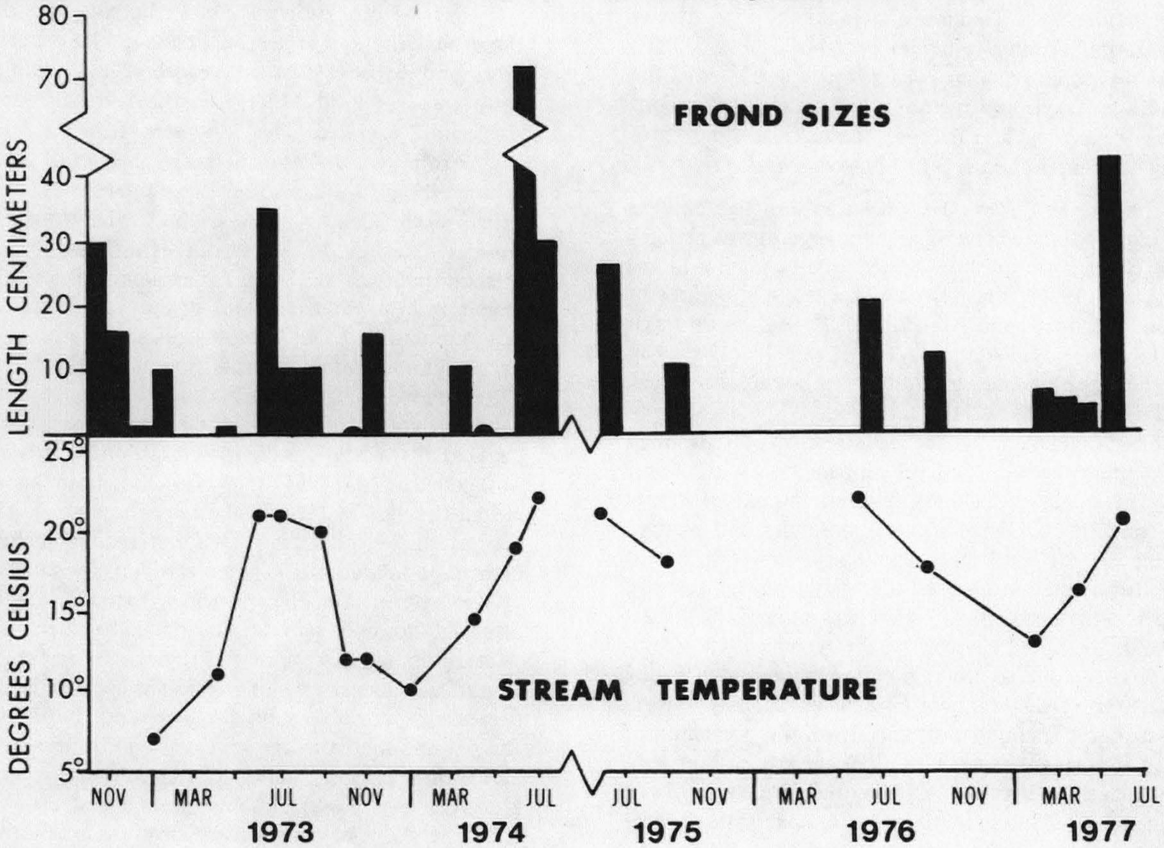


Figure 3. Relationship of *Boldia* frond length to water temperature in Lower Barton Creek, N.C. (semicircles designates occurrence of basal structure without erect fronds).

DISCUSSION

No evidence has been obtained to indicate a benefit to the snail resulting from its association with the alga. However, the apparent affinity of *Boldia* monospores for manganese-rich substrates may be a key to the development and perpetuation of this assocition. Parker *et al* (1973) reported an obligate association between a freshwater green alga *(Monostroma)* and black rocks rich in iron and manganese.

The evolution of the *Blodia*-snail association described herein is speculative; however, we have noted that the habitat requirements of the snails, *Leptoxis (Mudalia)* and *Oxtrema,* are very similar to those of *Boldia. Leptoxis* is restricted to flowing water saturated with oxygen, as is *Boldia. Oxytrema* can inhabit water of lower oxygen content, but *Boldia* has never been found under these conditions which may explain the preference of *Boldia* for the *Leptoxis (Mudalia) dilatata* and *L. carinata* complex.

Snails of the Pleuroceridae have left a fossil record dating to the lower Cretaceous Period with approximately 55 genera known through the Tertiary Period. *Oxytrema* is well represented from this period (Henderson, 19350; however, *Leptoxis (Mudalia)* is not know from the fossil record (D.W. Taylor, Pacific Marine Station, Dillion Beach, pers. comm.).

The red algae are an ancient group of plants based on the presence of phycobilin pigments, the absence of flagellated reproductive cells, and their presence in the marine fossil record (Smith, 1933; Dixon, 1973). It is not surprising that *Boldia* is not known in the fossil record because of its delicate, emphemeral frounds, which make fossilization unlikely.

The southern Appalachian and Ozark Mountains have existed as continously exposed land masses since the close of the Paleozoic Era. As such, they represent the oldest areas of North America with the possibility of a constant habitat. Steyermark (1959) stated that most endemic plant species of North America are restricted to geographic regions free from continental flooding or glaciation. Consequently, the abundance of endemic plants in the southern Appalachians, Cumberland Plateau, and the Ozarks is understandable. *Boldia* represents another example of this distributional pattern.

We hypothesize that *Boldia* represesnts an ancient form of Rhodophyta comparable to ancestral forms which have inhabited headwater streams of the southern Appalachians since Lower Cretaceous time. With similar environmental requirements as represented by present snail-algal associtions, and these conditions constantly satisfied by souther Appalachian streams, it is assumed that ancestral *Boldia* utilized shells of ancient pleurocerid snails as substrates fro the attachment and development of monospores. This substrate associtiion may have been accidental initially; however, if an alga with stream habitat requirements attached to a snail with similar requirements, its chances of remaining in headwater locations and thus within a refugium would be improved. The relationship apparently narrowed from snails of the Pleuroceridae to *Oxytrema* is known from the fossil record prior to the Pleistocene and because of the more compatible present day habitat requirements of *Leptoxis (Mudalia).*

During the Eocene Epoch, the Mississippi Embayment was reduced and the coastal plains began to emerge. It was perhaps because of changes in the drainage systems beginning in the Eocene (Ross, 1971) that *Boldia* exists in its present distribution. The Teays and Appalachian Rivers, ancestors of the New, Tennessee, and Alabama Rivers, were involved in an intricate exchange of streams to produce the present drainages, as detailed by Ross (1969, 1971).

Migration of divides and subsequent stream capture resulted from differential weathering of the Appalachian Mountains. The uplift of the Appalachians resulted in eastern slopes generally consisting of limestone and dolomite and western slopes composed of sandstones and quartizites. Downward weathering accelerated erosion of the eastern slopes, causing the divide to migrate westward as it was reduced in height (Hack, 1969). Erosional changes of the Appalachian Mountains resulted in an altered drainage system as westerly flowing streams were captured by the enlarging eastern drainage basin.

Stream capture, resulting primarily from the breaching of divides and subsequent reversal of drainage patterns, is thus the most plausible way to explain the present isolated populations of *Blodia.* Such chnges in drainage systems were primarily occurrences of the Cenozoic Era (Hack, 1969; Ross, 1967, 1971). Numerous regions of the southern Appalachians exist where divide migration and stream capture may have been instrumental in expending the range of *Boldia.* The Montgomery, Giles, and Craig County area of Virginia contains headwater streams of the New, Roanoke, and James Rivers and may have been a central point for distributing the alga about the state. The Boone region of North Carolina is surrounded by interlacing headwaters of the New, Yadkin, and Tennessee Rivers which flow in various directions to different drainages. In northern Georgia, headwater streams of the Tennessee, Alabama, and Savannah River systems are in close proximity to one another and stream piracies among them have occurred frequently (Ross, 1971).

The advance of Pleistocene glaciers never reached the locations of present day *Boldia* populations, and the alga may have inhabited streams of the northern Appalachians prior to glaciation. The use of the southern Appalachians as a botanical refugium during glacial advances has been noted by Braun (1951) and the similar condition of the Ozarks has been discussed by Steyermark (1959). Erosion following the Pleistocene glaciation resulted in the transfer of western drainage streams to the atlantic system (Ross, 1969). The modern drainage of southern Appalachian rivers and the present distribution of snail-algal associations probably date to the late Pleistocene.

A central location for the distribution of *Boldia* may have been the upper reaches of the Tennesee and New River drainages in southwestern Virginia and eastern Tennessee. The algal population in Scott County, Tennessee best represents a relic population because of the continous habitat offered by the Cumberland Plateau and the Association at this site of *Boldia* with the older snail genus *Oxytrema.*

Waterfowl are known to contribute to the dispersal of numerous genera of algae (Schlichting, 1958). Various ducks, coots and herons have been observed at *Boldia* habitats; however, it is doubtful that, if ingested, the alga could remain viable until excreted. The prostrate nature of *Boldia* during much of the year makes dispersal by the feet of birds unlikely, as does the alga's lack of desiccation resistant reproductive structures (Schlichting, pers. comm.). Therefore, while spores are easily washed downstream, it is difficult to envision any mechanism other than snails for explaining the alga's ability to remain in headwater locations or move upstream to establish new populations. The habitat in South Carolina at Ramsey Creek did not contain snails during our study. It receives 2.5 - 5.0 cm more precipitation per month than any other *Boldia* habitat (Smith, 1968). The original collection site in Ramsey Creek (Dillard, 1967) presently lacks *Boldia;* however, *Boldia* was collected one-half mile downstream in an area which lacked the alga in 1967 (Dillard, pers. comm.). These observations suggest that *Boldia* cannot readily maintain a permanent population site in streams which lack snails.

Monospores are the principal method of downstream dispersal; however, as spores or thallus fragments move downstream, dissolved oxygen tends to decrease as depth increases. Runoff also increases with a resultant increase in siltation and decrease in available sunlight. Increased runoff often lowers the pH and increases the nutrient load, especially in lowland agricultural areas; under such conditions green algae, bluegreen algae, and diatoms abound and usually eliminate red algae. Also, lowland stream locations are more susceptible to floodng and water temperatures are often higher than in upland streams. These conditions are less favorable for downstream expansion by *Boldia.*

The impoundment of rivers and steams not only limits acceptable habitats of *Boldia* but makes existence in mountain refugia difficult for both snail and alga. Notable was the impoundment of the North River in Alabama and the elimination of the *Boldia* population reported by Deason and Nichols (1970). Although human activities contribute to a decrease in potential habitats, the environmental requirements of *Boldia* are naturally limiting. Locations which satisfied the habitat requirements of *Boldia* are naturally limiting. Locations which satisfied the habitat requirements of *Boldia* may have existed to a greater extent in the past, but dramatic changes in lowland habitats outside the refugia of mountain streams have kept more extensive distributons in check.

The preceding discussion of the evolution and present distribution of the *Boldia*-snail association is admittedly speculative. However, we hope that this information will stimulate subsequent studies, including physiological culture studies to further identify the ecological characteristics of the *Boldia*-snail association.

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