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Phylogenetic relationships of the North American cyprinid subgenus *Hydrophlox*

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Notropis is one of the largest genera of North American fishes and is composed of a number of morphologically diagnosed subgroups; however, the validity of many has not been tested in a phylogenetic framework. One such subgroup is the subgenus *Hydrophlox*, which is composed of brilliantly colored species that engage in the symbiotic reproductive behavior of nest association. Although they have long been recognized as a cohesive group due to their nuptial coloration and fin tuberculation, very little is known about the relationships of species within *Hydrophlox*. We tested the monophyly of *Hydrophlox* using a mitochondrial marker (ND2) and two nuclear markers (ITS1 and RH), with Maximum Parsimony and Bayesian inference approaches. A well supported clade of "core" *Hydrophlox* was recovered and is composed of five taxa: *Notropis chiliticus, Notropis rubricroceus, Notropis lutipinnis, Notropis chlorocephalus,* and *Notropis chrosomus*. *Hydrophlox s.l.* is paraphyletic with respect to three taxa: *Notropis baileyi, Notropis leuciodus* and *Notropis nubilus*. While there was some discordance among the individual marker topologies, a combined evidence analysis recovered a topology that incorporated elements from all single-gene trees. Our analyses suggest that *Hydrophlox* is composed of five nominal species and additional undescribed diversity exists within this clade.

1. Introduction

Cyprinidae is the largest family of freshwater fishes in North America, encompasing over 50 genera. Nearly all of the approximately 300 North American species are members of subfamily Leuciscinae (Mayden, 1991; Nelson, 1994; Berra, 2001; Simons et al., 2003). Fossil evidence indicates that cyprinids have inhabited North America for over 31 million years (Cavender, 1991), and due to their significant diversification, resolving North American cyprinid relationships has been difficult (Cunha et al., 2002; Simons et al., 2003).

North American cyprinids exhibit astonishing taxonomic, morphological, behavioral, and ecological diversity, and have only recently become the focus of phylogenetic studies (Simons and Mayden, 1998; Broughton and Gold, 2000; Cunha et al., 2002; Simons et al., 2003; Schonhuth et al., 2008; Bufalino and Mayden, 2010). Of particular phylogenetic interest is the genus *Notropis*, which has been construed to include at least six recently segregated genera, and contains nearly 100 species (Gilbert, 1978; Bortone, 1989; Mayden, 1991; Warren et al., 1994; Wood et al., 2002) divided into three subgenera (*Notropis* [20 species], *Alburnops* [8 species], *Hydrophlox* [8 species]), at least three species groups (*Notropis texanus* species group [8 members], *Notropis volucellus* species group [10 members], *Notropis dorsalis* [6 species]) and a group which contains approximately 21 species whose relationships within *Notropis* are currently unresolved (Swift, 1970; Bortone, 1989; Mayden, 1991; Warren et al., 1994; Raley and Wood, 2001; Wood et al., 2002). *Notropis* has been the focus of few published molecular phylogenetic analyses (Bielawski and Gold, 2001; Raley and Wood, 2001; Schonhuth and Doadrio, 2003; Berendzen et al., 2008), all but the most recent using only mtDNA sequences (cyt*b*) or allozymes. The monophyly of genus *Notropis* has not been resolved, and not surprisingly, there is taxonomic volatility within and among subgenera and species groups.

Many members of the subgenus *Hydrophlox* are noted for their intense nuptial coloration and are among the 30% of North American minnows that engage in nest association (Outten, 1961; Johnston and Page, 1992; Clayton, 2000). This reproductive strategy, considered a specialized version of broadcast spawning, occurs when one species spawns in the prepared substrate (nest) of a host species (Johnston and Page, 1992). Despite years of observations, (e.g. Raney, 1947; Wallin, 1992; Fletcher, 1993; Johnston, 1994a,b; Johnston and Kleiner, 1994; Cochran, 2000), many aspects of this symbiotic relationship remain unknown. Understanding phylogenetic relationships among a monophyletic clade of minnows that engage in nest association provides a solid framework from which to begin investigations of the evolution of nest association in North American minnows.

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1.1. Taxonomic history and distribution

Since its description by David Starr Jordan (Jordan and Brayton, 1878), *Hydrophlox* has contained as many as 33 taxa (Swift, 1970). Characteristics such as nuptial coloration, size, and habitat were often used to include taxa within the subgenus. Swift (1970) redefined *Hydrophlox* and designated three species groups (Table 1) containing the following species: *Notropis rubricroceus* (Cope 1868), the type; *Notropis chiliticus* (Cope 1870); *Notropis chlorocephalus* (Cope 1870); *Notropis lutipinnis* (Jordan and Brayton 1878); *Notropis baileyi* Suttkus and Raney 1955; *Notropis leuciodus* (Cope 1816); *Notropis chrosomus* (Jordan 1877); *Notropis nubilus* (Forbs 1878); and *Notropis rubellus* (Agassiz 1850). *Hydrophlox* species were united by uniserial tubercles on pectoral fin rays, fine breeding tubercles over most of the body and head, and bright red, orange, and or yellow nuptial coloration. Although a cladistic analysis was not performed, Swift (1970) proposed relationships within and among his designated species groups of *Hydrophlox* based on distribution, ecology, and morphological characters (Fig. 1). Swift (1970) determined that members of the *texanus* species group were not closely related to *Hydrophlox* species; however, the placement of *Hydrophlox* within *Notropis* remains unknown as no sister-relationship hypotheses have been explicitly proposed.

Members of *Hydrophlox* are primarily found in fast-flowing streams of the southeastern United States (Fig. 2). Many are allopatric with a few notable exceptions: *N. baileyi* and *N. chrosomus* are sympatric in portions of the Alabama River system; *N. leuciodus* is sympatric with populations of *N. rubricroceus* in the French Broad River system and eastern portions of the Tennessee River drainage. Hybridization has been observed between *N. baileyi* and *N. chrosomus* (Boschung and Mayden, 2004) and also for *N. chiliticus* and *N. chlorocephalus* in areas where *N. chiliticus* populations have been introduced (Menhinick, 1991).

Notropis lutipinnisand *N. chlorocephalus* have been considered subspecies by some authors (Menhinick, 1991), based primarily on an apparent intergrade zone in the Broad River system in North Carolina. Wood and Mayden (1992), using allozyme data, found evidence for a polyphyletic *N. lutipinnis* in which Broad River system populations of *N. lutipinnis* are sister to *N. chlorocephalus*. They additionally found evidence for three diagnosable forms within *N. lutipinnis*, two of which remain undescribed.

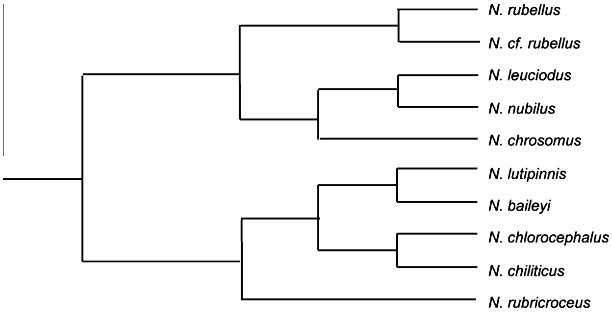

Fig. 1. Relationships of *Notropis* (*Hydrophlox*) inferred from Swift 1970.

Notropis nubilus was removed from the genus *Dionda* by Swift (1970) based on breeding coloration, tuberculation on body and head, large uniserial tubercles on pectoral rays, scalloped dorsolateral scales, low circumferential body scale count, and sharp predorsal line; however gut morphology, breeding biology, and distribution may not support this placement (Glazier and Taber, 1980; Fowler et al., 1984). In their examination of *Cyprinella*, Schonhuth and Mayden (2010) included *N. nubilus* in their analysis, the only such inclusion in a phylogenetic analysis of this species; however, the focus of their study was *Cyprinella* and placement of *N. nubilus* within *Hydrophlox* was not tested.

Mayden and Matson (1988), Humphries and Cashner (1994), and Bielawski and Gold (2002) found *N. rubellus* to be more closely allied with subgenus *Notropis* based on allozymes, morphology, and mtDNA, respectively. Even in Swift's (1970) designation, *N. rubellus* is distinct from all other *Hydrophlox* (Table 1). Swift also noted that *N. rubellus* exhibited considerable variation throughout its range and he suggested further examination before definitive inclusion into *Hydrophlox*. Currently, the *N. rubellus* complex contains five taxa: *N. rubellus* (Agassiz, 1850), *Notropis percobromus* (Cope, 1871), *Notropis micropteryx* (Cope, 1868), *Notropis suttkusi* Humphries and Cashner, 1994, and three undescribed forms allied with subgenus *Notropis* (Humphries and Cashner, 1994; Wood et al., 2002; Berendzen, et al., 2008; Berendzen et al., 2009).

The objectives of this study were to: (1) test the monophyly of *Hydrophlox* with one mitochondrial DNA (mtDNA) and three nuclear DNA (nucDNA) markers by including all putative members of the subgenus with representatives from multiple populations; (2) determine possible sister relationships between *Hydrophlox* and other members of the *Notropis* genus by selecting outgroup taxa from a broad sample of members of *Notropis*; and (3) investigate the relationships within *Hydrophlox* to account for cryptic or undescribed diversity and establish a hypothesis from which to explore the evolution of nest association within this group of colorful minnows.

Table 1
Species groups and character designations from Swift (1970) for members of the subgenus *Hydrophlox*.

Species group	Member taxa	Uniting characters
rubricroceus	*N. rubricroceus* (Cope, 1868) *N. chiliticus* (Cope, 1870) N. chlorocephalus (Cope, 1870) *N. lutipinnis* (Jordan and Brayton, 1878) *N. baileyi* Suttkus and Raney, 1955	Specialization of small, cool streams, with a gradient and turbulent habitats. Long fins; deep compressed body; crowded predorsal scales; rounded dorsolateral scales; high body circumference scale counts (25–30); high circumference caudal peduncle scale counts (12–18); strong predorsal stripe; lateral band present above and below lateral line canal; bright red, orange or yellow breeding colors with no iridescence
leuciodus	*N. leuciodus* (Cope, 1868) *N. chrosomus* (Jordan, 1877) *N. nubilus* (Forbes, 1878)	Adapted to fast waters in small to medium sized streams and steady flow habitats. Lower predorsal, body circumference, and caudal peduncle circumference scale counts; scalloped dorsolateral scales; thin predorsal stripe; unpigmented area above lateral line; melanophores on lateral line scales above and below lateral line: *N. leuciodus* with strong dashes, *N. chrosomus* and *N. nubilus* with "squarish blotches"; iridescent breeding coloration (though absent in *N. nubilus*)
rubellus	*N. rubellus* (Agassiz, 1850) and others	Terete body with well marked lateral canal; shares black lateral stripe, strong predorsal stripe, crowded predorsal scales, and high body circumference scale counts with rubricroceus species group. Distinct characters are: high anal ray count (9–12); high vertebral count; dorsal origin far behind pelvic origin; sharp snout; no caudal spot; small fins